selectivity for chemical modification, PET has become an excellent material for use in nanotechnology applications. In particular, PET membranes have been used to build very small pores with nanometer-scale diameters, so called ''nanopores''. Several interesting phenomena have been observed in PET nanopores, such as ionic current rectification, reverse rectification due to divalent cations, and nanoprecipitation. However, understanding the physical basis behind such phenomena is still a challenge. We have used molecular dynamics (MD) simulations to study the ionic transport properties of PET nanopores, including the conduction of KCl under different pH conditions and the effect of divalent ions on the ionic conduction and nanoprecipitation. To carry out these simulations, we have developed a protocol to build PET nanopores: First, we constructed a periodic model of bulk PET; then, we created a PET nanopore by removing atoms from a conical region and patching the exposed ends with benzoic groups, the PET surface reproducing the surface charge observed in experiments; finally, the PET nanopores are solvated and simulated under a variety of voltage biases using different ionic species, such as K(+1), Cl(-1), Ca(2+) and HPO4(2-) ions. We applied the protocol and found that it resulted not only in good agreement with experimental data, but also provided an atomic description of the ion dynamics in PET nanopores. Specifically, we observed the enhancement of ionic current due to the surface charge, the permanent binding of Ca(2+) ions to the PET surface, and the dynamics of HPO4(2-) ions inside PET nanopores.

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Nanopore Unzipping Of Ultra-long Dna Repeats For Single-molecule Mutation Detection

Zhiliang Yu, Ben McNally, Amit Meller.
Boston University, Boston, MA, USA.

Rolling-circle amplification (RCA) is an isothermal method for the hybridization-triggered enzymatic synthesis of hundreds to millions repeats of small, single-stranded, circular DNA. Using RCA, we create tandem repeats of a DNA sequence from human genome source, serving as a signal amplifier for ultrasensitive detection of specific nucleic acids mismatches. Solid-state nanopores have been shown to be an extremely useful tool in probing and characterizing biopolymers on the single molecule level. In our recent study[1] sub-2 nm solid state pores have been successfully utilized to unzip small DNA duplexes, and detect base mismatches. Here we demonstrate for the first time that kilo-base RCA products can be characterized using solid-state nanopores, allowing us to enhance mismatch detection sensitivity and accuracy by hybridization with oligos' containing the consensus sequence. This study is an important milestone for the realization of single nucleotide polymorphism and for nanopore sequencing methodologies, demonstrating the feasibility of sequential unzipping, and translocation of extremely long ssDNA molecules.

1. McNally, B., Wanunu, M. & Meller, A. (2008) Electro-mechanical unzipping of individual DNA molecules using synthetic sub-2 nm pores. *Nano Letters* **ASAP article 10.1021/nl802218f**, in press.

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Salt Dependence Of RNA Translocations Through Solid State Nanopores

Michiel van den Hout, Gary M. Skinner, Onno D. Broekmans, Cees Dekker, Nynke H. Dekker.
Delft University of Technology, Delft, Netherlands, Netherlands.

Solid state nanopores have become a powerful tool to probe structural features of single biopolymers. Here, molecules are passed through a nanometer sized hole by a strong electrical field, causing a small change in the ionic current through the pore. Where previous studies have mostly focused on studying DNA, we present translocation results for single RNA molecules. In particular, we present current blockades of double-stranded RNA molecules at varying concentrations of background salt. Similar to what was found for DNA, our preliminary results suggest a crossover from current blockades at high salt (1M KCl) to current enhancements at low salt concentration (0.1 M KCl). This can be explained by an increasing contribution from the counter-ions screening the RNA backbone as the background salt concentration is decreased. These experiments demonstrate the strength of solid state nanopores in studying RNA, and pave the way towards unraveling more complex RNA structures through the use of solid state nanopores.

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Fabrication And Characterization Of Tunable, Low Stress Al_2O_3 Nanopores For The Electronic Detection Of Biomolecules

Murali Venkatesan, Sukru Yemenicioglu, Brian Dorvel, Rashid Bashir.
University of Illinois at Urbana Champaign, Champaign, IL, USA.

Understanding the biophysics governing single molecule transport through solid state nanopores is of fundamental importance in working towards the goal of genome sequencing using nanopore based sensors. Here, we present a simple process for the fabrication and characterization of novel, low stress, low noise aluminum oxide nanopores for biomolecule detection. Aluminum oxide has numerous attractive properties including high mechanical hardness, low surface charge, chemical inertness to strong acids and excellent dielectric properties from DC to GHz frequencies.

Device fabrication involved the use of Atomic Layer Deposition and Deep Reactive Ion Etching tools to form low stress, mechanically robust aluminum oxide membranes. High temperature process steps were avoided to allow for possible process integration with metal nano-electrodes and optical probes. The nanometer sized pores themselves were formed through Field Emission Gun Transmission Electron Microscope (FEG-TEM) based sputtering. We demonstrate the precise size tunability of these structures in the nanometer regime and examine the physics governing pore contraction in aluminum oxide. Diffraction patterns reveal polycrystallinity localized to the pore region post sputtering suggesting localized heating and possible thermal annealing under the electron beam. Film composition and thickness were characterized. In addition, we examine the surface charge properties of these structures as a function of buffer pH and molarity. The single molecule sensing ability of this novel structure was tested using dsDNA. Electrical characterization revealed a significant reduction in membrane capacitance and reduced high frequency dielectric noise relative to existing silicon nitride and silicon dioxide topologies. These improvements can greatly enhance device performance by improving sensitivity and signal-to-noise ratio. In summary, our work provides a novel yet simple approach to fabricate tunable, low stress chemically functionalizable nanopores for the detection of biomolecules.

3330-Pos Board B377

Asymmetric Spectral Characteristic of Ion Currents in Conical Nanopores

Matthew Powell, Gael Nguyen, Craig Martens, Zuzanna Siwy.
University of California, Irvine, Irvine, CA, USA.

The noise analysis of ion current signals through single nanopores is a critical problem in using nanoporous systems for biosensing. We have examined the noise characteristics of ion currents in single asymmetric polymer nanopores. These pores are conical in shape with openings of several nanometers at the cone tip. The pore walls are negatively charged at pH 8 due to the presence of carboxyl groups with density of one group per square nanometer. These conically shaped pores are cation selective and rectify the current with preferential direction for cation flow from the narrow entrance of the pore to the wide opening of the pore. With our electrode configuration, the average currents for negative voltages are higher than the average currents for positive voltages. We have found that the noise characteristics for the positive and negative currents are very different. The time signals were examined through power spectra analysis and Hurst analysis. At low salt concentration, the transient behavior of currents flowing in the direction from the narrow opening towards the wide opening show a power spectra with distinct 1/f behavior, where f is the frequency. The Hurst analysis of these currents reveals a deterministic component in the current behavior. In contrast, the spectra of currents from the wide opening towards the narrow opening show a thermal noise characteristic. We will discuss how these differences in the transient signals of ion current in conical pores reflect differences in the electrochemical potential of cations in the nanopores, and how they can be important for biosensing.

3331-Pos Board B378

Direct Probing of DNA/Nanopore Interactions Using Optical Tweezers

Allison Squires, Meni Wanunu, Amit Meller.
Boston University, Boston, MA, USA.

Solid-state nanopores can be used to analyze the structure of long double-stranded DNA molecules and to probe their interactions with proteins. This method utilizes the native biopolymers' charge to electrically draw the molecules from the *cis* to the *trans* side of the pore. In small pores (<5 nm) the transport time of the biopolymer is determined by a balance of the electrical field and the frictional force resulting from interactions with the pore walls and hydrodynamic drag. Despite the central importance of biopolymer dynamics in virtually all nanopore applications, to date there have been no direct measurements of force in small pores during biopolymer transport. Here, we use optical tweezers to dynamically manipulate a λ-DNA molecule threaded through a <5nm pore while simultaneously recording force and ionic current. To characterize the interaction strength in the pore, we measure force/velocity profiles as a function of the applied voltage and ionic strength. By comparing experiments using different-sized pores, we quantify the relative contribution of interactions to the overall translocation dynamics. These measurements provide basic insight into the principles governing translocation in the interaction-dominated regime.

3332-Pos Board B379

Conductivity of Room Temperature Ionic Liquids in Single Nanopores

Matt Davenport, Ken Shea, Z. Siwy.
University of California, Irvine, Irvine, CA, USA.

Drawing inspiration from ionic selectivity of biological channels, we have explored the nature of the transport of ions through single nanopores of diameters comparable to the transported ions. As a test system, we used single nanopores and ions of room temperature ionic liquids (RTILs). RTILs are liquids composed entirely of charged species and contain no solvent. Their constituent ions can be very bulky with diameters ranging from tenths of an angstrom to greater than 10 Å. In recent years, RTILs have garnered a wealth of attention, especially in regard to their physical and chemical properties; however, most, if not all, studies thus far have focused on bulk characteristics, leaving their properties on the nanoscale ripe for investigation.

Our study focused on a select few RTILs, and on how the size of their cations and anions affects the conductivity of the liquid through our pores. Single nanopores of controlled geometry and of various diameters, ranging from a few nanometers to a few hundred nanometers, were prepared in polyethylene terephthalate foils. High voltages (-5V to + 5V) were applied across the membrane and the resulting ionic currents were recorded, allowing us to calculate the conductivity of the RTILs. Conductivity of the liquids in sub-10 nm pores was found several times smaller compared to the bulk values. Our results indicate that steric effects outweigh the contributions of electrostatic interactions of the ions with the pore walls in determining the conductivity of an RTIL in nanopores. While our experiments were motivated by biological channels whose openings are comparable in size to that of the ions in the system, they have allowed us to examine physical properties, primarily the conductivity, of certain RTILs. These studies represent important steps in the characterization of RTILs on the nanoscale.

3333-Pos Board B380

Fluidic Diodes in Nano- and Microscale Pores to Detect Drug Aggregation

Erik C. Yusko, Ran An, Michael Mayer.
University of Michigan, Ann Arbor, MI, USA.

We present a novel method of producing ion current rectification (ICR) in nano- and micro- scale pores that does not require electrical double layer overlap within a pore. ICR in nanopores has typically been constrained in two ways: i) at least one dimension or component of the channel must be on the order of the Debye screening length and ii) charge asymmetry must be induced within the pore. Asymmetric channel geometries, modified surface charges, and asymmetric bulk ion concentrations are commonly applied to produce the necessary charge asymmetry. In the method presented here, ICR is dependent on electroosmotic flow (EOF) to position two solutions of different conductance within a pore. Substrates containing pores with diameters of 10 nm, 30 nm, and 500 nm were used to separate the two different solutions. We have achieved rectification factors ranging from 2-15, as a function of solution properties, in conical and cylindrical pores with diameters much larger than the Debye screening length. Thus, ICR can be achieved at higher ionic strengths (here up to 300 mM), in pores with large diameters (here up to 3 μm), and in pores without patterned surface charges. We also present a phenomenon unique to the two-solution system that we introduce here. Dissolving a drug in one solution, which contains 75%, DMSO, and then moving the molecules through the pore into a purely aqueous solution leads to detectable aggregation of the drug in the pore. The aggregate typically proceeds to the point of blocking the pore (>85% reduction in current) and subsequently ejects from the pore. We have investigated the aggregation and subsequent clearance of the aggregate from the pore and propose a mechanism for the cyclic process.

3334-Pos Board B381

Nanopipette surface modification for biosensing

Paolo Actis, Senkei Umehara, Nader Pourmand, Miloslav Karhanek.
UC Santa Cruz, Santa Cruz, CA, USA.

We have developed a sensitive nanopipette assay for the detection and functional analysis of proteins in a microbial sample. This unique, label-free biosensor is inexpensive, easy to fabricate and versatile. The nanopipette size, geometry, and surface chemistry for attaching a biomarker, antibody or protein probe were previously optimized using both experiments and modeling to provide detectable signals in real time, in a very small reaction volume (attoliters). In this phase, the goal was to modify the surface chemistry procedure so that only a restricted area of the nanopipette tip was functionalized for probe attachment. Preliminary experiments demonstrate the sensitivity and selectivity of the nanopipette with specific protein targets of yeast cells and fungi. We have further developed biophysical and kinetic models that help us to interpret and explain phenomena underlying the current signals obtained from the nanopipette system. These models characterize the physical, chemical, electrical and thermodynamic forces responsible for molecular interactions (ion-surface and probe-target) at the nanopipette tip. The models provide several feedback parameters related to actual experimental conditions, which are used for further improvements of the detection scheme. In the next phase, the functionalized nanopipette will be applied to the study of living cells as a diagnostic tool to detect protein expression.

3335-Pos Board B382

Detection Of Nerve Agent Hydrolytes In An Engineered Nanopore

Deqiang Wang, Qitao Zhao, Dilani A. Jayawardhana, Xiyun Guan.
University of Texas, Arlington, Arlington, TX, USA.

We report a stochastic nanopore sensing method for the detection of organophosphorus nerve agent hydrolysis products. By employing an engineered α-hemolysin single pore embedded in a planar lipid bilayer as the stochastic sensing element and β-cyclodextrin as a host molecule, trace amounts of soman and cyclosarin hydrolytes could be detected, with detection limits of 53 nM and 102 nM, respectively. Importantly, sarin, tabun, and VX hydrolysis products, as well as other common pesticides, do not interfere with detection of the analytes. The method offers the potential as a rapid and sensitive sensing technique for use in on-site analysis of nerve agents in environmental monitoring applications at the single-molecule level.

3336-Pos Board B383

Transport Of Antibiotics Through OmpF Mutants Studied By Proteoliposome Swelling And Single-channel Reconstitution Techniques

Ekaterina M. Nestorovich[1], Anna Rossovsky[1], John Pang[1],
Mathias Winterhalter[2], Sergey M. Bezrukov[1].
[1]NICHD, NIH, Bethesda, MD, USA, [2]Jacobs University, Bremen, Germany.

We use site directed mutagenesis to study molecular mechanisms of beta-lactam antibiotic transport facilitated by the bacterial porin OmpF. First, we measure interaction of antibiotics with OmpF mutants, D113A, R82A, R42A, and E117A, reconstituted as single channels in bilayer lipid membranes (BLM). Second, we estimate antibiotic diffusion rates through these channels by measuring the swelling rates of proteoliposomes whose membranes contain wild type OmpF or these mutants.

Just as a wild type, R82A and R42A mutants studied by the BLM technique interact with OmpF showing clear time-resolved blockages of the current through their trimeric pores. D113A and E117A mutants demonstrate no time-resolved blockages but show some high-frequency excess noise. Two most probable explanations for the origin of this high-frequency noise component are that it is generated by: (i) fast irresolvable antibiotics transport events or (ii) events of antibiotic binding to the channel somewhere close to the opening of the channel without subsequent antibiotic translocation. In order to distinguish between these two scenarios we compare antibiotic translocation rates through OmpF mutants using reconstituted proteoliposomes. With this approach we are able to rule out the ''fast transport event'' scenario. D113A- and E117A-containing liposomes show significantly smaller swelling rates compared with the liposomes containing wild type and R82A and R42A mutants. These results are in accord with our theoretical rationalization of the constructive role of solute-channel interactions in channel-facilitated membrane transport.

3337-Pos Board B384

Mechanisms Of Selective Transport Through Nano-channels: Theory Vs. Experiment

Anton Zilman.
Los Alamos National Laboratory, Los Alamos, NM, USA.

Functioning of living cells requires selective molecular transport, which is provided by transport channels that are able to selectively transport certain molecular species while filtering others, even similar ones. Such channels can selectively transport their specific molecules in the presence of vast amounts of non-specific competition. In many biological channels, efficient and selective transport occurs without direct input of metabolic energy and without transitions from an 'open' to a 'closed' state during the transport event. Examples include selective permeability of porins and transport through the nuclear pore complex. Mechanisms of selectivity of such channels have inspired design of artificial selective nano-channels, which mimic the selective biological channels and are built upon the same principles(e.g [3]).

Precise mechanisms of selective transport through such nano-channels are still unknown. I present a theoretical model to explain the of selectivity of transport through nano-channels, which contains only two essential ingredients: i) transient trapping of the cargoes inside the channel (e.g. due to binding inside the channel) ii) competition between the transported molecules for the limited space inside the channel [1,2]. The theory provides a mechanism for selectivity based on the differences in the kinetics of transport through the channel between different molecules. The theory explains how the specific molecules are able to filter out the non-specific competitors - and proposes a mechanism for sharp molecular discrimination. The theoretical predictions [1,2] account for previous experimental results [3] and have been verified in ongoing experiments.