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3338-Pos Board B385
Aptamer-Encoded Nanopore For Single-Molecule Detection In Sensing Of Biomedical And Bioterrorist Agents
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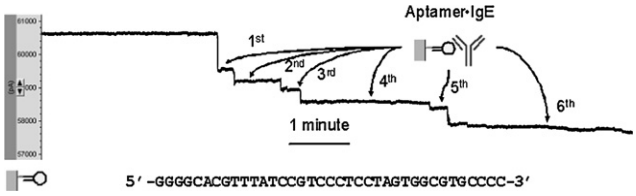
University of Missouri, Columbia, MO, USA.
Various solid nanopores have been constructed with the advantage of changeable pore size over the protein nanopore. In principle, these solid nanopores, once functionalized in the lumen, should be able to capture individual target molecules. One should be able to observe this process from the binding-produced discrete current blocks. However, direct observation of single molecule binding to solid nanopores has been rarely reported. Here we for the first time report the aptamer encoded nanopore for single-molecule biosensing. Aptamers are short DNA or RNA sequences that can fold into specific conformations to bind their target proteins with high affinity and high selectivity. Comparing with antibodies, aptamers are durable in severe environment such as high temperature and extreme pH values. In particular, aptamers are advantageous in nanopore applications due to their smaller volume than their target proteins, allowing target-generated current block to be visualized. In this report, we demonstrate an aptamer-encoded nanopore for single molecule detection of IgE and bioterrorist agent ricin.



3339-Pos Board B386
Label-Free Electrical Biosensing Using Functionalized Nanopipettes
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Nanopipettes are recognized as a versatile tool for engineering and life science studies. Among a variety of applications of nanopipette technology, we are particularly interested in developing a fully electrical sensing platform using nanopipette probes. The dimensions of our nanopipette, comparable to the size of many proteins and macromolecules, make it suitable for sensing biomolecules in general. Molecular interaction on the nanopipette tip are transduced into electrical signals based on changes in size, electrical charges and structures of the nanoscopic pore region.
Here we report the development of nanopipettes that specifically detect their non-labeled target molecules. To reliably identify the interacting molecules, the nanopipettes were individually functionalized by modifying the surface with antibodies. Then two different nanopipettes, one as a probe nanopipette with attached molecules specific to the target molecules (i.e. antigens) and the other as a control nanopipette, were immersed in the same bath solution to perform voltammetric measurement. The addition of target molecules tended to cause a lasting reduction in current flowing through the probe nanopipette, which was large enough to distinguish the probe nanopipette from the control. These results indicate that the target molecules were captured by antibodies specific to them, present only on the probe nanopipette surface, and that this process partially narrowed the pore. The tested target molecules included cancer biomarker proteins, therefore this system could be readily optimized for diagnostic use.

3340-Pos Board B387
Escape Dynamics of DNA from a Nanopore under the Influence of an AC Bias
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Monitoring the escape of ssDNA from a protein nanopore provides new insight into the dynamics of DNA translocation and a direct means of measuring progress of the escape. New instrumentation has been developed that allows for simultaneous application of an AC and DC bias across a protein nanopore. Initial measurements with the system have focused on the capture and release of ssDNA tails attached to DNA hairpins. Polynucleotide tails attached to 24 nucleotide DNA hairpins are threaded into the beta-barrel of an alpha-hemolysin channel under the influence of a strong DC driving voltage. After the capture of the hairpin, the DC voltage is turned off and the subsequent escape of the hairpin is directly monitored via an AC bias. Escape times were measured as a function of AC amplitude (20 to 250 mV), AC frequency (60-200 kHz), DC drive voltage (0 to 100 mV), and temperature (-10°C to 20°C). The applied AC voltage has been shown to play a significant role in the DNA/nanopore interaction. The results are well described by a one-dimensional diffusion model across an asymmetric, periodic potential.

3341-Pos Board B388
Ion-Regulated Assembling Of The G-Quadruplex Aptamer - A Nanopore Single-Molecule Study
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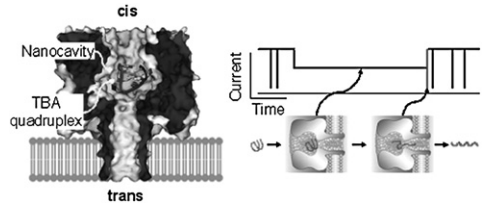
University of Missouri, Columbia, MO, USA.
Guanine-rich DNA and RNA can form high order G-quadruplexes through guanine-guanine base-pairs. G-quadruplexes in genome actively participate in gene regulation; and synthetic G-quadruplexes *in vitro* are potent pharmaceuticals, biosensors and bricks of nanostructures. Here we report on the development of a nanopore encapsulating single-molecule method for exploring how cations regulate the folding and unfolding of the G-quadruplex formed by the thrombin-binding aptamer (GGTTGGTGTGGTTGG). The signature blocks in the nanopore revealed that the G-quadruplex formation is cation-selective. The selectivity sequence is $K^+ > NH_4^+ \sim Ba^{2+} > Cs^+ \sim Na^+ > Li^+$, and G-quadruplex was not detected in Mg^{2+} and Ca^{2+}. Ba^{2+} can form a long-lived G-quadruplex with TBA, but the capability is affected by the cation-DNA interaction. This cation-selectivity is correlated with the G-quadruplex volume, which varies with cation species. Although the Na^+- and Li^+-quadruplexes feature similar equilibrium properties, they undergo radically different pathways. The Na^+-quadruplex folds and unfolds most rapidly, while the Li^+-quadruplex performs both reactions at the slowest rates. This research is beneficial for constructing fine-tuned G-quadruplex-based biosensors. The methodology in this work is also useful for investigating protein-G-quadruplex interactions.



3342-Pos Board B389
Folded and Unfolded Single Proteins Analyzed by Their Solid State Nanopore Translocation Dynamics
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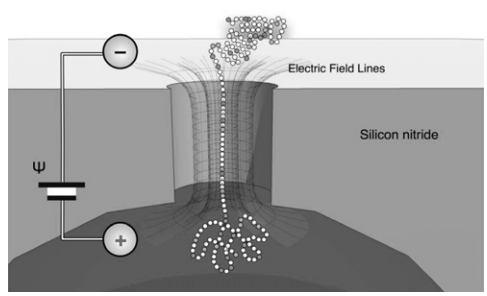
The translocation of biological polymers through individual nanometer-scale pores is vital to cellular function and has great potential for technological applications in protein or nucleic acid measurements and identification. Research into this area has been focussed on characterizing the physics of translocation through voltage-biased nanopores and exploiting it to identify or sequence biological polymers. Here we show that the DNA-calibrated translocation signals of ß-lactoglobulin and histidine-containing phosphocarrier protein match



quantitatively with that predicted by a simple sum of the partial volumes of the amino acids present in the pore when it stalls due to its primary charge. Our analysis suggests that the majority of the protein molecules were linear or looped during translocation suggesting that physiologically relevant potentials can unfold proteins. Our results suggest that the nanopore translocation physics and signals are sensitive enough to distinguish between proteins based on the excluded volume of a local segment of the polypeptide chain and the primary sequence of charges.

3343-Pos Board B390

Controlled Molecular Transport through Nanofilters with Tapered and Cylindrical Pores

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Many applications in molecular separation and sensing technology now require devices with uniformity at the nanometer scale over macroscopic areas. Advanced methods for fabrication and manipulation of such artificial tools can greatly increase process speed, selectivity and efficiency. In this work, we present a new synthesis technique for creating ~mm2 arrays of uniformly tapered nanopores. We investigate the effect of pore size (50-800nm), geometry and surface functionalization on diffusion rates of biomolecules through synthesized membranes. Results are compared against state-of-the-art polycarbonate track etched (PCTE) membranes and other filter technologies. Mass transfer rates are shown to increase up to 15x with tapered geometries compared to cylindrical geometries. Experimental results are supported with molecular calculations.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-ABS-407641.

3344-Pos Board B391

Quantized ionic conductance in nanopores

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We study ion transport through nanopores via molecular dynamics calculations. Due to the confined geometry and large local field of a single ion, the nanoscale atomic configurations of species influence the ionic conductance. In particular, hydration layers that form around ions in aqueous solution create a series of energy barriers to ion transport. As an ion enters the pore, these hydration layers have to be partially broken due to steric restrictions of the pore. The breaking of the layers proceeds in a highly nonlinear, step-like fashion, giving rise to a strong nonlinear dependence of the electrostatic energy barrier on the pore diameter and therefore also a step-like conductance. We discuss this effect as well as the conditions under which it may be experimentally observed.

3345-Pos Board B392

Effect of Valence and Concentration of Counterions on Electrophoretic Mobility of DNA in a Solid-State Nanopore

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Controlling the electrophoretic mobility of DNA in a solid-state nanopore is critical to the development of the nanopore technology for sequencing DNA because, under typical experimental conditions, DNA moves through a nanopore too fast for its sequence to be detected. One could expect that increasing the electrostatic screening of the DNA charge in a nanopore would reduce the force driving DNA through and consequently the DNA translocation velocity. In free solution electrophoresis experiments, increasing either the valence or the concentration of counterions in an electrolyte was shown to affect mobility of DNA. Through extensive all-atom molecular dynamics simulations, we investigated the feasibility of controlling electrophoretic mobility of DNA in a solid-state nanopore. In our simulations, a double stranded DNA molecule is placed in the center of a 3-nm-radius nanochannel. The system is solvated in an electrolyte containing either Na(+), Mg(2+), spermidine(3+) or spermine(4+) ions. An external electric field is applied and the resulting displacement of DNA is recorded. We have found that the valence and concentration of counterions can dramatically alter the electrophoretic mobility of DNA in a nanopore. In monovalent or divalent electrolytes, increasing the concentration was found to decrease the electrophoretic mobility, whereas in spermidine and spermine electrolytes, the direction of the DNA motion could be reversed. Analysis of the interaction between DNA and the surrounding electrolyte revealed that the reduction of the electrophoretic mobility is caused not only by the presence of counterions, but also by the hydrodynamic drag of an electro-osmotic flow near the DNA surface.

3346-Pos Board B393

Translocation Studies of Single Strand-DNA Oligomer Complexes with ds-DNA Markers Using Solid-State Nanopores

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We carried out solid-state nanopore experiments on designed single-stranded DNA molecule complex with double-stranded segments. We have designed short oligomers of single-stranded DNA of about 130 bases long each with 12-bases long sticky ends that are complimentary to those on one end of other oligomers to form ds-DNA regions by Watson-Crick base-pairing in these regions. Such a design facilitates the formation of a chain of single strands DNA with ds-DNA regions interspersed. In order to slow down the translocation speed of these complexes through solid-state nanopores that could enable one to identify the ds-DNA region markers in the blockage current signal during translocation, we have attached these ss-DNA complexes with a polystyrene bead on one end. We present the results of our preliminary studies that show that the signature of these ds-DNA region markers could be identified.

3347-Pos Board B394

Control of Ionic Transport through an Ionic Transistor based on Gated Single Conical Nanopores

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Control of ionic transport through nanofludic systems is a topic of scientific interest both for the ability to create novel devices as well as for the practical understanding of how to replicate the function of membrane protein channels. Because nanopores have large surface to volume ratios, modification of the effective surface charge of a nanopore plays a large role in the nature of the ion transport through it. To this effect, we have prepared a novel ionic transistor from single conical nanopores in polymer films. Control of the ion current through these single conical nanopore transistors is achieved through the deposition of an electrically insulated gold thin film ''gate'' electrode on the side of the polymer film with the small nanopore opening. By changing the electric potential applied to the ''gate,'' the current through the device can be changed from the rectifying behavior of a typical conical nanopore, to the almost linear behavior seen in cylindrical nanopores. This ion current tuning can be achieved with gate voltages that are lower than 1 V. The mechanism for this change in transport behavior is thought to be the enhancement of concentration polarization due to the increase of the effective surface change that occurs with increasing ''gate'' bias. Application of this transistor system for directing and amplifying ionic and molecular fluxes in nanofluidic devices will be discussed.

3348-Pos Board B395

Sifting out Methylated DNA with Synthetic Nanopores

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Methylation of cytosine residues in DNA produces 5-methylcytosine, changing the protein binding affinity of the sequence, hence altering the organization and expression of the surrounding DNA. The pattern of methylation often silences genes, which physiologically orchestrates processes like differentiation, and pathologically leads to cancer. However, current methods for detecting methylation are either limited in resolution and sensitivity or are too expensive and time-consuming with current technology. Here, we report measurements of the permeation of methylated DNA through a synthetic nanopore, using an electric field to force single molecules to translocate one-at-a-time. For pores <3.0 nm in diameter_comparable to the DNA helix_we found an electric field threshold for permeation of methylated DNA that depends on the methylation pattern.