

BIOFUNCTIONALIZATION OF GRAPHENE FOR BIOSENSING AND IMAGING

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Introduction

Graphene is single layer carbon crystal that emerged as a novel nanomaterial and demonstrated many exceptional features including excellent conductivity, high surface-to-volume ratio, remarkable mechanical strength and biocompatibility. Graphene has attracted increasing attentions from physics, chemistry and biomedical fields. Recently, functionalized graphene has been successfully used in many biomedical and bioassay applications and manifested its promising potentials in these fields (1).

Undoubtedly, well understanding of the interactions between graphene and biomolecules is one important key to fulfill the potential of graphene in biological applications. Here, we chose DNA, a core component in many fundamental life processes involving life proliferation, metabolism and evolution, as a target biomolecule, to study the interactions between DNA and functionalized graphene.

Besides, exploration of graphene with intracellular analyzing, living cell imaging and *in situ* monitoring are not wide-open and still remain at a very early stage.

Experimental

Functionalization of graphene: Graphene was produced in mass quantities through the thermal expansion of graphite oxide to yield single graphene sheets. The graphene were then functionalized by mixing with nitric acid and sulfuric acid (1:3 v/v) and sonicating in a water bath sonicator for two hours at 40°C. The mixture was then washed using deionized water and centrifuged at 1000 rpm for 10 to 30 minutes to remove the residual acids in the supernatant. The washing step was repeated until the pH of the supernatant was > 6.

Preparation of DNA-graphene samples: DNA-graphene samples were prepared in various concentration and buffers for the different types of experiments. The NMR

samples were prepared in 99% D₂O buffer to decrease water interference in ¹H NMR data collection.

Instruments and measurements: All fluorescence and anisotropy measurements were carried out at room temperature on a Safire 2 microplate reader (TECAN, Switzerland). Proton NMR spectra were obtained at 15°C using a Varian 600-Inova spectrometer equipped with a triple resonance probe and pulse field gradients. Circular dichroism data were collected on an Aviv Model 410 spectropolarimeter (Lakewood, NJ). The CD wavelength scans were recorded between 200 and 330 nm at 20°C. Bio-Rad Power PAC 300 was used to run gel electrophoresis and a NucleoVision imaging workstation (NucleoTech, USA) employed to take the gel images. Thermal profile studies were performed on a Roche Lightcycler real time PCR system (Basel, Switzerland).

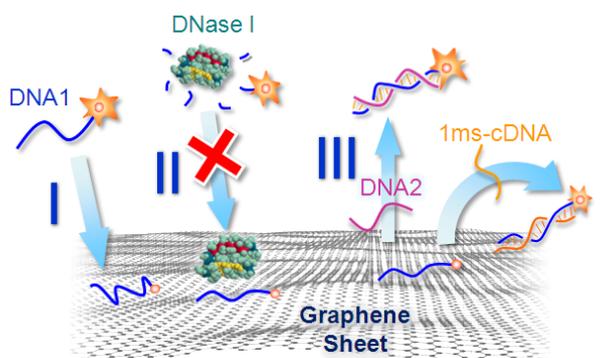
Results and Discussion

Interaction of DNA and Graphene

Our study revealed that the single-stranded DNA can be effectively and promptly adsorbed onto functionalized graphene via hydrophobic and pi-stacking interactions (Fig. 1). In contrast, the double-stranded DNA presents much weaker interaction with graphene. Interestingly, the adsorbed 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 adsorbed 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 sensitivity, improved selectivity and biostability.

To investigate the enzymatic cleavage protection effect on single-stranded DNA after interaction with functionalized graphene, DNA1 was incubated with

functionalized graphene in PBS buffer overnight to allow complete interaction. The samples were then treated with DNase I. As shown in Fig. 2, free DNA1 was partially digested after a 20 minute incubation with DNase I. After incubation for 60 minutes the DNA1 band was invisible indicating complete enzymatic hydrolysis of the single-stranded DNA. In contrast, there was no detectably hydrolysis of the single-stranded DNA in the presence of graphene after 60 minutes.



DNA1: FAM-5'-AAT CAA CTG GGA GAA TGT AAC TG-3'
 DNA2: 5'-CAG TTA CAT TCT CCC AGT TGA TT-3'
 1ms-cDNA: 5'-CAG TTA CAT TCT GCC AGT TGA TT-3'

Fig. 1. Cartoon illustration of the constraint of DNA molecules on functionalized graphene and its effects. I) The single-stranded DNA can be effectively constrained on the surface of graphene via adsorption. II) DNase I can digest free DNA but not graphene bound DNA. III) The constrained DNA show improved specificity response toward target sequences that can distinguish the complementary and single mismatch targets.

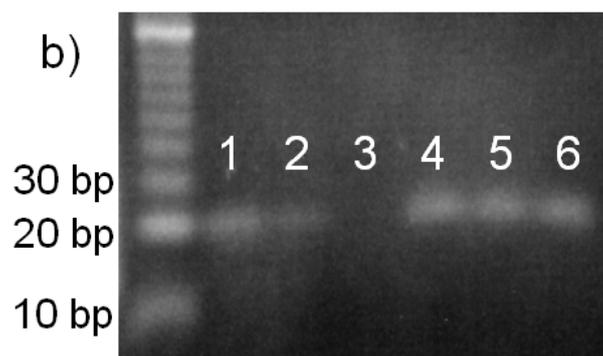


Fig. 2. Image of the gel electrophoresis of DNA and DNA-graphene with and without DNase I treatment. Lane 1: DNA only; Lane 2 and 3: DNA treated with DNase I for 20 (lane 2) and 60 (lane 3) minutes; Lane 4: DNA and graphene; Lane 5 and 6: DNA and graphene treated with DNase I for 20 (lane 5) and 60 (lane 6) minutes.

Combining the enzyme cleavage protection, the further applications of DNA-graphene nano-scaffold in biomedical field including imaging and drug delivery are fascinating. This study connects one core biomolecule with a very important and unique nanomaterial, and paves a new avenue for graphene based applications in biotechnology and biomedical fields.

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

We report the demonstration of cellular delivery and *in situ* molecular probing in living cells by employing graphene oxide nanosheets (GO-nS) as DNA cargo and sensing platform (Fig. 3). Due to the particular interaction between GO and DNA molecules, aptamer/GO-nS nanocomplex (aptamer/GO-nS) was designed and used as a real time biosensing platform in living cell systems. As well known, aptamer is an artificial oligonucleotide receptor derived from *in vitro* selection with highly specificity and affinity to a given target. Adenosine triphosphate (ATP) aptamer has been well studied for its sequence, structure, configuration and functions in the past decades due to the significance of ATP in living systems. Herein, ATP-ATP aptamer recognition was taken as a model system to elucidate (1) GO-nS can serve as transporter of DNA aptamer into living cells; (2) GO-nS shows efficient protection of oligonucleotides from enzymatic cleavage during the delivery to inter or intra cellular spaces; (3) GO-nS can act as sensing platform with high fluorescence quenching efficiency and realize the real time target monitoring in living cells. The successful delivery to molecular targets in living cells suggest that graphene oxide could be a good vehicle to transport gene into cells, acting as protector for the loading gene from enzymatic cleavage as well as *in situ* molecular probing in living cells.

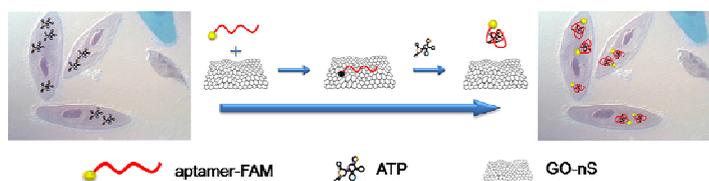


Fig 3. Schematic illustration of *in situ* molecular probing in living cells by using aptamer/GO-nS nanocomplex.

Reference:

1. Shao Y., Wang J., Wu H., Liu J., Aksay I.A., Lin Y. Graphene based electrochemical sensors and biosensors: A Review. *Electroanalysis*, **22** (2010)1027-1036