

Functionalization of Graphene and Graphene Oxide for Biosensing and Imaging

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Abstract—Recent advances in our group about graphene bioapplications are discussed. In particular, the functionalization of graphene and graphene oxide, biosensing and bioimaging by using graphene-based nanomaterials, and some fundamental studies of graphene and graphene oxide have been summarized.

I. INTRODUCTION

Graphene, emerging as a single-layer carbon crystal, is attracting increasing attention from the physical, chemical, and biomedical fields as a novel nanomaterial with many exceptional features including excellent electrical conductivity, high surface to volume ratio, remarkable mechanical strength, and biocompatibility.[1-3] Recently, functionalized graphene has been used in many biomedical and bioassay applications and shows promising potentials in these fields [4]. In our group, we have successfully prepared functionalized graphene and graphene oxide containing a variety of features with different specificities, and further utilized them for biosensing, bioimaging, and many other applications as well as fundamental research. This paper selectively summarizes these investigations conducted in our lab including descriptions of how such graphene and graphene oxide can be functionalized, and how such functionalized nanomaterials can be used for a variety of applications.

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II. MATERIALS AND EXPERIMENTAL

A. Electrochemical Experiments

Cyclic voltammetric (CV) and amperometric measurements were performed with electrochemical instruments (CHI620 and CHI 824, Taxis). A three-electrode system consisting of glassy carbon electrode, Ag/AgCl electrode, and Pt wire served as the working electrode, the reference electrode, and counter electrode, respectively. A 10 mL electrochemical cell was used for all electrochemical experiments. Cyclic voltammetric measurements were performed at the potential range of 0-1.0 V with a 50 mV/s scanning rate. For amperometric experiments, the electrode was held at a designated potential and the solution containing analytes was injected until the baseline became stable and flat.

B. Graphene-DNA Assay

Samples were spun and separated in an Eppendorf 5804 centrifuge (Eppendorf, Germany) and images recorded with a Sony a-300 digital camera (Sony, Japan). Fluorescence and anisotropy measurements were recorded on a Tecan Safire 2 microplate reader (TECAN, Switzerland). DNA agarose gel electrophoresis was powered with a Bio-Rad Power PAC 300 (Bio-Rad, Hercules, CA) with gel images recorded on a NucleoVision imaging workstation (NucleoTech, San Mateo, Calif).

Graphene sheets were produced in mass quantities starting with the chemical oxidation of graphite flakes into graphite oxide followed by thermal expansion to yield single-layered, but wrinkled, sheets. Detail synthesis and characterization information have been reported [5-6]. 5 mg of graphene was mixed with 2.5 mL 99.9% nitric acid and 7.5 mL 99.99% sulfuric acid and sonicated in a water bath for 2 hours at 40 °C. The mixture was transferred into 50 mL of deionized water and centrifuged at 1000 rpm for 10 minutes. Following supernatant removal, the precipitant was resuspended in 30 mL of deionized water and centrifuged again at 1000 rpm for 20 minutes. This washing step was repeated until the pH of the supernatant was > 6. After the final wash the precipitate (functionalized graphene) was suspended in deionized water at a concentration of 1.0 mg/mL. Prior to use this functionalized graphene solution was sonicated for 5 minutes.

Graphene solutions of 17 ug/mL were prepared in PBS buffer

(10 mM phosphate, 138 mM NaCl and 2.7 mM KCl) by sonicating diluted stock solutions for 2 minutes prior to use to allow effective graphene dispersal. Various DNAs were introduced at a final concentration of 200 nM, mixed well, and allowed to sit overnight at room temperature to allow complete adsorption onto the graphene surface. The DNA1-FAM-graphene solution was incubated for 30 min with random sequence DNA (50 nM) to saturate the free binding surface of functionalized graphene. One hundred microliter of the mixture was then transferred into a microplate and single-stranded cDNA solutions serially added with final concentrations ranging from 2.0 nM to 500 nM. The fluorescence intensity of the samples were monitored immediately after each addition and 45 minutes later using excitation at 490 nm and emission at 520 nm. The fluorescence spectra were obtained with excitation at 490 nm and emission ranging from 502 to 649 nm.

III. RESULTS AND DISCUSSION

A. Graphene Based Electrodes for Enhanced Detection of NADH

Nicotinamide adenine dinucleotide (hydrogen) (NADH) is an important biomolecule because it is a cofactor of dehydrogenases. NADH is electroactive. However, NADH generally has a large over-potential at the surface of conventional electrodes which adversely affects the sensitivity and selectivity.[7] Therefore, nanomaterials such as metal nanoparticles and CNTs have been widely studied as electrode-modifying materials to decrease the over-potential and to increase the electrochemical response to greatly improve the sensitivity and to avoid interference from the biological samples. In this work, functional graphene sheets (FGS) was coated on electrode surface and the resulted electrode was used for NADH detection. The FGS-coated glassy carbon electrodes (GCEs) were prepared by casting $\sim 6 \mu\text{L}$ of FGS suspension in ethanol onto clean electrodes and dried at room temperature. The suspensions were prepared by ultra-sonication and they were stable at room temperature for weeks. Before coating, the GCEs were polished with $0.3 \mu\text{m}$ alumina slurries and then sequentially ultra-sonicated in ethanol and water for 2-3 min and dried. Prior to the electrochemical experiments, the modified electrodes were carefully rinsed with water to remove any loosely attached portion of the FGSs and dried under nitrogen stream. A C/O ratio of ~ 10 in the FGSs was confirmed by X-ray photoelectron spectroscopy (XPS).

FGS exhibited electrocatalytic response to NADH. Figure 1 shows the CVs of the FGS-modified electrode in a 50 mM PBS containing 5 mM NADH and without NADH (first cycle of CV was recorded) in comparison to the electrochemical behavior of NADH at a bare GCE. The oxidation peak of NADH is observed both at the bare and the FGS-modified

GCEs at 0.65 and 0.23 V, respectively, with a substantial negative shift of ~ 0.42 V and a two-fold increase in the oxidation current. An oxidation peak is not observed in the absence of NADH. The electrochemical behavior of NADH was irreversible at the FGS-modified electrode. The upper left inset of Figure 1 shows the amperometric responses of the FGS-modified electrode and the bare electrode to the increasing NADH concentration at 0.3 V. As expected from the CV data, the amperometric responses to successive addition of NADH at the bare GCE are negligible at 0.3 V (Fig. 1, upper left inset, blue curve). Whereas, the FGS-modified electrode is responsive to the increasing NADH concentration at this potential as demonstrated by the steady-state signals in a few seconds (Fig. 1, upper left inset, red curve). A linear electrochemical response of the FGS-modified electrode to a wide range of NADH concentration was obtained (Fig. 1, upper right inset) and sub- μM level of NADH is detectable at the FGS-modified electrode.

The highly encouraging results reported here point the potential of graphene for developing dehydrogenase-based amperometric biosensors and to the need to research the mechanisms of the electron transfer reaction of redox proteins with modified graphenes in more detail.

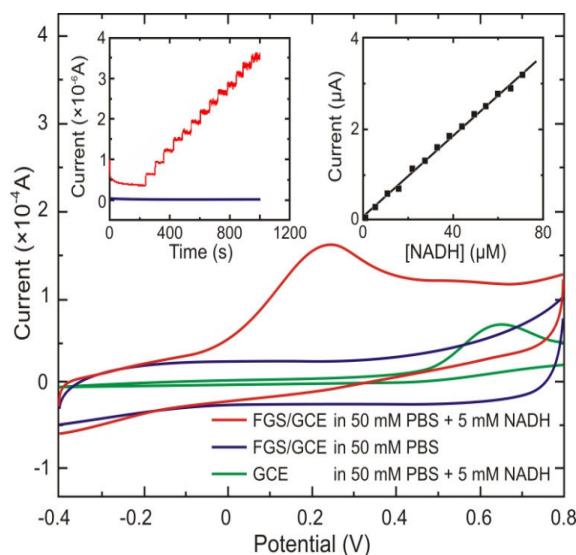


Fig. 1. The CVs of FGS-modified electrodes in a 50 mM PBS containing 5 mM NADH and in the absence of NADH. The CV of a bare GCE in 5 mM NADH. Scan rate: 50 mV/s. The electrolyte solution was degassed with a nitrogen stream. The upper left inset is the i - t curve with increasing NADH concentration at the FGS-modified (red) and bare GC electrode electrodes. The potential was held at 0.3 V. the upper right inset

B. Nitrogen-Doped Graphene and Its Application in Electrochemical Biosensing.

Chemical doping with foreign atoms is an effective method to intrinsically modify the properties of host materials. Among them, nitrogen doping plays a critical role in regulating the electronic properties of carbon materials. In this study, we report a facile strategy to prepare N-doped graphene by using nitrogen plasma treatment of graphene synthesized via a chemical method.[8] Meanwhile, a possible schematic diagram has been proposed to detail the structure of N-doped graphene (Fig. 2). By controlling the exposure time, the N percentage in host graphene can be regulated, ranging from 0.11 to 1.35%. Moreover, the as prepared N-doped graphene has displayed high electrocatalytic activity for reduction of hydrogen peroxide and fast direct electron transfer kinetics for glucose oxidase. The N-doped graphene has further been used for glucose biosensing with concentrations as low as 0.01 mM in the presence of interferences. This work is anticipated to open a new possibility in the investigation of N-doped graphene and promote the application in addressing various electrochemical issues.

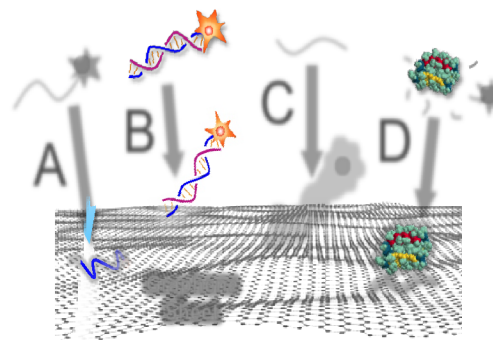


Fig. 3. Schematic illustration of the constraint of DNA molecules on functionalized graphene and its effects. a) The single-stranded DNA can be effectively constrained on the surface of graphene via adsorption. b) The constrained DNA show improved specificity response towards target sequences that can distinguish the complementary and single-mismatch targets. c) DNase I can digest free DNA but not

Our results indicate that the strong and fast adsorption of single-strand DNA onto graphene (Scheme 3A). This efficient absorption is due to the hydrophobic interaction and π -stacking effects between nucleosides, fluorescent dye and aromatic regions of graphene. Meanwhile, the fluorescence intensity of DNA was dramatically decreased, providing an easy and sensitive approach to characterize the interactions between DNA and graphene. In addition, graphene can efficiently quench various fluorescence labelings in a wide wavelength range. The fluorescence intensity can be quenched to lower than 1/300 of original signal, demonstrating that the graphene has higher quench efficiency on fluorescent dyes comparing to carbon nanotube. This exceptional high fluorescent quench efficiency may derive from the excellent electronic transference and conductivity of graphene. In contrast, the double-stranded DNA (DNA1+cDNA) has a much weaker interaction with functionalized graphene (Scheme 3B). In addition, the adsorption of single-stranded DNA can be reversed by adding complementary DNA. In addition, the DNA base-pairing induced desorption is faster and much more efficient than DNA absorbed on carbon nanotube surface. This feature may owe to the fact that the single-stranded DNA would wrap around carbon nanotube to form a stable and tight hybrid, thus decreasing the probability and tendency of hybridizing with complementary DNA. On the other hand, when single-stranded DNA is adsorbed onto two-dimensional graphene surface, it can not yield a wrap coil structure with graphene to form a more stable complex. In this situation, it can be more easily and efficiently desorbed from graphene surface via hybridizing with its complementary

C. DNA-Graphene Optical Nano-biosensor

Our study revealed the fast, efficient and reversible interaction between DNA and graphene. Interestingly, the protection of adsorbed DNA from enzymatic cleavage was also discovered. Furthermore, a facile, robust and sensitive DNA assay has been established based on DNA-graphene optical nano-biosensor [9].

sequence. These unique features of functionalized graphene, including high efficient DNA sorption, complementary DNA induced desorption and exceptional fluorescence quenching ability, will advance future biomedical applications using graphene.

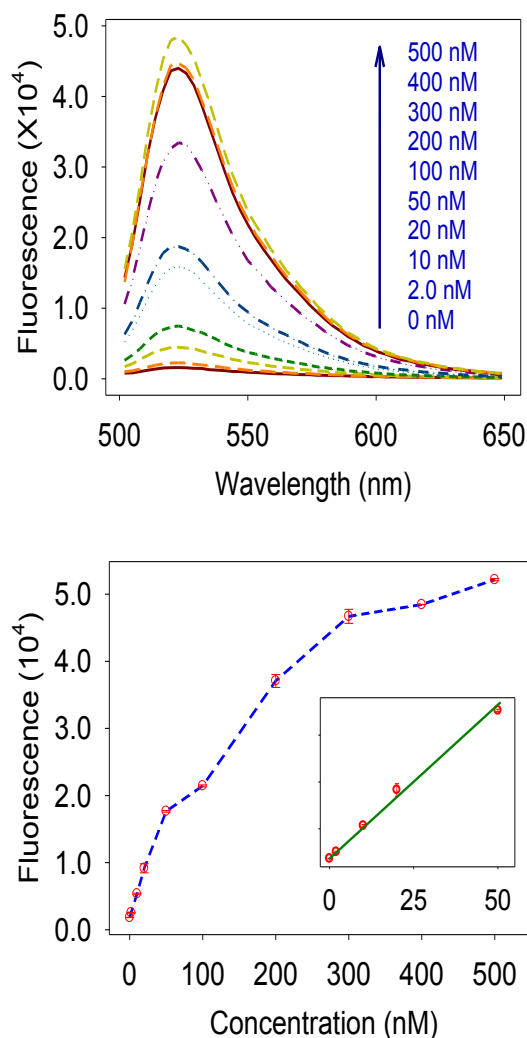


Fig. 4. The novel DNA assay based on DNA-graphene nano-biosensor. The fluorescence intensity of sample was plotted as the function of the concentration of complementary DNA. Inset: The linear response range is up to 50 nM.

Another interesting finding is the protection of DNA from enzymatic cleavage after non-covalent adsorption on functionalized graphene (Scheme 3D). The protection of DNA may due to the steric hindrance effect to DNase I, which prevents the DNA binding and cleavage sites of DNase I from accessing to DNA sequence and ceases the sequential

enzymatic digestion. This interesting feature is encouraging for many bioassays and biomedical applications requiring robust DNA probes and efficient DNA delivery in complex biological samples.

A novel nano-biosensor for DNA assay was developed by taking advantage of the reversible adsorption and desorption of the single-stranded DNA on functionalized graphene (Scheme 3C). In this assay, the DNA1-graphene complex after overnight incubation and autoassembly was used as the nano-biosensor. As shown in Fig. 4, the fluorescence intensity was increased after adding higher concentration of cDNA. The detection limit is 0.5 nM and the linear detection range is up to 50 nM. The high quench efficiency of graphene towards various fluorescence dyes will benefit the multiplex assay design. Comparing with other DNA detection methods, this novel assay provides excellent sensitivity and selectivity without sophisticated probe design and elaborate dye-quencher pairing.

In summary, the interactions between DNA and graphene were studied and some interesting features were revealed. The single-stranded DNA can be effectively and promptly adsorbed onto functionalized graphene via hydrophobic and π -stacking interactions. In contrast, the double-stranded DNA presents much weaker interaction with graphene. Interestingly, the absorbed single-stranded DNA can be effectively protected from enzymatic cleavage, which is encouraging for biomedical applications involving complex cellular and biofluids samples. In addition, the absorbed DNA can be desorbed from graphene surface via hybridizing with complementary DNA. Based on the unique features of DNA-graphene interactions, a DNA-graphene optical nano-biosensor has been demonstrated for DNA assay with facile design, excellent sensitive, improved selective and biostability. Considering the low cost and large scale production of graphene, these findings will promote applying graphene in both fundamental researches and practical applications.

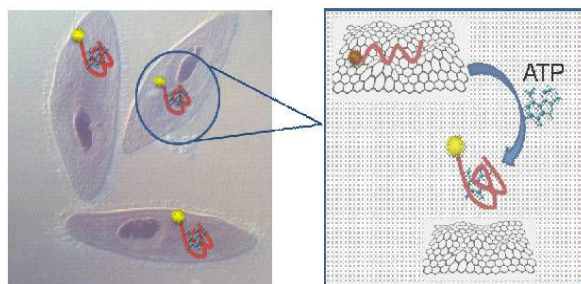


Fig. 5. Schematic illustration of in situ molecular probing in living cells by using aptamer/GO-nS nanocomplex

D. Aptamer/Graphene Oxide Nanocomplex for In Situ Molecular Probing in Living Cells.

We report the illustration of cellular delivering and in situ molecular probing in living cells by using graphene oxide nanosheets (GO-nS) as DNA cargo and sensing platform (Fig. 5). Due to the particular interaction between GO and DNA molecules, aptamer/GO-nS nanocomplex was designed and employed to demonstrate the dramatic DNA delivering, enzymatic cleavage protecting and biosensing capabilities of GO-nS in living cells. The results show that GO-nS could successfully transfer DNA aptamer into living cells, efficiently protect oligonucleotides from enzymatic cleavage during the delivery as well as selectively and simultaneously monitoring intra-cellular target (ATP and GTP) in situ.[10] The as-shown advantages of graphene oxide will enable it to be a robust candidate for many biological fields, such as DNA and protein analyzing, gene and drug delivering, intracellular tracking, and in vivo monitoring, etc.

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