MspA’s constriction. Our results indicate that MspA has high signal-to-noise and single nucleotide sensitivity for nanopore sequencing.

924-Pos  Board B724
Characterizing and Controlling the Motion of ssDNA in a Solid-State Nanopore
Binquan Luan, Gustavo Stolovitzky, Hongbo Peng, Stefan Harrer, Philip Waggner, Stan Pochapsky, Stephen Rossnagel, Glenn Martyna.

Essential to all nanopore-based DNA sequencing technologies is the ability to control motion of a single-stranded DNA (ssDNA) at single-base resolution. Experimental studies show that the average translocation speed of DNA driven by a biasing electric field can be affected by ion concentration, solvent viscosity or temperature. Despite of slowing down the average translocation speed, the instantaneous motion of DNA is too diffusive to allow each DNA base to be paused in front of a sensor suite for measurement. Using extensive all-atom molecular dynamics simulations, we study the diffusion constant, friction coefficient, electrophoretic mobility, and effective charge of ssDNA in a solid-state nanopore. Simulation results show that the spatial fluctuation of ssDNA in one nanosecond is comparable to the spacing between neighboring DNA bases, which makes the sensing of a DNA base very difficult. We demonstrate that the recently proposed DNA transistor could potentially solve this problem by electrically trapping ssDNA inside the DNA transistor (Appl. Phys. Lett. 91, 153103, 2007) and ratcheting ssDNA base-by-base in a biasing electric field (Phys. Rev. Letts. 104, 238103, 2010). We observed different types of translocations of ssDNA when increasing the biasing electric field. The simulated translocation of ssDNA in the DNA transistor was theoretically characterized using Fokker-Planck analysis.

925-Pos  Board B725
Hybrid Biological/Solid-State Nanopores
Adam Hall, Andrew Scott, Divr Rotem, Kunal Mehta, Hagan Bayley, Cees Dekker.

Biomolecular translocation through the bacterial nanopore α hemolysin (αHL) has been suggested as a possible platform for next-generation sequencing technology. In this method, a single protein pore is inserted in an otherwise impermeable lipid membrane and an applied electric field is used to drive charged biomolecules like DNA through it. Information like nucleotide sequence could then conceivably be read from the molecule as it translocates linearly to the other side. One challenge to the incorporation of this system into wafer-scale device architectures, however, is its reliance on a delicate lipid bilayer for mechanical support. The solid-state (SS) nanopore—composed of a single, fabricated aperture in a thin, solid-state membrane—offers a potential solution with demonstrated durability and integration potential. But, this system lacks the atomically resolved interior structure and genetic engineering ability of the protein pore. Here we present a potential solution that consists of a combination of the two systems, wherein a single αHL protein pore is inserted directly into a SS nanopore with orientation control, yielding a robust hybrid system.

926-Pos  Board B726
Engineering Biological Nanopore MspA for Sequencing DNA
Aleksei Aksimentiev, Swati Bhattacharya, Anthony Ho.

The sequence of a long DNA molecule can be determined, in principle, by measuring the ionic current blockade the molecule produces as it permeates a nanometer-diameter pore in a thin insulating membrane. The difficulties in realizing this idea in practice are common to both biological and synthetic nanopores: the pore geometry does not permit isolation of a single nucleotide and the DNA molecule moves too fast through the nanopore for its sequence to be determined by the ionic current measurement. Here, we report our progress in engineering biological pore MspA for sequencing applications. It has been experimentally shown that DNA strands immobilized inside the MspA pore produce ionic current blockades that permit identification of a single nucleotide substitution in the DNA sequence [doi:10.1073/pnas.1001831107]. Through all-atom molecular dynamics simulations we investigate the molecular origin of such extreme sensitivity of the ionic current to the sequence and orientation of DNA strands. Furthermore, we demonstrate the feasibility of reducing the rate of DNA transport by introducing point mutations in the MspA structure. Spanning tens of microseconds, our simulations provide the most detailed account of the atomic-scale mechanics of DNA and ion transport through biological nanopores.

927-Pos  Board B727
A Nanopore Sensor for Single Molecule Detection of Circulating MicroRNAs in Lung Cancer Patients
Yong Wang, Dali Zhe, Qiu Lin, Michael Wang, Li-Qun Gu.

Developing new technologies for cancer screening and early diagnosis is a critical issue for saving cancer patients’ lives. Our recent effort has led to the first nanopore biosensor in clinical disease detection. The targets are microRNA (miRNAs), a class of short (~18-24 nt) non-coding RNAs molecules that regulate gene expression at the post-transcriptional level. Aberrant expression of miRNAs has been found in all types of tumors. Thus miRNAs have been recognized as potential cancer biomarkers. Most notably, specific miRNAs are released from the primary tumor into blood circulation, making the detection of circulating miRNAs profile a powerful tool for noninvasive cancer detection, diagnosis, staging, and monitoring. Guided by designed programmable oligonucleotide probe, single miRNA molecules captured in the nanopore produce a signature current signal that function fingerprints, enabling us to identify a specific miRNA and quantify its concentration. The prototype of nanopore sensor has demonstrated the capability to discriminate single nucleotide differences between miRNAs (single nucleotide polymorphisms, SNPs). In clinical tests, the nanopore has shown the power to differentiate miRNA levels in blood from lung cancer patients and healthy people. This simple, sensitive, label-free technique requires no amplification for miRNA detection as in the RT-PCR method, has the potential for noninvasive and cost-effective early diagnosis of lung cancer. If validated in clinical trial, the nanopore sensor will become a system available to monitor cancer patients and to screen high risk populations for early diagnosis of cancers which will potentially save the lives of millions.

928-Pos  Board B728
Ultrathin Solid-State Nanopores as Biomolecular Discrimination Elements
Meni Wanunu.

Molecular-sized holes through ultrathin membranes are simple yet powerful reporters of biomolecular structure. In the regime where the nanopore size approaches the critical dimension of a biomolecule, nanopores are extremely useful as sensors with discrimination capabilities. In this presentation, I will show how synthetic nanopores with a length comparable with lipid membranes enable a precise discrimination among small biomolecules. I will demonstrate how these pores can detect a specific microRNA extracted from a biological sample, as well as examples in which discrimination among biomolecules was achieved. Also, I will describe the use of nanopores as discrimination among biomolecules that have slight chemical alterations.

929-Pos  Board B729
Micro-RNA Detection Using Nanopore Force Spectroscopy with MspA

The importance of small regulatory molecules known as micro-RNA (miRNA) has become increasingly prominent, in part due to their potential clinical use as biomarkers for the early-detection of various cancers. The current methods of miRNA detection involve microarrays or qPCR, each with distinct applications. We present an orthogonal and single molecule technique that has the potential to electronically detect miRNA. The technique relies on the nanopore MspA, a transmembrane pore that allows single-stranded DNA, but not double-stranded DNA, to be electrophoretically driven across a membrane. To detect miRNA we use a DNA probe that is threaded through MspA and bound with streptavidin that is too large to pass through the pore. The chosen probe anneals to miRNA and forms a double-strand that must be sheared to clear the pore of the DNA probe. We are able to specifically detect miRNA-probe duplexes using force spectroscopy and by reading the ionic current passing through MspA threaded with the DNA probes. This detection technique may provide a complementary means of examining miRNA.