[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.

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

Robert Szoszkiewicz[1], Lorin Milescu[2], Julio M. Fernandez[3].
[1]Kansas State University, Manhattan, KS, USA, [2]Harvard University, Medical School, Boston, MA, USA, [3]Columbia University, New York, NY, USA.

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.

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

Jeffrey G. Forbes[1], Wanxia L. Tsai[1], Richard J. Wittebort[2], Kuan Wang[1].
[1]NIAMS/NIH/DHHS, Bethesda, MD, USA, [2]University of Louisville, Louisville, KY, USA.

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.

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

Michael R. Falvo[1], Nathan Hudson[1], Daniel C. Millard[2], E. Timothy O'Brien III,[1], Richard Superfine[1].
[1]University of North Carolina, Chapel Hill, NC, USA, [2]Georgia Institute of Technology, Atlanta, GA, USA.

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

Yan Gu[1,2], Indira H. Shrivastava[1], Susan G. Amara[1], Ivet Bahar[1].
[1]University of Pittsburgh, Pittsburgh, PA, USA, [2]University of Science and Technology of China, Hefei, Anhui, China.

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

Benjamin P. Coleman, Samira Sarrami, Hugo R. Arias.
MIdwestern University, Glendale, AZ, USA.

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