Capturing a DNA duplex under near-physiological conditions

Huijuan Zhang,[1,2] Wei Xu,[3] Xiaogang Liu,[1,3] Francesco Stellacci,[1,4] and John T. L. Thong[1,5,a)]

[1]*Advanced Materials for Micro- and Nano-Systems Program, Singapore-MIT Alliance, National University of Singapore, 4 Engineering Drive 3, Singapore 117576*
[2]*Institute of Microelectronics, A*STAR, 11 Science Park Road, Singapore 117685*
[3]*Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543*
[4]*School of Engineering, Ecole Polytechnique Fédérale de Lausanne, STI IMX SUNMIL, CH-1015 Lausanne, Switzerland*
[5]*Department of Electrical and Computer Engineering, National University of Singapore, 4 Engineering Drive 3, Singapore 117576*

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We report *in situ* trapping of a thiolated DNA duplex with eight base pairs into a polymer-protected gold nanogap device under near-physiological conditions. The double-stranded DNA was captured by electrophoresis and covalently attached to the nanogap electrodes through sulfur-gold bonding interaction. The immobilization of the DNA duplex was confirmed by direct electrical measurements under near-physiological conditions. The conductance of the DNA duplex was estimated to be 0.09 μS. We also demonstrate the control of DNA dehybridization by heating the device to temperatures above the melting point of the DNA. © *2010 American Institute of Physics*. [doi:10.1063/1.3505152]

There has been considerable interest in DNA detection and electrical measurement for a wide range of potential applications in nanotechnology.[1–14] Optical detection based on gold (Au) and luminescent nanoparticles offers high detection sensitivity and selectivity but typically suffers from limited use in highly multiplexed detection and in situations of turbid fluidic environment. Electrical probing-based detection has recently emerged as an alternative approach to optical detection because it is compatible with aqueous media and allows direct signal readout in multiplexed format. However, real-time conductance analysis of an individual double-stranded DNA (dsDNA) requires stringent electronic contact of the DNA to devices.[13–21] Among many electrical probing techniques, nanogaps as contact electrodes with nanometer separation have been intensively used for studying the conductance of DNA.[22–25] It should be noted that the hybridization of DNA strands typically occurs in buffer solution of high ionic strength as the negative phosphate groups on the backbone of the DNA require a proximate condensation of positively charged counter ions.[26] The inevitable presence of background ionic current due to the buffer solution often interferes with electronic signal read-out and hinders direct conductance measurement of the dsDNA in solution.[27–30] Therefore, it has been challenging to carry out reliable conductance measurement of DNA duplexes in aqueous solutions under near-physiological conditions (i.e., high ionic strength).[31] Here we demonstrate *in situ* trapping of a thiolated DNA duplex with eight base pairs into a polymer-protected sub-2 nm gold nanogap. More significantly, we also demonstrate the ability to carry out, under near-physiological conditions, direct conductance measurements of the captured DNA duplex and monitor the change in conductance as the dsDNA melts.

The polymer-protected gold nanogap devices were fabricated through a process of electromigration and concurrent self-aligned polymer ablation in a polymethylmethacrylate (PMMA)-coated constricted electrode structure using a simple slow voltage sweep technique as previously reported.[32,33] In a typical experiment, we first patterned electrodes with a constriction by electron beam lithography. Metal layers of 18-nm-thick Au and 2-nm-thick Cr (as an adhesion layer) were thermally evaporated and lifted-off in acetone to create the Au electrodes. A PMMA layer of ~100 nm thick was then spin-coated onto the device as the

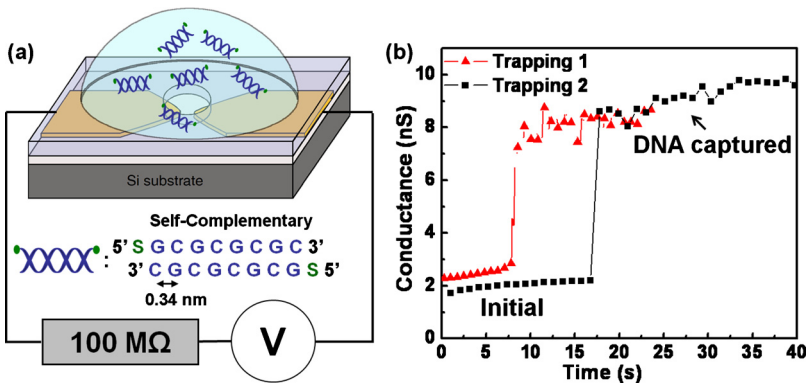

FIG. 1. (Color online) (a) Schematic of an electrophoresis setup for DNA trapping. The DNA 5′-SH-GCGCGCGC-3′ is self-complementary. The sub-2 nm nanogap is exposed to DNA solution through a PMMA hole opening (~100 nm in diameter) and the rest of the device remains protected by the polymer. Applied bias was 1 V. 100 MΩ is the series resistor to limit the electric field after the capture of one dsDNA to avoid multiple trapping events. (b) *In situ* monitoring of dsDNA trapping: two typical conductance vs time curves of DNA trapping (trapping 1 and 2 experiments are marked by triangles and squares, respectively). The step conductance increase indicates successful capturing of a dsDNA duplex.

a)Electronic mail: elettl@nus.edu.sg.

protection layer. Subsequently, a voltage sweep at a rate of 1 mV/7 s was applied to the bowtie structures at room temperature by using a Keithley 4200 parameter analyzer. The sweep was used to stress the constriction till tunneling current was detected (when conductance $<2e^2/h$). As a consequence, a sub-2 nm gap and a self-aligned hole in the PMMA (~100 nm in diameter), exposing the electrode tips, were simultaneously formed. A single-stranded oligonucleotide (5′-SH-GCGCGCGC-3′) was purchased in disulfide form from Sigma-Aldrich Ltd. The sequence of the DNA molecules was designed to be self-complementary to form a duplex structure upon hybridization. The length (~2.7 nm) (Ref. 34) of eight base pairs is slightly larger than the narrowest separation of the nanogap (~2 nm) fabricated by electromigration, to ensure that the nanogap can be bridged by the dsDNA. The DNA strands were hybridized in phosphate buffered saline (PBS) buffer solution (0.1 M sodium chloride, 10 mM sodium phosphate, and *p*H 7) by annealing at 85 °C for 5 min, followed by slow cooling to room temperature (27 °C) to form a duplex structure.[35] The melting temperature (T_m) of the self-complementary DNA duplex was determined to be 32 °C by using a SHIMADZU UV-2450 spectrophotometer.

The capture of an individual DNA duplex between the nanogap electrodes was achieved by electrostatic trapping [Fig. 1(a)].[22,36,37] A 10 μl droplet of dsDNA solution (10 μM in PBS solution) was first added to the PMMA-protected nanogap. Upon applying 1 V of bias between the electrodes with a 100 MΩ series resistor, a DNA duplex is polarized by the electric field and attracted to the nanogap. The trapping of the DNA duplex enables current flow through the circuit, resulting in most of the bias voltage being dropped across the series resistor. The probability of trapping a second DNA duplex is thus greatly reduced due to the reduced electric field between the electrodes.[22] It should also be noted that since both ends of the DNA double-helix are terminated with the thiol group, it is possible for both ends to be bound to the same electrode. In this case, the dsDNA does not bridge the gap, and the trapping electric field remains until such time that a dsDNA does get trapped and bridges the gap. As shown in Fig. 1(b), we observed a large steplike increase in the conductance when a dsDNA was captured. We attribute the increase in the conductance to the trapping of the dsDNA between the electrodes. Figure 1(b) shows two typical *in situ* trapping experiments of conductance measurements obtained from two different devices as a function of time. For example, in the second trapping experiment, the initial conductance of ~2 nS measured from 0 to 16 s is attributed to current flow between the nanogap electrodes that are selectively exposed to the ionic electrolyte solution through the PMMA hole structure. After 17 s, we observed a sharp increase in conductance (to 9 nS), indicating the capture of the dsDNA.

The conductance of the captured dsDNA was then electrically characterized in the same buffer solution after removing the series resistor. The captured DNA duplex is covalently bonded to the Au electrode through thiol-Au bonding as illustrated in Fig. 2 (inset, right). Figure 2 also shows a typical IV characteristic of the DNA duplex from −200 to 200 mV at a ramp rate of 5 mV/50 ms, demonstrating Ohmic behavior of the DNA. The dsDNA conductance of 0.09 ± 0.02 μS was obtained from 15 sample measurements

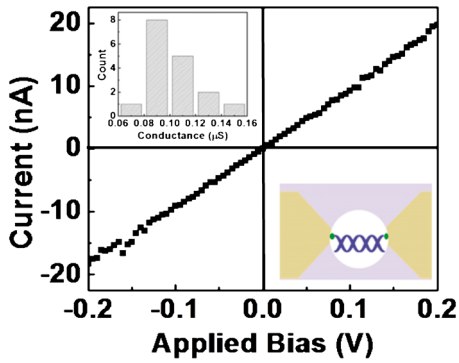

FIG. 2. (Color online) *I-V* characteristics of a dsDNA molecule in PBS buffer solution. The applied bias was from −200 to 200 mV at a ramp rate of 5 mV/50 ms. The inset at right corner is a schematic of dsDNA in a nanogap. Once a dsDNA molecule is trapped, the dsDNA is then immobilized to the nanogap electrode by thiol-gold bonding and formed a bridge between the nanogap so that it is possible to measure the conductance of the dsDNA in solution. Inset at the upper left corner: A histogram of dsDNA conductance measured from 15 samples.

as shown in Fig. 2 (inset, left), which is consistent with the value of 0.1 μS reported by Xu and co-workers.[28] The deviation in the conductance probably originates from multiple causes, including the spatial arrangement of DNA duplex, the variation in counter ions attached on the backbone of the DNA, and hydrogen bonding interaction.[38] The ionic current from solution that affects the measured conductance is ruled out as the polymer-protected nanogap device limited this current to tens of picoamperes.

Why is a DNA duplex conductive in aqueous solution? In theory, the hybridization of π_z orbitals in dsDNA could lead to conducting behavior.[26] According to Cuniberti and co-workers,[39] the conduction observed could result from a strong perturbation of the electronic system mediated by the dissipative water environment. The perturbation may modify the low-energy electronic structure of the DNA strand and induce new energy states, which could induce increased conduction. It has been reported that DNA conductance exhibits an exponential increase up to 10^6 times with increased humidity as the current does not flow through the DNA itself but flows through the water layer adsorbed at the DNA backbone.[40] Our experimental results show that upon drying or immersion in de-ionized water, the conductance of the captured DNA duplex is significantly reduced (Fig. 3). We propose that the conduction is not only contributed from the DNA duplex that bridges over the nanogap electrodes but also from the counter ions attached to the backbone of the DNA and from water molecules absorbed via ionic

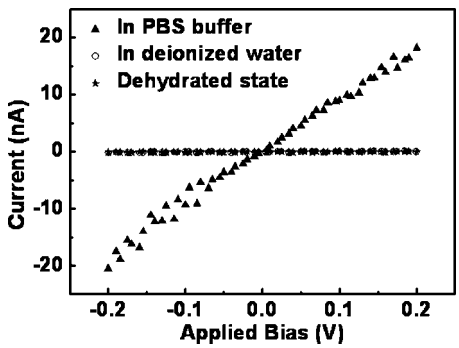

FIG. 3. *I-V* characteristics of a DNA duplex in PBS buffer solution (triangle), de-ionized water (dot) and dehydrated state (star). Note that the data set of the dehydrated state overlaps with that of the de-ionized state.

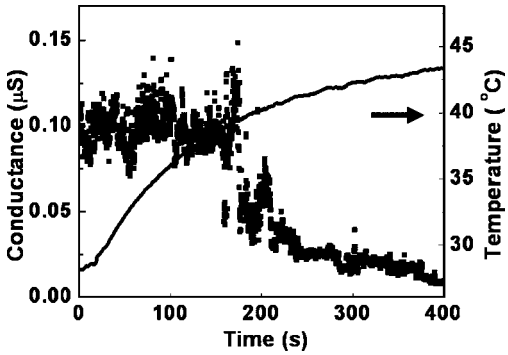

FIG. 4. *In situ* conductance measurement of the dsDNA upon melting. The applied bias is 0.1 V. A steplike conductance drop was observed due to denaturing of the DNA duplex structure.

conduction.[41] It is observed that the current is only in the order of tens of picoampere with significant background noise when the DNA is dried or immersed in de-ionized water, suggesting nonconducting behavior of the captured DNA under these conditions. In de-ionized water, the ds-DNA would be denatured due to the absence of counter ions, which are necessary to hold the negatively charged oligonucleotide backbones together. In the case of dehydrated samples, there are no counter ions or water molecules to create conduction paths.

To confirm that the DNA duplex is indeed trapped between the electrodes, *in situ* DNA melting experiments were carried out. Upon melting, the rigid rodlike[41,42] DNA duplex denatures and no longer bridges the nanogap, thus resulting in a conductance drop of the device. A thin-film heater was placed underneath the dsDNA-trapped nanogap device, and the temperature of the device was monitored with a thermocouple. The conductance of the DNA duplex in solution was monitored *in situ* at an applied bias 100 mV as it was being heated. From the time interval of 0 to 150 s, the conductance of ~ 0.1 μS is largely due to the current flow through the captured-DNA duplex [Fig. 4]. Over the time interval of 150–200 s, we observed the decreased conductance of the device, indicating the occurrence of denaturing process of the DNA duplex as the temperature of the device (>40 °C) exceeded the DNA melting temperature (~ 32 °C) [Fig. 4].

In summary, the polymer-protected sub-2 nm nanogap device enables *in situ* monitoring of electrical trapping of a dsDNA. The conductance of the captured-DNA duplex was electrically characterized and showed Ohmic-like conductance of 0.09 $\mu S \pm 0.02$ μS, which is consistent with the literature value of 0.1 μS. Additionally, *in situ* DNA melting experiments and conductance measurements were carried out in buffer solution to provide direct evidence of trapping of the DNA duplex between the electrodes. Further investigations of the conductance of DNA duplex with different lengths and base pairs using the polymer-protected nanogap device are currently underway in our laboratory.

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[1]S. W. Chung, D. S. Ginger, M. W. Morales, Z. Zhang, V. Chandrasekhar, M. A. Ratner, and C. A. Mirkin, Small **1**, 64 (2005).
[2]S. J. Park, T. A. Taton, and C. A. Mirkin, Science **295**, 1503 (2002).
[3]O. Akbulut, J.-M. Jung, R. D. Bennett, Y. Hu, H.-T. Jung, R. E. Cohen, A. M. Mayes, and F. Stellacci, Nano Lett. **7**, 3493 (2007).
[4]A. A. Yu, T. A. Savas, G. S. Taylor, A. Guiseppe-Elie, H. I. Smith, and F. Stellacci, Nano Lett. **5**, 1061 (2005).
[5]S. A. Claridge, H. W. Liang, S. R. Basu, J. M. J. Fréchet, and A. P. Alivisatos, Nano Lett. **8**, 1202 (2008).
[6]B. Y. Lee, S. M. Seo, D. J. Lee, M. Lee, J. Lee, J.-H. Cheon, E. Cho, H. Lee, I.-Y. Chung, Y. J. Park, S. Kim, and S. Hong, Lab Chip **10**, 894 (2010).
[7]W. Xu, X. Xue, T. Li, H. Zeng, and X. Liu, Angew. Chem. Int. Ed. **48**, 6849 (2009).
[8]X. Xue, W. Xu, F. Wang, and X. Liu, J. Am. Chem. Soc. **131**, 11668 (2009).
[9]F. Wang, D. Banerjee, Y. Liu, X. Chen, and X. Liu, Analyst (Cambridge, U.K.) **135**, 1839 (2010).
[10]X. Liu, Y. Zhang, D. K. Goswami, J. S. Okasinski, K. Salaita, P. Sun, M. J. Bedzyk, and C. A. Mirkin, Science **307**, 1763 (2005).
[11]X. Xue, F. Wang, and X. Liu, J. Am. Chem. Soc. **130**, 3244 (2008).
[12]F. Wang and X. Liu, Chem. Soc. Rev. **38**, 976 (2009).
[13]F. Wang, Y. Han, C. S. Lim, Y. Lu, J. Wang, J. Xu, H. Chen, C. Zhang, M. Hong, and X. Liu, Nature (London) **463**, 1061 (2010).
[14]Z. Ma, Y. Hong, M. Zhang, and M. Su, Appl. Phys. Lett. **95**, 233101 (2009).
[15]Y. Okahata, T. Kobayashi, K. Tanaka, and M. Shimomura, J. Am. Chem. Soc. **120**, 6165 (1998).
[16]H. W. Fink and C. Schonenberger, Nature (London) **398**, 407 (1999).
[17]A. Y. Kasumov, M. Kociak, S. Gueron, B. Reulet, V. T. Volkov, D. V. Klinov, and H. Bouchiat, Science **291**, 280 (2001).
[18]X. D. Cui, A. Primak, X. Zarate, J. Tomfohr, O. F. Sankey, A. L. Moore, T. A. Moore, D. Gust, G. Harris, and S. M. Lindsay, Science **294**, 571 (2001).
[19]M. Magoga and C. Joachim, Phys. Rev. B **56**, 4722 (1997).
[20]S. N. Yaliraki, M. Kemp, and M. A. Ratner, J. Am. Chem. Soc. **121**, 3428 (1999).
[21]F. Moresco, L. Gross, M. Alemani, K. H. Rieder, H. Tang, A. Gourdon, and C. Joachim, Phys. Rev. Lett. **91**, 036601 (2003).
[22]D. Porath, A. Bezryadin, S. de Vries, and C. Dekker, Nature (London) **403**, 635 (2000).
[23]X. Liang and S. Y. Chou, Nano Lett. **8**, 1472 (2008).
[24]K. H. Yoo, D. H. Ha, J. O. Lee, J. W. Park, J. Kim, H. Y. Lee, T. Kawai, and H. Y. Choi, Phys. Rev. Lett. **87**, 198102 (2001).
[25]A. J. Storm, J. van Noort, S. de Vries, and C. Dekker, Appl. Phys. Lett. **79**, 3881 (2001).
[26]R. G. Endres, D. L. Cox, and R. R. P. Singh, Rev. Mod. Phys. **76**, 195 (2004).
[27]G.-J. Zhang, G. Zhang, J. H. Chua, R.-E. Chee, E. H. Wong, A. Agarwal, K. D. Buddharaju, N. Singh, Z. Gao, and N. Balasubramanian, Nano Lett. **8**, 1066 (2008).
[28]B. Xu, P. Zhang, X. Li, and N. Tao, Nano Lett. **4**, 1105 (2004).
[29]H. Zhang, C.-L. Wong, Y. Hao, R. Wang, X. Liu, F. Stellacci, and J. T. L. Thong, Nanoscale **2**, 2302 (2010).
[30]H. van Zalinge, D. J. Schiffrin, A. D. Bates, W. Haiss, J. Ulstrup, and R. J. Nichols, ChemPhysChem **7**, 94 (2006).
[31]N. Mano, H. H. Kim, Y. Zhang, and A. Heller, J. Am. Chem. Soc. **124**, 6480 (2002).
[32]H. Zhang, R. J. Barsotti, C.-L. Wong, X. Xue, X. Liu, F. Stellacci, and J. T. L. Thong, Small **5**, 2797 (2009).
[33]H. Zhang, C. V. Thompson, F. Stellacci, and J. T. L. Thong, Nanotechnology **21**, 385303 (2010).
[34]M. Mandelkern, J. Elias, D. Eden, and D. Crothers, J. Mol. Biol. **152**, 153 (1981).
[35]S. M. Freier, D. D. Albergo, and D. H. Turner, Biopolymers **22**, 1107 (1983).
[36]A. Bezryadin and C. Dekker, J. Vac. Sci. Technol. B **15**, 793 (1997).
[37]A. Bezryadin, C. Dekker, and G. Schmid, Appl. Phys. Lett. **71**, 1273 (1997).
[38]R. N. Barnett, C. L. Cleveland, A. Joy, U. Landman, and G. B. Schuster, Science **294**, 567 (2001).
[39]R. Gutiérrez, S. Mandal, and G. Cuniberti, Nano Lett. **5**, 1093 (2005).
[40]T. Kleine-Ostmann, C. Jördens, K. Baaske, T. Weimann, M. Hrabe de Angelis, and M. Koch, Appl. Phys. Lett. **88**, 102102 (2006).
[41]M. E. Tuckerman, D. Marx, and M. Parinello, Nature (London) **417**, 925 (2002).
[42]D. J. Maxwell, J. R. Taylor, and S. M. Nie, J. Am. Chem. Soc. **124**, 9606 (2002).