nature nanotechnology

FOCUS | REVIEW ARTICLE

PUBLISHED ONLINE: 3 FEBRUARY 2016 | DOI: 10.1038/NNANO.2015.307

Graphene nanodevices for DNA sequencing

Stephanie J. Heerema and Cees Dekker*

Fast, cheap, and reliable DNA sequencing could be one of the most disruptive innovations of this decade, as it will pave the way for personalized medicine. In pursuit of such technology, a variety of nanotechnology-based approaches have been explored and established, including sequencing with nanopores. Owing to its unique structure and properties, graphene provides interesting opportunities for the development of a new sequencing technology. In recent years, a wide range of creative ideas for graphene sequencers have been theoretically proposed and the first experimental demonstrations have begun to appear. Here, we review the different approaches to using graphene nanodevices for DNA sequencing, which involve DNA passing through graphene nanopores, nanogaps, and nanoribbons, and the physisorption of DNA on graphene nanostructures. We discuss the advantages and problems of each of these key techniques, and provide a perspective on the use of graphene in future DNA sequencing technology.

DNA sequencing is an extremely rapidly evolving methodology to read off the sequence of bases in a genome. Given its role in human physiology and development, such sequence information is expected to significantly impact diagnosis and treatment of disease, ultimately facilitating personalized medicine where the right treatment can be applied to individuals. The progress towards cheaper and faster sequencing has been very impressive since the Human Genome Project[1] first sequenced the human genome. That project was largely carried out using the classical Sanger method[2], a process in which DNA strands are synthesized starting from a known primer sequence and terminated by a specific dideoxy deoxyribonucleoside triphosphate (dNTP), such that the last base in the sequence is known. DNA strands are then size separated by gel electrophoresis for reading off that last base. The Sanger procedure is time consuming due to the slow throughput with DNA fragment separation in gels. The need for cheaper and faster techniques drove both scientists and companies to work on new sequencing technologies[3,4]. Second-generation sequencers involved *in vitro* amplification of DNA strands and their clustering onto dedicated surfaces as well as sequencing by synthesis[5], where fluorescently tagged nucleotides are added by a polymerase, which enables a signal for each base to be instantly read off. These improvements substantially increased the degree of parallelism and reduced reagent volumes, leading to much faster and cheaper sequencing. These methods, however, came at the cost of significantly lower read lengths (typically ~100 bp) compared with the Sanger method (>500 bp)[6].

Yet newer sequencing methods, based on nanotechnology approaches, now focus on single-molecule long-read-length sequencing without any amplification or labelling. For example, Pacific Biosciences uses an array of zero-mode waveguides where each waveguide reads the base sequence by detecting the incorporation of single fluorescent nucleotides in DNA synthesis in real time[7]. This technology is particularly useful for *de novo* sequencing, as it allows long strands (on average several kbp long) to be read. Although sizeable error rates (~13%) have been reported[8], these errors are random, in contrast to context-specific errors (for example, palindromic sequences or GC-rich contents) that are generally observed in other techniques, such that multiple lower-quality base calls can be aligned to derive high-quality (*de novo*) sequence data[9,10]. Another interesting innovation recently emerged from Oxford Nanopore Technologies that built a sequencing device based on biological nanopores[11]. In such nanopore sequencing, one detects the base-dependent changes in the ionic current while a DNA molecule passes through the pore. This powerful technique allows for amplification- and label-free detection that can be scaled up for high-throughput sequencing. The technology was even developed into a portable device that could be ideal for direct use in health centres. First studies report that high-confidence alignments can resolve single-nucleotide variations and that the base reads are up to 85% accurate (that is, they have a very large 15% error on each base calling, but the accuracy seems to be improving rapidly)[12]. Further development towards next-generation sequencing devices is eagerly awaited, and there is a need for new approaches.

Graphene, a single layer of carbon atoms arranged in a 2D hexagonal lattice, is providing new opportunities. Since its discovery in 2004[13,14], interest in this material has increased dramatically[15] due to the fact that it combines a number of unique properties: it is atomically thin, stronger than steel[16], highly flexible[17], stretchable, and transparent[18], has tunable optical properties[19], is impenetrable to ions, and is an excellent thermal[20] and electrical conductor. It has attracted major attention for electronic applications due to its extremely high charge carrier mobilities, even at room temperature (1×10^5 cm^2 V^{-1} s^{-1})[21]. Graphene can be produced cheaply in large areas, thus allowing upscaling in a cost-efficient manner. Given the special properties of graphene and its wide range of potential applications[22], one may ask whether graphene provides novel opportunities for nanodevices for DNA sequencing. Indeed, this is the case, and this is the focus of this Review.

Many different concepts have recently been proposed to sequence DNA using the special properties of graphene, as summarized in Fig. 1. Graphene's atomically thin and ion-impermeable structure, for example, represents the ultimate membrane for nanopore-based sequencing (Fig. 1a), where each base of a DNA molecule will block the ionic current through a tiny nanopore in the thin graphene sheet slightly differently. Other innovative proposals employ graphene's conductive properties. As shown in Fig. 1b, each base residing within a nanosize gap within a graphene layer may lead to a different tunnelling current across the gap because of the different electronic level structure of the bases. Alternatively, one can monitor the in-plane current through a graphene nanoribbon with a nanopore through which a DNA molecule traverses (Fig. 1c), as different bases are predicted to modulate the nanoribbon current differently. Finally, a range of techniques rely on changes in graphene currents as a result of physisorption of DNA to the graphene surface (Fig. 1d). This Review provides an overview of the various theoretical proposals for graphene-based DNA sequencing and discusses the first experimental efforts in this direction.

Kavli Institute of Nanoscience Delft, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands. *e-mail: c.dekker@tudelft.nl

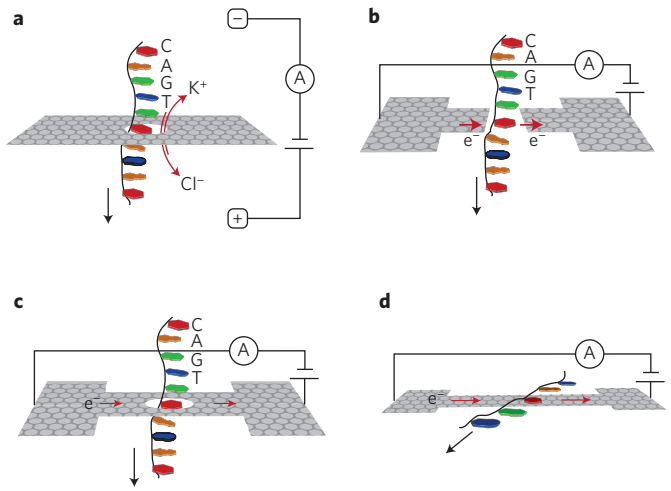

Figure 1 | Four new concepts using graphene nanostructures for DNA sequencing. a, Detection of changes in the ionic current through a nanopore in a graphene membrane due to the passage of a DNA molecule. **b**, Modulations of a tunnelling current through a nanogap between two graphene electrodes due to presence of a DNA molecule. **c**, Variations in the in-plane current through a graphene nanoribbon due to traversal of a DNA molecule. **d**, Changes in a graphene current due to the physisorption of DNA bases onto the graphene.

Ionic current detection through a graphene nanopore

First, we discuss DNA sequencing with graphene nanopores. The principle of nanopore sensing using ionic currents is quite elegant: an impermeable membrane containing a nanometre-sized hole is sandwiched between two compartments containing an electrolytic solution. When a voltage is applied across the membrane, an ionic current is induced through the pore. As DNA is strongly negatively charged, it can be driven in a head-to-tail fashion through the nanopore by an electric field. While the molecule translocates, it excludes ions from the pore volume, resulting in a temporal decrease in the ionic current. The magnitude and the duration of the current blockade provide information on the diameter and length of the molecule, respectively. For sequencing, each nucleotide should block the ionic current in a unique way that is dependent on its molecular size and shape. Nanopore sequencing is pursued with biological and solid-state nanopores.

Biological nanopores in cell membranes control the transport of molecules from one compartment to the other, and researchers have studied these systems for decades by measuring ion transport[23] and polymer translocations[24,25]. Nucleic acid translocations through α-haemolysin pores in lipid membranes were measured nearly two decades ago[26], motivated by the idea to read the consecutive bases of single-stranded DNA molecules in a linear fashion. Since these early days, the nanopore field has grown tremendously, and excitingly, DNA sequencing with nanopores has indeed been realized[27,28]. Solid-state nanopores present some interesting advantages over their biological counterparts, such as high stability, control over pore diameter and channel length, lower sensitivity to external parameters such as pH, temperature, salt concentration, and mechanical stress, and, importantly, they are well suited for massive upscaling and device integration on chip[29]. However, solid-state nanopores also have some disadvantages, such as the lack of true atomic control and increased noise levels. Indeed, so far, DNA sequencing has been realized with biological nanopores, but not yet with solid-state nanopores.

One of the most fascinating new developments has been the employment of graphene nanopores for DNA sequencing. Even monolayer graphene is impermeable to ions, and due to its strength, graphene can form a freestanding membrane, facilitating the ideal atomically thin membrane for nanopore measurements. The sensing resolution of monolayer graphene has the potential to attain its theoretical optimum, as the effective thickness of the graphene is only ~0.6 nm in solution due to ionic screening[30,31], which is the same length scale as the distance between two adjacent bases (~0.6 nm) of a single-stranded DNA molecule. Although it is not yet known whether single-base resolution can be achieved, this could highly simplify signal processing. This would present a significant advantage compared with the longer pore channels that are present in conventional silicon nitride pores and in protein pores, where complex signal deconvolution and processing is needed, because the ionic signal originates from several neighbouring nucleotides in the relevant volume of the pore. Another important advantageous property of graphene is that it is electrically conductive, which opens up the possibility to monitor an in-plane current through the membrane when the DNA molecule translocates.

Theorists have studied whether indeed DNA sequencing is possible with ionic current detection through graphene nanopores. Molecular dynamics simulations have been performed to study the movement of DNA molecules through a graphene pore, to evaluate in what way this affects the ionic current[32–34]. Early on, it was found that poly(AT) and poly(GC) can be distinguished at a bias voltage of 1 V (ref. 32). However, the simulations also exposed some problems with the approach, as they revealed that the bases move stochastically through the pore, which would lead to sequencing errors. Also, the current blockades were predicted to be strongly dependent on the local conformation of the DNA bases inside the pore resulting in a strong overlap of the current blockades for the different bases[32,33]. Interestingly, hydrophobic adhesion of bases to the graphene surface right next to the pore was found to significantly reduce the possible single-stranded DNA conformations[33]. These simulations suggested that the best 'stepwise' translocations may occur with a three-layer graphene sheet, such that collective binding and unbinding of the bases on both sides of the membranes is possible, while fluctuations in the DNA base orientations inside the pore are minimized[33].

In 2010, three independent groups published experimental data of double-stranded DNA translocations through graphene nanopores[30,35,36]. Their approaches were equivalent: 525-nm-diameter pores (Fig. 2a,b) were made with a transmission electron microscope in a freestanding graphene membrane on top of a larger hole in a silicon nitride membrane. A large current blockade (that is, the DNA sensor signal) was measured for DNA translocations compared with conventional silicon nitride solid-state pores due to the atomically thin membrane[30,36] (Fig. 2c). The signal amplitude was shown to be further maximized by minimizing the pore diameter[31]. In a next step towards sequencing, single-stranded DNA was detected. To do so, the attractive hydrophobic π–π stacking interactions between the nucleobases and graphene were overcome by applying a hydrophilic coating to the graphene to prevent attachment of the DNA to the graphene and the associated clogging of the pore[37]. In another experimental report, the opposite approach was taken and the adsorption and desorption of DNA bases on the graphene was in fact exploited to slow down DNA during translocation. Indeed, longer translocation times were found for single-stranded DNA (~5.5 μs nt^{-1}) compared with double-stranded DNA (~0.4 μs bp^{-1}) in a graphene/Al_2O_3/graphene sandwich device (Fig. 2d), where the slower translocation is likely to be caused by a stick–slip interaction[38].

A general challenge for DNA sequencing with solid-state nanopores is the fast translocation time of the DNA molecules, which typically traverse the pore at a speed of 0.01–1.00 μs per base depending on the conditions, which is orders of magnitude too fast given that measurements are generally performed at a bandwidth of only ~100 kHz, which is limited by the high noise in the ionic current[39].

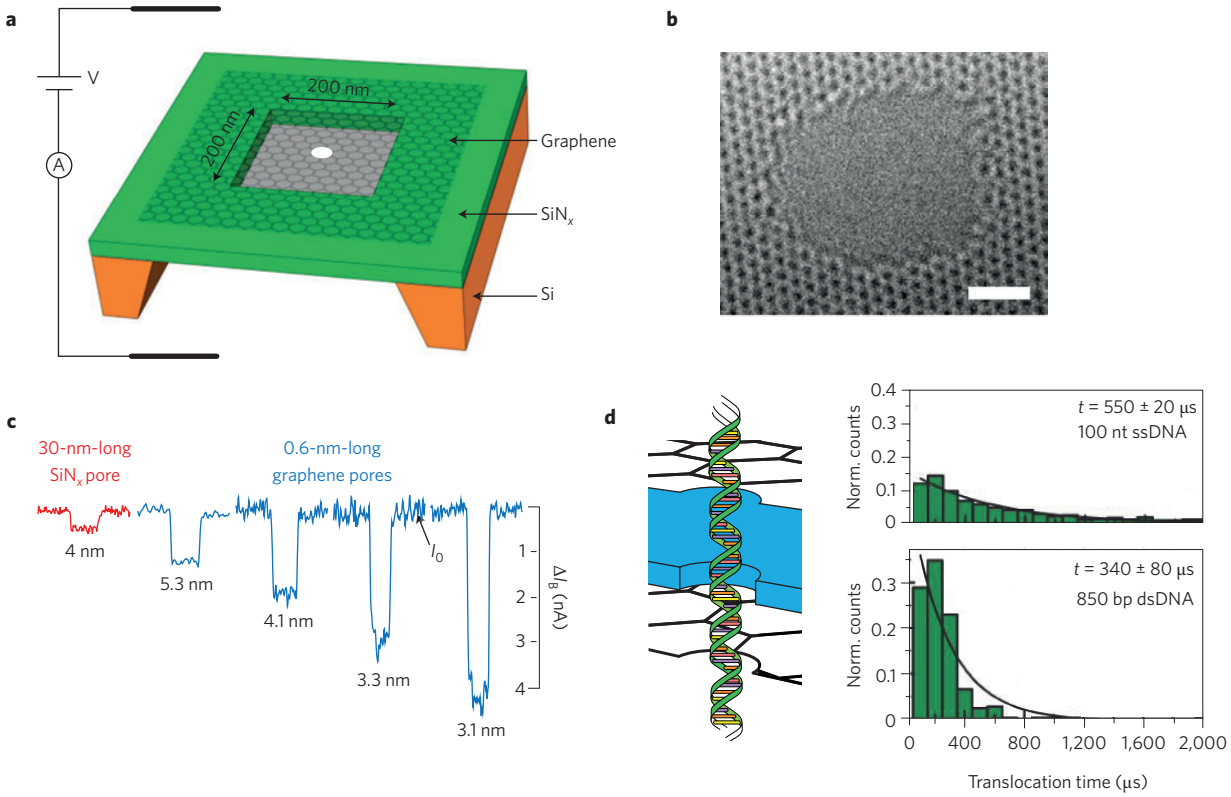

Figure 2 | DNA detection with ionic current measurements through graphene nanopores. a, Schematic of a typical graphene nanopore device layout, where a small nanopore is created in a graphene membrane that is freestanding over a hole (100–1,000 nm; a 200 × 200 nm aperture is shown in the figure) in a silicon nitride membrane on a silicon chip. **b**, Transmission electron microscopy (TEM) image (80 kV) of a 3 nm nanopore with clean and crystalline edge drilled in STEM mode at 600 °C. Scale bar, 1 nm. **c**, Double-stranded DNA current blockades (I_B) are larger for graphene nanopores (blue) than for SiN_x pores (red) due to their thin membranes. The largest blockade signals were measured with the smallest pores of ~3 nm. I_0 is the open pore current. **d**, Single-stranded DNA (ssDNA) translocations through nanopores in a membrane of stacked layers of graphene/Al_2O_3/graphene have shown that ssDNA does translocate slower than double-stranded DNA (dsDNA) due to interactions between the aromatic groups in the DNA bases and the graphene. Figures adapted with permission from: **a**, ref. 30, Nature Publishing Group; **b**, ref. 37, Nature Publishing Group; **c**, ref. 31, NAS; **d**, ref. 38, Wiley.

Also, the DNA molecule's movement is not completely confined, leading to positional fluctuations and variation in translocation velocity[40]. As temporal signals are interpreted into spatial information, this could be a serious problem for ionic current detection. Graphene nanopores particularly exhibit high low-frequency $1/f$ noise, which is probably of mechanical origin[41]. It may be possible to suppress this noise by reducing the area of the freestanding graphene[31], or by the use of multilayered structures[36,42,43]. Glass-based substrates may furthermore represent a good improvement, as low dielectric materials reduce the capacitive noise[44].

The solid-state nanopore field is still pushing towards base-discriminating measurements on DNA molecules that move through the pore more slowly, and graphene pores may contribute to these technical advances. It, however, remains a significant challenge to reach single-base resolution given the fast translocation times, the conformational fluctuations, the stochastic translocation of the bases, and the high noise levels. Various groups now look for alternative read-out schemes that are different from ionic current detection, by utilizing the intrinsic conductivity of graphene, as explained below.

Tunnelling across a graphene nanogap

We will now discuss DNA sequencing based on tunnelling across a graphene nanoslit. The concept is to measure a tunnelling conductance across two closely spaced graphene electrodes, and to monitor the variations of the current as a DNA molecule passes through the slit. Transmission spectra for tunnelling electrons depend on the electronic structure of the nucleotide and on the coupling of the nucleotide eigenstates to the graphene edges. A distinctive tunnelling current will be observed when the molecular energy level of a base falls within the voltage bias window of the two electrodes. When the molecular eigenlevels are far away from the electrochemical potentials of the graphene edges, tunnelling will be off-resonant and the tunnelling currents will be small. Graphene can be particularly useful in this set-up, because its single-atom thickness facilitates the detection of a single nucleotide that resides in the tunnelling gap. And perhaps most importantly, graphene can represent both the membrane and the electrodes at the same time because of its electrical properties. This greatly eases the fabrication of devices, as the nanogap and the electrodes are automatically aligned in the same plane (Fig. 3a)[45].

This idea was first proposed in 2010, with numerical simulations showing that sequencing should be possible for small gap sizes (1–2 nm)[45]. Similarly, simulations for graphene electrodes embedded within a silicon nitride nanopore reported base-specific detection[46]. Density functional theory (DFT) and non-equilibrium Green's function (NEGF) studies were utilized to study how transport across graphene nanoslits is modulated due to the presence of DNA bases in the slit. Indeed, a DFT–NEGF study on a gap in a zigzag-edged graphene nanoribbon, which is a nanostructured narrow graphene strip with perfect zigzag edges (Fig. 3b), predicted the possibility of base discrimination[47]. However, another study indicated that only the G base can be well distinguished from the other three due to quantum interference effects[48] that may occur from

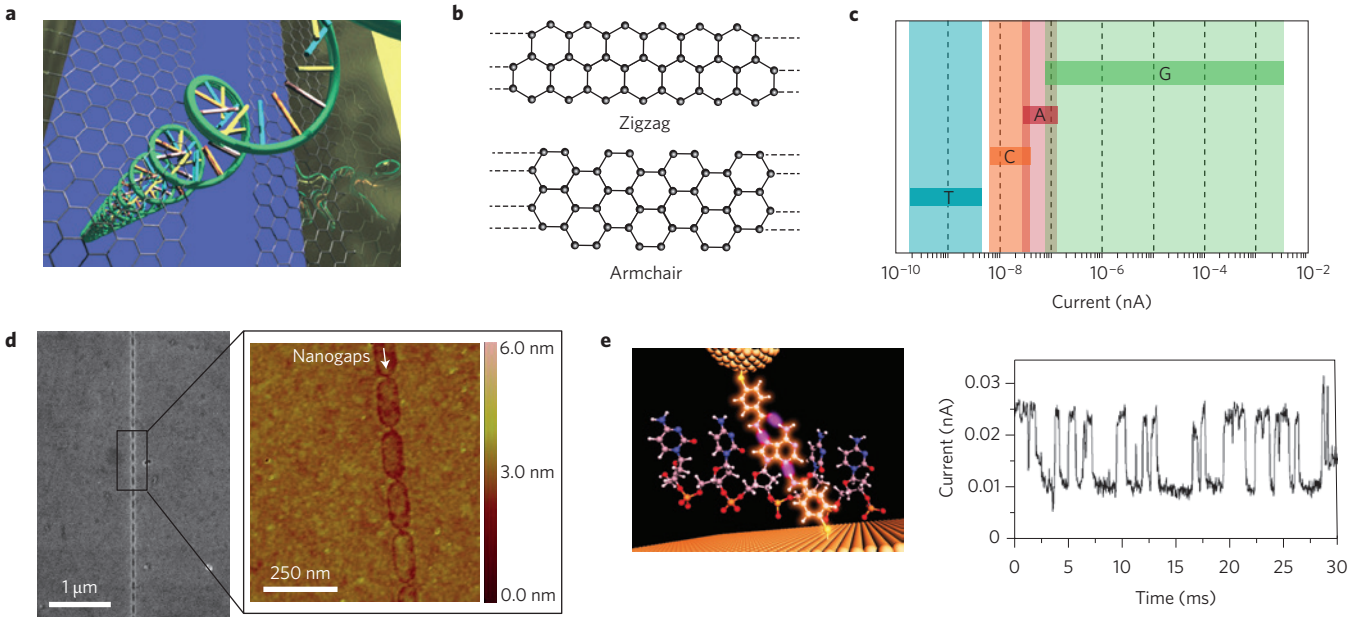

Figure 3 | Graphene nanogaps for DNA sequence detection. a, Artist's impression of a single-stranded molecule (backbone in green, bases in alternating colours) that translocates through a gap in graphene. **b**, Schematic image of two different edge geometries of graphene: zigzag and armchair. **c**, Theoretical calculations predict that the four DNA bases can be distinguished from the tunnelling currents across a graphene nanogap. The currents are very small (10^{-10}–10^{-3} nA) and are widely spread, but show little overlap. **d**, Scanning electron microscopy (left) and atomic force microscopy (right) images of an array of graphene gaps (1–10 nm) on silicon dioxide made with electron beam lithography and oxygen plasma etching. **e**, Left: Artist's impression of tunnelling electrodes functionalized with recognition agents (benzamide groups) that bind to a single DNA base in the centre. Right: Current spikes produced when deoxyadenosine 5′-monophosphate (dAMP) nucleotides were introduced between the tunnelling electrodes. Figures adapted with permission from: **a**, ref. 45, American Chemical Society; **c**, ref. 49, American Chemical Society; **d**, ref. 64, Wiley; **e**, ref. 52, Nature Publishing Group.

the rotation of bases and due to Fano-type resonances caused by energetic coupling between the discrete energy state of the DNA base and the continuous energy states of the graphene electrode.

As the tunnelling current is exponentially sensitive to changes in distance and orientation, large fluctuations in the tunnelling currents can be expected[45,48–50]. The tunnelling current distributions for the four DNA bases are therefore predicted to be broad (variations over orders of magnitude), yet with little overlap (Fig. 3c)[49]. Functionalization of the electrodes, for instance by hydrogenation or by attachment of one of the nucleobases, may provide a way to hold the molecule in a preferred orientation relative to the electrodes, thereby significantly reducing current fluctuations. Such passivation of the electrode edges is also suggested to promote coupling[50,51], and it may slow down the translocation speed of the DNA, allowing more time for measuring each individual base[50]. The idea of this 'recognition tunnelling' originates from successful experiments performed to slow down DNA while it moves through a gap[52–54] (Fig. 3e). Many efforts were focused on measuring DNA with metallic tunnelling electrodes embedded in silicon nitride pores[52,55–59], and indeed, some sequence information could be extracted when the DNA was pulled through the gap by an electric field[56,57].

So far, no DNA sequencing experiments using tunnelling through graphene gaps have been reported. However, stable nanogaps of 1–2 nm in few-layer graphene were formed through feedback-controlled electroburning, where heat due to the high current densities locally burns the graphene, and transport through contacted single molecules between the electrodes was measured[60–62]. Other approaches involved beam-based techniques such as helium ion beam lithography[63], and arrays of graphene nanogaps (1–10 nm) were fabricated using electron beam lithography and oxygen plasma etching (Fig. 3d)[64]. There are some significant challenges for this approach, as the tunnelling currents will be small due to the low density of states in graphene, fluctuations will be large due to base fluctuations (position and orientation), and the Brownian motion of ions and water molecules may induce additional noise. Furthermore, as the DNA is electrophoretically driven through the gap, its translocation speed will again be very high, which will make it even more difficult to resolve sequence information. Nevertheless, in view of the promising theoretical proposals and the successes made with fabricating tunnelling electrodes embedded in solid-state nanopores, interesting experimental results on DNA detection using graphene nanogaps may be expected in the near future.

In-plane transport of a graphene nanoribbon with a nanopore

The electrical properties of graphene can be exploited in a more direct way for DNA sequencing by monitoring the current through a narrow graphene nanostructure that contains a nanopore through which a DNA molecule translocates. Graphene is a gapless semiconductor[65], but when structuring the graphene into a nanometre-sized ribbon, its properties change depending on the edge profiles. Theoretical studies show that an armchair ribbon will be semiconducting[66–69] and that a zigzag-edged ribbon is metallic with a current profile that peaks at the edges[66,69–71]. Both armchair and zigzag nanoribbons have been proposed to present promising platforms for DNA sequencing in a large number of theoretical reports[72–79], and experimentalists have begun to explore this approach[80–85].

Similar results were obtained from various theoretical calculations, where electronic transport was studied using DFT and NEGF for different types of ribbon (width ~3 nm and pore diameter ~1.5 nm) in the absence and presence of each of the four DNA nucleobases[72–79].

The nanoribbon current was found to be modulated due to electrostatic interactions between the nucleotides and the graphene pore, causing a change in the local density of states in the graphene near the pore. Base specificity (that is, different nanoribbon currents when different bases are inserted in the pore) is

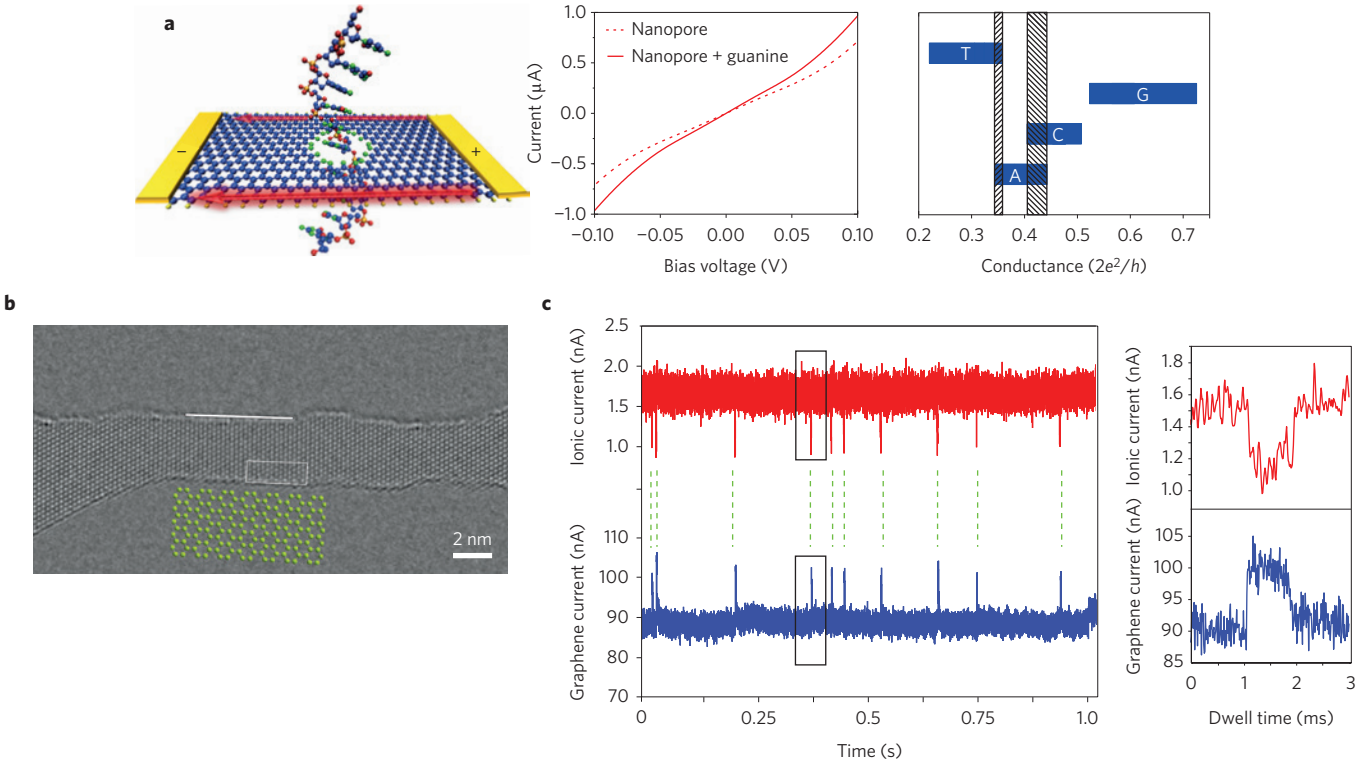

Figure 4 | Graphene nanoribbons with a nanopore for DNA sequencing. a, Left: Schematic view of a metallic zigzag graphene nanoribbon with a nanopore, where current flows mostly around the zigzag edges (red arrows). Middle: A guanine DNA base in the nanopore is shown to induce a (base-specific) ~μA modulation of the edge current. Right: The four different bases yield very different current modulations. Variations in base rotation result in a spread of the conductance modulations. Shaded areas mark the regions of overlap. **b**, TEM image of a nanoribbon in monolayer graphene, sculpted at 300 keV at 600 °C and imaged at 80 keV at 600 °C. The graphene was heated to preserve the single crystallinity. The white line indicates an armchair edge. The atomic structure model of the armchair edge highlighted by the white rectangle is shown by the green dots. **c**, Simultaneously recorded ionic current (red) and electrical current (blue) through a ~100-nm-wide graphene nanoribbon with a 10 nm pore during translocations of double-stranded DNA (graphene source–drain voltage 20 mV). Zoomed-in views of correlated event highlighted by black rectangles are shown in panels on the right. Figures adapted with permission from: **a**, ref. 74, American Chemical Society; **b**, ref. 85, American Chemical Society; **c**, ref. 80, Nature Publishing Group.

attributed to the different coupling strengths of the bases with the graphene nanoribbon.

The first DFT study on a graphene nanoribbon with a nanopore was published in 2010[72], where the authors calculated the current through a hydrogen-terminated armchair ribbon with a nanopore. By integrating over the density of states in the presence and absence of the respective DNA bases, this device could discriminate between the four different bases, a result that was found to be insensitive to strand orientation relative to the membrane. Similar calculations were done on a metallic nanoribbon[73], where the location of the pore was varied between the middle and the edge of the ribbon, and it was proposed that a ribbon with a pore located at the edge will be more suitable for DNA detection. Calculations have shown that edge currents in zigzag ribbons may be beneficial for DNA detection[74,86] (Fig. 4a). Base-distinct current variations were found, on the order of ~1 μA at 100 mV bias, much larger than what can be expected for armchair-edged ribbons where these edge currents are absent. These results were, however, contradicted by a self-consistent DFT study on zigzag-edged graphene nanoribbons[75] that showed that the respective bases can only be distinguished when transport is conducted away from the Fermi level. In another interesting study, nanoribbons with a finite width were compared with quantum point contact structures, which essentially are ribbons in the limit of zero width[76]. These point contacts were found to exhibit a greater sensitivity than armchair-edged ribbons provided that the carrier density is enhanced, for example by gating[76]. Another more complex device, consisting of two nanoribbons stacked on top of one another to form a small overhang (~3 nm) with a nanopore (~1.5 nm diameter)[78], yielded again base discrimination. Calculations performed on multilayered structures that facilitate multiple measurements on the same molecule, showed that a cross-correlation analysis between different nanopore scans of the same DNA molecule can yield an enhanced signal-to-noise ratio[79]. Graphene nanoribbons with a nanopore were also proposed to be able to distinguish whether DNA is methylated or not, a crucial biomarker for epigenetics. Methylated and non-methylated bases were shown to lead to characteristic differences in transport through a graphene nanoribbon with a 0.5-nm-wide hydrogenated pore[77].

Although the results of these theoretical studies are exciting, it has to be noted that most calculations on nanoribbons and on nanogaps were performed on simple model systems. The effect of ions and solvent molecules were typically not included and the DNA phosphate backbone was often assumed to be neutral in charge. In more realistic studies, where molecular dynamics simulations were used to model different DNA coordinates, with water molecules and ions included, base distinction appeared to be more difficult[87,88]. Also, the nanoribbon and nanopore edges were considered to be of either armchair or zigzag type, whereas in practice they may consist of a mixture of armchair and zigzag edges.

Experimentally, monolayer graphene nanoribbons can be produced in various ways. The most common techniques include electron beam lithography, (scanning) transmission electron microscopy ((S)TEM), and scanning tunnelling microscopy (STM) lithography. Alternatively, chemical techniques that involve unzipping of

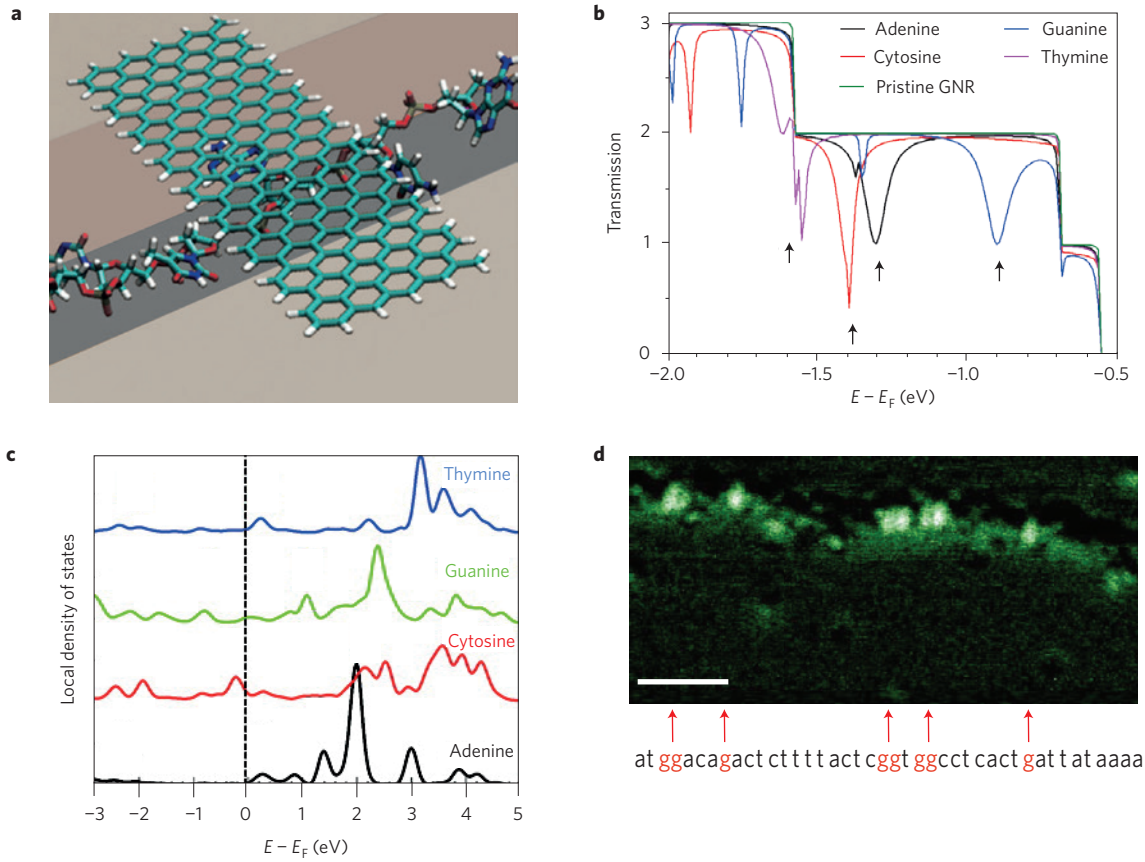

Figure 5 | DNA detection methods based on DNA physisorption. a, Schematic of a nanochannel device with an armchair graphene nanoribbon (GNR) along which a single-stranded DNA passes. DNA bases temporarily adsorb on the graphene while moving through the channel. **b**, DFT results for the structure in **a** show that base-varying conductance dips appear due to Fano resonance (black arrows) as a result of such DNA adsorption. **c**, DFT calculations for single DNA bases adsorbed on to graphene show different tunnelling conductances due to their differences in local density of states. **d**, STM image of single-stranded DNA molecules on a Cu(111) surface. The guanine sites are indicated by red characters in the bottom sequence, and by the red arrows. Scale bar, 5 nm. Figures adapted with permission from: **a**, ref. 101, Nature Publishing Group; **b**, ref. 102, RSC; **c**, ref. 106, American Chemical Society; **d**, ref. 107, Nature Publishing Group.

carbon nanotubes or 'bottom up' assembly of ribbons with the use of molecular precursors have been used[89]. Freestanding graphene nanoribbons (of sub-10 nm widths) were made using STEM[82–85]. It was shown that when the graphene is heated to >600 °C, it can be sculpted with near atomic precision, while maintaining pristine defect-free graphene[84]. At such elevated temperatures, self-repair is mediated by mobile carbon adatoms that constantly repair the defects caused by the electron beam. One can control the shape of the edges by cutting along specific crystallographic directions (Fig. 4b)[85]. Crystalline ribbons were also obtained using Joule heating, where a large voltage (~3 V) is applied across the ribbon, leading to local heating (>2,000 K) due to the high current densities. This heating recrystallizes the edges of the nanoribbon that rearrange along either a zigzag or armchair profile[90]. With that approach, armchair ribbons down to 0.7 nm in width were made, which were highly conducting and could sustain microampere currents at low voltages[82,83].

First experimental results on DNA translocation through graphene nanoribbons with nanopores were reported in 2013 (Fig. 4c)[80]. For an electron-beam-patterned ~100-nm-wide ribbon with a pore size of ~10 nm, simultaneous current drops in the ionic current and signals in the graphene ribbon during DNA translocation events were presented. These graphene current modulations were however caused by a nonlocal capacitive coupling of the DNA molecules to the ribbon, similar to the field effect described in ref. 91, whereas the effect evaluated in the theoretical proposals is induced by a change in local density of states at the pore. It is to be expected that smaller ribbons will exhibit much higher sensitivity. The currents through graphene nanoribbons are relatively large (much larger than the ionic currents in nanopore measurements and the predicted tunnelling currents across graphene nanogaps), and the resistance will only be on the order of the quantum resistance (that is, much smaller than that of nanopores and nanogaps). Accordingly, it can be expected that it is possible to carry out measurements at much higher bandwidths. This implies that one can potentially measure DNA-sequence information much faster, possibly even at the normal translocation speed of the DNA molecule, which would present a major advantage over conventional nanopore measurements. Given the sizeable efforts to fabricate well-defined small graphene nanostructures, it can be expected that DNA translocation experiments with nanoribbon–nanopore devices will be performed in the coming years, resolving whether one can indeed sequence DNA with this approach.

Detection methods based on DNA adsorption

The strong binding interactions between the aromatic groups of DNA bases and graphene have prompted researchers to find ways to exploit these interactions for a range of DNA sequencing applications based on current modulations in graphene due to DNA physisorption, or on measurements that rely on differences in electrochemical

activity, graphene field-effect transistors (FETs), and optical detection on adsorption and desorption of DNA molecules.

The nature of the binding of DNA bases to graphene is complex. Several mechanisms have been discussed, including π–π stacking, electrostatic, van der Waals, and hydrophobic interactions[92]. The main contribution is attributed to π–π bonding, which explains why single-stranded DNA binds more strongly to graphene than double-stranded DNA where the bases are hydrogen bonded and stacked within the helical structure[93,94]. The interaction strengths of the different bases with graphene vary as they depend on the polarizability of the DNA bases[93,95]. Both theoretical and experimental studies report that G binds most strongly to graphene while A, T, and C have lower and similar interaction strengths[93,96–100].

The non-covalent adsorption of DNA bases to graphene was suggested to induce modulations in the current through graphene nanostructures (Fig. 5a)[101–104]. To explore its use for DNA sequencing, the effects of DNA base adsorption on a graphene nanoribbon were calculated with DFT and NEGF[101]. The stacking interactions were found to be sufficiently strong to modulate the current and simultaneously sufficiently weak to allow detachment and subsequent attachment of the next base of a DNA molecule that was passing through the armchair nanoribbon (Fig. 5a). The interactions were shown to result in base-dependent conductance drops, due to Fano resonances (Fig. 5b)[102]. A second report demonstrated that T, G, and C bases that were adsorbed on a graphene ribbon altered the electric current through the ribbon, while a clear signature was lacking for A (ref. 103). It has to be noted that it will be extremely challenging to make ribbons that are narrow enough, such that only a single nucleotide can adsorb at the same time. It is likely that this will only be feasible with ribbons that are fabricated bottom up through chemical synthesis[105]. Base-dependent changes in the local density of states in graphene were confirmed in STM spectroscopy experiments. Calculations of the local tunnelling conductance through DNA bases that were physisorbed on graphene showed distinct peaks (Fig. 5c)[106], and STM spectroscopy on a Cu(111) surface was shown to be able to distinguish G bases within a single-stranded DNA molecule (Fig. 5d)[107].

A wide variety of experimental studies have been reported that exploit graphene–DNA interactions to determine sequence variations, using electrochemical, FET, and fluorescent detection schemes. Although most of these approaches are not suitable for actual *de novo* sequencing, they have succeeded in measuring DNA mismatches (for example, single or double DNA base mismatches). Graphene is well suited for electrochemical detection methods due to its high electrical conductivity, large surface area, and very fast heterogeneous electron transfer[108]. Single-nucleotide polymorphisms (SNP) were detected[109,110] with electrochemical impedance spectroscopy, where the charge transfer between the solution and the graphene is modified by adsorption or desorption of molecules on the surface. SNPs are sequence variations where a single nucleotide in the genome differs from the wild-type genome. They are widely studied as they relate to many diseases, such as cancer and Alzheimer's disease[4]. Electronic measurements on single-stranded DNA adsorption on graphene were also performed in a biochemical FET set-up, where the effect of DNA adsorption and hybridization on the source–drain current in graphene sheets was measured on variation of a gate potential[111,112]. Not surprisingly, single-stranded DNA was found to act as a negative gating agent that increased the hole density in graphene[113,114]. DNA hybridization to immobilized single-stranded DNA probes on chemical vapour deposition graphene could be used to detect single base mismatches[114]. Multiple DNA targets and various mismatched DNA strands were also selectively detected with fluorescence microscopy[115–118]. Fluorescent dyes attached to single-stranded DNA probes adsorbed to a graphene surface were efficiently quenched by graphene oxide, while after hybridization to complementary or mismatched strands, the fluorescent signals reappeared in the double-stranded DNA. A large number of studies have been reported on biosensing with graphene and graphene oxide (sensing amino acids, peptides, glucose, and more), and the interested reader is referred to ref. 119 for an extensive overview.

Adsorption of DNA onto sensitive nanographene structures, such as nanoribbons, can potentially lead to base-specific information. One major advantage in these adsorption studies is that base fluctuations in position and angle are minimized, which could lead to lower noise in the measurements. Further exploration of the approaches described above will reveal whether these techniques may indeed lead to actual DNA sequencing.

Outlook

Many efforts have been directed at developing new DNA sequencing techniques that benefit from graphene's special properties. In this Review, we highlighted the most prominent approaches involving graphene nanopores, nanogaps, nanoribbons, and physisorption on graphene nanostructures.

Despite the clear progress in the nanopore-sensing field, we believe that ionic current detection will not be the ultimate approach that will lead to DNA sequencing using graphene nanodevices. Major challenges remain in slowing down the DNA during translocation, reducing the stochasticity in the translocation velocity, reducing conformational fluctuations of the bases residing within the pore, and lowering noise levels. More promising, in our view, is to employ the conductive properties of graphene, that is, monitoring modulations in the currents running through a graphene nanostructure on interaction with DNA bases. We have discussed a number of theoretical studies that calculated the variations of tunnelling currents across a gap between two graphene electrodes due to the presence of DNA bases residing within that gap. While these theoretical results on simple model systems were promising, no experimental studies on graphene nanogaps for DNA sequencing have been reported so far, probably because of the significant experimental challenges involved (creating and maintaining a few-nanometres gap between graphene electrodes, slowly traversing DNA through it in a controlled way, and performing tunnelling current measurements while base, water, and ion fluctuations yield significant tunnelling current noise). Results on metallic tunnelling electrodes embedded in silicon nitride nanopores[56,57] are encouraging, however, and similar experiments using graphene electrodes are to be expected.

Many theoretical studies on graphene nanoribbons that contained a small nanopore showed that such ribbon devices can electronically discriminate different bases that occupy the pore, thus providing sequencing information if a DNA strand is led through the nanopore. An advantage over tunnelling current detection is that the currents in the nanoribbons are much larger, likely to yield higher signal-to-noise ratios, and that a lower resistance results in faster relaxation times in the electronic circuit, such that one can potentially carry out measurements at much higher bandwidths. It is to be expected that experiments on narrow graphene nanoribbons will resolve the abilities for base discrimination in the near future.

Electrochemical and fluorescent monitoring of adsorption and desorption of DNA on graphene surfaces has already demonstrated discrimination of local DNA sequence variations, such as SNPs. According to several theoretical studies, DNA base adsorption onto the surface of a graphene nanoribbon may even lead to base-distinct current modulations. Fabrication of very narrow crystalline graphene nanostructures is, however, extremely challenging.

This emphasizes the more general point that atomic engineering of graphene will be key to success in realizing graphene-based DNA sequencing devices. The nanodevices that are most promising for DNA sequencing feature narrow graphene nanostructures

with crystalline edges that probe the presence of DNA through detection of a tunnelling current or an in-plane nanoribbon current. Fabrication of such nanostructures with atomic-scale control is crucial, but poses quite a challenge. Patterning graphene at elevated temperatures (>600 °C) provides a way to minimize defects to preserve graphene's crystallinity[84]. Narrow ribbons with crystalline edges were also produced through Joule heating[83,90], where a voltage of ~2–3 V applied across a ribbon resulted in a local heating of 2,000 K, leading to recrystallization of the edges[90]. Alternatively, narrow bottom-up graphene nanoribbons that are chemically synthesized with perfect zigzag or armchair edges may represent the ultimate approach for ultrasensitive graphene devices[105]. For a more detailed perspective on the importance of defects in graphene nanostructures, the reader is referred to ref. 120.

Another challenge in many DNA sequencing approaches is to control the motion of the DNA molecule while it translocates through or along the graphene nanostructure. Many different solutions are being explored. Lower temperatures and higher buffer viscosities help a bit. Recently, a viscosity gradient, involving an ionic liquid BmimPF6 on the *cis* side and a 2 M KCl solution on the *trans* side, was used to lower the DNA translocation speed by two orders of magnitude[121]. A very different approach is to employ a polymerase or helicase enzyme to open the double-stranded DNA helix and slowly ratchet one of its strands through the pore channel[27,28]. Such protein–graphene hybrids or DNA origami–graphene structures[122–124] could provide means to control the motion of DNA molecules. Yet another alternative is to use plasmonics to control a DNA molecule in a nanopore[125,126]. In this approach, gold nanoantennas around a graphene nanopore are used to trap the DNA in a plasmonic hot spot right at the pore, introducing a 'physical knob' to switch the motion of the DNA through the pore on or off. Moreover, Raman spectroscopy on the DNA bases in the plasmonic hot spot at the pore can provide sequence information while the DNA molecule is stepped through the pore[125,127].

Graphene is a special material that offers unexpected opportunities. While this Review described a number of promising concrete proposals to sequence DNA with graphene nanodevices, the coming years may witness even more different approaches, for example, involving DNA in graphene liquid cells[128] or DNA translocation through carbon nanotubes[129,130]. Given the significant efforts on single-molecule sequencing and the fabrication of graphene nanostructures, we are hopeful that DNA sequencing with graphene will indeed materialize.

Received 10 April 2015; accepted 23 November 2015; published online 3 February 2016

References

1. Lander, E. S. *et al.* Initial sequencing and analysis of the human genome. *Nature* **409,** 860–921 (2001).
2. Sanger, F., Nicklen, S. & Coulson, A. R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl Acad. Sci. USA* **74,** 5463–5467 (1977).
3. Shendure, J. & Ji, H. Next-generation DNA sequencing. *Nature Biotechnol.* **26,** 1135–1145 (2008).
4. Metzker, M. L. Sequencing technologies — the next generation. *Nature Rev. Genet.* **11,** 31–46 (2010).
5. Ansorge, W. J. Next-generation DNA sequencing techniques. *New Biotechnol.* **25,** 195–203 (2009).
6. Steinbock, L. J. & Radenovic, A. The emergence of nanopores in next-generation sequencing. *Nanotechnology* **26,** 074003 (2015).
7. Levene, M. J. *et al.* Zero-mode waveguides for single-molecule analysis at high concentrations. *Science* **299,** 682–686 (2003).
8. Quail, M. A. *et al.* A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics* **13,** 341 (2012).
9. Ferrarini, M. *et al.* An evaluation of the PacBio RS platform for sequencing and *de novo* assembly of a chloroplast genome. *BMC Genomics* **14,** 670 (2013).
10. Sharon, D., Tilgner, H., Grubert, F. & Snyder, M. A single-molecule long-read survey of the human transcriptome. *Nature Biotechnol.* **31,** 1009–1014 (2013).
11. Mikheyev, A. S. & Tin, M. M. A first look at the Oxford Nanopore MinION sequencer. *Mol. Ecol. Resour.* **14,** 1097–1102 (2014).
12. Jain, M. *et al.* Improved data analysis for the MinION nanopore sequencer. *Nature Methods* **12,** 351–356 (2015).
13. Novoselov, K. S. *et al.* Electric field effect in atomically thin carbon films. *Science* **306,** 666–669 (2004).
14. Novoselov, K. S. *et al.* Two-dimensional atomic crystals. *Proc. Natl Acad. Sci. USA* **102,** 10451–10453 (2005).
15. Geim, A. K. & Novoselov, K. S. The rise of graphene. *Nature Mater.* **6,** 183–191 (2007).
16. Lee, C., Wei, X., Kysar, J. W. & Hone, J. Measurement of the elastic properties and intrinsic strength of monolayer graphene. *Science* **321,** 385–388 (2008).
17. Schedin, F. *et al.* Detection of individual gas molecules adsorbed on graphene. *Nature Mater.* **6,** 652–655 (2007).
18. Nair, R. R. *et al.* Fine structure constant defines visual transparency of graphene. *Science* **320,** 1308 (2008).
19. Pereira, V., Castro Neto, A. & Peres, N. Tight-binding approach to uniaxial strain in graphene. *Phys. Rev. B* **80,** 045401 (2009).
20. Balandin, A. A. *et al.* Superior thermal conductivity of single-layer graphene. *Nano Lett.* **8,** 902–907 (2008).
21. Morozov, S. *et al.* Giant intrinsic carrier mobilities in graphene and its bilayer. *Phys. Rev. Lett.* **100,** 016602 (2008).
22. Ferrari, A. C. *et al.* Science and technology roadmap for graphene, related two-dimensional crystals, and hybrid systems. *Nanoscale* **7,** 4598–4810 (2015).
23. Neher, E. & Sakmann, B. Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature* **260,** 799–802 (1976).
24. Colombini, M. Pore size and properties of channels from mitochondria isolated from *Neurospora crassa*. *J. Membrane Biol.* **53,** 79–84 (1980).
25. Krasilnikov, O., Sabirov, R., Ternovsky, V., Merzliak, P. & Muratkhodjaev, J. A simple method for the determination of the pore radius of ion channels in planar lipid bilayer membranes. *FEMS Microbiol. Lett.* **105,** 93–100 (1992).
26. Kasianowicz, J. J., Brandin, E., Branton, D. & Deamer, D. W. Characterization of individual polynucleotide molecules using a membrane channel. *Proc. Natl Acad. Sci. USA* **93,** 13770–13773 (1996).
27. Cherf, G. M. *et al.* Automated forward and reverse ratcheting of DNA in a nanopore at 5-Å precision. *Nature Biotechnol.* **30,** 344–348 (2012).
28. Manrao, E. A. *et al.* Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase. *Nature Biotechnol.* **30,** 349–53 (2012).
29. Dekker, C. Solid-state nanopores. *Nature Nanotech.* **2,** 209–215 (2007).
30. Garaj, S. *et al.* Graphene as a subnanometre trans-electrode membrane. *Nature* **467,** 190–193 (2010).
31. Garaj, S., Liu, S., Golovchenko, J. A. & Branton, D. Molecule-hugging graphene nanopores. *Proc. Natl Acad. Sci. USA* **110,** 12192–12196 (2013).
32. Sathe, C., Zou, X., Leburton, J.-P. & Schulten, K. Computational investigation of DNA detection using graphene nanopores. *ACS Nano* **5,** 8842–8851 (2011).
33. Wells, D. B., Belkin, M., Comer, J. & Aksimentiev, A. Assessing graphene nanopores for sequencing DNA. *Nano Lett.* **12,** 4117–4123 (2012).
34. Liang, L. *et al.* Theoretical study on key factors in DNA sequencing with graphene nanopores. *RSC Adv.* **3,** 2445–2453 (2013).
35. Schneider, G. F. *et al.* DNA translocation through graphene nanopores. *Nano Lett.* **10,** 3163–3167 (2010).
36. Merchant, C. A. *et al.* DNA translocation through graphene nanopores. *Nano Lett.* **10,** 2915–2921 (2010).
37. Schneider, G. F. *et al.* Tailoring the hydrophobicity of graphene for its use as nanopores for DNA translocation. *Nature Commun.* **4,** 2619 (2013).
38. Banerjee, S. *et al.* Slowing DNA transport using graphene–DNA interactions. *Adv. Funct. Mater.* **25,** 936–946 (2014).
39. Rosenstein, J. K., Wanunu, M., Merchant, C. A., Drndic, M. & Shepard, K. L. Integrated nanopore sensing platform with sub-microsecond temporal resolution. *Nature Methods* **9,** 487–492 (2012).
40. Plesa, C., van Loo, N., Ketterer, P., Dietz, H. & Dekker, C. Velocity of DNA during translocation through a solid-state nanopore. *Nano Lett.* **15,** 732–737 (2015).
41. Heerema, S. J. *et al.* 1/*f* noise in graphene nanopores. *Nanotechnology* **26,** 074001 (2015).
42. Venkatesan, B. M. *et al.* Stacked graphene-Al_2O_3 nanopore sensors for sensitive detection of DNA and DNA–protein complexes. *ACS Nano* **6,** 441–450 (2012).
43. Banerjee, S. *et al.* Electrochemistry at the edge of a single graphene layer in a nanopore. *ACS Nano* **7,** 834–843 (2013).
44. Lee, M.-H. *et al.* A low-noise solid-state nanopore platform based on a highly insulating substrate. *Sci. Rep.* **4,** 7448 (2014).
45. Postma, H. W. C. Rapid sequencing of individual DNA molecules in graphene nanogaps. *Nano Lett.* **10,** 420–425 (2010).

46. Zhao, Q. *et al.* Nanopore-based DNA analysis via graphene electrodes. *J. Nanomater.* **2012,** 1–5 (2012).
47. Zhang, H. *et al.* Detection of nucleic acids by graphene-based devices: a first-principles study. *J. Appl. Phys.* **115,** 133701 (2014).
48. Jeong, H. *et al.* Quantum interference in DNA bases probed by graphene nanoribbons. *Appl. Phys. Lett.* **103,** 023701 (2013).
49. Prasongkit, J., Grigoriev, A., Pathak, B., Ahuja, R. & Scheicher, R. H. Transverse conductance of DNA nucleotides in a graphene nanogap from first principles. *Nano Lett.* **11,** 1941–1945 (2011).
50. Prasongkit, J., Grigoriev, A., Pathak, B., Ahuja, R. & Scheicher, R. H. Theoretical study of electronic transport through DNA nucleotides in a double-functionalized graphene nanogap. *J. Phys. Chem. C* **117,** 15421–15428 (2013).
51. He, Y. *et al.* Enhanced DNA sequencing performance through edge-hydrogenation of graphene electrodes. *Adv. Funct. Mater.* **21,** 2674–2679 (2011).
52. Huang, S. *et al.* Identifying single bases in a DNA oligomer with electron tunnelling. *Nature Nanotech.* **5,** 868–873 (2010).
53. Lindsay, S. *et al.* Recognition tunneling. *Nanotechnology* **21,** 262001 (2010).
54. Krishnakumar, P. *et al.* Slowing DNA translocation through a nanopore using a functionalized electrode. *ACS Nano* **7,** 10319–10326 (2013).
55. Ivanov, A. P. *et al.* DNA tunneling detector embedded in a nanopore. *Nano Lett.* **11,** 279–285 (2011).
56. Tsutsui, M., Taniguchi, M., Yokota, K. & Kawai, T. Identifying single nucleotides by tunnelling current. *Nature Nanotech.* **5,** 286–290 (2010).
57. Tsutsui, M. *et al.* Single-molecule sensing electrode embedded in-plane nanopore. *Sci. Rep.* **1,** 46 (2011).
58. Bagci, V. M. K. & Kaun, C.-C. Recognizing nucleotides by cross-tunneling currents for DNA sequencing. *Phys. Rev. E* **84,** 011917 (2011).
59. Chen, X., Rungger, I., Pemmaraju, C. D., Schwingenschlögl, U. & Sanvito, S. First principles study of high-conductance DNA sequencing with carbon nanotube electrodes. *Phys. Rev. B* **85,** 115436 (2012).
60. Prins, F. *et al.* Room-temperature gating of molecular junctions using few-layer graphene nanogap electrodes. *Nano Lett.* **11,** 4607–4611 (2011).
61. Nef, C. *et al.* High-yield fabrication of nm-size gaps in monolayer CVD graphene. *Nanoscale* **6,** 7249–7254 (2014).
62. Sadeghi, H. *et al.* Conductance enlargement in picoscale electroburnt graphene nanojunctions. *Proc. Natl Acad. Sci. USA* **112,** 2658–2663 (2015).
63. Island, J. O. *et al.* Fabrication of hybrid molecular devices using multi-layer graphene break junctions. *J. Phys. Condens. Matter* **26,** 474205 (2014).
64. Cao, Y. *et al.* Building high-throughput molecular junctions using indented graphene point contacts. *Angew. Chem.* **124,** 12394–12398 (2012).
65. Wallace, P. The band theory of graphite. *Phys. Rev.* **71,** 622–634 (1947).
66. Fujita, M., Wakabayashi, K., Nakada, K. & Kusakabe, K. Peculiar localized state at zigzag graphite edge. *J. Phys. Soc. Jpn* **65,** 1920–1923 (1996).
67. Wakabayashi, K., Fujita, M., Ajiki, H. & Sigrist, M. Electronic and magnetic properties of nanographite ribbons. *Phys. Rev. B* **59,** 8271–8282 (1999).
68. Ezawa, M. Peculiar width dependence of the electronic properties of carbon nanoribbons. *Phys. Rev. B* **73,** 045432 (2006).
69. Brey, L. & Fertig, H. Electronic states of graphene nanoribbons studied with the Dirac equation. *Phys. Rev. B* **73,** 235411 (2006).
70. Nakada, K., Fujita, M., Dresselhaus, G. & Dresselhaus, M. Edge state in graphene ribbons: nanometer size effect and edge shape dependence. *Phys. Rev. B* **54,** 17954–17961 (1996).
71. Sasaki, K.-i., Murakami, S. & Saito, R. Gauge field for edge state in graphene. *J. Phys. Soc. Jpn* **75,** 074713 (2006).
72. Nelson, T., Zhang, B. & Prezhdo, O. V. Detection of nucleic acids with graphene nanopores: *ab initio* characterization of a novel sequencing device. *Nano Lett.* **10,** 3237–3242 (2010).
73. Ouyang, F.-P., Peng, S.-L., Zhang, H., Weng, L.-B. & Xu, H. A biosensor based on graphene nanoribbon with nanopores: a first-principles devices-design. *Chinese Phys. B* **20,** 058504 (2011).
74. Saha, K. K., Drndić, M. & Nikolić, B. K. DNA base-specific modulation of microampere transverse edge currents through a metallic graphene nanoribbon with a nanopore. *Nano Lett.* **12,** 50–55 (2012).
75. Avdoshenko, S. M. *et al.* Dynamic and electronic transport properties of DNA translocation through graphene nanopores. *Nano Lett.* **13,** 1969–1976 (2013).
76. Girdhar, A., Sathe, C., Schulten, K. & Leburton, J.-P. Graphene quantum point contact transistor for DNA sensing. *Proc. Natl Acad. Sci. USA* **110,** 16748–16753 (2013).
77. Ahmed, T., Haraldsen, J. T., Zhu, J.-X. & Balatsky, A. V. Next-generation epigenetic detection technique: identifying methylated cytosine using graphene nanopore. *J. Phys. Chem. Lett.* **5,** 2601–2607 (2014).
78. Sadeghi, H. *et al.* Graphene sculpturene nanopores for DNA nucleobase sensing. *J. Phys. Chem. B* **118,** 6908–6914 (2014).
79. Ahmed, T. *et al.* Correlation dynamics and enhanced signals for the identification of serial biomolecules and DNA bases. *Nanotechnology* **25,** 125705 (2014).
80. Traversi, F. *et al.* Detecting the translocation of DNA through a nanopore using graphene nanoribbons. *Nature Nanotech.* **8,** 939–945 (2013).
81. Lu, Y., Merchant, C. A., Drndić, M. & Johnson, A. T. C. *In situ* electronic characterization of graphene nanoconstrictions fabricated in a transmission electron microscope. *Nano Lett.* **11,** 5184–5188 (2011).
82. Puster, M., Rodríguez-Manzo, J. A., Balan, A. & Drndić, M. Toward sensitive graphene nanoribbon-nanopore devices by preventing electron beam-induced damage. *ACS Nano* **7,** 11283–11289 (2013).
83. Qi, Z. J. *et al.* Correlating atomic structure and transport in suspended graphene nanoribbons. *Nano Lett.* **14,** 4238–4244 (2014).
84. Song, B. *et al.* Atomic-scale electron-beam sculpting of near-defect-free graphene nanostructures. *Nano Lett.* **11,** 2247–2250 (2011).
85. Xu, Q. *et al.* Controllable atomic scale patterning of freestanding monolayer graphene at elevated temperature. *ACS Nano* **7,** 1566–1572 (2013).
86. Chang, P.-H., Liu, H. & Nikolić, B. K. First-principles versus semi-empirical modeling of global and local electronic transport properties of graphene nanopore-based sensors for DNA sequencing. *J. Comput. Electron.* **13,** 847–856 (2014).
87. Lagerqvist, J., Zwolak, M. & Di Ventra, M. Influence of the environment and probes on rapid DNA sequencing via transverse electronic transport. *Biophys. J.* **93,** 2384–2390 (2007).
88. Feliciano, G. T. *et al.* Capacitive DNA detection driven by electronic charge fluctuations in a graphene nanopore. *Phys. Rev. Appl.* **3,** 034003 (2015).
89. Jia, X., Campos-Delgado, J., Terrones, M., Meunier, V. & Dresselhaus, M. S. Graphene edges: a review of their fabrication and characterization. *Nanoscale* **3,** 86–95 (2011).
90. Qi, Z. J. *et al.* Electronic transport of recrystallized freestanding graphene nanoribbons. *ACS Nano* **9,** 3510–3520 (2015).
91. Xie, P., Xiong, Q., Fang, Y., Qing, Q. & Lieber, C. M. Local electrical potential detection of DNA by nanowire-nanopore sensors. *Nature Nanotech.* **7,** 119–125 (2012).
92. Oliveira Brett, A. M. & Chiorcea, A.-M. Atomic force microscopy of DNA immobilized onto a highly oriented pyrolytic graphite electrode surface. *Langmuir* **19,** 3830–3839 (2003).
93. Gowtham, S., Scheicher, R., Ahuja, R., Pandey, R. & Karna, S. Physisorption of nucleobases on graphene: density-functional calculations. *Phys. Rev. B* **76,** 033401 (2007).
94. Akca, S., Foroughi, A., Frochtzwajg, D. & Postma, H. W. C. Competing interactions in DNA assembly on graphene. *PLoS ONE* **6,** e18442 (2011).
95. Lee, J.-H., Choi, Y.-K., Kim, H.-J., Scheicher, R. H. & Cho, J.-H. Physisorption of DNA nucleobases on h-BN and graphene: vdW-corrected DFT calculations. *J. Phys. Chem. C* **117,** 13435–13441 (2013).
96. Antony, J. & Grimme, S. Structures and interaction energies of stacked graphene–nucleobase complexes. *Phys. Chem. Chem. Phys.* **10,** 2722–2729 (2008).
97. Varghese, N. *et al.* Binding of DNA nucleobases and nucleosides with graphene. *ChemPhysChem* **10,** 206–210 (2009).
98. Das, A. *et al.* Binding of nucleobases with single-walled carbon nanotubes: theory and experiment. *Chem. Phys. Lett.* **453,** 266–273 (2008).
99. Umadevi, D. & Sastry, G. N. Quantum mechanical study of physisorption of nucleobases on carbon materials: graphene versus carbon nanotubes. *J. Phys. Chem. Lett.* **2,** 1572–1576 (2011).
100. Le, D., Kara, A., Schröder, E., Hyldgaard, P. & Rahman, T. S. Physisorption of nucleobases on graphene: a comparative van der Waals study. *J. Phys. Condens. Matter* **24,** 424210 (2012).
101. Min, S. K., Kim, W. Y., Cho, Y. & Kim, K. S. Fast DNA sequencing with a graphene-based nanochannel device. *Nature Nanotech.* **6,** 162–165 (2011).
102. Cho, Y., Min, S. K., Kim, W. Y. & Kim, K. S. The origin of dips for the graphene-based DNA sequencing device. *Phys. Chem. Chem. Phys.* **13,** 14293–14296 (2011).
103. Song, B., Cuniberti, G., Sanvito, S. & Fang, H. Nucleobase adsorbed at graphene devices: enhance bio-sensorics. *Appl. Phys. Lett.* **100,** 063101 (2012).
104. Bobadilla, A. D. & Seminario, J. M. Assembly of a noncovalent DNA junction on graphene sheets and electron transport characteristics. *J. Phys. Chem. C* **117,** 26441–26453 (2013).
105. Cai, J. *et al.* Atomically precise bottom-up fabrication of graphene nanoribbons. *Nature* **466,** 470–473 (2010).
106. Ahmed, T. *et al.* Electronic fingerprints of DNA bases on graphene. *Nano Lett.* **12,** 927–931 (2012).
107. Tanaka, H. & Kawai, T. Partial sequencing of a single DNA molecule with a scanning tunnelling microscope. *Nature Nanotech.* **4,** 518–522 (2009).
108. Davies, T. J., Hyde, M. E. & Compton, R. G. Nanotrench arrays reveal insight into graphite electrochemistry. *Angew. Chem. Int. Ed.* **44,** 5121–5126 (2005).
109. Bonanni, A. & Pumera, M. Graphene platform for hairpin-DNA-based impedimetric genosensing. *ACS Nano* **5,** 2356–2361 (2011).

110. Zainudin, N., Mohd Hairul, A. R., Yusoff, M. M., Tan, L. L. & Chong, K. F. Impedimetric graphene-based biosensor for the detection of *Escherichia coli* DNA. *Anal. Methods* **6,** 7935–7941 (2014).
111. Heller, I. *et al.* Identifying the mechanism of biosensing with carbon nanotube transistors. *Nano Lett.* **8,** 591–595 (2008).
112. Heller, I. *et al.* Influence of electrolyte composition on liquid-gated carbon nanotube and graphene transistors. *J. Am. Chem. Soc.* **132,** 17149–17156 (2010).
113. Lin, J. *et al.* Gating of single-layer graphene with single-stranded deoxyribonucleic acids. *Small* **6,** 1150–1155 (2010).
114. Dong, X., Shi, Y., Huang, W., Chen, P. & Li, L.-J. Electrical detection of DNA hybridization with single-base specificity using transistors based on CVD-grown graphene sheets. *Adv. Mater.* **22,** 1649–1653 (2010).
115. Lu, C., Yang, H., Zhu, C., Chen, X. & Chen, G. A graphene platform for sensing biomolecules. *Angew. Chem.* **121,** 4879–4881 (2009).
116. He, S. *et al.* A graphene nanoprobe for rapid, sensitive, and multicolor fluorescent DNA analysis. *Adv. Funct. Mater.* **20,** 453–459 (2010).
117. Balapanuru, J. *et al.* A graphene oxide-organic dye ionic complex with DNA-sensing and optical-limiting properties. *Angew. Chem.* **122,** 6699–6703 (2010).
118. Tao, Y., Lin, Y., Huang, Z., Ren, J. & Qu, X. DNA-templated silver nanoclusters-graphene oxide nanohybrid materials: a platform for label-free and sensitive fluorescence turn-on detection of multiple nucleic acid targets. *Analyst* **137,** 2588–2592 (2012).
119. Hu, Y., Li, F., Han, D. & Niu, L. *Biocompatible Graphene for Bioanalytical Applications* (Springer, 2014).
120. Vicarelli, L., Heerema, S. J., Dekker, C. & Zandbergen, H. W. Controlling defects in graphene for optimizing the electrical properties of graphene nanodevices. *ACS Nano* **9,** 3428–3435 (2015).
121. Feng, J. *et al.* Identification of single nucleotides in MoS_2 nanopores. *Nature Nanotech.* **10,** 1070–1076 (2015).
122. Wei, R., Martin, T. G., Rant, U. & Dietz, H. DNA origami gatekeepers for solid-state nanopores. *Angew. Chem.* **124,** 4948–4951 (2012).
123. Bell, N. A. W. *et al.* DNA origami nanopores. *Nano Lett.* **12,** 512–517 (2012).
124. Plesa, C. *et al.* Ionic permeability and mechanical properties of DNA origami nanoplates on solid-state nanopores. *ACS Nano* **8,** 35–43 (2014).
125. Jonsson, M. P. & Dekker, C. Plasmonic nanopore for electrical profiling of optical intensity landscapes. *Nano Lett.* **13,** 1029–1033 (2013).
126. Nam, S. *et al.* Graphene nanopore with a self-integrated optical antenna. *Nano Lett.* **14,** 5584–5589 (2014).
127. Belkin, M., Chao, S.-H., Jonsson, M. P., Dekker, C. & Aksimentiev, A. Plasmonic nanopores for trapping, controlling displacement, and sequencing of DNA. *ACS Nano* **9,** 10598–10611 (2015).
128. Chen, Q. *et al.* 3D motion of DNA–Au nanoconjugates in graphene liquid cell electron microscopy. *Nano Lett.* **13,** 4556–4561 (2013).
129. Liu, H. *et al.* Translocation of single-stranded DNA through single-walled carbon nanotubes. *Science* **327,** 64–67 (2010).
130. Siwy, Z. S. & Davenport, M. Making nanopores from nanotubes. *Nature Nanotech.* **5,** 174–175 (2010).

Acknowledgements

We thank L. Vicarelli, H. W. Zandbergen, and C. Plesa for useful discussions. We acknowledge funding received from the Netherlands Organisation for Scientific Research (NWO/OCW) as a part of the Frontiers of Nanoscience program, and from the European Union Seventh Framework Programme under grant agreement no. 604391 (Graphene Flagship).

Additional information

Reprints and permission information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to C.D.

Competing financial interests

The authors declare no competing financial interests.