Detection of the O6-Carboxymethylguanine DNA Adduct in Colorectal Cancer Using a Graphene Field-Effect Transistor-Based Biosensors

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ABSTRACT— Efforts to understand genetic diseases and mutations in biological systems are the most important driver of research development in medical and biomolecular sciences. Rapid, sensitive, accurate, and cost-effective biomolecule analysis is particularly important in diagnosis and treatment. The discovery of graphene as a new nanomaterial with a carbon structure with a single atom thickness due to its unique electronic, mechanical, thermal, and optical properties has opened a new topic in research in various biomedical sciences and the production of biosensors for biomolecule analysis. In this research, a biosensor based on a graphene field-effect transistor (GFET) is used to detect DNA with optimal accuracy and sensitivity, which can be a basis for making DNA detection tools. In the studied structure, using non-equilibrium Green function equations and Poisson equation, we study the electron transfer in graphene field-effect transistors. Then, by examining the interaction between nucleotide bases (C, G, A, T) and O6-carboxymethylguanine related to the colorectal cancer DNA sequence to detection of mutation will be identified by GFET, and their binding energy determined.

KEYWORDS: Nano Biosensor, Graphene Field-Effect Transistors, Gene Mutation

I. INTRODUCTION

DNA is a biological molecule that contains genetic information [1]. Analysis and quantification of biomolecules and detection of a specific play sequence in DNA and genetic mutations are critical in clinical diagnosis and treatment, which helps to prevent or cure disease and genetic diagnosis of diseases [2]. One way of diagnosis is optical and electrochemical biosensors, with biomedical applications, which have become increasingly important in recent years [3]. One of the electrochemical biosensors for DNA detection is the graphene field-effect transistor (GFET). Graphene has been widely used due to its excellent electrical [4], mechanical [5], and chemical properties [6]. Regarding electrical properties [7], graphene exhibits exceptionally high electron mobility [8]. In GFET, the conductivity of graphene varies based on the amount of external electric field applied to a FET [9]. An approach for the development of a graphene-based biosensor was described experimentally by Lain-Jong Li *et al.* [10] in which due to the sensitivity of GFET to external electric fields, the sensitivity is achieved by applying probe molecules on the surface of the graphene channel. Cheng Wang *et al.* [11] using Target oligonucleotides and DNA aptamers have reported a conductivity-based graphene nanosensor that can detect unlabeled and specific

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small molecules. Kiana Aran *et al.* [12] discrimination of single-point mutations in DNA via Cas9 immobilized on a GFET has been reported. L. Chen, G. *et al*. [13] have proposed an experimental transistor of GFET with DNA function as a biosensor for quantitative measurement of VEGF165.

In this study, we model a GFET for DNA detection by measuring the current-voltage characteristics. To do that, we solved the non-equilibrium Green function equation and the Poisson equation. Also, we discuss the adsorption of double-strand DNA on graphene using the DFT method and molecular docking and introduce the interactions in the DNA-based GFET device to determine the function of DNA strands on GFET to detect specified DNA and electronic phenomena.

II. Device Model and Simulation Approach

The structure of the modeled GFET device is shown schematically in Fig. 1. The figure shows that a graphene nanoribbon (GNR) with semiconductor properties forms the transistor channel [7]. The oxide layer is considered silicon dioxide (SiO_2). Ultimately, an ohmic contact is made between the channel and each of the reservoirs, and the transistor behaves like a regular MOSFET [14]. The gate electrode is separated from the body and other parts of the system using an oxide layer.

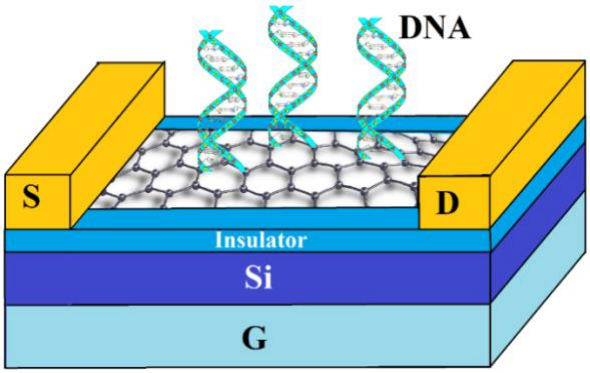

Fig. 1. A schematic view of the GFET sensor.

The source and drain regions at both ends of the channel have a number of 10 unit cells ($N_{s,d}$=10, with a width of 4.26 nm), and the number of cells per channel under the gate region is determined by the N_channel, which examines its various values.

The dielectric layers below the channel are considered to be silicon dioxide with a dielectric constant k=3.9 and thickness of 1.5 nm [15]. The charge and current passing through the channel are determined using quantum transfer conditions using the non-equilibrium working formalism (NEGF), with the nearest neighbor of Hamiltonian and the three-dimensional Poisson conditions, and solving self-compatibility equations [16].

The non-equilibrium Green function, NEGF, is defined by the following equations [17]:

$$G(E) = [(E + i\eta)\mathrm{I} - H - U - \Sigma_s - \Sigma_d]^{-1}, \quad (1)$$

where E is the energy in eV and η assumed to be 10^{-5} eV. H is the tight-binding Hamiltonian of the system. 'I' is the unit matrix, U is the potential energy value, Σ_s and Σ_d are self-energies due to the coupling of the channel with the right and left electrodes (source and drain). Solving Eq. 1, the following relationships are used to compute the electron (n) and hole (p) densities:

$$n = 2e \int_{-\infty}^{+\infty} \frac{dE}{2\pi} G^n(E)$$

$$p = 2e \int_{-\infty}^{+\infty} \frac{dE}{2\pi} G^p(E). \quad (2)$$

where the correlation functions for electrons, G^n and holes, G^p are:

$$G^n = G\,\Sigma^{in}\,G^{\dagger}.G^p = G\,\Sigma^{out}\,G^{\dagger} \quad (3)$$

where Σ^{in} and Σ^{out} are the in-scattering and out-scattering functions of the contacts, respectively. The retarded Green function 'G' is calculated using the recursive Green function algorithm. Finally, a self-consistent approach is used to gain potential, where an initial guess is proposed for the potential energy profile, then a new U is obtained using the Poisson equation. The current of the device is calculated as follows [17]:

$$\mathrm{I} = \frac{2e}{h} \int_{-\infty}^{\infty} dE\, T(E)[\, f_s(E) - f_d(E), \quad (4)$$

where $T(E) \equiv Trace\,[\,\mathbf{\Gamma}_s\, G\, \mathbf{\Gamma}_d\, G^{\dagger}]$, is the transmission function, $\mathbf{\Gamma}_{s(d)}$ is the level-broadening function of the channel due to the

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source (drain) contact, and $f_{s(d)}(E)$ is the Fermi distribution function of the source and drain. The solving process is fully explained in our previous paper [18].

III. INTERACTIONS OF DNA WITH GRAPHENE DEVICE

DNA is a long polymer made from repeating units called nucleotides. There are four types of nucleotides in DNA: adenine (A), guanine (G), cytosine (C), and thymine (T) [19]. Determining the order of the constituent bases of DNA is called DNA sequencing. This sequence will determine the genes, and the genes will determine each person's unique traits [20]. Nucleotide sequences in DNA are altered as a result of a phenomenon called a mutation. Depending on how a particular mutation changes the genetic structure of an organism, it can be harmless, beneficial, or even harmful [21]. In a kind of mutation, only a single nucleotide changes in a gene sequence, so only one codon is affected [12]. Therefore, the identification of mutations is of particular significance.

In order to evaluate the use of graphene in biosensors for DNA detection, in this work, we investigated the effects carry out by DNA adsorption on the electronic transfer properties of a graphene sheet. This study is divided into the interaction between graphene with: (i) nucleotide bases and (ii) DNA sequence.

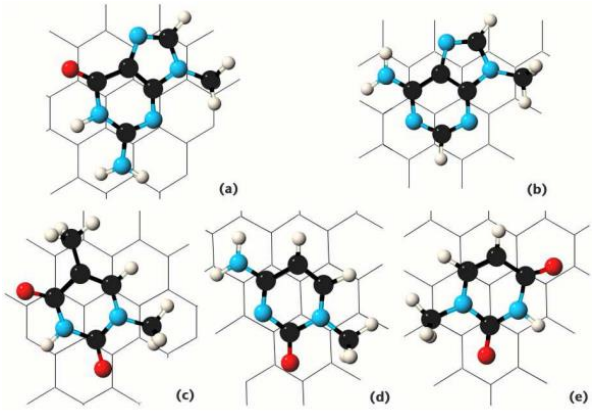

Fig. 2. The schematic interaction between the graphene and DNA bases in equilibrium geometry (a) guanine, (b) adenine, (c) thymine, (d) cytosine, and (e) uracil [22].

To evaluate the GFET performance considering the nucleotide bases (C, G, A, T), the DFT theory and the NEGF are used [23]. The interaction between the bases of the DNA molecule and the graphene plate has shown the reduction of the electrical conductivity due to Fano resonances. So, the T, G, A, and C bases, which are absorbed on the surface of a graphene ribbon, reduce the electric current of the graphene strip. To calculate the adsorption energy (the minimum interactive energy), E_{abs} (eV), and adsorption distance, d_{ads} (Å), first, an initial geometry with structure optimization is investigated. Later, in order to find the most stable system geometry, the interaction energy, E_{int}, between adenine (A), guanine (G), cytosine (C), or thymine (T) on the graphene sheet at different heights and orientations parallel to the graphene sheet was calculated using the following equation [23]:

$$E_{int}(h) = E_{sub-ads}(h) - E_{ref}, \quad (5)$$

where $E_{sub\text{-}ads}(h)$ is the energy of the adsorbent system for each distance (h) and E_{ref} is the total energy when the interaction between the substrate-adsorbate system is negligible. The basic interaction between DNA bases and graphene is shown in Fig. 2 [22].

Likewise, when considering a DNA sequence, it will be complex to understand the interaction between graphene and the DNA sequence [10]. Here we used molecular docking simulation to analyze the binding of graphene with the DNA sequences [24]. Molecular docking is one of the key tools in the structural study of biomolecules and drug design. The purpose of protein-ligand docking is to predict the proper binding site of a ligand to a protein with a known three-dimensional structure. When a molecule is attached to another molecule, to form a complex, the molecular docking method can predict the proper orientation of the molecule as well as the strength of the bond between the two molecules [25]. For this purpose, the structure of these two molecules must be known. Finding the best ligand orientation relative to the active site of the acceptor and estimating the binding energy are two important aspects of the docking algorithm, the software we can use is Autodock [26].

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For instance, colorectal cancers are caused by lifestyle and nutritional factors, aging, and genetic disorders. Premutagenic lesions of O6-methylguanine) O6-MeG) and O6-carboxymethyl guanine (O6-CMG), shown in Fig. 3(a)., commonly occur in human colorectal cancer cell lines [27]. One of the pathways that can lead to the formation of such lesions includes the initial nitrosation of amino acids such as glycine and its derivatives. N-nitrosoglycine is transformed into diazoacetate or a lactone, which powerful mutagen capable of alkylating guanine in DNA to produce O6-CMG and O6-MeG. Evidence suggests that O6- CMG primarily causes G: C→A: T transition mutations (Fig 3(b)). According to the contents here, we are faced with two structures, O6-CMG and O6-MeG. To distinguish each of these structures, the GFET is proposed in this work. Once the binding energy between the different structures of DNA interaction and the GFET is determined, the structure will be distinguished by current variation.

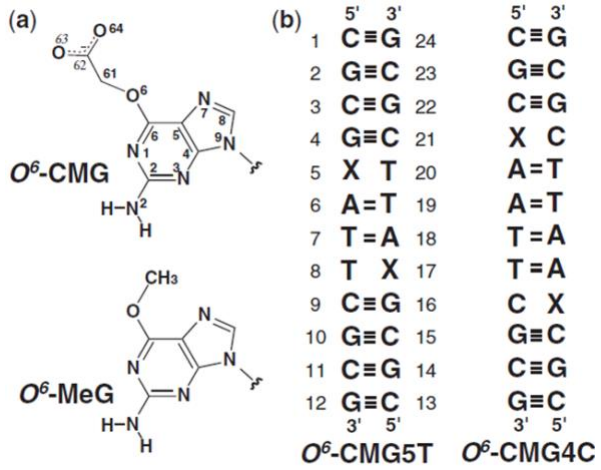


Fig. 3. Chemical structures of O6-CMG and O6-MEG, DNA duplexes (b). X indicates O6-CMG [26].

IV. Results and Discussion

We selected a graphene Nano-ribbon with a number of 13 unit cells, a width of 4.26 nm, and an armchair edge. The energy gap for this ribbon is about 0.72eV, which is suitable for a transistor application. The channel lengths have different values, N_channel=20, 22, and 24, and $N_{S,D}$=25, with considering the fixed amount of impurity atoms with doping 0.005 with a molar fraction of 0.01 (number of impure electrons per carbon atom). For a given channel length, gate voltage, and drain voltage, we solve the Green and Poisson equations self-consistently and obtain the potential along the channel [18]. The potential profiles for set V_G=1.6 V with a channel length of N_channel=24 is shown in Fig 4. Using the obtained potential in Hamilton, one can obtain the current [18].

The drain current increases linearly with the drain voltage and then saturates for a given gate voltage and channel length. As the transistor's behavior in sensing the DNA is important at different temperatures and channel lengths, it is necessary to examine the behavior without considering the DNA.

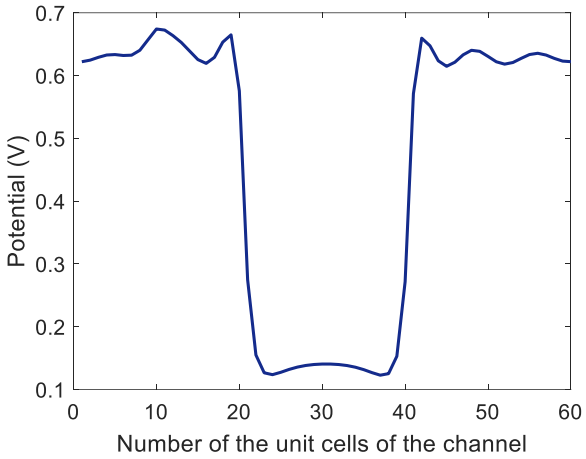
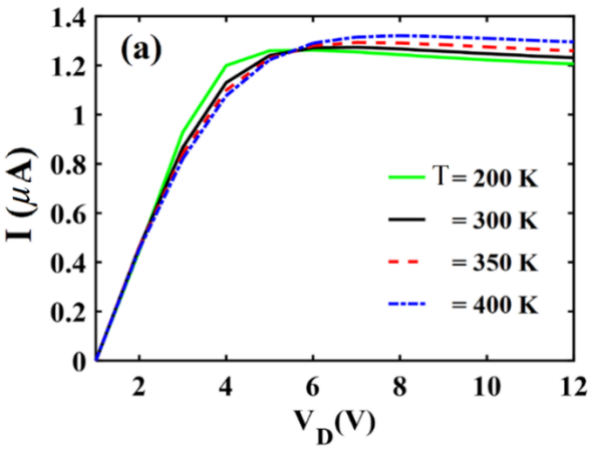


Fig. 4. The potential profiles for GFET with a channel length of N_channel=24.



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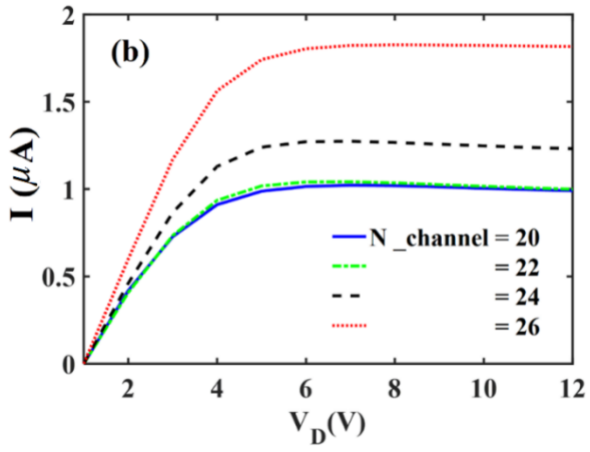

Fig. 5. The I–V characteristics of GFET in the absence of DNA, and V_G=1.6V, for different a) temperatures 200 to 400 K and b) channel lengths, at T=300K.

For different temperatures of T=200-400 K and different channel lengths N_channel=20, 22, 24, 26, the I-V diagrams are shown in Fig. 5. As depicted in the figure, the device is not so sensitive to the temperature and that means it can work as a sensor at different temperatures. Also, GFETs with longer channel length has higher saturated drain current.

Given the proposed nanostructure and nucleotide dimensions, the bond energy between each base (A, T, C, G) with graphene is obtained and shown in Table 1.

Table 1. Interaction Energy on Graphene-Nucleotides [23]

System	Interaction energy (Kcal/mol)
Graphene + Guanine	-22.49
Graphene + Adenine	-20.31
Graphene + Cytosine	-18.94
Graphene + Thymine	-18.40

Knowing the interaction Energy of Graphene-Nucleotides, one can calculate the drain current of the GFET. DNA sequencing acts as the negative gate voltage and decreases the current density and the conductivity of graphene. Figure 6. shows the effects of different nucleotides on the I-V characteristics of the GFET.

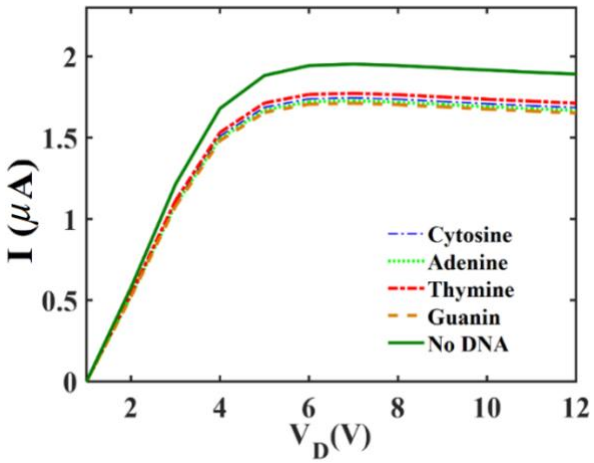

Fig. 6. The I–V characteristics of the GFET at V_G=1.6V, and N_channel=24, at T=300K in sensing of nucleobases (A, T, C, G).

To use the GFET to detect the DNA sequence of colorectal cancer and its mutation, the binding energy of O6-CMG (DNA1) and O6-MeG (DNA2), with graphene are calculated and depicted in Table 2.

Table 2. The interaction energy of the graphene with DNA1 and DNA2.

system	Interaction energy (Kcal/mol)
Graphene + DNA1	-44.2
Graphene + DNA2	-27.6

Using the interaction energy of the DNA1 (non-mutated) and DNA2 (mutated) with graphene, the I-V curve of the GFET with a channel length of 24, and at T= 300K is calculated and shown in Fig. 7. As expressed in the figure, there is about a 25% difference in the saturated currents of the cases without DNA, and with DNA1. Also, the saturated current difference of GFET between the mutated and non-mutated DNA is about 10%. This means these two cases can be distingue using GFET sensors easily.

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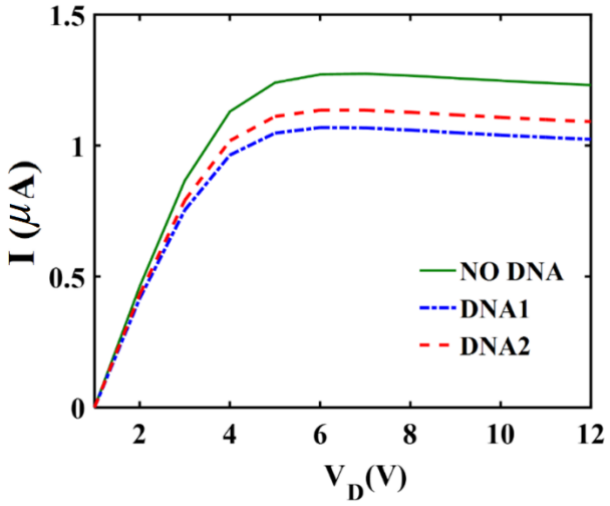

Fig. 7. The I–V characteristics of the GFET at V_G=1.6V, T=300K, and channel length N_channel=24 in the presence and absence of DNA1 and DNA2.

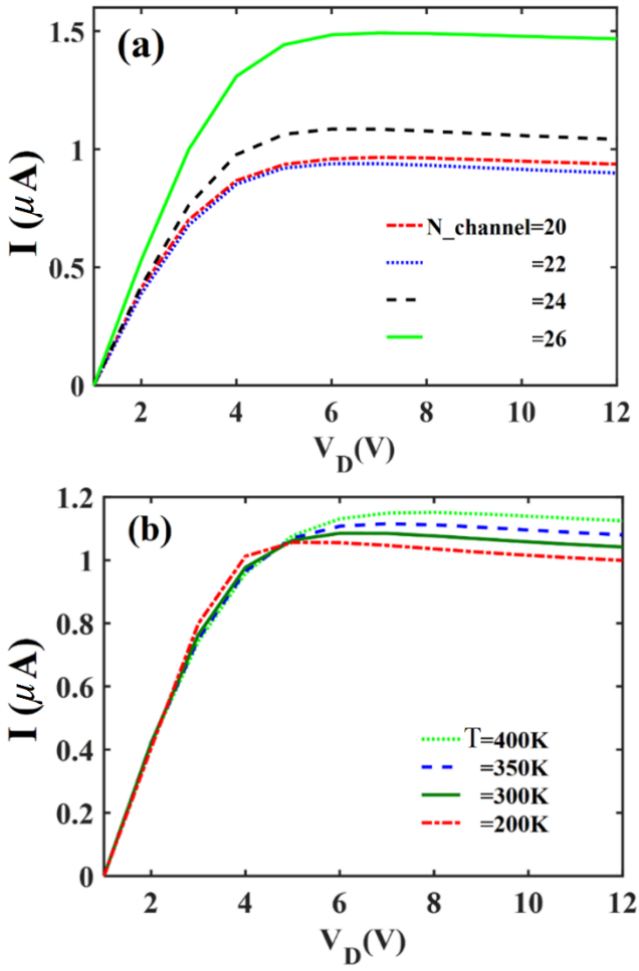

Fig. 8. The I–V characteristics of the GFET at V_G=1.6 V and in presence of DNA1 for devices with a) different channels N_channel=20, 22, 24, 26, at T=300K and b) with channel length N_channel=24 at different temperatures.

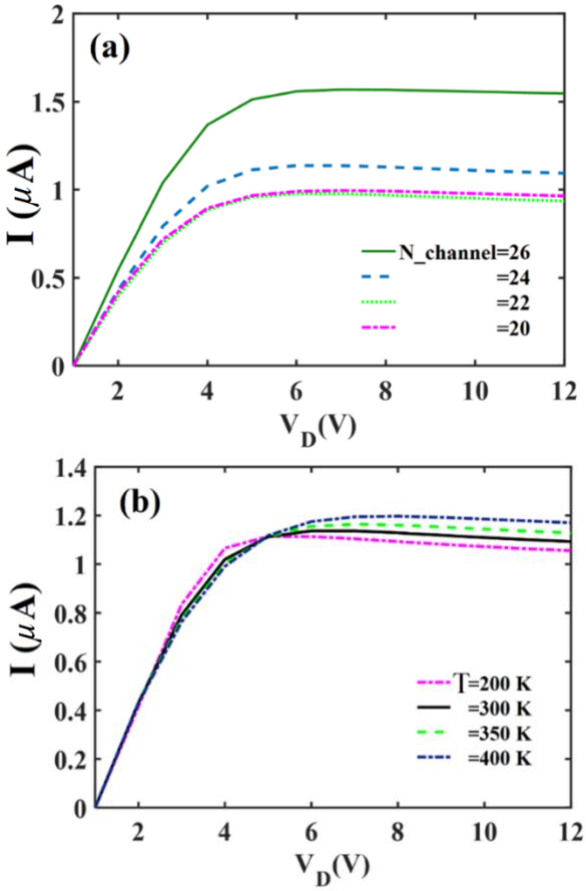

Fig. 9. The I–V characteristics of the GFET at V_G=1.6 V and in the presence of DNA2 for devices with a) different channels N_channel=20, 22, 24, 26, at T=300K and b) with channel length N_channel=24 at different temperatures.

The effects of the presence of DNA1 in GFETs with different channel lengths and at different temperatures are analyzed. The comparison of drains current in Figs. 5 and 8 for GFETs with different channel lengths shows that there is at least a 10% difference between the same devices with and without DNA1. Also, the increase in the channel length makes the differences higher. As shown in Fig. 8(b), the changing of the temperatures does not change the difference between the current of the devices with and without DNA.

In Fig. 9, The I-V characteristics of the GFET in presence of DNA2 for devices with different channel lengths, N_channel=20, 22, 24, and 26, and also for different temperatures are expressed. The current behaviors are as same as DNA1 in Fig. 8.

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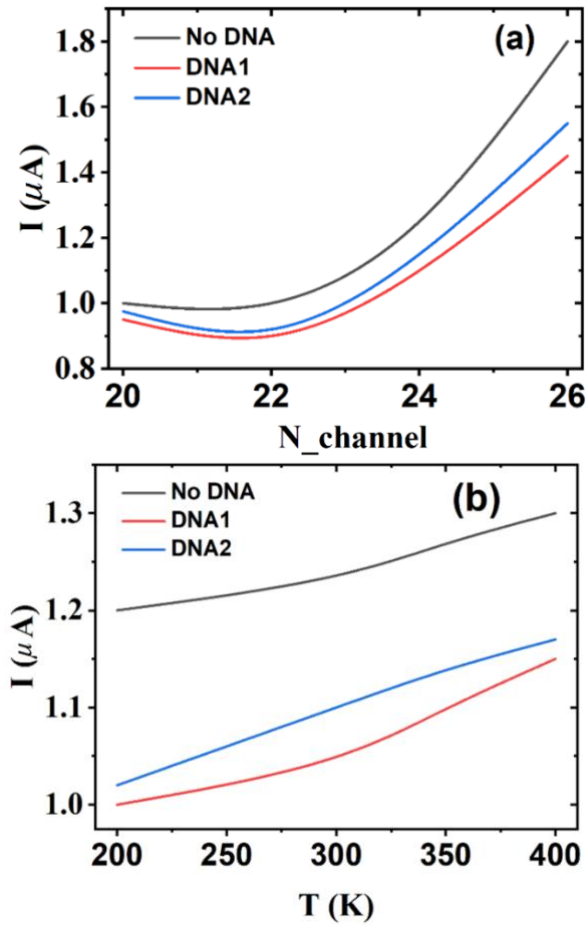

Fig. 10. The saturated drain current of GFET at V_G=1.6V in the absence and presence of DNA1 and DNA2, a) for different values of channel length at room temperature, b) with a channel length of 24, for different temperatures.

In Fig. 10 the saturated drain current of GFET at V_G=1.6V for different values of channel length and different temperatures in the absence and presence of DNA1 and DNA2 are expressed. Fig. 10(a). shows the current for the gate length of 20 to 26 at room temperature. As depicted in the figure, by increasing the gate lengths, the difference between the current for GFETs without DNA and with DNA1 (DNA2) increases from about 0.05 to 0.25 μA (0.35 μA). The 0.1 μA is the current difference between one base mutated and non-mutated DNA. For a large number of DNA bases, the current difference can be high enough to measure. It should be mentioned that we have a distinct DNA single-stranded probe (non-mutated and mutated various of a desirable gene). The designed device is sensitive to our selected sequence of DNA (gene) and can distingue between mutated and non-mutated samples. In a realistic situation, our probe only pairs with its complementary sequence. So, the mutated probe only detects mutated genes in the mixture of sample DNA.

The changing temperature does not change more the difference between the mutated and non-mutated currents (Fig. 10(b)) for different channel lengths, which means the GFETs can work as DNA sensors at different temperatures properly.

V. Conclusion

In this article, we modeled a GFET for the Detection of the mutation of O6-carboxymethylguanine related to colorectal cancer by measuring the current-voltage characteristics. To calculate the current, the non-equilibrium Green function equation and the Poisson equation are solved self-consistently, and the binding energy of double-strand DNA on graphene is obtained using the DFT method. The presence of DNA on the graphene layer changes the graphene conductivity and acts as a gate voltage. As DNA with a different mutation has a different sequence of nucleotides (C, G, A, T), it can change the graphene conductivity differently. There is almost a 25% difference in the saturated currents of the GFETs without DNA and with DNA1. Also, there is about a 10% difference in the saturated currents of the GFETs with DNA1 and DNA2. Increasing the channel length makes the device more sensitive and the differences between two currents (with/without DNA) is being higher. The changing of the temperatures does not change the sensitivity much.

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