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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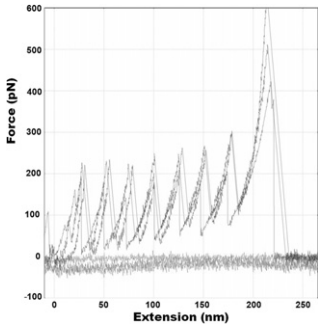
In North America, cardiac muscle diseases such as heart attacks and myopathies are on the rise. Contributing to work in this area, we have focused on a critical muscle protein called titin (connectin). Titin is responsible for all the passive force produced within muscle sarcomeres by acting as a molecular 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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Micro & Nanotechnology, Nanopores

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

in a free standing silicon nitride membrane. The translocation of CTPR proteins was measured in KCl solution at pH below and above its isoelectric point (pI), as well as with and without denaturing agent, Guanidine HCl. When a CTPR protein molecule transits through a nanopore driven by an applied voltage, it partially blocks the ions (K^+ and Cl^-) flow in the nanopore and generates a characteristic electric current blockage signal. The current blockage signal reveals information about the size, conformation, and primary sequence of the CTPR protein molecule. Previous translocation studies carried out with DNA have established that higher bias voltages result in shorter duration current blockages indicating that DNA translocates faster at a stronger electric field. However, CTPR translocation studies presented here show that longer duration current blockades were observed at higher bias voltages. We explain this surprising result by theoretical analysis of CTPR protein translocation in solid state nanopores. We discuss how the inhomogeneous distribution of the primary charge sequence of the CTPR proteins predicts translocation barriers that are proportional to the bias voltage. Larger barriers at higher bias voltages will result in longer translocation times, consistent with our experimental results.

3099-Pos

Quartz Nanopore Membranes for Low Noise Measurements of Ion Channel Conductance

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Planar lipid bilayer (PLB) apparatus provide an excellent platform for the study of isolated membrane proteins. The noise performance and bandwidth of PLB systems are poor relative to the state of the art in patch clamp/pipette apparatus. The problem is the relatively high capacitance of PLB systems relative to small area patch pipettes. At low frequencies (hundreds of Hz), the difference is small to non-existent. At higher frequencies, the noise becomes dominated by voltage noise from the amplifier acting on capacitance of the lipid bilayer and surrounding platform. With a much larger area, the noise for the PLB system rapidly exceeds that of the patch pipette. At intermediate frequencies (1 to 10's of kHz), the specific composition of the PLB platform can lead to an increase in noise due to dielectric loss [1].

We have developed a PLB system based upon a quartz nanopore membrane (QNM) with noise performance approaching the state of the art for patch clamp systems. Due to low dielectric loss, the QNM represents a significant advance in performance over the previously presented glass nanopore membrane [2] and provides for noise performance of ~200 fA at a 10 kHz bandwidth when coupled to a simple capacitive feedback amplifier. The resulting system has great immunity to vibration and electrical interference, without the need for a vibration isolation table and a large faraday shield. This new PLB platform will open up the potential for making very high bandwidth single channel measurements that were not previously possible.

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3100-Pos

Control of Salt Rejection by Surface Charge Patterning in Conical Polymer Nanopores

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Biological membrane protein channels show a variety of interesting transport properties, such as ionic and molecular selectivity. Studies on biological nanopores have shown that a pore's selectivity is due to both steric and electrostatic filtration of the ion or molecule that the pore is designed to transport. In the case of the aquaporin, the pore structure allows for the transport of water molecules at high flow rates without concurrent passage of ions. Careful preparation of an array of such salt rejecting channels would be useful in a variety of applications, in particular for desalination. To this effect, we have prepared "synthetic" salt rejecting channels from conical nanopores in polymer films. At low and moderate ionic strength, pores in polyimide and polyethylene terephthalate films are naturally cation-selective due to a native negative surface charge, and upon application of pressure, show salt rejection. The experimental data were supported by continuum modeling based on the Poisson-Nernst-Planck equations. The model also predicted that nanopores which contain a surface charge pattern consisting of a zone with positive surface charges next to a zone with negative surface charges should exhibit superior salt rejection capabilities compared to homogeneously charged pores. This improvement is due to the large potential barrier to ion transport created by the separation of cations and anions at the junction of the positively and negatively charged zones. Experimental and theoretical results are shown for both homogenous and surface charged patterned pores.

3101-Pos

Fabrication of Metallised Solid-State Nanopores Using Electrodeposition with Ionic Current Feedback

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In recent years, solid state nanopores fabricated in thin insulating membranes have been successfully employed as a new tool to detect and characterise the passage of DNA molecules. These nanopores circumvent some of the problems associated with protein channels, and offer the additional advantage of tunable pore size. Although several experiments have clearly demonstrated that modulations of ionic current during translocation of RNA or DNA molecules can be used to discriminate between polynucleotides, a key challenge with nanopores is to find methods to slow down and control the DNA translocation. It has been proposed that the presence of a metallic probe located at the nanopore can potentially enhance the electrostatic interaction between the DNA molecule and nanopore surface and hence reduce translocation times. Moreover, by applying an electric potential to the metallic nanopore it is possible to control the charge and ultimately allow for sorting and sizing of DNA fragments.

Here we report a novel method to fabricate these metallic nanopores with apparent diameters below 20 nm using electrochemical deposition and "on-line" ionic current feedback. Starting from large nanopores (diameter 100-200 nm) milled into gold silicon nitride membranes using a focused ion beam, we electrodeposit platinum onto the gold surface, reducing the effective pore diameter. By monitoring the ion current simultaneously, the electrodeposition process can be terminated at any pre-defined value of the pore conductance in a precisely controlled and reproducible way. Our approach is applicable to single nanopores as well as nanopore arrays, and can easily be extended to metal deposits other than Pt. In order to highlight their potential for single-molecule biosensing applications, we also show electrophoretic translocation of lambda DNA in a proof-of-concept experiment.

3102-Pos

Lipid Bilayers in Nanopores to Vary their Diameter, Characterize Amyloid-β Aggregates and Monitor the Activity of Membrane-Active Enzymes

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This research introduces the concept of coating the surfaces of nanopores with supported lipid bilayers for previously inaccessible nanopore-based assays. Current methods for shrinking nanopores with nanometer precision entail the use of specialized instruments such as focused ion beams or electron beams. Furthermore, altering the surface chemistry of nanopores currently requires multiple chemical steps and typically takes longer than one day. The method presented here modifies the surface chemistry of nanopores within 90 min by deposition of desired lipids with various chemical headgroups. This work also demonstrates the use of lipids with acyl chains of different lengths to shrink the diameter of a nanopore with sub-nanometer precision. Remarkably, the surface of bilayer-coated nanopores is non-fouling and makes it possible to detect aggregates of the "notoriously sticky" peptide, amyloid-β; the same nanopore without a bilayer clogged in every experiment. These non-fouling properties of nanopores coated with a fluid lipid bilayer made it possible to resolve single aggregates of amyloid-β and to characterize their true size distribution. Finally, this research took advantage of bilayer-coated nanopores to monitor the activity of the membrane-active enzyme, phospholipase D. Together the results presented here demonstrate that supported lipid bilayers can be used to alter the size and surface chemistry of nanopores reversibly. Moreover, bilayer-coated nanopores show promise to study membrane-active enzymes, membrane processes, as well as to perform nano-Coulter counter experiments on peptides that aggregate and adhere to surfaces such as amyloid-β.

3103-Pos

Nanopore-Based Sequence-Specific Detection of Duplex DNA for Genomic Profiling

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The ability of nucleic acids to form stable, sequence-specific complexes with foreign molecular probes has been exploited for a wide range of applications in life sciences, biotechnology, medicine, and forensics. Peptide nucleic acids (PNAs), nucleic acid analogs in which the negative sugar-phosphate backbone is replaced with a neutral peptide-like backbone, have been shown to display greater stability and sequence specificity to complementary ssDNA strands than natural DNA. This feature has been utilized in a number of applications