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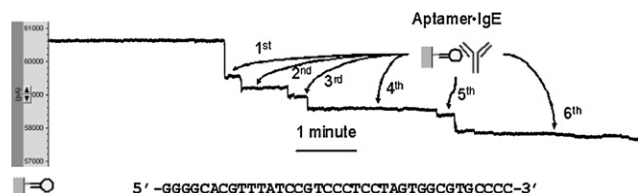
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Aptamer-Encoded Nanopore For Single-Molecule Detection In Sensing Of Biomedical And Bioterrorist Agents

Shu Ding, Changlu Gao, Li-Qun Gu.

University of Missouri, Columbia, MO, USA.

Various solid nanopores have been constructed with the advantage of changeable pore size over the protein nanopore. In principle, these solid nanopores, once functionalized in the lumen, should be able to capture individual target molecules. One should be able to observe this process from the binding-produced discrete current blocks. However, direct observation of single molecule binding to solid nanopores has been rarely reported. Here we for the first time report the aptamer encoded nanopore for single-molecule biosensing. Aptamers are short DNA or RNA sequences that can fold into specific conformations to bind their target proteins with high affinity and high selectivity. Comparing with antibodies, aptamers are durable in severe environment such as high temperature and extreme pH values. In particular, aptamers are advantageous in nanopore applications due to their smaller volume than their target proteins, allowing target-generated current block to be visualized. In this report, we demonstrate an aptamer-encoded nanopore for single molecule detection of IgE and bioterrorist agent ricin.



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Label-Free Electrical Biosensing Using Functionalized Nanopipettes

Senkei Umehara^{1,2}, Paolo Actis¹, Adam R. Rogers¹, Ronald W. Davis², Miloslav Karhanek¹, Nader Pourmand^{1,2}.

¹University of California, Santa Cruz, Santa Cruz, CA, USA, ²Stanford Genome Technology Center, Stanford University, Palo Alto, CA, USA.

Nanopipettes are recognized as a versatile tool for engineering and life science studies. Among a variety of applications of nanopipette technology, we are particularly interested in developing a fully electrical sensing platform using nanopipette probes. The dimensions of our nanopipette, comparable to the size of many proteins and macromolecules, make it suitable for sensing biomolecules in general. Molecular interaction on the nanopipette tip are transduced into electrical signals based on changes in size, electrical charges and structures of the nanoscopic pore region.

Here we report the development of nanopipettes that specifically detect their non-labeled target molecules. To reliably identify the interacting molecules, the nanopipettes were individually functionalized by modifying the surface with antibodies. Then two different nanopipettes, one as a probe nanopipette with attached molecules specific to the target molecules (i.e. antigens) and the other as a control nanopipette, were immersed in the same bath solution to perform voltammetric measurement. The addition of target molecules tended to cause a lasting reduction in current flowing through the probe nanopipette, which was large enough to distinguish the probe nanopipette from the control. These results indicate that the target molecules were captured by antibodies specific to them, present only on the probe nanopipette surface, and that this process partially narrowed the pore. The tested target molecules included cancer biomarker proteins, therefore this system could be readily optimized for diagnostic use.

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Escape Dynamics of DNA from a Nanopore under the Influence of an AC Bias

Daniel K. Lathrop¹, Geoffrey A. Barrall¹, Eric N. Ervin¹, Michael G. Keehan¹, Michael A. Krupka¹, Ryuji Kawano², Henry S. White², Andrew H. Hibbs¹.

¹Electronic Bio Sciences, San Diego, CA, USA, ²University of Utah, Salt Lake City, UT, USA.

Monitoring the escape of ssDNA from a protein nanopore provides new insight into the dynamics of DNA translocation and a direct means of measuring prog-

ress of the escape. New instrumentation has been developed that allows for simultaneous application of an AC and DC bias across a protein nanopore. Initial measurements with the system have focused on the capture and release of ssDNA tails attached to DNA hairpins. Polynucleotide tails attached to 24 nucleotide DNA hairpins are threaded into the beta-barrel of an alpha-hemolysin channel under the influence of a strong DC driving voltage. After the capture of the hairpin, the DC voltage is turned off and the subsequent escape of the hairpin is directly monitored via an AC bias. Escape times were measured as a function of AC amplitude (20 to 250 mV), AC frequency (60-200 kHz), DC drive voltage (0 to 100 mV), and temperature (-10°C to 20°C). The applied AC voltage has been shown to play a significant role in the DNA/nanopore interaction. The results are well described by a one-dimensional diffusion model across an asymmetric, periodic potential.

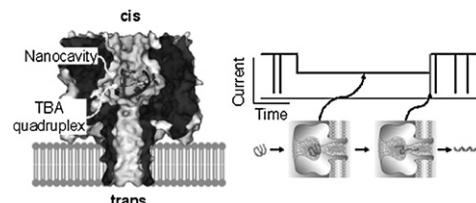
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Ion-Regulated Assembling Of The G-Quadruplex Aptamer - A Nanopore Single-Molecule Study

Ji Wook Shim, Qiulin Tan, Li-Qun Gu.

University of Missouri, Columbia, MO, USA.

Guanine-rich DNA and RNA can form high order G-quadruplexes through guanine-guanine base-pairs. G-quadruplexes in genome actively participate in gene regulation; and synthetic G-quadruplexes *in vitro* are potent pharmaceuticals, biosensors and bricks of nanostructures. Here we report on the development of a nanopore encapsulating single-molecule method for exploring how cations regulate the folding and unfolding of the G-quadruplex formed by the thrombin-binding aptamer (GGTTGGTGTGGTTGG). The signature blocks in the nanopore revealed that the G-quadruplex formation is cation-selective. The selectivity sequence is $K^+ > NH_4^+ \sim Ba^{2+} > Cs^+ \sim Na^+ > Li^+$, and G-quadruplex was not detected in Mg^{2+} and Ca^{2+} . Ba^{2+} can form a long-lived G-quadruplex with TBA, but the capability is affected by the cation-DNA interaction. This cation-selectivity is correlated with the G-quadruplex volume, which varies with cation species. Although the Na^+ - and Li^+ -quadruplexes feature similar equilibrium properties, they undergo radically different pathways. The Na^+ -quadruplex folds and unfolds most rapidly, while the Li^+ -quadruplex performs both reactions at the slowest rates. This research is beneficial for constructing fine-tuned G-quadruplex-based biosensors. The methodology in this work is also useful for investigating protein-G-quadruplex interactions.



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Folded and Unfolded Single Proteins Analyzed by Their Solid State Nanopore Translocation Dynamics

Jiali Li¹, David S. Talaga².

¹University of Arkansas, Fayetteville, AR, USA, ²Rutgers, The State University of New Jersey, Piscataway, NJ, USA.

The translocation of biological polymers through individual nanometer-scale pores is vital to cellular function and has great potential for technological applications in protein or nucleic acid measurements and identification. Research into this area has been focussed on characterizing the physics of translocation through voltage-biased nanopores and exploiting it to identify or sequence biological polymers. Here we show that the DNA-calibrated translocation signals of β -lactoglobulin and histidine-containing phosphocarrier protein match

