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Editorial | Open Access

Biological and Solid-State Nanopores for DNA Sequencing

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Abstract

History and current developments of nanopores for DNA sequencing are reviewed. Discussions are primarily carried out on the two major categories of the devices, biological and solid-state nanopores. Hybrid nanopores are also mentioned. Difficulties defying practical applications are demonstrated as well as some possible improvements. A brief prospect is given in addition.

Keywords: DNA sequencing; Biological nanopore; Solid-state nanopore; Blockage

Editorial

Upon their epochal finding, Watson and Crick found the radius of the double helix is about 1 nm [1]. A later research verified that the width of the DNA cross-section is between 2.2 nm and 2.6 nm in 0.2–10 mM Na^+ aqueous solutions [2]. As early as the discovery of this “nuclein” (DNA), Miescher had realized it is fundamentally a “multi-basic acid” [3]. Therefore, this macromolecule should be easily charged and moved by applied electric fields. According to the sketchy geometrical and chemical properties stated above, one may boldly imagine that the sequence of nucleobases in a certain DNA could be determined by electrophoretically driving a single-stranded DNA (ssDNA) through a pore with both nanometer-sized diameter and length, in the form of an unfolded string rather than a helix. Since the some physical or electrical properties of the pore (e.g., the through-pore electrical impedance) change with time as it is blockaded by or partially filled with different nucleotides (A, C, G or T) in the strand that is passing through, the sequencing information of the DNA could be decoded by recording and analyzing these changes. Compared with classical sequencing techniques, this “imaginary” approach seems quite advantageous due to the exclusion of time-consuming and costly PCR amplification step or chemical labeling and detecting. Although the idea sounds like a scientific fiction whilst DNA sequencing is still dominated by techniques based on the Sanger method [4], it has been being realized and developed for more than 15 years [5-7].

The first nanopore-structure for DNA sequencing is α-haemolysin (αHL) [5], an exotoxin produced by *Staphylococcus aureus* responsible for red blood cell’s lysis. This protein can insert itself, or the pore, onto lipid bilayers and therefore forms a trans-membrane channel with the diameter of 1.4 nm at the minimum [8]. Under certain applied voltage, intermittent drops (less than nano-amperes) in the ionic current though the channel were recorded and attributed to the blockage by the nucleotides. Engineered αHL mutants were later synthesized with the aim to optimize the identification of nucleotides [9,10]. Less than ten year after the first trial of αHL, also a protein from bacteria, *Mycobacterium smegmatis* porin A (MspA), which belongs to the well-known porins or channels in cellular membranes, was found to include a unique structure – a single goblet-shaped through hole with the narrowest part, a ~1nm-long, ~1nm-wide constriction, near the bottom end [11]. This particular structure is obviously beneficial for the spatial resolution of short segments of an ssDNA through the pore and thus MspA was artificially mutated and adopted as a nanopore for DNA sequencing [12]. In addition, another prion, modified outer membrane protein G (OmpG) was reported to possess potentials to be used as DNA-detecting nanopore though the analyte was not ssDNA but a common nucleotide, adenosine diphosphate (ADP) [13].

However, all these nanopores discussed above are membrane proteins that may be subject to denaturation and degradation resulting from multiple factors in the environment, such as temperature, pH value, the existence of reactive or poisonous chemicals, etc. Moreover, sizes of the biological channels are not easily controlled even though limited modification could be achieved through protein engineering [9,12,13]. Solid-state materials with high processability, tunable surface properties, low chemical reactivity, satisfactory durability and good biological compatibility seem to be alternatives in the nanopore-based sequencing for DNA. Materials that are frequently used in electronics, such as silicon, silicon nitride, have turned out to be major options for solid-state nanopores. An extra advantage for semiconductor-based nanopores is the prospective to incorporate their possible commercial manufacture into the microelectronic assembly lines.

The earliest investigation on solid-state nanopores for DNA sequencing was reported in 2001 [14]. A double-stranded DNA (dsDNA) containing 500 base pairs was transferred through a 5 nm silicon nitride pore fabricated by “ion-beam sculpting”. Two year later, silicon or silicon-based silicon dioxide pores with diameter down to 2~3 nm were drilled by the electron beam of a commercial transmission electron microscope (TEM), which is actually a well-known cause for specimen damage under TEM whereas the researcher utilized it to produce nanopores [15]. Up to now, the majority of reported nanopores for DNA sequencing are manufactured by these two methods [6,16]. Just as biological membranes (e.g. the lipid bilayer) sustain the channel proteins, solid-state nanopores need to be opened on suspended solid-state ultra-thin films and the fabrication of films with nano-scale thickness, which involves multiple nanotechnologies [7], should be accomplished prior to the pore-drilling. Nanopores have also been drilled on graphene, the ultra-thin two-dimensional material

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that is theoretically down to one atom-thick, and the corresponding DNA translocation was recorded [17,18].

While last few years have seen significant developments in solid-state nanopores, progresses in their protein-based counterparts never cease. The biological nano-channels are still showing better performances in many aspects, especially the reduced translocation velocity (will be discussed in the next paragraph), than their solid-state competitors [7]. There also have been endeavors to functionalize the interior surface of solid-state nano-channels with biological molecules in order to improve the selectivity in DNA-sensing [19]. Biological-solid-state hybrid nanopores have also been studied to sense proteins [20].

However, challenges are preventing nanopore sequencing from practical applications. The primary obstacle, as mentioned or indicated in most research papers and reviews in this area, is the extremely high velocity of DNA traveling through nanopores. This makes measurements and recording quite difficult. Approaches to obtain recordable and decipherable signals primarily include two categories: increasing the detention time of nucleotide in the channel (reducing the speed) and improving the measurement precision. Enormous efforts are devoted to the former. During the last 15 years, the DNA translocation speed in biological nanopores has been reduced by almost four orders [7]. Researchers are also working on novel enhanced measurement techniques. The lower section of a αHL mutant was verified to include at least two detecting sites, at each of which a respective distinguishable signal in the through-pore ionic current can be generated by DNA blockage [21]. This means a single nucleotide can be detected twice as the DNA travels through the channel and the recognizability of the bases could be significantly improved. Nano-scale electrodes were embedded in solid-state nanopore and the tunneling current, which flows between the two electrodes perpendicularly to the conventional through-pore ionic current, was recorded and the current patterns were ascribed to the events of DNA blockages [22]. Besides of modifications of the nanopore, it has been proposed, although not achieved yet, that by a certain exonuclease at the entrance of the pore, the assayed DNA could be cleaved into single nucleotides, which are then driven through the nanopore in the order of the original chain; the resolution would be therefore substantially improved [10]. The commercialization of nanopore sequencing has been initialized [7].

Nanotechnology, a comprehensive and fast developing area far beyond conventional medical and life studies, becomes extraordinarily involved in DNA sequencing, as well as it does in many other biological sciences [23]. In fact, nanopore sequencing is much more than about biological sciences plus nanotechnology. As indicated in the discussions, technologies including signal processing, pattern recognition, etc., also play critical roles in this rather new but progressively advancing area. Studies in this highly interdisciplinary field may not only provide a fast, low-cost and productive next-generation tool to decode genomic information, hopefully, in the near future, but promote further developments in the related sciences and technologies.

It should be noted at the end of this essay, although nanopore sequencing uses nanotechnologies to assay DNA, it does not belong to the “DNA nanotechnology”, which is about the fabrication of synthetic nucleic acid structures (may not limited to DNA or RNA chains) using the self-assembling DNA as the major engineering material [24].

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