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Membrane platforms for biological nanopore sensing and sequencing

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In the past two decades, biological nanopores have been developed and explored for use in sensing applications as a result of their exquisite sensitivity and easily engineered, reproducible, and economically manufactured structures. Nanopore sensing has been shown to differentiate between highly similar analytes, measure polymer size, detect the presence of specific genes, and rapidly sequence nucleic acids translocating through the pore. Devices featuring protein nanopores have been limited in part by the membrane support containing the nanopore, the shortcomings of which have been addressed in recent work developing new materials, approaches, and apparatus resulting in membrane platforms featuring automatability and increased robustness, lifetime, and measurement throughput.

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Introduction

In Coulter counting, cells in an electrolyte suspension travel through a small pore, causing transient decreases in the pore's ionic conductance proportional to cell volume that allows the cells to be rapidly counted and measured by size [1]. Since the initial development of this concept, devices with reduced pore size have been used to detect bacteria, viruses, and particles under 100 nm in diameter. The ultimate size limit is represented by nanometer-scale pores into which only ions and small molecules can fit, causing pA to nA-scale fluctuations in measured current [2•].

Nanopores can be made in a 'top-down' approach, where they are etched in thin membranes of semiconductor materials and graphene [3–5], or a 'bottom-up' approach in which pores are biological membrane proteins [6] or are synthesized from organic molecules, DNA, or peptides [7–10]. Although inorganic nanopores have advantages for technological applications in terms of temperature, solvent compatibility, robustness, and ability to be integrated with semiconductor electronics, biological nanopores have been explored the longest and have the best results till date, as a result of their easily made and modified, highly reproducible structures that enable repeatable current measurements.

The natural function of many biological nanopores is to facilitate transport of material or solution across cellular membranes to maintain cellular homeostasis or as a pathogenic mechanism. Genetic cloning and bacterial expression allows mass production and high precision modification, enabling production of molecular sensors capable of specific detection of a wide range of analytes at the single molecule level, discrimination between highly similar molecules, and quantitative measurement of the concentration of several species simultaneously [11••].

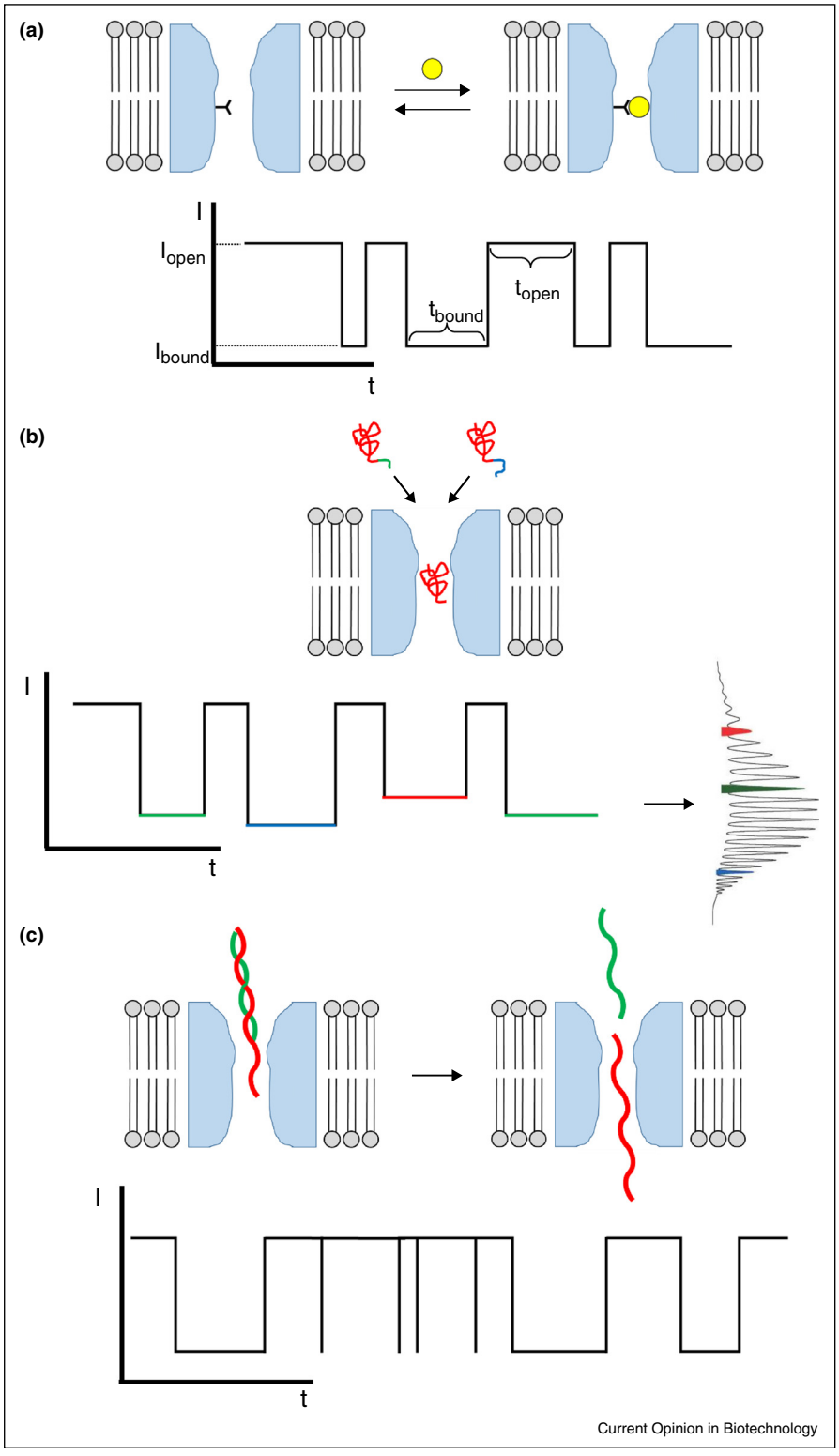
Protein nanopore measurement of DNA and RNA as the polymer is drawn through the pore has shown sequential steps in pore conductance, indicating that the bases along the polymer backbone can be detected and the sequence determined. Although other genome sequencing technologies have been progressing at an incredibly rapid pace, the modest instrumentation and consumable requirements of nanopore measurements, combined with their potential for long read lengths and single molecule measurement, have made this technique a subject of considerable excitement and research activity.

Practical sensors based on measurements of biological nanopores must take into account the membrane environment housing the nanopore. Artificial lipid bilayer membranes historically have been fragile, short-lived, and manually formed, suitable only for laboratory-bound scientific experimentation. A number of new approaches and devices have addressed many of these shortcomings [12•]; these developments, combined with the relatively simple low cost instrumentation required for nanopore measurements, point to a bright future for nanopore sensing technologies, and initial commercialization of nanopore DNA sequencing platforms have already begun.

Sensing with protein nanopores

The first demonstration of nanopore sensing utilized the pore protein alpha-hemolysin (αHL) from *Staphylococcus aureus*, engineered with binding sites to increase the

Figure 1



(a) (Top) Schematic of a biological nanopore in a lipid bilayer membrane. Binding of an analyte within the pore causes a step reduction in current (bottom) until the analyte unbinds and the process is repeated. Statistical analysis of the times before analyte binding (t_{open}) and times bound to analyte (t_{bound}) allow determination of the analyte concentration and bound lifetime, respectively. **(b)** (Top) PEG polymers of different length cause blockages in nanopore current of different magnitudes (bottom), able to resolve differences in length of one polymer unit. **(c)** (Top) Small nanopores can pass single stranded NA but not double stranded. The presence of target NA of a specific sequence (red strand) can be