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INVITED ARTICLE

Graphene nanoelectrodes for biomolecular sensing

Pawel Puczkarski,[b] Jacob L. Swett,[b] and Jan A. Mol[a]
Department of Materials, University of Oxford, Oxford OX1 3PH, U.K.

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Nanoscale biosensor technology has attracted considerable attention with its promise of revolutionizing techniques ranging from biological interfaces to rapid pathogen detection to enabling DNA data storage. Many approaches, such as nanopore sequencing, have been explored and are already achieving tremendous success; however, new sensing modalities and architectures are emerging that are envisioned for the next generation of ever more capable biosensors. These novel devices, combined with advances in machine learning, are moving concepts from simulation to experimentation and demonstration. In recent years, rapid advances have been made and many architectures have been put forward for novel approaches to biomolecular sensing using nanoelectronics, including the advent of tunnel junctions as a sensing platform. With high accuracy, sensitivity, and affordability, these sensors are predicted to drive a shift to personalized medicine and rapid diagnostics in real-time anywhere in the world. Here we give an overview of tunneling sequencing and its application in biomolecular sensing and provide a perspective on the use of scalable tunneling sequencing methods utilizing graphene as the active component.

I. TUNNELING-BASED SEQUENCING

The first successful demonstration of translocation and sensing of DNA and RNA strands through an alpha-hemolysin pore was reported by Kasianowicz et al. just over two decades ago in 1996.[1] Since then, many advances have been made toward reading the sequence of bases that encode the genetic instructions for life. These approaches, collectively termed nanopore sequencing, began first with biological nanopores followed by synthetically made solid-state nanopores. Now a mature technology, biological nanopore sequencing is capable of sequencing long unlabeled DNA strands with high accuracy and with miniaturized devices that offer both portability and affordable sequencing.[2] However, limitations resulting from the finite dimensions of both biological and solid-state nanopores remain.[3] Even nanopores in atomically thin membranes formed from 2D materials, such as graphene and MoS_2, suffer from these limitations as the effective channel length is increased by stray electric fields.[4,5] Sensitivity of the nanopore is also decreased by the formation of an electrical double layer and noise caused by the mechanical vibrations of the membrane.[6,7]

To continue to advance the accuracy, miniaturization, and affordability of these devices a paradigm shift is envisioned from the ionic current blockade to quantum electron tunneling-based sequencing (see Fig. 1).[8] In this configuration, analytes must pass between two electrodes placed just nanometers apart, known as a tunnel junction, allowing electrons to tunnel from one electrode to the other via the analyte molecule through a potential barrier that particles cannot surmount classically. Tunneling sequencing relies on probing the differences in the electronic structure of the bases and other small biomolecules as they move sequentially through the nanogap between the nanoelectrodes.[8]

Tunneling sequencing of DNA was first investigated using electron tunneling in a scanning tunneling microscope (STM),[9,10] where the analyte was firmly adsorbed onto a Cu(111) surface without any possibility of controlled translocation under the STM tip.[9] Realization of DNA imaging demanded the development of an aggregation-preventing approach for the deposition of DNA molecules on the metallic surface, and imaging was achieved via cryogenic STM measurements of double-stranded DNA. This pioneering study revealed the existence of a transport gap surrounded by peaks that result from electrons tunneling in a double-barrier system—one tunnel barrier between the STM tip and the DNA molecule and a second barrier between the molecule and the substrate.[11]

The discovery of a distinct electronic fingerprint of one of the DNA bases (guanine) allowed for its identification in a single-stranded DNA (ssDNA) and the partial sequencing of DNA with a known nucleotide order by STM.[12] Guanine could be distinguished from the other three bases in a topographic measurement at a fixed bias voltage, i.e., without the need for full tunneling spectroscopy, enabling fast sequencing. With additional development, full sequencing of short DNA strands has now also been realized.[13]

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[a]Address all correspondence to this author.
e-mail: jan.mol@materials.ox.ac.uk
[b]These authors contributed equally to this work.
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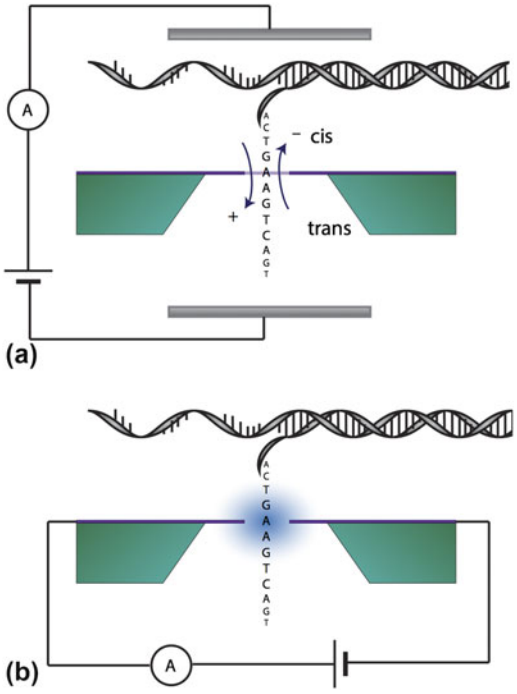


FIG. 1. Schematic illustrating the difference between (a) traditional solid-state nanopores sequencing by trans-membrane conductance of an ionic solution and (b) tunneling sequencing where the bases are read via transverse conductance (in-plane current).

An important conceptual advancement in DNA sequencing was the proposed use of nanopores integrated with tunnel junctions—first described by Zwolak and Di Ventra.[14] Tunnel junctions with sufficiently small probing areas should be able to analyze electronic fingerprints of single nucleotides in a DNA strand independent of the influence of the nearest neighboring nucleotides. If nanoscale fabrication challenges can be overcome, a robust integrated nanopore-tunnel junction device would combine advantages of nanopores and tunneling sequencing into a single device. Namely, control over the motion of DNA strands via the applied trans-membrane bias voltage from nanopore sequencing and high accuracy based on the tunneling current variations from the different electronic structure of bases.[15]

With this vision in mind, tunnel junctions have been fabricated via a variety of approaches. Tunnel junctions aligned with nanopores were prepared on insulating membranes by means of transmission electron beam ablation lithography[16] and high-resolution focused ion beam (FIB) milling.[17] In an alternative approach, electrodes embedded in long quasi-one-dimensional nanochannels were used to detect individual DNA strands.[18] Mechanically controllable break junctions have also been used to distinguish between solutions of single nucleotides.[19] Solutions containing thymine (T), cytosine (C), and guanine (G) give rise to different conductance values corresponding to the HOMO–LUMO gaps of the monophosphates when measured using tunneling sequencing.[19] Analyses of nucleotide monophosphates and short DNA strands have also been performed using electromigrated tunneling electrodes embedded in nanopores, showing a bimodal current peak distribution for strands consisting of two different bases (guanine and thymine).[20]

A study comparing the ionic and tunneling current recorded simultaneously in a gold-tunnel junction that was co-aligned with a FIB milled nanopore showed that two different classes of events can be detected[21]: (i) a short signal (with a mean dwell time of 0.3 ms) corresponding to fast DNA translocation only detected in the ionic current; and (ii) a long signal (4.7 ms) due to a decreased DNA translocation rate and an improved signal-to-noise ratio (SNR) due to nonspecific bonding to the tunnel junction. Mean dwell times recorded for the second class of events by both methods were in good agreement, indicating a common origin of signals detected by tunneling and ionic current, thus demonstrating the efficacy of the approach.[21] To achieve an acceptable SNR, it is often necessary to only allow a small portion of the tunnel junction to be exposed to maximize the signal from the analyte of interest. In liquid environments electronic noise in tunnel junctions can be a serious limitation; however, it can be reduced by coating the devices with a few nanometers of a passivation layer such as SiO_2[22,23] or Al_2O_3.[24]

Alternate experimental configurations of tunnel junctions have been realized in parallel to the development of metal tunnel junctions. These include carbon nanowire tunnel junctions aligned with nanopores and patterned via electron beam induced deposition from organic precursors.[25,26] This approach, which allows for the fabrication of electrodes aligned with nanopores, is perceived as a straightforward alternative capable of being adapted to multiple geometries. Recently, new approaches are being conceived which build on these foundations. One such approach which utilizes graphene as the ultimately thin version of carbon nanowire has already shown early promises in a variety of biosensing applications.

II. GRAPHENE BIOSENSORS

Due to graphene's remarkable properties, such as atomic thinness, high conductivity, and chemical stability[27] in both air and liquid, it has attracted considerable attention for applications in biosensing.[28–30] The ability to synthesize graphene via scalable methods[31,32] and pattern it with atomic precision has further motivated many researchers to incorporate it as the active component in biomolecular sensors. These sensing approaches span from graphene field-effect transistors[33,34] to trans-electrode membranes[29] to tunnel junctions,[35] all of which exploit unique properties of the

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material. Among these, configurations with nanogaps and nanoconstrictions offer the impressive capability to probe analytes electronically at the atomic scale with single-molecule sensitivity and high-time resolution.

Many techniques have been developed for fabricating nanoscale features in graphene. These can generally be classified into the paradigmatic categories of top-down and bottom-up fabrication methods, with much of the focus by the research community on the former. Bottom-up approaches include graphene nanoribbons,[36] DNA templated synthesis,[37] and block co-polymer lithography.[38] Within the bottom-up methods, graphene nanoribbons with the capability to specify edge shape, dimensions,[39] functionalization,[40] and doping[41] have shown the most promise, particularly when used in conjunction with graphene nanogaps. Top-down approaches include electron beam lithography (EBL) patterning,[42] FIB milling,[43] transmission electron microscopy sculpting,[44] scanning probe methods,[45] and feedback-controlled electroburning.[46]

Of the top-down fabrication approaches, EBL and feedback-controlled electroburning have been developed the most for graphene tunnel junctions. In particular Cao et al. have demonstrated the successful fabrication of arrays of tunnel junctions in single-layer graphene using EBL followed by oxygen plasma etching.[42] Briefly, a dashed line of 5 nm width was patterned in Poly(methyl methacrylate) (PMMA) using ultrahigh-resolution EBL, which was then developed and patterned via reactive ion etching to transfer the pattern to the graphene resulting in lines of nanoelectrodes ranging in width from 1 to 10 nm. These electrodes were also functionalized by carboxylic acid end groups and terpyridyl ligands to facilitate specific binding interactions. EBL which has been demonstrated for nanoscale patterning of graphene for other nanoscale quantum applications[47] offers a possible scalable solution for the fabrication of nanogaps; however, consistency and yield will need to improve for the research to continue to progress.

Feedback-controlled electroburning[46] is a technique, which as the name suggests, allows for real-time *in situ* monitoring of the graphene sculpting on the nanoscale (see Fig. 2). The ingenuity of the feedback mechanism is that the process can be tailored in response to variations in the graphene before the tunnel junction is produced and can respond accordingly as the process rapidly evolves. This provides a mechanism to controllably and consistently produce a high yield of either constrictions or nanogaps with the desired nanoscale dimensions.[47,48] Many groups have demonstrated variations on the technique[48–50] showing its efficacy on both single-layer graphene and few-layer graphene,[46] at room temperatures, in ambient conditions, and in vacuum,[49] with applications from single-molecule devices[50] to single-electron transistors.[51] Briefly, to sculpt graphene on the nanoscale via feedback-controlled electroburning, micron-scale ribbons of graphene are formed and

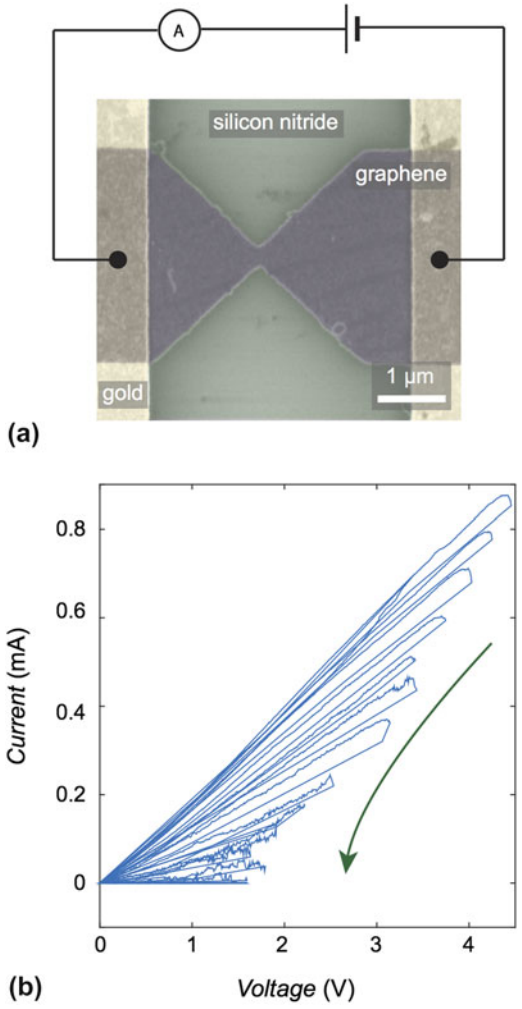


FIG. 2. (a) False-color scanning electron micrograph of a graphene constriction on a silicon-nitride substrate. The graphene is contacted via gold leads. (b) Typical current–voltage traces recorded during feedback-controlled electroburning of a graphene nanogap.

notched [see Fig. 2(a)], typically via a lithography process followed by oxygen etching. This notched portion forms a constriction which when a bias voltage is applied between the two ends of the ribbon joule heating will occur at the narrowest point. When sufficiently high current densities are achieved, joule heating can cause graphene at the edges of the constriction to react with oxygen at high temperatures. This causes a gap to open controllably at the edges of the constriction which in turn modifies the conductance across the ribbon. This change in conductance is sensed via the feedback mechanism and the current is abruptly reduced. The bias voltage is then ramped again and the process proceeds until the desired endpoint is achieved. Fabricating features in this manner avoids a catastrophic rupture of the constriction, allowing for a nanometer-scale gap or constriction to be formed, as desired.

Initially, graphene was explored as a solid-state analogue to tremendously successful biological nanopores

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for DNA sequencing[29] where trans-membrane conductance of ionic current through a nanometer-scale aperture was monitored while the DNA translocated electrophoretically through the pore. This showed feasibility, however, encountered difficulties, such as strong graphene DNA interactions[52] and challenges in reproducibly creating pores.[53] Recently, several new schemes utilizing graphene nanogaps and nanoconstrictions and transverse (in-plane) measurements have been explored (see Fig. 3). These include monitoring the in-plane current through a constriction with a localized nanopore either within[54,55] or near the pore,[56] bilayer graphene constrictions,[57–59] detecting electronic changes from localized strain due to a DNA translocating through a nanopore in a constriction,[59] changes in constriction current due to physisorption of analytes on the graphene surface[60] or from a channel below,[61] and modulations in tunneling current through a graphene nanogap.[62] The breadth of configurations demonstrate the versatility of nanoscale features in graphene exploiting quantum phenomena. In many of these device architectures, the graphene electrodes are placed between the two chambers and the DNA is typically translocated through the nanopore via an applied bias across the device. Confinement of the translocating DNA relative to the electrodes ensures that each base is read sequentially. However, the confinement can be achieved through other means, such as channels.[61]

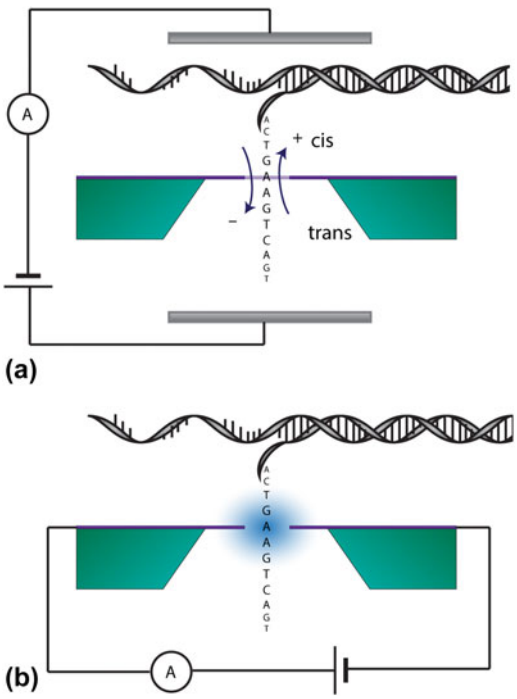


FIG. 3. Schematic demonstrating the organization of types of nanopore sequencing starting from the delineation between biological and solid-state, trans-membrane and transverse conductance (see Fig. 2) and finally different geometries for transverse conductance-based sequencing.

Of these approaches graphene tunnel junctions offer the most viable routes to an experimental demonstration. The approach consists of fabricating graphene nanogaps co-aligned with a pore within the substrate material, via a method such as feedback-controlled electroburning. The graphene electrodes, which form a tunnel junction, would then be passivated entirely except for in the vicinity of the nanogap where the DNA is electrophoretically driven through. This is similar, in principle, to how single molecules are probed currently[46,63,64] but done sequentially with the addition of an aperture for translocation.

The predicted advantages of transverse conductance sequencing methods using graphene parallel those of biological nanopores include long read lengths, real-time data, label-free direct molecular analysis, the capability to detect methylation and other epigenetic markers,[65] portability, and scalability via parallelization.[66] Due to their solid-state nature and unique-material properties, graphene nanoelectrodes also offer further hypothesized benefits, including stability and durability,[67] tunable gap width,[48] atomic thinness,[68] the ability to tune sensitivity via functionalization,[69,70] being planar and flexible,[71] and the promise of leveraging semiconductor manufacturing techniques for further miniaturization and low-cost production of biosensors at wafer scale.

III. FUNCTIONALIZATION

Tuning the width of graphene nanogaps provides a straightforward way to discriminate between species of interest due to differences in size; however, given the range of biomolecules of interest with similar sizes, more refinement is desired. One such method of tailoring the graphene is through functionalization, which ranges from noncovalent[72] to covalent bonding.[73] Covalent bonding has several configurations from common graphene edge terminations to the possibility of specifically designed functionalization such as tether molecules[74] or nucleobases[70] currently used in other systems.

Functionalization of the nanogaps and nanoconstrictions could help to facilitate bonding of various degrees between analytes of interest while also stabilizing edges and promoting preferred orientations of molecules for detection. Noncovalent bonding typically takes the form of π–π interactions, van der Waals forces, and hydrogen bonding. These most commonly occur when the analyte of interest bonds to the graphene such as in physisorption-based detection methods[60] or when the fluid containing the analyte interacts with the graphene. Common covalent functionalization includes hydroxylation and hydrogenation,[75] which are suspected to occur in many formation methods of graphene nanogaps and nanoconstrictions, to silicon termination.[76] Recently, there has been a growing interest in the specific functionalization of the edges of tunnel junctions and graphene

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nanogaps to permit tailoring of tunnel junctions for molecular recognition.

Molecular recognition using functionalized electrodes[77] relies on the formation of strong bonds between reader molecules (sometimes also called adapter molecules or tethering molecules depending on molecule type and configuration) and the electrodes (e.g., by thiol chemistry for gold or palladium electrodes[78]) and weak noncovalent interactions between the reader molecules and the analyte.[79] The formation of a bond between the reader and analyte molecules gives rise to a current peak with a time duration and amplitude that are characteristic of that specific interaction.[79] This approach improves DNA reads by decreasing the contact resistance of the junction and by limiting the number of possible molecular orientations of the DNA strand in the gap.[80] Slowing down DNA translocation also enhances the SNR by allowing for a longer signal averaging interval.[81] This can be achieved by functionalization of the tunnel junction with a reader molecule that weakly binds to the DNA, acting as a 'molecular velcro'.[82,83]

Molecules that specifically bind to a given DNA base can also increase the accuracy of the DNA sequencing. This can be attained through the use of van der Waals interaction (π–π stacking) with the nucleotides.[84] The tunneling current through these interactions depends on the strength of the bonding with the bases, allowing for differentiation between molecules in the gap with either two or three hydrogen bonds.[85] The differences in the strength of the bonding can also modulate the dwell time of a base in a junction facilitating detection. This approach subtly differs from solely detecting the change in tunneling current amplitude and can provide additional signals for differentiating the bases.[83,85]

IV. GENOMICS, PROTEOMICS, AND GLYCOMICS

To date, much of the focus for tunnel junctions has been on DNA sequencing applications[86]; however, methylation of DNA, which contributes to differential gene expression, can also be probed.[87] Methylated DNA gives rise to a higher tunneling current than its nonmethylated form in a MCBJ due to a better energetic alignment with the electrodes Fermi level.[88] The potential for tunnel junctions can also be extended beyond DNA entirely.

In addition to genomics, the fields of glycomics (the detection and analysis of carbohydrates) and proteomics (the detection and analysis of proteins) have prospects for tunnel junctions.[5] The detection of analytes at the single-molecule level is particularly important in the case of proteins, which—contrary to DNA molecules—cannot be amplified by a polymerase chain reaction.[5] Moreover, proteins and peptides often do not have any net charge, or the charge they do posses is not evenly distributed over the structure, hindering the possibility of dragging them electrophoretically through tunnel junctions. These issues may be overcome by the functionalization of neutral peptides with short and charged DNA oligomers by click chemistry.[89] Negatively charged oligomers can act as a molecular thread and exert a dragging force on the protein forcing it to translocate through the tunnel junction. The biggest challenge in protein sequencing, however, is in the expected range of variability in the sequencing signal. Whereas ssDNA is a linear chain constructed of only four different bases, proteins are composed of twenty fundamental building-blocks, termed amino acids. To identify proteins it is therefore necessary to develop a method capable of discriminating between these amino acids.

Recently, 8 out of 20 amino acids were detected in a gold MCBJ through which short peptides could be partially sequenced.[90] A higher selectivity, resulting in the identification of 18 out of 20 amino acids was achieved through functionalization with molecules that form weak, noncovalent bonds to the amino acids.[91] This also enabled the differentiation between various forms of the amino acids, including methylated and nonmethylated forms, L- and D-enantiomers, and isobaric amino acids.[91]

Carbohydrates are characterized by a complex secondary structure that cannot be resolved with simple mass spectrometry and therefore require bulky NMR techniques. While recognition tunneling using functionalized metal tunnel junctions is viable method to identify nanomolar concentrations of some saccharides,[74] the identification of the large variety of different carbohydrates is still being developed as it will demand the collection of an entire library of fingerprints for the variety of building blocks found in carbohydrates. Nevertheless, recognition tunneling may provide additional information about the structure of linear polysaccharides on the single-molecule level that cannot be obtained using other techniques.[74]

V. MACHINE LEARNING

Signals collected in experiments with tunnel junctions are typically analyzed by the characteristic current spikes produced with a particular focus on amplitude and duration. However, the relatively large distribution of values of these parameters makes the analysis cumbersome. Specifically, the overlap between peaks and the convolution of many signals recorded for different DNA bases and biomolecules can hinder their analysis and lower the identification accuracy. One possible solution to this issue is the application of machine learning methods for the analysis of the wide variety of features in the signals from tunnel junctions.[92] In particular, Support Vector Machines (SVMs)[81] have attracted a growing interest recently. It has been demonstrated that for single nucleotide solutions, the simultaneous analysis

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of basic parameters, such as duration and amplitude of individual peaks and groups of peaks, with additional analysis of less intuitive parameters obtained by Fourier and wavelet analysis of these signals, can increase the accuracy of identification.[93] These parameters were further analyzed with an SVM to identify the subset of parameters providing the highest accuracy of assignment. The outcome of the SVM analysis is a set of properties that can be used to assign a characteristic signal to the nucleotide with the highest accuracy.[78] It has also been demonstrated that the clustering of data from a SVM can lead to the increased accuracy of analysis, in particular, by taking into account the parameters describing contextual information (groups of peaks) and not only the single-peak properties.[93]

A similar SVM approach has also been used for the analysis of tunneling experiments using mixtures of different amino acids, increasing the accuracy of amino acid assignment.[91] SVMs trained on amino acids did not recognize them as constituents in peptide sequences, showing that there is a difference between a signal generated by free amino acids and the same amino acids in the short peptide chain. Nevertheless, applying the SVM to short peptides in a separate experiment showed it to be highly accurate even though the peptides were differing by a single amino acid.[93] Machine learning was also used to analyze data from scanning tunneling spectroscopy performed on mononucleotide ssDNA in an STM configuration.[94] Parameters used for this analysis contained HOMO and LUMO values and band gap energy, along with transition voltage, tunneling barrier, and electron effective mass, all at positive and negative biases. These parameters were analyzed with a modified naive-Bayes classifier leading to an increase in base calling accuracy compared to an analysis performed taking into account only HOMO and LUMO values.[94]

VI. OUTLOOK

With the further introduction of machine learning techniques to both the fabrication of graphene biosensors and the interpretation of their signals, progress can be expected to continue at a rapid pace. Tunnel junction devices, due to their high sensitivity and ability to be tailored toward many biological analytes of interest can be expected to have an important role along with a host of additional sensing modalities in biosensors of the future. This is likely to include being utilized in implantable devices, which will be enabled by robust solid-state devices. These sensors will also allow for real-time diagnostics at concentration levels that currently are not possible and drive a shift toward personalized medicine. This capability would allow for the detection of indicators of deteriorating health earlier on, enabling a shift toward proactive healthcare that is tailored to the patient. Along the way toward this vision, the devices would, if successful, offer an alternative approach to achieving the $100 genome[95] and usher in an era of ubiquitous DNA sequencing.[96]

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REFERENCES

1. J.J. Kasianowicz, E. Brandin, D. Branton, and D.W. Deamer: Characterization of individual polynucleotide molecules using a membrane channel. *Proc. Natl. Acad. Sci. U. S. A.* **93**(24), 13770 (1996).
2. J. Clarke, H-C. Wu, L. Jayasinghe, A. Patel, S. Reid, and H. Bayley: Continuous base identification for single-molecule nanopore DNA sequencing. *Nat. Nanotechnol.* **4**(4), 265 (2009).
3. 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**(10), 1146 (2008).
4. S. Garaj, S. Liu, J.A. Golovchenko, and D. Branton: Molecule-hugging graphene nanopores. *Proc. Natl. Acad. Sci. U. S. A.* **110**(30), 12192 (2013).
5. S. Lindsay: The promises and challenges of solid-state sequencing. *Nat. Nanotechnol.* **11**(2), 109 (2016).
6. H. Arjmandi-Tash, L.A. Belyaeva, and F. Schneider: Single molecule detection with graphene and other two-dimensional materials: Nanopores and beyond. *Chem. Soc. Rev.* **45**, 476 (2016).
7. J. Li, Y. Zhang, J. Yang, K. Bi, Z. Ni, D. Li, and Y. Chen: Molecular dynamics study of DNA translocation through graphene nanopores. *Phys. Rev. E* **87**(6), 62707 (2013).
8. M. Di Ventra and M. Taniguchi: Decoding DNA, RNA and peptides with quantum tunnelling. *Nat. Nanotechnol.* **11**(2), 117 (2016).
9. H. Tanaka: Scanning tunneling microscopy imaging and manipulation of DNA oligomer adsorbed on Cu(111) surfaces by a pulse injection method. *J. Vac. Sci. Technol., B: Microelectron. Nanometer Struct.–Process., Meas., Phenom.* **15**(3), 602 (1997).
10. H. Tanaka, C. Hamai, T. Kanno, and T. Kawai: High-resolution scanning tunneling microscopy imaging of DNA molecules on Cu(111) surfaces. *Surf. Sci.* **432**(3), 611 (1999).
11. E. Shapir, H. Cohen, A. Calzolari, C. Cavazzoni, D.A. Ryndyk, G. Cuniberti, A. Kotlyar, R. Di Felice, and D. Porath: Electronic structure of single DNA molecules resolved by transverse scanning tunnelling spectroscopy. *Nat. Mater.* **7**(1), 68 (2008).
12. H. Tanaka and T. Kawai: Partial sequencing of a single DNA molecule with a scanning tunnelling microscope. *Nat. Nanotechnol.* **4**(8), 518 (2009).
13. S. Huang, J. He, S. Chang, P. Zhang, F. Liang, S. Li, M. Tuchband, A. Fuhrmann, R. Ros, and S. Lindsay: Identifying single bases in a DNA oligomer with electron tunnelling. *Nat. Nanotechnol.* **5**(12), 868 (2010).
14. M. Zwolak and M. Di Ventra: Electronic signature of DNA nucleotides via transverse transport. *Nano Lett.* **5**(3), 421 (2005).

https://doi.org/10.1557/jmr.2017.256 Published online by Cambridge University Press

15. S. Agah, M. Zheng, M. Pasquali, and A.B. Kolomeisky: DNA sequencing by nanopores: Advances and challenges. *J. Phys. D: Appl. Phys.* **49** (2016).
16. M.D. Fischbein and M. Drndic: Sub-10 nm device fabrication in a transmission electron microscope. *Nano Lett.* **7**(5), 1329 (2007).
17. T. Maleki, S. Mohammadi, and B. Ziaie: A nanofluidic channel with embedded transverse nanoelectrodes. *Nanotechnology* **20**, 105302 (2009).
18. X. Liang and S.Y. Chou: Nanogap detector inside nanofluidic channel for fast real-time label-free DNA analysis. *Nano Lett.* **8**(5), 1472 (2008).
19. M. Tsutsui, M. Taniguchi, K. Yokota, and T. Kawai: Identifying single nucleotides by tunnelling current. *Nat. Nanotechnol.* **5**(4), 286 (2010).
20. M. Tsutsui, S. Rahong, Y. Iizumi, T. Okazaki, M. Taniguchi, and T. Kawai: Single-molecule sensing electrode embedded in-plane nanopore. *Sci. Rep.* **1**, 46 (2011). doi: 10.1038/srep00046
21. 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**(1), 279 (2011).
22. A. Arima, M. Tsutsui, T. Morikawa, K. Yokota, and M. Taniguchi: Fabrications of insulator-protected nanometer-sized electrode gaps. *J. Appl. Phys.* **115**(11), 114310 (2014).
23. T. Morikawa, K. Yokota, M. Tsutsui, and M. Taniguchi: Fast and low-noise tunnelling current measurements for single-molecule detection in electrolyte solution using insulator-protected nanoelectrodes. *Nanoscale* **9**, 4076 (2017).
24. N. Muthusubramanian, E. Galan, C. Maity, R. Eelkema, F.C. Grozema, and H.S.J. Van Der Zant: Insulator-protected mechanically controlled break junctions for measuring single-molecule conductance in aqueous environments. *Appl. Phys. Lett.* **109**(1), 13102 (2016).
25. P.S. Spinney, S.D. Collins, D.G. Howitt, and R.L. Smith: Fabrication and characterization of a solid-state nanopore with self-aligned carbon nanoelectrodes for molecular detection. *Nanotechnology* **23**(13), 135501 (2012).
26. A.P. Ivanov, K.J. Freedman, M.J. Kim, T. Albrecht, and J.B. Edel: High precision fabrication and positioning of nanoelectrodes in a nanopore. *ACS Nano* **8**(2), 1940 (2014).
27. A.K. Geim: Graphene: Status and prospects. *Science* **324**, 1530 (2009).
28. N. Mohanty and V. Berry: Graphene-based single-bacterium resolution biodevice and DNA transistor: Interfacing graphene derivatives with nanoscale and microscale biocomponents. *Nano Lett.* **8**(12), 4469 (2008).
29. S. Garaj, W. Hubbard, A. Reina, J. Kong, D. Branton, and J.A. Golovchenko: Graphene as a subnanometre trans-electrode membrane. *Nature* **467**(7312), 190 (2010).
30. Y. Yang, X. Yang, X. Zou, S. Wu, D. Wan, A. Cao, L. Liao, Q. Yuan, and X. Duan: Ultrafine graphene nanomesh with large on/off ratio for high-performance flexible biosensors. *Adv. Funct. Mater.* **27**, 1604096 (2016).
31. X. Li, C.W. Magnuson, A. Venugopal, J. An, J.W. Suk, B. Han, M. Borysiak, W. Cai, A. Velamakanni, Y. Zhu, L. Fu, E.M. Vogel, E. Voelkl, L. Colombo, and R.S. Ruoff: Graphene films with large domain size by a two-step chemical vapor deposition process. *Nano Lett.* **10**(11), 4328 (2010).
32. S. Bae, H. Kim, Y. Lee, X. Xu, J-S. Park, Y. Zheng, J. Balakrishnan, T. Lei, H.R. Kim, Y.I. Song, Y-J. Kim, K.S. Kim, B. Özyilmaz, J-H. Ahn, B.H. Hong, and S. Iijima: Roll-to-roll production of 30-inch graphene films for transparent electrodes. *Nat. Nanotechnol.* **5**(8), 574 (2010).
33. X. Dong, Y. Shi, W. Huang, P. Chen, and L.J. Li: Electrical detection of DNA hybridization with single-base specificity using transistors based on CVD-grown graphene sheets. *Adv. Mater.* **22**(14), 1649 (2010).
34. Y. Ohno, K. Maehashi, and K. Matsumoto: Chemical and biological sensing applications based on graphene field-effect transistors. *Biosens. Bioelectron.* **26**(4), 1727 (2010).
35. C.E. Malec and D. Davidović: Transport in graphene tunnel junctions. *J. Appl. Phys.* **109**(6), 064507 (2011).
36. J. Cai, P. Ruffieux, R. Jaafar, M. Bieri, T. Braun, S. Blankenburg, M. Muoth, A.P. Seitsonen, M. Saleh, X. Feng, K. Mullen, and R. Fasel: Atomically precise bottom-up fabrication of graphene nanoribbons. *Nature* **466**(7305), 470 (2010).
37. A.N. Sokolov, F.L. Yap, N. Liu, K. Kim, L. Ci, O.B. Johnson, H. Wang, M. Vosgueritchian, A.L. Koh, J. Chen, J. Park, and Z. Bao: Direct growth of aligned graphitic nanoribbons from a DNA template by chemical vapour deposition. *Nat. Commun.* **4**, 1 (2013).
38. D. Choi, C. Kuru, C. Choi, K. Noh, S-K. Hong, S. Das, W. Choi, and S. Jin: Nanopatterned graphene field effect transistor fabricated using block Co-polymer lithography. *Mater. Res. Lett.* **2**(3), 131–139 (2014).
39. X. Li, X. Wang, L. Zhang, S. Lee, and H. Dai: Chemically derived ultrasmooth graphene nanoribbon semiconductors. *Science* **319**(5867), 1229 (2008).
40. D.W. Boukhvalov and M.I. Katsnelson: Chemical functionalization of graphene. *Molecules* **21**, 344205 (2009).
41. B. Huang, Q. Yan, G. Zhou, J. Wu, B.L. Gu, W. Duan, and F. Liu: Making a field effect transistor on a single graphene nanoribbon by selective doping. *Appl. Phys. Lett.* **91**(25), 1 (2007).
42. Y. Cao, S. Dong, S. Liu, L. He, L. Gan, X. Yu, M.L. Steigerwald, X. Wu, Z. Liu, and X. Guo: Building high-throughput molecular junctions using indented graphene point contacts. *Angew. Chem., Int. Ed.* **51**(49), 12228 (2012).
43. Y. Zhou, P. Maguire, J. Jadwiszczak, M. Muruganathan, H. Mizuta, and H. Zhang: Precise milling of nano-gap chains in graphene with a focused helium ion beam. *Nanotechnology* **27**(32), 325302 (2016).
44. B. Song, G.F. Schneider, Q. Xu, G. Pandraud, C. Dekker, and H. Zandbergen: Atomic-scale electron-beam sculpting of near-defect-free graphene nanostructures. *Nano Lett.* **11**(6), 2247 (2011).
45. K. Zhang, Q. Fu, N. Pan, X. Yu, J. Liu, Y. Luo, X. Wang, J. Yang, and J. Hou: Direct writing of electronic devices on graphene oxide by catalytic scanning probe lithography. *Nat. Commun.* **3**, 1194 (2012).
46. 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**(11), 4607 (2011).
47. J. Moser and A. Bachtold: Fabrication of large addition energy quantum dots in graphene. *Appl. Phys. Lett.* **95**(17), 58 (2009).
48. C.S. Lau, J.A. Mol, J.H. Warner, and G.A.D. Briggs: Nanoscale control of graphene electrodes. *Phys. Chem. Chem. Phys.* **16**(38), 20398 (2014).
49. C. Nef, L. Posa, P. Makk, W. Fu, A. Halbritter, C. Schonenberger, and M. Calame: High-yield fabrication of nm-size gaps in monolayer CVD graphene. *Nanoscale* **6**(6), 7249 (2014).
50. S. Lumetti, A. Candini, C. Godfrin, F. Balestro, W. Wernsdorfer, S. Klyatskaya, M. Ruben, and M. Affronte: Single-molecule devices with graphene electrodes. *Dalton Trans.* **45**(42), 16570 (2016).
51. P. Puczkarski, P. Gehring, C.S. Lau, J. Liu, A. Ardavan, J.H. Warner, G.A.D. Briggs, and J.A. Mol: Three-terminal graphene single-electron transistor fabricated using feedback-controlled electroburning. *Appl. Phys. Lett.* **107**(13), 133105 (2015).

https://doi.org/10.1557/jmr.2017.256 Published online by Cambridge University Press

52. B.S. Husale, S. Sahoo, A. Radenovic, F. Traversi, P. Annibale, and A. Kis: SsDNA binding reveals the atomic structure of graphene. *Langmuir* **26**(23), 18078 (2010).
53. G. Goyal, Y.B. Lee, A. Darvish, C.W. Ahn, and M.J. Kim: Hydrophilic and size-controlled graphene nanopores for protein detection. *Nanotechnology* **27**, 1 (2016).
54. 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**(12), 939 (2013).
55. A. Girdhar, C. Sathe, K. Schulten, and J-P. Leburton: Graphene quantum point contact transistor for DNA sensing. *Proc. Natl. Acad. Sci. U. S. A.* **110**(42), 16748 (2013).
56. A. Balan, M. Drndi, M. Puster, and J.A. Rodrı: Toward sensitive graphene by preventing electron beam-induced nanoribbon-nanopore damage. *ACS Nano* **7**(12), 11283 (2013).
57. H. Sadeghi, L. Algaragholy, T. Pope, S. Bailey, D. Visontai, D. Manrique, J. Ferrer, V. Garcia-Suarez, S. Sangtarash, and C.J. Lambert: Graphene sculpturene nanopores for DNA nucleobase sensing. *J. Phys. Chem. B* **118**(24), 6908 (2014).
58. J. Prasongkit, G.T. Feliciano, A.R. Rocha, Y. He, T. Osotchan, R. Ahuja, and R.H. Scheicher: Theoretical assessment of feasibility to sequence DNA through interlayer electronic tunneling transport at aligned nanopores in bilayer graphene. *Sci. Rep.* **5**, 17560 (2015).
59. E. Paulechka, T.A. Wassenaar, K. Kroenlein, A. Kazakov, and A. Smolyanitsky: Nucleobase-functionalized graphene nanoribbons for accurate high-speed DNA sequencing. *Nanoscale* **8**(4), 1861 (2016).
60. D. Le, A. Kara, E. Schröder, P. Hyldgaard, and T.S. Rahman: Physisorption of nucleobases on graphene: A comparative van der Waals study. *J. Phys.: Condens. Matter* **24**, 424210 (2012).
61. S.K. Min, W.Y. Kim, Y. Cho, and K.S. Kim: Fast DNA sequencing with a graphene-based nanochannel device. *Nat. Nanotechnol.* **6**(3), 162 (2011).
62. H.W.C. Postma: Rapid sequencing of individual DNA molecules in graphene nanogaps. *Nano Lett.* **10**(2), 420 (2010).
63. J.O. Island, A. Holovchenko, M. Koole, P.F.A. Alkemade, M. Menelaou, N. Aliaga-Alcalde, E. Burzurí, and H.S.J. van der Zant: Fabrication of hybrid molecular devices using multi-layer graphene break junctions. *J. Phys.: Condens. Matter* **26**(47), 474205 (2014).
64. A. Barreiro, H.S.J. Van Der Zant, L.M.K. Vandersypen, and H.S.J. van der Zant: Quantum dots at room temperature carved out from few-layer graphene. *Nano Lett.* **12**(12), 6096 (2012).
65. J. Shendure and H. Ji: Next-generation DNA sequencing. *Nat. Biotechnol.* **26**(10), 1135 (2008).
66. Editorial: Building a better nanopore. *Nat. Nanotechnol.* **11**(2), 105 (2016).
67. 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 free-standing monolayer graphene at elevated temperature. *ACS Nano* **7**(2), 1566 (2013).
68. K.S. Novoselov, A.K. Geim, S.V. Morozov, D. Jiang, Y. Zhang, S.V. Dubonos, I.V. Grigorieva, and A.A. Firsov: Electric field effect in atomically thin carbon films. *Science* **306**, 666 (2004).
69. K. Sint, B.Y. Wang, and P. Kral: Selective ion passage through functionalized graphene nanopores. *J. Am. Chem. Soc.* **130**(49), 16448 (2008).
70. 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**(29), 15421 (2013).
71. A. Reina, X. Jia, J. Ho, D. Nezich, H. Son, V. Bulovic, M.S. Dresselhaus, J. Kong, K.S.K.S. Kim, Y. Zhao, H. Jang, S.Y. Lee, J.M. Kim, J-H. Ahn, P. Kim, J-Y. Choi, and B.H. Hong: Large-scale pattern growth of graphene films for stretchable transparent electrodes. *Nano Lett.* **9**(1), 30 (2009).
72. G.F. Schneider, Q. Xu, S. Hage, S. Luik, J.N.H. Spoor, S. Malladi, H. Zandbergen, and C. Dekker: Tailoring the hydrophobicity of graphene for its use as nanopores for DNA translocation. *Nat. Commun.* **4**, 2619 (2013).
73. V. Georgakilas, M. Otyepka, A.B. Bourlinos, V. Chandra, N. Kim, K.C. Kemp, P. Hobza, R. Zboril, and K.S. Kim: Functionalization of graphene: Covalent and non-covalent approaches, derivatives and applications. *Chem. Rev.* **112**(11), 6156 (2012).
74. J. Im, S. Biswas, H. Liu, Y. Zhao, S. Sen, S. Biswas, B. Ashcroft, C. Borges, X. Wang, S. Lindsay, and P. Zhang: Electronic single-molecule identification of carbohydrate isomers by recognition tunnelling. *Nat. Commun.* **7**, 13868 (2016).
75. D. Cohen-Tanugi and J.C. Grossman: Water desalination across nanoporous graphene. *Nano Lett.* **12**(7), 3602 (2012).
76. J. Lee, Z. Yang, W. Zhou, S.J. Pennycook, S.T. Pantelides, and M.F. Chisholm: Stabilization of graphene nanopore. *Proc. Natl. Acad. Sci. U. S. A.* **111**(21), 7522 (2014).
77. J. He, L. Lin, P. Zhang, and S. Lindsay: Identification of DNA basepairing via tunnel-current decay. *Nano Lett.* **7**(12), 3854 (2007).
78. S. Chang, S. Sen, P. Zhang, B. Gyarfas, B. Ashcroft, S. Lefkowitz, H. Peng, and S. Lindsay: Palladium electrodes for molecular tunnel junctions. *Nanotechnology* **23**(42), 425202 (2012).
79. S. Lindsay: Biochemistry and semiconductor electronics—The next big hit for silicon? *J. Phys.: Condens. Matter* **24**(16), 164201 (2012).
80. 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**(3), 1070 (2010).
81. J.N. Pedersen, P. Boynton, M. Di Ventra, A-P. Jauho, and H. Flyvbjerg: Classification of DNA nucleotides with transverse tunneling currents. *Nanotechnology* **28**, 015502 (2016).
82. P. Krishnakumar, B. Gyarfas, W. Song, S. Sen, P. Zhang, P. Krstić, and S. Lindsay: Slowing DNA translocation through a nanopore using a functionalized electrode. *ACS Nano* **7**(11), 10319 (2013).
83. S. Lindsay, J. He, O. Sankey, P. Hapala, P. Jelinek, P. Zhang, S. Chang, and S. Huang: Recognition tunneling. *Nanotechnology* **21**, 262001 (2010).
84. S. Biswas, S. Sen, J. Im, S. Biswas, P. Krstic, B. Ashcroft, C. Borges, Y. Zhao, S. Lindsay, and P. Zhang: Universal readers based on hydrogen bonding or π–π stacking for identification of DNA nucleotides in electron tunnel junctions. *ACS Nano* **10**(12), 11304 (2016).
85. 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**(5), 297 (2009).
86. Y. Wang, Q. Yang, and Z. Wang: The evolution of nanopore sequencing. *Front. Genet.* **5**, 1 (2015).
87. R. Cortini, M. Barbi, B.R. Caré, and C. Lavelle: The physics of epigenetics. **88**, 025002 (2016).
88. 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 (2011).
89. S. Biswas, W. Song, C. Borges, S. Lindsay, and P. Zhang: Click addition of a DNA thread to the N-termini of peptides for their translocation through solid-state nanopores. *ACS Nano* **9**(10), 9652 (2015).
90. T. Ohshiro, M. Tsutsui, K. Yokota, M. Furuhashi, M. Taniguchi, and T. Kawai: Detection of post-translational modifications in single peptides using electron tunnelling currents. *Nat. Nanotechnol.* **9**(10), 835 (2014).

https://doi.org/10.1557/jmr.2017.256 Published online by Cambridge University Press

91. Y. Zhao, B. Ashcroft, P. Zhang, H. Liu, S. Sen, W. Song, J. Im, B. Gyarfas, S. Manna, S. Biswas, C. Borges, and S. Lindsay: Single-molecule spectroscopy of amino acids and peptides by recognition tunnelling. *Nat. Nanotechnol.* **9**(6), 466 (2014).
92. P. Krstic, B. Ashcroft, and S. Lindsay: Physical model for recognition tunneling. *Nanotechnology* **26**(8), 84001 (2015).
93. S. Chang, S. Huang, H. Liu, P. Zhang, F. Liang, R. Akahori, S. Li, B. Gyarfas, J. Shumway, B. Ashcroft, J. He, and S. Lindsay: Chemical recognition and binding kinetics in a functionalized tunnel junction. *Nanotechnology* **23**, 235101 (2012).
94. L.E. Korshoj, S. Afsari, S. Khan, A. Chatterjee, and P. Nagpal: Single nucleobase identification using biophysical signatures from nanoelectronic quantum tunneling. *Small* **13**, 1603033 (2017).
95. E. Singer: The $100 genome. *MIT Technology Review* (2008). Available at: https://www.technologyreview.com/s/409919/the-100-genome/ (accessed April 15, 2017).
96. Y. Erlich: A vision for ubiquitous sequencing. *Genome Res.* **25**(10), 1411 (2015).

https://doi.org/10.1557/jmr.2017.256 Published online by Cambridge University Press