representations of helical repeat proteins have been found to be well-behaved and highly stable, and facilitate energy dissection using simple nearest-neighbor models.
In contrast, little progress has been made towards understanding the folding of β-sheet containing repeat. To determine the folding stability and cooperativity of these proteins, and to better understand the sequence determinants of structure and stability within these ubiquitous families, we have initiated studies on a series of LRR proteins of both naturally occurring and consensus-designed sequences. We find the LRR proteins PP32 and LC1 to be well-behaved, and to fold in a highly cooperative transition that is consistent with a two-state mechanism. NMR H/^{2}H exchange shows the repeating β-strands on the concave surface of both proteins to be more protected than the rest of the molecules, and can be regarded as an exchange-resistant core, whereas the terminal caps and convex structural elements are more labile. However, truncations and sequence substitution demonstrate that the caps significantly influence stability and kinetics.
To further simplify our analysis of β-sheet containing repeat protein folding, we designed consensus LRR sequences. On their own, these constructs are unfolded and/or aggregated. By fusing these consensus sequences with naturally occurring LRR protein YopM, we have obtained solublized, folded arrays that exhibit increased stability and drastically decreased unfolding and refolding rates with repeat number. Further studies are needed to dissect the complex folding pathways taken by these constructs.

2952-Pos Board B57
The Structural and Functional Role Fo the Sole Tryptophan Residue in the Human Acidic Fibroblast Growth Factor
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Abstract:
Fibroblast Growth Factor-1 (FGF-1) is a 16kDa heparin binding protein, which has been associated with a variety of important functions including angiogenesis and wound repair. In order for FGF-1 to enter the cell it must interact with the FGF-1 receptor on the cell surface. One vital residue involved in the binding of FGF-1 to the receptor is tryptophan 121 (W121). This study aims to examine the role of W121 on the conformation and functionality of FGF-1. Site-directed mutagenesis will be used to incorporate mutations at position 121. The effect of these mutations will be characterized using various biophysical techniques including fluorescence, CD, ITC, and mutli-dimesional NMR spectroscopy. As FGFs are involved in many crucial cellular processes, the gain from this study is expected to provide useful information on the regulation of the FGF signaling process.

2953-Pos Board B58
Folding Mechanism Revealing of PGB1 by FRET and Molecular Simulation
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Immunoglobulin-binding B1 domain of streptococcal protein G (PGB1) is a small (56 residues) protein with an α-helix (from Ala23 to Asp 36) lying on top of two pairs of anti-parallel β-sheets, β-hairpin 1 (from Met1 to Ala20) and β-hairpin 2 (from Glu42 to Glu56), covering the hydrophobic core. PGB1 contains no disulfide bonds in its structure and makes it an excellent model protein for folding study. Our simulation results showed that the α-helix and β-hairpin 2 had interacted prior than β-hairpin 1 in early folding stage. Similar experimental results can be observed by monitoring the folding intermediates of PGB1, which are performed by an over-critical refolding process, by using fluorescence resonance energy transfer (FRET) technique, the technique is a precisely optical technique which can reveal the distance difference within angstrom scale. The FRET analysis of PGB1 also indicated that the distance between α-helix and β-hairpin 2 remained approximately unchanged in all folding intermediates. However, the distance between β-hairpin1 and β-hairpin 2 decreased during the folding process. Therefore both simulation and FRET analysis were in consistence. The molecular dynamics of PGB1 during its folding process can be demonstrated.

Membrane Protein Functions

2954-Pos Board B59
Probing Mechanism for the Enhancement of Uptake of Fatty Acid into Cells by the Membrane Protein CD36
Su Xu, Michael Kirber, Nasi Huang, Kellen Brunaldi, James A. Hamilton.
CD36 is a membrane protein found in various cell types including adipocytes and endothelial cells. Physiological studies have suggested that CD36 is associated with insulin-resistant diabetes, however, the mechanism remains poorly understood. It is well recognized as a receptor for multiple ligands such as collagen and thrombospondin 1, and possibly acts as a catalyst to enhance the rate of transmembrane movement of fatty acid. To separate the two independent events transport across the plasma membrane and the subsequent intracellular metabolism, we have applied biophysical approaches and metabolic analyses to cells in vitro. Our results using cultured cells (HEK293 cells overexpressing CD36 and 3T3-L1 adipocyte cells) as well as mice adipocyte cells isolated from a CD36 null mouse showed that fatty acids diffuse through the plasma membrane rapidly with or without CD36. In HEK 298 cells, which normally synthesize triglycerides very slowly and to a limited extent, expression of CD36 enhanced the rate and extent of synthesis. Even in the presence of CD36, incorporation into triglycerides is a much slower process (min) relative to the transmembrane movement (sec), indicating that the rate-limiting step of the regulation of fatty acid uptake by CD36 is intracellular metabolism. Lastly, by PCR array analysis of 84 proteins, we have identified several enzymes involved in human fatty acid metabolism with gene expression levels altered by overexpression of CD36. Taken together, our results showed that CD36 increases fatty acid uptake by enhancing triglycerides synthesis rather than acting as a membrane transporter, but as yet by unidentified molecular mechanisms.

2955-Pos Board B60
Membrane Transport of CO_2 and H_2S: No Facilitator Required
Florian Zocher, John C. Mathai, Andreas Missner, Mark L. Zeidel, **Peter Pohl**.
The observation that some membranes and epithelia have no demonstrable gas permeability suggested that membrane channels may be involved in CO_2 transport. Both aquaporins and Rhesus proteins were reported to serve as pathways for CO_2 and H_2S. In contrast, we show here that membrane lipid has such high CO_2 and H_2S permeabilities that the presence of a protein channel does not enhance the flux. Therefore we reconstituted aquaporins into lipid bilayers and used scanning microelectrodes to monitor pH in the immediate vicinity of planar lipid bilayers. The lower limits of lipid bilayer permeabilities to CO_2 and to H_2S were equal to 3.2 ± 1.6 cm/s[1] and 0.5 ± 0.4 cm/s[2], respectively. We also observed that the CO_2 flux through the lipid bilayer decreases several fold when the rate of CO_2 formation from HCO_3^- was not augmented by carbonic anhydrase (CA). Experiments with epithelial cell monolayers grown on permeable support revealed the same result. Inhibition of CA transformed these otherwise highly CO_2 permeable cell monolayers into CO_2 barriers. Finally we tested the CO_2 permeability of the epithelium of the mammalian bladder. It was impermeable to CO_2 even after uroplakin knock-out. We found that the lack of intrinsic intracellular CA activity of these epithelial cells hampers the CO_2 exchange between blood and urine.
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2956-Pos Board B61
F65S Mutation in RhAG is Associated with Decreased Ammonia Flux Through Overhydrated Stomatocytic Erythrocytes
Sandrine Genetet, Pierre Ripoche, Julien Picot, Sylvain Bigot, Jean Delaunay, Corinne Amari-Alla, Yves Colin, **Isabelle Mouro-Chanteloup**.
The Overhydrated Stomatocytic (OHSt) erythrocytes of four patients, characterized by the F65S mutation (Bruce et al, Blood 2009) located in the pore of the ammonia channel Rh Associated Glycoprotein (RhAG), were studied. We have previously demonstrated that the equivalent substitution (F74L) in the non-erythroid analogue RhCG resulted in a reduction of the ammonia influx of 50% in transfected HEK293 cells.
Ghosts prepared by hypotonic lysis of OHSt and control (Ctl) erythrocytes, resealed in the presence of a pH-sensitive probe (pyranine), exhibited echinocytic morphology. Images of 1667 (Ctl) and 1998 (OHSt) echinocytes, visualized by light microscopy, allowed the determination of average ghost diameters: 5.81 ± 0.2 μm (Ctl) and 5.83 ± 0.06 μm (OHSt). RhAG densities, as determined by flow cytometry, were similar for Ctl and OHSt (78 000 to 80 000 copies/cell).