when studying protein binding of small-molecule ligands taken from compound libraries dissolved in organic solvents, as is usually the case during screening or drug development.
Here we introduce a new ITC competition assay that overcomes this limitation, thus allowing for a precise thermodynamic description of high- and low-affinity protein-ligand interactions involving poorly water-soluble compounds. We discuss the theoretical background of the approach and demonstrate some practical applications using examples of both high-affinity ($K_D < 1$ nM) and low-affinity ($K_D > 100$ μM) protein-ligand interactions.
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1253-Pos
Binding of A Natural Sterol to the Osh4 Protein of Yeast and Membrane Attachment
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Osh4 is an oxysterol binding protein homologue found in yeast that is essential for the intracellular transport of sterols and for cell life. It has been proposed that Osh4 acts as a lipid transport protein, capable of carrying sterols from the endoplasmic reticulum to the plasma membrane (PM).
Molecular dynamics (MD) simulations were used to analyze the binding of ergosterol to the Osh4 protein on an atomic level. During the course of 25-ns simulations, the sterol molecule remained tightly bound to the binding pocket of Osh4. These simulations revealed ergosterol binding was aided by both water-mediated interactions between the 3-hydroxyl (3-OH) group of ergosterol and surrounding polar residues as well as direct hydrogen binding between ergosterol and the Trp^{46} and Gln^{96} residues. Analysis of the interaction energy between ergosterol and Gln^{96} shows distinctly different states (−9.3 kcal/mol, −7.3 kcal/mol, and −4 kcal/mol), where the highest energy state was encountered 77% of the time.
In order to study how the Osh4 protein attaches to the PM, possible lipid binding sites were investigated through the use of a docking program as well as MD simulations. A model compound consisting of a phosphocholine lipid head group truncated at the C2 carbon solvated the Osh4 protein in conjunction with water. Protein/lipid interactions were observed and used to determine the proper orientation and placement of the protein with respect to a model membrane. We also developed a model yeast membrane containing ergosterol using CHARMM-GUI, and key membrane properties were investigated using data collected from a 60-ns MD simulation (areas per lipid, density profiles, and lipid dynamics). Ultimately, understanding how Osh4 attaches to the PM will lead to a clear understanding on how this protein transports sterols *in vivo*.

1254-Pos
Splitting of the Allosteric Function in Human Hemoglobin with an Altered Alpha1Beta1 Interface
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Previously, it was shown that human semihemoglobins, i.e., hemoglobin dimers of the form (alpha)(beta) in which only one subunit, either alpha or beta, contained a functional heme group while the complementary apo subunit lacked heme, were sensitive to allosteric effectors, which caused modulation of their affinity for oxygen (Tsuneshige, A. et al. (2004) J. Biol. Chem. 279, 48959-48967). The presented evidences contradicted the classic tenet of the ''two-state'' model of allostery in which modulation of the affinity for oxygen could only be achieved by a tetrameric hemoglobin adopting the high-affinity ''R'' or the low-affinity ''T'' conformations in a reversible fashion.
For the present study, we have prepared a hemoglobin molecule in which the residues alpha104Cys and beta112Cys were chemically modified following a reaction of the sulhydryl groups with a thiopyridyl reagent. These two residues are present in the alpha1beta1 interface, thus we expected that their chemical modifications would impair drastically the intradimeric communication. Surprisingly, oxygenation curves at different pH values, or in the presence of the allosteric efector inositol hexakisphosphate (IHP) showed striking common characteristics: symmetric shape, presence of cooperative binding of oxygen, and a corresponding decrease in overall oxygen affinity in response to acidic conditions or the presence of IHP. Moreover, affinities for oxygen at low and high saturation levels were both affected in similar fashion under any solution condition. These results strongly suggest that the modified hemoglobin behave like dimers exhibiting allosteric properties.

1255-Pos
Contrasting Effects of Halides on the Structure and Function of A Multimeric Allosteric Protein
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We have used two halide salts, namely, sodium chloride and sodium iodide, and studied their impact on the oxygenation characteristics of adult human hemoglobin (Hb). Previous studies in our group showed that both the halide salts exerted similar effects on the Hb function at concentrations below 0.1 M, i.e., an overall decrease in the affinity for oxygen, as a result of a decrease in the affinity at low oxygenation levels. However, as the halide concentrations increased, while chloride continued producing a progressive but rather diminished effect, iodide reverted its effects on Hb: the overall affinity for oxygen rather increased. Careful analysis of the oxygenation curves revealed that while the affinity for oxygen decreased at high oxygen saturation levels, the affinity at low oxygen concentration increased markedly. These effects reached a plateau at a concentration of 2 M, but even more surprisingly, cooperativity was never canceled. The results hinted at the possibility that iodide ions were splitting the tetrameric Hb molecules into asymmetric dimers. Dimers have been and still are considered non-cooperative, high oxygen-affinity systems. Yet, our present data clearly contrast with the previous tenet since cooperativity index showed values as high as 1.6 in the presence of 2 M NaI. Determination of molecular weight by size exclusion chromatography, and the study of oxygenation characteristics of symmetric nickel-iron Hb hybrids in the presence of sodium iodide showed that in fact the tetrameric Hb splits into two dimers that, strikingly, remain allosterically functional.

1256-Pos
Exploring the Conformational Space for the Interactions of Aromatic Residue Analogs with Biologically Important Saccharides
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Proteins interacting with carbohydrate ligands are getting a great deal of attention because of its important role in various biological processes. The crystal structures of several lectin-saccharide complexes have shown the presence of an aromatic residue in the binding site. The C-H hydrophobic patch of saccharide ''stacks'' against pi-cloud of aromatic residues forming CH-pi interactions, which are governed by dispersive and charge transfer interactions. The energetics of saccharide - aromatic residue interactions are dictated by their mutual position-orientations. It is conceivable that there exist low-energy position-orientations other than those found in the limited number of crystal structures of protein-carbohydrate complexes known to date. Hence, we have explored the conformational space for the interactions of 3-methylindole (3-MeIn), *p*-hydroxytoluene (p-OHTol) and toluene (Tol) (analogs of tryptophan, tyrosine and phenylalanine, respectively) with six saccharides. A Monte Carlo conformational search method was used to explore the features of the molecular potential energy surfaces. We found that the saccharides are densely populated above and below the pi-cloud of the aromatic ring of the amino acid residue but not along the edges. Clustering of conformers indicate the aromatic residues are spread out when interacting with C-H atoms as compared to that with -OH groups. The saccharides were capable of sliding on the surface of the aromatic residue. Four C-H groups can simultaneously participate in CH-pi interaction in 3-MeIn systems owing to its larger surface area. The β-D-Galactose and β-L-Fucose have been found to interact only through their b- and a-faces, respectively. Our ability to understand molecular flexibility through conformational search will further lead to advances in the design of drugs and to understand the advantages of selective choice of aromatic residues viz., tryptophan, tyrosine or phenylalanine in different carbohydrate binding proteins.

1257-Pos
From SPRI (Surface Plasmon Resonance Imaging) Affinity Capture Analysis Up to On-Chip MALDI-MS/MS Analyte Identification
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Multiplex format SPRi analysis allows direct visualization and thermodynamic analysis of biomolecular interactions, and is advantageously used for ligand-fishing of captured bio-molecules on multiple immobilized receptors. Mass spectrometry is a powerful tool for structural characterization and identification. Therefore, the combination of SPRi and MS into one concerted procedure is of a great interest for functional and structural analysis in the fields of proteomics, drug-discovery or diagnostic. We have implemented an on-chip MALDI analysis in which affinity captured bio-molecules are directly detected from the SPR-sensor surface.
The model presented was based on antigens-antibodies interactions. Antibodies, Anti-Beta-lactoglobulin, Anti-ovalbumin and a reference, were arrayed on biochip functionalized with a patented SAM-NHS surface chemistry and the SPR experiments were performed using a MS buffer. A mixture of label free antigens, Beta-lactoglobulin and ovalbumin, was injected and analyte capture was followed in real time. Following the collection of kinetic constants (Kon and Koff) information, the biochip was removed and each spot was submitted