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Challenges in fabricating graphene nanodevices for electronic DNA sequencing

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Abstract

Graphene-based electronic DNA sequencing techniques have received significant attention over the past decade and are hoped to provide a new generation of portable, low-cost devices capable of rapid and accurate DNA sequencing. However, these devices are yet to demonstrate DNA sequencing. This is partly due to complex fabrication requirements resulting in low device yields and limited throughput. In this paper, we review the challenging fabrication of graphene-based electronic DNA sequencing devices. We will place a particular focus on common fabrication challenges and look toward the development of high-throughput, high-yield fabrication of these devices.

Introduction

Often referred to as the blueprint to life, deoxyribonucleic acid, or DNA, contains the set of instructions required for the development and functioning of all living organisms. Given the importance of this molecule, it is hardly surprising that there has been significant interest in the development of low-cost methods capable of rapidly reading the sequence of nucleobases in a DNA molecule.[1–4] The development of such a DNA sequencing technique promises to revolutionize modern healthcare with applications ranging from rapid disease diagnosis and treatment to biosecurity and fundamental biological research.[5,6]

Many methods to sequence DNA have been proposed, however, a technique known as sequencing by synthesis has come to dominate the current market.[7] This approach typically works by adding and subsequently detecting fluorescently labelled nucleotides to a short fragment of the DNA. This process is repeated for many copies of the strand and the sequence is reconstructed by connecting overlapping regions. While this technique has been significantly optimized over the past decade, the relatively complex sample preparation, short read lengths, and expensive reagents required, place a fundamental limit on the minimum speed and cost that can be achieved using this sequencing method.[8,9] Moreover, as sequencing by synthesis relies on reconstructing the DNA sequence from short read fragments it is not suited for *de novo* sequencing or sequencing DNA containing large regions of repetition.

An alternate sequencing technique that has recently gained significant traction is nanopore-based DNA sequencing.[9–11] These devices consist of a nanometer-sized hole in an impermeable membrane that separates two chambers of an electrolytic solution [Fig. 1(a)]. When a voltage is applied across the membrane, ions flow through the nanopore resulting in a measurable current. Due to its negative charge, DNA can be electrophoretically driven through a nanopore by the application of an electric field.[12] The presence of DNA in the pore blocks the passage of ions leading to a change in the ionic current. Due to the unique properties of each nucleobase (such as size and shape), these current changes are base specific, thus enabling sequencing of DNA as it translocates through the pore.[13–15] Given the simple sample preparation and long read lengths of nanopore sequencing, this technology has the potential to provide low-cost, portable devices capable of rapid DNA sequencing.[9] Indeed, nanopore sequencing has already experienced significant success and devices based on this technology are now commercially available.[16–18]

Despite the success of nanopore sequencing, these devices suffer from several limitations. In particular, nanopore sequencing is typically pursued using biological nanopores, which have a fixed shape, limited stability, and are not suited to a massive upscaling in production.[10] One solution currently being explored is to create nanopores in solid-state materials[10] such as SiO_2, SiN_x and Al_2O_3. However, these pores are typically tens of nanometers thick resulting in ionic current blockades that are a convolution of the 10–100 nucleotides residing within the pore at a given time.[19] For this reason, there has been significant interest in creating nanopores in two-dimensional materials, such as graphene, for DNA sequencing.[19–22] However, fast DNA translocation rates through these nanopores have hindered single-base resolution due to large parasitic capacitance signals at high sampling frequencies.[23]

Given graphene's conductive properties, DNA sequencing techniques relying on detecting changes in the current in a

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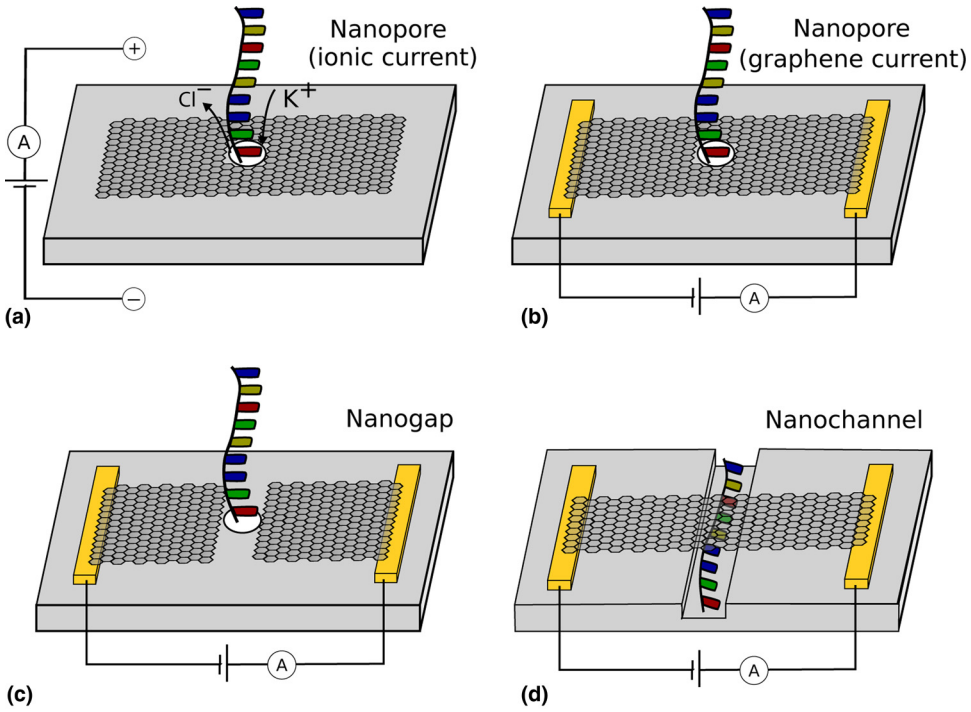


Figure 1. (a) Schematic of ionic current DNA sequencing using a graphene nanopore. (b–d) Schematics of DNA sequencing utilizing the conductive properties of graphene as; (b) DNA translates through a nanopore in the graphene, (c) DNA translates through a nanogap in the graphene and (d) DNA translates through an adjacent nanofluidic channel.

graphene nanostructure as DNA translates through a nearby nanopore or nanochannel have also been suggested.[19] In particular, three device geometries have thus far been proposed: (i) monitor the graphene current as DNA translocates through a nanopore in the graphene ribbon[24,25] [Fig. 1(b)], (ii) monitor the tunnelling current across a graphene nanogap as DNA translates through the gap[26] [Fig. 1(c)] and (iii) monitor the graphene current as DNA translates through an adjacent nanofluidic channel[27] [Fig. 1(d)]. Importantly, utilizing the conductive properties of graphene offers the potential for high bandwidth measurements, thus enabling DNA sequencing without the need to slow DNA translocation. Additionally, unlike ionic current-based sequencing, these devices can easily be multiplexed as the current through each nanostructure can be read independently. Despite their significant promise, DNA sequencing utilizing graphene-based electronic devices has yet to be demonstrated. This has been partly due to the complex fabrication requirements of these devices that has resulted in low device yields and limited throughput.[28–31] This paper reviews the general fabrication principles of graphene-based electronic sequencing devices. While each published study will inevitably use a different device design and fabrication procedure, particular focus will be given to common fabrication challenges as well as highlighting some of their potential solutions. It is hoped that this will help drive an increase in the fabrication yield and throughput of future devices hence providing a significant step toward sequencing. It is noted that reviews focusing on the mechanisms behind changes in the graphene current as DNA translates through a nearby nanopore are available elsewhere.[19,32]

Graphene nanopores

DNA sequencing devices consisting of a nanopore within a graphene nanoribbon (GNR) will be discussed first. These devices function by detecting changes in the graphene current as DNA translocates through the pore in a direction perpendicular to the graphene plane [Fig. 1(b)]. These devices should not be confused with ionic current-based graphene nanopore sequencing devices that have been extensively described elsewhere.[19–22] A schematic of the typical geometry for these devices is shown in Fig. 2(a). In particular, these devices typically consist of a SiN_x window that is suspended over a SiO_2 layer on a silicon substrate. Graphene and metal contact electrodes are patterned on the SiN_x with a passivation layer covering the metal electrodes. A nanopore in the graphene and underlying SiN_x window enables DNA translocation. These devices have been studied in many computational works and indeed nucleobase-specific changes in the graphene current have been predicted.[24,33–36] Furthermore, several experimental studies have detected DNA translocation through a graphene nanopore via changes in the graphene current.[25,30,37] However, fabrication throughput and device yield, as well as

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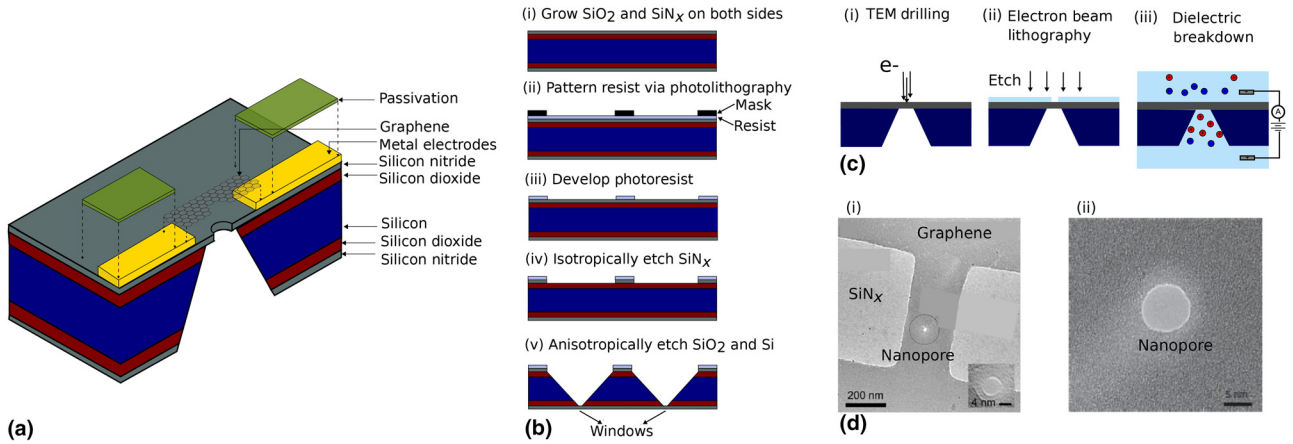


Figure 2. (a) Schematic representation of graphene nanopore electronic DNA sequencing devices. (b) Schematic of the steps involved in forming SiN_x windows. (c) Schematic of nanopore creation via (i) TEM drilling, (ii) electron beam lithography followed by reactive ion etching and (iii) delectric breakdown. (d) TEM images of a nanopore within a GNR created via TEM drilling.[25,38] Figures adapted with permissions from (d) Nature publishing group[25] and American Chemical Society.[38]

experimental challenges such as graphene wetting and electrostatic discharge continue to hinder the development of these devices towards DNA sequencing.[30]

This section will begin by discussing the fabrication of suspended SiN_x structures. These structures are necessary to enable nanopore formation and are also relevant for the graphene nanogap detectors discussed in the section "Graphene nanogaps." The procedure for fabricating SiN_x windows has been described elsewhere[39] and typically consists of growing a SiO_2 layer on a high resistivity silicon <100> substrate then depositing low-pressure chemical vapor deposition (LPCVD) SiN_x films, followed by photolithography and subsequent etching [Fig. 2(b)]. Care should be taken to ensure etching along the correct crystal orientations. A protective layer made of an easily removed polymer may be used during etching to protect features on the front side of the wafer from leakages caused by defects when etching. The SiO_2 film is used to reduce capacitive coupling between the metal electrodes and silicon substrate and is generally between 0.1 and 5 μm thick. One result of this thick SiO_2 layer is that, due to its compressive stress, the resulting SiN_x windows can have an uneven surface topography.[28] This can be partially reduced via thermal annealing, however, this may result in bulging of the window. Several studies have attempted to overcome these issues by growing thin SiN_x films on highly insulating substrates without a SiO_2 layer[40] or with a poly-Si[41] layer, however, these devices may exhibit increased noise due to capacitive coupling to the substrate.

Metal electrodes are used to enable electrical contact to graphene nanodevices. Although not strictly necessary, patterning contact electrodes prior to graphene transfer is preferable as it exposes graphene to less processing. Metal electrodes are fabricated using photolithography coupled with electron beam lithography (for large and small feature sizes, respectively) followed by metal deposition and subsequent liftoff. Electron beam lithography is used to pattern electrodes over the SiN_x window with local alignment markers written during this step to allow for later alignment of graphene nanostructures. Note that patterning resist over the SiN_x window requires a higher dose than supported parts of the chip due to the decreased contribution of backscattered electrons. These metal electrodes must be passivated to (i) reduce faradaic currents resulting from electrochemical reactions between the electrodes and ionic solution[28] and (ii) reduce noise resulting from capacitive coupling to the ionic solution. Several materials have been used for passivation such as SiO_2/SiN_x.[42] Al_2O_3,[25] TiO_2,[28] h-BN[30] and various polymeric films. Care should be taken to ensure proper adhesion of the passivation to metal electrodes.[43] In general, there are two considerations to be made regarding the thickness of the passivation layer. First, the film must be thick enough to avoid pinholes in the passivation. This thickness is largely dependent on the deposition process (e.g. atomic layer deposition, electron beam evaporation, CVD, photoresist spincoating) and the chosen dielectric. Second, the passivation thickness should be thick enough to reduce noise resulting from capacitive coupling to the ionic solution. Although dependent on the dielectric used and the device geometry, the authors have found that dielectric thicknesses greater than 500 nm are required to significantly reduce this noise. Electrode passivation is routinely performed after graphene transfer and patterning to ensure that the metal electrodes can be fully passivated as well as nonactive areas of the graphene.

Perhaps, the most challenging aspect of fabrication is forming the nanopore. These nanopores are typically formed using high-energy electron irradiation from a transmission electron microscope (TEM)[25,30,37,38] [Figs. 2(c)(i) and 2(d)]. However, this technique requires expensive equipment, expert

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training, and is time consuming. As such, techniques to reliably and rapidly form nanopores in graphene, SiN_x, and other two-dimensional materials continue to be researched. Other techniques to create nanopores in SiN_x membranes include electron beam lithography followed by reactive ion etching[44] [Fig. 2(c)(ii)] and focused ion beam milling, including gas field ion sources such as helium ion microscopes and eutectic sources. While these techniques are only capable of reproducibly creating nanopores with diameters on the order of 15 nm and larger, nanopores shrinking methods can be used to reduce the diameter of these pores[45] to sizes compatible with DNA sequencing applications. An alternate technique to scalably fabricate nanopores in SiN_x relies on electric field-induced breakdown. Namely, pores with subnanometer precision can be fabricated in SiN_x via feedback-controlled dielectric breakdown [Fig. 2(c)(iii)] whereby a large electric field is applied across the membrane while monitoring the leakage current.[46] Techniques to control the position and number of nanopores created via dielectric breakdown continue to be developed.[47,48] Such formation of SiN_x nanopores could then be followed by graphene transfer and nanopore formation by applying pulsed electric fields across the membrane[49] to create self-aligned graphene nanopores. Alternately, this pulsed electric field breakdown technique could be used to create nanopores in freestanding graphene membranes, however, a coating layer would be required to avoid DNA sticking to the graphene prior to translocation.[49]

Despite several promising studies demonstrating DNA detection via changes in the graphene current as DNA translates through a pore in the graphene,[25,30,37] recent works continue to cite low fabrication throughput and device yield as significant problems.[30] This is no doubt in part due to the many fabrication steps required, each of which need to be optimized to return an acceptable yield. Additionally, nanopore formation using a TEM is a time-consuming process which significantly limits device throughput. Techniques to scalably create nanopores in graphene and SiN_x, some of which were highlighted above, will need to continue to be developed if this sequencing approach is to continue to advance over the coming years.

Graphene nanogaps

DNA sequencing techniques based on graphene nanogaps will now be discussed. These devices function by detecting changes in the tunnelling current across a graphene nanogap as DNA translocates through the gap in a direction perpendicular to the graphene plane[50] [Fig. 1(c)]. Similar to the geometry discussed for graphene nanopores, these devices consist of a graphene nanogap aligned with a nanopore in the underlying SiN_x window. While it may be possible to use graphene as both the membrane and electrode material,[26] such a device would likely have limited control over the position of DNA during translocation.[51] This is a particularly important consideration given the exponential dependence of tunnelling current on gap distance. Graphene nanogap sequencing devices have been studied in several computational works where nucelobase-specific changes in the tunnelling current were predicted due to different coupling strengths between the graphene and the molecular orbitals of each base.[26,52–55] Additionally, experimental works have detected and discriminated nucleotides via changes in the tunnelling current in a scanning tunnelling microscope[56–59,60] and metal nanogaps.[60–65] No attempts to detect DNA using tunnelling currents across a graphene nanogap have been published to date.

Several techniques to fabricate graphene nanogaps have been demonstrated that could conceivably be used for nanogap-based DNA sequencing.[66–69] One such technique is feedback-controlled electroburning.[66,70,71] Here, a voltage ramp is applied across a GNR while the current is simultaneously recorded. Large current densities in the graphene induce Joule heating which can lead to oxidation or sublimation of carbon atoms at the edges.[71] When this happens, the graphene resistance increases resulting in a drop in the current [asterisk in Fig. 3(a)] at which point the voltage is ramped back to zero. Repeating this process allows for the controlled formation of a nanogap across a GNR [Figs. 3(a) and 3(b)]. Recently, graphene nanogaps have also been fabricated via mechanically controlled break junctions.[67] Here, graphene was patterned on a flexible phosphor bronze substrate and loaded into a three-point bending configuration [Fig. 3(c)]. The graphene current is monitored during bending until a sharp drop in the conductance is observed signifying the formation of a nanogap. Another method that has been used to create graphene nanogaps relies on capillary force-induced breaking of suspended graphene.[68,72] In this technique, graphene is suspended across a trench in the substrate, and broken due to the surface tension of the solvent during the drying process [Fig. 3(d)].

One of the major challenges in the fabrication of these devices is the alignment of graphene nanogaps with an underlying SiN_x nanopore. Graphene can be fabricated into a constriction to enable the controlled positioning of a graphene nanogap via the electroburning[70] [Fig. 3(b)] and mechanically controlled break junction[67] techniques discussed above. Patterning of graphene into such geometries is generally done via electron beam lithography followed by etching with alignment of features down to 20 nm readily obtained using local alignment markers. Care should be taken with alignment marker position near the SiN_x window. However, alignment on the order of a few nanometers would likely be required to ensure that a single tunnel junction is reproducibly aligned with a nanopore. Moreover, formation of graphene nanogaps via feedback-controlled electroburning and mechanical-controlled break junctions have to date only been demonstrated on supported or semi-supported substrates and these techniques will need to be developed for suspended graphene membranes compatible with DNA sequencing. It is noted that DNA translocation through graphene nanogaps was recently demonstrated by aligning a "micropore" in a polymeric film with the nanogap.[72] By doing this, the authors were able to detect DNA passing though the nanogap via changes in the ionic current, however, simultaneous monitoring of the graphene tunnelling current was not reported. While such a nanogap/micropore

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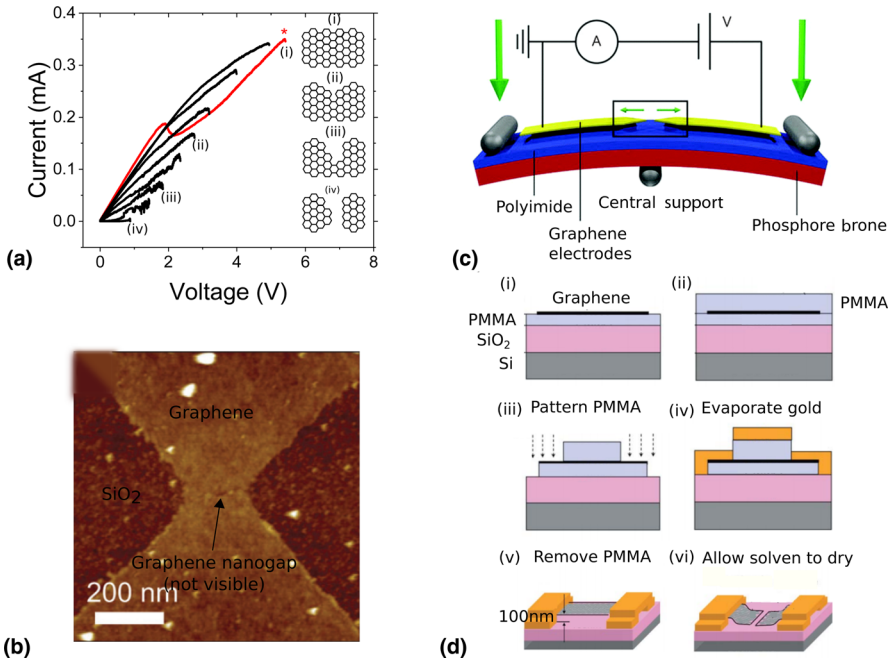


Figure 3. (a) An example of the electroburning process. The first trace is shown in red. The asterisk shows the point where the graphene resistance increases indicating the onset of electroburning. The insets show schematics of the nanogap formation process during electroburning. (b) An atomic force microscope image of a graphene nanogap formed via electroburning on a SiO_2 substrate.[70] The graphene has been patterned into a constriction to enable controlled positioning of the nanogap. (c) Schematic of the mechanical controlled break junction setup to form graphene nanogaps.[98] (d) Schematic of an example fabrication process to produce a graphene nanogap via capillary force induce breaking.[68] Figures adapted with permissions from (b) PCCP Owner Societies,[70] (c) Royal Society of Chemistry [relevant citation added above] (d) AIP Publishing.[68]

assembly may be useful for preliminary studies into DNA detection using graphene tunnelling currents, it is likely that sequencing devices will require more stringent control over the position of DNA as it translates through the nanogap.

The integration of electric and fluidic components is another challenging aspect in the development of graphene-based electronic sequencing techniques. For the nanopore and nanogap devices discussed above, similar fluidic designs to that used in typical ionic current nanopore experiments can be envisioned. These fluidic designs can be broadly classified into three classes; (i) a fluidic reservoir approach, (ii) a microfluidic flow cell approach, and (iii) a nanocapillary approach. For the proposed sequencing devices, electrical connections will need to be incorporated to enable measurements of the graphene current. For the fluidic reservior and microfluidic approaches this could be done by wirebonding to a printed circuit board or the incorporation of pins within the fluidic cell to directly contact the chip. Microfluidic cells have the advantage over fluidic reserviors in that they enable liquids to be pumped through the nanopore enabling pore wetting and fluidic transfer. This is a particular problem for the devices considered here since graphene is not compatible with commonly used techniques that render a nanopore hydrophilic such as Piranha solution and oxygen plasma treatment.[30] The use of nanocapillaries to fluidically contact devices[75] is an appealing approach as it may enable easy electrical connection. However, it is yet to be seen whether devices can be effectively contacted and the fluid localized at the nanopore without wetting of the electrical contacts. Additionally, a nanocapillary is not suited to multiplexed measurements.

While promising results have been reported on the detection and discrimination of nucleobases via tunnelling currents in a scanning tunnelling microscope and metal nanogaps, fabrication of graphene nanogaps for tunnelling current sequencing is yet to be reported. Indeed, several studies on metal nanogaps integrated within a nanopore have cited significant difficulties in the fabrication process,[28,29,42] which will likely remain for graphene-based devices. Promisingly, several scalable techniques to create graphene nanogaps have already been reported, however, these techniques will need to be developed for suspended graphene structures compatible with DNA seqeuncing. Moreover, the alignment of graphene nanogaps with a SiN_x nanopore to enable controlled DNA translocation poses a significant challenge in the future development of these devices.

Graphene nanoribbons

The last devices that will be discussed here consist of a GNR adjacent to a nanofluidic channel [Fig. 1(d)]. The proposed device works by detecting changes in the graphene current due to adsorption of DNA as it translates through the nanofluidic channel. A schematic of the proposed device geometry is shown in Fig. 4(a) with the envisioned devices consisting of

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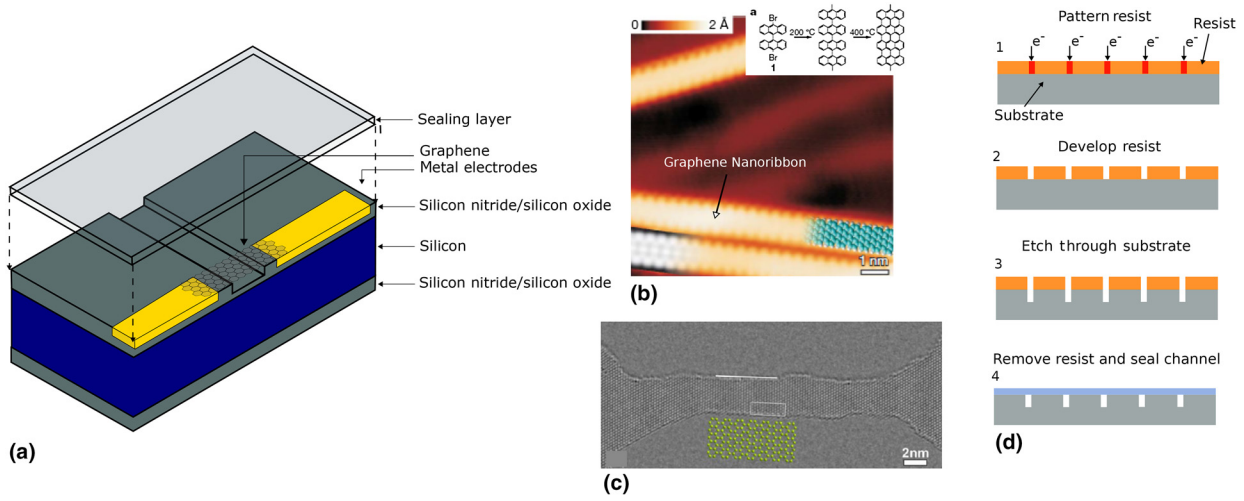


Figure 4. (a) Schematic of the proposed device geometry. (b) Scanning tunnelling microscopy image of GNRs created via bottom-up synthesis. The inset shows the reaction scheme for the precursor monomers into GNRs.[73] (c) Transmission electron micrograph image of a GNR which has been sculpted using high-energy electrons produced in a TEM.[74] (d) Schematic representation of the process to create nanofluidic channels in a substrate via electron beam lithography. Figures adapted with permissions from (b) Nature publishing group,[73] (c) American Chemical Society.[74]

a GNR bridging a nanofluidic channel on a SiN_x (or SiO_2) film. A sealing layer is placed over the nanochannel to enable fluid flow. These devices have been studied computationally where it was predicted that $\pi - \pi$ stacking between nucleobases and a nanometer-wide graphene ribbon can lead to base-specific changes in the graphene current due to Fano resonances.[27,76] Fabrication of devices capable of measuring the current in a nanometer-wide graphene ribbon while DNA translates through an adjacent nanochannel is yet to be demonstrated.

One of the major challenges in the fabrication of these devices is creating GNRs with widths on the order of 1 nm to ensure a limited number of nucleobases interact with the graphene at a given time. One technique to reliably create nanometer-wide graphene ribbons is via bottom-up synthesis. This method relies on the surface-assisted coupling of molecular precursors followed by their subsequent cyclodehydrogenation[73] [Fig. 4(b)]. The controlled alignment of these nanoribbons with electrodes and a nanofluidic channel is likely to be a challenge, however, this could potentially be overcome by developing self-alignment techniques such as dielectrophoretic deposition. Similar alignment issues may also be encountered with several other GNR fabrication techniques such as nanowire lithography[77] and unzipping of carbon nanotubes.[78] GNRs with widths on the order of 20 nm have been fabricated via electron beam lithography,[79] and their widths further reduced via gas-phase oxidation.[80] However, it is difficult to produce widths below 10 nm using this technique due to edge roughness introduced during electron beam lithography. Direct patterning of GNRs can also be achieved using energetic charged particles from a TEM[74,81] [Fig. 4(c)] or helium ion microscope.[82]

The fabrication of nanofluidic channels as well as DNA translocation through these channels have been described elsewhere.[83,84] Techniques such as electron beam lithography,[85–87] interferometric lithography,[88] and nanoimprint lithography are commonly used to create nanofluidic channels.[89–91] Briefly, these techniques generally involve patterning of a resist followed by etching of the substrate [shown in Fig. 4(d) for electron beam lithography]. These techniques create open nanofluidic channels that must be sealed to enable fluid flow. This can be done via, anodic bonding of a cover slide to the substrate. Metal electrodes may need to be patterned into the substrate to ensure a planar surface that allows for nanochannel sealing. This can be achieved by etching the substrate an appropriate thickness prior to metal deposition.[92] Note that further studies may need to be done to determine the channel dimensions required to ensure interaction between the DNA and graphene.

Finally, challenges in graphene transfer and contamination will be discussed. While this is clearly an important consideration for all the graphene-based sequencing techniques, it is discussed in this section as contamination may pose a significant issue for these devices as they rely on DNA adsorbing to graphene. Depending on the source of graphene, different graphene transfer methods may be used such as substrate etching, wedge transfer[93] and bubble transfer.[94] These techniques leave graphene suspended in an aqueous solution which can then be captured on the desired substrate. A polymeric film over the graphene is typically used during the transfer process to avoid the breaking of graphene. Graphene contamination may occur due to residues of this polymeric film, or other films introduced during lithographic steps. Such residues can be (partially) removed via current annealing[95] and thermal annealing.[96] Current and thermal annealing may also be used to remove atmospheric contamination on graphene, however, the surface will become quickly

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recontaminated when exposed to atmosphere. Techniques such as electrochemically cleaning graphene in solution have been studied and could potentially be used in situ to avoid graphene recontamination.[97]

While the idea to sequence DNA using changes in the current through a GNR due to physisorption of DNA translating through an adjacent nanochannel was first suggested in 2011,[27] there are yet to be any reports on the fabrication of these devices. This has likely been due to challenges in the fabrication of GNRs with widths on the order of 1 nm and their alignment with contact electrodes and a nanofluidic channel. Techniques such as dielectrophoretic deposition could potentially be used to align graphene and scalably produce devices, however, significant work will need to be done on the development of such techniques.

Conclusion and outlook

Graphene-based electronic DNA sequencing devices have received significant attention from the research community over the past decade. These devices are hoped to not only deliver on the advantages of ionic current nanopore sequencing, but also provide single base resolution, high bandwidth sampling, easy multiplexing and the potential for scalable manufacturing. In particular, three device designs have been suggested; (i) monitor the graphene current as DNA translates through a pore in the graphene, (ii) monitor the tunnelling current across a graphene nanogap as DNA translates through the gap and (iii) monitor the current through a GNR as DNA translates through an adjacent nanochannel. Despite the significant promise of these sequencing techniques, DNA sequencing using these devices is yet to be demonstrated. This has been partly due to the complex fabrication requirements of these devices resulting in low fabrication yields and limited throughput. Indeed, this will need to be addressed if DNA sequencing using these devices is to be realized. In particular, the development of robust, wafer-scale processes for substrate and electrode fabrication will be required. Additionally, techniques to scalably fabricate nanostructures in graphene, such as gaps and pores, as well as the alignment of these structures will need to be advanced. Given the relative infancy of graphene and the rapid development of nanofabrication techniques the authors are hopeful that such techniques will indeed by developed in the coming years. If this does indeed happen, it would represent a significant step toward graphene-based electronic DNA sequencing being realized.

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References

1. J. Shendure and H. Ji: Next-generation DNA sequencing. *Nat. Biotechnol.* **26**, 1135–1145 (2008).
2. M.L. Metzker: Sequencing technologies—the next generation. *Nat. Rev. Genet.* **11**, 31–46 (2009).
3. D. Deamer, M. Akeson, and D. Branton: Three decades of nanopore sequencing. *Nat. Biotechnol.* **34**, 518–524 (2016).
4. E.E. Schadt, S. Turner, and A. Kasarskis: A window into third-generation sequencing. *Hum. Mol. Genet.* **19**, R227–R240 (2010).
5. A.L. Norris, R.E. Workman, Y. Fan, J.R. Eshleman, and W. Timp: Nanopore sequencing detects structural variants in cancer. *Cancer Biol. Ther.* **17**, 246–253 (2016).
6. P. Stankiewicz and J.R. Lupski: Structural variation in the human genome and its role in disease. *Annu. Rev. Med.* **61**, 437–455 (2010).
7. E.R. Mardis: Next-generation DNA sequencing methods. *Annu. Rev. Genom. Hum. Genet.* **9**, 387–402 (2008).
8. M.J.P. Chaisson, R.K. Wilson, and E.E. Eichler: Genetic variation and the de novo assembly of human genomes. *Nat. Rev. Genet.* **16**, 627–640 (2015).
9. D. Branton, D.W. Deamer, A. Marziali, H. Bayley, S.A. Benner, T. Butler, M. Di Ventra, S. Garaj, A. Hibbs, X. Huang, S.B. Jovanovich, P.S. Krstic, S. Lindsay, X.S. Ling, C.H. Mastrangelo, A. Meller, J.S. Oliver, Y.V. Pershin, J.M. Ramsey, R. Riehn, G.V. Soni, V. Tabard-Cossa, M. Wanunu, M. Wiggin, and J.A. Schloss: The potential and challenges of nanopore sequencing. *Nat. Biotechnol.* **26**, 1146–1153 (2008).
10. C. Dekker: Solid-state nanopores. *Nat. Nanotechnol.* **2**, 209–215 (2007).
11. Y. Wang, Q. Yang, and Z. Wang: The evolution of nanopore sequencing. *Front. Genet.* **5**, (2015). Article no. 449.
12. J.J. Kasianowicz, E. Brandin, D. Branton, and D.W. Deamer: Characterization of individual polynucleotide molecules using a membrane channel. *Proc. Natl. Acad. Sci. USA* **93**, 13770–13773 (1996).
13. G.M. Cherf, K.R. Lieberman, H. Rashid, C.E. Lam, K. Karplus, and M. Akeson: Automated forward and reverse ratcheting of DNA in a nanopore at 5a precision. *Nat. Biotechnol.* **30**, 344–348 (2012).
14. E.A. Manrao, I.M. Derrington, A.H. Laszlo, K.W. Langford, M.K. Hopper, N. Gillgren, M. Pavlenok, M. Niederweis, and J.H. Gundlach: Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase. *Nat. Biotechnol.* **30**, 349–353 (2012).
15. B.M. Venkatesan and R. Bashir: Nanopore sensors for nucleic acid analysis. *Nat. Nanotechnol.* **6**, 615–624 (2011).
16. A.S. Mikheyev and M.M.Y. Tin: A first look at the oxford nanopore MinION sequencer. *Mol. Ecol. Resour.* **14**, 1097–1102 (2014).
17. M. Jain, H.E. Olsen, B. Paten, and M. Akeson: The oxford nanopore MinION: delivery of nanopore sequencing to the genomics community. *Genome Biol.* **17**, (2016). Article no. 239.
18. M. Jain, S. Koren, K.H. Miga, J. Quick, A.C. Rand, T.A. Sasani, J.R. Tyson, A.D. Beggs, A.T. Dilthey, I.T. Fiddes, S. Malla, H Marriott, T. Nieto, J.O. Grady, H.E. Olsen, B.S. Pedersen, A. Rhie, H. Richardson, A.R. Quinlan, T.P. Snutch, L. Tee, B. Paten, A.M. Phillippy, J.T. Simpson, N.J. Loman, and M. Loose: Nanopore sequencing and assembly of a human genome with ultra-long reads. *Nat. Biotechnol.* **36**, 338–345 (2018).
19. S.J. Heerema and C. Dekker: Graphene nanodevices for DNA sequencing. *Nat. Nanotechnol.* **11**, 127–136 (2016).
20. C.A. Merchant, K. Healy, M. Wanunu, V. Ray, N. Peterman, J. Bartel, M.D. Fischbein, K. Venta, Z. Luo, A.T.C. Johnson, and M. Drndic: DNA translocation through graphene nanopores. *Nano Lett.* **10**, 2915–2921 (2010).
21. G.F. Schneider, S.W. Kowalczyk, V.E. Calado, G. Pandraud, H.W. Zandbergen, L.M.K. Vandersypen, and C. Dekker: DNA translocation through graphene nanopores. *Nano Lett.* **10**, 3163–3167 (2010).
22. S. Garaj, W. Hubbard, A. Reina, J. Kong, D. Branton, and J.A. Golovchenko: Graphene as a subnanometre trans-electrode membrane. *Nature* **467**, 190–193 (2010).
23. J.K. Rosenstein, M. Wanunu, C.A. Merchant, M. Drndic, and K.L. Shepard: Integrated nanopore sensing platform with sub-microsecond temporal resolution. *Nat. Methods*, **9**, 487–492 (2012).
24. T. Nelson, B. Zhang, and O.V. Prezhdo: Detection of nucleic acids with graphene nanopores: Ab initio characterization of a novel sequencing device. *Nano Lett.* **10**, 3237–3242 (2010).
25. F. Traversi, C. Raillon, S.M. Benameur, K. Liu, S. Khlybov, M. Tosun, D. Krasnozhon, A. Kis, and A. Radenovic: Detecting the translocation of DNA through a nanopore using graphene nanoribbons. *Nat. Nanotechnol.* **8**, 939–945 (2013).

https://doi.org/10.1557/mrc.2018.187 Published online by Cambridge University Press

26. H.W.C. Postma: Rapid sequencing of individual DNA molecules in graphene nanogaps. *Nano Lett.* **10**, 420–425 (2010).
27. S.K. Min, W.Y. Kim, Y. Cho, and K.S. Kim: Fast DNA sequencing with a graphene-based nanochannel device. *Nat. Nanotechnol.* **6**, 162–165 (2011).
28. K. Healy, V. Ray, L.J. Willis, N. Peterman, J. Bartel, and M. Drndić: Fabrication and characterization of nanopores with insulated transverse nanoelectrodes for DNA sensing in salt solution. *Electrophoresis* **33**, 3488–3496 (2012).
29. A. Fanget, F. Traversi, S. Khlybov, P. Granjon, A. Magrez, L. Forró, and A. Radenovic: Nanopore integrated nanogaps for DNA detection. *Nano Lett.* **14**, 244–249 (2013).
30. S.J. Heerema, L. Vicarelli, S. Pud, R.N. Schouten, H.W. Zandbergen, and C. Dekker: Probing DNA translocations with inplane current signals in a graphene nanoribbon with a nanopore. *ACS Nano* **12**, 2323–2633 (2018).
31. Z. Yuan, C. Wang, X. Yi, Z. Ni, Y. Chen, and T. Li: Solid-state nanopore. *Nanoscale Res. Lett.* **13**, (2018). Article no. 56.
32. H.S. Kim and Y.-H. Kim: Recent progress in atomistic simulation of electrical current DNA sequencing. *Biosens. Bioelectron.* **69**, 186–198 (2015).
33. K.K. Saha, M. Drndić, and B.K. Nikolić: DNA base-specific modulation of microampere transverse edge currents through a metallic graphene nanoribbon with a nanopore. *Nano Lett.* **12**, 50–55 (2011).
34. F.-P. Ouyang, S.-L. Peng, H. Zhang, L.-B. Weng, and H. Xu: A biosensor based on graphene nanoribbon with nanopores: a first-principles devices-design. *Chin. Phys. B.* **20**, 058504 (2011).
35. S.M. Avdoshenko, D. Nozaki, C.G. da Rocha, J.W. González, M.H. Lee, R. Gutierrez, and G. Cuniberti: Dynamic and electronic transport properties of DNA translocation through graphene nanopores. *Nano Lett.* **13**, 1969–1976 (2013).
36. A. Girdhar, C. Sathe, K. Schulten, and J.-P. Leburton: Graphene quantum point contact transistor for DNA sensing. *Proc. Natl. Acad. Sci. USA* **110**, 16748–16753 (2013).
37. M. Puster, A. Balan, J.A. Rodrguez-Manzo, G. Danda, J.-H. Ahn, W. Parkin, and M. Drndić: Cross-talk between ionic and nanoribbon current signals in graphene nanoribbon-nanopore sensors for single-molecule detection. *Small* **11**, 6309–6316 (2015).
38. M. Puster, J.A. Rodrguez-Manzo, A. Balan, and M. Drndić: Toward sensitive graphene nanoribbon–nanopore devices by preventing electron beam-induced damage. *ACS Nano* **7**, 11283–11289 (2013).
39. A.W. Grant, Q.-H. Hu, and B. Kasemo: Transmission electron microscopy windows for nanofabricated structures. *Nanotechnology* **15**, 1175–1181 (2004).
40. M.-H. Lee, A. Kumar, K.-B. Park, S.-Y. Cho, H.-M. Kim, M.-C. Lim, Y.-R. Kim, and K.-B. Kim: A low-noise solid-state nanopore platform based on a highly insulating substrate. *Sci. Rep.*, **4**, (2014). Article no. 7448.
41. I. Yanagi, T. Ishida, K. Fujisaki, and K.i Takeda: Fabrication of 3-nm-thick si3n4 membranes for solid-state nanopores using the poly-si sacrificial layer process. *Sci. Rep.*, **5**, (2015). Article no. 14656.
42. A.P. Ivanov, E. Instuli, C.M. McGilvery, G. Baldwin, D.W. McComb, T. Albrecht, and J.B. Edel: DNA tunneling detector embedded in a nanopore. *Nano Lett.* **11**, 279–285 (2011).
43. Y. Temiz, A. Ferretti, Y. Leblebici, and C. Guiducci: A comparative study on fabrication techniques for on-chip microelectrodes. *Lab. Chip.* **12**, 4920–4928 (2012).
44. D.V. Verschueren, W. Yang, and C. Dekker: Lithography-based fabrication of nanopore arrays in freestanding SiN and graphene membranes. *Nanotechnology* **29**, 145302 (2018).
45. W. Asghar, A. Ilyas, J. Billo, and S. Iqbal: Shrinking of solid-state nanopores by direct thermal heating. *Nanoscale Res. Lett.* **6**, 372 (2011).
46. H. Kwok, K. Briggs, and V. Tabard-Cossa: Nanopore fabrication by controlled dielectric breakdown. *PLoS One* **9**, e92880 (2014).
47. S. Pud, D. Verschueren, N. Vukovic, C. Plesa, M.P. Jonsson, and C. Dekker: Self-aligned plasmonic nanopores by optically controlled dielectric breakdown. *Nano Lett.* **15**, 7112–7117 (2015).
48. Y. Wang, C. Ying, W. Zhou, L. de Vreede, Z. Liu, and J. Tian: Fabrication of multiple nanopores in a SiNx membrane via controlled breakdown. *Sci. Rep.* **8**, (2018). Article no. 1234.
49. A.T. Kuan, B. Lu, P. Xie, T. Szalay, and J.A. Golovchenko: Electrical pulse fabrication of graphene nanopores in electrolyte solution. *Appl. Phys. Lett.* **106**, 203109 (2015).
50. P. Puczkarski, J.L. Swett, and J.A. Mol: Graphene nanoelectrodes for biomolecular sensing. *J. Mater. Res.* **32**, 3002–3010 (2017).
51. J. Lagerqvist, M. Zwolak, and M. Di Ventra: Fast DNA sequencing via transverse electronic transport. *Nano Lett.* **6**, 779–782 (2006).
52. J. Prasongkit, A. Grigoriev, B. Pathak, R. Ahuja, and R.H. Scheicher: Transverse conductance of DNA nucleotides in a graphene nanogap from first principles. *Nano Lett.* **11**, 1941–1945 (2011).
53. J. Prasongkit, A. Grigoriev, B. Pathak, R. Ahuja, and R.H. Scheicher: Theoretical study of electronic transport through DNA nucleotides in a double-functionalized graphene nanogap. *J. Phys. Chem. C* **117**, 15421–15428 (2013).
54. Y. He, R.H. Scheicher, A. Grigoriev, R. Ahuja, S. Long, Z. Huo, and M. Liu: Enhanced DNA sequencing performance through edge-hydrogenation of graphene electrodes. *Adv. Funct. Mater.* **21**, 2674–2679 (2011).
55. R.G. Amorim, A.R. Rocha, and R.H. Scheicher: Boosting DNA recognition sensitivity of graphene nanogaps through nitrogen edge functionalization. *J. Phys. Chem. C* **120**, 19384–19388 (2016).
56. T. Ohshiro and Y. Umezawa: Complementary base-pair-facilitated electron tunneling for electrically pinpointing complementary nucleobases. *Proc. Natl. Acad. Sci. USA* **103**, 10–14 (2005).
57. S. Chang, J. He, A. Kibel, M. Lee, O. Sankey, P. Zhang, and S. Lindsay: Tunnelling readout of hydrogen-bonding-based recognition. *Nat. Nanotechnol.* **4**, 297–301 (2009).
58. H. Tanaka and T. Kawai: Partial sequencing of a single DNA molecule with a scanning tunnelling microscope. *Nat. Nanotechnol.* **4**, 518–522 (2009).
59. S. Chang, S. Huang, J. He, F. Liang, P. Zhang, S. Li, X. Chen, O. Sankey, and S. Lindsay: Electronic signatures of all four DNA nucleosides in a tunneling gap. *Nano Lett.* **10**, 1070–1075 (2010).
60. M. Di Ventra and M. Taniguchi: Decoding DNA, RNA and peptides with quantum tunnelling. *Nat. Nanotechnol.* **11**, 117–126 (2016).
61. M. Tsutsui, M. Taniguchi, and T. Kawai: Transverse field effects on DNA-sized particle dynamics. *Nano Lett.* **9**, 1659–1662 (2009).
62. M. Tsutsui, M. Taniguchi, K. Yokota, and T. Kawai: Identifying single nucleotides by tunnelling current. *Nat. Nanotechnol.* **5**, 286–290 (2010).
63. M. Tsutsui, S. Rahong, Y. Iizumi, T. Okazaki, M. Taniguchi, and T. Kawai: Single-molecule sensing electrode embedded in-plane nanopore. *Sci. Rep.* **1**, (2011). Article no. 46.
64. M. Tsutsui, K. Matsubara, T. Ohshiro, M. Furuhashi, M. Taniguchi, and T. Kawai: Electrical detection of single methylcytosines in a DNA oligomer. *J. Am. Chem. Soc.* **133**, 9124–9128 (2011).
65. P. Pang, B.A. Ashcroft, W. Song, P. Zhang, S. Biswas, Q. Qing, J. Yang, R.J. Nemanich, J. Bai, J.T. Smith, K. Reuter, V.S.K. Balagurusamy, Y. Astier, G. Stolovitzky, and S. Lindsay: Fixed-gap tunnel junction for reading DNA nucleotides. *ACS Nano* **8**, 11994–12003 (2014).
66. F. Prins, A. Barreiro, J.W. Ruitenberg, J.S. Seldenthuis, N. Aliaga-Alcalde, L.M.K. Vandersypen, and H.S.J. van der Zant: Room-temperature gating of molecular junctions using few-layer graphene nanogap electrodes. *Nano Lett.* **11**, 4607–4611 (2011).
67. S. Caneva, P. Gehring, V.M. Garca-Surez, A. Garca-Fuente, D. Stefani, I.J. Olavarria-Contreras, J. Ferrer, C. Dekker, and H.S.J. van der Zant: Mechanically controlled quantum interference in graphene break junctions. arXiv:1803.05642 March 2018.
68. H.M. Wang, Z. Zheng, Y.Y. Wang, J.J. Qiu, Z.B. Guo, Z.X. Shen, and T. Yu: Fabrication of graphene nanogap with crystallographically matching edges and its electron emission properties. *Appl. Phys. Lett.* **96**, 023106 (2010).
69. A. Bellunato, S.D. Vrbica, C. Sabater, E.W. de Vos, R. Fermin, K.N. Kanneworff, F. Galli, J.M. van Ruitenbeek, and G.F. Schneider: Dynamic tunneling junctions at the atomic intersection of two twisted graphene edges. *Nano Lett.* **18**, 2505–2510 (2018).
70. C.S. Lau, J.A. Mol, J.H. Warner, and G.A.D. Briggs: Nanoscale control of graphene electrodes. *Phys. Chem. Chem. Phys.* **16**, 20398–20401 (2014).
71. M. El Abbassi, L. Pósa, P. Makk, C. Nef, K. Thodkar, A. Halbritter, and M. Calame: From electroburning to sublimation: substrate and environmental effects in the electrical breakdown process of monolayer graphene. *Nanoscale* **9**, 17312–17317 (2017).

https://doi.org/10.1557/mrc.2018.187 Published online by Cambridge University Press

72. H.N. Patel, I. Carroll, R. Lopez, S. Sankararaman, C. Etienne, S. R. Kodigala, M.R. Paul, and H.W.C. Postma: DNA-graphene interactions during translocation through nanogaps. *Plos One* **12**, e0171505 (2017).
73. J. Cai, P. Ruffieux, R. Jaafar, M. Bieri, T. Braun, S. Blankenburg, M. Muoth, A.P. Seitsonen, M. Saleh, X. Feng, K. Mllen, and R. Fasel: Atomically precise bottom-up fabrication of graphene nanoribbons. *Nature* **466**, 470–473 (2010).
74. Q. Xu, M.-Y. Wu, G.F. Schneider, L. Houben, S.K. Malladi, C. Dekker, E. Yucelen, R.E. Dunin-Borkowski, and H.W. Zandbergen: Controllable atomic scale patterning of freestanding monolayer graphene at elevated temperature. *ACS Nano* **7**, 1566–1572 (2013).
75. C.E. Arcadia, C.C. Reyes, and J.K. Rosenstein: In situ nanopore fabrication and single-molecule sensing with microscale liquid contacts. *ACS Nano* **11**, 4907–4915 (2017).
76. Y. Cho, S.K. Min, W.Y. Kim, and K.S. Kim: The origin of dips for the graphene-based DNA sequencing device. *Phys. Chem. Chem. Phys.* **13**, 14293 (2011).
77. J. Bai, X. Duan, and Y. Huang: Rational fabrication of graphene nanoribbons using a nanowire etch mask. *Nano Lett.* **9**, 2083–2087 (2009).
78. L. Jiao, L. Zhang, X. Wang, G. Diankov, and H. Dai: Narrow graphene nanoribbons from carbon nanotubes. *Nature* **458**, 877–880 (2009).
79. M.Y. Han, B. zyilmaz, Y. Zhang, and P. Kim: Energy band-gap engineering of graphene nanoribbons. *Phys. Rev. Lett.* **98**, 206805 (2007).
80. X. Wang and H. Dai: Etching and narrowing of graphene from the edges. *Nat. Chem.* **2**, 661–665 (2010).
81. M.D. Fischbein and M. Drndić: Electron beam nanosculpting of suspended graphene sheets. *Appl. Phys. Lett.* **93**, 113107 (2008).
82. D.C. Bell, M.C. Lemme, L.A. Stern, J.R. Williams, and C.M. Marcus: Precision cutting and patterning of graphene with helium ions. *Nanotechnology* **20**, 455301 (2009).
83. D. Xia, J. Yan, and S. Hou: Fabrication of nanofluidic biochips with nanochannels for applications in DNA analysis. *Small* **8**, 2787–2801 (2012).
84. C. Duan, W. Wang, and Q. Xie: Review article: Fabrication of nanofluidic devices. *Biomicrofluidics* **7**, 026501 (2013).
85. A. Hibara, T. Saito, H.-B. Kim, M. Tokeshi, T. Ooi, M. Nakao, and T. Kitamori: Nanochannels on a fused-silica microchip and liquid properties investigation by time-resolved fluorescence measurements. *Anal. Chem.* **74**, 6170–6176 (2002).
86. S.L. Levy, J.T. Mannion, J. Cheng, C.H. Reccius, and H.G. Craighead: Entropic unfolding of DNA molecules in nanofluidic channels. *Nano Lett.* **8**, 3839–3844 (2008).
87. R. Riehn, R.H. Austin, and J.C. Sturm: A nanofluidic railroad switch for DNA. *Nano Lett.* **6**, 1973–1976 (2006).
88. D. Xia, Z. Ku, S.C. Lee, and S.R.J. Brueck: Nanostructures and functional materials fabricated by interferometric lithography. *Adv. Mater.* **23**, 147–179 (2010).
89. L.J. Guo, X. Cheng, and C.-F. Chou: Fabrication of size-controllable nanofluidic channels by nanoimprinting and its application for DNA stretching. *Nano Lett.* **4**, 69–73 (2004).
90. X. Liang, K.J. Morton, R.H. Austin, and S.Y. Chou: Single sub-20 nm wide, centimeter-long nanofluidic channel fabricated by novel nanoimprint mold fabrication and direct imprinting. *Nano Lett.* **7**, 3774–3780 (2007).
91. X. Liang and S.Y. Chou: Nanogap detector inside nanofluidic channel for fast real-time label-free DNA analysis. *Nano Lett.* **8**, 1472–1476 (2008).
92. T. Maleki, S. Mohammadi, and B. Ziaie: A nanofluidic channel with embedded transverse nanoelectrodes. *Nanotechnology* **20**, 105302 (2009).
93. G.F. Schneider, V.E. Calado, H. Zandbergen, L.M.K. Vandersypen, and C. Dekker: Wedging transfer of nanostructures. *Nano Lett.* **10**, 1912–1916 (2010).
94. Y. Wang, Y. Zheng, X. Xu, E. Dubuisson, Q. Bao, J. Lu, and K.P. Loh: Electrochemical delamination of CVD-grown graphene film: toward the recyclable use of copper catalyst. *ACS Nano* **5**, 9927–9933 (2011).
95. J. Moser, A. Barreiro, and A. Bachtold: Current-induced cleaning of graphene. *Appl. Phys. Lett.* **91**, 163513 (2007).
96. M. Ishigami, J.H. Chen, W.G. Cullen, M.S. Fuhrer, and E.D. Williams: Atomic structure of graphene on SiO_2. *Nano Lett.* **7**, 1643–1648 (2007).
97. W. Fu, L. Feng, G. Panaitov, D. Kireev, D. Mayer, A. Offenhusser, and H.-J. Krause: Biosensing near the neutrality point of graphene. *Sci. Adv.* **3**, e1701247 (2017).
98. L. Sun, Y.A. Diaz-Fernandex, T.A. Gschneidtner, F. Westerlund, S. Lara-Avila, and K. Moth-Pouslen: Single molecule electronics: from chemical design to functional devices. *Chem. Soc. Rev.* **43**, 7378–7411.

https://doi.org/10.1557/mrc.2018.187 Published online by Cambridge University Press