

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/263944946>

Effects of Graphene Nanopore Geometry on DNA Sequencing

ARTICLE *in* JOURNAL OF PHYSICAL CHEMISTRY LETTERS · APRIL 2014

Impact Factor: 7.46 · DOI: 10.1021/jz500498c

CITATIONS

8

READS

30

8 AUTHORS, INCLUDING:



Zhisen Zhang

Xiamen University

13 PUBLICATIONS 98 CITATIONS

[SEE PROFILE](#)



Jia-Wei Shen

Hangzhou Normal University

23 PUBLICATIONS 555 CITATIONS

[SEE PROFILE](#)



Hans Agren

KTH Royal Institute of Technology

867 PUBLICATIONS 18,754 CITATIONS

[SEE PROFILE](#)



Yaoquan Tu

KTH Royal Institute of Technology

70 PUBLICATIONS 633 CITATIONS

[SEE PROFILE](#)

Effects of Graphene Nanopore Geometry on DNA Sequencing

Zhisen Zhang,^{†,⊥} Jiawei Shen,^{‡,⊥} Hongbo Wang,[§] Qi Wang,^{*,†} Junqiao Zhang,[†] Lijun Liang,^{*,†,||}
Hans Ågren,[¶] and Yaoquan Tu^{||}

[†]Department of Chemistry and Soft Matter Research Center, Zhejiang University, Hangzhou 310027, People's Republic of China

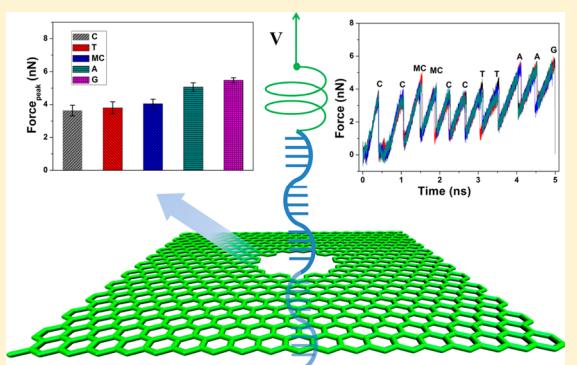
[‡]School of Medicine, Hangzhou Normal University, Hangzhou 310016, People's Republic of China

[§]College of Automation, Hangzhou Dianzi University, Hangzhou 310018, People's Republic of China

[¶]Division of Theoretical Chemistry and Biology, School of Biotechnology, KTH Royal Institute of Technology, SE-10691 Stockholm, Sweden

Supporting Information

ABSTRACT: In this Letter we assess the effect of graphene nanopore geometries on DNA sequencing by considering DNA fragments including A, T, C, G, and 5-methylcytosine (MC) pulled out of graphene nanopores of different geometries with diameters down to ~ 1 nm. Using steered molecular dynamics simulations it is demonstrated that the bases (A, T, C, G, and MC) can be identified at single-base resolution through the characteristic peaks on the force profile in a circular graphene nanopore but not in nanopores with other asymmetric geometries. Our study suggests that the graphene nanopore surface should be modified as symmetrically as possible in order to sequence DNA by atomic force microscopy or optical tweezers.



SECTION: Physical Processes in Nanomaterials and Nanostructures

INTRODUCTION

DNA sequencing with nanopores has attracted much attention ever since DNA translocation through the biological nanopore α -hemolysin was first demonstrated.¹ In nanopore DNA sequencing, a negatively charged DNA molecule is electrophoretically driven through the nanopore and its sequence is read off through measuring the reduction of the ion current during the DNA translocation through the pore. Such DNA sequencing with nanopores provides a promising technology for cheap and fast DNA sequencing free of enzyme-dependent amplification and fluorescent labeling steps.^{2,3}

While both biological and solid-state nanopores can be used for DNA sequencing, the latter technique⁴ offers a number of advantages, such as superior mechanical properties,^{5,6} multiplex detection,⁷ and high stability to complex environments.⁸ Significant progress has been made in DNA sequencing with solid-state nanopores.^{9–11} However, conventional nanopores are of several nanometers in thick and, as a result, they are occupied by many DNA bases during the sequencing, making it difficult to detect a single-stranded DNA (ssDNA) molecule at single-base resolution.

It has been demonstrated that nanopores fabricated from graphene sheets can be made extremely thin, even one-atom-thick,¹² and structurally robust,¹³ something that has opened a new chapter in DNA sequencing with nanopores with a resolution that allow identification of single nucleotides. DNA translocation through a nanopore can be recognized by

measuring the blocked current,^{14–19} which has increasingly been used in DNA detection in recent years.^{20–22} However, compared with traditional thick solid-state nanopores, graphene nanopores with diameter down to ~ 5 nm in experiments (or ~ 2 nm in simulations) have not achieved current signals with appropriate resolution for nucleotide identification.^{18,21} Up to now, the effect of pore geometries on DNA translocation through graphene nanopores remains unclear.

In this Letter, we report studies on the effect of graphene nanopore geometries on DNA sequencing, using all-atom steered molecular dynamics (SMD) simulations and thereby extending earlier studies of DNA detection with nanopores using this technique.^{23,24} As shown in Figure 1A, an ssDNA molecule translocates through a graphene nanopore in a ratchet-like way when it is pulled through the pore. By characterizing the force profile acting on the ssDNA, we are able to distinguish the nucleotides and 5-methylcytosine (MC, a methylated form of cytosine that may be involved in the regulation of gene transcription) passing through a circular nanopore with the diameter of ~ 1 nm.

System Setup. A graphene sheet was placed in the $x-y$ plane with the center of mass in the Cartesian coordinate origin (0, 0, 0). A circular nanopore was constructed by

Received: March 11, 2014

Accepted: April 18, 2014

Published: April 18, 2014



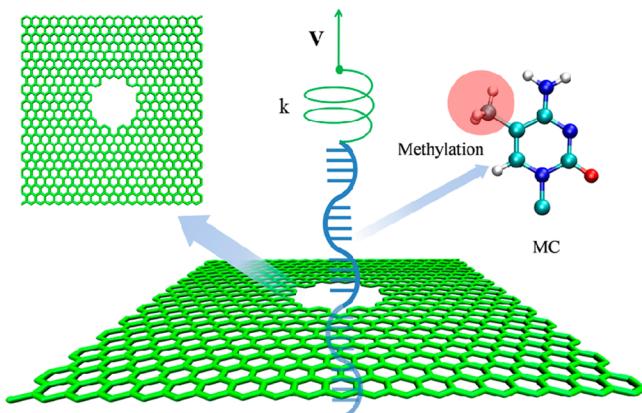


Figure 1. Scheme of the ssDNA sequencing with a graphene nanopore in SMD simulations. A harmonic string with force constant k is attached to the ssDNA and is pulled at a constant velocity V . The red area shows the difference between 5-methylcytosine (MC) and cytosine (C).

deleting the atoms with their coordinates satisfying $x^2 + y^2 < R^2$, where R is the radius of the graphene nanopore. Nanopores of different geometries but with the same area as the circular nanopore, including rhombic, square, triangle pores (see Figure 2 and Figure S1) were constructed. A model ssDNA molecule with sequence CCMC MCTT AAGG was constructed by using the Hyperchem software (Version 7.0, Hypercube, Inc.). Each system consists of an ssDNA molecule and a graphene nanopore of specific geometry with water molecules. All simulations were performed using the GROMACS 4.5.2 package. The DNA was modeled by the CHARMM27 force field. All the carbon atoms in the graphene sheet were set to be neutral with the Lennard-Jones parameters $\sigma_{CC} = 3.85 \text{ \AA}$ and $\epsilon_{CC} = -0.439 \text{ kcal/mol}$.^{25,26} All atoms including hydrogen atoms were represented explicitly. The cutoff for the nonbonded van der Waals interaction was set by a switching function starting at 10.0 \AA and reaching zero at 12.0 \AA . The time step of 2.0 fs is used in all the simulations with the bonds involving H atoms kept fixed. The Langevin method was employed to maintain the temperature at 298.0 K and the pressure at 101.3 kPa .

For each system, the model DNA was initially placed close to a nanopore of particular geometry and was solvated in a box of $50 \times 47 \times 180 \text{ \AA}^3$ with TIP3P water molecules. The system then underwent a 5000-step energy minimization before the

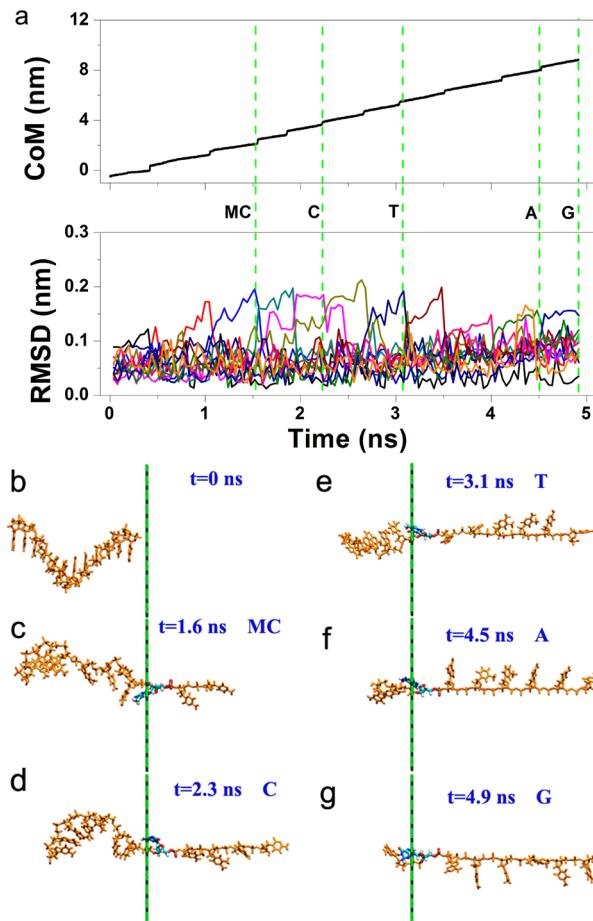


Figure 3. Conformations of the ssDNA with different nucleotides passing through the circular graphene nanopore in the SMD simulation (b, c, d, e, f, g) and the evolution of the corresponding RMSD value for each nucleotide and the Center-of-Mass (CoM) of the ssDNA (a). The green dashed line is the time at which a nucleotide starts to pass through the nanopore.

SMD simulation. In the SMD simulation, a harmonic spring with elastic coefficient of $6000 \text{ kJ/(mol nm}^2\text{)}$ was attached to the center of mass (CoM) of the first nucleotide of the ssDNA molecule and was pulled through the graphene nanopore at a velocity of 2 nm/ns .²³ In order to increase the accuracy, the SMD simulation was repeated four times for each system.

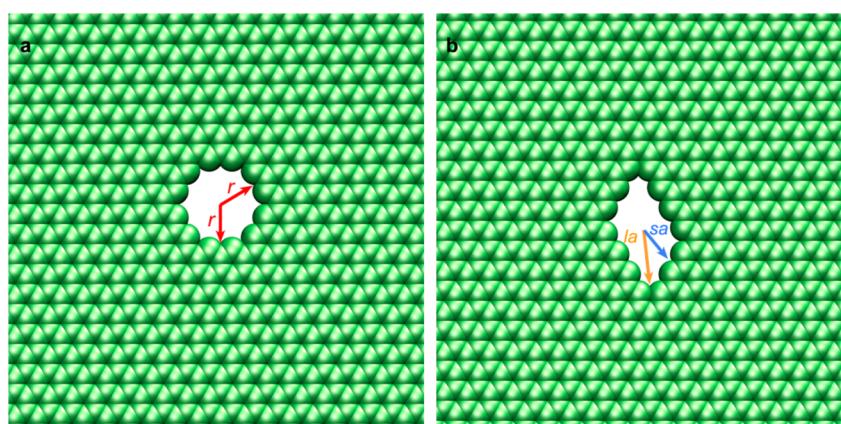


Figure 2. Graphene nanopores of different geometries. Shown in the figures are a circular nanopore (a) and a rhombic nanopore (b). r is the radius of the circular nanopore. sa is the short axis and la is the long axis of the rhombic nanopore.

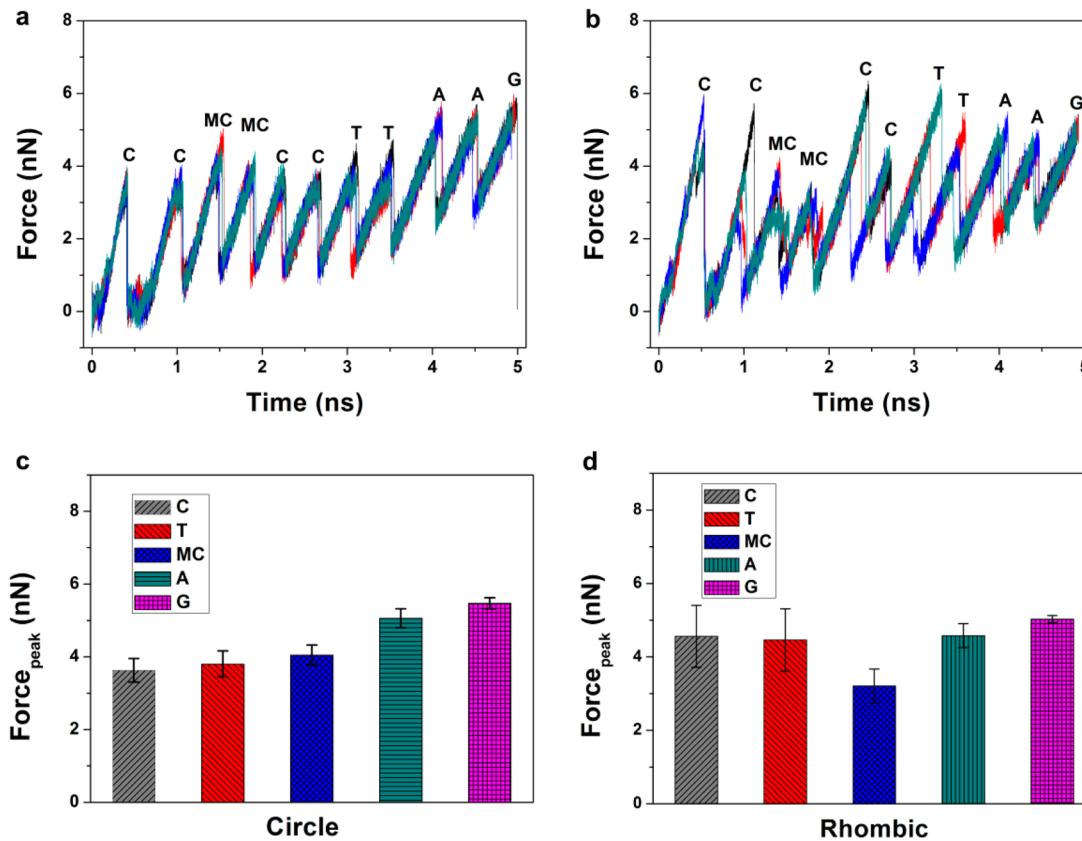


Figure 4. Force profiles when the ssDNA is pulled through the circular graphene nanopore (a) and the rhombic graphene nanopore (b). The force peak values for different nucleotides are extracted from the force profiles and are shown for the circular nanopore (c) and the rhombic nanopore (d).

RESULTS AND DISCUSSION

The process of the ssDNA (CCMC MCCC TTAAG) translocation through the circular graphene nanopore is shown in Figure 3. Four basic nucleotides (A, T, C, G) and 5-methylcytosine (MC) were found to translocate through the nanopore in a similar way, with the translocation process exhibiting a base-by-base ratcheting fashion. This phenomenon has been observed and studied by many groups.^{27,28} Luan et al. pointed out that the ratcheting motion could potentially enhance the signal-to-noise ratio for DNA sensing. In this “ratcheting” motion, the ssDNA translocation could be significantly slowed down, and the DNA detection time could be extended accordingly.

We note that MC passes through the nanopore in the same way as the other basic nucleotides, which means that MC can be identified with the ~1 nm graphene nanopore. The force peak value can be read base-by-base on the force curve as a function of pulling time. The changes of the root-mean-square deviation (RMSD) value of each nucleotide and the center of mass (CoM) of the ssDNA in the pulling process are shown in Figure 3a. Due to the ratcheting motion, the CoM demonstrates a stepwise change in the pulling process. As can be seen from the change of the RMSD values, each nucleotide threading the nanopore undergoes a significant conformational change. The angle between the base plane and that of the graphene nanopore changes significantly in order to facilitate the base to pass through the nanopore since the diameter of the nanopore was only ~1 nm.

We used SMD simulations to study how the model DNA with the sequence CCMC MCCCCT TAAG was read off by

graphene nanopores of different geometries. As shown in Figure 4 and Figure S2 (Supporting Information), there are 11 force peaks in the DNA translocation through a graphene nanopore of a particular geometry. This implies that each nucleotide through a graphene nanopore can be read off from the force profile directly. With the circular graphene nanopore, the characteristic peaks for the two pyrimidine bases (C and T) are lower than those of the purine bases (A and G). We attribute this difference in the characteristic peaks to the nucleotide geometries - the pyrimidine bases have six-membered rings composed of four carbon atoms and two nitrogen atoms, whereas the purine bases are five- and six-membered heterocyclic compounds. This makes the volume of the purine bases much larger than that of the pyrimidine bases and leads to the observed difference in the characteristic peaks when the ssDNA threads the nanopore. In the same way, the volume of MC is larger than C since the hydrogen atom in C is mutated into the methyl group. Thus, the peak value of MC is larger than C. The force peak values averaged over the same nucleotides from the four simulations were calculated and are shown in Figure 4 and Figure S3 as well. The force peaks of C, T, A, G are 3.63 ± 0.32 nN, 4.05 ± 0.32 nN, 5.06 ± 0.26 nN, and 5.47 ± 0.15 nN, respectively. We can see that the peak forces can be distinguished from one another for A, G, C, T and MC with the circular graphene nanopore. To better identify each nucleotide, the pulling process was simulated more times. As seen in Figure S5, the force peaks of C, T, A, G are 3.53 ± 0.23 nN, 3.85 ± 0.26 nN, 4.96 ± 0.31 nN, and 5.57 ± 0.18 nN, respectively, when they were averaged over 10 trajectories. This indicates that the error bars of the characteristic force peaks can

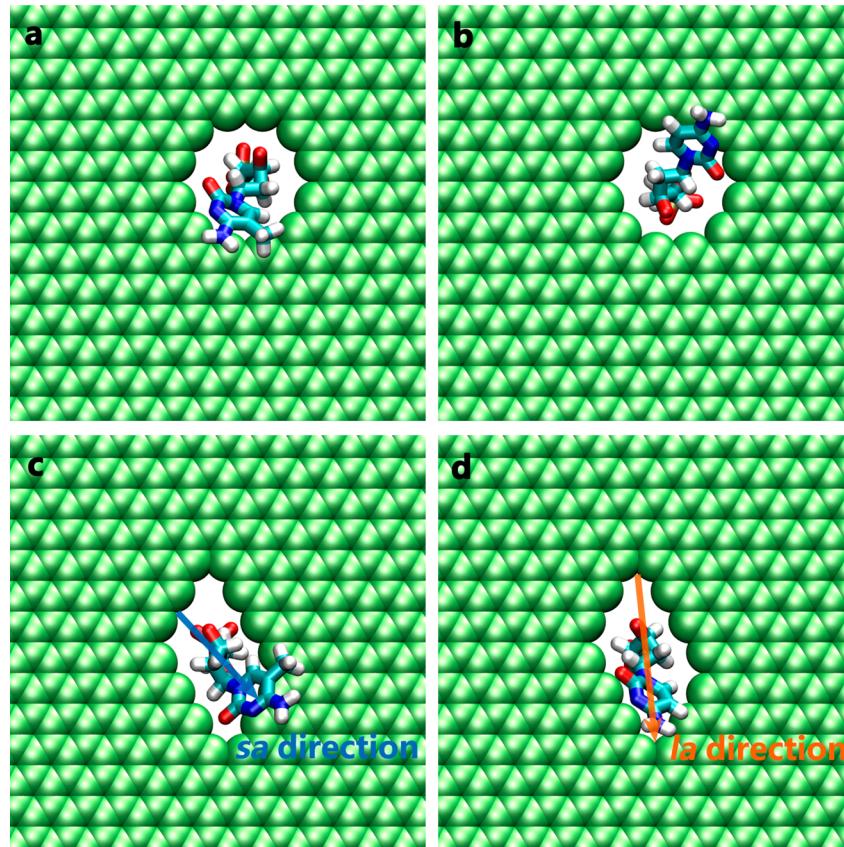


Figure 5. Illustration of nucleotides passing through the circular graphene nanopore (a–b) and the rhombic graphene nanopore (c–d). In the circular nanopore, there is only a small difference in the force curves for the same nucleotides to be pulled through the in different directions (a and b) since the pore is axisymmetric. In contrast, a significant difference in the force curves can occur for the same nucleotides being pulled through the rhombic nanopore in different directions; (c) from the *sa* direction of the rhombic nanopore; (d) from the *la* direction of the rhombic nanopore.

be decreased by simulating the pulling process more times, and that the bases can be identified better accordingly.

As we can see from Figure 4b and Figure S2, the peak forces are not the same even for the same nucleotides in the graphene nanopores other than the circular one. Taking the rhomboid as an example, the force peak value is ca. 6.0 nN for the first C but is ca. 4.1 nN for the second. As a result, the error bar for the averaged peak forces as indicated in Figure 4d is very large (ca. 0.9 nN). As seen in Figure S2a, the peak force values for different nucleotides are almost the same in the triangular nanopore. Compared with the circular nanopore, the effective area of the triangular nanopore for the ssDNA to pass through is much smaller, leading to that it is difficult for all the bases to pass through the nanopore. Thus, neither the rhombic nor the triangular graphene nanopore is able to discriminate the nucleotides. The ratcheting-fashion could increase the characteristic peaks, and thus improve the signal-to-noise ratio. However, it could not guarantee the nucleotides to be identified clearly.

As illustrated in Figure 5 and Figure S4, the direction of the phosphodiester bond to a single nucleotide can be considered as the translocation direction of the nucleotide. The fact that the conformation of the nucleotide with phosphodiester bond is long and narrow makes it is much easier for the nucleotide to pass through the nanopore along the *la* direction than along the *sa* direction. Accordingly, the force value is much smaller when the single nucleotide is pulled through the nanopore along the *la* direction. Due to the thermal fluctuation and conformational

change, the ssDNA can thread the nanopore in different orientations.¹⁴ Thus, as shown in Figure 4d, the error bar of the force peak value in the rhombic nanopore is very large, which makes it impossible to read the DNA sequence accurately in that case. On the other hand, the circular nanopore is axisymmetric, and thus the force peak of a base through it is almost orientation independent, leading to that the error bar is relatively small. As some studies have pointed out, DNA sequencing is very sensitive to the orientation of a nucleotide in a pore with certain geometry.^{14,15,29} Our study demonstrates that the orientation of a nucleotide is much more sensitive to an asymmetric nanopore than to an axisymmetric nanopore.

In summary, we have studied DNA translocation dynamics in graphene nanopores of different geometries by steered molecular dynamics simulations. We found that the ssDNA passes through the nanopores with an area of $\sim 0.785 \text{ nm}^2$ in a ratchet-like, base-by-base fashion, which is sequence specific. While bases bound together with phosphoester can always thread a graphene nanopore of specific geometry we find in our simulations that the four basic nucleotides (A, T, C, G) and 5-methylcytosine can be identified by the characteristic peaks on the force profile only with circular graphene nanopores within a certain range of diameters. The force peak follows $G > A > MC > T > C$ and is dependent on the geometry of the base, which means that bases of different geometries could be identified with this method. In addition, the identification of bases can be improved by repeating the SMD more times. To thread small nanopores such as those studied in this work, the

conformation of the base needs to change substantially. The effective area of a nanopore for the ssDNA to pass through is also an important factor. Our simulations also reveal that the force peak could change with the orientation of a nucleotide passing through the nanopore with a sensitivity that is much larger for an asymmetric nanopore than for an axisymmetric nanopore. Our simulations thus suggest that an axisymmetric nanopore is better suited to DNA sequencing via force profiles than an asymmetric nanopore and that the graphene nanopore surface should be modified to become as axisymmetric as possible in order to utilize atomic force microscopy or optical tweezing for DNA sequencing.

■ ASSOCIATED CONTENT

§ Supporting Information

Graphene nanopores of different geometries; force curve as a function of the simulation time when the ssDNA was pulled through graphene nanopores of different geometries; peak forces of different nucleotides extracted from the force curves for all the systems; Snapshots of nucleotides passing through the graphene nanopores with different shapes; force peak values for different nucleotides (circle nanopore) extracted from 10 trajectories. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Authors

*Fax: +86-571-87951895. E-mail address: qiwang@zju.edu.cn (Q.W.).

*E-mail address: lijunl.michael@gmail.com (L.L.).

Author Contributions

[†]Z.Z. and J.S. contributed equally to this manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 21273200 and 21074115), MOE (J20091551), Zhejiang Provincial Natural Science Foundation of China (Nos. Y14B030033, LQ12F05001, and LY13F04006), and Zhejiang University (2011XZZX002, 2011QNA3014). The computations were performed on resources provided by the Swedish National Infrastructure for Computing (SNIC) at the parallel computer centre (PDC), through the project “Multiphysics Modeling of Molecular Materials”, SNIC 020/11-23.

■ REFERENCES

- (1) Kasianowicz, J. J.; Brandin, E.; Branton, D.; Deamer, D. W. Characterization of Individual Polynucleotide Molecules Using a Membrane Channel. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 13770–13773.
- (2) Branton, D.; Deamer, D. W.; Marziali, A.; Bayley, H.; Benner, S. A.; Butler, T.; Di Ventra, M.; Garaj, S.; Hibbs, A.; Huang, X.; et al. The Potential and Challenges of Nanopore Sequencing. *Nat. Biotechnol.* **2008**, *26*, 1146–1153.
- (3) Venkatesan, B. M.; Bashir, R. Nanopore Sensors for Nucleic Acid Analysis. *Nat. Nanotechnol.* **2011**, *6*, 615–624.
- (4) Dekker, C. Solid-State Nanopores. *Nat. Nanotechnol.* **2007**, *2*, 209–215.
- (5) Hozumi, A.; Cheng, D. F.; Yagihashi, M. Lamination of Alumina Membranes to Polymer Surfaces: Thick, Hard, Transparent, Crack-Free Alumina Films on Polymers with Excellent Adhesion. *ACS Appl. Mater. Interfaces* **2011**, *3*, 2224–2227.
- (6) Striemer, C. C.; Gaborski, T. R.; McGrath, J. L.; Fauchet, P. M. Charge- and Size-Based Separation of Macromolecules Using Ultrathin Silicon Membranes. *Nature* **2007**, *445*, 749–753.
- (7) Kim, M. J.; Wanunu, M.; Bell, D. C.; Meller, A. Rapid Fabrication of Uniformly Sized Nanopores and Nanopore Arrays for Parallel DNA Analysis. *Adv. Mater.* **2006**, *18*, 3149–3153.
- (8) Yang, Y.; Liu, R.; Xie, H.; Hui, Y.; Jiao, R.; Gong, Y.; Zhang, Y. Advances in Nanopore Sequencing Technology. *J. Nanosci. Nanotechnol.* **2013**, *13*, 4521–4538.
- (9) Hong, J.; Lee, Y.; Chansin, G. A.; Edel, J. B.; Demello, A. J. Design of a Solid-State Nanopore-Based Platform for Single-Molecule Spectroscopy. *Nanotechnology* **2008**, *19*, 165205.
- (10) Chen, Z.; Jiang, Y. B.; Dunphy, D. R.; Adams, D. P.; Hodges, C.; Liu, N. G.; Zhang, N.; Xomeritakis, G.; Jin, X. Z.; Aluru, N. R.; et al. DNA Translocation through an Array of Kinked Nanopores. *Nat. Mater.* **2010**, *9*, 667–675.
- (11) dela Torre, R.; Larkin, J.; Singer, A.; Meller, A. Fabrication and Characterization of Solid-State Nanopore Arrays for High-Throughput DNA Sequencing. *Nanotechnology* **2012**, *23*, 385308.
- (12) Novoselov, K. S.; Geim, A. K.; Morozov, S. V.; Jiang, D.; Zhang, Y.; Dubonos, S. V.; Grigorieva, I. V.; Firsov, A. A. Electric Field Effect in Atomically Thin Carbon Films. *Science* **2004**, *306*, 666–669.
- (13) Fischbein, M. D.; Drndic, M. Electron Beam Nanosculpting of Suspended Graphene Sheets. *Appl. Phys. Lett.* **2008**, *93*.
- (14) Wells, D. B.; Belkin, M.; Comer, J.; Aksimentiev, A. Assessing Graphene Nanopores for Sequencing DNA. *Nano Lett.* **2012**, *12*, 4117–4123.
- (15) Nelson, T.; Zhang, B.; Prezhdo, O. V. Detection of Nucleic Acids with Graphene Nanopores: Ab Initio Characterization of a Novel Sequencing Device. *Nano Lett.* **2010**, *10*, 3237–3242.
- (16) Lv, W.; Chen, M.; Wu, R. a. The Impact of the Number of Layers of a Graphene Nanopore on DNA Translocation. *Soft Matter* **2013**, *9*, 960–966.
- (17) Liang, L.; Cui, P.; Wang, Q.; Wu, T.; Agren, H.; Tu, Y. Theoretical Study on Key Factors in DNA Sequencing with Graphene Nanopores. *RSC Adv.* **2013**, *3*, 2445–2453.
- (18) Sathe, C.; Zou, X. Q.; Leburton, J. P.; Schulten, K. Computational Investigation of DNA Detection Using Graphene Nanopores. *ACS Nano* **2011**, *5*, 8842–8851.
- (19) Aksimentiev, A. Deciphering Ionic Current Signatures of DNA Transport through a Nanopore. *Nanoscale* **2010**, *2*, 468–483.
- (20) Garaj, S.; Hubbard, W.; Reina, A.; Kong, J.; Branton, D.; Golovchenko, J. A. Graphene as a Subnanometre Trans-Electrode Membrane. *Nature* **2010**, *467*, 190–U173.
- (21) Merchant, C. A.; Healy, K.; Wanunu, M.; Ray, V.; Peterman, N.; Bartel, J.; Fischbein, M. D.; Venta, K.; Luo, Z. T.; Johnson, A. T. C.; et al. DNA Translocation through Graphene Nanopores. *Nano Lett.* **2010**, *10*, 2915–2921.
- (22) Schneider, G. F.; Kowalczyk, S. W.; Calado, V. E.; Pandraud, G.; Zandbergen, H. W.; Vandersypen, L. M. K.; Dekker, C. DNA Translocation through Graphene Nanopores. *Nano Lett.* **2010**, *10*, 3163–3167.
- (23) Qiu, H.; Guo, W. Detecting ssDNA at Single-Nucleotide Resolution by Sub-2-nanometer Pore in Monoatomic Graphene: A Molecular Dynamics Study. *Appl. Phys. Lett.* **2012**, *100*, 083106–083104.
- (24) Luan, B.; Afzali, A.; Harrer, S.; Peng, H.; Waggoner, P.; Polonsky, S.; Stolovitzky, G.; Martyna, G. Tribological Effects on DNA Translocation in a Nanochannel Coated with a Self-Assembled Monolayer. *J. Phys. Chem. B* **2010**, *114*, 17172–17176.
- (25) Liang, L.-J.; Wang, Q.; Wu, T.; Sun, T.-Y.; Kang, Y. Contribution of Water Molecules in the Spontaneous Release of Protein by Graphene Sheets. *ChemPhysChem* **2013**, *14*, 2902–2909.
- (26) Liang, L.-J.; Wu, T.; Kang, Y.; Wang, Q. Dispersion of Graphene Sheets in Aqueous Solution by Oligodeoxynucleotides. *ChemPhysChem* **2013**, *14*, 1626–1632.

- (27) Luan, B. Q.; Peng, H. B.; Polonsky, S.; Rossnagel, S.; Stolovitzky, G.; Martyna, G. Base-By-Base Ratcheting of Single Stranded DNA through a Solid-State Nanopore. *Phys. Rev. Lett.* **2010**, *104*.
- (28) Peng, H. B.; Luan, B. Q.; Harrer, S.; Polonsky, S.; Rossnagel, S.; Martyna, G.; Stolovitzky, G. Base-by-base Ratcheting of Single Stranded DNA through a Solid-State Nanopore: The DNA Transistor. *Abstr. Pap. Am. Chem. Soc.* **2010**, *240*.
- (29) Avdoshenko, S. M.; Nozaki, D.; Gomes da Rocha, C.; González, J. W.; Lee, M. H.; Gutierrez, R.; Cuniberti, G. Dynamic and Electronic Transport Properties of DNA Translocation through Graphene Nanopores. *Nano Lett.* **2013**, *13*, 1969–1976.