

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.

References:

1. Puchner, E.M., et al., Mechanoenzymatics of titin kinase. *Proc Natl Acad Sci U S A*, 2008, 105(36): p. 13385-90.
2. Puchner, E.M., et al., Comparing proteins by their unfolding pattern. *Biophys J*, 2008, 95(1): p. 426-34.

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 supported 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

was performed. Correlated imaging and high resolution mapping allowed unprecedented insights into dynamic membrane structure and behaviour.

3321-Pos Board B368

Biophysics of the Hyaluronan-Rich Pericellular Coat

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The articular cartilage consists of a complex extracellular matrix (ECM), which is subject to a high mechanical loading. To counteract the ongoing abrasion, a specialized cell type is embedded within the ECM. These so called chondrocytes constantly renew the ECM. To live and even divide in such a mechanically challenging environment, chondrocytes are protected by a several micron thick pericellular coat (PCC). This coat is of vital biological importance for example in cell proliferation and migration, but also in diseases like osteoarthritis or with age.

The PCC consists mainly of water. Thus it remains invisible in all light microscopy techniques. Where the individual components and even their molecular interactions are well understood, much less is known about the mesoscopic structure of the PCC, which in turn is vital to understand force transductions.

In order to analyze the molecular architecture of the PCC, we established a new set of tools to measure and manipulate the PCC on living cells. The micromechanical profile was measured with position-sensitive passive microrheology. In contrast to other mechanical techniques applied to the PCC, it is independent of the adjacent cell body and enables the measurement in the *z* direction normal to the cell surface. The observed profiles show a decreasing viscoelasticity within the PCC correlating to a decreasing concentration of the polymer backbone (hyaluronan).

Further, we demonstrate that the PCC expression depends on the cell's interactions with the ECM. In order to precisely control adhesion, we employed nanostructured surfaces. Chondrocytes not only change their adhesion areas in respect to the ligand density, but also the size of their PCC. The modified PCC size could also be related to changes in its mechanical properties analyzed in ongoing experiments.

3322-Pos Board B369

Functional Mapping of Single Molecules and Gels using Atomic Force Microscopy

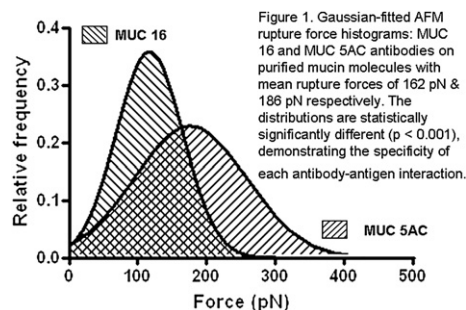
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We have used Atomic Force Microscopy (AFM) to map the spatial distribution of epitopes in the peptide core of mucin glycoconjugates, and also their glycosylation. Using AFM in a physiological environment, and tips functionalised with antibodies against MUC-gene specific epitopes in the peptide core, and also with lectins which recognise terminal sugars, we mapped both single molecules and complex macromolecular structures.

From the simultaneous topographic and force-spectroscopy data, the force-volume maps, we directly quantified the localization, number, and rupture force of recognition bonds on different epitopes of mucin molecules (Figure 1). The specificity of the interactions was confirmed using antibody-blocking peptides, and blocking sugars, and as expected, we observed a loading rate dependence of the unbinding strength.

We have extended these single molecule measurements to more complex physiological gel systems, such as the soft gel layer of the tear film. Binding experiments confirmed the relative abundance of two sugars, previously established by chemical analysis of purified mucins. This comprehensive approach to single molecules and their macromolecular assembly provides an insight into extracellular configuration and packaging of mucins in a gel, and points towards new applications of AFM force mapping.



3323-Pos Board B370

Electroporetic Identification Of Cancer Cells By Afm And Fluorescence Techniques

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We carried out a comparative study of cancer cells and normal cells by AFM and found that there exists a sufficient electroporetic difference between them. We measured the population and size of the "bulges" on cell surface, possibly indicating a step directly preceding electroporation, and of the pores upon repeatedly applying an electric field (0 ~ 2 kV). We found that the bulge population of the cancer cell was much higher than that of the normal cell at low electric potentials (0 ~ 500 V), while the pore size of the cancer cell was much bigger by several times. This phenomenon is believed to be due to the elastic difference of the cell membrane, as is actually confirmed by our force measurement that the cancer cell was softer than the normal cell. We also carried out supplemental studies by fluorescence microscopy and electron microscopy, which also supported our AFM results.

3324-Pos Board B371

Optical trapping of coated microspheres

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In an optical trap, micron-sized dielectric particles are held by a tightly focused laser beam. The optical force on the particle is composed of an attractive gradient force and a destabilizing scattering force. We hypothesized that using anti-reflection-coated microspheres would reduce scattering and lead to stronger trapping. We found that homogeneous silica and polystyrene microspheres had a sharp maximum trap stiffness at a diameter of around 800 nm - the trapping laser wavelength in water - and that a silica coating on a polystyrene microsphere was a substantial improvement for larger diameters. In addition, we noticed that homogeneous spheres of a correct size demonstrated anti-reflective properties. Our results quantitatively agreed with Mie scattering calculations and serve as a proof of principle. We used a DNA stretching experiment to confirm the large linear range in detection and force of the coated microspheres and performed a high-force motor protein assay. These measurements show that the surfaces of the coated microspheres are compatible with biophysical assays.

Micro & Nanotechnology: Nanopores

3325-Pos Board B372

Urea-Induced Conformational Changes in dsDNA Probed by Solid-State Nanopores

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Urea is an uncharged, polar molecule used ubiquitously as a structural denaturant for proteins, a hydrogen bond destabilizer in nucleic acids, and to increase the resolution of similar DNA fragments in electrophoretic processes. Despite its widespread use, the precise effects of varying urea concentrations on dsDNA structure are unknown. Solid-state nanopores in the range 2.7 - 4.5 nm have recently been shown to accurately gauge the effective diameter of dsDNA in aqueous solutions[1]. This DNA sizing method involves measurement of the reduction of a high ionic-strength electrolyte current as DNA transverses the pore. In this study, we systematically vary urea concentrations in the electrolyte solution (0 - 8.6M) and measure its effects on the effective DNA cross-section. Using an 800 bp dsDNA fragment, our studies reveal that the mean DNA effective diameter increases by as much as 0.6 nm. Further, the effective DNA diameter as a function of urea concentration follows a sigmoidal trend, indicative of cooperative binding. This interpretation is also supported by the translocation dynamics of the DNA as a function of urea concentration. We will present our experimental results along with a simple model to explain these observations.

[1]Wanunu, M., Sutin, J., McNally, B., Chow, A. & Meller, A. (2008) DNA Translocation Governed by Interactions with Solid State Nanopores. *Biophys J* 95, in press.

3326-Pos Board B373

Atomic Scale Description of Ionic Behavior in Polymer Nanopores

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Polyethylene terephthalate (PET) is a carbon-based polymer commonly used in plastic containers. Due to its high melting point, mechanical strength, and