PERSPECTIVE

Nanopore-Based Biosensors: The Interface between Ionics and Electronics

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ABSTRACT Techniques for translating the binding or the activity of single molecules directly into electrical signals are of interest for both fundamental and applied science. A paper in this issue describes experiments in which the ionic current through a biological nanopore is employed both to control and to monitor the attachment of individual DNA polymerase enzymes to their binding site on a single DNA molecule. This Perspective briefly sketches some of the factors that ultimately limit the performance of such nanoscale sensors, emphasizing in particular the interface between nanofluidic systems and external control electronics.

Many of the most exciting developments in recent years in biological nanoscience have resulted from the advent of techniques that enable probing the internal dynamics of individual molecules or the interactions between single molecules. As highlighted in a recent review,[1] the most widely employed of these techniques exploit the transduction of a molecular-scale event—a binding event, a conformation change, or a chemical reaction—into a macroscopically observable mechanical or fluorescent signal.

An alternative strategy is to develop approaches that directly translate the activity of a single molecule into an *electrical* signal. The very notion instantly invokes properties most commonly associated with electronic systems: speed, massive parallelization, and integration of a wide array of functions into a single system. Generating a measurable electrical signature from a molecular-scale event taking place in warm salty water, however, represents a substantial challenge. The most successful approach to date is based on the concept of the Coulter counter used to count and size particles. As illustrated in Figure 1a, a small hole, or pore, is opened between two reservoirs containing an electrolyte. A voltage difference is then applied between the reservoirs using two electrodes, resulting in an electrical (ionic) current flowing between the reservoirs. Because the pore represents the main hindrance to the free flow of ions, the magnitude of the electrical current is essentially determined by the size of the pore. Any obstruction of the free flow of ions through the pore results in a corresponding reduction in the current. If the pore has nanometer-scale dimensions, that is, dimensions comparable to the size of a single large molecule, the system becomes capable of detecting the passage of single molecules as they are driven through the pore. Both the magnitude and the duration of the blocking event can reveal information about the nature of the molecule traversing the nanopore.[2,3] Interestingly, while the molecule being detected must be charged in order to be driven through the nanopores by an electric field, its contribution to the electrical current is minimal and is normally dwarfed by that of the much more mobile small ions. It is this leveraging effect that ultimately lends this approach its sensitivity.

Preparing two reservoirs separated by a membrane containing a single pore with nanometer dimensions represents a major technical challenge, and top-down methods for doing so in a controlled and reproducible manner have been developed only recently.[4] Fortunately, nature has provided us with alternatives, of which the most widely used is the protein α-hemolysin. This protein reproducibly assembles in a lipid bilayer to form a narrow channel only 1.4 nm in diameter at its smallest constriction. This makes it eminently suitable for studying single-stranded DNA, which just fits inside the bore of this biological nanopore. The detection of single-stranded DNA translocating through α-hemolysin was first demonstrated by Kasianowicz *et al.* in 1996.[5] The decade since has witnessed a remarkably rapid rise in the sophistication of this type of experiment,[2,3] as evidenced by the recent demonstration that modified α-hemolysin nanopores can discriminate between individual DNA bases through analysis of their different electrical signatures.[6]

Electrical Control of Molecular Binding and Unbinding. The experiments of Wilson and co-workers described in this issue[7] employ α-hemolysin as a tool to study a distinct phenomenon, the binding of the enzyme DNA polymerase to a double-stranded-DNA/single-stranded-DNA junction that is held in close proximity to the nanopore. In this approach, the externally applied

See the accompanying Article by Wilson *et al.* on p 995.

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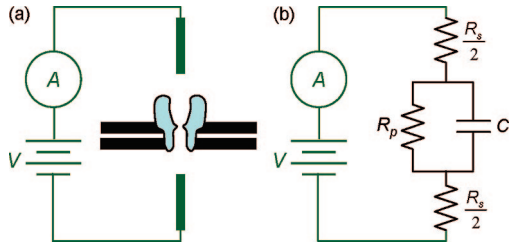

Figure 1. (a) Schematic diagram of the experiment. A voltage is applied across the electrodes *via* an external circuit, and the ionic current flowing through the nanopore is monitored. The presence of an analyte near the entrance or inside the nanopore causes this current to decrease. (b) Equivalent circuit for the ionic part of this system. R_s, R_p, and C represent the resistance of the solution, the resistance of the pore, and the capacitance of the lipid bilayer in which the nanopore is embedded, respectively. Molecular-scale events at the pore correspond to temporary changes in the value of R_p.

potential serves two purposes. First, it drives the ionic current that is used to passively monitor the presence or absence of DNA polymerase at its binding site on the DNA, as described above. Second, the applied potential is employed to switch the DNA molecule between two configurations. In the first, the so-called "fishing" configuration, the binding site for DNA polymerase is exposed to the reservoir containing the enzyme, which allows the latter to bind to the DNA; however, the ionic current in this configuration cannot be employed to detect whether an enzyme is bound. In the second, "probing" configuration, new enzyme molecules are no longer able to bind, but the presence of an already-bound enzyme can be detected *via* the ionic current. Repeatedly switching between these two states in an automated fashion allows Wilson *et al.* to accumulate detailed statistics on DNA polymerase binding. Note that the electrical signal is used to control the *spatial configuration* of the DNA (fishing *vs* probing); it is assumed that the presence of an electric field does not otherwise influence the binding properties of the enzyme during fishing.

Variations on this approach have been used by several authors to study biomolecular binding and to explore corresponding sensor concepts.[8−11] Two key factors for the broad applicability of such devices are the time resolution that is achievable and the total rate at which data can be acquired. Below, some of the fundamental limitations to the performance of these systems are considered. While the specific properties of α-hemolysin nanopores are employed here, many of the same considerations apply for other nanotechnology-based electrical biosensors.

Speed Bump. The ability to acquire extensive statistics in limited time and the ultimate time resolution of the detection system are limited by the speeds at which the potential can be switched and the ionic current measured. An important limitation arises quite naturally from the fact that the system is not entirely electronic in nature: the electrical current may be carried by electrons in the control electronics, but the loop is closed by ions in the fluid. Unfortunately, even highly concentrated salt solutions are poor electrical conductors compared to metals.

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Figure 1b shows a simple electrical circuit diagram that represents the flow of ions through the system sketched in Figure 1a. This circuit consists of three separate components: the resistance of the solution between the external electrodes and the membrane, R_s; the resistance of the nanopores itself, R_p; and the capacitance of the thin lipid bilayer that separates the two reservoirs and in which the nanopore is embedded, C. Under the salt conditions employed by the authors, the α-hemolysin nanopore has a resistance $R_p \sim 10$ GΩ, and this value changes by 14% upon DNA polymerase binding. This resistance is much larger than the solution resistance, R_s. The latter is dominated by the radial current profile in the vicinity of the membrane; for a circular membrane with a radius $r = 100$ μm, this gives a resistance $R_s \sim \rho/4r \sim 500$ Ω, where $\rho \sim 0.2$ Ωm is the resistivity of the 0.3 M KCl solution (about 10^8 times higher than the resistivity of copper). Finally, the capacitance of the lipid bilayer is ~2 μF/cm^2, yielding $C = \pi r^2$(2 μF/cm^2) ~ 1 nF.

The first fundamental limitation is set by the time it takes for a voltage step, V_{step}, applied through the external electrodes to polarize the membrane. This occurs on a time scale R_sC, which for the $r = 100$ μm lipid bilayer translates into a rise-time of ~0.5 μs. During this transient, an excess current flows through the system to charge the membrane. At very short times, this charging current has a magnitude V_{step}/R_s, which is much greater than the steady-state current given by $V_{step}/(R_s + R_p) \sim V_{step}/R_p$. On a time scale R_sC, the target signal is thus obscured by the charging transient, temporarily "blinding" the measurement system. This estimate of 0.5 μs is quite encouraging, but it represents an optimistic scenario. Addi-

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tional resistance and stray capacitance elsewhere in the system, the intrinsic response of the detection electronics, as well as additional, deliberately applied filtering, may all conspire to degrade this time resolution dramatically.

The simple analysis above has a second, more subtle consequence. In a measurement system capable of approaching the relaxation time scale R_sC, the current at short times (V_{step}/R_s) is much larger than the steady-state current (V_{step}/R_p). The magnitude of the charging transient can thus completely dwarf the signal to be detected. The detection electronics must have sufficient dynamic range to handle the initial transient without saturating, which usually translates into using a lower gain and, correspondingly, achieving a lower detection sensitivity.

In practice, perhaps the most important limitation on the achievable speed is that the noise level of the current detection circuitry must be sufficiently low to allow for detection of the small differences in current levels caused by the binding of DNA polymerase. Increasing the bandwidth of the measurement translates into higher noise levels, reducing the ability to detect small changes in the ionic current. The maximum achievable bandwidth is a complex function of the properties of the detection electronics, the values of the nanopore parameters in Figure 1b, any additional noise introduced by the nanopore, and the interplay between these factors. This problem is too involved to discuss here; for details, the reader is referred to recent analyses for the case of solid-state nanopores.[12,13] An important point that is often overlooked but worth emphasizing is that the nanopore functions as a transducer for voltage noise generated by a current-to-voltage converter. Minimizing the surface area of the membrane, and correspondingly its capacitance C, may under certain circumstances reduce the high-frequency noise levels at the detector.

Miniaturization and the Electron/Ion Interface. Another reason for scaling down the system dimensions is to improve the throughput of a single-molecule-based detection system by operating multiple detectors in parallel. The arithmetic is simple: a relatively modest array of 32 × 32 independently addressable nanopores could translate into a 1000-fold increase in data acquisition rate; however, scaling down single-molecule electrical sensors entails additional complications related to the ionic−electronic interface.

Conceptually, multiplexing entails the creation of an array of nanopores, each of which should be independently addressable for electrical measurements. The individual nanopores can be connected to a common macroscopic reservoir on one side, but must be addressable on the other side by an individual electrode, as sketched in Figure 2. This poses several challenges: how does one fabricate the individual wells and electrodes? To address this challenge, many solutions are possible,[14−16] including the recent interfacing of α-hemolysin with lipid-bilayer-modified silicon nanowire electrodes.[17] How does one ensure that the majority of the channels have a single nanopore,[18−20] and if successful, how does one handle the massive amounts of data generated by such an array operating at high frequency?

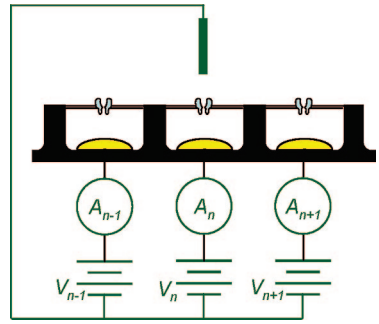

Figure 2. Schematic showing multiplexed nanopores.

Even before these fabrication problems, some basic issues regarding the electronic−ionic nature of the system must be resolved. So far, the nature of the interface between the electronic and ionic parts of the system has not been considered. In order to pass a constant (dc) current across the interface, a mechanism must exist for transferring charge between electrons in the electronics part and ions in the electrolyte part of the system. In other words, electrochemical reactions must take place at the interface. In a conventional measurement, such as in the work of Wilson *et al.*, these reactions take place at macroscopic electrodes that are located far from the nanopore. A typical system is the reaction of chloride ions (Cl^-) with a silver electrode (Ag) to form silver chloride (AgCl) and release an electron. This process is reversible, such that a Ag wire pretreated with Cl can be used to convert electrons into Cl^- ions. Such an electrode has a finite lifetime, as is easily seen by considering the extreme limit: an electrode can no longer act as a source of Cl^- once all of the Cl^- stored at its surface has been used. In experiments with nanoscale systems and macroscopic electrodes, this is not an issue since the low current levels that are used translate into long depletion times for the electrodes; however, in the case of a microscopic electrode embedded in a small reservoir[15] below a nanopore, this may become an important limitation.

One way to circumvent this is through the use of a so-called redox mediator: molecules that can be reversibly switched between two charge states while remaining in solution, thus only interacting with the electrode through the transfer of electrons. A typical mediator is the ferrocyanide/ferricyanide couple, which is often employed in glucose sensors. If present at sufficiently high concentrations, such mediators can provide the needed electron transfer at the interface. The drawback is that the addition of mediators to the solution can complicate preparation and, at least for the case of biological studies, create a system that is far removed from physiological conditions.

An extreme solution to the interface problem is to apply such large potentials (~1 V) that water itself starts to break down at the electrode surfaces to generate H^+ or OH^- ions. While this may be a useful approach when the electrodes are located far from the region of interest (as is the case in most electrophoresis measurements), this poses an obvious problem for a microscopic electrode embedded in a small reservoir; the reaction has the side effect of causing the pH of the solution in the small reservoir to change, once again removing the system from physiological conditions.[21]

Another way to solve the electronic/electrolyte interface problem is to forego electrochemical reactions altogether and to employ alternative current (ac) signals for detection. For small ac amplitudes, the interface between a conducting electrode and a liquid behaves as a capacitor (physically, electrons in the metal form one "plate" of this capacitor and ions accumulating at the surface of the electrode form the other plate). This allows an ac signal to flow across the electronic/electrolyte interface without electrochemistry taking place. The time resolution of such an approach is set by the frequency at which the current is driven. Not surprisingly,

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the properties of ionic conductors rear their head again and complicate the measurement.

Referring once again to Figure 1b, there are two pathways for current to flow through the nanopore system when an ac voltage V_{app} with frequency f is applied across the membrane.[22] First, an ohmic current V_{app}/R_p flows through the nanopore itself. Second, a displacement current $2\pi fCV_{app}$ flows across the membrane, 90° out of phase with the ohmic component. The magnitude of this displacement current increases linearly with frequency, such that it becomes comparable to the ohmic current at $f = f_c = 1/2\pi R_pC$. For $f > f_c$, this additional component increasingly dominates the total current and ultimately masks the desired signal completely. What makes this particularly relevant is the very high resistance of the pore, R_p: for the case of the $r = 100$ μm lipid bilayer discussed above, $R_pC \sim 10$ s and the crossover frequency at which the capacitance signal becomes relevant is only $f_c \sim 0.02$ Hz. Operating at a typical frequency of 1 kHz then yields a capacitive current that is 50 000 times larger than the current through the pore! Reducing the radius of the membrane, however, reduces its capacitance and thus shifts f_c to higher frequencies. A lipid bilayer with $r = 10$ nm yields $f_c = 2$ MHz. This could allow operation at frequencies in the megahertz range, with correspondingly higher time resolution. Miniaturization of these systems to the nanoscale may thus ultimately be driven by the need to interface the electrolytic and electronic parts of the system.

OUTLOOK

The intersection of nanofluidics, electronics, and single-molecule biological assays presents a fertile field for fundamental investigations and potential applications, yet the experimental challenges span a broad range of disciplines.[23] This Perspective has focused primarily on some of the complexities that arise when attempting to interface electronics with ionic systems. Meeting these challenges will represent a major step in the development of fully integrated nanoscale electrical biosensors.

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