corresponded to the number of induced rotations. We observed initial unwinding frequencies ranging from 0.02 Hz to 0.1 Hz, which decreased exponentially as the unbraiding process progressed. Using hydrodynamic equations, we estimated torques of up to 140 pN nm.

3087-Pos
Investigating the Interaction Between Folic Acid and Folate Binding Protein at the Single Molecule Level
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The folic acid-folate receptor recognition interaction has been exploited for targeted drug delivery, yet the detailed mechanism of this binding is unknown. Here, atomic force microscopy was employed to measure the folic acid-folate binding protein interaction. After conjugating a flexible poly(ethylene glycol) linker to folic acid and attaching this to an AFM tip, a controlled mechanical force was applied to disrupt the binding interaction. The amount of force required to cause rupture at various force loading rates were measured. The rupture force dependency on the loading rate characterizes the energy landscape of the single molecule interaction between folic acid and folate binding protein. Control experiments were performed to ascertain the specificity of the FA-FBP single molecule interaction. The use of dynamic force spectroscopy to investigate the details of this binding interaction provides new insight to guide the design of folate receptor-targeting molecules.

3088-Pos
Isopeptide Bonds Block the Mechanical Extension of Pilins in Gram-Positive Bacteria
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Pathogenic bacteria use long, fibrous structures known as pili to attach to host cells. In Gram-positive bacteria, the pili shaft is formed by the covalent polymerization of the so-called major pilin protein. Accessory pilins, usually with adhesive properties, are located along the pilus shaft interspersed with the major pilin, and/or at the tip of the pilus. In this work, the mechanical behavior of Spy0128, the major pilin from *Streptococcus pyogenes* (M1 serotype), has been studied by single-molecule force spectroscopy. Spy0128, which is composed of two domains, was sandwiched between I27 protein modules, and the resulting heteropolyprotein was pulled using an atomic force microscope. In this kind of experiments, the mechanical response of the I27 modules produces a characteristic fingerprint that identifies the traces where the pilin must have been subject to force. In those successful traces, no unfolding events corresponding to the pilin modules were detected, even when the protein was pulled at forces close to 1 nN. The possibility of the pilin modules extending at very low forces was ruled out by estimating the initial extension before the first I27 unfolding event. Therefore, Spy0128 is the most mechanically stable protein identified so far. In order to test whether the mechanical resilience of Spy0128 is caused by two intramolecular isopeptide bonds (one per domain) described by X-ray crystallography and mass spectroscopy, mutant variants of Spy0128 that block the formation of the isopeptide bonds were also studied. Different to wild-type, the mutant domains unfolded at around 200 pN, implying that the isopeptide bonds further stabilize protein domains that are mechanically stable *per se*. The findings presented here provide new insights into the mechanical architecture of pili from pathogenic Gram-positive bacteria.

3089-Pos
Multi-Scale Modeling of Force Propagation in Proteins Under Mechanical Stress
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Single-molecule measurements of how proteins respond to applied force can provide valuable clues to their structure-function relationship, particularly for proteins whose role *in vivo* relies on an ability to resist or sense force. Interpretation of such measurements relies heavily on theoretical and computational modeling; however, the brute-force approach, molecular dynamics simulation at atomic resolution, is only feasible for timescales orders of magnitude shorter than those appropriate to experiments. Thus coarse-graining is essential for accessing experimentally relevant timescales. However, almost all coarse-grained protein models to date have been designed for the explicit purpose of studying protein folding or normal mode flexibility, and are not capable of supplying quantitative predictions about response to large applied forces. We develop a new procedure for using force measurements from all-atom molecular dynamics simulations to parameterize a coarse-grained model specifically designed for studying force response. This model has the novel feature of using the flexible Morse potential as a basis function for describing non-bonded interactions. We test the model by using it to study the kinetics of ubiquitin rupture under quasi-equilibrium forcing, and compare with experimental results.

3090-Pos
Measuring Adhesion Forces Between Influenza Virus and Living Cells
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Influenza virus belongs to a wide range of viruses that are enclosed in a lipid envelope. The major spike protein of the viral envelope hemagglutinin (HA) binds sialic acid (SA) residues of glycoproteins on the plasma membrane of the host cells. This represents the first step of infection and requires multiple simultaneous interactions since the affinity between one single HA-SA pair is estimated to be very low (10-13 M-1). The attachment of influenza virus particles to living host cells was characterised on the level of single molecules using optical tweezers and atomic force spectroscopy. Unbinding events where analysed and revealed a multimodal rupture force distribution. This suggests sequential binding of multiple receptors. Treatment of the cells with neuraminidase (NA) which cleaves terminal sialic acid residues leads to a decrease of the binding probability by >50 %. This indicates a specific interaction between hemagglutinin and sialic acid unravelled by force measurements.

3091-Pos
Fusion of Biomimetic 'Stealth' Probes into Lipid Bilayer Cores
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The ability to specifically and non-destructively incorporate inorganic structures into or through biological membranes is essential to realizing full bio-inorganic integration. However, molecular delivery and interfaces to inorganic objects, such as patch-clamp pipettes, generally rely upon destructive membrane holes and serendipitous adhesion, rather than selective penetration and attachment to the bilayer. In fact, materials greater than a few nanometers have not been shown to penetrate lipid bilayers without disrupting the continuity of the membrane. I will discuss the development of nanofabricated probes that spontaneously insert into the hydrophobic membrane core by mimicking the hydrophobic banding of transmembrane proteins, forming a well-defined bio-inorganic lateral junction. These biomimetic 'stealth' probes consist of hydrophilic posts with 2-10 nm hydrophobic bands formed by molecular self-assembly, and are easily fabricated onto a variety of substrates including silicon wafers, nanoparticles, and AFM tips.
By fabricating this architecture onto AFM probes, we directly measured the penetration behavior and adhesion force of different molecular functionalities within the bilayer. Following insertion, stealth probes remain anchored in the center of the bilayer, while purely hydrophilic probes have no preferred location. The strength of the stealth probe adhesion varies greatly between short and long chain alkane functionalizations, indicating that chain mobility, orientation, and hydrophobicity all contribute to stability within the bilayer. In addition, the consequences of geometric factors such as band thickness and the presence of multiple bands on interface stability have been established. By selectively choosing the desired properties of the hydrophobic band, it is possible to tune the failure tension of the interface from values comparable to that of pristine lipid vesicles to only a fraction of the strength. Finally, the ability to transfer the stealth probe behavior to other platforms (e.g. nanoparticles for drug delivery) will be discussed.

3092-Pos
Effect of Cell Mechanics on the Transient Force Response of a Molecular Bond and the Derived Kinetic Off-Rate
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Molecular force has a fundamental role in cellular motility. The correct knowledge of the transient history of molecular force is essential for accurate estimation of kinetics parameters through force spectroscopy. Currently, it is assumed that the molecular force is instantaneously equal to the externally applied force. In this work we predict via analytical models and simulation of two cells attached by a single bond that cell mechanics and hydrodynamics modulates the externally applied force such that the instantaneous bond force is not equivalent. Various mechanical models (solid, elastic, viscoelastic) of cells and microvilli are considered over relevant ranges of loading rates (10^2-10^5 pN/s) and force magnitudes (0-200 pN). Specifically it is demonstrated that both microvillus extension and tether formation decrease the pulling force imposed on the adhesive bonds leading to a prolonged bond lifetime. It is demonstrated that modulation of molecular force leads to inaccurate estimation of kinetic off-rate. In particular, it is shown that the applied force is not instantaneously

experienced by the adhesive bond even when the cell and microvilli are modeled as solid materials. It is further shown that microvillus elasticity plays a role in bond behavior characterization.

3093-Pos

Does Calcium Interact with Titin's Immunoglobulin Domain in Cardiac Muscle?

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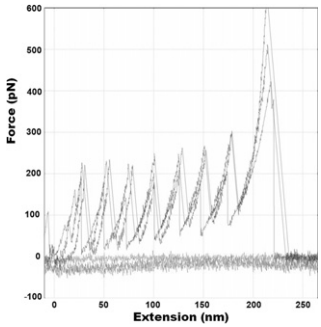
In North America, cardiac muscle diseases such as heart attacks and myopathies are on the rise. Contributing to work in this area, we have focused on a critical muscle protein called titin (connectin). Titin is responsible for all the passive force produced within muscle sarcomeres by acting as a molecular spring preventing muscle over-extension. By adjusting the length of titin's extensible region, a muscle can vary its elastic properties and thus passive force capability. The calcium dependent elasticity of titin has largely been attributed to the PEVK domain, however this mechanism has only been able to explain a tiny contribution of the passive force regulation observed. We propose that other elements in titin, namely the immunoglobulin (Ig) domains, might hold the key to explaining titin's remaining calcium regulated passive force. Fluorescence spectroscopy and atomic force microscopy revealed a change in the microenvironment of the I27 protein with calcium addition.



The application of a mechanical force may trigger the exposure of new binding sites that were buried, therefore Ig domain unfolding may modulate its resting length, elasticity and ligand binding properties, all of which are important for passive force regulation.

3094-Pos

Single-Molecule Kinetics Under Force: Probing Protein Folding and Enzymatic Activity with Optical Tweezers

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Weak non-covalent bonds between and within single molecules govern many aspects of biological structure and function (e.g. receptor-ligand binding, protein folding) In living systems, these interactions are often subject to mechanical forces, which can greatly alter their kinetics and activity. My group develops and applies single-molecule manipulation techniques to explore and quantify these force-dependent kinetics. We have developed a variety of optical tweezers techniques, such as high-resolution 3D position tracking using interference imaging (0.2 nm resolution in z, 1 nm in x-y) [1,2], active feedback for longterm stability in trap height and focus (1-2 nm stability) [2], and intensity modulation imaging for quantifying high-frequency fluctuation above the acquistion rate of a detector (power spectrum measurements above 100 kHz can be made with a slow camera) [3]. We have used these methods to quantify the force-dependent unfolding and refolding kinetics of proteins, including the cytoskeletal protein spectrin in collaboration with E. Evans [4], and the A2 domain of the von Willebrand factor blood clotting protein in collaboration with T. Springer [5]. Furthermore, we have studied the kinetics of the ADAMTS13 enzyme acting on a single A2 domain, and have shown that physiolgical forces in the circulation can act as a cofactor for enzymatic cleavage, regulating hemostatic activity [5].

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3095-Pos

Nanopores as Biosensors: DNA Sequencing and Chiral Discrimination

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Biosensors are stochastic sensors inspired by biology. They are potentially important for many applications in bionanotechnology, from DNA sequencing to single-molecule detection and even chiral discrimination. While the macromolecular properties of the individual components i.e. sensor and analyte are well-characterised, the intricacies of their interaction are less well understood. For full exploitation of biomolecules as stochastic sensors, detailed knowledge of their interactions with other biological and chemical species is desirable. Thus, we have performed a series of molecular dynamics simulations of the bacterial toxin, alpha hemolysin (aHL) and derivative model pores to address issues such as the mechanism of DNA transport through the pore, and the molecular basis of chiral discrimination when the protein is fitted with a molecular adapter (in this case the cyclic molecule, beta cyclodextrin (bCD)).

We study the orientational discrimination of the DNA molecule by restraining the DNA at one end, inside the protein barrel, and applying an electric field. Simulations of the wildtype protein and mutants give good agreement with published experimental data and allow us to explore the molecular basis of discrimination. Our simulations of a model pore (the aHL barrel with only selected sidechains included), allow us to probe the mechanism of DNA threading into the pore once it has already entered the vestibule of the protein. Our results indicate that only key sidechains are required for the interaction with the DNA molecule, and thus have important implications for the future design of engineered protein pores.

Our third set of simulations explores the ability of pores fitted with bCD to discriminate between the enantiomers of ibuprofen. We have used simplified models of pores with full atomistic representation of the bCD and ibuprofen molecules to capture the subtleties of their interaction under an applied external field.

3096-Pos

Modeling of Ionic Currents in a Semiconductor Nanopore

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In this work we are modeling behavior of ionic solution, fully dissociated in water, in the nanopore in a solid-state semiconductor membrane, measuring ionic concentrations and fluxes depending on the voltage applied to the system and geometry of the nanopore. The model is based on the Nernst-Planck and Poisson's equations. Boltzmann statistics is used for charge carrier concentrations in the solution, and Fermi-Dirac statistics is employed to govern electrons and holes concentrations in the semiconductor material. Our approach can be used in modeling semiconductor nanopore membranes with arbitrary internal structure, although the most of results are obtained for a heavily doped n-Si membrane.

3097-Pos

Facilitated Polypeptide Translocation through a Protein Pore

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Facilitated translocation of proteins through a transmembrane protein pore is a ubiquitous and fundamental process in biology. Protein translocation machineries possess various binding sites within the pore interior, but a clear mechanistic understanding of how the interaction of the polypeptides with the binding site alters the underlying kinetics is still missing. Here, we employed standard protein engineering and single-channel electrical recordings to obtain detailed kinetic information of polypeptide translocation through the *staphylococcal* α-hemolysin (αHL) transmembrane pore, a robust, tractable, and versatile β-barrel protein. Binding sites comprised of rings of negatively-charged aspartic acid residues, engineered at different positions within the β barrel, produced significant alterations in the functional features pf the protein pore, facilitating the transport of cationic polypeptides from one side of the membrane to the other. The translocation of polypeptides through the engineered protein pore was dependent on the position of the binding site, the length of the polypeptide as well as its hydrophobic index.

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3098-Pos

Solid-State Nanopore Translocation of Idealized Helical Repeat Proteins

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We report on the translocation of consensus tetratricopeptide repeat (CTPR) proteins with 10 and 20 repeats through single solid-state nanopores formed