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
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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
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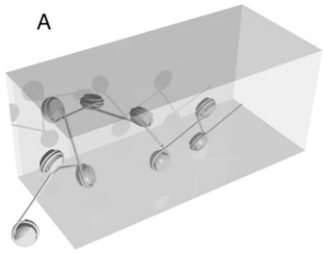
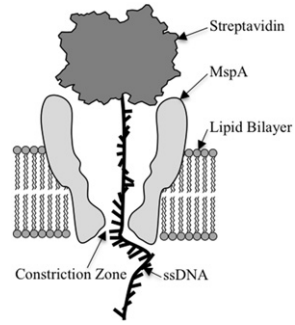
Nanochannels with a diameter of about 100nm2 are a novel method for stretching DNA for genomic investigations. Such devices are implemented through standard nanolithography in fused silica. The elongation of DNA results from an interplay of steric and entropic effects. Previous applications of nanochannel 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
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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
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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.

By reduction of their contact area, we were able to reduce the measurement noise sufficiently to perform single molecule translocation and even nanopore force spectroscopy experiments. We applied this technique to the translocation of DNA hairpin molecules, but also to a G-quadruplex DNA structure, which has not been characterized using nanopore force spectroscopy before. From the data, the unfolding rates of these DNA structures are extracted and compared with those obtained with other single molecule techniques.

3116-Pos

Studying Voltage Dependent Noise in Polymer and Solid State Nanopores

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Studying the noise properties of ion currents in nanopores can improve detection limits for nanopore sensors as well as give insight into behavior of transport at the nanoscale. We focused on the so-called 1/f noise that is observed in the low frequency regime of the ion current power spectra. We found that 1/f noise in single conically shaped nanopores in polymer films exhibits voltage-dependent noise properties, which are not observed for cylindrical pores. The current passing through the nanopore in the low conductance state shows equilibrium 1/f noise, similar to the noise observed in solid state nanopores. Equilibrium fluctuations are defined as the voltage independent power spectrum magnitude normalized by the current squared. The high conductance state causes the 1/f noise to increase exponentially with increased applied voltage, showing a non-equilibrium 1/f noise. Therefore we can switch between the equilibrium and non equilibrium behavior simply by adjusting the voltage. The current in the high conductance state is about 5 times higher then the current in the low conductance state but the noise at 1 Hz is over 100 times higher. Cylindrically shaped nanopores in polymer and solid-state films do not show current rectification and show equilibrium 1/f noise. We discuss these results and give a comparison of the nanopore noise in these various systems. We hypothesize that the non-equilibrium current fluctuations originate from structural fluctuations of flexible polymer pores. The hypothesis is tested by comparison of noise properties between polymer and silicon nitride pores studied at different electrolyte concentrations.

3117-Pos

Slowing DNA Translocation through Nanopores using Organic Salt Solutions

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One of the key challenges to nanopore DNA sequencing is to slow down DNA translocation. Here, we report that the translocation velocities of various DNA homo- and co-polymers through protein pores could be significantly decreased by using electrolyte solutions containing organic salts. Using a butylmethylimidazolium chloride solution instead of the commonly used KCl solution, DNA translocation rates on the order of hundreds of microseconds per nucleotide base were achieved. The much enhanced resolution of the nanopore coupled with the different event blockage amplitudes produced by different nucleotides permits the convenient differentiation between various DNA molecules.

3118-Pos

Electrophysiological Method for Quantification of the Number of phi29 DNA Packaging Nanopore in Planar Bilayer Membrane

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Bacterial virus phi29 uses one of the strongest DNA packaging nanomotors to package its micron-length genomes into a pro-capsid. After re-engineering, whether the DNA nano-motor can be used to pump drugs, DNA, RNA or other therapeutic molecules into specifically targeted cells represent a great challenge in nanomedicine. We have recently successfully embedded the connector, a core component of phi29 DNA package motor, into a planar bilayer membrane (BLM). Under an electric field, double-stranded DNA translocated through the connector channel.

The application of phi29 connector array as a stochastic sensor requires knowledge of the number of channels on each membrane. We herein report a method for precise counting of the number of channels on each membrane by electrophysiological approach. Generally, the number of channels is determined by the conductance of total channels and conductance per single channel. Using a derived empirical equation, we can calculate the conductance per single channel at any salt concentration using conductivity of respective conducting buffer. The total conductance of total channels can be measured by ionic current through all the channels under an applied ramp voltage. Comparison of calculated and true values established this as a feasible, reliable and reproducible approach.

3119-Pos

Ultrathin Nanopores for Nucleic Acid Analysis

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Over the past decade, synthetic nanopores in solid-state membranes have gained reputation as platforms for studying the biophysical properties of biopolymers. In this presentation, the precise fabrication of ultrathin nanopores and their utility for analyzing complex nucleic acid samples will be discussed, with major emphasis on their resolution capabilities for different biopolymers. The membrane thickness was found to play an important role on the signal obtained from different biopolymers. These findings are critical for developing ultrasensitive nanopore assays which profile biopolymer structure, important for genomics and other biophysical studies.

3120-Pos

Single Channel Sensing of dsDNA using the Membrane- Adapted Nanopore of phi29 DNA Packaging Motor

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The bacteriophage phi29 DNA-packaging motor, geared by six pRNA molecules, contains a truncated cone shape connector channel that is 3.6nm in diameter at its narrow end and 6nm in diameter at its wide end. This channel allows dsDNA to enter and exit the virus procapsid during virus maturation and infection, respectively. We modified the genes that code for the core of the phi29 DNA packaging motor in order to change the amino acid sequence of its protein for membrane incorporation. The modified connector was reconstituted into liposomes and fused into a planar lipid bilayer membrane. Distinctive current jumps were found for each connector insertion. The conductance of each connector channel was measured and found to be uniform (4.8nS in 1M KCl). The membrane embedded connector channel was found to be able to translocate dsDNA. The translocations were recorded as blockage events of the current. The blockages were equal for each of the individual channels, generating a clean, homogenous and uniform signal representing DNA translocation.

The connector channel is larger than the previously studied ion channels, which could only let ssDNA pass. The robust property of the connector in ion and dsDNA tranlocation has extensive potentials in microelectromechanical sensing, microreactors, gene delivery, drug loading and DNA sequencing. Single molecule and low concentration sensing can be achieved using this membrane embedded connector system. Additionally, the available crystal structure of the connector protein makes it easy to modify the channel for specific applications and the established large scale purification procedure of the connector will facilitate its practice.

3121-Pos

Fingerpriting of DNA and RNA using the Channels of Bacteriophage phi29 DNA Packaging Motor

Farzin Haque, Peng Jing, Jia Geng, Chris Stites, Peixuan Guo.
University of Cincinnati, Cincinnati, OH, USA.

Living systems contain a wide variety of nanomachines and highly-ordered structures of macromolecules that could serve as modules, tool boxes or building blocks in nanotechnology. The ingenious design of the bacteriophage phi29 DNA packaging motor with an elegant and elaborate channel has inspired its application for single molecule detection and sensing. The central component of the phi29 motor is the connector composed of twelve copies of the protein gp10, which form a dodecamer channel. The connector after incorporation into a lipid bilayer can serve as a detector for extremely sensitive, reliable, and precise sensing and fingerprinting of ions and macromolecules at the single molecule level (Nature Nanotechnology, in press). Double stranded and single stranded DNA can be electrophoretically driven through the channel in a concentration and voltage dependent manner. Information about the structure, length and conformational dynamics can then be deduced by their characteristic dwell time during translocation and by their relative percentage in current blockades. This protein nanopore system with explicit engineering capability has potential technological applications such as rapid DNA sequencing, gene therapy and controlled drug delivery.

3122-Pos

Inhibition of the Voltage-Gated Sodium Current and Opening of Nanopores By Ultra -Short Electric Pulses

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Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA, USA.

Exposure of mammalian cells to high-voltage, ultra-short electric pulses (USEP) leads to formation of membrane nanopores and alters multiple physiological processes, including function of voltage-gated channels. However, it is not known if USEP affect the channels directly, or the effects are mediated