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CONFERENCE PROGRAM

30 September 2018 – Sunday	
12:00-17:00	Registration
	School on Molecular Mechanisms of Aging and Age-Related Diseases.
13:00-13:15	Introductory lecture. Valentin Gordeliy “Old and New Ideas on Ageing”
13:20-13:45	Vladimir Anisimov "Gerontology in Russia: yesterday, today, tomorrow?"
13:50-14:50	Vladimir Anisimov “Aging and Age-Related Diseases”
14:55-15:55	Alexey Golubev “The main theories of the spans of life and aging must be mutually consistent”
15:55-16:25	Coffee Break
16:25-17:25	Olga Bocharova “Mechanisms of development, problems of early diagnostics, methods of treatment of neurodegenerative diseases on the example of Alzheimer's disease”
17:30-18:30	Claudio Francheschi “Inflammaging as a conceptual framework for aging and age-related diseases”

1 October 2018 – Monday	
08:00-09:55	Registration and welcome coffee
	Opening of the conference.
10:00-10:10	Welcome address from MIPT rectorate and the Board of the Research Center for Molecular Mechanisms of Aging and Age-Related Diseases of MIPT.
10:15-10:25	Raymond Stevens "Bridge Institute – Converging the Arts and Sciences for Scientific Integration"
10:30-10:40	James Liu "iHuman Institute – Virtual Human, Imaging Across the Scales"
10:45-10:55	Norbert Kucerka "Welcome address from JINR"
11:00-11:20	Keynote lecture: Sebastian Schmidt "Multi methods platform for analyses in Life Science"
11:25-11:50	Alain Milon "Structure and dynamics of receptor-bound neuropeptides"
11:55-12:20	Vladan Rankovic "Towards the optical cochlear implant: viral gene transfer into auditory neurons"
12:25-12:50	James Liu "Structural Biology Study of Human Cannabinoid Receptors"
12:55-13:20	Norbert Dencher "Challenge the «Free Radical Theory of Ageing» and the «Aβ peptide extracellular plaque hypothesis» of Alzheimer's disease"
13:20-14:50	Lunch
14:50-15:15	Mike Hanson "GPCR Consortium: Four years of progress in structural biology"

15:20-15:40	Vladimir Sokolov "Simultaneous analysis of multiple fluorescent proteins using acoustic flow cytometry"
15:45-16:20	Keynote lecture: Dieter Willbold "Structural characterization of Aβ species, their assemblies and intermediates – and how to develop a successful treatment strategy for Alzheimer's disease"
16:25-16:50	Wolfgang Voos "Molecular mechanisms of mitochondrial protein homeostasis in health and disease"
16:55-17:20	Adrian Mancuso "Serial crystallography at the European XFEL: First results and present opportunities"
17:25-17:50	Piotr Bregestovski "Light - efficient tool for control and analysis of nervous system"
17:50-18:25	Coffee Break and Poster Session
	School on Molecular Mechanisms of Aging and Age-Related Diseases.
18:25-19:25	Vladimir Skulachev "Identification of the first genes involved in the aging and anti-aging programs"
19:30-20:30	Leonid and Natalia Gavrilovy "Biodemography of Aging and Longevity"

2 October 2018 – Tuesday	
09:00-09:25	Thomas Gensch "Flavin-binding fluorescent proteins as genetically encoded photosensitizers"
09:30-09:55	Adam Round "Serial-serial crystallography, SAXS-WAXS, SPI and other future opportunities at the SPB/SFX instrument of the European XFEL"
10:00-10:25	Ivan Cornella-Taracido "Chemical biology approaches to interrogate the membrane proteome"
10:25-10:50	Coffee Break and Poster Session
10:50-11:25	Keynote lecture: Karl-Erich Jaeger "A novel membrane-bound phospholipase from the human pathogen *Pseudomonas aeruginosa*"
11:30-11:55	Albert Guskov "Electron microscopy of biomembranes and their components"
12:00-12:25	Thomas Hauss "Modulation of lipid membrane structure and dynamics in the presence of amyloid-ß peptide"
12:25-14:00	Lunch
14:00-14:25	Marat Yusupov "Application of the ribosome-inactivating small molecules"

14:30-14:50	Natalya Dudkina "Cryo-electron microscopy as a tool for studying biological macromolecules"
14:55-15:30	Keynote lecture: Markus Sauer "Single-molecule localization microscopy. Where next?"
15:35-16:00	Djordje Musil "Fragment-based Approach in the Drug Discovery Process"
16:00-16:25	Coffee Break and Poster Session
16:25-16:50	Francisco Rodriguez-Valera "Diversity of rhodopsins revealed by metagenomics"
16:55-17:20	Mike Heilemann "Counting subunits within membrane receptor complexes using single-molecule localization microscopy"
17:25-17:50	Martin Engelhard "Microbial Halorhodopsins: Light-Driven Chloride Pumps"
17:50-18:25	Coffee Break and Poster Session
	School on Molecular Mechanisms of Aging and Age-Related Diseases.
18:25-19:25	Joachim Altschmied "Telomerase – not only a «nuclear weapon»"
19:30-20:30	Yegor Yegorov "How to treat aging?"

3 October 2018 – Wednesday	
09:00-09:25	Alexander Molochkov "Gauge theory: protein topology and dynamics"
09:30-09:55	Roman Efremov "Proteins and membranes: born to be together. A computational view"
10:00-10:25	Alexander Gorskiy "Random network viewpoint on the creation of membrane inhomogenieties"
10:25-10:50	Coffee Break and Poster Session
10:50-11:25	Keynote lecture: Raymond Stevens "Efforts to model the pancreatic beta cell"
11:30-11:55	Volker Busskamp "Restoring visual function by optogenetics and non-coding RNAs"
12:00-12:25	Konstantin Lukyanov "Probes for high-photostability super-resolution fluorescence microscopy of live cells"
12:25-14:00	Lunch
14:00-14:25	Valentin Gordeliy "New Rhodopsins"
14:30-14:50	Stanislav Kukla "Duolink PLA Technology – A Powerful Tool for Study of Endogenous Protein Function"
14:55-15:30	Keynote lecture: Alexey Rak "Integrating novel biophysical approaches in the rational structure based drug discovery"

15:30-15:55	Coffee Break and Poster Session
15:55-16:20	Judith Haendeler “How caffeine fires up the powerhouses of the cells”
16:25-16:50	Manuel Etzkorn “Membrane interactions as modulators in protein aggregation and signaling”
16:55-17:20	Gleb Bourenkov “Towards the time-resolved synchrotron serial crystallography at PETRAIII”
17:25-17:50	Yurii Krupyanskii “Biocrystallization of the nucleoid of bacteria under stress. Possible XFEL studies”
17:50-18:25	Coffee Break and Poster Session
	School on Molecular Mechanisms of Aging and Age-Related Diseases.
18:25-19:25	Boris Vanyushin “Epigenetics and aging”
19:30-20:30	Vladimir Chupin “Lipid peroxidation and aging”
20:30-21:00	Round table: concluding remarks and discussion

4 October 2018 – Thursday	
09:00-09:25	Oxana Galzitskaya "Should the Treatment of Amyloidosis Be Personified? Molecular Mechanism of Amyloid Formation by Abeta Peptide and Its Fragments"
09:30-09:55	Andrei Gilep "Protein Family Based Approach in Drug Discovery: Cytochrome P450"
10:00-10:25	Alexander Popov "X-ray data collection strategy and radiation damage"
10:30-10:55	Wei Liu "GPCR structural studies by CryoEM and MicroED"
11:00-11:25	Gordon Leonard "Structural Biology at the ESRF: present and future"
11:25-11:50	Coffee Break and Poster Session
	Young scientists section
11:50-14:00	1. Vitaly Shevchenko "Optogenetic Tools: Hunting for the Rhodopsins " 2. Zhou Fang "Determination of an Underneath Binding in the Transmembrane Domain of Smoothened Receptor" 3. Jing Jing Wang "Activation and allosteric modulation of muscarinic acetylcholine M4 receptor " 4. Peng Yao "Structural Basis of G Protein Couple Receptor Polypharmacology" 5. Kate White "STMS:Cell – Spatiotemporal Multi-scale Modeling of Eukaryotic Cells" 6. Aleksandra Luginina "Effects of mono- and divalent cations on GPCR stability" 7. Anastasiia Gusach " Optimization of G protein-coupled receptor expression for functional studies" 8. Dmitry Zabelskiy "Functional and Structural Characterization of the OLPVR1 Viral Rhodopsin from Giant Virus in Antarctica" 9. Kirill Kovalev "Structural and functional features of the light-driven sodium pump KR2" 10. Ivan Maslov "Exploration of a conformational landscape for a membrane protein via single-molecule fluorescence microscopy" 11. Egor Marin "Successful GPCR structure determination using PAL XFEL"
14:05-14:20	Anton Chugunov "«Achilles Heel» of the Bacterial Membranes: Computational Modeling of Lipid II — Target for Novel Antibiotics"
14:25-14:40	Ivan Gushchin "Transmembrane Signal Transduction in Two-Component Systems"
14:40-16:00	Lunch
16:00-22:00	Social event

5 October 2018 – Friday	
09:00-09:25	Plamena Angelova “α-synuclein aggregation as a trigger for neuronal death in PD or how physiology becomes pathology”
09:30-09:55	Dmitry Melnikov "Basic topology of curves, polygons and long molecules"
10:00-10:25	Ulrike Alexiev “Membrane protein diffusivity and nanodynamics"
10:30-10:50	Demidenko Artem ”Modern and classic integral membrane proteins purification techniques”
10:50-11:15	Coffee Break and Poster Session
11:15-11:50	Keynote lecture: Ernst Bamberg “Rhodopsin based Optogenetics”
11:55-12:20	Christoph Fahlke “Molecular physiology and pathophysiology of K+ coupling in glutamate transporter”
12:25-12:50	Antti Niemi “Proteins and Time Crystals”
12:50-14:10	Lunch
14:10-14:45	Keynote lecture: Andrei Lupas “Axial helix rotation in transmembrane signal transduction”
14:50-15:15	Raul Gainetdinov “Trace Amines and Their Receptors”

15:20-15:45	Olga Sokolova “Multiple roles of cytoplasmic domains in voltage-gated ion channels”
15:50-16:15	Sergei Strelkov “Structure of intermediate filaments”
16:20-16:45	Igor Chizhov “A second wind: Photocycles of retinal proteins”
16:45-17:10	Coffee Break and Poster Session
17:10-17:35	Vladimir Chupin “Lipidic Nanocarriers for Drug Delivery”
17:40-18:05	Vadim Cherezov “A Decade of GPCR Structural Biology”
18:10-18:35	Georg Bueldt “Early Optogenetics transferring Bacteriorhodopsin into the Inner Mitochondrial Membrane and Observing Protein Synthesis at a Ribosome by Optical Tweezers”
18:40-19:05	Vanessa Maybeck “Engineering the Cell Membrane for Bioelectronics”
19:10-19:30	Conclusion remarks
19:30-22:00	Farewell party

PREFACE

It is for the fourth time MIPT holds the international conference BIOMEMBRANES in Dolgoprudny, Moscow District, Russia. The conference traditionally gathers world leaders from various fields of modern integrative structural biology with a major focus on scientific challenges in the field of biomembranes and membrane proteins and their role in aging and age-related diseases. A significant part of the conference program is devoted to structural methods, including rapidly developing cryoEM, XFELs and super-resolution fluorescent microscopy.

In BIOMEMBRANES'18, we draw particular attention to the transfer of scientific results to applications, and therefore, several talks are devoted to optogenetics and medical research. We also invited outstanding speakers from the international pharmaceutical industry who, in particular, will emphasize the role of structural biology in drug design.
We agreed with our partners that this conference is the first in a series of international events "Virtual Human - Imaging Across Scales". Following Dolgoprudny, consecutive events will be held later this year in Shanghai and California. Next year, Jülich/ Düsseldorf may also join this list.
The International School "Mechanisms of Aging and Age-Related Diseases" is taking place as a satellite event of the conference. The goal of the school is to give to the students and young researchers a general introduction to modern ideas in aging research. It was inspired by Prof. Vladimir Anisimov and further supported by Prof. Vladimir Skulachev, Prof. Boris Vanyushin, Prof. Claudio Franceschi and other prominent scientists who are actual speakers of the School.
The Conference is organized by the Laboratory for Advanced Studies of Membrane Proteins, the Laboratory for Structural Biology of GPCRs, the Laboratory for Chemistry and Physics of Lipids, the Laboratory for Structural Analysis and Engineering of Membrane Systems and the Laboratory for Ageing and Age-Related Neurodegenerative Diseases which are parts of the Research Center for Molecular Mechanisms of Aging and Age-Related Diseases at MIPT. Several research centers co-organize BIOMEMBRANES'18: the Research Center Jülich, the Bridge Institute at the University of South California, iHuman Institute at ShanghaiTech and the Joint Institute for Nuclear Research.
The Conference is greatly supported by the MIPT rector board, The Russian Academic Excellence Project 5-100, the Russian Foundation for Basic Research and our sponsors.
We greatly acknowledge scientists who are giving talks, presenting their posters and sharing the results of their beautiful research with the participants of the Conference and the School. Certainly, we are very grateful to all the participants for their active role. This conference is for you and we are happy if you enjoy it.

The Organizing Committee, the Program Committee

LECTURES

Structure and dynamics of receptor-bound neuropeptides

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G-protein-coupled receptors (GPCR) play an essential role in human physiology. They are prominent pharmacological targets and the understanding of molecular mechanisms underlying their activity is fundamental for the design of new drugs. Since a decade, a number of 3D structures have been elucidated, but sparse in complex with peptide agonists. We will present two examples of the characterization by liquid-state NMR of the structure and dynamics of neuropeptides bound to their receptor, dynorphin and ghrelin.

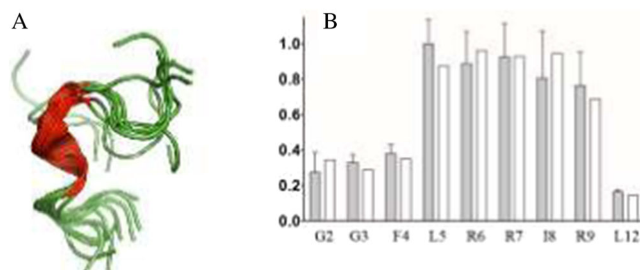

Fig. 1 (A) Receptor-bound conformation of dynorphin 1-13: a helical conformation is formed between F4 and R9; (B) order parameter profile of dynorphin N–H bonds in the receptor-bound state. *Grey:* experimental data; *white:* calculated S^2 profiles from molecular dynamics simulations of dynorphin–receptor complexes. Note that both the N- and C-termini remain flexible in the receptor-bound state

Dynorphin

The opioid receptor family contains four subtypes MOP, DOP, KOP and NOP, each activated by specific neuropeptides. The kappa-opioid receptor (KOP) mediates the actions of opioids with hallucinogenic, dysphoric and analgesic activities. Its endogenous agonist is dynorphin A, a 17 amino acid–long peptide, of sequence YGGFLRRIRPKLKWDNQ. The KOP 3D structure was first solved in 2012, KOP being in a complex with an antagonist, JDTic (1). More recently, a new KOP structure was solved, in the presence of a nanobody mimicking G-proteins and an agonist (2). These two structures reveal the major structural changes associated with KOP activation by an agonist and provide a wealth of detailed information on the molecular interactions driving these conformational changes. However, they do not fully explain the activation mechanism by dynorphin itself.

Using NMR, we characterized the structure and dynamics of dynorphin 1–13 (a shorter, fully active analogue of dynorphin A) bound to KOP, and in the absence of G-protein (3). Using transferred NOE experiments we observed the formation of a helical conformation extending from residue L5 to R9 (Fig. 1 (A)). ^{15}N relaxation rate measurements provided a profile of N–H vector order parameters along the peptide sequence (Fig. 1 (B)). This is a direct measure of the conformational flexibility of dynorphin bound to KOP. It confirmed the flexibility of the last four residues, which was expected in the context of the "message-address" concept (4). We also found that the first four residues retained a high degree of internal motion when bound to KOP. This was much less expected since it was known that the receptor–peptide recognition is extremely dependent on the exact sequence in the "message" part of opioid peptides, YGGFL. It provided the first accurate quantification of internal dynamics of a neuropeptide in its receptor-bound state. Receptor and peptide dynamics are known to be essential parameters of GPCRs activation (5) and one should consider this residual dynamics as an essential feature of the receptor activation mechanism.

Dynorphin is known to bind to KOP in its G-protein decoupled state with a 10-fold reduced affinity compared to its G-protein-coupled, fully activated state. It is thus expected that both the structure and dynamics of dynorphin should be different in the activated state. Based on the recent publication of the 3D structure of KOP bound to an agonist and a nanobody (2), we could extend our NMR observations to the ternary complex dynorphin—KOP—nanobody, and we will present the results during the BIOMEMBRANES'18 conference.

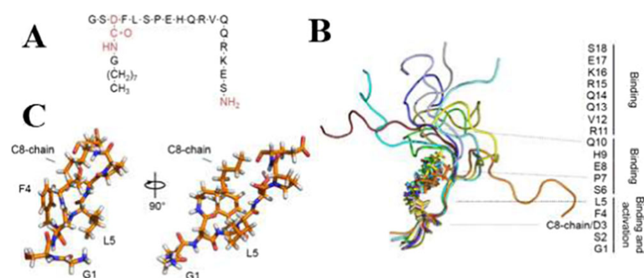

Fig. 2 (A) Peptide sequence of the Ghrelin analogue used in the present study. The differences with the natural sequence are highlighted in red. (B) Ghrelin receptor-bound conformation determined from trNOE data. (C) It is characterized by a well-defined hydrophobic core formed by the acyl chain (which is essential for ghrelin's activity) and the Phe4 and Leu5 side chains

Ghrelin

Ghrelin is a lipopeptide hormone involved in phenomena such as appetite, growth hormone secretion and reward-seeking behaviors. It is the natural ligand of the growth hormone secretagogue receptor (GHSR). A strategy similar to the one presented above was applied to the Ghrelin–GHSR pair. The perdeuterated receptor reconstituted into lipid nanodiscs after expression in *E. coli* (6) allowed studying Ghrelin in its bound state by liquid state NMR. We performed transferred ^{1}H NOE experiments to determine the hormone's conformation and we measured ^{15}N transverse relaxation to decipher the conformational flexibility along its sequence. Consistent with pharmacological data, the Ghrelin amino-terminal part folds with its acyl chain to form a hydrophobic core essential for GHSR binding and activation. In contrast, the carboxy-terminal part remains flexible and may participate in the binding with the receptor through electrostatic interactions. Furthermore, we combined NMR data with molecular dynamics simulations to build an ensemble of Ghrelin–GHSR complexes and thus reproduce the order parameter profile determined experimentally.

This approach provides the first active structure of Ghrelin and a novel insight into the molecular mechanisms responsible for its activity with respect to the activation of GHSR. Here again, the results were obtained in the absence of G-proteins, and extension of this work will involve using a ternary complex peptide—receptor—G protein (or a mimic of G proteins). Considering the pharmacological relevance of Ghrelin and its receptor, our results may lead to the design of new drugs with applications in the treatment of obesity, diabetes and addiction.

1.Wu, H., D. Wacker, M. Mileni, V. Katritch, G. W. Han, E. Vardy, W. Liu, A. A. Thompson, X.-P. Huang, F. I. Carroll, S. W. Mascarella, R. B. Westkaemper, P. D. Mosier, B. L. Roth, V. Cherezov, and R. C. Stevens. 2012. Structure of the human κ-opioid receptor in complex with JDTic. Nature 485:327.
.Che, T., S. Majumdar, S. A. Zaidi, P. Ondachi, J. D. McCorvy, S. Wang, P. D. Mosier, R. Uprety, E. Vardy, B. E. Krumm, G. W. Han, M. Y. Lee, E. Pardon, J. Steyaert, X. P. Huang, R. T. Strachan, A. R. Tribo, G. W. Pasternak, F. I. Carroll, R. C. Stevens, V. Cherezov, V. Katritch, D. Wacker, and B. L. Roth. 2018. Structure of the nanobody-stabilized active state of the kappa opioid receptor. Cell 172:55-+.
3.O'Connor, C., K. L. White, N. Doncescu, T. Didenko, B. L. Roth, G. Czaplicki, R. C. Stevens, K. Wuthrich, and A. Milon. 2015. NMR structure and dynamics of the agonist dynorphin peptide bound to the human kappa opioid receptor. Proc Natl Acad Sci U S A 112:11852-11857.
4.Schwyzer, R. 1986. Estimated conformation, orientation, and accumulation of dynorphin A-(1-13)-tridecapeptide on the surface of neutral lipid membranes. Biochemistry 25:4281-4286.
5.Hilger, D., M. Masureel, and B. K. Kobilka. 2018. Structure and dynamics of GPCR signaling complexes. Nat Struct Mol Biol 25:4-12.
6.Damian, M., S. Mary, M. Maingot, C. M'Kadmi, D. Gagne, J. P. Leyris, S. Denoyelle, G. Gaibelet, L. Gavara, M. Garcia de Souza Costa, D. Perahia, E. Trinquet, B. Mouillac, S. Galandrin, C. Gales, J. A. Fehrentz, N. Floquet, J. Martinez, J. Marie, and J. L. Baneres. 2015. Ghrelin receptor conformational dynamics regulate the transition from a preassembled to an active receptor:Gq complex. Proc Natl Acad Sci U S A 112:1601-1606.

Towards the optical cochlear implant: viral gene transfer into auditory neurons

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Although cochlear implants based on electrical stimulation of spiral ganglion neurons (SGNs) restore speech comprehension in most users, it is technically limited in frequency resolution due to the spread of current. The aim of the Göttingen Cochlear Optogenetics Program is to overcome this limitation by replacing electrical stimulation with spatially confined optogenetic stimulation of SGNs. To this end, we have successfully established virus-mediated transduction of SGNs with channelrhodopsins in rodents, followed by optogenetic stimulation of the auditory pathway. Red-shifted fast Chrimson version enabled SGN firing at near-physiological rates and activated several stages of the auditory pathway. Approximations of the spatial spread of cochlear excitation in the inferior colliculus in response to suprathreshold optical and electrical stimuli suggested a better frequency resolution for optogenetic than for electrical stimulation. We further characterized the induced percept by activation of neurons in the primary auditory cortex and analyzed a behavioral response in virus-injected gerbils based on a shuttle box paradigm. Behavioral thresholds for light intensity were found to be < 2mW, close to the threshold of neurons in the auditory cortex, and thresholds for light pulse duration were as short as 0.1 ms. Finally, we developed and inserted the first multi-channel LED-cochlear implant which enabled optogenetic stimulation of rodent SGNs. In conclusion, optogenetic stimulation of channelrhodopsin-transduced SGNs leads to physiological and behavioral responses with properties that promise potential for future application in clinical hearing restoration.

Structural biology study of human cannabinoid receptors

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In humans, GPCRs signal in response to a diverse array of stimuli including light molecules, hormones and lipids, where these signals affect downstream cascades to impact both health and disease states. Yet, despite their importance as therapeutic targets, detailed molecular structures of only ~ 50 unique GPCRs have been determined to date. A key challenge to their structure determination is adequate stable protein expression and crystallization. I will present the methods and technologies for obtaining human cannabinoid receptor CB_1 which are suitable for crystallization.
I will also present the crystal structures of human CB_1 in complex with different stabilizing ligands, designed, synthesized and characterized for the structural studies. The structure of the antagonist bound CB_1-AM6538

complex reveals an expansive and complicated binding pocket network consisting of multiple sub-pockets and channels to various regions of the receptor. The three-arm ligand structure is common to CB_1 antagonists and inverse agonists and may be critical for stabilizing the inherent flexibility of the native receptor in a non-signaling conformation. The structures of the agonist-bound CB_1-AM11542 and CB1-AM841 complexes (Hua et al., Nature 547(7664):468-471, 2017) uncovered significant conformational changes in the overall structure, relative to the antagonist-bound state (Hua et al., Cell 167(3): 750-762, 2016), including a 53% reduction in the volume of the ligand-binding pocket and an increase in the surface area of the G protein binding region. In addition, a "twin toggle switch" of $Phe200^{3.36}/Trp356^{6.48}$ is experimentally observed and appears to be essential in receptor activation. The structures reveal important insights into the activation mechanism of CB_1 and provide a molecular basis for predicting the binding modes of Δ^9-THC, the endogenous and synthetic cannabinoids. The plasticity of the binding pocket of CB_1 appears to be a common feature among certain class A GPCRs. These key findings should inspire the design of chemically diverse ligands with distinct pharmacological properties.

1. T. Hua, K. Vemuri, S. Nikas, R. Laprairie, Y. Wu, L. Qu, M. Pu, A. Korde, S. Jiang, J.H. Ho, GW. Han, D. Ding, X. Li, H. Liu, S. M. Hanson, S. Zhao, L. Bohn, A. Makriyannis, R Stevens. Liu ZJ. Crystal Structure of agonist-bound Human Cannabinoid Receptor CB1. Nature, 547(7664):468-471, 2017
2. T. Hua, K. Vemuri, M. Pu, L. Qu, GW. Han, Y. Wu, S. Zhao, W. Shui, S. Li, A. Korde, R. Laprairie, E. Stahl, J. Ho, N. Zvonok, H. Zhou, I. Kurareva, B. Wu, Q. Zhao, M. Hanson, L. Bohn, A. Makriyannis, R Stevens. ZJ. Liu. Crystal Structure of the Human Cannabinoid Receptor CB1. Cell, 167(3):750-762, 2016

Challenge the "free radical theory of ageing" and the "Aß peptide extracellular plaque hypothesis of Alzheimer's disease"

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We are searching for the common biology of health and diseases. If there is any, we might be able to understand and to manipulate those factors and mechanisms that determine, e.g. human life span and—even more important—health span as well as age-associated diseases. There is a wealth of information and strong scientific data accumulated in recent years that biological membranes are this common link. Therefore, with the unravelling of the structure, dynamics and physiology of all different cellular membranes at the molecular/atomic level in the near future, we can understand most of the vital cellular processes and can cure malfunctioning. Fortunately, we are close to the "Scientific Grail" of knowledge on biological membranes. This will be exemplified focusing on the involvement of membranes in ageing as well as in the neurodegenerative disease Alzheimer's dementia (AD). Biological membranes, the breakthrough in the evolution of cellular life, have developed into the largest and most active organelles/organs in every cell/organism. They perform energy-conversion, e.g. oxidative phosphorylation (OxPhos) in mitochondria and photosynthesis in chloroplasts, as well as signal transduction, but are also involved in numerous diseases as a primary trigger and/or target. Our research interest is especially devoted to mitochondrial membranes. Why mitochondria? They are enveloped even by two structurally and functionally different membrane systems. The surface area of the inner mitochondrial membrane in humans is 14000 m^2. By OxPhos protein complexes/supercomplexes residing therein, ~70 kg ATP per day are synthesized. In addition, mitochondria are involved in apoptosis (the process of programmed cell death), regulation of cellular metabolism including synthesis of certain steroids and lipids, and processing and storage of calcium and copper, but are also the main generators and targets of free radicals and reactive oxygen species [1, 2]. Therefore, mitochondria play a central role for the cell in almost all organisms.

Ageing and calorie restriction modulate the mitochondrial proteome, the protein oxidation profile and the metabolism in defined brain areas

Ageing is the progressive loss of function accompanied by decreasing fertility as well as increasing morbidity and finally mortality with advancing age. The causes and mechanisms of ageing are still enigmatic. Therefore, understanding molecular processes underlying ageing remains a challenge. The process of ageing is controlled by a complex network of pathways in which those related to mitochondrial functions are of paramount importance. We are applying a variety of techniques to unravel the molecular mechanisms of ageing as well as of Alzheimer's and Parkinson's disease and the involvement of biological membranes [1, 3-7].

It is well established that mitochondria are one of the most adversely affected organelles during ageing. There is increasing evidence that mitochondria even have a key role in causing ageing. Many theories are trying to explain the cause of ageing [8,9]. The "free radical theory of ageing" postulated by D. Harman in 1956 [10] is currently still the most popular ageing theory.
Age-dependent changes in the cellular proteome (amount and interaction of proteins, post-translational modifications, enzymatic activities) are currently considered as targets and even triggers of ageing and of age-associated diseases. Therefore, we have investigated the role of the mitochondrial proteome in conserved mechanisms of ageing. The observed modulation of the mitochondrial proteome, OxPhos supercomplex (respirasome) architecture and activity by age, reactive oxygen species (ROS) and nutrition gives insight into the involvement of mitochondrial metabolism in life and health span. Our focus is on brain ageing. Age-dependent changes of defined brain areas were analysed [3-6]. Among different regions, the hippocampus is distinct as compared to the cortex [3] and striatum (as well as to the heart and liver).
The mitochondrial and cellular proteome of various ageing model organisms with defined mitochondrial aetiology of ageing, at different age and/or nutritional status, were analysed by 2D native/SDS-PAGE [11,12], by mass spectrometry and by enzyme activity measurements. It is well established that controlled reduction of the intake of calories results in prolongation of health and life span. By age and by calorie restriction (CR), induced changes in the composition, amount and oxidative status of mitochondrial proteins in specific rat brain areas and liver were analyzed. Pronounced age- and CR-related changes in the abundance of MF_oF_1

ATP synthase as well as of OxPhos complexes and supercomplexes (natural assemblies of the complexes I, III and IV into stoichiometric entities, such as $I_1III_2IV_{0-4}$) were observed in all tissues and organisms studied. In liver tissue, CR lowers, e.g. OxPhos complex I assembly and complex IV activity as well as promotes the formation of OxPhos supercomplexes [13,14]. Obtained data allow to understand the interplay between protein activity, energy conversion, oxidative stress and ageing as well as intervention by CR. Overall, CR aids minimization of ROS formation in rat liver mitochondria, especially via modulation of the properties of OxPhos complex IV, while the antioxidant response is less involved [13].

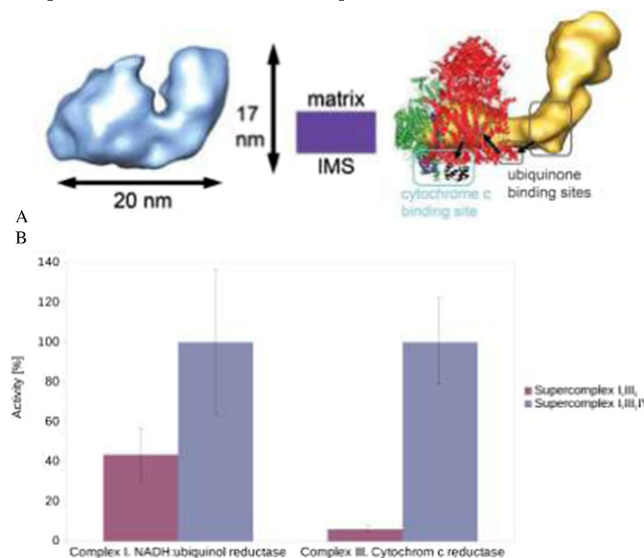

Fig. 1 (A) Side view of the 3D map of supercomplex $I_1III_2IV_1$ as seen along the membrane plane. The location of the membrane in the side view is displayed in purple. IMS: intermembrane space. The three individual complexes as they would assemble to form supercomplex $I_1III_2IV_1$. The electron microscopic 3D map of complex I filtered to 5.0 nm is shown in yellow, the X-ray structure of complex III_2 (PDB ID 2A06) is displayed in red, and the X-ray structure of complex IV (PDB ID 1OCC) is shown in green (from ref. [1]). (B) Comparison of activity of the isolated bovine heart OxPhos supercomplex I_1III_2 and $I_1III_2IV_1$. For complex I, the inhibitor (rotenone)-specific NADH:ubiquinol reductase activity, and for complex III, the inhibitor (antimycin)-specific cytochrome c reductase activity, were determined. Complex I in supercomplex I_1III_2 displayed only about half the activity of that in supercomplex $I_1III_2IV_1$. Complex III was highly active in supercomplex $I_1III_2IV_1$, but in supercomplex I_1III_2 showed only minor cytochrome *c* reductase activity. Data plotted from ref. [15]

With the advent of the first 2D and 3D structures of active supercomplexes composed of OxPhos complexes I and III as well as of I, III and IV [1,15,16] (Fig. 1 (A)) also their superior enzymatic activities were observed [15]. The activities of OxPhos-complexes assembled as supercomplexes are 2 to > 15 [4,6,15] times higher than those of the respective individual complexes (Fig. 1 (B)); therefore, not solely the amount but also the proportion of the individual OxPhos complexes vs. specific OxPhos supercomplexes and the activity of OxPhos (super)complexes determine the overall performance of the respiratory chain/of ATP generation. This has to be considered for a systems biological description of energy transformation as well as for elucidation of the molecular basis of ageing and age-associated neurodegenerative diseases. Alterations occur during ageing in fungi, worms and rats, as well as in human cells and affect mitochondrial structure and function. The observed age-associated alterations in the amount and composition of super-assemblies [1,3] will have a strong impact on energy [4,13,15] and ROS [17] metabolism. They play a significant role in the physiology of ageing as well as in the pathophysiology of Alzheimer's and Parkinson's disease [1,3-6]. Unfortunately, the knowledge of age- and disease-induced modulation of the membrane lipidome (amount, composition, oxidative status of the membrane lipids) is currently poor, but urgently required since lipids are irreplaceable as, e.g. membrane protein environment and second messengers.

In contradiction to the predictions of the "free radical theory of ageing", mitochondrial proteins of the rat cortex exhibited less oxidative modifications (protein carbonylation) in aged rats compared to young rats (Monika Frenzel und Norbert A. Dencher, unpublished results 2011). Supporting this initial observation, by our recent large-scale iTRAQ proteomics analysis, no pronounced increase in protein oxidation during ageing both in brain and heart mitochondria was observed (Carina Ramallo Guevara, Ansgar Poetsch und Norbert A. Dencher, unpublished results 2016), challenging the "Mitochondrial Free Radical / Reactive Oxygen Species Theory of Ageing" (in the strict sense of elevated levels of oxidatively damaged proteins at advanced age as cause of cell death/ impairment). However, beyond doubt, free radicals, ROS and RNS (reactive nitrogen species) (and therefore antioxidants too) are of uppermost importance for cell survival and disease onset [18].

Cell organelles and membranes as targets of Alzheimer's disease-triggering amyloid beta peptides

Alzheimer's disease is one of many age-associated diseases, but the most common dementia in elderly (60–80% of all dementia in the currently about 47 million cases worldwide). The highest risk factor for AD is age. If no efficient therapy is developed soon, 120 million AD patients have been predicted for the year 2050. Therefore, it is important to understand the molecular mechanisms leading to this currently incurable neurodegenerative disease. It is most likely that AD is triggered by amyloid-β (Aβ) peptides with 38 to 43 amino acids that are derived from the amyloid precursor protein APP residing in membranes.

Different mechanisms of the contribution of Aβ peptides in AD have been proposed. There are still discussions on whether Aβ fibrils, which form extracellular plaques, or amyloid beta monomers and oligomers are the major contributor in the pathogenesis of AD. According to the "amyloid hypothesis", aggregates of amyloid fibrils that are deposited outside neurons in dense formations (senile or neuritic plaques) are the causative agent of AD, possibly in combination with neurofibrillary tangles (NFTs). Neuron loss, vascular damage and dementia follow Aβ peptide deposition. However, cases of AD without plaques or their presence in non-demented people have also been reported. Contrary to the extracellular plaque hypothesis, still favoured by most researchers in the field, even Aß monomers are bioactive via insertion into membranes [7, 19-22].

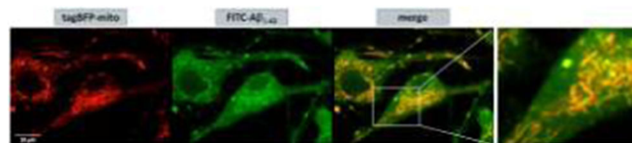

Fig. 2 Intracellular localization of $A\beta_{1-42}$ peptide. OLN-93 cells showed a pronounced colocalization of FITC labeled $A\beta_{1-42}$ (green) and mitochondria (tagBFP-mito, red) 15 h after treatment, triggered/induced by currently not understood events (Victoria Decker, Tobias Meckel and N.A.D. unpublished data 2016)

In order to demonstrate cellular and organelle trafficking of Aß peptides, to identify their target(s) and to analyze their deleterious

effects on cell and membrane function, in our studies, Aß$_{42}$ peptide monomers/small oligomers were externally applied to mammalian cells (human neuroblastoma cell line and rat oligodendroglia cell line). (In general, for meaningful translation of knowledge gained on cell cultures to tissues, especially in respect to metabolism and to oxidative stress responses, the oxygen conditions in the cell culture should be comparable to the levels in human body tissues, e.g. 5% in the brain and not the atmospheric 21% oxygen usually administered [7,23]). Monomeric/oligomeric peptides entered cells, as proven in our investigation by confocal fluorescence microscopy with Aβ$_{42}$ peptides labeled with one of four different fluorophores. We were able to track in time and space the pathway of the Aß peptides from the outside of the cell across the plasma membrane to internal target membranes of specific organelles. In this way, for both cell lines, we did prove that fluorescently labeled Aβ peptides in minutes co-localized with the plasma membrane (Podolyak E.Y., unpublished results 2018) and thereafter entered the cells and trafficked to organelles, e.g. predominantly to endosomes/lysosomes [7]. Although often it has been proposed that mitochondria are a central target of Aß peptides (as summarized in the informative review by Pagani and Eckert, ref. [24]), only on rare occasions we could observe pronounced co-localization of Aß peptides and mitochondria, and solely in rat oligodendroglia cell line (Fig. 2).

However, even in the predominant cases without any visible co-localization, mitochondrial parameters and metabolism were affected upon application of Aß peptides, indicating that minor amounts of the Aß peptides are bioactive in this organelle, e.g. by forming channels. The deep insertion of Aß peptides into the lipid bilayer, that subsequently induced membrane perturbations, was verified by neutron scattering [20-23], AFM force spectroscopy [22] and fluorescence polarization. All our results are in line with and do indicate at the molecular level that Aß peptides intercalate into membranes, perturb the structure of the lipid bilayer, modulate lipid dynamics, induce membrane fusion and, in this manner, will lead to malfunctioning and finally to death of cells. These data challenge the "Aß peptide extracellular plaque hypothesis of AD". Monomeric/oligomeric Aß peptides affected numerous physiological cell parameters, such as ROS concentration, protein oxidation (carbonylation), viability (necrosis/apoptosis), mitochondrial membrane potential, membrane viscosity as well as mitochondrial DNA amount [7]. Therefore, we predict that medical interventions to disassemble extracellular Aß peptide plaques in human brains, leading to the release of monomeric/oligomeric Aß peptides, will be counterproductive, even harmful. Hopefully, the gained knowledge of Aß peptide–induced changes in the biochemical and biophysical properties of membranes contribute to our understanding of the pathology of Alzheimer's disease as well as being a clue for early diagnosis and efficient therapy.

1. Seelert H, Dani DN, Dante S, Hauss T, Krause F, Schäfer E, Frenzel M, Poetsch A, Rexroth S, Schwassmann HJ, Suhai T., Vonck J., Dencher N.A. From protons to OXPHOS supercomplexes and Alzheimer's disease: structure dynamics–function relationships of energy-transducing membranes. *Biochimica Et Biophysica Acta (BBA)-Bioenergetics* 1787.6 (2009): 657-671.
2. Lichtmannegger J, Leitzinger C, Wimmer R, Schmitt S, Schulz S, Kabiri Y, Eberhagen C, Rieder T, Janik D, Neff F, Straub BK, Schirmacher P, DiSpirito AA, Bandow N, Baral BS, Flatley A, Kremmer E, Denk G, Reiter FP, Hohenester S, Eckardt-Schupp F, Dencher NA, Adamski J, Sauer V, Niemietz C, Schmidt HHJ, Merle U, Gotthardt DN, Kroemer G, Weiss KH, Zischka H. Methanobactin reverses acute liver failure in a rat model of Wilson disease. *The Journal of clinical investigation* 126.7 (2016): 2721-2735.
3. Frenzel M, Rommelspacher H, Sugawa MD, Dencher NA. Ageing alters the supramolecular architecture of OxPhos complexes in rat brain cortex. *Experimental gerontology* 45.7-8 (2010): 563-572.
4. Kuter K, Kratochwil M, Berghauzen-Maciejewska K, Głowacka U, Sugawa MD, Ossowska K, Dencher NA. Adaptation within mitochondrial oxidative phosphorylation supercomplexes and membrane viscosity during degeneration of dopaminergic neurons in an animal model of early Parkinson's disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1862.4 (2016): 741-753.
5. Kuter K, Kratochwil M, Marx SH, Hartwig S, Lehr S, Sugawa MD, Dencher NA. Native DIGE proteomic analysis of mitochondria from substantia nigra and striatum during neuronal degeneration and its compensation in an animal model of early Parkinson's disease. *Archives of physiology and biochemistry* 122.5 (2016): 238-256.
6. Wernicke C, Hellmann J, Zięba B, Kuter K, Ossowska K, Frenzel M, Dencher NA, Rommelspacher H. 9-Methyl-β-carboline has restorative effects in an animal model of Parkinson's disease. *Pharmacological Reports* 62.1 (2010): 35-53.
7. Džinić, Tamara, and Norbert A. Dencher. Oxygen concentration and oxidative stress modulate the influence of Alzheimer's disease Aβ1–42 Peptide on Human Cells. *Oxidative medicine and cellular longevity* 2018 (2018).
8. Skulachev VP, Bogachev AV, Kasparinsky FO. Concept of aging as a result of slow programmed poisoning of an organism with mitochondrial reactive oxygen species. *Principles of Bioenergetics*. Springer, Berlin, Heidelberg, 2013. 305-353.
9. Parkhitko AA, Favorova OO, Khabibullin DI, Anisimov VN, Henske EP. Kinase mTOR: regulation and role in maintenance of cellular homeostasis, tumor development, and aging. *Biochemistry (Moscow)* 79.2 (2014): 88-101.
10. Harman D. Aging: a theory based on free radical and radiation chemistry. *Journal of gerontology.* (1956) 11, 298-300
11. Dani, Diksha, and Norbert A. Dencher. Native-DIGE: A new look at the mitochondrial membrane proteome. *Biotechnology Journal: Healthcare Nutrition Technology* 3.6 (2008): 817-822.
12. Diksha D, Dencher NA. Native DIGE: Efficient tool to elucidate protein interactomes. *Difference Gel Electrophoresis. Humana Press,* New York, NY, 2018. 53-68.
13. Dani D, Shimokawa I, Komatsu T, Higami Y, Warnken U, Schokraie E, Schnölzer M, Krause F, Sugawa MD, Dencher NA. Modulation of oxidative phosphorylation machinery signifies a prime mode of anti-ageing mechanism of calorie restriction in male rat liver mitochondria. *Biogerontology* 11.3 (2010): 321-334.
14. Kim SE, Mori R, Komatsu T, Chiba T, Hayashi H, Park S, Sugawa MD, Dencher NA, Shimokawa I. Upregulation of cytochrome c oxidase subunit 6b1 (Cox6b1) and formation of mitochondrial supercomplexes: implication of Cox6b1 in the effect of calorie restriction. *Age* 37.3 (2015): 45.
15. Schäfer E, Seelert H, Reifschneider NH, Krause F, Dencher NA, Vonck J. Architecture of active mammalian respiratory chain supercomplexes. *Journal of Biological Chemistry* 281.22 (2006): 15370-15375.
16. Schäfer E, Dencher NA, Vonck J, Parcej DN. Three-dimensional structure of the respiratory chain supercomplex $I_1III_2IV_1$ from bovine heart mitochondria. *Biochemistry* 46.44 (2007): 12579-12585.
17. Maranzana E, Barbero G, Falasca AI, Lenaz G, Genova ML. Mitochondrial respiratory supercomplex association limits production of reactive oxygen species from complex I. *Antioxidants & redox signaling* 19.13 (2013): 1469-1480.
18. Feniouk BA, Skulachev VP. Cellular and molecular mechanisms of action of mitochondria-targeted antioxidants. *Current aging science* 10.1 (2017): 41-48.
19. Dante S, Hauss T, Dencher NA. Insertion of externally administered amyloid β peptide 25− 35 and perturbation of lipid bilayers. *Biochemistry* 42.46 (2003): 13667-13672.
20. Dante S, Hauß T, Brandt A, Dencher NA. Membrane fusogenic activity of the Alzheimer's peptide Aβ (1–42) demonstrated by small-angle neutron scattering. *Journal of molecular biology* 376.2 (2008): 393-404.
21. Dante S, Hauß T, Steitz R, Canale C, Dencher NA. Nanoscale structural and mechanical effects of beta-amyloid (1–42) on polymer cushioned membranes: a combined study by neutron reflectometry and AFM Force Spectroscopy. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1808.11 (2011): 2646-2655.

22. Barrett MA, Trapp M, Lohstroh W, Seydel T, Ollivier J, Ballauff M, Dencher NA, Hauß T. Alzheimer's peptide amyloid-β, fragment 22–40, perturbs lipid dynamics. *Soft Matter* 12.5 (2016): 1444-1451.
23. Džinić T, Hartwig S, Lehr S, Dencher NA. Oxygen and differentiation status modulate the effect of X-ray irradiation on physiology and mitochondrial proteome of human neuroblastoma cells. *Archives of physiology and biochemistry* 122.5 (2016): 257-265.
24. Pagani L, Eckert A. Amyloid-Beta interaction with mitochondria. *International Journal of Alzheimer's Disease* 2011 (2011).

Structural characterization of amyloid beta species, their assemblies and intermediates—and how to develop a successful treatment strategy for Alzheimer's disease

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Oligomeric amyloid β (Aβ) is suspected to be the most toxic species in Aβ aggregation and responsible for the development and progression of Alzheimer's disease (AD). We have investigated their formation by AUC, SANS and NMR spectroscopy [1-3]. I will summarize some of our results and also report the first high-resolution structure of the Aβ(1–42) fibril that will possibly allow rational drug design in the future [4]. Development of compounds that are able to eliminate already formed, toxic Aβ oligomers is very desirable. In recent years, we have developed all-D-enantiomeric peptides that have proven to be able to directly and specifically eliminate toxic Aβ oligomers in vitro. Such all-D-peptides combine the advantages of small molecules with the specificity of peptides. They are orally available and non-immunogenic. The in vivo proof of concept for "D3", the lead compound of this development, was accomplished in several treatment studies [5-8]. Here, we describe in vivo efficacy of the improved D3 derivative "PRI-002" (alias RD2) [9-12]. PRI-002 is able to reverse the cognitive deficit and to significantly reduce Aβ pathology even in old-aged transgenic AD mice with full-blown pathology and deficits, even after oral administration. PRI-002 has proven to be fully blood-brain barrier penetrable and demonstrated target engagement in vitro and in vivo, in particular by showing significant reduction of Aβ oligomers in the brains of RD2-treated compared to placebo-treated mice. The correlation of Aβ elimination in vivo and the reversal of cognitive deficits in old-aged transgenic mice are in support of Aβ oligomers being relevant not only for disease development and progression but also for Aβ oligomers as a promising target for the causal treatment of AD. I will summarize preclinical efficiency data with successful in vivo proof-of-concept in four treatment studies in three different transgenic animal models in three different laboratories. Based on very favourable properties in preclinical and safety and toxicology, we started a first-in-human phase I clinical trial to show safety also in humans. I will report the first clinical data.

1. Dammers et al., Biophys. J. 112, 1621-1633 (2017).
2. Dammers et al., Chem. Science. 8, 4996-5004 (2017).
3. Wolff et al., Sci. Rep. 7, 2493 (2017).
4. Gremer et al., Science 358, 116-119 (2017).
5. Funke et al., ACS Chem. Neurosci. 1, 639-648 (2010).
6. Brener et al., Sci. Rep. 5, 13222 (2015).
7. Jiang et al., PLoS One 10, e0128553 (2015).
8. Elfgen et al., Eur. J. Pharm. Sci. 107, 203-207 (2017).
9. Leithold et al., Pharm Res. 33, 328-336 (2016).
10. van Groen et al., Sci. Rep. 7, 16275 (2017).
11 Kutzsche et al., Molecules 22, 1693 (2017).
12 Schemmert et al., Mol. Neurobiol. *in press* [2018]

Molecular mechanisms of mitochondrial protein homeostasis in health and disease

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Mitochondria represent essential components of eukaryotic cells, providing energy and molecules for the cellular metabolism, as well as participating in crucial signaling processes. Mitochondrial dysfunction has been implicated in a wide array of human pathologies, ranging from diabetes, cancer and, in particular, neurodegenerative diseases. It is clear that the maintenance of mitochondrial integrity and function, summarized by the term "homeostasis", is playing an essential for cellular survival. A detailed biochemical characterization of the molecular mechanisms underlying mitochondrial protein homeostasis is therefore crucial for the understanding and treatment of human disease. In respect to the endosymbiotic origin of mitochondria, protein homeostasis is based on a balanced equilibrium between protein biogenesis and the removal of damaged polypeptides from the organelle. In particular, under stress conditions, this equilibrium will become unbalanced, resulting in mitochondrial dysfunction and the correlating diverse pathologies. Based on the biological importance of the organelle, cells have evolved specific biochemical processes to maintain mitochondrial protein homeostasis [1]. On the level of individual polypeptides, mitochondria contain a set of molecular chaperones and ATP-dependent proteases that may directly repair and prevent an accumulation of misfolded polypeptides. If this primary defense level is overcome, organellar damage can be prevented by (i) a sequestration and neutralization of aggregated polypeptides in specific intramitochondrial protein quality control compartments (IMiQ) [2]; (ii) a genetic upregulation of the expression of protein quality control enzymes, called mitochondrial unfolded protein response (mtUPR); and (iii) a removal of terminally damaged mitochondria by a specific form of autophagy, called mitophagy.
In my presentation, I will give examples of typical mitochondrial damage control processes. First, I will discuss how cellular aggregates, in this case β-amyloid peptide aggregates, formed in patients with Morbus Alzheimer, might influence mitochondrial biogenesis processes [3]. Second, I will show how mitochondria are able to sequester intra-mitochondrial aggregates, and third, I will review the signaling processes involved in the specific degradation of damaged mitochondria, a process involved in the etiology of Morbus Parkinson.

1. Voos, W. *et al.* Quality control at the mitochondrion. *Essays Biochem.* **60**, 213 (2016).
2. Bruderek, M. *et al.* IMiQ: a novel protein quality control compartment protecting mitochondrial functional integrity. *Mol. Biol. Cell* **29**, 256 (2018).
3. Cenini, G. *et al.* Amyloid β-peptides interfere with mitochondrial preprotein import competence by a co-aggregation process. *Mol. Biol. Cell* 27, 3257 (2016).

Serial crystallography at the European XFEL: first results and present opportunities

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Fig. 1 The SPB/SFX Instrument in preparation for a serial crystallography experiment

Serial crystallography at X-ray Free-Electron Lasers (XFELs) [1] has proven to be a valuable addition to the array of structure determination techniques presently available, particularly for time-resolved studies involving either photo-sensitive systems [2,3], radiation damage–sensitive sample [4] or potentially for systems involving the mixing of substrate with a system under study [5]. The European X-ray Free-Electron Laser [6] presents a new and expanded capability to perform serial crystallography experiments. This is not just because additional XFEL sources create a higher availability of experimental time but also because the European XFEL offers the highest repetition rate of XFEL pulses of all XFELs with orders more pulses per unit time.
In this presentation, I will outline the experimental capabilities of the single particles, clusters and biomolecules and serial femtosecond crystallography (SPB/SFX) instrument [7] of the European XFEL (EuXFEL), an instrument designed to predominantly support structural biology applications. I will show a selection of results from the first serial femtosecond crystallography experiments at the EuXFEL which demonstrate that we can successfully exploit the megahertz repetition rate of EuXFEL for structural biology [8,9] and give insights into future experiments that may be performed at the SPB/SFX instrument.

1. Chapman, H. N. *et al.* Femtosecond X-ray protein nanocrystallography. *Nature* **470,** 73 (2011).
2. Nango, E. *et al.* A three-dimensional movie of structural changes in bacteriorhodopsin. *Science* **354,** 1552–1557 (2016).
3. Tenboer, J. *et al.* Time-resolved serial crystallography captures high-resolution intermediates of photoactive yellow protein. *Science* **346,** 1242–1246 (2014).
4. Suga, M. *et al.* Native structure of photosystem II at 1.95 Å resolution viewed by femtosecond X-ray pulses. *Nature* **517**, 99–103 (2014).
5. Schmidt, M. Mix and Inject: Reaction Initiation by Diffusion for Time-Resolved Macromolecular Crystallography. *Advances in Condensed Matter Physics* **2013,** 1–10 (2013).
6. Tschentscher, T. *et al.* Photon Beam Transport and Scientific Instruments at the European XFEL. *Applied Sciences* **7,** 592–35 (2017).
7. Mancuso, A. P., Reimers, N., Borchers, G., Aquila, A. & Giewekemeyer, K. *Technical design report: Scientific instrument Single Particles, Clusters, and Biomolecules (SPB)*. (European XFEL, 2013).
8. Wiedorn, M., Oberthuer, D., et al, Nature communications, accepted.
9. Grünbein, M., et al, Nature communications, **9**, 3487 (2018).

Light-efficient tool for control and analysis of the nervous system

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In recent years, three new areas were developed, which use the light for investigation the functions of cells, to control neuronal activity and neuronal networks, to measure the concentration of ions and other cellular compounds and to control the behaviour of living organisms, as well as to search for new ways to treat certain diseases. These areas are *optogenetics*, *optosensorics and optopharmacology*.
Optogenetics—using of photosensitive transmembrane bacterial proteins capable to cause excitation or inhibition of cellular activity under illumination by a different wavelength.
Optosensorics—using specific genetically encoded biosensors for non-invasive analysis of intracellular, concentration of ions and other cytoplasmic components. Biosensors are macromolecular protein constructs that have fluorophore groups capable to change selectively fluorescence upon interaction with specific ions, molecular groups, or proteins. Main categories of genetically encoded biosensors are as follows: (a) consisting of a single fluorescent protein sensitive to a specific molecule or ion (single FP-sensors); (b) consisting of two fluorescent proteins and working either on the principle of ratiometric analysis or resonance energy transfer from the donor fluorescent protein to the acceptor (FRET-sensors).
Optopharmacology—is based on the ability of certain molecules (azobenzenes, spiropiranes, diarylethenes) to change their conformation upon illumination with the light of a specific wavelength. Optopharmacological compounds are chemical constructs, which consist of the following: (i) photoswitch, capable to change conformation or charge distribution upon illumination; (ii) molecule capable specifically modulate a function of a target protein, i.e. agonists or antagonists, and, in some cases, (iii) anchor molecule (frequently, maleimide, capable of forming covalent bonds with cysteines), for increasing photochrome specificity. Photochromic molecules represent unique tools for spatially and temporally precise control of numerous biological processes, including neuronal activity. Different types of photoswitchable regulators have been designed and characterized for a large number of ligand-gated receptors in the mammalian nervous system [1,2].
Recent achievements and future perspectives of optopharmacology in the light-induced modulation of receptor-operated channels, particularly, Cys-loop receptors, as well as molecular designs of photoswitches will be discussed.

1. Kramer, R.H., Mourot, A. and Adesnik, H., (2013). Optogenetic pharmacology for control of native neuronal signaling proteins. *Nature neuroscience*, *16*(7), p.816-823.
2. Bregestovski, P., Maleeva, G. and Gorostiza, P., 2018. Light-induced regulation of ligand-gated channel activity. *British journal of pharmacology*, *175*(11), pp.1892-1902.

Flavin-binding fluorescent proteins as genetically encoded photosensitizers

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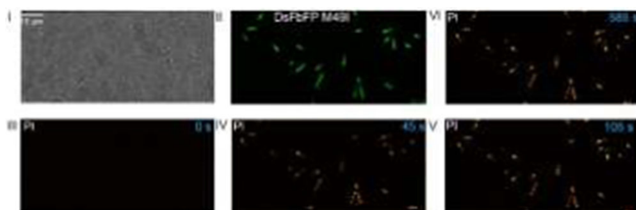

Fig. 1 Images of DsFbFP M49I expressing *E. coli* cells before blue light irradiation (I, bright field; II, DsFbFP fluorescence emission; III propidium iodide (PI) fluorescence emission) and PI emission images after different blue light exposures (λ_{illu} = 460–490 nm; P = 102 mW cm^{-2}) (IV, 45 s; V, 105 s; VI, 585 s; adapted from reference [9])

Genetically encoded fluorescent labels, sensors and activators based on fluorescent proteins from the family of green fluorescent proteins (GFP) have revolutionized live cell imaging since 1994—the year of ground-breaking studies by the labs of Martin Chalfie and Roger Tsien [1,2]. More than 10 years ago, the palette of fluorescent proteins had been extended by a widespread, small-protein domain found in many sensory and photosensory proteins, the so-called LOV-domain (LOV: light-oxygen-voltage). LOV-domains bind noncovalently—often with very high affinity—endogenous flavins, most prominently flavin mononucleotide (FMN). LOV-domains enter a relatively simple photocycle upon blue-light illumination that lasts for seconds to days, where the signaling state characterized by the formation of a covalent bond between the chromophore and a nearby cysteine is formed in microseconds. The conformational changes in the signaling state lead to a change of interactions with other protein and LOV dimerization probability.

Since more than 10 years, modified LOV-domains (flavin-binding fluorescent proteins (FbFPs)) have been used as fluorescent reporters [3,4,5,7,8], where the single conserved cysteine residue is mutated that is responsible for the transient covalent bond to flavin chromophore in the signaling state. FbFPs show good fluorescence properties (Φ_{fl} = 0.15–0.45) and offer the same advantages over fluorescent organic dye molecules as fluorescent proteins from the GFP protein family, e.g. targeting to specific cell types and cell compartments, control of expression onset and level and possibility of chronic observations. While FbFP fluorescence is limited to the blue-green part of the spectrum and their brightness is lower compared to GFPs, FbFPs are superior under conditions of limited O_2 levels, i.e. in bacterial cell cultures or certain cell tissues (e.g. tumors) due to the fact, that GFP-like fluorescent proteins need O_2 for chromophore formation, while FbFPs need only the presence of FMN or similar flavins, which is given for all cell types and organisms tested so far.

Despite the term "fluorescence" in their name, FbFPs show another prominent activity based on their rich photophysics, i.e. they usually have a sizeable if not high triplet-state formation quantum yield and therefore can generate reactive oxygen species (ROS) like singlet molecular oxygen (O_2 $^1\Delta_g$) or superoxide radical ($O_2^{\cdot-}$). ROS react with and modify proteins, lipids RNA/DNA and other essential molecules of cells eventually leading to cell killing [6,9,10]. By doing so, certain FbFP variants with high ROS yields are highly toxic to cells under blue light illumination.

In this contribution, data on the phototoxicity of a number of FbFPs towards bacteria and mammalian cells as well as relevant photophysical parameters with low to high photodamaging capabilities will be presented. The relevant fluorescence microscopy and optical spectroscopy techniques will also be described.

1. Chalfie, MGFP: Lighting up life (Nobel Lecture). *Angew. Chem. Int. Ed.* 48: 5603-5611 (2009).

2. Tsien, R.Y. Constructing and exploiting the fluorescent protein paintbox (Nobel Lecture). *Angew. Chem. Int. Ed.* 48: 5612-5626 (2009).

3. Drepper, T., Eggert, T., Circolone, F., Heck, A., Krauß, Guterl. J.-K., Wendorff, M., Losi, A., Gärtner, W. and Jaeger, K.-E. Reporter proteins for in vivo fluorescence without oxygen. *Nat. Biotechnol.* 25:443-445 (2007).

4. Drepper, T., Gensch, T., and M. Pohl, M. Advanced in vivo applications of blue light photoreceptors as alternative fluorescent proteins. *Photochem. Photobiol. Sci.* 12:1125-1134 (2013).

5. Wingen, M., Potzkei, J., Endres, S., Casini, G., Rupprecht, C., Fahlke, C., Krauss, U., Jaeger, K.-E., Drepper, T., and Gensch, T. The photophysics of LOV-based fluorescent proteins – new tools for cell biology. *Photochem. Photobiol. Sci.* 13:875-883 (2014).

6. Torra, J., Burgos-Caminal, A., Endres, S., Wingen, M., Drepper, T., Gensch, T., Ruiz-González, R., and Nonell, S. Singlet oxygen photosensitisation by the fluorescent protein Pp2FbFP L30M, a novel derivative of Pseudomonas putida flavin-binding Pp2FbFP. *Photochem. Photobiol. Sci.* 14:280-287 (2015).

7. Wingen, M., Jaeger, K.-E., Gensch, T., and Drepper, T. Novel thermostable flavin-binding fluorescent proteins from thermophilic organisms. *Photochem. Photobiol.* 93:849-856 (2017).

8. Rupprecht, C., Wingen, M., Potzkei, J., Gensch, T., Jaeger, K.-E., and Drepper, T. A novel FbFP-based biosensor toolbox for sensitive in vivo determination of intracellular pH. *J. Biotechnol.* 258:25-32 (2017).

9. Endres, S., Wingen, M., Torra, J., Ruiz-González, R., Polen, T., Bosio, G., Bitzenhofer, N., Hilgers, F., Gensch, T., Nonell, S., Jaeger, K.-E., and Drepper, T. An optogenetic toolbox of LOV-based photosensitizers for light-driven killing of bacteria. *Sci. Rep.* (2018) *under review.*

10 Shu, X., Lev-Ram, V., Deerinck, T.J., Qi, Y., Ramko, E.B., Davidson, M.W., Jin, Y., Ellisman, M.H., Tsien, R.Y. A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms. *PLoS Biol.* 9:e1001041 (2011).

Serial-serial crystallography, SAXS-WAXS, SPI and other future opportunities at the SPB/SFX instrument of the European XFEL

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The European X-ray Free-Electron Laser (XFEL) [1] along with its first instruments has successfully completed the first round of experiments. This facility as a world leading source of ultrabright, ultrashort, coherent X-ray pulses now continues its development to facilitate more ambitious experiments at the operational instruments as well as additional instruments with more diverse capabilities. Thanks to the high repetition rate and multiplexing, simultaneous experiments can be undertaken. This not only means multiple instruments in operation simultaneously but as only a tiny fraction of the intensity in each pulse interacts with the sample with the remaining majority of the intensity can be reused. By refocusing the pulses to a second interaction region to perform another SFX experiment we can more efficiently use the FEL pulses delivered to the Single Particles, Clusters and Biomolecules and Serial Femtosecond Crystallography (SPB/SFX) Instrument [2].

This lecture will present the key ideas and the technical design for serial-SFX as well as the use of the downstream detector to extend the range of length scales observable at the SPB/SFX instrument. I will also outline the facilities supporting users from preparation to execution of their experiments showing how we aim to facilitate the user's science at the European XFEL facility.

1. Abela, R. et. al. XFEL: The European X-ray Free Electron Laser - technical design report (2006) https://doi.org/10.3204/DESY_06-097.

2. Mancuso, A. P., Aquila, A., Borchers, G. et al. Technical design report scientific instrument single particles, clusters, and biomolecules. SPB (2013) https://doi.org/10.3204/XFEL.EU/TR-2013-004.

Atomistic details of the mechanisms of membrane remodelling by phospholipase A in bacteria: implications for virulence adaptation and biofilm formation

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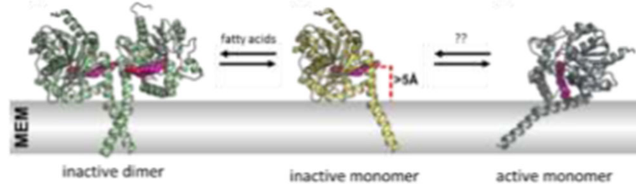

Fig. 1 Phospholipase activity of PlaF located at the cytoplasmic membrane (MEM) of *P. aeruginosa* is regulated by dimerization. Fatty acids (C11 and C14) are labeled in purple; a bound detergent molecule (β-octyl-glucoside) in orange

Phospholipases A, conserved among all domains of life, have essential functions for every living organism including membrane and energy homeostasis pinpointing them at the interface of cell sensing and metabolism. However, the mechanisms by which bacterial pathogens use intracellular phospholipase A to alter their own membranes and adapt themselves for virulence are largely unknown.

Here, we describe PlaF [1], a phospholipase A located in the cytoplasmic membrane of the opportunistic human pathogen *Pseudomonas aeruginosa*. We have identified this enzyme as one of the major virulence factors in a *Drosophila melanogaster* infection model as well as an important determinant affecting bacterial biofilm formation. A combination of in vivo lipidomic analysis and in vitro enzymology showed that PlaF modifies the membranes by hydrolyzing bacterial phospholipids. In vivo cross-linking and in vitro protein-protein interaction experiments indicated a regulation of PlaF activity through reversible dimerization. Furthermore, the observed negative feedback regulation by fatty acid products that induce dimerization suggested a role of PlaF in membrane turnover. Using comparative proteomics, we have identified a number of proteins linked to biofilm formation and virulence whose function is likely regulated by PlaF-mediated modulation of the membrane phospholipid composition.

The solved X-ray structure of dimeric PlaF is a unique structure of a full-length membrane protein revealing for the first time interactions of N-terminal transmembrane helices and their role for protein dimerization and for localization to the membrane. Structural analysis and molecular dynamic simulations of PlaF in a phospholipid bilayer allowed us to propose a mechanism for PlaF activation induced by monomerisation followed by a structural rearrangement of monomeric PlaF at the membrane surface which results in accessibility of the active site cavity for membrane phospholipids (Fig. 1).

1. Kovacic F, Bleffert F, Caliskan M, Wilhelm S, Granzin J, Batra-Safferling R, Jaeger KE (2016) A membrane-bound esterase PA2949 from *Pseudomonas aeruginosa* is expressed and purified from *Escherichia coli*. FEBS Open Bio. 6(5):484-493

Structural studies on SLC1 transporters

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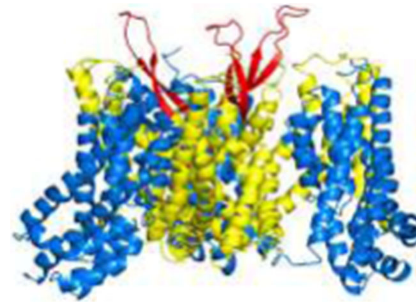

Fig. 1 The general organization of ASCT2 trimer: scaffold and transport domains in yellow and blue respectively; the β-hairpin elements responsible for viral recognition are highlighted in red

SLC (Solute Carrier) transporters form one of the largest superfamilies of membrane-embedded transporters, comprised of 52 families, with more than 400 unique proteins [1]. These transporters are crucial for maintaining homeostasis of numerous chemicals (amino acids, metal ions, vitamins, organic ions, sugars, etc.) within mammals, and malfunctioning of these transporters usually causes severe complications, rendering these transporters as possible drug targets [2]. The SLC1 family has a prominent role in the homeostasis of glutamate—a major neurotransmitter in higher eukaryotes. In mammals, this family consists of seven members, five of which are capable to pump glutamate against its gradient using the energy stored in the sodium gradient (excitatory amino acid transporters, EAAT1-5, [3]) and two transporters perform the neutral amino acid exchange (ASCT1-2, [4]). Intriguingly, the expression of the ASCT2 transporter is linked to the proliferation of many kinds of cancer [5], and furthermore, it serves as a recognition partner for many retroviruses [6]. To understand how these transporters work and what are the structural determinants of their malfunction and associated diseases, the structures of these transporters are essential. The structural biology has been instrumental in improving our understanding of SLC1 family in many aspects. During the past decade, about 20 structures of homologous proteins GltPh and GltTk have been published (reviewed in [7]), which revealed the general organization of these transporters, and suggested the novel (elevator-like) transport mechanism and resolved the tight coupling between the transported substrate and sodium ions. Recently, we have published the first structure of the human ASCT2 transporter [8], which has provided the first glimpses into the structural basis of viral recognition and substrate exchange.

1.Perland, E. & Fredriksson, R. Classification systems of secondary active transporters. *Trends in Pharmacological Sciences* **38,** 305–315 (2017).
2.Lin, L., Yee, S. W., Kim, R. B. & Giacomini, K. M. SLC transporters as therapeutic targets: emerging opportunities. *Nat Rev Drug Discov* **14,** 543–560 (2015).
3.Vandenberg, R. J. & Ryan, R. M. Mechanisms of glutamate transport. *Physiological Reviews* **93,** 1621–1657 (2013).
4.Kanai, Y. *et al.* The SLC1 high-affinity glutamate and neutral amino acid transporter family. *Mol. Aspects Med.* **34,** 108–120 (2013).

5.Liu, Y. *et al.* The role of ASCT2 in cancer: A review. *Eur. J. Pharmacol.* (2018). https://doi.org/10.1016/j.ejphar.2018.07.007

6.Shimode, S., Nakaoka, R., Shogen, H. & Miyazawa, T. Characterization of feline ASCT1 and ASCT2 as RD-114 virus receptor. *J. Gen. Virol.* **94,** 1608–1612 (2013).

7.Arkhipova, V., Guskov, A. & Slotboom, D. J. Analysis of the quality of crystallographic data and the limitations of structural models. *J. Gen. Physiol.* **149,** 1091–1103 (2017).

8.Garaeva, A. A. *et al.* Cryo-EM structure of the human neutral amino acid transporter ASCT2. *Nat. Struct. Mol. Biol.* **25,** 515–521 (2018).

Modulation of lipid membrane structure and dynamics in the presence of amyloid-ß peptide

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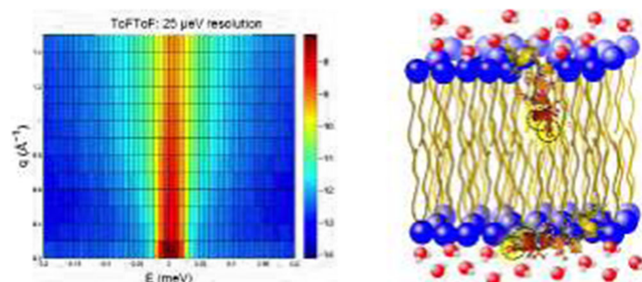

Fig. 1. A typical QENS spectrum (right, here ToFToF, MLZ, FRM2, Garching, Germany) to determine the molecular dynamics in a lipid membrane in the presence of Aβ peptides, which are sketched in the left graph

Hallmarks of Alzheimer's disease (AD) are extracellular amyloid plaques found in the brain tissue of patients died by suffering AD. These plaques are composed mainly of amyloid-β peptide (Aβ), composed of 39–43 residues long. According to the amyloid hypothesis, the predominantly followed concept in AD research, the accumulation of Aβ in the brain is the primary influence driving AD pathogenesis. In recent years, more and more findings point to the fact that monomeric Aβ or small oligomers may be a crucial factor in neurodegeneration typical of AD. The direct interaction of the Aβ peptide with the lipid membrane causes membrane disruption as well as may affect signaling and metabolic pathways in the brain. We investigated lipid membranes doped with Aβ, and fragments of it with different neutron-scattering techniques. In SANS experiments, we were able to demonstrate a membrane fusogenic activity of Aβ [1] and neutron diffraction revealed detailed structural details on the location of Aβ fragments in lipid membranes of various compositions [2-4]. Quasi-elastic neutron scattering (QENS) was used to study dynamical changes induced by the peptide [5-7]. To observe the lipid dynamics in a large time window from pico- to nanoseconds, the experiments were performed on neutron spectrometers with different energy resolutions. With this, we could discriminate short-range intrinsic lipid dynamics and long-range diffusion. The lipid diffusion was altered due to amyloid-β interaction and the observed changes differ in the fluid or gel phase, respectively. For example, in membranes doped with the Aβ(22–40) fragment, the in-plane Brownian diffusion of lipids near the phase transition temperature was slowed down in comparison to the pure membrane.

1. S. Dante, T. Hauß, A. Brand, N.A. Dencher, Membrane fusogenic activity of the Alzheimer's peptide Aβ(1–42) demonstrated by small-angle neutron scattering, *Journal of Molecular Biology*, **376**, 393-404 (2008).

2. Dante S., T. Hauss, N.A. Dencher, β-amyloid 25 to 35 is intercalated in anionic and zwitterionic lipid membranes to different extents, *Biophysical Journal*, **83**, 2610-2616 (2002).

3. Dante S., T. Hauß, N.A. Dencher, Insertion of externally administered amyloid β peptide 25-35 and perturbation of lipid bilayers, *Biochemistry*, **42**, 13667-13672 (2003).

4. Dante S., T. Hauß, N.A. Dencher, Cholesterol inhibits the insertion of the Alzheimer's peptide Aβ(25-35) in lipid bilayers, *European Biophysics Journal With Biophysics Letters*, **35**, 523-531 (2006).

5. Buchsteiner A., T. Hauß, S. Dante, N.A. Dencher, Alzheimer's disease amyloid-beta peptide analogue alters the ps-dynamics of phospholipid membranes, *Biochim Biophys Acta*, **1798**, 1969-1976 (2010).

6. Buchsteiner A., T. Hauß, N.A. Dencher, Influence of amyloid-beta peptides with different lengths and amino acid sequences on the lateral diffusion of lipids in model membranes, *Soft Matter*, **8**, 424-429 (2012).

7. Barrett M.A., M. Trapp, W. Lohstroh, T. Seydel, J. Ollivier, M. Ballauff, N.A. Dencher, T. Hauß, Alzheimer's peptide amyloid-beta, fragment 22-40, perturbs lipid dynamics, *Soft Matter*, **12**, 1444-1451 (2016).

Application of the ribosome-inactivating small molecules

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The ribosome is a major target for small-molecule inhibitors. We used X-ray crystallography to determine 16 high-resolution structures of the full 80S ribosome from *Saccharomyces cerevisiae* in complexes with 12 eukaryote-specific and 4 broad-spectrum inhibitors. All inhibitors were found associated with messenger RNA and transfer RNA binding sites. The study defines common principles of targeting and resistance and provides insights into their mode of action.

We also describe the structure of a newly synthesized cycloheximide analogue in complex with the ribosome. This inhibitor induces cell death in a wide range of in vitro-cultivated human cancer cell lines. It is bound to the E-site tRNA CCA-end binding pocket formed by 25S rRNA forming interactions with residues of the protein eL42. This is an unusual feature as so far inhibitory small molecules targeting the ribosome have mostly been described making contact with rRNA.

Aminoglycosides are chemically diverse, broad-spectrum antibiotics that target functional centers within the bacterial ribosome to impact all four principle stages (initiation, elongation, termination and recycling) of the mechanism of translation. The propensity of aminoglycosides to induce miscoding errors that suppress the termination of protein synthesis supports their potential therapeutic interventions in human diseases associated with premature termination codons (PTCs).

We have determined high-resolution crystal structures of the 80S ribosome with paromomycin, geneticin (G418), gentamicin and TC007 and showed multiple aminoglycoside-binding sites within the large and small ribosomal subunits. Multivalent interactions of the aminoglycosides suggest that their chemical composition and distinct modes of interaction with the ribosome will have an influence on PTC read-through efficiency.

Single-molecule localization microscopy: where next?

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Single-molecule sensitive super-resolution microscopy techniques such as *d*STORM and PALM provide microscopic images with subdiffraction spatial resolution. This enables new insights into how proteins are organized in a cellular context, with a spatial resolution approaching virtually the molecular level [1]. Because of their intrinsic single-molecule sensitivity, they allow quantitative access to cellular structures, for example how proteins are distributed and how they interact with other biomolecules. Ultimately, it is even possible to determine protein numbers in cells and the number of subunits in a protein complex. Thus, they can pave the way toward a better understanding of how cellular function is encoded at the molecular level. Here, we demonstrate how single-molecule localization microscopy can be used advantageously for subdiffraction-resolution fluorescence imaging, discuss current limitations and point out future prospects.

For example, super-resolution imaging of protein distributions in larger intact tissue volumes with preserved fine structure remains so far challenging. We demonstrate that 3D-*d*STORM in the red spectral range with a high-NA water immersion lens and optimized staining procedures can be used to map protein distributions with $\sim 20 \times 20 \times 60$ nm^3 resolution in cryosections with a thickness of 25 µm. We recorded thousands of neuronal subcompartments aberration-free in volumes of up to $28 \times 30 \times 14$ µm^3 in 90 min. Using highly specific antibodies, we measured protein distributions and clusters with distinct size, number and density in different brain regions. In addition, we developed a new multicolor localization microscopy method that enables quantitative multidimensional *d*STORM. We show how single-molecule sensitive super-resolution microscopy methods can be used successfully in clinical day-to-day diagnostics of cancer diseases to improve next-generation personalized immunotherapies.

1. Sauer, M, Heilemann, M (2017) Single-molecule localization microscopy in eukaryotes. *Chem Rev*, 117:7478-7509.

Fragment-based approach in the drug-discovery process

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In modern industrial drug discovery, there is a need for rapid development of small-molecule agents against therapeutic targets. Fragment-based methods enable identification of weak-affinity ligands amenable to fast optimization. During the past two decades, these methods have developed into a promising strategy in drug discovery, where a variety of biochemical and biophysical techniques can be employed for fragment library screening and for characterization of fragment hits. The hits derived from fragment-based screening have typically a higher ligand efficiency than an average HTS hit. This gives a better starting point for hit optimization and lead discovery. Here, we report the results of the fragment screening campaigns for selected targets from Merck's drug discovery program.

Novel rhodopsins from metagenomic screenings

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Rhodopsin-coupled molecular machineries are the adaptive response of heterotrophs to the free, unlimited, energy source represented by the solar light. Rhodopsins are currently classified into two main protein families: type-1 rhodopsins, encoded by bacteria, archaea and viruses and, type-2, by eukaryotes. In this line, it is not surprising that rhodopsins are diversified into an astounding variety and that this diversity has found application in developing optogenetic tools. Rhodopsins used in optogenetics come from pure cultures of microbes. Nevertheless, culture is a rather inefficient (slow and unreliable) tool for isolating novel microbes. Metagenomics and single-cell genomics nowadays are the most effective instruments to get insights about microbes that, if not replacing culture, are allowing major advances. Taking benefit from recent sampling campaigns [1–3], we coupled protein similarity search and Hidden Markow Models in order to identify and subsequently classify rhodopsins from marine metagenomic data. We were able to retrieve 7285 proteins that were grouped into 174 clades. Moreover, we discovered novel groups of xanthorhodopsins, hybrid pump rhodopsins and rhodopsins that currently have an unpredictable function. Four clades come from mesopelagic metagenomes and the activity of their protein might be related to low-light conditions. Moreover, mesopelagic proteins, in particular, might represent the focus of future researches aimed at identifying more sensitive molecules that might have several potential applications.

1. Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G, et al. Structure and function of the global ocean microbiome. Science (80-) 2015; 348.
2. Duarte CM. Seafaring in the 21st century: The Malaspina 2010 circumnavigation expedition. Limnol Oceanogr Bull . 2015. , 24: 11–14
3. Haro-Moreno JM, López-Pérez M, de la Torre JR, Picazo A, Camacho A, Rodriguez-Valera F. Fine metagenomic profile of the Mediterranean stratified and mixed water columns revealed by assembly and recruitment. Microbiome 2018; 6: 128.

Counting subunits within receptor complexes using single-molecule localization microscopy

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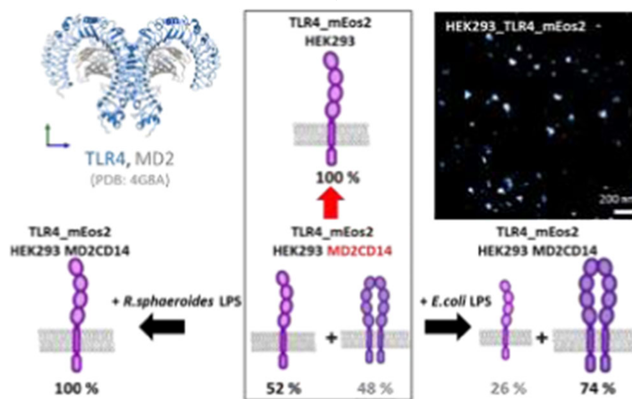

Fig. 1 Quantitative super-resolution imaging of Toll-like receptor 4 in HEK293 cells reveals the receptor dimerization upon specific stimuli. Resting TLR4 (middle, bottom) is partially dimeric and requires co-receptors MD2/CD14. TLR4 responds specifically to different bacterial ligands (lipopolysaccharides, LPS), either leading to receptor dimers or monomers and the activation of a specific down-stream signaling cascade

Knowledge of assembly and subunit architecture of macromolecular complexes in a cellular context is essential to infer their biological function. Fluorescence microscopy has become increasingly popular for quantifying molecular numbers in the cellular environment. However, at high protein densities, the spatial resolution limit of ~200 nm in conventional (diffraction-limited) microscopy hampers direct observation of single

protein complexes. Super-resolution fluorescence techniques present a powerful solution to bypass this limit. Single-molecule localization microscopy (SMLM) is particularly well suited; as next to high-resolution images of cellular structures, it potentially provides quantitative information on the detection of single, protein-conjugated fluorescent labels [1]. Here, we address the question how membrane proteins organize into functional molecular units in their native cellular environment. For this purpose, we combined single-molecule super-resolution imaging in combination with a photokinetic analysis of fluorophore blinking events and extract protein stoichiometries from super-resolved membrane protein clusters. The analysis of fluorophore blinking events in an SMLM experiment offers a promising route to probe oligomeric states in protein complexes [2-4]. We demonstrate the practical applicability of this approach by quantifying the oligomerization states of several membrane proteins tagged with the mEos2 fluorescent protein [3, 5]. We further applied this method to investigate the stoichiometry of membrane receptors in functional complexes and its changes with respect to specific ligands. We found that Toll-like receptor 4 (TLR4) organizes into clusters with one or two receptor molecules [6]. We further showed that lipopolysaccharides (LPS), secreted from different bacteria, shift the population of TLR4 towards either monomers or dimers.
The combination of SMLM and photokinetic analysis should be robust and broadly applicable to counting co-localized molecules in vivo and in vitro.

1.M. Sauer, M. Heilemann, Single-molecule localization microscopy in eukaryotes. *Chemical reviews* **117**, 7478-7509 (2017); published online EpubJun 14 (10.1021/acs.chemrev.6b00667).
2.S. Avilov, R. Berardozzi, M. S. Gunewardene, V. Adam, S. T. Hess, D. Bourgeois, In cellulo evaluation of phototransformation quantum yields in fluorescent proteins used as markers for single-molecule localization microscopy. *PloS one* **9**, e98362 (2014)10.1371/journal.pone.0098362).
3.F. Fricke, J. Beaudouin, R. Eils, M. Heilemann, One, two or three? Probing the stoichiometry of membrane proteins by single-molecule localization microscopy. *Scientific reports* **5**, 14072 (2015); published online EpubSep 11 (10.1038/srep14072).
4.S. H. Lee, J. Y. Shin, A. Lee, C. Bustamante, Counting single photoactivatable fluorescent molecules by photoactivated localization microscopy (PALM). *Proceedings of the National Academy of Sciences of the United States of America* **109**, 17436-17441 (2012); published online EpubOct 23 (10.1073/pnas.1215175109).
5.G. Hummer, F. Fricke, M. Heilemann, Model-independent counting of molecules in single-molecule localization microscopy. *Molecular biology of the cell* **27**, 3637-3644 (2016); published online EpubNov 7 (10.1091/mbc.E16-07-0525).
6.C. L. Kruger, M. T. Zeuner, G. S. Cottrell, D. Widera, M. Heilemann, Quantitative single-molecule imaging of TLR4 reveals ligand-specific receptor dimerization. *Science signaling* **10**, (2017); published online EpubOct 31 (10.1126/scisignal.aan1308).

Microbial halorhodopsins: light-driven chloride pumps

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Halorhodopsins have been identified in various bacterial and archaeal species. Besides the group of archaeal halorhodopsins, distinct chloride-transporting rhodopsin groups have recently been identified in other organisms like Flavobacteria or Cyanobacteria. Originally halorhodopsin was discovered in *H. salinarum*. However, halorhodopsin from *N. pharaonis* is the best-studied homologue because of its facile expression and purification and its advantageous properties, which was the reason to introduce this protein as neural silencer into the new field of optogenetics. Here, the functional and structural characteristics of halorhodopsins are described [1]. Results will be discussed in light of common principles underlying the mechanism of ion pumps and sensors.

1.Engelhard, C.; Chizhov, I.; Siebert, F.; Engelhard, M. Microbial halorhodopsins: light-driven chloride pumps. *Chem Rev* 2018, https://doi.org/10.1021/acs.chemrev.7b00715.

Gauge theory: protein topology and dynamics

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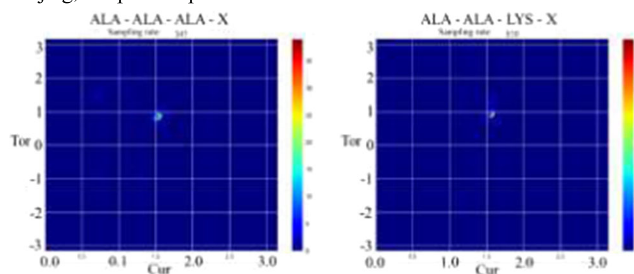

Fig. 1 Distribution of number of particular sequences of four amino acids with respect to values of torsion and bending angles. X is an arbitrary amino acid.

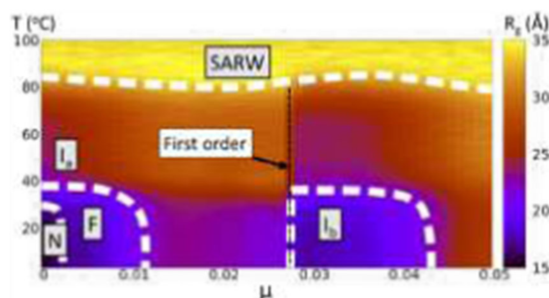

Fig. 2 The myoglobin radius of gyration (R_g) phase diagram: Collapsed native state (N). Unfolding of F-helix (F). Two molten globules (Ia and Ib) with first order-like transition line between them. Self-avoiding random walk phase (SARW).

In the case of a complex system, it is often impractical if not impossible to accurately describe physical phenomena. Whenever any adequate solution of the underlying fundamental equations is out of reach, one may instead focus on a few key variables and construct a so-called effective theory for those. For example, even a practical high precision, first-principles quantum chemistry calculation is never concerned with the behavior of the subnuclear quarks and gluons, instead it employs variables at a higher level of hierarchy. In general, an effective theory description can also be highly complicated, even for a fairly simple system [1]. However, in many circumstances and in particular when the physical system admits symmetries and (approximate) separations of scales, the reduced set of variables can be treated on their own right. Their physical behavior then becomes tractable, in terms of an effective energy functional, as a useful approximation.
The folding and unfolding of a protein is an example of a highly complex physical process where an effective theory description

should have value: The (un)folding of an individual protein can proceed along diverse pathways, but the underlying mechanism is always governed by the same universal biophysical and biochemical principles [2–5]. This makes an effective theory description of the (un)folding processes viable.

The Landau-Ginsburg-Wilson (LGW) approach [6, 7] is a systematic methodology to try and develop an effective theory description. The approach is useful in the modelling of those phase properties in a material system, that have a universal character. Here, we shall propose how an LGW approach could be developed to analyze the phase diagram of globular proteins under variable thermodynamical circumstances, when the ambient temperature and acidity as the external environmental variables.

The corresponding field theory model is based on local symmetry of proteins that dynamically define tertiary structure of proteins [8]. Protein local symmetry is defined by amino-acids and protein backbone bonds structure. The covalent bond between amino acids central carbon atom C_a and carbon in the carboxyl group C' has very low rotation energy. Rotation energy of the rest of the bonds in the amino-group is high and neighbor C and all peptide bond atoms between them are in the same plane. Taking it into account, we can consider the protein backbone as a freely rotating discrete chain of such planes. A such chain formally can be described by a discreet (1+1) manifold with U(1) local symmetry. Correspondingly, the gauge phase can be associated with rotational angle of the C_a and C' bond. In the water medium, the protein local U(1) symmetry is broken due to hydrophobic and hydrophilic forces, what lead to the amino acids alignment inside (hydrophobic) or outside (hydrophilic) of the protein secondary structure. Thus, the considered above local symmetry breaking and chirality folds the freely rotating constant bending chain into to the correct protein structure according to amino-acids and media interaction. Within the LGW approach, the corresponding local fundamental interactions are integrated in effective dynamical degrees of freedom that are the bending and torsion of the chain treated as dynamical fields. This idea is supported by the observation of the strong correlation between sequences of four amino acids and values of bending and torsion angles (see Fig.1).

We have found that LGW approach can describe universal aspects of protein phase diagram in the T-pH plane (see Fig.2). In the case of myoglobin, we reproduce all the major features of its phase diagram with R_g as the probe, from very low T values to above 100 °C and for pH values from neutral down to pH = 4. In particular, the instability of F-helix, the transition to molten globule I_a, the unfolding of the remaining helices and the appearance of the second molten globule I_b are all in line with experimental observations. The LGW approach even predicts the unfolding of I_b, with decreasing pH but since we have not attributed a chemical potential to the low-pK_a aspartic acid and glutamic acid we do not observe the decay of helical structure when pH tends to 2.

We conclude that the LGW approach shows great promise to model and describe the universal aspects of equilibrium and near-equilibrium thermodynamics of proteins.

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1. R.P. Feynman, F. Vernon, *Ann. Phys.* **24** 118 (1963)
2. O.B. Ptitsyn, *Vestn. Akad. Nauk SSSR* **5** 57 (1973)
3. J.D. Bryngelson, P.G. Wolynes, *PNAS (USA)* **84** 7524 (1987)
4. A.V. Finkelstein , E.I. Shakhnovich *Biopolymers* **28** 1681 (1989)
5. P.G. Wolynes, J.N. Onuchic, D. Thirumalai, *Science* **267** 1619 (1995)
6. K.G. Wilson, J. Kogut, *Phys. Repts.* **12** 75 (1974)
7. N. Goldenfeld, *Lectures on phase transitions and the renormalization group* (Addison-Wesley, Reading, 1992).
8. Niemi A.J., Theor Math Phys, **181**, 1235 (2014)

Proteins and membranes: born to be together—a computational view

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Motivation and aim. Apart from the barrier role traditionally attributed to them, cell membranes fulfill the equally important task of providing an effective accommodation of numerous external agents, including membrane proteins (MPs). Molecular mechanisms of such processes have been poorly studied, but recently it has been shown that the most important property of lipid bilayers is their dynamic heterogeneous character—the work of these complex mesoscopic systems depends critically on their local structural features (up to ~ 1 nm), hydrophobic and electrical properties, and so on. Moreover, when analyzing membranes, it is necessary to take into account the parameters of their dynamic behavior at times from 10 ps, including stochastic phenomena. The totality of these factors represents the so-called "membrane response," i.e., active reaction of the water-lipid medium to interacting external molecules. Thus, the study of protein-membrane systems is possible only if mutual influence (adaptation) of partners at the molecular level is taken into account. One of the most informative methods for solving such problems is atomistic computer modeling.

Results. A computational approach to the analysis of the structural and dynamic parameters of all components of model membranes—MPs, lipids, water and ions—has been developed. It is established that local changes in the membrane environment play an important role in the binding of membrane-active peptides and peripheral MPs, causing specific clustering of lipids and initiating the formation of defects in the membrane. It is shown for the first time that lipids make a significant contribution to the free energy of spontaneous dimerization of MPs. The detailed balance of various energy contributions strongly depends on the composition of the membrane and the amino acid sequence of the protein. The assumption is made that the process of association of transmembrane alpha-helices in lipid bilayers has a predominantly entropic character.

Conclusion. MPs and their water-lipid environment equally determine the nature of the biological behavior of cell membranes, mutually strongly affecting each other and responding to external influences in a self-consistent manner.

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Random network viewpoint on the creation of membrane inhomogenieties

Gorskiy A.

I will discuss the aspects of the membrane deformations by the statistical physics tools. The membrane is represented by the topological network and the Laplacian of the network is the discrete approximation of the membrane Laplacian. We apply the recently discovered phase transitions in the networks for the processes of the bubble and hole creations on the membrane. The dependence of the transition type on the local and global structure of the model network will be discussed. The mechanisms of the spontaneous discrete symmetry breaking on the membrane worldsheet will be mentioned

Towards a model of the human pancreatic beta cell

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A key scientific challenge in biology and chemistry is the integration of data across the different scales—molecular, cellular, and whole body In a collaborative open source effort with several different groups across California and Shanghai, we are workings towards a multi-scale model of the human pancreatic beta cell at atomic resolution Such a model will be useful for advancing the field of structure-based drug design from the protein scale to the cellular scale and provide us with better feedback in the understanding and design of new medicines to treat type II diabetes. We invite all groups interested in this effort to join called the Pancreatic Beta Cell Consortium https://dornsifeuscedu/bridge-institute/pancreatic-beta-cell-consortium/

Optogenetic vision restoration and regeneration of photoreceptor outer segments

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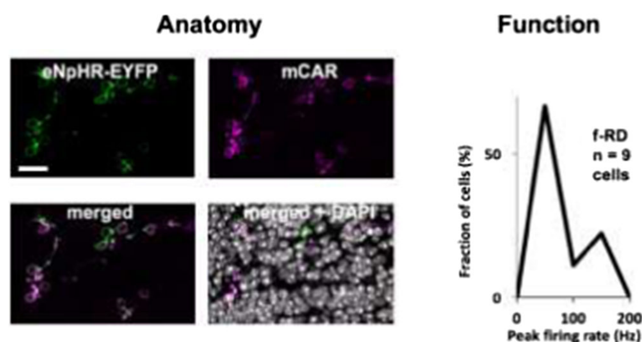

Fig. 1 Halorhopsin-expressing and thereby light-sensitive cones in 495-day-old blind mouse retina. (Left) Representative immunostainings of eNpHR-EYFP (green) and mouse cone arrestin (mCAR, magenta) positive cones in a fast-retinal degeneration mouse model (f-RD). Nuclei are stained with DAPI (white). Scale bar, 20μm. (Right) Quantification of light responses recorded from retinal ganglion cells by microelectrode arrays upon eNpHR stimulation of persisting cones in a 495 days old f-RD retina

Loss of vision caused by hereditary diseases often starts with the degeneration of rod photoreceptors causing night blindness. Subsequently, the cone photoreceptors, important for daylight, color and high acuity vision, become structurally damaged as they lose their light-sensitive antenna, so-called outer segments[1]. However, the non-functional cones can persist and be reactivated for almost 500 days in mouse models of retinal degeneration (Fig.1).
We showed that one can optogenetically reactivate persisting cone photoreceptor cell bodies by overexpressing the archaebacterial Halorhodopsin eNpHR[2]. Resensitized photoreceptors activated all retinal cone pathways tested and resulted in the activation of cortical circuits. Former blind mice showed visually guided behaviors. In addition, we demonstrated that Halorhodopsin also activated photoreceptors in human *post-mortem* retinas. Our approach is currently assessed for clinical translation. Additionally, we have shown that other hyperpolarizing optogenes with optimized properties drove cone photoreceptor cell bodies in retinal degeneration mouse models[3]. The downside of optogenetic vision restoration strategies is that high light intensities are required for stimulation and the optogene's activity range is narrow. Therefore, adaptation to different light levels and amplifying the overall light intensity will require additional technical devices[4]. Therefore, we also wondered whether it would be possible to regenerate cone outer segments. We showed that two non-coding miRNAs, miR-182 and miR-183, prevented the loss of outer segments in mice[5]. These miRNA species were also essential for the formation of small outer segments resulting in light responses in stem-cell-derived retinal cultures. We demonstrated that miR-182- and miR-183- and their regulated genes are required for cone outer segment maintenance and functional outer segment formation in vitro. In summary, the regeneration and preservation of cone outer segments represents an early intervention strategy whereas optogenes only require persisting retinal cell types to render these cells artificial photoreceptors[1]. Both approaches are promising to restore some visual functions in degenerated retinas[6].

1.Klapper, S.D., Swiersy, A., Bamberg, E. & Busskamp, V. Biophysical properties of optogenetic tools and their application for vision restoration approaches. *Front Syst Neurosci* **10**, 74 (2016).
2.Busskamp, V. et al. Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. *Science* **329**, 413-417 (2010).
3.Chuong, A.S. et al. Noninvasive optical inhibition with a red-shifted microbial rhodopsin. *Nat Neurosci* **17**, 1123-1129 (2014).
4.Scholl, H.P. et al. Emerging therapies for inherited retinal degeneration. *Sci Transl Med* **8**, 368rv366 (2016).
5.Busskamp, V. et al. miRNAs 182 and 183 are necessary to maintain adult cone photoreceptor outer segments and visual function. *Neuron* **83**, 586-600 (2014).
6.Roska, B. & Sahel, J.A. Restoring vision. *Nature* **557**, 359-367 (2018).

Probes for high-photostability super-resolution fluorescence microscopy of live cells

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Advanced fluorescence microscopy of live cells strongly suffers from low phototability of fluorescent proteins (FPs) as well as phototoxic effects induced by excitation light. This is especially true for super-resolution fluorescence microscopy (nanoscopy) that often relies on very strong illumination to detect single molecules and/or induce photoswitching. Here, we describe our recent progress towards low-phototoxic and high-photostability imaging.
Earlier we demonstrated that bleaching of green FPs can result from light-induced electron transfer from the excited chromophore to external electron acceptors [1]. To enhance photostability, this process can be suppressed in different ways [2], e.g., by using cell media depleted of redox-active vitamins or containing antioxidants. Also, we found that some point mutations block electron transfer within the protein, leading to increased photostability. This approach enabled us to develop a highly photostable EGFP variants.
Reversible switchable optical fluorescent transition (RESOLFT) nanoscopy uses scanning optical scheme similar to that for stimulated emission depletion (STED). At the same time, illumination intensities in RESOLT are several orders of magnitude lower compared to STED, because RESOLT relies on reversibly switchable fluorescent proteins (rsFPs). Using optimized red protein FusionRed as a template, we developed new rsFPs that require green-orange light for photoswitching in RESOLFT [3]. This allowed us to avoid cell illumination with phototoxic violet-blue light, which is inevitable for all previously available rsFPs.
In spite of the undoubted importance, common labeling techniques based on covalently bound fluorescent proteins or dyes have certain limitations, such as insufficient photostability, labeling density, and rate of fluorescence development. To solve these problems, we suggested two methods based on *reversible* binding of a fluorescent molecule with target protein. First approach was based on specific binding of a fluorogenic dye with a protein host—mutants of *E. coli* lipocalin Blc [4]. Fluorogen within the

protein showed a strong increase of fluorescence quantum yield, thus ensuring visualization of intracellular localization of the target protein fused to Blc. Fast exchange of the cell-permeable dye between cell and medium led to a high apparent photostability of the fluorescence signal. At low concentrations of the fluorogen, events of its binding can be detected at a single-molecule level, allowing reconstruction of super-resolved images.

Another approach utilized reversible heterodimerization of artificial alpha-helixes (K/E helixes), one of which is attached to a target protein and another—to a fluorescent protein. Coexpression of such proteins in a cell resulted in their association. We tested different pairs of K/E-helixes and selected ones that ensure colocalization of the target and fluorescent proteins and, at the same time, fast exchange of the fluorescent protein in this complex. This exchange resulted in enhanced apparent photostability, when only a small part of the cell was illuminated, for example, in the case of high-resolution confocal microscopy of a selected cell region, or in a regime of total internal reflection fluorescence (TIRF). We took advantage of this exchangeable probe for tracking of clathrin-coated vesicles—a model where number of target protein (clathrin) molecules is strictly limited.

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1. Bogdanov, A.M. *et al.* Green fluorescent proteins are light-induced electron donors. *Nat Chem Biol.* **5,** 459-61 (2009).
2. Acharya, A., *et al.* Photoinduced Chemistry in Fluorescent Proteins: Curse or Blessing? *Chem Rev.* **117,** 758-795 (2017).
3. Pennacchietti, F. *et al.* Fast reversibly photoswitching red fluorescent proteins for live-cell RESOLFT nanoscopy. *Nat Methods.* **15,** 601-604. (2018).
4. Bozhanova, N.G. *et al.* Protein labeling for live cell fluorescence microscopy with a highly photostable renewable signal. *Chem Sci.* **8,** 7138-7142 (2017).

Integrating novel biophysical approaches in fragment-based lead discovery workflow: MST and nDSF for screening and validation

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Fragment-based lead discovery has proved to be an effective alternative to high-throughput screenings in identifying chemical matter that can be developed into robust lead compounds. The search for optimal combinations of biophysical techniques that can correctly and efficiently identify and quantify binding can be challenging due to the physicochemical properties of fragments. In order to minimize the time and costs of screening, optimal combinations of biophysical techniques with maximal information content, sensitivity, and robustness are needed. Here, we present an approach utilizing automated microscale thermophoresis (MST) affinity screening to identify fragments active against human kinase. MST in concert with nDSF identified multiple hits that were confirmed by X-ray crystallography but not detected by orthogonal methods. Furthermore, MST and nDSF also provided information about ligand-induced aggregation and protein denaturation. The technique delivered a large number of binders while reducing experimentation time and sample consumption, demonstrating cost- and time-effectiveness of MST to execute and maximize the efficacy of fragment screening campaigns.

How caffeine fires up the powerhouses of cells

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Cardiovascular functionality decreases with age. Interestingly, recent studies have shown a protective effect of caffeine on the cardiovascular system. We have shown that concentrations of caffeine detectable in serum after moderate coffee consumption enhanced migratory capacity of endothelial cells (EC), which critically depends on mitochondrial function. Therefore, we wanted to identify the causal, molecular link between caffeine and mitochondrial energy metabolism. Thus, EC were transfected with standard procedures and migration was determined in a scratch wound assay. To specifically analyze mitochondrial and nuclear p27, we designed lentiviruses expressing these organelle-targeted proteins and transduced different cardiovascular cells. ATP production and oxygen consumption were measured in mitochondria isolated from hearts of wildtype and p27-deficient littermates. Microarray analyses were performed with RNA from hearts of wildtype and p27-deficient littermates.

Surprisingly, we found that caffeine induces the translocation of p27/Kip1 (p27) into the mitochondria. Reducing p27 levels with siRNA inhibited basal- and caffeine-induced migration. To investigate the effects of p27 localization on mitochondrial energy metabolism and migration we expressed mitochondrially and nuclear targeted p27 (mito p27/nuc p27) in EC. While expression of nuc p27 decreased basal migration, mito p27 increased migration, and mitochondrial ATP production. Similarly, only overexpression of mito p27, but not nuc p27, rescued the complete loss of migratory capacity induced by knockdown of p27. To investigate the link between caffeine and p27 in vivo, we performed microarray analysis of hearts from wildtype and p27-deficient mice treated with caffeine in their drinking water. Caffeine-induced expression of genes involved in mitochondrial energy metabolism and biogenesis only in wildtype mice demonstrating a crucial role for p27 in enhanced mitochondrial function. Moreover, complex I-induced respiration was significantly reduced in heart mitochondria from p27-deficient mice. Caffeine increased oxygen consumption in wildtype mice, but not in their p27-deficient mice [1].

Taken together, caffeine improves mitochondrial functionality in a mitochondrial p27-dependent in manner in the endothelium and in the heart.

1.Ale-Agha, N. *et al.* CDKN1B/p27 is localized in mitochondria and improves respiration-dependent processes in the cardiovascular system-New mode of action for caffeine. *PLoS Biol* **16**, e2004408, https://doi.org/10.1371/journal.pbio.2004408 (2018).

Nanocrystallization of bacteria nucleoid at stress conditions

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Survival of living organisms in constantly changing environmental conditions is possible due to universal hereditary strategies of adaptation to various types of stress, based on structural, biochemical and genetic rearrangements. One of the strategies implemented in bacterial cells is related to the protection of the nucleoid from unfavourable environmental conditions by binding of DNA to specific histone-like proteins, the main one being the protein Dps (DNA binding protein from starved cells), and condensation of DNA with DPS in nanocrystalline complex which was recently discovered in gram-negative bacteria *E. coli* that are subject to 48-hour starvation [1]. Nanocrystallization (or biocrystallization) of nucleoid

helps to protect the nucleoid from damage and resume the activity of the bacterial cells later, upon improvement of the external conditions. Thus, in the bacteria *E. coli* after 48 h of starvation, the nucleoid together with a stress-induced protein Dps forms highly ordered, tightly packed DNA–Dps co-crystals [1]. The structure of these crystals within a cell was studied using cryoelectron microscopy and tomography [1], as well as by use of synchrotron radiation on macromolecular crystallography station ID-23-1[1]. However, the DNA conformation within these complexes has remained unsolved. One of the reasons why it was difficult to solve the conformation is the small size of the nanocrystals of the nucleoid within a cell ≤ 400 nm. We expect that with the use of the XFEL radiation, we can obtain further structural information and our ultimate target is the crystallographic data to 2.5 Å resolution. Fresh (48 h of starvation) sample usually contain 10^{9} cells/ml. We have not yet tried to isolate the crystals after cell lysis by gradual centrifugation. However, we are confident that we can perform such sample preparation

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1.Sinitsyn D.O. et al., Biocrystallization of bacterial nucleoid under stress. *Russian Journal of Physical Chemistry B,* **11,** 833 (2017)

Should the treatment of amyloidosis be personified? Molecular mechanism of amyloid formation by abeta peptide and its fragments

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Our studies of a number of amyloidogenic proteins and peptides allowed us to propose our own model for the formation of a fibril structure [1-3]. We found that the main building block of fibril of any morphology is the ring-like oligomer. The interaction of ring-like oligomers with each other in different ways for the investigated proteins allows us to explain their polymorphism. In the literature, there is an opinion that when developing therapeutic agents against amyloidosis, a personified approach should be used, since it is shown that fibril formations in different patients can morphologically differ. In this connection, the discovery of a general mechanism explaining the formation of fibrils from ring-like oligomer structures could facilitate the development of generic drugs. However, in our opinion, the main attention should be paid not to polymer formations of proteins/peptides in the form of fibrils or even to oligomer aggregates, but to physicochemical, genetic and other reasons leading to destabilization of native protein/peptide molecules and triggering the fibril formation process. The studies were supported by the Russian Science Foundation (grant numbers 14-14-00536 and 18-14-00321).

1. Galzitskaya, O. & Selivanova, O. Rosetta Stone for Amyloid Fibrils: The Key Role of Ring-Like Oligomers in Amyloidogenesis. *J Alzheimers Dis.*, **59(3):785-795**, (2017).
2. Selivanova, O. *et al.* The Mechanism Underlying Amyloid Polymorphism is Opened for Alzheimer's Disease Amyloid-β Peptide. *J Alzheimers Dis.* **54(2):821-30** (2016).
3. Selivanova, O. *et al.* To Be Fibrils or To Be Nanofilms? Oligomers Are Building Blocks for Fibril and Nanofilm Formation of Fragments of Aβ Peptide. *Langmuir* **34(6):2332-2343**, (2018).

Protein family based approach in drug discovery: cytochrome P450

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Cytochromes P450 (P450) are membrane bound hemeproteins involved in biosynthesis of bioactive molecules and metabolism of drugs. Impairment of the expression or activity of P450s can cause breast and prostate cancer, resistant hypertension, chronic kidney disease, etc. These enzymes are also involved in the biotransformation of most foreign substances, including 70–80% of clinically used drugs. Moreover, this type of enzymes plays an essential role in metabolic pathways of different pathogens. Therefore, P450s are important targets for generation of new drugs for treatment of cancer, cardio-metabolic and endocrine disorders as well as drug-resistant forms of tuberculosis and mycosis. However, these enzymes are challenging target for rational drug design: membrane nature of this hemeprotein; requirement of redox partners for functional assay; high dynamic flexibility of substrate access channel and the active site; co-translational folding (inability to refold P450). As a result, it is impossible to predict substrate or inhibitor from primary structure of P450s.

Based on our scientific progress, we have developed a unique technological platform designed for P450-targeted drug discovery based on integration of scientific achievement in structural biology and enzymology as well as the use of bioinformatics, experimental and technological practices for synthesis of the molecules with desired properties. Our research is focused on understanding of the molecular mechanism of substrate recognition, binding and catalysis of sterols and eicosanoids modifying P450s as well a structure-functional study of mycobacterial cytochromes P450 involved in biosynthesis and metabolism of bioactive lipids. This research provides valuable tool for P450 targeted rational drug design.

In the field of structural analysis of P450s, we collaborate with SGC (Toronto), MIPT (Dolgoprudny), ILL (Grenoble), and NIAID NIH.

X-ray data collection strategy and radiation damage

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X-ray macromolecular crystallography is the core method to obtain the information about 3-D protein structure. However, crystal structure determinations of biological macromolecules are limited by the availability of sufficiently sized crystals with sufficient diffraction power and by the crystal destruction due to radiation damage. Success of difficult crystallographic experiments using weakly diffracting macromolecular crystals critically depends on a proper choice of the data collection protocol that is governed by the experimental purpose, by the characteristics of the X-ray beam and instrumentation used to collect data and by the samples properties. Radiation damage is a critical factor limiting data quality and being able to predict the absorbed dose and mitigate the effects of exposure to X-rays is very important.

Over the last decade, we developed strategy program BEST [1] and several additional methods and software programs for crystal diffraction recognition, characterisation and radiation damage parameters determination [2, 3]. BEST applies a statistical model for expressing the results of the diffraction experiment as functions of experimentally variable parameters. Based on an initial analysis of a few diffraction images, the experimental parameters are quantitatively optimized and the conditions determined, under which the required data statistics are reached with minimum radiation dose. To describe the radiation damage, two-parametric model is used which accounts for both the average intensity decay and radiation-induced non-isomorphism. BEST implementations of radiation damage-driven data collection optimization are being successfully used in everyday practice at the synchrotron beamlines over the last years.

Here, we will discuss some new ideas and further BEST developments that can be applicable to the most difficult structural projects, were poor diffraction is inherent and the necessary amount of structural information may not be attained using a single sample. The method should be applied for optimal data collection from the sets of micro crystals, for data collection at ambient temperature and for data collection with limited possibility for registration of the diffraction pattern. Scientific challenge here was the development of method that provides the ability to automatically recognize and rank the series of single diffraction patterns collected during the low-dose mesh scan. This is carried out using our new programs DOZOR and MeshBest [4]. Another scientific challenge is the development of new model to describe the increase in the systematic intensity measurements errors under progressing radiation dose and the application of this model of radiation induced non-isomorphism to the optimal planning of serial crystallography measurements.

1. Bourenkov, G.P. & Popov, A.N. (2010). *Acta Cryst.* **D66**, 409-419
2. Leal R., Bourenkov G.P., Svensson O., Spruce D., Guijarro M. & Popov A.N., *J. Synch. Radiation* (2011), **18(3)**,381-386
3. Leal R., Bourenkov G., Russi S. & Popov A., (2013)., *J. Synchrotron Rad.* **20**, 14–22
4. Melnikov I. A., Svensson O., Bourenkov G., Leonard G. A. and Popov A. N. *Acta Cryst* (2018), **D74**, 355-365.

Structural biology at the ESRF: present and future

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The ESRF's facilities for Structural Biology comprise 6 end-stations for macromolecular crystallography (MX) [1], one end-station for BioSAXS experiments [2] and one end-station based around a Titan Krios cryo-electron microscope. These are supplemented by a number of support laboratories including the ID29-S "Cryobench" facility for in crystallo optical spectroscopy [3] and a facility for the high-pressure cryo-cooling and/or derivatisation of crystals of biological macromolecules [4]. This talk elucidates the instrumentation and experimental possibilities available on these end-stations, with a particular emphasis on their use in the study of the three-dimensional structures of membrane proteins.
The ESRF is currently preparing the upgrade of its accelerator and storage ring as part of the ESRF Extremely Brilliant Source (EBS) project (see http://www.esrf.fr/about/upgrade for details). This presentation will thus also look ahead to the evolution of ESRF facilities for Structural Biology post-2020, focusing on the possibilities for time-resolved MX that ESRF-EBS X-ray beams will facilitate.

1. Mueller-Dieckmann, C. *et al.* The status of the macromolecular crystallography beamlines at the European Synchrotron Radiation Facility. *Eur. Phys. J. Plus* **130: 70** (2015); https://doi.org/10.1140/epjp/i2015-15070-0.
2. Pernot, P., *et al.* Upgraded ESRF BM29 beamline for SAXS on macromolecules in solution. *J. Synchrotron Rad.* **20**, 660-664 (2017).
3. von Stetten, D. *et al. In crystallo* optical spectroscopy (icOS) as a complementary tool on the macromolecular crystallography beamlines of the ESRF. *Acta Cryst.* **D71**, 15-26 (2015).
4. Lafumat, B., *et al.* Gas-sensitive biological crystals processed in pressurized oxygen and krypton atmospheres: deciphering gas channels in proteins using a novel `soak-and-freeze' methodology. *J. Appl. Cryst.* **49**, 1478-1487 (2016).

Transmembrane signal transduction in two-component systems

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Allosteric and transmembrane (TM) signaling are among the major questions of structural biology. Here, we review and discuss signal transduction in four-helical TM bundles, focusing on histidine kinases and chemoreceptors found in two-component systems. Previously, piston, scissors, and helical rotation have been proposed as the mechanisms of TM signaling. We discuss theoretically possible conformational changes and examine the available experimental data, including the recent crystallographic structures of bacterial nitrate/nitrite sensor histidine kinase NarQ [1] and archaeal phototaxis system NpSRII:NpHtrII [2]. We show that TM helices can flex at multiple points and argue that the various conformational changes are not mutually exclusive, and often are observed concomitantly, throughout the TM domain or in its part [3]. The piston and scissoring motions are the most prominent motions in the structures, but more research is needed for definitive conclusions.
The study was supported by the Russian Science Foundation (project № 18-74-10053).

1. Gushchin, I. *et al.* Mechanism of transmembrane signaling by sensor histidine kinases. *Science* (eaah6345). **356** (2017).
2. Ishchenko, A. *et al.* New Insights on Signal Propagation by Sensory Rhodopsin II/Transducer Complex. *Scientific Reports* (41811). **7** (2017).
3. Gushchin, I. & Gordeliy, V. Transmembrane Signal Transduction in Two-Component Systems: Piston, Scissoring, or Helical Rotation? *BioEssays* (1700197). **40** (2018).

"Achilles heel" of the bacterial membranes: computational modeling of lipid II—target for novel antibiotics

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Motivation and Aim: Antibiotics (AB) resistance is a major threat to global health, so development of novel AB classes is urgently needed. Lantibiotics are natural compounds that effectively control bacterial populations, thus study of the molecular mechanisms of recognition of their targets in bacterial membrane may drive creation of novel AB. Many lantibiotics (exemplified by their prototypic member nisin) target lipid II—unique molecule of the bacterial membranes that limits synthesis of the cell wall, which is vital for bacteria. The "core" of the recognition pattern is lipid II's pyrophosphate group, which is unlikely to evolve and thus represents engaging pharmaceutical target. Our purpose is to build realistic model of lipid II in its native environment, decipher molecular mechanism of its recognition by membranoactive antibiotics and design novel compounds "capturing" lipid II in bacterial membranes—prototypic antimicrobials relieved of the AB resistance.
Methods and Algorithms: We use molecular dynamics (MD) to model native-like bacterial membranes with dedicated phospholipids and lipid II content. Original surface mapping technique [1] reveals the perturbation of the membrane by lipid II. Tracing of intermolecular hydrogen bond formation and *Energy of the probe atoms pair* approach reveal the pharmacophore of lipid II recognition by nisin.
Results: Previously, we have shown that highly-flexible lipid II molecule introduces an "amphiphilic pattern" into the surrounding bacterial membrane—probable initial stage of lipid II recognition by the

extracellular agents [2]. Our current results establish a pyrophosphate recognition pharmacophore in the nisin molecule. We discover unique features of pyrophosphate chemical and spatial structure, which render it as promising target for further AB development.
Conclusion: These results may be employed for further study of lipid II targeting by antimicrobial (poly)cyclic peptides and for design of novel AB prototypes.
Acknowledgements: This work was supported by the Molecular and Cell Biology Program of the Russian Academy of Sciences, RFBR grant #16-04-00578 and within the framework of a subsidy by the Russian Academic Excellence Project "5-100". Access to computational facilities of the Supercomputer Center "Polytechnical" at the St. Petersburg Polytechnic University is greatly appreciated.

1. Koromyslova A.D., Chugunov A.O., Efremov R.G. Deciphering fine molecular details of proteins' structure and function with a Protein Surface Topography (PST) method. *J. Chem. Inf. Model.* **54**, 1189-1199 (2014);
2. Chugunov A., Pyrkova D., Nolde D., Polyansky A., Pentkovsky V., Efremov R. Lipid-II forms potential "landing terrain" for lantibiotics in simulated bacterial membrane. *Sci. Rep.* **3**, 1678 (2013).

Alpha-synuclein aggregation as a trigger for neuronal death in PD or how physiology becomes pathology

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Aging-related neurodegenerative diseases are characterized by the occurrence of misfolded proteins. Parkinson's disease (PD) is characterized by the formation of Lewi bodies, formed by aggregated alpha-synuclein. Toxicity of alpha-synuclein mainly depends on the type of aggregate it forms. Aggregation of alpha-synuclein affects neuronal calcium homeostasis and mitochondrial function and leads to transition metal-dependent ROS production and lipid peroxidation [1, 2, 3].
Alpha-synuclein monomers are constantly formed throughout life and play important role in physiological regulation of mitochondrial function [4]. When aggregated, alpha-synuclein that is directly interacting with the ATP synthase in physiology, switches to pathology via the adoption of a toxic gain of function. This results in the opening of the mitochondrial permeability transition pore and ultimately leads to neuronal loss [5]. Suppression of the rates of lipid peroxidation by different strategies restores physiological calcium signalling in human cellular PD models. Furthermore, prevention of ferroptosis by iron chelators, lipid soluble antioxidants, deuterated PUFAs or by ferrostatin-1: each is a promising approach towards alpha-synuclein- or dopamine-induced neuronal toxicity.

1. Angelova PR et al. Ca^{2+} is a key factor in α-synuclein-induced neurotoxicity. J. Cell Science (2016)
2. Deas E, N Cremades, PR Angelova et al. Alpha-synuclein oligomers interact with metal ions to induce oxidative stress and neuronal death in Parkinson's disease. Antioxidants & Redox Signaling (2015)
3. Angelova PR et al. Lipid peroxidation is essential for α-synuclein-induced cell death. J Neurochem, (2015)
4. Ludtmann MH, PR Angelova et al. Monomeric alpha-synuclein exerts physiological role on brain ATP Synthase Activity. J Neurosci. (2016)
5. Ludtmann MH *, PR Angelova* et al. Direct interaction of oligomeric α-synuclein with ATP synthase opens the permeability transition pore in PD. Nature Commun. (2018)

Basic topology of curves, polygons, and long molecules

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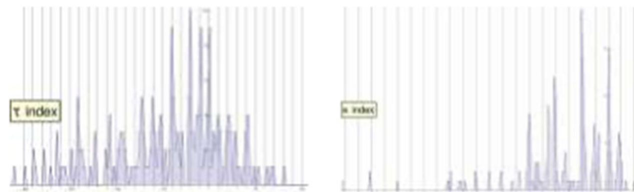

Fig. 1 Distribution of values of the torsion (left) and curvature (right) indices for 218 selected proteins from PDB

In the coarse graining approach, proteins can be represented by polygons connecting the positions of Ca atoms. The Frenet description of curves in threedimensional space, and its discrete form, can be applied to introduce standard topological characteristics for protein molecules [1]. We show that this description introduces a new classification of proteins in terms of a pair of discrete indices quantized in units of Pi (see Fig. 1 and Ref. [2]). We explain the connection of these protein indices with the standard topological invariants of curves, e.g., self-linking number, and with a field theory model invoking solitons [3].

1. Hu S., Lundgren M., Niemi A. J., Phys. Rev. E 83 (2011) 061908
2. Lundgren M., Krokhotin A., Niemi. A. J., Phys. Rev. E 88 (2013) 042709
3. Danielsson U. F., Lundgren M., Niemi A. J., Phys. Rev. E 82 (2010) 021910

Comparing modern and classic techniques for integral membrane protein purification

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Membrane proteins play key roles in fundamental biological processes, such as transport of molecules, signaling, energy utilization, and maintenance of cell and tissue structures. About 30% of the genes determined by genome sequencing encode membrane proteins, and these proteins comprise more than 50% of the current drug targets. Despite their importance, our knowledge of the structure and function of membrane proteins at the molecular level lags far behind that for soluble proteins [1].
Integral membrane proteins exist in a lipid environment of biological membranes (biomembrane), but the available techniques for purifying, handling, and analyzing them were optimized for water-soluble proteins in an aqueous environment. To be able to handle and study membrane proteins, they must be dispersed in an aqueous solution. This is usually accomplished by adding a detergent that solubilizes the biomembrane and forms a soluble complex with the lipids and membrane proteins [1].
The report is dedicated to comparison of different approaches for purification of recombinant integral membrane proteins with different affinity tags (6His and GST) including detergent screening on 96-well filter plates - His MultiTrap FF (28400990) or HP (28400989) and GST MultiTrap 4B (28405500) - for reproducible, high-throughput screening and rapid parallel purification [2, 3]. With this technology, wide range of chromatography conditions could be estimated in parallel for several proteins giving significant impact for rapid purification protocol development or

optimization. This approach could be valuable both for fundamental proteomics studies and for RnD phase in pharma or biotech. In GE HCLS (www.gelifesciences.com) study, the approach was successfully used for optimizing capture stage for six different histidine-tagged membrane proteins [2]. On the next stage upscaling multistep chromatography using GE HCLS chromatography systems, media and reagents was performed [2]. Preservation of enzymatic activity during detergent screening using this approach shown for GST-tagged *E. coli* integral membrane protein in other case study [3].

1. GE Healthcare Handbook "Purifying Challenging Proteins Principles and Methods" 28-9095-31 AA
2. His MultiTrap FF and His MultiTrap HP. GE Healthcare Data file 11-0036-63 AB
3. Glutathione S-transferase (GST) Gene Fusion System. GE Healthcare Data file 28-9622-84 AA

Molecular physiology and pathophysiology of K^+ coupling in glutamate transporter

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Excitatory amino acid transporters (EAATs) are secondary-active transporters that are crucial for the termination of glutamatergic synaptic transmission and for the maintenance of low resting glutamate levels. They transport glutamate from the synaptic cleft and its surrounding into neuronal and glial cells via alternating inward- and outward-directed rigid-body motions of the transport domain, either bound to (i) glutamate, three Na^+, and one H^+, or (ii) one K^+ (1). The complex transport stoichiometry permits the establishment of low resting glutamate concentrations to prevent glutamate excitotoxicity. Related prokaryotic transporters harness only Na^+ or H^+ gradients, suggesting that glutamate-bound translocation has undergone extensive evolutionary optimization in order to increase transport rates.
We combined molecular dynamics (MD) simulations of Glt_{Ph} and human EAAT1 with experiments on Glt_{Ph}, EAAT1, and EAAT2 to identify the K^+-coupling mechanism of EAATs. Moreover, we studied how impaired K^+-bound re-translocation in mutant EAAT1 is responsible for a human genetic disease, migraine with aura including hemiplegia (2).

1. Zerangue, N., & Kavanaugh, M. P. (1996). Flux coupling in a neuronal glutamate transporter. Nature, 383, 634.
2. Kovermann, P. *et al.* Impaired K^+ binding to glial glutamate transporter EAAT1 in migraine. Sci Rep 7, 13913

Proteins and time crystals

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In this talk, we first describe what are classical time crystals, in a Hamiltonian setting. We argue that time crystals are commonplace, in the case of linear molecules, and we continue to propose that proteins can be good examples where the dynamics is basically that of a time crystal. We show simple numerical examples of polypeptides, where time crystal behavior seems to be present.

Axial helix rotation in transmembrane signal transduction

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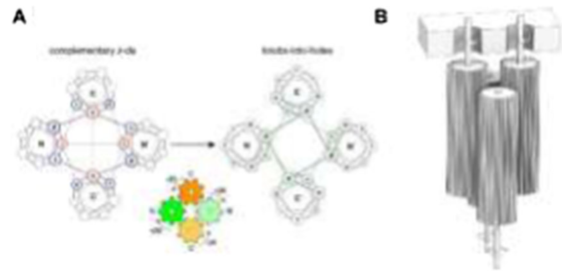

Fig. 1 **a** Schematic cross section through four-helical coiled coils, showing complementary x-da packing (left) vs. knobs-into-holes packing (right). The two packing modes can be interconverted by rotating adjacent helices by 26° in opposite directions, as illustrated by the cogwheel diagram. **b** Schematic view of the cogwheel model for signal transmission

The mechanism(s) by which extracelullar stimuli are transmitted from the sensory domains of transmembrane receptors to the effector domains are still substantially under debate. Whereas the most widespread model for propagation of the stimulus is the piston model, in which the axial displacement of one receptor subunit relative to the other activates the effector domain, we have put forward the cogwheel model (Fig. 1) [1], in which axial rotation of the helices between two coiled-coil packing modes [2] leads to the activation of the effector domain by a constrain-and-release mechanism [3]. In this mechanism, the coiled-coil backbone of the receptor sequesters the catalytic effector domains in an inactive conformation, until axial rotation of its helices releases the effector domains to assume a catalytically productive conformation. We have recently shown that this model applies not only to histidine kinases, but also to adenylyl cyclases [4].

1. Hulko, M. *et al*. The HAMP domain structure implies helix rotation in transmembrane signaling. *Cell* **126**, 929–940 (2006).
2. Lupas, A. N., Bassler, J. & Dunin-Horkawicz, S. The Structure and Topology of α-Helical Coiled Coils. *Subcell. Biochem*. **82**, 95–129 (2017).
3. Ferris, H. U. *et al*. Mechanism of regulation of receptor histidine kinases. *Structure* **20**, 56–66 (2012).
4. Bassler J, Schultz JE, Lupas AN. Adenylate cyclases: Receivers, transducers, and generators of signals. *Cell. Signal*. **46**, 135–144 (2018).

Trace amines and trace amine-associated receptors (TAARs)

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Trace amines are endogenous compounds classically regarded as composing β-phenylethyalmine, p-tyramine, tryptamine, p-octopamine, and some of their metabolites. They are also abundant in common foodstuffs, and can be produced and degraded by the constitutive microbiota. The ability to utilize trace amines has arisen at least twice, with unrelated receptor families present in invertebrates and vertebrates. The term trace amine was coined to reflect the low tissue levels in mammals, however, in invertebrates relatively high levels are present where they serve the role of an invertebrate adrenergic system involved in “fight or flight” responses. Vertebrates express a family of receptors termed trace amine-associated receptors (TAAR). Humans possess 6 functional receptors: TAAR1, TAAR2, TAAR5, TAAR6, TAAR8 and TAAR9. With the exception of TAAR1, TAAR are expressed in olfactory epithelium neurones, where

they detect diverse ethological signals including predators, spoiled food, migratory cues, and pheromones. Outside the olfactory system TAAR1 is the most thoroughly studied with both central and peripheral roles. In the brain, TAAR1 has been identified as a novel therapeutic target for several neuropsychiatric disorders, including schizophrenia, depression, and addiction. In the periphery, TAAR1 shows potential as a novel therapeutic target for diabetes and obesity, and may also regulate immune functions. Recent advances in understanding physiological relevance and pharmacological potential of human TAARs will be discussed. Through this, a picture emerges of an exciting field on the cusp of significant developments, with the potential to identify new therapeutic leads for some of the major unmet medical needs in several areas including neuropsychiatry and metabolic disorders.
This work was supported by the Russian Science Foundation (Grant no. 14-50-00069).

Diverse roles of cytoplasmic domains in Kv ion channels

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EAG channels play a major role in many physiological processes, including cardiac repolarization and cell proliferation. The non-domain-swapped structure of eag1 and hERG suggests that the mechanism of voltage-dependent gating is quite different from the classical mechanical lever model. Molecular aspects of hERG voltage-gating have been quite extensively studied. S4-S5 linker (S4-S5L) has been shown to act as a ligand binding to the S6 gate (S6 C-terminal part, S6T) and stabilizing it in a closed state. N-Cap, the N-terminal extremity of the channel has been suggested to interact with S4-S5L to modulate channel voltage-dependent gating. As a result, N-Cap deletion results in drastic acceleration of channel deactivation. Here, we addressed if these two major mechanisms of voltage-dependent gating are conserved in KV10 channels. (i) Using cysteine bridges and S4-S5L mimicking peptides, we showed that the ligand/receptor model is conserved in KV10.2, suggesting this model as a hallmark of slowly activating channels, as opposed to fast activating channels. (ii) Truncation of the N-Cap and/or the PAS domain prevented channel trafficking to the membrane, as opposed to hERG channel in which N-Cap and PAS domain truncation mainly affected channel gating. Altogether, our results suggest a conserved ligand/receptor (allosteric) model of voltage gating, but divergent roles in N-Cap and PAS domains among EAG channels.

Structure of intermediate filaments: progress and challenges

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The three-dimensional structure of intermediate filaments (IFs), also known as nanofilaments, is a fundamental, still largely unresolved problem of structural molecular biology. Functional studies of various IF protein classes are currently florishing, which is not surprising due to their fundamental importance and scores of disease-related mutations found in IF proteins. Yet the understanding of their 3D structure is clearly lagging behind, despite a long history of research. As I will explain, recently there has been a substantial progress on the atomic structure of the signature central domain of IF proteins which forms a segmented coiled-coil dimer. This progress is due to both experimental crystallographic studies and in silico structure prediction. Likewise, new data are becoming available on the atomic structure of the tetramer, the solution species observed for cytoplasmic IF classes. At the same time, the available evidence on higher-order architecture of IFs is still lacking sufficient detail, with intrinsic disorder and heterogeneity being the major challenges. I will discuss the recent developments, based on a synergistic use of various experimental approaches, that contribute to progress in the field.

Lipidic nanocarriers for drug delivery

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At present time, nanoparticles are increasingly used in the targeted delivery of drugs, genetic material, and diagnostic of different diseases. The formation of nanocarriers based on amphiphilic properties of the compounds that form the nanocarriers. Usually, it is the phospholipids, detergents, and some other compounds.
Several promising small-molecule drugs and genes previously deemed less than useful due to problems of stability, solubility, and nonspecific toxicity can now be delivered to the intended sites of action with the help of nanocarriers like micelles, nanoparticles, and liposomes.
Advantages of the nanocariers include the following:

- Improved solubility of the encapsulated drugs
- Prevention of chemical and biological degradation under storage conditions of agents and during patient administration`
- Reduction of the nonspecific side-effects and toxicity of encapsulated drugs, thus improving their efficacy and therapeutic index
- Versatility when chemically modified with attached specific surface ligands for targeting
- Compatibility with biodegradable and nontoxic materials
- Methods of producing nanocarriers, their use in drug and gene delivery and diagnostics are discussed.

1. Chupin, V., de Kroon, A. I., de Kruijff, B. Molecular architecture of nanocapsules, bilayer-enclosed solid particles of cisplatin. Journal of the American Chemical Society, 126, 42 (2004).
2. Ivanova, E. A., et al. Synthesis of bivalent neogalactolipids via modified Staudinger reaction. RSC Advances, 2, 11 (2012).
3. Nguen, H. Q., et al. Development and characterization of nanoparticles prepared from the mixture of triterpenoids and amphiphilic meso-arylporphirins. Russian Journal of Bioorganic Chemistry, 41, 2 (2015).
4. Pattni B.S., Chupin V.V., Torchilin V.P. New Developments in Liposomal Drug Delivery. Chem. Rev. 115, 19 (2015).

A decade of GPCR structural biology

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G protein-coupled receptors (GPCRs) are cellular gatekeepers that regulate a variety of physiological processes in the human body and serve as attractive pharmaceutical drug targets. Structure-function studies of this superfamily have been enabled in 2007 [1, 2] by multiple breakthroughs in technology that included receptor stabilization [3], crystallization in a membrane environment [4], and microcrystallography [5]. This talk will summarize key advancements in our understanding of the mechanisms of ligand recognition, allosteric modulation and signal transduction across the membrane, contributed by the last 10 years of structural studies of GPCRs [6]. Recent advancements in X-ray free electron lasers and cryo-electron microscopy open up new opportunities and promise to further accelerate structure-function studies of the whole GPCR superfamily [7, 8].

1. Cherezov, V. et al. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. *Science* **318**, 1258 (2007).
2. Rasmussen, S.G.F. et al. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature* **450**, 383 (2007).
3. Rosenbaum, D.M. et al. GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. *Science* **318**, 1266 (2007).
4. Cherezov, V. Lipidic cubic phase technologies for membrane protein structural studies. *Curr Opin Struct Biol* **21**, 559 (2011).
5. Cherezov, V. et al. Rastering strategy for screening and centring of microcrystal samples of human membrane proteins with a sub-10 micrometer size X-ray synchrotron beam. *J R Soc Interface* **6 (Suppl 5)**, S587 (2010).
6. Katritch, V., Cherezov, V., Stevens, R.C. Structure-function of the G protein-coupled receptors superfamily. *Annu Rev Pharmacol Toxicol* **53**, 531 (2013).
7. Stauch, B., Cherezov, V. Serial Femtosecond crystallography of G protein-coupled receptors. *Annu Rev Biophys* **47**, 377 (2018).
8. Ishchenko, A., Gati, C., Cherezov, V. Structural biology of G protein-coupled receptors: new opportunities from XFELs and cryoEM. *Curr Opin Struct Biol* **51**, 44 (2018).

Early optogenetics transferring bacteriorhodopsin into the inner mitochondrial membrane and observing protein synthesis at a ribosome by optical tweezers

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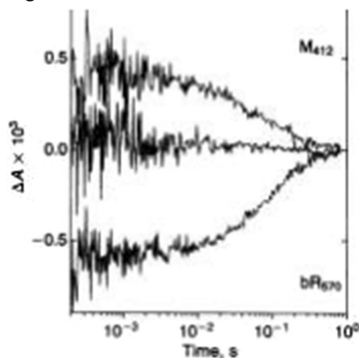

Fig. 1 Light-induced changes in absorbance (ΔA) measured at 412 nm (M intermediate of bR) for bR incorporated into the IM. When M state is decreasing (upper trace), ground state of bR measured at 570 nm (lower trace) is increasing. The middle trace depicts a measurement at 412 nm of a sample where no bR is incorporated into the IM.

Optogenetic study: The light-driven proton pump bacteriorhodopsin (bR) from Halobacterium salinarum has been genetically transferred into the Inner Mitochondrial membrane (IM) of the eukaryotic cell Schizosaccharomyces pombe, where the archaebacterial proton pump replaces or increases the proton gradient usually formed by the respiratory chain. For targeting and integration, as well as for the correct orientation of bR in the IM, the bacterioopsin gene (bop) was fused to signal sequences of IM proteins. Retinal was added to the culture medium. Northern and Western blot analysis proved that all hybrid gene constructs containing the bop gene and a mitochondrial signal sequence were expressed and processed to mature bR. Fast transient absorption spectroscopy showed photocycle activity of bR integrated in the IM by formation of the M intermediate. Experiments with the pH-sensitive fluorescence dye 2',7'-bis(2-carboxyethyl)-5 (and -6)-carboxyfluorescein revealed bR-mediated proton pumping from the mitochondrial matrix into the intermembrane space. Glucose uptake measurements under anaerobic conditions showed that yeast cells containing photoactive mitochondria need less sugar under illumination. In summary, our experiments demonstrate the functional genetic transfer of a light energy converter to a naturally non-photoactive eukaryotic organism.

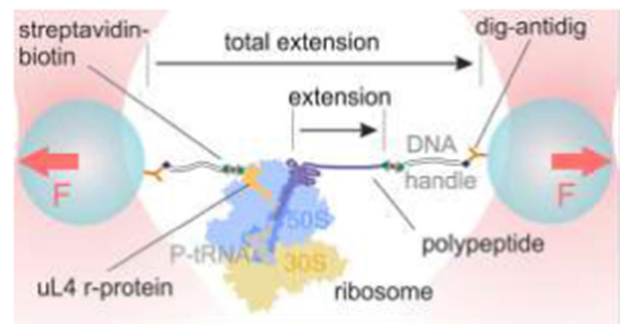

Fig. 2 By dual trap optical tweezers where the ribosome is linked to one polystyrene bead (F) and the nascent polypeptide chain to second one.

Protein synthesis, a single molecule study: Protein biosynthesis is inherently coupled to co-translational protein folding. Folding of the nascent chain already occurs during synthesis and is mediated by spatial constraints imposed by the ribosomal exit tunnel as well as self-interactions. How co-translational protein folding and the rate of synthesis are linked to a protein's amino acid sequence is still not well defined. Here, we follow synthesis by individual ribosomes using dual-trap optical tweezers and observe simultaneous folding of the nascent polypeptide chain in real time. We show that observed stalling during translation correlates with slowed peptide bond formation at successive proline sequence positions and electrostatic interactions between positively charged amino acids and the ribosomal tunnel. We also determine co-translational folding sites initiated by hydrophobic collapse for an unstructured and two globular proteins while directly measuring initial co-translational folding forces. Our study elucidates the intricate relationship among a protein's amino acid sequence, its co-translational nascent-chain elongation rate, and folding.

1. Hoffmann, A., Hildebrandt, V., Heberle, J & Büldt,G. Photoactive mitochondria: *In vivo* transfer of a light-driven proton pump into the inner mitochondrial membrane of Schizosaccharomyces pombe. Proc. Natl. Acad. Sci. USA. 91, 9367-9371 (1994)
2. Wruck, F., Katranidis, A. Nierhaus, K.H., Büldt, G. & Hegner. M. Translation and folding of single proteins in real time. Proc. Natl. Acad. Sci. USA. 114 (22) 4399-4407 (2017)

Engineering the cell membrane for bioelectronics

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Degeneration or errant behavior of electrogenic cells, such as neurons and muscle, are critical components of various diseases and disabilities. In order to correct these deficiencies, a variety of

devices have been developed to interface with the body, sending and receiving electrical signals that can be processed by cells. These hybrid systems composed of synthetic electronics and living cells form the basis of bioelectronics. Through the use of genetic engineering we can now also engineer the cells themselves to improve bioelectronics systems (Fig. 1). By combining light-sensitive protein channels and pumps, or fluorescent reporters we can decouple bioelectronics stimulation and recording to prevent artefacts. We also show a higher spatial specificity of manipulating single cells in a network using optogenetics than using electrical stimulation from extracellular electrodes[1]. Combining optogenetics with our network patterning expertise, we are now designing externally controllable neuronal networks with defined connectivity. To increase spatial specificity of optical manipulation even further, we are pursuing several strategies to restrict optogenetic actuation to specific neuronal structures, such as the axon or dendrites.

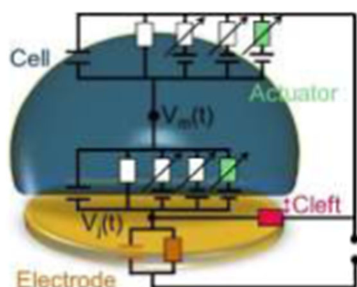

Fig. 1 Electrical model of a bioelectronic system overlaid on the schematic of a cell and extracellular electrode. The cell membrane can be genetically modified to improve properties of the membrane resistance and cleft resistance

1. Maybeck, V. et al. An evaluation of extracellular MEA versus optogenetic stimulation of cortical neurons. Biomedical Physics & Engineering Express (2016). https://doi.org/10.1088/2057-1967/2/5/055017

YOUNG SCIENTISTS SESSION

Determination of an underneath binding in the transmembrane domain of smoothened receptor

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Smoothened receptor (SMO) is a key component in the Hedgehog (Hh) signal transduction pathway[1]. Pharmaceutical developments on SMO have resulted in FDA-approved drugs for the treatment of basal-cell carcinoma, which unfortunately, were soon associated with drug resistance[2-3]. There are several ligands that, like Allo-1, remained the antagonism on drug-resistant mutant but their binding and related mechanism was unknown. Following our successive efforts in the structural study of SMO, we report herein the determination of an underneath binding for Allo-1 using integrative chemical and computational approaches such as photoaffinity probe based chemoproteomic study, computational modeling and structure activity relationship (SAR) analysis[4]. This mode reveals the molecular base of Allo-1 binding and paves the way for the design of new generation of ligands for cancer treatment.

Activation and allosteric modulation of muscarinic acetylcholine M4 receptor

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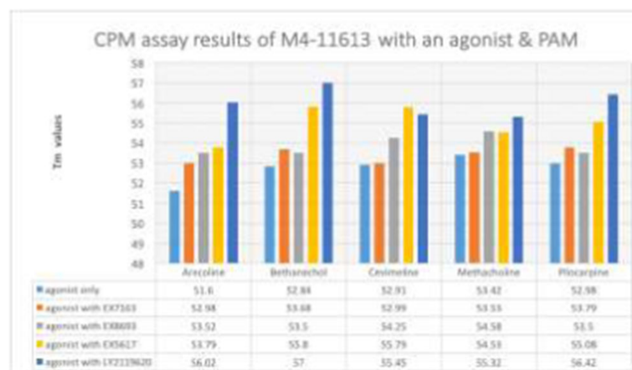

Fig. 1. The Tm values of M4 protein binding with different agonist and PAM groups in CPM assay

Muscarinic acetylcholine 4 receptor (M4 mAChR) provides valuable target for the treatment of multiple central nervous system disorders, including schizophrenia, Alzheimer's disease (AD) and Huntington's disease (HD). Both orthosteric agonists and positive allosteric modulators (PAM) of M4 have been reported as promising ligands that not only have antipsychotic effects, but can also improve cognitive impairment and motor dysfunction. The M4 mAChR is of particular interest with regards to its involvement in schizophrenia because it has been implicated in the regulation of dopamine levels in the brain. Current efforts have now focused on modulators that bind to allosteric sites on mAChRs due to the high homology of the orthosteric binding sites among all muscarinic receptors, allowing these compounds to display unprecedented subtype selectivity. The structure of M4 in complex with orthosteric inverse agonist was solved. However, recent studies show that allosteric modulators hold potential to develop subtype-specific and pathway-specific therapeutics compared with orthosteric ligands. The binding modes and molecular mechanism of M4 allosteric modulation are still unknown. In this study, we have screened different combinations of agonists and allosteric modulators to stabilize the receptor (Figure 1) and obtained some crystal hints. Hope we can solve the high-resolution crystal structures of M4 in complex with different allosteric modulators, and also perform the pharmacology studies of M4, to offer important insights into the activation mechanism and allosteric modulation of M4 receptor and provide new perspectives for the development of novel M4 ligand drugs for several severe disorders of the central nervous system. These achievements improve the strategic design of small molecules for lead identification and optimization targeting allosteric binding sites of GPCR.

1. Weixing Shen, Joshua L. Plotkin et al. M4 Muscarinic Receptor Signaling Ameliorates Striatal Plasticity Deficits in Models of L-DOPA-Induced Dyskinesia. null, Volume 90, Issue 5, 2016, 1139.
2. Carrie K Jones, Michael Bubser et al. Muscarinic and Nicotinic Acetylcholine Receptor Agonists and Allosteric Modulators for the treatment of Schizophrenia. Neuropsychopharmacology Reviews 37, 16-42 (2012).
3. David M. Thal, Bingfa Sun et al. Crystal structures of the M1 and M4 muscarinic acetylcholine receptors. Nature 1–6 (2016).

Structural basis of G protein couple receptor polypharmacology

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Polypharmacology is the next generation of disease treatment strategy, which offers more effective drugs that are less toxic and have fewer side effects. However, polypharmacological drugs, which simultaneously act on multiple targets, remain extremely difficult to develop, though maybe not for long according to the structural study of GPCR, which is a family of many important drug targets. Apparently, the key lies in obtaining a better understanding of the three-dimensional structure of receptor proteins. Dr. Yao Peng and her team's research results show the high-resolution atomic structure of the human serotonin receptor 2C (5-HT2C), an important G-protein-coupled receptor (GPCR) drug target for the treatment of schizophrenia and drug abuse, and recently was central to the development of the anti-obesity drug Belviq. This is an important step towards realizing the full potential of polypharmaceuticals. The ability to observe atomic-level structures of receptors like 5-HT2C will revolutionize drug development. The three-dimensional structural images of 5-HT2C were presented in active and inactive states, as well as the structural basis of GPCR polypharmacology being deciphered in her research. Such detailed data of key receptors will help drug hunters design new polypharmacology drugs, revolutionizing the treatment of disease.

STMS: cell—spatiotemporal multi-scale modeling of eukaryotic cells

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The construction of a predictive model of an entire eukaryotic cell that describes its dynamic structure from atomic to cellular scales is a grand challenge at the intersection of biology, chemistry, physics, digital media and computer science. This task requires both an enormous amount of data, as well as integrative computational methods.[1] There is no such model available that can fully represent the complexity and scope of an entire cell. Therefore, we are using STMS-Cell (spatiotemporal multi-scale modeling of eukaryotic cells) an integrated approach that brings together diverse disciplines to create functional models. For our proof-of-principle work, we have selected to focus on pancreatic β cells as our target, because of their relatively simple anatomy (e.g., without the axons and dendrites of neurons). The wealth of β-cell experimental data available in the literature, especially from electron tomography and transcriptomics, and their well-characterized physiology make β cells an ideal platform for initiating the STMS-Cell platform. Additionally, diabetes is a worldwide problem affecting hundreds of millions of people with increasing patient numbers every year. As a first step, we are defining the ultrastructure components, and identify proteins expressed in β cells and their quantities using a combination of X-ray tomography, cryo-electron tomography, mass-spec proteomic analysis. Additionally, we will determine how different glucose or GPCR agonist treatments effect the cellular architecture and protein expression. Our ultimate goal is to integrate these data using both computational and mathematical modeling approaches. These STMS-Cell models will represent a sophisticated convergence of our understanding of cellular structure and function, revolutionize biological discovery, open new dimensions of research, and accelerate advancements in healthcare.

1. Singla J. *et al.*, Opportunities and challenges in building a spatiotemporal multi-scale model of the human pancreatic β-cell. (2018) *Cell.* 173(1):11-19.

Effects of mono- and divalent cations on GPCR stability

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G protein-coupled receptors (GPCRs) constitute the largest superfamily of proteins in eukaryotic organisms, which transmit signals across the cell membrane and regulate many physiological processes. Because of their involvement in variety of diseases, GPCRs have been extensively studied during the last several decades; yet their molecular mechanisms of ligand recognition and signal transduction are not fully understood.
The importance of sodium in ligand binding and GPCR function was first reported over 40 years ago[1]. However, only recently high-resolution GPCR structures helped to reveal a sodium binding site coordinated by D2.50 and other conserved in class A GPCRs residues[2-3]. It has been shown that sodium stabilizes the inactive state, and suggested that it plays a key role in receptor activation[4]. Analysis of the available GPCR structures demonstrates that Na^+ binding coordination varies among GPCR subfamilies, calling for more detailed studies.
In this work, we report effects of mono- and divalent cations on GPCR stability. Experiments, performed on the wild type receptor and sodium site mutants, revealed Na^+ ion selectivity and affinity.
The work was supported by the Russian President Grant for Governmental support of Young Scientists (project no. MK-5184.2018.4)

1. Pert, C. B. et al. Opiate Agonists and Antagonists Discriminated by Receptor Binding in Brain. *Science* 182, 1359–61 (1973).
2. Liu, W. et al. Structural Basis for Allosteric Regulation of GPCRs by Sodium Ions. *Science* 337, 232–6 (2012).
3. Fenalti, G. et al. Molecular control of delta-opioid receptor signaling. *Nature* 506, 191 (2014).
4. Zhang C. et al. High-resolution crystal structure of human protease-activated receptor 1, *Nature* 492, 387-92 (2012). This work was supported by the Russian Science Foundation (project no. 16-14-10273).

5. Katritch, V. et al. Allosteric sodium in class A GPCR signaling. *Trends Biochem Sci*. 39, 233–44 (2015).

Optimization of G protein-coupled receptor expression for functional studies

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During the last decade, progress in structure-function studies of G protein-coupled receptors (GPCRs) has spurred an increased interest in rational drug discovery[1]. GPCRs are responsible for the intracellular signal transduction eliciting specific cellular responses. They regulate majority of physiological processes and, therefore, are involved in a variety of pathologies from allergic reactions to color blindness, cardiovascular diseases, and cancerogenesis. About 30% of all approved drugs target GPCRs, making this protein superfamily highly popular for drug design applications[2]. Initially designed compounds are tested via ligand screening assays that require properly folded and functional receptor to be expressed on the plasma membrane of eukaryotic cells[3].
In this work, we have systematically studied several factors that can influence expression level and protein trafficking in several mammalian and insect cell lines. Infection timing, transfection protocol, and regulating regions were optimized for specific GPCR targets, and the effects of N-terminal tags as well as introns have been tested. The optimal combination was used for further steps in the ligand-screening pipeline. This work was supported by the Russian Science Foundation (project no. 16-14-10273).

1.Hauser, A. S., Attwood, M. M., Rask-Andersen, M., Schiöth, H. B. & Gloriam, D. E. Trends in GPCR drug discovery: New agents, targets and indications. *Nat. Rev. Drug Discov.* **16,** 829–842 (2017).
2.Sriram, K. & Insel, P. A. GPCRs as targets for approved drugs: How many targets and how many drugs? *Mol. Pharmacol.* mol.117.111062 (2018). https://doi.org/10.1124/mol.117.111062
3.Zhang, R. & Xie, X. Tools for GPCR drug discovery. *Acta Pharmacol. Sin.* **33,** 372–384 (2012).

Functional and structural characterization of the OLPVR1 viral rhodopsin from giant virus in Antarctica

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Microbial rhodopsins are light-driven membrane protein superfamily, which members were found in all kingdoms of life and have extremely diverse functions[1,2]. Recently, proteorhodopsin genes were found in genome of Organic Lake Phycodna virus, that inhabits shallow hypersaline Organic Lake in Antarctica[3]. Viral rhodopsins are classified into two distinct subfamilies, namely group I and group II, that have low similarity with other microbial rhodopsin families, and therefore are of high interest to investigate[3,4]. In this work, we report a comprehensive study of Organic Lake Phycodna viral rhodopsin 1 (OLPVR1), which is the member of the viral rhodopsin group I. OLPVR1 was expressed in *E. coli* and purified with metal affinity chromatography (Ni-NTA) and SEC, that allowed production of the highly purified protein, which was used for functional tests and crystallization trials. Being incorporated in soy bean liposomes OLPVR1 has been observed to change the pH of the media upon illumination. Photocycle studies have shown that the protein has an extremely long photocycle (about 10 s) in DDM and DMPC-MSP1D1 nanodiscs. OLPVR1 was successfully crystallized *in meso* in monoolein and monopalmytolein lipids, and the crystals gave X-ray diffraction up to 1.6 Å and 1.4 Å respectively (ESRF, ID23-1 and ID30B). High resolution structure of OLPVR1 reveals that the protein has an unusual binding pocket with monoolein molecule inside that directly interact with OLPVR1 hydrogen bond network in cytoplasmic/extracellular part of the protein. Such lipid-protein interaction has not been observed for the rhodopsins previously and could be functionally relevant. Ion channeling, patch-clamp and site-directed mutagenesis studies of OLPVR1 are currently in progress and are aimed to clarify the possibilities for the protein to be used as an optogenetic tool.

1.Gushchin, I. & Gordeliy, V. *Microbial rhodopsins. Subcellular Biochemistry* **87,** (2018).
2.Béjà, O. *et al.* Bacterial Rhodopsin : Evidence for a New Type of Phototrophy in the Sea. *Science (80-.).* **289,** 1902–1906 (2000).
3.Yutin, N. & Koonin, E. V. Proteorhodopsin genes in giant viruses. *Biol. Direct* **7,** 34 (2012).
4.López, J. L. *et al.* Microbial and viral-like rhodopsins present in coastal marine sediments from four polar and subpolar regions. *FEMS Microbiol. Ecol.* **93,** 1–9 (2017).

Structural and functional features of the light-driven sodium pump KR2

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Rhodopsins are the most universal biological light-energy transducers and abundant phototrophic mechanisms evolved on Earth. They are found in all the kingdoms of life and have a remarkable diversity and potential for biotechnological applications. Recently, the first cationic non proton sodium-pumping rhodopsin KR2 from *Krokinobacter eikastus*, which pumps Na^+ only at physiological pH, but H^+ at acidic pH, was discovered and functionally and structurally characterized[1–3]. However, the existing structures of KR2 are contradictory and the crystals used for the investigations were grown at low pH. Therefore the mechanism of Na^+ pumping is not yet completely understood.
We solved the high-resolution structure of KR2 using the crystals grown at physiological pH, representing its Na^+ pumping state. We successfully crystallized and solved several atomic resolution structures of the KR2 and its mutants in functionally important states. The structures shed light on the sodium pumping mechanism of KR2 and helped us to show that oligomerization of the microbial rhodopsin is essential for its biological function. The studies also demonstrate the rearrangements in the protein with pH decrease. The precise structure provides new insights into the

mechanisms of microbial rhodopsins and opens the way to a rational design of novel optimized cation pumps for optogenetics[4].

1.Inoue, K. *et al.* A light-driven sodium ion pump in marine bacteria. *Nat. Commun.* **4,** (2013).
2.Kato, H. E. *et al.* Structural basis for Na + transport mechanism by a light-driven Na + pump. *Nature* **521,** 48–53 (2015).
3.Gushchin, I. *et al.* Crystal structure of a light-driven sodium pump. *Nat. Struct. Mol. Biol.* **22,** (2015).
4.Grimm, C., Silapetere, A., Vogt, A., Bernal Sierra, Y. A. & Hegemann, P. Electrical properties, substrate specificity and optogenetic potential of the engineered light-driven sodium pump eKR2. *Sci. Rep.* **8,** 9316 (2018).

Exploration of a conformational landscape for a membrane protein via single-molecule fluorescence microscopy

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Membrane proteins are challenging research objects, but also important drug targets. Emerging structural studies provide essential static information about protein organization and protein-drug interaction interfaces. On the other hand, mechanistic understanding of membrane protein function requires the information about its conformational landscape and dynamics. This type of information is hardly accessible by the majority of ensemble-based methods, but can be resolved via single-molecule fluorescence spectroscopy.
To get information about number of conformational states, their relative population and kinetics for a membrane protein we analyzed patterns of single-molecule fluorescence (intensity, lifetime and anisotropy) from protein-induced fluorescence enhancement (PIFE)-compatible fluorophores attached to specific sites of the proteins-of-interest. For this study, we optimized protocols of (1) membrane protein expression and purification, (2) specific fluorescent labeling of protein-of-interest and (3) membrane protein reconstitution in nanodisc. To expand the observation time for each protein we (4) immobilize it on the surface of microscopy slide. We also (5) perform experiment with proteins freely diffusing in solution in nanodisc to have a control against immobilization artifacts. This work was supported by the Russian Science Foundation (project no. 16-14-10273).
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Successful GPCR structure determination using PAL XFEL

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Membrane proteins play an important role in signal transduction across the cell membrane in many organisms [1–3]. Their structural studies have recently become more accessible due to advancements in their expression, stabilization and crystallization, as well in computational methods of stability prediction, facilitating above mentioned steps [4]. In the past decade, serial femtosecond crystallography (SFX) using X-ray free electron lasers (XFELs) enabled structure determination of previously challenging G protein-coupled receptor (GPCR) structures [5], paving the way to structure-based drug design and understanding of GPCR activation mechanisms [6]. However, currently only 5 XFELs are available for SFX experiments, among which, to our knowledge, only 3 have reported at least one successful example of proof-of-concept on protein structure determination, including recently published EuXFEL structures [7]. Here, we report the first to our knowledge successful GPCR structure determination using the NXI beamline at PAL XFEL [8]. The data were collected at 30 Hz within less than 12 h using an LCP injector [9] for microcrystal delivery, resulting in a 2.5 Å resolution structure. This outlines the ability of PAL XFEL to facilitate novel structure determination projects, with potential for improvement in near future.This work was supported by the Russian Science Foundation (project no. 16-14-10273)

1. Gushchin, I. & Gordeliy, V. Microbial Rhodopsins. In: *Membrane Protein Complexes: Structure and Function. Subcellular Biochemistry*, vol 87, Harris J., Boekema E. (eds), Springer, Singapore (2018). https://doi.org/10.1007/978-981-10-7757-9_2
2. Gushchin, I. *et al.* Mechanism of transmembrane signaling by sensor histidine kinases. *Science* **356,** eaah6345 (2017).
3. Volkov, O. *et al.* Structural insights into ion conduction by channelrhodopsin 2. *Science* **358,** eaan8862 (2017).
4. Popov, P. et al. Computational design of thermostabilizing point mutations for G protein-coupled receptors. *eLife* 7, e34729 (2018). https://doi.org/10.7554/eLife.34729.002
5. Katritch, V., Cherezov, V. & Stevens, R. C. Structure-Function of the G Protein–Coupled Receptor Superfamily. *Ann. Rev. Pharma. Toxicol.* 53, 531–556 (2013).
6. Stauch B, Cherezov V (2018) Serial Femtosecond Crystallography of G Protein-Coupled Receptors. *Annu. Rev. Biophys.* 47: 377-397
7. Park, J., Kim, S., Nam, K.-H., Kim, B. & Ko, I. S. Current status of the CXI beamline at the PAL-XFEL. *J. Korean Phys. Soc.* 69, 1089–1093 (2016).
8. Weierstall, U. et al. Lipidic cubic phase injector facilitates membrane protein serial femtosecond crystallography. *Nat. Commun.* 5, 3309 (2014). https://doi.org/10.1038/ncomms4309
9. Grünbein, M. L. et al. Megahertz data collection from protein microcrystals at an X-ray free-electron laser. *Nat. Commun.* 9, 3487 (2018). https://doi.org/10.1038/s41467-018-05953-4

THE INTERNATIONAL SCHOOL MECHANISMS OF AGING AND AGE-RELATED DISEASES

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Gerontology in the Russian Federation: yesterday, today, tomorrow

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There are several milestones in the history of Russian gerontology. First of all, this is a book by Ilia Metschnikoff «Etudes sur la nature humaine: Essai philosophie optimiste» (1903), where he introduced the term «gerontology» and put the cornerstone of the scientific discipline in biology and physiology of aging. In the 20-ies of the XX century the works of

N.A. Belov, A.A. Bogdanov, I.I. Schmalhausen, S.A. Voronov raised the question on the possible increase in the life span of animals and humans. The 30-40-ies are characterized by the development of the first national gerontological schools in this country—in Kiev and Kharkov (A.A. Bogomolets, A.V. Nagorny, I.N. Bulankin) and in Leningrad (Z.G. Frenkel, E. S. Bauer, V.G. Baranov). In 1938 in Kiev there took place the first scientific conference on aging. In 1957 in Leningrad, Z.G. Frenkel and I.I. Likhnitskaya organized the first in this country City Scientific Society of Gerontologists and Geriatricians. In 1958, there was established Research Institute of Gerontology of the USSR Academy of Medical Sciences in Kiev. In 1963, in Kiev, there took place the first All-Union Conference (Congress) on Gerontology and Geriatrics. A long-term All-Union comprehensive research program in gerontology and geriatrics was elaborated and coordinated by the Joint Research Council in Human Physiology of the USSR Academy of Sciences and Academy of Medical Sciences during the period from 1981 to 1990. This period is characterized by active development of gerontology in the many regions of the country—in Leningrad, Moscow, Tbilissi, Kishinev, Minsk, etc. Of great importance appeared to be workshops «Basic problems of aging» organized by N.M. Emanuel (1970-1984). The Group (Laboratory) of Mechanisms of Aging was organized by V.M. Dilman in 1973 at the Institute of Experimental Medicine in Leningrad. Four All-Union Congresses were held in 1972, 1976, 1982 and 1988. In 1990 in Kiev the first issue of the All-Union journal "Problems of Aging and Longevity" saw the light. Major steps of Russian gerontology development up to middle 80-ies of the last century were described by Yu.K. Duplenko (1985).

Desintegration of the USSR resulted in the collapse of all former All-Union structures and actual closure of systematic studies in gerontology and geriatrics on the territory of the Russian Federation (Anisimov et al., 2011). In 1992, St. Petersburg Institute of Bioregulation and Gerontology was organized in St. Petersburg, in Samara - Research Institute "International Centre for the Problems of the Aged" (1996), in Moscow—Research Institute of Gerontology (1997). In 1994, in St.Petersburge, the Russian Gerontological Society was founded which united of leading scientists and specialists in gerontology and geriatrics, demography and behavior, social workers etc. around the country irrespective of their agencies belonging. In 1995, Gerontological Society was registered as the Gerontological Society of Russian Academy of Science (RAS), and in 1997 at the 16th IAG Congress it became a member of IAG. The Society publish the journal "Advances in Gerontology" since 1997. Journals "Clinical Gerontology," "Gerontology and Geriatrics," "Old Generation," "Psychology of Maturity and Ageing" are regularly also published in the country, as well a number of scientific books, textbook and manuals are published in the field of gerontology and geriatrics (Kotelnikov et al., 1997; Melentiev & Yarygin et al., 2003, 2005, Tkacheva et al, 2018).

Over 300 scientific conferences and symposia, including about 50 international ones have been organized since the date of Gerontological Society foundation in 1994. Among them it is worth mentioning such significant events as The Russian Congresses of Gerontologists and Geriatrists was held in 1999 (Samara), 2003 (Moscow) and 2012 (Novosibirsk), the 2nd European Congress on Biogerontology, 2000, St. Petersburg; the 6th European Congress of Clinical Gerontology, 2002, Moscow; the 6th European Congress of the International Association of Gerontology and Geriatrics, 2007, St. Petersburg;; European school on oncology «Cancer in the Aged: Advances and Prospects», 2001, Moscow, annual International Forum "Old Generation," St. Petersburg etc. (Mikhailova et al., 2005)

In 2012 at Novosibirsk Congress new Russian Association of Gerontology and Geriatrics (RAGG) has been organized focused on geriatric problems and service for elderly. In 2017 and 2018 RAGG held two Congresses of Geriatrics in Moscow.

Teaching of gerontology in Russia has been included into curricula since 1993, and speciality "physician-geriatrist" was adopted by the resolution of the RF Ministry of Health and RF Ministry of Education № 33 of 1995. The system of personnel training in gerontology in the USSR takes its beginning from the onset of postgraduate course. In 1986, there was set up the first in Russia Chair of Geriatrics in Leningrad State Institute for Postgraduate Education where the Department of Geriatrics has been launched since 1980. Alongside training of medical workers in gerontology and geriatrics there has been undertaken personnel training in social sphere starting from 1992. It has been conducted in compliance with the state educational standards of higher professional education on specialties "Social work" and "Social pedagogy". «Social work» standard has a special discipline «Social gerontology» therein. Demographic aspects are included into the course "Social politics," the issues of pensioners' rights protection—into the course "Legal coverage of social work," gender issues—into the course «Genderology and Feminology». According to the state educational standard, professional social work embraces population social protection, work with different social, age, gender and ethnic groups, individuals in need of social assistance and protection. Basic curricula include humanitarian, socio-economic and natural sciences. This multidisciplinary training has an integrative character. Postgraduate courses for social workers in the field of gerontology are open in many national universities. International schools in gerontology launched in Russia gave a new impulse to personnel training. Bearing in mind acute interest at the national level towards geriatric oncology and contribution of Russian gerontologists to the development of this issue, In 2002–2017 in St. Petersburg there were held six International schools on gerontology and geriatrics organized by the International Institute on Aging—UNO (Malta), and The Gerontological Society of RAS.

On the initiative of the Gerontological Society a scientific specialty «Gerontology and Geriatrics—medical and biological sciences» has been introduced into the official list of specialties of the Russian Federation in 2001. More 300 dissertations were prepared and defended on the new specialty. It is worth noting that researchers from Ukraine, Belarus, Kazakhstan and Uzbekistan, Syria, Ecuador, Sweden got their Ph.D.and DSc. degrees from the dissertation councils in Russia.

The primary objectives of the Gerontological Society consist in promoting the development of gerontology and related fields of physiology and biology; integrating research results with practice; establishing and maintaining contacts with scientific gerontological institutions of former Soviet Union and other countries and with international non-governmental scientific organizations; organizing and convening meetings to exchange and discuss research and practical issues; assisting Society members in improving their professional skills and research activities; providing research and methodological assistance in teaching gerontology and geriatrics at higher schools and those for paramedical personnel; membership in international scientific associations and participating in the international meetings; fostering and distributing knowledge and recent scientific achievements in the field of Society's activity.

Gerontological Society of RAS started in 1994 with 7 regional branches and about 100 members at present joints 50 regional branches and about 2500 members. The bulk of investigation carried out by Russian researchers according to certain directions is quite substantial, but unfortunately their publications are rather rare in leading international journals. Many of them do not meet the requirements of such journals due to weak methodological basis, thus they cannot contribute to the development of the issue they dwell on. At the same time, some of them could have undoubtedly shown up-to-date professional level should their research be supported and their laboratories up-graded.

Demographic situation in Russia (decreased birth rate, increased proportion of old people in the structure of population, especially in big cities, such as Moscow, St. Petersburg, Ekaterinburg and other, unprecedented decrease of expected life span, decreased number of people of the working age and their premature aging) and unfavorable demographic prognosis for the coming decades, put forward not only the issue of health in Russia, but its economic and political safety. Recent Order of the Government of the Russian Federation of February 5, 2016 No. 164-R "Strategy of actions in the interests of citizens of the older generation in the Russian Federation until 2025" as well announced by the President of the Russian Federation National Demography Project inspired optimism on a fate of gerontology in the country.

1. Anisimov V.N., Khavinson .Kh., Mikhailova O.N. Biogerontology in Russia: from past to future // Biogerontology, 2011. Vol.12. No.1. P.47-60.
2. Mikhailova, O.N., Anisimov, V.N., and Sidorenko, A.V. 2005, Development of Gerontology in Russia: the Role of International Collaboration, Firm Kosta, St. Petersburg.
3. Duplenko, Yu.K. 1985, Ageing. Essays of the Problem Development, Nauka, Leningrad.
4. Kotelnikov, G.P., Yakovlev, O.G., and Zakharova, N.O. 1997, Gerontology and Geriatrics (Textbook),
5. Samara Publishing House, Moscow, Samara.
6. Golubev, A.G. 2017, Biology of Life Span and Aging, N-L Publ., St.Petersburg.
7. Melentyev, A.S., and Yarygin, V.I. 2005, Manual on Gerontology and Geriatrics, Vol.1, Introduction
8. into Clinical Gerontology, GOETAR-MED, Moscow.
9. Melentyev, A.S., and Yarygin, V.I. 2003, Manual on Gerontology and Geriatrics, Vol. 4. Clinical
10. Geriatrics, GOETAR-MED, Moscow.
11. Geriatrics. National Manual. 2018. Eds. O.N. Tkacheva et al. GOETAR-MED, Moscow.

Aging and age-related diseases: prevent, postpone, or threat?

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Conventional medicine increases life expectancy by preventing death from age-related diseases. This increases the number of elderly patients, which, in the final analysis, places a heavy burden on the state and society as a whole. Anti-aging medicine will slow the aging and onset of age-related diseases (Blagosklonny, 2014; Vaiserman. 2017). The experience accumulated by leading domestic and foreign institutions that provide medical and social assistance to citizens of the older generation makes it possible to determine the main priorities in the field of aging for the coming decade (World Report on Aging, 2015):

• Healthy aging to increase life expectancy;
• Maintaining and restoring mental health;
• Inclusion and participation of the elderly in society and the labor market;
• Quality assurance and maintenance of social protection systems;
• Safe aging of the house and in society;
• Unequal aging and age-related inequalities;
• Biogerontology: from mechanisms to impacts.

In an open letter published on April 4, 2006 on the site of LongevityScience, signed by 57 leading scientists of the world (http://imminst.org/cureaging/. 2006), it was noted that in laboratory animals (nematodes, fruit flies, mice, etc.) it was possible to achieve a slowing down of aging and prolongation active life. Therefore, based on the generality of the fundamental mechanisms of aging, there is reason to believe that it is possible to slow the aging process in humans. Expansion of our knowledge of aging will allow better to confront such exhausting organism pathologies related to aging, like cancer, cardiovascular diseases, type II diabetes and Alzheimer's disease.
Therapy, based on the knowledge of the fundamental mechanisms of aging, will contribute to better counteracting these age pathologies. Intensification of research on the fundamental mechanisms of aging and the search for ways to slow it down can lead to much larger dividends than when directly confronting age-related pathologies. As the mechanisms of aging become more and more clear, effective means of intervention in this process can be developed. This will allow a significant number of people to extend a healthy and productive life.

Many leading gerontologists support the position that it is time not only to consider the therapeutic possibilities of treating age-associated diseases, but also to initiate clinical studies with the ultimate goal of increasing the life expectancy (and, of course, longevity) of the human population, respecting the medical principle *primum non nocere* (Blagosklonny, 2014; Longo et al., 2015; Kennedy & Partridge, 2017). The most promising areas are:

• Pharmacological suppression of the growth hormone axis / IGF-1;
• Restriction of protein intake and starving diets;
• Pharmacological suppression of the mTOR-S6K pathway;
• Pharmacological regulation of some sirtuin proteins and use of spermidine and other epigenetic regulators;
• Pharmacological suppression of inflammation;
• Long-term use of metformin.

In 2000, the US National Aging Institute launched the Intervening Testing Program (ITP), which uses mice to test substances with the potential to increase life expectancy and slow the progression of diseases and dysfunctions (Nadon et al., 2016). Such effects include pharmacological agents, nutraceuticals, foods, diets, food additives, plant extracts, hormones, peptides, amino acids, chelating agents, and antioxidants. Interventions that require intensive forms of administration, such as daily injections or administration with a probe, are not will be investigated in the framework of ITP. Among the drugs that are still being tested are resveratrol, curcumin, green tea extract, N-acetylcysteine, simvastatin, etc. In Western Europe in recent years, this kind of experiments are almost not performed, perhaps because of their complexity, duration and high cost. In Russia at the international level, a very limited number of institutions are currently engaged in research into the carcinogenic potential and geroprotective activity of pharmacological drugs. In Russia standard protocol for testing of potential geroprotectors was developed and for many years successfully applies (Anisimov et al., 2007, 2013). The described testing technique was used in the study of the biological activity of more than 30 pharmacological (Anisimov et al., 2012).
Aging has been slowed and healthy lifespan prolonged with genetic, dietary, and pharmacologic approaches in many model organisms: yeasts, worms, fruit flies, insects, short-living fishes, birds, rodents (mice, rats and hamsters), minipigs, dogs, and monkeys. Recent experimental studies demonstrate that medications targeting aging (antioxidants, calorie restriction mimetics, autophagy inductors, etc.) can substantially promote health and extend lifespan (Anisimov 2006; Spindler, 2012; Blagosklonny 2014; Longo et al 2015; Vaiserman 2017). Pharmacologically targeting aging appears to be more effective in preventing age-related pathology compared with treatments targeted to particular pathologies. The development of new anti-aging drugs represents a great opportunity for the pharmaceutical and healthcare industries. However, if people will better survive into later life and live longer, the increase of incidence of age-associated diseases, cardiovascular, diabetes type 2, and cancer including will be a great challenge for the mankind. The search adequate models for selection more effective and safe methods of life extension became the most hot spot in biology of aging.
There are at least two accepted definitions for compounds applicable to pharmacological intervention into the aging: a) *anti-aging drugs*, which presumably are able to reverse the aging process (rejuvenation) and b) *geroprotectors,* which being administered leads to prevention of premature aging and/or slows down or postpone aging. Spindler (2012) introduced term "longevity therapeutics" for drugs would intervene in the process of aging to extend mean and/or maximum life span, maintain physiological function, and mitigate the onset and severity of a broad spectrum of age-associated diseases in mammals. Vaiserman (2017) subdivided potentially geroprotective agents into several groups: those demonstrating anti-aging effect, but without any evidence of life span increase; drugs which increase life span reducing

incidence of age-associated pathology, and agent which extend lifespan because they suggested to reverse the aging process itself. While laboratory animals are similar to humans in some respects (such as patterns of aging at the molecular, cellular/tissue, and physiological levels, responses to hazardous exposures), there is a growing pool of experimental evidence indicating important differences (genetic, metabolic, ontogenetic etc.) among mammalian species that make valid interpretation and extrapolation of the animal experiments to humans difficult. Issues of concordance of responses between rodent species and between rodents and humans—as well as repeatability and site-specificity—are important considerations in evaluating laboratory animal results (Vaiserman, 2017).

In 2003, the US National Institute of Aging, NIH, started the Aging Interventions Testing Program (ITP), which proposed to test compounds with the potential to extend lifespan and to delay (postpone) age-associated diseases and dysfunctions (Nadon et al, 2016). Among such means are pharmacological drugs, nutriceutics, food products, diets, food additives, plant extracts, hormones, peptides, amino acids, chelating agents, antioxidants, etc. In the framework of the ITP aspirin, nordihydroguaretinic acid, nitrofluorodiprophen, rapamycin, resveratrol, and some other drugs were studied. Priority was paid to preparations which are easily available, have a reasonable price and can be administered with food (preferentially) or with drinking water. An ITP protocol includes two phases. During the first phase, the capability of the drug to increase lifespan is studied. In addition, other parameters, such as the animal's activity in young and old age, metabolic hormone levels and T-lymphocyte levels are also studied. During the second phase, drugs shown promising results are studied more intensively to reveal canidates for further clinical studies. Behavioral and cognitive experiments, measurement of the oxidation level and pathomorphological studies of the dead animals take place during the second phase.

In 2000, an international program (project) on the assessment of efficacy and safety of geroprotectors has been' suggested (Nadon et al, 2016). Its activity could be carried out under the control of the United Nations Program on Aging, World Health Organization and the International Association of Gerontology and Geriatrics (Anisimov & Sidorenko, 2018). The aim of this program is the preparation of international critical reviews by an expert working group providing systems and guidance of evidence relating to the activity and efficacy of geroprotective drugs. Experts could give recommendations for additional studies, if required. The categorization of an agent is a matter of scientific judgment that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

Table 1 Summary on some most significant effects of promising geroprotectors observed in rodents (↑ - increase; ↓- decrease; *Ala-Glu-Asp-Gly)

Parameters	Metformin	Rapamycin	Melatonin	Epitalon*
Life span	↑	↑	↑	↑
Antioxidant potential	↑	↑	↑	↑
Susceptibility to insulin	↑	↑	↑	↑
Low-density lipids	↓	↓	↓	↓
Resistance to stress	↑	↑	↑	↑
Reproductive function	↑	↑	↑	↑
Cognitive and learning capacity	↑	↑	↑	↑
Physical endurance	↑	↑	↑	↑
Age-related pathology	↓	↓	↓	↓
Cancer risk	↓	↓	↓	↓

Group 1: The drug is a geroprotector for *humans*. This category will given to drugs with *sufficient evidence of lifespan increase* in humans. Evidence is confirmed by epidemiological multicenter randomized studies;

Group 2: This category includes drugs for which, at one extreme, the degree of evidence of geroprotective activity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data, but for which there is evidence of lifespan extension in model animals.

Group 3**:** The drug is *not classifiable as to its geroprotective effect in humans*. This category is used most commonly for agents for which the evidence of geroprotective effect is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Group 4: The drug is probably *not a geroprotector* in humans. This category is used for drugs for which there is *evidence suggesting lack of lifespan extension in* humans and in experimental animals.

Publication of the results received by the expert group would assist national and international health institutions to plan and perform programs of rehabilitation and prevention of premature aging, as well as to make a decision about risk-benefit ratios of such programs. Experts in the working groups need to develop a scientific report about the evidence of geroprotector efficacy and safety of the drugs. They should not give any recommendations directly to national or international health institutions about regulation or legislation of drug usage, this remains the exclusive priority of this organizations. Currently, there is no substance which could be evaluated as a group 1 agent (i.e., geroprotector activity of the drug had been proved in humans). Drugs that could be in group 2 are probably metformin, rapamycin, melatonin, pineal peptide preparations epithalamin and Ala-Glu-Asp-Gly (epitalon). There are numerous data confirming the geroprotective effect of these drugs in animal experiments and, in some cases, in clinical studies (Table 1). These drugs are probably the most reliable candidates for testing in multicenter randomized clinical studies (Anisimov, 2001, 2006, 2010, 2015)

The evaluation of the safety of a drug in rodents is a crucial aspect of its preclinical trials. Long-term assays for carcinogenicity in rodents are an integral method which evaluates toxicity and some adverse effects of the drug being tested (Neff et al, 2013). Combination in one study both safety and geroprotective potential of drugs significantly decreases the cost of the study. GeroScope is an in silico project that can aid prediction of novel anti-aging drug from existing human gene expression data (Aliper et al, 2016). The design of the majority of studies in the field was found to suffer from confounds and defects. Accordingly, there is the need to create standard guidelines for testing such drugs and for evaluation of life extension potential as well as other late effects, tumor development including. Guideline for the testing should include such significant points as animals models, regime of testing and biomarkers/endpoints. The system of experimental preclinical study of such drugs could include a study on their effects on biomarkers of aging, lifespan and the development of various age-associated pathologies, especially tumors. The study should be conducted in rats and mice (inbred, outbreed or genetically modified animals) treated by drugs in different doses for their whole life (Anisimov et al, 2012, 2013, 2014). The ultimate goal in this field is the choice of geroprotectors for studies in humans. To achieve these goals, the international standards for preclinical and clinical studies of agents for pharmacological interventions into the aging, as well as for evaluation of results of such studies, should be developed. In the coming years, the perspective direction could be the development of new biomarkers, based mostly on biochemical and genetic methods, for short-term screening of such drugs. At present, cooperative studies on anti-aging drugs and geroptotectors conducted in various laboratories could be promising.

1. Aliper A, Belikov AV, Garazha A, et al. Aging (Albany NY). 2016;8: 2127-52.

2. Anisimov VN. Exp Gerontol. 2001; 36: 1101-36.

3. Anisimov VN. Current Drug Res 2006; 7: 1485-504.
4. Anisimov V.N. Oncotarget. 2015; 6: 39398-407.
5. Anisimov VN, Sidorenko AV. Aging (Albany NY) 2018; 10: 863-867.
6. Anisimov VN, Popovich IG, Zabezhinski MA, et al. Biochim Biophys Acta 2006;1757:573-89.
7. Anisimov VN, Popovich IG, Zabezhinski MA. Methods Mol Biol. 2013; 1045: 145-60.
8. Anisimov VN, Ukraintseva SV, Yashin AI. Nature Rev Cancer. 2005; 5: 807-19.
9. Anisimov VN, Zabezhinski MA. Popovich IG, et al. Expert Opinion Drug Discovery. 2012; 7: 85-95.
10. Blagosklonny MV. Cell Death Dis. 2014; 5: e1552. https://doi.org/10.1038/cddis.2014.520.
11. Kennedy BK, Partridge L. Aging (Albany NY). 2017; 9: 1090-5.
12. Longo VD, Antebi A, Bartke A, et al. Aging Cell. 2015; 14: 497-510.
13. Montesano R, Bartsch H, Vainio H, et al. IARC Sci Publ No 83. Lyon: IARC, 1986.
14. Nadon NL, Strong R, Miller RA, Harrison DE. EBioMedicine. 2016; https://doi.org/10.1016/j.ebiom.2016.11.038.
15. Neff F, Flores-Dominguez D, Rayan DP, et al. J Clin Invest. 2013; 123: 3272-91.
16. Spindler SR. Age (Dordr). 2012; 34: 111-20.
17. Vaiserman AM (Ed). Anti-Aging Drugs: From Basic Research to Clinical Practice. London: The Royal Society of Chemistry, 2017.
18. World Report on Ageing and Health. Geneva: WHO. 2015.

Biodemography of aging and longevity: testing the hypothesis of lifespan limit

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Biodemography of aging is a scientific approach based on using demographic data and methods for getting insights into biological mechanisms of aging. Traditionally biodemographic studies are focused on studying and explaining three empirical observations:
1. The Gompertz law of mortality (exponential growth of failure rate with age).
2. The compensation law of mortality (mortality convergence at older ages).
3. Late-life mortality deceleration and mortality plateaus (deviations from the Gompertz law to lower levels at older ages).

The latter observation is closely related to actively debated topic of the limit to human lifespan. This topic received a lot of attention after 2017 publication in *Nature* suggesting that the limit to human lifespan exists and is equal to 115 years [1]. This publication was followed by a flurry of comments, most of which rejected the idea of lifespan limit.
It should be noted that the idea of no limit to human lifespan dominated the field of gerontology and biodemography over the last 25 years. This topic got particular attention after publication, testing the hypothesis of human lifespan limit through the analysis of mortality trajectories at advanced ages [2]. It was suggested that mortality force (hazard rate) should go to infinity while approaching the limit and hence accelerates compared to the exponential growth with age (the Gompertz law). After finding out that the observed mortality slows down rather than accelerates compared to the Gompertz law, it was concluded that there is no evidence for human lifespan limit [2]. Wilmoth further elaborated on this topic and analyzed trends in maximum reported age at death (MRAD) by year of death and year of birth [3]. He suggested that there should be no increase in MRAD if lifespan limit hypothesis is correct. At the time of his study, MRAD demonstrated very strong growth both across calendar time of death and year of birth [3, 4] suggesting that there is no limit to human lifespan. This conclusion was reinforced further by studies of mortality trajectories, which showed mortality deceleration rather than mortality acceleration at advanced ages for many countries [5–7]. Thus, earlier studies demonstrated the so-called "old-age mortality deceleration," "mortality leveling-off," and "mortality plateaus," when death rates at extreme old ages do not grow as fast as at younger ages.
Later some new developments in the research of old-age mortality emerged. Study of more recent and more reliable data on US mortality demonstrated that mortality continues to grow exponentially with age even at extreme old ages [8, 9]. It should be emphasized that the USA has the largest number of survivors to age 100 years compared to other advanced economies. This finding cannot be explained by low quality of US data, because age misreporting is associated with lower rather than higher estimates of old-age mortality and hence with more expressed mortality deceleration [8, 10]. In another study, the Gompertz-like mortality trajectories were found for old-age mortality in Australia, Canada, and the USA [11]. This means that the chances of exceptional survival are smaller than assumed earlier. However, recent publication in *Science* [12] demonstrated flat mortality after age 105 years for Italian population adding controversy to this topic.
Another recent observation is that mortality of centenarians does not decrease noticeably in recent decades, despite a significant decline in mortality of younger age groups [13–15]. Thus, the projected estimates of old-age survival may be lower than formerly believed.
These recent studies of old-age mortality suggest the need of revisiting the limit to human lifespan agenda. Supercentenarians (persons surviving to age 110 years) may be a useful model for testing the lifespan limit hypothesis. Although mortality of supercentenarians was analyzed earlier [16], the conclusions about mortality trajectories were based on visual inspection of graphs rather than quantitative analysis. Previous studies of supercentenarians analyzed longevity records by year of death and did not account for the existence of non-extinct birth cohorts [1, 17].
In this study, we tested the limit to lifespan hypothesis using two existing methods: (1) evaluating the shape of mortality trajectories at extreme old ages [2, 3] and (2) analyzing the pace of growth for maximum age at death in subsequent birth cohorts [3]. Hazard rates of supercentenarians were measured by actuarial estimate (equivalent to central death rate estimation) applying standard method implemented in the Stata package. Accelerating mortality trajectory at advanced age and no progress in maximum lifespan growth in subsequent birth cohorts should confirm the limit to lifespan hypothesis.
Two sets of data on supercentenarians were analyzed: International Database on Longevity and Gerontology Research Group database. We found that for extinct birth cohorts, maximum reported age at death is increasing over time in subsequent cohorts. However, the pace of growth is slower for cohorts born after 1879 compared to earlier born cohorts. Comparison of two competing models of mortality, the Gompertz model and the exponential ("no-aging") model, revealed that for older birth cohorts (born before 1885), exponential model demonstrated the best fit (according to the Akaike goodness-of-fit criterion) while for more recent birth cohorts (1885+), the Gompertz model showed the best fit. Comparison with mortality of 1898 US birth cohort revealed that mortality of supercentenarians born after 1885 has steeper slope with age compared to mortality below age 110 years, which suggests possible mortality acceleration. Slow progress in maximum reported age at death and mortality acceleration at extreme ages for more recent birth cohorts may indicate that possibility of temporary limit to human lifespan cannot be completely excluded.
Continuous exponential growth of mortality at extreme old ages represents a challenge to many aging theories, including the evolutionary theory that explains senescence by declining force of natural selection with age. New ideas are needed to explain why exactly the same exponential pattern of mortality growth is observed not only at reproductive ages, but also at very old post-reproductive ages (up to 106 years and beyond), long after the force of natural selection becomes negligible (when there is no room for its further decline).
Another important development in the field of biodemography is a discovery of long-term "memory" for early-life experiences in longevity determination. Siblings born to young mothers have

significantly higher chances to live to 100 years, and this finding, confirmed by two independent research groups, calls for its explanation [18–20]. Even the place and season of birth matters for human longevity, as studies found [21, 22]. Beneficial longevity effects of young maternal age are observed only when children of the same parents are compared, while the maternal age effect often could not be detected in between-families' studies, presumably being masked by between-family variation [23]. Finally, large sex differences are found in longevity predictors for males and females, suggesting higher importance of occupation history for male centenarians (farming), and higher importance of home environment history (radio in household) for female centenarians [22].

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1. Dong, X., Milholland, B., & Vijg, J. (2016). Evidence for a limit to human lifespan. *Nature, 538*, 257-259.
2. Gavrilov, L. A., & Gavrilova, N. S. (1991). *The Biology of Life Span: A Quantitative Approach*. New York: Harwood Academic Publisher.
3. Willmoth, J. R. (1997). In search of limits. In K. W. Wachter, & C. E. Finch (Eds.), *Between Zeus and the Salmon. The Biodemography of Longevity* (pp. 38-64). Washington, DC: National Academy Press.
4. Wilmoth, J. R., Deegan, L. J., Lundstrom, H., & Horiuchi, S. (2000). Increase of maximum life-span in Sweden, 1861-1999. *Science, 289*(5488), 2366-2368, https://doi.org/10.1126/science.289.5488.2366.
5. Horiuchi, S., & Wilmoth, J. R. (1998). Deceleration in the age pattern of mortality at older ages. *Demography, 35*, 391-412.
6. Thatcher, A. R. (1999). The long-term pattern of adult mortality and the highest attained age. [Article]. *Journal of the Royal Statistical Society Series a-Statistics in Society, 162*, 5-30.
7. Thatcher, A. R., Kannisto, V., & Vaupel, J. (1998). *The Force of Mortality at Ages 80 to 120.* (Vol. 5, Monographs on Population Aging). Odense: Odense University Press.
8. Gavrilov, L. A., & Gavrilova, N. S. (2011). Mortality measurement at advanced ages: A study of the Social Security Administration Death Master File. *North American Actuarial Journal, 15*(3), 432-447.
9. Gavrilova, N. S., & Gavrilov, L. A. (2015). Biodemography of Old-Age Mortality in Humans and Rodents. [Article]. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences, 70*(1), 1-9, https://doi.org/10.1093/gerona/glu009.
10. Preston, S. H., Elo, I. T., & Stewart, Q. (1999). Effects of age misreporting on mortality estimates at older ages. [Article]. *Population Studies-a Journal of Demography, 53*(2), 165-177, https://doi.org/10.1080/00324720308075.
11. Bebbington, M., Green, R., Lai, C. D., & Zitikis, R. (2014). Beyond the Gompertz law: exploring the late-life mortality deceleration phenomenon. *Scandinavian Actuarial Journal, 3*, 189-207.
12. Barbi, E., Lagona, F., Marsili, M., Vaupel, J. W., & Wachter, K. W. (2018). The plateau of human mortality: Demography of longevity pioneers. [Article]. *Science, 360*(6396), 1459-1461, https://doi.org/10.1126/science.aat3119.
13. Drefahl, S., Lundstrom, H., Modig, K., & Ahlbom, A. (2012). The era of centenarians: mortality of the oldest old in Sweden. [Letter]. *Journal of Internal Medicine, 272*(1), 100-102, https://doi.org/10.1111/j.1365-2796.2012.02518.x.
14. Gavrilov, L. A., Gavrilova, N. S., & Krut'ko, V. N. (2017). The Future of Human Longevity. *Gerontology, 63*(6), 524-526.
15. Robine, J. M., & Cubaynes, S. (2017). Worldwide demography of centenarians. [Article]. *Mechanisms of Ageing and Development, 165*, 59-67, https://doi.org/10.1016/j.mad.2017.03.004.
16. Gampe, J. (2010). Human mortality beyond age 110. In H. Maier, J. Gampe, B. Jeune, J.-M. Robine, & J. W. Vaupel (Eds.), *Supercentenarians.* (pp. 219-230). Heidelberg, Dordrecht, London, New York: Springer.
17. Vijg, J., & Le Bourg, E. (2017). Aging and the Inevitable Limit to Human Life Span. [Article]. *Gerontology, 63*(5), 432-434, https://doi.org/10.1159/000477210.
18. Gavrilov, L. A., & Gavrilova, N. S. (2012). Biodemography of Exceptional Longevity: Early-Life and Mid-Life Predictors of Human Longevity. *Biodemography and Social Biology, 58*(1), 14-39, https://doi.org/10.1080/19485565.19482012.19666121. PMID: 22582891 PMCID: PMC13354762, https://doi.org/10.1080/19485565.2012.666121.
19. Gavrilov, L. A., & Gavrilova, N. S. (2013). Determinants of exceptional human longevity: new ideas and findings. . *Vienna Yearbook of Population Research, 11*, 295-323.
20. Jarry, V., Gagnon, A., & Bourbeau, R. (2013). Maternal age, birth order and other early-life factors: a family-level approach to exploring exceptional survival. *Vienna Yearbook of Population Research, 11*, 263-284.
21. Gavrilov, L. A., & Gavrilova, N. S. (2011). Season of birth and exceptional longevity: comparative study of American centenarians, their siblings, and spouses. *Journal of aging research, 2011*, 104616.
22. Gavrilov, L. A., & Gavrilova, N. S. (2015). Predictors of Exceptional Longevity: Effects of Early-Life and Midlife Conditions, and Familial Longevity. *North American Actuarial Journal, 19*(3), 174-186.
23. Barclay, K., & Myrskyla, M. (2018). Parental age and offspring mortality: Negative effects of reproductive ageing may be counterbalanced by secular increases in longevity. [Article]. *Population Studies-a Journal of Demography, 72*(2), 157-173, https://doi.org/10.1080/00324728.2017.1411969.

TERT—not only a nuclear weapon

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Telomerase has originally been described as a nuclear ribonucleoprotein complex that counteracts telomere erosion, the shortening of the ends of chromosomes, which occurs during every cell division due to the "end-replication-problem" ultimately resulting in senescence and aging. Over the last decade, it has been realized that at least the catalytic subunit of Telomerase, Telomerase Reverse Transcriptase (TERT) is not only present in the nucleus, but also in the mitochondria, the powerhouses of cells [1]. These organelles contain their own DNA, which, however—due to its circular topology—does not have telomeres.

Interestingly, TERT is upregulated upon injury or after exercise in the heart, an organ consisting to a large part of post-mitotic, non-dividing cells, suggesting that in the heart TERT has other functions than telomere protection [2].

To investigate the role of mitochondrial TERT in aging and age-related diseases of the cardiovascular system, we have created a unique mouse model containing TERT exclusively in the mitochondria (mitoTERT mice).

Phenotypically, mitoTERT mice displayed no differences in cardiovascular parameters at baseline when compared to TERT-deficient and wildtype littermates. Mechanistically, mitochondrial TERT was sufficient to compensate for TERT-deficiency in mitochondrial respiration in the heart. Moreover, while TERT-deficient animals showed worse outcome after myocardial infarction compared to wildtype littermates, mitochondrial TERT was sufficient to rescue this defect and infarct size was even smaller than in wildtype littermates. Along the same line, mitochondrial TERT protected cardiomyocytes from apoptosis and rescued the defect of fibroblasts to differentiate into myofibroblasts, a mechanically strong,

contractile cell type, which is needed after myocardial infarction to replace dead cardiomyocytes. Furthermore, mitochondrial TERT improved the migratory capacity of endothelial cells, which is required to restore a functional endothelium after damage by arterial closure. Taken together, it seems reasonable to develop strategies to increase TERT levels or activity to counteract the age-related decline of heart function or to ameliorate the outcomes after myocardial infarction.

1. Haendeler, J. *et al.* Mitochondrial telomerase reverse transcriptase binds to and protects mitochondrial DNA and function from damage. *Arteriosclerosis, thrombosis, and vascular biology* **29**, 929-935 (2009).
2. Zurek, M., Altschmied, J., Kohlgrüber, S., Ale-Agha, N. & Haendeler, J. Role of Telomerase in the Cardiovascular System. *Genes* **7**, 29.

Hypothesis about the method of treating aging with the help of mitolytics.

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In 2015, the concept of senolytics appeared. Senescent cells have a SASP phenotype and secrete various pro-inflammatory factors in the environment that have a harmful local and general effect [1]. These factors form a positive feedback: the more senescent cells accumulated, the more intensely age the surrounding cells.

In experiments on mice, it was shown that selective elimination of senescent cells can improve the general condition of animals and promote rejuvenation, as many body parameters are moved back in time. Unfortunately, the use of such drugs in humans is apparently too toxic, although clinical trials began in 2018. The search for senolytics was based on the study of the features of the work of genes in senescent cells. Those ways, which are strengthened, can serve as a target of therapy.

There were well-founded opinions that removing all senescent cells would lead not to rejuvenation, but to degeneration: for example, the destruction of non-replaceable nerve cells would lead to loss of function; the destruction of senescent niches of stem cells will lead to a disruption in the function of stem cells. On the other hand, senolytic application, limited in the place of holding, will certainly find its place in rejuvenating therapies of the future.

One of the distinctive features of cell aging, in addition to SASP, is the increasing dysfunction of mitochondria (SAMD) [2], which is partially capable of determining SASP. In senescent cells, the mass of mitochondria increases, they release more ROS, the number of mutations in mitochondrial DNA increases.

I propose the concept of the destruction not senescent cells, but aging mitochondria.

It is known that there are mechanisms by which cells are able to get rid of dysfunctional mitochondria with the help of mitophagy. If we learn how to manage this process, then we can intensify the purification of cells from aging mitochondria, decrease SASP and produce an action similar to that of senolytics, but applicable to all types of cells.

How can we affect mitophagy?

The natural mechanism of triggering mitophagy, apparently, is the following. The aging mitochondria acquires over time errors in the structure, due to the oxidative damage of the components of the electron transfer chain and DNA mutations. This leads to an increase in the production of ROS. In response, a synthesis of the uncouplers is triggered in the cell, which attempts to "turn off" the defective mitochondria and transfer it to a state of organelle devoid of membrane potential and, in the course of time, make it a target for mitophagy (a particular variant of autophagy). The retrograde response of the nucleus, in addition to the uncouplers, includes the export of telomerase to mitochondria, which probably regulates the work of mitochondrial genes in order to normalize or "turn off" the mitochondria. Apparently, mitochondria, which do not produce ATP and lack a membrane potential, acquire targets for mitophagy.

We can use the natural mechanism of elimination of aging mitochondria. So, in a cell there is a population of mitochondria, differing in the potential of the inner membrane. It can be assumed that the lower the potential, the worse (older) mitochondria. Our task is to eliminate mitochondria with low membrane potential. Assuming that we have uncouplers that act proportionally to their concentration and are capable of reducing potential, we will be able to accelerate the mitophagy of mitochondria with low potential, thereby changing the mitochondrial population towards rejuvenation.

Uncouplers have some other pluses that can treat aging. Uncoupling leads to increased metabolism, when energy sources are intensively consumed. Cells begin to empty the deposits of excess materials and look for new ones, for example, glucose. Increased autophagy and the rate of metabolism of cellular components certainly should have a rejuvenating effect.

At the level of the organism, the uncoupling should resemble the action of restriction of nutrition, which is known for its rejuvenating ability. Increasing the expenditure of energy substrates should lead to weight reduction and a decrease in glucose level (are directed against obesity and type 2 diabetes).

Another act of uncoupling is an increase in temperature. This should lead to an intensification of the synthesis of chaperones and, thereby, to an improvement in the quality control over conformations. Uncoupling is associated with increased oxygen consumption, which locally can reduce the partial pressure of oxygen and lead to the induction of HIF and subsequent events associated with regeneration.

It is possible that one of the candidates for uncouplers are various fatty acids, including oxidized ones, which have a number of suitable properties. The problem needs to be studied.

1. Kirkland, J.L. &. Tchkonia, T. Cellular Senescence: A Translational Perspective. EBioMedicine 21-28, 21 (2017).
2. Korolchuk, V. I., Miwa, S., Carrol, B. & von Zglinicki, T. Mitochondria in cell senescence: is mitophagy the weakest link? EBioMedicine 7-13, 21 (2017).

Epigenetics and ageing

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In my opinion epigenetics is the science on the inheritable organism properties that are not associated with changes in the DNA nucleotide sequence. The main epigenetical mechanisms in the cell are enzymatic DNA methylation (I call it *secondary genetic code*), various enzymatic modifications of histones (*histone code*) and gene silencing with non protein coding RNAs (siRNA and others). DNA methylation is performed in the cell by specific enzymes DNA-methyltransferases that selectively transfer methyl group from S-adenosyl-*L*-methionine (SAM) to DNA cytosine or adenine bases with formation there of 5-methylcytosine (m^5C) and N^6-methyladenine (m^6A) residues, respectively. DNA methylation in the cell is involved in the control of all genetical processes including such as transcription, cell differentiation, DNA replication, recombination, gene transpositions, DNA repair, X chromosome inactivation (sex differentiation).

Cytosine DNA methylation is essential for DNA secondary structure and DNA interactions with various proteins including transcription factors. The main cytosine DNA methylation sites in eukaryotes are CpG and CpNpG sequences. For some proteins, DNA methylation is a block for DNA binding but for others it is an obligatory event. Therefore, DNA methylation is the mechanism of up- or down regulation of transcription.

In fact, we discovered tissue [1, 2] and age [2, 3] specificity of DNA methylation in eukaryotes and suggested first that DNA methylation is a mechanism of regulation of gene expression and cellular differentiation. We first established that DNA methylation declines with age [2, 3], it was observed in fish (Pacific salmon) and mammals (rats, cows). Later it was found to be true for mice and humans. Thus, we conclude that global genome (DNA) demethylation is a general biological ageing phenomenon**.** Today DNA methylation, in particular CpG methylation, is considered as "biological clock" as so called DNAm aging corresponds well to chronological age. There are many data indicating that despite to global DNA demethylation some genes unmethylated at CpG sites are going to be newly methylated during ageing. The age DNA demethylation or unmethylation is mainly concerned with moderately and highly reiterated DNA sequences and palindromes [4] that may code for protein uncoding RNAs including siRNA responsible for regulation of RNA-directed DNA methylation and epigenetical gene silencing. Distortions in DNA methylation lead to premature ageing and various inheritable diseases. Significant inhibition of DNA-methyltransferases or knock-out of their genes resulted in death. So, there is no life without proper DNA methylation in the cell.

DNA methylation is more or less flexible process. In this DNA modification, at least, three players take place—DNA itself, DNA-methyltransferase and SAM. So, DNA methylation in cell depends on all stages of formation of these components. Besides, respective DNA sites in chromatin formed should be available for DNA modification. Therefore, the structural organization of chromatin is also essential for this genome modification. DNA methylation can be modulated (or possibly to some extent even controlled) by hormones, some biologically active peptides and antioxidants. For example, ionol (BHT) introduced by Harman as a geroprotector increased slightly the m^5C content in DNA but sharply (by many times) induced total DNA-methyltransferase activity in rat liver [5].

We have to keep in the mind that DNA methylation is only one but key element in the whole family of regulatory epigenetical mechanisms mentioned above. All they are closely associated together, interdependent and well coordinated. For instance, DNA methylation often depends on respective histone modifications and vice versa. Therefore, it is no doubt that changes in histone modifications as well as in gene silencing by respective RNAs can be involved in ageing also.

Anyway, ageing is definitely genome reprogramming that, in particular, is associated with rearrangements of DNA methylation pattern [6, 7] and chromatin remodeling. Practically the use of all known old and modern means (diet, antioxidants, peptides, hormones, vitamins and others) are more or less blind and only partial very limited attempts to an efficient increase of lifespan. For more cardinal success in this field, the radical genome reprogramming associated with the intelligent delicate gene addressed genome editing is needed.

1. Vanyushin B.F., Tkacheva S.G., Belozersky A.N. Rare bases in animal DNA. *Nature,* **225**, 948-949 (1970).
2. Berdyshev G.D. et al. Nucleotide composition of DNA and RNA of somatic tissues of Pacific salmon and its changes during spawning. *Biokhimiya,* **32,** 988-993 (1967).
3. Vanyushin B.F. et al. The 5-methylcytosine in DNA of rats. Tissue and age specificity and the changes induced by hydrocortisone and other agents. *Gerontologia* (Basel), **19**, 138-152 (1973).
4. Romanov G.A., Vanyushin B.F. Methylation of reiterated sequences in mammalian DNAs. Effects of the tissue type, age, malignancy and hormonal induction. *Biochim. Biophys. Acta,* **653**, 204-218 (1981).
5. Vanyushin B.F. et al., Butylated hydroxytoluene modulates DNA methylation in rats. *Eur. J. Biochem.,* **256**, 518-527 (1998).
6. Pogribny I.P., Vanyushin B.F. Age-related genomic hypomethylation. *In:* Epigenetics of Aging, Springer, p. 11-27 (2010).
7. Ashapkin V.V., Kutueva L.I., Vanyushin B.F. Aging epigenetics: accumulation of errors and more. *In:* Aging. Exploring a Complex Phenomenon. CRC Press, London, New York, p. 175– 205 (2018).

POSTER SESSION

Analysis of dynamics in an intrinsically disordered protein using NMR relaxation by grouping residues in segments

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The twenty-first century made us discover that a significant amount of proteins is at least partially disordered in their functional state. Even membrane proteins can have intrinsically disordered regions: it was found that the phosphorylation of the disordered C-terminal domain of connexin43 regulates gap junctions in cells [1]. However, a precise description of the dynamics of intrinsically disordered proteins (IDPs) and regions is required to understand their interactions with other proteins.

In our recent study [2], we used multi-field NMR relaxation data to study modes and timescales of dynamics in an archetypal IDP, a C-terminal domain of the nucleoprotein of Sendai virus (NT). NT has 124 amino acid residues and is almost completely unfolded: only 17 residues show a transient helical secondary structure. We found that the optimal description of measured relaxation rates requires three timescales: fastest (1), tens of picoseconds; intermediate (2), 0.5–1.5 ns; slow (3), 5–25 ns. Precisely, we modeled the rotational autocorrelation function of the backbone N–H bond vector in each residue with a sum of three exponentially decaying terms, $C(t) = \sum_{k=1..3} A_k e^{-t/\tau_k}$, each term having its relative amplitude A_k and correlation time τ_k. The slowest timescale (τ_3) was assigned to chain-like or "segmental" motions that affect multiple residues at the same time. We remarked that a sequence profile of the τ_3 correlation time has very flat regions, as if multiple residues effectively share the same slow correlation time, but in our previous study [2], we analyzed each residue individually. In our new model, we consider each residue as a part of a variable-length "segment" of residues which share the same slowest timescale correlation time τ_3, all other parameters being fitted individually for a residue. For each residue in NT, we do a statistical comparison of models with segments of increasing length (starting from three amino acids) being centered on that residue, and determine at which length the fit of relaxation data becomes significantly worse than that for a shorter segment. We find that the optimized segment length is considerably longer than the minimal length of 3 residues in the regions of NT where the slow correlation time sequence profile is flat. Finally, we show that the segmental model where multiple residues share the same slow correlation time allows us to calculate this correlation time with higher precision while still fitting well the relaxation data.

1. Grosely, R. *et al.* Effects of phosphorylation on the structure and backbone dynamics of the intrinsically disordered connexin43 C-terminal domain. *J. Biol. Chem.* **288**, 24857–24870 (2013).
2. Abyzov, A. *et al.* Identification of dynamic modes in an intrinsically disordered protein using temperature-dependent NMR relaxation. *J. Am. Chem. Soc.* **138**, 6240−6251 (2016).

The specificity of carboxypeptidase T from *Thermoactinomyces vulgaris* may be altered by changing the hydrophobicity of S1′ binding pocket

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Metallocarboxypeptidase T from *Thermoactinomyces vulgaris* (CPT) is an enzyme with wide substrate selectivity that is able to cleave off as positively and negatively charged, as (preferably) hydrophobic amino acid residues from C-terminal of peptide substrates. The mutagenesis data and 3D structures of CPT suggest that the side chains of Leu254 and Leu211 are involved in the recognition of negative charges and hydrophobic substrates; therefore, mutations Leu254Asn and Leu211Gln have been introduced. Replacement of hydrophobic Leu254 by hydrophilic Asn reduces effectivity of the catalysis of hydrophobic substrates twofold and completely eliminates the ability of the mutant to cleave the negatively charged substrates. Replacement of hydrophobic Leu211 by hydrophilic Gln leads to conversion of CPT to the carboxypeptidase with preferable selectivity to positively charged substrates. Crystallographic data shows that *N*-sulfamoulamidoglutamic acid (SGlu), the transition-state analog of glutamine substrate, is bound in the wild-type CPT in the same pose as *N*-sulfamoylarginine (SArg) and *N*-sulfamoyl leucine (SLeu), but is bonded with L254N CPT in a non-productive manner. As the X-ray analysis demonstrated, L211Q mutation leads to unfavorable interaction of hydrophilic side chain of Gln211 with hydrophobic side chain of transition-state analogs of Leu- and Phe-containing substrates. Computer simulation of the CPT complexes with SArg and SLeu demonstrated that change in Gibbs partial energy of the interaction of interface amino acid residues with ligands was at maximum in the case of Leu254 and Leu211. Calculated dissociation constants for these complexes as well as complexes of the wild-type CPT with SArg, SPhe, and SLeu were in a good agreement with experimental Ki data. The work was supported by the Russian Scientific Foundation [Project number 17-14-01256].

The models of bio-membranes at different levels of organization

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By this investigation, we obtained some data about the structural and functional changes of biomembranes under the actions of exogenous or endogenous substances. As the experimental models imitated the biomembranes of organelles and cells of animal origin, the fragments of rabbit sarcoplasmic reticulum (FSR) (the main Ca^{2+} depot at muscle cells) and mouse erythrocytes were used. As the exogenous substance, the plant methylxanthine caffeine, synthetic regulator of plant growth melafen, and synthetic hybrid antioxidants IHFANs (I) were used. As endogenous substances, bovine serum albumin (BSA) and human serum albumin (HSA) were used. HSA was fully freed from adsorbed hydrophobic ligands. The free fatty acids (FFA) from membranes of intracellular organelles (FSR) were extracted by HSA. FSR contains 0.65 mg lipids/1 mg protein and FFA less 2%. The treatment FSR by HAS decreased the passive permeability of FSR for Ca^{2+}, and changes of Mg^{2+} and caffeine influence Ca^{2+} release from FSR. The liberation of FSR from FFA leads to increasing work efficiency of SR Ca^{2+} - pump ATPase SERCA-2. Thus, Ca^{2+} accumulated to Ca^{2+} - depot so intensively. Similar extraction of FFA by albumins from membranes can take part and in the animal body tissues and cells. Thus, it may be possible to regulate Ca^{2+} accumulation and Ca^{2+} release to or from SR Ca^{2+} - depot by additions or extractions of certain ligands to or from albumins. The albumin's binding with exogenous synthetic substances I was tested by using the registration of the intensity of intrinsic fluorescence of BSA, which contains 2 triptophane residues in hydrophobic regions of molecule. I (10^{-18} to 10^{-10} M) preserve the destruction of BSA molecule by absorbing its (I) alkyl tails at albumin surface. But the "loosening" of molecule BSA structure occurred, when the large concentrations (10^{-5} to 10^{-3} M) of I. For the study of influences of exogenic synthetic substances to animal cells, the experiments with melafen and insulated native erythrocytes were provided by the test of spontaneous hemolysis and hemolysis initiated at hypo and hyper osmotics. Melafen at all range of concentrations (10^{-7} to 10^{-3} M) caused the small additional hemolysis and did not protect against spontaneous hemolysis. The measurements of hemolysis at hyper osmotic conditions (3–4 M of NaCl) imitated the properties of biomembranes at bed environment. The erythrocyte's hemolysis significantly varies in the presence of melafen over a wide range of concentrations (10^{-9} to 10^{-3} M). Melafen at concentrations 10^{-12} M and 10^{-11} M do not change the general picture of hemolysis of erythrocytes. Thus, endo- and exogenous substances may regulate the biomembrane's properties.

Electron-transfer protein–protein complex formation in higher plants, green alga, and cyanobacteria

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Simulation of protein association dynamics is crucial for understanding their functionality. In Brownian dynamics, proteins are considered as rigid bodies subjected to electrostatic and random Brownian forces [1]. Brownian dynamics is not so computationally expensive as molecular dynamics, thus allowing exhaustive sampling of relative orientations of protein molecules approaching each other in a virtual reaction space [2]. Long-range electrostatics is the major factor affecting molecule orientation on encounter [3]. Sampling and density-based clustering of encounter complexes allow finding if all of them constitute a single group, or if they can be classified into several distinct clusters, and obtain characteristics of such groups (clusters). BD and cluster analysis together provide revealing the most popular orientations in encounter complex and in combination with molecular dynamics used to simulate the transformation of encounter complexes provide complete reconstruction of protein–protein interaction over large temporal and spatial scales. We performed four series of numerical experiments for a comparative study of protein–protein complex formation of cytochrome f and plastocyanin from two species of cyanobacteria (*Phormidium laminosum* and *Nostoc* sp.), higher plants, and green algae *Chlamydomonas reinhardtii*. According to our results, in green algae and higher plants, plastocyanin finds itself in side-on orientation upon encountering complex formation while in cyanobacteria *Nostoc* head-on orientation of plastocyanin molecule is dominant. Further transformation of encounter complexes in higher plants and green algae is associated with rotation of plastocyanin around electrostatic anchor joint constituted by R184K185K187 loop on cytochrome f and D42E43D44E45 loop on plastocyanin. On the contrary, *Nostoc* cytochrome f is subject to major conformational changes: G188E189D190 loop bends toward plastocyanin molecule without any significant rotation of plastocyanin molecule.
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1. Kovalenko, I.B. et al. Computer simulation of protein-protein association in photosynthesis (2011). https://doi.org/10.1051/mmnp/20116704
2. Kovalenko, I.B. et al. A novel approach to computer simulation of protein-protein complex formation (2009). https://doi.org/10.1134/S1607672909040127
3. Kovalenko, I.B. et al. The role of electrostatic interactions in the process of diffusional encounter and docking of electron transport proteins (2016). https://doi.org/10.1134/S1607672916030066

Plasma and nuclear membrane localization of paraoxonase 2 in different types of malignancies

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PON2 belongs to the paraoxonase protein family that consists of lactone hydrolyzing enzymes with different substrate specificities. Unlike other members of this family, PON2 demonstrates substantial antioxidant activity, localizes predominantly inside the cell, and is ubiquitously expressed in all human tissues. Previously, it was proposed that the main function of paraoxonases is a defense against pathogens such as *Pseudomonas aeruginosa*; however, recent findings highlight an important role of PON2 in the protection from oxidative stress, inhibition of apoptosis, and progression of various types of malignancies. In the current study, we performed bioinformatic analysis of RNA and DNA sequencing data obtained from tumor samples from more than 10,000 patients with 31 different types of cancer and determined expression levels and mutations of the PON2 gene. Next, we investigated the intracellular localization of PON2 in multiple cancer cell lines and identified proteins that interact with PON2 using LC-MS/MS mass spectrometry. Our data indicate that the high PON2 expression correlates with worse prognosis for patients with multiple types of solid tumors and favors the possibility that PON2 when localized on the nuclear envelope and endoplasmic reticulum may protect cancer cells from unfavorable environmental conditions and chemotherapy.
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Molecular modeling of cytokinin signaling components from potato *Solanum tuberosum* L.

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Cytokinins are the class of phytohormones that act using multistep phosphorelay (MSP) system. MSP has evolved from the two-component system (TCS) and consists of three types of proteins: transmembrane receptor–sensor hybrid histidine kinase (HK), histidine-containing phosphotransfer protein (HPt), and nuclear response regulator (RR). Publication of *Solanum tuberosum* genome sequence [1] and availability of crystal structures of plant MSP's main elements in protein database (PDB) [2] opened the way to construct spatial structures of potato cytokinin signaling elements, using the homology modelling method. All functional domains of all three potato (Phureja cultivar) cytokinin receptors (StHK2, StHK3, and StHK4) were modelled. Extracytosolic sensor modules (included dimerization interface and CHASE domain, consisting of PAS and PAS-like subdomains) were built as dimers in the complex with cytokinin ligand. Cytosolic HisKA domains were also created in dimeric form. Models of H_ATPase domains were obtained as complexes with ATP and Mg^{2+} ions. Receiver domains (RD) were created as a monomers and hetero-oligomers with phosphotransfer protein StHPt1a as a counterpart, in the presence of Mg^{2+} ions in both cases. Receiver-like domains were modelled too.
The results of the modelling provide insight into some important structural features of the potato cytokinin signaling. First of all, it shows structural conservation as well as deviations from known structures of plant MSP systems and bacterial TCS, manifested mainly in loop insertions and amino acid variability in some regions. Also, we showed spatial positions of some key amino acid replacements in canonical motifs and single amino acid polymorphism in Désirée cultivar as compared to Phureja. And finally, we prepared the basis for investigation of processes that may play a major role in *Solanum tuberosum* cytokinin signaling: ligand binding, ATP binding and hydrolysis, phosphotransfer, involvement of metal ions, dimerization of proteins, and their interactions with downstream or upstream partners. Some methods and results of this work, as well as related computational and experimental data on the potato cytokinin signaling system, are described in detail in the recent article [3].
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1. The Potato Genome Sequencing Consortium. Genome sequence and analysis of the tuber crop potato. *Nature*. **475**, 189–195 (2011).
2. Pekárová B. *et al.* Structural aspects of multistep phosphorelay-mediated signaling in plants. *Molecular plant*, **9(1)**, 71-85 (2016).
3. Lomin S.N. *et al.* Cytokinin perception in potato: new features of canonical players. *Journal of Experimental Botany*, **69(16)**, 3839–3853 (2018).

Self-organized 3D neural organoids derived from Down syndrome IPS cells are representative model of early stages of Alzheimer's disease

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Alzheimer's disease (AD) is the most common form of dementia [1]. Individuals with Down syndrome (DS) are the biggest risk group of AD (more than seven million people). Nowadays, IPS cell models are applied to study AD mechanisms. Self-organized 3D organoid is the most reliable method for in vitro disease modeling. In this report, we studied self-organized 3D neural organoids, derived from DS IPS cells. We compared beta-amyloid secretion and gene expression profiles of AD-related genes in DS and normal karyotype organoids.
We observed secretion upregulation of two isoforms of beta-amyloid (bA40 and bA42) in the DS organoids compared to normal karyotype organoids. In addition, DS organoids demonstrate significant shift in bA40/bA42 ratio. Gene expression levels of APP, BACE2, RCAN1, DYRK1A and GFAP and CREB1 showed confident differences between cells derived from DS and normal karyotype groups. These genes are involved in beta-amyloid processing and AD mechanisms [3,4].
Thereby, we consider self-organized 3D neural organoids derived from DS IPS cells as reliable cell models of early stages of Alzheimer disease.
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1. A Burns, S Iliffe. Alzheimer's disease, BMJ, 2009.
2. R.L.Webb and M.P. Murphy. B-Secretases, Alzheimer's disease, and Down syndrome, Current Gerontology and Geriatrics Research, 2009.
3. G. Verdile, S.E. Gandy, R.N. Martins. The role of presenilin and its interacting proteins in the biogenesis of Alzheimer's beta amyloid, Neurochem Res, 2007.
4. M. Smolarkiewicz, T. Skrzypczak, P. Wojtaszek. The very many faces of presenilins and the γ-secretase complex, Protoplasma, 2013.

Structural studies of glutamate transporter homologue from *Pyrococcus horikoshii*

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Glutamate is the most abundant excitatory neurotransmitter in vertebrate neural system. There are two main families of proteins which control the glutamate transport: excitatory amino acid transporters (EAATs) and vesicular glutamate transporters (VGLUT). EAATs are sodium-dependent proteins which move glutamate from the synaptic cleft back to neurons and glial cells [1]. Irregularities in expression or functioning of these proteins are suspected to be involved to a big variety of diseases from Alzheimer's disease to schizophrenia [2]. Protein Glt_{Ph} from archea *Pyrococcus horikoshii* shows 37% homology with EAATs and occurs as a common model for EAAT studies [3]. There are several crystal structures of this protein with the best resolution 2.97 Å [4]. All these structures were obtained via in surfo crystallization method. In this work, we presented 2.6-Å resolution structure of Glt_{Ph} from archea *Pyrococcus horikoshii* that was obtained using in meso approach. The protein was expressed in *Escherichia coli* and crystallized in monoolein lipidic cubic phase. The more detailed structure opens the way to performing precise molecular dynamics studies to understand better the principles of functioning of glutamate transporters and discover the mechanisms of its medically relevant mutations.

1. Zerangue, N., & Kavanaugh, M. P. (1996). Flux coupling in a neuronal glutamate transporter. Nature, 383(6601), 634.
2. Kim, A. H., Kerchner, G. A., & Choi, D. W. (2002). Blocking excitotoxicity. In CNS neuroprotection (pp. 3–36). Springer, Berlin, Heidelberg.
3. Machtens, J. P., Kortzak, D., Lansche, C., Leinenweber, A., Kilian, P., Begemann, B., ... & Fahlke, C. (2015). Mechanisms of anion conduction by coupled glutamate transporters. Cell, 160(3), 542–553.
4. Boudker, O., Ryan, R. M., Yernool, D., Shimamoto, K., & Gouaux, E. (2007). Coupling substrate and ion binding to extracellular gate of a sodium-dependent aspartate transporter. Nature, 445(7126), 387.

Analysis of hyaluronic acid metabolism gene expression profiles in neurodegenerative disorders using transcriptome analysis methods

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Connections between alteration of extracellular matrix composition and the emergence and development of various pathologies have been detected for many diseases, but the mechanisms of these alterations have been poorly studied in the context of inflammatory states of the central nervous system. Understanding molecular mechanisms for regulating composition of the matrix, its interaction with cells, is considered promising for development of new therapeutic strategies that promote regeneration and synaptic plasticity. At the moment, distribution of the enzymes of metabolism of hyaluronic acid, a crucial component of the extracellular matrix, which is considered an endogenous ligand that activates intracellular signaling cascades through interaction with a number of receptors, among which are Toll-like receptors (TLR-2, TLR-4) and CD44 receptors along the nervous system cells, remains an open question. Earlier, our group showed that genome-wide transcriptome analysis allows making reliable predictions about system's behavior and estimating changes in particular metabolic pathways [1–3]. The goals of this research were to analyze different aspects of hyaluronic acid metabolism and to draw up a list of involved genes; to choose appropriate genome-wide transcriptome arrays of patients diagnosed with Parkinson and Alzheimer disease from GEO database; to process these data using DEG (differential gene expression according to *T*-test) analysis and WGCNA (weighted gene correlation network analysis) to trace variations in chosen gene expression profiles.

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1.Ivliev, A. E., T Hoen, P. A. C., Borisevich, D., Nikolsky, Y. & Sergeeva, M. G. Drug repositioning through systematic mining of gene coexpression networks in cancer. *PLoS One* 11, 1–19 (2016).
2.Ivliev, A. E., 't Hoen, P. A. C., van Roon-Mom, W. M. C., Peters, D. J. M. & Sergeeva, M. G. Exploring the transcriptome of ciliated cells using in silico dissection of human tissues. *PLoS One 7*, (2012).
3.Ivliev, A. E., 't Hoen, P. A. C. & Sergeeva, M. G. Integrated systems and technologies coexpression network analysis identifies transcriptional modules related to proastrocytic differentiation and sprouty signaling in glioma. (2010). https://doi.org/10.1158/0008-5472.CAN-10-2465

Investigation of interactions of agitoxin-2 and its derivatives with hybrid potassium channels

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The development of peptide blockers of potassium channels is one of the promising directions for the creation of drugs for the treatment of autoimmune and neurological diseases associated with the abnomal functioning of Kv1.x channels (x = 1,3,6).

Agitoxin-2 (AgTx2) isolated from the venom of the scorpion *Leiurus quinquestriatus hebraeus* is a high-affinity peptide blocker of several potential-dependent potassium channels Kv1.x. Introduction of point mutations allows one to make significant changes in toxin affinity and to increase its selectivity toward porous domain of a particular channel Kv1.x. Based on the analysis of published data and results of molecular modeling, a series of point mutations was proposed, four derivatives of AgTx2 were obtained, and their properties were studied.

Using the bioengineered fluorescent detection system based on *Escherichia coli* cells expressing the hybrid potassium channels KcsA-Kv1.x (x = 1,3,6) in their plasma membrane [1, 2], the activity of the AgTx2 derivatives was investigated by measuring the peptide ability to bind to these channels. The dissociation constants of the peptide channel complexes were determined by the method of competitive inhibition of binding. The obtained data allowed one to refine the interaction interface of AgTx2 with Kv1.x channels and characterize the effect of the

introduced mutations on the interaction profile of the constructed peptide blockers with the pore region of channels Kv1.x (x = 1, 3, 6).
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1. Kudryashova, K. et al. Fluorescent system based on bacterial expression of hybrid KcsA channels designed for Kv1.3 ligand screening and study. *Analytical and Bioanalytical Chemistry,* **405**, 2379–2389 (2013). https://doi.org/10.1007/s00216-012-6655-6
2. Kuzmenkov A.I. et al. Variability of potassium channel blockers in Mesobuthus eupeus scorpion venom with focus on Kv1.1: an integrated transcriptomic and proteomic study. *J. Biological Chemistry,* **290,** 12195–12209 (2015). https://doi.org/10.1074/jbc.M115.637611
3. Nekrasova O.V. et al. Complexes of peptide blockers with Kv1.6 pore domain: molecular modeling and studies with KcsA-Kv1.6 channel. *J Neuroimmune Pharmacology*, **12**, 260–276 (2017). https://doi.org/10.1007/s11481-016-9710-9

The mechanisms of the reduced basal and stimulated by the agonists of luteinizing hormone receptor production of testosterone in aging male rats

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Aging leads to a weakening of the steroidogenesis in the testes, resulting in a deficiency of steroid hormones and the dysfunctions of the reproductive, cardiovascular, and other systems. However, the mechanisms of this are poorly understood, and the approaches to correct age-associated impairment of testicular function are not sufficiently developed. The aim of this work was to study the stimulating effects of human chorionic gonadotropin (hCG), a homologue of luteinizing hormone (LH), and TP03, a low-molecular-weight agonist of LH receptor (LHR), on testosterone (T) production and the expression of LHR and steroidogenesis proteins in young (3 months) and aging (15 months) male Wistar rats, and to investigate activity of the adenylyl cyclase (AC) system in the testicular membranes of these animals. The treatment with hCG (100 IU/rat/day) and TP03 (15 mg/kg/day) was carried out for 3 days. In the testicular membranes of aging rats, the stimulation of AC by hCG and guanine nucleotide was decreased, indicating a weakening of the coupling between LHR and G_s protein, the main components of the AC system regulating the steroidogenesis. In aging rats, the plasma T level and the expression of the *Star*, *Cyp11a1*, and *Cyp17a1* genes encoding cholesterol-transporting StAR protein and the cytochromes $P450_{scc}$ and P450-17α in the testes were decreased, while the expression of the *Lhr*, *Hsd3B*, and *Hsd17B* genes encoding LHR and dehydrogenases 3β-HSD and 17β-HSD did not change significantly. The $P450_{scc}$ catalyzes the conversion of cholesterol to pregnenolone, 3β-HSD is responsible for the conversion of pregnenolone into progesterone, and P450-17α and 17β-HSD convert progesterone into 17-hydroxyprogesterone, androstenedione, and T. With increasing age, the stimulating effect of hCG and TP03 on T production was weakened, despite the different mechanisms of their action. The treatment of both young and aging rats with hCG led to an increase in the *Star*, *Cyp11a1*, and *Hsd3B* expression. In addition, in aging rats, the *Hsd17B* expression was increased, and in young rats the *Cyp17a1* and *Hsd17B* expression was reduced. The treatment of young rats with TP03 led to an increase in the *Star* and *Cyp17a1* expression, and TP03 treatment of aging rats increased the *Star* and *Hsd17B* expression. Thus, in the testes of aging rats, the coupling between LHR and G_s protein and the sensitivity of LHR to different types of agonists were weakened, which leads to a decrease in the hCG- and TP03-induced production of T, and the basal and LHR agonist-stimulated levels of gene expression for the key steroidogenic proteins were changed in a specific manner.
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Studying of exon–intron gene structure using spliceosome blockade in neuronal culture

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Alternative splicing provides the mRNA diversity in cells by exclusion of introns and some exons from the pre-mRNA. To investigate exon–intron structure of genes expressed in neurons, we applied spliceosome blocker pladienolide B to hippocampal neuronal culture.
Neurons were obtained from 0–1-day-old rat pups and transferred to poly-lysine–coated plate. At 15–16 days, in vitro control solution or pladienolide B (250 nM) was added to culture for 5 h. Then, the cultured medium was replaced by ExtractRNA reagent (Evrogen). Purified RNA was cloned to cDNA using reverse transcription and analyzed by PCR and by whole-genome transcriptome sequencing.
PCR with primers to *c-fos* gene and subsequent gel electrophoresis revealed that pladienolide B application induces production of five PCR products of c-fos instead of one product in control conditions. Alone PCR product corresponds to a region of mature c-fos mRNA while several bands seem to be expressed from pre-mRNA.
For whole-genome transcriptome sequencing, RNA was analyzed by Agilent 2100 Bioanalyzer and rRNA was depleted by a NEBNext R rRNA Depletion Kit (Human/Mouse/Rat). Then, we prepared cDNA libraries using an Ion Total RNA-Seq Kit v2 for Whole Transcriptome Libraries (Ion Torrent, Life Technologies). Sequencing was performed on an Ion Proton sequencer.
Reads were aligned and analyzed using HISAT2 program. We have shown intron accumulation after pladienolide B treatment in comparison with control group.
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1. Ghosh, A. & Anderson, D. Enantioselective total synthesis of pladienolide B: a potent spliceosome inhibitor. *Org Lett.* **14,** (2012)
2. Mauger, O *et al.* Targeted intron retention and excision for rapid gene regulation in response to neuronal activity. *Neuron.* **92,** (2016)

Model implantation of photosensitive cells reveals necessary cluster size needed for pacing of the heart culture

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Treatment of bradyarrhythmia is a challenge of modern medicine. Tissue engineering (TE) methods are promising in the therapy of this disease. The major approach of TE is a creation of cell groups in the myocardium which acts as a source of the whole heart stimulation. Those cells can be driven by photosensitive proteins [1] externally. Alternatively, the cells could be triggered by the internal pathway, by membrane pacemaking proteins. Such clusters can form by cell transplantation [2] or by local delivery of viruses with the appropriate genes. Regardless of the cluster formation method, the amount of cell which is needed for excitation remains a question.
To study this problem, a model implantation of photosensitive ChR2-HL-1 cells [3] in a culture of neonatal rat ventricular cardiomyocytes (NRVCM) was carried out. The cells of this line

were implanted in the form of single cells, small clusters (10–100 cells), and big spheroids (> 2000 cells). Immature (6 h after seeding) and mature (48 h after seeding) cultures of NRVCM were used as substrates for implantation.
Optic stimulation experiments showed low pacing efficiency of single photosensitive cells incorporated in NRVCM culture. At a cell density of 20–80 cell/mm^2, it was possible to detect only single irregular excitation waves as a response to optical stimulation. Small cluster implantation was efficient in terms of photostimulation on immature heart culture. In this case, clusters effectively created cell contacts, demonstrating response to optical stimulation. Implantation of the same clusters on mature monolayers resulted in little incorporation of photosensitive cells into the heart culture and the absence of stimulation. Similarly, spheroid seeding was efficient only on immature culture. However, they were affected by shear stress that caused their detachment and subsequent cessation of the stimulation.
The results obtained in the research show that the number of cells in a cluster and its interaction with target tissue are crucial for the creation of the biological pacemaker. The cell number that is needed for reliable stimulation can be estimated as 100 cells. Moreover, it was shown that interaction with ECM is important for cell contact formation.
The work was supported by the RSF grant no. 16-15-10322.

1. Volkov, O., *et al.* Structural insights into ion conduction by channel rhodopsin 2. *Science.* **358,** (2017).
2. Chepeleva, E.V., *et al.* Analysis of biological compatibility of polylactide nanofibrous matrix vitalized with cardiac fibroblasts in a porcine model. *Genes and cells.* **12(4),** (2017).
3. Agladze, N. N., *et al.* Synchronization of excitable cardiac cultures of different origin. *Biomaterials science.* **5.9,** 1777-1785. (2017).

Structural study of magnetic alginate membranes with $CoFe_2O_4$ nanoparticles for biomedical applications

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Currently, alginate is one of the most commonly used biopolymers in science, nutrition, and agricultural life, mainly because of its excellent gel and viscosity. The industrial use of the initial alginate was as an adhesive, but has recently been widely used as thickening agents, emulsifiers, gelling agents, and film. The combination of use of magnetic particles and alginate can lead to new biomedical and biotechnological applications, including targeted drug delivery, magnetic cell separation, enzyme immobilization, magnetic resonance imaging (MRI), and hyperthermia treatment.
The sodium alginate morphology can be modified by the action of divalent cations, such as calcium, inducing three-dimensional special networks [1–3]. The inclusion of magnetic nanoparticles will affect the polymer network modifying different properties of the membrane [4]. Earlier results on investigations of the gelation process of low-viscosity sodium alginate with calcium chlorite dehydrate have shown SANS to be a very useful method [5].
In the present work are presented preliminary structural investigations of alginate membranes doped with $CoFe_2O_4$ nanoparticles and the effect of their cross-linking with $CaCl_2{\cdot}2H_2O$ using atomic force microscopy (AFM), scanning electron microscopy (SEM), X-ray diffraction (XRD), and small-angle X-ray scattering (SAXS).

1. Draget K.I., Smidsrød O., Skjåk-Bræk G., *Alginates from algae from polysaccharides and polyamides in the food industry*, A. Steinbuchel, S. K.Rhee, Wiley-Blackwell, London, 2005.
2. Sikorski P., Mo F., Skjåk-Bræk G. and Stokke B.T., Biomacromolecules **8(7)** 2098 (2007).
3. Braccini I. and Pérez S., Biomacromolecules **2**, 1089 (2001).
4. Balasoiu M., Lebedev V.T., Orlova D.N., Bica I., Raikher Yu.L., Journal of Physics: Conference Series 351, 012014(9) (2012).
5. Badita C.R., Aranghel D., Radulescu A, Anitas E.M., Romanian Report in Physics 68(3), 1115 (2016).

Using tag-mediated labelling and FRET approaches to investigate ligand-induced dynamics of GPI-anchored membrane receptor in living cell

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New labelling approaches and fluorescence methods for probing dynamics of protein–protein interaction at the plasma membrane of living cells are developing nowadays. In our research, we compared two ways of labelling of glycosylphosphatidylinositol (GPI)-anchored receptor T-cadherin and used two ways of Förster resonance energy transfer (FRET) detection to obtain information about ligand-induced dynamics of the receptor. Both ligands of T-cadherin, low-density lipoproteins (LDL) and high-molecular weight adiponectin, are involved in the pathogenesis of cardiovascular diseases, but the molecular mechanisms remain unclear.
We cloned several constructs of T-cadherin: with sequence of fluorescence proteins (tagYFP or mTFP1) and with sequence of small tag S6 peptide. Enzyme-mediated fluorescence labelling of this small tag in T-cadherin revealed a number of advantages compared to labelling by fluorescent proteins. It minimized distortion of protein properties by fluorescent labelling since small tag (S6 peptide sized 12 amino acids flanked by one amino acid linker) and fluorophores used in this case are much smaller than fluorescent proteins (2.5 kDa vs 27 kDa). Using cell-impermeable fluorescent substrate, we restricted labelling to surface-exposed proteins of living cells. Synthetic fluorescent dyes are not prone to dimerization, unlike many fluorescence proteins. Moreover, it gave superior control of the donor to acceptor ratio.
Analysis of acceptor photobleaching by laser scanning confocal microscopy has shown increase of FRET efficiency in the presence of serum with two ligands. For measurement of dynamics of sensitized acceptor emission during addition of ligands, we used confocal microscopy and flow cytometry. We found that LDL induced formation of short-lived T-cadherin dimers, and the high-molecular weight adiponectin induced formation of stable complexes of T-cadherin. The T-cadherin–adiponectin complex did not trigger calcium response and inhibited LDL-induced calcium signalling. We suggest that almost all T-cadherin on the membrane is bound with adiponectin under physiological conditions but in pathological conditions it interacts with LDL which trigger intracellular signalling.
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Close-up on mitochondria with surface-enhanced Raman spectroscopy

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Selective and sensitive investigation of the electron transport chain (ETC) components in functional mitochondria is essential for fundamental biophysical research and for the development of new medical diagnostic methods. At present, fluorescent microscopy, absorption spectroscopy, and registration of O_2 consumption provide only indirect information about the redox state of ETC complexes in mitochondria. For the first time, we suggest a novel label-free approach based on the surface-enhanced Raman spectroscopy (SERS) to monitor conformational changes and the redox state of cytochrome *c* in ETC of functional mitochondria [1]. We demonstrated that various hierarchical silver (Ag) nanostructures provide highly intensive enhancement of Raman scattering of cytochrome *c* in mitochondria without affecting their morphology and respiration [2]. The recorded SERS spectra reveal a set of characteristic peaks at 750, 1127, 1170, 1371, 1565, 1585, and 1638 cm^{-1}, resulting from the normal group vibrations of the pyrrole rings, methine bridges, and side radicals in the heme *c* molecules. We showed that SERS spectra of cytochrome *c* in mitochondria are sensitive to the activity of the electron transport, rate of ATP synthesis, mitochondrial membrane potential, mitochondria morphology, and activity of ETC complexes. For the first time, we demonstrated that SERS spectra of cytochrome *c* in functional heart mitochondria differ for healthy and spontaneously hypertensive rats. This opens new possibilities for the development of SERS-based diagnostical tools for the prediction of the development of cardiovascular diseases.
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1. Brazhe N.A. et al. Probing cytochrome c in living mitochondria with surface-enhanced Raman spectroscopy. *Scientific Reports*, **5,** 13793 (2015) | https://doi.org/10.1038/srep13793
2. Sarycheva A.S., Brazhe N.A. *et al.* New nanocomposites for SERS studies of living cells and mitochondria. *J. Mater. Chem. B*, **4**, 539–546 (2016) | https://doi.org/10.1039/c5tb01886b

The effect of chromophoric group modifications on the spectral properties of proteorhodopsin from *E. sibiricum* (ESRh)

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A new proteorhodopsin (ESRh)—light-driven proton pump from the psychrotrophic bacterium *Exiguobacterium sibiricum*—has Lys-96 as a proton donor moiety to the Schiff base that distinguishes ESRh from related retinal proteins, bacteriorhodopsin (BR) and xanthorhodopsin (XR), in which the donor function is performed by Asp residues with a carboxyl side chain.
Light-induced changes in retinal proteins are associated with charge redistribution in the excited retinal chromophore and are driven by isomerization of the chromophore moiety around a "critical" double bond. Thus, the chromophore molecule modification is a promising approach to the structure–function relationship study in such pigments. In the present study, for the estimation of the influence of the chromophoric group structure on the functional properties of ESRh, expressed in *Escherichia coli*, we used the following modification types of natural *all-E*-retinal: analogs, modified at the ring—4-oxoretinal, 3,4-didehydroretinal, 5,6-dihydro-5,6-epoxyretinal, and 4-fluorophenylretinal; analog, modified at the polyenic chain—13-desmethylretinal, analogs with altered length of polyenic chain—C22, C25, C15; the acyclic retinal derivative (without the ionone ring).
The effects of chromophoric group structures on the functional properties of proteorhodopsin from *E. sibiricum* (ESRh) were studied. ESRh retinal binding site was found as preserving the similar stereo- and spatial restrictions on the chromophore structure during the retinal protein reconstitution process (except for C25 analog). It was revealed that the structure peculiarities of the chromophore analog molecules affect the optical parameters of ESRh and BR pigment families in similar ways.
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1. Belikov, N. E. *et al.* The effect of the chromophoric group modification on the optical properties of retinal proteins. *Mendeleev Commun.* **28**, 406–408 (2018).

Disfunction of mitochondria and membrane permeability in sepsis under the influence of microbial metabolites

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Mitochondrial disfunction is the main factor in the development of multiple organ failure in sepsis. In this work, the involvement of microbial metabolites in the induction of mitochondrial disfunction and alterations of membrane permeability was studied. Although microbial metabolites are necessary for normal metabolism and homeostasis, however, in excessive concentrations, they can exert toxic effects on several functions and the organism, including influencing the permeability of membranes and the blood–brain barrier. In sepsis, the concentrations of microbial phenolic acids in the blood increase tens of times. Previously, we have shown that these compounds affect the mitochondrial respiration and ROS production in liver mitochondria and neutrophils. Under normal physiological conditions, phenolic acids were effective predominantly at high concentrations. The goal of the present work was to study the influence of phenolic acids on the induction of mitochondrial permeability transition pore, oxidative phosphorylation, and the activity of mitochondrial dehydrogenases under conditions typical for the pathogenesis of sepsis. For this, the influence of phenolic acids of microbial origin on the functions of mitochondria under the conditions of acidosis and deficiency of the oxidation substrate accompanying the development of sepsis was investigated. These conditions sharply activated the action of benzoic, phenylpropenic, phenylpropionic, and phenylacetic acids on the induction of the mitochondrial pore, oxidation of NADH, and oxidative phosphorylation in isolated liver mitochondria. Phenolic acids decreased the threshold concentrations of calcium ions required to open mitochondrial pore, and when the medium was acidified, their effect at the same concentrations was almost two times higher than at norm. However, the most effective factor that enhanced the effect of phenolic acids was the deficit of the oxidation substrate. Preincubation of mitochondria with phenolic acids in the absence of an oxidation substrate was accompanied by a significant inhibition of the dehydrogenases of the tricarboxylic acid cycle. The obtained data indicate the potential role of phenolic acids of microbial origin in the development of mitochondrial

dysfunction and activation of mitochondrial pore opening in the inflammatory process and sepsis.

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A study of the interaction of the new antituberculous drug delamanid with liposomes and rat liver mitochondria

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Delamanid (Del) has recently been approved for the treatment of multidrug- and extensively drug-resistant tuberculosis. The antibacterial effect of this compound has been well studied. Del inhibits the biosynthesis of mycolic acids of cell wall of *Mycobacterium tuberculosis* [1]. An important step in the introduction of a new antibacterial preparation into medical practice is the study of its influence on the eukaryotic cell, in order to reveal its possible side effects in humans and animal organisms. At the same time, data on the interaction of Del with cells of eukaryotic organisms are scarce: these drugs were introduced into practice not a long time ago and in quite a hasty manner [2]. This study has been made to determine the interaction of Del with artificial membranes (liposomes) and mitochondria isolated from rat liver.

Differential scanning calorimetry showed that Del (10 mol%) changes the thermotropic phase behavior of DMPC, decreasing the temperature of the main phase transition. Del at concentrations of 10–50 μM does not practically induce the release of the fluorescent probe sulforodamine B from the dye-loaded lecithin liposomes, that is, Del is not a membrane-permeabilizing agent. At the same time, dynamic light scattering revealed that Del at a concentration of 10 μM induces aggregation of liposomes in the suspension.

A study on rat liver mitochondria showed that Del inhibits the mitochondrial respiration at the ADP-stimulated (V_3) and the DNP-stimulated (uncoupled, V_{DNP}) states. In addition, the sensitivity of the permeability transition pore to calcium ions in mitochondria was increased by Del. Possible mechanisms of action of Del are discussed.

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1. Yang, JS. *et al.* Delamanid, bedaquiline, and linezolid minimum inhibitory concentration distributions and resistance-related gene mutations in multidrug-resistant and extensively drug-resistant tuberculosis in Korea. *Ann Lab Med.* **38,** (2018).
2. Liu Y. *et al.* Delamanid: from discovery to its use for pulmonary multidrug-resistant tuberculosis (MDR-TB). *Tuberculosis.* **111**, (2018).

Structural analysis of the nuclear and mitochondrial membranes of rat hepatocytes in experimental hyperthyroidism by electron microscopy

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Hyperthyroidism, excess thyroid hormone, promotes a hypermetabolic state associated with a variety of abnormalities of liver function [1]. The pathogenesis of hepatic dysfunction in thyrotoxicosis is unknown, but has been attributed to mitochondrial dysfunction [2]. Here, we studied the effect of altered thyroid function on ultrastructural changes in mitochondria and nucleus in a rat model of hyperthyroidism. Wistar rats were administered 100 μg thyroxin per 100 g body weight for 5 days. As expected, serum triiodothyronine (T3) and thyroxine (T4) increased by three and four times, respectively. The morphologic features of the nuclei and mitochondria of hyperthyroid rat hepatocytes were assessed by electron microscopy. In hyperthyroidism, the configuration of both nuclear membranes was altered in 65% of the total number of analyzed nuclei, with invagination and parallel arrangement, which was not registered in the control. In hyperthyroid rat liver, 58% of the mitochondria exhibited a decrease in the number of cristae and were in the swollen state. In 69% of the profiles, the swollen mitochondria included rounded mono- or multilayer membrane structures, called lamellar bodies (LBs). Most of LBs (67%) were located in the perinuclear mitochondria, while their number was reduced toward the cell membrane. The swollen mitochondrial cristae were transformed into lamellar membranes for five sequential stages: (1) the cristae changed their orientation, getting directed radially toward the center of the mitochondria; (2) a rounded monomembrane lamella was formed in the center of the mitochondria; (3) the lamella near the mitochondrial membrane became a multimembrane; (4) the lamellar structures were separated from the inner membrane of the mitochondria, causing protrusions but not tearing it apart; (5) the lamellae exit the mitochondria without disturbing the membrane integrity. One can assume that lamellar structures formed in hyperthyroidism in the liver tissue take part in the excretion of catabolism products and in the removal of excessive thyroxine from the organism.

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1. Choudhary A. and Roberts I. Thyroid storm presenting with liver failure. *J Clin Gastroentrol*;**29**:318–321 (1999).
2. Upadhyay G. *et al.* Severe hyperthyroidism induces mitochondria-mediated apoptosis in rat liver. *Hepatology.* **39**(4):1120-30 (2004).

Expression, purification, and NMR study of the transmembrane domains of the insulin-like receptor family

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Receptor tyrosine kinases (RTKs), single-spanning membrane receptors, play one of the central roles in the process of transmission of biochemical signals inside the cells. Malfunction of RTKs results in many human diseases: developmental, oncogenic, neurodegenerative, immune, cardiovascular, etc. Nevertheless, despite the comprehensive studies of RTKs [1, 2] not only the activation mechanisms but also the role of the lipid environment in the receptor activation are not clear. This is also true for the family of insulin-like receptors that belongs to the RTK. This family has two distinctive structural features: (1) all insulin-like receptors exist in the plasma membrane as covalent dimers cross-linked via extracellular ligand-binding domains, and (2) the presence of a proline residue in the first third of the single-span transmembrane (TM) domain that probably influences its mobility, including *cis-trans* isomerization of the peptide bond in front of the TM proline.

We developed and optimized high-performance systems of cell-free expression and purification of TM fragments for all three receptors of the insulin-like receptor family (InsR, IRR, IGF1R) for biochemical and biophysical studies. The system allows obtaining milligram quantities of the receptor TM fragments with isotope labeling more than 95%. We produce

the ^{15}N-isotope–labeled samples for NMR studies, which subsequently make it possible to characterize in detail spatial structure, dynamics, and kinetic parameters of conformational rearrangement for these TM fragments. The TM fragments were solubilized in detergent micelles, 40–60-kDa supramolecular membrane-mimicking complexes, which allow acquiring proper high-resolution NMR spectra. As a preliminary result, a slow conformational transition due to the *cis-trans* isomerization of the peptide bond in front of the proline residue was observed in the NMR spectra of the insulin-like receptor TM domains in micellar environment.
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1. Bocharov, E.V. *et al.* Helix-helix interactions in membrane domains of bitopic proteins: specificity and role of lipid environment, *Biochim. Biophys. Acta – Biomembranes*. **1859**, 561-576 (2017)
2. Bocharov, E.V. *et al.* Conformational transitions and interactions underlying the function of membrane embedded receptor protein kinases. *Biochim. Biophys. Acta – Biomembranes*. **1859**, 1417-1429 (2017)

K(ATP)- channels contribute in the decrease of Ca2+- transient in the mouse atria by NaHS

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Hydrogen sulfide (H_2S) is an important endogenous gaseous mediator that is synthesized from L-cysteine by cystathionine-γ-lyase s in the myocardium [1]. H_2S has a negative inotropic effect. It was shown that K(ATP)- channels and L-type of Ca^{2+}- channels are its main targets in the rat and frog myocardium [2, 3, 4]. The change in the intracellular Ca^{2+} concentration is a key factor that ensures the contraction and relaxation of cardiomyocytes. In this work, we have investigated the effect of H_2S on Ca^{2+}- transient in the mouse atria and the role of K(ATP)- channels in this effect. The experiments were performed on the left atria of laboratory mice. The registration of Ca^{2+}- transients was performed by fluorescence microscopy. The fluorescent calcium indicator (Fluo4-AM) was used for the recordings of the intensity of Ca^{2+}- transients. Sodium hydrogen sulfide (NaHS, 300 μM) was used for exploring the effects of exogenous H_2S. The application of NaHS led to a significant decrease of Ca^{2+}- transients. Glibenclamide (50 μM) was used as the blocker of K(ATP)- channels and it caused an increase in the amplitude of Ca^{2+}- transient in cardiomyocytes. In the next set of experiments, treatment with glibenclamide was carried out immediately after the incubation with the fluorescent dye. The NaHS effect was eliminated against the background of the preliminary blocking of K(ATP)- channels. Thus, H_2S can reduce the amplitude of Ca^{2+}- transient through the activation of K(ATP)- channels, which leads to a shortening of the action potential of cardiomyocytes and, as a consequence, to a decrease of Ca^{2+}- transients.

1. Geng, B. *et al*. Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol. *Biochemical and biophysical research communications,* **318**(3), (2004).
2. Sun, Y. G. *et al*. Hydrogen sulphide is an inhibitor of L-type calcium channels and mechanical contraction in rat cardiomyocytes. *Cardiovascular research*, **79**(4), (2008)
3. Abramochkin, D. V. Modulation of sinoatrial node pacemaker activity by carbon monoxide and hydrogen sulfide. *Doklady Biological Sciences*. **453**(1). Springer US, (2013).
4. Khaertdinov, N. N. *et al*. Hydrogen sulfide in regulation of frog myocardium contractility. *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology*, **7**(1), (2013).

Signal transduction mechanism via transmembrane domains of bitopic receptors studied by high-resolution NMR

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The human epidermal growth factor receptor (EGFR) and growth hormone receptor (GHR) serve as excellent models of bitopic receptors of type I to illustrate how ligand-induced conformational rearrangements and specific dimerization of extracellular domains lead to the allosteric activation of the cytoplasmic domains, resulting in signal propagation across the membrane. Dysregulated signaling from these receptors has been shown to play significant roles in promotion of a number of human diseases, and inhibitors of these receptors have been among the most successful examples of targeted therapies to date. Many essential aspects of EGFR and GHR signal transduction at the molecular level have been elucidated lately. Nevertheless, there are several issues yet to be resolved, including the particular role of single-span helical transmembrane domain (TMD) and flexible juxtamembrane regions in the receptor activity switching in terms of an apparent loose coupling between structural rearrangements of the extracellular and intracellular regions. We experimentally determined the alternative dimeric conformations of the EGFR and GHR TMDs in different membrane-mimicking environments using high-resolution NMR spectroscopy combined with MD relaxation in explicit lipid bilayer. Based on the available mutagenesis data, observed conformations correspond to the dormant and active states of both receptors. Fine adaptation of intermolecular polar and hydrophobic contacts that we found to accompany the different EGFR TMD dimerizations (observed in detergent micelles or in lipid bicelles) suggests that certain membrane properties can govern the TMD helix–helix packing and, thus, their alteration can trigger the receptor state, whereas two distinct dimeric modes of GHR TMD (coexisting in micellar environment) revealed the functional role of juxtamembrane region rearrangements in alternation between protein–protein and protein–lipid interactions that can be initiated by ligand binding. It was observed the TMD helix–helix packing diversity appears in favor of the lipid-mediated rotation-coupled activation mechanism, which implies that the sequence of structural rearrangements of EGFR and GHR domains is associated with perturbations of the lipid bilayer in the course of ligand-induced receptor activation, considering the receptor together with its lipid environment as a self-consistent signal transduction system.
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Structural insights into initial steps of Alzheimer disease via mutation-induced conformational variability of APP transmembrane domain

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Alzheimer disease (AD) is the most common cause of neurodegenerative disorder and may contribute to 60–70% of cases of dementia (according to the WHO report). Despite some progress in the study of the AD molecular mechanisms of development, the initial steps of the pathogenesis are still puzzling. Amyloid Aβ-peptides forming plaques in brain during AD are the products of sequential intramembrane cleavage of a single-span membrane amyloid precursor protein (APP). A lot of mutations associated with AD familial forms were found in the APP transmembrane (TM) domain and juxtamembrane (JM) regions. The pathogenic mutations presumably affect structural–dynamic properties of the APP TM domain, e.g., changing its conformational stability, lateral dimerization, and intermolecular interactions, which can result in enhanced and alternative cleavage by γ-secretase in membrane. We designed highly productive systems of bacterial and cell-free expression and easy purification procedure for $^{13}C/^{15}N$-isotope–labeled APP JM-TM fragments of different length (corresponding to the sequential cleavage steps of APP) and with familial AD mutations, as well as the several TM fragments of γ-secretase. The fragments were solubilized in membrane-mimicking complexes (detergent micelles and lipid bicelles), which allows acquiring proper high-resolution NMR spectra despite low sample stability and aggregation. Molecular dynamics relaxation of obtained NMR structures of the fragments in hydrated explicit lipid bilayers provided a detailed atomistic picture of the intra- and intermolecular interactions. The mutant APP JM-TM fragments are shown to be promising objects for elaboration of the molecular aspects of γ-secretase proteolysis, e.g., "Australian" (APP L723P) mutation is identified to be associated with autosomal-dominant, early-onset AD. We detected enhanced flexibility and partial unfolding of the N- and especially C-terminal regions of the TM helix of L723P mutant compared to wild-type peptide, which can facilitate the APP proteolysis in the ε-site and switch between alternative ("pathogenic" and "non-pathogenic") cleavage cascades. These findings suggest a straightforward mechanism of the pathogenesis associated with this mutation, and serve as a clue for understanding the molecular-level events associated with APP sequential proteolysis resulting in accumulation of pathogenic forms of amyloid Aβ-peptides.

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SIRT1 and SIRT6 as possible markers of thymus aging

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Sirtuins control energy homeostasis and may be the determining factors of the organism aging. Sirtuins differ in tissue specificity, subcellular localization, enzyme activity, and targets [1]. SIRT1 is the most studied member of the sirtuin family; it is involved in histone deacetylation and, therefore, in the apoptosis process. Some researchers suggest that this function can be used in cancer therapy [2]. Moreover, SIRT1 inhibits lipid accumulation in adipocytes and improves insulin sensitivity, which is crucial for patients with type 2 diabetes mellitus [3]. SIRT6 is identified in some articles as a tumor suppressor that regulates aerobic glycolysis in cancer cells. The appearance of intensified glycolysis and the tumor growth in SIRT6-deficient cells indicate that SIRT6 plays a role both in the tumor formation and in its activity maintenance [4].

The data on the link between age-related involution of thymus and sirtuin activity is lacking. In this thesis, we present the preliminary stage results. Thymic tissue samples of patients who died from non-infectious diseases and/or immunodeficiency (they all died of cardiovascular diseases, cardiac malformation, or hypoxia) from extreme age groups were selected: 1, the first year of life ($n = 6$); 2, 60–79 years ($n = 5$); 3, 80–99 years ($n = 5$). Immunofluorescent staining and morphometric analysis in ImageJ were conducted. The relative area (RA) and the mean gray value (average brightness, AB) of the SIRT1 and SIRT6 expressions were estimated. According to the ANOVA, there were significant differences in the groups by the SIRT6 RA and AB with $p = 0.005$ and $p = 0.011$, respectively. The mean RA-SIRT6 in group 1 was 0.59 (95% CI 0.49–0.70); in group 2, 0.40 (95% CI 0.20–0.60); in group 3, 0.30 (95% CI 0.17–0.43). The mean AB-SIRT6 in group 1 was 4.24 (95% CI 3.71–4.78); in group 2, 13.11 (95% CI 4.47–21.75); in group 3, 7.98 (95% CI 5.42–10.53). These results confirm the possible role of sirtuins in thymus regression. It is planned to expend the samples, to add the samples of 5–9-year-old, 10–14-year-old, 15–24-year-old, and 25–44-year-old patients, and conduct the real-time PCR for the quantitative evaluation of protein expression.

1. Mei, Z. *et al.* Sirtuins in metabolism, DNA repair and cancer. *J. Exp. Clin. Cancer Res.* **35,** 182 (2016).
2. Chao, S.-C. *et al.* Induction of sirtuin-1 signaling by resveratrol induces human chondrosarcoma cell apoptosis and exhibits antitumor activity. *Sci. Rep.* **7,** 3180 (2017).
3. Rahman, S. & Islam, R. Mammalian Sirt1: insights on its biological functions. *Cell Commun. Signal.* **9,** 11 (2011).
4. Desantis, V. Lamanuzzi, A. & Vacca, A. The role of SIRT6 in tumors. *Haematologica* **103,** 1–4 (2018).

The advantages of the GI-XSW technique for investigation of protein Langmuir monolayers formed from the polydisperse solutions

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The grazing incidence X-ray standing waves (GI-XSW) technique combines the potential of X-ray scattering and spectroscopy. The advantages of this technique enable it to obtain the distribution of atoms of a certain type in structural studies of thin films and monolayers. The main principle of GI-XSW is to record the yield of secondary radiations (especially fluorescence) in the interaction of X-rays with matter under the conditions of formation of an XSW. The angular dependence of the specular component intensity and the fluorescence yield signal from atoms of a specified type in the sample are simultaneously recorded.

In this work, the GI-XSW technique was used for investigation of the structural features of Langmuir monolayers of a lysozyme protein (HEWL) on the surface of a water subphase. These monolayers were fabricated from the polydisperse solutions which contain lysozyme oligomers formed under the crystallization conditions. The results of small-angle X-ray scattering indicate that the addition of a precipitant causes the protein molecules to be assembled into dimers and octamers [1]. In this work, potassium chloride (KCl) was used as a precipitant favorable for oligomer formation at the initial crystallization stage.

It was shown that the thickness of the resulting protein layer is twice the diameter of an individual lysozyme molecule and matches the diameter of the octamer. Thin layers of the precipitant ions (K^+ and Cl^-) form directly under the protein monolayer. These results are confirmed by the previous study of the same films deposited onto solid substrates by the Langmuir-Schaeffer technique. Based on these results, it can be indicated that the precipitant ions interact with the protein monolayer.

Thus, the GI-XSW technique allows detecting directly the positions of precipitant atoms relative to the protein layer, and it can be useful for obtaining additional information on the principles of the interactions between proteins and salt ions.

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1. Kovalchuk, M. V. et al. Investigation of the initial crystallization stage in lysozyme solutions by small-angle X-ray scattering. Crystal Growth and Design **16**, 1792–1797 (2016).
2. Kovalchuk, M. V. et al. Modification of the Langmuir–Schaefer method for fabrication of ordered protein films. Crystallography Reports **62**, 632–638 (2017).

Protein expression with intact N-terminal sequence

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N-terminal amino acid sequence of proteins is frequently used by sorting machinery of the eukaryotic cell to transport protein to different cell compartments such as the mitochondria, nucleus, cell membrane, or ER [1].
Proteins with targeting sequence expressed in *Escherichia coli* may lose parts of this sequence by specific (f-Met) and unspecific N-terminal degradation if the following amino acid is suitable. One of the ways to protect this sequence is to hide it behind other amino acids, which are later cleaved by protease during purification.
SUMO-tag is usually used for enhancing functional protein expression [2]. After protein purification, SUMO is cleaved by protease ULP-1. One of the useful properties of this system is low sensitivity to amino acid sequence after cleavage site allowing the production of a variety of proteins in *E. coli* with desired N-terminus, like targeting sequence.
Here, we have used SUMO-tag system to produce N-terminally tagged FbFP and GFP for injection in eukaryotic cells to study protein sorting.

1. Kunze, M. & Berger, J. The similarity between N-terminal targeting signals for protein import into different organelles and its evolutionary relevance. *Front. Physiol.* **6,** 259 (2015).
2. Panavas, T., Sanders, C. & Butt, T. R. in 303–317 (Humana Press, Totowa, NJ, 2009). https://doi.org/10.1007/978-1-59745-566-4_20

3D structure of natural tetrameric form of human butyrylcholinesterase obtained by cryo-electron microscopy

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Human butyrylcholinesterase (BChE) is a stoichiometric bioscavenger of toxic organophosphates. It can be used as an antidote to protect acetylcholinesterase, and is a protein of choice for development of detoxification biocatalysts for clinical applications. Despite the number of different monomeric structures of recombinant human BChE obtained to date, all attempts to obtain an atomic structure of the natural glycosylated tetrameric BChE were unsuccessful.
Here, we present for the first time the 3D structure of the natural tetrameric form of human butyrylcholinesterase, obtained by Cryo-EM technique at a final resolution of 7.6 Å. The tetramer has a C2 symmetry, with all the subunits arranged in a propeller-like mode, where the two opposite BChE subunits disposed higher the other two subunits. Such subunit organization is in contrast with the proposed previously "flat" model of subunit arrangement in BChE tetramer. The electron density showed that C-terminal tails of all the subunits tightly interact with each other and form a left-handed superhelix around the PRAD-peptide, supporting rigidity of the tail. The tail is situated in the center of the tetramer and is oriented nearly perpendicular to the tetramer "plane". It was also observed that the subunits in the tetramer have different contacts with neighbouring subunits. This allows the consideration of the tetramer as a dimer of dimers, which is additionally strengthened by the C-terminal tail interactions.
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Interaction of hyaluronic acid with dermal membrane: a molecular dynamics simulation study

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Hyaluronic acid (HA) is a naturally occurring polyanionic polysaccharide that consists of *N*-acetyl-d-glucosamine and β-glucoronic acid. It is present in the intercellular matrix of most vertebrate connective tissues especially skin where it has a protective, structure stabilizing, and shock-absorbing role. The unique viscoelastic nature of HA along with its biocompatibility and non-immunogenicity has led to its use in a number of clinical applications. When applied to the skin, HA provides beneficial effects such as skin hydration, elasticity regeneration, and improved wound healing [1].
The major limitation for active compound penetration through the skin is overcoming the most outer layer of the non-viable epidermis—stratum corneum (SC). SC serves as a rate-limiting lipophilic barrier against the uptake of chemical and biological toxins as well transepidermal water loss. The structure of SC is organized as stacked bilayers of ceramides in a splayed chain conformation with cholesterol associated with the ceramide sphingoid moiety and free fatty acids associated with the ceramide fatty acid moiety [2].
The mechanisms of HA-mediated skin penetration, however, are still poorly understood. In the present study, we propose the mechanism by which HA can penetrate the SC barrier with and without (dimethyl sulfoxide) DMSO which is often used in cosmetics. We have carried out the coarse-grained molecular dynamics simulations of model SC together with the following: (1) DMSO; (2) DMSO with HA in different concentrations; (3) pure HA also in different concentrations.
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1. Witting, M. et al. Interactions of hyaluronic acid with the skin and implications for the dermal delivery of biomacromolecules. *Mol. Pharm.* **12**, 1391–1401 (2015).
2. Gupta, R. & Rai, B. Molecular dynamics simulation study of skin lipids: effects of the molar ratio of individual components over a wide temperature range. *J. Phys. Chem. B* **119**, 11643–11655 (2015).

Proteases in in meso crystallization

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During the last decade, crystallization approach that uses water-soluble fusion proteins appended to membrane proteins for in meso crystallization allowed obtaining the structures of the beta2-adrenergic receptor and plenty of other G protein-coupled receptors [1]. Having *Halobacterium salinarum* bacteriorhodopsin (bR) as a model, we have applied this approach to rhodopsins. The fusion of bR with BRIL (thermostabilized apocytochrome b562 RIL) was expressed in *Escherichia coli*, purified and crystallized as described earlier [2]. However, BRIL was missing in the structure of the fusion protein solved at 2-Å resolution. We have not observed proteolytic products in the sample used for the crystallization, and prolonged incubation of the solubilized protein at room temperature has not shown considerable proteolysis.

We have carried out experiments with the bR-BRIL fusion protein to access its stability in the lipid phase. They confirmed that, although the protein is relatively stable in detergent solution for several weeks upon its incorporation into the mesophase, BRIL is cut off within 3 days. We have investigated several reagents known to inhibit different types of proteases and found out that addition of 2 mM EGTA efficiently prevents the degradation of the fusion protein in the phase. The nature of the protease inhibitor allows us to assume that the sample proteolysis is carried out by co-purified metalloproteases.

Generally, the crystallization handbooks recommend considering the addition of protease inhibitors during purification and crystallization of proteins [3]; however, additional components in a crystallization mixture could hamper crystallization. Present experiments make it clearly visible that principal changes in the environment of a membrane protein during in meso crystallization considerably change its proteolytic stability. Therefore, one should not only access degradation of the target protein in a sample intended for in meso crystallization, but also control the proteolysis in the crystallization media activated by an influence of the lipid media itself, especially when dealing with fusion proteins.

1. Cherezov, V. *et al.* High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. *Science* **318,** 1258–1266 (2007).
2. Bratanov, D. *et al.* An approach to heterologous expression of membrane proteins. The case of bacteriorhodopsin. *PLoS One* **10,** (2015).
3. McPherson, A. Current approaches to macromolecular crystallization. *European Journal of Biochemistry* **189,** 1–23 (1990).

Effect of dimebon on lifespan extension based on *C. elegans* model

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Initially marketed in Russia as a treatment for hay fever in the 1980's, Dimebon (Latrepirdine) has shown to have neuroactive, neuroprotective and cognitive enhancing properties in a number of in vitro/in vivo studies (1). This "old" drug is actively studying now for repositioning of its original targets especially towards geroprotective capacity. The main pipeline of these research has focused on study in mice, and very little known about Dimebon anti-aging effect in C. elegans model.

In this study, the life-extension effect of Dimebon was tested on Caenorhabditis elegans in comparison with well-known geroprotectors such as Metformin, Reserpine and Harmane. Dimebon and all reference compounds were tested in synchronized C. elegans in liquid media following by standard protocols (2). Two series of experiments with 0.1 μM and 10 μM concentrations of drugs were set up with the same stock of nematodes and monitored during 35 days of experiment. Moreover, these drugs were tested on resistance to anti-oxidative stress induced by hydrogen peroxide at 10 μM concentration. The number of survived animals in the presence of tested drugs was registered two times per week and compared with the control group without drug treatment. Our study revealed that the life-span of nematodes was increased by 21.4% in presence of Dimebon at 10 μM concentration, while in presence of Harmane at the same concentration increase was only by 14.2%, as compared to the non-treated nematodes. The effect of Dimebon on life-span was pronounced even at lower concentration while demonstrating of life-extension up to 18 % at the 0.1 μM concentration. Known geroprotectors, such as Harmane and Reserpine were less effective though extended life-span to 7% and 9% at the same concentration, respectively. In series of experiments on anti-oxidative stress resistance, 34% and 17% more nematodes were remained alive in the presence of Dimebon compared with Metformin and Reserpine, respectively, at 5 hours of incubation in the presence of 10 μM H2O2. After 23 hours of H2O2 exposure, 21% and only 11% of nematodes were still alive in presence of Demibon and Reserpine, respectively, whereas all nematodes were dead in the metformin presence. The results of our C. elegans model study support the vision of the Dimebon as a potential geroprotective drug, thus opening new avenue for our ongoing work focusing on the discovery of mechanism the of Dimebon action in the life extension.

1. Lermontova N.N. et al. Dimebon improves learning in animals with experimental Alzheimer's disease. Bull Exp Biol Med. 2000; 129:544–546.
2. Ekaterina Lashmanovaa et al. Fucoxanthin increases lifespan of Drosophila melanogaster and Caenorhabditis elegans. Pharmacological Research (2015), 2280241

Microfluidic flows of photometabolites acting on chlorophyll fluorescence reveal cell-to-cell communications in *Chara*

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Intracellular communications in giant algal cells are mediated by cytoplasmic streaming that compensates for physical limitations on distances accessible for diffusion. Long-distance signaling and metabolite transmission in individual cells were studied by measuring actual chlorophyll fluorescence *F'* on microscopic areas of *Chara* internodes upon local illumination of a remote cell region located 1–4-mm upstream in the cytoplasmic flow [1–3]. When a narrow (diam. 400 μm) 30-s light pulse was applied, it induced the wave-like *F'* transients propagating through the shaded cell parts in the direction of liquid flow at a rate comparable to the velocity of cytoplasmic streaming. This approach is now applied to analyzing the intercellular transmission of photoassimilates and endogenous reductants between the branchlet internodes of *Chara corallina* (*australis*). Unlike earlier techniques based mostly on microinjection of fluorescein-labeled chemicals, pulse-amplitude–modulated microfluorometry combined with the precisely positioned fiber-optic illumination provides a noninvasive assay for detecting natural

metabolites that were produced at physiological concentrations by illuminated chloroplasts and crossed the membrane barrier between the contacting internodes.

A significant part of regulatory solutes exported from brightly illuminated chloroplasts into the streaming fluid was found to leave the intracellular rotational flow and enter the neighboring internode after a distinct delay required to cross the internodal complex. Under transcellular experimental configuration, the delay in *F'* peak formation (counted from the *F'* peak position for intracellular measurements) was independent on separation distance between the local light source and the point of *F'* detection. This fixed delay can be ascribed to the diffusion of solutes through the plasmodesmata, which allows the estimates of their permeability to photosynthetic metabolites. The occurrence of bulk fluid flow through the plasmodesmata is another alternative to be taken into consideration. Mild changes in external osmolarity did not disturb the cytoplasmic streaming and chloroplast photosynthetic activity (as assessed from the quantum yield of PSII-driven electron flow) but resulted in reversible closing of the plasmodesmal route. Thus, the cell-to-cell transmission of photometabolites across the nodal complex of *Chara* is sensitive to external cues and is subject to dynamic regulation.

1. Bulychev, A. & Komarova A. Photoregulation of photosystem II activity mediated by cytoplasmic streaming in *Chara* and its relation to pH bands. *BBA – Bioeneregetics (386-395).* **1858,** (2017).

2. Komarova, A. *et al.* Cyclosis-mediated long-distance communications of chloroplasts in giant cells of Characeae. *Funct. Plant Biol. (236-246).* **45,** (2018).

3. Bulychev, A. & Rybina A. Long-range interactions of *Chara* chloroplasts are sensitive to plasma-membrane H^+ flows and comprise separate photo- and dark-operated pathways. *Protoplasma* (2018).

Mitochondrial network dynamics examined with photoconvertible Dendra2-protein

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The balance between fusion and fission processes forms the phenotype of mitochondrial network and is crucial for its proper function. Pathologies of mitochondrial network dynamics are associated with ageing-related diseases of the cardiovascular system and the nervous system.

To study the dynamics of mitochondrial networks via fluorescence microscopy we produced human embryonic kidney (HEK) cells stably expressing photoconvertible fluorescent protein Dendra2 [1] targeted to mitochondria. Therefore, we transduced HEK cells with a lentiviral vector and isolated a single highly expressing cell clone to produce a genetically homogeneous population with a constant expression level of Dendra2.

We have achieved high-precision photoconversion of Dendra2 in parts of the mitochondrial network of a single cell and observed redistribution of photoconverted signal across the cell in time-lapsed measurements. For quantitative examination of mitochondrial network morphology we used algorithms of image processing implemented in MiNa ImageJ plugin [2] and suggested a quantitative approach for analyzing these dynamic data based on colocalization analysis.

As starting point for experiments aimed at analyzing mitochondrial dysfunction we induced changes in the mitochondrial network with oxidative stress (initiated with H_2O_2) and a mitochondrial membrane-potential uncoupler (CCCP, carbonyl cyanide m-chlorophenylhydrazon).

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1. Chudakov, D. M., Lukyanov, S. & Lukyanov, K. A. Using photoactivatable fluorescent protein Dendra2 to track protein movement. *Biotechniques* **42,** 553–565 (2007).

2. Valente, A. J., Maddalena, L. A., Robb, E. L., Moradi, F. & Stuart, J. A. A simple ImageJ macro tool for analyzing mitochondrial network morphology in mammalian cell culture. *Acta Histochem.* **119,** 315–326 (2017)

Using principal component analysis of lipid molecule conformations to quantitatively describe membrane behavior

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Molecular dynamics simulations provide atomic-level information about the processes occurring in biological membranes. Typically, lipid molecules exhibit complex conformational changes occurring on the timescales from femtoseconds to hundreds of nanoseconds. While most of the structural analyses of molecular dynamics simulations of lipid molecules focus either on average properties of the whole membrane or on particular structural characteristics such as dihedral angles, we apply principal components analysis (PCA) to study the conformations of individual lipid molecules [1]. The approach results in identification of major collective motions and comprehensive quantitative characterization of the molecule's conformational space and characteristic time scales of motions in molecular dynamics simulations. Furthermore, it provides a simple framework for comparison of different simulations of membrane systems. The Python-based software performing the analysis – PCALipids – could be found via link: https://github.com/membrane-systems/PCAlipids.

We illustrate the approach with several examples. First, we compare the simulations of DOPC bilayers conducted using eight commonly used high resolution force fields, and PCA provides quantitative estimates of similarities and differences between them [1]. Second, we use PCA to compare all-atom and coarse grained (Martini) simulations of lipids. We find that CG simulations capture 75 to 100% of the major collective motions, and result in 5-7 fold faster sampling. Also, we show that CG simulations overestimate short range interactions and overall flexibility of lipid molecules [2]. Third, we study the influence of cholesterol and temperature on lipid molecule conformations. We show that the phase transitions induced by temperature and cholesterol are different, consistent with experimentally measured data [3].

We expect that our results will be useful for further development and improvement of AA and CG force fields, in particular via elimination of identified differences in lipid behavior, and for quantitative description of lipid conformations in different phases.

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1. Buslaev, P. *et al.* Principal component analysis of lipid molecule conformational changes in molecular dynamics simulations. *JCTC(12(3)),***1019-1028***,* (2016)

2. Buslaev, P., Gushchin, I. Effects of coarse graining and saturation of hydrocarbon chains on structure and dynamics of simulated lipid molecules. *Scientific Reports* **7,** (2017).

3. Marsh, D. Liquid-ordered phases induced by cholesterol: A compendium of binary phase diagrams. *Biochimica et Biophysica Acta (1798).* **688-699,** (2010).

Recombinant analogue of mambalgin-2 from *Dendroaspis polylepis* inhibits proliferation of glioblastoma cells

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Mambalgin-2 from the venom of *D. polylepis* is a potent inhibitor of ASIC channels. We have developed a system for bacterial production of recombinant analogue of mambalgin-2. Acid-sensing ion channels (ASICs) and other members of the ENaC/Deg family are known to form chimeric channel in high-grade glioblastomas but not in normal astrocytes or low-grade malignancies [1]. Usually that hybrid channel is formed by ASIC1a and α or γENaC subunits [2]. Inhibition of this channel by several toxins, for example by psalmotoxin-1 (PcTX-1), leads to decrease in glioblastoma cells proliferation and migration [3].
Here we investigated the influence of mambalgin-2 on glioma U251 MG cells and glioblastoma A172 cells. Using qPCR technique, we found that both cell lines posses ASICs 1a, 2a, 2b, 3 and 4 subunits as well as α and γ subunits of EnaC channels. Incubation of U251 MG cells with 1 μM of mambalgin-2 significantly reduced cell viability—by 20% (24 and 48 h incubation) and by 40% (72 and 96 h incubation). Influence of mambalgin-2 on A172 viability became prominent (70 %) after 72 h incubation. An analysis of the dose-response curve revealed the concentration-dependent mode of the Mambalgin-2 action with EC_{50} $\sim 0.6 \pm 0.01$ nM and $\sim 10 \pm 1.6$ nM for U251 MG and A172 cells, respectively. Effect of mambalgin-2 on U251 MG and A172 cell growth was comparable with effect of potent ASIC antagonist amiloride. Our data revealed new functional properties of Mambalgin-2 and point on the toxin as perspective drug for treatment of glioblastomas.
The study was supported by the Russian Foundation of Basic Research (Project № 18-34-00497).

1. Berdiev, B.K. *et al.* Acid-sensing ion channels in malignant glioblastomas. *J Biol Chem, 278(17),* **15023-15034,** (2003).
2. Kapoor, N. *et al.* Interaction of ASIC1 and ENaC subunits in human glioblastoma cells and rat astrocytes. *Am J Physiol Cell Physiol, 300(6),* **1246-1259,** (2011).
3. Rooj, A.K. *et al.* Glioblastoma-specific cation conductance regulates migration and cell cycle progression. *J Biol Chem, 287(6),* **4053-4065,** (2012).

Noradrenaline switch mesenchymal stem/stromal cells to proinflammatory phenotype

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Inflammation is a typical pathological process induced by pathogenic factors. Inflammation includes destruction by pathogenic agent and regeneration. Mesenchymal stem/stromal cells (MSC) are important members of inflammation and regeneration. MSC are part of a majority of tissues of organisms. MSC are capable to differentiate in several types of cells therefore MSC play an important role in reparation and regeneration of injuries. MSC also regulates inflammation, because of MSC influence on activity of cells of immune system by secreting of cytokines and inducing an anti-inflammatory effect. Hormones and neuromediators regulate functional activity of MSC. Noradrenaline, which controls secretory activity and differentiation of MSC, is one of the important neuromediators for MSC. Earlier, we showed the interesting phenomenon of switching of intercellular signaling, activating by adrenergic receptors: noradrenaline stimulates beta-adrenergic receptors in MSC and induces heterologous sensitization of alpha 1A-adrenergic receptors.
In our work, we studied the influence of noradrenaline on secretory activity MSC, associating with regulation of inflammation. Using NanoString we examined changing of expression above 700 cytokines and other proteins, associating with regulation of immune reaction. Using PanCancer Immune Profiling Panel we showed noradrenaline induces elevation of expression RNA of many proinflammatory cytokines, those receptors and members of signaling pathways, activating by these cytokines, and decreasing expression of antiinflammatory molecules. Also we explored changing of expression of 17 proinflammatory cytokines using BioRad BioPlex. We observed that stimulation by noradrenaline leads to increasing of expression of proinflammatory cytokines, such as IL-6, IL-8, G-CSF, IFN-G, and MCP-1. Repeated stimulation alpha 1A-adrenergic receptors by noradrenaline did not change expression of cytokines. Therefore stimulation of beta-adrenergic receptors, but not alpha1A adrenergic receptors leads to switching MSC to proinflammatory phenotype.
Thus, we established that the stimulation by noradrenaline induced elevation of secretion of pro inflammatory cytokines and cells were more sensitive to these molecules. MSC switched from anti-inflammatory to proinflammatory phenotype. The work was supported by the grant of the president of the Russian Federation for the state support of young Russian scientists—candidates of sciences MK-3167.2017.7, Russian Science Foundation (RSF) grant 14-15-00439.

Identification of abnormalities in mice with transglutaminase type 3 mutation

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During the differentiation program keratinocytes from the basal layer of epidermis move upward through the granular layer, where they undergo the process of cornification, to the stratum corneum, the outermost layer of the epidermis, consisting of terminally differentiated dead cells. Cornification involves the replacement of the plasma membrane by the cornified envelope comprised of an inner protein envelope covalently attached to an outer lipid envelope. Transglutaminase type 3 has a well-documented role in cornified envelope assembly being important for skin barrier formation. In the hair follicle cells transglutaminase type 3 cross-links small proteins such as trichohyalin and involucrin that are necessary to maintain intercellular connections and sheath structures of the hair follicle. Mutant mice with the transglutaminase 3 mutation (we/we mutants) are an invaluable tool for investigating the morphogenesis abnormalities. The mutation causes no obvious visual defects of hair growth, but immunohistochemical analysis that we performed in the present work revealed abnormalities in the development of the epidermis and hair follicles. More serious abnormalities in epidermis stratification and hair follicle morphogenesis were found in double mutants we/we wal/wal, thus the mutant we gene has a modifying effect on the wal gene amplifying alopecia in double homozygous mice. Mice with the we/we wal/wal genotype become almost hairless at the age of 21 days, with short hairs remaining on the muzzle. There were significantly fewer hair follicle primordial in this mutant compared with we/we and wild type. We identified invalid placodes, positive for ectodysplasin A1 receptor, nuclear β-catenin, and LEF1, which failed to invaginate, produced a double basal-like layer of epidermal cells, and lacked cylindrical keratinocytes. We also observed DP-like groups of intensively stained cells by alkaline phosphatase in the absence of visible signs of folliculogenesis in the epidermis. Multiple organogenesis abnormalities identified in our study support a notion that transglutaminase 3 contributes to keratinocyte differentiation and epidermis and hair follicle development.

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Multi-scale imaging highlights a critical role of spectrin in hearing development and deafness

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The cuticular plate of cochlear hair cells (HCs) is a specialized region that helps convert sound-induced mechanical vibration into electrical signals. While spectrins are essential to many HC functions and therefore hearing, their structural organization remains poorly understood. Here we investigate the structures and organizations of αII- and βII-spectrin in rodent HCs with super-resolution fluorescence imaging methods. We find that αII- and βII-spectrin form ring-like structures that wrap around the circumference of stereocilia rootlets and are spaced nearly evenly along their core regions. Functionally, we find both αII- and βII-spectrin rings associate with mice with hearing disability in noise model and aging model and that βII-spectrin in HCs directly participate in hearing formation. And specifc deletion of βII -spectrin in HCs resulted in rapid HC degeneration and profound deafness in mice. Thus, we conclude that the organized structures spectrin in the cuticular plate may provide the mechanical support to withstand the vibrations of the stereocilia during the mechanotransduction of auditory stimuli, and thereby play a critical role for the development of hearing.

1. Bennett, V. & Baines, A. J. Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. Physiol Rev 81, 1353-1392, https://doi.org/10.1152/physrev.2001.81.3.1353 (2001).
2. Furness, D. N., Mahendrasingam, S., Ohashi, M., Fettiplace, R. & Hackney, C. M. The dimensions and composition of stereociliary rootlets in mammalian cochlear hair cells: comparison between high- and low-frequency cells and evidence for a connection to the lateral membrane. J Neurosci 28, 6342-6353, https://doi.org/10.1523/JNEUROSCI.1154-08.2008 (2008).
3. D'Este, E., Kamin, D., Gottfert, F., El-Hady, A. & Hell, S. W. STED nanoscopy reveals the ubiquity of subcortical cytoskeleton periodicity in living neurons. Cell Rep 10, 1246-1251, https://doi.org/10.1016/j.celrep.2015.02.007 (2015).

Design of new potential anti-aggregatory agents

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The creation of new anti-aggregatory agents with reduced side effects is very important task for bioorganic and medicinal chemistry in connection with the necessity of the prevention and the treatment of cardio-vascular disorders, which are the main reason of the infarction, the stroke and pathological thromboses. We had done earlier the design and the synthesis of the library of potential anti-aggregatory agents, 5-substituted 3-pyridylisoxazoles, based on the scaffold containing pyridyne and isoxazole rings connected with C-C bond in position 3 of isoxazole ring [1]. All compounds demonstrated the anti-aggregatory activity, and their mechanism of action was studied [1]. Our experiments allowed to identify the role of tested 5-substituted 3-pyridylisoxazoles as the thromboxane A_2 receptor antagonists.

We studied the relative selectivity of a series of most active compounds with respect to the platelet membrane receptors—ADP, platelet-activated factor (PAF), adrenaline and thrombin receptors. The influence of the substituent nature in positions 3 and 5 in 5-substituted 3-pyridylisoxazole molecule on the anti-aggregatory activities was shown [2, 3].
Another modification of the scaffold included the introduction the aryl moiety in position 3 and aryl or hetaryl moieties in position 5 of isoxazole ring. The synthetic approaches for the preparation of these possible prototypes of anti-aggregatory agents were developed, and a series containing 6 compounds was synthesized. Some compounds from this series were tested on the anti-aggregatory activity using arachidonic acid as an inductor of human platelets aggregation in human washed platelets suspension.
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1. Demina, O. V., Khodonov, A. A., Sinauridze E. I., Shvets V. I., Varfolomeev S. D. *Rus. Chem. Bull*. (2014), 2092.
2. Demina, O. V., Belikov, N. E., Varfolomeev, S. D., Shvets, V. I., Khodonov, A. A. *Russian Pat.* No 2565754 (2015).
3. Demina, O. V., Belikov, N. E., Varfolomeev, S. D., Khodonov, A. A. *Rus. Chem.Bull*. (2018), *in print*.

Epigenetic alterations in ischemic penumbra in the rat cerebral cortex induced by photothrombotic stroke

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In ischemic stroke vascular occlusion and ATP depletion rapidly induce necrosis and tissue infarct. During following hours cell damage propagates to surrounding tissue and forms the transition zone (penumbra) that is potentially salvageable. Previously, we showed up-regulation of several dozens proteins involved in cell signaling, apoptosis, cytoskeleton remodeling, proteolysis in the penumbra after photothrombotic stroke (PTS, experimental stroke model) in the rat cerebral cortex. To study mechanisms that regulate protein expression in PTS-induced penumbra, we explored some histone modifications that regulate transcriptional activity of the genome. PTS was induced in the rat cerebral cortex by local laser irradiation (Ø3mm) after administration of Rose Bengal, which does not penetrate cells and remains in the bloodstream. Laser irradiation caused local vessel occlusion and tissue infarct. Control: non-irradiated contralateral cortex. At 1, 4, or 24 h after photothrombosis the 1.5 mm width ring penumbra around Ø3mm infarct core was cut out, homogenized, and incubated with fluorochromes Cy3 or Cy5. Antibody microarrays that, except other proteins, contain antibodies against epigenetic proteins were incubated with fluorochromed samples and scanned on the microarray scanner at 532 and 635 nm. The experimental to control ratios characterized the difference in protein levels between photothrombotic and control tissues. The levels of histone H3 acetylated on lysine 9 and/or phosphorylated of serine 10 were reduced in the penumbra 1, 4, or 24 h after PTS. Deacetylation of histone H3 leads to chromatin condensation and suppression of transcriptional activity that finally contributes to apoptosis, which prevails in penumbra. This effect could be associated with over-expression of histone deacetylase HDAC1, HDAC2 and HDAC4 after PTS. Immunohistochemistry and western blotting confirmed the upregulation of HDAC1 and HDAC2. Histone acetyltransferases HAT1 and PSAF were also upregulated in the penumbra, but lesser and later. The expression of histone methyltransferase SUV39H1 was not changed significantly in the penumbra. Thus, histone deacetylases may serve as potential targets for anti-stroke therapy, and their inhibitors may be prospective neuroprotective anti-stroke agents. Supported by the Russian Science Foundation (grant no. 18-15-00110) and Russian Ministry of Education and Science (research organization grant no. 6.4951.2017/6.7).

The dynamics of changes in the cellular and intracellular distribution of sirtuins 1, 2 and 6 in early post-stroke recovery

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Sirtuins are the III class histone deacetylases with their activity associated with regulation of important cellular processes including metabolism, cell growth, apoptosis, autophagy, and genetic control of aging. We studied the cellular and intracellular distribution of Sirt1, Sirt2, and Sirt6 in an early recovery period after phototrombotic stroke (PTS) in mice.
Sirt1 was present both in the nuclei and cytoplasm of cortical and hippocampal neurons and astrocytes in the brain of sham-operated animals. We have observed an increase in the level of SIRT1 in astrocytes during the period of scarring (7 and 14 days after PTS), which is probably because of the ability of the protein to regulate the scar formation in neurodegenerative processes.
Sirt2 was detected exclusively in the cytoplasm of the neurons, but not in the astrocytes in the brain of the mice. We have not observed SIRT2 transition into the neuronal nuclei at the investigated times after the stroke (3, 7, 14 and 21 days after PTS). The immunoreactivity of Sirt2 in the cortical neurons in the ipsi- and contralateral hemispheres of mice 3 days after PTS was increased, which is probably caused by the ability of SIRT2 to induce the expression of pro-inflammatory cytokines.
We have not observed changes in the level and intracellular localization of Sert6 at the investigated times after the PTS in the brain cells.
Thus, we can conclude that Sirt1 and Sirt2 are important intracellular regulators in ischemia/reperfusion but with opposite functional effects. Sirt1 is mainly neuroprotective by protecting neurons from damage and decreasing in the penumbra at early reperfusion times, whereas the Sirt2 expression grows during this period, which, on the opposite, contributes to the damage and death of brain cells.
The study was supported by the Russian Foundation for Basic Research (project no. 16-04-01135).

The fatty acid-modified peptide fragment of leptin as the effective regulator of leptin signaling pathways in the hypothalamic neurons in the conditions of the in vitro and in vivo

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Leptin produced by the adipose tissue is the key regulator of food intake and energy expenditure. The main target of leptin is the hypothalamic arcuate nuclei, in which the leptin receptors are expressed. Activating these receptors, leptin triggers the 3-phosphoinositide cascade, which includes the enzyme Akt kinase, and the STAT3/5-dependent cascades. In obesity, the regulatory effects of leptin on hypothalamic neurons are reduced due to the impaired transport of leptin through the blood-brain barrier (BBB) and to the dysfunctions in the hypothalamic leptin system. When leptin is used to treat obesity, peripheral leptin resistance is increased and leptin transport through the BBB is weakened, which results in a low effectiveness of leptin therapy. In this regard, there is a need to develop new regulators of leptin signaling that easily penetrate the BBB and do not provoke leptin resistance. Based on molecular models of leptin binding to its receptor, the C-terminal region of leptin molecule responsible for the regulatory effects of leptin was identified. We synthesized two lipophilic derivatives of the leptin fragment 116-122 (Ser-Cys-Ser-Leu-Pro-Gln-Thr) and studied their biological activity in the in vitro and in vivo. Synthesis of the peptide 116-122 was carried out using the BOC/Bzl strategy and *Nα-tert*-butyloxycarbonyl-protected amino acids. The peptide 116-122 was modified with the myristoyl or palmitoyl groups at the N-terminus by the treatment of N-terminal serine residue with the activated esters of myristic or palmitic acid. In the primary cultured hypothalamic neurons, both lipidated peptides increased the Ser^{473}-phosphorylation of Akt-kinase and Tyr^{705}-phosphorylation of the transcription factor STAT3, the key effector components of the leptin signaling, but the myristoylated peptide was more soluble and therefore used in the in vivo experiments. Long-term intranasal administration of the peptide Myr-116-122 (9 days, a daily dose of 100 μg/rat) to male Wistar rats with diet-induced obesity significantly reduced the food intake, which resulted in a decrease in the abdominal and epididymal fat mass. The peptide treatment led to normalization of the glucose and insulin levels and to improvement of glucose and insulin sensitivity. Thus, the myristoylated leptin fragment 116-122 activates the leptin signaling in hypothalamic neurons and its intranasal administration to obese rats has the hypophagic and metabolism-improving effects, which indicates the prospects for the use of lipidated fragments of leptin to treat metabolic diseases.

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Orphan receptor tyrosine kinase of the insulin receptor family takes shape: structural study by small-angle X-ray scattering and AFM

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Insulin receptor-related receptor (IRR), an orphan receptor tyrosine kinase of the insulin receptor family, attracts the attention due to its unusual properties. Since its discovery in 1989, no endogenous ligands for IRR have been identified despite significant efforts that included the genome analysis. Activation of IRR can be achieved by increasing the extracellular pH value and finally postulated that IRR is an alkali sensor that functions in the kidney as an extracellular pH detector. The insulin receptor, as well as the closely related insulin-like growth factor 1 receptor (IGF-1R), and IRR consist of two covalently coupled monomers and, thus, require a specific mechanism for signal transduction across the plasma membrane. Since the activation of the IRR is determined by its ectodomain, the isolation and study of the structure of the ectodomain IRR is fundamental basis of the IRR alkali sensing mechanism. This is the first report with 3D model of IRR extracellular part under neutral and alkaline pH by two complementary methods of structural biology: small-angle X-ray scattering and atomic force microscopy.
We show that the shape of ectodomain is completely different than the published three-dimensional structure of the IR ectodomain, which is a lambda shaped symmetrical head-to-tail complex of two disulphide-linked monomers. Unexpectedly, our experimental scattering curves of IRR ectodomain under pH 7.4 and pH 9.0 is almost identical. It can be interpreted that the alkalinization of the medium and activation of the receptor are accompanied by local rearrangements and rotations inside macromolecule structure that do not alter its overall shape.

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PECVD grown SiO_x based memristor devices

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Since traditional "charge"-based non-volatile memory (Flash) will face the potential scaling challenge below the 10 nm node with reliability and power consumption issues, there is a huge drive toward developing alternative concepts. Among those the resistive switching (RS) memory, or resistive random access memory (ReRAM), is one of the promising candidates. RS-based memory may be integrated with the processors on the same chip that would greatly increase the speed and decrease the power consumption, and potentially can replace both dynamic RAM and flash memory. Furthermore, RS devices represent the implementation of the so called memristors (resistor with memory), which can be used to design combined computing circuits thus enabling not only to store and process information on the same physical platform, but also to allow massively parallel computations in a simple crossbar array architecture.
Alternatively, neuro- and synaptic electronics is an interesting application for ReRAM that aim to build artificial synaptic devices emulating the computations performed by biological synapses. These emerging field of research potentially has better efficiency in solving specific problems and outperform real-time processing of unstructured data than conventional von Neumann computational systems.
There have been numerous studies of different materials exhibiting reversible resistance switching characteristics for synapse-like electronic device development. But many of them are difficult to integrate with complementary metal-oxide semiconductor (CMOS) fabrication. Silicon oxide (SiO_x) has long been used as gate dielectrics for MOS field-effect transistors in CMOS technology, and it is appealing to use it as a functional material for memristor devices [1].
In this work, SiO_x layers were grown by plasma-enhanced chemical vapor deposition (PECVD) technique. We present the detailed study of the complex correlation between the stoichiometry of the film (which includes Si, O, N, H) and the functional properties of RS devices based on Pt/SiONH/TiN structures. We establish the actual elemental and chemical composition of such "SiO_x" based layers using the combination of techniques (RBS/ERDA, XPS, FTIR) and identify the critical role of both N and H on the electrical properties. Although the functional properties of PECVD "SiO_x" based RS memory devices are found to be satisfactory when fabricated at optimized parameters ($V_{form} \leq 5V$, $V_{SET/RESET} = -1.2/+1.4V$, $>10^5$ switching cycles at $t_p = 100$ ns for devices 5 μm in diameter), we conclude that the richness of the composition in this class of materials would hinder their integration with standard CMOS technology due to difficulty in controlling exact stoichiometry affecting device performance.
1.Hsieh C.C. et al. Review of Recent progress on Neural Electronics and Memcomputing Applications in Intrinsic SiO_x-Based resistive Switching Memory. Chapter 11 from the book *Memristor and Memristive Neural Networks*, INTECH 2018. https://doi.org/10.5772/intechopen.68530

High-yield expression of eukaryotic light-driven pump from *L.maculans* in LEXSY and its spectroscopic characterization

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Leptosphaeria maculans is a fungal pathogen identified as a causal agent of blackleg disease, which is one of the most devastating diseases of *Brassica* crops worldwide [1]. In 2005, the *L.maculans* genome was found to carry a bacteriorhodopsin-like protein gene, which is the first microbial rhodopsin found in fungi. The *Leptosphaeria* rhodopsin (LR) was confirmed to covalently bind retinal chromophore and to have a light-driven proton transport activity, similar to bacteriorhodopsin [2]. Despite the interest to LR functional properties, there is no structural information available at the moment, which may be also important for optogenetic applications purposes as the LR has a eukaryotic origin. We demonstrate, for the first time, high-yield expression of the shortened (49-313) and full-length (1-313) constructs of LR in LEXSY expression system. Using optimized protocol with Ni-NTA and SEC purification steps, we were able to obtain the purified protein with high yield (3.5 g/L of culture) and peak ratio (A280/A540) of 1.4 on average. The purified protein shows BR-like photocycle kinetics during flash-photolysis experiments which in good agreement with previously reported results for protein expressed in *P. pastoris* [2,3]. Besides that, we demonstrate that LR expressed in LEXSY has proton pumping activity in liposomes, which confirms that expression in LEXSY allows production of the functionally active protein. Both shortened and full-length LR constructs were successfully crystallized in meso with needle-like crystals about 50–100 μm long that diffracted up to 2.5 Å, although dataset has poor quality.

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1.Robin, A. H. K. *et al.* Leptosphaeria maculans Alters Glucosinolate Profiles in Blackleg Disease–Resistant and -Susceptible Cabbage Lines. *Front. Plant Sci.* **8,** 1–15 (2017).
2.Waschuk, S. A., Bezerra, A. G., Shi, L. & Brown, L. S. Leptosphaeria rhodopsin: Bacteriorhodopsin-like proton pump from a eukaryote. *Proc. Natl. Acad. Sci.* **102,** 6879–6883 (2005).
3.Legkun, G., Okhrimenko, I., Chupin, V. & Gordeliy, V. Membrane protein targeting into mitochondria. *Biochim. Biophys. Acta - Bioenerg.* **1857,** e125 (2016).

Ca^{2+} uptake and permeability transition in heart and skeletal muscle mitochondria: a comparative study

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Mitochondria of various organs and tissues of animals in the energized state are able to accumulate high concentrations of Ca^{2+} from the cytoplasm. The absorption of Ca^{2+} by mitochondria plays an important role in regulating energy metabolism of the cell [1]. Achieving Ca^{2+} concentration in the matrix above a certain limiting level results in the release of these ions from the mitochondria. The maximal release of Ca^{2+} from the matrix of these organelles is observed upon induction of the mitochondrial pore (MPTP) [1,2]. At present, the participation of Ca^{2+} transport systems and mitochondrial pore in the development of cardiovascular diseases, neurodegenerative processes, muscular dystrophies, neurodegenerative processes, etc. has been shown [1].

In this work, we performed a comparative study of the kinetics of Ca^{2+} uptake and induction of MPTP in mitochondria of the heart and skeletal muscles of Wistar rats. It was found that heart mitochondria are characterized by a higher rate of Ca^{2+} uniport compared to skeletal muscle mitochondria. It is assumed that the observed differences in calcium uptake rate are due to the different ratio of the regulatory and channel subunits of mitochondrial calcium uniporter (MCU) [3]. The Ca^{2+} uptake by organelles is accompanied by the generation of H_2O_2, in this case the production rate of H_2O_2 in mitochondria of skeletal muscles is lower compared to heart mitochondria. It was found that heart mitochondria are characterized by more than twice the calcium retention capacity compared to skeletal muscle mitochondria. The MPTP inhibitor CsA increased the calcium capacity of the organelles, however in this case the capacity of skeletal muscle mitochondria was almost five times lower than that for the heart mitochondria. We assume that the observed differences in effectiveness of MPTP induction may be due to different amounts and proportions of the main MPTP proteins (cyclophilin D and adenylate translocator).

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1. Griffiths, E. & Rutter, G. Mitochondrial calcium as a key regulator of mitochondrial ATP production in mammalian cells. *Biochim. Biophys. Acta*. **1787**, 1324–1333 (2009).
2. Dubinin, M. *et al*. Induction of calcium-dependent nonspecific permeability of the inner membrane in liver mitochondria of mammals and birds: a comparative study. *Biologicheskie Membrany*. **32**, 328–337 (2015).
3. Murgia, M. & Rizzuto, R. Molecular diversity and pleiotropic role of the mitochondrial calcium uniporter. *Cell Calcium*. **58**, 11-17 (2015).

Structure modeling of the human double-pore GIP-complex

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Almost all mitochondrial proteins are encoded by nuclear genes, synthesized on cytosolic ribosomes and then imported across the outer membrane of mitochondria by molecular multisubunit machinery, termed the translocase of the outer membrane (TOM) complex [1]. TOM-complex is conventionally subdivided in two parts: stable, pore-making GIP (general import pore)-complex consisted of five subunits (TOM40, TOM22, TOM5, TOM6, and TOM7) and surface receptors for mitochondrial preproteins (TOM70, TOM20) characterized by dynamic association with other TOM components. A high-resolution atomistic structure of the entire assembly is currently absent.
In this study, we aim at obtaining the atomic-resolution structural model of the human GIP, a core part of the TOM-complex. The modeling of the atomistic 3D-structure of the human GIP-complex was carried out in a stepwise fashion where the first step was the structure modeling of individual TOM components using the combination of the I-TASSER protocol and MEMOIR algorithm followed by the refinement with the ModRefiner program. Next, the human TOM components were structurally superimposed onto the respective ones of the homological yeast GIP-complex which structural model has been obtained recently using integrative modeling platform [2]. In the third step, a three-staged computational docking protocol GalaxyRefineComplex – FlexPepDOCK – ROSETTADOCK was applied.

This strategy has resulted in a two-pore structural model of the human GIP-complex where two TOM22 proteins were bound with high affinity, caused by significant shape- and electrostatic complementarities, to two pore-forming TOM40 molecules.

1. Van der Bliek, A.M., Sedensky, M.M., Morgan, P.G. Cell biology of the mitochondrion. *Genetics (843-871)*. **207**. (2017).
2. Dudko, H.V. & Veresov, V.G. Double-pore structure model of yeast GIP-complex. *Proceedings of the International Conference "Molecular, Membrane and Cellular basis of Functioning of Biosystems", Minsk, Belarus (22)*. (2018).

Neurotrauma: Expression of signaling proteins in axotomized crayfish ganglia

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Neurotrauma leads to people disability and death, especially men. However, effective neuroprotectors capable of protecting neurons in the first hours after nerve damage are absent so far. We believe that modulation of signaling pathways that control cell death may exert neuroprotective action. To explore molecular mechanisms of axotomy-induced neurodegeneration, we used a novel model object: the axotomized abdominal ganglia of the crayfish ventral nerve cord (VNC). VNC contains six ganglia (500-1000 neurons each) connected by nerves. After transection of all connectives one can get six bilaterally axotomized ganglia. Control—the undamaged VNC. Using Western blot, we studied the difference in expression of various signaling proteins belonging to different cellular subsystems in the VNC ganglia after axotomy. We used antibodies against apoptosis executor caspase 3, histone deacetylase HDAC1 that epigenetically regulates protein biosynthesis, cytoskeleton-associated protein cofilin, and PINK1 and parkin that are involved in mitochondrial quality control. After axotomy the VNC ganglia and control VNC were incubated in physiological saline 1 or 3 h at room temperature. Then control and axotomized ganglia were homogenized on ice and proteins were extracted. Immunoblotting showed that the level of HDAC1 in VNC ganglia increased 1.5-fold as compared with the control level ($p < 0.05$) at 3 h (but not 1 h) after axotomy. This leads to suppression of the transcriptional activity and to inhibition of protein synthesis. We did not observe significant influence of axotomy on levels of caspase 3, cofilin, and PINK1 in VNC ganglia. Possibly, a 3-h interval was too short for inducing apoptosis. However, the expression of parkin increased almost 3 times at 1, but not 3 hours after the axotomy. Since parkin participates in mitophagy, one can assume that axotomy suppresses protein synthesis and stimulates mitophagy in the crayfish nervous system. The biochemistry of crayfish nerve cells is generally similar to that in mammals. So, one can suggest that nerve injury can inhibit protein synthesis and induce mitophagy in the mammalian nervous tissue.
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Virtual screening of novel ligands of cytochromes P450

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The most appropriate way to construct drugs with high efficiency but low side-effects is to test them towards the range of medically important molecular targets. The cytochrome P450 (CYPs) super family is responsible for different significant metabolic functions in human body, playing

crucial role in both endogenous and exogenous substrate metabolism [1]. That is why information about CYPs interaction with possible ligands is of a great importance for «smart» drug-design.
We created an automated protocol for virtual screening of possible CYPs ligands based on Autodock Vina software [2] and Python RDKit library. The tool was used for searching of novel ligands of CYPs among endogenous steroids derivatives – effective anticancer agents [3-4] – with modifications in A, B, C and/or D rings.
In our research we tested different cytochromes P450 with great clinical significance: CYP19, CYP17, CYP11A1, CYP51 etc. For in silico experiments, we used model—presented in the Protein Data Bank.
Using our tool allowed us to pick out "essential" CYPs' ligands from a group of molecules. Protocol application for virtual screening of sterol derivatives showed that there are 17-substituted androstanes, androstane D-lactones and estrane derivatives which bind with high efficiency in the active site of CYPs.
In vitro screening of the ligands founds and identification of enzymatic reactions products are the aims of our further work.

1. Dejong, A. *et al.* The Cytochrome P450 Superfamily Complement (CYPome) in the Annelid Capitella teleta. *PLoS One.* **9(11),** (2014).
2. Trott, O. *et al.* AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput Chem.* **31(2),** (2010).
3. Jovanović-Šanta, S. *et al.* Antihormonal potential of selected D-homo and D-seco estratriene derivatives. *Steroids*. **97,** (2015).
4. Ajduković, J. *et al.* 17 (E)-Picolinylidene androstane derivatives as potential inhibitors of prostate cancer cell growth: Antiproliferative activity and molecular docking studies. *Bioorg. Med. Chem.* **21(23),** (2013).

Effect of homocysteine and its derivatives on oxidative stress in rat pituitary GH3 cells

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Endogenous thiols are a group of compounds that contain SH groups and provide a redox balance of cells. Changes in their concentrations lead to various abnormalities in the body systems. Hyper-homocysteinemia (HHcy) is a disease due to elevated homocysteine plasma levels [1]. HHcy is a well-known risk factor for cardio-vascular diseases, neurodegenerative disorders, common pregnancy complications, etc [2]. Oxidative stress is one of the mechanisms of homocysteine action, however in the body tissues homocysteine rapidly oxidized to homocystine and homocysteine thiolactone [1]. The aim of the study was detection of the effect of homocysteine and its derivatives—homocystine and homocysteine thiolactone on oxidative stress in GH3 rat pituitary cells, which secreting growth hormone and prolactin using fluorescent dye DCF.
The experiments were performed on the GH3 culture of rat pituitary cells. Analysis of active oxygen forms was performed using a DCF fluorescent dye (the cells were incubated in dye-containing solution for 20 min). In control conditions the dye luminescence was observed, which is associated with oxidation of DCF with endogenous hydrogen peroxide (H2O2). To analyze the effect of homocysteine (300 μM) and its derivatives—homocystine (300 μM) and homocysteine thiolactone (300 μM) on the level of oxidative stress, cells were incubated in substance containing medium for 20 min or 24 h (acute or chronic incubation, respectively). All dye luminescence intensity values in these cases (after incubation with substance) were significantly higher than in the control conditions.
It was shown that not only homocysteine but also its metabolites - homocystine and homocysteine thiolactone, cause an increase in the production of active forms of oxygen by both acute and chronic incubation, which leads to the development of oxidative stress. In GH3 cells, it can lead to a decrease in the secretion of growth hormone, and as a result to disorders of growth and development in prenatal hyperhomocysteinemia.
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1. Ansari, R., Mahta, A., Mallack, E. & Luo, J. Hyperhomocysteinemia and Neurologic Disorders : a Review. *J Clin Neurol* **10,** 281–288 (2014).
2. Gaifullina, A. S. *et al.* Homocysteine augments BK channel activity and decreases exocytosis of secretory granules in rat GH3 cells. *FEBS Lett.* **590,** 3375–3384 (2016).

A new approach for the synthesis of biologically important nucleotides using a thermostable multi-enzymatic cascade

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We propose a new approach for conversion of D-pentoses into biologically important purine nucleotides using enzymes from thermophilic microorganisms: ribokinase and phosphoribosylpyrophosphate synthetase (PRPPS) were used for the conversion of *D*-pentoses into 5-phosphates that were further converted into 5-phospho-α-*D*-pentofuranose 1-pyrophosphates, adenine phosphoribosyltransferase (APRT) catalyzed the condensation of the pyrophosphates with adenine and its derivatives. The recombinant enzymes were produced using the *E. coli* expression system and their substrate specificity towards different heterocyclic bases and activity dependence on different factors were investigated.
Crystallization of the enzymes was performed using the capillary counter-diffusion technique. 3D structure of the ribokinase was solved at 2.87 Å resolution. Structure of the APRT and complex PRPPS/ADP/SO_4^{2-} were solved and deposited into RCSB Protein Data Bank with PDB entries 6fsp (at 2.6 Å resolution) and 5t3o (at 2.2 Å resolution), respectively. The determination of the structure for enzymes complexed with substrate can play a critical role in understanding the reasons behind its narrow substrate specificity. In silico modelling of protein-ligand interaction by molecular docking via simulations of molecular dynamics was performed for complex APRT/PRPP/Mg^{2+} and PRPPS/ATP/ribose-5-phosphate. Crystallization serves as the basis for the rational search for mutant forms of the enzyme with desired properties, including those having broader substrate specificity, which is of great interest for biotechnological applications.
The work was supported by the Russian Science Foundation (project no.14-50-00131).

1. Esipov, R.S. *et al.* A cascade of thermophilic enzymes as an approach to the synthesis of modified nucleotides. *Acta Naturae* (*160–168*). **8**, (2016).
2. Sinitsyna, E.V. *et al.* Crystallization and preliminary x-ray diffraction study of recombinant adenine phosphoribosyltransferase from the thermophilic bacterium *Thermus thermophilus s*train HB27. *Crystallogr. Rep*. *(580–583)*. **62**, (2017).
3. Abramchik Y.A. *et al.* Crystallization and preliminary X-Ray diffraction study of recombinant ribokinase from *Thermus Species* 2.9. *Crystallogr. Rep. (976–980)*. **61**, (2016).
4. Timofeev V.I. *et al.* Crystal structure of recombinant phosphoribosylpyrophosphate synthetase 2 from *Thermus thermophilus* HB27 complexed with ADP and sulfate ions. *Acta Crystallogr F Struct Biol Commun.* (369-375). **73**, (2017).

Potential protein factors for cross-talking between mycoplasmas and environment

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Mollicutes or mycoplasmas are a specialized clade of Gram-positive bacteria that lack a cell wall and have significant genome reduction. Most known Mollicutes are parasites, and some are saprophytes. Mycoplasma infections can lead to acute inflammatory episodes, but most are chronic, suggesting that these bacteria have developed strategies to avoid host`s immune system response. *Mycoplasma gallisepticum* is a cause agent of avian chronic respiratory disease. It is known that *M. gallisepticum* is a parietal parasite and can infect eukaryotic cells. Possible ways of interaction between pathogen and host are through membrane, secreted, surface-associated molecules or vesicles. In this work, we focused on description, analysis of secreted, surface-associated, and membrane proteins of *Mycoplasma gallisepticum* using mass spectrometry approach.
We compared protein abundance in all localization fractions with abundance the same proteins in total proteome samples. Proteins that were statistically significantly (p value < 0.05) enriched in secreted, surface-associated and membrane fractions were accepted like positive results. There were 24, 20, and 173 proteins, respectively. All secreted proteins have predicted signal peptide and identified peptides do not cover N-terminus of proteins. Using conserved domain analysis, secreted proteins were divided into four groups: immunoglobulin-binding proteins, serine proteases (family S8) and two groups of proteins with uncharacterized function. Some genes that code proteins with uncharacterized function are clustered in operons. There were only three groups among surface-associated proteins: variable lipoprotein family proteins (VlhA), putative peptidases (DUF31) and proteins with uncharacterized function. Abounded proteins in membrane fraction were various. We confirmed that identified proteins were secreted using mMaple fluorescent fusion proteins system. These results can help to elucidate molecular mechanisms both of infection and escape host's immune system response to mycoplasmas.
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The utility of sulfonamide group in the design of bifunctional neuraminidase inhibitors

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Neuraminidase (NA) is a membrane protein of influenza virus that plays an essential role in release of progeny virions from host cell surface and constitutes an important target for treatment. Frequent NA mutations confer resistance to its inhibitors (zanamivir, oseltamivir) and make it urgent to develop new medicines [1]. Detection of a so-called 430-cavity nearby the active site of NA provided an opportunity to develop bifunctional inhibitors which could simultaneously interact with the active site and the abovementioned cavity. A crucial factor for the inhibitor's binding in the NA active site is an interaction with the arginine triad. The required linker of a bifunctional inhibitor should mediate this interaction and provide a stable chemical bond between functional fragments. In this work, the utility of a sulfonamide-based linkage and its conformational properties have been studied by molecular modeling.
Sulfonamides from ZINC database were used in molecular docking experiments with the Lead Finder software [2]. Compounds capable of forming hydrogen bonds between the -SO_2-N= group and the arginine triad were selected for further analysis. It was found that the sulfonamide-based linker could interact optimally at the boundary between the active site and the 430-cavity, allowing functional fragments of diverse structure to occupy these important binding sites. These results demonstrate that proposed linker may be a universal component to construct bifunctional inhibitors out of various structural fragments. To test the hypothesis, several models of zanamivir derivatives containing -SO_2-NH-(CH_2)n-linker were built, in which zanamivir (first functional fragment) was localized in the NA active site while the second, hydrophobic, fragment is localized in the 430-cavity.
Molecular modeling has shown that sulfonamide linkers may be successfully used for the design of bifunctional NA inhibitors targeted towards both the active site and the 430-cavity.
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1. Hussain M. *et al*. Drug resistance in influenza A virus: the epidemiology and management. *Infect. Drug Resist.(121-134).* **10**, (2017).
2. Stroganov O.V. *et al.* Lead finder: an approach to improve accuracy of protein-ligand docking, binding energy estimation, and virtual screening. *J. Chem. Inf. (2371-2385).* **48**, (2008).

Blue light causes the activation of apoptotic genes in the pigment epithelium of the human retina

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Age-related macular degeneration (AMD) is the third cause of vision loss after cataract and glaucoma in the world. AMD is a complex multifactorial disease with demographic, genetic and environmental risks. The reasons for this are the gradual process of aging of the body and the eyes in particular, trauma, infectious, inflammatory eye diseases, high myopia, hereditary causes, and transferred ophthalmic operations. All of the above causes the relevance of the investigation of the mechanisms of the action of light of various spectra on cells, tissues and the organism as a whole, and the definition of safe exposure regimes that provide treatment for various types of diseases, taking into account the individual recovery capabilities of the organism.
Pigment epithelium performs a huge number of functions. Dysfunction or developing dystrophic processes in the pigment epithelium may subsequently lead to severe results.
The greatest changes in the transcriptional activity of the investigated genes in the cells of the pigment epithelium were observed after their irradiation with blue light for 10 min, especially the genes-markers of oxidative stress and genes-markers of necrosis. So an hour after irradiation, the concentration of mRNA of genes involved in the development of oxidative stress of GPX2, CYBB, DUOX1, DUOX2, NOX5, and HMOX1 increased. An increase in the expression of the gene-markers of necrosis—FOXI1 and RAB25—was also observed. If we look at the dynamics of the response to blue light, we observed a decrease in the activity of the cell death markers, however, the markers of oxidative stress appeared to be elevated on the third day after irradiation. For example, for the genes GPX5, PTGS1 and APOE, a constant elevated level of transcription was observed. Interestingly, on the third day, the mRNA concentration increased for a large number of genes involved in the development of oxidative stress, in particular for the genes GPX2, GSTP1, DUOX2, PTGS1, APOE, MT3, SOD3, ALOX12, NOX4, UCP2, BNIP3, DHCR24, HSPA1A , SIRT2, HSP90AA1, POLRMT, TFAM. The study was carried out with the financial support of the RFBR and the Government of the Moscow Region within the framework of the scientific project No. 17-44-500740 and with the support of the president's grant MK-1880.2017.7.

Study of the patch-clamp electrophysiological properties of cardiomyocytes derived from patient-specific iPSCs

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It is known that the basis of the majority of cardiac tachyarrhythmias are circulating excitation wavelength, known as reentri [1] Since our experimental model was cardiomyocytes and monolayers of cardiomyocytes obtained by the method of cell reprogramming, it was necessary to study their main characteristics and to compare the characteristics of cells carrying a mutation along HERG channels with cells from a healthy individual. The patch-clamp data confirmed that the formation of functional ventricular cardiomyocytes of the ISMA6L line (health line) begins on the twelfth day after the onset of differentiation. The shape corresponds to the ventricular action potential shortened duration on the 12th day, and further it tends to increase up to 400 ms by day 28 and then remains unchanged. The action potential duration is short in cardiomyocytes derived from if31-5 line with a mutation in IKr and then reaches stable duration (more than 600 ms) by the 27th day of differentiation. The remaining ion currents, such as INa, ICaL-type, were fixed already in the early stages of differentiation (on the 14th day).

1. Antzelevitch C (2001) Basic mechanisms of reentrant arrhythmias. Current Opinion in Cardiology 16: 1-7.
2. Davidenko JM, Pertsov AM, Baxter WT, Salomonsz R, Cabo C, et al. (1993) High-resolution mapping of action-potential duration gradients in isolated sheep epicardial muscle. Circulation 88: 327-327.

Functional adenine nucleotide translocator is required for maintenance of mitochondrial network in yeast

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Mitochondrial network is dynamically adjusted to the metabolic state of the cell. In particular, the length of mitochondrial filaments is determined by the balance of mitochondrial membranes fission and fusion, which is sensitive to NTP levels and mitochondrial transmembrane potential. However, in a model organism—baker's yeast *Saccharomyces cerevisiae*—the depletion of mitochondrial DNA (mtDNA) exerts only a mild effect on mitochondrial network structure. This is surprising because mtDNA encodes core proteins of respiratory chain and ATP-synthase. Moreover, mtDNA-minus (*rho0*) cells are unable to respire and have low mitochondrial transmembrane potential. We speculated that some nuclear-encoded mitochondrial transporters play a key role in the shaping of mitochondrial network. We produced a yeast strain with down-regulated major ATP/ADP antiporter, Pet9p. Mitochondria isolated from this strain showed negligible adenine translocator (ANT) activity. We found, that in contrast to *rho0* cells, cells with suppressed ANT activity had abnormal mitochondrial structure under respiratory conditions. In particular, significant fraction of the cells showed aggregated and hyperfused morphology of mitochondrial network. Surprisingly, the repression of *PET9* did not significantly affect mitochondrial fusion rate in yeast zygotes. Furthermore, we did not find any inhibition of mitochondrial fission in this strain. At the same time, segregation of mitochondria in ANT-deficient yeast cells was strongly biased towards accumulation of mitochondria in the mother cells. In budding yeast mitochondrial quality control relies on segregation of functional and damaged mitochondria between daughter and mother cells, respectively. Thus, we suggest that adenine nucleotide translocator can be a key factor determining the segregation of functional/dysfunctional mitochondria between the dividing cells. Our results suggest that mitochondrial inner membrane transporters can contribute to coupling of mitochondrial network morphology to ATP production in the mitochondrial matrix.

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Light-induced structure changes in Bacteriorhodopsin D96N by SAXS

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The results of SAXS measurements of changes of gyration radius of bacteriorhodopsin bR D96N containing purple membranes (PMs) during light-induced M-state were compared with its ground state. SAXS measurements were done on the X-ray instrument Rigaku, MIPT, Dolgoprudny, Russia [1] and on the ESRF facility, BM29 beamline, Grenoble, France [2].
Previous researches had shown tertiary structure changes during bR M-state [3] and probably radii gyration changes. However, it was still unclear if these radii changes were the pure effect of light exposure or caused by chemical M-state binding. For checking this hypothesis, bR mutant D96N with extremely elongated M-state was used instead of binding chemicals. In addition, it was observed how a state of aggregation of PMs influences light-induced changes.
In this study, purple membranes were observed in aggregated and non-aggregated states and under different pH conditions. It was found that the strongest changes could be observed at alkaline pH values higher than 8.5. For non-aggregated PMs, there were no changes of the gyration radius. This fact approves the hypothesis that previously measured in literature radii gyration changes were caused by binding chemicals. For aggregated PMs the light-induced changes at lower Q-range of scattering curves were noticed. The gyration radii systematical changes were found about 2 Å. This indirectly approves the hypothesis of light-induced structural changes of large PM aggregates, but the mechanisms of the effect are being discussed.

1. Murugova, T. N., et al. "Low resolution structural studies of apoferritin via SANS and SAXS: the effect of concentration." *Journal of optoelectronics and advanced materials* 17.9-10 (2015): 1397-1402.
2. Pernot, Petra, et al. "Upgraded ESRF BM29 beamline for SAXS on macromolecules in solution." *Journal of synchrotron radiation* 20.4 (2013): 660-664.
3. Sass, H. J., et al. "The tertiary structural changes in bacteriorhodopsin occur between M states: X-ray diffraction and Fourier transform infrared spectroscopy." *The EMBO journal* 16.7 (1997): 1484-1491.
4. Zimányi, László, et al. "Singular value decomposition with self-modeling applied to determine bacteriorhodopsin intermediate spectra: analysis of simulated data." *Proceedings of the National Academy of Sciences* 96.8 (1999): 4408-4413.

The prenatal hyperhomocysteinemia promoted high sensitivity to 4-aminopyridine -induced epileptiform activity in hippocampal slices of the rat

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Homocysteine, a thiol-containing amino acid derived from dietary methionine through demethylation. The breakage of metabolism due to genetic alteration in metabolic enzymes or deficiency in cofactors may lead to hyperhomocysteinemia [1]. Abnormal accumulation of homocysteine during pregnancy induces learning deficits in offspring at early postnatal development [2]. It was shown, that HHCy can contribute to seizures in patients with Down syndrome, depression and in alcohol withdrawal [1,3,4]. The goal of this study was to estimate the sensitivity of hippocampal slices of newborn rats with prenatal hyperhomocysteinemia (pHHCy) to 4- aminopyridine–induced seizure-likes events (SLE). Experiments were performed on slices of Wistar rats during second and third postnatal weeks (P9-19) using extracellular field electrodes in the CA3 pyramidal cell layer of hippocampus. To determinate the threshold of 4-aminopiridine for generation of SLE the convulsant was added by increasing doses. Pups with pHHCy were born from females received daily methionine with food. In control group application of 15-35 μM 4- aminopyridine induced a gradual increase of the frequency of multiunit activity of hippocampus neurons. In concentration of 50–75 μM 4-aminopyridine induced SLE in 75% cases ($n = 15$) after 4.3±0.9 min of perfusion. In slices prepared from the hippocampus of rats with pHHCy the elevation of background neuronal firing was observed and application of 15–35 μM 4-aminopyridine induced SLE in 88% of the cases with 6.0 ±0.6 min of seizure onset ($n = 26$). Our findings indicate that pHHCy significantly lowers the threshold of 4-aminopyridine–induced SLE. It is known that homocysteine and its metabolites are potent agonists of NMDA-receptor, which are linked with epileptogenesis. It is possible that pHHCy can induce the hyperexcitability of neuronal network of immature hippocampus by stimulating NMDA-receptors and changing the electrophysiology property of neurons. This work was supported by RFFI no. 18-015-00423.

1. Ansari, R. *et al.* Hyperhomocysteinemia and Neurologic Disorders: a Review. *J Clin Neurol.***10**, 281-288 (2014).
2. Baydas, G. *et al.* Effects of maternal hyperhomocysteinemia induced by high methionine diet on the learning and memory performance in offspring. *Int. J Dev. Neurosci.* **25** 133-139 (2007).
1. Brustolin, S et al. Genetics of homocysteine metabolism and associated disorders. *Braz J Med Biol Res.* **43**, 1–7 (2010).
2. Bleich, S et al. Plasma homocysteine is a predictor of alcohol withdrawal seizures. *Neuroreport.* **21**;2749-52 (2000).

Identification of novel trace amine-associated receptor-1 ligands among clinically used drugs

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Trace amine-associated receptor-1 (TAAR1) belongs to G protein-coupled receptors superfamily and responds to trace amines such as β-phenylethylamine (β-PEA), tyramine, and octopamine. It represents an attractive potential mediator of certain aspects of movement, cognitive, and emotional control and thus can serve as potential molecular target for neuropsychiatric disorders such as schizophrenia and depression [1]. Investigation of TAAR1 requires development of powerful ligand tools for specific and biased modulation of their functions in vivo. For this purpose, we aimed to identify effective and potent ligands of TAAR1 including agonists (full/partial) and antagonists for biological and pharmacological applications.
For this purpose, we have used a method based on bioluminescence resonance energy transfer technology (BRET) described in previous work [2]. Transfections were carried out by Lipofectamine 2000 according to standard protocol. HEK293T cells were transfected with hTAAR1 and EPAC biosensor cDNA and were split into 96-well plates at 10^5 cells per well. On the following day, 70 ul of PBS was added to each well followed by addition of 10 ul of a 50 uM coelenterazine-h solution and 10 ul of a 2 mM IBMX solution. After 10-min incubation, either 10 ul of vehicle or 10 ul of compound at concentration 10 uM in PBS was added, and the plate was then placed into a Mithras LB943 reader (Berthold Technologies, Bad Wildbad, Germany) by using special BRET filter pair (480 nm—coelenterazin-h and 530 nm—YFP). The BRET signal is determined by calculating the ratio of the light emitted at 530 nm to the light emitted at 480 nm. For EC_{50} evaluation, dose-response curves were analyzed.
As the first step, we have analyzed several commercially available compound libraries including FDA-approved drug library. Several full and partial agonists of human TAAR1 have been identified. Most of them belong to alpha2-adrenergic receptor ligands, such as naphazoline (EC_{50} 0.6 nM), guanfacine (EC_{50} 20 nM), and guanabenz (EC_{50} 10 nM). Also, we have identified some psychoactive drugs acting through TAAR1 signaling pathway such as maprotiline (EC_{50} 450 nM) and clonidine (EC_{50} 80 nM). Thus, TAAR1 activity may contribute to some therapeutic and/or aversive effects of these prescription drugs and the obtained results shed new light on molecular mechanisms of drug activity.

1. Sotnikova, T. *et al*. Trace amine-associated receptors as emerging therapeutic targets. *Mol Pharmacol (229-35)*. **76(2)**, (2015).
2. Espinoza, S. *et al.* BRET approaches to characterize dopamine and TAAR1 receptor pharmacology and signaling. *Methods Mol Biol. (107-22).* **964,** (2013).

Resonance Raman spectroscopy to investigate changes in cytochromes under inflammation and metabolic disorders

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Cytochromes are key components of mitochondrial electron transport chain (ETC). Redox state, conformation, and number of cytochrome hemes are related to mitochondrial functional state. Pathology-driven disturbances in electron transfer at the level of any cytochrome lead to increased formation of reactive oxygen species (ROS), ETC uncoupling, and disruption of ATP synthesis, which in turn can provoke apoptosis. Raman spectroscopy (RS) allows label-free noninvasive determining conformation and redox state of cytochromes. Earlier, we showed an increased amount of reduced B-type cytochromes in skeletal myocytes from patients with obesity and metabolic disorders [1]. This indicated an overload of ETC with electrons due to an excess of NADH, FADH2, and overreduced quinone pool, which can lead to increased generation of ROS.
Large amount of proinflammatory cytokine, tumor necrosis factor (TNFα), is known to be produced in adipose and muscle tissue in the case of obesity, which stimulates the inflammatory process and is considered to be a key link in the development of insulin resistance. Therefore, we hypothesized that the effect of pure TNFα on mitochondria in cells will be similar to that observed in obesity.
We investigated the effects of two different proinflammatory cytokines: TNFα and interferon (IFNγ) on the cell line (oral mucosal progenitor cells, OML-PC) with RS. Laser wavelength 532 nm allows to obtain resonance RS from B-type and C-type cytochromes, but not from cytochromes A. We demonstrated that both cytokines caused inflammation and leaded to oxidative stress. However, they had a completely different effect on mitochondrial ETC and lipid content. Effects of TNFα were very similar to those observed in skeletal myocytes in obesity. Increased reduction of cytochrome B without any changes in cytochrome C may be explained by the increase in electron flow to quinone pool due to metabolic shift towards fatty acid catabolism observed under these pathological conditions.

We propose a noninvasive label-free approach to investigate alterations in mitochondrial cytochromes under inflammation in cell lines using Raman spectroscopy. This approach can be used to study inflammation and other age-related diseases in model systems. Authors acknowledge financial support from RFBR (grant mol_a 18-34-00503\18).

1. Brazhe N.A. et al. Raman probing of lipids, proteins, and mitochondria in skeletal myocytes: a case study on obesity. *J Raman Spec.* 48(9), (2017)

Studies of the sensory domain of histidine kinase QseC

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The gastrointestinal tract is one of the most complex heterogeneous environments in mammals, where most of microbial flora is located in the large intestine [1]. This microbial flora is necessary for a mammal to correctly assimilate nutrients [2]. Among bacteria, there may be pathogens, whose interaction with host can be a cause of various diseases. Due to the high density and diversity of bacteria in the gastrointestinal tract, they can interact with each other and with the host through various signaling systems [3].
One of such systems is a quorum-sensitive signaling system that includes two proteins: QseC and QseB. In this two-component system, QseC is a bacterial adrenergic receptor that activates virulence gene transcription through QseB. QseC is able to interact with adrenalin and noradrenalin and also with bacterial autoinducer 3 (AI-3) [4]. Elucidating the role of this two-component system in bacterial virulence and characterization of its signaling mechanism can contribute to development of new antibiotics.
Here, we studied the sensory domain of QseC. Four different genetic constructs have been prepared: two containing only the QseC sensor domain and two containing a fusion of QseC sensor domain and a fluorescent LOV domain. We determined the stabilizing buffer components and showed that membrane-proximal charged α-helix fragments are important for protein stability. We also showed that fluorescent chimeric constructs can be used to track protein expression and purification as well as its interaction with ligands.

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1. Berg, R. D. The indigenous gastrointestinal microflora. *Trends Microbiol.* **4,** 430–435 (1996).
2. Hooper, L. V. Commensal Host-Bacterial Relationships in the Gut. *Science.* **292,** 1115–1118 (2001).
3. Krell, T. *et al.* Bacterial Sensor Kinases: Diversity in the Recognition of Environmental Signals. *Annu. Rev. Microbiol.* **64,** 539–559 (2010).
4. Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C. & Sperandio, V. The QseC sensor kinase: A bacterial adrenergic receptor. *Proc. Natl. Acad. Sci.* **103,** 10420–10425 (2006).

Closer look at unbinding transition of phosphatidylcholine membranes induced by calcium ions

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Calcium ions occupy a special place in biological processes, regulating the most important physiological and biochemical processes in an organism [1]. The presence of calcium ions with low concentration in the solution causes a transition of multilamellar vesicles (MLVs) of phosphatidylcholine membranes from the "bound" to "unbound" state—unbinding transition—and the spontaneous formation of unilamellar vesicles (ULVs) [2]. The complex analysis of unbinding transition of 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC) multilamellar vesicles (MLVs) was done for calcium ion concentration range of 0–1.0 mM by small-angle neutron scattering (SANS), densitometry, dynamic light scattering (DLS), and atomic force microscopy (AFM).
The critical Ca^{2+} ion concentrations for DMPC (10 mg/ml) in $CaCl_2 \cdot 2H_2O/D_2O$ solvent were determined from the SANS data as 0.33(3) mM in $P_{\beta'}$ and 0.37(2) mM in L_α phases during heating; 0.56(3) mM in $L_{\beta'}$ and L_α phases during cooling. However, a local decrease in the volume of DMPC molecules occurs before the unbinding transition: 1035 Å^3 at $C_{Ca^{2+}}$ = 0.25 mM ($L_{\beta'}$, 10 °C) and 1120 Å^3 at $C_{Ca^{2+}}$ = 0.2 mM (L_α, 55 °C). Spontaneous formation of ULVs from MLVs was observed by SANS at $C_{Ca^{2+}}$ = 0.3 mM in both phases with the bilayer thickness of 4.12(3) nm and 3.64(4) nm in $P_{\beta'}$ and L_α phases, respectively. These values are in good agreement with bilayer thicknesses of the MLVs of DMPC in D_2O, obtained by passing a solution through a polycarbonate membrane with pores of 100 nm. The decrease in the area of the DMPC molecule in the formed system confirms the fact that the binding of the Ca^{2+} ions to oxygen from PO_4^- moieties of the lipid headgroup leads to reorientation of the P^-–N^+ dipole into the region of the intermembrane space.
In addition, not all MLVs are converted to ULVs, which was observed using DLS and AFM methods. Thus, size distribution of DMPC MLVs (1 mg/ml) by intensity has a bimodal form at $C_{Ca^{2+}} \geq 0.4$ mM ($L_{\beta'}$) and at $C_{Ca^{2+}} \geq 0.6$ mM (L_α). Peak positions were calculated as 66.5 nm (STD = 8 nm) and 553.0 nm (STD = 181 nm) at 20 °C; 80.9 nm (STD = 17 nm) and 497.2 nm (STD = 198 nm) at 55 °C.

1. Gennis, R. B. Biomembranes: molecular structure and function. Springer-Verlag New York Inc., New York (1989)
2. Gorshkova, Yu.E. et al. Structure and phase transitions of DMPC multilamellar vesicles in the presence of Ca^{2+} ions. Journal of Surface Investigation **11(1)** (2017). https://doi.org/10.1134/S1027451016050499

Membrane mimetic materials in protocell design and modeling

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Both structural and functional protocell models require compartmentalization of the reaction medium provided by membraneous or membrane-mimetic structures, which are necessary for reaction-diffusion processes, and hence, morphogenesis of the protocell ultrastructure. Formation of the complex membranous structures differing from the minimal surfaces requires a non-equilibrium state of the multiphase microheterogeneous system. Since the diffusion transport during morphogenesis within the cellular membrane-bounded microreactors is mostly a physical phenomenon and is not genetically controlled, it is possible to perform biomembrane and biomorphogenesis modeling using different membrane-mimetic systems, regardless of their chemical nature. The Turing model of morphogenesis describes the interactions between an abstract activator and inhibitor, rather than of specific biomacromolecules. The only requirement here is the possibility of the physical (in particular, rheological) modeling in the framework

of the similarity theory and the dynamical analogy principles. Formation of the biomorphic structures in membrane-mimetic systems (including protocells) can be controlled and determined by the similarity of the processes resulting from the physical and chemical membrane activity independent from its chemical composition. According to the basic physical and chemical principles, such membrane-mimetic systems can demonstrate a number of emergent complex properties, characteristic for biomembranes, such as charge and ion separation, selective transmembrane ion transport, maintenance of the concentration gradients and ion concentration oscillations, electrochemical potential generation (including photoinduced membrane potentials in the presence of photocatalytic centers), gas transport and assimilation, redox reactions involving adsorbed substrates and immobilized catalysts, osmotic properties, biomimetic analog of endocytosis based on the surface phenomena binary division of the membranous structures after achieving a critical specific surface area, and membrane coalescence. Considering the fundamental difference of the chemical compounds abundant at the early Earth from the current chemical composition of the living organisms, almost any type of the Fendler membrane mimetic materials can be suitable for protocell modeling, provided that an appropriate energy-rich external factor produces non-equilibrium conditions necessary for initiation of the morphogenetic processes and activation of the cascade chemical reactions across the membrane boundaries. The above principle combines structural and functional approaches to the protocell modeling and significantly extends the range of substances suitable for protocell design.

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Some features of HSP70 expression and content in peripheral blood of patients with obliterating arteriosclerosis

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It is known that chronic inflammation is associated with age-related diseases like atherosclerosis and neurodegenerative diseases. It was shown that HSP70 plays an essential role in the process of chronic inflammation. Mechanisms of HSP70 involvement in the development of diseases remain controversial, although in atherosclerosis, HSP70 were thought to act as autoantigens and trigger both cell- and antibody-mediated immune responses. A considerable increase of extracellular pool of HSP70 and level of antibody to the protein in serum of peripheral blood obtained from patients with arteriosclerosis was also shown. In this work, we investigated alterations of HSP70 serum content and expression in peripheral immune cells of patients with obliterating arteriosclerosis.

The obtained results testify to significant differences between healthy donors and patients in a number of parameters connected with HSP70. In particular, our data demonstrated an increased content of inducible HSP70 in lymphocytes isolated from peripheral blood of the patients as compared with healthy donors. Our preliminary results indicated also the presence of considerable level of surface HSP70 on lymphocytes obtained from a number of patients in contrast to healthy donor lymphocytes having no surface HSP70. Additionally, our data confirm the results of other authors concerning an increased serum level of extracellular HSP70 and antibody to the protein in the blood of the patients. An essential addition to the literature data is connected with those demonstrated in our study—a significant positive correlation of the measured parameters mentioned above with the level of vessel calcification that reflects the development of arteriosclerosis.

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Interference of a D-enantiomeric peptide with hetero-association of amyloid-β oligomers and prion protein

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects millions of people worldwide. One hallmark of the disease is the aggregation of amyloid-β (Aβ) into soluble oligomers and insoluble fibrils. Several studies indicate that oligomers rather than fibrils are the most toxic species in disease propagation. Aβ oligomers bind with high affinity to membrane-associated prion protein (PrP), leading to toxic signalling across the cell membrane, which makes this interaction an attractive therapeutic target. Here, we studied the Aβ-PrP interaction in detail. Our data show that full-length, soluble human (hu) PrP(23-230), as well as huPrP(23-144) lacking the globular C terminal domain, bind to Aβ oligomers to form large complexes beyond the megadalton size range. Following purification by sucrose density gradient ultracentrifugation, the Aβ and huPrP contents in these hetero-assemblies were quantified by RP-HPLC. The Aβ/PrP molar ratio in the assemblies showed some variation in a narrow range depending on the molar ratio of the initial mixture. Specifically, the molar ratio in the assemblies of about 4 Aβ to 1 huPrP in presence of an excess of huPrP(23-230) or huPrP(23-144) suggests that four Aβ units are required to form one huPrP binding site. The Aβ binding all D-enantiomeric peptide RD2D3 competed with huPrP for Aβ oligomers in a concentration-dependent manner. Our results highlight the importance of Aβ oligomer-specific multivalent interactions and demonstrate that compounds can be identified which compete with PrP for these interactions.

1. Rösener, N.S. *et al.* A D-enantiomeric peptide interferes with hetero-association of amyloid-β oligomers and prion protein. *Journal of Biological Chemistry,* (2018), in press, https://doi.org/10.1074/jbc.RA118.003116jbc.RA118.003116.

Investigation of the influence type of solvents (H_2O and D_2O) on the formation of oligomers in lysozyme solution by small-angle X-ray scattering

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The effect of protonated (H_2O) and deuterated (D_2O) water on the structure of the crystallization solution of lysozyme was studied. The structure of the solutions was investigated by small-angle X-ray and neutron scattering methods. At the initial stage of crystallization of lysozyme under crystallization conditions of tetragonal symmetry system crystal growth, it is shown that when the H_2O solvent is replaced by D_2O, dimers and octamers are formed corresponding to the structure of the future crystal in the same way as it happens in the case of the H_2O solvent.

Tetramer, hexamer, and higher-order oligomers were not detected under any conditions, while in the pure protein solution, in which

crystallization cannot occur, only the monomers of the lysozyme protein are detected. The concentration of octamers increases with increasing protein concentration and a decrease in temperature [1–3]. The dependence of the content of oligomers in lysozyme crystallization solution on temperature was studied. The measurements were carried out in the temperature range from 30 to 5 °C in steps of 1 °C. Replacing H_2O to D_2O leads to an increase in the percentage of oligomers in the crystallization solution, similar to a decrease in temperature ~ 8 °C, which is in good agreement with the data on protein solubility in H_2O and D_2O [4].

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1. Kovalchuk M.V. et al., Cryst. Growth Des. Investigation of the initial crystallization stage in lysozyme solutions by small-angle X-ray scattering (2016). V. 16, P. **1792**
2. Boikova A.S. et al. Acta Cryst. D73. Octamer formation in lysozyme solutions at the initial crystallization stage detected by small-angle neutron scattering (2017). V. V. 62. № 3. P. **591**
3. Dyakova, Yu.A. et al. Crystallography Reports. Small-angle X-ray scattering study of conditions for the formation of growth units of protein crystals in lysozyme solutions.(2017). P. **364**
4. Gripon C. et al. J. Cryst. Growth. Lysozyme solubility in H_20 and D_20 solutions: a simple relationship (1997). V. 177. P. **238**

Triphenylphosphonium-based antioxidants slow down stress-induced senescence in the human cells

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Modern scientific research proves the existence of an interrelationship between mitochondria damage, telomeric DNA shortening, and aging. The intracellular imbalance of reactive oxygen species (ROS), mainly produced by disturbed mitochondria, damages proteins, lipids, and DNA. Importantly, such endogenous oxidative stress impairs the mitochondria itself and guanine-rich, irreparable telomeric DNA. In turn, telomere shortening decreases mitochondrial biogenesis. Antioxidants could synergistically fight against cellular aging by protecting mitochondria and telomeres from ROS abundance. It has been shown that some antioxidants could slow down accelerated telomere shortening, occurring under oxidative stress.

In this work, triphenylphosphonium-based ROS scavengers (MitoQ as studied reference) were investigated by Flow-FISH method for impact on telomeres in MRC5 cells. Antioxidants attenuated speed of telomere shortening under 21% O_2 atmosphere after a week incubation in comparison with control not-treated cells. Not studied earlier compound has shown higher effect than MitoQ. Mitochondria health in antioxidant-treated cells was checked by the following fluorescent probes: Mitotracker (network morphology, absence of the stress-induced thread–grain transition), TMRM (mitochondria membrane potential), MitoSOX (absence of reactive oxygen species generation from mitochondria).

Magnification of the antioxidants influence on telomeres will allow to increase their anti-aging effectiveness.

1. Passos, J., Von Zglinicki, T. Mitochondria, telomeres and cell senescence. *Experimental Gerontology* **40**, 466–472 (2005).
2. Saretzki, G., Murphy, M., Von Zglinicki, T. MitoQ counteracts telomere shortening and elongates lifespan of fibroblasts under mild oxidative stress. *Aging cell* **2(2)**, 141–143 (2003).

Role of Iowa mutation on the structure and dynamics of APP transmembrane domain of Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative disorder among the elderly. Annually, more than 5 million new cases are registered, characterized by memory loss, intellectual disability, and widespread deterioration of cognitive abilities. The disease causes the death of neurons with the formation of senile (amyloid) plaques formed during proteolysis of amyloid precursor protein (APP). The presence of mutations in the genes of *APP*, *PSEN1*, and *PSEN2* leads to the early onset of AD and accounts for the most severe cases.

In this study, we determined the spatial structure of the transmembrane domain of APP (Gln686-Lys726) with Iowa mutation (D694N). The peptide was solubilized in the detergent micelles consisted of dodecylphosphocholine. The structure of the peptide comprises two α-helical regions (juxtamembrane Lys687-Asn694 and transmembrane Lys699-Leu723) connected by the mobile loop region Val695-Asn698. Data on the intramolecular mobility was obtained based on the values of rotational correlation times. A comparison was made with the wild-type peptide [1]. The mutation has no significant effect on the mobility and the structure of APP. Replacement of a charged aspartic acid to an uncharged asparagine can lead to the significantly smaller repulsion from the negatively charged membrane. We also assume that the absence of the electrostatic interaction between COO^- of Asp694 and NH_3^+ of Lys698 groups makes the juxtamembrane helix more mobile. Presumably, this can provide the more effective intramembrane proteolysis of APP by the γ-secretase and the increase in production of β-amyloids.

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1. Nadezhdin, K. D., Bocharova, O. V, Bocharov, E. V & Arseniev, A. S. Structural and dynamic study of the transmembrane domain of the amyloid precursor protein. *Acta Naturae* **3,** 69–76 (2011).

Study of visual pigment rhodopsin supramolecular organization in photoreceptor membrane by SAXS and SANS

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The visual pigment rhodopsin is a prototypical member of a large G-protein-coupled receptor (GPCR) family, which plays a key role in all regulatory processes of living organisms. Like many other membrane receptors, GPCRs are known to form dimers and high-order oligomers in membranes. However, the supramolecular organization of rhodopsin in photoreceptor

membranes (PM) is discussed. AFM images of the native rod outer segment (ROS) disk membranes showing the rows of rhodopsin dimers provide a demonstration of their possible supramolecular organization [1]. At the same time, there are a number of works, which present the evidence of a rhodopsin monomeric state in the photoreceptor membranes [2].
We have investigated the rhodopsin supramolecular organization in the PM in the solution by SAXS and SANS methods. A comparative analysis of two different samples was performed: the sample contained the photoreceptor discs (PD) in buffer solution, and the sample contained ROS including the PD packed in an orderly manner. In the latter case, the sample corresponding to native package of the photoreceptor discs in the ROS should reveal a regularity of structural components under such analysis. It was shown that the packing density of the rhodopsin molecules in the photoreceptor membrane is unusually high [4]. In addition, the thickness of the membrane and the peak position, which we assume as the average distance between the rhodopsin molecules in the plane of membrane, remains the same independently from the experiment conditions. The rhodopsin supramolecular organization in photoreceptor membrane is discussed.

1. Fotiadis, D. *et al.,* Rhodopsin dimers in native disc membranes. *Nature (127-128)*, **421,** (2018).
2. Yasuda, S. *et al.,* Spatial arrangement of rhodopsin in retinal rod outer segment membranes studied by spin-labeling and pulsed electron double resonance. *Biochem. Biophys. Res. Com. (134-137),*. **425**(2), (2012).
3. T. Feldman *et al.,* Study of visual pigment rhodopsin supramolecular organization in photoreceptor membrane by small-angle neutron scattering method with contrast variation. *Doklady Biochemistry and Biophysics (420–423).* **465**, (2015).

Serotonin induced heterologous sensitization of alpha1A-adrenergic receptors in mesenchymal stem/stromal cells

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Mesenchymal stem/stromal cells (MSC) are identified in many tissues of an organism and play an important role in reparation, regeneration, and homeostasis. Hormones and neuromediators regulate functional activity of MSC. Noradrenaline is one of the key functional regulators of MSC. Earlier, we observed that all main isoforms of adrenergic receptors are expressed in MSC, but majority of them are not intraconnected with calcium-dependent system of intracellular singling. Beta-adrenergic receptors influence this connection. Stimulation of beta-adrenergic receptors/Gs - protein/adenylyl cyclase/cAMP leads to an increased expression of alpha1A-adrenergic receptors and sensitivity to noradrenaline after 6 h.
In this work, we investigated alternative to noradrenaline adenylyl cyclase inducers that are capable of increasing MSC sensitivity to catecholamines. We studied signaling pathways that can be induced by main neuromediators and then selected neuromediators whose receptors activate adenylyl cyclase, such as dopamine (DRD1, DRD5), histamine (HRH2), serotonin (HTR4, HTR6, HTR7), and adenosine (A2b, A2a). We found, using PCR, that mRNA expression of receptors A2a, A2b, DRD1, DRD5, HRH2, HTR6, and HTR7 are present in MSC. We stimulated MSC and after 6 h observed an increased sensitivity to noradrenaline. We found that serotonin like noradrenaline increases sensitivity to noradrenaline. Histamine, adenosine, and dopamine do not affect MSC. In order to establish the mechanism of this phenomenon, we examined changes of alpha1A-adrenergic receptor expression after stimulating MSC with serotonin. Using western blotting, we showed that preincubation with serotonin leads to an elevated expression of alpha1A-adrenergic receptors in MSC after 6 h. Besides, we have done an inhibitor analysis singling pathway which is associated with influence of serotonin. Inhibition of adenylyl cyclase and proteinkinase A decreases sensitivity of MSC to noradrenaline.
We defined that serotonin and noradrenaline regulate interconnection of adrenergic receptors and calcium singling in MSC. The work was supported by the Grant of the President of the Russian Federation for the state support of young Russian scientists - candidates of sciences MK-3167.2017.7, Russian Science Foundation (RSF) grant 14-15-00439, RFBR 18-015-00421 a.

The ALDH+ and SORE6+ cell subpopulations analysis in triple negative breast cancer cell lines

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Cancer stem cells (CSCs) are a subpopulation of tumor cells with strong tumor initiation capacity and resistance to chemotherapy. Due to these properties, CSCs have become a prospective target for cancer therapy. Targeting CSCs seems especially promising in aggressive oncologies, e.g., triple negative breast cancer (TNBC), which is characterized by aggressive metastasizing and lack of efficient therapy [1]. However, correct identification of CSCs remains a challenge. Currently, several markers of breast cancer stem cells have been established and widely used but still no consensus has been reached on which criteria are more specific and relevant. For example, $CD44^{+}CD24^{-/low}$ phenotype and aldehyde dehydrogenase 1 (ALDH+) expression are both considered as breast cancer stem cell markers and applied together or separately to isolate and enrich CSC subpopulations. However, the overlapping between these two subpopulations is extremely low which raises the demand for other prospective approaches [2, 3]. Lentivirus reporter SORE6 has been recently developed as a new tool to detect CSCs [4]. This construct encodes the destabilized fluorescent protein mCherry gene under control of Sox2/Oct4-sensitive promoter. In our study, we used ALDH+ method to detect CSCs as well as SORE6 reporter to compare the size of CSCs fraction in several TNBC cell lines. We found low correlation in percentage of ALDH+ and SORE6+ cell populations for the majority of TNBC cell lines. The correlation between the size of ALDH+ fraction and TNBC subtype was revealed and for SORE6 method, there was no evidence of such link. We studied the expression of Nanog, the key stemness regulator, in SORE6+ subpopulation of several cell lines to further fully characterize SORE6 reporter.
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1.de Mendonça Uchôa, D. *et al.* Expression of cancer stem cell markers in basal and penta-negative breast carcinomas – a study of a series of triple-negative tumors. *Pathol. - Res. Pract.* **210,** 432–439 (2014).
2.Liu, Y. *et al.* Lack of correlation of stem cell markers in breast cancer stem cells. *Br. J. Cancer* **110,** 2063–2071 (2014).
3.Horimoto, Y. *et al.* Combination of cancer stem cell markers CD44 and CD24 is superior to ALDH1 as a prognostic indicator in breast cancer patients with distant metastases. *PLoS One* **11,** e0165253 (2016).
4.Tang, B. *et al.* A flexible reporter system for direct observation and isolation of cancer stem cells. *Stem Cell Reports* **4,** 155–169 (2015).

Amino acid linkers optimization for the FusionRed-based voltage sensor enhancement

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Visualization of electrical activity in living cells represents an important challenge in context of basic neurophysiological studies. Previously, we described new voltage-sensitive bimolecular fluorescence complementation constructs based on monomeric red fluorescent proteins (FP) [1]. We modified the reporter domain of the VSFP-Butterfly1.2 voltage sensor [2] in order to improve signal detection and red-shift and its spectral sensitivity. Originally, the reporter part of this sensor was built with two FPs (yellow mCitrine and red mKate) forming a FRET pair. These FPs were attached to the N- and C-termini of a voltage-sensitive domain (VSD) which had been made as a chimera of voltage-activated potassium channel Kv3.1 and *Ciona intestinalis* voltage-sensitive phosphatase.
In order to develop a new design of voltage sensors, we used insertion-into-cpFP topology as a basis for interdomain communication. Two non-fluorescent portions of red FP FusionRed were linked to the N- and C-termini of VSD in a way to provide free interaction of regions, which formed a polypeptide chain in the original fluorescent protein. Our experiments successfully demonstrated that the design of reporter domain based on VSD insertion into circularly permuted red FP can be used for voltage imaging. Moreover, we demonstrated that engineering of the protein by changing polypeptide linker length between VSD and FP allowed to change contrast and polarity of the response. We found that the linker reduction by more than seven amino acids changed the polarity of the response to the opposite side. Interestingly, the response contrast increased with the shortening and lengthening of the amino acid chain from the point of polarity reversal, but the maximum contrast was observed with linker length of 20 amino acids.

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1. Kost LA, Nikitin ES, Ivanova VO, Sung U, Putintseva EV, Chudakov DM, et al. (2017) Insertion of the voltage-sensitive domain into circularly permuted red fluorescent protein as a design for genetically encoded voltage sensor. PLoS ONE 12(9): e0184225. https://doi.org/10.1371/journal.pone.0184225
2. Akemann W, Mutoh H, Perron A, Park YK, Iwamoto Y, Knöpfel T. Imaging neural circuit dynamics with a voltage-sensitive fluorescent protein. J Neurophysiol. 2012;108: 2323–37. https://doi.org/10.1152/jn.00452.2012

Effect of amphipathic helices on the mechanical properties of the lipid bilayer

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Epsin protein plays an important role in topological rearrangements of cellular membranes in endocytosis and mitosis. Recruitment of epsin to the plasma membrane facilitates its local invagination. Shallow insertion of the protein into the lipid bilayer (LB) thought to be crucial for protein curvature sensing/generation now is disputed [1]; the impact of protein crowding is considered as the dominant force that provides membrane deformation [2]. Nevertheless, the exact mechanism of the influence of epsins on the mechanical and geometrical properties of LB remains unclear. We suppose the inserting of H0 helix of the epsin N-terminal homology (ENTH) domain is enough to provide strong membrane-protein interaction and to induce bending stress in LB leading to established accumulation of ENTH in the convex region of the membrane. To clarify this issue, we studied the ability of polypeptide forming H0 alone to reveal ENTH-like curvature-driven redistribution on the surface of LB. According to Le Chatelier's principle, such curvature-driven redistribution of both ENTH and H0 should be associated with the reduction of membrane bending rigidity in a protein size-dependent manner. Here, we compare the impacts of synthetized polypeptides—analogs of H0 amphiphilic helix and ENTH on geometry and mechanical properties of model lipid bilayers. We used lipid nanotubes (NT) pulled from planar bilayers as a model of a curved membrane [3]. We showed that H0 and ENTH redistribution between flat membrane and curved NT resulted in similar reduction of effective bending modulus of LB and NT narrowing. Our result confirms a significant role of amphipathic helices in peripheral protein curvature sensing and generation of plasma membrane. While at high protein coverage, the effect of crowding should be taken into consideration as well.

1. McMahon, H. T., & Gallop, J. L. (2005). Membrane curvature and mechanisms of dynamic cell membrane remodelling. *Nature*, *438*(7068), 590.
2. Stachowiak, J. C., Schmid, E. M., Ryan, C. J., Ann, H. S., Sasaki, D. Y., Sherman, M. B., ... & Hayden, C. C. (2012). Membrane bending by protein–protein crowding. *Nature cell biology*, *14*(9), 944.
3. Башкиров, П. В. (2007). Мембранные нанотрубки, вытянутые из бислойной липидной мембраны (БЛМ), как модель для исследования механических свойств сильно изогнутых бислоев. *Биол. мембраны, 24*, 183-192.

Knowledge-based prediction of protein-ligand binding affinities

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Binding of small molecules to proteins is driven by thermodynamic laws and can be described using the notion of free energy, whose minimum should correspond to the system's most stable conformation. However, its rigorous computation is very computationally challenging. This has lead to an extensive development of new approximate methods. Our approach, called Convex-PL [1], is a knowledge-based scoring function based on the idea that the information about protein-ligand interactions can be extracted from the geometry of known complexes in the form of pairwise inter-atomic potentials. We suppose these potentials to be of a free shape, accepting that they can be decomposed into a polynomial basis with unknown expansion coefficients, which are then deduced from structural data by solving an optimization problem. However, such approaches are unable to fully estimate the binding free energy due to the lack of the information about the environment and unknown entropic contributions. Due to these reasons, knowledge-based scoring functions, which perform quite good at finding near-native poses [2–4], are less reliable at binding affinities estimations. To overcome this problem, we created a solvent model and trained a linear ridge regression model to predict binding constants from a set of descriptors consisting of the original Convex-PL score, radial distribution functions of the solvent probe atoms, and a ligand flexibility measure. Finally, we assessed Convex-PL using data from the D3R Grand Challenge 2 submissions and the CASF 2013 Benchmark [5]. Here, Convex-PL was outperforming other scoring functions in the pose prediction exercise and ranked second in the binding affinities prediction and virtual screening tests. This study was partially supported by the Ministry of Education and Science of the Russian Federation (grant no. 6.3157.2017/PP).

1. Kadukova, M. & Grudinin, S. Convex-PL: a novel knowledge-based potential for protein-ligand interactions deduced from structural databases using convex optimization. *J. Comput.-Aided Mol. Design.*, 2017
2. Grudinin, S. et al. Predicting binding poses and affinities in the CSAR 2013–2014 docking exercises using the knowledge-based Convex-PL potential. *J. Chem. Inf. Model.*, 2015

3. Grudinin, S. et al. Predicting binding poses and affinities for protein-ligand complexes in the 2015 D3R Grand Challenge using a physical model with a statistical parameter estimation. *J. Comput. Aided Mol. Des.*, 2016
4. Kadukova, M. & Grudinin, S. Docking of small molecules to farnesoid X receptors using AutoDock Vina with the Convex-PL potential: lessons learned from D3R Grand Challenge 2. *J. Comput. Aided Mol. Des.*, 2017
5. Li, Yan, et al. Assessing protein–ligand interaction scoring functions with the CASF-2013 benchmark. *Nature protocols* 13.4, 2018

The effect of suction process on the growing of gas bubble with convective acceleration

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The concentration distribution around growing gas bubble in the blood and biotissues of divers who ascend to the surface too quickly is obtained by the Mohammadein and Mohamed model [1] for variant and constant ambient pressure through the decompression process. The mathematical model describing this problem consists of four main equations: mass, convective diffusion, Fick's and Laplace's equations. The mathematical model is solved analytically to obtain the concentration distribution around a growing gas bubble in biotissues. The growth of gas bubble is affected by initial concentration difference ΔC_0, diffusivity of gas in tissue D_T, the constant K_d at decompression, surface tension σ, and initial void fraction ϕ_0. The relation between the growth of gas bubble $R(t)$ and time t is obtained from the definition of the concentration distribution around a growing gas bubble in biotissues. The relation between the growth of gas bubble $R(t)$ and time t is studied under the effect of two different values of initial void fraction ϕ_0 and critical bubble radius R_c. The present model is compared with the Mohammadein and Mohammed model [1].

1. S. A. Mohammadein, K. G. Mohamed. Growth of gas bubbles in the biotissues with convective acceleration. Int. J. of Biomath. , **7 (6)** (2014).

Detergent-free solubilization of human potassium channels expressed in eukaryotic cells

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Membrane protein structural studies represent a great challenge despite recent advances in the development of new solubilizing agents and strategies. One of the promising approaches is the use of recently described membrane lipodiscs (SMALPs). The method is based on the ability of the amphipathic polymer poly(styrene-co-maleic acid) (SMA) to cut small discoidal structures out from the lipid bilayer. The use of SMALPs allows not only to exclude detergents from the purification process, but also to preserve the native lipid envelope around the hydrophobic part of the protein to some extent [1].
This work is devoted to the use of SMALPs for the solubilization and purification of human potassium channels from the eukaryotic cell line. COS-1 cells were transfected with plasmids containing potassium channels hKCNH5 and hKCNQ1 and fusion protein hKCNQ1-KCNE1 sequences augmented with the C-terminal affinity tags. The cells were harvested 2 days after transfection and either fractionated or used intact for direct protein solubilization. COS-1 cell membrane fraction rapidly dissolves in the presence of SMA copolymer with the formation of nanosized particles. The particle diameter is about 10–14 nm according to dynamic light scattering. The ultracentrifugation and immunoblotting show that SMA effectively solubilizes hKCNH5 and hKCNQ1 proteins from both membrane fraction and whole cells. On the other hand, most of the hKCNQ1-KCNE1 fusion protein remains in the pellet yet some traces could be detected in the supernatant.
SMA-solubilized proteins were stable enough to be purified on the affinity resin. The purified protein samples were applied onto the EM grids, negatively stained with 1% uranyl acetate, and studied in a transmission electron microscope JEM 2100. Micrographs were analyzed with EMAN 2.12 software. Micrographs show pure and homogeneous samples with particles size of 10–11 nm for hKCNQ1, 11–12 nm for hKCNQ1-KCNE1, and 12–14 nm for hKCNH5. Class averages of proteins contain tetrameric particles confirming correct subunit assembly. The obtained data showed that SMALPs promote the protein isolation and purification and can be used for further structural studies of potassium channels.
This work is supported by the RFBR grant № 18-504-12045. The electron microscopy was carried out using an analytical electron microscope JEM 2100 (JEOL, Japan) as part of a Unique Scientific Facility "Three-Dimensional electron microscopy and spectroscopy" of the Biological Faculty of Moscow State University.

1. Jamshad, M. et al. Surfactant-free purification of membrane proteins with intact native membrane environment. Biochem. Soc. Trans. (2011). 813.

The role of inner mitochondrial membrane in aging and age-related diseases

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It is well known that in the organs that need large amounts of ATP, mitochondria are located rightly around the myofibrils and cristae, and these are located as "coins stack." Also, it has been found that for up to 4 days of hypoxia, the cristae of plant mitochondria become parallel packing [1], and during hypoxia on the fifth to seventh day after coronary artery ligation in the muscle fibers of the myocardium, it revealed a large number of mitochondria with densely packed cristae [2]. However, the function of the conformational changes occurring in the mitochondria during the synthesis of ATP remains incomprehensible. On the basis of numerous published data and our own experimental data, we have developed a mechano-chemiosmotic model of coupling electron transfer to ATP synthesis [3], where the electron transfer along ETC, proton transfer, transport of cations, cyclic low-amplitude swelling-shrinkage, and ATP synthesis are coupled processes. According to this model, an asymmetric contact of dimers of opposite cyt bc_1 complexes is formed in the intracristal space during shrinkage of organelles, which is a mechanical regulator of electron transfer from [2Fe-2S] cluster to heme c_1 and ROS production. The formation of ROS was experimentally demonstrated in hypoxia recently [4]. Moreover, we offer a new mechanism of ATP synthase functioning, where the lysines and arginines of ATP synthase play an important role. Thus, the change in the structure of the inner membrane of mitochondria, causing swelling-shrinkage of the intracristae space, is an electron transfer regulator of ROS (reactive oxygen species) production and ATP synthesis. In conclusion, considering the relationship between ROS and mitochondrial structure, it is possible to develop therapeutic strategies against aging and age-related diseases such as type 2 diabetes, cancer, herpes zoster, and Alzheimer's disease.

1. B.B. Vartapetian, I.N. Andreeva, I.P. Generozova, L.I. Polyakova, I.P. Maslova, Y.I. Dolgikh, A.Y. Stepanova. Functional electron microscopy in studies of plant response and adaptation to anaerobic stress, Ann Bot. 91 (2003) 155-172.
2. V.B. Saprunova, I.M. Solodovnikova, L.E. Bakeeva. The cytochrome c oxidase activity in mitochondria of cardiomyocytes of isolated cardiac tissue under long-term hypoxic incubation. Tsitologiia. 50 (2008) 268-274. Russian.

3. E.A.Kasumov, R.E. Kasumov, I.V. Kasumova, A mechano-chemiosmotic model for the coupling of electron and proton transfer to ATP synthesis in energy-transforming membranes: a personal perspective, Photosynth Res., 123 (2015) 1-22.
4. G. Sgarbi, G. Gorini, F. Liuzzi, G. Solaini, A. Baracca, Hypoxia and IF_1 expression promote ROS decrease in cancer cells, Cells. 7 (2018) pii: E64. https://doi.org/10.3390/cells7070064.

Antibacterial/hemolytic activity of long-length cyto-insectotoxins is gained due to the cumulative effect of its parts

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Understanding the interaction of membrane-active peptides with biomembranes is essential for drug discovery, especially novel antimicrobial peptides [1]. The latter are believed to be recruited from polycationic peptides, abundantly present in insect and snake venoms [2]. Cyto-insectotoxin 1a (CIT1a) is a 69-residue-long peptide isolated from *Lachesana tarabaevi* spider venom [3]. The peptide features broad-spectrum antibacterial activity, affecting both Gram-positive and Gram-negative microorganisms, as well as being highly toxic to insect and human blood cells.
In the current work, based on computational analysis of the structure-function data of membrane-active peptides, we designed and synthesized four 20-residue peptides, spanning the whole amino acid sequence of CIT1a and tested their antibacterial/hemolytic properties. All of the peptides show lower antibacterial/hemolytic activity compared to CIT1a. This suggests (1) the activity of CIT1a is amplified via cumulative effect of its fragments and (2) the obtained peptides can be considered as templates for design of new antibacterial substances due to their low toxicity against human erythrocytes.
In addition, taking into account the activities of the obtained peptides, we address the question of prediction of hemolytic activity of linear (void of disulfide bonds) peptides based on coarse-grained molecular dynamics and our statistical analysis of a group of polycationic peptides, whose hemolytic properties had been determined and currently available online http://crdd.osdd.net/raghava/hemolytik/.

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1. Dubovskii, P. V., Volynsky, P. E., Polyansky, A. A., Chupin, V. V., Efremov, R. G. & Arseniev, A. S. Spatial structure and activity mechanism of a novel spider antimicrobial peptide, *Biochemistry.* ***45***, 10759-10767 (2006).
2. Dubovskii, P. V., Vassilevski, A. A., Kozlov, S. A., Feofanov, A. V., Grishin, E. V. & Efremov, R. G. Latarcins: versatile spider venom peptides, *Cell Mol Life Sci.* ***72***, 4501-22 (2015).
3. Vassilevski, A. A., Kozlov, S. A., Samsonova, O. V., Egorova, N. S., Karpunin, D. V., Pluzhnikov, K. A., Feofanov, A. V. & Grishin, E. V. Cyto-insectotoxins, a novel class of cytolytic and insecticidal peptides from spider venom, *Biochem J.* ***411***, 687-696 (2008).

SAXS studies of apoferritin in different pH with consideration of dimers

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Apoferritin is a spherical shell of ferritin, the main storage of iron in an organism. This is a widespread protein, which can be found in organisms all over the world. It is well known that apoferritin may be used in drug delivery systems [1] and could be used as a cancer indicator or for identification of biological age. Apoferritin is an efficient adjuvant for anti-virus therapy.
However, the mechanism of how apoferritin arranges iron transfer inside and outside the molecule is not clarified yet. One of the possible reasons is the pH value of the solvent. The work [2] is about investigation of apoferritin by SAXS in different pH values and does not consider dimerization effect. In this work, we present SAXS results of apoferritin measurements obtained on the instrument BM29 (ESRF, Grenoble, France) at pH values 4.8, 7.5, and 9.0. We observed that the protein is stable near the isoelectric point pI. 3D low-resolution structures are compared with those in [2, 3]. In addition, analogous SANS data is presented. We show that even small amounts of dimers significantly impacted the recovered structures. Lower pH values require additional technical solutions for SEC-SAXS, where we can observe significant changes of structural organization of apoferritin.

1. Z. Zhen et al., "RGD-modified apoferritin nanoparticles for efficient drug delivery to tumors," no. 6, pp. 4830–4837, 2013.
2. M. Kim et al., "PH-dependent structures of ferritin and apoferritin in solution: disassembly and reassembly," Biomacromolecules, vol. 12, no. 5, pp. 1629–1640, 2011.
3. D. V. Zabelskii et al., "Ambiguities and completeness of SAS data analysis: investigations of apoferritin by SAXS/SANS EID and SEC-SAXS methods," J. Phys. Conf. Ser., vol. 994, no. 1, 2018.

Axotomy-induced ultrastructural alterations and Ca^{2+}-dependent necrosis and apoptosis of the crayfish mechanoreceptor neuron and glial cells

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Traumatic damage to the brain or spinal cord is among the main causes of people death and disability. Severe nerve injury such as axotomy induces neuron degeneration and death of surrounding glial cells. Neuroglial interactions maintain mutual survival of neurons and glia. To study ultrastructural changes and signaling processes involved in axotomy-induced cell death, we used a simple neuroglial preparation—crayfish stretch receptor (CSR). CSR contains a single mechanoreceptor neuron (MRN) surrounded by the glial envelope, which consists of 10–20 glial layers that are alternated with collagen layers. CSRs were isolated using the novel technique, which does not disrupt MRN connection to the abdominal ganglion. After control registration of MRN firing, its axon was transected at 6–10 mm from the soma. Electron microscopy showed that the transected axon end was sealed by glia and collagen. As a result, the ion flux through the cut axon end was not significant, and axotomized MRN could fire 6–10 h after isolation. The axotomized axon completely lost microtubules at a distance of 30–50 μm from the transection site. In this area, axonal mitochondria swelled, their cristae were destroyed, and matrixes were partially lost. Axonal microtubular bundle became regular at 200–300 μm. In this region, axonal mitochondria shrank and contained dark matrix and local edema. Near the transected axon end, strong edema developed in the glial envelope. Glial and collagen layers deformed and

swelled. Glial ER and mitochondria swelled and formed numerous large vesicles. Mitochondrial cristae fragmented to small vesicles. Axotomy caused death of glial cells not only near the transection site, but also cells remote at several millimeters. Thus, axon integrity is necessary for glia survival. After CSR isolation, free Ca^{2+} level that was visualized by fluo-4 gradually increased in the MRN perikaryon and glial envelope. Necrotic and apoptotic remote glial cells (RGC) that surround the 2-mm proximal axon segment (4–8 mm from the transection site) were visualized by propidium iodide and Hoechst 33342. Triple $[CaCl_2]_o$ increased axotomy-induced apoptosis of RGC. Application of ionomycin or thapsigargin confirmed involvement of Ca^{2+} influx, or inability of ER Ca^{2+}-ATPase (SERCA) in axotomy-induced apoptosis or necrosis of RGC. Application of Cd^{2+} or ryanodine showed that Ca^{2+} influx through plasma membrane or release from ER contributed to axotomy-induced RGC necrosis. This suggests the involvement of Ca^{2+} in apoptosis and necrosis of remote glial cells surrounding the axotomized neuron. Supported by Minobrnauki RF (grants 6.4951.2017/6.7 and 6.6324.2017/8.9).

The effects of fullerene derivatives on the model lipid membranes

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Water-soluble fullerene derivatives have a wide spectrum of biological activity. Carboxyfullerenes are known to have photosensitizing properties [1] and can be applied in photodynamic therapy [2]. A supramolecular complex of fullerene–aminohexanoic acid with hexonium can penetrate the blood–brain barrier and might be used as a delivery system for various drugs to the brain [3]. C_{60} complex with polyvinylpyrrolidone (C_{60}/PVP) demonstrates a strong antiviral activity [4].
The aim of this work was to examine the effects of five different fullerene derivatives on the properties of model lipid membranes, in particular, the phase transition temperature of membrane-forming lipids, the boundary potential at the water/membrane interface, and the ability to form ion-conductive pores in lipid bilayers. We examined supramolecular complexes of fullerene–aminohexanoic acid (IEM-2197) and fullerene–malonic diacid with hexonium (IEM-2143), C_{60} complex with γ-cyclodextrine (C_{60}/(γ-CD)$_2$) and C_{60}/PVP.
We used the electrophysiological methods of recording of K^+–nonactin and fullerene derivative-induced currents through bilayers formed by Montal and Mueller [5] from dioleylphosphocholine (DOPC) and differential scanning microcalorimetry of dipalmitoylphosphocholine (DPPC) liposomes.
IEM-2143 induced ion-conductive pores in DOPC bilayers bathed in 0.1 M KCl (pH 7.4) at the 100-times lower concentrations than IEM-2197 and C_{60}/PVP (2 mg/ml). C_{60}/(γ-CD)$_2$ did not increase membrane conductance up to 20 mg/ml. The introduction of IEM-2197, C_{60}/(γ-CD)$_2$, and C_{60}/PVP did not significantly affect the phase behavior of DPPC at the equimolar ratio to lipid. The phase transition temperature decreased with the increasing concentration of IEM-2143. The data are in agreement with the assumption of deeper penetration of IEM-2143 into bilayer than other tested compounds. The addition of the tested derivatives did not affect K^+–nonactin current. This indicates that the agents do not affect membrane boundary potential.

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1. Yang, X.L., Fan, C.H., Zhu, H.S. *Toxicology in vitro* (2002). https://doi.org/10.1016/S0887-2333(01)00102-3
2. Asada, R.1., Liao, F., Saitoh, Y., Miwa N. *Mol Cell Biochem.* (2014). https://doi.org/10.1007/s11010-014-1968-8
3. Piotrovskiy, L.B., *et al*. *Doklady biochemistry and biophysics.* (2016). https://doi.org/10.1134/S1607672916030030
4. Piotrovskiy, L.B., Kiselev, O.I. *Fullerenes in biology.* (2006). *P.89*-94.
5. Montal, M., Muller, P. *Proc Nat Acad Sci USA*. **69**, (1972).

Is the root cause of aging located inside or outside the cell membrane?

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The protracted search for a clue to the aging enigma is related to the fact that the solution is looked for at levels where one can see only the consequences of aging, but not its root cause. The cellular level is located in the middle of the structural hierarchy of the systems of the body. Therefore, if cells are inherently capable of unlimited self-maintenance, the causes of aging should be looked for at the upper levels of the hierarchy—outside the cell membrane. Otherwise, the cause of aging is hidden at the subcellular levels—inside the cell membrane.
Usually, we have considered cellular aging to be the primary process. As cells are not capable of unlimited self-maintenance even under ideal conditions in vitro, most researchers consider them to be the root cause of aging. Then, aging of the whole organism seems to be an inevitable consequence of the aging of its cells. However, analysis of the accumulated data shows that the decrease and loss of mitotic potential of cells in vitro (which are considered to be among the main signs of senescence) may be a consequence of differentiation, which is programmed to block the mitotic ability. The natural microenvironment of somatic stem cells, which keeps them at the stage of stem cells with unlimited mitotic potential, is disturbed in cell cultures. Thus, true cellular aging should be seen as a process related to their functioning in an aging organism or in an inadequate culture condition, and such aging is largely reversible [1]. That is why some molecular and cellular biologists have started discussing the issue of moving the root cause of aging beyond the cell membrane (e.g., [2] and references at [3]).
If we take into account the fact that vitality of really sustainable systems and modes is possible only in certain limited range of ambient condition, then the control theory and systems approach are sufficient both to discover the root cause of aging and to understand the underlying mechanisms of its implementation [3, 4]. Since outside adequate functioning modes determined by the suitable environment, even immortalized cells and ageless hydras start aging "according to Gompertz" (see references at [3]), similar to the mortality pattern of humans.

1. Rando, T.A. & Chang H.Y. Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. *Cell*. **148,** (2012).
2. Olovnikov, A.M. Lunasensor, infradian rhythms, telomeres, and the chronomere program of aging. *Ann. N. Y. Acad. Sci*. **1057,** (2005).
3. Khalyavkin, A.V. Phenoptosis as genetically determined aging influenced by signals from the environment. *Biochemistry (Moscow).* **78,** (2013). https://doi.org/10.1134/S0006297913090058
4. Khalyavkin, A.V. & Krut'ko, V.N. How regularities of mortality statistics explain why we age despite having potentially ageless somatic stem cells. *Biogerontology.* **19,** (2018). http://rdcu.be/vNyb

Photochromic labels as a new challenge for nanobiotechnology

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A promising way for the new hybrid photoactive/photocontrollable systems and materials design consists in the covalent binding of the photochromic probes via their covalent "immobilization" on various substrates, e.g., polymers, lipids, proteins, and quantum dots. Developing the new generation of photochromic probes containing substituents with appropriate functional group type will be required for the implementation of this procedure. Indoline spirobenzopyrans are one of the most studied photochromic compound classes. The structure of possible target substrates defines the nature of the reactive anchor group. Spectral properties and photochemical parameters of spirobenzopyrans depend significantly on the nature of the substituent present in the defined part of the molecule; hence, the targeted variation of substituents' nature allows to search directly for new photochromes with given photochemical properties and various stimulus-responsive structural elements.

Previously, we have developed a new synthetic method for the key carbonyl precursor—5′-formyl-6-nitrospiropyran derivative by direct formylation of 6-nitrospiropyran—in one step with 86% yield under the Duff reaction conditions. The synthetic application potential of these precursors for targeted modification of the photochrome molecule at 5′-position has been significantly broadened by the application of well-known synthetic procedures (Wittig and Horner-Emmons olefination; nucleophilic addition to the carbonyl group with a family of reagents, possessing an active methyl or methylene groups; reductive amination; [3+2]cycloaddition reaction; and others).

We developed a number of new photochromic probes and labels on the 5′-substituted spirobenzopyran scaffold. All the types of labels were prepared by the effective synthetic approach which included the direct modification of the spiropyran molecule. The choice of the target-reactive group or "molecular address" was determined by type and nature of the target structure. The following conjugation procedure variants were used: (a) covalent binding of the probe molecule with a target binding site by self-recognition principle (bacteriorhodopsin); (b) noncovalent binding of the probe molecule with target by the recognition of "molecular address" part, which was introduced in label (TxA_2 receptor inhibitors); (c) covalent binding of the probe molecule with target by terminal selectively reactive group (HS group for CdSe quantum dots); (d) covalent binding of the probe molecule with target by terminal reactive group.

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Development of coarse-grained models of *P. aeruginosa* lipopolysaccharides with different antigen chains

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Pseudomonas aeruginosa is one of the most widespread species of Gram-negative bacteria, which is the leading causative agent in the resuscitation departments. *P. aeruginosa* has a whole set of mechanisms for the development of antibiotic resistance. For example, resistance to cationic aminoglycosides (for example, gentamicin) is due to the modification of lipopolysaccharides (LPS) resulting in negative charge decrease of these molecules.

LPS of *P. aeruginosa* outer membrane consist of three structural components: lipid A, core oligosaccharide and antigen chain. Most *P. aeruginosa* strains produce two distinct forms of antigen chain, one a neutrally charged homopolymer of D-rhamnose (A band) and the other a heteropolymer of three to five distinct sugars in its repeat units (B band).

We created coarse-grained (CG) models of LPS molecules with A and B band antigen chains using the MARTINI force field. The base model of the rough LPS molecule, consisting only of lipid A and oligosaccharide, was adopted from [1]. To optimize the CG parameters of LPS antigen chains, we performed a full atomic molecular dynamics (MD) simulation using CHARMM36 force field during 500 ns. Previously published work [2] confirms high conformational mobility of the rhamnose antigen chain. To provide insight into the details of conformation arrangement of antigen chains with different chemical compositions, we simulated and created CG models of about 1 μs.

Developed CG models of LPS molecules with different antigen chains will be used to construct models of the *P. aeruginosa* outer membrane. Different lengths and chemical compositions can be the critical point of LPS membrane interaction with antimicrobial compounds like the previously created CG model of polycationic phthalocyanine [3].

1. Oosten, B. Van & Harroun, T. A. *A MARTINI extension for Pseudomonas aeruginosa PAO1 lipopolysaccharide* (2016). https://doi.org/10.1016/j.jmgm.2015.12.002

2. Galochkina, T. et al. *Conformational dynamics of the single lipopolysaccharide O-antigen in solution* (2016). https://doi.org/10.1002/cphc.201600524

3. Orekhov, P. S. et al. *Molecular mechanism of uptake of cationic photoantimicrobial phthalocyanine across bacterial membranes revealed by molecular dynamics simulations* (2018). https://doi.org/10.1021/acs.jpcb.7b11707

Molecular dynamics studies of nanosized systems combined with neutron scattering experiments

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Molecular simulation studies are performed within the research activities of molecular dynamics (MD) modeling group of neutron optics sector in the Department of Neutron Investigations of Condensed Matter, Frank Laboratory of Neutron Physics (FLNP), Joint Institute for Nuclear Research (JINR), thereby outlining the international research collaborations closely performed with Japanese groups (Keio University, Waseda University, RIKEN, etc.). The examples have to cover a general issue on "Computer Design for New Drugs and Materials," which demonstrate the efficient use of computer MD in both classical (conventional) and quantum chemical methods implementations. A special attention has been paid to the lipid structure evaluation concerning the peculiar properties of lipid membranes revealed by combining use of MD simulations and neutron scattering experiments. Some aspects of our recent studies include computer modelling of nonequilibrium chemo-electronic conversion of water adsorption on the surface of yttria-stabilized zirconia.

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1. N. Kučerka, E. Dushanov, Kh. Kholmurodov, J. Katsaras, and D. Uhríková (2017). Calcium and zinc differentially affect the structure of lipid membranes. Langmuir, 33 (12), pp 3134–https://doi.org/10.1021/acs.langmuir.6b03228

2. Kholmirzo T. Kholmurodov (Ed). "Computer design for new drugs and materials. Molecular dynamics of nanoscale phenomena", (Leading Scientist, Frank Laboratory of Neutron Physics, Joint Institute of Nuclear Research, Dubna, Moscow Region, Russia), ISBN: 978-1-53612-082-0, Nova Science Publishers, New York (2017), 231 p.
3. Tomobe K, Yamamoto E, Kholmurodov K, Yasuoka K (2017). Water permeation through the internal water pathway in activated GPCR rhodopsin. PLoS ONE 12(5): e0176876. https://doi.org/10.1371/journal.pone.0176876.
4. Kh Kholmurodov, S Majumder et.al. IOP Conf. Series: Journal of Physics: Conf. Series 848 (2017) 012012 https://doi.org/10.1088/1742-6596/848/1/012012: Journal of Physics: Conf. Series 848 (2017) 012012 https://doi.org/10.1088/1742-6596/848/1/012012

The effect of energy metabolism level of erythrocytes on their oxygen membrane permeability

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With the development of some pathological processes in the human body, the level of cell metabolism and consequently the level of adenosine triphosphate (ATP) decrease. As a result, various abnormalities occur in cell structures, including cytoplasmic membranes [1]. The authors of [2] showed that the ATP level affects the deformability of the erythrocyte membrane. M.V. Fock et al. [3] found that the nonspecific permeability of erythrocyte membranes for oxygen is regulated automatically by a special mechanism, and this automatism can be disturbed by the development of pathological processes in the body. However, at present, the relationship between the level of energy metabolism (the ATP concentration) and the permeability of erythrocyte membranes for oxygen has not been sufficiently investigated. The aim of the work is to investigate the correlation between the level of ATP in erythrocytes and the permeability of their membranes for oxygen. For this purpose, we use a unique device, KINOX Gamma-4, which allows us to evaluate the permeability of erythrocyte membranes by the rate of their saturation in special conditions. To obtain erythrocytes with a reduced level of energy metabolism, we used an inhibitor—2-deoxyglucose—which is able to rapidly enter erythrocytes and competitively inhibit the formation of glucose-6-phosphate, the central reaction in the processes of glycolysis. The incubation of erythrocytes with 2-deoxyglucose at a concentration of 160 mM resulted in the disappearance of 90% of the original ATP in erythrocytes in 2.5 h [4]. The experiments conducted showed that if the initial state of human erythrocytes is regarded as normal (that is, the blood is taken from a healthy donor), the addition of the above-mentioned inhibitor leads to an increase in the rate of saturation of the erythrocytes with oxygen. If the red blood cells were taken from the body of a person with a disease (for example, diabetes mellitus), then depending on the initial state of its energy metabolism, the saturation rate will vary differently with the addition of an inhibitor. Thus, we show the correlation between the level of ATP in erythrocytes and the permeability of their membranes for oxygen.

1. Kumar, R. *et al.* Changes in erythrocyte membrane in type-2 diabetes mellitus with and without dyslipidemia, *J Diabetes Metab* **2**(6) (2011).
2. Wan, J. *et al.* Red blood cell dynamics: from cell deformation to ATP release, *Integr. Biol.* **3**(10), 972–981 (2011).
3. Fok, M.V. *et al*. Effect of erythrocyte-membranes oxygen permeability decreasing during oxygenation, *Biofizika* **34** (5), 901-904 (1989).
4. Kondo, T. & Beutler, E. Depletion of red cell ATP by incubation with 2-deoxyglucose. *J Lab Clin Med*. **94**(4): 617-23 (1979).

Conformational analysis of sialic acid in its *p*-nitrophenyl and lactose derivatives bound to influenza membrane neuraminidase active site

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One of the major membrane glycoproteins of influenza virus—neuraminidase—plays a key role in virus replication cycle by cleaving sialic acid from host cell surface facilitating release of virions and spread of infection. The external arrangement of neuraminidase makes it a primary target for antiviral therapeutic strategies but results in permanent evolutionary pressure by a host immune system and high mutational frequency. This phenomenon leads influenza to rapid developing of viral resistance to modern therapeutics constituting an emerging challenge in medicine.
The promising inhibitor design approach relies on precise mimicking of substrate's transition state structure during enzyme catalysis. Recent development workflow of neuraminidase inhibitors which had not produced effective candidate molecules was based on the assumption of substrate's terminal sialic acid following $^3S_1 \rightarrow {}^3H_4$ conformational path during catalysis revealed in experiments with *p*-nitrophenyl derivative but not the native oligosaccharide substrate [1]. In this work, using molecular modeling, we demonstrate that substrate's *p*-nitrophenyl derivative undergoes alternative conformational transitions compared to native trisaccharides, thus hiding true conformational requirements for transition-state mimicking inhibitors.
Comparative analysis has been made on reconstructed free energy landscapes for both 3′-sialolactose and *p*-nitrophenol α-D-sialoside bound to H5N1 influenza neuraminidase by the molecular modeling of Michaelis complex formation using nonparametric Bayesian clustering of glycan structures coupled with metadynamics [2]. *p*-Nitrophenyl derivative demonstrated ^{25}B state as a major conformation for a sugar ring structure not seen in a complex of neuraminidase with a native 3′-sialolactose substrate which exhibits strong stabilization of intermediate state between $^5S_1$ and B_{14} while prohibiting the majority of other possible conformations. Design of effective antivirals might benefit if it takes into account geometric properties of $^5S_1$, B_{14}, and closest possible E_4 transition state for a core structure of candidate molecules.

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1. Colombo C. et al. Design and synthesis of constrained bicyclic molecules as candidate inhibitors of influenza A neuraminidase. *PloS one* **13**, e0193623 (2018)
2. Kirilin E.M., Švedas V.K. Study of the conformational variety of the oligosaccharide substrates of neuraminidases from pathogens using molecular modeling. *Moscow University Chemistry Bulletin* **73**, *39–45 (2018)*

Study of the interaction of products of mutant endothelin-1 and eNOS genes with vascular membranes, as the cause of arterial hypertension

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Endothelin-1 and eNOS play an important role in the regulation of vascular tone. Based on this, the research theme was formed: how the mutant forms affect the tone of the vessels.
To carry out this study, a PCR-RFLP analysis was conducted to find out which mutant forms are most often found in patients with hypertension. The data was processed in SPSS.
In the future, it is planned to obtain the most frequently encountered mutant forms and study their interaction with the vascular membranes.

Live-cell super-resolution microscopy with blue fluorescent protein TagBFP

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Localization-based super-resolution microscopy enables visualization of cellular structures and processes, such as cytoskeleton or plasma membrane dynamics, in live cells at the subdiffraction level. Generally, this technique requires special fluorophores capable of photoactivation or photoswitching. Here, we report unexpectedly high photoactivity of TagBFP [1] and successfully applied this common blue fluorescent protein for localization-based super-resolution microscopy.
Previously, we revealed blinking behavior of some red fluorescent proteins, including TagRFP—precursor of TagBFP—that allows to dramatically improve the resolution [2]. Then, we tested the similar conditions on TagBFP-actinin-transfected live cells. As we proposed, under continuous 405-nm illumination, TagBFP has demonstrated fluorescence intensity fluctuations in a millisecond scale, which can be further processed by a recently reported analytical approach [3]. As a result, the super-resolved images of alpha-actinin were obtained.
Interestingly, we found that TagBFP partially converts into a red state under common imaging conditions. The photoconversion efficiency was not high enough to perform localization-based super-resolution microscopy. However, this observation should serve as a caution when performing multicolor imaging using 405-nm laser. Blue-to-red photoconversion of TagBFP was also detected in protein solution in vitro. After prolonged 405-nm illumination, a noticeable increase in TagBFP absorbance at ~555 nm has been observed that matches well with TagRFP spectral characteristics.
In conclusion, we, for the first time, showed the use of TagBFP in live-cell super-resolution microscopy without need of external chemicals or intense laser illumination. Besides, the phenomenon of its blue-to-red photoconversion was revealed.

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1.Subach, O. M. *et al.* Conversion of red fluorescent protein into a bright blue probe. *Chem. Biol.* **15,** 1116–1124 (2008).
2Klementieva, N. V. *et al.* Intrinsic blinking of red fluorescent proteins for super-resolution microscopy. *Chem. Commun.* **53,** 949–951 (2017).
3.Gustafsson, N. *et al.* Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations. *Nat. Commun.* **7,** 12471 (2016).

Activation of acid-sensing channels stimulates cell type-specific calcium entry in rat cerebellum

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Acid-sensing ion channels (ASICs) are proton-gates cation channels which belong to superfamily of amiloride-sensitive degenerin/epithelial Na^+ channels (DEG/ENaC) [1]. ASICs are involved in many physiological processes such as nociception and pain, synaptic function, and plasticity. Activation of ASICs causes neuronal depolarization sometimes associated with direct or indirect Ca^{2+} entry and response of individual neuron which strongly depends on subunit composition of expressed ASIC channels [2].
Several studies have shown that in the central nervous system, ASICs also play an important role in pathological states associated with extracellular acidification and neuronal death (ischemia, epileptic seizures, etc.). Particularly, the calcium influx driven by activation of ASICs seems to be an important factor for determining neuron's viability [3].
Here, we report that activation of ASICs by local application of acidic solution (pH 4.5) causes cell type-specific Ca^{2+} entry in acute cerebellar slices from young rats (p10-15) measured with OregonGreen-488. Local puff of acidic solution led to robust, transient increase in $[Ca^{2+}]_i$ in cerebellar granule cells layer (100% of cells) while only occasional responses were obtained in Purkinje cell layer (15% of all cells). The responses in granule cells were then further investigated for their sensitivity to pharmacological substances. It was found that calcium entry caused by acids was not affected by the presence of tetrodotoxin, ionotropic glutamate receptor blockers (APV, CNQX), or aspirin (up to 2 mM). However, the amplitude of response was partially inhibited by flurbiprofen (72% and 45% from control by 2 and 4 mM, respectively) and completely blocked by 100 μM of amiloride (nonselective ASIC blocker).
These results show for the first time that within the same brain structure, the effect of extracellular pH level lowering of neuronal $[Ca^{2+}]_i$ might be cell type-specific. This finding should be taken into account when acidosis-related neuronal damage or design of neuroprotective drugs is considered.

1.Osmakov, D. I., Andreev, Y. a. & Kozlov, S. a. Acid-sensing ion channels and their modulators. *Biochem.* **79,** 1528–1545 (2015).
2.Gründer, S. & Pusch, M. Biophysical properties of acid-sensing ion channels (ASICs). *Neuropharmacology* **94,** 9–18 (2015).
3.Ortega-Ramírez, A., Vega, R. & Soto, E. Acid-sensing ion channels as potential therapeutic targets in neurodegeneration and neuroinflammation. *Mediators Inflamm.* **2017,** (2017).

Lipid bilayer deformations regulate functioning of gramicidin A channel and determine its clustering

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Gramicidin A (gA) is a well-known peptide forming ion-conducting channels in a lipid membrane by transbilayer dimerization. gA channel is characterized by conductance, dimerization probability, and channel lifetime. The channel characteristics depend on elastic properties of the lipid bilayer (thickness, spontaneous curvature, etc.).
We studied how gA dimer is formed from two initially isolated monomers in the framework of theory of elasticity of liquid crystals adopted to lipid membranes. Because gA monomer is shorter than the lipid monolayer thickness, gA deforms lipid bilayer in its vicinity. If two monomers are close enough, their induced deformations overlap and monomers interact with each other. We calculated the interaction energy of monomers at different distances and obtained channel lifetime and probability of dimer formation. The results were compared with the experimental data [1, 2]. The features of monomer interaction could explain the long persistence of gA channels in planar bilayer after fusion with gA-containing liposomes [3] without assumptions of internal gating. Monomer interactions predict gA clustering at surface concentration of $C_{crit} = 0.05\ nm^{-2}$, which is in good agreement with the experimental data [4].

1. Goulian, M., et al. Gramicidin channel kinetics under tension. *Biophysical Journal* 74.1 (1998): 328-337.
2. Lundbæk, Jens A., and Olaf S. Andersen. Spring constants for channel-induced lipid bilayer deformations estimates using gramicidin channels. *Biophysical journal* 76.2 (1999): 889-895.

3. Jones, Tyson L., et al. Gramicidin channels are internally gated. *Biophysical journal* 98.8 (2010): 1486-1493.
4. Diociaiuti, Marco, et al. Aggregation of gramicidin A in phospholipid Langmuir–Blodgett monolayers. *Biophysical journal*82.6 (2002): 3198-3206.

Single cysteine containing RNase barnase and DARPin G3 as components of "molecular constructor" for addressed toxin production

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At present, targeted toxins capable to selectively eliminate cancer cells are most often produced as fusion proteins in bacteria. The principal disadvantage of the method is the lack of flexibility, when it is impossible to alternate targeted toxin functionality by its components shuffling, like in a "molecular constructor." The investigation demonstrates a new approach to targeted toxin production on the example of such proteins as the toxin RNase barnase and the protein DARPin G3 targeted to HER2/neu receptor. By substituting the single amino acid in their structures for cysteine and performing the simple click ligation reaction, which results in the disulfide bond formation and in the covalent protein linkage, targeted toxins with various functionalities can be easily generated. The main expected result of the research is the practical realization of the idea of targeted toxin modular construction using the methods of click chemistry and genetic engineering, allowing easily to choose and introduce rationally into their structure the various functional modules, changing the toxin functionality in wide range.
Here, the results concerning the production of the single cysteine containing proteins barnase and DARPin G3 in *Escherichia coli* are presented.
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Oxytocin/vasopressin receptors in trigeminal nociceptive system and their role in migraine pathology

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It is known that hormone oxytocin has antinociceptive action in mammalians [1]. Thus, it was clearly shown that oxytocin is able to prevent migraine pain [2]. However, the mechanisms of this analgetic effect of oxytocin are still unknown. In this work, we study the migranic pain signaling in rat trigeminal system, and its regulation by oxytocin.
It was observed that oxytocin (1 uM) application causes the strong Ca-response wave in cultured trigeminal neurons of rats (P10-12). Together with it, oxytocin prevented Ca signaling evoked by activation of GABA pain-transducing receptors in neurons. During electrophysiological registration of trigeminal nerve meningeal afferent activity in dura mater of adult rats (P40-42), we have observed the increasing effect of oxytocin (1 and 10 uM) on spontaneous action potentials in peripheral terminals. Using the method of polymerase chain reaction in real time (real-time PCR), we have discovered the high level of oxytocin receptors mRNA both in trigeminal neurons and meningeal tissue of rats. More of it, the significant level of vasopressin receptor mRNA was shown there, indicating that oxytocin effects could be realized via both types of membrane receptor pathways in trigeminal system, as it was shown earlier for other sensory neurons [3]. The cellular mechanisms of oxytocin/vasopressin regulation of migraine pain are the matter of our intensive study.
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1. Tracy, L.M. *et al.* Oxytocin and the modulation of pain experience: implications for chronic pain management. Neurosci. Biobehav. Rev., **55,** (2015)
2. Philips, W.J. *et al.* Relief of acute migraine headache with intravenous oxytocin: report of two cases. J. Pain Palliat. Care Pharmacother., **20(3),** (2006)
3. Schorscher-Petcu, A. *et al.* Oxytocin-induced analgesia and scratching are mediated by the vasopressin-1A receptor in the mouse. J. Neurosci, **30(24)**, (2010) https://doi.org/10.1523/JNEUROSCI.1594-10.2010.

Visualization of G-protein-coupled receptors using photoactivated localization microscopy (PALM)

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G-protein-coupled receptors (GPCRs) transduce a variety of extracellular signals and thus play a crucial role in cell biology. After activation by a ligand, GPCRs undergo conformational changes, leading to dissociation or rearrangement of a heterotrimeric G-protein complex [1]. GPCRs and G-proteins are localized to the plasma membrane and organized into signaling platforms, such as nanodomains. This organization is important for both fidelity and efficacy of signal transduction. As the size of these nanodomains is below the diffraction limit of light microscopy, important aspects of G-protein signaling can only be ascertained by techniques whose spatial resolution is on the order of tens of nanometers.
In order to observe G-protein signaling events with high resolution, we aimed to use techniques of advanced optical microscopy. Specifically, we wanted to combine the technique of single-photon polarization microscopy (1PPM) with photoactivated localization microscopy (PALM). 1PPM has previously been shown to allow observations of G-protein activation. PALM (relying on photoswitchable fluorescent molecules and serial photoswitching) allows precise localization of individual fluorescent molecules even in dense populations, and thus high spatial resolution [2, 3].
Within our project, we employed techniques of cell biology, optical engineering, and advanced image analysis in order to assess the ability of two constructs (Galphai1-L91-rsEGFP2 and GAP43-rsEGFP2-Galphai1) to report on G-protein signaling with spatial resolution better than the diffraction limit of regular optical microscopy.

1.Kobilka, B. K., G protein coupled receptor structure and activation. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2007,** *1768* (4), 794-807.
2.Scarselli, M.; Annibale, P.; Gerace, C.; Radenovic, A., Enlightening G-protein-coupled receptors on the plasma membrane using super-resolution photoactivated localization microscopy. *Biochemical Society Transactions* **2013,** *41* (1), 191.
3.Scarselli, M.; Annibale, P.; McCormick, P. J.; Kolachalam, S.; Aringhieri, S.; Radenovic, A.; Corsini, G. U.; Maggio, R., Revealing G-protein-coupled receptor oligomerization at the single-molecule level through a nanoscopic lens: methods, dynamics and biological function. *The FEBS Journal* **2016,** *283* (7), 1197-1217.

Sigma 1 receptor restores dendritic spines of medium spiny neurons in Huntington's disease in vitro model

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Huntington's disease (HD) is an incurable autosomal dominant inherited neurodegenerative disorder caused by accumulation of mutant Huntingtin protein (mHtt). HD is characterized by progressive atrophy of the striatum as a result of degeneration in medium spiny neurons (MSN) which starts with the elimination of postsynaptic protrusions called dendritic spines. While precise link between accumulation of mHtt and selective loss of MSN remains elusive, a lot of evidence implicate that mHtt disturbs Ca^{2+} homeostasis and affect Ca^{2+} regulation in MSN. For instance, mHtt binds to type 1 inositol (1,4,5)-trisphosphate receptor (InsP3R1) and increases its sensitivity to activation by inositol (1,4,5)-trisphosphate which leads to depletion of ER Ca^{2+} level and subsequent dysregulation of Ca^{2+} balance in MSN. This contributes to the cortico-striatal synaptic dysfunction which is a key component of early pathology of HD and morpologically indicated as a decrease in density of dendritic spines. Recent studies suggest sigma 1 receptor (S1R) as a therapeutic target for the treatment of HD. S1R is a resident ER membrane protein involving in the modulation of Ca^{2+} homeostasis and has multiple neuroprotective properties. The aim of the present study is to test the hypothesis that S1R may overcome dendritic spine loss observed in HD MSN. The study was carried out on the cortico-striatal co-cultures established from mouse model of HD (FVB-Tg (YAC128)53Hay/J; Jackson Laboratory). Co-cultures establish from wild-type (WT) mice from the same litter were used as a control. Administration of highly selective S1R agonists prevents spine elimination in HD MSN and restores synaptic connections between cortical and striatal neurons up to the WT level. Moreover, downregulation of S1R significantly decrease spine density in both WT and YAC128 cultures indicating an important role of this protein in calcium regulation and spine stability. Based on these findings, we support the idea that S1R may be considered as perspective target for the treatment of HD.

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Location of the general anesthetics in model membranes

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General anesthetics is a wealthy group of chemical compounds acting on the central nervous system by affecting consciousness and behavior. Aliphatic alcohols and alkanes are one of the most common representants of this family. Despite the profound research devoted to explanation of the mechanism of their acting, the problem has not been figured out satisfactorily. According to a plausible hypothesis, it is believed that anesthetic effect is induced on a molecular level in cell membrane while affecting its functional properties through structural changes [1, 2].
We have prepared and scrutinized model biological membranes consisting of DOPC lipids, loaded with labelled or unlabeled decane molecules at two different concentrations. Employing the small-angle neutron diffraction method, we have reconstructed structural profiles of examined bilayers, identified their structural changes caused by the introduction of decane, and determined the exact location of decane molecules. The plausibility of experimental data evaluation has been corroborated by the system's thermodynamics, and further also by the independent molecular dynamics simulations. The obtained results agree consistently with experimental findings, and support the notion of general anesthetic effect we conclude in our presentation.

1. Mourtisen, Ole G. *Life - as a matter of fat*. Springer, Berlin, Heidelberg. ISSN 978-3-540-23248-3, (2005).
2. Cantor, Robert S. The lateral pressure profile in membranes: a physical mechanism of general anesthesia. *Biochemistry (2339–2344).* **36,** (1997).

Bacteriorhodopsin electronic structure and its modification during *all-trans* → *13-cis* photoisomerisation

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Photoisomerisation of retinal is the fastest reaction in the nature and this is the common reaction for retinal-binding proteins—visual rhodopsins and microbial rhodopsins. Electronic structure defines spectral and kinetic properties of this reaction.
For understanding how electronic structure changes during photoisomerisation, we chose the 3D structure of bacteriorhodopsin (BR) as a commonly used model object. We have done QM/MM analysis of the BR structure before and after illumination (structures are obtained from SFX-studies [1]). We found that the electronic structure of the BR was crucially changing during excitation and electron density has highly shifted to Schiff base for excited state compared to ground state. We suggest that polar aromatic residues Y185 and W86 and W182 share electron density with retinal as a result of their affinity to electron, which is located on retinal. Mutations in the active center, Y185F and Y185F/W182F, impact the ground state delocalization of electron density from the retinal to the protein environment, especially from amino acid residues that has been mutated. These mutations also lead to the shift of the calculated maximum of absorption. Opposite to tyrosine and tryptophan residues, non-polar phenylalanine residues cannot accept electron from retinal and it is the main cause of these changes.
Results of this study will be used for experimental studies based on the recombinant BR expression system in *E. coli* [2]. It could be important for further development of new spectral-shifted forms of retinal-binding proteins and for understanding the mechanism of regulation photoisomerisation of retinal.

The research is carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University [3]. The work is ongoing with the support of RFBR grant 17-00-00167K (KOMFI 17-00-00166).

1. Nakane, Takanori, et al. "Membrane protein structure determination by SAD, SIR, or SIRAS phasing in serial femtosecond crystallography using an iododetergent." *Proceedings of the National Academy of Sciences* 113.46 (2016): 13039-13044.
2. Smitienko, O. A., et al. "Femtosecond and picosecond dynamics of recombinant bacteriorhodopsin primary reactions compared to the native protein in trimeric and monomeric forms." *Biochemistry (Moscow)* 82.4 (2017): 490-500.
3. Sadovnichy, V., et al. AT (2013)."Lomonosov": supercomputing at Moscow State University." *Contemporary High Performance Computing: From Petascale toward Exascale* (2013): 283-307.

SANS investigations of biological objects on a YuMO spectrometer: results and possibilities

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Structural studies play a key role in soft matter research, due mostly to the crystallography realized on synchrotron beam lines. Small-angle neutron scattering on the other hand is appealing to structural investigations of hydrogen-rich matter because of special features of neutrons. In particular, there are differences both in the size and in the sign of scattering length density for hydrogen and deuterium that allow for employing the labeling and/or contrast variation methods.
Here, we present some results and possibilities of the "YuMO" small-angle neutron scattering spectrometer (IBR-2, JINR, Dubna, Russia) [1], such as structural behavior of lipid membranes under different conditions (temperature, pressure, humidity), structural studies of complex lipid membranes, and their additional components including bacteriorhodopsin, DNA, various viruses [2], phycobiliproteins of the cyanobacterium *Acaryochloris marina* [3], and other proteins to name but a few. Useful information about data treatment and experimental approach as well as examples using SANS facility for soft matter will be given [4, 5].

1. Kuklin, A., Islamov, A. & Gordeliy, V. Scientific reviews: two-detector system for small-angle neutron scattering instrument. *Neutron News.* **16(3),** (2005).
2. Zabrodskaya, Y. *et. al.* The amyloidogenicity of the influenza virus PB1-derived peptide sheds light on its antiviral activity. *Biophysical chemistry.* **234,** (2018).
3. Golub, M. *et. al.* Solution structure and excitation energy transfer in phycobiliproteins of Acaryochloris marina investigated by small angle scattering. *Biochimica et Biophysica Acta (BBA)-Bioenergetics.* **1858(4),** (2017).
4. Zabelskii, D. *et al.* Ambiguities and completeness of SAS data analysis: investigations of apoferritin by SAXS/SANS EID and SEC-SAXS methods. *Journal of Physics: Conference Series.* **Vol. 994, No. 1,** (2018).
5. Kuklin, A. *et. al.* Neutronographic investigations of supramolecular structures on upgraded small-angle spectrometer YuMO. *Journal of Physics: Conference Series* **Vol. 848, No. 1,** (2017).

DDM solubilization-based protocol for isolation and purification of spinach chloroplast ATP synthase

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Multisubunit membrane protein ATP synthases play a critical role in the bioenergetic cycle of all organisms. A number of genetic and aging-related diseases are associated with mutations in ATP synthase genes or a partial loss of the dimeric integrity of the complex [1, 2]. A clear understanding of structure-function dependences may suggest possible ways of treatment. Despite the fact that tremendous effort in structure determination for the ATP synthase protein family was made, function-related questions remain so far partially unanswered [3, 4]. One of the possible reasons for doubt is using strong detergents for solubilization, which can significantly affect the integrity and functionality of the complex.
Here, we present a high-yield protocol for isolation and purification of spinach chloroplast ATP synthase which uses only a mild DDM detergent for solubilization and stabilization of the complex, a multistep ammonium sulfate salting out procedure, and size exclusion chromatography as purification steps. Mass spectrometry and SAXS approaches were used to validate the integrity and purity of the complex.
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1. R. Kucharczyk *et al.*, Mitochondrial ATP synthase disorders: molecular mechanisms and the quest for curative therapeutic approaches. *Biochim. Biophys. Acta - Mol. Cell Res.* **1793** (2009), pp. 186–199.
2. N. G. Rampello, M. Stenger, B. Westermann, H. D. Osiewacz, Impact of F1Fo-ATP-synthase dimer assembly factors on mitochondrial function and organismic aging. *Microb. cell (Graz, Austria)* (2018), https://doi.org/10.15698/mic2018.04.625.
3. P. Turina, D. Samoray, P. Gräber, H+/ATP ratio of proton transport-coupled ATP synthesis and hydrolysis catalysed by CF0F1-liposomes. *EMBO J.* (2003), https://doi.org/10.1093/emboj/cdg073.
4. A. Hahn, J. Vonck, D. J. Mills, T. Meier, W. Kühlbrandt. Structure, mechanism, and regulation of the chloroplast ATP synthase. *Science* (2018), https://doi.org/10.1126/science.aat4318.

ATR-FTIR spectroscopy reveals spectral features of exosomal membrane valuable for drug delivery systems development

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Exosomes are naturally produced membrane-derived vesicles. They may be found almost in any biological fluids; therefore, their toxicity is minor. Nowadays, exosomes have begun to be explored for use as drug delivery systems able to increase circulation time in blood and biocompatibility of compounds [1]. Due to their structure, they can transfer hydrophobic, hydrophilic, and amphiphilic drugs. Additionally, exosomes could facilitate drug targeting and help to avoid immune response.
The aim of this work is to study spectral properties of exosomal membrane in order to find out any unusual features able to become an analytical valuable. FTIR spectroscopy seems to be an adequate and useful method for exosomal structure investigation. As a model for first studies, we used the so-called synthetic exosomes. These are liposomal vesicles with addition of BSA protein. We have successfully obtained linear dependence between protein-lipid content of synthetic exosomes and peak integral intensity in IR spectra. Utilizing the dependence, it is possible to determine protein-to-lipid ratio in natural exosomes. In the next step, we studied a trigger drug release in low-frequency alternative magnetic field, using complexes of exosomes with magnetic nanoparticles. These particles, being attached to the surface of the lipid membrane, can oscillate in magnetic field changing its permeability; thus, the drug incorporated in the exosome can easily

be released. Such an effect has been already demonstrated for liposomal complexes with magnetic nanoparticles [2]. We have investigated changes in bilayer fluidity using the combination of FTIR spectroscopy and classical fluorescent spectroscopy. BODIPY-fluorescent label B9PPC was added into exosomes in advance. It is known that lipid membrane loosening could be evidenced by decrease of fluorescent polarization signal. We have obtained complex dependence between fluorescent polarization signal and exposure time in magnetic field. We are able to claim for sure that exosome membrane could be loosed by magnetic nanoparticles after exposure in magnetic field.
This research is supported in part by RSF-14-13-00731 grant.

1. Kim, M.S. Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. *Nanomedicine Nanotechnology, Biol. Med.* **12,** 655–64 (2016).
2. Kutsenok, E.O. *et al.* The study of the influence of low-frequency alternative magnetic field on the complexes of liposomes with magnetic nanoparticles by fluorescent methods. *Bionanotox.* Crete, Greece (8-15 May 2016).

Refinement of molecular complexes using geometrical constraints

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Molecular structure refinement methods aim to optimize conformations of molecules in a complex with respect to each other. The optimization algorithms are used to get rid of possible steric clashes and to take into account flexibility of molecular complexes [1]. However, due to the extremely rugged shape of the energy landscape, there is a problem of precise algorithm settings. As a consequence, algorithm tuned for one specific energy function might not be working well with the other energy functions. Here, we present steady and fast rigid body refinement approach, which works with arbitrary force fields and does not require any parameter tuning with respect to the energy function. To circumvent the "curse of energy landscape," we determine geometrical constraints in the conformational space during the optimization process. These constraints are defined using rapid calculations of the root-mean-square deviation, as a distance metric in the conformational space [2, 3]. We applied our method for molecular complexes of various types and demonstrated its efficiency using both classical force fields, e.g., Lennard-Jones or CHARMM, as well as statistics-based scoring functions [4, 5]. We showed that due to the geometrical constraints, our method remains stable and successfully resolve soft, moderate, and large steric clashes in molecular complexes. The refinement method will be made available as a SAMSON State Updater of the SAMSON software platform at http://www.samson-connect.net.

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1. Mirzaei, Hanieh, et al. "Rigid body energy minimization on manifolds for molecular docking." *Journal of chemical theory and computation* 8.11 (2012): 4374-4380.
2. Neveu, Emilie, et al. "RapidRMSD: rapid determination of RMSDs corresponding to motions of flexible molecules." *Bioinformatics* 1 (2018): 9.
3. Popov, Petr, and Sergei Grudinin. "Rapid determination of RMSDs corresponding to macromolecular rigid body motions." *Journal of computational chemistry* 35.12 (2014): 950-956.
4. Popov, Petr, and Sergei Grudinin. "Knowledge of native protein–protein interfaces is sufficient to construct predictive models for the selection of binding candidates." *Journal of chemical information and modeling* 55.10 (2015): 2242-2255.
5. Neveu, Emilie, et al. "PEPSI-Dock: a detailed data-driven protein–protein interaction potential accelerated by polar Fourier correlation." *Bioinformatics* 32.17 (2016): i693-i701.

The role of hydrodynamic forces on nuclear pore assembly

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In current models of nuclear pore (NP), the participation of DNA and lipids is not assumed. The task of this study was to select ternary complexes (TC): zwitterionic liposomes with the addition other lipids, DNA, and Mg^{2+} to create holes in a double lipid bilayer similar to NP but without proteins, which are believed basic constructional element in modern models of NP. The creation of pores between spaced lipid bilayers is the main obstacle of such models, but this problem is easy to overcome in our model.
In the system, large unilayer vesicles (LUVs)+DNA+Mg^{2+} inside giant liposome (GUV)> 10 μm in diameter, there are many places where TC locates near the double lipid bilayer. As one bilayer, there may be a lipid bilayer of LUV1 containing TCs. The second bilayer of another liposome—LUV2 (1–5 μm in diameter)—could be inside GUV. Another case was when TC is located inside GUV and LUV2 is outside GUV, but near to the TC. Liposomes of such size are more convenient to observe by a visible microscope. By this method, we obtained the formation of short-term channels between the TC and the liposome outside of the GUV but close to the TC. In addition, we observed emissions of particles containing DNA from GUV, resulting in the movement of the GUV on the distance at a multiple of their size. This indicates that a high energy of fusion between TC and the lipid bilayer is unleashed in this process. Nuclear pores serve as sites of initiation of transcriptions in a cell, because ssDNA is the best site of transcription initiation. Not all TC reach nuclear membrane and transform into pores; many of them located far from the nuclear envelope also enhance transcription. The TC can form aggregates that are the basis "transcription factories."

Glycophorin A transmembrane helices dimerization: the role of the lipid environment

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Transmembrane (TM) α-helical domains are common structural elements of membrane proteins. They play an important role in functioning of membrane receptors and ion channels, so protein-protein interactions in the lipid environment are involved into the functioning of the most vital membrane systems. It is known that lipid environment properties modulate the activity of receptor tyrosine kinases and other proteins [1]. However, the molecular details of this process are still not clear. Glycophorin A here represents a model of a very stable TM dimer. Its TM domain is well studied, including the effect of several point mutations. In the present work, we study the behavior of monomers and dimers of human glycophorin A TM domain and its two mutant forms, G83A and T87V, in a lipid environment using molecular dynamics simulations. The comparison of free energy profiles, residual contributions, and lipid environment properties was done. It was found that the dimerization displays prominent entropic character, and the membrane plays an important role in the association of TM α-helices of glycophorin A. We conclude that selected mutations utilize two different mechanisms of the dimer weakening: T87V strongly changes protein-protein contacts making impossible hydrogen bonding between monomers, and G83A slightly

changes dimer geometry resulting in non-favorable protein-lipid contacts coupled with the redistribution interfacial protein-protein interactions [2]. It was also shown that both monomers and dimers of all three forms of glycophorin A have lipid binding sites in the hydrophobic region of the bilayer. These interactions with lipid acyl chains are involved in the process of TM domains interactions, as we found that monomers "feel" each other in terms of changes of interaction free energy on large distances when there is no direct protein-protein contacts. Thus, the lipid membrane plays an active role in dimer formation.
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1. Bocharov E.V. *et. al.* Helix-helix interactions in membrane domains of bitopic proteins: specificity and role of lipid environment. *Biochim. Biophys. Acta.* **1859**(4), 561-576, (2017).
2. Kuznetsov A.S., Volynsky P.E., Efremov R.G. Role of the lipid environment in the dimerization of transmembrane domains of glycophorin A. *Acta Naturae*, **7**(4), 122-127 (2015).

The pattern of *Prkcz* gene expression is regulated epigenetically and depends on cellular identity

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Realization of memory mechanisms requires synergic functioning of plenty intracellular molecular systems, but until recently, neither of them was shown to be selectively involved in the learning process only. However, in the last decade, the neuron-specific product of *Prkcz* gene (protein kinase Mζ) was characterized as the necessary and sufficient molecule for memory formation and maintenance. Multi-promoter organization of *Prkcz* gene enables to selectively transcribe either short (PKMζ) or long (PKCζ) isoform depending on the tissue type. However, the precise mechanism of the region-specific *Prkcz* expression is still uncharacterized.
The main idea of this study was to uncover and extend the principles and mechanisms of tissue-specific expression of *Prkcz* gene. Initial experiments were carried out on the PC12 cell line derived from rat pheochromocytoma that can be easily differentiated either into chromaffin cells or cells with a neuron-like phenotype at various conditions. Using real-time PCR method, we compared the expression levels of short neuron-specific PKMζ transcripts and long non-neuronal PKCζ transcripts in the differentiated cell cultures. The presence of both transcripts was detected in the naïve cell cultures. Under continuous (5 days) treatment with nerve growth factor (NGF), the PC12 cultures underwent transformation into cells with a neuron-like phenotype. Analysis of gene expression revealed a significant enhancement of the PKMζ transcript quantity in the differentiated cell cultures. The number of non-neuronal PKCζ transcripts did not change after NGF treatment. In contrast, application of dexamethasone (Dex) triggered differentiation of PC12 into chromaffin cells similar to those in adrenal glands. Morphological changes induced by Dex were accompanied by selective enhancement of the PKCζ transcripts quantity rather than the PKMζ. Consistent with the previous studies, our data demonstrate that pattern of the *Prkcz* gene expression can be determined by cellular identity. It is widely accepted that cell type-specific gene expression programs are coupled with epigenetic modifications of chromatin. Therefore, we assumed that expression of the *Prkcz* isoforms can be regulated epigenetically. To test this, the mature primary cultures of rat cortical neurons that consistently expressed only the PKMζ isoform were treated with a histone deacetylase inhibitor trichostatin A (TSA). We observed that the TSA-induced histone hyperacetylation downregulated the expression of the neuronal PKMζ and triggered massive production of a non-neuronal PKCζ transcript.
Together, our data may indicate existence of the *Prkcz* promoter competition driven by epigenetic rearrangements that requires further investigation.
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Neonatal proinflammatory stress induces selective increase in IL-6 expression in adult rat hippocampus

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Early stress is believed to underlie depressive disturbances in adults. Modeling effects of early stress on the development of depressive behavior in rats is a relevant experimental approach to study pathogenesis of affective diseases. Recently, we have shown that 3-month-old Wistar rats neonatally injected with Gram-negative bacteria lipopolysaccharide (LPS, days 3 and 5) demonstrate depressive-like behavior and disturbances in hippocampal synaptic plasticity [1, 2]. However, the mechanisms of the neonatal proinflammatory stress (NPS) effects remain obscure. Clinical studies report that increase in proinflammatory mediators, in particular IL-6, in the blood of patients is a biomarker of major depression. In addition, disturbances, the main neuroendocrine route of stress response, are seen in depressive patients.
This study aimed at the effects of NPS and "behavioral stress" (forced swim tests, FST) on the expression of genes associated with neuroinflammation (IL-1b, IL-6, TNFa, Sall1, fractalkine (CX3CL1) and its receptor (CX3CR1)) and stress response (corticotropin-releasing hormone (CRH) and its receptors (CRHR1, CRHR2), glucocorticoid (GR) and mineralocorticoid receptors (MR)) in the hippocampus and neocortex of male Wistar rats. NPS as well as FST stimulated expression of IL-6 mRNA and increase of its protein level in the hippocampus. Notably, expression of TNFa mRNA decreased after FST in the hippocampus of the rats subjected to NPS, while a decline in MR mRNA expression was demonstrated in the neocortex of NPS rats.
The data suggest that NPS induces a hippocampus-specific development of chronic neuroinflammation in adult rats expressing depression-like behavior which may underlie disturbances of hippocampal synaptic plasticity and behavioral abnormalities in adult animals. Chronic changes in functioning of the hypothalamo-pituitary-adrenal axis may also contribute to the development of depressive features.
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1.Tishkina, A. *et al.* Neonatal proinflammatory challenge in male Wistar rats: effects on behavior, synaptic plasticity, and adrenocortical stres sresponse. *Behav. Brain Res.* **304,** 1–10 (2016).
2.Onufriev, M. V. *et al.* Neonatal proinflammatory stress induces accumulation of corticosterone and interleukin-6 in the hippocampus of juvenile rats: potential mechanism of synaptic plasticity impairments. *Biochem.* **82,** 275–281 (2017).

Compensatory upregulation of neurotrophin signaling rescues neuroblastoma cells from death after KIT knockdown

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Receptor tyrosine kinase KIT is expressed by considerable number of neuroblastoma (NB) cells and it is also expressed by several other cancers. KIT-positive NB cells form an aggressive subset of tumor cells. KIT inhibition is considered as a promising approach for NB treatment. Still, the major problem of targeted anti-cancer therapy is acquisition of drug resistance by malignant cells. Here, we report that KIT knockdown by small hairpin RNA in model NB cells resulted in inhibition of proliferation and induction of apoptosis. Signaling pathways analysis based on genome-wide microarray gene expression data revealed that in NB cells, growth-related pro-survival pathways are activated. Amongst others, there was an increase in expression of neurotrophins (NGF and BDNF) and their receptors. We showed that exogenous neurotrophins rescued NB cells from apoptosis and partially restored their proliferation. We identified ERK2 as the major component of compensatory signaling. Inhibition of ERK2 in KIT-downregulated cells resulted in enhanced cell death. Overall, we show how neuroblastoma cell can compensate KIT signaling downregulation by employing other pro-survival pathways.

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The prove of Na,K-ATPase and Src kinase direct interaction and the role of glutathionylation in complex formation

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Na,K-ATPase maintains the homeostasis of Na^+ and K^+ in animals. Na,K-ATPase is redox sensitive and can be glutathionylated under hypoxia and ischemia. Glutathionylation inhibits hydrolytic activity of the enzyme. Na,K-ATPase is also the receptor for cardiotonic steroids, in particular, ouabain. One of the main ways of signal transmission upon binding of ouabain is the activation of Src kinase. Src is supposed to form a functional complex with Na,K-ATPase, though direct interaction of these proteins has not been shown yet. In complex with Na,K-ATPase, Src is inactive, but it gets activated upon binding of ouabain to Na,K-ATPase [1].

Earlier, in SC-1 murine fibroblast and human embryonic kidney HEK293 cells, we observed that not only binding of ouabain, but also exposure to hypoxia results in the activation of Src, while binding of ouabain under hypoxia, in contrast, leads to Src inactivation. We supposed that these effects could be caused by changes in the receptor function of Na,K-ATPase under hypoxia as a consequence of Na,K-ATPase glutathionylation [2].

In the current work, we investigated the influence of glutathionylation on the receptor function of Na,K-ATPase. First, with isothermal titration calorimetry using Na,K-ATPase purified from duck salt glands, we compared the ability of non-glutahionylated and glutathionylated Na,K-ATPase to bind ouabain and did not find any significant difference. Next, we checked if glutathionylation of Na,K-ATPase could alter its interaction with Src. For the first time, we could observe the direct interaction between Src and Na,K-ATPase. Using the microscale thermophoresis (MST) approach, we have shown that NT-647-labeled human recombinant Src forms a complex with Na,K-ATPase purified from duck salt glands with K_d of 0.2 μM, while the presence of oxidized glutathione (GSSG) in the buffer prevents the protein complex formation. We have also measured the activity of Src in complex with Na,K-ATPase. Keeping Src concentration constant and varying Na,K-ATPase concentration, we found that upon rising of Na,K-ATPase concentration, Src activity was also growing. Adding of GSSG to the reaction mixture resulted in significant increase in Src activity, suggesting that glutathionylation of cysteine residues located in the interface of proteins' interaction leads to disturbance of Src binding to Na,K-ATPase that could explain the change in receptor function of Na,K-ATPase in hypoxia.

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1. Cui, X. & Xie, Z . *Molecules*. (2017). https://doi.org/10.3390/molecules22060990.
2. Petrushanko, I. et al. *Redox Biol.* (2017). https://doi.org/10.1016/j.redox.2017.05.021

Liposomal form of fluoroquinolones: how to control physico-chemical properties?

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Development of drug delivery systems is one of the most important tasks of current state-of-art in biopharmaceutics. New active molecules able to fight with severe diseases, e.g., cancer and infectious treatment, usually possess limited bioavailability and a number of side effects [1]. One of the perspective approaches to overcome these drawbacks is to incapsulate active molecule into liposomes, lipid vesicles with membrane structure.

In current research, we have studied ways to control physico-chemical properties of liposomal form of two fluoroquinolones: levofloxacin (LLev) and moxiflocan (LMox). Both of these drugs are widely used in therapy of different infections including tuberculosis and ophthalmologic infections [2]. However, low bioavailability in sites of inflammation motivates to use liposomal drug delivery systems.

We have considered two approaches: via changing the liposomal composition (in a hope to increase drug loading efficacy) and via surface functionalization by chitosan derivatives (in a hope to stabilize the system in suspension, to vary total ζ-potential and size of vesicles, and to modulate drug-release kinetics). The methodology of the study included UV spectroscopy, ATR-FTIR spectroscopy, and DLS method, as one of the most informative for liposomal drug formulation [3].

We have found that 20% of cardiolipin in liposome composition increase drug loading to 90% in the case of active loading strategy (VS liposome 100% DPPC). As chitosan derivatives, we have used PEG-chitosan with MW from 5 to 90 kDa and degree of modification from 5 to 25%. Complex formation leads to growth of hydrodynamic radius of vesicles and neutralization of ζ-potential. ATR-FTIR spectroscopy data reveals main binding sites, phosphate and carbonyl groups of lipids, and investigate drug location in membrane. Changes in membrane depend on membrane composition and polymer composition.

Complex formation with chitosan derivatives, as we found, modulates drug release: in 0.02 M PBS solution pH 7.4, release is significantly prolonged and in 0.02 M sodium acetate buffer solution pH 5.5, release is accelerated.

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1. Russell, D. G. *Mycobacterium tuberculosis*: here today, and here tomorrow. *Nat. Rev. Mol. Cell Biol.* **2,** 569–77 (2001).
2. Le-Deygen, I. M., Skuredina, A. A. & Kudryashova, E. V. Drug delivery systems for fluoroquinolones: new prospects in tuberculosis treatment. *Russ. J. Bioorganic Chem.* **43,** 487–501 (2017).
3. Deygen, I. M. *et al.* Novel prodrug of doxorubicin modified by stearoylspermine encapsulated into PEG-chitosan-stabilized liposomes. *Langmuir* **32,** 10861–10869 (2016).

Sodium nitroprusside increases the activity of rat trigeminal nerve

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Nitric oxide (NO) is an endogenous gaseous messenger molecule, involved in a variety of physiological and pathological processes [2]. NO controls physiological functions through cellular and molecular mechanisms, neurotransmission and vascular tone, regulates steps synthesis of proteins, and produces their post-translational modifications [1]. The interaction between NO and superoxide anion ($O2^{-}•$) produce the potent oxidant peroxynitrite ($ONOO^{-}$) that can lead to oxidative damage, nitration, and *S*-nitrosylation of biomolecules including proteins, lipids, and DNA [1]. Moreover, participation of NO in the pathology of many forms of primary headaches (including migraine) was shown [2]. The aim of this work was to study the effects of the NO donor—sodium nitroprusside (SNP)—on the activity of the rat primary afferents of the trigeminal nerve. Experiments were performed at the trigemino-vascular system of rats (P35-45) where isolated hemiskull with intact dura mater, dural vessels, and nerve, was used which allowed to study molecular mechanisms underlying first steps of a headache [3]. In control conditions, trigeminal nerve displays spontaneous action potentials which were recorded extracellularly with suction electrode. SNP in low concentration (100 mkM) does not induce significant effects on the activity of the trigeminal nerve—the frequency of action potentials was $28.5 \pm 2.8\%$ compared to that of control (n=3). Two hundred mkM of SNP leads to the significant increase of the action potential frequency up to $279.3 \pm 54.7\%$ compared to that of the control ($n = 4$, $p < 0.05$). An inactivated SNP with light does not change the activity of the trigeminal nerve ($88.5 \pm 23\%$ of control; $n = 3$). The obtained data indicated the involvement of the NO in activation of the trigeminal nerve, which may underlie the pathophysiology of a migraine.

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1. Förstermann, U. & Sessa, W. *Nitric oxide synthases: regulation and function. European heart journal* (2012). https://doi.org/10.1093/eurheartj/ehr304
2. Pradhan, A. et. al. *Targeted nitric oxide synthase inhibitors for migraine. Neurotherapeutics* (2018). https://doi.org/10.1007/s13311-018-0614-7
3. Shatillo A., et. al. *Cortical spreading depression induces oxidative stress in the trigeminal nociceptive system. Neuroscience* (2013). https://doi.org/10.1016/j.neuroscience.2013.09.002

Anomalous diffusion in complex heterogeneous media

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Earlier studies [1, 2] showed that the anomalous transport mode may occur in dense membranes, for example, particle transport in living cells. For observing trajectories of labeled particles, fluorescent correlation microscopy is commonly used; it is the so-called single-particle tracking experiments. The mean square of the particle displacement obtained from the experiment shows the deviation from the normal Gaussian diffusion regime. Typically, such diffusion mode that occurs due to the complex cellular organization, however, is not limited to it. To consistently construct a model describing this effect, it is necessary to take into account the following factors: the heterogeneity of the medium, the mechanisms of movement in each phase of this medium, the times at which observation occurs, and so on. Earlier, it was shown that such an approach can be realized by the Monte Carlo method using the CTRW (continuous time random walk) model. The transport of a point-like particle in a disordered two-dimensional medium with traps was studied for the case of a diffusion-ballistic regime. The particle hopping distributions thus obtained belong to the class of stable distributions, which corresponds to the regime of anomalous diffusion. Furthermore, it was shown that the characteristic parameters of this distribution depend on the distribution of traps, as well as the velocity of the particles trapped.
Since the formulation of a similar problem in the one-dimensional case admits an exact analytic solution, we use it for qualitative comparison and verification of simulation results. The corresponding Fokker-Planck equation was obtained, the distribution of the probability density of particle detection was found, and the distribution by particle displacements was calculated. Thus, one of the possible mechanisms of particle movement in traps was revealed, leading to an abnormal transfer regime. Such a conclusion agrees qualitatively with the results of analytical calculations in the one-dimensional case.

1. Javanainen M. *et al.* Anomalous and normal diffusion of proteins and lipids in crowded lipid membranes. *Faraday Discussions (161)*, **397**, (2013).
Campagnola, G. *et al.* Superdiffusive motion of membrane-targeting C2 domains. *Sci Rep,* **5**, 17721 (2015).

Toward the first orphan GPCR structure

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G protein-coupled receptors (GPCRs) are the most common targets of the neuropharmacological drugs in the central nervous system (CNS). Until now, there are still ~120 GPCRs with unknown endogenous ligands and are thus classified as orphan GPCRs. With high potential of being novel therapeutic targets, drug discovery toward this group of GPCRs is hampered by the lack of structural understanding and the limited knowledge of endogenous ligand. To uncover the atomic-resolution structure of an orphan GPCR and to provide hint for deorphanization, we performed structural analysis using x-ray crystallography, along with ligand/probe discovery. Through iterative construct design and optimization, we have obtained diffraction-quality crystals. We also identified several hit ligands and antibody that can be developed as probe for deorphanization.

Markers of gene expression imbalance for the model of Down syndrome in vitro

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Down syndrome (DS) is the most common form of human genetic disorder. In most cases, it is caused by the presence of an extra 21st chromosome. This disease causes a wide range of pathologies, such as cognitive development disorder, congenital heart disease, predisposition to myeloid leukemia, or Alzheimer’s disease [1]. In order to investigate the mechanisms of the onset of these pathologies, relevant cell models are also needed. One of such relevant models is induced pluripotent stem cells (IPSC) from individuals with DS, which can be differentiated into neural direction.

Development of genome editing technologies, such as CRISPR/Cas9 system, allows us to investigate some important genes on the 21st chromosome (RCAN1, DYRK1A, RUNX1, etc.) which can play a key role in the generation of gene expression imbalance. However, this requires the development of an independent system for quantifying the level of gene expression imbalance [2].
In this study, we analyzed several transcriptomes of IPS-derived neurons (4 from DS individuals and 4 from normal karyotype), IPSCs (2 from DS individuals and 2 from normal karyotype), and fibroblasts (2 from DS individuals and 2 from normal karyotype). Then, we chose 16 genes with differentiated expression shown by the transcriptome analysis and tested their expression level in several independent IPSC lines by qPCR. As a result, we identified at least five genes (DNMT1, GINS2, MCM2, MCM5, MCM7) with confident differences between cells derived from DS and normal karyotype groups. These genes happen to take part in transcription regulation processes. We suppose that this will help us to study mechanisms for Down syndrome in the future.
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1. R.L.Webb and M.P. Murphy. B-Secretases, Alzheimer's disease, and Down syndrome, Current Gerontology and Geriatrics Research, 2009.
2. Antonarakis S.E. Down syndrome and the complexity of genome dosage imbalance // Nature Reviews Genetics. 2017. Vol. 18, № 3. P. 147–163.

Spider toxin inhibits gating pore currents in mutant sodium channels

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Excitability in the nervous system and muscles is based on the functioning of voltage-gated sodium channels. These integral membrane proteins contain two domains with discrete functionalities. The central pore domain (PD) is responsible for current flow and ion selectivity, while voltage-sensing domains (VSDs) are necessary for the voltage dependence of channel activation and inactivation. However, in some cases, an aberrant leak current through the VSD occurs. This current is triggered by certain mutations in VSDs and is known as "gating pore current". Hypokalemic periodic paralysis (HypoPP) characterised by episodes of muscle weakness due to the inactivation of sodium channels in skeletal muscle fibres is associated with gating pore currents [1]. We have shown recently that Hm-3 toxin from the crab spider *Heriaeus melloteei* can inhibit gating pore currents through VSD-I of mutant skeletal muscle voltage-gated sodium channel $Na_V1.4$ associated with HypoPP [2]. We suggest that similar toxins, so-called gating modifiers, can also be efficient against gating pore currents through different VSDs. To test this possibility, we produced several spider toxins in a prokaryotic expression system and tested them on mutant sodium channels.
Synthetic genes encoding spider toxins were cloned into a bacterial expression vector. Toxins were produced as fusion proteins with thioredoxin in *Escherichia coli*. The proteins were separated by affinity chromatography and cleaved by cyanogen bromide to release the toxins from thioredoxin; purification was achieved through reversed-phase HPLC. Recombinant spider toxins were tested on $Na_V1.4$ channels with mutations in VSDs provoking gating pore currents. Channels were expressed in *Xenopus laevis* oocytes and ion currents were recorded using the two-electrode voltage clamp technique. As a result, we report here that PaurTx-3 toxin from the tarantula *Paraphysa scrofa* inhibits gating pore currents through VSD-II of $Na_V1.4$ associated with periodic paralysis.
This work was funded by the Molecular and Cell Biology Program of the Russian Academy of Sciences.

1.Matthews, E. *et al.* Voltage sensor charge loss accounts for most cases of hypokalemic periodic paralysis. *Neurology* **72,** 1544–1547 (2009).
2.Männikkö, R. *et al.* Spider toxin inhibits gating pore currents underlying periodic paralysis. *Proc. Natl. Acad. Sci.* 201720185 (2018). https://doi.org/10.1073/pnas.1720185115

Smart ligand-sensitive monolayers based on modified liposomes for biomedical applications

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Liposomes are well-known nanoagents for many applications such as modelling of membrane proteins and targeted drug and gene delivery [2, 3]. However, modern biomedical techniques require design of nanocomplexes with adjustable characteristics such as ligand sensitivity or modulated affinity [1]. In this work, we studied feasibility of using liposomes to form controllable ligand-sensitive monolayers.
To produce specific and stable nanoagents, we varied a number of liposomal properties such as size, membrane lipid composition, and concentration. In addition, to facilitate liposome detectability, we load liposomes with fluorescent and magnetic markers. Also, to investigate affine interactions between the liposomes and biomodified surfaces, we conjugated liposomes with various biomolecules including biotin and folic acid.
The polysterene surfaces were coated with different adsorbed proteins to bind modified liposomes in order to model various biosurfaces including colloidal ones. In particular, 96-well plate surfaces, polystyrene-coated magnetic and nonmagnetic nanoparticles were modified with proteins in order to vary an effective area of model surfaces.
Our studies have resulted in improvement of monolayer key features such as specificity, self-assembly, and ligand-sensitive disassembly due to high adjustability of liposomal properties. As a result, modified liposomes appeared to be promising nanoagents to form smart biolayers and therefore to be used in a production of advanced nanomaterials.

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1.Nikitin M. P., Shipunova V. O., Deyev S. M. & Nikitin P. I. Biocomputing based on particle disassembly. *Nat. Nanotechnol.* **9,** 716–722 (2014).
2.Verchère A., Broutin I. & Picard M. Reconstitution of membrane proteins in liposomes. *Methods Mol. Biol.* **1635,** 259–282 (2017).
3.Daraee H., Etemadi A., Kouhi M., Alimirzalu S. & Akbarzadeh A. Application of liposomes in medicine and drug delivery. *Artif. Cells, Nanomedicine Biotechnol.* **44,** 381–391 (2016).

Production and structural study of the toll-like receptor 1 cytoplasmic domain

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Currently, special attention is paid to transmembrane proteins, such as ion channels and membrane receptors. From an applied point of view, membrane proteins (MP) are of interest as targets for medicines and as objects for biotechnological development.
The family of toll-like receptor (TLR) refers to the first type of membrane proteins. TLRs play a critical role in innate immunity as the first line of

host defense. The medical and biological significance of the TLR signaling is obvious, since the dysregulation of the TLR system causes various autoimmune diseases and septic shock, and some therapeutic strategies targeting TLRs have already emerged. Despite the fact that the general scheme of the operation of TLRs was known and even there are structures of individual fragments for some proteins of this family, the detailed mechanism of signal transmission inside the cell remains unclear. To date, structural problems were solved using methods of cryoelectron microscopy, X-ray diffraction analysis, and NMR spectroscopy, but NMR studies in environments simulating the membrane allow getting information about the structure of the protein, its mobility, and changes directly in the process of interaction with other proteins or ligands. With such data, it is possible to construct an accurate model of MP functioning and describe the process of signal transmission into the cell.
This work presents result of bacterial expression, purification, and assignment of NMR spectra of the intracellular domain of TLR1. The obtained data serves as a starting point for studying the mechanism of signal transmission inside the cell when TLR is activated.

2. Gushchin, I. & Gordeliy, V. *Microbial rhodopsins. Membrane protein complexes: structure and function* (2018). https://doi.org/10.1002/9783527610754
3. Friedrich, T. & Geibel, S. *et al.* Proteorhodopsin is a light-driven proton pump with variable vectoriality. *J. Mol. Biol.* 321, 821-838 (2002). https://doi.org/10.1016/S0022283602006964

Characterization of rhodopsin from Antarctic UV-resistant bacteria *Hymenobacter* sp. (LR1)

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Metagenomics revealed great number of putative rhodopsins. Several of them were characterized and proved to function as effective optogenetic tools. The putative microbial rhodopsin LR1 from *Hymenobacter* sp. was chosen for characterization [1] basing on the unusual properties of its recently discovered host bacterium, which is resistant to the cold Antarctic environment and UV radiation.
LR1 belongs to the class of proteorhodopsins and demonstrates high similarity with KR1, blue proteorhodopsin, and green proteorhodopsin, which function as outward proton pumps. The sequence alignment of LR1 revealed unique amino acids E77 and D88 corresponding to proton acceptor and proton donor in bacteriorhodopsin from *Halobacterium salinarum* at the D85 and D96 positions [2], respectively.
The *E. coli* expression system was used for LR1 expression. The expressed protein is stable; the yield of LR1 is allowed the further in vitro functional tests and crystallization. The spectrum of LR1 has two absorbance peaks in the visible area at 390 and 520 nm. The ratio of absorbance at 390 and 520 nm depends on the pH level and the concentration of sodium chloride. According to the SEC results, protein undergoes reversible oligomerization in response to the changes of its concentration and the pH level of the buffer solution. The photocycle of LR1 is significantly slower than that of characterized proteorhodopsins [3] with length of ca. 100 s for the sample of solubilized LR1 at 20 °C (pH 7.5, 100 mM NaCl) and more than ca. 100 s for protein reconstituted into asolectin liposomes at 10 °C (pH 7.5, 100 mM NaCl). The light-induced function of LR1 was not detected yet. The method of black lipid membrane did not reveal any active ion transport function. Several LR1 crystals were obtained, and the diffraction data set up to 5-Å resolution was collected. This work is supported by 16-15-00242 RSF.

1. Lyubaykina, N. & Okhrimenko, I. *et al.* Heterologous expression and purification of rhodopsin from radioresistant Antarctic bacterium. *FEBS Journal* 284, 251-252 (2017). https://doi.org/10.1111/febs.14174

Recombinant analogue of human membrane-tethered protein Lynx1 abolishes the impairment of neuronal plasticity caused by Aβ_{1-42} and JNK activation

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels associated with cognitive processes such as memory, attention, and learning. Many neurodegenerative diseases are accompanied with a cognitive impairment connected with dysfunction of nAChRs. Human membrane-tethered prototoxin Lynx1 modulates nAChRs in the brain areas important for learning and memory. Previously, we showed that the expression level of endogenous *Lynx1* in the cortex of transgenic 3xTg-AD mice modelling Alzheimer disease is decreased in comparison with that of wild-type mice [1]. Here, we demonstrate that β-amyloid peptide Aβ_{1-42} decreases mRNA expression of endogenous *Lynx1* in primary cortical neurons, and this diminishing is associated with an activation of c-Jun N-terminal kinase (JNK). Additionally, we show that *Lynx1* expression decrease, as well as blockade of long-term potentiation, underlying cellular mechanisms of synaptic plasticity, caused by Aβ_{1-42} may be abolished by incubation with water-soluble recombinant analog of Lynx1. Data obtained point to water-soluble Lynx1 as a promising tool for amelioration of cognitive processes in neurodegenerative diseases.

The work was supported by the Russian Science Foundation (project № 16-14-00102).

1. Thomsen M.S., Arvaniti M., Jensen M.M., Shulepko M.A., Dolgikh D.A., Pinborg L.H., Härtig W., Lyukmanova E.N., Mikkelsen J.D. *Lynx1 and Aβ1-42 bind competitively to multiple nicotinic acetylcholine receptor subtypes.* (2016). https://doi.org/10.1016/j.neurobiolaging.2016.06.009.

Monitoring of platelet membrane integrity, using vital staining

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Structural changes in human platelet may indicate various types of diseases. Granules play dramatic role in common platelet integrity, but platelet deficiency is mainly associated with membrane failure. We studied human platelets of blood donors, nearly after harvesting and during 5-day storage at 22 °C: platelets of patients with acute exogenous poisoning, patients with injuries of musculoskeletal system, patients with chronic diseases. We also studied donor platelets, treated with membrane damaging factors—400–600 μM hydrogen peroxide and 5–20% dimethyl sulfoxide. Platelets were analyzed with our original method (patent RF № 2485502), based on fluorochrome dyes' vital staining and fluorescent

microscopy. We valued the level of platelets with granules, %; mean fluorescence activity (MFA) of stained platelet cytoplasm, ft-candelas (all membrane structures, excluding granules). In donor plasma, nearly all population of discoid platelets had equal MFA, estimating 24.5±0.1 ft-candelas, as in platelets with granules, as in functionally inactive platelet without granules. Decay of MFA had mainly degenerative platelets with serious membrane damage. These cells estimated in average 12.8±4.6 ft-candelas after staining. Number of platelets with low membrane fluorescence gradually increased during a short storage of donor platelets—from 3.5±0.5% at 1 day to 44.2±3.0% at 4 days. As a result, over 5 days of storage, all discoid platelets did not contain granules and had very low MFA (11.8 ± 3.3 ft-candelas). A similar decay of cytoplasm membrane integrity was observed in the presence of high concentrations of hydrogen peroxide or dimethyl sulfoxide. In patients with injuries of the musculoskeletal system, MFA violations were not identified or detected in very small quantities (less than 5% of the entire population of discoid platelets), followed by a normal level of platelets with granules. In patients with acute exogenous poisonings in 60% cases, the proportion of membrane-damaged platelets was the same as that in donors, and only in 15% of cases was higher than 10%, although the total level of platelets with granules and adhesive activity was significantly decent. On the other hand, patients with chronic diseases in more than 30% cases had abundance of platelets with low MFA (3–5 times above the normal level). Vital staining allows to assess the integrity of platelet membranes in vitro and in vivo. In pathologies, the level of discoid platelets with damaged membranes can significantly vary. Monitoring the status of platelet membranes seems to be topical in patients with acute and chronic diseases.

Novel light-driven proton pump: functional and structural study

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Expanding optogenetic toolbox requires searching for the new light-sensitive ion pumps and channels and their thorough functional and structural study [1]. In the present work, we characterized yet unexplored rhodopsin (named NR1) from unique Gram-negative bacteria *Sphingomonas paucimobilis*. Found by our bioinformatics search [2], NR1 has close homology to bacteriorhodopsin (bR), but serine residue in position of the putative proton donor (in contrast to aspartic acid in bR) is a reason to expect different ion transport properties.
We have confirmed that the new rhodopsin performs as outwardly directed proton pump by measuring light-induced pH changes in *E. coli* (expressing NR1) suspensions and by direct recording of photocurrents through lipid bilayer with embedded NR1 by black lipid membrane (BLM) technique. We showed that the duration of photocycle in an acidic environment (pH 5.0–6.5) is about 100 ms which is a hundred times shorter than in neutral and basic pH. However, the duration of photocycle of bR is about 10 ms in a wide range of pH, so we can conclude that NR1 is rather slow and pH-sensitive proton pump.
Preliminary experiments of in meso crystallization resulted in rod-shaped crystals sized up to 150 µm. We obtained diffraction patterns with resolution up to 3.0 Å in one direction but it is not sufficient to analyze the protein structure and compare it to already known rhodopsins. Further optimization of crystal growth and harvesting is needed to reduce local flexibility and motion, and therefore to get perfect well-ordered protein crystals. This work is supported by 16-15-00242 RSF.

1. Okhrimenko, I. *et al.* Search of new optogenetics tools by means of structural and functional characterization of novel microbial rhodopsins which reproduce mutations of already known ones. *FEBS Journal.* Vol. 283, (2016).
2. Ushakov, A. *et al.* Knowledge-based prediction model for characterization of microbial rhodopsins for optogenetics. *FEBS Journal.* Vol. 283, (2016).

The cytochrome c complexes with cardiolipin monolayer formed at different surface pressures

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The work presents a systematic study of the complexes forming during the interaction of the cytochrome *c* (Cyt*c*) with the lipid monolayer: cytochrome *c*—natural bovine cardiolipin (BCL) containing polyunsaturated fatty acids and cytochrome *c*—tetraoleilcardiolipin (TOCL) resistant to peroxidation [1]. The adsorption kinetics and elastic properties of such protein-lipid complexes were investigated under different conditions and were found to be similar both phospholipids
In particular, the parameters of the kinetic curves are similar for both TOCL and BCL. This shows that lipid peroxidation of natural CL which occurs during its interaction with Cyt*c* does not occur in BCL monolayers or makes no effect on the incorporation of Cyt*c* into the film.
While protein concentration in the subphase rise the protein incorporation rate into monolayer increases, but the amount of penetrated molecules remains constant. This amount reduces linearly with the increasing of initial surface pressure of lipid monolayer.
Taken together with literature data, our results show that the conformation change of Cytc occurs during its adsorption on the water-air interface or incorporation into a CL monolayer on the water surface. Apparently, one half of the globule sphere becomes predominantly hydrophobic and the other hydrophilic and charged ("stratified" Cytc).

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1. Marchenkova M.A. *et al.* Cytochrome c complexes with cardiolipin monolayer formed under different surface pressure. *Langmuir (31 №45)*, ***12426***, (2015). https://doi.org/10.1021/acs.langmuir.5b03155.
2. Vladimirov Yu.A. *et al.* Molecular mechanisms of apoptosis. Structure of cytochrome c-cardiolipin complex. Biochemistry *(78 №10)*, ***1086***, (2013). https://doi.org/10.1134/S0006297913100027.

The complex analysis of X-ray mesh scans for macromolecular crystallography

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Modern synchrotron beamlines dedicated to macromolecular crystallography aim at enhancing the accuracy of diffraction measurements and to this end, a preliminary analysis of the sample is of high importance. In particular, the proper centring of a crystal in the X-ray beam and an accurate estimation of its size has a crucial impact on the final quality of the data. Whenever optical microscopy methods cannot provide sufficient accuracy in crystal centring, very often the case for membrane proteins buried in opaque lipidic mesophases, an X-ray mesh/grid scan is an indispensable tool.
The complex analysis of X-ray mesh/grid scans presented here includes methods and software programs for determining the dispositions and characteristics of protein crystals contained in a sample holder (centre coordinates, shapes/sizes, and diffraction quality). In the first step, mesh scan images recorded at the grid points in the sample area are analysed for the presence of protein diffraction. In the second step, mesh scan areas are grouped based on diffraction pattern similarity and a "crystal map" illustrating different crystal areas in the sample holder can be built. Finally, crystal positions and corresponding aperture choices can be extracted from the map for use in subsequent data collection strategies.

1. Melnikov I. A., Svensson O., Bourenkov G., Leonard G. A. and Popov A. N. *Acta Cryst* (2018), D74, 355-365.

Changes in functioning of glyceraldehyde-3-phosphate dehydrogenase during synucleinopathies

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α-Synuclein is a 140-a.a.r. protein expressed in the central nervous system, which is one of the main players in the development of Parkinson's disease (PD) and other synucleinopathies. A hallmark of this disease is the formation of protein aggregates called Lewy bodies and eventual neuronal death. N-terminal end of α-synuclein is responsible for binding to cell membrane [1]. Moreover, a lot of mutations found in familial cases of PD, including A53T, are located in the N-terminus, which allows speculating that disruption of membrane binding is crucial for the disease progression. It can lead to the disruption of energy metabolism, which is the main focus of this work.
We have shown previously that α-synuclein interacts with partially oxidized glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in vitro, leading to the inactivation of the enzyme which can be rescued with reducing agents such as DTT [2]. We obtained SH-SY5Y neuroblastoma cells with overexpressed WT or A53T α-synuclein, which were used for the estimation of GAPDH activity and lactate generation rate. The formation of amyloid aggregates was confirmed with thioflavin S staining and showed the presence of such aggregates in A53T α-synuclein-expressing cells, while in WT-expressing cells, amyloid structures were observed only after hydrogen peroxide and Fe^{2+}-induced stress. GAPDH activity was lowered in cells expressing α-synuclein A53T rather than in cells expressing WT α-synuclein compared to control, which implicates either difference in the interaction of GAPDH with WT and A53T α-synuclein monomers or interaction with different forms of α-synuclein, such as oligomers, protofibrils, fibrils, and other intermediate species. Lactate generation was measured after incubation of cell lysates with glycolytic substrates and revealed a significant decrease in A53T-expressing cells compared to others.

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1. Davidson W. *et al.* Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *J. Biol. Chem. (9443–9449).* **273,** (1998).
2. Barinova, K. *et al.* Binding of alpha-synuclein to partially oxidized glyceraldehyde-3-phosphate dehydrogenase induces subsequent inactivation of the enzyme. *Arch Biochem Biophys. (10-22).* **642,** (2018).

Design and synthesis of new amphiphilic polymers for specific interaction with streptavidin and solubilization of membrane proteins

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The study of membrane protein function is an important task. We proposed a new approach to study membrane proteins interaction with ligands using the method of surface plasmon resonance (SPR). The main advantages of using SPR over traditional methods are the possibility to directly investigate intermolecular interactions and analysis of the kinetic parameters of the binding process. One of the problems of using of SPR is solubility of membrane proteins in aqueous solutions. The problem of solubility of membrane proteins can be solved by using amphiphilic polymers such as A8-35 [1]. The second problem is binding membrane proteins to the surface of a commercially available chip such as a chip with immobilized streptavidin [2].
We synthesized a molecule which is capable to solubilize membrane proteins and interact with streptavidin. The molecule consists of amphiphilic polymers which have covalently attached to peptides which are capable of recognizing streptavidin. Peptides did not contain free sulfhydryl, amino, and carboxylic groups. Two ligand peptides, WSHPQFGG and GGGGCWHPQAGC (with intermolecular disulfide bridge), were obtained. Ligands were synthesized by the solid-phase method using Fmoc-protected amino acids. Both structures have the central amino acid sequence HPQ [3], characteristic of streptavidin ligands. The ligand WSHPQFGG showed a weak affinity for streptavidin. The ligand GGGGCWHPQAGC was covalently attached to the amphiphilic polymer A8-35. The binding of the construction of A8-35-GGGGCWHPQAGC with streptavidin and solubilization of membrane proteins will be studied in more detail.

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1. Popot, J.-L. *Membrane proteins in aqueous solutions. Biological and medical physics Biomedical Engineering* (Springer International Publishing, 2018). https://doi.org/10.1007/978-3-319-73148-3
2. Scopes, D. *et al.* Aβ oligomer toxicity inhibitor protects memory in models of synaptic toxicity. *Br. J. Pharmacol.* **167,** 383–392 (2012).
3. Schmidt, T. G., Koepke, J., Frank, R. & Skerra, A. Molecular interaction between the Strep-tag affinity peptide and its cognate target, streptavidin. *J. Mol. Biol.* **255,** 753–66 (1996).

Single-particle electron microscopy structure of voltage-gated K_VAP channel

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Voltage-gated K+ (K_V) channels are tetramers with four identical subunits, each with six transmembrane segments (helices S1–S6). Segments S5 and S6 form the central pore at the interface between the subunits, and helices S1–S4 of each subunit form the voltage-sensing domains (VSDs). The arginine-containing helix S4 represents the voltage-sensing element, which moves with respect to the membrane upon the changes in electric field. The structure of prototypical K_VAP channel (archaea *Aeropyrum pernix*) was determined earlier by crystallizing the channel in a complex with monoclonal Fab fragments attached to its VSDs [1]. However, the VSDs in the crystal were observed in non-native conformation. The cryo-EM provided an electron density map of this complex at limited 10.5-Å resolution [2]. This result was obtained before the revolution in the EM field; thus, determination of a high-resolution K_VAP structure with non-distorted VSDs is still actual. Here, we performed single-particle reconstruction from the EM images of purified K_VAP protein in detergent micelles without added Fab. First, we generated a preliminary structure from images of the negatively stained protein and used it as an initial model for the reconstruction from cryo-EM data set. Cryo-images of purified K_VAP were obtained at Titan Krios microscope in ThermoFisher user facility, Eindhoven, the Netherlands. The 51,347 particles of the channel were collected and the preliminary model was refined.

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1. Jiang, Y. *et al.* X-ray structure of a voltage-dependent K+ channel. *Nature*, (2003), **423**:33-41.
2. Jiang, Q.X. *et al.* Electron microscopic analysis of KvAP voltage-dependent K+ channels in an open conformation. *Nature*, (2004), **430**:806-10.

Changes of apoptosis and autophagy markers (membrane and nuclear) in cells of kidney cancer under the influence of Gratiola officinalis extract in vivo

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Background. Cancer is the leading cause of disability and death among the population. Therefore, the search for new treatments for cancer, including from plant material, selectively activating apoptosis, and blocking autophagy are relevant today [1, 2, 3].
The aim of the study was to determine the mechanisms of pathomorphosis of the transplanted kidney cancer in experiments in vivo under the influence of the flavonoid-containing extract of Gratiola officinalis.
Materials and methods. The experiment was carried out on 30 male Wistar rats, with a transplanted kidney cancer RA. The rats received the Gratiola officinalis extract orally or intramuscularly 72 h after the inoculation for 12 days at a dose of 110 mg/kg/day and then were removed from the experiment. The comparison group was made up of animals with a tumor, but without effect. Immunohistochemical markers of apoptosis (p53, bax, bcl-2, Fas-receptor, Fas-ligand), autophagy (LC3b), and proliferation (Ki67) markers were used in the study. The Cramer-Welch criterion (T) was used to compare the groups.
Results. The marked decrease in the expression of proliferation (Ki67) and high expression of apoptosis markers (p53, bax, CD95 (Fas/APO-1), FAS-ligand) were noted in tumor cells in both ways of administration of the Gratiola officinalis extract and, in the comparison group, the expression of apoptosis markers was absent. All the described changes were more pronounced at intramuscular administration. The expression of the marker of autophagy LC3b increased at oral administration and decreased at intramuscular administration of Gratiola officinalis extract.
Conclusion. There is a suppression of proliferation and activation of signaling and mitochondrial apoptosis pathways, as well as blocking of protector autophagy under the influence of Gratiola officinalis extract. The LC3b autophagy marker can be used as an additional criterion for evaluating therapeutic pathomorphosis of tumors.

1.N.A. Navolokin, et al. Effect of extracts of Gratiola officinalis and Zea mays on the tumor and the morphology of the internal organs of rats with transplanted liver cancer, *Russian Open Medical Journal*, *(1-2)*, **2**, (2012).
2.N.V. Polukonova, et al. A new extraction method of bioflavonoids from poisonous plant (Gratiola officinalis L.). *Russian Open Medical Journal*, *(3)*, **304**, (2014).
3.Navolokin, N.A. et al. Effect of flavonoid-containing extracts on the growth of transplanted sarcoma 45, peripheral blood and bone marrow condition after oral and intramuscular administration in rats. *Russian Open Medical Journal, (6-3)*, **e0304**, (2017).

The role of P2X3 receptors of hydrogen sulfide in rat trigeminal nerve

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H2S is a gasomediator, which is involved in a wide range of functions in the body: cardiovascular, endocrine, and nervous system. Recent studies indicate the participation of H2S in the nociceptive system [1]. The trigeminovascular system is one of the models for the study of nociception mechanisms. It is represented by trigeminal nerve endings, innervating vessels of the Dura mater and directly involved in the pathogenesis of primary headaches, such as a migraine [2]. However, the mechanisms of migraine pathogenesis are not fully understood. It is known that changes in the activity of ATP-gated P2X3 receptors of trigeminal neurons play an important role in the generation and transmission of chronic pain [3]. Therefore, the aim of this work is to study the involvement of P2X3 receptors in the effects of hydrogen sulfide in the rat trigeminal nerve.
The whole-cell configuration of the patch-clamp method was used to register the ion currents of P2X3 receptors on the culture of trigeminal neurons. As the donor of H2S was used hydrogen sulfide (NaHS). The results of our study showed that in the culture of trigeminal neurons, application of NaHS (100 mkM) caused a decrease in the amplitude of both fast and slow components of P2X3 receptor. This effect may be related to the reduction of disulfide bonds of the receptor subunits. Therefore, dithiothreitol (DTT), which is a reducing agent, was used. The perfusion of cells by the NaHS donor in the presence of DTT resulted in a more significant decrease in the response amplitude of both fast and slow components of P2X3 receptor. This data suggested that effect of NaHS does not relate to modification of the receptor subunits.
Thus, the results indicate an inhibitory effect of H2S donor on the pronociceptive effects of ATP.

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1. Koroleva K., et al. Receptor mechanisms mediating the pronociceptive action of hydrogen sulfide in rat trigeminal neurons and meningeal afferents. Frontiers in cellular neuroscience (2017). https://doi.org/10.3389/fncel.2017.00226
2. Messlinger K. Migraine: where and how does the pain originate? Experimental brain research (2009). https://doi.org/10.1007/s00221-009-1756-y
3. Гиниатуллин Р. А. Нейрофизиологические механизмы мигрени и новые принципы патогенетического лечения. Казанский медицинский журнал (2011).

Facilitation of NMR assignment of membrane proteins with limited stability by combinatorial selective labeling

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Assignment of backbone resonances is a crucial step in every protein NMR investigation. The conventional procedure of the backbone assignment requires acquisition of a set of 3D triple-resonance spectra for uniformly ^{13}C,^{15}N-labeled protein sample. The full set of 3D experiments for backbone assignment requires up to a week of measurements, thus limiting application of this method to the proteins with low stability. Combinatorial selective ^{13}C,^{15}N-labeling (CSL) provides alternative fast approach for backbone resonance assignment. Analysis of simplified NMR spectra (e.g., 2D HSQC/HNCO) provides information about the residue types in each dipeptide. To obtain this information, the CSL scheme is required, e.g., one needs to specify the number of labeled samples and the labeling pattern for each residue type in the samples.
In this work, we designed "CombLabel" algorithm that calculates sequence-specific schemes, based on the given stock of isotope-labeled AAs and a set of 2D NMR spectra that will be used. Theoretical calculations using literature examples revealed that CombLabel software outperformed the previously proposed algorithms by the amount of assignment information, which could be extracted from CSL and by the number of samples. The software was tested on the isolated voltage-sensing domain (VSD) of human Kv2.1 channel. CSL approach in combination with the limited data from standard triple-resonance NMR spectra resulted in assignment of ~ 70% of backbone resonances for the VSD.

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Structural and biophysical studies of a LOV domain-derived fluorescent protein

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Light-oxygen-voltage (LOV) domains are conserved parts of photoreceptors in plants, bacteria, and fungi that bind flavins as chromophore and detect blue light. Photoexcitation of the flavin chromophore induces conformational changes in the LOV domains that lead to activation of various effector domains, such as kinases, esterases, and DNA-binding motifs. In the past, LOV-domain variants have been developed as fluorescent reporter proteins (called flavin-based fluorescent proteins; FbFPs), which due to their ability to fluoresce under anaerobic conditions, fast folding kinetics as well as small size of ~ 12–16 kDa make them a promising reporter system for the quantitative real-time analysis of biological processes [1].
Here, we present a small thermostable flavin-based fluorescent protein CagFbFP derived from a soluble LOV domain-containing histidine kinase from a thermophilic bacterium *Chloroflexus aggregans*. The 107 amino acids long CagFbFP protein consists only of the conserved LOV core domain lacking the signalling-relevant helices A′α and Jα. The protein is thermostable with a melting point of about 66 °C that does not depend on NaCl concentration; it also can refold in solutions containing up to 500 mM NaCl. Unexpectedly, the crystal structure of CagFbFP determined at the resolution of 1.07 Å reveals alternative conformations of the key signaling residue glutamine 148. Molecular dynamics simulations show that the two conformations interconvert rapidly. Both crystal structure and small angle scattering data show that the protein is dimeric. Overall, the protein appears to be a promising model for structural studies of LOV domains and for application as a fluorescent reporter.
The study was supported by the Russian Foundation for Basic Research (project № 18-34-00742).

1. Wingen *et al.* The photophysics of LOV-based fluorescent proteins – new tools for cell biology. *Photochemical & Photobiological Sciences* (875-883). **13** (2014).

Comparison of size and other properties of lysine dendrimers and short lysine dendritic brush

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Lysine dendrimer of 5th generation [1, 2] and fragment of short lysine dendritic brush [3] with second-generation dendrons of the same molecular weight were studied by molecular dynamics simulation method. The size and structural properties of these two molecules were compared. It was demonstrated that dendritic brush has smaller size and more dense core than the dendrimer. At the same time, the shape of both molecules is close to spherical. Radial density profile for both molecules is not monotonous and has minimum near core of molecules. This minimum is wider and deeper for dendrimer than for dendritic brush. The shape of radial distributions of the end groups and of the total radial charge density of these molecules is rather similar but for lysine dendritic brush, both distributions are shifted closer to center of mass of molecule than for dendrimer. Thus, dendrimer has wider region of low density and low charge near its center of mass than dendritic brush and is more suitable for use for encapsulation and delivery of hydrophobic drugs.

1. Falkovich, S.; Markelov, D.; Neelov, I.; Darinskii, A. Are structural properties of dendrimers sensitive to the symmetry of branching? Computer simulation of lysine dendrimers. *J. Chem. Phys.* ***139***, 064903 (2013). https://doi.org/10.1063/1.4817337.
2. N. N. Sheveleva, D.A. Markelov, M. A. Vovk, M. E. Mikhailova, I.I. Tarasenko, I.M. Neelov, E. Lähderanta NMR studies of excluded volume interactions in peptide dendrimers, *Scientific Reports,***8**, 8916, (2018) https://doi.org/10.1038/s41598-018-27063-3
3. B. Okrugin, M. Ilyash, D. Markelov, I. Neelov Lysine dendrigraft nanocontainers. Influence of topology on their size and internal structure, Pharmaceutics **10**, 129 (2018). https://doi.org/10.3390/pharmaceutics10030129

Inorganic polyphosphate modulates electrophysiological activity of neurons and signal transduction in glial cells

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Inorganic polyphosphate (PolyP) is a biopolymer composed of tens to hundreds of orthophosphate residues linked by the phosphoanhydride bonds[1]. PolyP presents in all living organisms and plays numerous physiological functions. Micromolar concentrations of PolyP are known to be in mammalian brain[2]. However, the role of PolyP in neurons and glial cells is unclear. In this study, we explore physiological reactions of neurons and glial cells of crayfish stretch receptor on exogenous inorganic PolyP with different lengths. Extracellular recordings were used to observe electrophysiological reactions of neurons. It was shown that long (130 residues, 100 μM) and medium (60 residues, 100 μM) but not short (14 residues, 100 μM) PolyP induce increase of action potential frequency of neurons. Dual fluorescent staining with propidium iodide and Hoechst 33342 showed an increase of the level of glial cells necrosis after application of long and medium PolyP. Preliminary incubation of stretch receptor with inhibitor of P2 purinoceptors PPADS (100 μM) abolished toxic effect of PolyP on glial cells but did not influence electrophysiological reactions of neurons on PolyP.
The data obtained suggests that long PolyP can trigger the activation of signaling pathways regulating necrosis and apoptosis in glial cells via activation of purinergic receptors. But in neurons, electrophysiological reactions on PolyP probably connected with membrane depolarization and activation of voltage dependent Ca^{2+} channels due to negative charge of PolyP rather than with activation of ATP receptors.

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1.Pavlov, E. *et al.* Inorganic polyphosphate and energy metabolism in mammalian cells. *J. Biol. Chem.* **285,** 9420–9428 (2010).
2.Kumble, K. D. & Kornberg, A. Inorganic polyphosphate in mammalian cells and tissues. *J. Biol. Chem.* **270,** 5818–22 (1995).

The key role of mitochondrial ROS in the development of autocatalytic pathophysiological process at diabetes mellitus

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The aim of this work is to study the role of mitochondria in the development of pathophysiological disorders at diabetes mellitus, a disease that now is becoming a global epidemic[1]. At the root of the diabetes is the disruption of insulin receptor intracellular signaling cascades leading to a slowdown in the glucose assimilation by peripheral tissues and thus a sharp increase in glucose blood level. In the present work, main known aspects of the pathology development causing the tissue and organ damage were analyzed. As a result, two groups of parallel processes were identified. The first group includes biochemical processes, mainly associated with the imbalance of the reactive oxygen species (ROS) regulating system. The second group includes processes of a higher physiological level—the disruption of organs and body systems, such as liver and circulatory system. The main result of this work is an explanation of the biochemical and physiological reasons of the diabetic complications development. It is shown how the two above-mentioned groups of processes mutually reinforce each other due to the positive feedbacks provided by the increase in the ROS concentration caused by both their increased synthesis and the depletion of the cell antioxidant systems. The conducted analysis showed that a key source of ROS providing the beginning of pathological autocatalytic process is mitochondria. Mitochondrial ROS synthesis strongly increases in the conditions of periodic hypoxia-reoxygenation[2] arising due to complex microvessel diabetic damage. It is essential that mitochondrial ROS in turn significantly aggravates the vascular injury creating a vicious circle.
As a result of the work, a detailed scheme of diabetes development was constructed and the main elements of the autocatalytic cycle were indicated, showing the objectives for exact therapy. On the next step, amino acid glycine was considered as a potentially effective drug that weakens diabetic autocatalytic cycle. Based on glycine biological action and clinical trials it was proved that it really reduces oxidative stress and prevents the development of diabetic complications. Glycine action is provided by its direct action on mitochondria, immune cells and endothelium of blood vessels.

1.Dedov, I. I., Shestakova, M. V. & Vikulova, O. K. National register of diabetes mellitus in Russian Federation. *Diabetes Mellit.* **18,** 5 (2015).
2.Selin, A. A. *et al.* On the regulative role of the glutamate receptor in mitochondria. *Biol. Chem.* **397,** 445–458 (2016).

Design of pegylated lipoplex

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An important strategy for increasing the effectiveness of drugs and reducing their toxicity and side effects is the rational drug forms design. The most attractive strategy in terms of safety is the development of non-viral DNA and RNA delivery systems, for example, vectors based on cationic liposomes. Moreover, the presence of special modifications such as PEG-derivativeson on the outer surface of lipoplexes provides the prolongation effect in experiments in vivo.
The aim of this work was the optimization of PEG quantity that would create prolonged biological effect of lipoplex-based drugs but would not reduce their transfection activity.
Three alkyl derivatives of PEG2000 were synthesized at the first step of this project. The hydrophobic domain was formed by the residues of cholesterol, octadeciamine, or stearic acid. A collection of liposomes based on the lipotripeptide $OrnOrnGlu(C_{16}H_{33})_2$ was created with the addition of each of the synthesized compounds in an amount of 2, 4, 5, 6, 8, 10, and 15 mol % of the total lipotripeptide.
Examination of liposome/DNA or liposome/miRNA complexes transfection activity on HEK293 and HepG2 cell lines showed that the best results after 24 h of incubation were achieved with 2 and 6 mol % PEG-derivatives on the surface of liposomes for both cell lines. The transfection activity doubled after 48 h of incubation with modified lipoplexes. In addition, it was noted that the structure of the hydrophobic domain among synthesized compounds does not affect the ability of liposomes to deliver the genetic material into cells.
The results of cytotoxicity study for liposomal dispersions with different levels of PEG on the surface were obtained by MTT test. They indicated a decrease in the toxicity of liposomes with an increase in the proportion of the PEG presents in liposome composition. This phenomenon could be explained by decrease in the number of positive charges on the surface of cationic liposomes, which leads to decrease in their toxic effect on the cells.
Thus, it has been shown that insertion of hydrophobic PEG-derivatives in to the liposomes leads to a decrease in the cytotoxicity of the modified cationic particles. It also provides a slower penetration of the particles into the cells, prolonging their action. In the short run, pegylation decreases the efficiency of DNA transfection; however, in

the long run, the transfection efficiency of pegylated liposomes increases in comparison with the unmodified particles. The most effective transfection was achieved with the insertion of 4–6 mol % PEG in the liposomes. It should be also noted that the structure of thehydrophobic anchor in PEG-derivatives does not affect the physico-chemical and biological properties of the particles.

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Modeling of ATP synthase rotor ring protein-lipid complex

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ATP synthases are large multiprotein complexes found in all kingdoms of life and in cell membranes of prokaryotes as well as in mitochondria and chloroplasts of eukaryotes [1]. Generally, the complexes consist of two subunits, one of which is soluble and another is embedded in the membrane. The membrane part includes a so-called c-ring formed by 8 to 15 *c* subunits, which is rotating in the membrane during ATP synthesis. Highly detailed atomistic structures of isolated c-rings and whole ATP synthases have recently become available [2]. Here, we present a computational approach for obtaining models of c-ring-lipid complexes. The resulting models are stable in simulations, and positions of lipid molecules correspond well to those observed in experimental structures.

1. Walker J.E. The ATP synthase: the understood, the uncertain and the unknown. *Biochem. Soc. Trans.* (1-16). **41** (2013).
2. Kühlbrandt W. & Davies K.M. Rotary ATPases: a new twist to an ancient machine. *Trends Biochem. Sci.* (106–16) **41** (2016).

Comparison of various substitution matrices for amino acid alignment and homology search of microbial rhodopsins

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Alignment of amino acid sequences by means of dynamic programming is one of the popular sequence comparison methods. This work is focused on the finding of microbial rhodopsin family-specific scoring functions for global similarity matrix-based sequence alignment. We constructed a set of substitution matrices based on sequence (RHOD1, RHOD2) and structure (RHODS) alignment of microbial rhodopsins using methods described elsewhere [1]. We examined performance of developed substitution matrices along with several popular (BLOSUM, VTML) and rare (JTT, PHAT, PFASUM) substitution matrices for pairwise amino acid alignment of microbial rhodopsins in two kind of tests. First, we compared alignments obtained with the particular matrix with a reference alignment obtained from structural superposition of corresponding proteins. We found that all matrices demonstrate similar performance which was slightly better for family-specific matrices RHOD1, RHOD2, and RHODS. Second, we checked an ability of matrices under consideration to detect similarity between type-1 rhodopsins and novel heliorhodopsin discovered recently [2]. To this end, we tried to find probable heliorhodopsin homologues in SwissProt and PDB databases. In this test, matrices showed very different performance. While BLOSUM and VTML matrices were able to detect similarity with halorhodopsin in both databases, PFASUM matrices did it only in PDB and the rest matrices (RHOD1, RHOD2, RHODS, JTT, PHAT) failed the test.
The research is carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University [3]. The work is ongoing with the support of RFBR grant 17-00-00167K (KOMFI 17-00-00166).

1. Kuznetsov I.B., Protein sequence alignment with family-specific amino acid similarity matrices BMC Res. Notes 4 (2011).
2. Pushkarev A., et al. A distinct abundant group of microbial rhodopsins discovered using functional metagenomics. Nature 558 (2018).
3. Sadovnichy V., et al. "Lomonosov": supercomputing at Moscow State University in:, Contemp. High Perform. Comput. From Petascale Towar. Exascale (2013).

The role of acetylcholine in P2X7-gated mast cells activation

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Emerging evidence suggest implication of immune system and inflammation in migraine. Thus, patients with migraine revealed signs of systemic inflammation, such as elevated levels of several pro-inflammatory cytokines including interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-alpha (TNFα) [1, 2]. In the frame of the cholinergic anti-inflammatory pathway (CAP) theory, it has been shown that acetylcholine (ACh) via nicotinic receptors inhibited ATP-triggered release of the pro-inflammatory interleukin-1β [3]. However, it remains unclear whether it was inhibited at the P2X7 receptor level or at following stages.
To address this issue, in this project, we investigated ATP-induced activation of P2X7-gated channels on peritoneal mast cells and tested effect of ACh on opening of P2X7-gated channels. Freshly isolated mast cells were stimulated with ATP alone, or in the presence of ACh. To assess mast cells activation, we used a fluorescent dye YO-PRO1, which penetrates the cell membrane through opened P2X7-gated ion channels. Samples were analyzed using flow cytometry.
We found that ATP largely increased P2X7 receptor-mediated uptake of YO-PRO1 in mast cells. ACh also slightly increased YO-PRO1 uptake but this cholinergic agonist did not reduce ATP-induced dye uptake via P2X7 channels. Obtained data indicate that ATP efficiently opens P2X7 receptor large pore associated with activation of inflammasome. The lack of inhibitory effect of ACh suggests that its anti-inflammatory action is realized downstream of active P2X7-receptors.
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1. Wang, F. *et al*. Association of serum levels of intercellular adhesion molecule-1 and interleukin-6 with migraine. *Neurol Sci*. (2015) 36:535-540. https://doi.org/10.1007/s10072-014-2010-3.
2. Khaiboullina, S. *et al*. Cerebellar atrophy and changes in cytokines associated with the CACNA1A R583Q mutation in a Russian familial hemiplegic migraine type 1 family. *Front Cell Neurosci*. (2017) 11:263. https://doi.org/10.3389/fncel.2017.00263.
3. Hiller, S. et al. β-Nicotinamide adenine dinucleotide (β-NAD) inhibits ATP-dependent IL-1β release from human monocytic cells (2018) 19: 1-21. https://doi.org/10.3390/ijms19041126

Functional studies and spatial structure of new retinal-binding proteins

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Structural and functional characteristics of new unexplored microbial rhodopsins, as well as high-resolution data of the structure of these membrane proteins and their mutant variants, are the necessary input information for the development of concepts of the relationship between the structure and function of retinal-containing proteins, using molecular modeling techniques and improving bioengineering designs of photosensitive proteins for solving problems optogenetics and biophotonics.
To define the function and to obtain high-resolution structural information for new microbial rhodopsins particularly found by knowledge-based prediction bioinformatics search of microbial rhodopsins [1], we use the well-developed pipeline of methods of heterologous expression, isolation and purification, functional characterization of previously unexplored microbial rhodopsins, determination of their kinetic characteristics, and determination of their spatial structure of atomic resolution by X-ray diffraction. Under the study are unexplored rhodopsins of *Sphingomonas paucimobilis* and *Pantoea anthophila*, also various mutant forms of rhodopsin from Gram-positive bacteria *Exiguobacterium sibiricum* (ESR), previously obtained and characterized by the authors of the project [2], *Exiguobacterium sp.* AT1b and *Exiguobacterium* 7-3 from permafrost. Molecular modeling methods [3] were used to predict the structures and dynamic properties for amino acid substitutions, general stability, and thermal stability of unexplored rhodopsins. Based on the proteins' structures, spectral properties were calculated by QM/MM methods.
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1. Ushakov, A., et al. Knowledge-based prediction model for characterization of microbial rhodopsins for optogenetics. *FEBS Journal* (2016). Volume 283, P-03.03.2-014
2. Petrovskaya, L., et al. ESR—a retinal protein with unusual properties from Exiguobacterium sibiricum. *Biochemistry (Moscow)* (2015), 80, 688-700.
3. Nekrasova, O., et al. Complexes of peptide blockers with Kv1.6 pore domain: molecular modeling and studies with KcsA-Kv1.6. *J. Neuroimmune Pharmacol.* (2016), 12, 260-276

Probing the assembly dynamics and structure of styrene–maleic acid lipid particles by coarse-grained simulations

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Amphiphilic maleic acid-containing alternating copolymers composed of alternating maleic acid and styrene (SMA) account for a major recent methodical breakthrough in the study of membrane proteins. They were found to directly solubilize phospholipids and membrane proteins both from artificial and natural bilayers yielding discoidal SMA/lipid particles (SMALPs) [1].
SMA-encased nanolipoparticles are comprised of lipid or lipid/protein cores surrounded by a polymer belt and have, depending on the preparation routine, diameters of 10–30 nm. Within particular preparation, the size of particles is uniform, which renders them suitable for diverse experimental techniques, e.g., for cryo-electron microscopy and EPR measurements [2, 3].
Although many empirical studies indicate the great potency of SMA copolymers for membrane research, the mechanisms of their action remain obscure. It is unknown what factors account for the very assembly of SMA-encased lipid particles and why they have a uniform size.
We have developed a coarse-grained (CG) molecular model of SMA-polymers within the framework of the popular MARTINI CG force field. The obtained model was used to probe the behavior of SMA polymers with varying composition/charge/concentration in solution as well as their interaction with lipid membranes.
The results of the simulations indicate that SMA polymers with styrene/maleic acid ratios of 2:1 and 3:1 form SMALPs by different mechanisms: either via poration or via a mechanism similar to the microvesicle release. Also, we found that the SMA polymers tend to aggregate in solution into clusters of the specific size what might account for the uniform size of the SMALPs.

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1. Dörr, Jonas M., et al. The styrene–maleic acid copolymer: a versatile tool in membrane research. European Biophysics Journal 45.1 (2016). https://doi.org/10.1007/s00249-015-1093-y
2. Parmar, Mayuriben, et al. Using a SMALP platform to determine a sub-nm single particle cryo-EM membrane protein structure. BBA-Biomembranes 1860.2 (2018). https://doi.org/10.1016/j.bbamem.2017.10.005
3. Voskoboynikova, N., et al. Characterization of an archaeal photoreceptor/transducer complex from Natronomonas pharaonis assembled within styrene–maleic acid lipid particles. RSC Advances 7.81 (2017). https://doi.org/10.1039/C7RA10756K

The role of OsO_4 fixation in the contrast formation of cellular membrane structures

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For the investigation of intracellular structures with the aid of atomic force microscopy, traditional preparation methods for transmission electron microscopy are often used [1]. One of the drawbacks of this methods is a low contrast on AFM topography and phase images. So, for successful AFM study of cellular membrane structures, these protocols should be optimized. In the present work, the role of osmium tetroxide in AFM images contrast formation was investigated. OsO_4 acts as a lipid structures fixative and greatly affects cell mechanical properties. For purposes of the study, monolayers of fibroblasts cultures were fixed in glutaraldehyde and half of the samples were additionally fixed with OsO_4. After that, all of the samples were dehydrated in graded ethanols, embedded in Epon 812 epoxy resin, and cut on a microtome for subsequent AFM study.
On the samples fixed with OsO_4, a low contrast was observed both on topographical and phase images. The interior of cells was quite homogeneous. The only detectable ultrastructure features were cytoplasmic membranes and nuclear laminas. Samples prepared without OsO_4 showed noticeably higher contrast. Many intracellular organelles appeared such as endoplasmic reticulum, mitochondria, and cristae of an individual mitochondrion.

According to the results of the study, the protocols without OsO_4 fixation should be used during the biological sample preparation procedure for AFM investigation. The above-mentioned effect can be elucidated through the influence of osmium tetroxide on mechanical and chemical properties of cells membrane structures. OsO_4 increases the stiffness of lipid bilayers and their affinity to epoxy resins. But the issue of the influence of epoxy resin stiffness on AFM contrast remains open. An alternative to chemical fixation may be freeze-drying method [2] which keeps membranes intact and provides better contrast.

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1. Gulin, A., *et al.* Correlating microscopy techniques and ToF-SIMS analysis of fully grown mammalian oocytes. *Analyst.* **141.13,** 4121-4129. (2016)
2. Pogorelov, A. G., *et al.* The use of ToF-SIMS for analysis of bioorganic samples. *Biophysics.* **63.2,** 215-221. (2018)

Pharmacophore model for nisin/lipid II interaction in bacterial membrane

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Nisin is a 34-residue peptide lantibiotic produced by a lactic acid bacterium and has antibacterial action against a wide spectrum of Gram-positive bacteria. Nisin binds specifically to lipid II, a membrane-embedded precursor in peptidoglycan synthesis. The conservative chemical structure of lipid II's pyrophosphate (PPi) moiety—the binding site—makes nisin a candidate for future generation of antibiotics. 3D structure of nisin/lipid-II complex was solved by NMR in DMSO; however, the native one—in membrane—may fundamentally differ.
To explore the structural/dynamic characteristics of nisin and lipid II and to identify the mechanism of their recognition, molecular dynamics (MD) simulations were carried out for the following systems: lipid II in hydrated lipid bilayer and truncated nisin1-11 analogue in water—with and without PPi. Computational experiments revealed that the structure of lipid II is determined by the surrounding water-lipid milieu and differs considerably from its conformation in water and DMSO. Being solvent-accessible during all the simulation time, PPi moiety was found to adopt three distinct conformations, only two of which remained capable of nisin binding. In these states, PPi group forms the "binding pharmacophore" by exposing on the bilayer surface its two H-bond acceptors—oxygen atoms—at the unique distance that cannot be formed by surrounding polar heads of lipids. We suggest this feature to play a crucial role in high selectivity of lipid II/nisin recognition. Also, this indicates the membrane importance in accommodation of lipid-II to interaction with nisin. Nisin analogue was found to adopt five major states in aqueous solution. The in-house approach based on calculations of the energy of the probe oxygen atoms pair revealed that only one of the five states is competent for interaction with the target. This state ("recognition pharmacophore") is defined by specific configuration (closed-ring position) of the peptide, which is stabilized by four co-directional NH-vectors of ring A forming a unique H-bonding network. As a result, a new medium-guided induced fit mechanism of lipid II/nisin molecular recognition in model bacterial membrane was proposed.
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SAS investigations of size changes in lipid vesicles near temperature point of phase transition

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Biological cell membrane is composed of many lipids and membrane proteins. In this respect, lipid membranes provide good models for numerous biophysical studies.
The investigation of lipid membranes near the phase transition temperature point has a significant importance not only for theoretical findings [1] but also from a biological point of view because the cell membrane has an important role in biological processes [2].
In present work, we study unilamellar vesicles (ULV) of DMPC and DPPC lipids of different sizes, obtained by extrusion through 300- and 500-Å track membranes. During the phase transition, the internal radius and surfaces of a vesicle are enlarged, in connection with the change in the hydration of the lipid heads and the decrease of thickness of lipid membrane with temperature increment [3]. We show the relationship between the inner radii and thicknesses of the vesicles vs temperature near the phase transition point using SANS and SAXS. SANS measurements were performed on the small-angle spectrometer YuMO (IBR-2, JINR, Dubna, Russia) [4], SAXS was performed on BM29 (ESRF, Grenoble, France). Obtained results are discussed.

1. Gordeliy, V. I., Cherezov, V., & Teixeira, J. (2005). Strength of thermal undulations of phospholipid membranes. Physical Review E, 72(6), 061913.
2. Kharakoz, D. P. (2001) On a possible biological role of the phase transition liquid-solid in biological membranes. Usp. Biol. Khim, 333-364
3. Soloviov at all. (2014). Changes in the area per lipid molecule by P–V–T and SANS. Investigations in Macromolecular Symposia, Vol. 335, No. 1, pp. 58-61.
4. Kuklin, A. I., Islamov, A. K., & Gordeliy, V. I. (2005). Scientific reviews: two-detector system for small-angle neutron scattering instrument. Neutron News, 16(3), 16-18.

Comparative study of peroxidation intensity and resistance of erythrocyte membranes of mammals, birds, and reptiles

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The level of lipid peroxidation (LPO) in any healthy organism is relatively low and promotes the renewal of membrane structures of the cell. There are factors that initiate LPO. These factors lead to a change in intensity of LPO and result in development of various pathologies. The parameters of LPO are the content of conjugated dienes and malonal dialdehyde (MDA) in blood plasma, as well as in cells and organelles directly involved in oxygen transport and oxygen metabolism such as erythrocytes and mitochondria.

The bird organism is more susceptible to LPO under high intensity of their growth. Interestingly that LPO processes are influenced by the live weight and diet of bird. Especially, the last factor is important for agricultural birds. In addition, the level of lipid peroxidation decreases with inclusion in ration the substances which enhance the antioxidant system activity. The similar important factors for wild birds and animals are food rations, habitat conditions, especially temperature, and also the intake of various modulators of antioxidant activity.
As is known, reptiles have the hibernation ability. It gives more interest in LPO study. The period before and after hibernation is characterized by various biochemical, physiological, morphological, and behavioral changes of reptiles. Before the hibernation, the activity of enzymes decreases, changes in cell membrane are observed and, consequently, changes LPO activity. After the hibernation, all these processes are renewed. Formation of active oxygen species is increased. It leads to subsequent increase in LPO intensity. The antioxidant system begins to work later. It leads to a greater level of peroxidation intensity than before hibernation.
The purpose of the study was to compare the intensity of peroxide processes in erythrocytes of different species such as mammals, birds, and reptiles. Erythrocytes are the first element which participate in peroxide activation and reacts by changing their parameters such as membrane characteristics. To achieve the purpose, MDA content in erythrocytes, sorption capacity, and osmotic resistance of erythrocytes according to their hemolysis level in NaCl solutions of different concentrations were analyzed. The obtained results allow to conclude that the osmotic resistance of erythrocytes of snakes (*Natrix natrix*) is higher than the same parameter of hens (*Gallus gallus dom.*), pigeons (*Columba livia*), and rats (*Rattus norvegicus*). The sorption capacity of erythrocyte membranes of the snakes is much lower than that of the pigeons and hens. The study of MDA content in erythrocytes did not reveal significant differences between the animals. It was revealed inversely proportional relationship between MDA content in erythrocyte and sorption capacity of erythrocyte membranes—with the increases of MDA concentration the sorption capacity decreases.

Biosensor analysis of the native membrane protein interactions in the biological membrane's lipid model

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Nowadays, surface plasmon resonance (SPR) biosensors are widly using in the great range of intramolecular interactions analysis, for example, the protein-protein complex association and dissociation processes. The base methodological approach for the immobilization of the ligand protein on the surface of the optical chip of the biosensor is the covalent immobilization of the ligand macromolecules by the free chemically active groups [1]. During this process, it is possible to reduce the proteins' degrees of freedom. It could affect on the obtained experiment results—covalent immobilization of some cytochromes P450 can inhibit their ability to interact with their partners. Besides, working protocols for the chips dedicated for amino- or thiol-groups involved immobilization of membrane proteins does not include the formation of lipid membrane models imitating the natural environment of studied macromolecules.
This problem could be solved by using chips covered with hydrophobic dextran (in Biacore product line biosensors (GE Healthcare, USA) it is the L1-chips). This type of chips allows forming of lipid layer on its surface therefore making it possible to immobilize ligands included in the proteoliposomes. This approach provides an opportunity to ligand immobilization without any chemical modification and model the natural lipid environment of membrane proteins.
We have shown using the Biacore 3000 SPR-biosensor that human cytochrome b5 immobilized on the L1 chip using the proteoliposome approach can interact with human cytochrome P450 3A4. Equilibrium dissociation constant (KD) of these complex was near one order lower than KD shown for the same complex obtained in the experiments with cytochrome b5 covalently immobilized by amino-groups on the surface of the CM5 chip without lipid environment [2].

1. Nguyen H.H. *et al.* Surface plasmon resonance: a versatile technique for biosensor applications. *Sensors (Basel).* **15(5)**, 2015
2. Yablokov E. *et al.* Thermodynamics of interactions between mammalian cytochromes P450 and b5. *Archives of Biochemistry and Biophysics.* **619**, 2017

L-DOPA as an independent neurotransmitter in brain

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Ocular albinism 1 (OA1) receptor—atypical G-PCR with unknown ligand—until recently has been analyzed as a protein involved into pigmentation in the neurosensory retina only. Ten years ago, a new conception was offered shedding light to a possible ligand to OA1—L-DOPA [1]. These receptors are expressed in several brain regions in adults [2].
L-DOPA, up until more recent time, was supposed to play an exclusive role of dopamine's precursor, which is converted out of tyrosine under influence of tyrosine hydroxylase (TH) and involved into synthesis of melanin and dopamine. But latest evidence reports that L-DOPA is assumed to be an independent neurotransmitter as well [3].
Several monoamines, among which are serotonin, dopamine, norepinephrine, play a crucial role of morphogenic factors in brain [4–6]. Thus, we assume that L-DOPA morphogenically affects developing brain. There is no data on gene expression to DOPA's receptors in ontogenesis that is why we aim to reveal OA1 expression patterns in different brain regions.
Based on the data of OA1 expression in adult animals, the following rat brain structures were accepted for analysis: striatum, mediobasal hypothalamus, midbrain, and brain stem at different embryonic (E) and postnatal (P) days: E16, E18, E21, P3, P7, P15, and P30. The data was obtained via application of RT-PCR.
In all the brain structures, the receptors are identified even at E16 being an indirect proof of L-DOPA's morphogenic function.
In striatum, brain stem, and midbrain, the peak of expression is achieved immediately after birth, but to the end of the second week of life, expression level is decreasing.
Another pattern is observed in hypothalamus: the expression level is consistently high at embryonic days and is decreasing at the end of the first week after birth.
As expected, we actually registered a high OA1 expression level in the period of active morphogenesis in brain. This data allows us speculating on DOPA's function as a morphogene.

This issue will be examined in our following studies.
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1. Lopez VM, Decatur CL, Stamer WD, Lynch RM, Mckay BS. L-DOPA is an endogenous ligand for OA1. 2008;6(9).
2. Fukuda N, Naito S, Masukawa D, Kaneda M, Miyamoto H, Abe T, et al. Expression of ocular albinism 1 (OA1), 3, 4-dihydroxy-L-phenylalanine (DOPA) receptor, in both neuronal and non-neuronal organs. Brain Res [Internet]. Elsevier; 2015;1602:62–74. Available from: https://doi.org/10.1016/j.brainres.2015.01.020

3. Misu Y, Goshima Y, Miyamae T. Is DOPA a neurotransmitter? Trends Pharmacol Sci. 2002 Jun; 23(6):262-8.
4. Lauder, J.M. Serotonin and cardiac morphogenesis in the mouse embryo. Teratology. 1993. 47, 573-84.
5. Ugrumov M.V. Hypothalamic monoaminergic system in ontogenesis: development and functional significance. Int. J. Dev. Biol. 1997. V. 41. P. 809-816.
6. Gaspar, P., Cases, O. and Maroteaux, L. The developmental role of serotonin: news from mouse molecular genetics. Nat Rev Neurosci 2003. 4, 1002-12.

Structure-functional studies of a new autotransporter from *Psychrobacter cryohalolentis* K5[T]

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Autotransporters from Gram-negative bacteria comprise a large family of outer membrane proteins which consist of a surface-exposed passenger domain and a translocator domain with beta-structural fold [1]. AT877 is a new autotransporter protein from *P. cryohalolentis* K5[T] with an esterase passenger domain belonging to the GDSL lipase family. AT877 displays maximum hydrolytic activity on medium-chain *p*-nitrophenyl esters (C8-C10). By fusion of the coding sequences of its α-helical linker and translocator domain with that of the cold-active esterase EstPc, 10 type III domain of human fibronectin (10Fn3) or fluorescent protein mCherry, we have obtained a new system for the display of the recombinant proteins at the surface of the bacterial cells [2]. As it was shown by whole-cell ELISA, activity assays, and confocal microscopy, these proteins were successfully displayed on the cells of the recombinant *Escherichia coli*. Studies of the deletion variants of AT877 demonstrated that proteins which presumably contain from 4 to 7 beta-strands in their C-terminal domain are more active than the wild type in translocation of 10Fn3 to the cell surface. To increase the yield of the exposed protein, we have constructed hybrid passenger domains consisting of EstPc and 10Fn3 and demonstrated that N-terminal position of EstPc in the passenger significantly improves efficiency of the surface display of ^{10}Fn3 [3].

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1.Nicolay, T., Vanderleyden, J. & Spaepen, S. Autotransporter-based cell surface display in Gram-negative bacteria. *Crit Rev Microbiol* **41**, 109-123 (2015).
2.Petrovskaya, L. *et al.* Cell surface display of cold-active esterase EstPc with the use of a new autotransporter from Psychrobacter cryohalolentis K5T. *Extremophiles : life under extreme conditions* **19**, 161-170 (2015).
3.Petrovskaya, L. *et al.* Fusion with the cold-active esterase facilitates autotransporter-based surface display of the 10th human fibronectin domain in Escherichia coli. *Extremophiles : life under extreme conditions* **22**, 141-150 (2018).

Lipidomic analysis of different phenotypic forms of mycoplasmas

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Mycoplasmas are parasites of almost all living organisms. This is the bacteria with significant reductions of the genome and metabolism, and the lacking of a cell wall. They can persist in host for a long time without causing any symptoms. Recently, our group has isolated new morphological form of *Mycoplasma hominis* from the blood of people with inflammatory diseases of the urogenital tract. Although usually in humans, mycoplasmas colonize mainly epithelium cells of the urogenital and respiratory tract. In addition, we have shown that this form of *M. hominis* can be obtained in vitro, when the classical form is exposed to various stress conditions. These phenomena, including interaction with hosts, avoidance of immune response, and other types of communications with surrounding environment may occur due to changes in cell surface.
The aim of this work is studying the differences in the lipid composition of the mycoplasma cytoplasmic membrane for the classical cultivated form and a new discovered potentially persistent in the host organism. To identify common patterns of changes in the lipid composition between different phenotypic forms, we have investigated common trends of differences for two species of mycoplasmas, *M. hominis* and *M. gallisepticum*.
Classical and persistent forms of colonies were obtained in vitro for laboratory strains of both species. Lipids were extracted using MTBE-methanol method and studied by LC-MS using Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo). It is known that mycoplasmas are partially or totally incapable of fatty acid synthesis and depend on the host or the culture medium for their supply. Most lipids in mycoplasmas are contained in the cytoplasmic membrane or can be used as energy sources. The major differences in the composition of lipids between different phenotypes belong to the classes of glycerolipids and glycerophospholipids, which form the charge and fluidity of cytoplasmic membrane and are responsible for host-pathogen interactions, as well as to the classes of sphingolipids, which are not only contained in the membrane bilayer but are also abundant in neural tissue.

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Time-dependent intracellular localization of externally applied Alzheimer’s disease A$\beta_{1\text{-}42}$ peptide

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Although the extracellular plaque hypothesis of AD is still favored by most researchers, there is increasing evidence that intracellular accumulation of Aβ monomers/small oligomers is one of the earliest key events leading to synaptic and neuronal dysfunction[1].
Intracellular pool of Aβ has a double origin: generation from amyloid precurser protein (APP) inside the cells and uptake from the extracellular space[2,3]. Some mechanisms involved in Aβ internalization by different types of brain cells and consequences of Aβ uptake were described[4–6]. However, the precise mechanism of Aβ internalization by neurons still remains unclear.
In order to study cellular and organelle trafficking of Aβ, in our current studies, fluorescently labeled A$\beta_{1\text{-}42}$ peptide was disaggregated to form monomers/small oligomers that were externally applied to mammalian cells. By means of confocal microscopy for both human neuroblastoma (SH-SY5Y) and rat oligodendroglia (OLN-93), cell lines we studied, the cellular localization of A$\beta_{1\text{-}42}$ and its traffick to organelles depending on time and type of fluorescent label.

Since neurons are known to use different mechanisms than glia to internalize Aβ, the challenge ahead is to understand the significance of this diversity in the development and progression of AD as well as the links between Aβ internalization and cellular dysfunction leading to neurodegeneration.

1.Bayer, T. A. & Wirths, O. Intracellular accumulation of amyloid-Beta - a predictor for synaptic dysfunction and neuron loss in Alzheimer's disease. *Front. Aging Neurosci.* **2,** 8 (2010).
2.LaFerla, F. M., Green, K. N. & Oddo, S. Intracellular amyloid-β in Alzheimer's disease. *Nat. Rev. Neurosci.* **8,** 499–509 (2007).
3.Džinić, T. & Dencher, N. A. Oxygen concentration and oxidative stress modulate the influence of Alzheimer's disease A β 1–42 peptide on human cells. (2018). https://doi.org/10.1155/2018/7567959
4.Mohamed, A. & Posse De Chaves, E. Aβ Internalization by neurons and glia. *SAGE-Hindawi Access to Res. Int. J. Alzheimer's Dis.* (2011). https://doi.org/10.4061/2011/127984
5.Mandrekar, S. *et al.* Microglia mediate the clearance of soluble A through fluid phase macropinocytosis. *J. Neurosci.* (2009). https://doi.org/10.1523/JNEUROSCI.5572-08.2009
6.Snyder, E. M. *et al.* Regulation of NMDA receptor trafficking by amyloid-β. *Nat. Neurosci.* **8,** 1051–1058 (2005).

Computer-aided study of umifenovir binding by the influenza hemagglutinin

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Influenza hemagglutinin is the main protein of the viral membrane playing the crucial role in attaching the virus to a healthy cell for the subsequent fusion of the virus with the host cell membrane hence being attractive target for design of effective inhibitors.
In a recent study [1], structures of influenza hemagglutinin complexed with commercial antiviral drug umifenovir (Arbidol) were obtained by X-ray diffraction analysis. The model of the umifenovir action as an inhibitor was described based on the analysis of the static structures of the hemagglutinin-umifenovir complexes. As reported, the binding of inhibitor molecules in symmetric sites forms a network of non-covalent interactions that strengthen the high-conserved stem region of hemagglutinin prohibiting subsequent conformational changes required for infection to proceed. However, the newly determined umifenovir binding site is about 16 Å away from the position predicted earlier by mass spectrometry and computer simulation [2]. Moreover, only one of known four single-point mutations causing the resistance to umifenovir is located near both reported sites whereas the direct influence of the other ones is hard to hypothesize without additional analysis. Consequently, it will be informative to investigate the umifenovir-hemagglutinin binding by molecular dynamics and calculate free energy landscape of the process.
In this work, we have used the metadynamics to scan all surface of hemagglutinin for umifenovir binding sites. To restore free energy landscape and describe all possible binding modes, the so-called collective variables have been implemented—spherical coordinates of radius vector between centers of mass of protein and ligand while keeping the molecule close to protein surface by restraining coordination number. The set of collective variables allowed moving the ligand from one predicted site to another around the protein along with the complete free energy landscape on chosen set of collective variables. The obtained ensemble of umifenovir molecule orientations corresponding to the major minima on the landscape is used as a starting point to model hemagglutinin structural rearrangements at fusion with host's cell membrane to address contradictory results of static X-ray crystallographic data. This study was supported by the RFBR grant № 18-315-00390.

1. Kadam U., Wilson A. Structural basis of influenza virus fusion inhibition by the antiviral drug Arbidol. Proc. Natl. Acad. Sci. 114. **206-214,** (2017).
2. Leneva I.A. et al. Characteristics of Arbidol-resistant mutants of influenza virus: implications for the mechanism of anti-influenza action of Arbidol. Antiviral Research. 100. **399–406,** (2013).

Antioxidant properties of tetra nitrosyl iron complex

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Today, the main strategy in finding drugs for NO-therapy is studying of new classes of exogenous NO donors. It is known that sulfur-nitrosyl iron complexes stimulate vasodilation and have antitumor and antimetastatic activities [1].
The impact of tetra nitrosyl iron complex (TNIC) on formation of thiobarbituric acid reactive substances (TBARS) following the reaction of oxidized lipids with TBA in mice brain homogenate has been studied in the present work. It was shown that TNIC inhibits the formation of TBARS. The level of TBARS has decreased by 25% in the presence of 100 μM TNIC and by 65% in the presence of 1 mM TNIC.
A sensitive chemiluminescence method for the determination of antiradical activity of TNIC was applied. Luminol was used to increase quantum yields of chemiluminescence. Tert-butyl hydroperoxide was used as an initiator of lipid peroxidation. The antioxidant capacity was calculated from the changes in area under chemiluminescence kinetic curve. Chemiluminescence intensity has decreased by 77% at 100 μM concentration of TNIC, and by 99% at 1 mM.
Apparently, inhibition of lipid peroxidation and decrease in chemiluminescence intensity in the presence of TNIC are due to interaction of nitric oxide and its metabolites with lipid radicals resulted in breaking of free radical chain.
Thus, the conducted studies have shown that in the used model systems, TNIC is an effective antioxidant, which is an important addition to the previously studied biological activities of this compound.

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1. Aldoshin, S.M., Sanina, N.A. Functional nitrosyl iron complexes: a new class of nitric oxide donors to cure socially important diseases. Basic Sciences for Medicine: Biophysical Medical Technologies, Vol. 1, 72–102 (2015) [in Russian].

GSNO is the most perspective glutathione-related substance in maintaining heart contractile function under hypoxic conditions

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Severe hypoxia leads to decline in cardiac contractility and induces arrhythmic events in cardiomyocytes. There are a few main membrane proteins (RyR; SERCA; Na,K-ATPase; VGCCs; NCX), which are required for maintaining transmembrane ionic gradient and conducting the action potential of the cell. These proteins are redox sensitive, and onset hypoxia leads to them oxidative damage.

We demonstrate that thiol-containing compounds: N-acetylcysteine (NAC), glutathione ethyl ester (et-GSH), oxidized tetraethylglutathione (tet-GSSG), oxidized glutathione (GSSG), and S-nitrosoglutathione (GSNO), reduce negative effects of hypoxia on isolated rat cardiomyocytes due to redox regulation of ion transporting proteins and protection of them from irreversible oxidation. Efficacy of thiol-type compounds was evaluated by their ability to maintain normal calcium transients in cardiomyocytes under hypoxic conditions. In normoxia, calcium peaks possess an asymmetric shape with sharp upward rise and more delayed downward descent. Under hypoxia, the downward arm of Ca^{2+} transients is distorted and enlarged indicating the deficiency of Ca^{2+} extrusion system whereas Ca^{2+} influx system seems relatively intact. Preincubation of cardiomyocytes with 0.1 mM GSNO, 0.5 mM et-GSH, GSSG, tet-GSSG or with 10 mM NAC allows cells five times longer tolerate the hypoxic conditions and elicit regular Ca^{2+} transients in response to electric pacing. The shape of Ca^{2+} transients generated in the presence of GSNO and et-GSH under hypoxia was similar to that observed in normoxic control cardiomyocytes. Thiol compounds were investigated on the model of perfusing isolated rat heart in conditions of ischemia-reperfusion where GSNO also demonstrated maximal protective effect. Concentration of 0.05 μM GSNO accelerated the recovery of normal contractile function of isolated rat heart subjected to ischemia-reperfusion by 39%. Also, GSNO increased glutathionylation of tissue-specific Na,K-ATPase alpha-2 subunit, the principal ion-transporter of cardiac myocyte sarcolemma, which prevents Na,K-ATPase irreversible oxidation and regulates its function to support normal Ca^{2+} ion handling in hypoxic cardiomyocytes. Altogether, GSNO appears effective cardioprotector in hypoxic conditions worth further studies toward its cardiovascular application.

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Comparative analysis of serotonin 5-HT receptors from X-RAY, EM, MD

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Ion channels form a major class of integral transmembrane proteins involved in the regulation of fundamental cellular processes [1]. The main function of ion channels is the selective movement of ions through the membranes. The importance of research of channels' structures is underscored by the identification of numerous "channelopathies," caused by ion channel mutations [2]. However, the complicated structure of eukaryotic ion channels is a barrier to structure determination by experimental methods [3]. Thus, only one atomic X-ray structure of the pentameric ligand-dependent mammalian channel of the serotonin 5-HT3 receptor (pdb id 4PIR [4]) and one Cryo-EM structure (pdb id 6BE1 [5]) are known at the present. The actual goal is to compare the different structures of the serotonin 5-HT3 receptors and its models constructed using the homology modeling and molecular dynamics. The obtained models in open and closed states differ from each other in the zone of the inner vestibule. The pore radius in this region is larger in open state than that in closed and X-ray structure. The membrane part of the X-ray structure of 5-HT3 receptor and the closed state model form a zone of minimum pore radius in M2 spirals due to the oxides of the threonine hydroxyl groups. The hydrated sodium ions are unable to pass through this section of the 5-HT3 receptor channel, according to the molecular dynamics data obtained in our laboratory. Thus, the X-ray structure of the 5-HT3 receptor corresponds to the closed conformation, in contradistinction to Cryo-EM structure of the serotonin 5-HT3 receptor. The work was supported by the Russian Foundation for Basic Research (grant 16-34-60252).

1. Jegla T. et al. Evolution of the human ion channel set. *Comb Chem High Throughput Screen* (2009). https://doi.org/10.2174/138620709787047957
2. Spillane J. et al. Genetic neurological channelopathies: molecular genetics and clinical phenotypes. Neurogenetics (2015). https://doi.org/10.1136/jnnp-2015-311233
3. Bill R. et al. Overcoming barriers to membrane protein structure determination. *Nat Biotechnol* (2011) https://doi.org/10.1038/nbt.1833.
4. Hassaine G. et al. X-ray structure of the mouse serotonin 5-HT3 receptor. *Nature.* (2014) https://doi.org/10.1038/nature13552
5. Basak S. et al. Cryo-EM structure of 5-HT3A receptor in its resting conformation. *Nat Commun.* (2018). https://doi.org/10.1038/s41467-018-02997-4

CompoMug for prediction of stabilizing mutations in GPCRs

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Engineering of GPCR constructs with improved thermostability is a key for successful structural and biochemical studies of this transmembrane protein family, targeted by 40% of all therapeutic drugs. Here, we present a comprehensive computational approach for effective prediction of stabilizing mutations in GPCRs, named CompoMug[1], which employs sequence-based analysis, structural information, knowledge-based module, and a derived machine learning predictor. Tested experimentally on the serotonin 5-HT2C receptor target, CompoMug predictions resulted in ten new stabilizing mutations, with an apparent thermostability gain ~ 8.8 °C for the best single mutation and ~ 13 °C for a triple mutant. Binding of antagonists confers further stabilization for the triple mutant receptor, with total gains of ~ 21 °C as compared to wild-type apo 5-HT2C. The predicted mutations enabled crystallization and structure determination for the 5-HT2C receptor complexes both in inactive and active-like states[2]. Furthermore, CompoMug is applicable to different GPCR families, for example class F receptors, as well. Tested on the FZD4 receptor, eight new stabilizing mutations were identified, with the ~ 3 °C gain in apparent melting temperature for the best single-point mutation. Combination of top four point mutations improved crystal quality and allowed structure determination of the FZD4 receptor in the apo form[3]. While CompoMug already shows high 25% hit rate and utility in GPCR structural studies, further improvements are expected with accumulation of structural and mutation data. CompoMug is available at https://gitlab.com/pp_lab/CompoMug.git.

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1. Popov P., et al., Computational design of thermostabilizing point mutations for G protein-coupled receptors. *eLife* 7 (2018): e34729.
2. Peng Y., et al. 5-HT2C receptor structures reveal the structural basis of GPCR polypharmacology. *Cell* 172.4 (2018): 719-730
3. Shifan, Y. *et al.* Crystal structure of the Frizzled 4 receptor in a ligand-free state. *Nature In Press*

Unusual tandem HCN- and K_v-like channels of eukaryotic microorganisms

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Cyclic nucleotide-gated cation (HCN/CNG) and voltage-gated potassium (K_v) channels are known to be essential for signal transduction and regulation of membrane potential in a eukaryotic cell. Previously, the analysis of transcriptomes of unicellular eukaryotes revealed unusual homologs of HCN/CNG and K_v channels in dinoflagellates [1]. Further, we found such homologs among available transcripts of other eukaryotic microorganisms, i.e., oomycetes and chlorarachniophytes. The analysis of primary structure of translated transcripts showed that they possess a double set of six transmembrane segments, including pore-loops with GY(F)G-motif. Moreover, tandem homologs of HCN channels have two C-linkers connected with two cyclic nucleotide-binding domains (CNBDs). Remarkably, CNBD in the first repeat of tandem HCN-like channel is substantially reduced. The analysis of nucleotide and translated amino acid sequences encoding predicted tandem channels suggested that these unusual transcripts are the result of ancestral gene duplication or fusion, rather than the result of an error during the transcriptome assembly. The phylogenetic analysis of 377 eukaryotic and prokaryotic K_v- and HCN-like protein sequences showed that the tandem HCN-like channels of oomycetes and dinoflagellates have a common origin, whereas the tandem K_v-like channels could have evolved two or three times independently in the oomycete, dinoflagellate, and chlorarachniophyte lineages.

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1. Pozdnyakov, I.A. & Skarlato, S.O. Analysis of the dinoflagellate *Prorocentrum minimum* transcriptome: identifying the members of the voltage-gated cation channel superfamily. *Cell and Tissue Biology*. **9**, 483–492 (2015).

Lentiviral gene delivery to plasmolipin-expressing cells using Mus caroli endogenous retrovirus envelope protein

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Gene therapy is a promising method for treating malignant diseases. One of the main problems is target delivery of therapeutic genes. Here, we show that lentiviral vector particles pseudotyped with Mus caroli endogenous retrovirus (McERV) envelope protein can be used for selective transduction of PLLP-expressing cells. These particles were shown to be an effective tool for selective marking of PLLP expressing cells. Furthermore, a gene-encoding cytotoxic protein in McERV env-pseudotyped vector particles may be used. Here, we demonstrated that McERV env-pseudotyped vector particles are a promising tool for targeting of PLLP-expressing cells.

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Selective fluorescent probe for potassium Kv1.3 channel based on GFP-tagged agitoxin 2

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Engineering of fluorescent probes based on potassium channel blockers [1] gives a convenient tool for searching for natural high selective blockers of these channels and creating synthetic ones. This, in its turn, expands the opportunity to design efficient drugs that are able to correct excessive expression and activity of selected potassium channels.
We have produced three constructs of fluorescent probes eGFP-AgTx2 consisting of agitoxin 2 (AgTx2), peptide blocker of voltage-gated potassium Kv1 channels, and green fluorescent protein (GFP), which differ in linkers and presence/absence of hexa-histidine tag. By using the fluorescent test-system based on *Escherichia coli* cells expressing hybrid potassium channels KcsA-Kv1.x ($x = 1, 3, 6$) in their plasma membrane [2, 3], we have shown high selectivity of binding of these constructs to Kv1.3 channel, measured dissociation constants of these complexes, and studied their dependency on pH. We have also shown that the created fluorescent probes can be successfully used to detect Kv1.3-channel blockers in scorpion venoms and among individual substances and to estimate the dissociation constants of complexes between Kv1.3 channel and these blockers.
The analysis of structural features and properties of the created fluorescent probes allows us to conclude that GFP plays an essential role in the selectivity of interactions of these probes with Kv1.3 channel.

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1. Kuzmenkov, A. et al. Fluorescent protein-scorpion toxin chimera is a convenient molecular tool for studies of potassium channels. *Scientific reports* 6, 33314-33324 (2016). https://doi.org/10.1038/srep33314
2. Kudryashova, K. et al. Fluorescent system based on bacterial expression of hybrid KcsA channels designed for Kv1.3 ligand screening and study. *Analytical and Bioanalytical Chemistry* 405, 2379-2389 (2013). https://doi.org/10.1007/s00216-012-6655-6
3. Nekrasova, O. et al. Recombinant Kv channels at the membrane of Escherichia coli bind specifically agitoxin2. *J. Neuroimmune Pharmacology* 4, 83-91 (2009). https://doi.org/10.1007/s11481-008-9116-4

MRI-Based Perspective on "Virtual Organ"

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"Virtual Organ" aims to integrate cellular architecture, protein distribution, and function into organ structure. MRI will be used to explore the relationship between organs and their molecular organization. First, whole mice can be imaged by high-resolution MRI, which is a non-invasive method to detect anatomic structures and macroscopic physiological processes. Then, super-resolution imaging, which provides mesoscopic information including GPCR distribution and intercellular connections, can be used within the same animals and compared to MRI. The third step is to create a correlation between patient MRI data and mice data. From these experimental results, we can build a brain model to benefit personalized medicine, including GPCR-structure-based drug development.

Particularly, the primary contrasts in MRI are T2, T2*, T1, and proton density, which provide different information about the local environment in the voxel. Thus, a combination of cell density, cell structure, and extracellular structure will affect contrast. However, models of cell types can be used to create outputs of these expected effects, then up-scaled to create a "Per-Voxel" model [1]. Virtual Organ will bring models of cellular function to the whole-organ scale, building a bridge between the macroscopic aspects of an organ and the microscopic function of its components.
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1. Yu Y, Herman P, Rothman D L, et al. Evaluating the gray and white matter energy budgets of human brain function[J]. Journal of Cerebral Blood Flow & Metabolism, 2017: 0271678X17708691.

MMP-9 expression reduce by Crocetin in HCT-116 colorectal cancer cells

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Introduction: The role of matrix metalloproteinases (MMPs) in the degradation of extracellular matrix (ECM) during the metastatic process is important. One of the most vital cellular mechanisms in cancer development is matrix metalloproteinase 9 (MMP-9)-based cell migration and invasion. Crocetin ($C_{20}H_{24}O_4$), extracted from the saffron, inhibits proliferation of various types of carcinoma cell lines. We aimed to investigate the in vitro anti-proliferation and anti-invasion effect of crocetin on HCT-116 colorectal cancer cells.
Methods: Colorectal cancer cells treated dose-dependently (200, 400, 600, and 800 μM) by crocetin. MTT proliferation assay and RT-PCR were performed.
Results: Crocetin dose-dependently (200, 400, 600, and 800 μM) and significantly (in 800 μM was 40% inhibition; $p < 0.0001$) inhibited HCT-116 cell viability. RT-PCR demonstrated that crocetin significantly ($p < 0.0001$ in 200, 400, 600, and 800 μM) reduced mRNA expression levels of MMP-9 in comparison with untreated HCT-116 cells. Also, data revealed that these effects were dose-dependent.
Conclusion: These data demonstrate that crocetin may inhibit human colon cancer metastasis. Crocetin should be investigated further as a viable option in the treatment of colorectal cancer.
Keywords
CrocetinColon cancerInvasionMMP-9

Characterization of thermostable LOV-based fluorescent protein variants with mutations of a highly conserved glutamine

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Flavin-binding fluorescent proteins (FbFPs) are small in vivo reporters, derived from light oxygen voltage (LOV) domains of photoreceptors of plants, bacteria, and fungi. They bind flavins as chromophore and are responsible for blue light sensation. One of the key LOV domain residues is FMN-proximal glutamine, which is highly conserved in different species and plays a central role in signal propagation in wild-type proteins. Mutations of this glutamine are known to affect optical properties of FbFPs [1, 2]; however, at the moment, there are no experimental data on structural effects of these mutations on FMN environment
Here, we present biophysical and structural characterization of variants of a thermostable FbFP from thermophilic bacterium *Chloroflexus aggregans* (CagFbFP) with mutations of the conserved glutamine to all possible polar and charged amino acids. Absorption and fluorescence spectra revealed blue-shifted absorption and fluorescence-emission maxima of up to 9 nm for the mutated variants in comparison with CagFbFP. Thermostability studies demonstrated that the mutants are less stable than the initial construct, which could prevent structural studies of such mutations in case of non-thermostable FbFPs. Crystal structures of all six mutants were determined to a high resolution allowing to observe different variants of the chromophore-binding environment.
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1. Davari *et al.* Photophysics of the LOV-based fluorescent protein variant iLOV-Q489K determined by simulation and experiment. *J Phys Chem B* (3344–52). **120** (2016).
2. Khrenova *et al.* Theoretical characterization of the flavin-based fluorescent protein iLOV and its Q489K mutant. *J Phys Chem B* (5176–83) **119** (2015).

Stilbene derivative as a photosensitive compound to control the excitability of neonatal rat cardiomyocytes

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Substances that can be used as photosensitizers for cardiac tissue are very helpful in modeling various excitation patterns in a cardiac tissue culture and may have prospective use in the temporary and permanent ablation of unwanted excitation sources in the heart.
The aim of the present work is to study the effect of stilbene derivative c-TAB (2-{4-[(E)-2-(4-ethoxyphenyl) vinyl] phenoxy} ethyl) trimethylammonium bromide) [1] on the cardiomyocyte layers and voltage-gated ion channels in cardiac cells. C-TAB is a structural analogue to AzoTAB, reported previously as a photoswitch for cardiac and neural cells, in which the azobenzene moiety is replaced by a stilbene grouping. However, while AzoTAB reversibly enables the tuning of cardiomyocyte excitability to the desired degree [1, 2], it is noticeably toxic to the cells. Thus, it has no pharmacological prospects and cannot be used in tissue culture experiments for extended periods of time (i.e., several hours or more).
A replacement of the azobenzene moiety by a stilbene grouping makes c-TAB less toxic to living cells. C-TAB has been shown to successfully inhibit excitation in cardiac cells in both *trans-* and *cis-* forms. It was also shown that the nature of the excitability blockage in cardiac cells is in the modulation of voltage-gated ion channels. Under the action of c-TAB in both *trans-* and *cis-* forms, the fast sodium and calcium currents are suppressed, while the slow potassium currents and Ito increase. The inhibition under c-TAB is reversible and can be overturned easily by washing out the c-TAB, however, not by light illumination. The irradiation of cardiac cells with near-UV when the *trans-* form of c-TAB is applied changes reversible inhibition to a permanent one that cannot be overturned by a washout.

1. Patent: MIPT, RU Pat., RU 2515502 C1, 2012
2. Magome N., Kanaporis G., Moisan N., Tanaka K., Agladze K.// TISSUE ENGINEERING: Part A. 2011.V.17 #21 and 22.
3. Magome N., and Agladze K. // Physica D. 2010. V.239.P. 1560.

Numerical representation of GPCR–ligand interactions for machine learning

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Approximately 30% of the drugs on the market target G-protein coupled receptors (GPCRs), which are key players in many important biological processes[1]. Virtual ligand screening and in silico structure-based drug design are widely used to search of new drug candidates amongst chemical libraries. Here, we present a molecular descriptor aimed to digitize GPCR-ligand interaction as an input vector for machine learning. The descriptor encodes physico-chemical and structural information and consists of three parts. The first part corresponds to the structural interaction fingerprint (SIFt) and represents receptor-ligand interaction interface[2]. The second part corresponds to the radial distribution functions (RDF) of various atom pair types[3,4] capturing chemical and geometrical properties of the interaction interface. Finally, the third part corresponds to the 3D structure pharmacophore PHAP3PT3D[5] that captures ligand properties. We demonstrated the efficiency of the proposed descriptor on the angiotensin receptor type 1 (AT1R) in complex with different ligands. Overall, the descriptor consists of 8197 components and takes ~ 1 s to be computed for a single receptor-ligand complex. Given a set of ligands with various affinities to AT1R from the Chembl database[6], we composed a set of decoys[7] and formulated a machine-learning problem using computed descriptors as an input and affinity values as an output. We used support vector machine approach along with the fivefold cross-validation to obtain the optimal parameters of the predictor, resulting in the f1-measure of 0.89. Therefore, the proposed descriptor efficiently encodes information relevant for ligand binding to AT1R. Our method could be applied to other GPCRs in order to derive prediction models based on the structure affinity relationship data.

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1. Hauser, A. S. et al., Trends in GPCR drug discovery: new agents, targets and indications. *Nat. Rev. Drug Discov.* **16,** 829–842 (2017).
2. Deng, Z., Chuaqui, C. & Singh, J. Structural interaction fingerprint (SIFt): a novel method for analyzing three-dimensional protein–ligand binding interactions. *J. Med. Chem.* **47,** 337–344 (2004).
3. Kadukova, M. & Grudinin, S. Convex-PL: a novel knowledge-based potential for protein-ligand interactions deduced from structural databases using convex optimization. *J. Comput. Aided. Mol. Des.* (2017).
4. Popov, Petr, and Sergei Grudinin. Knowledge of native protein–protein interfaces is sufficient to construct predictive models for the selection of binding candidates. *Journal of chemical information and modeling* 55.10 (2015): 2242-2255.
5. Hinselmann, G. et al. jCompoundMapper: an open source Java library and command-line tool for chemical fingerprints. *J. Cheminform.* **3,** 3 (2011).
6. Gaulton, Anna, et al. The ChEMBL database in 2017. *Nucleic acids research* 45.D1 (2016): D945-D954.
7. Popov, Petr, and Sergei Grudinin. Eurecon: equidistant uniform rigid-body ensemble constructor. *Journal of Molecular Graphics and Modelling* 80 (2018): 313-319.

The role of $\beta(+)/\alpha(-)$ intersubunit transmembrane interface in $GABA_AR$ potentiation

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The type A receptors of γ-aminobutyric acid ($GABA_AR$) are the major inhibitory receptors in CNS. We investigated potentiation of $GABA_AR$ by different positive allosteric modulators: general anesthetic (etomidate, ETM), endogeneous neurosteroid (allopregnanolone, ALP), and fenamate (mefenamic acid, MFA). We combined electrophysiological and modeling approaches to study the interaction mechanisms of these substances with $GABA_AR$. Patch-clamp recordings showed that MFA strongly potentiates GABA-induced currents in acutely isolated rat Purkinje cells with $EC_{50} \cong 15$ μM [1]. We found that potentiating effects of MFA and ETM were non-additive suggesting that these drugs act through a common binding site. The data of mutational studies indicate that ETM efficacy strongly depends on mutations in α_1 M236 and β_2 M286. We built the homology model of open $\alpha_1\beta_2\gamma_2$ $GABA_AR$ based on the cryo-EM structure of α_1 GlyR and used Monte-Carlo energy minimization to optimize the drug-receptor complexes. Our models predict that both ETM and MFA form H-bonds with β_2 M2 R269 (19′) and N265 (15′), and strong van-der-Waals contacts with M286 and F289 (β_2 M3) and L232, P233, and M236 (α_1 M1) [1]. ALP enhances the ETM action on $GABA_AR$. Thus, our model predicts that ALP should also enhance the MFA action. Patch-clamp recordings showed that co-application with ALP (100 nM) provoked a left-shift of the MFA concentration-dependent curves. Data from mutational studies indicate that neurosteroids bind in the cytoplasmic part of the $\beta(+)/\alpha(-)$ interface. We found that ALP binds between M1 and M3 helixes and forms a hydrogen bond with $\alpha1$ Q242 and strong van-der-Waals contacts with α_1 W246, I239 and β_2 L297, L301 [2]. Interaction energy between MFA and ALP bound in the $\beta(+)/\alpha(-)$ interface is negligible. Therefore, each drug modulates $GABA_AR$ affecting some specific receptor mechanisms. ALP binding site is located near the receptor desensitization gate and may enhance MFA potentiation affecting the probability of the channel transition to the desensitized state. Earlier, we described three major pore constrictions located at the levels of −2′, 9′, and 20′ residues which diameter depends on the receptor functional state [3]. The first two correspond to the desensitization and activation gates. We suggested that the 20′ ring may also have a gaiting serve function. MFA binds closely to the ring 20′ and may prolong the $GABA_AR$ lifetime in the open state. Supported by RFBR grant 18-015-00038.

1. Rossokhin, A. & Sharonova, I. *The FEBS Journal 284 Supl. 1, abstract nnumber P.3.4-014* (2017). https://doi.org/10.1111/febs.14174.
2. Rossokhin, A. et al. *FEBS Open Bio 8, abstract number P.15-013-Mon* (2018). https://doi.org/10.1002/2211.
3. Rossokhin, A. & Zhorov, B. *J Comput Aided Mol Des 30(7):559* (2016).

X-Ray structural studies of membrane proteins at high pressure

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When studying proteins, the question often arises as to how small molecules (water, oxygen) or cations\anions are distributed in the cavities defined by a protein's three-dimensional structure. To answer this question, high-resolution structural information derived from macromolecular crystallography (MX) is often required.
In this regard, taking bacteriorhodopsin (BR) as target, we investigated the applicability of the "soak-and-freeze" method [1], which has successfully showed how water channels are arranged in some water-soluble proteins, to crystals of membrane proteins. "Soaking" of crystals of bacteriorhodopsin was carried out in an argon atmosphere pressurized at up to 2000 bar and the crystal structures determined and refined at 1.7 Å resolution. These revealed the presence of water molecules/ions not seen in previous crystal structures. The biological significance of these is now being analyzed.

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1. Lafumat B. et al. Gas-sensitive biological crystals processed in pressurized oxygen and krypton atmospheres: deciphering gas channels in proteins using a novel "soak-and-freeze" methodology. Journal of Applied Crystallography. – 2016. – T. 49. – №. 5. – C. 1478-1487.

The application of postnatal and iPS-derived cells for human hair follicle and skin reconstruction in vitro

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The obtaining of 3D organs with the purpose of their further transplantation is the most relevant approach in the modern cell biology and regenerative medicine. Human postnatal cells are often unable to maintain the normal morphogenesis in bioengineered tissues. IPSCs are able to be differentiated into multiple directions reproducing more "fetal" state. In this research, we will focus on human hair follicle (HF) reconstruction and the restrictions in human postnatal skin cells and iPSCs applications.
Skin fetal cells possess the ability to be self-organized in 3D conditions producing mature HF [1]. As human fetal tissues are scarcely available for research and therapy, we have analyzed the ability of human postnatal skin cells to induce folliculogenesis in culture. The combination of the HF dermal papilla cells (DPCs) and skin keratinocytes (KCs) in a hanging drop culture resulted in formation of organoids with HF-specific properties [2]. QPCR analysis and immunohistochemistry revealed upregulation of HF-specific markers: Lef1, P-cadherin, K75, and others. The morphological structure of aggregates was similar to HF at the germ stage of development. Unfortunately, we have not identified any signs of further morphogenesis. Due to the age of skin donors, one of the reasons for morphogenetic failure could be the inability of aged cells to respond to external stimuli. We have found out that mesenchymal cells obtained from investigated patients also did not differentiate into myofibroblasts under hypoxic conditions in presence of TGFβ that indicates age-related defects. Our results have revealed the necessity of neonatal or fetal cells for successful HF development in culture. For this aim, iPSCs were involved. BMP4 application allowed us to differentiate iPSCs to more fetal K18+ KCs or to mature KCs with K5 and K14 expression. DPCs were obtained through neural crest stage and expressed versican, smooth muscle actin, and other specific markers. The results of obtained DPCs transplantation demonstrated HFs induction in immunodeficient mice.
The further experiment with differentiation of pluripotent cells into hair follicle origins can be profitable in the therapy of skin and hair aging.

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1. Qiao, J. *et al*. Hair morphogenesis in vitro: formation of hair structures suitable for implantation. *Regen Med*. (2008). https://doi.org/10.2217/17460751.3.5.683.
2. Kalabusheva, E. *et al.* Hair germ model in vitro via human postnatal keratinocyte-dermal papilla interactions: impact of hyaluronic acid. *Stem Cells Int.* (2017). https://doi.org/10.1155/2017/9271869

Modern materials used in 3D-bioprinting and methods of their improvement for use in regenerative medicine

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Currently, 3D-bioprinting is widely developed, which finds its application in regenerative medicine, and for its successful improvement, materials other than those used in conventional 3D-printing are needed [1]. One of the main requirements for biomaterials is biocompatibility and biodegradability, the ability to perform certain functions for the required time without harm to the body. In order for the created material to meet these requirements, it is necessary to know what materials exist at the moment that are used in regenerative medicine.
In 3D-bioprinting, hydrogels are widely used, which are polymers that have a high ability to retain water and imitate the environment of the native tissue. Very promising are hydrogels consisting of methacrylated gelatin and silk proteins (the recombinant spidroin analog of the Nephila clavipes web strand and silk fibroin from the cocoons of the silkworm Bombyx mori) [2]. These polymers are characterized by high biocompatibility and biodegradability, which allows their use in tissue engineering. To replace bone defects, composite materials with mechanical properties similar to natural bone are used. To obtain these properties, zirconium oxide, silica, or bioglass is added to the framework containing the polymer (chitosan, polycaprolactan, polylactic acid, polyethylene glycol diacrylate, etc.). Such a framework has high mechanical properties and is able to stimulate tissue vascularization.
It is necessary to say that the success of the implant depends on the properties of the biomaterial. The ideal 3D biomaterial should be biocompatible, easy to print with an adjustable resorption rate, and morphologically imitate living tissue. With all the parameters, such biomaterial in the future will be able to give good results in the creation of 3D-printed structures.

1. Nagibovich O. A., Svistov D. V., Peleshok S. A., Korovin A. E., Gorodkov E. V. Application of 3D-printing technology in medicine. Clinical pathophysiology. Volume 23, Issue 3. 2017:14-22. (In Russ.)
2. Bessonov I.V., Kotliarova M.S., Kopitsyna M.N., Fedulov A.V., Moysenovich A.M., Arkhipova A.Y., Bogush V.G., Bagrov D.V., Ramonova A.A., Mashkov A.E., Shaitan K.V., Moisenovich M.M. Photocurable hydrogels containing methacrylated gelatin and spidroin or fibroin. Vestnik Moskovskogo universiteta. Seriya 16. Biologiya. 2018;73(1):29-33. (In Russ.)

Small angle scattering structural study of the NpSRII/HtrII complex

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The sensory rhodopsin II–transducer complex (NpSRII/HtrII) from *Natronomonas pharaonis* is a representative of archaebacterial photoreceptors. Two-component signaling systems (TCS) comprise photoreceptors along with chemoreceptors and sensor histidine kinases. NpSRII/HtrII requires dimerization for signal transduction and forms a trimer-of-dimers in cell membrane [1–3]. This complex is a widely used model system to study how TCS transfer signals across the membrane. Despite the structure of the truncated construct is available [1], the molecular mechanism of the signal transduction is to be revealed, since there is no full-length complex structure.

In this work, we study the low-resolution structure of NpSRII/HtrII using small angle X-ray scattering (SAXS). SAXS profiles were obtained on the BM29 beamline (ESRF) [4] and processed using the ATSAS software suite [5].

Calculated ab initio structure displays an elongated shape with a length-to-width ratio of ~5:1. It has a characteristic cavity along a central axis and reveals that the "O"-shaped conformation takes place from both the "O" and "Y" models of trimer-of-dimers formation [2]. The resulting complex length is ~325 Å, which is in a good agreement with the literature data [2]. The structure contains regions of possible contacts of HtrII dimers, which are located in the expected positions of the beginning and edge of the kinase control module and provide stability of the trimer.

Overall, our work provides key insights into the molecular mechanism of the full-length complex. It will advance in our understanding of TCS signal propagation.

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1.Gordeliy, V. I. *et al.* Molecular basis of transmembrane signalling by sensory rhodopsin II–transducer complex. *Nature* **419,** 484–487 (2002).
2.Orekhov, P. *et al.* Sensory rhodopsin I and sensory rhodopsin II form trimers of dimers in complex with their cognate transducers. *Photochem. Photobiol.* **93,** 796–804 (2017).
3.Ryzhykau, Y. L. *et al.* Trimers of dimers of SRII/HtrII full complex. Small angle scattering structural investigation. *FEBS J.* **284,** 154 (2017).
4.Pernot, P. *et al.* Upgraded ESRF BM29 beamline for SAXS on macromolecules in solution. *J. Synchrotron Radiat.* **20,** 660–664 (2013).
5.Petoukhov, M. V *et al.* New developments in the ATSAS program package for small-angle scattering data analysis. *J. Appl. Cryst* **45,** 342–350 (2012).

Doxorubicin-loaded folate liposomes for targeted drug delivery

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Cationic liposomes (CL) are promising delivery systems, due to ability to encapsulate both lipophilic and hydrophilic drugs. For targeted drug delivery, CL could be modified with folic acid, since tumor cells are characterized by folate receptors (FR) overexpression. Usually, ligands are introduced in liposomes by thin film technique (A). In this study, to covalently attach ligands only to the liposome surface, a new click-chemistry approach, namely post-modification technique (B) has been developed. The aim of the study was to obtain doxorubicin (DOX)-loaded folic acid-associated cationic liposomes (FLPs) by post-modification technique and to evaluate their cytotoxicity in vitro.

Folate-associated liposomes were obtained by both A (FLPs) and B (FLPs*) techniques and characterized in terms of mean size and ζ-potential values (Table 1). Liposomes without folic acid (LPs) were used as a control.

Sample	Mean D, nm	PI, %	ζ-potential, mV
FLPs	254	87	+47
FLPs*	135	93	+51
LPs	95	98	+58

Table 1. Physico-chemical parameters of the liposomes

HeLa, MCF-7, U-87 MG, and C6 cell lines were chosen because they are known to differ in FR numbers. The FLP accumulation and localization in the cells were evaluated by confocal microscopy as well as by flow cytometry. Liposome cytotoxicity was measured by MTT-test.

Both folate-associated liposomes (FLPs and FLPs*) were shown to have higher accumulation efficiency and enhanced cytotoxicity compared to those of LPs.

Zinc binding properties of human growth hormone

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Human growth hormone (hGH, somatropin) is a single chain polypeptide hormone consisting of 191 residues. Its main function is the stimulation of somatic and bone growth, as well as an increase in the size and mass of organs and tissues. In addition, it influences protein, carbohydrate, and lipid metabolism. Numerous effects caused by hGH are based on its ability to bind to specific receptors on the target cell surfaces. Unlike animal GHs, hGH can bind to prolactin, thus displaying a lactogenic activity. The hGH affinity to the prolactin receptor was shown to considerably increase (by more than 10,000 times) in the presence of zinc (Zn^{2+}) ions [1]. The hGH residues involved in Zn^{2+} binding are His^{18}, His^{21}, and Glu^{174}. Zn^{2+} was demonstrated to induce the hGH dimerization during which two Zn^{2+} ions associate per hGH dimer in a cooperative fashion. Replacement of potential Zn^{2+} ligands (His^{18}, His^{21}, and Glu^{174}) in hGH with alanine weakens both Zn^{2+} binding and hGH dimer formation [2]. Although much is known about Zn^{2+}-hGH interplay, many phenomena remain unexplained. For example, the mechanism of selective and reversible precipitation of hGH by Zn^{2+} is still unknown [3]. As well as the mechanism of hGH secretory granules formation which is of obvious biological significance.

In the current study, a set of hGH variants containing single and multiple mutations of residues important for Zn^{2+}-binding was prepared by genetic engineering methods. The somatogenic receptor binding properties of these variants were analyzed by surface plasmon resonance method. The Zn^{2+}-binding properties were studied by using the isothermal microcalorimetry technique. The Zn^{2+}-induced precipitation of hGH and its variants was investigated. The results obtained suggest the complex character of Zn^{2+}-hGH interaction.

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1. Cunnigham, B.C. *et al.* Zinc mediation of the binding of human growth hormone to the human prolactin receptor. *Science*, **250**, 1709–1712 (1990).
2. Cunningham, B.C. *et al.* Dimerization of human growth hormone by zinc. *Science* **253** (5019), 545-548 (1991).
3. Yang, T.H. et al. Effect of zinc binding and precipitation on structures of recombinant hGH and NGF. *J. Pharm. Sci.* **89** (11), 1480–1485 (2000).

Optical study of adenylyl cyclase signalling cascade in regulation of viscoelastic properties of human erythrocytes membrane

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Deformability of erythrocytes (red blood cells, RBC) is an intrinsic biomechanical property that determines their ability to pass through narrow blood vessels, which diameter is less than the linear size of the cells. Alterations of RBC deformability (RBC-D), which occur at various deceases, e.g., diabetes mellitus type I and II, arterial hypertension, sickle cell disease, etc., lead to the impairment of gas exchange processes that may result in tissue hypoxia and necrosis.
There are mechanisms of RBC-D regulation involving intercellular signalling cascades[1]. One can be an adenylyl cyclase (AC) cascade, which is directed at the synthesis of secondary messenger cAMP in response to external stimuli. An increase of the cytosolic cAMP activates protein kinases A leading to the phosphorylation of the peptide substrate. It results in the dissociation of the bonds between the cytoskeleton and the membrane protein complexes. This molecular system can be used for the fast adaptive increase of RBC-D to maintain the effectiveness of blood microcirculation.
In this work, we present our results of the optical study of the effects of adenylyl cyclase stimulation on the RBC-D using laser ektacytometry. Two different ways of the stimulation were studied: (1) stimulation via β-adrenergic receptor with epinephrine and (2) with membrane-permeable cAMP analog dibutyryl-cAMP (db-cAMP). Laser ektacytometry allows to quantitatively characterize RBC-D by analyzing the diffraction patterns obtained by illuminating a suspension of RBCs with a laser beam[2]. RBC are elongated under the shear flow in the microfluidic chamber. We observed that the RBC-D curve—the graph of RBC elongation index (EI) dependence on shear-stress in semi-log scale—located higher when AC was activated in comparison with intact RBC. The sum of the distances between points of the curves was used as a metrics for characterizing RBC-D changes: 0.337 ([epinephrine] 1 μM) and 0.361 ([db-cAMP] 2.5 μM). The results indicate that the adenylyl cyclase activation leads to the increase of RBC deformability.

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1.Muravyov, A. V. *et al.* Crosstalk between adenylyl cyclase signaling pathway and Ca2+ regulatory mechanism under red blood cell microrheological changes. *Clin. Hemorheol. Microcirc.* **45,** 337–345 (2010).
2.Priezzhev, A. V., *et al.* Laser ektacytometry and evaluation of statistical characteristics of inhomogeneous ensembles of red blood cells. *JQSRT.* **146,** 365–375 (2014).

Ionophoric effect of free bile acids. Study on isolated liver mitochondria

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Free bile acids (BA) are the family of amphiphilic molecules which have a free hydrophilic carboxyl group and a hydrophobic steroid nucleus with a different number of hydroxyl groups in their composition [1]. The effect of BA: lithocholic (LCA), deoxycholic (DCA), chenodeoxycholic (CDCA), ursodeoxycholic (UDCA) and cholic (CA), as inducers of the Ca^{2+} and K^+ transport through the inner membrane of isolated liver mitochondria has been studied in the present work. In experiments on succinate-fueled mitochondria, it was shown that the effect of hydrophobic BA: LCA, DCA, CDCA, and hydrophilic CA as inducers of Ca^{2+}-dependent cyclosporin A (CsA)-sensitive mitochondrial pore is not associated with modulation of inorganic phosphate effects. Among these BA, LCA contains only one hydroxyl group at the 3 carbon atom in the α-position. This causes the greatest hydrophobicity of LCA and its pronounced cytotoxicity. We have established that the uniqueness of the action of LCA is also in its ability to induce the release of Ca^{2+} from energized mitochondria in the presence of CsA. It was found that UDCA, which has a therapeutic potential, in contrast to these bile acids, is capable of inducing a Ca^{2+}-dependent CsA-insensitive permeability of the inner membrane of liver mitochondria. Such UDCA-induced permeability of mitochondria is observed only in the presence of potassium chloride in the medium and is blocked by inorganic phosphate. The uniqueness of the effects of UDCA is due to the presence of a hydroxyl group at the 7 carbon atom in the β-position. In experiments on deenergized liver mitochondria, it was shown that these BA are capable of inducing the release of Ca^{2+} from the matrix, and such an effect is not associated with damage of the inner membrane, since it is accompanied by the generation of $\Delta\psi$—the formation of the diffusion potential. BA-induced Ca^{2+} release from the liver mitochondrial matrix in the case of suppression of calcium uniport by ruthenium red leads to a decrease in the effectiveness of their action as inducers of Ca^{2+}-dependent permeability in mitochondria. Thus, depending on the structure of the molecule (the amount and localization of hydroxyl groups), BA are able to induce the transport of Ca^{2+} and K^+ through the inner mitochondrial membrane, which indicates their ionophore activity.

1. Khoroshavina, E. *et al.* Ursodeoxycholic acid, in contrast to other bile acids, induces Ca^{2+}-dependent cyclosporin A-insensitive permeability transition in liver mitochondria in the presence of potassium chloride. *Biologicheskie Membrany.* **33**, 344–352 (2016).

The investigation of the induced contractile activity of the mouse colon and the role of FFA3 in IBS

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Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder caused by bowel dysfunction and is defined as a variable combination of chronic or recurrent gastrointestinal symptoms by any structural or biochemical abnormalities[1]. Although the pathogenesis of IBS is not known, gut microbial activity is likely related to symptom severity. Recently, it has been shown that key microbial products, such as short-chain fatty acids (SCFA), have specific GPCRs, principally free fatty acid receptor 2 (FFA2, or GPR43) and FFA3 (GPR41)[2], which helped to clarify the mechanism of microbiota-related physiological responses, like the alteration of peristalsis and ion transport[3]. In this study, we established a IBS

mouse model, assessed of intestinal hypersensitivity and investigated FFA3 agonist effects on carbachol-induced motility of mouse colon.

Studies were performed on mice and were randomly assigned to either a control group or a model group, which received an intracolonic infusion of 1% acetic acid in saline. Sensitivity of the colon to balloon distention in adults was measured by grading their abdominal withdrawal reflex (AWR) and recorded contraction of mouse colon segments a length of 5 mm under isometric conditions.

AWR scores in the IBS model group at average volumes significantly were higher than in the control group ($n = 17$, $p < 0.05$). However, there were no significant differences at lower and higher volumes ($p > 0.05$), which can be associated with a high level of stimulation and an increase in the intensity of the response to stimulation. Application of 1 μM carbachol into the organ bath immediately and strongly contracted the colon segments followed by inhibition of spontaneous contractions. Against the background of pretreatment of segments with the FFA3-agonist butyric acid, carbachol-induced contractions of IBS-group were more potent than of control. These results suggest that FFA3-agonist, perhaps, prevented carbachol-induced circular muscle relaxation in IBS-group. Furthermore, it is emphasizing the potential of FFA3 as mechanisms involved in the pathology of IBS.

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1.Sandhu, B. K. & Paul, S. P. Irritable bowel syndrome in children: pathogenesis, diagnosis and evidence-based treatment. *World J. Gastroenterol.* **20,** 6013–6023 (2014).

2.Hurst, N. R., Kendig, D. M., Murthy, K. S. & Grider, J. R. The short chain fatty acids, butyrate and propionate, have differential effects on the motility of the guinea pig colon. *Neurogastroenterol. Motil.* **26,** 1586–1596 (2014).

3.Kaji, I. *et al.* Free fatty acid receptor 3 activation suppresses neurogenic motility in rat proximal colon. *Neurogastroenterol. Motil.* **30,** e13157 (2018).

The chain of dihedral angles: how does it rotate in condensed media?

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The mechanics of the conformational motions of chain molecules (peptides, lipids, etc.) in viscous medium are considered. We demonstrate that very complex representative point trajectory in the torsion angles' configuration space can be described in terms of simple geometrical representations. This approach leads to clear physical picture for collective conformational dynamics and understanding of MD simulation effects. Let us consider the next variables:

(1) $S = \sum_i \gamma_i \vec{r}_i^{\,2}$ - the energy-dissipation (ED) rate, γ_i - viscous friction coefficient, $\vec{r}_i$ - the velocity of i-node of the chain ($i = 1,2,...N$).

(2) $\frac{dU}{dt} = \sum_i \vec{r}_i \frac{\partial U}{\partial \vec{r}_i}$ - the rate of change of the potential energy (PE).

In the case of the viscous medium (water, etc.) inertial terms are negligible. Thermal fluctuations can be taken into account. Due to conservation energy law we can obtain

(3) $S + \frac{dU}{dt} = \sum_i \left(\frac{\partial U}{\partial \vec{r}_i} + \gamma_i \vec{r}_i \right) \vec{r}_i = 0$

Equations (1) and (3) can be rewritten in terms of standardized velocity components as follows:

(1a) $\sum_{i,\alpha} Z_{i,\alpha}^2 = 1; \quad \alpha = 1,2,3; \quad Z_{i,\alpha} = \dot{r}_{i,\alpha}\sqrt{\gamma_i/S};$

(3a) $\sum_{i,\alpha} \left\{ Z_i + \frac{1}{2\sqrt{S\gamma_i}} \frac{\partial U}{\partial \vec{r}_i} \right\}^2 = \frac{1}{4}$

Equation (1a) defines the hypersphere (HSP) of radius 1 with center at 0 in the space of the node velocities. Equation (3a) defines the manyfold of HSPs with radius 1/2 and the centers "O" whose distances from the zero point are ½ as well. The center "O" of HSP (3a) rotates around zero point inside HSP (1a) due to conformational dynamics of the chain molecule. The contact point of HSPs (1a) and (3a) moves on the surface of HSP (1a). Time dependence of the contact point coordinates specifies the trajectory of the representative point.

Two extremal principles for the relaxation of the stressed conformation states can be formulated [1]. The first is – «The rate of PE decrease is maximal at every moment in time». The second is – «The rate of ED is minimal at a given rate of PE decreasing».

In the asymptotic limit $N >> 1$, there exists a very useful rule for molecular chain conformation dynamics in viscous medium: "Dihedral angles' rotations occur in such a way that the average energy dissipation rate is uniformly distributed over all nodes of the molecular chain."

It demonstrates the collective effects for the rotations along the dihedral angles of the chain in a viscous medium, which arise from the action of friction forces only.

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1. Shaitan K.V. Variational principles in the mechanics of conformational motions of macromolecules in a viscous medium. Biophysics (Moscow), 2018, Vol. 63, No. 1, pp. 1–9. https://doi.org/10.1134/S0006350918010165

Localization of luciferase of fungus *Neonothopanus nambi*

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Luciferase promotes transformation of luciferin into the oxyluciferin molecule in the excited state, which in turn releases a quantum of visible light upon relaxation to the ground state. Luciferases are widely applied for in vitro and in vivo analysis of metabolites, monitoring of gene expression, drugs screening, bioimaging, etc. Fungal bioluminescence system is a scope of research for numerous of scientists for more than 50 years. The structure of fungal luciferin was recently elucidated in our laboratory [1] that led us to fungal luciferase discovery. This enzyme is a new and unique protein, which does not have significant homology with any of other described proteins. Also, transmembrane domain has been predicted by primary analysis of fungal luciferase sequence using bioinformatic methods. In this work, we have investigated intracellular localization of recombinant *Neonothopanus nambi* luciferase. For this purpose, we have made several genetic constructs, which have encoded full-size and truncated, without predicted transmembrane part, genes of *N. nambi* luciferase, fused with genes of fluorescent protein TagGFP2. These constructs were used for cotransfection of mammalian U2OS cells with subcellular localization vectors, contained fluorescent protein genes. We have performed immunohistochemistry analysis of these cells, and we suppose that these results will be very useful for future development of in vivo applications, based on *N. nambi* luciferase. This work was supported by the Russian Ministry of Education and Science, grant RFMEFI61317X0062.

1. Purtov, K. V. *et al.* The chemical basis of fungal bioluminescence. *Angew. Chem.* **54**, 8124-8128, (2015)

Superfused neocortex in vivo for exploration of the optical intrinsic signal

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Registration of the optical intrinsic signal (OIS) takes the special place among other techniques for neuroimaging. It is known that there are several mechanisms underlying the OIS associated with the hemodynamic and tissue scattering changes. Previously, we showed that, unlike adults OIS, tissue light scattering dominates the OIS in neonatal rats due to delayed hemodynamic response. However, the mechanisms underlying the tissue component are largely unknown. While in olfactory cortex, it is shown that swelling of the neuronal axons underlies the OIS, in the developing barrel cortex, the OIS has a postsynaptic nature. With the aim to explore the mechanisms of the OIS in the developing barrel cortex, we suggested to use the superfused neocortex in vivo that was originally developed for adult hippocampus (Khazipov and Holmes 2003) and showed its efficiency in neocortical research (Minlebaev et al., 2007). The experiments were done using the barrel cortex of neonatal rats in vivo (postnatal days P4–P7). The superfused chamber was placed over the barrel cortex and the OIS was evoked by the stimulation of the sensory vibrissae on the snout. Using the combination of the close IR highlight and the superfused cortical preparation, we have observed the evoked OIS that was not significantly different from the OIS that we have demonstrated previously, using similar wavelength of the light (Sintsov et al., 2017). Thus, we propose novel approach that shares the advantages of the in vitro and in vivo preparations and allows to explore the mechanisms underlying the OIS in in vivo, as well as creates the opportunity for the use of electrophysiological records in the active region of the brain.

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1. Khazipov, R. & Holmes, G.L. Synchronization of kainate-induced epileptic activity via GABAergic inhibition in the superfused rat hippocampus *in vivo*. *J Neurosci*. **5337-5341** (2003). https://doi.org/10.1523/JNEUROSCI.23-12-05337.2003.
2. Minlebaev M. *et al.* Network mechanisms of spindle-burst oscillations in the neonatal rat barrel cortex *in vivo*. *J Neurophysiol.* **692-700** (2007). https://doi.org/10.1152/jn.00759.2006
3. Sintsov M. *et al.* Developmental changes in sensory-evoked optical intrinsic signals in the rat barrel cortex. *Front. Cell. Neurosci.* **333–337** (2016). https://doi.org/10.1007/s12668-016-0359-x

Deuterium-reinforced fatty acids for combined treatment of neurodegenerative diseases: application for gene therapy

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Non-enzymatic autoxidation of polyunsaturated fatty acids damages lipid membranes and generates numerous toxic by-products implicated in neurodegeneration, aging, and other pathologies. The rate-limiting step of the process is the abstraction of bis-allylic hydrogen atoms.
We have earlier described an approach to inhibiting the chain reaction, based on deuteration of PUFAs at the bis-allylic sites (D-PUFAs) [1]. Even partial deuteration inhibits the oxidative chain reaction, producing observable biological effects [2, 3].
Conjugates of siRNA with lipophilic biomolecules have proved their efficiency in terms of delivery and cell penetration. Recently, DHA was reported as a better alternative to cholesterol for gene knockdown in neuronal tissues [4]. DHA is a natural constituent of neural cell membranes, it is actively internalized by neurons and other cell types, its metabolites have anti-inflammatory properties and DHA-siRNA conjugates are much less hydrophobic than those with cholesterol. However, compared to chemically stable cholesterol, DHA is readily susceptible to oxidation in physiological media.
Here, we propose a convenient click-chemistry-based approach to siRNA-D-PUFAs conjugates, in which fatty acid, on the one hand, facilitates gene delivery, and on the other, has its own therapeutic impact, which altogether may give rise to a synergetic therapy of age-related and neurodegenerative diseases.

1. M.S. Shchepinov, Rejuv. Res. 2007, 10, 47-59.
2. S.Hill et al. Free Rad. Biol. Med. 2011, 50, 130-138.
3. Lamberson C.R. et al. J. Am. Chem. Soc. 2014, 136, 838-841.
4. M. Nikan et al. Mol. Ther. Nucleic Acids. 2016, 5(8), e344.

Immunotherapeutic targeting of immunological synapse

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Immune checkpoint therapy has provided the recent breakthrough in treatment of previously incurable metastatic cancers [1]. Immune checkpoints are molecules that are expressed on the surface of immune cells and regulate their activity. Activation of effector lymphocytes occurs after formation of functional immunological synapse (IS) with target or antigen presenting cell. IS formation is a result of highly dynamic and coordinated activity of receptors, signaling molecules, cytoskeleton, and vesicular transport within the lymphocyte [2]. Immune checkpoints are integral parts of this dynamic process, but little is known about their actual roles. Although the overall immune response relies on the complex interplay between different immune and tumor cells, formation of competent IS is a crucial step for priming of lymphocytes and initiation of this process. Understanding of the molecular and spatiotemporal aspects of IS regulation by checkpoint molecules will aid in development more effective checkpoint therapy. Here, I will review recent data on IS regulation by checkpoint molecules and emerging concepts for their pharmacological targeting.

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1. Ribas, A. & Wolchok, J.D. Cancer immunotherapy using checkpoint blockade. *Science (1350-1355)*. **359**, (2018)
2. Dustin, M.L. & Groves, J.D. Receptor signaling clusters in the immune synapse. *Ann Rev. Biophys. (534-556).* **41,** (2012).

Mechanisms of modulation of $GABA_A$ receptor chloride channels by niflumic acid

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Niflumic acid (NFA) is a drug belonging to a class of fenamates used as non-steroidal anti-inflammatory drugs. Additionally, NFA produces modulation of a variety of ion channel responses including $GABA_A$ receptors in the brain. In this study, we investigated the modulation of GABA-

induced currents by NFA in Purkinje cells acutely isolated from young rat cerebellum. GABA-evoked currents were recorded using whole-cell patch-clamp and fast perfusion techniques. At holding potential, −70 mV NFA had a biphasic effect on responses elicited by 2 μM GABA (EC_{20}): it increased GABA currents in the concentration range from 3 to 100 μM and suppressed responses at concentrations higher than 100 μM. Inhibitory effect of NFA developed only after activation of the channel and was strongly voltage-dependent, suggesting that the blocking action of NFA is a result of NFA binding at the site located within $GABA_A$ receptor channel pore. The termination of GABA and NFA application was followed by a transient increase of the inward current—"tail" current, suggesting that NFA acts as a sequential open channel blocker, which prevents dissociation of agonist while the channel is blocked. Analysis of the block properties using the "double jump" protocol revealed a two-phase character of the interaction of the blocker with the channel: the first, fast phase of the block developed within a few milliseconds, hundreds of milliseconds were required for the development of the second phase. The Woodhull analysis revealed that NFA blocks $GABA_A$ receptor at the fractional electrical distances of 0.19 and 0.72 from the external membrane surface for early and late phases of inhibition, respectively. The surface binding site had a lower affinity for the NFA than the deeper. We have built homology model of the open $GABA_A$ receptor based on the X-ray structure of the glutamate-gated chloride channel and used powerful Monte-Carlo energy minimization approach to predict the NFA binding site. Using the method of molecular modeling, we have shown that there are at least three sites in the ion channel pore of $GABA_A$ receptor where NFA can suppress the ion permeation. Our observations imply that NFA has at least three separate binding sites on $GABA_A$ receptors in Purkinje cells. At the first proposed site, NFA acts as a positive allosteric modulator, two other binding sites located in $GABA_A$ receptor pore: one closer to the external part of the membrane and the second one significantly deeper in the pore.

NMR spectroscopy identifies binding interfaces in the complex of gating modifier toxin Hm-3 with first voltage-sensing domain of Na_V1.4 sodium channel

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Voltage-gated Na^+ (Na_V) channels contain domains that have discrete functionalities. The central pore domain allows current flow and provides ion selectivity, whereas peripherally located four voltage-sensing domains (VSD-I/IV) are needed for voltage-dependent gating. Certain mutations trigger a leak current through VSDs leading to various diseases. For example, hypokalemic periodic paralysis (HypoPP) type 2 is caused by mutations in the S4 voltage-sensing segments of VSDs in the skeletal muscle channel Na_V1.4. The gating modifier toxin Hm-3 (crab spider *Heriaeus melloteei*) inhibits leak currents through such mutant channels and represents useful hit for HypoPP therapy.
To study molecular basis of Hm-3 interaction with the VSD-I of Na_V1.4 channel, we expressed the isolated domain (~150 a.a., four transmembrane helices, S1-S4) in the cell-free system. Mixed micelles of zwitterionic detergents (DPC/LDAO 1:1) provided optimal conditions for NMR study. The limited stability of the VSD-I samples in this milieu (half-life time of ~24 h at 45 °C) prevents usage of classical assignment approach based on the 3D triple-resonance (^{1}H-^{13}C-^{15}N) experiments. Therefore, we used combinatorial selective labeling and extracted sequence-specific information from the 2D TROSY and HNCO spectra measured for several selectively ^{13}C,^{15}N-labeled samples. This provided straightforward assignment for ~50% of VSD-I backbone resonances.
NMR data show that Hm-3 partitions into micelles through a hydrophobic cluster formed by aromatic residues and reveal complex formation with VSD-I through electrostatic and hydrophobic interactions with the S3b helix and the S3–S4 extracellular loop. Two different hydrophobic interfaces on the Hm-3 surface are responsible for the interactions with the micelle and VSD-I. The model of the Hm-3/VSD-I complex was built using protein-protein docking guided by NMR restrains. Our data identify VSD-I as a novel specific binding site for neurotoxins on sodium channels.

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Simultaneous imaging of structurally different objects: pathogens, phagocytic cells lymphatic and blood vessels in whole-mount airway samples using advanced fluorescent microscopy

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Whole-tissue imaging is essential to acquire the entire information about the inflammatory process compartmentalization[1]. Neutrophils are the primary phagocytic cells that migrate fast from bone marrow via blood vessels to the site of inflammation[2]. Recent studies have demonstrated that neutrophils can also relocate from the site of inflammation toward lymph nodes via lymphatic vessels[3]. Here, we investigated fungal pathogen-induced neutrophil migrations using three-dimensional images of whole-mount conducting airway. Visualization of murine whole-mount conducting airway specimens was performed at different time points after inhalation of Alexa700-labeled fungal spores using a confocal laser scanning microscope Zeiss 780. Tissue samples were incubated with combination of antibodies: rat anti-Ly6G for neutrophil identification, goat anti-CD31 for blood vessels recognition, and rabbit anti-Lyve1 to distinguish lymphatic vessels. The combination of secondary donkey anti-rat-Alexa488, donkey anti-goat-Alexa555, and donkey anti-rabbit-Alexa647 antibodies was further applied. To visualize actin-rich epithelial and smooth muscle cells, samples were stained with Phalloidin-Atto425 and nuclei were identified with Hoechst33342. Simultaneous visualization of neutrophils, lymphatic vessels, and blood vessels was achieved by using of λ-mode followed by spectral unmixing. Quantitative analysis of neutrohils in blood and lymphatic vessels as well as in conducting airway walls was performed using Zen and Fiji software.

Thus, we followed the neutrophil migration in course of inflammatory response.

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1. Shevchenko, M. A. *et al.* Aspergillus fumigatus infection-induced neutrophil recruitment and location in the conducting airway of immunocompetent, neutropenic, and immunosuppressed mice. *J Immunol Res.* (2018). https://doi.org/10.1155/2018/5379085
2. Kohler, A. *et al.* G-CSF-mediated thrombopoietin release triggers neutrophil motility and mobilization from bone marrow via induction of Cxcr2 ligands. *Blood (4349-4357).* **117**, (2011). https://doi.org/10.1182/blood-2010-09-308387
3. Hampton, H. R., Bailey, J., Tomura, M., Brink, R. & Chtanova, T. Microbe-dependent lymphatic migration of neutrophils modulates lymphocyte proliferation in lymph nodes. *Nature Communications* **6**, (2015). https://doi.org/10.1038/Ncomms8139

Chronic ethanol ingestion induces glomerular filtration barrier proteins genes expression alteration and increases matrix metalloproteinases activity in the kidney

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Chronic alcohol ingestion-induced kidney structure and function alterations is very well known, but the precise underlying molecular mediators involved in ethanol-induced kidney abnormalities remain elusive. The aim of this study was to investigate the effect of chronic ethanol exposure on matrix metalloproteinases 2 and 9 and the glomerular filtration barrier proteins (nephrin and podocin), as well as vascular endothelial growth factor receptors 1 and 2 (VEGFRs) isoform gene expression in the kidney of rats. Sixteen male wistar rats with an initial body weight of 220 ± 10 g were divided into the following two groups: 1—control and 2—ethanol. Similar to our previous study, rats in the ethanol group received ethanol with a dose of 4.5 g/kg body weight (Merck KGaA, Darmstadt, Germany) saluted in tap water (20% *w*/*v*) intragastrically by gavage once a day, for 6 weeks. After 6 weeks of treatment, the results revealed a significant increase of matrix metalloproteinases 2 and 9 activity, as well as significant decrease of nephrin and podocin gene expression in the ethanol group, compared to that in the control group. These findings indicate that ethanol-induced kidney abnormalities may in part be associated with alteration in expression of nephrin and podocin, and increasing activity of matrix metalloproteinases 2 and 9 as key molecular mediators in the kidney function.

A long-term administration of low-molecular-weight agonist of luteinizing hormone receptor into male rats causes a steady increase in testosterone level and does not induce resistance to gonadotropins

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Luteinizing hormone (LH) and human chorionic gonadotropin (hCG), the endogenous agonists of LH receptor (LHR), are widely used in the assisted reproductive technologies and to treat the reproductive dysfunctions. However, their application in clinic is associated with a number of undesirable effects. Recently, on the basis of three-dimensional structure of the transmembrane domain of LHR and the allosteric site located within it, the low-molecular-weight agonists of LHR have been developed. The most interesting among them are thienopyrimidine derivatives, including 5-amino-*N*-*tert*-butyl-2-(methylsulfanyl)-4-(3-nicotinamido)phenyl) thieno[2,3-*d*]pyrimidine-6-carboxamide (TP03) synthesized earlier by us. The purpose of the study was a comparative study of the influence of long-term treatment of male Wistar rats with TP03 and hCG on the plasma T level and expression of LHR and the steroidogenesis proteins in the testes. The expression of the genes encoding LHR (*Lhr*), which binds to hCG and TP03, cholesterol-transporting StAR protein (*Star*), cytochrome $P450_{scc}$ (*Cyp11a1*), and 3β-hydroxysteroid dehydrogenase (*Hsd3b*) was studied. In the experiments, 3-month-old male rats treated with TP03 (i.p., 15 mg/kg/day) and hCG (s.c., 100 IU/rat/day) were used. During the 7-day treatment, hCG and TP03 significantly increased the production of T, but the dynamics of these effects was different. In the case of hCG, on the first day, an increase in T level was maximal (158 ± 15 nM) and then it reduced significantly. The TP3 effect was stable, reaching a maximum on the 7th day of the treatment. On the first day, the increase in T level caused by TP03 was four times lower than that for hCG, but on the 7th day, the steroidogenic effect of TP3 exceeded that of hCG. The weakening of steroidogenic effects of hCG was accompanied by a significant decrease in the *Lhr* gene expression in the testes, indicating its resistance to gonadotropins, while in the case of TP03, the *Lhr* expression on the 7th day was increased. Along with this, on the 7th day in the testes of hCG-treated rats, a compensatory increase in the *Star*, *Cyp11a1*, and *Hsd3b* expression was showed, while the TP03 increased the *Star* expression only. Thus, long-term treatment of male rats with TP03, unlike hCG, does not cause the resistance of the testes to gonadotropins and demonstrates a well-pronounced stimulating effect on T production, and this indicates that TP03 is a promising drug for activating the testicular steroidogenic function.

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Potential antibacterial drugs from unique collection of yeast-like fungi

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Antibiotic resistance phenomenon in pathogenic bacteria constantly challenges researches in finding new antimicrobials. Cell lysates and culture fluids of yeast and yeast-like fungi possess a huge potential in this area. Yeast strains demonstrated antagonistic activity towards such pathogens as *Listeria monocytogenes*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus aureus* [1–4]. In addition, the first widely used antibiotic penicillin was isolated from the *Penicillium fungi*.
We use the unique collection (created by Yarovoy B. F. and Stepanova V. P.) that contains over 2500 yeast and yeast-like fungi strains. Material was collected during research expeditions to various regions of USSR, including Kuril Islands, Kamchatka peninsula, and Sakhalin Island. 325 representatives of the collection have revealed resistance to elevated temperatures (37–52 °C) and salts of heavy metals (Cu, Cd, Co), and 4 representatives have demonstrated the ability to grow in the presence of constant β-irradiation [5]. Some of the strains exhibit antimicrobial activity.
The aim of our study is to examine culture fluids of strains for potential antibacterial activity with consecutive determination of the mechanism of action. Experimental high-throughput system allows for initial identification of cell processes (protein biosynthesis and SOS response) impaired by the presence of inhibitors [6].

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1. Fakruddin, M. et al, BMC Complementary and Alternative Medicine.17,64 (2017).
2. Roostita, L.B. et al, Adv J Food Sci Technol. 3, 6 (2011).
3. Rajkowska, K. et al. FoodTechnol Biotechnol. 50, 2 (2012).
4. Syal, P., Vohra, A., Intl J Microbiol Res. 5, 2 (2013).
5. Suslov, A.V. et al, Radiation Biology, Radioecology. 44, 5 (2004).
6. Osterman, I., et al, Antimicrob Agents Chemother. 60, 12 (2016).

The effect of erythropoietin receptors agonist on ultrastructural organization of primary hippocampal cultures after hypoxia modeling

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The aim of the investigation was to determine the neuroprotective effect of carbamylated darbepoetin (CdEPO) on ultrastructural organization of primary hippocampal cells after hypoxia modeling.
Hippocampal cells were obtained from C57Bl/6 mice embryos (E14). An acute normobaric hypoxia was modeled on day 14 of culture development in vitro. CdEPO in concentration of 50 ng/ml and 100 ng/ml were applied according to following schemes: 40 min before hypoxia or 6 and 24 h after hypoxia. Ultrastructural features of primary hippocampal cells were evaluated on day 7 after hypoxia modeling by examining ultrathin cultural sections with a Morgagni 268D transmission electron microscope (FEI Company, USA).
The studies revealed that different CdEPO concentrations and its application modes did not change the number of synaptic contacts compared to the sham culture. However, depending on the method of CdEPO administration and regardless to its concentration, the cell ultrastructure was insignificantly changed.
A high number of degeneration areas were detected in cultures with CdEPO application before hypoxia. In axons, the vesicles with changed size were often found. Moreover, osmiophilic bubbles with double membrane were observed in processes. In many cells, the rough endoplasmic reticulum is small or it has a reduced amount of ribosomes on its surface. Generally, there was a large amount of destroyed ER network. Vesicles with unknown origin were found in the dendritic spines (presumably, there were ER cisterns). In large dendrites, mitochondria had partially destroyed cristae, predominantly with normal osmiophility and regular form.
Synapses with a well-defined PSD were observed in cultures with CdEPO application 6 and 24 h after hypoxia. Synaptic vesicles had normal size and osmiophility. A moderate amount of ribosomes on the ER were observed in the cell body compared to the sham cultures. Mitochondria in the cell body were close to normal parameters. A few amounts of mitochondria had enlightened matrix and partially destroyed cristae. Mitochondria with modified shape (by narrowing in the middle) were occasionally observed in the processes.
In conclusion, CdEPO application contributes to preservation of primary hippocampal cells ultrastructure after hypoxic influence.
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Structural basis of Human GPR55 for diabetes and obesity therapies

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Human GPR55 is a class A G-protein coupled receptor (GPCR), expressed in a wide range of organs and tissues, including brain and spinal cord, spleen, adrenals and bone, adipocytes, gastrointestinal tract, and islets [1, 2]. Recent studies have revealed the important role of GPR55 in insulin secretion, food intake and energy homeostasis [3], cancer cell proliferation, migration, and apoptosis [4], indicating GPR55 is of great importance in future diabetes, obesity, and cancer therapies. However, the structure and signaling of GPR55 is not fully studied. Therefore, we aim to determine the structure of GPR55 through crystallization in lipidic cubic phase (LCP) and study functions and signaling of GPR55 more thoroughly with the solved structure. We have successfully purified stable constructs of GPR55 for further crystallization, aiming to solve the structure within 1 year. The structure of human GPR55 will provide insights into the functional studies and guide the design of new selective ligands for diabetes, obesity, and cancer therapies

1. Sawzdargo, M *et al* Identification and cloning of three novel human G protein-coupled receptor genes GPR52, Psi GPR53 and GPR55: GPR55 is extensively expressed in human brain *Mol Brain Res* **64**, 193-198 (1999)
2. Ryberg, E *et al* The orphan receptor GPR55 is a novel cannabinoid receptor *British journal of pharmacology* **152**, 1092-1101 (2007)
3. Liu, B, Song, S, Jones, P M & Persaud, S J GPR55: from orphan to metabolic regulator? *Pharmacol Therapeut* **145**, 35-42
4. Ford, L A *et al* A role for L-α-lysophosphatidylinositol and GPR55 in the modulation of migration, orientation and polarization of human breast cancer cells *British journal of pharmacology* **160**, 762-771 (2010)

SAXS and densimetry studies of DMPC/POPE mixture: morphology or structure changes?

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Phosphatidylcholines and phosphatidylethanolamines are the main components of cytoplasmic membranes of mammals [1]. DMPC and POPE lipids with close points of the main phase transition at 24 and 25 °C (Avanti Lipids) were studied. The SAXS curves were obtained for the multilayer vesicles (MLV) aqueous solution of DMPC/POPE mixture (1.5%/1.5% wt/wt) at 20 °C on the BM29 facility (ESRF, Grenoble, France). The SAXS curves have multiple diffraction peaks corresponding to the repeat distance of 61.4 and 65.3 Å (similar to pure POPE and DMPC MLV lattice parameters).
Measurements of density vs temperature dependence of the same DMPC/POPE MLV aqueous solutions on the Anton Paar DMA5000M density meter (JINR, Dubna, Russia) in the temperature range of 20–30 °C show the presence of phase transition at temperature of 23.8 °C, coinciding with the point of the main phase transition for pure DMPC vesicles. For aqueous 3% wt/wt solution of pure POPE MLV, a density jump occurs at temperature of 25.47 °C, which coincides with the data of DSC [2]. The first heating-cooling cycle also exhibits feeble density jumps at 24.12 °C during heating and 23.53 °C during cooling. During the second and the third heating-cooling cycles, only the phase transition at 23.8 °C was observed while the studied solution maintained visually stable. These data may indicate either the appearance of heterogeneities in the lipid MLV bilayer of the DMPC/POPE mixture or the MLV alteration—both morphological and structural.

1. Navas, B. *et al.* Composition dependence of vesicle morphology and mixing properties in a bacterial model membrane system. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **1716.1,** (2005).
2. Zweytick, D. *et al.* Studies on lactoferricin derived E. coli membrane active peptides reveal differences in the mechanism of N-acylated versus non-acylated peptides. *Journal of Biological Chemistry* (2011).

Evaluation of the possibility of simultaneous using membrane biosensors and vital dyes in determining the sensitivity of 2D and 2.5D systems based on cancer cell lines to oncotoxic drugs

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Aiming to determine toxic effects at the cellular level, modern studies shift to the use of vital dyes and biosensors, since they allow us to evaluate the kinetics of the reaction. Kinetics can serve as an additional prognostic criteria, allowing one to take into account more parameters that

help to evaluate the effect produced. One of the currently known apoptotic pathways deeply depends on the participation of mitochondria. Therefore, simultaneous measurment of plasma membrane and mitochondrial membrane activity gives one a powerfull tool for further investigations in the area of apoptotic pathways and anti-cancer drug testing. Data obtained from 2.5D and 3D systems acquire special value, since these systems are closer in reactions to in vivo. Yet, signal detection in such systems faces difficulties due to the size of 2.5D objects and high density of cells within (e.g., in spheroids).

The aim of this work was to develop a biosensor-based system suitable for the analysis of the resistance of cancer cell lines to the toxic effects of anicancer drugs in both 2D and 2.5D and 3D cellular systems. We aimed to test our system of simultaneous detection of the state of cellular and mitochondrial membranes in 2D and 2.5D cellular systems on various cancer lines, as well as to perform a primary analysis of the kinetic curves obtained in this analytical system. As a detector of the functional state of mitochondrial membrane, a commercially available biosensor Mitotracker Orange was used. Its fluorescence intensity is directly related to the potential on the mitochondrial membrane. To assess the state of the cell membrane, we used a commercially available vital dye Fluorescein diacetate. Esterase activity is required to activate its fluorescence, and cell-membrane integrity is required for intracellular retention of the fluorescent product. Modelling of anticancer drugs treatment, such as DMSO and doxorubicin, allowed us to obtain and compare data on the kinetics of functional state changes of both cellular and mitochondrial membranes in 2D and 2.5D systems for various cancer cell lines.

The results obtained with such system suggest that in 2.5D culture, the response to toxic effects occurs more slowly, accompanied by increased tolerance to toxic effects of anticancer drugs. In 2.5D, the relative sensitivity of cells to toxic effects depends on the position of the cell in the spheroid. For instance, difference in tolerance for B16 cell line depending on the position in 2.5D object was 5% in concentration, and delay time in 2D vs 2.5D was 50–100% (200–300% vs 3D). This confirms the diversity in reactions to the anticancer agent between 2D, 2.5D, and 3D systems that cannot only be explained only by the difference in cell density.

Thus, the proposed system is a new tool for investigating the drug resistance of solid tumor models (e.g., spherions) and provides an opportunity for further analysis of the signaling pathways involved in launching apoptosis in cancer cells.

Chitosan-mannose conjugate as a functionalizing agent for liposomal form of fluoroquinolones: synthesis and properties

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Infectious diseases are one of the top ten leading causes of death in the world. Fluoroquinolones are the group of antibacterial drugs that are widely used in the treatment of many diseases, however, like other drugs, cause a number of side effects [1]. In addition, to achieve a more effective therapeutic effect, drug delivery systems are currently used. One of the perspective drug delivery systems for fluoroquinolones is liposome. These lipid vesicles are biocompatible and easy to obtain, but still there is a request for functionalize liposomal surface to improve biopharmaceutical properties of liposomal drugs.

Chitosan is a natural biopolymer, which is actively used in industry and previously in our lab, the beneficial strategy of liposomal properties managing via complex formation with various chitosan derivatives was developed [2, 3]. It is well-known that alveolar macrophages, typical host for *Mycobacterium Tuberculosis*, possess valuable number of mannose-binding receptors [4] on the surface; thus, current research is dedicated to study chitosan-mannose conjugate as a functionalizing agent for liposomal form of fluoroquinolones including synthesis, properties, and influence on the membrane.

The technique of production of chitosan particles modified by D-mannose is developed. The synthesis is based on soaking chitosan polymer (5 kDa and 90 kDa) in acetic acid, adjusting the pH value to 9.0 and adding D-mannose to obtain Schiff base. It was found that depending on the molecular weight of the biopolymer, it is possible to regulate the degree of modification of amino groups. Properties of liposomal vesicles covered by obtained polymer were studied by FTIR and NTA.

These polymers are promising for modifying the surface of liposomes to give the system desired properties.

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1. Dube D. *et al* Tuberculosis: from molecular pathogenesis to effective drug carrier design. *Drug Discovery Today* **17**, 760–773 (2012).
2. Deygen, I. M. *et al.* Novel prodrug of doxorubicin modified by stearoylspermine encapsulated into PEG-chitosan-stabilized liposomes. *Langmuir* **32**, 10861–10869 (2016).
3. Deygen I.M., Kudryashova E.V. New versatile approach for analysis of PEG content in conjugates and complexes with biomacromolecules based on FTIR spectroscopy *Colloids Surf., B: Biointerfaces* **141**, 36-43 (2016).
4. Filatova L.Yu *et al.* Targeted delivery of anti-tuberculosis drugs to macrophages: targeting mannose receptors. *Russ. Chem. Rev.* **87**, 374-391 (2018).

Personalized ionic models of cardiomyocytes on the basis of molecular data and genetic algorithms

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A number of pathological conditions such as heart failure or atrial fibrillation result in a change of protein expression in cardiomyocytes and, as a consequence, affect action potential (AP) waveform, conduction velocity, and restitution properties. These pathological or personal non-pathological AP differences are usually impossible to account for in mathematical models, hindering clinical application of computer simulations. We developed a novel approach to solve this problem using cap analysis of gene expression (CAGE) [1] and genetic algorithms (GA) [2].

Donor hearts ($n = 8$) with no recorded history of cardiovascular diseases were procured for biopsy collection of atrial and ventricular tissue. In order to account for relative ionic channels conductivities differences in heart tissue samples, we have measured mRNA expression level via CAGE technique [1]. In order to find absolute values of ionic channels conductivities, we developed a modification of GA [2]. GA convergence speed was increased by Levy flight augmented mutation operator, i.e., distance of mutation was distributed according to Cauchy distribution, while direction was uniformly distributed in 15-dimensional parametric space. AP waveform dependence on cycle length measured via optical mapping technique was used as input data for GA. O'Hara-Rudy model [3] with conductivities rescaled by GA was used for cardiomyocytes electrophysiology simulations. Benchmark tests with simulated AP as input data showed that modified algorithm is capable to find ionic current conductivities with amplitude exceeding 0.1 μA/μF within 20% error, while error of ionic currents with amplitude exceeding 1 μA/μF was within 12%.

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1. Murata M. *et al.* Methods Mol Biol. 1164:67-85. (2014); https://doi.org/10.1007/978-1-4939-0805-9_7
2. Bot CT, Kherlopian AR, Ortega FA, Christini DJ, Krogh-Madsen T. *et al. Frontiers in Physiology.* (2012). https://doi.org/10.3389/fphys.2012.00421
3. O'Hara T, Virág L, Varró A, Rudy Y. PLoS Comp. Biol. 7(5):e1002061 (2011)

Interactions between ergosterol biosynthesis (ERG) and ergosterol transport (LAM) genes in yeast Saccharomyces cerevisiae

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Sterols play a key role in the regulation of the physico-chemical properties of the plasma membrane of eukaryotic cells. The major yeast sterol ergosterol is synthesized in endoplasmic reticulum (ER); its molar fraction is from 5% in internal cell membranes to 40% in the plasma membrane (PM). The transport of sterols between the plasma membrane and the endoplasmic reticulum is crucially important for cells, but its molecular mechanisms and regulation are still unclear. LAM genes are found in most eukaryotic organisms, including humans, they encode proteins that contain transmembrane and ergosterol-binding domains. ERG genes encode proteins of ergosterol biosynthesis, erg2-erg6 realizing the last five steps of sterol modification; its disruption is not lethal. Here, we investigated the genetic relationships between erg2-erg6 and lam2 genes.
It was known that erg2-erg6 mutations affect the sensitivity to hyperosmolar conditions. We confirmed that data then shown that lam disruption also affects on growth in hyperosmolar conditions. LAM2 encodes major Lam protein with strongest high salt sensitivity. We obtained panels of strains with erg2-erg6 disruption in lam2 null (lam2) or LAM2 overexpression (LAM2) background. Then compared growth speed of single and double mutants on normal and high osmolarity conditions. We found a genetic interaction between ERG4 and LAM2 genes. We assume that the ergosterol precursors accumulating in erg4 are less well transported between ER and PM.

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Antidiabetic action of water-soluble pentamino acid derivatives of fullerene C_{60}

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Type 2 diabetes mellitus (T2DM) is a heterogeneous metabolic disorder characterized by a chronically elevated blood glucose concentration which arises from a combination of insufficient insulin secretion and a reduced sensitivity of target cells and tissues to insulin. Free radical oxidation, nonenzymatic protein glycation, and polyol pathway are considered among the main factors of the pathogenesis of complications associated with diabetes.
In this work, we have investigated the effect of eight water-soluble pentamino acid fullerene derivatives (WPDFs) on the therapeutic target for T2DM in vitro: aldose reductase (AR), formation of advanced glycated end products (AGE), and the process of lipid peroxidation (LP). It was found that all tested compounds possess pronounced antioxidant activity. The most active antioxidant is the GI-400. Derivatives GI-349, -364, -377, -380, and -400 significantly inhibit aldose reductase at a concentration of 10^{-4} M. The most effective inhibitors are GI-349 and GI-400, inhibiting AR by 51 and 40%, respectively. The mechanism of inhibition of aldose reductase by the compounds GI-349 and GI-400 was determined. It was shown that GI-349 noncompetently inhibits aldose reductase with $Ki = 8.47 \times 10^{-5}$ M, and GI-400 is a competitive inhibitor of aldose reductase with $Ki = 1.53 \times 10^{-5}$ M.
We also investigated the effect of a number of WPDFs on the non-enzymatic protein glycation process in vitro. It is shown that the studied WPDFs have antiglycating activity, and IC_{50} has been determined. The most effective compounds are GI-349 and GI-400 with IC_{50} of 30.0 and 15.8 μM respectively.
The most effective compound GI-400 has been investigated in rat's model of T2DM. This fullerene derivative led to the normalization of complex morphological and functional parameters (blood glucose level, a number of important biochemical parameters, liver and pancreas structure), which testifies to its pronounced anti-diabetic effects. After treatment of GI-400 in rats with induced T2DM with stable hyperglycemia, the blood glucose level decreases to the level of intact rats on the 14th day.
The work was financially supported by the program of fundamental research of the Presidium of the Russian Academy of Sciences "Nanostructures: Physics, Chemistry, Biology and Technology Basics."

KIT downregulation in AML cells increase sensitivity to kinase inhibitors

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Earlier, we suggested MAPK1/2 as a key regulator, responsible for malignant blood cell survival after treatment with anti-cancer drugs. In this study, we used MAPK1/2 small-molecular inhibitors to evaluate the response of leukemia cells to anti-cancer therapeutic agents treated with exogenous cytokines and growth factors. Here we demonstrated the increase in sensitivity of KIT-positive cells to anti-KIT small hairpin RNA when MAPK inhibitors were added. Also, we showed that inhibitors increase sensitivity of leukemia cells to anti-cancer drugs or to KIT downregulation. We suggest the strategy to reduce stress-response mechanisms existing in malignant myeloid cells; by that, they can avoid apoptosis and survive during the therapy. We provide evidence that targeting KIT in AML cells may be insufficient for successful treatment and other inhibitors targeting possible compensatory mechanisms should be used. The results were obtained within the program of fundamental research for state academies for the 2013–2020 years (no. 01201363823). Flow cytometry experiments were supported by the RFBR grant (project no. 17-04-01555). All viability assays were performed within RSF grant (project no. 14-14-01089-П).

Effect of stimulation of medial septal neurons on the expression of genes in rat hippocampus

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It is well known that cholinergic input from the medial septum is critical for generation of rhythmic neuronal activity in the hippocampus. It is also known that acetylcholine can change gene expression. However, it is still

not clear whether an increase in the activity of cholinergic septal neurons may lead to the long-term effects by changing gene expression in the regions where these neurons project. Here, we studied the effect of medial septum stimulation on gene expression in the ventral and dorsal parts of the hippocampus in both brain hemispheres. Three groups of animals were examined. In two groups, the medial septum was stimulated by rectangular pulses and the focal potentials were recorded in the CA1 field. In one of these groups, after recording the baseline, we induced long-term potentiation (LTP) in synapses formed by neurons of the medial septum on the neurons of the hippocampal CA1 area. In the second group, we stimulated septal neurons without LTP induction. The third group of animals was a control group without electric stimulation. We found very strong differences between the response of the ipsi- and contralateral hippocampus to the stimulation of the septum. In the intact contralateral (both dorsal and ventral) hippocampus, none of the septal stimulations altered gene expression. In contrast, in the ipsilateral hippocampus, which was injured by the inserted recording electrode, stimulation of the septum resulted in an increase in the expression of some genes not only in the dorsal hippocampus (where there was injury) but also in the ventral part of it. It turned out that stimulation of the septum led to a very strong potentiation of the expression of inflammation-related genes (Il1β, Tnf, Ccl2, Il6) in the dorsal but not ventral hippocampus. The expression of Ngf and Bdnf genes was enhanced by septal stimulation in the dorsal and ventral regions of the ipsilateral hippocampus. Among the immediate early genes (c-Fos, Arc, and Cyr61), only Arc expression increased in response to septal stimulation in both dorsal and ventral regions of the ipsilateral hippocampus. It should be noted that the potentiating effect of septum stimulation was always stronger in the dorsal hippocampus. The data obtained suggest that the examined types of septal neuron activity cannot induce strong changes in gene expression, however, in combination with other types of stimuli, such as inflammation, may act as a powerful modulator of gene expression. Supported by the RSF grant no. 16-15-10403.

Clones of adaptive NK cells can be obtained by stimulation with IL-2 and K562-mbIL21

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Cytomegalovirus (CMV) infection can cause accelerated differentiation of NK cells which are characterized by the phenotype $CD56^{dim}CD57^{+}NKG2A^{-}NKG2C^{+}CD16^{bright}KIR^{+}$ and possess features of adaptive cells. Some studies have shown that they can have a significant antitumor potential, due to changes in the hierarchy of receptors controlling functions of natural killer cells. The goal of this work was to obtain and analyze clones derived from NK cells expressing the NKG2C receptor. NK cells were obtained by negative magnetic separation of PBMC from healthy donors. Four collections of clones were made by sorting single cells in 96-well plates. For stimulation, the NK-MACS medium supplemented with 100 U/mL of IL-2 and the 2×10^3 K562-mbIL21 feeder cells (kindly provided by Dr. Dean A. Lee) were used. The clones of cells from $CD56^{bright}NKG2C^{-}$, $CD56^{bright}NKG2C^{+}$, $CD56^{dim}CD57^{-}NKG2C^{-}$, $CD56^{dim}CD57^{-}NKG2C^{+}$, $CD56^{dim}CD57^{+}NKG2C^{-}$, and $CD56^{dim}CD57^{+}NKG2C^{+}$ subsets were obtained. The proportion of $NKG2C^{+}$ cells in initial NK cell populations of these individuals ranged from 14 to 48%. The highest frequency of clone generation was observed in subpopulations of $CD56^{bright}$ cells. In two collections (both from CMV^{+} donors), the frequency of clone generation was higher in $CD56^{bright}NKG2C^{+}$ subset, in two others frequencies did not differ between $NKG2C^{+}$ and $NKG2C^{-}$ subpopulations. The cloning efficiency in $CD56^{dim}NKG2C^{+}$ subpopulations was higher in three donors out of four; however, in one of the CMV-positive donors, it was lower, compared to the subpopulations of $CD56^{dim}NKG2C^{-}$. Importantly, the survival of clones from the $NKG2C^{+}$ subpopulations, like a total number of cells in the clone, in most cases was higher than that in the corresponding $NKG2C^{-}$ subpopulations. Phenotype of 92 clones that demonstrated high proliferative potential was analyzed. Twenty of them were from the $CD56^{dim}CD57^{+}NKG2C^{+}$ subpopulation. Based on the presence of surface expression of NKG2C and CD57 and the absence of expression of NKG2A, a selection of clones that potentially possessed the properties of adaptive NK cells was carried out. We found 12 clones satisfying these criteria, which accounted for 60% of the total number of clones analyzed from the $CD56^{dim}CD57^{+}NKG2C^{+}$ subset. Most of these clones had reduced or absent NKp30 and NKp46 receptor expression. The HLA-DR and granzyme B expression levels were increased compared to the $CD56^{dim}CD57^{-}NKG2C^{+}$ subset. Thus, it can be concluded that NK cells with memory-like phenotype are capable of proliferating in response to stimulation with IL-2/K562-mbIL21. This work was supported by the Russian Science Foundation, grant no. 16-15-00309.

Complex biochemical and biophysical study of CYP136 from *M. tuberculosis*

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Tuberculosis is a severe infectious disease usually caused by the bacterium *Mycobacterium tuberculosis*. Mycobacterial genome codes for 20 cytochrome P450s (MtbCYPs). Existing data indicate an important role of mycobacterial CYPs in pathogenicity and viability of pathogen, so developing CYP-specific inhibitors is a promising strategy to develop novel anti-TB drugs. CYP136 from *M. tuberculosis* is a poorly studied protein; CYP136 is the closest neighbor to mycobacterial lanosterol 14-alpha demethylase (CYP51), one of a few functionally characterized MtbCYPs. In the present work, we report molecular cloning, heterologous expression, and purification of MtbCYP136. Unlike other soluble bacterial CYPs, MtbCYP136 needs detergent for its purification, indicating on possible association with membrane. Studies of MtbCYP136 interactions with different ligands using UV-visible spectroscopy show efficient binding of azolecontaining compounds, widely used as cytochrome P450 inhibitors. Dissociation constants (Kd) for interaction were estimated. Clotrimazole and econazole bind to CYP136 with high affinity ($Kd = 0.09$ μM and $Kd = 1.7$ μM, respectively). We also have shown that the enzyme is probably involved in lipid metabolism. In order to measure parameters of binding of different ligands independently of spectral changes, we use isothermal calorimetry (ITC). Enthalpy, entropy, and Gibbs energy of interaction of CYP136 with azoles were estimated. Circular dichroism (CD) spectra of CYP136 show the presence of properly folded protein containing alpha-helical and beta-sheet regions. Protein stability was measured using differential scanning calorimetry (DSC). Thermal unfolding profile revealed two melting temperatures. Dynamic light scattering (DLS) study demonstrated that protein is predominately in monomeric form. Cytochrome P450 require the presence of redox partner for catalysis. By now, only few data are available on interaction of mycobacterial CYPs with their native redox partners. We analyzed parameters of binding of potential redox partners from *M. tuberculosis* to CYP136 using ITC. The effect of interaction with redox partner on thermal stability of CYP136 has been studied using DSC.This work was supported by joint research grant of Belarusian Republican Foundation for Fundamental Research (X18P-098) and Russian Foundation for Basic Research (18-54-00030)

1. Molecular cloning, heterologous expression, isolation and ligandbinding properties of the Mycobacterium tuberculosis cytochrome CYP136. Proceedings of the NASB. Series of chemical sciences. 4, (2012).

Signals of migraine pain from meningeal trigeminal fibers activated by ATP and serotonin: new model in NEURON environment

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Migraine pain is likely triggered by release of nociceptive substances from multiple meningeal mast cells forming neuro-immune synapses with trigeminal nerve fibers (Kilinc et al., Neuropharmacology, 2017). In this project, in NEURON environment, we modelled nociceptive firing in trigeminal nerve fibers activated by the powerful algogen ATP (Yegutkin et al., Purinergic Signal 12:561–574, 2016) released from meningeal mast cells. We considered multiple processes determining the fate of ATP in the extracellular space and kinetic properties of ATP-gated P2X3 receptors as well as a set of potassium and sodium channels in nerve fibers. We generated a range of models for nociceptive firing in trigeminal nerves and identified key factors, which can contribute to persistent and bursting spiking activity, which is supposed to be proportional to migraine pain. These factors include co-expression of weakly desensitizing subtype of ATP-gated P2X receptors, repetitive release of ATP, branching morphology of nerve terminals and functional peculiarities of sodium Nav1.8 channels. As serotonin plays also a powerful pro-nociceptive role in trigeminal fibers (Kilinc et al., Neuropharmacology, 2017), we also considered a co-release of serotonin from mast cells together with ATP and potential interaction of two algogens on nerve terminals. Our model not only explains key events underlying migraine pain but also provides a novel tool to explore *in silico* variable conditions predisposing to pathological trigeminal pain signalling. Supported by RFBR KOMFI grant 17-00-00053, Program of Competitive Growth of KFU, and was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities number 2.8303.2017/8.9.

1. Kilinc, E. *et al.* Serotonergic mechanisms of trigeminal meningeal nociception: Implications for migraine pain. *Neuropharmacology* (2017). https://doi.org/10.1016/j.neuropharm.2016.12.024
2. Yegutkin, G. G., Guerrero-toro, C., Kilinc, E. & Koroleva, K. Nucleotide homeostasis and purinergic nociceptive signaling in rat meninges in migraine-like conditions. *Purinergic Signal.* **12,** 561–574 (2016).

Discreteness of charge effects in binding of cationic membrane-active peptides to lipid bilayer

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Antimicrobial, cytotoxic and cell-penetrating peptides are diverse in their amino acid sequences, but share common physicochemical features like short length, high net charge and amphipathic conformation in membrane bound state (Almeida, Biochim Biophys Acta 1838:2216–2227, 2014). Nonspecific electrostatic interactions of basic residues of a peptide with anionic membrane lipids and amphipathicity mediate initial binding of such peptide to plasma membranes. At the same time, numerous membrane-active peptides function when they are localized at high concentrations on the lipid membrane (Nguyen et al., Trends Biotechnol 29:464–472, 2011). Elevated surface concentrations facilitate membrane permeation of cell-penetrating peptides or lead to self-assembly of structures that disrupt membrane integrity in the case of antimicrobial and cytotoxic peptides. Dissecting the role of electrostatic interactions in this functional peptide condition is important to understand the reason why the majority of such peptides bear high positive charge (Torrent et al., Angew Chem 123:10874–10877, 2011). Previously, we found that partitioning of highly cationic cell-penetrating peptide EB1 (Lundberg et al., FASEB J 21:2664–2671, 2007) into lipid membranes demonstrates saturation. Here we investigate how this behavior of EB1 peptide partitioning depends on the charge of the membrane. We observed saturation of peptide binding to liposomes that comprises 5%, 10% and 25% of negatively charged lipids (POPC/POPG lipid mixture). We found that the surface peptide concentration needed for saturation increases with the amount of anionic lipids in a membrane, while the total surface charge (bound peptide plus anionic lipids) is positive and also increases for saturated membranes with a higher portion of negatively charged lipids. We demonstrated that to describe observed saturation, the discreteness of charge effects should be considered (Mulgrew-Nesbitt et al., Biochim Biophys Acta 1761:812–826, 2006) that may by qualitatively addressed by numerical solution of Poisson-Boltzmann equations with atomic models. The work was supported by RFBR grant no. 18-34-00992.

1. Almeida, P. F. Membrane-active peptides: binding, translocation, and flux in lipid vesicles. *Biochim. Biophys. Acta*, **1838**, 2216-2227 (2014)
2. Nguyen, L. T. *et. al.* The expanding scope of antimicrobial peptide structures and their modes of action. *Trends Biotechnol.*, **29**, 464-472 (2011)
3. Torrent, M., *et. al.* The generation of antimicrobial peptide activity: a trade-off between charge and aggregation?. *Angew Chem*, **123**, 10874-10877 (2011)
4. P. Lundberg, *et. al.* Delivery of short interfering RNA using endosomolytic cell-penetrating peptides. FASEB J. **21** 2664-2671 (2007)
5. Mulgrew-Nesbitt, *et. al.* The role of electrostatics in protein–membrane interactions. *Biochim. Biophys. Acta* **1761**, 812-826 (2006)

Detection of a Calreticulin-Like Channel Protein with Properties of the ATP-Sensitive Potassium Channel in the Rat Liver Endoplasmic Reticulum

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Earlier, in our laboratory, the 57-kDa protein isolated from the rat liver mitochondria and reconstituted into bilayer lipid membrane (BLM) was shown to form ATP-sensitive potassium channels with conductivity of 10 pSm (Mironova et al., Membr Cell Biol 10(5):583–591, 1997). MS MALDI TOF/TOF analysis showed that the structure of the channel protein is similar to the precursor of calreticulin, which is widely distributed in the endoplasmic reticulum. The question has arisen as to whether the channel can present in the endoplasmic reticulum. Using a method of water-ethanol extraction from the rat liver endoplasmic reticulum, the protein with the properties of the mitochondrial ATP-sensitive potassium channel was isolated and purified. To verify that the isolated protein is localized in the endoplasmic reticulum, analysis of the organelle fraction purity by western blot was carried out. The membrane fraction antibody cocktail (Abcam, UK) that contained primary antibodies targeting specific organelle markers was used. It was found that the obtained fraction of microsomes is pure and not contaminated with other organelles. In addition, this experiment proves that the studied protein is localized in the endoplasmic reticulum. MS MALDI TOF/TOF analysis revealed that the structure of the isolated protein, as well as that of the mitochondrial protein, is similar to the precursor of calreticulin. We found that the isolated protein has no structural similarity to any of the known ion transport proteins. When the protein is reconstituted into the BLM, its minimum conductivity is 10 pSm, as well as it is capable of forming channels with multilevel

conductivity and possesses selectivity for cations. It was obtained that physiological concentrations of ATP inhibit the channel protein activity, which allows it to be attributed to the ATP-sensitive channels. The selective blocker of the mitochondrial ATP-sensitive potassium channels, 5-hydroxydecanoate, at the concentration of 300 μM blocks the activity of the isolated protein, which indicates that the mitoK_{ATP}-like protein can also be localized in the endoplasmic reticulum. The work was supported by the Russian Science Foundation (RSF) (project no. 16-15-00157).

1. Mironova, G. *et al.* ATP-dependent potassium channel from rat liver mitochondria: inhibitory analysis, channel clusterization. *Membr. Cell. Biol.*, 10(5):583-91 (1997).

Alanine mutation of Glu125 and Asp649 enhance the effectivity of catalysis and modulate secondary substrate specificity of oligopeptidase B from *S. proteamaculans*

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Oligopeptidase B (OpdB) is a peptidase from the family of prolyl oligopeptidases. All members of the family have an unusual seven-bladed β-propeller N-terminal domain that impedes penetration of protein molecules with MM over 3 kDa into a cavity at the interface with C-terminal catalytic domain where the active center is located. The protozoan OpdBs are the most studied enzymes and the only ones with obtained spatial structures. The crystal structures of *Leishmania major* and *Trypanosoma brucei* OpdB demonstrate the importance of the interdomain interface and particularly five salt bridges between the catalytic and propeller domains for enzyme activation. It is intriguing that four from five functionally important interdomain salt bridges found in protozoan OpdBs are not conserved in known gamma-proteobacterial enzymes including the object of our study OpdB from *Serratia proteamaculans* (PSP). Using molecular modelling and site-directed mutagenesis, we performed the search of alternative factors stabilizing active conformation of PSP in the absence of the aforementioned protozoan interdomain salt bridges. Analysis of homology-based model of PSP closed form showed that charged amino acid residues D649, K655, R658, and K600, surrounding catalytic His652, participate in forming inter- and intra-domain polar contacts with residues of N-terminal β-propeller domain E75-R658, E96-R658, E96-K660, and K655-D649 salt bridges and D649-E125 H-bond. Site-directed mutagenesis proved the crucial role of the E75-R658, E96-R658, and E96-K660 salt bridges in stabilization active conformation of catalytic center since their elimination caused enzyme inactivation. The role of the other contacts is more complicated: mutations D649A and E125A increased effectivity of catalysis and modulated secondary substrate specificity. A similar effect was observed in the presence of Ca2+. We postulated that Ca2+ coordinates carboxyl groups of S2-substrate binding center residues and affects their interaction with amino groups of Arg or Lys residues from either the same protein or substrate P2 position and investigated how Ca2+ influences the activity of PSP carrying the single mutations K655A, D649, and E125 and their combinations. In addition, we performed MD simulations of PSP homology-based model and analyzed the relative stabilities of polar contacts described above. The work was supported by the Russian Science Foundation: project no. 17-14-01256.

Structure of SMA 3000-stabilized lipodisks: a computer modelling study

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Solubilization of membrane proteins by a styrene-maleic acid (SMA) copolymer has recently been reported as a promising tool for membrane protein extraction (Bagrov et al., Biophys 61(6):942–949, 2016; Dörr et al., Eur Biophys J 45:3–21, 2016; Jamshad et al., Nano Res 8(3):774–789, 2014; Knowles et al., JACS 131(22):7484–7485, 2009). Such procedure results in formation of structures termed lipodisks (or SMALPs). Lipodisks allow extracting membrane proteins without the usage of detergents, thus providing native lipid environment for membrane proteins. The simplicity of the method indicates it as an outstanding technique for membrane protein solubilization. However, the mechanism of lipodisk formation is still unknown on an atomic level. In this work, we propose a stable full-atom lipodisk model prepared for long molecular dynamics simulations. The resulting lipodisk model contained 60 molecules of POPC (30 molecules in each membrane leaflet) and was around 9 nm in diameter. The lipodisk was built using 29 SMA molecules (around 36 monomers each) with 1:3 maleic acid/styrene ratio. Molecular models make it possible to evaluate the physicochemical properties of membranes (cooperativity parameter, the area per lipid, electron density profile) that are hard to measure in lipodisks and compare them with the characteristics of native membranes. Also, such models can be used to study the influence of lipodisk environment on the structure of the extracted proteins. This work was supported by the RFBR grant 18-504-12045.

1. Bagrov, D. V. *et al.* Characterization of Lipodisc Nanoparticles Containing Sensory Rhodopsin II and Its Cognate Transducer from *Natronomonas pharaonic* (2016). *Biophys,* 61(6), 942–949. https://doi.org/10.1134/S0006350916060063
2. Dörr, J. M. *et al.* The styrene–maleic acid copolymer: a versatile tool in membrane research (2016). *Eur Biophys J,* 45:3–21. https://doi.org/10.1007/s00249-015-1093-y
3. Jamshad, M. *et al.* Structural analysis of a nanoparticle containing a lipid bilayer used for detergent-free extraction of membrane proteins (2014). *Nano Research*, *8*(3), 774–789. https://doi.org/10.1007/s12274-014-0560-6
4. Knowles, T. J. *et al.* Membrane proteins solubilized intact in lipid containing nanoparticles bounded by styrene maleic acid copolymer (2009). *Journal of the American Chemical Society*, *131*(22), 7484–7485. https://doi.org/10.1021/ja810046q

The effect of triclosan on the functioning of marsh frog (*Pelophylax ridibundus* (Pallas, 1771)) liver mitochondria

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Triclosan (TCS) is a synthetic drug with a broad spectrum of antimicrobial activity. This agent is widely used in many personal care products. It has long been believed that TCS is harmless to human. However, evidence has been recently accumulated that toxic effects of TCS are also manifested in eukaryotes. We have recently shown that this agent is capable of inducing polymorphic phase transitions in the membrane of lecithin liposomes and the inner membrane of mitochondria, which disrupts the packing of the membrane and leads to its permeabilization (Belosludtsev et al., Biochim Biophys

Acta 1860:264–271, 2018). Given the widespread use of TCS, the tendency to the accumulation of this agent in aqueous ecosystems and sediments is not surprising, where they persist for a long period of time (Brausch and Rand, Chemosphere 82:1518–1532, 2011). In this work, we investigated the effect of TCS on the permeability of the inner membrane of mitochondria isolated from the marsh frog liver (*Pelophylax ridibundus* (Pallas, 1771)) as well as on the parameters of respiration and oxidative phosphorylation of liver mitochondria of these animals. We found that TCS induces the swelling of the organelles of these animals, insensitive to the mitochondrial pore inhibitor cyclosporin A. This corresponds to the data obtained earlier on rat liver mitochondria (Belosludtsev et al., Biochim Biophys Acta 1860:264–271, 2018). It can be assumed that the basis of this effect of TCS is also the formation of lipid pores in the inner mitochondrial membrane. In addition, we studied the effect of TCS on the effectiveness of oxidative phosphorylation and the activity of respiratory chain complexes of marsh frog liver mitochondria. It was found that TCS at a concentration of 20 μM effectively suppresses the succinate-fueled respiration of the organelles and also inhibits the activity of II, II+III, and I+III respiratory chain complexes without affecting the activity of other complexes. The toxic effect of TCS on membranes and membrane systems of organisms inhabiting the aquatic environment is discussed. The work was supported by the Ministry of Education and Science of Russian Federation (no. 17.4999.2017/8.9) and the Russian Foundation for Basic Research (no. 18-315-00033) and by a grant of Mari State University (no. 2018-03b).

1. Belosludtsev, K. *et al*. Study of the mechanism of permeabilization of lecithin liposomes and rat liver mitochondria by the antimicrobial drug triclosan. *Biochim. Biophys. Acta*. **1860**, 264–271 (2018).
2. Brausch, J. & Rand, J. A review of personal care products in the aquatic environment: environmental concentrations and toxicity. *Chemosphere.* **82**, 1518-1532 (2011).

Spatial distributions of Na^+ and K^+ around charged carboxyl groups in solution environment and in Na_vMs channel selectivity filter

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It is now apparent from crystal structures that side chains of a glutamic acid residues that comprise an E/E/E/E selectivity filter of bacterial sodium and eukaryotic calcium channels are directed into the pore lumen. It is suggested that specific coordination of ions and water molecules with oxygen atoms of charged carboxyl groups of these glutamate residues facilitates an ion discrimination in the channel pore. Impotence of coordination geometry for ion selectivity is also supported by several observations that glutamate-to-aspartate mutations in the filter sufficiently affect channel selectivity. Results of molecular dynamics simulations (MD) suggests that the potassium ion does not fit into E/E/E/E ring with water molecules coordinated in the same favorable geometric arrangement as the sodium ion does, which ensures Na^+ vs K^+ discrimination by Na_vMs channel (Corry et al., JACS 134:1840−1846, 2011). Different characters of ion-carboxylate coordination are observed depending on ion type in solution systems. With the use of X-ray absorption, it was revealed that sodium ion binds charged oxygen of carboxyl group more tightly than potassium in acetate salts solutions (Aziz et al., Phys Chem Lett 112:12567–12570, 2008). At the same time, MD simulations showed that both direct ion binding and solvent-separated coordinated configurations play an important role in distinct thermodynamics of potassium and sodium acetate solutions (Hess et al., PNAS 106:13296–13300, 2009). In this work, we used MD to obtain spatial distributions of sodium and potassium ions around charged carboxyl groups in different environments. In the case of glycine in solution, we found that ion-carboxylate coordination states (bidentate and monodentate) are occupied sufficiently different by sodium and potassium; sodium behavior resembles one reported for calcium. We obtained similar results for ion coordination with charged carboxyl groups located on the surface of soluble proteins. Finally, we modeled corresponding coordination states for carboxyl groups in a dense environment of a selectivity filter of bacterial voltage-gated sodium channel Na_vMs. The work was supported by RFBR grant no. 18-34-00992.

1. Corry, B. *et. al.* Mechanism of Ion Permeation and Selectivity in a Voltage Gate Sodium Channel. *JACS*, **134,** 1840−1846 (2011).
2. Aziz, E *et. al.* Cation-Specific Interactions with Carboxylate in Amino Acid and Acetate Aqueous Solutions: X-ray Absorption and ab initio Calculations. *Phys Chem Lett.*, **112,** 12567–12570 (2008).
3. Hess, B., *et. al.* Cation specific binding with protein surface charges. *PNAS,* **106**, 13296 –13300 (2009).

Parkinson's disease dependent alterations of phenotype of peripheral blood lymphocytes

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Parkinson's disease (PD) is one of the most common neurodegenerative diseases. In recent years, there are growing evidences that the pathogenesis of this disease is connected with regional and peripheral immune processes. Currently, the association of clinical signs of PD with different characteristics of patient immune status is actively being searched. In the framework of this problem, we perform a study of functional state of immune cells, in particular, phenotype of peripheral blood lymphocytes isolated from a group of patients with Parkinson's disease in comparison with a group of healthy donors. Twenty-nine healthy donors and 27 patients with PD participated in the study. Flow cytometric analysis was used for determination of profile of plasma membrane surface markers on lymphocytes in subpopulations of mononuclear cells isolated from peripheral blood of the participants of the study. As a result, some differences were revealed between the groups of patients and healthy donors. In particular, the content of CD4+ lymphocytes in CD3+ cell population was higher in PD group in comparison with healthy donors (68.6% and 62.5%, respectively, $p = 0.05$), whereas the part of CD8+ lymphocytes was decreased in the group of patients (23.3% and 29.3%, respectively, $p = 0.04$). Thus, we registered for PD a bias towards T helper turn (the ratio CD4+/CD8+:3.0 and 2.1, respectively, $p = 0.04$). Besides, our results demonstrated an increased amount of central memory T cells with CD3+CD197+ phenotype in population of lymphocytes from PD patients (78.8% in comparison with 69.8%, $p = 0.04$). On the contrary, the content of differentiated effector memory T cells (CD197-45RA+27-(TEMRA)) was decreased in the PD group (59% against 80%, $p = 0.04$). In addition, the registered in our experiments level of expression of CD57—a marker of terminal differentiation of T and NK cells—was lower in the PD patients for CD3+CD56− (6.9% in comparison with 13.4%, $p = 0.01$) and CD3+56+ (66.8% in comparison with 77.2%, $p = 0.05$) subpopulations. Also, for CD3+56+ subpopulation of NKT cells, we detected an increase of amount of HLA-DR-positive cells for the PD group (15.2% against 6.1%, $p = 0.02$) that indicated a higher level of activation of these cells in the course of the disease. As well as for NKT cells, a tendency to activation was registered in the PD group for NK cells by detection of level of NKG2C activating receptor (15.9% in comparison with 7.5, $p > 0.05$). Thus, our results confirmed a relationship between development of Parkinson's disease and some peripheral immune processes. This work was supported by the Russian Science Foundation, grant no. 16-15-10404.

Kilohertz serial crystallography with wide-bandwidth X-ray beam

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Serial crystallography with a polychromatic X-ray beam allows data collection with much shorter exposure times compared to the synchrotron experiments with monochromatic X-rays (Meents et al., Nat Commun, 2017). The usage of a polychromatic X-ray beam with two orders of magnitude higher number of photons spread over a large energy range leads to many more Bragg reflections measured in a single diffraction shot of a crystal at a certain orientation. Therefore, much fewer orientations and crystals are required to obtain a full dataset. The new integrating detectors, which were developed for experiments at X-ray free-electron lasers and are able to handle high photon count rates and high framing rates, provide an opportunity to perform serial crystallography experiments with polychromatic X-rays in a very short time compared to experiments with a monochromatic beam. Using the fast scanning Roadrunner II fixed-target goniometer (Roedig et al., Nat Methods 14:805–810, 2017) in combination with the Jungfrau detector, we collected serial crystallographic data from two model protein samples at a frame rate of 1 kHz and exposure times down to 1 μs at beamline ID09 at the ESRF. Diffraction experiments were performed using a multilayer monochromator, which provides X-rays with an energy spread of 2.7% (FWHM). The total data collection time for a complete dataset using this new method was only 2 min. The *CrystFEL* open-source software suite for serial crystallography was used for data processing (White et al., J Appl Crystallogr 45:335–341, 2012). The high quality of the resulting data in addition to the fast data collection times shows the potential of using this method to collect ultra-low-dose room-temperature datasets of redox-sensitive proteins and to study irreversible processes such as enzymatic reactions at microsecond time resolution.

1. Meents, A. *et al.* Pink-beam serial crystallography. *Nat. Commun.* (2017). https://doi.org/10.1038/s41467-017-01417-3.
2. Roedig, P. *et al.* High-speed fixed-target serial virus crystallography. *Nat. Methods* **14**, 805–810 (2017).
3. White, T. A. *et al.* CrystFEL: a software suite for snapshot serial crystallography. *J. Appl. Crystallogr.* **45**, 335–341 (2012).

Interaction of the domain with unknown functions of telomere-binding protein TRF2 with nuclear lamina

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Different types of premature ageing disease progeria caused by the mutation of the *LMNA* gene are accompanied by abnormalities of the nuclear membrane and shortening of telomeres. Evidently, there is some mechanism attaching telomeres to the nuclear envelope but it is still unknown. Wood et al. (Nat Commun 5:6467, 2014) reported interactions between lamin A/C and telomere-binding protein TRF2. TRF2 has a domain with unknown functions. The homology between its amino-acid sequence and some sequences of rod domains of laminas may point out that this domain is responsible for the interaction of TRF2 and telomeres with the nuclear envelope. The polypeptide corresponding to the domain with unknown functions (udTRF2) was expressed using transformed bacterial strain *Escherichia coli* RosettaBlue (DE3)-pET32a-udTRF2 and then was isolated from soluble fraction of cell lysate by ammonium sulfate precipitation and ion-exchange chromatography. Polyclonal antibodies against udTRF2 were raised in guinea pigs and used for co-immunoprecipitation with the nuclear lamina extract isolated from nuclei of mouse liver cells. Anti-udTRF2 antibodies were incubated with protein A-sepharose for 3 h at 4 °C. The nuclear lamina extract was incubated with udTRF2 for 7 h at 4 °C, then added to protein A-sepharose with antibodies and incubated for 14 h at 4 °C (sample 1). As a control, protein A-sepharose was incubated with the udTRF2 solution (sample 2); protein A-sepharose with antibodies was incubated with the udTRF2 solution (sample 3) and with an extract of lamina (sample 4). Immunoblotting with antibodies to TRF2 (antiTRF2, Abcam), lamins C, which is a splice-variant of lamin A, and lamin B showed that lamins C and B partially bound to udTRF2 immobilized on sepharose, while in control sample 4 lamins remained in the supernatant. Thus, we have shown that udTRF2 domain can bind lamins in vitro. Ability of interaction of TRF2 through its udTRF2 domain with lamin B1 was detected for the first time, but its functional significance is not yet known. This work was supported by the Russian Foundation for Basic Research (no. 15-04-01857 and 16-34-00714), Russian Science Foundation (no. 15-15-20026), and RAS "Molecular and cell biology" granting programme.

1. *Wood, AM et al.* TRF2 and laminA/C interact to facilitate the functional organization of chromosome ends. *Nat. Commun.* **5,** ncomms6467 (2014)
2. *Voronin AP et al.* A telomere-binding protein (TRF2/MTBP) from mouse nuclear matrix with motives of an intermediate filament-type rod domain. *J Anti Aging Med.* **6,** (2003).

Influence of stabilizing amphiphiles on the integrity of fluid, gel, and liquid-ordered phase antitumor liposomes loaded with lipophilic prodrug in the bilayer

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Liposomes like other nanoparticles became covered with proteins very rapidly upon contacts with blood plasma. Liposome protein interactions could lead to destabilization of the membrane. Thus, we incorporated in liposomes several amphiphilic molecules that are assumed to be able to stabilize membrane in blood plasma: phosphatidylinositol (PI), ganglioside GM_1, polyethylene glycol 2000 conjugate with dioleoylphosphatidylethanolamine (PEG-PE), and an acidic oligopeptide conjugate with dioleoylphosphatidylethanolamine (CMG-PE). Liposomes of 100-nm diameter were loaded with 10 mol% of Mlph-DG, dioleoylglycerol ester of melphalan (chemotherapeutic alkylating agent). Liposome stability in serum *ex vivo* was evaluated by the fluorescence increase detection upon encapsulated self-quenched calcein leakage. Our results show that the best stabilization of nanosized liposomes with fluid lipid bilayer was achieved by incorporation of ganglioside GM_1 or CMG-PE, in liposome membrane. Even small amounts (2 mol%) of these molecules protected liposomes from the destructive action of plasma proteins at least for 24 h. The increase in percentage of these molecules (up to 10) resulted in widened water channels, or cracks, and appeared in the bilayer due to interactions of proteins with bulky negatively charged residues of amphiphiles protruding outwards. The inclusion of PI (10 mol%) ensured stabilization of the

liposomal formulation in serum only for the first 4 h. The presence of PEG-lipid conjugate in the membrane of fluid-phase liposomes promoted dissociation of the components from the bilayer as we have seen by wave-like calcein release, presumably through micelle formation. This can result in both loss of a water-soluble drug and a lipophilic (pro)drug. Gel-phase membranes with amphiphiles were even less stable in serum as compared to the fluid-phase ones with PEG-PE or even with 10 mol% of GM_1 and CMG-PE. We consume that such behavior in serum is due to fragile membrane with fluid inclusions that cannot stand the pressure of protein corona and its incorporation. In accordance with known data and argumentation, cholesterol-containing liquid-ordered bilayers supplemented with 10% of PEG-lipid (and loaded with Mlph-DG in our case) show good stability in serum, though calcein release rate exceeds observed for fluid lipid bilayer with GM_1 or CMG-PE. The work was supported by the Russian Foundation for Basic Research (project no. 16-04-01585 A)

NMR study of human Lypd6 and Lypd6b proteins acting on nicotinic acetylcholine receptors

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Several endogenous ligands of nicotinic acetylcholine receptors (nAChRs) belonging to the Ly-6/uPAR family were discovered in higher animals. These proteins share structural homology with 'three-finger' snake α-neurotoxins, specific inhibitors of nAChRs. Some of these proteins (e.g., Lynx1, Lypd6) are membrane-tethered via GPI-anchor and co-localize with nAChRs modulating their functions in the brain. Others (SLURPs) are secreted and act as autocrine/paracrine hormones in epithelium. Lypd6 and Lypd6b are expressed in the brain and unlike other human Ly6/uPAR proteins have additional long N- and C-terminal sequences flanking the 'three-finger' LU-domain. Lypd6 increases the amplitude of nicotine-induced calcium currents in mouse trigeminal ganglion neurons. Lypd6 of *Danio rerio* fish is involved in the regulation of Wnt/β-cathenin signaling pathway, and the knockdown of Lypd6 leads to the impairment of embryonic development. Expression of Lypd6b in *Xenopus laevis* oocytes increases the sensitivity of nAChRs to acetylcholine and their desensitization rate. In the present work, the ^{13}C,^{15}N-labeled variants of water-soluble LU domains of human Lypd6 and Lypd6b were produced in *E. coli* and studied by NMR. The proteins adopt typical 'three-finger' fold and possess three loops (I, II, III) protruding from the β-structural core. In contrast to other Ly6/uPAR proteins, the Lypd6 and Lypd6b contain only one β-sheet formed by five strands and involving residues form all three loops. Loops I and III, stabilized by additional disulfide bonds, accommodate two 'unexpected' α-helical elements. C-terminal regions (87-95) of Lypd6 and Lypd6b demonstrate conformational heterogeneity in solution possibly connected with cis-trans isomerization of Leu85-Pro86 bond going with characteristic time ~ 0.1 s. The exchange motions were also observed in the adjacent β-strands. ^{15}N-relaxation data revealed significant ps-ns mobility in the unstructured C-terminal region of both proteins and in the Lypd6b loop II. The determined overall rotation correlation time (~ 5.5 ns at 30 °C) confirmed monomeric state of the proteins in solution. Obtained spatial structure of Lypd6 permitted to model its interactions with the α4β2 neuronal nAChR. Protein-protein docking and molecular dynamics revealed tight contacts between loop I of Lypd6 and the entrance to the agonist binding site that is located between the α4 and β2 subunits of the receptor. The work was supported by the Russian Science Foundation (project no.16-14-00102).

Volume-sensitive outwardly rectifying anion channel in mouse peritoneal macrophages

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Primarily, functional stress of the organism affects the responses of cells of the innate, and only later of the adaptive immunity. Macrophages are specialized mezenchymal cells of the innate immunity system, which are widespread in the organism and active against microbes, cancer cells, alien, and toxic substances. The phagocytosis is the basic function of macrophages and is supposedly accompanied with fluctuations in cellular volume. Therefore, ion transporting systems activated by volume changes should play a major role in macrophage functions. Cellular swelling is known to activate a number of channels and transporters with the volume-sensitive outwardly rectifying (VSOR) anion channel playing a central role in the regulatory volume decrease machinery which is important for keeping cell integrity. However, biophysical properties and physiological functions of VSOR in macrophages have not been described thus far. In the present experiments, we studied the phenotype of the VSOR channels in mouse peritoneal macrophages. When inflated by using slightly hypertonic pipette solution, cells responded with visible swelling accompanied by a robust activation of anionic currents with VSOR phenotype. The half-activation time of VSOR activation was 9.8 ± 0.8 min ($n = 5$). The macroscopic current had a prominent outward rectification and inactivated at depolarized potentials over + 60 mV. Current density at the peak of activation reached 783 ± 70 pA/pF at + 100 mV ($n = 20$). The selectivity experiments showed no effect of replacement in bath solution of 135 mM Na^+ to 135 mM $NMDG^+$. However, when 135 mM Cl^- anions were equimolarly replaced with $glutamate^-$, we observed a shift of reversal potential ΔE_{rev} by 28.3 ± 3.2 mV ($n = 5$), which corresponds to the permeability ratio $P_{Glu}/P_{Cl} = 0.28 \pm 0.05$ ($n = 5$). The macroscopic swelling-activated anion current in peritoneal macrophages was sensitive to VSOR blockers with a sensitivity order tamoxifen > DCPIB > phloretin. The functional role of the volume-sensitive outwardly rectifying anion channel in cells of innate immunity system in general, and in phagocytosis in particular, remains poorly understood, especially with respect to the reactive oxygen species, a well-known VSOR activator. It is also expected that VSOR channels expressed in peritoneal macrophages may serve as a perspective target for search of new immunomodulators of next generation which could be effective not only at the level of lymphocytes (adaptive immunity), but also on the phagocytosis level (innate immunity).

Phospho-specific antibodies for studying the function of LPAP

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TCR signaling cascade is tightly controlled by a number of kinases and phosphatases which in turn are regulated by diverse network of adaptor proteins. Among such molecules, there is a small transmembrane protein LPAP (lymphocyte phosphatase-associated phosphoprotein) that forms a stable complex with the phosphatase CD45 (Schraven et al., J Biol Chem 269:29102–29111, 1994). It also interacts with the coreceptor CD4 and the kinase Lck (Veillette et al., J Biol Chem 274:14392–14399, 1999). However, the role of LPAP in these interactions and TCR signaling in general is not known. Formulating hypotheses concerning LPAP based on its structure is difficult because LPAP has no protein homologues and its three dimensional structure is unresolved. In the previous work, we showed that LPAP has four phosphorylation sites and is represented by at

least five proteoforms both in Jurkat cell line and in primary T cells (Filatov et al., Clin Transl Immunol 4:e44, 2015; Kruglova et al., PLoS One 12:e0182468, 2017). The pattern of LPAP phosphorylation changed when the cells were activated by various stimuli. Since phosphorylation is widely used in the regulation of TCR signaling, site-specific time-resolved studies of LPAP phosphorylation may be a key to its role in the cell. The aim of this work has been to generate phospho-specific antibodies against individual LPAP phosphorylation sites and apply them for the analysis of LPAP proteoforms in different cells and under different stimulatory conditions. We used four phospho-peptides of LPAP which included phosphosites Ser-99, Ser-153, Ser-163, or Ser-172. BALB/c mice were immunized with peptides coupled to BSA in Freund's adjuvant. Sera were collected and tested for reactivity and specificity in ELISA and Western blot. With these antibodies, we have shown the LPAP phosphorylation kinetics of individual sites under PMA and TCR stimulation. Moreover, we have been able to study LPAP phosphorylation in primary cells. This work was supported by the Russian Foundation for Basic Research grant no. 18-34-00705.

1. Schraven, B. et al. LPAP, a novel 32-kDa phosphoprotein that interacts with CD45 in human lymphocytes. J. Biol. Chem. 269, 29102–11 (1994).
2. Veillette, A., Soussou, D., Latour, S., Davidson, D. & Gervais, F. G. Interactions of CD45-associated protein with the antigen receptor signaling machinery in T-lymphocytes. J. Biol. Chem. 274, 14392–9 (1999).
3. Filatov, A., Kruglova, N., Meshkova, T. & Mazurov, D. Lymphocyte phosphatase-associated phosphoprotein proteoforms analyzed using monoclonal antibodies. Clin. Transl. Immunol. 4, e44 (2015).
4. Kruglova, N. A., Meshkova, T. D., Kopylov, A. T., Mazurov, D. V & Filatov, A. V. Constitutive and activation-dependent phosphorylation of lymphocyte phosphatase-associated phosphoprotein (LPAP). PLoS One 12, e0182468 (2017).

Low-resolution structure of modular nanotransporters obtained by small-angle X-ray scattering method

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Modular nanotransporters (MNTs) are chimeric proteins which serve to transport locally acting drugs to cancer cell nucleus (Gilyazova et al., Cancer Res 66(21):10534–10540, 2006). Polypeptide chain of MNT consists of several modules, each responsible for the specific step of the drug delivery (Slastnikova et al., EJNMMI Res 2(1):59, 2012). Ligand module interacts with the receptor overexpressed on cancer cell surface, then due to receptor-mediated endocytosis MNTs come to the endosomal compartment. After that, MNTs escape endosomes, interact with importin dimer, and finally are translocated into the nucleus. Until recently, there was no information about MNT structure. Using small-angle X-ray scattering method coupled to size exclusion chromatography (Brennich et al., J Vis Exp 119, 2017), we obtained some parameters of MNT chimeric proteins (Rg = 3.7 and 3.9 nm for MNT-αMSH and MNT-EGF respectively). Using program Dammin (from ATSAS package (Franke et al., J Appl Crystallogr 50(Pt 4):1212–1225, 2017), we have obtained low-resolution structure of MNT. We speculate that N- and C-termini of the protein are distanced, what gives an ability for different modules not to interfere with each other. This information is key full for further MNT improvement while going towards clinical trials.

1. Gilyazova D. G. et al. Targeting cancer cells by novel engineered modular transporters. Cancer Res 2006, 66(21):10534-10540.
2. Slastnikova T. A. et al. Modular nanotransporters: a versatile approach for enhancing nuclear delivery and cytotoxicity of Auger electron-emitting 125I. EJNMMI Res 2012, 2(1):59.
3. Brennich, M. E et al. Online Size-exclusion and Ion-exchange Chromatography on a SAXS Beamline. J Vis Exp 2017;(119).
4. Franke D. et al. ATSAS 2.8: a comprehensive data analysis suite for small-angle scattering from macromolecular solutions. J Appl Crystallogr. (2017), 50(Pt 4):1212-1225.

New inward H^+ pumps xenorhodopsins

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Xenorhodopsins are a subfamily of retinylidene protein that function as light-gated inward proton pump. A number of proteins have been recently reported, representing various types of organisms (Inoue et al., Nat Commun 7:13415, 2016; Shevchenko et al., Sci Adv 3:e1603187, 2017). The photocycle of *Parvularcula oceani* XeR is 200 ms, while the protocycle of *Nanosalina* XeR lasts 29 ms. It was shown that NsXeR can produce large transmembrane currents (up to 1.4 pA/pF) in model systems, such as HEK293, NG-108. Further, NsXeR was capable in activating the neurons at their maximum firing frequency (Bamberg et al., Biophysical Journal 114:669A, 2018). We are looking for the new members of the XeR subfamily, emerging from various organisms living in common and exotic environments. XeR constructs fused with fluorescent protein were heterologously expressed in human neuroblastoma cells SH-SY5Y. Whole-cell voltage-clamped experiments were conducted. New *Aliterella atlantica* XeR showed inward current. Level of protein surface expression was also estimated by immunostaining flow cytometry. Proteins with low level of surface expression showed no current in patch clamp experiments. Transport activity, however, was demonstrated by pH changes in *Escherichia coli* suspension. This work was carried out with support from and as part of the programme of the Centre for AI Science and Technology at the Moscow Institute of Physics and Technology.

1.Inoue, K. *et al*. A natural light-driven inward proton pump. *Nat. Commun.* **7**, 13415 (2016).
2.Shevchenko, V. *et al.* Inward H+ pump xenorhodopsin: Mechanism and alternative optogenetic approach. *Sci. Adv.* **3**, e1603187 (2017).
3. Bamberg, E. *et al.* Improved Microbial Rhodopsins for Ultrafast Red-Shifted Optogenetics. Biophysical Journal. **114**, 669A (2018)

The role of Ca^{2+} and NF-κB in NO generation in photosensitized neurons and glial cells

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In photodynamic therapy (PDT), stained cells are damaged by light in the presence of oxygen. Following intense oxidative stress kills cells. PDT is used in oncology, in particular, in neurooncology for destruction of brain tumors. However, it also damages surrounding healthy neurons and glial cells. NO is a putative extracellular neuroglial mediator that can regulate survival and death of neurons and glia. Previously, we showed the participation of NO in PDT-induced death of sensory neurons and satellite glial cells in the isolated crayfish stretch receptor (CSR). CSR is a simple model object consisting of a single mechanoreceptor neuron (MRN) surrounded by satellite glial cells. It was photosensitized by alumophthalocyanine photosens and irradiated by a diode laser (670 nm). Dynamics of NO generation was studied using the fluorescence probe DAF-2DA (4,5-diaminofluorescein diacetate). NO may be produced by Ca^{2+}-dependent neuronal NO synthase or by Ca^{2+}-independent inducible NO synthase (iNOS). Fourfold increase in the extracellular $CaCl_2$ concentration, or application of calcium ionophore ionomycin, or tBuBHQ, an inhibitor of Ca^{2+}-ATPase (SERCA), which prevents pumping out Ca^{2+} and causes its accumulation in the cytosol, enhanced PDT-induced NO production in MRN and glia. Oppositely, Cd^{2+} that blocks Ca^{2+} channels in the plasma membrane inhibited PDT-induced NO generation. Nifedipine, a selective blocker of L-type Ca^{2+} channels, also reduced PDT-induced NO generation in MRN and glia. Therefore, Ca^{2+} penetration through calcium channels in the plasma membrane, mainly through L-type channels, contributed to PDT-induced NO production in neurons and glia. Except Ca^{2+}-dependent nNOS, PDT can putatively induce NO production by inducible NOS synthase (iNOS) through the Ca^{2+}-independent pathway. Selective inhibition of iNOS by SMT suppressed NO generation that indicated the significant involvement of iNOS in NO production. The rapid expression of iNOS in photosensitized cells can be mediated by NF-κB, which is known to be stimulated by PDT. In fact, NF-κB activator prostratine increased PDT-induced NO production in MRN and glia relatively control. On the contrary, parthenolide, an inhibitor of NF-κB, significantly decreased PDT-induced NO generation. This confirms the involvement of iNOS and NF-κB in PDT-induced NO generation in MRN and glial cells. Thus, both Ca^{2+}-dependent nNOS and Ca^{2+}-independent iNOS were involved in NO production in photosensitized crayfish neurons and glial cells. This work was supported by the grants of PFBR (no. 15-04-05367) and Russian Ministry of Education and Science (no. 6.6324.2017/8.9 and no. 6.4951.2017/6.7).

Response of leukemia cells to KIT downregulation via activation of pro-apoptotic and pro-survival signaling pathways

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Acute myeloid leukemia (AML) is one of the most frequent blood malignancies. For elderly patients, prognosis is poor with low rate of remissions and huge number of relapses. One of the most common event in AML blast cells is overexpression of gene coding receptor tyrosine kinase KIT. KIT seems to be an attractive target for anti-leukemia treatment, but the input of non-mutant KIT upregulation in AML still remains unclear. We used RNA interference (RNAi) technique to determine the mechanisms by which AML cells respond to KIT downregulation. We observed slowdown of cell growth when KIT is repressed; also the dysregulation of genes coding cyclins and cyclin-associated kinases was demonstrated. By performing a genome-wide microarray-based screening of gene expression, we showed that KIT downregulation is followed by dramatic changes in signaling pathway activation profile. Most of them are responsible for cell death and suppression of cell growth. However, the huge number of activated pathways are associated cell survival, avoiding apoptosis, and proliferation stimulation. Among them, signaling pathways associated with ERK kinases are found to be compensatory upregulated when KIT is suppressed. Signaling pathways associated with cytokines production and conversion were also found upregulated in response to KIT knockdown. The results were obtained within the program of fundamental research for state academies for the 2013–2020 years (no. 01201363823). Gene expression analysis experiments were supported by the Russian Science Foundation (project no. 14-14-01089-П). Cell viability assays were performed within the RFBR grant (project no. 170401555).

LCP crystallization of A_{2A} adenosine receptor bound to amilorides

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G protein–coupled receptors play a pivotal role in signal transduction. Recent advances in their structure determination allowed to reveal important features of allosteric binding sites available for drug design (Lu and Zhang, J Med Chem, 2018; Gutierrez-de-Teran et al., Structure 2175:21–12, 2013). However, the mechanism of allosteric modulation is still poorly understood and lacks structural explanation. Amilorides are the well-known allosteric modulators for adenosine receptor. We performed extensive screening analysis of crystallization conditions for A_{2A}-StaR (b562RIL) (Robertson et al., Neuropharmacology (60-1)36, 2011) adenosine receptor bound to amilorides using FRAP (Fenalti et al., Methods Enzymol (557-)417, 2015). We found out that salts of monovalent ions at low pH increase protein diffusion in LCP. Our results demonstrated the conditions, which are more suitable for crystallization of adenosine receptor bound to allosteric modulator using LCP.

1. Lu, S. & Zhang, J. Small molecule allosteric modulators of G protein-coupled receptros: drug-target interactions. *Journal of Medicinal Chemistry* (2018). https://doi.org/10.1021/acs.jmedchem.7b01844
2. Hugo Gutierrez-de-Teran *et al.* The role of a sodium ion binding site in the allosteric modulation of the A2A adenosine G protein-coupled receptor. *Structure (21-12)*. **2175**, (2013).
3. Robertson, N. *et al.* The properties of thermostabilised G protein-coupled receptors (StaRs) and their use in drug discovery. *Neuropharmacology (60-1)*. **36,** (2011).
4. Fenalti, G. *et al.* Fluorescence Recovery After Photobleaching in Lipidic Cubic Phase (LCP-FRAP): A precrystallization assay for membrane proteins. *Methods in Enzymology (557-.)*. **417,** (2015).

Bacteriorhodopsin from *Exiguobacterium sibiricum*/amphipathic polymers complexes: functional studies

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Retinal-containing membrane proteins are present in many domains of life. They use light energy for a wide range of functions, for instance, ion transport or photosensing. They are used for development of many applications including the optogenetic control of cell and tissue. Amphipathic polymers are frequently used for obtaining complexes with membrane proteins. Amphipathic polymers keep membrane

proteins in their native state, stable, and water-soluble in the absence of detergents or phospholipids. This helps to study membrane proteins via many biophysical techniques. We obtained complexes of bacteriorhodopsin from bacteria *Exiguobacterium sibiricum* (ESR) (Petrovskaya et al., Biochem 80(6):688–700, 2015) with two types of amphipathic polymers—A8-35 and PMAL-C12 (Popot, Annu Rev Biochem 79(1):737–775, 2010). Stability of complexes was checked by UV/VIS spectroscopy. It was shown that complexes were stable over months at room temperature and under light irradiation. Average sizes of complexes were measured by dynamic light scattering. The mean radius was about 2.5 nm. The photocycles and the spectra of ESR intermediates in complexes with amphipathic polymers were measured by laser-induced time-resolved spectroscopy. The mean time of photocycle for both complexes was longer than that for ESR in *n*-dodecyl-β-D-maltoside micelles (0.7 s and 0.1 s correspondingly). This work was supported in part by the RFBR grant 17-00-00167K (KOMFI 17-00-00166, 17-00-00165, 17-00-00164) and the Ministry of Education and Science of the Russian Federation (grant no. 6.3157.2017/PP).

1. L. E. Petrovskaya *et al.*, "ESR — A retinal protein with unusual properties from Exiguobacterium sibiricum," *Biochem.*, vol. 80, no. 6, pp. 688–700, Jun. 2015.
2. J.-L. Popot, "Amphipols, Nanodiscs, and Fluorinated Surfactants: Three Nonconventional Approaches to Studying Membrane Proteins in Aqueous Solutions," Annu. Rev. Biochem., vol. 79, no. 1, pp. 737–775, Jun. 2010.

Guanidine hydrochloride fixed bacteriorhodopsin in the intermediate state of its photocycle

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Conformational changes of membrane proteins are part of their function and, in particular, transport mechanism. We investigated conformational changes of photosensitive protein bacteriorhodopsin from purple membranes. High-resolution diffraction of electrons (Glaeser et al., Biophys J 50:913–920, 1986), neutrons (Dencher et al., Proc Natl Acad Sci USA 86:7876–7879, 1989) and X-ray (Koch et al., EMBO J 10:521–526, 1991; Dencher et al., Photochem Photobiol 54:881–887, 1991) showed that photoinduced conformational changes of bacteriorhodopsin occur while transitioning from BR568 into the ground state BR412 though the intermediate state M412 that corresponds the state of the proton pump BR. We found a significant difference in thicknesses of purple membranes (dark-adapted $T = 50.3$ Å and light-adapted $T = 44.7$ Å) by small-angle neutron scattering, YuMO (JINR, Dubna, Russia) (Kuklin et al., Neutron News 16:16–18, 2005) and small-angle X-ray scattering (Murugova et al., J Optoelectron Adv Mater 17:1397–1402, 2015). Investigations of 2% *w/w* of purple membranes at pH 9.6 in 100 mM guanidine hydrochloride showed that the M-state of bacteriorhodopsin was fixed for several hours that subsequently requires direct interaction between guanidine hydrochloride and purple membranes caused by light.

1.Glaeser, R. M., Baldwin, J., Ceska, T. A. & Henderson, R. Electron diffraction analysis of the M412 intermediate of bacteriorhodopsin. *Biophys. J.* **50,** 913–20 (1986).
2.Dencher, N. A., Dresselhaus, D., Zaccai, G. & Büldt, G. Structural changes in bacteriorhodopsin during proton translocation revealed by neutron diffraction. *Proc. Natl. Acad. Sci. U. S. A.* **86,** 7876–9 (1989).
3.Koch, M. H. *et al.* Time-resolved X-ray diffraction study of structural changes associated with the photocycle of bacteriorhodopsin. *EMBO J.* **10,** 521–526 (1991).
4.Dencher, N. A. *et al.* Proton translocation and conformational changes during the bacteriorhodopsin photocycle: Time-resolved studies with membrane-bound optical probes and X-ray diffraction. *Photochem. Photobiol.* **54,** 881–887 (1991).
5.Kuklin, A. I., Islamov, A. K. & Gordeliy, V. I. Scientific Reviews: Two-Detector System for Small-Angle Neutron Scattering Instrument. *Neutron News* **16,** 16–18 (2005).
6.Murugova, T. N. *et al.* Low resolution structural studies of apoferritin via SANS and SAXS: The Effect of concentration. *J. Optoelectron. Adv. Mater.* **17,** 1397–1402 (2015).

Controlled release of doxorubicin from magnetoliposomes under low-frequency magnetic field

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One of the most described way to induce controlled release from magnetoliposomes (MLs) is magnetic hyperthermia. However, the method is inconvenient due to application of high-frequency magnetic field with high intensity during a long time. Our research deals with the non-heating low-frequency alternating magnetic field (LF-AMF). MNPs incorporated into liposomes can respond to an external magnetic field by mechanical rotation (Brown relaxation) and thus destabilize liposomes and induce the release of encapsulated drugs or biomolecules. The aim of this study was to formulate doxorubicin-loaded magnetoliposomes (MLs-DOX) for theranostic application and to show the efficiency of LF-AMF application in controlled drug release.
Results and Discussion
Under LF-AMF exposure of 15 min, the MNPs in MLs firstly aggregated into clusters, then these clusters rotated and destroyed the MLs membranes (data from TEM). *In vitro* DOX release from MLs under AMF exposures was 1.5–2 times more effective than that in control experiment (total release for 6 h under AMF and without was 45% and 25%, respectively). Cellular internalization was enhanced with MLs-DOX under LF-AMF. It was shown, under 15-min exposure to AMF, free DOX was around the cell nucleus and lipids were located in cytoplasm as aggregates, in the case of non-treated MLs-DOX, there was no free DOX in the cells. The effect depended on AMF parameters. Thus, we showed the possibility of new approach for remote-controlled drug release from liposomes. Confocal microscopy showed that untreated MLs-DOX localized in cytoplasm or lisosomas during 3 h. Release of DOX under LF-AMF led to higher toxicity of MLs-DOX according to MTT test on 4t1 cells. Free DOX accumulated in cell nucleus of spheroids during 45 min, while MLs "penetrated" into spheroids during 3–4 hr of incubation. Incubation of the AMF-treated MLs showed the accumulation of DOX in spheroids, while there were no DOX without exposure to AMF in spheroids. Grants support: RSF 14-13-00731 and RFBR 16-33-01023

Effects of neonatal pro-inflammatory stress on the expression of genes associated with neuroinflammation and stress reactivity in the neocortex and hippocampus of juvenile rats

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Depressive disorders are among most common mental diseases. The induction of a depressive-like behavior in adult animals by the injection of Gram-negative bacteria lipopolysaccharide (LPS) in neonatal age is a widely used approach to model depressive-like states in rodents. Activation of the neuroinflammation as well as disturbed stress reactivity appears to be key triggers of the depressive state in this model. In this study, we tried to identify changes in the expression of genes associated with neuroinflammation and the response to stress in juvenile rats preceeding the manifestation of depressive-like behavior. We investigated the delayed effect of neonatal pro-inflammatory stress (NPS) on the expression of genes involved in the development of neuroinflammation (IL-1b, IL-6, TNF-a, Sall1, fractalkine (CX3CL1) and its receptor (CX3CR1)) and in stress response (corticotropin-releasing hormone (CRH) and its receptors (CRHR1, CRHR2), glucocorticoid (GR) and mineralocorticoid (MR) receptors) in the frontal and somatosensory cortex as well as in the dorsal (DH) and ventral (VH) hippocampus of male and female 30-day-old Wistar rats. NPS induced a decrease in the expression of fractalkine in the DH and an increase in the expression of fractalkine and its receptor genes in the VH of males but not females. NPS increased the expression of GR in the VH of juvenile males without affecting respective expression in the DH. Notably, NPS increased the expression of GR in the DH of juvenile females without affecting its expression in the VH. Thus, we found no signs of activation of genes involved in neuroinflammation in experimental animals. However, changes in the expression of fractalkin and its receptor suggest that the properties of immune system cells in the CNS of experimental animals have changed. In addition, changes in CRH and GR expression may also point to mechanisms of a depressive-like behavior in adult animals. These effects may underlie the predisposition to depression of experimental animals, differences between male and female rats reflecting gender-related specificity. Supported by RSF grant no. 14-25-00136 (stress, depression) and RAS (remote hippocampal damage)

Molecular modeling of agitoxin 2 complex with Kv1.1 binding site

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Voltage-gated potassium channel Kv1.1 is widely distributed in the central nervous system and involved in the development of neuroinflammatory diseases such as multiple sclerosis, stroke and trauma (Tian et al., Chem Biol Drug Des 83:1–26, 2014). Detailed studies of channel interactions with peptide blockers are the basis for rational design of peptide ligands with improved properties to treat channel-associated diseases. Agitoxin 2 (AgTx2) is a representative of a large family of peptide blockers with a CSα/β structure (cysteine-stabilized α-helix-β-sheet) possessing high affinity to Kv1 channels (Kuzmenkov et al., Biochem (Mosc) 80:1764–99, 2015). A goal of the current study was to create a structural model of AgTx2 complex with KcsA-Kv1.1 channel and analyze in detail the binding interface. KcsA-Kv1.1 chimera represents the KcsA channel bearing a blocker binding site of Kv1.1 and allows one to reproduce blocker interactions with the Kv1.1 binding site in bacteria (Kudryashova et al., Anal Bioanal Chem 405:2379–2389, 2013) followed by characterization of the complex features by molecular modeling (Novoseletsky et al., Acta Naturae 8:35–46, 2016). 3D structure of the complex was generated by homology modeling method using charybdotoxin complex with Kv1.2-2.1 hybrid potassium channel (pdb-code 4JTA (Banerjee et al., Elife 2:e00594, 2013) as a template. Trajectory of molecular dynamic simulation was performed using the Gromacs processing package in all-atom force field opls-AA and analyzed to choose the optimal conformation of AgTx2 in the Kv1.1 binding site. Analysis of formed hydrophobic and stacking interactions, hydrogen and ionic bonds was performed for representative frames using the Platinum software. Estimation of binding contacts between channel and toxin residues allowed identification of key residues involved in the binding as well as possible mutation sites for changing selectivity against Kv1.1 channel. The results of the binding free energy calculation are in a good agreement with the measured experimental value. This work was supported by the Program for fundamental research of the Presidium of Russian Academy of Sciences.

1.Tian, C. *et al.* Potassium channels: Structures, diseases, and modulators. *Chem. Biol. Drug Des.* **83,** 1–26 (2014).
2.Kuzmenkov, A. I.. *et al.* Diversity of Potassium Channel Ligands: Focus on Scorpion Toxins. *Biochem. (Mosc).* **80,** 1764–99 (2015).
3.Kudryashova, K. S. *et al.* Fluorescent system based on bacterial expression of hybrid KcsA channels designed for Kv1.3 ligand screening and study. *Anal. Bioanal. Chem.* **405,** 2379–2389 (2013).
4.Novoseletsky, V. N. *et al.* Modeling of the Binding of Peptide Blockers to Voltage-Gated Potassium Channels: Approaches and Evidence. *Acta Naturae* **8,** 35–46 (2016).
5.Banerjee, A. *et al.* Structure of a pore-blocking toxin in complex with a eukaryotic voltage-dependent K+ channel. *Elife* **2,** e00594 (2013).

The effect of oregano essential oil on BALB/c mice longevity

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It is well known that bioactive substances of plant origin affect the state of health and are widely used as medications. In this regard, plant essential oils (EOs) are of particular interest due to the wide range of their biological effects in the organism. Despite the high activity of the oregano EOs, there is no data on its influence on macroorganisms. In the present work, we studied the effect of oregano EOs at two different doses on mortality of BALB/c mice. Mice were given oregano with drinking water at a daily dose of about 0.3 μg and 0.03 ng throughout their life. The control group drank drinking water without oregano. Based on the data on the animals' longevity in each of the three groups, we built the mortality curves. The equation of the form $y = Ax + b$ (A and b—linear regression coefficients, y—mortality, x—age in days) was used for mathematical processing of the results of those sections of curves where mass death of animals occurred. The calculated values of death rate and time of latent period as well as median and maximum lifespan are presented in Table 1.

Parameter	Control ($y = 0.37x - 204.2$)	EO at 0.3 μg per day ($y = 0.32x - 208.2$)	EO at 0.03 ng per day ($y = 0.41x - 232.9$)
Rate of death	0.37 ± 0.01	0.32 ± 0.02	0.41 ± 0.02
Median lifespan, days	687	807	690
Maximum lifespan, days	822	963	812
Latent time, days	552	651	568

Table 1 Mathematical and statistical processing of mortality curves of BALB/c mice in control and in the case of oregano EO consumption at different doses.

It was found that oregano EOs at 0.03 ng per day demonstrated slight effects closed to control. However, EOs of oregano at 0.3 μg per day increased both median and maximum lifespan by 17% in comparison to the control group. The rate of death in the case of 0.3 μg oregano EOs per day decreased by 14% compared to control. Estimated time of the onset of animal death (latent time) also increased by 12% in the group that obtained water with higher amount of oregano EOs. As we consider, the phenomenon of extending lifespan of animals exposed to oregano can be not only the result of directional influence oregano on aging mechanisms, but rather a consequence of a reduced risk of development or/and a more favorable course of age-related diseases.

Conformational study of an archaeal photoreceptor/transducer complex from *Natronomonas pharaonis* assembled in lipid nanoparticles using EPR spectroscopy

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The transmembrane protein signaling complex *Np*SRII/*Np*HtrII plays a key role in negative phototaxis of the halophilic archaeon *Natronomonas pharaonis* (Klare et al., Eur J Cell Biol 90, 2011; Klare et al., Febs Lett 564:219–224, 2004). Photon absorption induces transient structural changes in the photoreceptor sensory rhodopsin II (*Np*SRII) (Wegener et al., Embo J 20:5312–5319, 2001), which are conducted to the transducer *Np*HtrII. The subsequent signal propagates along the cytoplasmic part of *Np*HtrII to the intracellular signaling pathway (Orekhov et al., Plos Comput Biol 11, 2015) that modulates the rotation of the flagellum. We studied conformation and dynamics of *Np*SRII/*Np*HtrII after reconstitution in cell membrane–mimicking nanoparticles, namely, styrene–maleic acid lipoprotein particles (SMALPs) (Voskoboynikova et al., Rsc Adv 7:51324–51334, 2017) and lipid nanodiscs [6]. The size and shape of assembled lipid nanoparticles were characterized by atomic force microscopy (AFM). We used continuous-wave (cw) electron paramagnetic resonance (EPR) spectroscopy to analyze the protein dynamics through tracking the residual motion of spin-labeled side chains. We determined the interspin distance distributions between labels by pulse EPR experiments and compared the results with *in silico* spin-labeling rotamer analyses based on available X-ray crystallographic data. Our data indicate that *Np*SRII/*Np*HtrII complexes reconstituted in both types of nanoparticles retained their structural integrity and functionality. This work was supported by the German Research Foundation (DFG, STE640/14), the Federal Ministry of Education and Research of Germany (BMBF, 05K14MPA), and the Russian Foundation for Basic Research (RFBR) (Project No. 18-504-12045).

1. Klare, J. P., Bordignon, E., Engelhard, M. & Steinhoff, H. J. *Eur J Cell Biol* **90**, (2011).
2. Klare, J. P. *et al. Febs Lett* **564**, 219-224, (2004).
3. Wegener, A. A., Klare, J. P., Engelhard, M. & Steinhoff, H. J. *Embo J* **20**, 5312-5319, (2001).
4. Orekhov, P. S. *et al. Plos Comput Biol* **11**, (2015).
5. Voskoboynikova, N. *et al. Rsc Adv* **7**, 51324-51334, (2017).

Adrenaline induces calcium signal in neurons and astrocytes

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Adrenaline (ADR) is present in the mammalian brain and it is known that brain tissue can synthesize ADR *in vitro*. It plays an important role in increasing blood flow to muscles, output of the heart, pupil dilation response, and blood sugar level. In addition, ADR regulates motor coordination, learning and memory, and the sleep-wake cycle. However, little is known about the influence of ADR on calcium signal in neurons or astrocytes. In the present work, the effect of ADR on Ca^{2+} signaling in neurons and astrocytes was studied. The intracellular calcium and mitochondrial membrane potential from neurons and astrocytes were monitored after addition of different concentrations of ADR. We observed that the Ca^{2+} signal is likely to be initiated at least in part by the metabolism of ADR by monoamine oxidase, which produces reactive oxygen species and then stimulates the activation of phospholipase C. Subsequently, release of Ca^{2+} from the endoplasmic reticulum via the inositol 1,4,5-trisphosphate receptor mechanism is involved. Removal of the extracellular Ca^{2+} did not inhibit the ADR-induced Ca^{2+} signal, while pre-treatment with thapsigargin, which depletes the ER stores, did abolish the Ca^{2+} signal, suggesting that the signal is dependent on intracellular calcium stores. These findings suggest that ADR has an effect on calcium signaling in neurons and astrocytes and may contribute to understanding the physiological role of ADR in CNS.

Peptide-based regulation of brain cell plasmatic membrane receptor systems functional activity

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Neuroreceptors are the main component of cell-to-cell signaling system in the brain. As a rule, they are located on cell plasmatic membrane and activated by the specific corresponding ligands. The molecular regulation of different receptor systems' functional activity let to configure the external signal perception and to adjust the following cell answer. One of the above regulating mechanisms is based on conformational changes in receptor structure following specific binding of additional molecule called as allosteric modulator. In this study, we showed that small endogenous as well as synthetic peptides are able to modulate the functional activity of different receptor systems in the brain. Such peptides as Semax, Selank, and proglyprol (well-known novel peptide drugs with a broad range of activities in the central nervous system (Kolomin et al., Neuroscience and Medicine 4:223–252, 2013)) affect the binding of several tritium-labeled ligands to corresponding receptors (for example, GABA, Glu, Ach, dopamine, and other). The specific interactions of the peptides are complicated and in all probability not limited by the binding to own orthosteric sites. For the Semax and some of its derivative peptides, the existence of allosteric (to other neuromediators) binding sites or multiply cooperative binding sites was shown. We also hypothesized that one of the molecular mechanisms which underlies Selank anxiety effects can be associated with subtype and subunit selective dose-dependent allosteric modulation of GABA receptors. The joint action of Selank and benzodiazepines regulates the activity of GABA binding in specific manner, which is not cumulative and differs from either substance individually. It was also proposed that Semax, proglyprol, and some inherent structural components of Semax form an integrated family of bioactive peptides working simultaneously at different sites in the brain. This family was termed as "synacton." One peptide is able to regulate activity of several different neuroreceptors in unique dose-dependent nonlinear manner. At the same time, the functional activity of receptor may be regulated by several peptides. The composition of synacton is dynamically changed depending on the activity of the target cell proteolytic enzymes. All the above data shows that in a brain the endogenous biologically active peptides form the unique regulatory system based on peptide allosteric regulation of neuroreceptor system functional activity. The study was supported by the Russian Science Foundation, grant no. 16-14-00077, and the Program of the Presidium of Russian Academy of Sciences "Molecular and Cell Biology and Postgenomic Technologies."

1. Kolomin, T. et al. A New Generation of Drugs: Synthetic Peptides Based on Natural Regulatory Peptides. *Neuroscience & Medicine* **4**, 223-252 (2013).

Observation of the interaction between Glp-1 and Glp-1R by paramagnetic relaxation enhancement NMR

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The glucagon-like peptide-1 receptor (Glp-1R) is a member of the class B family of G protein–coupled receptors (GPCRs). The Glp-1R transmembrane domain (TMD) structure in complexes with allosteric modulators and the full-length Glp-1R structure in complex with a peptide agonist were solved by X-ray crystallography, as was the cryo-EM structure of active Glp-1R in complex with a G protein (Zhang et al., Nature, 2017; Jazayeri et al., Nature 546(7657):254–258, 2017; Song et al., Nature 546(7657):312–315, 2017). In spite of the availability of the aforementioned crystal structures, lack of complementary dynamics information has limited the structure-activity analyses of these important polypeptide hormone-receptor systems. Whether Glp-1 peptide can interact with the transmembrane domain of the Glp-1R is still unknown. Here we studied dynamics and the interaction between Glp-1 peptide and Glp-1R TMD by site-specific 19F paramagnetic relaxation enhancement (PRE). First, 19F-probes were introduced near the orthosteric binding site of Glp-1R by conjugation of 2,2,2-trifluoroethanethiol (TET) (Susac et al., Angew Chem Int Ed Engl 54(50):15246–15249, 2015) with cysteine-SH groups and site-directed spin labeling was used to introduce a nitroxide spin label at the 8, 31 position of Glp-1 peptide, respectively. Then, nitroxide spin-labeled Glp-1 were used to study the interactions with Glp-1R TMD by 19F-NMR experiments. Initial NMR studies suggest that 19F signals near the orthosteric binding site attenuated by spin-labeled Glp-1, while the 19F signals of the intracellular side were less affected. Additionally, nitroxide-labeled glucagon has similar effects.

1. Zhang, Y., et al., Cryo-EM structure of the activated Glp-1 receptor in complex with a G protein. Nature, 2017.
2. Jazayeri, A., et al., Crystal structure of the Glp-1 receptor bound to a peptide agonist. Nature, 2017. 546(7657): p. 254-258.
3. Song, G., et al., Human Glp-1 receptor transmembrane domain structure in complex with allosteric modulators. Nature, 2017. 546(7657): p. 312-315.
4. Susac, L., et al., In-Membrane Chemical Modification (IMCM) for Site-Specific Chromophore Labeling of GPCRs. Angew Chem Int Ed Engl, 2015. 54(50): p. 15246-9 into ion conduction by channelrhodopsin 2. *Science (80-.).* **358,** (2017).

Structural and functional study of human full-length glucagon-like peptide 1 receptor GLP-1R

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Glucagon-like peptide 1 (GLP-1) is a peptide hormone secreted from three major tissues in humans, enteroendocrine L cells in the distal intestine, α cells in the pancreas, and the central nervous system, which exerts important actions in the management of type 2 diabetes mellitus and obesity. The function of GLP-1 is mediated through interactions with the glucagon-like peptide 1 receptor (GLP-1R), a class B G protein–coupled receptor (GPCR). Class B G protein–coupled receptors are composed of an extracellular domain (ECD) and a seven-transmembrane (7TM) domain, and their signaling is regulated by peptide hormones. However, it has been very challenging to obtain a full-length class B GPCR structure at atomic resolution, probably because of the dynamic nature and lack of ligands that exist to stabilize the different domains at the same time. Recently, a complex structure of GLP-1R bound to GLP-1 and Gs protein was determined by Cryo-EM (Zhang et al., Nature, 2017) and a crystal structure of GLP-1R bound to peptide 5 was determined by X-ray (Jazayeri et al., Nature, 2017). However, because of the limited resolution, the molecular interactions between ligand and protein are not very clear. In our study, we are aiming at solving the high-resolution structure of the full-length GLP-1R and correlating the structure to function. We have developed a series of stable constructs which when combined with certain ligands generated a stable full-length GLP-1R sample that is sufficient for crystallization studies. Ultimately, we devote to determine the high-resolution (> 3.5 A) structure of full-length GLP-1R which will lead to more efficacious drugs to treat T2DM and obesity.

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1.Zhang, Y., et al., *Cryo-EM structure of the activated GLP-1 receptor in complex with a G protein.* Nature, 2017.
2.Jazayeri, A., et al., *Crystal structure of the GLP-1 receptor bound to a peptide agonist.* Nature, 2017.

Specific changes in heart and liver mitochondrial membranes under conditions of the respiratory system supercomplex formation

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In this work, by three independent methods, various types of the process of protein and lipid segregation in mitochondrial membranes were studied, which take place under the conditions of the oxidative phosphorylation system supercomplex formation at weak osmotic stress (Krasinskaya et al., FEBS Lett 167:176–180, 1984). The initial stages of protein-lipid separation were investigated using a fluorescent pyrene probe (Yaguzhinsky et al., Biophysics 62:415–420, 2017). A significant drop in the probe concentration in the vicinity of proteins has been found under hypotonic conditions. That is interpreted as the consequence of known effect of increased lipid hydration under the influence of osmotic pressure (Pfeiffer et al., Biochim Biophys Acta 1609:148–152, 2003). The second type of protein-lipid interaction weakening has been shown by the means of small-angle neutron scattering (experiments were conducted on mitochondria that retain functional activity). This method makes possible not only to determine the distance between the centers of the cristae membranes on the entire lipid-protein membrane, but also to separately investigate the lipid component of the membrane. It is shown that in mitochondria incubated simultaneously under hypoxic and hypotonic stress conditions, the protein component under the influence of osmotic pressure is partially separated from the lipid component. The difference in the thickness of the protein and lipid membrane regions is about 50 Å in heart mitochondria. The third method used was atomic force microscopy. With it, the strongest type of protein-lipid separation in mitochondrial

membranes was demonstrated. Together, this data strongly supports the assumption that under conditions of functional supercomplex formation, there is a significant separation of protein and lipid membrane components which reflects the formation of a structural supercomplex.

1.Krasinskaya, I. P., Marshansky, V. N., Dragunova, S. F. & Yaguzhinsky, L. S. Relationships of respiratory chain and ATP-synthetase in energized mitochondria. *FEBS Lett.* **167,** 176–180 (1984).
2.L S. Yaguzhinsky, Y A. Skorobogatova & S V. Nesterov. Functionally significant low-temperature structural alterations in mitochondrial membranes of homoiothermic animals. *Biophysics* **62,** 415–420 (2017).
3.Pfeiffer, H., Binder, H., Klose, G. & Heremans, K. Hydration pressure and phase transitions of phospholipids. II. Thermotropic approach. *Biochim. Biophys. Acta* **1609,** 148–152 (2003).

Two types of quinol:periplasmic e-transporter oxydoreductases

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Quinol:cytochrome c oxidoreductase was first described as one of the mitochondrial complexes (complex III) and later was found as a component of many prokaryotic redox chains both respiratory and phothosynthetic. Another name, bc_1-complex, reflects the cytochrome composition of the complex—two-heme cytochrome *b* and cytochrome c_1. The complex also contains Fe–S subunit and cytochrome *b* subunit may exist as a whole polypeptide or as two separate subunits. The complex fulfills reduction of cytochrome *c* or other periplasmic mobile electron transporters at the expense of quinol oxidation and is well studied in structural and evolutionary aspects. There is another class of cytochrome complexes that fulfill the same function. The operons of this class have homology to the operons of three-subunit membrane molybdopterin oxidoreductases and contain additional genes encoding two cytochrome *c* and two more polypeptides. Since these two classes have different origins, it seems very interesting to examine points of their emergence and subsequent evolution. For this end, dendrograms based on the amino acid sequences of the two classes of complexes are compared with total phylogeny depicted from dendrograms based on universal molecular clocks. Usually, 16s-rRNA is used as such a clock. Here, concatenated amino acid sequences of seven universal proteins not prone to lateral gene transfer are used to construct a tree for a representative set of prokaryotes. The tree contains the same bacterial and arhaeal phyla as the 16s-rRNA tree, but it defines a short period with irresolvable branching order that looks like a burst of branching. Main bacterial phyla (Proteobacteria, Actinobacteria, Bacilli, Cyanobacteria) and some minor ones contain bc_1 complexes. The structure of the clusters on the dendrogram for these complexes is congruent to the structure of the phyla on the total phylogenetic tree. But there are some mixed clusters that comprises the complexes from several bacterial phyla. The hosts of such complexes belong to the members of some orders of δ-Proteobacteria and some sparsely occupied phyla (Acidobacteria, Plactomycetes, and Verrucomicrobia). The bc_1 operons of the members of these clusters are rather various. The alternative complexes are homologous to three-subunit molybdopterin-containing complexes, one of which can be considered a precursor. In the genomes of δ-Proteobacteria, there are operons that gradually acquire genes encoding additional subunits: five-heme cytochrome c, one-heme cytochrome *c*, a duplicate of the membrane subunit, and one more membrane subunit. Full operons containing all indicated additional genes can be found in the genomes of such bacterial phyla as Bacterodetes, Acidobacteria, Chloroflexi, Plactomycetes, Verrucomicrobia, and some others.

Engineering of a monomeric thermostable LOV domain-derived fluorescent protein

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Fusing of targets with fluorescent proteins is a widely used tool in molecular biology. Nowadays, the most commonly used fluorescence reporters are GFP-like proteins (green fluorescent proteins) (Specht et al., Annu Rev Physiol 79:93–117, 2017). However, GFP-like reporters have several limitations: they cannot be applied in low-oxygen environments, need time for chromophore maturation after translation, and are of a relatively big size (MW 26.9 kDa) (Tsien, Annu Rev Biochem 67:509–544 1998; Drepper et al., Nature Biotech 25:443–445, 2007). Previously, we investigated small thermostable flavin-based fluorescent protein CagFbFP (MW 12.4 kDa) derived from a soluble LOV domain-containing histidine kinase from the thermophile *Chloroflexus aggregans* (Hanada et al., Int J Syst Evol Microbiol 45:676–681, 1995). It shows high stability in a wide range of conditions (temperature, pH, urea); however, small-angle X-ray scattering (SAXS) studies showed that it is dimeric in solution. This might affect correctness of *in vivo* studies with the protein as a fluorescent reporter. To engineer a monomeric variant, the high-resolution crystallographic structure that we obtained previously was analyzed and several mutants were prepared. The monomeric mutants were expressed, purified, and verified with using size exclusion chromatography (SEC), SAXS, and thermal shift assay.

1. Specht, E. A., Braselmann, E. & Palmer, A. E. A Critical and Comparative Review of Fluorescent Tools for Live-Cell Imaging. *Annu Rev Physiol* **79**, 93–117, (2017).
2. Tsien, R. Y. The green fluorescent protein. *Annu. Rev. Biochem*. **67**, 509–544 (1998).
3. Drepper, T. *et al*. Reporter proteins for in vivo fluorescence without oxygen. *Nature Biotech*. **25**, 443–445 (2007).
4. Hanada, S. *et al.* Chloroflexus aggregans sp. nov., a filamentous phototrophic bacterium which forms dense cell aggregates by active gliding movement. *International Journal of Systematic and Evolutionary Microbiology* **45**, 676-681 (1995).
5. Hanada, S *et al.* Chloroflexus aggregans sp. nov., a filamentous phototrophic bacterium which forms dense cell aggregates by active gliding movement. *Int. J. Syst. Bacteriol*. **45**, 676–681 (1995).

Light-driven sodium pump KR2 electrophysiological characterization

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Optogenetics as a new field of knowledge actively searches for the new tools. Nowadays, rhodopsins are the most common tools used in the field. Bacterial sodium pump found in 2013 KR2 is one of the most promising protein for suppression of neuronal action potentials. Proteins' structure determination helps to find out possible ways of active or passive ion transport through membrane (Inoue et al., Nat Com, 2013; Guschin et al., Nat Str Mol Biol, 2015; Kato et al., Nature, 2015; Guschin et al.,

FEBS J, 2016). However, more complete understanding of ion-translocation mechanisms comes from the analysis of the protein function in cells in different intra- and extracellular conditions. In this work, we have obtained the dependence of the current generated by the sodium pump on the pH in SHSY-5Y cells. Genetic construct consists of humanized KR2-gene, hemagglutinin signal peptide, FLAG-tag for antibody staining, p2A self-cleavage peptide, and red fluorescence protein as a marker of expression. We conducted flowcytometry with SHSY-5Y cells transfected with KR2 and stained with FITC-antiFLAG antibody. It showed that KR2 is not fully expressed in plasmatic membrane. Thus, the genetic construct is to be improved to reach better protein trafficking to the cytoplasmic membrane. However, construct allowed to compare the protein pumping efficiency at different pH. In the sodium-containing pipette and bath solutions, the current value grows from 229.1 pA/nF at pH 6 to 1159.5 pA/nF at pH 7.4. Further pH growth causes falling of the current to 318.5 pA/nF at pH 9.

1. Inoue et al., A light-driven sodium ion pump in marine bacteria, Nat.Com., 2013
2. Guschin, Shevchenko, Polovinkin et al., Crystal structure of a light-driven sodium pump, Nat.Str.Mol.Biol., 2015
3. Kato et al., Structural basis for Na^+ transport mechanism by a light-driven Na^+ pump, Nature, 2015
4. Guschin, Shevchenko, Polovinkin et al., Structure of the light-driven sodium pump KR2 and its implications for optogenetics, FEBS J, 2016

The development of selective inhibitors of phosphotyrosine phosphatase 1B on the basis of 4-oxo-1,4-dihydrocinnoline structure and their application to treat diet-induced obesity

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One of the key causes of obesity and metabolic syndrome is a decrease in the activity of the hypothalamic leptin and insulin signaling pathways, which leads to a weakening of the central regulation of food intake and energy homeostasis. The reduced activity of their pathways may be due to the increased activity of protein phosphotyrosine phosphatase 1B (PTP1B), their negative regulator. The inhibition of PTP1B is one of the approaches to normalize the leptin and insulin signaling and to treat obesity-associated metabolic disorders. The aims of the work were to design and synthesize the selective PTP1B inhibitors on the basis of 4-oxo-1,4-dihydrocinnoline and to study their activity in the *in vitro* and their effect on the metabolic parameters and the hypothalamic signaling in rats with diet-induced obesity (DIO). Based on the three-dimensional structure of the catalytic and allosteric sites of human and rat PTP1B, the suitable structures of 4-oxo-1,4-dihydrocinnoline derivatives were selected and the corresponding compounds were synthesized. The ethyl-3-(hydroxymethyl)-4-oxo-1,4-dihydrocinnoline-6-carboxylate (PI4), ethyl-3-(hydroxymethyl)-4-dioxo-1,4-dihydro-1λ4-cinnoline-6-carboxylate (PI5), ethyl-1-benzyl-3-(hydroxymethyl)-4-oxo-1,4-dihydrocinnoline-6-carboxylate (PI6), and 1-benzyl-6-(ethoxycarbonyl)-4-oxo-1,4-dihydrocinnoline-3-carboxylate (PI7) had the specific PTP1B-inhibiting activity, but only PI6 was highly specific for PTP1B and did not affect the activity of T cell protein tyrosine phosphatase, the PTP1B-related enzyme. The treatment of rat hypothalamic neurons with PI6 induced the increase in the Thr^{308}-phosphorylation of Akt-kinase and the Tyr^{705}-phosphorylation of the transcriptional factor STAT3, the main targets for insulin and leptin, which indicates the activation of the insulin and leptin signaling in them. A single administration of PI6 (10 mg/kg, i.p.) to DIO rats led to an increase of the phosphorylated forms of Akt-kinase and STAT3 in the hypothalamus of obese animals. The long-term treatment of DIO rats with PI6 (10 days, 10 mg/kg/day) induced a decrease in the food intake and body weight and improved the glucose and insulin sensitivity and lipid profile. Thus, the PI6, a newly designed selective inhibitor of PTP1B, suppresses PTP1B activity and activates the insulin and leptin signaling pathways in the hypothalamus, normalizing the insulin- and leptin-mediated regulation of eating behavior and peripheral homeostasis in DIO, which allows us to consider PI6 a prototype for creating drugs to treat obesity and metabolic syndrome.

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Putative role of transmembrane domain dimerization in activation of proteins from the insulin receptor family

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Insulin receptor subfamily consists of insulin receptor (IR), insulin-like growth factor 1 receptor (IGF-1R), and insulin receptor-related receptor (IRR). The first two participate in cell metabolism, growth, and survival. Their dysfunction leads to pathological states associated with these processes. IRR acts as alkali sensor and takes part in the acid-base balance regulation. This subfamily belongs to receptor tyrosine kinases (RTKs), whose mechanism of activation usually requires dimerization involving their transmembrane (TM) segments. However, structural and dynamic aspects of the latter are still unclear because the related experimental or theoretical structural data are missing. The goal of this work was to construct molecular models of the IR subfamily TM dimers and propose their activation mechanism based on computational results. Molecular dynamics (MD) simulations of IR extra- and intracellular domains revealed that TM segments of the receptor can be considered rather autonomous upon dimerization in lipid membrane. Due to high degree of sequence homology, we propose the same result for IGF-1R and IRR, and further consider TM dimerization in lipid membrane without extra- or intracellular segments. Coarse-grained (CG) MD simulations of monomeric TM helices in lipid bilayer show high probability of their spontaneous dimerization. Another set of dimeric states was obtained using molecular hydrophobicity potential (MHP) surface complementary approach (PredDimer tool) (Polyansky et al., Bioinformatics 30(6):889–890, 2014). We studied models that were stable during MD calculations in hydrated lipid membrane. Association potential of mean force of stable models shows strong dimerization tendency of structures that have insignificant structural difference between IR and IGF-1R and with models after dimerization in CG. This structural resemblance of IR and IGF-1R TM dimer models can reflect similar activation mechanism. Based on the totality of the computational data, we assume that the models of TM dimers obtained for these two receptors represent their active states. The work was supported by the Russian Science Foundation (grant 18-14-00375) and the Russian Foundation for Basic Research (grant 18-54-15007)

1. Polyansky A.A. *et al.* PREDDIMER: a web server for prediction of transmembrane helical dimers. **Bioinformatics**. 30(6):889-90, (2014).

Activation of mammal prion protein specific aggregation by glycated β-casein

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Mammal prion protein (PrP^C) is localized on the outer side of the cell plasmatic membrane and plays an important role in the development of neurodegenerative diseases of amyloid nature. Prion protein is able to undergo an amyloid conversion spontaneously or under the influence of external factors, and, consequently, form very stable aggregates. The amyloidogenic molecule PrP^{Sc} is able to provoke similar amyloid changes in other proteins by direct interaction with them. Initially, the prion was believed to transmit an information about its secondary and tertiary structure only to molecules of the same protein, but now the information pool about cross-aggregation between PrP^{Sc} and other proteins has increased significantly. In particular, the interaction between the membrane-bound prion protein and the beta-amyloid peptide was suggested to be involved in the development of Alzheimer's disease. In our work, we investigated the role of protein glycation in the regulation of their interaction with prion protein, using beta-casein as the object for glycation. This food milk protein was shown to demonstrate chaperone-like activity; e.g., it is able to inhibit amyloidization and aggregation of PrP. However, after the beta-casein glycation with glucose and other compounds, its chaperone-like properties disappear. The glycated beta casein begins to aggregate and interacts specifically with the fluorescent dye on amyloid thioflavin T, thus forming ordered fluorescent spiral aggregates. The addition of glycated casein to prion protein leads to activation of the various ordered structures formation. Moreover, in the presence of thioflavin T, specific structures of stellate forms, consisting of spiral elements, are formed. Based on these observations, we concluded that prion protein works as a matrix for assembling ordered aggregates of glycated beta casein and, probably, other glycated proteins. The obtained data evidence the possibility of changes in the PrP and partner proteins interaction after glycation. It is also important to note that the increase of thioflavin T fluorescence due to interaction with glycated proteins must be taken into account for the case of using this compound as an indicator of amyloid structures formation.

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Sequence-based analysis of sodium binding pocket in GPCRs

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Sodium binding pocket plays an essential role in signalling and activation processes of GPCR; however, the detailed mechanism remains poorly understood. Sodium ion was observed in several recently solved atomic structures of GPCRs in inactive state, where it is coordinated by highly conserved motif of amino acid residues (Katritch et al., Trends Biochem Sci 39.5:233–244, 2014). Mutation of these residues might significantly change receptor behavior. In particular, D2.50N, S3.39A, and D7.49N point mutations were used to stabilize receptor conformation and facilitate its structure determination (Popov et al., eLife 7 e34729, 2018). Here, we analyzed sodium binding pockets in class A GPCRs using sequence-based methods. Firstly, we composed and curated multiple sequence alignments of α-, β-, γ-, and δ-branches of class A GPCRs (Fredriksson et al., Mol Pharmacol 63.6:1256–1272, 2003). We performed covariation analysis of sodium binding pocket residues and identified that 6.48–7.49 is one of the most correlated residue pairs, with 6.48W–7.49N and 6.48F–7.49D amino acid pairs observed in 63% and 14% of class A GPCRs, respectively. Interestingly, most of the receptors with 6.48F–7.49D amino acid pairs belong to the δ-branch of class A GPCRs, meaning that these receptors possess different sodium coordination pattern. By analyzing sequence profiles obtained with the Hidden Markov models, we observed GPCR groups with canonical sodium binding pocket in α- and γ-branches, while many of GPCRs from β- and δ-branches have altered sodium binding pockets. Furthermore, each branch contains receptors with significantly different amino acid motifs, compared to the canonical sodium binding pocket, suggesting that there are GPCRs with different and still unknown activation mechanisms.

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1. Katritch, Vsevolod, et al. "Allosteric sodium in class A GPCR signaling." *Trends in biochemical sciences* 39.5 (2014): 233-244.
2. Popov P., et al., "Computational design of thermostabilizing point mutations for G protein-coupled receptors." *eLife* 7 (2018): e34729.
3. Fredriksson, Robert, et al. "The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints." *Molecular pharmacology* 63.6 (2003): 1256-1272.

Matrices of on-chip Pt/TaOx/Ta resistive switching memory devices for future bioelectronic applications

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Future implantable neurointerface devices will require substantial computational and memory budget available in a very strict power envelope. Such capabilities are very difficult to provide using conventional memory architectures due to very high energy cost of memory interfaces. This problem can be circumvented by tightly coupling memory and computation, but this approach poses a difficulty—memories, which can be placed close to computation devices (e.g., static RAMs) which are typically of very low density. Fortunately, a set of emerging memory technologies can overcome this difficulty by providing very low power nanoscale storage cells, which can be integrated directly into computing electronics, paving the way to perform processing in memory. One of the most promising technologies is the non-volatile memory based on the reversible resistive switching (RS) phenomena in a thin film of functional material upon application of external electric field (voltage). Among different transition metal oxides serving a functional layer in RS devices, TaOx is one of the favorites (Lee et al., Nat Mater 10:625, 2011; Wedig et al., Nat Nanotech 11:67, 2016). In this work, sub-micrometer size cross-bar Pt/TaOx/Ta resistive switching (RS) devices were produced by magnetron sputtering technique at room temperature. The elemental and chemical composition of few-nanometer-thick functional TaOx (x~3) layer is revealed by the combination of Rutherford backscattering spectrometry and hard x-ray photoemission spectroscopy techniques. The proposed resistivity switching mechanism is based on the electrical drift of over-stoichiometric (wrt. Ta_2O_5) O ions. Such sub-micrometer size RS devices were integrated with 1000 × 1000 matrices of 90-nm CMOS transistors to ensure accurate control of the so-called compliance current across 1T-1R devices. For the optimal thickness, 6 nm for TaO_x layer 1T-1R devices in the matrix exhibit robust "forming-free" RS, with more than 10^{10} cycles under pulsed switching ($V_{on}/V_{off} = 2.2/-2.2$ V, 1 μs). The obtained matrices of 1T-1R devices will serve the basis for embedded non-volatile memory useful for future neurointerfaces under development. This work was carried out with support from and as part of the programme of the Centre for AI Science and Technology at the Moscow Institute of Physics and Technology.

1. M.-J. Lee, et al., A fast, high-endurance and scalable non-volatile memory device made from asymmetric Ta2O5−x/TaO2−x bilayer structures. Nat. Mater. 10 625 (2011).
2. Anja Wedig et al., Nanoscale cation motion in TaOx, HfOx and TiOx memristive systems. Nat. Nanotech. 11 67 (2016).

Membrane-derived virus-like particles as a transport vehicle for mRNA molecules

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Viruses utilize lipid bilayer of the eukaryotic cell membranes to envelope themselves and prepare for spreading among the surrounding cells. This phenomenon has been used by bioengineers for the development of transport vehicles (Magneot et al., Mol Therapy 19:1656–1666, 2011). These vehicles, termed virus-like particles (VLPs), resemble viruses, however lack viral genetic material. In our work, we created membrane-derived VLPs by the mean of fusing VSV-G protein of a zoonotic virus (Vesicular stomatitis virus) with the RNA-binding archaeal L7Ae protein of *Archeoglobus fulgidu*s. These VLPs demonstrated highly efficient delivery of mRNA molecules of EGFP to the recipient cells, including hard-to-transfect cell lines such as induced pluripotent stem cells (iPS cells) (Takahashi and Yamanaka, Cell 126:663–676, 2006) and monocytes. This VSVG-L7Ae VLP system is non-specific and easy to engineer in the laboratory. We hope that this transport tool can be of an interest for the researchers who work with mRNA delivery to various cell lines.

1. Magneot et al., Protein Transfer into Human Cells by VSV-G-induced Nanovesicles. *Mol.Therapy* **19**, 1656–1666 (2011).
2. Takahashi, K. & Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* **126**, 663–676 (2006).

Intranasal insulin administration normalizes the gene expression of antioxidant enzymes in the two-vessel ischemia and reperfusion in the rat brain

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The experimental and clinical studies of insulin influence on the brain functions suggest that insulin is a promising drug to treat the neurodegenerative and neurological diseases. The brain ischemia and ischemia-induced oxidative stress are the important components of the pathogenesis of these diseases. However, the protective effect of insulin on the brain ischemia and reperfusion has not yet been investigated. The aim of this work was to study the effect of intranasally administered insulin (I-I) on the expression of genes encoding the key antioxidant enzymes, such as catalase and superoxide dismutases of the types 1 and 2, in the cerebral cortex of rats in the conditions of the brain ischemia and reperfusion (IR). Two-vessel ischemia of the forebrain in four-month Wistar rats was caused by occlusion of the carotid arteries for 20 min in the combination with hypotension (Zorina et al., J Evol Biochem Physiol 54:246–249, 2018). After the blood flow recovery, the reperfusion of the brain was maintained within 1 h. The I-I (0.5 IU/rat) was administered to rats 1 h before the occlusion. The study of gene expression was performed using qRT-PCR, and the expression of the target genes (*cat*, *sod1*, *sod2*) was calculated by the $\Delta\Delta$CT method, using the 18S rRNA as a standard. In the cerebral cortex of rats with IR, the expression of the *cat*, *sod1*, and *sod2* genes was decreased by 52.4, 28.4, and 15.8%, respectively ($P < 0.05$), as compared with control (the sham-operated rats). The treatment of IR rats with I-I led to the normalization of the expression of these genes. It should be noted that the treatment of sham-operated animals with I-I did not affect significantly the expression of *cat*, *sod1*, and *sod2* genes in the cerebral cortex. Earlier, we showed that I-I reduced the development of brain lipid peroxidation in the conditions of IR (Zorina et al., J Evol Biochem Physiol 54:246–249, 2018). Based on the data obtained in the present study, it can be concluded that I-I prevents the lipid damage due to the improvement of the antioxidant enzymes production. The work is supported by the RFBR (no. 18-315-00285) and FASO (AAAA-A18-118012290427-7).

1. Zorina, I.I., Zakharova, I.O., Bayunova, L.V. & Avrova, N.F. Insulin Administration Prevents Accumulation of Conjugated Dienes and Trienes and Inactivation of Na , K -ATPase in the Rat Cerebral Cortex during Two-Vessel Forebrain Ischemia and Reperfusion. *J. Evol. Biochem. Physiol.* **54,** 246–249 (2018).

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