Graphene and Graphene Oxide Based Biosensor
For DNA Detection

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Contents

1 Introduction 2

2 Graphene Family Material 3

3 Material Properties Related to DNA Detection 5
  3.1 Surface area ................................................................. 5
  3.2 Surface Chemistry ........................................................... 6
  3.3 Electrochemistry ............................................................. 8

4 Graphene-Based Sensors 9
  4.1 Electrochemical DNA Sensors ............................................. 9
  4.2 Electronic Sensors ............................................................ 11
  4.3 Optical DNA Biosensors ................................................... 13

5 Conclusions and Outlook 15

Abstract
Owing their extraordinary electrochemical, electronic and optical properties, Graphene-based materials show huge potential to fabricate the biosensor to detect DNA (cDNA, ssDNA, dsDNA). There are three main types of graphene DNA sensors, electrochemical, electronic and optical. In this review, I will survey properties and applications of variety novel graphene DNA detecting sensors and offer insights on the underlying DNA detection mechanisms.

1 Introduction

High selective, rapid, fast responding and cost effective DNA detection biosensor is expected in disease diagnosis and treatment, cancer detection and gene mutation in recent years. Using nanomaterial to fabricate the biosensor drove the scientists attention.\(^{1-3}\) Graphene-family material (especially for graphene and graphene oxide) is really interesting in this type biosensor assembling due to its extremely high surface area, exceptional electronic and electrochemical properties.\(^4\) In this article, I aim to survey properties and applications of variety novel graphene DNA detecting sensors and offer insights on the underlying DNA detection mechanisms.

As a famous type of two-dimensional nanomaterial, graphene was first discovered in 2004 and won the Nobel Prize in 2010. The typical unique nanostructure is two-dimensional carbon sheet with honeycomb-like arrangement of atoms, shown in figure 1. Due to this special nanostructure, graphene owes the attractive electronic and electrochemical properties. It shows impressive carrier mobility and carrier density at room temperature.\(^5\) The electrochemical potential of graphene is 2.5 V in 0.1 PBS which is higher than most of the carbon based material.\(^6\) All those promising properties drove the attention of the scientists and companies on the work of developing electronic, electrochemical and optical DNA detection sensor based graphene-family material. How the DNA interacting with the graphene is also vary important in DNA detection. In this review, I will summarize the varies DNA detection sensors based on graphene family material and the involved interaction between
DNA sequences and graphene material. Some methods of the functionalization of graphene material could improving the performance of the sensors, which also will be mention in this article.

## 2 Graphene Family Material

As developing in the past decade, there are plenty of graphene-family material having been fabricated for variety applications. Graphene, few-layer-graphene (FLG), graphene oxide (GO) and reduced graphene oxide (rGO) are most advanced for bio-detection application.

Single layer graphene was first isolated from graphite using special adhesive tape in 2004 by a research group from Mechester University led by Geim. Then there are two main technologies becoming vary popular in graphene production, repeated mechanical exfoliation of graphite flakes and growth by chemical vapor deposition. The chemical process to fabricating graphene shows better biological performance might due to larger substrate areas than the other ways. The typical graphene nanostructure is already mentioned above.(shown in figure 1) While there are still some problems for pristine graphene that it can not be suspended in aqueous solution vary well, which is important in biological applications. Therefore, the other types of the graphene-family material are more interesting for DNA detection which will discuss in later sections. Few-layer-graphene was found as byproduct of the fabrication for pristine graphene. It is general that these ultrathin graphene film, 1–10 layers, on crystalline transition metals substrates might introduce some contamination into the products might showing unexpected thermal expansion or electronic behaviors.

Graphene oxide(GO) is a highly chemically modified graphene which containing oxygen functional groups and the chemical atom analysis shows the ratio of carbon to oxygen be around three to one. It is one of the most admired graphene nano-material for biological applications in recent years because it could be made as a stable, homogeneous GO aqueous suspension, which is really important in DNA detection field. Just like in figure 1, as a
highly oxidized product of graphene, the surface structure of GO is consists of a single layer graphene with oxygen functional groups such as carboxylate acid, epoxy and alcohol groups on the periphery and the carbon to oxygen ratio have been analyzed to be around three to one. Those functional groups provide pH dependent negative charge and colloidal stability.\textsuperscript{10–12}

There are also unmodified graphenic domains are hydrophobic and capable showing the interactions which both relative to DNA absorption.\textsuperscript{13} This could stabilize the DNA molecules in the solution which is a really crucial property for DNA detection. The layer number distribution of the GO sample sometimes have influence the performance of the sample, while there are seldom papers reported the detailed mechanism. There are three main methods to product GO are Brodie, Staudenmaier and Hummers Methods.\textsuperscript{11} All those methods are involved in introducing the way to oxidized the graphite via different oxides. For example, in Brodie and Staudenmaier methods, they use a oxide mixture of potassium permanganate and sulfuric acid.\textsuperscript{14} These ways of chemical exfoliation from graphite to GO provide outstanding process to produce a stable homogeneous GO aqueous suspension which could use to fabricate continuous high quality thin film. And also the large amount of chemically active oxygen defects give the sample the possibility of chemical functionalaized treatment to improve the biological application.
So called reduced graphene oxide has the surface that oxygen functional groups on original GO are partly removed by chemical or physical treatment. The main purpose of this reduction process is to restore the p-conjugated structure and the electrical conductivity. Even though the remaining functional group on the surface of rGO might lower the electronic properties via reducing the conductivity, the electronic performance of rGO-based biological sensors are benefit from the enhanced interaction between the remaining functional groups and the analyte.\(^\text{15}\) Thus the promising electrical conductivity and the chemical active defects make the rGO as a vary attractive material using for fabricate the electronic DNA sensors.\(^\text{1,2}\) The general reducing conditions for producing rGO is including two types of treatments, high temperature thermal treatment and chemical treatments with the reducing chemicals.\(^\text{16}\)

3 Material Properties Related to DNA Detection

As we mentioned above, there are some properties of the graphene-based material have huge influence on the performance of the graphene-based biological sensors. In this section, we will summary how those properties related to the biological application.

3.1 Surface area

In figure 1, the fundamental surface structure of graphene and graphene oxide are shown. For monolyer graphene, each carbon atoms are sp2-hybridized and are assigned on the surface by layers. All those atoms are exposed outside. According to the BET test results, the specific surface area of graphene could reach to 2630 m\(^2\) which is vary high comparing to other carbon-based material.\(^\text{11}\). This is at least one order of magnitude higher than other types of the nanomaterials.\(^\text{17}\) As for graphene oxide, 600–900 m\(^2\) g\(^{-1}\) of surface area was estimated. This value of surface area roughly close to the theoretical value which is 890 m\(^2\) g\(^{-1}\), even though it is depended on the degree of oxidation of the GO and also on its aggregation.
level.\textsuperscript{18} The BET surface area measurements for reduced graphene oxides yielded 466 m\textsuperscript{2}/g which is related to the degree of GO exfoliation before the reduction. There is an potential assumption being given to explain the decline of the surface area from graphene to GO and rGO that the aggregation of the graphene oxide upon the reduction.\textsuperscript{19}

\section*{3.2 Surface Chemistry}

The surface chemistry situation for those graphene-based material are different even before the functionalization due to the various surface structures and areas as we mentioned above. Therefore, They demonstrated particular properties of surface-interaction among small gas molecules to large biomolecules. There are two types interaction, covalent interation and non-covalent interaction. In my work, I mainly focused on the non-covalent interaction which is related to the DNA detection.

The surface of natural graphene is hydrophobic and the material has no significant solubility in most solvants.\textsuperscript{1,20} To get the possibility to fabricate the graphene-based devices in soluble way, some scientist established some methods to fuctionalize the surface via non-covalent interaction or covalent reaction to introduce some hydrophilic groups. The most general method based on covalent reaction in terms of increasing the solubility involves the oxidation of graphene to introduce the oxygenated species like carboxyl, epoxy and hydroxyl groups on the surface of graphene producing graphene oxide.\textsuperscript{20} One drawback of this method is the level of oxidation on surface can not be controlled.\textsuperscript{21} The non-covalent interactions of graphene are formed based on van der Waals forces or – stacking.\textsuperscript{20,22} One of the benifit of this type of interactions is that the surface structure of pristine graphene is preserved not like the covalent interaction cases. From small gas molecules to large molecular weight biomolecules, they all could be absorbed on the surface of graphene.\textsuperscript{23} Here we main focus on the DNA-graphene interaction. The nature of the interaction between the material surface and DNA bases are not trullly understood, it is still considered as non-covalent interaction. Several types of forces are studied and regarded as the main contribution of the interaction
including – stacking, electrostatic, van der Waals, and hydrophobic interactions. Among all these forces, – stacking is contributed the most which explained why single-stranded DNA shows stronger binding to graphene than double-stranded DNA where the intramolecular hydrogen bonding of bases are shown. In addition, The binding strength between the different bases with graphene surface vary as the difference of their polarizability.  

As product of graphene oxidation, graphene oxide shows better dispersability in variety solvants due to the surfaces of GO contains hydrophilic regions, by which means that the hydrogen bonding between surfaces and biomolecules or metal ions could form. Just like we mentioned above, the surface of GO could be viewed as two types of domains, oxidized regions that the carbon atoms are sp3 hybridized bonding with oxygen functional groups and the unoxidized regions that the carbon there are sp2 hybridized. One of the most important reaction of GO is its reduction because the product reduced graphene oxide shows higher electrical conductivity than GO. This reduction process could be accomplished via chemical, electrochemical or thermal reduction methods. And also due to high reactivity of chemistry of GO, there are many ways to functionalize the surface though chemical reactions which gives many possible modified structures utilizing for DNA detection and also other molecules binding shown in figure 2. For example, Lu and his coworker reported that they functionalized the GO surface with oligonucleotide molecular beacon (MB) which increasing the separation ability of ss DNA and dsDNA. This will talk more specifically later. GO demonstrates the balance between the reasonable dispersability in solvants and relative high non-covalent interaction domains, making this material as promising platform for high sensitive and selective detection of DNA as I talked. The general mechanism of DNA absorption on GO surface is roughly like for graphene, while the oxygenated species on the GO’s surface contribute to the binding too. But still there are some works indicated that they found the proof of DNA chemical grasping on GO. Hydrophobic forces and -stacking play the most crucial roles in the interactions which can overcome the electrostatic repulsion. After binding different types of DNA, the whole material demonstrates differ-
ent electrochemistry, electronic conducting or optical properties which could be detected via some specific measurements.¹

![Figure 2: Schematic illustration of GO-based electrodes for electrochemical applications. Copyright 2010 C. Da Chen.](2.jpg)

### 3.3 Electrochemistry

One popular type of DNA sensor is known as electrochemical sensor which is fabricated based on the detection of the change for electrochemistry properties after DNA absorption.¹ Therefore, to understand the electrochemistry properties of graphene-based material is important. It is possible to investigate the electrochemistry properties based on the results from the enormous amount of publishments on graphite and carbon based nanotubes. Because it was shown that there is no difference of electrochemistry properties from SWCNTs to graphite.³¹ Graphene shows a wide electrochemical potential of ca. 2.5 V in 0.1 M PBS (pH 7.0), which is comparable to the graphite.⁴ The charge-transfer resistance on graphene is much lower that of graphite electrodes.³² As for GO and rGO, they both exhibit high electrochemical capacitance with excellent cycle performance and rGO shows even higher electrochemical
capacitance and cycling durability than carbon nanotubes (CNTs). The specific capacitance was found to be 165 and 86 F/g for rGO and CNTs, respectively. Cyclic voltammetry of graphene oxide sheets exhibits significant reduction waves starting at 0.60 V (vs. Ag/AgCl reference electrode), reaching a maximum at 0.87 V.

4 Graphene-Based Sensors

With all these merits of graphene-based material, they have become crucial candidates for DNA sensing application in liquid situation. As I mentioned above, the variation of the amounts of different DNAs on the material surface will change the electrochemical, electronic, or optical behaviors which could be detected via some technologies to achieve the sensing job. Here, we provide the review of the sensors in three categories based on the parameters they detected, which are electrochemical, electronic and optical DNA sensors. Furthermore, many derivation graphene-based material obtained from the functionalization through the formation of donor-acceptor complex, these could improve the DNA sensor performance. The nanoparticles, organic compounds and biomolecules are utilized for functionalization.

4.1 Electrochemical DNA Sensors

Graphene has a large electrochemical potential window of ca. 2.5 V in 0.1 M PBS (pH 7.0) in solution, therefore the detection of the molecules with either high oxidation or reduction potentials becomes achievable. Graphene-based modified electrode is used for detection of DNA hybridization for electrochemical changes. This type of DNA sensors offer really fast response speed, high sensitivity and finest selectivity for the specific DNA hybridization. The main mechanism of novel electrochemical DNA sensor is that the DNA duplex (between the probe and target DNAs) formed from the ss DNA grasping on the electrode surface, which is labeled with an electrochemical indicator to recognize it. This immobilization of DNA onto the surface caused an increase in the electrochemical impedance value, a further increase in
electrochemical impedance value is observed after the hybridization. An interesting work demonstrated by Wang’s group that a supersandwich structure of biosensors which showed high sensitivity, the detection limit is 100fM. The capture probe modified electrode was denoted as cDNA/Au NPs/rGO/GCE where thiol is labeled at DNA.

Figure 3: Schematic illustration of the experimental protocol. The platform can easily load the ssDNA probe and the detection mechanism is affected by the ratio of polyaniline and graphene (P/G). (above) ds-DNA releases from the platform when the mass ratio of P/G is less than 1/20, (below) ds-DNA remains on the platform when the ratio of P/G is larger than 1/10.

But there is a disadvantage of this type of label-needed sensors that the electrochemical labels are involved making the architecture kind complex and the non-direct way to detect target DNA decreasing the sensibility. Furthermore, the intrinsic electrochemical activity of the nucleobases (primarily purine) at the GO-based electrodes provides the potential application for the label-free electrochemical detection of nucleobases. This new type of label-free DNA sensors has been proved to be one successful method to directly detect the target DNA which requiring no labeling process or external indicators. Generally, the multilayer composites architecture is used here. Peng and his coworker reported a high
sensitive label-free electrochemical DNA sensor interface based on Au nanoparticles/toluidine blue-GO composites thin film for the effective detection of MDR1 gene. The peak current was straightforward related to the concentration of the target DNA from $1.0 \times 10^{11}$M to $1.0 \times 10^{9}$M, with a detection limit of $2.95 \times 10^{12}$M. Ping and his coworker fabricated dynamic P/Gratio-based DNA sensor by deposition of polyaniline and pristine graphene nanosheet (P/Gratios) composites in different mass ratios, DNA probe and bovine serum albumin (BSA) layer by layer on the surface of a graphene-based electrode. It was capable to detect C-DNA in a range from $0.01$ pm to $1$ m through changing ratios of polyaniline to graphene and SNPs are also detectable for such sensor. The illustration of the detecting process is shown in figure 3.

### 4.2 Electronic Sensors

The single layer graphene is a semi-metal with high carrier mobility ($20,000$cm$^2$ V$^{-1}$ s$^{-1}$) and carrier density $10^{-13}$cm$^{-2}$ that are very promising for fabrication of electronic sensors. Graphene electronic sensors are generally referred as Field effect transistor (FET) which mainly applied its field-effect characteristics. The FET relies on an electric field to control the shape and conductivity of a channel of one type of charge carrier could based on graphene-based material. There are several principles could use to explain how this type FET working as sensors. One general guess is that the graphene conductance can be sensitively inflected by minute gating signals. Some other claimed that the doping effects, charge carrier scattering, or change of local dielectric environment could also be used to realize the graphene-based electronic detection. Mohanty and his coworker reported an electronic DNA sensor based on a microsized graphene oxide sheet. The probe ss DNA was physically absorbed on the GO sheet and the conductance of GO increased which means that the hybridization of the target ssDNA as a result of doping effect under dry condition. In addition, they also demonstrated that the hybridization of a pair of target and probe ss DNA produced hole doping effect. Demonstrated another assumption on other electronic sensor that the detection
(decrease of graphene conductance) is based on DNA induced n-doping on graphene instead of the field-effect and impurity screening mechanism.\textsuperscript{2}

![Figure 4: Schematic illustration of the graphene FET device operated by liquid gate\textsuperscript{37}](4.jpg)


Recent years, the operation of GFET sensors in aqueous condition has promoted for biosensing. Zheng and his coworker developed a novel PNA-functionalized G-FET biosensor based on CVD grown monolayer which could avoid the contamination and large number of defects on graphene surface. The author claimed that this device was used for the directional technique and high-affinity PNA-DNA hybridization for ultrasensitive, label-free, and highly specific detection of DNA. It showed a great sequence-specific affinity to target DNA and achieved an excellent DNA detection sensitivity as high as 10 fM.\textsuperscript{38} Chan and his coworker presented a graphene-based bio-field-effect transistor (bioFET) for the detection of avian influenza A virus subtype H7 gene based on CVD graphene and AuNPs. The scheme of the bioFET device is shown in figure 4. The probes conjugated to the AuNPs were applied to hybridize with the target gene in a sandwich assay format for signal amplification. This biosensor demonstrated lower limit of detect of 64 fM, which is the lowest record for graphene-based for DNA detection.\textsuperscript{37}
4.3 Optical DNA Biosensors

As I mentioned above, graphene oxide exhibits outstanding optical properties. Unlike the zero-gap graphene material, GO can fluoresce at a really wide range of wavelengths, from near-infrared to ultraviolet.\textsuperscript{33} This makes GO becoming a promising candidate as a fluorescence label for DNA detection. And also, GO is capable of quenching fluorescence. The quenching efficiency of GO is preferable than the traditional organic quenchers. Therefore, GO can play either a donor or acceptor role in fluorescence energy transfer (FRET).\textsuperscript{33} Some claimed that the ratio and type of its oxygenation of the surface could be used to control the optical characteristics of GO.\textsuperscript{39}

![Figure 5: Three possible mechanisms of hybridization between a probe DNA adsorbed by GO and its cDNA (target DNA). The oxygenated groups and defects on GO are not drawn for clarity of the figures. In all the cases, the probe DNA with a fluorophore label is preadsorbed and the cDNA is added afterward. The tendency of GO adsorbing ds-DNA is lower than that of the adsorption of ss-DNA. (A) Langmuir-Hinshelwood mechanism. (B) Eley-Rideal mechanism. (C) Displacement mechanism.\textsuperscript{40} Copyright 2013. Biwu Liu.](5.jpg)

An novel GO-based DNA biosensor was exhibited by Fei Liu and his coworker. The GO sheets were applied in an array format to recognize the specific target DNA-DNA hybridization.
bridization interation. When the probe DNA bond to the surface of GO is hybridized with the AuNPs labeled complementary DNA strand, then the fluorescence emission intensity of the GO is decreased remarkably. In this case the GO served as the energy donors and the AuNPs played the role in FET as the energy acceptor. There are still some cases that the GO is treated as quenchers. One example is from Wang that the planar GO surface of the sensor allows simultaneous quanching of multiple ssDNA probes labeled with different dyes leading a multicolor sensor for the detection of multiple target DNA. Lu and his coworker demonstrated similar principle works with dye-labeled DNA. A really interesting work did by Liu and his coworker was to determine a possible mechanisms of how DNA hybridization took place in the presence of GO via a fluorophore-labeled DNA probe with experimental proof and exhibited some other mechanisms. If the interaction based on the LangmuirHin-shelwood mechanism, the cDNA is also adsorbed followed by diffusion through the pathway on GO. When the cDNA achieves a probe DNA, a duplex is formed on the GO surface and then desorbed like in figure 5a. Another possible mechanism is the leyRideal mechanism, where the adsorbed probe DNA directly reacts with the target cDNA that is dissolved in the solution phase at the GO/water interface like in figure 5b. And another mechanism shown in figure 5c is also impact on the whole process, that some of the probe DNA could be displaced by the target cDNA into the solution phase to hybridize with the free cDNA in solution. For DNA detection, Huang and his coworker exhibited a GO based optical biosensor for DNA single-base mismatch study. By applying a 40-mer probe DNA (P1), both short (20-mer) and long (60-mer) targets led to much lower fluorescence signals than the complementary target T1 that was of the same length as P1. But still the exact mismatch location of the target DNA still cannot be determined via this way.
5 Conclusions and Outlook

Graphene series material become really promising candidates for a wide range of application from physic, chemistry to biography in a short history. As I reviewed above, the DNA sensors fabricating using graphene-based material especially graphene oxide exhibite really outstanding performance with many merits like high sensitivity and selectivity, wide detection range, quick responsibility and low cost. Each type of the DNA detection sensor demonstrates outstanding behavior to recognize varieties types of DNA strand with relative low concentration requirement.

But the exact mechanism of the each type of DNA sensor is poorly understood and critical experimental proof are lack. For the future development, how to solve the problems about graphene material production with highly controllable and scalable and to construct more efficient and direct detection structure of devices become more crucial. In witness the development of the graphene-based DNA sensors, we could predict that they will turn into a important series of DNA sensors in industry field someday in sooner future.

References


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