Application of nanostructures in aptamer based biosensors

Tibor Hianik[1], Gabriela Castillo[1], Maja Šnejdarková[2], Alexandra Poturnayová[2], Anna Porfireva[3], Gennady Evtugyn[3], Anna Miodek[4], Helene Dorizon[4], Hafsa Korri-Youssoufi[4], Andreas Ebner[5]

[1]Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynská dolina F1, 842 48 Bratislava, Slovakia, [2]Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji, Slovakia, [3]Analytical Chemistry Department, Kazan Federal University, 18 Kremlevskaya Street, Kazan, 42008, Russian Federation, [4]Institut de Chimie Moléculaire et de Matériaux d'Orsay, Université Paris-Sud, Bâtiment 420, 91405 Orsay, France, [5]Biophysics Institute, Johannes Kepler University Linz, Altenbergertrasse 69, 4040 Linz, Austria
tibor.hianik@fmph.uniba.sk

DNA and RNA aptamers are single stranded oligonucleotides with high affinity to proteins or other ligands that are similar to those of antibodies. The aptamers are selected *in vitro* by the SELEX method [1]. In solution, aptamers maintain an unique 3D configuration that contains specific binding site to the ligand. Aptamers can be easily modified by biotin, SH or amino- groups, leading to a variety of immobilization strategies on solid supports. Using simple molecular engineering based on DNA hybridization it is possible to develop aptamer dimers containing two binding sites like antibodies [2,3]. These aptamer dimers (aptabodies) are characterized by enhancing sensitivity to the analyte, for example to thrombin or cellular prions. We have shown that typical guanine quadruplexes that form binding site for thrombin are stable in aptamer dimers [4]. Currently there is increased interest in development of aptamer based biosensors (aptasensors) for detection of proteins and other molecules using various sensing methods, such us optical, acoustical and electrochemical [5,6]. Aptasensors could be used for fast and low cost medical diagnostics. The sensitivity of detection depends not only on the selectivity of binding site, but also on the supporting part added to the aptamer that serves for better immobilisation onto a solid support. Nanostructures such as carbon and ZnO nanotubes, graphenes, molecularly imprinted polymers, and that modified by calixarenes and dendrimers are of great advantage in aptamer immobilisation and also improve detection of ligands especially in combination with electrochemical methods.

In this contribution we report various immobilisation and detection strategies of proteins using nanostructured aptasensors. By means of multiwalled carbon nanotubes (MWCNTs) as an immobilization matrix we developed high sensitive biosensor for detection of human thrombin [2] and cellular prions (PrP^C) [7] in biological liquids. We have shown that immobilisation of aptamers and aptamer dimers at MWCNTs improved the sensitivity of the sensor for thrombin and allowed detection in a complex matrix such as blood plasma. By means of electrochemical quartz crystal microbalance method (EQCM) we performed comparative analysis of the sensitivity of DNA aptamers and antibodies specific to PrP^C immobilised on a surface of MWCNTs. We found that the limit of detection (LOD) for both aptamers (50 pM) and antibodies (20 pM) was comparable. Most recently we substantially improved the LOD using immobilisation of aptamers onto multilayer surface composed of MWCNTs with covalently attached polyamidoamine dendrimers (PAMAM) of fourth generation (G4) conjugated with ferrocene-1'-(N(3-butylpyrrole)butanamide) (Fe-NHP). Streptavidin-biotin conjugation served as linker with biotin-modified aptamer designed for specific prion recognition (Fig. 1a). Using cyclic voltammetry (CV) it has been possible to record reversible redox currents of the ferrocene with oxidation and reduction peaks corresponding to the potentials 0.24 mV and 0.17 mV (vs. Ag/AgCl reference electrode), respectively. The current decreased with increasing PrP^C concentrations form 1 pM to 10 μM and reaches saturation after 1 μM {Fig. 1b). The current decay was due to limitation of the electron exchange in the sensing layer. LOD was found to be 1.3 pM which is acceptable for practical applications. The sensor was tested also in a human blood serum with satisfactory recovery in average of 74 %. The interferences with BSA up to concentrations 10 μM were negligible.

Recently we developed new approach for aptamer immobilisation using electropolymerized layer of Neutral Red (NR) at glassy carbon electrode (GCE) onto which polycarboxylated thiacalix[4]arene has been adsorbed by electrostatic accumulation. NR and aminoterminated thrombin-specific aptamer were then covalently linked to the thiacalixarenes by EDC-NHS chemistry (Fig. 2) [8]. The NR reduction current recorded after 10 min incubation decayed with increased thrombin concentration due to limitation of the electron exchange in the surface layer. The aptasensor makes it possible to determine thrombin in concentration range 0.1–50 nM (LOD 0.05 nM) in blood serum without any alteration of the response in the presence of 100 fold excess of serum proteins.

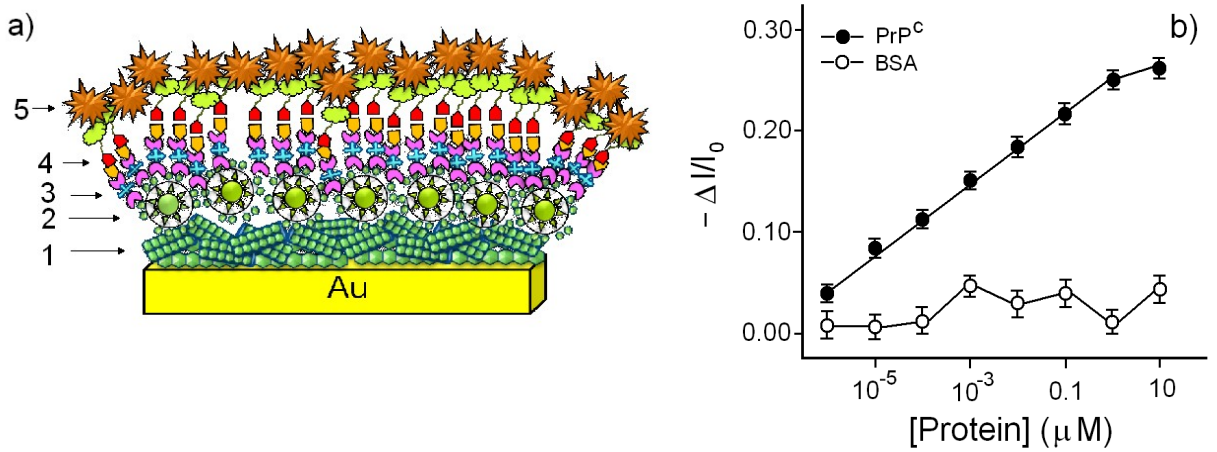

Fig. 1 a) The scheme of the biosensor based on MWCNTs-dendrimer- ferrocene-streptavidin layer with immobilised aptamers sensitive to PrP^C (1-MWCNT, 2-Dendrimer, 3-Fe-NHP, 4-Biotinylated aptamer connected to streptavidin, 5-PrP^C). b) Relative changes of the current peak corresponding to the ferrocene oxidation vs. concentration of PrP^C or bovine serum albumin (BSA), respectively ($\Delta I=I-I_0$, where I_0 and I are amplitudes of the current prior and after addition of the analyte, respectively).

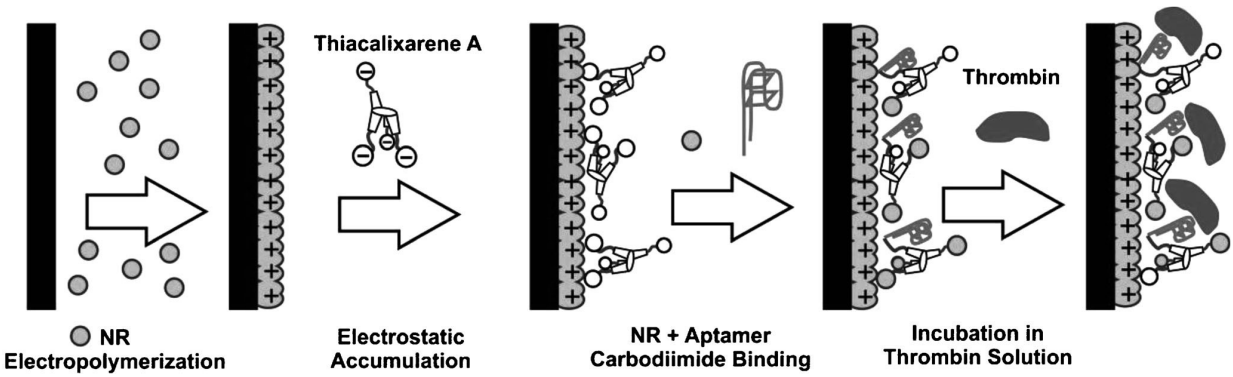

Fig. 2. General scheme of the aptasensor assembling for detection thrombin at glassy carbon electrode. Neutral Red (NR) is the electroactive probe [8].

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