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DOI
10.1038/NNANO.2015.307

Publication date
2016

Document Version
Accepted author manuscript

Published in
Nature Nanotechnology

Citation (APA)
Heerema, SJ., & Dekker, C. (2016). Graphene nanodevices for DNA sequencing. *Nature Nanotechnology*, *11*(2), 127-136. https://doi.org/10.1038/NNANO.2015.307

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Graphene nanodevices for DNA sequencing

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Abstract

Fast, cheap, and reliable DNA sequencing could be one of the most disruptive innovations of this decade as it will pave the way for personalised medicine. In pursuit of such technology, a variety of nanotechnology-based approaches have been explored and established including sequencing with nanopores. Due 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.

Introduction

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 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 included by a

polymerase, which enables to instantly read off a signal for each base. 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 to the Sanger method (> 500bp) [6].

Yet newer sequencing methods, based on nanotechnology approaches, now focus on single-molecule long-read-length sequencing without any amplification or labeling. 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 to read long strands (on average several kbp long). Although sizeable error rates (∼13%) have been reported [8], these errors are random, in contrast to context-specific errors (e.g. 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 which could be ideal for direct use in health centers. First studies report that high-confidence alignments can resolve single-nucleotide variations and that the base reads are up to 85% accuracy (i.e. yet 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, transparant [18], it has tunable optical properties [19], is impenetrable for ions, and it 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 x 10^5 cm^2/V s) [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 if 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 Figure 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 tunneling current across the gap because of the different electronic level structure of the bases. Alternatively one can monitor the inplane 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, see 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.

1. 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 nanometer-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 already nearly two decades ago [26], motivated by the idea to read the consecutive bases of a ss-DNA molecules in linear fashion. Since these early days, the nanopore field has grown tremendously, and excitingly, DNA sequencing with nanopores has indeed been realised [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, since the effective thickness of the graphene is only $\sim$0.6 nm in solution due to ionic screening [30, 31], which is the same length scale as the distance between two adjacent bases ($\sim$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 in comparison to the longer pore channels that are present in conventional SiN pores and in protein pores, where complex signal deconvolution and processing is needed, because the ionic signal originates from several neighboring 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 inplane 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 (MD) 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(A-T) and poly(G-C) can be distinguished at a bias voltage of 1V [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 ssDNA conformations [33]. These simulations suggested that the best 'stepwise' translocations may occur with a three-layer graphene sheet, such that collective binding/unbinding of the bases on both sides of the membranes is possible, whilst fluctuations in the DNA base orientations inside the pore are minimised [33].

In 2010, three independent groups published experimental data of double-stranded DNA translocations through graphene nanopores [35] [36] [30]. Their approaches were equivalent: 5-25 nm diameter pores (Fig. 2A, 2B) 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 (i.e. the DNA sensor signal) was measured for DNA translocations compared to conventional silicon nitride solid-state pores due to the atomically thin membrane (Fig. 2C) [30, 36]. The signal amplitude was shown to be further maximised by minimising 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 ssDNA ($\sim$5.5 μs/nt) compared to dsDNA ($\sim$0.4 μs/bp) in a graphene-Al_2O_3-graphene sandwich device (Fig. 2D), where the slower translocation is likely caused by a stick-slip interaction [38].

A general challenge for DNA sequencing with solid-state nanopores is the fast translo-

cation time of the DNA molecules, which typically traverse the pore at a speed of 0.01-1 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 $\sim$100kHz which is limited by the high noise in the ionic current [39]. 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 likely 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 readout schemes that are different from ionic current detection, by utilising the intrinsic conductivity of graphene, as explained below.

2. Tunneling across a graphene nanogap

We will now discuss DNA sequencing based on tunneling across a graphene nanoslit. The concept is to measure a tunneling 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 tunneling electrons depend on the electronic structure of the nucleotide and on the coupling of the nucleotide eigenstates to the graphene edges. A distinctive tunneling 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, tunneling will be off-resonant and the tunneling currents will be small. Graphene can be particularly useful in this setup because its single-atom thickness

facilitates the detection of a single nucleotide that resides in the tunneling 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 functions (NEGF) studies were utilised 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 nanometer ribbon (ZGNR), 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 Guanine base can be well distinguished from the other three due to quantum interference effects [48] that may occur from 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 tunneling current is exponentially sensitive to changes in distance and orientation, large fluctuations in the tunneling currents can be expected [45, 48–50]. The tunneling 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 tunneling' originates from successful experiments performed to slow down DNA while it moves through a gap [52–54]. Much efforts were focussed on measuring DNA with metallic tunneling 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 tunneling 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 like helium ion beam lithography [63], and arrays of graphene nanogaps (1-10 nm) were fabricated using e-beam lithography and oxygen plasma (Fig. 3D) [64]. There are some significant challenges for this approach, as the tunneling 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 tunneling electrodes embedded in solid-state nanopores, interesting experimental results on DNA detection using graphene nanogaps may be expected in the near future.

3. Inplane 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 nanometer-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 ribbons (width $\sim$3 nm and pore diameter $\sim$1.5nm) 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 (i.e. different nanoribbon currents when different bases are inserted in the pore) is 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 (Fig. 4A) [74,86]. Base-distinct current variations were found, in the order of $\sim$1 μA at 100mV bias, much larger than what can be expected for armchair edged ribbons where these edge currents are absent. These results were, however, contradicted by a self-consistent DFT study on zigzag GNRs [75] that showed that the respective bases can only be distinguished when transport is conducted away from the Fermi level. In another interesting study, ribbons with a finite length along the ribbon were compared to quantum point contact structures, which essentially are ribbons in the limit of zero length [76]. These point contacts were found to exhibit a greater sensitivity than armchair edged ribbons provided that the carrier density is enhanced, for example by gating [76]. Another more complex device, consisting of a two nanoribbons stacked on top of one another to form a small overhang ($\sim$3 nm) with a nanopore ($\sim$1.5 nm) [78], yielded again base discrimination. Calculations performed on multi-layered 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. 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 MD 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 e-beam lithography, (S)TEM, and STM lithography. Alternatively, chemical techniques that involve unzipping of carbon nanotubes or 'bottom up' assembly of ribbons with the use of molecular precursors have been used [89]. Freestanding graphene nanoribbons (of sub-10nm widths) were made using scanning transmission electron microscopy (STEM) to obtain narrow ribbons [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 ad-atoms 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 (> 2000 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 0.7nm 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 ribbons with nanopores were reported in 2013 [80] (Fig. 4C). For an e-beam patterned ∼100nm-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 [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 in the order of the quantum resistance (i.e. 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 through nanoribbon-nanopore devices will be performed in the coming years, resolving whether one can indeed sequence DNA with this approach.

4. 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 measurements that rely on differences in electrochemical activity, graphene FETs, and optical detection upon 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 ssDNA binds more strongly to graphene than dsDNA 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 it depends on the polarizability of the DNA bases [93, 95]. Both theoretical and experimental studies report that guanine 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 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 [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. Likely 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 tunneling 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 ssDNA molecule (Fig. 5D) [107].

A wide variety of experimental studies was 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 (e.g. single or double DNA base mismatches). Graphene is well fit 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 Alzheimers disease [4]. Electronic measurements on ssDNA adsorption on graphene were also performed in a biochemical FET setup, where the effect of DNA adsorption and hybridisation on the source-drain current in graphene sheets was measured upon variation of a gate potential [111, 112]. Not surprisingly, ssDNA was found to act as a negative gating agent that increased the hole density in graphene [113, 114]. DNA hybridisation to immobilised ssDNA probes on CVD graphene could be used to detect single base

mismatches [114]. Multiple DNA targets and various mismatch DNA strands were also selectively detected with fluorescence microscopy [115–118]. Fluorescent dyes attached to ssDNA 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 dsDNA. A large amount 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.

Summing up, 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 minimised, 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, i.e., monitoring modulations in the currents running through a graphene nanostructure upon interaction with DNA bases. We have discussed a number of theoretical studies that calculated the variations of tunneling 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 were reported so far, likely because of the significant

experimental challenges involved (creating and maintaining a few-nm gap between graphene electrodes, slowly traversing DNA through it in a controlled way, and performing tunneling current measurements while base, water, and ion fluctuations yield significant tunneling current noise). Results on metallic tunneling 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 tunneling current detection is that the currents in the nanoribbons are much larger, likely yielding higher signal-to-noise ratios and lower RC times, 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 tunneling current or an inplane nanoribbon current. Fabrication of such nanostructures with atomic-scale control is crucial, but poses quite a challenge. Patterning graphene at elevated temperatures ($>600^{\circ}$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 $\sim$2-3 V applied across a ribbon resulted in a local heating of 2000K, 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 ultra-sensitive graphene devices [105]. For a more detailed perspective on the importance of defects in graphene

nanostructures, the reader is referred to [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 2M 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 dsDNA 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 hotspot right at the pore, introducing a "physical knob" to switch the motion of the DNA through the pore on or off. Moreoever, 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.

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).

Figures

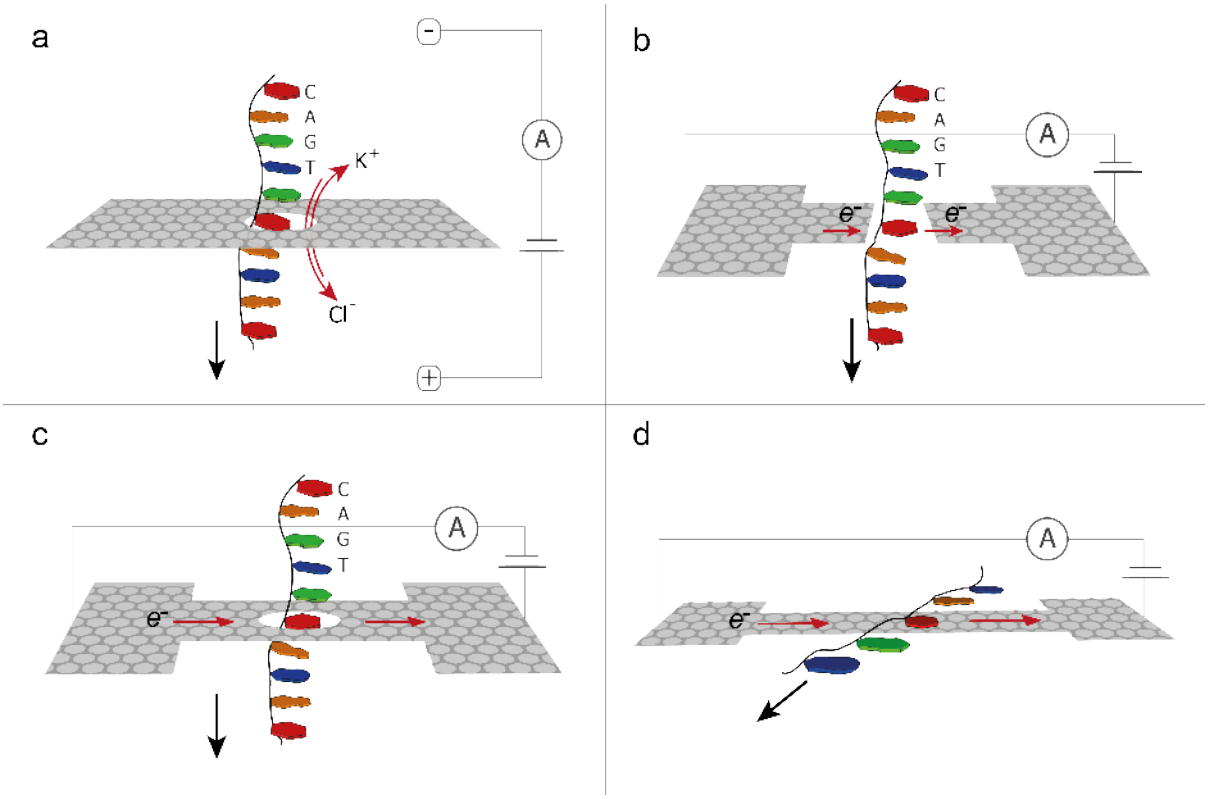

Figure 1: Four new concepts using graphene nanostructures for DNA sequencing. **(a)** Detection of changes in the ionic current through a nanopore in a graphene membrane due to the passage of a DNA molecule. **(b)** Modulations of a tunneling current through a nanogap between two graphene electrodes due to presence of a DNA molecule. **(c)** Variations in the inplane 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 on to the graphene.

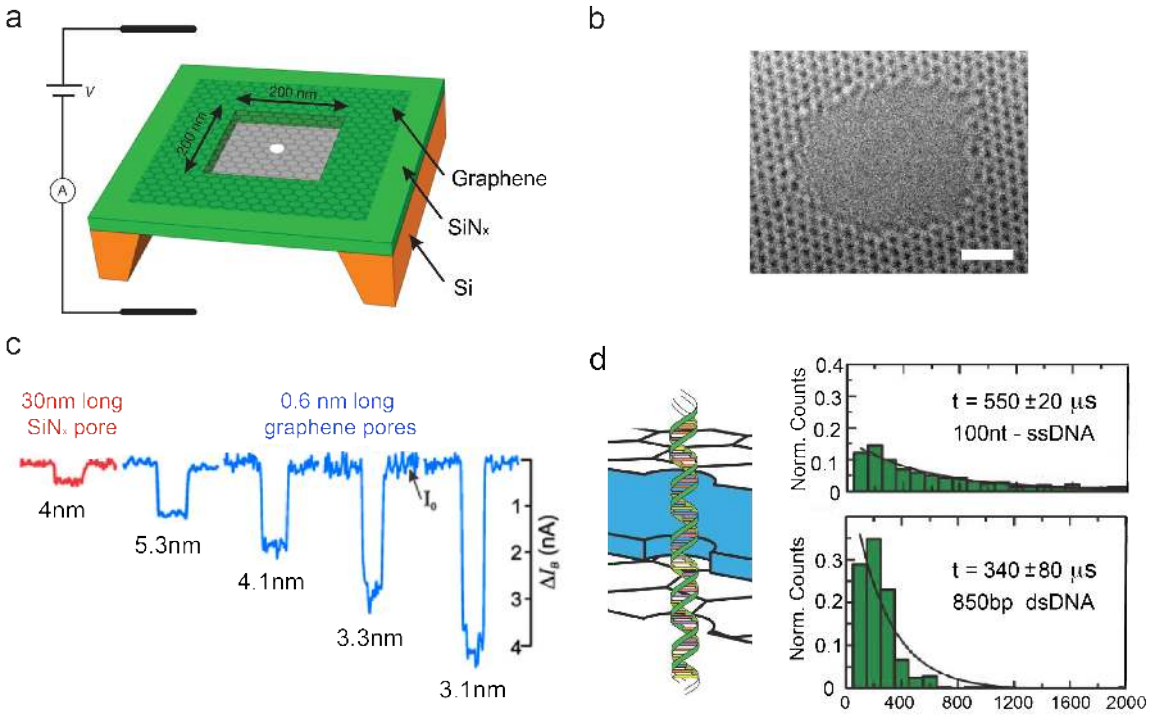

Figure 2: DNA detection with ionic current measurements through graphene nanopores. **(a)** Schematic of a typical graphene nanopore device layout, where a small nanopore is created in a graphene membrane that is freestanding over a hole (100-1000 nm) in a silicon nitride membrane on a silicon chip. Adapted from [30]. **(b)** TEM image (80 kV) of a 3 nm nanopore with clean and crystalline edge drilled in STEM mode at 600 °C (scale bar is 1 nm). From [37]. **(c)** dsDNA current blockades are larger for graphene nanopores (blue) than for SiN pores (red) due to their thin membranes. Largest blockade signals were measured with the smallest pores of ∼3nm. Adapted from [31]. **(d)** ssDNA translocations through nanopores in a membrane of stacked layers of graphene-Al_2O_3-graphene have shown that ssDNA does translocate slower due to interactions between the aromatic groups in the DNA bases and the graphene. Adapted from [38].

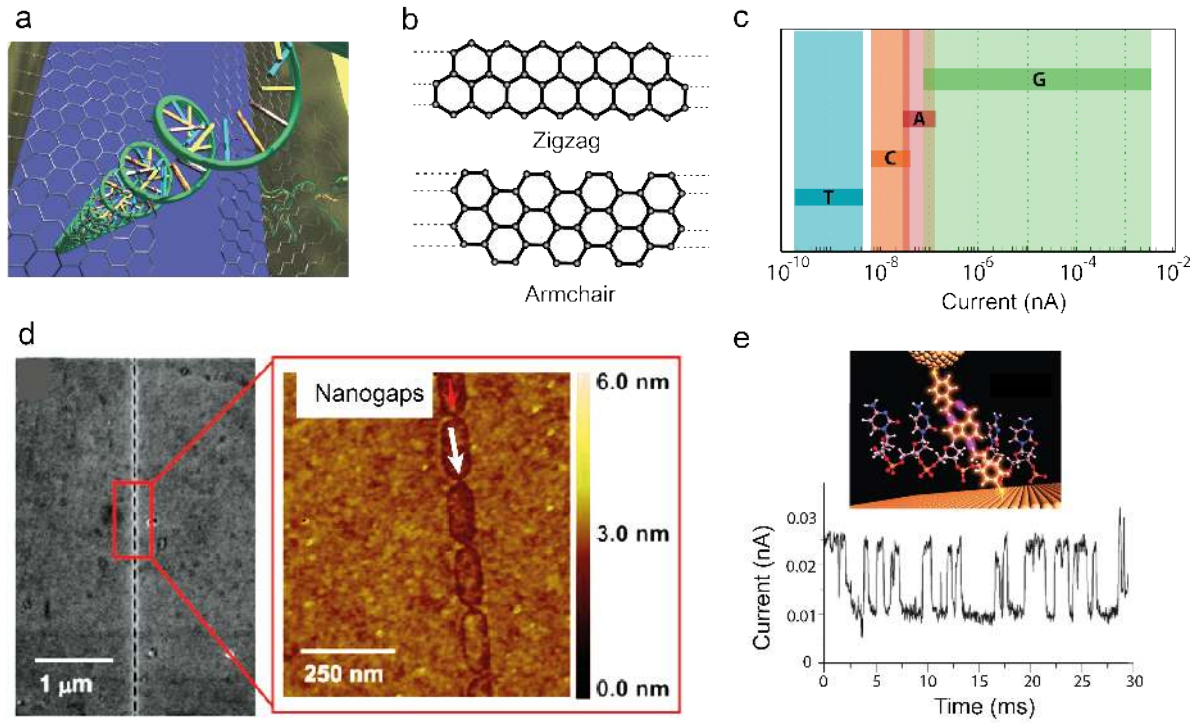

Figure 3: Graphene nanogaps for DNA sequence detection. **(a)** Artist impression of a ssDNA molecule (backbone in green, bases in alternating colors) that translocates through a gap in graphene. Adapted from [45]. **(b)** Schematic image of two different edge geometries of graphene: zigzag and armchair. **(c)** Theoretical calculations predict that the four DNA bases can be distinguished from the tunneling currents across a graphene nanogap. The currents are very small ($10^{-10} - 10^{-3}$ nA) and are widely spread, but show little overlap. Adapted from [49]. **(d)** SEM and AFM images of an array of graphene gaps (1-10nm) on silicon dioxide made with e-beam lithography and oxygen plasma etching. Adapted from [64]. **(e)** Top: artist impression of tunneling electrodes functionalized with recognition agents (benzamide groups) that bind to a single DNA base in the centre. Bottom: Current spikes produced when dAMP nucleotides were introduced between the tunneling electrodes. Adapted from [52].

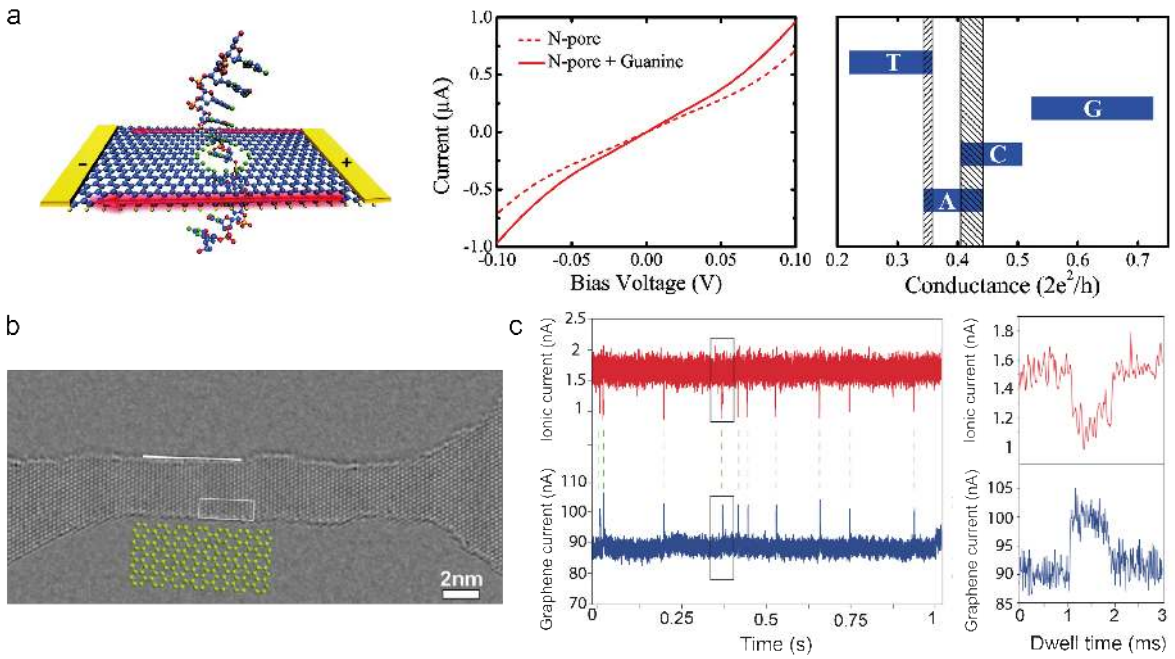

Figure 4: Graphene nanoribbons with a nanopore for DNA sequencing. **(a)** Left: schematic view of a metallic zigzag graphene nanoribbon with a nanopore, where current flows mostly around the zigzag edges (red arrows). Middle: A guanine DNA base in the nanopore is shown to induce a (base-specific) $\sim \mu$A 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. From [74]. **(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. From [85] **(c)** Simultaneously recorded ionic current (red) and electrical current (blue) through a ∼100nm wide graphene nanoribbon with a 10nm pore during translocations of dsDNA (graphene source drain voltage 20 mV). Adapted from [80].

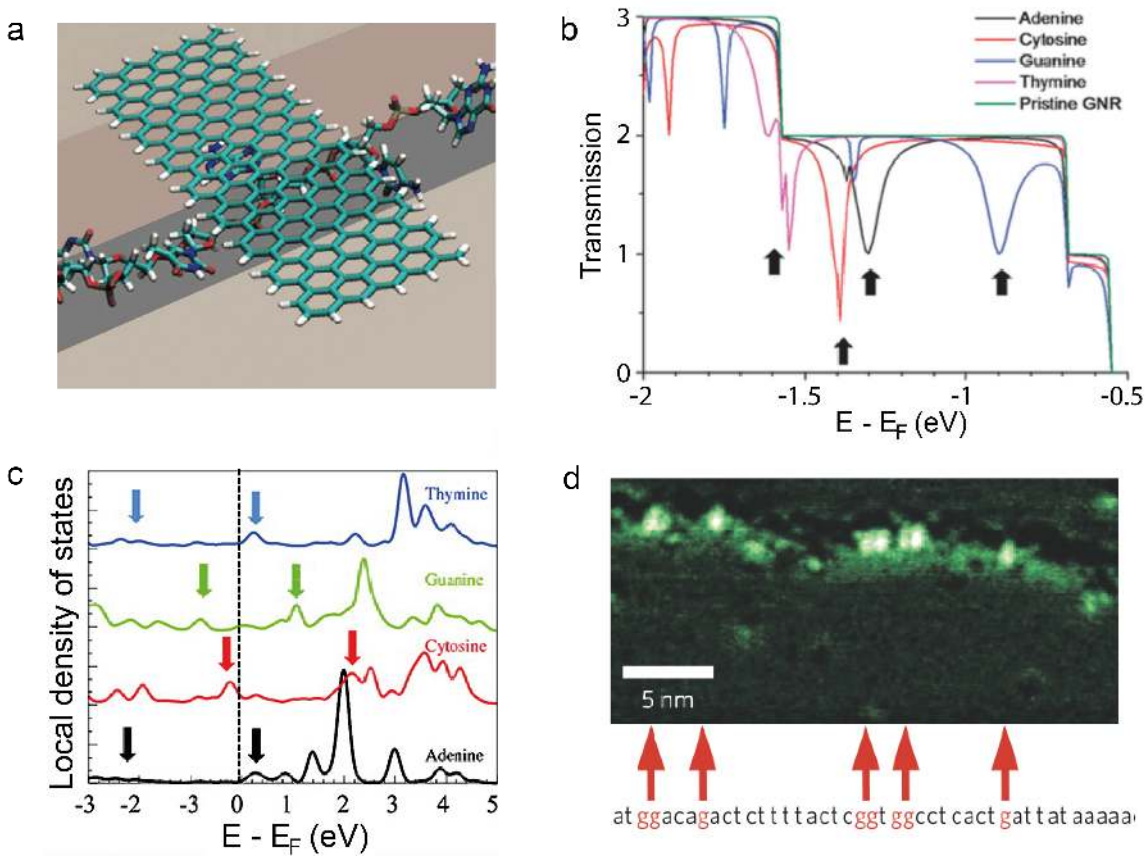

Figure 5: DNA detection methods based on DNA physisorption. **(a)** Schematic of a nanochannel device with an armchair graphene nanoribbon along which a ssDNA passes. DNA bases temporarily adsorb on the graphene while moving through the channel. Adapted from [101]. **(b)** DFT results for the structure of **a** show that base-varying conductance dips appear due to Fano resonance (black arrows) due to such DNA adsorption. Adapted from [102]. **(c)** DFT calculations for single DNA bases adsorbed on to graphene show different tunneling conductances due to their differences in local density of states. From [106]. **(d)** STM image of ssDNA molecules on a Cu(111) surface. The guanine sites are indicated by red characters in the bottom sequence, and by the red arrows. Adapted from [107].

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. *Proceedings of the National Academy of Sciences* **74**, 5463–5467 (1977).

[3] Shendure, J. & Ji, H. Next-generation DNA sequencing. *Nature biotechnology* **26**, 1135–45 (2008).

[4] Metzker, M. L. Sequencing technologies - the next generation. *Nature reviews. Genetics* **11**, 31–46 (2010).

[5] Ansorge, W. J. Next-generation DNA sequencing techniques. *New biotechnology* **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 (New York, N.Y.)* **299**, 682–6 (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 biotechnology* **31**, 1009–14 (2013).

[11] Mikheyev, A. S. & Tin, M. M. A first look at the Oxford Nanopore MinION sequencer. *Molecular Ecology Resources* **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 (New York, N.Y.)* **306**, 666–9 (2004).

[14] Novoselov, K. S. *et al.* Two-dimensional atomic crystals. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 10451–3 (2005).

[15] Geim, A. K. & Novoselov, K. S. The rise of graphene. *Nature materials* **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 (New York, N.Y.)* **321**, 385–8 (2008).

[17] Schedin, F. *et al.* Detection of individual gas molecules adsorbed on graphene. *Nature materials* **6**, 652–5 (2007).

[18] Nair, R. R. *et al.* Fine structure constant defines visual transparency of graphene. *Science (New York, N.Y.)* **320**, 1308 (2008).

[19] Pereira, V., Castro Neto, A. & Peres, N. Tight-binding approach to uniaxial strain in graphene. *Physical Review B* **80**, 045401 (2009).

[20] Balandin, A. A. *et al.* Superior thermal conductivity of single-layer graphene. *Nano letters* **8**, 902–7 (2008).

[21] Morozov, S. *et al.* Giant Intrinsic Carrier Mobilities in Graphene and Its Bilayer. *Physical Review Letters* **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 fromNeurospora crassa. *The Journal of Membrane Biology* **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 Microbiology Letters* **105**, 93–100 (1992).

[26] Kasianowicz, J. J., Brandin, E., Branton, D. & Deamer, D. W. Characterization of individual polynucleotide molecules using a membrane channel. *Proceedings of the National Academy of Sciences* **93**, 13770–13773 (1996).

[27] Cherf, G. M. *et al.* Automated forward and reverse ratcheting of DNA in a nanopore at 5-Åprecision. *Nature biotechnology* **30**, 344–8 (2012).

[28] Manrao, E. A. *et al.* Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase. *Nature biotechnology* **30**, 349–53 (2012).

[29] Dekker, C. Solid-state nanopores. *Nature nanotechnology* **2**, 209–15 (2007).

[30] Garaj, S. *et al.* Graphene as a subnanometre trans-electrode membrane. *Nature* **467**, 190–3 (2010).

[31] Garaj, S., Liu, S., Golovchenko, J. A. & Branton, D. Molecule-hugging graphene nanopores. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 12192–6 (2013).

[32] Sathe, C., Zou, X., Leburton, J.-P. & Schulten, K. Computational investigation of DNA detection using graphene nanopores. *ACS nano* **5**, 8842–51 (2011).

[33] Wells, D. B., Belkin, M., Comer, J. & Aksimentiev, A. Assessing graphene nanopores for sequencing DNA. *Nano letters* **12**, 4117–23 (2012).

[34] Liang, L. *et al.* Theoretical study on key factors in DNA sequencing with graphene nanopores. *RSC Advances* **3**, 2445 (2013).

[35] Schneider, G. F. *et al.* DNA translocation through graphene nanopores. *Nano letters* **10**, 3163–7 (2010).

[36] Merchant, C. A. *et al.* DNA translocation through graphene nanopores. *Nano letters* **10**, 2915–21 (2010).

[37] Schneider, G. F. *et al.* Tailoring the hydrophobicity of graphene for its use as nanopores for DNA translocation. *Nature communications* **4**, 2619 (2013).

[38] Banerjee, S. *et al.* Slowing DNA Transport Using Graphene-DNA Interactions. *Advanced Functional Materials* **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**, 487–92 (2012).

[40] Plesa, C., van Loo, N., Ketterer, P., Dietz, H. & Dekker, C. Velocity of DNA during Translocation through a Solid-State Nanopore. *Nano letters* **15**, 732–7 (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-Al2O3 nanopore sensors for sensitive detection of DNA and DNA-protein complexes. *ACS nano* **6**, 441–50 (2012).

[43] Banerjee, S. *et al.* Electrochemistry at the edge of a single graphene layer in a nanopore. *ACS nano* **7**, 834–43 (2013).

[44] Lee, M.-H. *et al.* A Low-Noise Solid-State Nanopore Platform Based on a Highly Insulating Substrate. *Scientific Reports* **4**, 7448 (2014).

[45] Postma, H. W. C. Rapid sequencing of individual DNA molecules in graphene nanogaps. *Nano letters* **10**, 420–5 (2010).

[46] Zhao, Q. *et al.* Nanopore-Based DNA Analysis via Graphene Electrodes. *Journal of Nanomaterials* **2012**, 1–5 (2012).

[47] Zhang, H. *et al.* Detection of nucleic acids by graphene-based devices: A first-principles study. *Journal of Applied Physics* **115**, 133701 (2014).

[48] Jeong, H. *et al.* Quantum interference in DNA bases probed by graphene nanoribbons. *Applied Physics Letters* **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 letters* **11**, 1941–5 (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. *The Journal of Physical Chemistry C* **117**, 15421–15428 (2013).

[51] He, Y. *et al.* Enhanced DNA Sequencing Performance Through Edge-Hydrogenation of Graphene Electrodes. *Advanced Functional Materials* **21**, 2674–2679 (2011).

[52] Huang, S. *et al.* Identifying single bases in a DNA oligomer with electron tunnelling. *Nature nanotechnology* **5**, 868–73 (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**, 10319–26 (2013).

[55] Ivanov, A. P. *et al.* DNA tunneling detector embedded in a nanopore. *Nano letters* **11**, 279–85 (2011).

[56] Tsutsui, M., Taniguchi, M., Yokota, K. & Kawai, T. Identifying single nucleotides by tunnelling current. *Nature nanotechnology* **5**, 286–90 (2010).

[57] Tsutsui, M. *et al.* Single-molecule sensing electrode embedded in-plane nanopore. *Scientific reports* **1**, 46 (2011).

[58] Bagci, V. M. K. & Kaun, C.-C. Recognizing nucleotides by cross-tunneling currents for DNA sequencing. *Physical Review 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. *Physical Review B* **85**, 115436 (2012).

[60] Prins, F. *et al.* Room-temperature gating of molecular junctions using few-layer graphene nanogap electrodes. *Nano letters* **11**, 4607–11 (2011).

[61] Nef, C. *et al.* High-yield fabrication of nm-size gaps in monolayer CVD graphene. *Nanoscale* **6**, 7249–54 (2014).

[62] Sadeghi, H. *et al.* Conductance enlargement in picoscale electroburnt graphene nanojunctions. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 2658–63 (2015).

[63] Island, J. O. *et al.* Fabrication of hybrid molecular devices using multi-layer graphene break junctions. *Journal of physics. Condensed matter : an Institute of Physics journal* **26**, 474205 (2014).

[64] Cao, Y. *et al.* Building High-Throughput Molecular Junctions Using Indented Graphene Point Contacts. *Angewandte Chemie* **124**, 12394–12398 (2012).

[65] Wallace, P. The Band Theory of Graphite. *Physical Review* **71**, 622–634 (1947).

[66] Fujita, M., Wakabayashi, K., Nakada, K. & Kusakabe, K. Peculiar Localized State at Zigzag Graphite Edge. *Journal of the Physical Society of Japan* **65**, 1920–1923 (1996).

[67] Wakabayashi, K., Fujita, M., Ajiki, H. & Sigrist, M. Electronic and magnetic properties of nanographite ribbons. *Physical Review B* **59**, 8271–8282 (1999).

[68] Ezawa, M. Peculiar width dependence of the electronic properties of carbon nanoribbons. *Physical Review B* **73**, 045432 (2006).

[69] Brey, L. & Fertig, H. Electronic states of graphene nanoribbons studied with the Dirac equation. *Physical Review 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. *Physical Review B* **54**, 17954–17961 (1996).

[71] Sasaki, K.-i., Murakami, S. & Saito, R. Gauge Field for Edge State in Graphene. *Journal of the Physical Society of Japan* **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 letters* **10**, 3237–42 (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 Physics 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 letters* **12**, 50–5 (2012).

[75] Avdoshenko, S. M. *et al.* Dynamic and electronic transport properties of DNA translocation through graphene nanopores. *Nano letters* **13**, 1969–76 (2013).

[76] Girdhar, A., Sathe, C., Schulten, K. & Leburton, J.-P. Graphene quantum point contact transistor for DNA sensing. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 16748–53 (2013).

[77] Ahmed, T., Haraldsen, J. T., Zhu, J.-X. & Balatsky, A. V. Next-Generation Epigenetic Detection Technique: Identifying Methylated Cytosine Using Graphene Nanopore. *The Journal of Physical Chemistry Letters* **5**, 2601–2607 (2014).

[78] Sadeghi, H. *et al.* Graphene sculpturene nanopores for DNA nucleobase sensing. *The journal of physical chemistry. B* **118**, 6908–14 (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 nanotechnology* **8**, 939–45 (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 letters* **11**, 5184–8 (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**, 11283–9 (2013).

[83] Qi, Z. J. *et al.* Correlating atomic structure and transport in suspended graphene nanoribbons. *Nano letters* **14**, 4238–44 (2014).

[84] Song, B. *et al.* Atomic-scale electron-beam sculpting of near-defect-free graphene nanostructures. *Nano letters* **11**, 2247–50 (2011).

[85] Xu, Q. *et al.* Controllable atomic scale patterning of freestanding monolayer graphene at elevated temperature. *ACS nano* **7**, 1566–72 (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. *Journal of Computational Electronics* **13**, 847–856 (2014). arXiv:1408.4300.

[87] Lagerqvist, J., Zwolak, M. & Di Ventra, M. Influence of the environment and probes on rapid DNA sequencing via transverse electronic transport. *Biophysical journal* **93**, 2384–90 (2007).

[88] Feliciano, G. T. *et al.* Capacitive DNA Detection Driven by Electronic Charge Fluctuations in a Graphene Nanopore. *Phys. Rev. Applied* **3**, 34003 (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**, 3510–20 (2015).

[91] Xie, P., Xiong, Q., Fang, Y., Qing, Q. & Lieber, C. M. Local electrical potential detection of DNA by nanowire-nanopore sensors. *Nature nanotechnology* **7**, 119–25 (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. *Physical Review 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. *The Journal of Physical Chemistry C* **117**, 13435–13441 (2013).

[96] Antony, J. & Grimme, S. Structures and interaction energies of stacked graphene-nucleobase complexes. *Physical chemistry chemical physics : PCCP* **10**, 2722–9 (2008).

[97] Varghese, N. *et al.* Binding of DNA nucleobases and nucleosides with graphene. *Chemphyschem: a European journal of chemical physics and physical chemistry* **10**, 206–10 (2009).

[98] Das, A. *et al.* Binding of nucleobases with single-walled carbon nanotubes: Theory and experiment. *Chemical Physics Letters* **453**, 266–273 (2008).

[99] Umadevi, D. & Sastry, G. N. Quantum Mechanical Study of Physisorption of Nucleobases on Carbon Materials: Graphene versus Carbon Nanotubes. *The Journal of Physical Chemistry Letters* **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. *Journal of physics. Condensed matter : an Institute of Physics journal* **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 nanotechnology* **6**, 162–5 (2011).

[102] Cho, Y., Min, S. K., Kim, W. Y. & Kim, K. S. The origin of dips for the graphene-based DNA sequencing device. *Physical chemistry chemical physics : PCCP* **13**, 14293–6 (2011).

[103] Song, B., Cuniberti, G., Sanvito, S. & Fang, H. Nucleobase adsorbed at graphene devices: Enhance bio-sensorics. *Applied Physics Letters* **100**, 063101 (2012).

[104] Bobadilla, A. D. & Seminario, J. M. Assembly of a Noncovalent DNA Junction on Graphene Sheets and Electron Transport Characteristics. *The Journal of Physical Chemistry C* **117**, 26441–26453 (2013).

[105] Cai, J. *et al.* Atomically precise bottom-up fabrication of graphene nanoribbons. *Nature* **466**, 470–3 (2010).

[106] Ahmed, T. *et al.* Electronic fingerprints of DNA bases on graphene. *Nano letters* **12**, 927–31 (2012).

[107] Tanaka, H. & Kawai, T. Partial sequencing of a single DNA molecule with a scanning tunnelling microscope. *Nature nanotechnology* **4**, 518–22 (2009).

[108] Davies, T. J., Hyde, M. E. & Compton, R. G. Nanotrench arrays reveal insight into graphite electrochemistry. *Angewandte Chemie (International ed. in English)* **44**, 5121–6 (2005).

[109] Bonanni, A. & Pumera, M. Graphene platform for hairpin-DNA-based impedimetric genosensing. *ACS nano* **5**, 2356–61 (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 letters* **8**, 591–5 (2008).

[112] Heller, I. *et al.* Influence of electrolyte composition on liquid-gated carbon nanotube and graphene transistors. *Journal of the American Chemical Society* **132**, 17149–56 (2010).

[113] Lin, J. *et al.* Gating of single-layer graphene with single-stranded deoxyribonucleic acids. *Small (Weinheim an der Bergstrasse, Germany)* **6**, 1150–5 (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. *Advanced materials (Deerfield Beach, Fla.)* **22**, 1649–53 (2010).

[115] Lu, C., Yang, H., Zhu, C., Chen, X. & Chen, G. A Graphene Platform for Sensing Biomolecules. *Angewandte Chemie* **121**, 4879–4881 (2009).

[116] He, S. *et al.* A Graphene Nanoprobe for Rapid, Sensitive, and Multicolor Fluorescent DNA Analysis. *Advanced Functional Materials* **20**, 453–459 (2010).

[117] Balapanuru, J. *et al.* A Graphene Oxide-Organic Dye Ionic Complex with DNA-Sensing and Optical-Limiting Properties. *Angewandte Chemie* **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. *The Analyst* **137**, 2588–92 (2012).

[119] Hu, Yuwei, Li, Fenghua, Han, Dongxue, 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**, 3428–35 (2015).

[121] Feng, J. *et al.* Identification of single nucleotides in MoS2 nanopores. *Nature Nanotechnology* (2015).

[122] Wei, R., Martin, T. G., Rant, U. & Dietz, H. DNA Origami Gatekeepers for Solid-State Nanopores. *Angewandte Chemie* **124**, 4948–4951 (2012).

[123] Bell, N. A. W. *et al.* DNA origami nanopores. *Nano letters* **12**, 512–7 (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 letters* **13**, 1029–33 (2013).

[126] Nam, S. *et al.* Graphene nanopore with a self-integrated optical antenna. *Nano letters* **14**, 5584–9 (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* (2015).

[128] Chen, Q. *et al.* 3D motion of DNA-Au nanoconjugates in graphene liquid cell electron microscopy. *Nano letters* **13**, 4556–61 (2013).

[129] Liu, H. *et al.* Translocation of single-stranded DNA through single-walled carbon nanotubes. *Science (New York, N.Y.)* **327**, 64–7 (2010).

[130] Siwy, Z. S. & Davenport, M. Making nanopores from nanotubes. *Nature nanotechnology* **5**, 174–5 (2010).