Microreviews in Cell and Molecular Biology 2016

Optical DNA Sequencing in a Nanopore Array

Key Words:
nanopore array, DNA sequencing, optical detection, Fluo-8, droplet interface bilayer, long-range DNA strand, Ca^{2+} flux

Abstract: Rapid progresses have been achieved in one of the most promising third-generation sequencing methods: nanopore sequencing during recent years. Optical nanopore sequencing shows visible potential as the latest method. By optically encoding the ionic flux through protein nanopores, such as α-haemolysin and MspA, in a single droplet interface bilayer, the discrimination and detection of nucleic acid sequences can be parallelized. Nanopore blockades can discriminate between DNAs with sub-picoampere equivalent resolution, and specific miRNA sequences can be identified by differences in unzipping kinetics. If completely developed, this method will greatly increase the speed of sequencing after overcoming the sacrifices in device size and cost.

Introduction

Nanopore protein is a tunnel set on cell membrane, just the protein in fluid mosaic model of cytomembrane, playing an important role in transmission. When taken from organism and placed on an impermeable membrane, which separates two chambers containing electrolytic solution, it may be utilized for single-molecule biological sensing, for example, DNA sequencing. In a typical nanopore measurement, the nanopore is the only passage for ion transport between the two chambers containing electrolytic solutions. A pair of Ag/AgCl electrodes is placed across the nanopore to form a closed electrical circuit. The ion migration through the nanopore is generated by the potential caused by the electrode reaction, and as a result, a sustainable current flow across the membrane arises. When a biomacromolecule migrates through the pore such as DNA strand, there will be a current blockage which can be detected by galvanometer. The signals of different basic groups are different, so the blockage amplitude and duration time can provide analytical information for molecular identifications.

This method using ionic current recording in planar bilayers with enzyme ratcheting of the DNA, is developed by Oxford Nanopore Technologies. It realizes low cost , fast speed, and great convenience to a great extent. However, because the readouts from each nanopore must be addressed separately, it is difficult to see how this significant increase in scale might be achieved without sacrifices in device complexity, size and cost.

Optical DNA sequencing can efficiently solve the problem in paralization.

Recent Progress

In optical DNA sequencing, nanopore ionic currents are converted into an optical signal. Nanopore array is constructed in droplet interface bilayer (DIB), at a density of 10^4 nanopores per mm^2 in a single DIB. The droplet is filled with Fluo-8 dyed Ca^{2+} aqueous and surrounded by a lipid membrane. When put on a voltage, The Ca^{2+} ions migrate and concentrate on the nanopores. A 'plume' of Ca^{2+} appears as a bright spot at the location of each pore in the bilayer. This provides an optical analogue of single channel recording (oSCR), so we can see the nanopores under microscope .

To prevent the DNA strand migrating too fast or too free to be detected, the DNA is immobilized by tethering to streptavi-din, mimicking a step in the translocation produced by a processive enzyme.

To determine the resolution, the relation between applied potential, fluorescence and equivalent currents using different homo-polymeric DNAs is examined. The current–voltage (I/V) relation in a planar lipid bilayer (PLB) which is the carrier of ionic current recording can be compared to the equivalent fluorescence–voltage (F/V) response in a DIB, and the relationship between the residual current and residual fluorescence is approximately linear. So we can estimate that the standard error in the mean signal from a particular blocking level in fluorescence measurements is equivalent.

During the process that single DNA strand migrating through α-haemolysin, the signal strength of fluorescence changes as below:(1)Ca^{2+} flows in the direction opposite to the DNA flow, and forms a complex with Fluo-8 to

become fluorescent, the most brilliant(2)DNA strand is driven into the pore, partially blocking the Ca2+ flux (3)the trapped DNA strand is released but almost completely blocks the optical signal (4)The fluorescence diminishes due to the near reversal of the Ca2+ flux at negative potentials.

In step(2), when different single DNA strands moving through the MspA nanopore, such as C65 (5′-biotin-CCCCCCCCCCCC-CCC-C35-CCTGTCTCCCTGCCG-3′) and A65 (5′-biotin-AAAAAAAAAAA-AAA-A35-CCTGTCTCCCTGCCG-3′) used in the journal, we can see obvious differences in fluorescence signals change along with time.

But if we put a miRNA strand (hybridized with a DNA probe to control the process) through α-haemolysin nanopore,the signal strength of fluorescence changes as below:(1)an open nanopore, the most brilliant (2)shows a decrease in fluorescence when the hybridized complex is captured and subsequently unzipped, quite dim (3)Following unzipping, when the DNA probe has translocated through the pore the miRNA remains in the vestibule, brilliant (4) The miRNA then translocates, dimmest (5) and the pore re-opens, brilliant like (1). So, just like current ionic detection, optical detection also needs different recording methods when experimenting.

Parallelization is not only the nanopores, but also the bilays, due to the increased likelihood of bilayer rupture for large-area DIBs at potentials more than 100 mV. By creating bilayer arrays between an agarose substrate and a micropatterned agarose chip, the parallelization of bilays can be increased.

Discussion

Optical DNA sequencing is almost the latest progress in nanopore sequencing, applying a very creative method to visualize the process of DNA strand migrating through the pore. The greatest progress it makes compared with electrical readout is the parallelization of detection, with no requirements for biomolecule labelling. It will extremely improve the speed of DNA-sequencing when developed mature.

Also, it conforms to the historical trend of the science development. Researches are more and more focusing on photon rather than electron. The former displays greater accuracy and potential, so is the optical detection.

However, potential is double-edged. It proves too much to be perfected. It remains difficult to see the strand and the migration process directly, for we cannot get the cross-section due to the limitation of technology. Now, most efforts are made to display the nanopore, resolution of basic groups is still lower than electrical method. Noise-signal ratio may be higher but not that satisfying.

The method is highly dependent on device, and easily limited by device. The time resolution can be improved by replacing the camera with a less sensitive, but faster detector. The balance between efficiency and sensitivity is difficult to achieve.

In the present configuration, the maximum period for data acquisition is limited to 30 min by the accumulation of Ca^{2+} in the droplet. A 30 min maximum duration places little restriction on most conceivable nanopore measurements, particularly if the goal is high-speed, high-throughput detection. In the present set-up, the total area can be imaged with present camera is $150 \times 150\ \mu m^2$. This gives a maximum theoretical throughput of 3,000 pores. Lens-free and macroscopic TIRF imaging are potential avenues for improvement.

There are still many attempts for experiment. Types of nanopores, method to control DNA strands, process of migration, even fluorescence, with these potential improvements in technology, optical recording from an array of nanopores should produce sequencing signals with a rate of considerable bases per mm2 per second, which could in principle produce a human genome sequence in minutes.

References

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