In this work we study the colicin E9 system and the PPIs that remodel during its translocation across the outer and inner membranes of *E. coli*. Through a series of *in-vitro* single molecule force spectroscopy experiments performed using an atomic force microscope, we identify a mechanism by which dissociation of the highly avid colicin E9-immunity protein interaction (fM affinity, half-life days) can take place via the application of small forces (<20 pN) during colicin translocation (Farrance et al. PLoS Biol, 2013). Such a mechanism could account for the disparity between measured lifetimes of colicin-immunity protein interactions and the timescale on which colicins kill (days vs. minutes) for which immunity protein release is a prerequisite.
Further and ongoing pulling experiments on other PPIs formed during colicin E9 translocation involving periplasmic proteins from the Tol complex also show interesting behaviour under applied forces. These results suggest that the PPI network formed during E9 translocation would be able to support and transduce significant forces and these may be crucial in understanding the mechanism by which colicins gain access to the cellular interior and ultimately kill their target.

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Single Molecule Force Spectroscopy Reveals the Molecular Mechanical Anisotropy of the FeS4 Metal Center in Rubredoxin
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Mechanical anisotropy is an important feature of materials. Depending on the pulling direction, a material can exhibit very different mechanical properties. Mechanical anisotropy on the microscopic scale has also been observed for individual elastomeric proteins. Depending upon the pulling direction, a protein can unfold via different mechanical unfolding pathways and exhibit different mechanical stability. However, it remains to be demonstrated if the concept of mechanical anisotropy can be extended to molecular scale for small molecular objects containing only a few chemical bonds. Here, we choose the iron-sulfur center FeS4 in rubredoxin as a model system to demonstrate the molecular level mechanical anisotropy. We used single molecular force spectroscopy to investigate the mechanical rupture of the FeS4 center along different pulling directions. The FeS4 cluster is a simple molecular object with defined three-dimensional structure, where a ferric ion and four coordinating cysteinyl ligands are arranged into a distorted tetrahedral geometry. Mutating two specific residues in rubredoxin to cysteines provides anchoring points that enable us to stretch and rupture the FeS4 center along five distinct and precisely controlled directions. Our results showed that mechanical stability as well as the rupture mechanism and kinetics of the FeS4 center are strongly dependent upon the direction along which it is stretched, suggesting that the very small FeS4 center exhibits considerable mechanical anisotropy. It is likely that structural asymmetry in the FeS4 cluster and the modulation of the local environment due to partial unfolding of rubredoxin are responsible for the observed mechanical anisotropy. Our results suggest that mechanical anisotropy is a universal feature for any asymmetrical three-dimensional structure, even down to a molecular scale, and such mechanical anisotropy can be potentially utilized to control the mechanochemical reactivity of molecular objects.

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Force Spectroscopy of Tip Link Proteins: A Study of Inner-Ear Biophysics
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Hair cells in the inner ear convert mechanical stimuli, in the vestibular and auditory systems, into electrical signals which can be processed by the brain. Hair cells are highly polarized with a unique elaboration of modified microvilli at their apical surface known as stereo cilia. Sensory stimuli such as sound waves of a particular frequency will result in oscillations of the stereociliary bundle at that frequency. Movements of this bundle couple mechanically to the channel. This force acts to gate the channel in turn resulting in a mechano-electrical transduction. The force is relayed to the channel by filaments known as tip links. Tip links are made of two atypical cadherins, protocadherin-15 and cadherin-23 at the lower and upper ends respectively. The tip link is held together by a non-covalent interaction between two anti-parallel pairs of EC domains. Although much has been discerned about the biophysics of this system the lack of adequate technology has prevented the direct measurement of many of these properties. We have developed molecular tools to facilitate the single molecule study of these properties. Self-assembled DNA nanoswitches are functionalized with protocadherin 15 and cadherin 23 fragments using the enzyme sortase. In order to preserve protein function, protein-DNA coupling is performed under physiological conditions. In this two-step process, a small synthetic peptide is first coupled to a DNA oligo. Next, utilizing the enzyme sortase, the protein is coupled to the DNA-peptide chimera under physiological conditions. This strategy front-loads all of the protein-incompatible chemistry so that it is performed on an oligo and a synthetic peptide, which are far more tolerant of non-physiological conditions. Once assembled, the nanoswitches are then used in an optical tweezer system to measure the rupture forces of the tip link under different conditions.

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Probing the Unbinding Kinetics of DNA-H-NS-DNA Protein Complexes by a High-Speed and High-Throughput Single-Molecule Pulling Assay using Atomic Force Microscopy
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We demonstrate an atomic force microscopy (AFM) pulling assay to measure the unbinding kinetics of DNA-protein interactions. Here we use this technique to investigate the interactions between DNA and the abundant, non-specific nucleoid-associated protein H-NS (Histone-like Nucleoid-Structuring protein). In particular, we probe the unbinding kinetics of the DNA-H-NS-DNA bridges under the influence of one environmental factor, Mg^{2+}-ions. In this experiment, biotinylated DNA molecules are attached on an avidin-coated mica surface. Then thiolated DNA molecules and H-NS proteins are added to the sample solution to form DNA-H-NS-DNA complexes. Individual DNA-protein complexes are then pulled by a gold-coated AFM tip. By measuring the force-distance curve, we gain information on the strength with which H-NS forms bridges between DNA molecules. Here, we probe both single DNA-H-NS-DNA complexes and sample many complexes by fast scanning of the AFM tip over the sample's surface and performing approach/pulling cycles for each position. This experimental approach leads to high-throughput measurements with single-molecule resolution and is widely applicable to other DNA-bridging proteins and receptor-ligand interactions.
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Instrument Free Biomolecular Interaction Measurement with DNA Nanoswitches
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The quantification of molecular interactions is a cornerstone of almost all aspects of biomedical research, from fundamental cell biology to drug discovery. Yet progress has been impeded by challenges implicit in current approaches: high-cost of equipment and reagents, low throughput of many approaches, and requisite technical expertise. Here we present a new method that solves these challenges, replacing standard equipment with a nanoscale molecular tool. This is accomplished by using DNA origami to place molecules on a strand of DNA effectively creating a ''molecular laboratory'' that allows us to control both local concentration and binding stoichiometry at a single-molecule level. The binding state of these molecules alters the geometry of the DNA, which can be easily readout using gel electrophoresis. This allows us to make standard binding kinetics measurements in a highly parallel way at low cost. Furthermore these DNA ''nanoswitches'' provide new capabilities such as the ability to readout the binding state of multi-component systems such as bispecific antibodies or allosteric drugs.

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Simultaneous Measurement of Forces and Currents using an AFM-FET Hybrid Sensor for Studying Single Biomolecular Interactions
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The majority of biological functions are carried out at the molecular level by interactions between various biomolecules. Because these interactions are often the target of pharmaceutical agents, drug screening techniques must