Probing the Transport of Ionic Liquids in Aqueous Solution through Nanopores

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titration, we created an abasic mapping, in 2M and 1M KCl, that details α-hemolysin's sensitivity with respect to electrical conductance as a homopolymer with a single abasic traverses through the pore. From our map, we are able to reveal the smallest (minimal) voltages that can reveal DNA translocation progress through pore, e.g., during enzyme-catalysis on the pore. These results are part of preliminary studies that aim to measure hydrolysis of DNA by the Exonuclease I (ExoI) of E. coli on the nanopore. ExoI is catalytically active in both 1 and 2 M KCl. In this research, we present voltages that make it possible to observe ExoI-catalyzed DNA hydrolysis on the nanopore in 1 and 2 M KCl, respectively.

1030-Pos Board B816

Mechanical Model of Cell Membrane Penetration by Vertical Nanowires

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For many therapeutic and scientific applications, direct access into a cell's interior is the key for delivering various biomolecules to alter cell behavior or for intracellular assays. However, the lipid membrane presents a challenging barrier that prevents biomolecular species from entering the cytosol.

The recent discovery of cell viability despite penetration by vertical nanowires (NW) has opened new avenues for direct intracellular access. Cells are found to be impaled onto small diameter nanowires without application of any external force. Vertical NW arrays have been reported to serve as a universal and efficient platform for introducing RNA, DNA, proteins and peptides into a broad range of cell types.

However, the cell membrane penetration mechanism by vertical nanowires is still unknown. Several recent experiments have shown that the penetration efficiency is greatly reduced with increasing NW diameter. Understanding the penetration mechanism is the key to optimizing the design of nanowires and developing advanced devices based on this technique.

In this work a mechanical model is developed to predict cell membrane penetration by vertical nanowires. The tension and strain on cell membranes due to indentation by nanowire are calculated using a model of axisymmetric deformation of elastic membrane indented by a probe with a hemispherical tip. The critical membrane rupture conditions are determined under different failure criteria, either tension or strain. The effects of NW radius, NW aspect ratio and cell membrane stiffness on penetration are investigated based on the mechanical model. Our results provide a practical guide to designing nanowires for applications in cell membrane penetration.

1031-Pos Board B817

Detection of Methylated DNA by Modified GP10 Nanopore

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We report the electrophysiology of a biological sensor for the detection of methylation state changes of DNA characteristic of carcinogenesis. This sensor is an engineered nanopore composed of a freestanding lipid bilayer containing the capsid portal protein GP10 and several mutants in a freestanding lipid bilayer. The measured conductance is on par with some of the largest biological nanopores, like those of the mechanosensitive channels and porins found in many prokaryotes. The variable region and the C-terminal crown both appear to play a role in restricting conductance. These two areas are known to interact with the viral DNA but remain unresolved in the crystal structure. We have used the C-terminal interaction as a basis for distinguishing dsDNA methylation state by engineering a methylated DNA binding domain onto the crown of GP10. Of the two methylated DNA binding domains tested, MBD2 and MeCP2, MeCP2 imparts greater stability to the nanopore possibly due to its increased ability to interact with DNA or other proteins. The engineered pore has the ability to electrically distinguish methylated and hydroxymethylated DNA from the unmodified form. We envision this sensor as a future tool for detecting the alterations in DNA methylation state commonly associated with carcinogenesis.

1032-Pos Board B818

Probing the Transport of Ionic Liquids in Aqueous Solution through Nanopores

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The permeation of water soluble molecules across cell membranes is controlled by channel forming proteins and particularly the channel surface determines the selectivity. An adequate method to study properties of these channels is electrophysiology and in particular analyzing the ion current fluctuation in the presence of permeating solutes provides information on possible interactions with the channel surface. The temperature-dependent transport of the ionic liquid 1-butyl-3-methyl-imidazolium chloride (BMIM-Cl) in aqueous solution is studied theoretically and experimentally. Using molecular dynamics simulations and ion-conductance measurements, the transport is examined in bulk as well as through a biological nanopore, OmpF and its mutant D113A. This investigation is motivated by the observation that aqueous solutions of BMIM-Cl drastically reduce the translocation speed of DNA or antibiotics through nanopores in electrophysiological measurements. This makes BMIM-Cl an interesting alternative salt to improve the time resolution. In line with previous investigations of simple salts, the size of the ions and their orientation adds another important degree of freedom to the ion transport, thereby slowing the transport through nanopores. An excellent agreement between theory and conductance measurements is obtained for wild type OmpF and a reasonable agreement for the mutant. Moreover, all-atom simulations allow an atomistic analysis revealing molecular details of the rate-limiting ion interactions with the channel.

[1] Mahendran KR et al, *J. Phys: Condens. Matter* **22** (2010) 454131.
[2] Niraj Modi, Pratik Raj Singh et al, J. Phys. Chem. Lett. 2 (2011) 2331-36.

1033-Pos Board B819

A Designed Polymer Detects the microRNAs Based on Protein Nanopore

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Abstract
MicroRNA (miRNA) is a kind of small non-coding RNA, which plays important role in modulating gene expression. And it is a new candidate for some disease diagnosis. However, it is difficult to detect because of the short length, low concentration and mixed with other components in cell. In our work, a synthesized polymer is design to detect miRNA based on protein nanopore. The positively charged polymer has high specificity to given kind of miRNA. Meanwhile, the capture rate in a site-direct mutated nanopore is several hundred folds higher than other kinds of probe from *trans* side. After binding of probe and miRNA, the complex causes signature event when associating with the pore. As the result, the polymer probe can detect low concentration of miRNA to tens of picomolar. According the side preference of nucleic acid, the unrelated ones in *trans* side will not interfere the detection. Besides, three members of miRNA family can be distinguished using the same probe by the properties of the signatures, even though there is only one or two bases difference in them.

1034-Pos Board B820

SSB Enhances Detection of ssDNA Translocation through Solid-State Nanopores

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The small diameter and secondary structure formation are major problems in nanopore-based analysis of hetero-sequence ssDNA/RNA. Here we report how binding of single-stranded binding protein (SSB) can both prevent secondary formation and increase diameter of ssDNA. SSB is a helix-destabilizing protein in virtue of its binding with high affinity to ssDNA and plays important roles in DNA replication, recombination and repair. E.coli SSB forms tetramers and binds every 35 nucleotides (nt) under conditions used in our experiments. We have translocated long (7.2 kb) and short SSB-coated ssDNA in the 37-100 nt range. For long SSB-coated ssDNA, current blockade levels are lower and last significantly longer than those of the free ssDNA, which is due to straightening of the globular structure. SSB-coated ssDNA molecules as short as 37-100 nt translocate much faster but are still easily detectable. We found translocation times of 0.92 ± 0.18 ms for 37-nt ssDNA/SSB and 1.40 ± 0.14 ms for 100-nt SSB/ssDNA. This is the first demonstration that ssDNA shorter than 100 nt can be detected using solid-state nanopore and applicable for future applications, such as ssDNA sizing or sequencing of the natural and long ssDNA, which forms complicated secondary structure.