

## Challenges in Third-Generation DNA Sequencing

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DNA sequencing is one of the leading precursors of the personalized medicine, i.e. for reading hereditary traits for predisposition to diseases which are coded in DNA, and thus to prevent, diagnose, and treat diseases. After the success of the Human Genome Project in 2003, which achieved reading a human genome for \$3B spent over thirteen years, the National Human Genome Research Institute has been supporting novel ideas to develop technologies under “\$1000 Genome” initiative and make human genome sequencing as affordable as a routine medical checkup. Due to these research initiatives over a course of ten years, the DNA sequencing cost has been reduced by about 100,000 times, but still requires development of fast, label-free and cheaper technologies, that can be massively produced and used.

The development of the DNA sequencing technology is a collective effort in nanoscience which integrates biology, chemistry, physics, and engineering, through interlaced experimental and theoretical/computational efforts. Particularly, the prospective of the so called physics-based third-generation methods is interesting, since they are intrinsically fast and can operate on a single DNA molecule [1]. A DNA is one-dimensional biopolymer, a sequence of four chemical moieties (nucleotide bases) - adenine, thymine, cytosine and guanine. A ssDNA polymer is negatively charged along its phosphorous backbone, with one elementary charge per monomer. The basic idea of these methods is electrical detection of the DNA sequences, either by reading the electron tunneling current measured across the pore transversally to the translocating DNA varies with a base passing through the pore between the electrodes [2-8] or by reading the ionic current through a pore while a single DNA polymer is translocated through a nanopore of molecular diameter or a nanogap by electrophoretic field, one base at a time [9-10]. The measurement and computation of the conductance of a single molecule is an attractive concept for molecular detection because single-molecule conductance may be governed at this length-scale by the molecule intrinsic electronic properties [1,11]. Detecting single chemical and biological molecules without using receptors or chemical interfaces is a paradigm shift in chemical sensing. Because the method requires fewer preparation steps and eliminates expensive ingredients like polymerases and ligases in the sequencing reaction. Successful nanopore sequencing would reduce the cost of genome sequencing tremendously. Nanopores can be an effective tool for confinement of DNA [12-21]. One of the main challenges of this concept is a need for the full control of the nucleobases localization and orientation as well as of DNA translocation rate while it is threading in its natural water and electrolyte environment through a nanopore [22]. Besides, the thermal fluctuations, Brownian motion, and both transversal and electrophoretic fields cause strong fluctuations of instantaneous mutual position of the DNA bases and electrodes, i.e. a large noise and poor signal-to-noise ratio in the transversal non-resonant tunneling conductance. For example, it is found that the variation in the conductance due to the geometry of the base relative the electrode can easily override the difference between different types of nucleotide [6,8]. Therefore, a full control of the DNA translocation and localization as it threads the nanopore becomes a primary concern for the DNA sequencing techniques using synthetic nanopores [23-25]. Theoretical calculations predicted many times increased signal-

to-noise ratio in the tunneling reads of the DNA if the electrodes are functionalized so as to promote the common non-resonant tunneling through the DNA nucleotides to a resonant one [26,27]. One of the most successful approaches to reduction of the noise, with significantly increased abilities for distinction of the tunneling electric signals belonging to various DNA bases is so called recognition tunneling of Stuart Lindsay from Arizona State University, based on the functionalization of the electrodes with organic molecular “readers” through which the electrodes are coupled to the DNA nucleotides with hydrogen bonding, strong enough to reduce the tunneling current width to the level of the bases resolution but weak enough to enable DNA translocation through the pore [28-30].

Nanopore technology is potentially capable of sequencing much longer continuous strands of DNA than other techniques. This capability is becoming increasingly important for studies of long-range genomic complexity to associate DNA rearrangements with cancer and other diseases. The first nanopore devices were based on pore-proteins but recently, synthetic pores in the solid state materials have been fabricated.

Concerning improvements of the DNA translocation control, there is a number of approaches. A growing interest in nanopores in grapheme [31-32] is certainly caused by high potential for the DNA bases reading resolution caused by atomic-size thickness of this material. Here, we mention in more details two very distinctive types of synthetic nanopores: A narrow carbon nanotube and a Paul nanotrap, both functioning in aqueous/electrolyte environment.

Single-walled carbon nanotubes (SWCNT) are excellent candidates for nanopore applications because they have attractive chemical, electronic, mechanical properties, and can be fabricated with various lengths and diameters. Significant advantages for nanofluidic applications are based on hydrophobic, almost frictionless internal surface of the SWCNT. As a consequence, ionic currents through an individual SWCNT is markedly different from those through the other type solid-state nanopores (SSN) or through a protein nanopore (e.g.  $\alpha$ -hemolysin). The magnitude of signal is much larger (nA) than in a conventional SSN (pA) due to a significant increase of electro-osmotic current caused by the perfect slip on the atomistically smooth internal surfaces [33-36]. When DNA molecules are present in a narrow SWCNT, the ionic current may increase, opposite to the expected ionic blockade in SSN and in protein nanopores [33]. In addition, a

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CNT may be much longer than the translocated DNA segments, and the whole DNA segment typically stays inside the CNT during the translocation, which can be related to the slow rise and random spikes in ionic current seen in experiments [33]. These distinctive features of the SWCNT nanopores, are documented and partially understood from both molecular dynamics and coupled Poisson-Nernst-Planck-Stocks equations. Matching various time and spatial scales across the whole experimental systems by large computations, a mechanism was proposed based on electro-osmotic flow resulting from trapped excess charges in the tube in conjunction with ideally slip internal tube surfaces [33-35] which is well fitted with the experimental results.

The aqueous Paul trap technique provides an alternative to the physically thin nanopores for DNA sequencing by creating a virtual nanoscale confinement region using a relatively large device. Its functionality is based on the quadrupole radio-frequency electric field, as experimentally demonstrated. The novel idea of confinement of an ion in an aqueous environment within a Paul-type quadrupole trap [37] offers increased electrical detection efficiency for heteropolymers confined within a nanopore regardless of detection scheme [38]. The DNA segment is confined in an "aqueous virtual pore" [39-41] which is formed by the electrophoretic, dielectrophoretic and viscosity forces. The long-time motion of particle is subject only to random, Brownian fluctuations. We find that the rms fluctuations determine the size of the AVP and can be modulated by adjusting external voltages and frequencies, in full agreement with experiment and theory [42-46]. Individual charged particles are dynamically confined into nanometer scale in space by radio-frequency voltages which are used to generate an alternating focusing/defocusing potential well in two orthogonal directions. The feasibility of an aqueous Paul trap using proof of principle planar device was demonstrated experimentally by the group of Mark Reed of Yale University [40]. Further miniaturization of the trap device might bring the realization of a few-nm virtual pore, which greatly relaxes many difficulties in fabrication and application of a physical nanopore and can lead to lab-on-a-chip systems controlling localization and translocation of a DNA polymer and other charged biomolecules submerged in water. The theoretical predictions indicate reduction of the mean rms fluctuations and therefore reduction of the size of a virtual pore with decreasing the trap and the charged particle size [39]. Fabrication of smaller traps has been done so far, with trapping of 10 nm fluorescent quantum dots.

The trapping, localization, and motion control of biomolecules by utilization of their charges as well as their recognition based on their molecular structure are of critical importance to various biotech and medical applications, which go far beyond the DNA sequencing. Crucial characteristics of the systems presented here is their multiscale character in both time and space, requiring both validating experiments and supercomputing hardware with spectrum of computational methods, ranging from quantum mechanics, classical molecular dynamics to continuum theories of micro/nano fluidics, described by systems of coupled nonlinear differential equations, and determined usually by irregular boundary conditions.

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