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.

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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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**3311-Pos Board B358**

**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

regions, the availability of key residues involved in cell-cell adhesion, and cadherin's mechanical response. The simulations also revealed the different mechanical strengths of type I and II adhesion complexes. The results illustrate the general principles of linker mediated elasticity of modular proteins relevant for cell-cell adhesion and sound transduction.

**3315-Pos Board B362**

**Direct and Model Free Calculation of Force Dependent Dissociation Rates and Free Energy Barriers from Force Spectroscopic Data**

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Force spectroscopy allows to test out the free energy landscapes of molecular interactions by repeatedly applying a load to the molecular interaction and detecting the rupture events. At present the dependency of the rupture forces on the pulling speed or the shape of the detected rupture force distributions are analyzed to get information about the underlying free energy landscape. But all of these models contain approximations and basic assumptions.

We present a fast and completely model free way to extract the force dependent dissociation rates and free energies directly from the force curve data. Using the Ni-NTA-His6 interaction as a model system and comparing the resulting parameters with results from other techniques, we demonstrate the correctness and practicability of this method.

The presented approach, which is applicable to any force spectroscopic methods, makes it possible to test or validate directly any energy landscape models without any basic assumptions.

**3316-Pos Board B363**

**Single-Molecule Force Spectroscopy Reveals the Function of Titin Kinase as Force Sensor**

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Conformation and dynamics of the polypeptide chain determines the function of proteins. Since an external force tilts the underlying energy landscape, AFM-based single-molecule force spectroscopy is an ideal tool to explore and control both the conformation and the dynamics of proteins as well as their force-induced functions.

In vertebrate muscle, the giant elastic protein titin is involved in strain sensing via its C-terminal kinase domain (TK) at the sarcomeric M-band and contributes to the adaptation of the muscle. Recently we could show by means of AFM-based single-molecule force spectroscopy, molecular dynamics simulations, and enzymatics that an external force activates the ATP binding of the auto-inhibited TK before unfolding the structural titin domains, and that TK can thus act as a biological force sensor [1].

Here, we introduce a new single-molecule mechanical pump-and-probe protocol to study the conformational changes during strain-induced activation. This allows for the experimental identification of the steps through which the auto-inhibition of TK is mechanically relieved at low forces, leading to the binding of the co-substrate ATP and priming of the enzyme for subsequent auto-phosphorylation and substrate turnover. The large statistics [2] of single-molecule pump-and-probe experiments allows us to estimate the on- and off-rates of the mechanically induced ATP binding.

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**3317-Pos Board B364**

**Chemical Diversity and Origin of Thioredoxin Catalysis Revealed by Force-clamp Spectroscopy**

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Understanding the chemical mechanisms by which enzymes attain their rate acceleration has been an object of intense research in the last decades. Single-molecule force spectroscopy has become a powerful tool allowing for direct manipulation of chemical reactions, thus providing a new perspective to study the kinetics and mechanisms involved in enzyme catalysis. In the present study, we have used force-clamp spectroscopy to show that by applying a stretching force to the substrate, disulfide reduction by the enzyme thioredoxin (Trx) can take place through different chemical pathways. In particular, we have used Trxs from four different kingdoms to demonstrate that three different catalytic mechanisms are widespread in nature. While all Trxs have developed a complex enzymatic mechanism that can be detected at low force, two distinct chemical mechanisms dominate at high forces. In the case of prokaryotic-origin Trxs, the high-force mechanism is force-accelerated and well-described by an SN2 reaction featuring a bond elongation of 0.17 Å. By contrast, for eukaryotic-origin Trxs such a mechanism is force-independent, which implies that the disulfide bond does not elongate at the reaction transition state. We propose that an ancestral Trx-like enzyme should exhibit the force-accelerated SN2 mechanism while showing little or no enzymatic mechanism. The emergence of the Trx binding groove through evolution is likely to be responsible for the appearance of the low-force enzymatic mechanism and also for the different catalytic behaviour in the high force-regime. Indeed, computational structural analysis and molecular dynamics simulations show that the Trx binding groove is significantly deeper and more restricted in eukaryotic Trxs than in prokaryotic Trx. Such a structural difference may have a direct effect on the chemical reaction mechanism observed at high force, favouring an electron transfer reaction over an SN2 reaction in eukaryotic Trxs.

**3318-Pos Board B365**

**A Single Molecule Study of Enzyme Inhibitor Interactions**

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Recently developed microscopic models by Dudko et al. were used to estimate the apparent kinetic and thermodynamic parameters in a single molecule force spectroscopy study of the carbonic anhydrase enzyme and a sulfonamide inhibitor. The most probable rupture force for the enzyme-inhibitor interaction demonstrates a nonlinear dependency on the log-loading rate. Estimates for the kinetic and thermodynamic parameters were obtained by fitting the nonlinear dependency to linear cubic potential and cusp potential models and compared to the Bell-Evans model. The reliability of the estimated parameters was verified by modeling the experimental rupture force distributions by the theoretically predicted distributions at rupture. We also report that an increase in the inhibitor tether length has a significant effect on the apparent kinetic and thermodynamic parameters while extending the length of the linkers which attach the enzyme to the surface has a minimal effect.

**3319-Pos Board B366**

**Single Molecule Force-Optical Spectroscopy of Annexin-V on Model Membranes**

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We have studied the diffusion dynamics and intermolecular interactions of annexin-V (A5) molecules on lipid bilayer in both monomeric and self-assembled 2D crystal domains using a correlated force-optical microscope. The A5 monomers bound to a fluid liquid bilayer diffuse in a random walk manner, and occasionally two A5 molecules collide and ''flirt'' with each other in a dance-like motion. The diffusion can be completely frozen by liquid-to-gel bilayer phase transition, permitting the measurement of interaction strength of single A5-lipid molecules. When A5 molecules self-assemble to form 2D crystal domain, the diffusion of A5 molecules inside the domain appears to be frozen, but the domain itself can move and change the shape on lipid bilayer during AFM imaging. Finally, the observed unbinding characteristics of A5 molecules in 2D crystal domain are discussed considering the A5-A5 and A5-lipid interactions in the 2D crystal phase.

**3320-Pos Board B367**

**Nanomechanical Properties of Lipid Bilayers by AFM-based Force Mapping**

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Solid suppported lipid membrane phase separation is of significant interest for the understanding of cell membrane structure and function. Here, we report an atomic force microscopy (AFM) based force mapping approach for the analysis of membrane phase separation. Simultaneous fluorescent imaging, topology and mapping of interaction forces of phospholipid bilayer rafts and membranes