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Recent advances of DNA sequencing via nanopore-based technologies

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Abstract This review briefly summarizes recent progress in nanopore DNA sequencing from the beginning of 2012 to July 2014. Although partial successes have been achieved in different types of nanopores, biological pores such as α-hemolysin and MspA afford most promising results.

Keywords DNA sequencing · Nanopore · α-Hemolysin · MspA · phi29 connector · Solid-state nanopore · Graphene · SWCNT

1 Introduction

A nanopore sensor can be regarded as a Coulter counter at the single-molecule level. After nearly 20 years' rapid development, nanopore-based sensing technology has become one type of important method for detecting various analytes such as metal ions [1, 2], organic small molecules [3, 4], biomacromolecules including oligonucleotides [5], peptides [6], and so on [7, 8]. Among all of those research efforts, DNA sequencing has drawn the most attention [9]. Many groups have actively engaged in this field; especially Bayley's group (http://www.nanoporetech.com/news/press-releases/view/39) in Oxford has fabricated the first commercial DNA sequencing device based on α-hemolysin (α-HL).

SPECIAL TOPIC: Nanopore for DNA Sequencing

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Nanopores are generally divided into two types: biological nanopores and solid-state nanopores. Biological nanopores include α-HL [10], *Mycobacterium smegmatis* porin A (MspA) [11], and phi29 connector [12]. Solid-state nanopores are mostly fabricated in silicon-based materials such as silicon and silicon nitride [13, 14]. Recently, new types of materials have also been used to fabricate nanopores. In particular, atomically thin two-dimensional (2-D) materials have been proposed to have base resolution in the ultimate fast DNA sequencing and thus induced intensive pursuit. Single layer and few layers of graphene [15], boron nitride [16], and molybdenum disulfide [17] were reported to be able to detect DNA molecules through the drilled nanopores. Another type of new nanopore was built from the assembly of DNA origami [18]. The high quality current signals and precise modification of any DNA bases of the origami endow the nanopore with great potential in molecular sensing and DNA sequencing. Interestingly, ultrashort single-walled carbon nanotubes (SWCNTs) have also been inserted into a lipid bilayer to construct a new type of nanopore [19]. DNA translocation was observed in the SWCNT nanopore, and chemical modification on the DNA strand could be sensitively detected by the nanopore.

In this review, we focus on the recent advances of DNA sequencing via nanopore-based technologies from January 2012 to July 2014.

2 Biological nanopore

2.1 α-HL

α-Hemolysin is the most extensively studied biological nanopore in the last 20 years. In 1996, Kasianowicz et al. [5] constructed the first α-HL nanopore platform and

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observed the translocation of individual DNA molecules. This work raised the curtain on nanopore DNA sequencing. Thereafter, tremendous efforts have been devoted to overcoming the two long-lasting problems that hinder the development of α-HL-based nanopore sequencer: bluntness of the base reading region and fast translocation of DNA through the channel. Bayley's group [20] has been leading the research in improving the spatial resolution of α-HL by site-directed mutagenesis, and three recognition sites were disclosed. As to the temporal control of the movement of DNA molecule inside α-HL, early studies have been focused on decreasing temperature [21, 22], introducing organic salts [23], adjusting viscosity [24], adding internal molecular brakes [25], and so forth, but only with limited success. The real breakthrough was achieved in 2012 by Akeson's group [26] who used phi29 polymerase to slow down the rapid translocation of DNA strands. Basically, the target DNA was hybridized with the primer and then bound to the phi29 polymerase, forming a ternary complex which was located right above the α-HL nanopore. During the replication process, the target strand served as a template and was slowly ratcheted through the nanochannel, at a typical rate of tens of milliseconds per nucleotide, which is long enough for current discrimination (Fig. 1). Yet, conditions need to be optimized to reduce repeat and miss reading.

Kumar et al. [27] proposed a novel nanopore-based sequencing by synthesis (Nano-SBS) platform. In this concept, the four nucleotides were labeled with different chemical tags, which could be released and translocated through the nanopore during the polymerase reaction, thus producing distinctive current blockades to allow nucleobase recognition. As an example, the authors used different

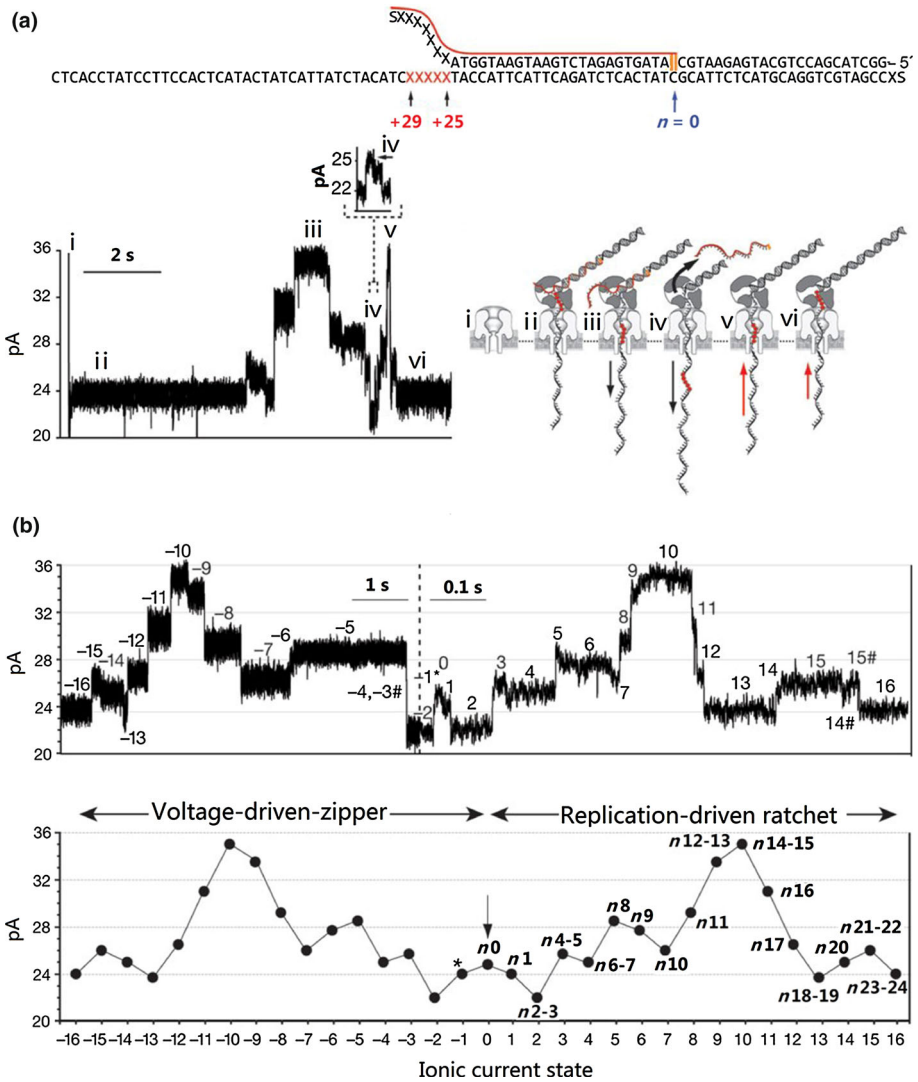

Fig. 1 (Color online) Control of DNA translocation through α-HL with phi29 polymerase. As the replication proceeds, the template DNA strand was ratcheted through the nanopore (**a**) and each current level corresponding to nucleotide incorporation could be resolved (**b**). Reprinted with permission from [26], Copyright 2012 Nature Publishing Group