Graphene as a subnanometre trans-electrode membrane

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Isolated, atomically thin conducting membranes of graphite, called graphene, have recently been the subject of intense research with the hope that practical applications in fields ranging from electronics to energy science will emerge1. The atomic thinness, stability and electrical sensitivity of graphene motivated us to investigate the potential use of graphene membranes and graphene nanopores to characterize single molecules of DNA in ionic solution. Here we show that when immersed in an ionic solution, a layer of graphene becomes a new electrochemical structure that we call a trans-electrode. The trans-electrode’s unique properties are the consequence of the atomic-scale proximity of its two opposing liquid–solid interfaces together with graphene’s well known in-plane conductivity. We show that several trans-electrode properties are revealed by ionic conductance measurements on a graphene membrane that separates two aqueous ionic solutions. Although our membranes are only one to two atomic layers thick, we find they are remarkable ionic insulators with a very small stable conductance that depends on the ion species in solution. Electrical measurements on graphene membranes in which a single nanopore has been drilled show that the membrane’s effective insulating thickness is less than one nanometre. This small effective thickness makes graphene an ideal substrate for very high resolution, high throughput nanopore-based single-molecule detectors. The sensitivity of graphene’s in-plane electronic conductivity to its immediate surface environment and trans-membrane solution potentials will offer new insights into atomic surface processes and sensor development opportunities.

We measured the ionic conductance of a 0.5 × 0.5 mm, chemical vapour deposition (CVD)-grown2,3, sheet of graphene mounted across the surface of a 200 × 200 nm aperture in a 250-nm-thick, free-standing, insulating SiNx layer on a Si substrate chip (Fig. 1). The electronic properties are measured across the sheet electrode, from one face to the other, hence ‘trans-electrode’. Spatially resolved micro-Raman spectra of the G, G’ peaks from the graphene showed it to consist of a mixture of one-layer and two-layer domains4,5, with a domain size of ≈10 μm. The chip-mounted membrane was inserted in a fluidic cell so that it separated two compartments, each subsequently filled with ionic solutions electrically contacted with Ag/AgCl electrodes. The small diameters of the polydimethylsiloxane (PDMS) seals in the fluidic cell precluded ionic solution from leaking around the edges of the graphene.

With 100 mV bias applied between the two Ag/AgCl electrodes, current measurements in a variety of chloride electrolytes show that the conductance across the graphene membrane is far below the nanosiemens level (Table 1). The highest conductances are observed for solutions with the largest cations, Cs and Rb, correlated with a minimal hydration shell that mediates their interaction with the graphene4,6. We attribute this conductance to ion transport through defect structures in the free-standing graphene. Contributions from electrochemical currents to and from the graphene can be ruled out (Methods). The observed conductances for different cations falls much faster than the solution conductivities on going from CsCl to LiCl (Table 1), suggesting an influence of graphene–cation interactions. Nevertheless, we cannot completely rule out ionic transport through graphene that is in contact with the chip surface. Small asymmetries and nonlinearities in the current–voltage (I–V) curves were observed in the data for Table 1 and elsewhere (for example, Fig. 2), reflecting asymmetrical properties of the graphene surfaces associated with its CVD growth1 or transfer to the chip.

A single nanometre-scale pore7 (produced by electron-beam drilling) in the graphene trans-electrode membrane increases its ionic conductance by orders of magnitude (Fig. 2). Experiments with known nanopore diameters and solution conductivities allow us to deduce graphene’s effective insulating thickness. The ionic conductance G of a pore of diameter d in an infinitely thin insulating membrane is given by

\[ G = \sigma d \]  

where \( \sigma = F(\mu_g + \mu_cl)C \) is the conductivity of the ionic solution, F is the Faraday constant, C is ionic concentration, and \( \mu_g(c) \) is the

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mobility of potassium \((i = K)\) and chloride \((i = Cl)\) ions used in our measurements. The linear dependence of conductance on diameter follows from the current density being sharply peaked at the pore’s perimeter for an infinitely thin membrane. For membranes thicker than the pore diameter, the conductance becomes proportional to the nanopore area. For finite but small thicknesses, we rely on computer calculations to predict the conductance.

In agreement with equation (1), the trans-electrode conductance of pores with diameters ranging from 5 to 23 nm (Fig. 3) exhibited a linear dependence of conductance on diameter measurements along different nanopore axes. Modelled conductances for a 2-nm-thick membrane (dotted line) and a 10-nm-thick membrane (dashed-dotted line) are presented for comparison.

DNA experiments also reveal the membrane thickness and the nanopore diameter. The results using a 5-nm pore in graphene and double-stranded DNA molecules are shown in Fig. 4. The insets show two single-molecule translocation events. In the event shown in the right inset, a molecule passes through the pore in an unfolded linear fashion. In contrast, the left inset shows an event in which the molecule is folded over on itself when it enters the pore, increasing the current blockade for a short time9. Each single-molecule translocation event can be characterized by two parameters: the average current drop, or blockade, and the duration of the blockade, which is the time it takes for the molecule to completely translocate through the pore. The scatter plot in Fig. 4 shows the value of these parameters for each of 400 DNA single-molecule events. The

| Table 1 | Trans-membrane conductance of as-grown graphene |
|---------|--------------------------|--------------------|------------------|
| Solution | Graphene conductance (pS) | Solution conductivity \((10^{-5} \, \text{S} \, \text{m}^{-1})\) | Cation hydration energy \(i \text{ (eV)}\) |
| CsCl    | 67 ± 2                  | 1.42               | 3.1             |
| RbCl    | 70 ± 3                  | 1.42               | 3.4             |
| KCl     | 64 ± 2                  | 1.36               | 3.7             |
| NaCl    | 42 ± 2                  | 1.19               | 4.6             |
| LiCl    | 27 ± 3                  | 0.95               | 5.7             |

The membrane separated two compartments, each containing only the ionic solutions indicated in column 1. Conductances were determined from voltage bias scans between +100 mV and −100 mV. All data shown here are from the same device, the graphene membrane of which was suspended across a 200 × 200 nm SiN frame. The absolute magnitudes of the conductances varied by a factor of two from membrane to membrane, but the general trend and order of conductance differences with the five solutions was invariant for all membranes.
characteristic shape of these data is similar to that obtained in silicon nitride nanopore experiments, where almost all the events, folded and unfolded, fall near a line of constant electronic charge deficit (e.c.d.): that is, regardless of how the otherwise identical molecules are folded, each blocks the same amount of ionic charge movement through the pore during the total time it takes each molecule to move through the pore. Such molecules pass through the pore uninhibited by sticking to the graphene surface. The few events that are encircled in the plot do not satisfy this condition, and their long translocation times indicate graphene–DNA interactions, which slow their translocation through the nanopore.

We compare the experimentally determined open pore and DNA blocked pore conductance with numerical solutions (as above, and see Methods), where the membrane thickness and the nanopore diameter are the fitting parameters. Using the observed mean current blockade $\Delta I = 1.24 \pm 0.08 \text{nA}$ during translocation of unfolded double-stranded DNA of diameter 2.0 nm (ref. 10), and the observed conductance of the pore when DNA is absent ($G = 105 \pm 1 \text{nS}$), we calculate that $L_T = 0.6 \pm 0.5 \text{nm}$, in excellent agreement with the value deduced above from open pore measurements alone. The pore diameter $d = 4.6 \pm 0.4 \text{nm}$ deduced from these calculations also agrees with the geometric diameter of 5.0 $\pm$ 0.5 nm obtained from transmission electron microscopy (TEM) of this pore.

The best fit value from both experiments, $L_T = 0.6 \text{nm}$, agrees with molecular dynamics simulations showing the graphene–water distance to be 0.31–0.34 nm on each side of the membrane. $L_T$ might also be influenced by the typical presence of immobilized water molecules and adsorbed ions in the Stern layer. On the other hand, theoretical studies argue against any immobilized water layer on graphene, and experimental measurements support an anomalously high slip between water and an internal curved carbon nanotube surface. Although very little is actually known about the surface chemistry of specifically adsorbed ions on single-atom-thick graphene layers, measurements of the ionic current through the inner volume of carbon nanotubes with diameters less than 1 nm (ref. 15) may indicate that ions are not immobilized on these graphitic surfaces at all. Our subnanometre values for $L_T$ support this view.

The extremely small $L_T$ value we obtain suggests that nanopores in graphene membranes are uniquely optimal for discerning spatial or chemical molecular structure along the length of a molecule as it passes through the pore. Although polymer translocation speeds and electronics bandwidth currently preclude a direct measurement of a nanopore’s spatial or geometric resolution limit, we can gain insight into the system’s limit by numerically modelling the resolution obtainable as a function of $L_T$.

The model uses a long, insulating, 2.2-nm-diameter cylinder symmetrically translocating through the centre of a 2.4-nm diameter nanopore. At one position along its length, the cylinder diameter changes discontinuously from 2.2 nm to 2.0 nm. Solving for the ionic conductance for this geometry as the discontinuity passes through the pore, we obtain the predictions shown in Fig. 5. The decreasing blockade (increasing conductance) of a pore is clearly seen as the large-diameter portion of the cylinder leaves the pore. The results of calculations for two $L_T$ values are shown. For the conservative $L_T = 1.5 \text{nm}$, the spatial resolution (defined as the distance over which the conductance changes from 75% of its greatest value to 25% of that value) is given by $\delta z = 7.5 \text{Å}$, whereas the best-fit value $L_T = 0.6 \text{nm}$ leads to $\delta z = 3.5 \text{Å}$. We conclude from our experiments and modelling that a pore in graphene is inherently capable of probing molecules with subnanometre resolution. Functionalizing the graphene nanopore boundary or observing its local in-plane electrical conductance during translocations may provide additional or alternative means of further increasing the resolution of this system.

We have demonstrated that an atomically thin sheet of graphene grown via CVD on the surface of a nickel substrate was spin-coated with a composite of spin-coated electrodes and the graphene nanopore boundary or observing its local in-plane electrical conductance during translocations may provide additional or alternative means of further increasing the resolution of this system.

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We have demonstrated that an atomically thin sheet of graphene can be fabricated into a new structure—a trans-electrode membrane—that attracts cations and anions to its opposing surfaces with subnanometre proximity. Interactions between anions and cations across the interface are mediated by the graphene and the high electric fields this interface supports. Owing to its extreme thinness, the graphene layer’s in-plane electronic conductance is also sensitive and available for probing the interfacial environment. With electrical contacts applied to the graphene electrode, this conductance can be measured even for very small area (<1 x 1 μm) membranes, making the trans-electrode a particularly interesting device for chemical sensing and surface electrochemistry studies. Surface chemical reactions can be probed at very few charged sites by ionic current measurements through a nanopore. In-plane electronic and nanopore ionic current measurements with trans-electrode devices would greatly extend this methodology. Many opportunities exist for modifying the properties of the trans-electrode device and its sensitivity (for example, by changing membrane thickness, doping and defects). The interactions at, and between, the two liquid–solid interfaces in graphene may well hold many surprises and applications.

After this Letter was submitted, the concept of using graphene nanopores to characterize DNA polymers appeared online. We believe that the present Letter provides the first realization of DNA translocation through atomically thin graphene.

**METHODS SUMMARY**

Graphene grown via CVD on the surface of a nickel substrate was spin-coated with an adhesion film of MMA/MAA (methyl-methacrylate/methacrylic acid) copolymer adhesion film. The nickel was etched away overnight in a 1 M HCl solution. The film was placed graphene side down across a 200-nm square aperture in the SiN$_4$ coating on a windowed Si chip (Fig. 1). The adhesion film was dissolved and washed away with acetone. Nanometre-scale pores in the graphene were electron-beam-drilled in a 200-kV JEOL 2010 transmission electron microscope. The fluidic cell was fashioned from polyether-ketone with PDMS fluidic seals on each side of the chip. Ionic current measurements through the as-produced graphene membranes or graphene membranes with nanopores were performed by standard electrophysiology methods.

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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**Author Contributions**

Graphene samples were grown by J.K. and A.R. Experiments and calculations were performed by S.G. Other activities, including data interpretation, conclusions and manuscript writing, were performed collaboratively at Harvard University by S.G., W.H., D.B. and J.A.G.

**Author Information**

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METHODS

Graphene preparation. Continuous large-scale graphene films were synthesized by CVD on 300-nm-thick nickel film evaporated on top of a Si/SiO2 wafer, using a recently published method. Raman spectroscopy, TEM and selected area diffraction studies show the graphene film to be of excellent quality and mostly strongly and irreversibly to the carrier chip's SiN coating as a free-standing portion of the graphene film, the chip was critical-point immersed in acetone, dichloroethane and finally isopropanol. To remove any residues from the graphene film, the chip was subsequently immersed in 33 wt% solution of KOH at room temperature for 1 min, then vigorously rinsed with isopropanol and ethanol. To avoid damage to the suspended free-standing portion of the graphene film, the chip was critical-point dried. Finally, the chip was loaded into a rapid thermal annealer and heated to 450 °C in a stream of gas containing 4% H2 in He for 20 min to drive off any remaining hydrocarbons. To avoid recontamination, the chip was immediately loaded into a transmission electron microscope for further processing.

A single nanometre-sized pore was drilled through the graphene membrane using a focused electron beam in a JEOL 2010 FEG transmission electron microscope operated at 200 kV acceleration voltage. Methods to form more reproducibly dimensioned nanopores in graphene are being developed, but we determined the nanopore size by electron microscope visualization in a well spread electron beam so as to keep the total electron exposure of the graphene membrane to a minimum. The reported nanopore diameter is an average of four measurements along different nanopore axes, as determined from calibrated transmission electron micrographs using DigitalMicrograph software (Gatan). The error bar represents the standard deviation of those measurements, and reflects irregularities and deviations of the pore perimeter from perfect circularity. If the chip or TEM holder had any contaminating organic residue, amorphous carbon was seen to visibly deposit under the electron beam. Such devices were discarded. After drilling the nanopore, the graphene nanopore chips that were not immediately investigated were kept under a clean vacuum of ~10^-10 torr.

Fluidic cell preparation. The chip-mounted graphene was inserted between the two half-cells of a custom-built microfluidic cassette made of polyether-ether-ketone. The two sides of the chip were sealed with PDMS gaskets. The opening of theasket that pressed against the graphene film on the Si/SiN carrier chip (Fig. 1) had an inside diameter of ~100 μm. Consequently, the gasket orifice was smaller than the dimensions of the graphene film (0.5 × 0.5 mm), and completely sealed off the graphene edge from the electrolyte. On the opposite side of the chip, the electrolyte is in contact with the graphene membrane only through the 200-nm-wide square window in the SiN membrane. Note that there is a large area difference between the two graphene faces in contact with the electrolyte (a circular area of 100 μm diameter versus a square 200 × 200 nm area). This difference in contact area may in part explain the small conductance asymmetries and nonlinearities in our graphene J–V curves.

The two half-cells were first filled with ethanol to facilitate wetting of the chip surface. The cell was then flushed with deionized water, followed by 1 M KCl solution with no buffer. To avoid any potential interaction between the graphene and the solutes which could affect the measurements of graphene thickness and DNA translocations, all the electrolytes were kept as simple as possible and were unbuffered. With the exception of solutions used in the experiment of Fig. 4, all solutions pHs ranged over only 0.2 pH units, from 5.09 to 5.29, as measured both before and after use in the described experiments. The pH of the solution used for the Fig. 4 experiment was adjusted to pH 10.4 with KOH just before use. Because the design of our microfluidic cassette maintained the solution largely out of contact with ambient atmosphere, the pH varied less than 0.2 pH units during the course of the Fig. 4 experiments.

Measurements. Ag/AgCl electrodes in each half-cell were used to apply an electric potential across the graphene membranes and to measure ionic currents. The current traces were acquired using an Axopatch 200B (Axon Instruments) amplifier, which was connected to an external eight-pole Bessel low-pass filter (type 90IP-LBL. Frequency cut-off) operating at 50 kHz. The analogue signal was digitized using a NI PCI-6229 DAQ card (National Instruments) operating at 250-kHz sampling rate and 16-bit resolution. The experiment was controlled through IGOR Pro software.

For DNA translocation measurements, the microfluidic cell was flushed with 3 M KCl solution at pH 10.4, containing 1 mM EDTA. High KCl concentration and high pH were found to minimize DNA–graphene interaction. We introduced 10-kilobase-pair restriction fragments of double-stranded λ-phage DNA molecules to the cis chamber. The DNA translocation events were analysed with MATLAB using a fitting function that consisted of multiple square pulses convoluted with an appropriate Bessel filter function to mimic the recording conditions.

Conductivities of all the solutions mentioned in the main text were measured using an Accumet Research AR50 conductivity meter, which had been calibrated using conductivity standard solutions (Alfa Aesar, product numbers 43405, 42695, 42679). All the fluidic experiments were performed under temperature-controlled laboratory conditions, at 24 °C.

To investigate the contribution from electrochemical (Faradic) currents, a large-area graphene film (~2 × 4 mm²) was transferred to a glass slide and contacted at one end with silver paint attached to a metallic clip over which wax insulation was placed. The exposed end of the film was immersed in 1 M KCl electrolyte with a Ag/AgCl counter electrode, and the electrochemical J–V curves were measured in the same voltage range as used in the trans-electrode experiments. After normalizing for the surface area, we conclude that any electrochemical currents in the trans-electrode devices were three orders of magnitude too small to account for the approximately picocamp currents measured through the as-grown graphene membranes in Table 1.

Simulations. The numerical simulations were performed using the COMSOL Multiphysics finite element solver in appropriate three-dimensional geometry with cylindrical symmetry along the axis of the nanopore. We solved the full set of Poisson–Nerst–Planck equations in the steady-state regime. In the range of physical parameters of interest (high KCl concentration and small applied voltage), the numerical simulation solution was found not to differ significantly from the solution of the Laplace equation with fixed conductance, which has significantly less computational penalty. A DNA molecule was modelled as a long stiff insulating rod of diameter 2 nm which threads through the centre of the nanopore. For lateral resolution calculations, we added a step of 2.2-nm diameter to the DNA model, and we calculated the change in the ionic current as the discontinuity is translocated through the centre of the pore. The total ionic current was calculated by integrating current density across the diameter of the nanopore.
The theory yields surprisingly simple, experimentally verifiable solutions, revealing that the ratio of the mean number of mediator molecules to that of the target molecules is a critical factor in setting the limit of noise suppression. When needed, noise suppression is a costly enterprise: a tenfold reduction in noise is possible only if 10,000 mediator molecules are produced for each target molecule. The cost of reducing variation is large in other realms of biology, as well. For example, the maintenance of constant body temperature in mammals (homeothermy) means that they consume around ten times more energy than do reptiles, which have varying body temperatures. Why spend this extra energy? After all, both types of animal thrive on Earth. The reason is that, because of homeothermy, mammals are not limited to being active only within narrow ranges of environmental temperature, a factor that is thought to have favoured their global expansion.

It remains to be seen, however, how widely such expensive negative-feedback loops are used in gene-regulatory networks to suppress fast fluctuations in mediator-molecule abundance. So far, several plasmids have been identified that produce mediator molecules at an amazing rate, which can indeed contribute to efficient control of copy numbers.

A second insight gained by Lestas et al. counters the idea that optimal function is attained when a network evolves to a sufficient complexity. On the contrary, increasing the number of components in the signalling circuitry introduces more opportunities for external noise to compromise transmission in the feedback circuitry — which, in our mythical analogy, will leave Athena starved of information. Taking these conclusions to the extreme, it may not be surprising that some mechanisms for plasmid copy-number control lack indirect signalling circuitries altogether. Such control can then be achieved by permitting replication when there is only a single free copy of the plasmid and by inhibiting replication when two plasmids bind to each other (pairing of the DNA sequences that initiate replication prevents the replication of both plasmids).

Plasmid fluctuations occur on a fast timescale. When fluctuations are slow, the uncertainty in the signal is reduced — Athena can collect more information and exert more precise control. Even without feedback, biochemical reaction networks of appropriate structure can exert absolute concentration control over some of the network components. When evolutionary gene duplication or slow environmental changes alter gene expression, the concentration of the gene product will be kept constant by such networks. Several biochemical and genetic networks may combine fluctuations at fast and at very slow timescales, which in turn must be examined by careful experiments, because networks have very different buffering capacities at fast and slow timescales. This may also explain why even a simple negative-feedback loop in a genetic circuit can efficiently reduce concentration heterogeneities in a cell population.

It will be interesting to compare the optimal network structures suited to suppress either slowly varying, inherited population heterogeneities, or fast fluctuations. Breaking a system down into a few reaction steps to be examined, while confining the properties of the rest of the signalling network in a general way, should lead to further insights into the operation of cellular networks. Moreover, knowing the limits of system performance will aid progress in biological engineering.

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**NANOTECHNOLOGY**

**Holes with an edge**

Hagan Bayley

Tiny holes have been drilled through individual layers of graphene — atomically thin sheets of carbon — using an electron beam. These nanopores might be useful for the ultrarapid sequencing of single DNA molecules.

The idea that DNA could be sequenced by running a strand through a tiny hole — a nanopore — and reading off the bases by electrical detection was suggested 14 years ago. Since then, significant progress has been made towards this goal, provoked by the US National Institutes of Health’s $1,000-genome challenge. Recent developments in base identification seemed to give the upper hand to protein nanopores. However, three papers now report that nanopores fabricated from graphene — sheets of carbon only one or a few atoms thick — might have crucial advantages for this application.

Graphene, a hugely extended aromatic molecule of fused six-membered carbon rings, is a material with extraordinary electrical and mechanical properties. On page 190 of this issue, Garaj et al. describe how they used an electron beam to bore holes ranging in diameter from 5 to 23 nanometres into graphene one or two layers thick. They then mounted the graphene in a chamber with an aqueous salt solution on each side of the film, and measured the current carried by the salt’s ions when a voltage was applied across electrodes immersed in the solutions. The conductances of the nanopores scaled with their diameters, as expected for pores for which the thickness is much less than the diameter. On the basis of the conductance values, the authors calculated that the effective insulating thickness of the graphene was only about 0.6 nm. This is much smaller than that of other materials that have been used for DNA analysis — 30 nm for typical silicon nitride pores, for example, and 10 nm for α-haemolysin protein pores.

Garaj et al. went on to measure the current carried by a graphene nanopore of diameter 5 nm while double-stranded DNA passed through it. They observed spikes in the current traces, which denoted current blockades corresponding to the transit of both folded and unfolded DNA. Similar blockades had previously been observed in analogous experiments with silicon nitride pores. A basic solution of high ionic strength ensured that translocation of only a minority of the DNA molecules was hindered by adherence to the graphene surface. Using the mean amplitude of the current blockades, the authors were again able to calculate the effective thickness of the graphene film, confirming it to be about 0.6 nm.

The graphene used by Garaj et al. was prepared by a process known as chemical vapour deposition (CVD). Writing in Nano Letters, Schneider et al. report similar data for the translocation of double-stranded DNA through graphene nanopores, but using films that were made by exfoliation (the removal of graphene sheets from bulk graphite). They say that such films have fewer defects than those made by CVD.

In contrast to Garaj and colleagues’ findings, Schneider and colleagues’ data suggest that the conductance of the pores scales with the square of the pore diameter. This indicates that Schneider and colleagues’ film was thicker than expected, perhaps because the authors coated it with 6-mercaptohexanoic acid to prevent DNA sticking to the graphene surface.

A third study of DNA translocation through graphene nanopores, also published in Nano Letters, is reported by Merchant and colleagues. They worked with CVD-produced graphene that had a thickness of 3–15 atomic layers (rather than just one or two layers, as used by Garaj et al.), containing pores 5–10 nm.
in diameter. Consistent with the other studies\(^1\~^{3}\), the authors observed current blockades associated with translocations of both folded and unfolded DNA, despite the high leak currents (perhaps caused by pinhole defects in the graphene) seen in this case.

So where do we stand with respect to the development of a graphene-nanopore device for DNA sequencing? In one manifestation of nanopore sequencing, single-stranded DNA would be sequenced by observing base-specific modulation of the ionic current as individual bases pass a recognition point in a pore\(^4\~^{6}\). A perceived advantage of graphene monolayers is that the entire thickness of a nanopore is comparable to the dimensions of a base, and might, therefore, form only one recognition point rather than participate in multiple contacts with DNA in the pore (Fig. 1). In the work considered here\(^6\), the double-stranded DNA moved at velocities of about 10 nanoseconds per base — too quickly to permit the resolution of current blockades arising from individual bases. Garaj et al.\(^1\) therefore resorted to computation to estimate the likely spatial resolution that could be achieved with a 2.4-nm pore at slower translocation speeds, and judged it to be as good as 0.35 nm — compatible with the identification of single bases.

But even if the translocation of single-stranded DNA through a pore can be slowed to a velocity of milliseconds per base, at which current blockades for individual bases should be measurable by ionic-current recording\(^2\), graphene-nanopore sequencing might still fall victim to one of several difficulties. For example, the graphene nanopores exhibit high levels of current noise\(^4\~^{6}\). This can be remedied, but only at the expense of thickening the device\(^6\). Furthermore, there is no experimental evidence that graphene nanopores will distinguish between different bases, and so the edge of the pore might need to be chemically modified to slow, pause and orient the translocating bases.

A second proposed means of base identification uses the quantum-mechanical phenomenon of electron tunnelling through DNA\(^1,2\), with graphene as a ‘trans-electrode’ (Fig. 1). Measurements of tunnelling currents or other electrical characteristics might allow extraordinarily rapid base identification at speeds of microseconds per base\(^1,2,13\). Crucially, both the ionic-current and tunnelling-current approaches for DNA sequencing are likely to need pores of 1.5 nm diameter or less (Fig. 1), much narrower than those so far described.

DNA sequencing using graphene nanopores will also undoubtedly require the application of new chemistry and physics. The nature of graphene surfaces, and especially that of the periphery of graphene nanopores, is poorly understood. Graphene surfaces are likely to be elastically corrugated\(^14\) and to contain various defects, such as those that cause the leak currents in the present work\(^14\). And what chemical groups are present around the periphery of graphene nanopores after exposure of the pores to air and water? Perhaps they resemble the melange of groups found in graphene oxide\(^15\): carboxylates, hydroxyls, epoxides, alkynes, dienes and more. Or maybe the electron beam that creates the pores induces structural reorganization of the peripheral groups, generating new configurations such as five-membered rings\(^16\). Either way, are these peripheral structures susceptible to further rearrangement or hydrolysis, which would cause the nanopores to be irreproducible or unstable? And can they be covalently modified to facilitate base recognition?

Importantly, the surfaces of the graphene might need passivation to make them chemically inert, and the hole might need to be chemically modified independently of the surface — a taxing challenge. In terms of physics, ultrarapid DNA sequencing will require large arrays of electrically addressable nanopores. This challenge might be more readily tackled by using nanoscale gaps in graphene ribbons\(^17\), rather than nanopores in graphene sheets\(^1\).

Single-molecule DNA sequencing by arrays of nanopores offers the possibility to obtain genome sequences in less time than it takes to unravel a stethoscope. This potentially revolutionary technology will be unrivaled when numerous genome sequences are required from a single person — to personalize cancer treatment, for example. The first experimental steps\(^1\) taken with graphene nanopores suggest a compelling approach for clearing the remaining hurdles in the implementation of nanopore DNA sequencing.

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The author declares competing financial interests. See online article for details.

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**Figure 1 | Proposed methods for DNA sequencing.** An attractive strategy for single-molecule DNA sequencing is to pass single-stranded DNA through a nanopore in a graphene monolayer. Here, the rings of carbon atoms in the graphene are depicted as hexagons, and the diameter of the nanopore is about 1.5 nm, corresponding to about 35 hexagonal units. The strand is moving from top to bottom in an applied electric potential, and each of the four DNA bases is shown in a different colour. The DNA could be sequenced by observing the flow of ions through the pore (vertical yellow shading) and recording the distinctive fluctuations of ionic current caused by each type of DNA base as it blocks the ionic flow. Alternatively, fluctuations in a transverse tunnelling current (horizontal yellow shading) carried through the graphene, and modulated by DNA passing through the pore, could be measured; the crocodile clips represent electrical connections. One possible problem is that single-stranded DNA can adhere to graphene, as shown. Three papers\(^4\~^{6}\) now report that fluctuations of ionic current can be measured when DNA passes through a graphene nanopore, although the resolution of the measurements is currently insufficient to detect and identify individual bases.

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