NANOBIOTECHNOLOGY

A new look for nanopore sensing

Coating solid-state nanopores with fluid lipid bilayers can reduce the translocation speeds of molecules and prevent the nanopores from clogging.

Tim Albrecht

Biological and solid-state nanopores have been explored for applications in single-molecule biosensing, protein detection and ultrafast, label-free DNA sequencing[1–3]. Nanopore-based sequencing, in particular, is expected to be an easy-to-use and portable technology that will be useful in point-of-care diagnostics. However, the lack of spatial resolution and molecular specificity associated with nanopores has hindered their use as tools for routine sensing and sequencing applications, as has the difficulty of controlling the speed at which molecules translocate through nanopores. Furthermore, there are problems with mechanical instability and clogging that have been only partly overcome so far[4–6].

Writing in *Nature Nanotechnology*, Michael Mayer of the University of Michigan and colleagues[7] at the University of Arkansas and the University of California, San Diego, report that they have addressed some of these problems by coating solid-state nanopores with a fluid lipid bilayer, which allows the surface chemistry to be tailored and pore diameters to be tuned (Fig. 1). By embedding specific molecular receptors into the lipid bilayer, proteins could be preconcentrated on the lipid and selectively translocated through the nanopore. The fluidity of the pore walls allowed for fine-tuning of the translocation speed, simply by choosing a lipid bilayer with the right viscosity, and prevented the nanopores from clogging.

The total speed of a nanopore sensor is determined by the translocation speed of an individual biomolecule through the nanopore and the time required to collect a statistically significant amount of data. Translocation frequency, which is the number of translocation events per unit time, depends on the concentration of the biomolecule and this typically cannot be varied at will. Concentrations that are too low result in a low translocation frequency and potentially very long measurement times, whereas concentrations that are too high may lead to overcrowding inside the pore, and possibly even clogging, owing to the simultaneous translocation of several biomolecules.

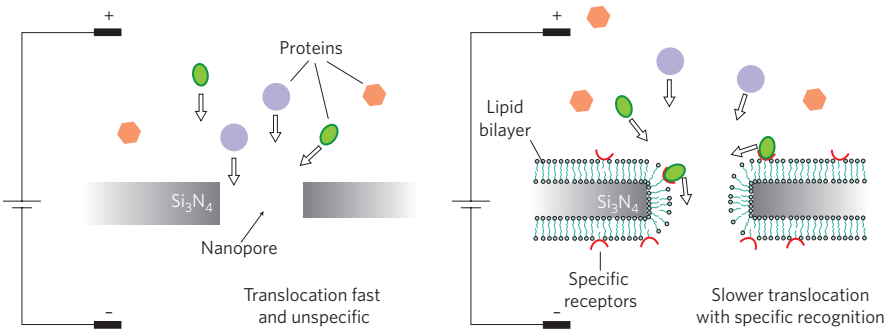

Figure 1 | Comparison of a conventional nanopore sensor and the fluid-coated nanopore sensor. Left: Schematic showing the conventional nanopore sensor. Translocation of proteins is fast and non-specific, and the overcrowding of biomolecules inside the nanopore often leads to clogging. Right: Nanopore sensors coated with a fluid lipid bilayer contain receptors that allow specific binding of proteins. Translocation is slower and individual translocation events can more easily be resolved. The fluidity of the pore walls also prevents clogging.

Similar but much more efficient nanopore sensors have been found in the olfactory systems of insects. Rather than attempting to detect extremely low concentrations of pheromones directly, the external skeleton of an insect contains nanopores that are coated with a mobile fluid lipid bilayer. The bilayer, which contains specific receptors, allows odorant molecules to bind and be preconcentrated before being transported to the olfactory neurons in the antennae of the insects.

Inspired by this, Mayer and co-workers made a nanopore that was 20–30 nm wide, and also 20–30 nm deep, in a silicon nitride membrane, and then coated it with a fluid lipid bilayer by exposing the membrane to a suspension of unilamellar liposomes. Similar to the receptors on the nanopores in insects, the bilayer contains biotin groups that act as specific molecular receptors for streptavidin, anti-biotin antibody fragments and other biotin-binding proteins. Although the solution concentration of the analytes of interest in this case is so small that they probably remain undetected, their high affinity to biotin leads to a significant preconcentration on the lipid-bilayer surface.

On application of an electric field across the nanopore membrane, the inherent mobility of the lipid bilayer on the solid support allows ions and proteins in solution, and notably surface-bound proteins, to be dragged through the nanopore to give a characteristic change in its conductance. Although proteins in solutions generally translocate very quickly (on the microsecond timescale) and are extremely difficult to detect, proteins that are bound to receptors in fluid walls are more easily detected. Because the translocation speed of receptor-bound proteins depends on the viscosity of the lipids, choosing the right lipid with the most suitable viscosity offers fine control over the translocation process. Lipids that are more viscous and have longer hydrophobic chains could slow down translocation speeds by up to two orders of magnitude.

Another advantage of using the fluid lipids is that a potentially large range of receptor molecules can be integrated into the bilayer and, depending on the target molecule in solution, a new level of molecular specificity can be introduced to nanopore-based sensing. Differentiating between protein species in classical nanopore sensors is a huge challenge because proteins with similar size and charge result in similar translocation

characteristics and are thus not specific enough, especially in complex mixtures. Finally, the fluidity of the lipid bilayer inside the nanopore also reduces the likelihood of pores being clogged by protein aggregates, addressing another major issue in nanopore sensing. Amyloid-beta proteins that are used as a model system were shown to translocate smoothly through the pores.

Mayer and colleagues[7] have essentially killed four birds with one stone with the new approach. The fluid lipids have allowed specific proteins from dilute solutions to be concentrated and translocated at frequencies much higher than expected from the solution concentration and without clogging the pores.

This new technology is not restricted to improving the sensing performance of nanopore devices. The idea of being able to deliver certain molecules, say proteins or enzymes, to a well-defined location on the nanopore could also be applied to assemble particular types of molecules, supramolecular structures or more complex molecular 'machines' at or inside the nanopore[8]. This may offer interesting prospects for the design of single-molecule devices or biological/solid-state hybrid structures potentially for many devices in parallel, say on a wafer scale.

The introduction of the 'fluid-wall technology' seems to be just the beginning of an exciting new development in nanopore research and, more generally, biological nanotechnology as a whole. ❐

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NANOELECTRONICS

Making light of electrons

Electrons have been channelled through graphene wires using the principles of optical guiding by fibre optic cables.

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Science and technology often rely on analogy to make progress. For example, photonic crystals are periodic materials that allow only certain wavelengths of light to propagate in them, in the same way that crystalline semiconductors allow electrons with only certain energies to move through them. Electronic counterparts of familiar optical components such as pinhole sources[1], interferometers[2], beam splitters and electron beam lenses[3,4] have also been built. These advances exploit the shared wave nature of electrons and photons, and have been made possible by developments in semiconductor materials growth and nanofabrication that allow the patterning of the potential energy landscape experienced by an electron on submicrometre scales and with extremely low disorder. Now, the electron has been made to mimic the photon in a new way, courtesy of graphene. Writing in *Nature Nanotechnology*, Charles Marcus and co-workers at Harvard and Purdue University report that they have guided electrons through narrow graphene channels by exploiting the same principles by which light is guided through a fibre optic cable[5].

Electronic waveguides (such as nanowires) typically work by creating a potential energy step that prevents mobile electrons from leaving the waveguide. However, an electronic analogue of the optical fibre has not been demonstrated. Optical fibres guide light through a core region (with a high refractive index) that is surrounded by cladding (with a lower refractive index). Light hitting the core–cladding interface bends and, for a shallow enough incident angle, is trapped in the core. This phenomenon, known as total internal reflection, allows the guiding of light over tens of kilometres without the need for signal regeneration.

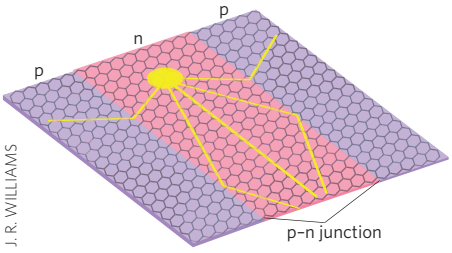

Figure 1 | Electrons from a point source (yellow circle) travel down a graphene p–n waveguide, consisting of two hole-rich areas (p) and a central, electron-rich area (n). Electrons incident on the p–n junction at glancing angles are reflected back into the n-type core, whereas those incident at larger angles escape into the p-type cladding. The unusual bend in the escaping trajectories is characteristic of a change in the sign of the index of refraction.

Now enter graphene. In this material, it is impossible to exclude electrons from some regions of space using simple potential energy steps, so there is a motivation for examining other ways of guiding electrons. Furthermore, the possibility of light-like guiding in graphene is somehow more obvious than it is in conventional semiconductors. This is because the relationship between the energy and wavelength of electrons in graphene is very similar to the corresponding relationship in photons, because electrons in graphene behave as if they were massless, in close analogy to massless photons. In retrospect, it is clear that light-like electron guiding could be done in conventional semiconductors as well, and perhaps that will be fruitfully explored in the coming years.

There are two possible approaches to achieve light-like guiding of electrons in graphene. First, in direct analogy to a fibre optic cable, one can construct an electronic 'cladding' with a lower density of carriers than in a corresponding 'core' to achieve so-called optical guiding. Or, more exotically, the cladding and core charge densities can be made opposite to one another, by arranging for one to carry holes and the other electrons, resulting in p–n guiding (Fig. 1). This gives the cladding an effectively negative index of refraction, a concept also borrowed from optics[6,7].

Marcus and collaborators simulate, then build, both types of guiding configuration in graphene wires. They focus on calculating and measuring the guiding efficiency, which is the portion of electrons injected at one end of the wire that successfully transit to the other end. A simulation of an idealized,