Several studies suggest that tau in AD brains may exhibit abnormal interactions with the neuronal cell membrane. We hypothesize that the lipid membrane can mediate tau pathology by templating tau to misfold into an assembly-competent conformation and subsequently nucleating tau to aggregate into fibrils. We used lipid monolayers at the air/water interface as a model membrane to probe tau-membrane interactions. We found that although tau (hTau40) is highly soluble and charged, it is also highly surface active. hTau40 exhibits strong association with negative DMPG lipids, while exhibiting weaker interactions with the positive DMTAP and neutral DMPC lipids. Thus, tau-membrane interactions are strongly mediated by electrostatic interactions. To identify the hTau40 domain that is responsible for its interaction with membranes, we measured the interaction between different tau constructs (K18 and K32) and lipid membranes. Additionally, X-ray scattering experiments were carried out to elucidate the structural details of tau associated with lipid membranes. Our data show that tau's C-terminal, microtubule binding domain, is responsible for its association with the lipid membrane and that these binding events disrupts the ordering and structure of the membrane. Our study suggests that the ''soft'' nature of tau can give rise to rich dynamic behaviors at interfaces, such as the physiological lipid membrane interface. Our data implicate that the inner leaflet of the cell membrane, enriched in negatively charged lipids, can potentially recruit tau in the cytoplasm, which may be critical in initiating the cascade of pathogenic misfolding and aggregation events in AD.

1248-Pos

Global Bilayer Properties can Modulate Membrane Protein Oligomerization

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While sequence dependent oligomerization of individual transmembrane α-helices has been studied to some extent in the recent years, the influence of the lipid bilayer properties on defined helix-helix interactions remains largely uncharacterized. To study the potential impact of changing bilayer properties on a defined transmembrane helix-helix interaction we have followed association of fluorescently labeled glycophorin A transmembrane peptides in model membranes by fluorescence spectroscopy. Changes in Förster resonance energy transfer strongly suggest that the lipid bilayer thickness does significantly influence the monomer-dimer equilibrium of the transmembrane domain. Furthermore, the presence of cholesterol in model membranes promotes self-association of transmembrane helices by modulating the bilayer thickness and -more importantly- by affecting lipid acyl chain ordering. In addition, changes in the lipid composition, which modulate lipid bilayer curvature elasticity and the lateral pressure profile, affect GpA dimerization. In conclusion, the findings show that the physical state of a membrane can be critically involved in controlling specific and promiscuous interactions of α-helical transmembrane domains, as *e. g.* involved in membrane protein folding and assembly as well as in transmembrane signaling.

1249-Pos

Effects of Post-Translational Modifications on the Structure and Stability of Human LDL

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LDL remodeling in vivo (by hydrolysis, oxidation, glycosylation, lipid transfer, drugs, etc.) may affect LDL entrapment in the arterial wall, which causes inflammation and promotes atherosclerosis. The molecular basis underlying the pro- or anti-atherogenic effects of modified LDL is unclear. To test whether LDL modifications lead to changes in LDL structure and stability, we used (i) myeloperoxidase and Cu2+ to produce LDL oxidized to various stages, (ii) phospholipase A2 (PLA2) to hydrolyze LDL phospholipids, (iii) beta-glucose to glycosylate apoB in LDL. Earlier we showed that heat denaturation of LDL is a kinetically controlled reaction that involves partial unfolding of the beta-sheet structure in apoB, protein dissociation, and changes in LDL morphology such as fusion and rupture. Here we test the effects of LDL modifications on these structural transitions.

Our results show that LDL oxidation leads to a gradual unfolding of the secondary structure in apoB (observed by far-UV circular dichroism, CD) and inhibits heat-induced LDL fusion (observed by turbidity, near-UV CD and electron microscopy). We propose that fusion inhibition results from modifications that increase surface-to-core ratio (e.g., transfer of polar lipids to LDL or lipolysis of apolar lipids), and/or from protein cross-linking upon advanced oxidation.

To assess the effect of PC hydrolysis, we hydrolyzed LDL phospholipids by PLA2, removed free fatty acids by albumin, and analyzed the structure and stability of modified LDL. CD spectroscopy showed no significant changes in the apoB secondary structure. Turbidity and electron microscopy showed that PC hydrolysis promotes LDL fusion, an effect that is reversed by albumin treatment. Consequently, free fatty acids promote lipoprotein fusion. Interestingly, glycosylation of apoB and LDL treatment with niacin also promote lipoprotein fusion. These results help understand molecular basis for LDL fusion in vivo and in vitro.

1250-Pos

Thermodynamics of Gndhcl Induced Unfolding of A Helical Membrane Protein in Mixed Micelles

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Mechanisms of folding and stability of membrane proteins are poorly understood. This is linked to the known difficulties to establish reversible denaturation conditions for these proteins. In this work, we describe the equilibrium unfolding of CopA, an 804 residues Cu^{+}-transporting ATPase from *Archaeoglobus fulgidus*. Guanidinium hydrochloride induced a reversible decrease in fluorescence quantum yield, far UV ellipticity, and the loss of ATPase and phosphatase activities. Refolding of CopA from this unfolded state led to recovery of full biological activity and all the structural features characteristic of the native enzyme. The unfolding process showed typical characteristics of a two state process with ΔG_{ow} 13 kJ mol ^{1} and m 4 kJ·mol ^{1}·M ^{1}. These seemly atypical values suggest the existence of non-detectable unfolding intermediates. Moreover, the C_m was 3 M and the ΔCp_w° 0.93 kJ·mol ^{1}·K ^{1}, giving account of the thermophilic character of this protein. Circular dichroism spectroscopic analysis of the unfolded state shows that most of the secondary and tertiary structure was disrupted. The fraction of Trp fluorescence accessible to soluble quenchers shifted from 0.48 in the native state to 0.96 in the unfolded state with a significant red shift of fluorescence Trp spectra. Also, hydrophobic patches in CopA, mainly located in the transmembrane region, were disrupted as indicated by the lack of fluorescence from the 1-aniline-8-naphtalenesulfonate probe at high concentration of denaturant. Nevertheless, the unfolded state had a small but detectable amount of residual structure, which might play a key role in both CopA folding and adaptation for working at high temperatures.

Protein-Ligand Interactions II

1251-Pos

Thermodynamics of Binding Silver Ion to Jack Bean Urease

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Jack bean urease (JBU; E.C. 3.5.1.5) has six identical subunits, which each subunit consists of a single kind of polypeptide chain containing 840 amino acid residues with relative molecular mass of 90770, excluding the two nickel ions per subunit. Inhibition of urease by heavy metal ions is important special in view of heavy metal ion pollution. Silver ion nearly is always listed as one of the strongest inhibitors. Silver ions coordinate to nitrogen- (histidine) and possibly oxygen- (aspartic and glutamic acids) containing functional groups in urease. Here, a thermodynamic study of silver ions by JBU was carried out at two temperatures of 27 and 37°C in Tris buffer (30 mM; pH 7.0) using an isothermal titration calorimetry. There is a set of twelve identical and non-interacting binding sites for silver ions. The intrinsic dissociation equilibrium constant and the molar enthalpy of binding are 185 μM and 16.7 kJ/mol at 27°C and 229 μM and 16.3 kJ/mol at 37°C, respectively. The molar entropy of binding is +15.7 J K ^{1} mol ^{1}at 27°C and +17.1 J K ^{1} mol ^{1} at 37°C. Hence, the binding process of silver ion to HBU is not only enthalpy driven but also it is entropy driven, which the role of entropy driven should be more effective by increasing the temperature.

1252-Pos

A New ITC Assay for Measuring Ultratight and Low-Affinity Protein-Ligand Interactions

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Isothermal titration calorimetry (ITC) is the gold standard for the quantitative characterisation of protein-ligand and protein-protein interactions.[1] However, reliable determination of the dissociation constant (K_D) is typically limited to the range 100 μM > K_D > 1 nM. Nevertheless, interactions characterised by a higher or lower K_D can be assessed indirectly, provided that a suitable competitive ligand is available whose K_D falls within the directly accessible window.[2] Unfortunately, the established competitive ITC assay requires that the high-affinity ligand be soluble at high concentrations in aqueous buffer containing only minimal amounts of organic solvent. This poses serious problems

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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[2] Sigurskjold, *Anal. Biochem.***2000**, *277*, 260.

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