increasing load. Little, however, is known about the mechanics of E-selectin bonds with its ligands. We tested the load-dependence of bond rupture for E-selectin, and its dependence on the history of load application, by using the distribution of load between multiple receptor-ligand bonds to create a complex loading history. Briefly, an E-selectin-coated bead was held in a laser trap and touched to the vertical surface of a bead coated with sialyl lewis A (sLe^a), allowing one or more bonds to form with the ligand-coated surface. The laser trap was deflected away from the trapped bead, applying a nearly instantaneous load. When multiple bonds were present, we were able to discern the rupture of each bond as a step displacement of the trapped bead away from the stationary bead. In this way we were able to monitor both the number of bonds and the lifetime of each bond. We assumed that the load was evenly distributed between bonds and were thus able to monitor bond lifetimes across complex loading histories as the bonds ruptured asynchronously. Our data suggests that E-selectin/sLe^a bonds behave as catch-slip bonds with critical forces of approximately 35 pN. Further, the lifetime of single bonds is similar to the lifetime of bonds that have previously shared load with others. This implies that E-selectin/sLe^a bonds do not display strong force history-dependence. Rather, the bond lifetime is determined solely by the instantaneous load on the bond.

194-Pos Board B73

How Protein Materials Balance Strength, Robustness And Adaptability

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Proteins constitute crucial building blocks of life, forming biological materials such as hair, bone, spider silk or cells, which play an important role in providing key mechanical functions to biological systems. We present molecular dynamics simulations combined with theoretical modeling, used here to develop predictive multi-scale models of the deformation and fracture behavior of protein materials, capturing atomistic, molecular, meso- and microscopic scales. Through explicitly considering the hierarchical architecture of protein constituents, including the details of their chemical bonding, our models are capable of predicting their mechanical behavior across multiple length- and time-scales, thereby providing a rigorous structure-property relationship. We exemplify the approach in the analysis of two model protein materials, spider silk and intermediate filaments, representing beta-sheet and alpha-helix rich protein structures, respectively. Spider silk is a protein material that can reach the strength of steel, despite the predominant weak hydrogen bonding. Intermediate filaments are an important class of structural proteins responsible for the mechanical integrity of eukaryotic cells, which, if flawed, can cause serious diseases such as the rapid aging disease progeria or muscle dystrophy. For both examples, we present a multi-scale analysis that enables us to understand the structural basis of how these materials balance strength, robustness and adaptability through formation of hierarchical molecular and supermolecular features, and how structural flaws associated with genetic diseases contribute to the failure of these materials to provide biological function. We explain why the utilization of hierarchical features in protein materials is vital to their ability to combine seemingly incompatible properties such as strength and robustness, and adaptability. We discuss the implications of our work for the science of multi-scale interactions in biophysics, and how this knowledge can be utilized to develop de novo bioinspired nanomaterials via a bottom-up design.

195-Pos Board B74

Single-Molecule Mechanics of the Muscle Protein Myomesin

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Muscle contraction is mediated by molecular motors that interact between the thin and the thick filaments of muscle cells (actin and myosin, respectively). This leads to misalignment of the filaments within the sarcomeres, the otherwise highly ordered contractile subunits of muscle fibers. In order to maintain or restore the alignment, filament-crosslinking molecules must feature suitable elastic properties to tolerate and counteract tension forces. The M-band protein myomesin in the center of the sarcomere is such a crosslinker; it dimerizes and has binding sites for myosin and titin, the giant half-sarcomere spanning protein. Recent studies revealed that myomesin exhibits a so far unknown folding motif of immunoglobulin (Ig) domains that are connected by α-helices (Pinotsis et al. 2008. EMBO J, 27(1):253-64); these presumably contribute to myomesin's physiological function.

Here, we report AFM force spectroscopy studies of myomesin which support the assumption that the Ig domain-connecting α-helices augment the protein's overall tertiary structure elasticity with a previously unobserved secondary structure elasticity due to α-helix unfolding and refolding at forces around 25pN. This particular behavior would allow myomesin to stay quite rigid up to an external tension force of about 25pN, while at higher forces the unfolding of the (fast refolding) α-helices elongates the protein and thus protects the (slowly refolding) Ig domains from denaturation.

196-Pos Board B75

Bending rigidity of type I collagen homotrimer fibrils

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Normal type I collagen is an $\alpha1(I)_2\alpha2(I)$ heterotrimeric triple helix, but $\alpha1(I)_3$ homotrimers are also found in fetal tissues and various pathological conditions, e.g., causing bone fragility and reducing tendon tensile strength. It remains unclear whether $\alpha1(I)_3$ homotrimers alter mechanical properties of individual fibrils or affect tissues by altering their organization at a higher level. To address this question, we investigated how homotrimers affect fibril bending rigidity. Homotrimer fibrils have been shown to be more loosely packed so that we expected them to be more susceptible to bending. However, confocal imaging of *in vitro* fibrillogenesis revealed straight, spear-like homotrimer fibrils and curved heterotrimer fibrils. Surprisingly, homotrimer fibrils were more rigid despite being thinner and more hydrated. To quantify fibril rigidity, we analyzed their shape by Fourier decomposition, determined the correlation function for the direction along each fibril, and calculated the distribution of local fibril curvature. The fibril persistence length of homotrimers was 3 ~ 10 times longer than for homotrimers. These persistence length values indicated much higher bending rigidity of homotrimer helices. We conjectured that the increased rigidity might be related to stabilization of the region surrounding the mammalian collagenase cleavage site. In heterotrimers, this region is known to be the most flexible along the helix. We corroborated this hypothesis by probing the susceptibility of the collagenase cleavage site to MMP-1. Dissection of the observed effects revealed an increased stability of the homotrimer helix at this site. We argue that the loss of the $\alpha2(I)$ chain reduces type I collagen flexibility within the region most vulnerable to bending, thereby increasing the overall bending rigidity of the helix and fibrils. Higher fibril rigidity may alter tissue mechanics not only directly but also by changing the tissue scaffold architecture.

197-Pos Board B76

AFM Manipulation Of Small Fibrin Networks

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The mechanical properties of fibrin networks, the primary structural component of blood clots, are of great interest both from a biophysical and biomedical perspective. Macroscopic rheological studies have shown that, like other biopolymer gels, fibrin exhibits non-linear elasticity known as strain stiffening. The microscopic origins of this behavior are not well understood, however. We studied fibrin network mechanical properties using a combination fluorescence/atomic force microscope (AFM) system to quantitatively manipulate and visualize small (10-30 segments) two dimensional fibrin networks suspended over micropatterned channels. This setup enabled evaluation of the strain and orientation of each fiber in the network during AFM stretching manipulations as well as acquisition of force data. In other AFM manipulation work, we have shown that, like the macroscopic gels which they comprise, individual fibrin fibers exhibit significant strain stiffening. Our results show strain stiffening of individual fibers plays a significant role in the response of the overall network. In particular, strain stiffening affects the distribution of strain, reducing strain concentrations and spreading it more equitably throughout the network. In physiological contexts, this may act as a mechanism for strengthening the network and reducing the chance of mechanical failure (embolism). Our experimental data was compared to model networks of both linear and strain stiffening fiber segments.

198-Pos Board B77

Probing Structure and Mechanics of Yeast Prion Proteins with Optical Tweezers

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The relevance of amyloid fibers to a variety of severe human disorders and interest in using amyloid fibers as nanowire materials demands a better understanding of their molecular structures and mechanical properties. In Saccharomyces cerevisiae, protein Sup35, or its N-terminal fragment (NM) can assemble into a range of amyloid fibers with different underlying protein conformations. The structural diversity of Sup35 amyloids gives rise to a range of yeast prion phenotypes, referred to as weak [PSI+] or strong [PSI+]. This research uses combined optical trapping and fluorescence imaging to explore the mechanics, structure, and structural diversity of NM amyloid fibers. Fibers were reconstituted from purified fluorescently-labeled NM protein at 4°C and 37°C, which populate different protein conformations. These fibers induce strong

[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