[PSI+] and weak [PSI+] respectively when transferred in non-prion yeast cells ([psi−]). Tethers were made by attaching one end of the fiber to a cover slip and the other end to a fluorescently labeled polystyrene bead. Optical tweezers were used to obtain force-extension curves for single fibers. Simultaneously, fiber deformation was imaged with an intensified fluorescence camera utilizing an interlaced fluorescence and trapping laser chopping method developed in our lab to slow the trap accelerated photobleaching. Imaging served to confirm the single fiber assay and to identify fiber structure and boundary conditions. The force extension curves were fit to an appropriate wormlike chain model in order to characterize contour length, persistence length, and axial stiffness of individual fibers. Inhomogeneities were identified in the fiber structure in the form of point defects (hinges) that greatly reduce fiber bending stiffness. Furthermore, data for fibers reconstituted at 4°C and 37°C have shown differences in the mechanical properties indicating that distinct structures result in different intermolecular and intramolecular interactions of prion proteins.

199-Pos Board B78

Dwell Time And Maximum Likelihood Analysis Of Single Molecule Disulfide Bond Reduction Events While Under A Stretching Force

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We study the effects of force on the enzymatic disulfide bond reduction by human thioredoxin (hTRX) in an engineered polyprotein with precise number of disulfide bonds. Single polyprotein molecules are stretched by a cantilever of the Atomic Force Microscope (AFM) in the force-clamp (FC-AFM) mode. Each single disulfide reduction is accurately detected from stepwise increases in the molecule's length vs time (FC traces). Previous FC-AFM studies with *E. Coli* thioredoxin have proposed two simultaneously occurring disulfide reduction mechanisms producing the overall reduction rate to decrease and then increase with increasing pulling force. In contrast, for the human thioredoxin (hTRX) the overall reduction rate only decreases with a pulling force up to a plateau at forces larger than 300 pN. Here, at each clamping force (100 pN - 400 pN) we collect a large number (> 500 events) of long (>50 s) FC traces. We analyze the data by exponential fits to the ensemble of FC traces and logarithmic histograms of the times elapsed to the actual reduction events (dwell times). Our results demonstrate two force decelerated reduction pathways in 100 pN - 200 pN merging into one apparent pathway in 300 pN - 400 pN. The faster pathway is strongly force dependant and predominates at low forces. The latter one is slower and very weakly force dependant. Next, we apply the maximum likelihood methods (MLM) to fit the FC dwell-time sequences. The MLM confirms the presence of two independent reaction pathways in the whole set of investigated forces. We attribute the faster pathway to a Michaelis-Menten type mechanism with a force-dependant catalytic step. We speculate that the mostly force-independent pathway may represent an electron-tunneling mechanism of reduction.

200-Pos Board B79

Intrinsically Disordered Titin PEVK as a Molecular Velcro: Salt-Bridge Dynamics and Elasticity

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It is increasingly recognized that many proteins are intrinsically disordered and do not have a unique compact structure as those found in globular proteins. Titin is a giant modular protein (3-4 MDa) found in the muscle sarcomere that is comprised of both globular and disordered modules. The elastic titin PEVK segment, with tandem repeats of ~28 residue modules, plays a major role in the passive tension of skeletal and heart tissues. We have proposed based on AFM studies of a cloned titin PEVK fragment, that salt-bridges play a central role in the elasticity of this PEVK polyelectrolyte. We have engineered a construct of 15 repeats of a single titin 28-residue PEVK module (human exon 172). The 50 kDa polyprotein shows well-resolved NMR spectra in dilute solution and in highly concentrated gels. Both chemical shifts and sequential NOE's indicate the presence of polyproline II helices. From long-range NOE's, we observed, for the first time, stable K to E salt-bridges with non-random pairings. Simulated annealing with NMR restraints yielded a manifold of plausible structures for an exon 172 trimer showing many salt-bridges. Steered molecular dynamics simulations (SMD) were done to study how the manifold of salt-bridges evolves during the stretching experiment. Repeated SMD simulations at slow velocity (0.0005 nm/ps) show force spectra consistent with experimental AFM force spectra of the polyprotein. SMD shows that salt-bridges occur even at high degrees of stretch and that these short range interactions are in integral part of the mechanical properties of PEVK. We propose that the long-range, non-stereospecific nature of electrostatic interactions provide a facile mechanism to tether and untether the flexible chains, which in turn affect elasticity as well as control the accessibility of protein-protein interaction to these nanogel-like proteins.

201-Pos Board B80

AFM Mechanical Studies Of A Novel Form Of The Biopolymer Fibrin: Elastomeric Sheets

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Fibrin is a gel-forming biopolymer that constitutes the supporting fiber network structure of blood clots within the vasculature. The structure and mechanical properties of these fiber networks have been extensively studied for decades, inspired both by their unusual materials properties as well as their profound biomedical importance. We have recently observed a previously unreported alternate form of polymerized fibrin: two dimensional sheets of molecular thickness. Structural data revealing the sheet structure collected with atomic force microscopy (AFM), SEM and TEM will be presented. When prepared on micropatterened surfaces, the fibrin sheets spontaneously polymerized to span channels or holes in the underlying substrate. Using a combination fluorescence/AFM system, we have manipulated the suspended sheets and collected strain and force data. Our results show that fibrin sheets are a novel biological material: continuous elastomeric films capable of supporting reversible strains well in excess of 100% with an elastic modulus in the few MPa range.

Molecular Simulations of Membranes & Membrane Proteins

202-Pos Board B81

Substrate translocation pathway in glutamate transporter: Insights from molecular simulations

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Glutamate transporters are membrane proteins found in neurons and glial cells, which play a critical role in regulating cell signaling by clearing glutamate released from synapses. While extensive biochemical and structural studies have shed light onto different aspects of glutamate transport, the time-resolved molecular mechanism of substrate (glutamate or aspartate) translocation, or the sequence of events occurring at the atomic level after substrate binding and before its release intracellularly, remain to be elucidated. We identify an energetically preferred permeation pathway of about 23 Å between the helix HP1b on the hairpin HP1 and the transmembrane helices TM7 and TM8, using the high resolution structure of the transporter from Pyrococcus horikoshii (GltPh) in steered molecular dynamics simulations. Detailed potential of mean force calculations along the putative pathway reveal two energy barriers encountered by the substrate (aspartate) before it reaches the exit. The first barrier is surmounted with the assistance of two conserved residues (S278 and N401) and a sodium ion (Na2); and the second, by the electrostatic interactions with D405 and another sodium ion (Na1). The observed critical interactions and mediating role of conserved residues in the core domain, the accompanying conformational changes (in both substrate and transporter) that relieve local strains, and the unique coupling of aspartate transport to Na+ dislocation provide new insights into methods for modulating substrate transport.

203-Pos Board B82

Interaction of Novel Ibogaine Analogs With The Human α3β4 Nicotinic Receptor

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This work is an attempt to characterize the binding site and the inhibitory activity of ibogaine analogs on the human $\alpha3\beta4$ nicotinic acetylcholine receptor (h$\alpha3\beta4$). In this regard, we used [^{3}H]ibogaine equilibrium binding and Scatchard-plots, [^{3}H]ibogaine and [^{3}H]epibatidine competition binding, and ibogaine-induced inhibition of Ca^{2+} influx approaches. The results indicate that: (1) there is one high-affinity binding site for [^{3}H]ibogaine, (2) ibogaine inhibits the h$\alpha3\beta4$ with higher potency than that for the $\alpha1\beta2\gamma\delta$ AChR, (3) ibogaine interacts with different conformations of the h$\alpha3\beta4$ with the indicated affinity (or potency) sequence: Desensitized > Resting > Open, (4) [^{3}H]ibogaine competition experiments indicate that ibogaine and 18-MAC, among ibogaine analogs, and imipramine and dextromethorphan, among other known noncompetitive antagonists, have the highest affinities for the h$\alpha3\beta4$ ion channel, and

(5) [^{3}H]epibatidine competition experiments indicate that ibogaine analogs interact with the agonist sites with very low affinities. Interaction of 18-MAC with the hα3β4 ion channel could be important for its anti-addictive property.

204-Pos Board B83
Noble Gas Anesthetics and Immobilizers Show Different Binding Distributions to KcsA Channel
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Neuronal ion channel is prominent candidate of molecular target anesthesia, but still not yet identified. Using KcsA potassium ion channel as model, anesthetic-protein interactions are investigated. We choose xenon, krypton and argon as anesthetics, which have simple structure. Neon and helium were also studied, which are structurally similar to anesthetics but do not have the anesthetic effects predicted by the Overton-Meyer rule (nonimmobilizers). Using computer simulation these binding sites of KcsA are searched. From the noble gas-KcsA complex structure we discuss binding characteristics of anesthetic and nonimmobilizer. **Methods:** 1k4c (PDB) was used as KcsA structure. Cavities in KcsA was searched with alpha-site finder (geometric search) in Molecular Operating Environment 2007.0902 (MOE, Chemical Computing Group, Canada), that is candidates of binding site of noble gas. Obtained dummy atom from alpha-site finder was used as initial position. Noble gas binding position was searched with energy minimization around initial position. MMFF94x was used for forcefield. **Results:** Binding energy of Xe, Kr, Ar were -8 to -4 kcal mol^{-1}, whereas Ne and He were -2 kcal mol^{-1}. Xe, Kr, Ar bound to gating region first, then they distributed to inter-helical space of transmembrane region. Ne bound to inter-helical space first, then to the gating region. Energy gaps of inter-helical sites were small, so noble gas was consider to be possible to transit from site to site with thermal energy. We considered that inter-helical binding have small position specificity (nospecific binding). Ne and He binding distributed inter-helical sites, the energy gaps were further small. They showed nonspecific binding. Anesthetics and nonimmobilizers of noble gases show different binding distribution to KcsA. We speculate that pharmacological difference of anesthetic and nonimmobilizer originates from the difference in binding distribution of these substances.

205-Pos Board B84
Ligand Induced Conformational Changes in GPCRs: Insight Into the Activation of Rhodopsin and β-adrenergic Receptors
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Signal transduction in GPCRs is initiated by ligand binding at the extracellular domain of the receptor. Recent experimental evidences indicate that structurally different ligands with varied efficacies stabilize distinct receptor conformations. Understanding the relationship between ligand structure and the stabilized receptor conformation is critical in designing GPCR drugs with functional selectivity for a particular signaling pathway. We recently developed a computational method (LITiCon) to study the ligand induced transmembrane conformational changes in GPCRs. Using this method, we have predicted the active conformation of bovine rhodopsin stabilized by the full agonist all-trans retinal. The major conformational changes upon activation are the straightening of the TM6 kink and tilting of the intracellular end of TM5 towards TM6. These predictions are in agreement with the recently published crystal structure of ligand-free opsin, which is believed to be in a partially active conformation. We then study the conformational changes in human β-adrenergic receptors induced by full and partial agonists as well as inverse agonists. In the predicted conformation of the β_2-adrenergic receptor stabilized by the full agonist norepinephrine, the three serines on TM5 come inside the binding pocket and the extracellular end of TM6 tilts towards TM3. These changes lead to shrinking of the norepinephrine binding pocket thus tightening the protein-ligand contacts. A new HB between N293 on TM6 and the β-OH of norepinephrine is formed in the norepinephrine stabilized conformation, which was not possible in the inactive conformation. Virtual ligand screening of the inactive receptor conformation shows higher selectivity for antagonists compared to agonists, whereas that of the norepinephrine stabilized conformation shows higher selectivity for agonists compared to antagonists. These results along with new insights into the ligand specificities between β_1 and β_2 receptor subtypes will be presented.

206-Pos Board B85
Structural Determinants Of Antibiotic And β-lactamase Diffusion Through Bacterial Porins
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General diffusion porins such as OmpF and OmpC, located in the outer membrane of bacteria, represent the main entry point for different classes of antibiotics. Bacteria can resist the action of antibiotics by underexpressing and/or mutating porins. Nowadays the problem of bacterial resistance calls for new antibiotics.
Another way bacteria exhibit resistance is by expressing enzymes that degrade antibiotics, such as β-lactamase that act on β-lactam antibiotics. Inhibitors of such enzymes are prescribed in combination with antibiotics to block β-lactamase and let antibiotics to reach their target. Again, β-lactamase inhibitors have to diffuse through porins in order to reach their target. Understanding how antibiotics and β-lactamase inhibitors diffuse through porins would help to design new molecules with improved permeation properties, solving this problem of resistance.
To investigate the diffusion process of molecules through bacterial porins we used classical MD simulations using OmpF in monomeric and trimeric form. Indeed, as showed experimentally, diffusion is controlled mainly by interaction at the molecular scale. However the high level of accuracy of MD represents also a limitation for simulations to reach the typical time scale of diffusion, from microsecond to millisecond. To overcome this problem we used an acceleration scheme, metadynamics, that allow extending simulations time to biological time scale.
From MD simulations we identified the structural determinants that play a key role in the diffusion process : (i) Flexibility of the molecule diffusing and porin (ii) particular localisation of charged residues (iii) presence of hydrophobic pockets. Further, we observed reciprocal influence of each monomer, in particular in the external loops and the constriction region. We compared diffusion of different antibiotics through various classes of porins, to understand better the problem of bacterial resistance to antibiotics.

207-Pos Board B86
Conformational Transitions and Proton Conduction in the Multidrug Efflux Pump AcrB
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The increasing problem of multi-drug resistance (MDR) in cancer therapy or bacterial infections is to a large degree caused by multi-drug efflux pumps in pathogenic cells. In Escherichia coli, a major resistance mechanism against antibiotics is based on a tripartite multi-drug export complex comprising the inner membrane translocase AcrB, the membrane-fusion protein AcrA and the outer-membrane channel TolC. AcrB functions as the engine of this complex, using proton motive force to expel a wide variety of unrelated toxic compounds such as antibiotics, disinfectants or detergents. The molecular mechanisms of how proton conduction through AcrB is coupled to drug expulsion are not fully understood yet. Here we report a combination of normal mode analysis (NMA) and molecular dynamics (MD) simulation to investigate conformational transitions occurring in the AcrB reaction cycle and to identify residues crucial for proton conduction. In the crystallographic structure of AcrB each monomer is trapped in a different conformation, representing consecutive states in the transport mechanism. Applying the elastic network NMA variant of minimum action pathway (Kim et al. 2002), we computed transitions between these states. The resulting c-alpha trajectories were then converted back to all atom in an approach of steered energy minimization. We also performed multi-copy MD simulations of AcrB embedded in a phospholipid/water environment using the GroMACS simulation package. Mapping the proton conduction pathway was done on the basis of protein-internal water dynamics and monitoring their frequency of forming hydrogen bonds to adjacent residues.
References:
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208-Pos Board B87
Dynamics Of Water Molecules In Bacteriorhodopsin Mutants
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Water molecules are essential for the functioning of proton-pumping proteins. Bacteriorhodopsin is a light-driven proton pump whose reaction cycle is accompanied by changes in the interactions between the protein and the retinal chromophore with water molecules. Of particular importance is the formation of a chain of water molecules that mediate the reprotonation of the retinal Schiff base from the Asp96 residue. Asp96 is replaced by histidine in channelrhodopsin-1 (G. Nagel et al, Science 296, 2395-2398, 2002), and by glutamate in *Neurospora* rhodopsin (Y. Fan, L. Shi & L. S. Brown, FEBS 581, 2557-2561, 2007). Significant effects of mutating Asp96 on the proton-pumping kinetics of bacteriorhodopsin, and effects of mutating the corresponding residues in other retinal proteins, have been documented. To understand how replacement of