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.

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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.

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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:
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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.

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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.

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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.

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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