

Research Highlights

Highlights from the latest articles in nanomedicine



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Single molecule DNA synthesis can be detected electronically

Evaluation of: Cockroft SL, Chu J, Amarin M, Reza Ghadiri M: A single-molecule nanopore device detects DNA polymerase activity with single-nucleotide resolution. *J. Am. Chem. Soc.* 130(3), 818–820 (2008) – Venkatesan & Bashir

Rapid and reliable genome sequencing is essential in many aspects of biology and medicine. Significant strides have been made towards reducing the cost and time of sequencing (see review by Dorvel and Bashir on page 2). Recent developments in the use of nanopore sensors promise to bring us closer to the goal of single molecule sensing. Bayley *et al.* proposed to combine exonuclease digestion of DNA with the single molecule sensing capabilities of protein nanopores to sense individual bases [1]. Iqbal *et al.* demonstrated the ability to experimentally detect single nucleotide polymorphisms in a target sequence using functionalized solid state nanopores [2].

More recently, Cockroft *et al.* demonstrated the ability to monitor the base by base activity of DNA polymerase at the single molecule level using an α -hemolysin (α -HL) nanopore [3]. The authors electrophoretically thread and capture a single strand of DNA–polyethylene glycol (PEG) copolymer terminated at one end in a biotin–streptavidin group within a protein nanopore. The protein nanopore is formed by inserting a single α -HL protein into an artificial lipid bilayer. Under an applied transmembrane potential, a steady and characteristic ionic current flows through the pore. Biomolecule transport (ssDNA and PEG) results in molecule-specific current blockades, causing characteristic shifts in nanopore conductance. The DNA–PEG complex

is threaded through the pore from its free 3'-ssDNA terminus and then trapped by hybridizing the free end with a primer of specific length. Baseline ionic current was recorded both before and after the introduction of DNA polymerase. Single nucleotide primer extensions resulted in successive displacements of the template DNA strand, thereby altering the proportion of ssDNA to PEG within the protein pore. Single base incorporation successfully resulted in distinct, detectable changes in nanopore conductance. Primer extension could be halted in the absence of the required base. Using this technique, the authors demonstrate a simple temporal approach to decode nine consecutive bases in a template strand of interest. The single base resolution achieved using this technique coupled with the ability to regulate DNA transport in single base steps makes this a novel and potentially viable nanopore-mediated DNA sequencing approach. This technique is also applicable to the study of biopolymers and their binding kinetics with specific proteins.

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Sequencing a single viral genome a reality

Evaluation of: Harris TD, Buzby PR, Babcock H *et al.*: Single-molecule DNA sequencing of a viral genome. *Science* 320, 106 (2008) – **Dorvel & Bashir**

In 2003, the cost of sequencing the human genome was \$2.7 billion. Since then, sequencing technology has become quicker, faster and cheaper. Using the latest short read sequencing technology, DNA pioneer James Watson's genome was recently acquired at the bargain price of \$1.5 million. Although short read sequencing strategies have proven successful, their current cost and DNA library preparation complexity still may limit their applicability to human genome resequencing. With a reference genome in place, lower cost strategies for sequencing have been proposed; in particular, strategies that utilize even shorter read lengths and higher parallelism.

In this article the authors have devised a single molecule sequencing approach based upon sequencing by synthesis, demonstrating it on the M13 viral genome. The authors designed a

simple and fast method of DNA library preparation, resulting in poly(dA) templates tagged with Cy3 fluorophore that hybridize to poly(dT) oligonucleotides randomly attached on glass coverslips. Cy5 labeled C, T, A or G nucleotides were added in successive cycles, Cy5 emission monitored on each strand, then Cy5 chemically cleaved off for the next cycle. The authors go into detail about how this ideology can be used for reducing error, as well as how they overcome the lasting problem of homopolymer detection. Monitoring over 280,000 strand positions simultaneously with Cy3 fluorescence, the M13 genome was deciphered with an average read length of only 23 bases and a coverage of more than 150 \times . By merging innovative biotechnology with computer science, the authors demonstrate a highly parallel, asynchronous synthesis method with incredible success in detecting insertions, substitutions and deletions. Although a viral genome is far from the estimated 6 gigabases of the diploid human genome, the progress made by the authors makes the coveted goal of the '\$1000 genome' that much closer to reality.

Modeling of nanobiosensors is critical to improving their performance

Evaluation of: Nair PR, Alam MA: Screening-limited response of nanobiosensors. *Nano Lett.* 8, 1281–1285 (2008) – **Elibol & Bashir**

Field effect sensors for bio-chemical detection were demonstrated over 35 years ago [1] using a standard transistor-like structure with a 2D planar surface. The gate was replaced with an analyte specific coating, and the target molecule modulated the current flow in the active area between the source and the drain electrode. The advent of nanotechnological developments allowed the fabrication of nearly 1D active areas with cross-sectional dimensions at the nanometer scale; the active surface was rolled into a cylinder, increasing the surface to volume ratio. Experiments using devices

fabricated with either bottoms-up [2] or top-down [3] fabrication methodologies reported a dramatic increase in the sensitivity along with other puzzling properties. This was the first step towards realizing ultra-sensitive real-time, label-free sensors that could be multiplexed and densely integrated to yield a cost-effective bio-molecule monitoring platform. However, an adequate theoretical framework to explain these results has been lacking, hindering the further development and optimization for the wide-range applications of this technology.

Nair and Alam previously tackled the problem of providing device design guidelines by considering the electrostatics of the system [4]. They have also explained the increased sensitivity observed with the nano-biosensors by

considering the diffusion-capture kinetics of the target molecules [5], which showed geometry-dependent properties – diffusion of the target molecules to the device is enhanced as the dimensionality of the active area decreases. In this more recent work [6], they combine these two approaches to provide a coherent theoretical framework and explain some of the puzzling properties seen with the nano-biosensors. Very importantly, the model accurately predicts the logarithmic dependence of device response on the target molecule concentration. The elegant analytical solutions provide important insight into the steady state and transient sensor-response dependence on parameters such as analyte concentration, buffer ionic concentration and pH. The

model can easily be modified and expanded for the modeling and optimization of more complex situations, which will allow the accurate prediction of sensor performance and further optimization of nano-biosensors for novel applications.

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Microchips capture rare cancer cells from blood

Evaluation of: Nagrath S, Sequist LV, Maheswaran S *et al.*: Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature* doi:10.1038/nature06385 (2007) (Epub ahead of print)
– Park & Bashir

Recent work of Toner and colleagues (Nagrath *et al.*) shows a pioneering and very promising method for isolating circulating tumor cells (CTCs) in peripheral blood from cancer patients using a microfluidic device. CTCs are cells that detach from a primary tumor and find their way in the bloodstream. CTCs are indicative of the presence of tumors and are believed

to seed a secondary tumor. Hence, successful isolation of viable CTCs can be a potential alternative of invasive biopsy and cancer monitoring. Also, isolation and capture of CTCs can enable their molecular characterization, facilitating the further discovery and understanding of bio-markers and metastasis of cancer. For isolation of CTCs, Toner and colleagues developed a microfluidic device consisting of an array of micro-posts and functionalized with these posts with antibodies specific for CTCs. Whole blood sample from a patient was directly injected into the device with a controlled flow rate and CTCs in the blood are captured as they interacted with these posts,

whereas other blood cells pass through the micro-post array. Then, CTCs attached to the micro-post array were fluorescently labeled and counted. The developed CTC-chip can detect CTCs in over 99% of cases in 116 blood samples and 98.5% of the captured CTCs were observed to be viable. In addition, and very importantly, the CTC count was shown to be closely related to the tumor volume and was shown to be an accurate indicator for early decision-making in cancer treatment. This technique can provide a robust and versatile platform for monitoring cancer and residual disease and also for studying key biological determinants of blood-borne metastases.