Real-time detection of deoxyribonucleic acid bases via their negative differential conductance signature

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In this Brief Report, we present a method for the real-time detection of the bases of the deoxyribonucleic acid using their signatures in negative differential conductance measurements. The present methods of electronic detection of deoxyribonucleic acid bases are based on a statistical analysis because the electrical currents of the four bases are weak and do not differ significantly from one base to another. In contrast, we analyze a device that combines the accumulated knowledge in nanopore and scanning tunneling detection and which is able to provide very distinctive electronic signatures for the four bases.

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I. INTRODUCTION

The deoxyribonucleic acid (DNA) molecule is built up of four bases: adenine (A), guanine (G), cytosine (C), and thymine (T), which are attached to sugar and a phosphate backbone. A DNA base together with the sugar and phosphate backbone molecules forms a nucleotide, while the sugar and DNA base is referred to as a nucleoside. A double-strand DNA is formed by pairing complementary bases (A-T and G-C) of nucleotide sequences. The DNA is the molecule with the highest density of information, which encrypts the genetic code of any living organism, including the humans. Therefore, the decoding of DNA sequence of bases is of paramount importance for understanding life and for detecting and healing the potential diseases. Presently, the sequencing of a single human genome takes a few months and is a high-cost procedure, exceeding a few millions of dollars. The huge amount of information contained in the human genome is encoded in more than 3×10^9 base pairs that must be detected and interpreted.

Since the diameter of the DNA helical structure is on the nanometer scale, nanotechnologies are now involved for the electrical detection of DNA bases using several techniques based on DNA translocation through nanopores, which include the measuring of the ionic blockade current through the nanopore, the detection of the transverse nanopore current, and the measurement of voltage fluctuations in a capacitor across the nanopore (see the review in Ref. [1]). All these detection methods rely on averaging many measurements for the identification of the base sequence since the electronic signals produced by the four bases are weak and quite similar. This low contrast between the electronic signatures of different bases makes their identification difficult and, so, a statistical approach is needed. Solutions to alleviate this problem include the identification of DNA base pairing via the distance decay of the tunneling current between the tip of a scanning tunneling microscope (STM) and the substrate [2], a method that requires more than one functionalized reading head to identify all DNA bases, or the use of an alternating electronic field in a nanopore capacitor [3], which detects the hysteresis of the bases of the DNA strand that moves back and forth through the nanopore; such an oscillatory movement increases the detection time.

In what follows, we propose a method to detect in real time the base sequence of a single-strand DNA molecule that translocates through a nanopore. This method combines the nanopore architecture with the phenomena of field emission and tunneling, specific for STM techniques. The net result is an enhancement of about four orders of magnitude of the detected current signal from the pA level (specific to the nanopore detection method via the ionic blockade current) up to a few tens of nA and the allocation to each base of a very distinctive pattern of the differential conductance, which jumps from negative to positive values.

II. DNA BASE DETECTION METHOD

The method presented in this Brief Report relies on pulling the single-strand DNA molecule through a nanopore with a diameter of a few nanometers fabricated in a membrane terminated by two sharp electrodes. A dc field transverse to the membrane translocates the DNA through the nanopore, via the applied force F, due to the fact that the backbone of the DNA [the black right edge in Fig. 1(a)] is always negatively charged. The two sharp electrodes are used to collect the electrical signal perpendicular to the DNA backbone axis. In contrast to methods based on the measurement of the transverse current through the nanopore, the sharp electrodes used in this detection scheme are similar to STM tips and thus are supposed to emit/collect field-emitted electrons that overcome the work function of the electrodes and the DNA bases. A schematic representation of the measuring device is illustrated in Fig. 1(a).

In this device, the electrons are emitted from the sharp electrode after overcoming its work function ϕ, pass through a generic DNA base B (B can be either A, G, C, or T) that is

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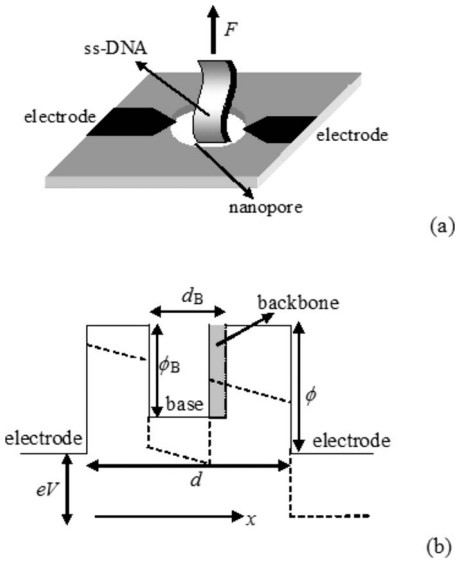

FIG. 1. (a) Schematic illustration of the nanopore device configuration for detecting the DNA bases and (b) the energy-band distribution used to model it in the absence (solid line) and presence (dashed line) of an applied voltage.

located in the nanopore at that moment, and are then collected by the other sharp electrode. The electron energy potential distribution that is used to model the device is represented in Fig. 1(b). The nucleotide, with width d_B, is divided into the phosphate backbone and the nucleoside because base-related work functions could only be extracted from STM measurements [4] performed on nucleosides. The backbone part of the nucleotide is insulating and is modeled as a barrier with the same height as the work function of base B (in nucleoside) denoted by ϕ_B. This model is in agreement with the STM measurements on DNA in [5]. The presence of the phosphate backbone renders the energy potential distribution asymmetric. The diameter of the DNA nucleotide takes values given in Ref. [1], whereas the width of the backbone barrier is assumed to be $d_{bb}=5$ Å [6]; then, the nucleoside has a width d_B-d_{bb}. The nanopore diameter is denoted by d.

So, the DNA base detection proposed in this Brief Report is based on the sequence of two tunneling processes between the nanopore edges and the base, the nanopore device being equivalent to a resonant tunneling diode (RTD) type device that works with field-emitted electrons. Since resonant tunneling is involved in the functioning of this device, the resulting current is larger than in usual STM measurements and the electric signatures of the four bases become more dissimilar.

The electrical signal and, in particular, the work function of the four DNA bases are different and, hence, unique, as follows from the theoretical work in Ref. [7] and from the STM measurements of nucleosides in Ref. [4]. Although the electronic signatures of the A, G, C, and T bases are quite difficult to calculate, these bases behave—at least when field emission occurs—as potential barriers with different heights. This fact allows modeling the electron transport through the device in Fig. 1(a) with the simple energy-band configuration depicted in Fig. 1(b).

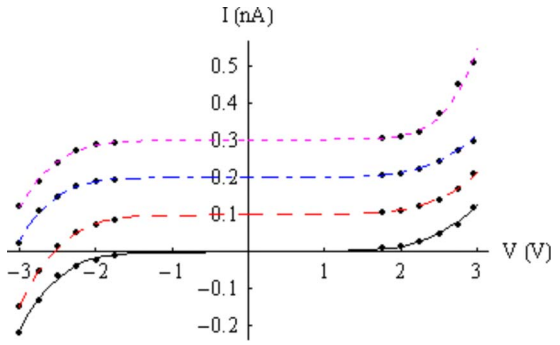

FIG. 2. (Color online) Fitting curves of the experimental data in Ref. [5].

More precisely, in order to extract the ϕ_B values from the experimental STM I-V characteristics of the different nucleosides in Fig. 3a in Ref. [4], we have fitted these data with

$$I(V) = V/R + aV^2 \exp(-b/V). \tag{1}$$

The first term in the right-hand side of Eq. (1) represents the contribution of a series resistance R and the last term is a typical Fowler-Nordheim characteristic, which describes a one-dimensional field emission from a barrier of height ϕ_B, which assumes a triangular shape in the presence of an applied electric field. So, DNA bases can be modeled as potential barriers with heights ϕ_B for field-emitted electrons, their work function being obtained from the expression

$$b = \frac{4L}{3}\frac{\sqrt{2m_0}}{e\hbar}\phi_B^{3/2}, \tag{2}$$

where L is the STM tip-sample distance and m_0 is the free-electron mass. The fitting curves are represented in Fig. 2 with solid line for A, dotted magenta line for C, dashed red line for G, and dashed-dotted blue line for T; the points on all these curves representing I-V experimental data extracted from Fig. 3a in Ref. [4]. The characteristics for C, G, and T have been raised with 0.1 nA, 0.2 nA and, respectively, 0.3 nA in order to render them readable. The forward (backward) polarization data were fitted with a series resistance value of 500 Ω (555 Ω) for A, 1000 Ω (500 Ω) for C, 454 Ω (666 Ω) for G, and 1000 Ω (100 Ω) for T, while the corresponding a parameters in Eq. (1) are 0.34 nS/V (0.95 nS/V), 5.51 nS/V (1.15 nS/V), 0.35 nS/V (0.75 nS/V), and 0.27 nS/V (1.35 nS/V). As follows from Eq. (2), the work functions are determined from the b parameters of the fit, which take for positive (negative) polarizations the values 9.7 V (11.2 V) for A, 16 V (12.2 V) for C, 10.1 V (10 V) for G, and 9.3 V (12.9 V) for T. The different values of the fitting parameters for the two polarizations are probably due to specific interactions of the bases with the substrate. Since in our proposed device the bases are not placed on the substrate, we use in the simulations the average value between the work functions obtained from data at positive and negative polarizations. These average work functions are $\phi_A=1.74$ eV, $\phi_T=1.81$ eV, $\phi_C=2.12$ eV, and $\phi_G=1.7$ eV for an estimated $L=0.66$ nm.

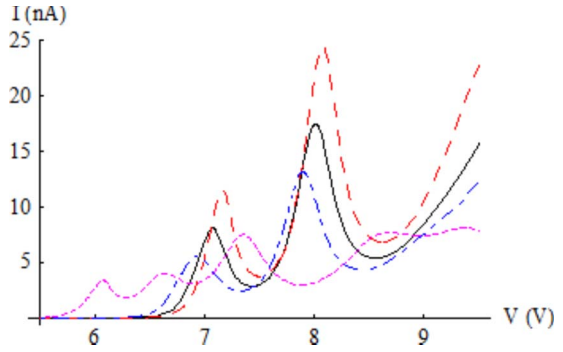

FIG. 3. (Color online) *I-V* characteristics of different bases that translocate through the nanopore: A (solid line), C (dotted magenta line), G (dashed red line), and T (dashed-dotted blue line).

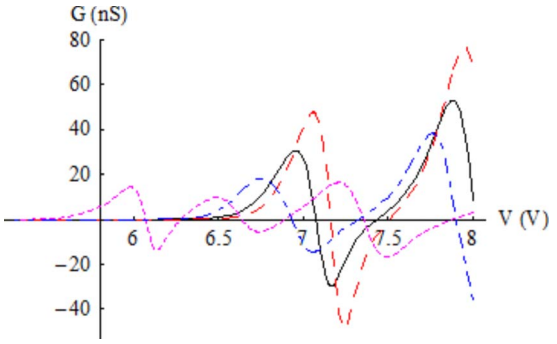

FIG. 4. (Color online) Differential conductances of the A, C, G, and T bases obtained from the respective characteristics in Fig. 2.

With these experimentally determined parameters for the four DNA bases, we have modeled the *I-V* characteristic through the nanopore configuration in Fig. 1 for $d=2.7$ nm. The current was calculated using the Landauer formalism at room temperature,

$$I(V) = (2e/h)\int T(E,V)[f_1(E) - f_2(E)]dE, \tag{3}$$

where $f_{1,2}(E)$ are the Fermi-Dirac distribution functions of the two electrodes situated at potentials 0 and $-eV$ and $T(E,V)$ is the transmission coefficient of the electrons that are emitted from one electrode, pass through one base, and are then collected by the opposite electrode. This transmission coefficient is calculated by solving the time-independent Schrödinger equation for the electron wave function Ψ,

$$\left[-\frac{\hbar^2}{2m}\nabla^2 + V_{pot}(V)\right]\Psi = E\Psi, \tag{4}$$

in a one-dimensional potential profile that varies along the x direction, as depicted in Fig. 1(b). More specifically, in the absence of an applied voltage the potential energy $V_{pot}(0) = V_{pot0}$ in the three regions between the two electrodes separated by distance d (vacuum, base, and backbone+vacuum) is steplike and equals to ϕ, $\phi-\phi_B$, and ϕ, while in the presence of an applied voltage V the potential profile acquires a triangular shape $V_{pot}(V)=V_{pot0}-eVx/d$ for $0\leq x\leq d$. Although a triangular potential profile is a simplification of the real situation since it does not include, for example, space-charge effects, the good agreement in Fig. 2 between experimental data and the Fowler-Nordheim model based on a one-dimensional triangular potential model suggests that such a potential describes satisfactorily the reality.

The results of the simulations are represented in Fig. 3 with solid line for A, dotted magenta line for C, dashed red line for G, and dashed-dotted blue line for T. The series of peaks that appear in all *I-V* characteristics indicates the formation of resonant energy levels in the quantum well of the structure represented in Fig. 1(b), the quantum well being the DNA nucleoside that translocates through the nanopore at the moment the electrical measurement is performed. The DNA bases have different current characteristics due to both different work functions and different diameters (different quantum well depths and widths). Since the electrical measurement can be done in a much shorter time than the time it takes for a nucleotide to be pulled through the nanopore (this translocation time is about 1 ms [1]), no confusion about which nucleosides contribute to the signal is possible.

From Fig. 3 it follows that C has a measurable current for significantly lower voltages than the other bases, whereas the current peaks for T, A, and G appear at increasing voltages. Note that a detection method that relies on observing peaks in current rather than differences in current values is potentially more precise. In order to further increase the differences between the electric signatures of the four bases, one can measure their differential conductance. The simulations of this parameter G obtained from the curves in Fig. 3 are illustrated in Fig. 4 with the same line type. As expected, the negative differential conductances take both positive and negative values; the negative peaks of G offering a clear means of identification the four DNA bases.

In real experiments of DNA translocation through nanopores, the obtained signal has fluctuations due to counterions in the solution or those attached to the DNA backbone, as well as due to charge hopping between base pairs [8]. As a consequence, the response is characterized through a standard deviation around the mean value. In carefully engineered nanopores, such as α-hemolysin pores, this standard deviation can be lower than 10% around the mean current value, for all four bases [9]. If we assume that the proposed device works in optimal conditions and that the current has a standard deviation of about 15% then the work functions for the four bases calculated from the STM currents as described previously would have a corresponding standard deviation of about 2% around the mean value given above. (Note that the larger STM current deviation in Ref. [4] includes effects that are not present in nanopore translocation experiments such as nonuniformities of the nucleoside film deposited on the substrate.) If we assume that the probability P of detecting a base has a Gaussian shape centered around the value calculated with the mean ϕ_B, then from Fig. 4 we can identify the voltages V that should be applied on the nanopore device in order to optimally identify the four bases. For example, as suggested by Fig. 4, C can be identified for applied voltages below 6.3 V, for which it is the only base to respond, but it can also be optimally identified for $V=6.75$ V, as shown in Fig. 5(a). In this case, the conductance value associated to C has a maximum normalized probability P (the probability is

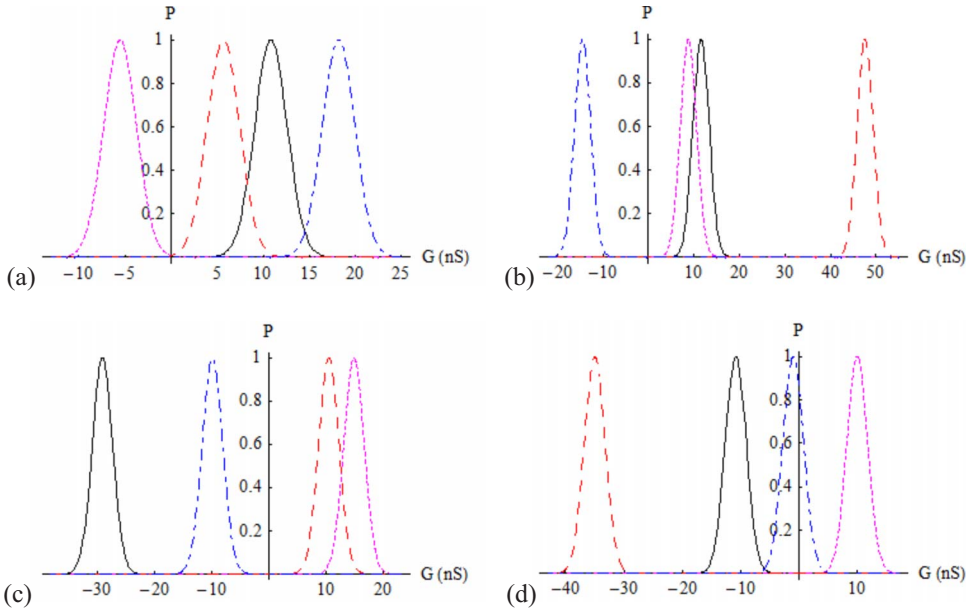

FIG. 5. (Color online) Normalized probabilities of differential conductance values for A (solid line), C (dotted magenta line), G (dashed red line), and T (dashed-dotted blue line) at (a) $V=6.75$ V, (b) $V=7.05$ V, (c) $V=7.15$ V, and (d) $V=7.3$ V.

normalized so that its maximum value is 1) at −6 nS, whereas the conductance values of the other three bases have positive values. The conductance associated with C can take values between −11 nS and 0 nS with lower probabilities due to the fluctuations in the environment and DNA interactions. In a similar manner, T (A, G) can be best identified for $V=7.05$ V ($V=7.15$ V, $V=7.3$ V), case in which the conductances of the four bases take the values illustrated in Fig. 5(b) [Figs. 5(c) and 5(d)]. For these voltages, the conductance of one base has an interval of possible values that is completely separated from the intervals of variations in the conductances of the other three bases. In this way, one can precisely identify each base by sweeping the applied voltage between 6.75 and 7.3 V and calculating the corresponding differential conductances. This interval can be reduced to 6.75–7.15 V because G can also be optimally identified (simultaneously with T) from the measurement at $V=7.05$ V, as shown in Fig. 5(b). The results in Fig. 5 (if C is identified at voltages lower than 6.3 V) show that the identification process of DNA signatures for all bases can accommodate even larger fluctuations than the 15% value considered in the simulations.

The results in this Brief Report show the feasibility of this device for real-time detection of all DNA bases. In specific environmental conditions, the tunnel barrier heights can be modified with respect to the values used in this Brief Report; but the intrinsic distinguishability of the DNA basis should be maintained, as discussed in Ref. [10].

III. CONCLUSIONS

In conclusion, the differential conductance signature, especially for negative values of the differential conductance, is a sure path in the electronic identification of the four bases of DNA. These bases can be precisely identified from the differential conductances at specific applied voltages even if fluctuations in the environment and the DNA interactions are included. We have to note that very recently but in a different context, in a molecular nanodevice containing DNA immobilized across gaps formed by gold electrodes, negative differential resistance was found at room temperature [11].

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