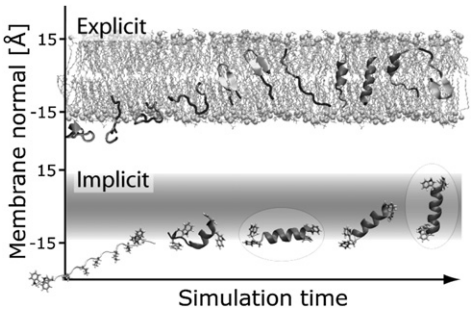
a two-stage pathway, where helical segments fold at the phase boundary before inserting, due to the large energetic penalty associated with de-solvating exposed peptide bonds.
The adsorption, folding and membrane insertion of a model peptide (WALP) was studied via microsecond-timescale molecular simulations at atomic resolution. Both an implicit model and an explicit lipid bilayer setup were used. The implicit simulations generally follow the theoretically predicted two-stage pathway. The vastly increased sampling yields fully converged thermodynamic properties such as the free-energy of folding and membrane insertion. In contrast, the explicit bilayer simulations show that after spontaneous adsorption the peptide immediately crosses the polar interface to locate at the hydrophobic membrane core. Remarkably, there is no interfacial state and the dominant configurations are deeply inserted unfolded and beta-hairpin conformers. The native trans-membrane helix formed for several hundred nanoseconds is not stable. At present the reasons for this unexpected behavior remain unclear.



1709-Pos Board B553
Forces that stabilize membrane proteins
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Understanding the energetics of molecular interactions is essential to addressing many of the central quests of biochemistry including protein structure prediction and design, relating structure to function, mapping evolutionary pathways, learning how mutations cause disease and drug design. Hydrogen bonding and Van der Waals packing are two of the fundamental molecular forces that govern the protein structure and function. Because of technical challenges, however, there have been no quantitative tests of these forces in the context of large membrane proteins. While hydrogen-bonding has been widely regarded as an important force in a membrane environment because of the low dielectric constant of membranes and a lack of competition from water, our recent double-mutant cycle analysis shows that the average contribution of eight interhelical side-chain hydrogen-bonding interactions in bacteriorhodopsin is unexpectedly modest, providing only 0.6 kcal of energy per a mol of interaction on an average, which is quite similar to the strengths measured in soluble proteins. Van der Waals packing, on the other hand, is also thought to be important, as highlighted in number of transmembrane helix dimerization motifs that drive strong helix-helix association in the absence of polar residues by providing tight knob-into-hole interactions. Van der Waals strength, reported by the slope of the correlation found between the thermodynamic stability changes that we measured in six individual cavity-creating large-to-small hydrophobic side-chain mutants of bacteriorhodopsin and the increase in cavity size observed in the refined crystal structures of the corresponding mutant proteins, is indeed quite significant and also very similar to the contributions observed in a soluble protein. Weak hydrogen-bonding and significant Van der Waals packing should be reflected in considerations of membrane protein folding, dynamics, design, evolution and function.

1710-Pos Board B554
Dna-protein Coupling Perspective In Studies Of Unfolding/folding Transitions Of A Protein
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DNA-Protein perspective in studies of unfolding/folding transitions (UFT) accounts for three arguments, both practical and theoretical. Firstly, both experimental and computational research show that properties of protein in unfolded state depend on the molecule length and not its amino acid composition - amino acid profile is not important. Meanwhile, the longer is the protein, the closer is its amino acid composition to the average genome-wide abundance of amino acids (agw aaa). Hence, it might be that the lessening of the importance of amino acid diversity in unfolded protein is consonant to degeneracy of the Universal genetic code (ugc). We show that agw aaa-profile follows closely the ugc-degeneracy profile, while other natural profiles fail.
Secondly, behind the central dogma of biology, that protein is an end product of the DNA-mRNA-Protein line, there is an assumption that common, universal features might exist in mechanisms of untwisting/unwinding of DNA material and UFT of Protein. We focus therefore on the transverse, residue-component of protein in a search of fast and high precision returns to the folded conformation.
Thirdly, the idea of intrinsic mechanical fitness factor, that natural variation and selection give rise to functional design, acknowledges that despite protein does not reproduce itself it possesses some fitness form-factor. Free energy landscape perspective produces such intrinsic score function. Along with serving as a fitness factor for natural variation and selection of DNA material, protein does possess a fitness factor of its own. In a spirit of the Protein-DNA coupling perspective, we project in a self-similar or hierarchical fashion this proposition to the residue level by introducing a residue fitness factor. Contributing to this approach is an experimental knowledge, that for each residue, there is a protein for which it is a key-residue during UFT.

1711-Pos Board B555
Stability of the Spinach Aquaporin (SoPIP2;1) in Detergent Solution and Lipid Membranes
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SoPIP2;1 is one of the major integral proteins in spinach leaf plasma membranes. It is member of the aquaporin family and a highly selective water transporter. Due to this specific characteristic SoPIP2;1 is a candidate for use in industrial membranes water-filtration applications. For this purpose it is very important to characterize whether the protein is well incorporated and stable in the different membranes to be used for the industrial water-filtration supports. Circular Dichroism (CD) and Fluorescence spectroscopy techniques have been used for the characterization of the protein and the protein-membrane complex. The secondary structure of SoPIP2;1 has been analyzed in buffer containing detergent (OG) and in membranes formed by *E.coli* lipids, DPhPC or different phospholipid mixtures. We have also tested how alkane solvents (C_{10}, C_{14} and C_{16}) affect the SoPIP2;1 structure since these solvents are used during the industrial water-filtration membrane preparation. SoPIP2;1 secondary structure is predominantly α-helical in the different environment analyzed as it is expected for the members of the aquaporin protein family. The protein shows high structural stability in detergent solutions. Thermal unfolding experiments show that SoPIP2;1 is irreversible unfolded at temperatures around 58°C. The incorporation of the protein into the different membranes has been performed using different methods and the resulting complex was tested with the techniques mentioned above. It was observed that after shaking the samples in presence of alkane solvent small changes are induced in the SoPIP2;1 structure which still have to be studied whether they could affect the protein functionality. This is the first time that different physicochemical properties of SoPIP2;1 are characterized which are prerequisites for devising an optimal protein-membrane complex for the water-filtration system with technological applications.

1712-Pos Board B556
Structural and membrane binding properties of the Prickle PET domain
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The planar cell polarity (PCP) pathway is required for fetal tissue morphogenesis as well as for maintenance of adult tissues in animals as diverse as fruit flies and mice. One of the key members of this pathway is Prickle (Pk), a protein that regulates cell movement through its association with the Dishevelled (Dsh) protein. Pk presents three LIM domains and a PET domain of unknown structure and function. Both the PET and LIM domains control membrane targeting of Dsh, which is necessary for Dsh function in the PCP pathway. Here, we show that the PET domain is monomeric and presents a nonglobular conformation with some properties of intrinsically disordered proteins. The PET domain adopts a helical conformation in the presence of 2,2,2-trifluorethanol (TFE), a solvent known to stabilize hydrogen bonds within the polypeptide backbone, as analyzed by circular dichroism (CD) and NMR spectroscopy. Furthermore, we found that the conserved and single tryptophan residue in PET, Trp 536, moves to a more hydrophobic environment when accompanied with membrane penetration and that the protein becomes more helical in the presence of lipid micelles. The presence of LIM domains, downstream of PET, increases protein

folding, thermostability, and tolerance to limited proteolysis. In addition, pull-down and tryptophan fluorescence analyses suggest that the LIM domains physically interact to and regulate membrane penetration of the PET domain. The findings reported here favor a model where the PET domain is engaged in Pk membrane insertion, whereas the LIM domains modulate this function.

1713-Pos Board B557

Can An Autotransporter Protein Truly Transport Itself Across A Lipid Bilayer?

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Autotransporter (AT) proteins are synthesized with an N-terminal signal sequence, which is cleaved after secretion through the inner membrane, a passenger domain (the mature protein), and a C-terminal porin domain. The porin domain in inserted in the outer membrane (OM) and the passenger domain is secreted through this pore. OM secretion does not require ATP nor a proton gradient, therefore the driving force for efficient secretion remains unknown, nor is it known what prevents premature folding of the passenger in the periplasm. There is an ongoing debate in the literature about the role, if any, of interactions between AT proteins and periplasmic chaperones and/or OM proteins like Omp85 on AT secretion. We are developing an in vitro system to test the autonomy of AT secretion, using the model AT pertactin from Bordetella pertussis. By mixing unfolded pertactin passenger+porin domains with lipid vesicles, we will test whether the purified protein is competent for secretion across a membrane. While this does not exclude the possibility of chaperone interactions and the participation of other proteins in vivo, it would show that ATs have the capacity to cross a membrane independently, strengthening the importance of β-sheet formation as a potential driving force for OM secretion. Vesicles consisting of PC, 10% PG, and 1% NBD-PC were made; the fluorophore NBD is spread equally in the inner and outer membrane of the vesicles. Vesicle formation was confirmed by fluorescence microscopy, and quenching experiments. We have successfully expressed the isolated pertactin porin domain, and refolded it. Future work will determine the insertion of passenger+porin into vesicles, and the extent of transport, if any.

1714-Pos Board B558

3D solution structure of the C-terminal Chromodomain of the Chloroplast Signal Recognition Particle and its interaction with cpSRP 54 peptide

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Chloroplasts use chloroplast signal recognition particle (cpSRP) pathway to import important cargo like light harvesting chlorophyll protein (LHCP). cpSRP is unique among SRPs in being devoid of RNA. cpSRP consists of an evolutionarily conserved 54-kDa subunit (cpSRP54) and an unique 43-kDa subunit (cpSRP43). cpSRP43 subunit has four-ankyrin repeat domain at the N terminus and a C-terminal chromo domain (CD). The C-terminal CD of cpSRP43 has been shown to provide interaction sites for the cpSRP54 subunit. In addition, the chromodomain in the cpSRP43 subunit is also believed to be important for the formation of the transit complex with LHCP. Also, recent work on cpSRP 43 protein has shown that it mimics the shape and charge distribution of SRP RNA, that's missing from this system. In this context, we embarked on the structural characterization of the C-terminal CD using a variety of biophysical techniques including multidimensional NMR spectroscopy. Far UV circular dichroism spectrum of CD shows that the backbone of the protein is predominantly in the helical conformation. 1H-15N HSQC spectrum of CD is well- dispersed suggesting that the protein is structured. Complete resonance assignments (1H, 15N and 13C) in CD have been accomplished using a variety of triple resonance experiments. Chemical shift index plots show that CD is an alpha + beta protein. A detailed analysis of the three-dimensional solution structure of CD and its interaction with the cpSRP 54 peptide will be presented. The three-dimensional solution structure of CD provides valuable insights into the molecular mechanism underlying the post-translational transport and integration of LHCP on the thylakoid membrane.

1715-Pos Board B559

Functional Properties of Slow Skeletal Troponin T Isoforms in Cardiac Muscle Regulation

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Since human slow skeletal troponin T (HSSTnT) mRNA expression has been shown to be upregulated in end-stage heart failure, it is important to understand its possible role in cardiac muscle regulation. At least three SSTnT isoforms have been found to exist in slow skeletal muscle: HSSTnT1 (+ Exons 5 and 12), HSSTnT2 (+5, −12), HSSTnT3 (−5, −12) and HSSTnT4 (−5, +12, only found at mRNA level). Porcine papillary skinned fibers displaced with HSSTnT1, 2 or 3 and reconstituted with HCTnI-TnC complex showed a decrease in the Ca^{2+} sensitivity of force development compared to adult human cardiac troponin T (HCTnT3). The extent of TnT displacement was analyzed by measuring the unregulated tension at pCa 8.0 after TnT treatment. The maximal recovered force was increased in fibers displaced with HSSTnT1 and 3. In contrast, HSSTnT4, showed an increase in the Ca^{2+} sensitivity of force development and no change in maximal force recovered compared to HCTnT3. Using a reconstituted system, actomyosin ATPase activity containing different HSSTnT isoforms was determined. None of the HSSTnTs were found to alter the ATPase activation in the presence of Ca^{2+}. In contrast, HSSTnT2 and 3 showed an increase in the ATPase inhibition at 0.8 μM [Tn] in the absence of Ca^{2+}. All of the HSSTnTs were less soluble than HCTnT3 at three different pHs, with the HSSTnT4 having the lowest solubility. Circular dichroism experiments performed in the presence of 1M KCl, revealed structural changes between HSSTnTs and HCTnT3. Proteolytic digestion assays are being carried out to determine the susceptibility of HSSTnTs to proteolysis. These results suggest that HSSTnT isoforms may play distinct functional roles in muscle regulation and the molecular mechanism may lie in their physical-chemical properties. Supported by NIH HL-042325 and AR-050199 and AHA 0825368E.

Membrane Protein Structure I

1716-Pos Board B560

Structure Of Gamma-secretase Reveals An Active Site Facing An Internal Membrane Cavity

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The integral membrane protein complex gamma-secretase catalyzes the final step in the production of amyloid beta-peptides involved in the pathogenesis of Alzheimer's disease. Using negative-stain single-particle electron microscopy we have determined the structure of a native-like 500kDa gamma-secretase complex comprising presenilin, nicastrin, APH-1, and PEN-2 that is fully catalytically active. Antibody labeling of the extracellular domain of nicastrin was employed to ascertain the topology of the reconstruction. Active site labeling with a gold-coupled transition state analog inhibitor demonstrates that gamma-secretase contains a single active site facing a large conical internal cavity. This cavity, surrounded by a ~35Å thick transmembrane protein wall, extends from the extracellular side of the membrane to past the membrane centre, where it narrows to finally close at the cytoplasmic side. Based on our structure we suggest a model for gamma-secretase function, in which a hydrophobic transmembrane helix substrate is hydrolyzed by catalytic aspartyl moieties at the interface of a water-accessible internal cavity away from the surrounding lipid environment.

1717-Pos Board B561

A Helix-to-Coil Transition in the Transmembrane Dimer of the Amyloid Precursor Protein is Required for Proteolysis by γ-Secretase

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Processing of amyloid precursor protein (APP) by γ-secretase is the last step in the formation of the Aβ peptides associated Alzheimer's disease. MAS solid-state NMR spectroscopy is used to establish the structural features of the transmembrane (TM) and juxtamembrane (JM) domains of APP in membrane bilayers that facilitate proteolysis. Using peptides corresponding to the APP TM and JM regions (residues 618-660), we target the glycines within the TM domain, as well as residues at the γ- and ϵ-cleavage sites. We find that GxxxG motifs involving Gly625, Gly629 and Gly633 mediate TM helix homodimerization, and that the TM helix breaks at the transition point near the ϵ-cut site. We show that the insertion of three consecutive leucines at the transition point in APP695 inhibits ϵ-cleavage leading to a low production of Aβ peptides and an accumulation of the α- and β- C-terminal fragments. The leucine insertion extends the TM domain by one helical turn, whereas an insertion of three glycines does not, demonstrating that the helix-to-coil transition is required for γ-secretase processing. Our data support a progressive cleavage mechanism for APP proteolysis that depends on the helix-to-coil transition at the TM-JM boundary and unraveling of the TM α-helix.