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.

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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.

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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.

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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.

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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