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.

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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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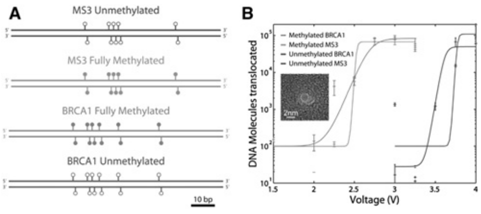
Methylation of cytosine residues in DNA produces 5-methylcytosine, changing the protein binding affinity of the sequence, hence altering the organization and expression of the surrounding DNA. The pattern of methylation often silences genes, which physiologically orchestrates processes like differentiation, 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.



Shown below are the results from a comparison of fully methylated and unmethylated versions of the BRCA1 and MS3 genomic DNA fragments(A). Using quantitative PCR, we measured the number of DNA molecules which translocated through a 1.8nm nanopore at a given voltage(B). Unmethylated MS3 and BRCA1 translocate above 3.77V and 3.61V, respectively while the thresholds for fully methylated MS3 and BRCA1 are 2.53V and 2.69V respectively.

3349-Pos Board B396

Static and Dynamic Investigations of RecA-DNA Complex with a Solid-State Nanopore

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In recent years, artificial nanometer-sized pores (nanopores) have been successfully employed as a new tool to detect the passage of DNA molecules. When a molecule is driven through the nanopore by an externally applied electric field, it blocks part of the ionic current, resulting in a temporal current blockade. So far, only translocations of bare DNA have been reported. In this work, we extend this to protein-coated DNA molecules by including the well-studied RecA protein, which plays an essential role in the central steps of homologous recombination. To accomplish its DNA strand exchange activities, RecA polymerizes onto DNA to form a stiff helical nucleoprotein filament. These filaments translocate through solid-state pores, as we demonstrate unambiguously. Additionally, we use the nanopore system in conjunction with an integrated optical tweezer, which allows us to insert the RecA-DNA complex into the nanopore. We will discuss results as they pertain to both the charge structure and conformation of the complex. This work also lays the groundwork for future experiments on sequential screening of proteins, say transcription factors, which locally coat DNA.

3350-Pos Board B397

Controlled DNA Translocation Through a Nanopore Membrane with Different Electrostatic Landscapes

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We present computational modeling of a DNA translocation through a nanopore membrane with different electrostatic landscapes. The simulated membrane-DNA system is immersed in a biased electrolyte solution to induce DNA translocation. Thin electrically tunable membrane composed of two layers of n-type and p-type semiconductor materials is used to obtain distinct electrostatic potential landscapes in the nanopore. A simple charges-and-springs model is used to model single stranded DNA molecule. Electrostatic potential landscapes in the nanopore with one and more potential extrema are compared, and their effect on DNA translocation is studied. We show that electrostatic potential landscape in the nanopore controls the translocation of DNA through the nanopore. In particular, we specify different conditions under which DNA translocates through the nanopore in one-nucleotide-at-a-time fashion, can be pulled back and forth as well as paused in the nanopore.

3351-Pos Board B398

What Is The Nature Of Interactions Between DNA And Nanopores Fabricated In Thin Silicon Nitride Membranes?

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Manipulating the drag force acting on charged biopolymers, such as DNA, during their passage through solid-state nanopores is critical for various single-molecule applications for biotechnology (e.g., sequencing, genotyping, DNA/protein interactions). We have recently shown for solid-state nanopores in silicon nitride (SiN) that small variations in the nanopore diameter, temperature, and voltage, strongly affect the biopolymer's average sliding velocity, indicating that in this size regime DNA transport is governed by DNA/pore attraction[1]. However, the exact nature of these interactions has remained to date unknown. To elucidate the character of these nanopore/DNA interactions, we have performed two independent types of experiments: First, we investigated the capture rate of DNA as a function of temperature. Surprisingly, our temperature measurements reveal anti-Arrhenius behavior, suggesting a two-step capture process. Second, we mapped the spatial distribution of charged biopolymer in the vicinity of the SiN membrane, using a custom confocal microscope equipped with a nano-positioning stage. Our measurements reveal a salt-dependent enhancement of DNA concentration in the vicinity of the SiN. These combined results suggest that electrostatic DNA/pore and DNA/membrane interactions are present between the weakly positive SiN surface and the negatively-charged DNA. In this presentation, these results are discussed and a model that explains these and previously reported observations is presented.

[1] Wanunu, M. Sutin, J. McNally, B. Chow, A. and A. Meller (2008) DNA Translocation Governed by Interactions with Solid State Nanopores, Biophys. J., 95 (11), in press.

3352-Pos Board B399

Nucleotide Identification and Orientation Discrimination of DNA Homopolymers Immobilized in a Protein Nanopore

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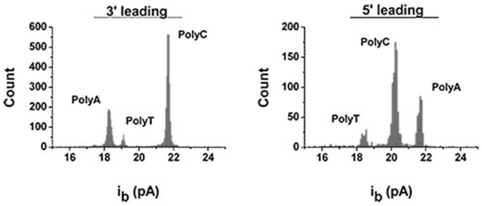
Nanopores have been used as extremely sensitive resistive pulse sensors to detect analytes at the molecular level. There has been interest in using such a scheme to rapidly and inexpensively sequence single molecules of DNA. To establish reference current levels for adenine, cytosine, and thymine nucleotides, we measured the blockage currents following immobilization of single-stranded DNA polyadenine, polycytosine, and polythymine within a protein nanopore in chemical orientations in which either the 3′ or the 5′ end enters the pore. Immobilization resulted in low-noise measurements, yielding sharply defined current distributions for each base that enabled clear discrimination of the nucleotides in both orientations. In addition, we find that not only is the blockage current for each polyhomonucleotide orientation dependent, but also the changes in orientation affect the blockage currents for each base differently. This dependence can affect the ability to resolve polyadenine and polythymine; with the 5′ end entering the pore, the separation between polyadenine and polythymine is double that observed for the 3′ orientation. This suggests that, for better resolution, DNA should be threaded through the 5′ end first in nanopore DNA sequencing experiments.



Bioinformatics & Structure Prediction

3353-Pos Board B400

Combining Protein-protein Interaction Networks with Structures

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Recent studies have shown that proteins involved in cancer can exhibit a different network topology than that of non-cancer proteins (Jonsson et al). Here, we study the interface and network properties of the cancer and non-cancer proteins in the human protein-protein interaction network. The interfaces of hub proteins are predicted by PRISM web-server (Aytuna et al.; Ogmen et al.). We classify hub proteins as singlish-interface or multi-interface according to the number of distinct binding interfaces of the hub proteins (Kim et al). The interactions of the singlish interface hubs and multi-interface hubs are the so-called transient and permanent interactions, respectively. We analyze the interface properties (such as interface area, amino acid composition of the interface, and conservation of the interface) of these transient and permanent interactions. We observe different patterns of interface properties. Based on these, we can distinguish the interface properties of cancer and non-cancer proteins. In order to include structural information in the topological analysis, we relate the interface properties to the network properties. Interestingly, we find that the singlish-interface hubs correspond mostly to hub-bottlenecks (hubs with high betweenness value), constituting dynamic components of the network, whereas the multi-interface hubs correspond mostly to the hub-nonbottlenecks (hubs with low betweenness value).

Kim PM, Lu L, Xia Y, Gerstein M: Relating 3D structures to protein networks provides evolutionary insight Science 2006, 314: 1938-1941.

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Ogmen U, Keskin O, Aytuna AS, Nussinov R, Gursoy A: PRISM: protein interactions by structural matching. Nucleic Acids Res 2005, 33(Web Server issue):W331-336.