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

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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

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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

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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

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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.

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

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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.