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REVIEW

Development of Biological Nanopore Technique in Non-gene Sequencing Application

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Abstract: Nanopore technique is a low-cost, ultrafast method for single-molecule level analysis without labels. Nanopore technique was first proposed more than 20 years ago and exhibited an excellent potential in DNA sequencing. So far, the commercial development of nanopore strand-sequencing as a portable device has been realized. Meanwhile, a remarkable number of studies have demonstrated that nanopores represent versatile single-molecule sensors for a wide range of molecules. Here, we mainly review the development of nanopore technique based on the interface interactions between biological pore and analytes such as proteins/peptides to obtain kinetic and thermodynamic structural information at single-molecule level. As examples, a large number of biological molecules and metal ions could be quantitatively detected by nanopore analysis, allowing its development for the future biotechnologies and medicine applications. Besides, the electrochemical measurement system is crucial for the nanopore technique. Therefore, we focus on advancements in relative software and ultralow current instrumentations with a high-bandwidth.

Key Words: Nanochannel; Single-molecule detection; Electrochemical analysis; Ultralow current amplifier; Review

1 Introduction

Nanopore analysis technology is an effective method for single-molecule detection. In 1996, α-hemolysin nanopores were firstly used in the detection of nucleic acids and opened the floodgates to researches based on nanopore[1]. Due to its lack of labeling and amplification, biological nanopores have shown excellent potential in DNA sequencing. So far, the commercial portable nanopore strand-sequencing device is available. Recent studies have demonstrated that nanopore represents a versatile single-molecule sensor for a wide range of molecules. When single molecule traverses through a nano-scale pore under an electric field, the properties of the analytes such as length, mass and structure can be obtained according to the current amplitude and duration of blockade current. Compared with other single molecule analytical methods such as atomic force microscope and optical tweezers, nanopore technique can be used for the detection of biological molecule without specific immobilization, amplification and labels. Therefore, various biological membrane proteins, including mycobacterium smegmatis porin A (MspA)[2,3], phi29[4], ClyA[5], FhuA[6], lysenin[7], CsgG[8], SP1[9] and aerolysin[10] have been used in pore-based sensing methods, and aerolysin nanopores have shown excellent resolution for short oligonucleotides[11–15]. With the development of microfabrication technologies, a variety of solid-state nanopore fabrication approaches along with a broad collection of supporting membrane materials have been exploited, for instance, the solid-state nanopores fabricated with silicon[16–19], TiO_2[20], Al_2O_3[21] and graphene[22,23] have been prepared by ion beam sculpting[24], electron beam construction with TEM[25], or atomic layer deposition[26]. Solid-state nanopore has been the subject of intensive studies recently for its advantages including tunable pore size[27], easy modification[28,29],

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mechanical robustness and integration compatibility with sophisticated electronics and optical readout system. Several reviews have been published whereby the readers may have a tutorial and detailed overview[30,31]. Herein, it will not be deeply discussed. Besides, electrochemical detection system is also very crucial. Under the promotion of nanopore technique, relative software and ultralow current instrumentations with high-bandwidth are well designed[32].

In this article we mainly review the use of nanopore technique based on the interface interactions between biological pore and analytes such as protein/peptide to obtain kinetic and thermodynamic information at single-molecule level (Fig.1). For some analytes that are hard to detect directly, two kinds of approaches are typically used for the nanopore-based analysis. One relies on the modification of the biological nanopore such as mutation of the nanopore and immobilization of suitable receptor on the inner surface of nanopore. When analyte interacts with a specific site within the nanopore, a significant blocking current signal is generated. For instance, cyclodextrins decorated with polyamine was used as recognition element in α-HL pore for highly sensitive and selective detection of Cu^{2+} [33]. The second approach uses specific mutual interaction of target-probe molecule without constructing a binding site in the nanopore interior. For example, DNA probes combined with a nanopore electrochemical sensor can sensitively detect pathogenic DNA[34], micro RNA[35] and so on. These two methods have significantly expanded the application scope of nanopore technique, and the range of analytes that can be detected with nanopores now spans small molecules, organic polymers and metal ions. The nanopore technique has shown excellent potential in life science area, biotechnologies and medicine applications.

2 Analysis of protein/peptide

Protein is the fundamental substance of life, and associates with various activities in cells. Thus it's crucial to investigate interactions between protein-protein at single-molecule level. Recently, some important properties of protein have been well studied based on nanopore technology, such as its stability in solution, conformation changes, folded states, enzymatic process and so on[36].

Compared with DNA, protein/peptide has more complex structures and charge distribution, which brings great challenges to nanopore technology. Therefore, researchers designed model molecules to simplify complex structures of protein/peptide for the primary study. In 2003, researchers analyzed and successfully identified individual peptides containing different repeats of the sequence (Gly−Pro−Pro) as they traversed the pore based on signature transit time and blockage current[37]. Further study accomplished the detection of different components of $(Gly-Pro-Pro)_n$ (n = 1, 2, 3) in complex solution (Fig.2)[38]. Researchers detected different configurations of protein/peptide, such as α-helix, β-hairpin[39]. Based on analysis of characteristic signals, they found that the

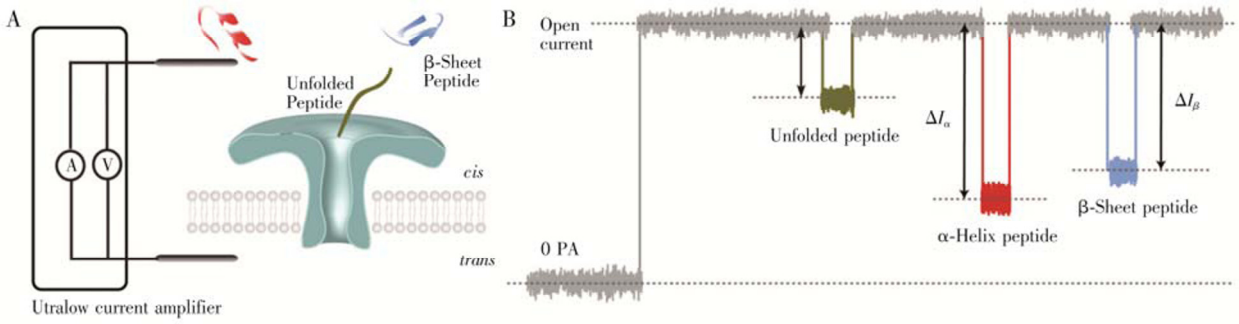

Fig.1 Principle of biological nanopores for single molecule analysis. (A) Schematic of the α-hemolysin pore embedded in a lipid membrane. Unfolded and simple α-helical or β-sheet forming peptides can readily translocate. (B) Specific blockade current could be recorded depending on the unique conformation of different analytes

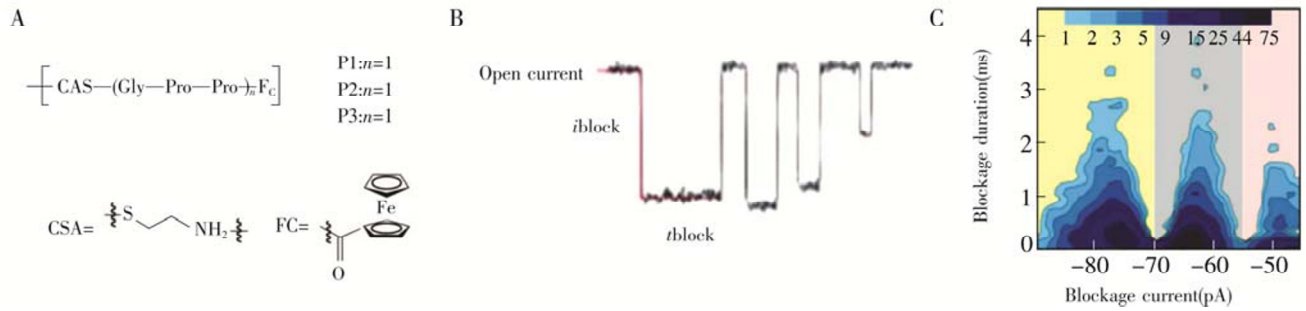

Fig.2 (A) Structural formula of peptides used in this research; (B) Representation of signals obtained in a single molecule detection experiment; (C) Contour plots of current transient of mixture peptides P1, P2 and P3[38]