917-Pos Board B717
Transcription Activation via Transcriptional Bursting
Zach Hensel, Haidong Feng, Bo Han, Christine Hatem, Xuefang Liu, Jin Wang, **Jie Xiao**.
Transcription factors (TFs) often regulate their own expression; monitoring TF production in real time will help elucidate autoregulatory mechanisms. However, many TFs are expressed at low levels and are difficult to detect. Further, labeling TFs with fluorescent proteins risks disrupting their regulatory functions. Here, we report a novel strategy, Co-Translational Activation by Cleavage (CoTrAC), to monitor autoregulation of a transcription factor, λ repressor cI, in live *E. coli* cells. CI becomes fully functional upon co-translational cleavage from a membrane-targeted fluorescent reporter, which can be counted individually. Using this strategy, we discovered that cI activates its own expression via transcriptional bursting_the production of multiple mRNA molecules in brief, well-separated periods of time. CI increases not only the frequency but also the size of transcriptional bursts. Negative autoregulation by cI decreases the burst frequency, but not the burst size. These results link activation of gene expression by a TF to burst-like transcription.

Micro and Nanotechnology; Nanopores I

918-Pos Board B718
Single-Molecule Signatures for Characterization and Regulation of Nucleic Acids Unzipping in a Nanopore
yong wang, Li-qun Gu.
The nanopore has been developed as a molecule force microscope to explore the unzipping of nucleic acids. The double-stranded DNA (dsDNA) such as hairpin with an overhang trapped in the nanopore can generate long current blocks. By measuring the voltage-dependent block duration, the unzipping kinetics as well as the force and energy involved in the double strands hybridization can be characterized. However, few studies have presented convincing characteristic current patterns for unzipping occurrence. In this report, we uncovered such a signature current pattern that can electrically track the entire unzipping process, from the time course of unzipping to the motion pathway of unzipped single-stranded DNA. With the signature signals, the release of DNA without unzipping as well as DNA trapping directionality can also be distinguished. Quantitative analysis of signature signals showed that the overhang of a DNA is more favorable to the nanopore compared to the blunt end. Therefore the overhang is not only an unzipping driver, but also a controller of DNA trapping orientation, regulating the unzipping in a programmable manner. Discrimination of unzipping signatures not only gives precise insight into the unzipping mechanism, but more importantly in biosensors, the unzipping signature can act as a single-molecule marker that ensures both selectivity and sensitivity.

919-Pos Board B719
Electrostatic Trapping of Polymers During Translocation Through a Semiconductor Nanopore
George T. Nelson, Maria Gracheva.
The idea that DNA can be sequenced by threading it through a nanopore and reading the resulting ionic current is a promising one, but one that has not yet been realized due to some significant roadbloacks. One such issue is the speed of translocation, where it is difficult to slow the translocation enough to achieve a meaninful signal to noise ratio. Our proposed solution to this problem is to create a nanopore in a semiconductor membrane that contains a p-n junction. In this way, it should be possible to create a voltage difference across the pore that can momentarily trap DNA base pairs as they move down the electrophoretic gradiant. Presented herein is a computational method for modeling single-stranded DNA molecules as they undergo translocation through a semiconductor nanopore constructed as described above. The results of this Langevin dynamics indicate how effective this type of nanopore is at reducing translocation times. Furthermore, this simulation provides a better understanding of how to optimize our nanopore for the eventual long term goal of sequencing DNA base pairs based upon their blocked ionic current signature.

920-Pos Board B720
Unidirectional Translocation of DNA Through the Phi29 Connector Channel
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When channel diameter is reduced to the nm range (<10 nm), the interaction between the solution and channel wall starts to dominate ionic flow. An interesting phenomenon, ion current rectification, has thus been well studied, which has found its application in logic gates and diodes in fluidic circuits. Similar to the ion current rectification, we have found DNA transport through the connector channel experienced a one-way traffic mechanism. Using NTA-Ni Nanogold, we characterized the DNA transport directionality by means of single channel recording, i.e., DNA can only go through the Phi29 connector channel from the N-terminal narrow end to the C-terminal wide end. The uni-directional control in dsDNA transportation would potentially provide a novel system with a natural gate to control dsDNA loading and gene delivery in bioreactors, liposomes, and other nano-machines. The findings also forecast a ratchet or valvular mechanism of the viral DNA packaging motor.

921-Pos Board B721
Mapping the Sensing Zone of Alpha-Hemolysin Using Immobilized DNA Containing a Single Abasic Residue
Eric N. Ervin, John J. Watkins, Geoffrey A. Barrall, Prithwish Pal, Michael G. Keehan, Andrew D. Hibbs.
As efforts to sequence individual strands of DNA using biological nanopores continue to evolve, it has become increasingly important to understand the nature of the DNA-nanopore interaction. More specifically, characterizing the "sensing" zone inside the nanopore is an important part of understanding how nucleotide differentiation occurs. In order to characterize the sensing zone of the biological nanopore alpha-Hemolysin (αHL), we have examined how an adenine homopolymer containing a single abasic site blocks current flow through an αHL pore as the position of the abasic site is moved sequentially through the pore. In these experiments, single stranded DNA (ssDNA) oligonucleotides with a biotin (BTN) group at the 3' terminus were allowed to complex with streptavidin. Due to the inability of the large streptavidin to translocate through the αHL, the attached oligonucleotide could be electrophoretically immobilized inside the pore. Creating and capturing numerous oligonucleotides in which the single abasic site was positioned progressively farther from the BTN termination (e. g. 7 adenines from the BTN end, 8 adenines from the BTN end, etc.) allowed a blocking current map of the αHL lumen to be developed. This blocking current map of αHL yielded a single peak, which is approximately two nucleotides in width, centered midway through the αHL pore where the beta-barrel meets the vestibule. This single peak corresponds to the primary sensing zone of αHL. Further experiments have demonstrated that both the width and location of the primary sensing zone can be altered by creating mutant αHL proteins in which the residues inside the αHL pore have been changed. These studies should prove integral in creating a class of mutant αHL pores capable of discriminating between single nucleotides in ssDNA.

922-Pos Board B722
Simulation of Ionic Current Through the Nanopore in a Double Layered Semiconductor Membrane
Alexey Nikolaev, **Maria E. Gracheva**.
Starting with a description of an electrostatic model of a double-layered semiconductor membrane immersed in an electrolyte solution, we provide a comparison of the electric potential and ionic concentrations in a nanopore for different nanopore geometries (double-conical, single-conical, cylindrical) and for various voltages applied to the membrane. Voltage-current characteristics for ionic currents, as well as their rectification ratios are calculated using a simple ion transport model. The rectification ratio is found to be a linear function of potential variation in the pore. Based on our calculations, we find that the double layered semiconductor membrane with a single-conical nanopore with a narrow opening in the n-Si layer exhibits the largest range of available potential variations in the pore, and thus, may be better suited for control of polymer translocation through the nanopore.

923-Pos Board B723
Determining the Resolution of Biological Porin Mspa for Nanopore Sequencing
Elizabeth A. Manrao, Ian M. Derrington, Mikhail Pavlenok, Michael Niederweis, Jens H. Gundlach.
Nanopore sequencing has the potential to become a fast and low-cost DNA sequencing platform. The ion current passing through a small pore identifies the sequence of single stranded DNA (ssDNA) electrophorically driven through the constriction. A mutant pore protein, MspA, derived from Mycobacterium smegmatis forms a short and narrow channel ideal for resolving single nucleotides. To study the resolution, we immobilize ssDNA within the pore using a biotin-NeutrAvidin complex. Each base, adenine, cytosine, thymine, and guanine, produces a distinct current signature and methylated cytosine is distinguishable from unmethylated cytosine. Single heteronucleotide substitutions within homopolymer ssDNA are used to characterize the resolution of the pore. Using single nucleotide polymorphisms, we show that single nucleotides within heteromeric ssDNA can be identified when the substitution is held in