Solid-state nanopores

The passage of individual molecules through nanosized pores in membranes is central to many processes in biology. Previously, experiments have been restricted to naturally occurring nanopores, but advances in technology now allow artificial solid-state nanopores to be fabricated in insulating membranes. By monitoring ion currents and forces as molecules pass through a solid-state nanopore, it is possible to investigate a wide range of phenomena involving DNA, RNA and proteins. The solid-state nanopore proves to be a surprisingly versatile new single-molecule tool for biophysics and biotechnology.

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Have you ever struggled to pass a length of thread through the eye of a needle? If you have, you will know that it is difficult, especially if the needle's eye is small. However, advances in nanotechnology now allow single polymer molecules to be threaded through nanopores that measure just a few nanometres across. Although the passage of molecules through nanopores is commonplace in biology, it is only recently that researchers have been able to coax single DNA molecules through solid-state nanopores. This article reviews progress in this still young and rapidly expanding field, with a particular emphasis on engineered nanopores in silicon materials.

The biological cell is filled with all types of nanopores that control the trafficking of ions and molecules in and out of the cell and among the subcellular structures: examples include the ion channels that conduct ions across the cell surface; the nuclear membrane pores that control the passage of messenger RNA from the cell nucleus into the cytosol; the proteins that are secreted across pores in the membranes of cell organelles; and viruses, which dump their genomes into cells via pores that they insert into the cell membrane. Some of the transport through pores is passive, although most of it is actively controlled. Elucidating the physical processes involved in nanopore transport phenomena will remain a fertile field of research for years to come. Pioneering work on single-ion channels started decades ago with electrophysiology experiments that could measure the flow of ions through single nanopores in cell membranes[1].

In the 1990s, it was proposed that it might be possible to use nanopores as sensors for DNA[2]. If DNA could be transferred through a nanopore in a linear fashion, this might serve as a device to read off the sequence of DNA in an ultrafast way, which is of obvious interest for genomics applications. The first experimental results were reported in 1996 by Kasianowicz and co-workers at NIST on the biological pore α-haemolysin[3], which has become the protein of choice for this type of research. More recently, non-biological solid-state nanopores have opened up an even wider range of new research areas, but let us first review the wealth of results that have been obtained for α-haemolysin.

BIOLOGICAL NANOPORES

α-haemolysin is a protein secreted by *Staphylococcus aureus* bacteria as a toxin, which forms nanopores that spontaneously insert themselves into a lipid membrane. It features a transmembrane channel with a width of 1.4 nm at its narrowest point (see Fig. 1a.)[4], which allows ions to pass at a high ionic conductance of about one nS for typical conditions of one molar salt. An applied voltage of 100 mV across the membrane leads to a current of ~100 pA, which can be readily measured. A decade ago it was proposed that DNA can thread such pores, and that this would perhaps allow its sequence to be read off. The small constriction of this pore allows the passage of single-strand DNA (ssDNA), but not double-strand DNA (dsDNA) because, with a diameter of 2.2 nm, it is too wide. The idea of the basic experiment is simple: DNA is a highly charged molecule, so it can be driven through the nanopore in a linear head-to-tail fashion by an electric field (Fig. 1b). When the DNA enters the nanopore, the ionic current is reduced because part of the liquid volume that carries the ionic current is occupied by the DNA (similar to the Coulter counters used for counting and sizing particles).

Experiments on the traversal of polynucleotides through an α-haemolysin pore were first published ten years ago[3]. The data demonstrated that one could probe the traversal of individual ssDNA molecules, shown from analysis of electrical signals (the presence of DNA reduces the ion current by an order of magnitude) and from quantitative polymerase chain reaction analysis.

A host of follow-up experiments revealed that simple measurements of the passage of molecules through the nanopore (a process called translocation) could actually address a range of interesting properties of these molecules. Molecules were found to travel fast; for typical conditions, a 100 nucleotide string of C bases in ssDNA will move through in about 0.1 ms, yielding a residence time of about 1 μs per base (ref. 5). However, ssRNA molecules containing only A bases (polyA) move an order of magnitude slower than polyC, polyT and polyG molecules because polyA RNA has a secondary structure which needs to be deconstructed before it can pass through the pore[6]. As a result of the asymmetric structure of α-haemolysin, entry of a DNA molecule to the *cis* side (top in Fig. 1a) is easier than insertion at the *trans* (bottom) side[7]. This was attributed to a higher entropic barrier for entry or electrostatic repulsion of DNA from negative charge at the bottom side.

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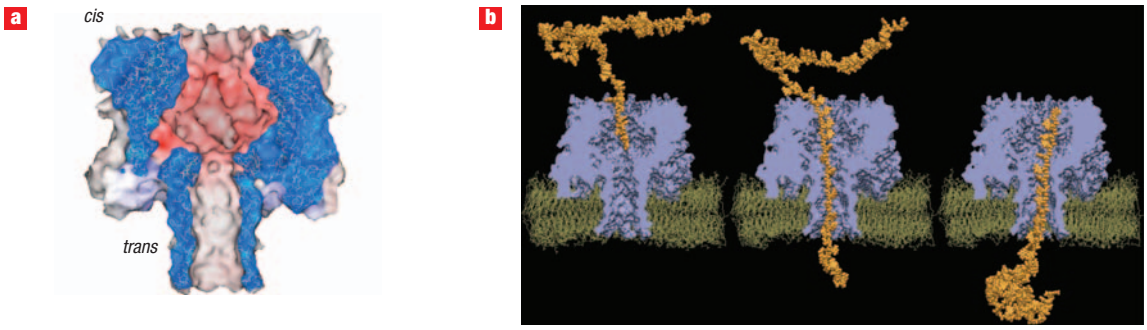

Figure 1 DNA translocation through a biological nanopore. α-haemolysin is a transmembrane protein that contains a pore that is approximately 1.4 nm wide at its narrowest point, which allows single-stranded DNA or RNA to move in and out of cells. **a**, The cross-sectional structure of α-haemolysin. The *trans* side is located towards the cytosol in the cell, whereas the *cis* side points outwards. Reproduced from ref. 11. **b**, Snapshots of a ssDNA molecule passing through such a nanopore. The DNA molecule enters the cavity at the *cis* side (left). Subsequently, the membrane voltage drives the DNA through the pore towards the *trans* side (middle, right). These snapshots are the result of a molecular dynamics simulation. Image courtesy of Aleksei Aksimentiev.

As intuitively expected, a larger applied voltage will both increase the frequency and decrease the duration of each translocation event because the molecules will move at higher velocity. The event frequency has been reported to be exponentially dependent on the voltage[7]. At constant voltage, the speed that the DNA moves through the pore is constant for all lengths — with long DNA taking an equivalently longer time to translocate — except for very short DNA (less than 12 bases), which moves at a higher velocity[5]. Translocation appears to be a thermally activated process as indicated by an exponential temperature dependence of the translocation time[8].

In experiments on DNA translocation, an α-haemolysin pore acts as an stochastic sensor which typically detects the passage of a few thousands molecules. Analyses involve plotting the induced change in current versus the translocation time in a scatter plot (an example for a solid-state nanopore is given in Fig. 3b), where each point represents the translocation of a single DNA molecule. With this type of plot even subtle differences between different populations of molecules can be discerned. For example, one can distinguish whether a ssDNA molecule enters the pore with its 3' end or its 5' end[9]. Remarkably, a 3'-threaded DNA molecule moves a factor of two slower than a 5'-threaded molecule. Molecular dynamics simulations suggested that this is due to a tilt orientation of the bases towards the 5' end at the point where the nanopore confines the DNA most stringently.

Given the motivation to develop translocation of DNA through α-haemolysin as a sequencing method, a particularly appealing early result was that the current signals were found to be different when comparing a homopolymer, made up of 100 identical bases, with another homopolymer of different type, for example comparing polyA and polyC RNA[6,8]. A difference could also be seen within an RNA molecule with a heterosequence of $A_{30}C_{70}$. The observation that one could distinguish A and C bases in the electrical signal led to optimism about the use of nanopores for sequencing. However, it was subsequently realized that the signal-to-noise ratio was insufficient to ever reach single-base resolution in translocation experiments under typical conditions. The main reason is that translocation through α-haemolysin occurs at such a fast rate: during the ~1 μs that one base spends at the narrowest constriction, the number of ions that pass the pore is in the order of 100. Statistical fluctuations in this number overwhelm the subtle differences between the four different types of bases. Sequencing by straightforward DNA translocation through α-haemolysin thus seems unrealistic. However, this is not the end of the story at all and alternative strategies for sequencing with nanopores are being developed (see Outlook section).

A great advantage of protein nanopores is that they can be chemically engineered with advanced molecular biology techniques such as mutagenesis[10]. It is therefore possible to make well-defined local changes in the structure, for example, by exchanging one amino acid group for another, or adding a small organic molecule at a specific molecular site. Using such techniques, a variety of α-haemolysin biosensors were developed, such as ones with hydrophobic groups that bind organic molecules[11] and with a biotin-labelled chain that can probe biotin-binding proteins[12]. Similarly, the addition of a nucleotide oligomer makes it possible to probe the hybridization of complementary DNA: if the sequences match, duplex binding will occur and the DNA translocation will be halted until, after a while, the DNA is unzipped and translocates through the pore[13]. Unzipping has also been studied extensively in experiments on ssDNA hairpins[14,15] and on ssDNA with a dsDNA end[16], again achieving discrimination at single-base resolution. Recently, translocation through anthrax pores[17], which have a structure very similar to α-haemolysin, has been studied.

FABRICATION OF SOLID-STATE NANOPORES

Although biological pores have proved to be very useful for a range of interesting translocation experiments, they do exhibit a number of disadvantages such as fixed size and limited stability. Typically the pores, and also, in particular, their embedding lipid bilayer, can become unstable if changes occur in external parameters such as pH, salt concentration, temperature, mechanical stress, and so on. Fabrication of nanopores from solid-state materials presents obvious advantages over their biological counterpart such as very high stability, control of diameter and channel length, adjustable surface properties and the potential for integration into devices and arrays. Various routes have been explored to meet the challenge of fabricating pores with true nanometre dimensions.

Etching a hole into an insulating layer has been one way to create pores. Researchers have also etched holes in glass slides, albeit with larger dimensions, for electrophysiological measurements on cells[18,19]. A more delicate technique has been to guide the etching so that it occurs preferentially along one linear defect path that was created by shooting a single high-energy heavy-metal ion through a polymer layer[20]. Importantly, one can monitor the ion current across the layer during the etching, which provides feedback for when to

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stop etching. In this way, pores with nanometre dimensions, down to 2 nm, have been made. However these pores typically have a narrow opening angle of ~1°, which is non-ideal for translocation of polynucleotides. The surface of the etched pore can be modified, for example, by coating with a gold layer. This changes the electrostatic boundary condition of the pore and provides a template to add further organic layers through thiol self-assembly techniques.

Five years ago, Golovchenko and co-workers at Harvard reported a novel technique — ion beam sculpting — by which they could fabricate single nanopores in thin SiN membranes with true nanometre control[21]. Working with SiN and SiO_2 materials provides an advantage as these materials have been extremely well-developed and studied over the course of fifty years of microelectronics. The Harvard group used a dedicated ion beam-machine (Fig. 2a) that uses a focused ion beam to mill a tiny hole in the membrane. Feedback from ion detectors below the membrane provided signals to indicate when to stop the milling. Interestingly, they observed that, depending on the ion rate and temperature, pores could enlarge and shrink, allowing the fine-tuning of pores in the nanometre range. Pores with nanometre dimensions were made in this way and provided a starting point for DNA translocation measurements.

Our group at Delft has pursued another approach (Fig. 2b)[22]. Again, using the techniques from Si microfabrication, free-standing membranes of Si, SiN, or SiO_2 can be made. Subsequently, we can pattern holes in these using electron-beam lithography and subsequent etching. A typical diameter of such a hole is 20 nm, and this could be reduced to sub-10 nm if needed. In imaging these holes with a transmission electron microscope (TEM), we discovered that high-intensity wide-field illumination with electrons slowly modified the nanopore size. Large holes, with a diameter greater than the membrane thickness, grew in size, whereas small holes shrunk. Note that this provides a way to fine-tune the nanopores to a small size with subnanometre resolution and with direct visual feedback from a commercial TEM. This process of glassmaking at the nanoscale occurs as the SiO_2 membrane flows to decrease the surface tension and so slowly reshapes around the hole[23]. If electron-beam exposure is stopped, the structure will be entirely frozen and stable. It has also been shown that a pore can be directly drilled in a membrane by a locally focused electron beam in a TEM[22,24–26], making laborious preparatory electron-beam lithography of larger nanopores unnecessary. Again, such pores can be modified with the wide-field TEM illumination.

It is clear that the fabrication of pores with true nanometre dimensions has greatly advanced in recent years. Nanopores have now been fabricated with subnanometre control and with diameters as small as 1 nm. In addition to opening up the way to single-molecule measurements, this also leads to other applications, such as the fabrication of nanopore-based metal electrodes for electrochemistry[27]. Other groups are pursuing nanopores in multilayer membrane materials that may provide additional electronic probes for detection of DNA[28].

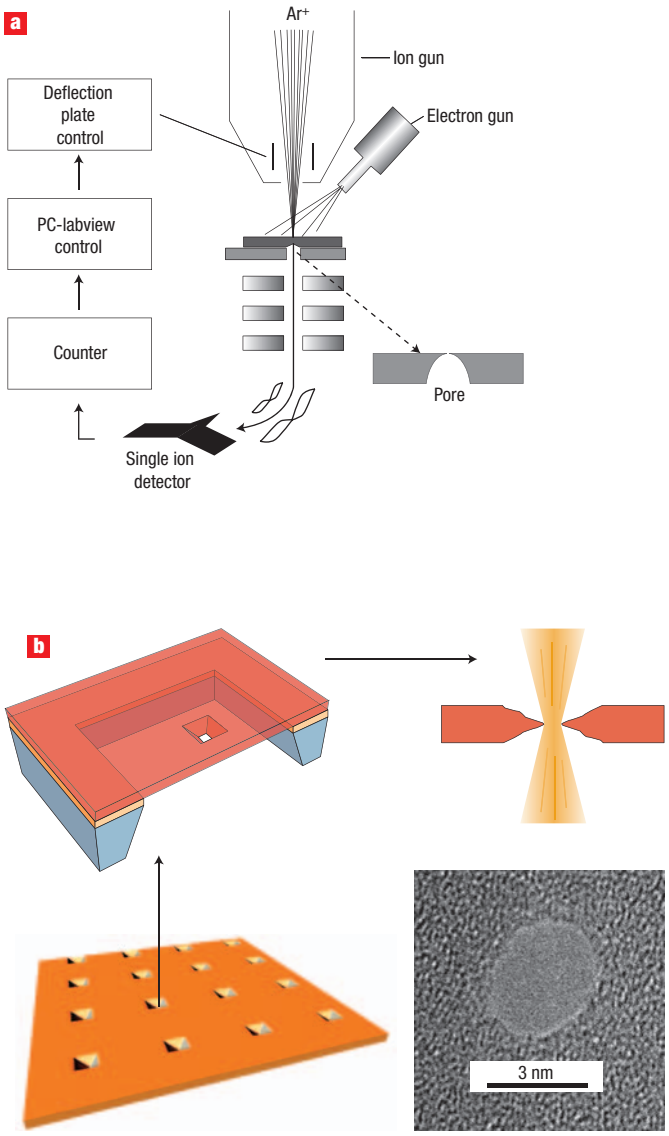

Figure 2 Fabrication of solid-state nanopores. Pores with nanometre-size diameters can be made with a variety of techniques within a thin silicon-based membrane. **a**, The focused-ion-beam technique developed by the Harvard group[21]. When the ions drilling the hole penetrate through the membrane, the beam is shut down. **b**, The TEM technique developed at Delft[22]. An electron beam drills a hole in the membrane. The result can be directly monitored in the electron microscope and the electron beam can also be used to enlarge or shrink the nanopore in a controlled way. The figure at the bottom right shows a typical TEM image of a nanopore (with a diameter of 3 nm in this example).

IONIC CONDUCTION OF SOLID-STATE NANOPORES

When these solid-state nanopores are immersed in a liquid with a monovalent salt, their ion conduction can be measured. Under good conditions, linear current–voltage characteristics can be measured up to a high voltage (1 V), and the pore conductance obtained matches that expected from the bulk conductivity of the liquid and the geometry of the nanopore. Typical measurements are taken at one molar salt. At low salt (below ~0.1 M), the effect of the negatively charged walls of SiO_2 sets in and the lowered pore conductance is dominated by the counter ions that screen the walls. Measurements of the salt dependence of the nanopores' ionic conduction shows that this picture describes the data well, provided that the chemical reactivity of the silicon dioxide surface is accounted for[29].

Asymmetric current–voltage curves are not uncommon and can have multiple causes. For polymer nanopores, it has been reported that the sign and magnitude of the applied voltage can cause the pore size to vary[30]. The cause of asymmetries in Si-based nanopores, however, remains less clear. Interesting effects have been observed when the ion concentration differs on both sides of the nanopore. Resistance changes and asymmetric current–voltage curves were observed and interpreted to result from the nanopore's charged

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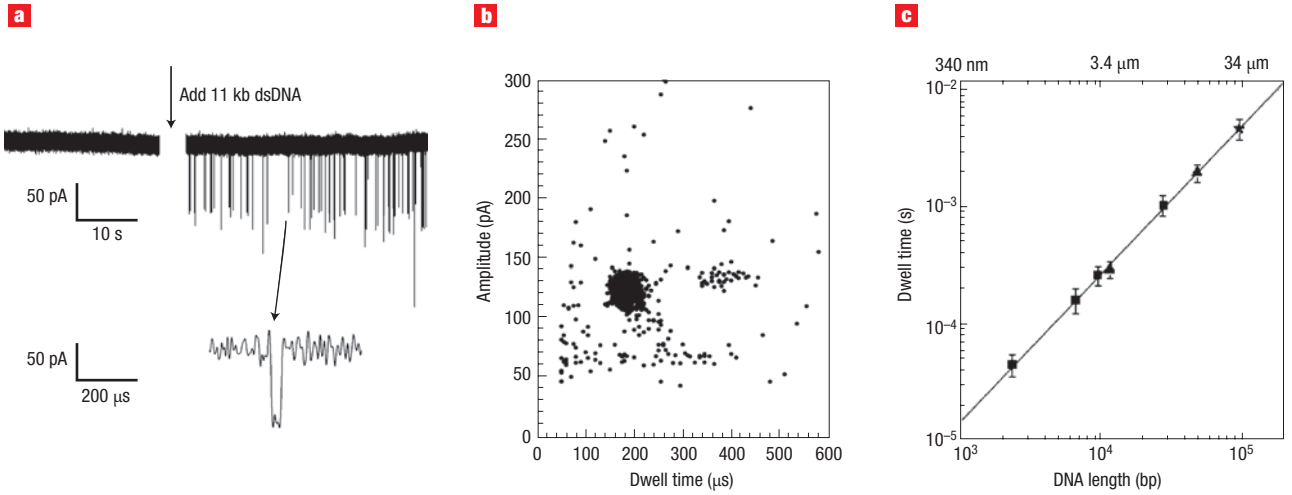

Figure 3 Translocation of dsDNA through a solid-state nanopore. **a**, An example of the ion current versus time measured for a nanopore. When 11 kbp dsDNA is added, downward spikes appear. Each spike indicates an event where a single DNA molecule traverses the 10 nm nanopore. The bottom panel zooms in on one event. **b**, A scatter plot where the amplitude of the current change is plotted versus the time of the DNA translocation. Each point is a separate translocation event. This particular example is the data for 11 kbp circular nicked dsDNA which traverses the 8 nm pore in about 200 μs without any complicated folding phenomena. On average each traversal creates a current dip of 120 pA at the applied voltage of 120 mV. **a** and **b** reproduced with permission from ref. 37. Copyright (2005) APS. **c**, Translocation time versus the contour length of the dsDNA. Surprisingly, it was found that long dsDNA molecules display a nonlinear, power-law dependence $\tau \approx L^{\alpha}$ with an exponent α=1.27. Reproduced with permission from ref. 39. Copyright (2005) ACS.

walls and the distribution of electric fields within the asymmetric conical-shaped pore[31].

Nanopores can exhibit undesirable phenomena such as a large variability in conductance[32] and significant noise, both as a function of time and among samples. Theoretical work on biological pores suggests that switching between different pore conformations generates 1/f noise[33], and experiments on polymer nanopores have provided evidence for this[34]. Recently, our Delft group identified a different noise source of nanopores[35], namely nanometre-sized gaseous bubbles — so-called nanobubbles. Conductance profiles show strong variations in both the magnitude of the conductance and in the noise when a single nanopore is scanned through the focus of an infrared laser beam multiple times. Differences of up to five orders of magnitude were found in the current power spectral density. A simple model of a cylindrical nanopore that contains a nanobubble explained the data well. Nanobubbles have been observed before in other experiments but the reason for their thermodynamic stability is still under debate (for a review see ref. 36).

DNA TRANSLOCATION THROUGH SOLID-STATE NANOPORES

The main aim of researchers working with solid-state nanopores has been to measure the translocation of individual DNA molecules. The first current traces that indicated dsDNA in a solid-state nanopore were reported by the Harvard group[21]. More extensive measurements were presented by Storm *et al.* from our group. Similar to the α-haemolysin experiments, molecules pass the nanopore in a linear fashion as evidenced from monitoring the conductance of a voltage-biased pore. Contrary to α-haemolysin, this is also possible with dsDNA and long molecules of up to 100 kilobases.

The basic experimental result is illustrated in Fig. 3a: when 11 kbp-long dsDNA is added to the negatively biased reservoir, downward spikes appear in the ionic current through the pore. Each spike represents a single-molecule event where an individual DNA molecule translocates the nanopore. For each event, one can characterize the amplitude by which the current is reduced as well as the duration of the blockage. Plotting amplitude versus duration produces a scatter plot. Figure 3b shows such a scatter plot for circular 11 kbp dsDNA which displays a simple distribution around a single point. More complicated distributions are often observed and careful analysis of scatter plots has proven quite helpful to disentangle different types of translocations.

DNA with different lengths can be identified from the different durations of translocation. Indeed, it was shown that individual DNA molecules within a mixture containing different size molecules could be distinguished[37], providing a demonstration of DNA sizing that is analogous to the well-known gel electrophoresis technique but without the need to stain the DNA. Moreover, the nanopore technique requires much less material than existing techniques — as few as 1,000 molecules.

A narrow pore only allows the linear passage of DNA in a head-to-tail fashion. In wider pores, however, it has been observed that DNA can traverse in a folded manner[37,38]. Long DNA molecules form a randomly folded coil. For such a configuration, the translocation of DNA can start at a point somewhere from the side of such a coil. If this happens, two double-strand pieces of DNA enter the pore, followed by one piece when one of the two pieces has encountered its end. Indeed this was observed. More complex phenomena with up to five parallel pieces of a single dsDNA molecule were also detected.

Another interesting polymer-physics effect concerns the translocation velocity. Experiments have shown that long (say 10 kbp) DNA does not traverse a wide solid-state nanopore in a simple linear fashion at constant speed. Instead, it was found that the translocation time grows as a power of the DNA length, $\tau \approx L^{\alpha}$ with an exponent α=1.27 (ref. 39). This could be theoretically understood to result from the hydrodynamic drag on the section of the DNA polymer outside the pore being the dominant force counteracting the electrical driving force. This leads to a power-law scaling with an exponent of 1.22, which is in good agreement with the data[39].

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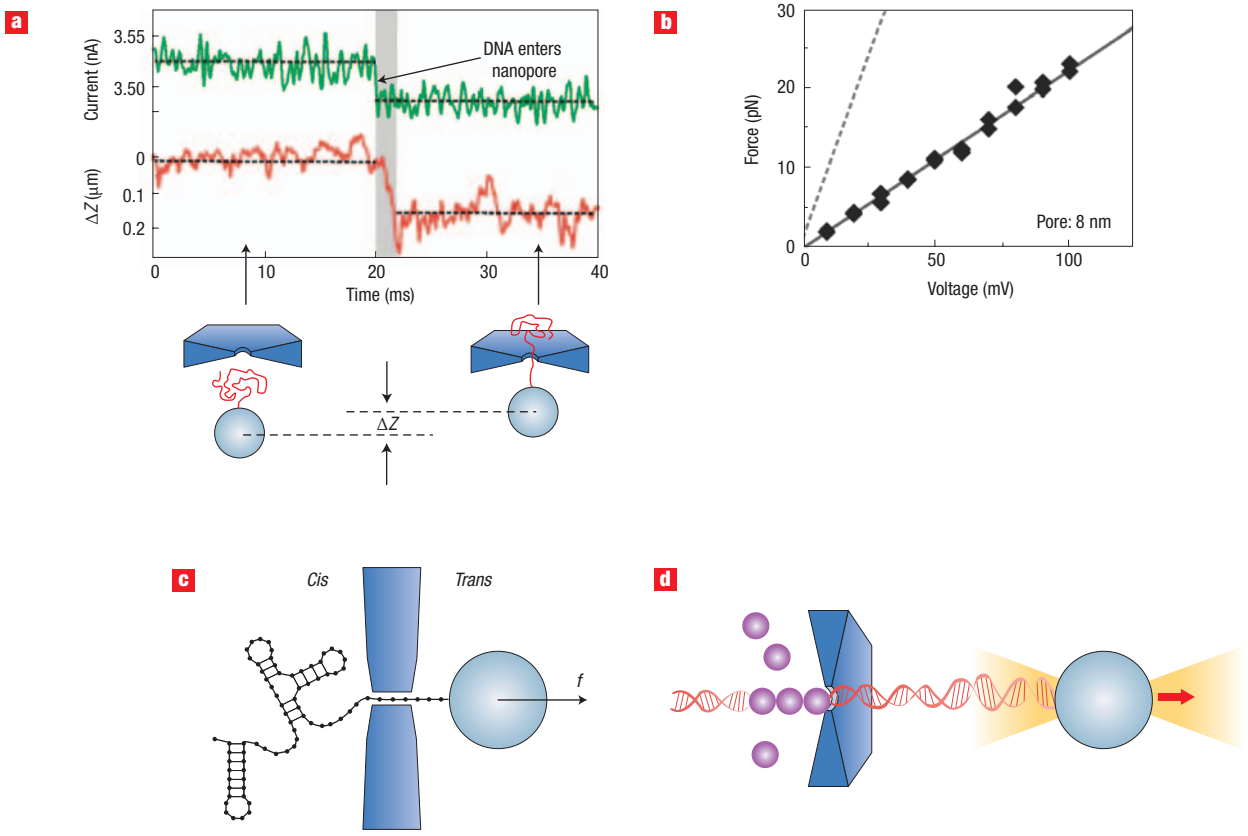

Figure 4 New developments. **a**, Force measurements can be executed on a single dsDNA molecule. DNA is attached to a bead that is controllably held in an optical tweezer. With an applied voltage, the DNA is drawn into the nanopore and as a result the bead is displaced from its equilibrium position in the trap. The displacement is a measure of the force that is acting on the DNA molecule in the nanopore. Reproduced from ref. 48. **b**, The measured force on a dsDNA molecule in an 8 nm nanopore at 20 mM KCl. The dashed line represents the force expected for DNA with a charge of 2 electrons per bp. Reproduced from ref. 48. **c**, A sketch of RNA unfolding by sequential unzipping of its hairpins, which can be monitored by pulling it through a nanopore with an applied force, *f*. It has been theoretically calculated that the signatures of individual hairpins should be discernable[55,56]. **d**, A sketch of a possible experiment where the (dis)association of DNA-binding proteins is measured. This type of experiment is interesting from the point of view of basic science, and possibly also DNA sequencing. Much thought is given to strategies where locally binding probes are a measure of the local sequence, which may be measured in nanopores.

New phenomena were also discovered for DNA translocation at low salt concentrations. Surprisingly, at 100 mM salt, the insertion of DNA does not lead to suppression of the ionic current, but to an enhancement[40,29]! In fact, the salt dependence of the magnitude of the current change showed a sign reversal when the KCl concentration was close to 0.4 M. This remarkable finding can be understood from a simple model where current decreases result from the partial blocking of the pore and current increases are attributed to motion of the counter ions that screen the charge of the DNA backbone[29].

A variety of other interesting experimental results are now appearing as the field is growing. For example, the velocity of translocating DNA can be slowed down by an order of magnitude if the salt concentration is increased, the driving voltage is decreased and the viscosity of the fluid is increased by the addition of glycerol and lowering of the temperature[41]. By melting DNA at high pH, the translocation of ssDNA can be observed[42]. For very small nanopores (~2 nm), it has been reported that ssDNA can pass, whereas dsDNA is blocked from passing because of its larger diameter[43]. Modelling of the translocation process has been initiated with extensive molecular dynamics simulations[44]. For small pores, a voltage threshold for dsDNA translocation was found, which was associated with the stretching transition that occurs in dsDNA near 60 pN (ref. 45), suggesting that dsDNA can be drawn through very narrow pores when strongly driven.

FORCE SPECTROSCOPY WITH NANOPORES

The rich variety of experimental observations of DNA translocation events through nanopores has increasingly led to questions about the basic physical understanding of the translocation process. What are the forces acting on the DNA and how large are they? What is the role of the counter charges? How about hydrodynamic couplings? What role does entropy play on DNA entering the hole[46]? And so on.

New experimental probes — beyond the straightforward ionic conductance — are needed to address such questions. Our group has used optical tweezers for direct force measurements on DNA in nanopores[47]. Here, one end of the DNA is attached to a bead, which is trapped in the focus of an infrared laser. Subsequently, we can insert individual DNA molecules into a single nanopore and arrest the DNA during voltage-driven translocation. The force acting on the DNA then pulls the bead away from the centre of the optical trap, an effect that can be measured with high accuracy using the reflected light from the bead. These experiments directly

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measure the force on a DNA molecule in a solid-state nanopore (Figure 4a).

The force acting on a single dsDNA was found to rise linearly with the applied voltage, at a rate of 0.23 pN mV^{-1} (Fig.4b)[48]. Interestingly, this value is entirely independent of the KCl salt concentration as measured from 20 mM to 1 M. The force acting on a DNA molecule thus does not depend on salt, which is even more remarkable when remembering that, as mentioned above, the induced change in the ion current actually reverses sign in this range of salt concentrations. The value of 0.23 pN mV^{-1} is a factor of four lower than that expected from a naive calculation of the electrostatic force acting on DNA, which considers a DNA charge of two electrons per base pair because of the phosphate groups on the backbones. The force data have been interpreted as indicating that bound counter ions lead to an effective DNA charge of 0.50 electrons per base pair[48]. A similar value was found from the salt dependence of the current change[29]. Although this appears to nicely coincide with an early ion-condensation prediction of 0.48 electrons per base pair by Manning[49], caution is needed because a full model needs to incorporate not only the statics but also the dynamics of ion motion along DNA as well as hydrodynamic coupling.

The application of a laser to solid-state nanopores has also led to other uses. A nanopore acts as a sensitive thermometer with single nanometre dimensions because it measures the temperature-dependent conductance of the ionic liquid[50]. A laser heats up the liquid at its focal point, and its three-dimensional intensity profile was mapped out by scanning the nanopore, similar to tomography. This new technique does not rely on any optical elements and allows quantitative measurement of optical intensity or temperature distributions in aqueous environments with nanometre resolution.

New methods of force spectroscopy can also be investigated without using optical tweezers. Now that the linearity of the force versus applied voltage has been established and the force magnitude is calibrated, one can use this to simply vary the applied voltage and thus the applied force. Meller and co-workers have worked out a technique where the applied voltage is shut off as soon as the current drops due to the insertion of a DNA molecule[15]. Subsequently, the voltage can be ramped and the response of the molecule monitored.

OUTLOOK: THE S-WORD AND MORE

What more can we expect from solid-state nanopores? First and foremost, how about the big S-word, sequencing[51]? If sequencing with nanopores turns out to be feasible, the impact will be huge because it will remove the need for chemical modification, amplification and surface adsorption of the DNA. Single-molecule DNA sequencing would open up the reading of the genome of single cells and generally act as the key technology in genomics.

As mentioned above, the prospects of simply monitoring the ionic current itself during straightforward translocation are not good, mainly because the DNA moves through the pore too fast. However, optical tweezers can pull DNA through a nanopore at arbitrarily slow speeds, so this approach is worth further research.

Many alternative ideas for sequencing with nanopores exist. Theoretical calculations indicate that sequence information can be extracted from a tunnelling current through the DNA bases as measured between two transverse nanoelectrodes located right at the nanopore[52], though this claim has been disputed[53]. Another idea is to measure changes in the mutual capacitance as DNA molecules pass through a nanopore in a semiconducting multilayer[28] rather than a nanopore in a single insulating membrane. Antisense DNA oligomers may be located near the nanopores by self-assembly chemistry and may be able to probe certain sequences along a passing DNA molecule. Sequence-dependent optical probes may be added and subsequent readout at the pore may then signal the sequence. These and other ideas await experimental verification that will show whether sequencing is feasible and competitive.

The impact of solid-state nanopores is, however, by no means determined by sequencing applications. A range of both technological and scientific opportunities lie ahead. Separation, sizing and sorting can be based on molecular mass, charge, hydrophobicity, stereochemistry and so on. DNA size determination and haplotyping (deducing the constitution of an individual chromosome) are obvious first applications. Sensors can be built with solid-state nanopores — similar to that already demonstrated with α-haemolysin — by incorporating a biomolecular recognition complex (a biotin, antisense DNA or antibodies) into the pore. Further technological challenges include the integration of pores into nanofluidics[54], electronic circuitry and optoelectronic detectors, the incorporation of biological pores with solid-state pores, and the chemical functionalization of nanopores.

There are also plenty of scientific questions that can be addressed in experiments with solid-state nanopore. How are the counterions organized around the DNA, and what is their mobility and coupling to the surrounding liquid? What is the effect of the lateral confinement on the translocating molecule? Are there effects of water in the nanoscale confinement of the pore? What is the effect of the chemical composition, charge state and hydrophilicity/phobicity of the nanopore walls? What is the ultimate detection limit of local structures along the DNA: can one detect a single bound protein, a triple-strand structure, a knot, a hairpin, a mismatched base and so on? Theoretical calculations indicate that it will be feasible to unzip individual hairpins in a ssRNA molecule by slowly pulling the molecule through the pore and measuring the associated force (Fig. 4c)[55,56]. The great advantage of the nanopore technique is that — in contrast to the conventional optical tweezer techniques — one can unzip the hairpins sequentially, thus unravelling the RNA structure in a linear fashion along the molecule.

This illustrates that a nanopore can also be used as a local force actuator. This may open up new experiments where, for example, the biophysics of DNA-processing molecular motors can be studied because it is possible to follow the motor action in real time as it processes the DNA[57]. In a similar example the polymerization of DNA-binding proteins can drive DNA through a pore if its size is large enough to pass the DNA but too small to pass the proteins (Fig. 4d). Monitoring this process with a nanopore may yield parameters such as the polymerization speed and stalling forces.

It is also possible to translocate non-polynucleotide polymers, in particular to observe the passage of single protein molecules[58,59]. This raises the intriguing possibility that nanopores may provide a new route to unfolding proteins in a controlled way. Finally, the ability to fabricate pores with true nanoscale dimensions may find applications that are very different from biomolecular detection. For example, they have been used as a template for nanoelectrodes with diameters down to 2 nm (ref. 24), and have recently been used to explore new regimes for electrochemistry[60]. Larger solid-state pores can act as chip-based probes and are promising alternatives to conventional patch-clamping for electrophysiology on cells.

Given this long list of new opportunities, as well as the recent progress in the field, solid-state nanopores are set to remain an active area of research for years to come.

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References

1. Sakmann, B. & Neher, E. (eds). *Single Channel Recording* 2nd edn (Plenum, New York, 2005).
2. Deamer, D. W. & Akeson, M. Nanopores and nucleic acids: prospects for ultrarapid sequencing. *Trends Biotechnol.* **18,** 131–180 (2000).

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3. Kasianowicz, J. *et al*. Characterization of individual polynucleotide molecules using a membrane channel. *Proc. Natl Acad. Sci. USA* **93,** 13770–13773 (1996).
4. Song, L. *et al*. Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. *Science* **274,** 1859–66 (1996).
5. Meller, A., Nivon, L. & Branton, D. Voltage-driven DNA translocations through a nanopore. *Phys. Rev. Lett.* **86,** 3435–3438 (2001).
6. Akeson, M., Branton, D., Kasianowicz, J. J., Brandin, E. & Deamer, D. W. Microsecond time-scale discrimination among polycytidylic acid, polyadenylic acid, and polyuridylic acid as homopolymers or as segments within single RNA molecules. *Biophys. J.* **77,** 3227–3233 (1999).
7. Henrickson, S. E. *et al*. Driven DNA transport into an asymmetric nanometer-scale pore. *Phys. Rev. Lett.* **85,** 3057–3060 (2000).
8. Meller, A., Nivon, L., Brandin, E., Golovchenko, J. & Branton, D. Rapid nanopore discrimination between single oligonucleotide molecules. *Proc. Natl Acad. Sci. USA* **97,** 1079–1084 (2000).
9. Mathe, J. *et al*. Orientation discrimination of single-stranded DNA inside the -hemolysin membrane channel. *Proc. Natl Acad. Sci. USA* **102,** 12377–12382 (2005).
10. Bayley, H. *et al*. Engineered Nanopores in *NanoBiotechnology* (eds Niemeyer, C. M. & Mirkin, C. A.) (Wiley-VCH, Weinheim, 2005).
11. Gu, L. Q. *et al*. Stochastic sensing of organic analytes by a pore-forming protein containing a molecular adapter. *Nature* **398,** 686–690 (1999).
12. Movileanu, L. *et al*. Detecting protein analytes that modulate transmembrane movement of a polymer chain within a single protein pore. *Nature Biotechnol.* **18,** 1091–1095 (2000).
13. Howorka, S., Cheley, S. & Bayley, H. Sequence-specific detection of individual DNA strands using engineered nanopores. *Nature Biotechnol.* **19,** 636–639 (2001).
14. Vercoutere, W. *et al*. Rapid discrimination among individual DNA hairpin molecules at single-nucleotide resolution using an ion channel. *Nature Biotechnol.* **19,** 248–252 (2001).
15. Mathe, J. *et al*. Nanopore unzipping of individual DNA hairpin molecules. *Biophys. J.* **87,** 3205–3212 (2004).
16. Sauer-Budge, A. F. *et al*. Unzipping kinetics of double-stranded DNA in a nanopore, *Phys. Rev. Lett.* **90,** 238101 (2003).
17. Halverson, K. M. *et al*. Anthrax biosensor, protective antigen ion channel asymmetric blockade, *J. Biol. Chem.* **280,** 34056–34062 (2005).
18. Schmidt, C., Mayer, M. & Vogel, H. A chip-based biosensor for the functional analysis of single ion channels. *Angew. Chem. Int. Edn* **39,** 3137–3140 (2000).
19. Fertig, N. *et al*. Microstructured glass chip for ion-channel electrophysiology. *Phys. Rev. E* **64,** 040901 (2001).
20. Siwy, Z. & Fulinski, A. Fabrication of a synthetic nanopore ion pump. *Phys. Rev. Lett.* **89,** 198103 (2002).
21. Li, J. *et al.* Ion-beam sculpting at nanometre length scales. *Nature* **412,** 166–169 (2001).
22. Storm, A. J. *et al*. Fabrication of solid-state nanopores with single-nanometre precision. *Nature Mater.* **2,** 537–540 (2003).
23. Storm, A. J. *et al*. Electron-beam-induced deformations of SiO_2 nanostructures. *J. Appl. Phys.* **98,** 014307 (2005).
24. Heng J. B. *et al*. Sizing DNA using a nanometer-diameter pore. *Biophys. J.* **87,** 2905–2911 (2004).
25. Krapf, D. *et al*. Fabrication and characterization of nanopore-based electrodes down to 2 nm. *Nano Lett.* **6,** 105–109 (2006).
26. Zandbergen, H. W. *et al.* Sculpting nano-electrodes with a transmission electron beam for electrical and geometrical characterization of nanoparticles. *Nano Lett.* **5,** 549–553 (2005).
27. Lemay, S. G. *et al*. Lithographically fabricated nanopore-based electrodes for electrochemistry. *Anal. Chem.* **77,** 1911–1915 (2005).
28. Gracheva, M. E. *et al*. Simulation of the electric response of DNA translocation through a semiconductor nanopore-capacitor. *Nanotechnology* **17,** 622–633 (2006).
29. Smeets, R. M. M. *et al*. Salt-dependence of ion transport and DNA translocation through solid-state nanopores. *Nano Lett.* **6,** 89–95 (2006).
30. Siwy, Z. *et al*. Rectification and voltage gating of ion currents in a nanofabricated pore. *Europhys. Lett.* **60,** 349–355 (2002).
31. Siwy, Z. *et al*. Asymmetric diffusion through synthetic nanopores. *Phys. Rev. Lett.* **94,** 048102 (2005).
32. Ho, C. *et al*. Electrolytic transport through a synthetic nanometer-diameter pore. *Proc. Natl Acad. Sci. USA* **102,** 10445–10450 (2005).
33. Bezrukov, S. M. & Winterhalter, M. Examining noise sources at the single-molecule level: $1/f$ noise of an open maltoporin channel. *Phys. Rev. Lett.* **85,** 202–205 (2000).
34. Siwy, Z. & Fulinski, A. Origin of $1/f\alpha$ noise in membrane channel currents. *Phys. Rev. Lett.* **89,** 158101 (2002).
35. Smeets, R. M. M. *et al*. Nanobubbles in solid-state nanopores *Phys. Rev. Lett.*, **97,** 088101 (2006).
36. Attard, P. Nanobubbles and the hydrophobic attraction. *Adv. Colloid Interface Sci.* **104,** 75 (2003).
37. Storm, A. J. *et al*. Translocation of double-strand DNA through a silicon oxide nanopore. *Phys. Rev. E* **71,** 051903 (2005).
38. Li, J. *et al*. DNA molecules and configurations in a solid-state nanopore microscope. *Nature Mater.* **2,** 611–615 (2003).
39. Storm, A. J. *et al.* Fast DNA translocation through a solid-state nanopore. *Nano Lett.* **5,** 1193–1197 (2005).
40. Chang, H. *et al*. DNA-mediated fluctuations in ionic current through silicon oxide nanopore channels *Nano Lett.* **4,** 1551–1556 (2004).
41. Fologea, D. *et al*. Slowing DNA translocation in a solid-state nanopore. *Nano Lett.* **5,** 1734–1737 (2005).
42. Fologea, D. *et al.* Detecting single stranded DNA with a solid state nanopore, *Nano Lett.* **5,** 1905–1909 (2005).
43. Heng, J. B. *et al.* The electromechanics of DNA in a synthetic nanopore. *Biophys. J.* **90,** 1098–1106 (2006).
44. Aksimentiev, A. *et al.* Microscopic kinetics of DNA translocation through synthetic nanopores. *Biophys. J.* **87,** 2086–2097 (2004).
45. Heng, J. B. *et al*. Stretching DNA using the electric field in a synthetic nanopore. *Nano Lett*. **5,** 1883–1888 (2005).
46. Muthukumar, M. Polymer escape through a nanopore. *J. Chem. Phys.* **118,** 5174–5184 (2003).
47. Keyser, U. F., van der Does, J., Dekker, C. & Dekker, N. H. Optical tweezers for force measurements on DNA in nanopores. *Rev. Sci. Instr.* **77,** 105105 (2006).
48. Keyser, U. F. *et al.* Direct force measurements on DNA in a solid-state nanopore. *Nature Phys.* **2,** 473–477 (2006).
49. Manning, G. S. Limiting laws and counterion condensation in polyelectrolyte solutions I. Colligative properties. *J. Chem. Phys.* **51,** 924–933 (1969).
50. Keyser, U. F. *et al*. Nanopore tomography of an objective focus. *Nano Lett.* **5,** 2253 (2005).
51. Service, R. The race for $1000 genome. *Science* **311,** 1544–1546 (2006).
52. Lagerqvist, J., Zwolak, M. & Di Ventra, M. Fast DNA sequencing via transverse electronic transport. *Nano Lett.* **6,** 779–782 (2006).
53. Zhang X.-G. *et al*. First-principles transversal DNA conductance deconstructed. *Biophys. J.* **91,** L04–L06 (2006).
54. Craighead, H. G. Future lab-on-a-chip technologies for interrogating individual molecules. *Nature* **442,** 387–393 (2006).
55. Gerland, U., Bundschuh, R. & Hwa, T. Translocation of structured polynucleotides through nanopores. *Phys. Biol.* **1,** 19–26 (2004).
56. Bundschuh, R. & Gerland, U. Coupled dynamics of RNA folding and nanopore translocation. *Phys. Rev. Lett.* **95,** 208104 (2005).
57. Kafri, Y., Lubensky, D. K. & Nelson, D. R. Dynamics of molecular motors and polymer translocation with sequence heterogeneity. *Biophys. J.* **86,** 3373–3391 (2004).
58. Siwy, Z. *et al.* Protein biosensors based on biofunctionalized conical gold nanotubes. *J. Am. Chem. Soc.* **127,** 5000–5001 (2005).
59. Han, A. *et al.* Sensing protein molecules using nanofabricated pores. *Appl. Phys. Lett.* **88,** 093901 (2006).
60. Krapf, D. *et al.* Experimental observation of nonlinear ionic transport at the nanometer scale. *Nano Lett.* **6,** 2531–2535 (2006).

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Competing interest statement

The author declares that he has no competing financial interests.

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