allow bacteria to interact with and colonize various surfaces (1). In the present study, we have used atomic force microscopy (AFM) for both imaging and pulling on Tfp from *P. aeruginosa* (PAO1) and from its PilA, PilT, and PilB mutants. A single pilus filament was mechanically stretched and the resulting force-extension profiles were fitted using the worm-like-chain (WLC) model. The statistical distributions obtained for contour length, persistence length, and number of pili per bacteria pole, were used to evaluate the mechanical properties of a single pilus and the biogenesis functions of different proteins (PilA, PilT) involved in its assembly and disassembly. Importantly, the persistence length value of ~ 1 μm measured in the present study, which is consistent with the curvature of the pili observed in our AFM images, is significantly lower than the value of 5 μm reported earlier by Skerker *et al.* (2). Our results shed new light on the role of mechanical forces that mediate bacteria-surface interactions and biofilm formation.

References:
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3310-Pos Board B357

Pathogenic Mutations alter the Mechanical Stability of Polycystin-1 Immunoglobulin PKD Domains

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Mutations in polycystin-1 can cause Autosomal Dominant Polycystic Kidney Disease (ADPKD), which is a leading cause of renal failure. The available evidence suggests that PC1 acts as a mechanosensor, receiving signals from the primary cilia, neighboring cells, and extracellular matrix. polycystin-1 is a large membrane protein that has a long N-terminal extracellular region (about 3000 aa) with a multimodular structure including sixteen Ig-like PKD domains, which are targeted by many pathogenic missense mutations. The mechanical properties of the wild-type PKD domains are known; however, nothing is known about the effects of pathogenic mutations on the biophysical properties of PKD domains. Here we investigated the mechanical properties of several pathogenic missense mutants on the first Ig-like domain (PKD-d1) by using a combination of protein engineering and single-molecule atomic force spectroscopy. We found that missense mutations alter the mechanical unfolding and refolding pathways of PKD-d1 resulting in distinct mechanical phenotypes. Using a simple 2-state energy diagram we find that point mutations can affect the free energy of mechanical unfolding/refolding and the position of the transition state. Depending on the pulling speed we find that a mutation can increase or decrease the unfolding forces. This indicates that the pathogenic mutants can affect the PKD domain's normal response to external mechanical forces and may help us understand the molecular mechanisms underlying ADPKD.

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Calibration Of Optical Tweezers In Viscoelastic Media

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The application of optical traps to manipulate or monitor intracellular processes in in vivo systems calls for precise procedures both to evaluate the characteristics of the trap and the viscoelastic properties of the intracellular medium. Here, we verify experimentally the authenticity of a calibration procedure, specifically developed for use in calibration of optical tweezers in complex, viscoelastic media (Fischer and Berg-Sørensen, J. Opt. A: Pure Appl. Opt. 9, S239 (2007)). We demonstrate the application of the procedure in i) water and in ii) entangled F-actin without cross-linkers. As the theoretical background of the procedure is essentially convenient formulations of the fluctuation-dissipation theorem, we name the procedure as the ''FDT-method''. The analysis of the measurements in water may be validated directly: We can compare the results for the spring constant to results obtained with well-established methods and those for the viscoelastic properties to theoretical predictions. In the actin solution, we may similarly compare our results with results of microrheology experiments in the literature.

3312-Pos Board B359

Understanding Receptor Kinetics And Mechanics In Phagocytosis Uptake Using Deformable Polyelectrolyte Microcapsules As Force Sensors

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Macrophages play a dominant role in early host defenses against infection. Their defense consists of the ingestion of unwanted objects via an actin-driven membrane deforming process called phagocytosis. Understanding the receptor kinetics that trigger phagocytosis and the tightly controlled mechanics that drive this extremely expedient event is difficult. Here we present a new method of measuring phagocytotic kinetics and mechanics using deformable, mechanically calibrated polyelectrolyte microcapsules - a hollow shell approximately 4.5 μm in diameter. IgG biofunctionalized capsules are readily ingested by macrophages. The forces exerted by the cell during uptake are measured by visualization of the deformed capsules throughout uptake. We have established the lower limit of phagocytotic forces by identifying which capsules of which strength collapse during phagocytosis. We have found capsules that buckle at 130-150nN as measured by AFM, deform and then buckle during phagocytosis. Using this method, we can monitor subtle changes in the capsule shape throughout the event, including the classic squeezing deformation that arises from a contractile actin belt that travels up around the particle as it is consumed. This method can be extended to unravel the roles of the diverse molecular species involved in phagocytosis including several different myosin motors, actin binding proteins and other signaling molecules. Using drugs or molecular biology to interfere with certain molecules, the resultant change in the deformation sequence sheds light on the suppressed molecule's role. In our first series of experiments, PI3-Kinase inhibitor LY294002 has been applied to the macrophages. These preliminary experiments have shown that microcapsules that typically collapse become extremely deformed, no longer collapse. We interpret these results to indicate that PI3-Kinase is required for the closure of the phagocytotic cup.

3313-Pos Board B360

Direct Observation of Protein Complex Disassembly by Single Molecule Force Spectroscopy

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In recent years, single molecule force spectroscopy has opened unique possibilities to investigate the mechanical properties of single protein molecules. So far, experiments have focused on the mechanical behavior of a protein's tertiary structure. For a large number of proteins, however, multiple folded protein molecules are arranged into a multi-subunit complex. How the quaternary structure of such a complex responds to force is not clear. Here, we present a toolkit to study the mechanical properties of dimeric and trimeric protein complexes by single molecule force spectroscopy. We apply these methods to two different model systems:

The dimeric α-crystallin domain is the building block of Hsp 16.5 from Methanococcus janashii. We can directly observe that this dimer dissociates at ~ 200 pN into two metastable monomeric subunits, which subsequently unfold independently.

The two most N-terminal domains of human titin are assembled into an antiparallel complex by telethonin in the Z-disk region of the sarcomere. We show that the Ig domains of titin are stabilized in the presence of telethonin, and that this stabilization is optimized to provide a high level of mechanical strength in the sarcomere. The dissociation force of the titin-telethonin complex exceeds 600 pN, making it one of the highest rupture forces known to date.

Single molecule force spectroscopy allows us not only to measure directly the dissociation forces of protein complexes, but also to observe the mechanical hierarchy of the involved building blocks. Building on these results, future experiments will attempt to observe the assembly of protein complexes using single molecule force spectroscopy.

3314-Pos Board B361

Calcium Dependent Dynamics and Forced Unbinding of Cadherins

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Members of the cadherin superfamily of proteins have been implicated in selective cell adhesion, signaling, and more recently in mechanical processes such as sound transduction. While it is well known that the extracellular repeats of cadherin proteins mediate cell-cell adhesion in a calcium-dependent manner, the molecular mechanisms behind the influence of calcium in cadherin's adhesion dynamics and its mechanical response are not well understood. In addition, the architecture and strength of adhesion complexes formed by different members of the cadherin family remain debated. Here we report molecular dynamics simulations of classical cadherins type I (C-cadherin) and type II (Cadherin-8 and Cadherin-11) involving systems with up to 355,000 atoms. The simulations show how calcium ions control the structural integrity of cadherin's linker