Double-functionalized nanopore-embedded gold electrodes for rapid DNA sequencing

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We have studied the effect of double-functionalization on gold electrodes for improving nanopore-based DNA sequencing. The functionalizing molecular probes are, respectively, capable of temporarily forming hydrogen bonds with both the nucleobase part and the phosphate group of the target DNA, thus potentially minimizing the structural fluctuations of a single-stranded DNA molecule passing between the gold electrodes. The results of our first-principles study indicate that the proposed setup yields current signals that differ by at least 1 order of magnitude for the four different nucleic acid bases, thus offering the possibility to electrically distinguish them. © *2012 American Institute of Physics*. [doi:10.1063/1.3673335]

The human genome is to a large extent shared between individuals, yet the DNA sequences of different persons do exhibit variations which can be of importance for risk assessments regarding the development of certain hereditary diseases and eventually even for the optimal course of medical treatment.[1,2] Statistical studies comparing genomes of a large number of individuals (and linking them to their medical history) are crucial for a better understanding of various diseases and could be regarded as a prerequisite for a directed development of genome-based personal medicine.[3]

The currently used standard technology for DNA sequencing[4] is well established, and as such reliable and reasonably fast. However, the cost factor remains as an obstacle for truly large-scale whole-genome studies.[5,6] The drive towards orders of magnitude less expensive technologies for DNA sequencing has motivated research on radically new approaches for reading out the genomic information.[7,8] Nanopore-based electronic DNA sequencing has attracted significant interest in this regard.[9,10] In 1996, Kasianowicz *et al.*[11] demonstrated that single-stranded DNA (ssDNA) or RNA molecules can be electrophoretically driven through a biological nanopore and can be detected due to their blocking of the ionic current while translocating. Since then, the prospects of DNA sequencing research have increasingly shifted towards solid-state nanopores, which possess the advantage that they have higher chemical, thermal, and mechanical stability than their biological counterparts. Furthermore, they might allow the insertion of nanopore-embedded electrodes. Then, as the nucleotides of DNA move through the nanopore one after the other, they will pass between two such opposing embedded electrodes, across which a bias voltage is applied. The resulting time-dependent transverse tunneling current could then ideally directly reveal the DNA sequence.[12,13]

A major difficulty for DNA sequencing with nanopore-embedded electrodes is the achievement of single-base resolution, which requires (due to the sub-nanometer spacing of adjacent nucleobases) extremely sharp electrodes. To overcome this problem, the creation of nanogaps in atomically thin graphene and subsequent utilization of the thus created graphene edges as electrodes has been suggested,[14] and this idea was recently evaluated from first principles.[15] In the present work, we follow a different route, namely, that of functionalizing the embedded electrodes with suitable probe molecules. This approach could solve the single-base resolution problem too, and in addition address some of the biggest challenges of nanopore-based DNA sequencing, e.g., controlling the DNA velocity as well as its structural orientation during the translocation process. He *et al.*[16] demonstrated how functionalization of one of the two nanopore-embedded gold electrodes using hydrogen bond mediated tunneling signals can be used for systematic DNA sequencing. At the same time, another study[17] showed that cytosine can be a promising molecular probe to attach to gold electrodes so that the probe and nucleic acid bases interact through weak hydrogen-bonds. The extent of hydrogen-bonding between the probe and the target molecule is weak enough so that the ssDNA molecule can continue to slide through the nanopore while driven electrophoretically. The covalent bond between the gold electrode and the thiol-based probe is strong enough so that the latter remains firmly attached to the gold surface and will not be ripped away by the ssDNA molecule passing by.

Here, we studied a more sophisticated scenario, namely, that of functionalizing both gold electrodes, however, with two different molecules,[18] in such a way that one of the chemical probes is intended to interact with the base part of the DNA nucleotides, whereas the other will be interacting with the phosphate group. Thus, the main objective was to see whether we could use two different molecular probes for interacting with both sides of the ssDNA through hydrogen bonds, to achieve better control on the DNA movement as well as on its structural orientation. It was recently shown from molecular dynamics simulations[19] that such a

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formation of temporary weak hydrogen bonds can considerably reduce the fluctuations in the electronic output signal.

Based on our previous study,[17] we have chosen cytosine as the molecular probe intended to bind to the nucleobase part of DNA, whereas in our search for a suitable chemical probe to bind to the DNA phosphate group, we focused on molecules containing amine groups ($-NH_2$). Thus, we required a chemical probe which possesses both -thiol as well as -amine functional groups, so that the -thiol group could be anchored to the gold electrode, while the amine group interacts with the DNA phosphate group. We examined $HSCH_2CH(NH_2)_2$, $HSCH_2CH(NH_2)(COOH)$, and $HSC(NH_2)_2$. Our preliminary calculations to determine the most stable pairing geometries between the probe and the target DNA phosphate group revealed that $HSC(NH_2)_2$ (thiourea or thiocarbamide) bind reasonably well with the DNA phosphate group and were thus deemed to be very promising for further studies. Thiourea or thiocarbamide is a well-known compound and widely available. Thus, we chose thiocarbamide as a chemical probe which can bind to DNA phosphate group through weak H-bonding. All starting geometries were evaluated at the B3LYP/6-31 G* level of theory[20,21] using the GAUSSIAN 09 package.[22]

A schematic illustration of the relevant elements of the proposed device is shown in Figure 1. The computational model of our proposed nano-scale device is divided into three parts: left electrode, a central scattering region, and right electrode. Each electrode consists of six layers of Au (111) where the first three outer layers are kept rigid as in bulk for the calculations. The central scattering region consists of three inner layers of gold (111) from each electrode, the attached chemical probes, and the target nucleic acid base of the ssDNA. We used the density functional program SIESTA (Ref. 23) for structural relaxations. To reduce computational cost, we used single-zeta with polarization (SZP) orbitals for Au and double-zeta polarization (DZP) for H, C, N, O, P, and S atoms. We used the local density approximation (LDA) for the exchange-correlation potential for both the electrodes and the molecules. The transport properties of the electrode–molecule–electrode device were calculated using TRANSIESTA,[24] which allows for first-principles quantum modeling of molecules under non-equilibrium conditions. This method is based on the non-equilibrium Green's function (NEGF) technique that has been well implemented in SIESTA, in such a way that the density matrix of the system is calculated self-consistently even when the system is subjected to an external bias.

In order to evaluate whether the four nucleotides adenine (A), guanine (G), cytosine (C), and thymine (T) can be electrically distinguished, we have carried out transport calculations with these nucleotides while located between the double-functionalized gold electrodes (Figure 1). Specifically, the resulting current signal was calculated at both 0.1 V and 0.25 V bias, as shown in Figure 2. The transmission curves calculated at zero bias are provided in Figure 3. The zero-bias transmissions are somewhat higher for purine bases (A, G) when compared with the pyrimidine bases (C, T). This is due to the different structures of the two general types of nucleotides. The extent of coupling between the chemical probes and the nucleotides are quite different from one to another, but it is maximal for purine bases and minimal for pyrimidine bases. The functionalizing chemical probe $SC(NH_2)_2$ interacting with the DNA phosphates group seems to be similar for all the nucleotides. The extent of interaction between the cytosine chemical probe and the DNA nucleotides are different from one to other. This is due to the structural differences between the four DNA bases. Our current versus voltage plot calculated for the device involving both the probes is shown in Figure 2. The current calculated at 0.1 V bias is at its maximum for A followed by G and T and at its minimum for C. The calculated current at 0.1 V bias for A is three orders of magnitude higher than G, which is in turn 100 times higher than that of T, which finally is almost one order of magnitude larger than C. Hence, the difference in current calculated at 0.1 V bias is found to be variant enough to distinguish in principle all four nucleotides from each other. We have calculated the current for our device ranging from 0.1 V to 0.25 V, and our results show that at 0.1 V bias we could distinguish the purine and pyrimidine bases but at 0.25 V bias the calculated current is quite similar for C and T.

In summary, then, we have shown that double-functionalized nanopore-embedded gold electrodes with two different molecular probes attached could lead to an improvement for distinguishing the four DNA nucleic acid

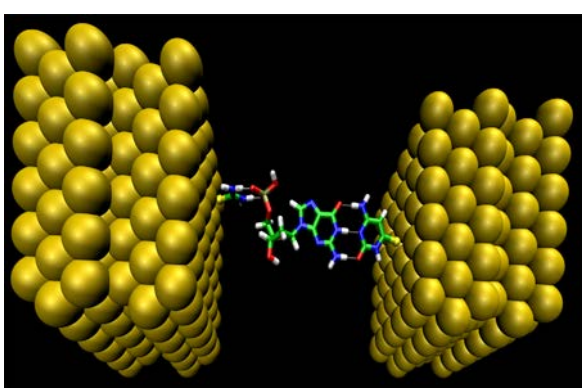

FIG. 1. (Color online) Schematic illustration of the proposed device setup with left and right gold electrodes, and a central scattering region containing the two different probe molecules anchored to the gold electrodes, the target nucleic acid base of ssDNA, and a portion of the semi-infinite gold electrodes (see also cover image of this issue).

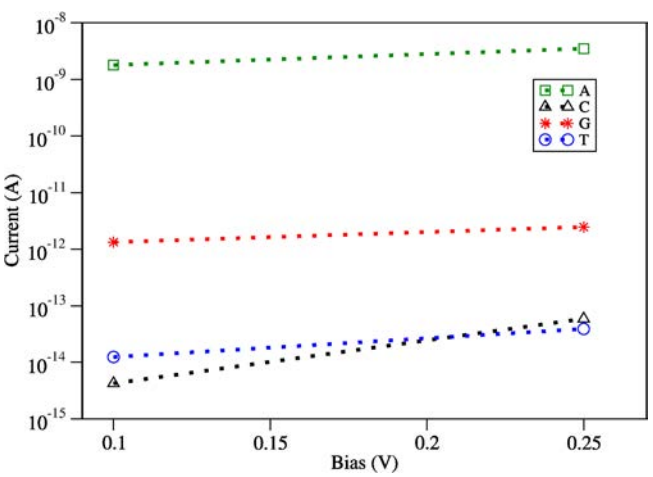

FIG. 2. (Color online) The current calculated at 0.1 and 0.25 V bias, plotted for all four nucleotides.

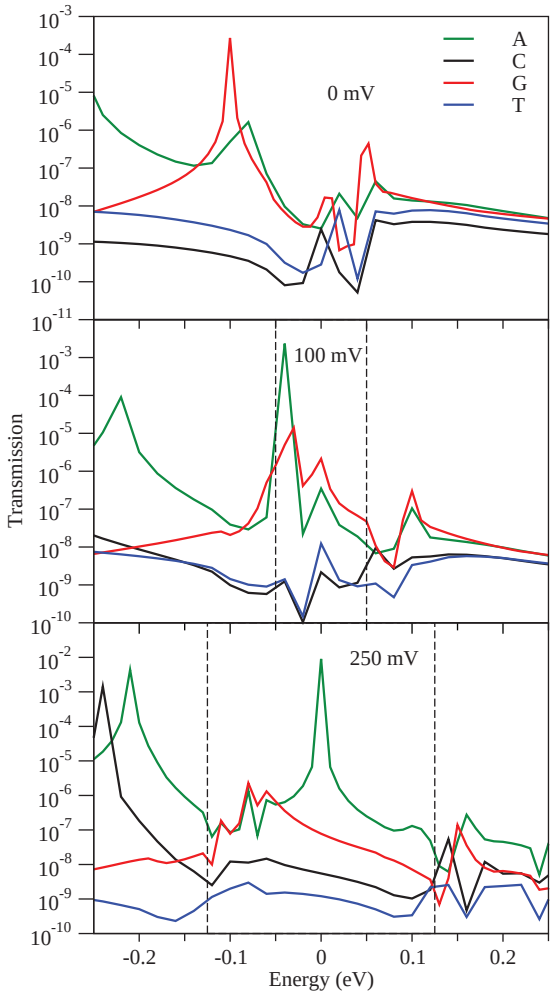

FIG. 3. (Color online) Transmission functions plotted on a logarithmic scale for the four target nucleic acid bases at a bias of $V = 0$ mV, 100 mV, and 250 mV. The Fermi level has been aligned to zero. The voltage window of $\pm V/2$ is indicated by the vertical dashed lines.

bases separately compared with the case where only one electrode were to be functionalized. The hydrogen bonding interaction between the cytosine chemical probe and the target nucleic bases was found to be maximal for purine bases and minimal for pyrimidine bases. The current calculated at 0.1 V bias was found to be in principle capable of distinguishing between the four DNA nucleotides.

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