Nanostructure-Based Fluorescent Biosensors

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Abstract

We have studied and investigated the development of a fluorescent biosensor based on graphene oxide (GO) for the analysis of interaction between a fluorophore FAM (Carboxyfluorescein)-labeled single-stranded DNA with its complementary single-stranded DNA oligonucleotide (target). The graphene oxide adsorbs the FAM-labeled single stranded DNA (probe) and quenches its fluorescence. Upon addition of the complementary single stranded DNA oligonucleotide, the probe hybridizes to its target [1] thus producing a double-stranded DNA which detaches from the GO. The release of the double helix leads to the recovery of dye fluorescence that can be monitored by fluorimetric techniques.

Introduction:

Graphene oxide (GO) is a single-atom-thick and two-dimensional carbon material. It is the oxidized form of graphene, with "O" functional groups attached to the sp^2 C basal plane and edges [2]. GO has tremendous attractive and great remarkable electronic, mechanical, and thermal properties[3]. When compared with other nanomaterials GO has some superior properties, high quenching efficiency, good water dispersibility (hydrophilic) due to the presence of the "O" functional groups. These properties make GO a promising nanomaterial for biological applications, including biosensors.

In this study we examined the GO by using Atomic Force Microscopy(AFM) to characterize the GO interaction with ssDNA.

Materials and methods:

Pristine GO flakes were prepared using a modified Hummers method and dispersed in water with a concentration of 0.5 mg/mL. Then we tested the GO with buffered solution containing 20mM Tris-HCl pH 7.5, 100mM NH4Cl, 5mM KCl, 10mM Mg(CH3COO)2. The samples were prepared by drop casting, the GO and DNA with buffered solution[1] on 300 nm SiO2/Si(100) at room temperature. The biomolecules were allowed to adhere to the SiO2 surface for 5-10 minutes. AFM was performed in air in tapping mode using a Veeco Digital D5000 system.

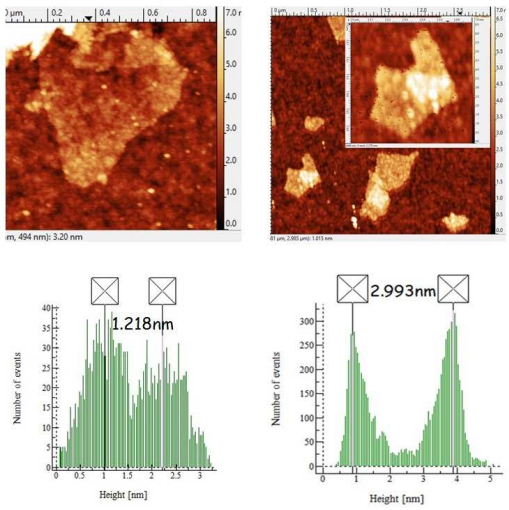

Figure 1: AFM height image of GO sheets deposited on SiO2 substrates (left); AFM height image of DNA-GO complex (right) by histogram analysis.

Samples characterization by AFM:

We used atomic force microscopy (AFM) to characterize the GO- DNA complex. Figure 1 (left) shows the AFM image of the GO flakes, whose thickness (about 1.2 nm) is reported in the histogram analysis. Figure 1 (right) shows the typical AFM

image of the DNA-GO complex, where the bright areas on the GO surface might be due to the adsorption of DNA. In this complex the thickness is about 3nm.

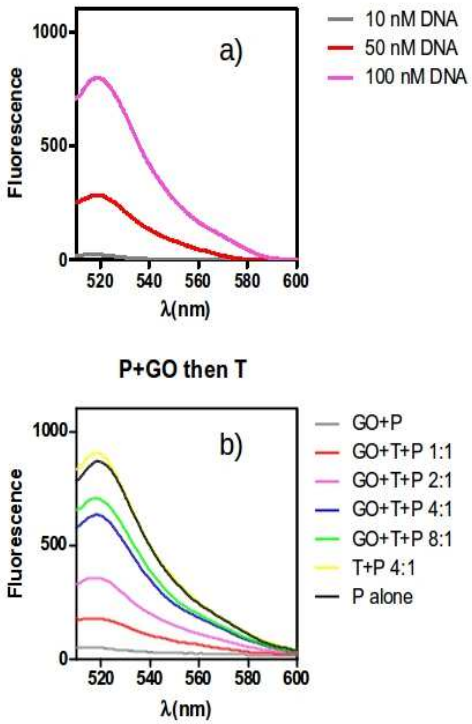

Figure 2:Fluorescence emission spectra (excitation at 495nm) of ssDNA at different conditions**: a)Increasing amounts of fluorescently labeled** ssDNA in Tris-HCl buffer with different concentrations; **b)** Fluorescently labeled ssDNA(100nM) in Tris-HCl buffer + GO in the presence of increasing amounts of target DNA (T).

Spectrofluorimetric analyses of DNA-GO complexes

The fluorescence spectra of ssDNA (5'-FAM-cttgtggaagatgggcaagaagactggagg-3') and ssDNA-GO were measured to monitor the adsorption of ssDNA on GO. As can be seen from Figure 2(a), in the absence of GO the fluorescently labeled ssDNA shows strong fluorescence emission. Furthermore, this emission is proportional to the concentration of ssDNA in solution.
However, in the presence of GO, up to ~97% quenching of the fluorescence emission was observed (Figure 2b). This observation indicates that GO can strongly adsorb ssDNA and can efficiently quench its fluorescence.
The fluorescently labeled ssDNA-GO complex displayed significant fluorescence enhancement upon addition of complementary target DNA oligonucleotide (Figure 2b). This recovery of fluorescence increases with increasing concentration of the target DNA added to the mixture.

Conclusions:

GO can be used as a platform for fast, sensitive, and selective detection of biomolecules. The low cost and large production scale of GO make it a promising material for devising biosensors compared to other nanomaterials.

References:

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