in a free standing silicon nitride membrane. The translocation of CTPR proteins was measured in KCl solution at pH below and above its isoelectric point (pI), as well as with and without denaturing agent, Guanidine HCl. When a CTPR protein molecule transits through a nanopore driven by an applied voltage, it partially blocks the ions (K^+ and Cl^-) flow in the nanopore and generates a characteristic electric current blockage signal. The current blockage signal reveals information about the size, conformation, and primary sequence of the CTPR protein molecule. Previous translocation studies carried out with DNA have established that higher bias voltages result in shorter duration current blockages indicating that DNA translocates faster at a stronger electric field. However, CTPR translocation studies presented here show that longer duration current blockades were observed at higher bias voltages. We explain this surprising result by theoretical analysis of CTPR protein translocation in solid state nanopores. We discuss how the inhomogeneous distribution of the primary charge sequence of the CTPR proteins predicts translocation barriers that are proportional to the bias voltage. Larger barriers at higher bias voltages will result in longer translocation times, consistent with our experimental results.

3099-Pos

Quartz Nanopore Membranes for Low Noise Measurements of Ion Channel Conductance

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Planar lipid bilayer (PLB) apparatus provide an excellent platform for the study of isolated membrane proteins. The noise performance and bandwidth of PLB systems are poor relative to the state of the art in patch clamp/pipette apparatus. The problem is the relatively high capacitance of PLB systems relative to small area patch pipettes. At low frequencies (hundreds of Hz), the difference is small to non-existent. At higher frequencies, the noise becomes dominated by voltage noise from the amplifier acting on capacitance of the lipid bilayer and surrounding platform. With a much larger area, the noise for the PLB system rapidly exceeds that of the patch pipette. At intermediate frequencies (1 to 10's of kHz), the specific composition of the PLB platform can lead to an increase in noise due to dielectric loss [1].

We have developed a PLB system based upon a quartz nanopore membrane (QNM) with noise performance approaching the state of the art for patch clamp systems. Due to low dielectric loss, the QNM represents a significant advance in performance over the previously presented glass nanopore membrane [2] and provides for noise performance of ~200 fA at a 10 kHz bandwidth when coupled to a simple capacitive feedback amplifier. The resulting system has great immunity to vibration and electrical interference, without the need for a vibration isolation table and a large faraday shield. This new PLB platform will open up the potential for making very high bandwidth single channel measurements that were not previously possible.

[1] R. A. Levis, et al, Methods in Enzymology, 293, pp. 218-266, 1998
[2] R. J. White, et al, J. Am. Chem. Soc., 129, pp. 11766-11775, 2007

3100-Pos

Control of Salt Rejection by Surface Charge Patterning in Conical Polymer Nanopores

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Biological membrane protein channels show a variety of interesting transport properties, such as ionic and molecular selectivity. Studies on biological nanopores have shown that a pore's selectivity is due to both steric and electrostatic filtration of the ion or molecule that the pore is designed to transport. In the case of the aquaporin, the pore structure allows for the transport of water molecules at high flow rates without concurrent passage of ions. Careful preparation of an array of such salt rejecting channels would be useful in a variety of applications, in particular for desalination. To this effect, we have prepared "synthetic" salt rejecting channels from conical nanopores in polymer films. At low and moderate ionic strength, pores in polyimide and polyethylene terephthalate films are naturally cation-selective due to a native negative surface charge, and upon application of pressure, show salt rejection. The experimental data were supported by continuum modeling based on the Poisson-Nernst-Planck equations. The model also predicted that nanopores which contain a surface charge pattern consisting of a zone with positive surface charges next to a zone with negative surface charges should exhibit superior salt rejection capabilities compared to homogeneously charged pores. This improvement is due to the large potential barrier to ion transport created by the separation of cations and anions at the junction of the positively and negatively charged zones. Experimental and theoretical results are shown for both homogenous and surface charged patterned pores.

3101-Pos

Fabrication of Metallised Solid-State Nanopores Using Electrodeposition with Ionic Current Feedback

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In recent years, solid state nanopores fabricated in thin insulating membranes have been successfully employed as a new tool to detect and characterise the passage of DNA molecules. These nanopores circumvent some of the problems associated with protein channels, and offer the additional advantage of tunable pore size. Although several experiments have clearly demonstrated that modulations of ionic current during translocation of RNA or DNA molecules can be used to discriminate between polynucleotides, a key challenge with nanopores is to find methods to slow down and control the DNA translocation. It has been proposed that the presence of a metallic probe located at the nanopore can potentially enhance the electrostatic interaction between the DNA molecule and nanopore surface and hence reduce translocation times. Moreover, by applying an electric potential to the metallic nanopore it is possible to control the charge and ultimately allow for sorting and sizing of DNA fragments.

Here we report a novel method to fabricate these metallic nanopores with apparent diameters below 20 nm using electrochemical deposition and "on-line" ionic current feedback. Starting from large nanopores (diameter 100-200 nm) milled into gold silicon nitride membranes using a focused ion beam, we electrodeposit platinum onto the gold surface, reducing the effective pore diameter. By monitoring the ion current simultaneously, the electrodeposition process can be terminated at any pre-defined value of the pore conductance in a precisely controlled and reproducible way. Our approach is applicable to single nanopores as well as nanopore arrays, and can easily be extended to metal deposits other than Pt. In order to highlight their potential for single-molecule biosensing applications, we also show electrophoretic translocation of lambda DNA in a proof-of-concept experiment.

3102-Pos

Lipid Bilayers in Nanopores to Vary their Diameter, Characterize Amyloid-β Aggregates and Monitor the Activity of Membrane-Active Enzymes

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This research introduces the concept of coating the surfaces of nanopores with supported lipid bilayers for previously inaccessible nanopore-based assays. Current methods for shrinking nanopores with nanometer precision entail the use of specialized instruments such as focused ion beams or electron beams. Furthermore, altering the surface chemistry of nanopores currently requires multiple chemical steps and typically takes longer than one day. The method presented here modifies the surface chemistry of nanopores within 90 min by deposition of desired lipids with various chemical headgroups. This work also demonstrates the use of lipids with acyl chains of different lengths to shrink the diameter of a nanopore with sub-nanometer precision. Remarkably, the surface of bilayer-coated nanopores is non-fouling and makes it possible to detect aggregates of the "notoriously sticky" peptide, amyloid-β; the same nanopore without a bilayer clogged in every experiment. These non-fouling properties of nanopores coated with a fluid lipid bilayer made it possible to resolve single aggregates of amyloid-β and to characterize their true size distribution. Finally, this research took advantage of bilayer-coated nanopores to monitor the activity of the membrane-active enzyme, phospholipase D. Together the results presented here demonstrate that supported lipid bilayers can be used to alter the size and surface chemistry of nanopores reversibly. Moreover, bilayer-coated nanopores show promise to study membrane-active enzymes, membrane processes, as well as to perform nano-Coulter counter experiments on peptides that aggregate and adhere to surfaces such as amyloid-β.

3103-Pos

Nanopore-Based Sequence-Specific Detection of Duplex DNA for Genomic Profiling

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The ability of nucleic acids to form stable, sequence-specific complexes with foreign molecular probes has been exploited for a wide range of applications in life sciences, biotechnology, medicine, and forensics. Peptide nucleic acids (PNAs), nucleic acid analogs in which the negative sugar-phosphate backbone is replaced with a neutral peptide-like backbone, have been shown to display greater stability and sequence specificity to complementary ssDNA strands than natural DNA. This feature has been utilized in a number of applications

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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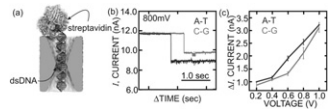
We report a new method for trapping a single molecule of double-stranded DNA (dsDNA) in a solid-state nanopore in SiNx membrane and describe the prospects for sequencing it. It is possible to trap a single dsDNA molecule in a nanopore <3nm in diameter by first applying a voltage larger than this threshold 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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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

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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