chemical shift perturbations at several residues, suggesting potential binding. We will discuss these results and their implications for future efforts in K-Ras drug discovery.

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Database of Ca Protein-Ligand Binding Gibbs Energies, Enthalpies, Entropies, Volumes, and Crystal Structures

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We have designed, synthesized and determined the binding thermodynamics of over 700 aromatic sulfonamides to the family of 12 human carbonic anhydrase (CA) isoforms. The proteins were cloned and expressed in bacterial and human cell cultures and affinity-purified in large quantity sufficient for ITC and crystallography. The binding affinities were determined by the thermal shift assay (FTSA, also termed ThermoFluor or differential scanning fluorimetry, DSF), a high-throughput method. The enthalpies and entropies of binding were determined by ITC, a medium throughput method, for a selection of compounds and CA isoforms. A correlation map between the compound chemical structure and the binding ΔG and ΔH was drawn. The map showed which structural features of the compounds generated the highest increments in exergonicity and exothermicity of compound binding. Furthermore, only some structural features were most useful in generating compounds that would selectively bind to cancer-expressing CA isoforms, but would not bind to essential for life human CA isoforms. Volumes and compressibilities of binding were determined by PressureFluor and densitometry for several compounds. Over 60 X-ray crystal structures showed the position of compounds bound in the enzyme active center. ITC was essential technique that enabled the dissection of unknown contributions from linked reactions such as buffer protonation to the binding reaction. Only after the subtraction of pH-dependent buffer contribution to the enthalpy of binding, the *intrinsic* Gibbs energies and enthalpies of binding were obtained. All methods that determine the binding reaction, such as FTSA, ITC, SPR, thermophoresis, and enzymatic inhibition methods would provide only the *observed* thermodynamics of binding that is pH and buffer-dependent. It was important to calculate the true (intrinsic) parameters and use them in the structure-thermodynamics correlation maps.

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Comparison of the RGD- and AGDV-Containing Peptide Interactions with the Platelet Integrin Alphaiibbeta3

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The C-terminal AGDV-containing dodecapeptide (γC-12) sequence of the fibrinogen γ-chain mediates binding of soluble fibrinogen to the activated integrin αIIbβ3, leading to platelet aggregation. However, the RGD-containing peptide sequences located in two places in the fibrinogen Aα-chain inhibit this process and contribute to αIIbβ3 binding when fibrinogen is immobilized and when it is converted to fibrin. Although the interaction of αIIbβ3 with various RGD-containing and γC-12 peptides has been studied extensively, there is still not a comprehensive characterization of their binding to the active open and inactive closed conformations of αIIbβ3. Here, we combined experimental and computational approaches to compare kinetics, thermodynamics and structural details of cycloRGDFK (cRGDFK) and γC-12 binding to αIIbβ3. Using an optical trap-based single-molecule technique, we measured the probability of peptide binding to αIIbβ3 as a function of αIIbβ3-peptide interaction time and extracted first-order binding/unbinding rates and binding affinity constants (K_b), which indicate that cRGDFK binds to αIIbβ3 tighter than γC-12 (K_b=0.46×10^{-14}cm^2 and K_b=0.3×10^{-14}cm^2, respectively). Next, we performed docking modeling and implicit solvent MD simulations on GPUs using the NMR-determined solution structures of the peptides, and crystallographically resolved structures of the αIIbβ3 conformers. The results showed that cRGDFK and γC-12 bound to the αIIbβ3 at the physiological binding site with similar strength for the open and closed αIIbβ3 conformations. The Gibbs free energy (ΔG) of the αIIbβ3-peptide complexes, calculated using Umbrella Sampling technique, indicated that the overall thermodynamic stability of the complex is higher for cRGDFK than for γC-12 (ΔG=16.1 kcal/mol and ΔG=13.5 kcal/mol, respectively). These results account for our previous observations that the affinity of RGD for αIIbβ3 is greater than AGDV and support our hypothesis that the RGD motifs promote the interaction of αIIbβ3 with immobilized fibrinogen and fibrin.

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Brushed Polyethylene Glycol and Phosphorylcholine as Promising Grafting Agents against Protein Binding

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The grafting of linear polyethylene glycol (PEG) is a common strategy in ascribing the stealth effect to nanostucture against protein adsorption for biomedical applications. However, PEG may still evoke side effects *in vivo* that compromises the circulation and efficacy of PEGylated drugs or nanocarrier. Recently, we demonstrated the synthesis of brushed phosphorylcholine (PC) as biomimetic alternative to linear PEG for the grafting of superparamagnetic iron oxide nanoparticles (IONP). Compared to brushed PEG (bPEG), brushed PC (bPC) rendered even better suspendability, stability, biocompatibility and cellular distribution. To further examine the structures of bPEG and bPC and directly test their antifouling properties against proteins, discrete molecular dynamics (DMD) simulations were performed. We found that brushed polymers were more rigid than the linear PEG while bPEG and bPC ligands displayed distinct globular and cylindrical morphologies. Grafting either bPEG or bPC onto IONPs led to different characteristics of the grafting layer, namely ligand coverage, height, and conformational strains both laterally and vertically. Upon mixing both bPEG and bPC onto the same IONP surface while maintaining the same grafting density, a conformational relaxation of the bPEG was observed in DMD simulations, as corroborated by fluorescence quenching of Cy5 attached to bPEGs in the experiment. Both bPEG- and bPC-grafted NPs displayed antifouling against human serum albumin (HSA), with an increased grafting density giving rise to enhanced protein avoidance. Our results suggest that, with a stronger repulsion to HSA and the capability to a higher grafting density due to its cylindrical shape, bPC is more advantage than both linear and brushed PEG for grafting NPs with minimal protein binding. These new structural and energetic insights offer a general guidance for NP synthesis and anti-fouling applications employing branched polymers.

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Insights into a Low Promiscuous Aminoglycoside Modifying Enzyme, Aminoglycoside *N3* Acetyltransferase-VIa

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Aminoglycoside modifying enzymes (AGMEs) are plasmid-encoded enzymes found in resistant bacteria. AGMEs covalently modify their substrates and thus render the drug ineffective for the bacteria. Aminoglycoside (AG) antibiotics are bactericidal agents used to treat various bacterial diseases like tuberculosis and meningitis. However, the clinical effectiveness of these drugs has been drastically affected by the emergence of AGMEs. More than 50 different AGMEs are known, having variable levels of substrate promiscuity. However, no correlation has been confirmatively observed between the sequence or structure of an AGME and its substrate profile. We aim to understand the molecular principles underlying this ligand selectivity by deciphering the thermodynamic, structural and dynamic properties of enzyme-ligand complexes. Kinetic, thermodynamic and structural properties of the aminoglycoside *N3* acetyltransferase VIa (AAC-VIa) are described. Despite having significant sequence similarity to highly promiscuous acetyltransferase, AAC-VIa can modify only 5 aminoglycosides, with a ~4-fold difference in the k_{cat} values. Thermodynamic studies determined the binding of ligands to be enthalpically driven and entropically unfavorable. Unlike other AGMEs, the formation of binary and ternary complexes was accompanied by a net deprotonation of the enzyme, ligand or both. Also, the values for heat capacity change (ΔCp) were within the range of protein-carbohydrate interactions. Another significant difference was observed in the structure of AAC-VIa and other AGMEs in solution. Analytical ultracentrifugation (AUC) studies showed that AAC-VIa exists in a monomer-dimer equilibrium, with more dimeric form appearing with increasing concentrations of the enzyme. Binding of ligands drive the enzyme to a more monomeric form. Also, dimer formation is achieved mainly through polar interactions. Crystal structures of different complexes of the enzyme showed