in vitro and *in vivo* to 'tag' specific sequences. Moreover, the ability of PNA to locally displace one of the strands in double-stranded DNA (dsDNA), thus forming a P-loop, makes PNA an ideal candidate for dsDNA sequence detection. Here, we demonstrate a purely electrical detection method of short (8mer) sequences in dsDNA. Sub-5nm solid-state nanopores have recently demonstrated their capability in sizing DNA molecules as they translocate across the pore. Based on this finding, we show for the first time that short dsDNA sequences can be detected, label-free, on the single molecule level. We find that a ~3.5 kbp long dsDNA 'tagged' with short PNA probes induces distinct secondary blockade levels in excess of those found on typical dsDNA molecules. Additionally, tagged molecules displayed significantly increased translocation times - and an increase in the distribution of those times. Furthermore, we demonstrate the ability to statistically discriminate between multi-tagged DNA and untagged DNA. We thus have established a foundation for the development of a radically new single-molecule platform for ultra-fast pathogen and mutation diagnostics[1], ultimately impacting our ability to effectively respond to emerging infections or disease development on a personal level.

1) Singer, A., et al, (2009) "Nanopore-based sequence-specific detection of duplex DNA for genomic profiling" *Journal of the American Chemical Society* (under review).

3104-Pos

Discriminating Bases by Stretching Double-Stranded DNA in a Nanopore

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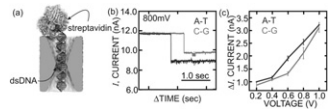
We report a new method for trapping a single molecule of double-stranded DNA (dsDNA) in a solid-state nanopore in SiNx membrane and describe the prospects for sequencing it. It is possible to trap a single dsDNA molecule in a nanopore <3nm in diameter by first applying a voltage larger than this threshold and forcing the molecule to translocate through the pore. If the electric field is then rapidly switched to a value below threshold, the DNA becomes trapped for seconds in the pore, compared to a sub-millisecond translocation if the field is maintained above threshold. Moreover, if the duration in the trap is commensurate with the bandwidth we can discriminate distinct signatures of C-G and A-T base-pairs by simply measuring the pore current. Molecular dynamics simulations of these experiments reveal that, when trapped, the dsDNA is stretched in the pore in a specific tilted orientation, depending on the orientation of the leading nucleotides, while the B-form canonical structure is preserved outside the pore. Finally, we show using streptavidin bound biotinylated dsDNA (Fig. 1a) that it's possible to discriminate stretched basepairs in the trapped configuration (Fig. 1b,c) between A-T and C-G.



3105-Pos

Single Stranded DNA Translocation in Small Solid State Nanopores

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University of Arkansas, Fayetteville, AR, USA.

We present the translocation of single stranded DNA (ssDNA) through ion beam sculpted solid state nanopores. Several lengths of ssDNA, 5386, 1079, 132, and 100 bases long, were measured in nanopores under denaturing and non-denaturing conditions by varying pH and temperature. Small nanopores, 3-4 nm in diameter, were used to slow the translocation times of 1079 base ssDNA molecules. Double stranded DNA (dsDNA) with the lengths of 5386 and 1079 bases were also measured with the same nanopores to serve as a control. The current drop amplitude and translocation time of ssDNA and dsDNA of the same length are compared. In addition, translocation of the 1079 base pair double strand section from the PhiX174 genome was verified by PCR amplification and gel electrophoresis, a first for ion beam fabricated pores. We also discuss the implications that differentiation between ssDNA and dsDNA and the slowing of ssDNA translocation have on the development of nanopore based DNA sensing applications.

3106-Pos

Towards Ultra-Fast DNA Sequencing using Nanopores and Parallel Optical Readout

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Dramatically reducing the cost of DNA sequencing will revolutionize the healthcare system by enabling patient genomes to be determined in routine procedures. This belief has resulted in large scale investments in alternative sequencing methodologies. One of the most promising techniques to emerge is nanopore sequencing, where individual biomolecules are electrophoreticaly threaded through nanoscale pores. We are developing a novel, nanopore based DNA sequencing platform, which revolves around the unzipping of converted DNA in a solid-state nanopore[1]. Sequence information is attained with fluorescent probes attached to the DNA using a custom 2-color wide-field mode of detection. The key advantages of this single-molecule method are enzyme free readout processes, and massive parallelization using Total Internal Reflection (TIR) optics. Here we report, for the first time, on our ability to identify all 4 bases in an automated manner, with a high level of certainty and speed. This level of certainty is achieved as a result of the high signal to background in our custom TIR system, and the unzipping mechanism which un-quench the fluorophores at the time of detection. A key element to increasing the speed of sequencing with nanopore-based methods is massively parallelizing the readout. We demonstrate the feasibility of this by the simultaneous detection of optical unzipping events in multiple nanopores. These results strongly support the utility of nanopores in the field of DNA sequencing.

1) see: Branton, D. *et. al.* Nature Biotechnol. 26, 1146 (2008)

3107-Pos

Revealing Programmable Ion-Exchange in a G-quadruplex using the Nanopore Detector

Ji Wook Shim, Qiulin Tan, **Li-Qun Gu**.
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Guanine-rich DNA and RNA can form high order G-quadruplexes through metal ion-coordinated guanine-guanine base-pairs. G-quadruplexes in genome actively participate in gene regulation, and *in vitro* designed G-quadruplexes are potent pharmaceuticals, biosensors and building bricks of nanostructures. We have electrically visualized the trapping of a single G-quadruplex in the nanocavity enclosed by the alpha-hemolysin nanopore. The characteristic conductance blocks allowed us to discriminate between a folded G-quadruplex that is trapped in the nanocavity and an unfolded linear-form DNA that simply translocates through the nanopore [*J.Phys.Chem.B* 112, 8354-8360 (2008)]. This ability has enabled the study on the ion-selective folding/unfolding of a single G-quadruplex [*Nucl.Acids.Res* 37, 972-982 (2009)]. In this report, we uncover another important G-quadruplex process, **ion-exchange**, by examining the G-quadruplex formed by the thrombin-binding aptamer (TBA) in various designated ion mixtures. In the mixture of Na^+ and K^+, the G-quadruplex residing time in nanopore was prolonged and the occurrence of unfolded linear TBA translocation was reduced as the K+ concentration gradually increases from 0 mM to 500 mM, convincing that K^+ is highly sensitively binding with G-quadruplex, and the continuous K^+ exchange in G-quadruplex elongates the lifetime of G-quadruplex. In contrast, in the mixture of Li^+ and Na^+, the G-quadrpulex stays shorter in nanocavity and the occurrence of linear TBA was reduced to the similar level with that by pure Na^+, indicating that the Na^+ is highly preferred, compared to Li^+, to intrude into G-quaruplex after Li^+ leaves, and unfolds G-quadruplex into linear TBA. This research is to support the understanding of molecular kinetics tuned by environmental factors, and the result may apply for ion-regulating programmable biosensors and novel nanobiotechnology.

3108-Pos

A Novel Single Molecular Signature for Discriminating DNA Unzipping in a Nanopore

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Using nanopore for DNA unzipping has been extensively studied. By measuring the voltage-dependent duration of current blocks produced by the unzipping process, one can evaluate the force and energy involved in the double strands hybridization. However, in the real-time biosensing, other molecules co-existing in the mixture may also produce blocks in similar amplitude and duration. Therefore a characteristic current signature is needed to discriminate the unzipping signal from other blockades. Here we identify a novel molecular signature that can reveal sequential steps in DNA unzipping in the nanopore. When the double-stranded DNA (dsDNA) containing a single-stranded tag at the terminal is trapped in the α-hemolysin pore from the cis mouth, the tag initially sticks into the β-barrel by blocking the pore to Level 1 (15% of the full conductance, +150 mV), whereas the double-stranded section is stopped from entering due to its wider dimension than the entry of β-barrel. Once the unzipping occurs, the longer ssDNA (with the tag) first runs through the β-barrel and leaves the pore from the trans opening driven by the voltage, while the shorter ssDNA remain trapped in the nanocavity of the pore. This configuration gives rise to the less blocking Level 2 (38% of the full conductance). After waiting in the nanocavity for hundreds of microseconds, the shorter ssDNA ultimately traverses the β -barrel, switching the conductance back to Level 1. This unique molecular

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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University of Cincinnati, Cincinnati, OH, USA.
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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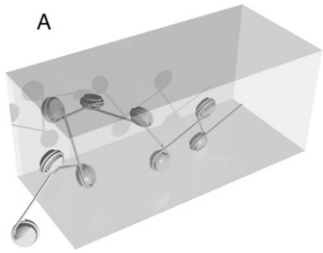
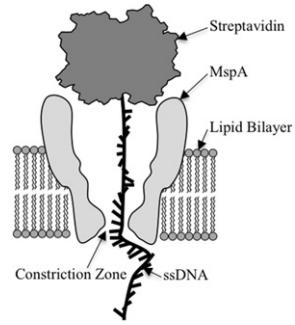
North Carolina State University, Raleigh, NC, USA.
Nanochannels with a diameter of about 100nm2 are a novel method for stretching DNA for genomic investigations. Such devices are implemented through standard nanolithography in fused silica. The elongation of DNA results from an interplay of steric and 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
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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.