1242-Pos
Interface Dynamics In Hub Proteins
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Dynamical properties of proteins may have a significant role in regulating protein-protein interactions. In particular, intrinsic disorder and disorder-order transitions have been claimed to be especially involved in the binding of proteins known as ''hubs'' [1], which are characterized by a high level of connectivity in protein-protein interaction (PPI) networks. We therefore started to investigate the dynamical properties of hubs through molecular simulations, to assess the role of conformational flexibility in promoting ''promiscuity'' of interactions.
For this study, a dataset of proteins with known structure and interaction partners was first prepared. To cope with the incompleteness of the interaction data contained in the Protein Data Bank (PDB), we mapped the PPI database IntAct [2], which collects interactions from a wide range of experimental techniques, onto the structure-based database PiSite [3]. The proteins were then partitioned into 'classes' with increasing number of interactions.
A preliminary survey of the dynamical properties of each class was done using two independent approaches, namely tCONCOORD [4] and the Gaussian Network Model [5]. For each complex, the interfaces were extracted using the POPSComp method [6,7]. The availability of information on both the dynamics and the interaction properties will allow to determine possible correlations between flexibility and binding diversity.
[1] Dosztnyi et al., J. Proteome Res., 5, 2985-2995 (2006).
[2] Kerrien et al., Nucleic Acids Res., 35, D561-D565 (2006).
[3] Higurashi et al., Nucleic Acids Res., 37, D360-D364 (2009).
[4] Seeliger et al., Structure, 15, 1482-1492 (2007).
[5] Bahar et al., Folding & Design, 2, 173-181 (1997).
[6] Fraternali et al., Nucleic Acids Res., 30, 2950-2960 (2002).
[7] Kleinjung et al., Nucleic Acids Res., 33, W342-W346 (2005).

Protein Folding & Stability: Interactions with Membranes & Lipids

1243-Pos
Resolving the Native Structure of *Escherichia Coli* OmpA
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Modification of the sorting signal (residues 163-169) of outer membrane protein A (OmpA) of *Escherichia coli* by amphiphilic, water-insoluble oligo-(R)-3-hydroxybutyrates (cOHB) enables it to form narrow pores of ~ 80 pS in planar lipid bilayers at room temperature. Here we show that additional modifications of the C-terminal domain of OmpA in the periplasm enable it to refold into large pores in the outer membranes. Both narrow and large pore conformers migrate as 30 kDa proteins on SDS-PAGE gels. OmpA isolated from outer membranes (M-OmpA) refolds into large pores of ~ 450 pS after incubation in micelles or planar bilayers at elevated temperatures (E_a 33.2 kcal/mol), whereas OmpA isolated from cytoplasmic inclusion bodies (I-OmpA) treated in the same manner continues to form only narrow pores. Western blot immunoassay using anti-OHB IgG and ^{1}H-NMR indicate that chymotrypsin-generated C-terminal segment 264-325 of M-OmpA contains cOHB, whereas the same segment of I-OmpA does not. Importantly, the narrow to large pore transition also fails to occur when M-OmpA is exposed to disulfide bond reducing agents. The results indicate that cOHB-modification of the sorting signal in the cytoplasm and of the C-terminal segment 264-325 in the periplasm as well as C_{290} -C_{302} disulfide bond formation in the periplasm are all necessary steps in folding OmpA to its native large pore structure. They further suggest that cOHB modification may be an important factor in protein targeting and protein folding.

1244-Pos
Spectroscopic Study of Anchoring Aromatic Residues in Membrane Proteins and Peptides: Applications to Protein Folding and Vesicle Disruption
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Aromatic amino acids play critical roles in the stability and function of membrane-associated proteins and peptides. Here, we apply the site-selective vibrational tool, UV resonance Raman spectroscopy, to probe changes in the structure and microenvironment of tryptophan residues in integral membrane proteins and membrane-associated antimicrobial peptides. Alterations in molecular interactions, such as hydrogen-bonding states, cation-pi interactions, and local polarity, of tryptophan residues accompany the association and folding of these membrane-bound proteins to synthetic lipid bilayers. These results reveal the diversity of molecular interactions that help guide the in vitro assembly of membrane proteins and peptides in vesicles, and provide molecular clues to the mechanisms of membrane protein folding and vesicle disruption.

1245-Pos
Combining Genomic Information with Molecular Dynamics Simulation to Model Two-Component Signal-Transduction Systems
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Bacteria, archaea and some Eukaryotes employ so-called two-component signal transduction systems (TCS) as a means of adaptation to cellular and environmental stimuli. Typically, these systems feature a membrane bound sensor histidine kinase (SK), which modulates its autokinase activity in response to the stimulus and a transcription factor/response regulator (RR), which accepts a phosphoryl group from the SK and in turn mediates a cellular response. The phosphoryl-transfer requires the formation of a SK/RR complex ruled by transient interaction. This and other transient protein complexes involved in signal transduction are difficult to resolve by experimental means, such as X-ray crystallography or NMR, as evidenced by a lack of structural representatives for many such systems in the protein database PDB. The presented work demonstrates how theoretical methods can close this gap. A genomic direct coupling analysis extracts protein-protein interaction contacts from protein sequence databases. This information is integrated with experimentally determined structures of the unbound proteins in molecular dynamical simulation to understand protein docking and predict the structure of the protein complex. The reliability of this approach is demonstrated by achieving crystal resolution accuracy when reconstituting the known sporulation phosphotransferase complex between Spo0B and Spo0F, which is related to the TCS phsphotransfer complex. We introduce a structural model for the complex of TCS proteins TM0853 with TM0468, consistent with all available experimental data.

1246-Pos
Computer Simulations of Alzheimer's Beta Amyloid Interactions with Multicomponent Lipid Bilayers
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Amyloidogenic protein unfolding and subsequent aggregation on cell surfaces are linked to many protein misfolding diseases, e.g., Alzheimer's (AD) and Parkinson's. Beta-amyloid (betaA), a 39 to 43 residue peptide, is released from neuronal membranes upon sequential proteolytic cleavages of a large transmembrane amyloid precursor protein by two secretases. An understanding of the conformational transitions and stability mediated by the lipid surface interactions is important for developing new strategies for the prevention and treatment of protein misfolding diseases. Using all-atom MD simulation techniques, we explore the initial folding and lipid insertion kinetics of betaA of both 40 and 42 residue long on the surfaces of well-defined lipid nanodomains with different cholesterol contents that mimic the neuronal lipid membranes. Several molecular clusters consisting of different initial conformations, alpha-barrel, beta-sheet and globular-coil, of betaA and stable mixed lipid bilayer in explicit solvent have successfully been constructed. The conformational transitions and stability of betaA on the lipid surface and in a partially inserted state on lipid nanodomains of different lipid compositions were systematically studied. The protein-induced membrane disruptions were examined by calculating the lipid order parameter, water permeability and bilayer thickness profiles of the lipid bilayers. The time-dependent secondary structure of betaA was used to gauge the unfolding events of the protein and its dependence on the lipid composition of the interacting bilayers. Our results revealed that the cholesterol content in the lipid bilayer strongly affects the initial lipid surface-induced unfolding and bilayer-insertion and stability behavior of betaA in our model bilayer systems. Our computer simulation data may provide useful computational insights on the controversial sensitization and protective roles of lipid membranes on the protein aggregation events in AD.

1247-Pos
Lipid-Membrane Mediated Tau Misfolding and Aggregation
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Neurofibrillary tangles comprise of aggregated tau protein are a pathological hallmark of Alzheimer's disease (AD). However, the molecular basis of the early tau aggregation events, such as the nature of the structural fluctuations that trigger the cascade of misfolding and aggregation events, are unknown.

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