signature functions as a marker, enabling us to recognize the binding of a dsDNA and its unzipping process, therefore is useful for discriminating different sources of blocks in real-time biosensing.

3109-Pos
Sensing and Actuation with a Native GP10 Nanopore
David Wendell.
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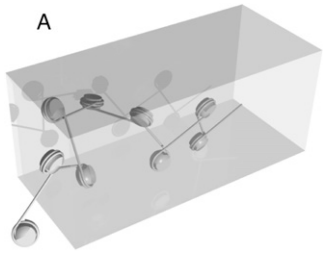
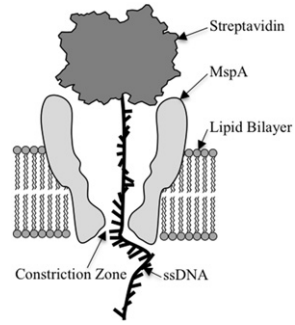
Previous GP10 nanopore studies have been limited to a c-terminal His-tag mutant. Here we show that native GP10 can incorporate into a lipid membrane and that the conductance appears to be restricted by both the variable region and the c-terminal crown, areas known to interact with the viral DNA but unresolved in the crystal structure. In addition to the electrophysiology of the channel, we explore the effects of the lipid membrane environment and show the discrimination of different lengths of dsDNA using dwell-time within the pore. We also present an engineered form of GP10 that is rendered photosensitive using a covalently attached azobenzene derivative. This attachment scheme allows us to modulate the conductance of the pore and control passage of dsDNA. Several biological nanopores have been engineered for stochastic sensing and DNA sequencing applications; the aperture size and electrical stability of GP10 makes it an equally attractive candidate for such endeavors.

3110-Pos
Epigenetic Analysis of Chromatin in Nanochannels
Diana E. Streng, Shuang F. Lim, **Robert Riehn**.
North Carolina State University, Raleigh, NC, USA.
Nanochannels with a diameter of about 100nm2 are a novel method for stretching DNA for genomic investigations. Such devices are implemented through standard nanolithography in fused silica. The elongation of DNA results from an interplay of steric and entropic effects. Previous applications of nanochannel stretching included sizing, restriction mapping, and observation of transcription factor binding.
We show here that nanochannels can also be used to map the site-specific epigenetic state of DNA. In particular, we show here that the concept by nanoconfinement can be extended to chromatin, or DNA complexed to histones, and that the stretching is within the range expected from the de Gennes theory. We also demonstrate that the location-resolved cytidine methylation state of DNA can be mapped by specific fluorescent labeling. We will discuss the basic operation of these technique, and the application to artificial substrates with predefined epigenetic marks.



3111-Pos
Prolonged Excursion of a Single Protein into a Synthetic Nanopore
David J. Niedzwiecki, Liviu Movileanu.
Syracuse University, Syracuse, NY, USA.
Nanopores drilled into silicon nitride were used as stochastic sensors to inspect protein analytes at the single molecule level. Measurements on the protein bovine serum albumin (BSA) revealed both short-lived current spikes, in the range of tens of microsends, and long-lived current blockades, in the range of seconds. The presence of long-lived current blockades suggests a strong interaction between BSA molecules and the nitride surface of the nanopore interior. The nature of these long duration interactions was explored under a variety of conditions. Single-channel current analysis indicated that this interaction does not follow a simple bimolecular kinetic pathway. We hypothesize that BSA enters the nanopore in a non-equilibrium state in order for such interactions to occur.

3112-Pos
Single Nucleotide Discrimination in Single Stranded DNA Immobilized within Biological Nanopore MspA
Elizabeth A. Manrao[1], Marcus D. Collins[1], Ian M. Derrington[1], Kyle W. Langford[1], Mikhail Pavlenok[2], Michael Niederweis[2], Jens H. Gundlach[1].
[1]University of Washington, Seattle, WA, USA, [2]University of Alabama, Birmingham, AL, USA.
Biological nanopores are currently being investigated as a fast, low cost DNA sequencing platform. Single stranded DNA (ssDNA) is electrophoretically driven through a protein pore as the ionic current through the constriction is measured. The porin MspA of Mycobacterium smegmatis was mutated to produce a channel highly suitable for nanopore DNA sequencing. To study the resolution of the mutated porin MspA, we immobilize ssDNA within the pore using a streptavidin 'anchor'. Each base, adenine, cytosine, thymine, and guanine, produces a distinct current signature when it is held within the nanopore. We examine homopolymer ssDNA with a single heteronucleotide substitution to determine the recognition site within MspA. Discrimination of a single base in a heteromeric ssDNA is performed with two single nucleotide polymorphisms (SNPs) where the polymorphism is positioned at the recognition site. Our results indicate that MspA has the ability to provide high-resolution single nucleotide discrimination.



3113-Pos
Hydrophobic Gating in Synthetic Nanopores
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In nature, nanopores play a critical role in a number of vital biological functions and understanding this role is just as critical. These pores can be ion selective based on their size and/or surface charge, but further functionality is achieved by modulating, or gating, their conductance state. The conductivity of a particular nanochannel can be controlled in a number of ways, including mechanically, chemically and electrically. By studying these phenomena in model systems, we may be able to take large steps towards understanding the underlying fundamental physics phenomena behind these mechanisms. Here, we present what we believe to be the first study to show the gating of a synthetic channel based on its hydrophobicity, which has been observed to be a natural gating mechanism in mechanosensitive channels.
Using nanopores prepared in polyethylene terephthalate (PET) by the track-etching technique, we show that it is possible to decorate the pore surface with hydrophobic chemical groups and that these significantly alter the properties of the pore. Prior to modification, aqueous electrolytic solutions are able to conduct readily through the pore, but afterwards, the pore demonstrates closed and open states. This behavior is also observed to be voltage dependent. Increasing voltage increases the probability of the pore to be in the open states. There is also a voltage range where the pore does not conduct at all. The hydrophobic gating was studied as a function of pore diameter and charge of the residual groups.

3114-Pos
Ion Channels in Nanoscale Apolipoprotein Bound Bilayers
Sourabh Banerjee, Crina M. Nimigean.
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Nanoscale apolipoprotein bound bilayers (NABBs) and similar nanolipoprotein particles have been used to purify and study complex transmembrane proteins in a native-like lipid environment. NABBs are stable, homogeneous discoidal lipid bilayers, approximately 10 nm in diameter, formed by a stoichiometric mixture of zebrafish apo A-I protein (zap1) and lipids. [1] We now report the use of NABB technology to study ion channels. As a proof-of-principle, we reconstituted a well-characterized potassium channel KcsA, containing a non-inactivating mutation E71A, into NABBs and evaluated transfer of channels from the discoidal NABBs to black lipid membranes (BLMs). The channels transferred readily from the NABBs to BLMs. Single channel recordings of KcsA E71A transferred from NABBs were identical to the channel transferred using liposomes. The electrical properties of the BLM were unaffected by NABBs in the absence of channels. Electron microscopy imaging was performed to further characterize NABBs containing KcsA and other potassium channels. The NABBs are thus an ideal platform for further functional assays of detergent-labile ion channels. [1] S. Banerjee, T. Huber, T.P. Sakmar. 2008. Rapid Incorporation of Functional Rhodopsin into Nanoscale Apolipoprotein Bound Bilayer (NABB) Particles. J. Mol. Biol. 377, 1067-1081.

3115-Pos
Nanopore Translocation Experiments in Microemulsion Droplets
Stephan Renner, Sandra Geltinger, Friedrich C. Simmel.
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We show that a novel bilayer formation technique based on microemulsion droplets introduced by Bayley and coworkers can be utilized to perform nanopore DNA translocation experiments. In this technique, a bilayer is formed between two touching emulsion droplets.