

*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<sup>1</sup>, 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

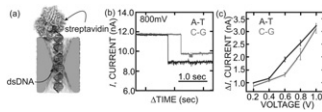
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We report a new method for trapping a single molecule of double-stranded DNA (dsDNA) in a solid-state nanopore in SiNx membrane and describe the prospects for sequencing it. It is possible to trap a single dsDNA molecule in a nanopore <3nm in diameter 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 electrophoretically 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<sup>1</sup>. 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<sup>+</sup> and K<sup>+</sup>, the G-quadruplex residing time in nanopore was prolonged and the occurrence of unfolded linear TBA translocation was reduced as the K<sup>+</sup> concentration gradually increases from 0 mM to 500 mM, convincing that K<sup>+</sup> is highly sensitively binding with G-quadruplex, and the continuous K<sup>+</sup> exchange in G-quadruplex elongates the lifetime of G-quadruplex. In contrast, in the mixture of Li<sup>+</sup> and Na<sup>+</sup>, the G-quadruplex stays shorter in nanocavity and the occurrence of linear TBA was reduced to the similar level with that by pure Na<sup>+</sup>, indicating that the Na<sup>+</sup> is highly preferred, compared to Li<sup>+</sup>, to intrude into G-quadruplex after Li<sup>+</sup> 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  $\alpha$ -hemolysin pore from the cis mouth, the tag initially sticks into the  $\beta$ -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  $\beta$ -barrel. Once the unzipping occurs, the longer ssDNA (with the tag) first runs through the  $\beta$ -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  $\beta$ -barrel, switching the conductance back to Level 1. This unique molecular