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.

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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.

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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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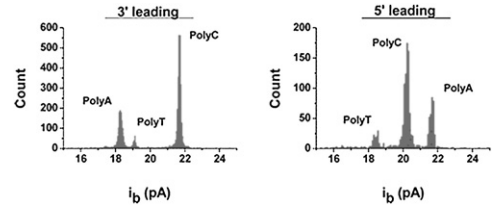
Nanopores have been used as extremely sensitive resistive pulse sensors to detect analytes at the molecular level. There has been interest in using such a scheme to rapidly and inexpensively sequence single molecules of DNA. To establish reference current levels for adenine, cytosine, and thymine nucleotides, 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).

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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.