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Short communication

Graphene oxide-based optical biosensor functionalized with peptides for explosive detection

Qian Zhang [a], Diming Zhang [a], Yanli Lu [a], Yao Yao [a], Shuang Li [a], Qingjun Liu [a,b,*]

[a] Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, PR China

[b] Cyber Innovation Joint Research Center, Zhejiang University, Hangzhou 310027, PR China

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ABSTRACT

A label-free optical biosensor was constructed with biofunctionalized graphene oxide (GO) for specific detection of 2,4,6-trinitrotoluene (TNT). By chemically binding TNT-specific peptides with GO, the biosensor gained unique optoelectronic properties and high biological sensitivity, with transducing bimolecular bonding into optical signals. Through UV absorption detection, increasing absorbance responses could be observed in presence of TNT at different concentrations, as low as 4.40×10^{-9} mM, and showed dose-dependence and stable behavior. Specific responses of the biosensor were verified with the corporation of 2,6-dinitrotoluene (DNT), which had similar molecular structure to TNT. Thus, with high sensitivity and selectivity, the biosensor provided a convenient approach for detection of explosives as miniaturizing and integrating devices.

1. Introduction

The detection of explosives, such as 2,4,6-trinitrotoluene (TNT) and 2,6-dinitrotoluene (DNT), is of great significance for applications ranging from environment conservation to public safety, and has received increasing attention throughout the world (Hwang et al., 2011; Gingras et al., 2013). A large amount of approaches, especially various sensors based on different technologies, have been developed for their detection. Among them, biosensors that emulating the olfactory systems have attracted tremendous attention for their portability and high sensitivity (Bielecki et al., 2012; Caygill et al., 2012). These biosensors mostly combine physicochemical sensors with sensitive materials such as cells and proteins separated from living organisms or cultured *in vitro*. Although these sensitive substances can greatly maintain their sensitivity, it is not easy for them to be preserved stably for long time and produced in quantity, which fuels the needs for more stable and cost-effective alternatives for specific and highly sensitive detection of explosives (Kim et al., 2011; Liu et al., 2014).

Peptides are biorecognition molecules that have different chemical diversity and various biological sensitivities. Besides, they can be chemically engineered to bind specific targets (Lakshmanan et al., 2012). Compared with other biological components, such as cells and proteins, peptides have long-time stabilities and sensitivities, and can form self-assembled hybrid conjugates with a variety of materials, including nanoparticles and nanosheets (Ruigrok et al., 2011). Furthermore, many peptide synthesis approaches have been emerged recently, to obtain and design target peptides according to specific demands (Cui et al., 2012). All these imply that peptides are ideal sensitive substances for biosensors. With more and more peptides of specificity or multi-selectivity determined, it promises various kinds of applications for peptides in the field of biosensors.

In this study, a novel label-free optical biosensor was constructed based on graphene oxide (GO) crosslinking with TNT-specific peptides. GO is a 2D structure material with many oxygen-containing groups on graphene single sheets. These polar oxygen-functional groups give GO unique optoelectronic properties and make it easily be modified by various species, such as nanoparticles, nucleic acids and proteins (Tao et al., 2013). Combining remarkable optoelectronic sensing properties of GO with the specific sensitivity of peptides, this biosensor could detect TNT through absorption spectra, showing a high-throughput approach for specific explosive detection.

* Corresponding author at: Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, PR China. Fax: +86 571 87951676.
E-mail address: qjliu@zju.edu.cn (Q. Liu).

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2. Materials and methods

2.1. Dispersion of GO nanosheets

As a kind of emerging two-dimensional carbon nanomaterials, GO contains oxygen-containing groups such as hydroxyl groups (–OH), carboxyl groups (–COOH) and epoxy groups on graphene single sheets (Zhu et al., 2010). These oxygen functional groups result in the hydrophily of GO and serve as sites for chemical modification. In this study, GO (~99% purity, 5 mg) made by Hummers method was purchased from XFNANO Materials Tech Co., Ltd. (Nanjing, China) and dispersed in deionized water (10 mL). The obtained solution was sonically oscillated in ice-water bath (0 °C) for 30 min and then centrifuged at 630 × *g* for 30 min to obtain the clear and well-dispersed GO supernatant (~0.5 mg/mL).

2.2. Carboxyl esterification of GO nanosheets

Through dehydration condensation reaction, stable covalent bonds can be formed between Carboxyl groups and amino groups (Ye et al., 2011). This reaction carried out by a two-step conjugation in aqueous solvents: Carboxylates (–COOH) first reacted with N-hydroxysuccinimide (NHS) in the presence of N-ethyl-N′-[3-(dimethylamino) propyl]carbodiimide (EDC), resulting in a semi-stable NHS ester. This ester then reacted to primary amines ($-NH_2$) to form stable covalent bonds. In the study, excessive EDC (5 mg) and NHS (5 mg) were successively added into the GO supernatant (8 mL) and sonically oscillated for 1 h to generate semi-stable NHS esters. Then, pH of the mixture supernatant was adjusted to 8.0 with sodium hydroxide (NaOH) for following functionalization.

2.3. Functionalization of GO with specific peptides

TNT-specific sequence peptides (WHWQRPLMPVSI) were synthesized by solid phase peptide synthesis (SPSS) (Jaworski et al., 2008; Sam et al., 2009). The peptides (dissolved in phosphate buffer solution, 0.145 mM, 0.1 mL) were added into the mixture (10 mL) obtained above, then preserved at 4 °C in the dark for a night for the crosslinking of the NHS easters with peptides. To remove unbound peptides, the final solution was centrifuged again at 11,750 × *g* for 30 min. The sediment was then redispersed in deionized water and the centrifugation process repeated several times to guarantee a relatively pure aqueous solution of peptide-functionalized GO.

2.4. Optical detection of TNT

TNT (4.40 mM) was diluted by anhydrous methanol at different orders of magnitude (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} mM) for optical detection. These solutions (20 μL) were respectively combined with the peptide-functionalized GO dispersions (200 μL) and the absorption spectra of these mixture solutions were monitored by Ocean Optics USB2000+ Spectrometer with the wavelength ranging from 200 nm to 400 nm (0.38 nm optical path). Methanol liquid (20 μL) was added into one functionalized GO dispersion (200 μL) as control trial. Every solution was repeatedly measured for four times.

Moreover, DNT and isoamyl acetate were chosen for comparison with TNT detection. DNT is also a kind of nitroaromatic explosives which has similar structure to TNT. While, isoamyl acetate has same number of carbon atoms as TNT, however, without the benzene ring. DNT (5.49 mM) and isoamyl acetate (7.68 mM) were also diluted by anhydrous methanol at six orders of magnitude (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} mM). Same operating steps were repeated for detection of DNT and isoamyl acetate. TNT, DNT, isoamyl acetate, and other reagents were all obtained from Sigma-Aldrich Co., LLC (St. Louis, MO USA).

3. Results

3.1. Optical detection of peptide-functionalized GO

Due to the presence of various oxygen-containing groups, GO has several structural models. It has been mostly identified that hydroxyl and epoxy groups were located on the basal plane, while carboxyl groups were at the sheet edges with smaller amounts of other groups (Chen et al., 2012). With the help of EDC and NHS, TNT-specific peptides could be crosslinked to GO nanosheets through dehydration condensation reactions between carboxyl groups and amino groups, producing peptide-functionalized GO. As shown in Fig. 1A, the crosslinking caused local structure changes of GO and resulted in changes of optical properties, which could be observed from UV absorbance spectra. Fig. 1B demonstrated that the UV spectrum of GO showed wavelength of maximum absorbance at ~235 nm due to the π–π^* of the C–C aromatic rings (Veerapandian et al., 2014). Compared to GO, the absorption peak of biofunctionalized GO had a red shift in the wavelength. Several reports have stated that shifts in absorbance peak were usually the external manifestation of changes in molecular structure of substances (Peet et al., 2007; Bakshi et al., 2014). Thus, peptides have been crosslinked to the GO and showed structural differences to the nanosheets.

3.2. Sensitivity of the biosensor for TNT detection

Peptides in this study were specifically sensitive to TNT, and could combine TNT molecules through partial charge-charge interactions or hydrogen bonding (Khan et al., 2004). As a result, when there were TNT molecules around the functionalized GO nanosheets, the peptide chains assembled on nanosheets would specifically capture TNT molecules, as described in Fig. 2A. Donor–acceptor interactions and π–π interactions would take place between peptide chains and TNT molecules, which might cause further changes in optical properties. Fig. 2B showed absorption responses of the biosensor to TNT at different concentrations. The addition of TNT changed absorption magnitude in the optical spectrum of the biofunctionalized GO, indicating that interactions might occur between TNT molecules and peptide chains on GO nanosheets. With TNT concentration increasing, from 4.40×10^{-9} mM to 4.40×10^{-4} mM, the corresponding absorbance increased as well, which might be due to the increasing formation of complexes of TNT and biofunctionalized GO. The obtained absorption peak values at ~238 nm were plotted against log concentration of TNT. The responses showed linear relationship and small standard deviation δ, indicating stability of the biosensor. The theoretical detection limit was ~4.40×10^{-12} mM in 3δ/slope.

3.3. Specificity of the biosensor for TNT detection

The selectivity of the biosensor was measured through comparison with detection of DNT and isoamyl acetate. It could be seen from Fig. 3A that DNT was a kind of nitroaromatic explosives which had very similar structure to TNT, containing a benzene ring with a methyl group and nitro groups at ortho and para position. Although containing seven carbon atoms as with GO, isoamyl acetate had no benzene ring and was very different to TNT and DNT as a kind of aromatic substances. The magnitudes of absorption peak at ~238 nm in spectrum were recorded in presence of TNT, DNT, isoamyl acetate and mixture of TNT and DNT. As

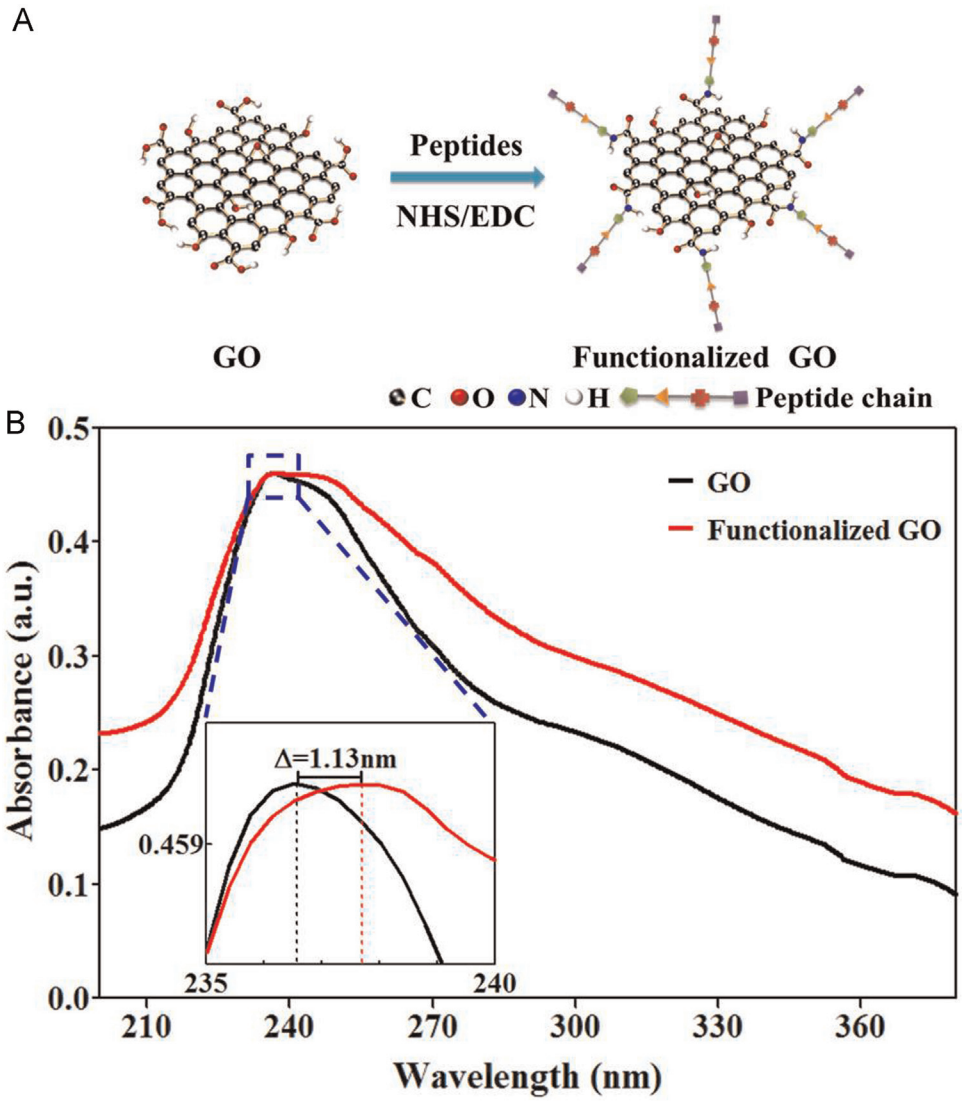

Fig. 1. Schematic of synthesis (A) and UV absorption spectra (B) of biofunctionalized GO. The inset shows that the absorption peak of biofunctionalized GO has a red shift in the wavelength.

shown in Fig. 3B, the increases of absorbance induced by TNT were higher than that of DNT and isoamyl acetate. For isoamyl acetate, the magnitude of absorbance peak showed little changes. This might due to that isoamyl acetate was very different from TNT in the structure and could not bind with the peptides on the GO nanosheets specifically. However, the biosensor had responses to DNT at high concentrations such as 5.49×10^{-5} mM and 5.49×10^{-4} mM, while DNT at low concentrations elicited no significant responses. Moreover, the responses to mixture of TNT and DNT, whose concentrations were respectively same as corresponding single solution, were a little higher than that to TNT, but still kept linear relationship as TNT did. As peptides used in this research were excessive, these results were in accordance with previous studies that these sequence-specific peptides could also respond to DNT due to the similar structure of DNT to TNT, but the response was much less obvious compared with TNT (Jaworski, 2009). Therefore, the biosensor displayed good specificities in the detection of low concentrations of explosives, indicating a good candidate for TNT-specific detection.

4. Discussion

In our study, an optical biosensor using peptides functionalized GO was constructed for specific detection of TNT. The results showed that addition of TNT could induce an absorption increase of the biosensor. Optical detection of TNT and mixture of TNT and GO at six concentrations used in this study were also performed. For clear comparison, only the spectra of maximum and minimum concentrations of TNT were shown in Fig. S1 (Supplementary Material), compared with detection results of these two concentrations of TNT with the biosensor. Absorption peaks of TNT and mixture of TNT and GO were different to that of mixture of biofunctionalized GO and TNT, and absorption increases of TNT and mixture of TNT and GO at ~238 nm compared with the control trials with methanol were smaller than that induced by addition of TNT into the biosensor, which indicated that TNT molecules might have little interaction with GO nanosheets. As a result, the absorption increases in TNT detection probably owed to interactions between biofunctionalized GO and TNT molecules.

The optical responses of the biosensor showed linear relationship against log concentrations of TNT. For further exploring the process involved in the detection of TNT with the biosensor and eliminating the influence induced by free TNT molecules in liquids, continuous variation experiments (Likussar and Boltz, 1971; Schmuck and Schwegmann, 2005) were also performed in this study. The molar sum of TNT and peptides was 7.5×10^{-8} mM,

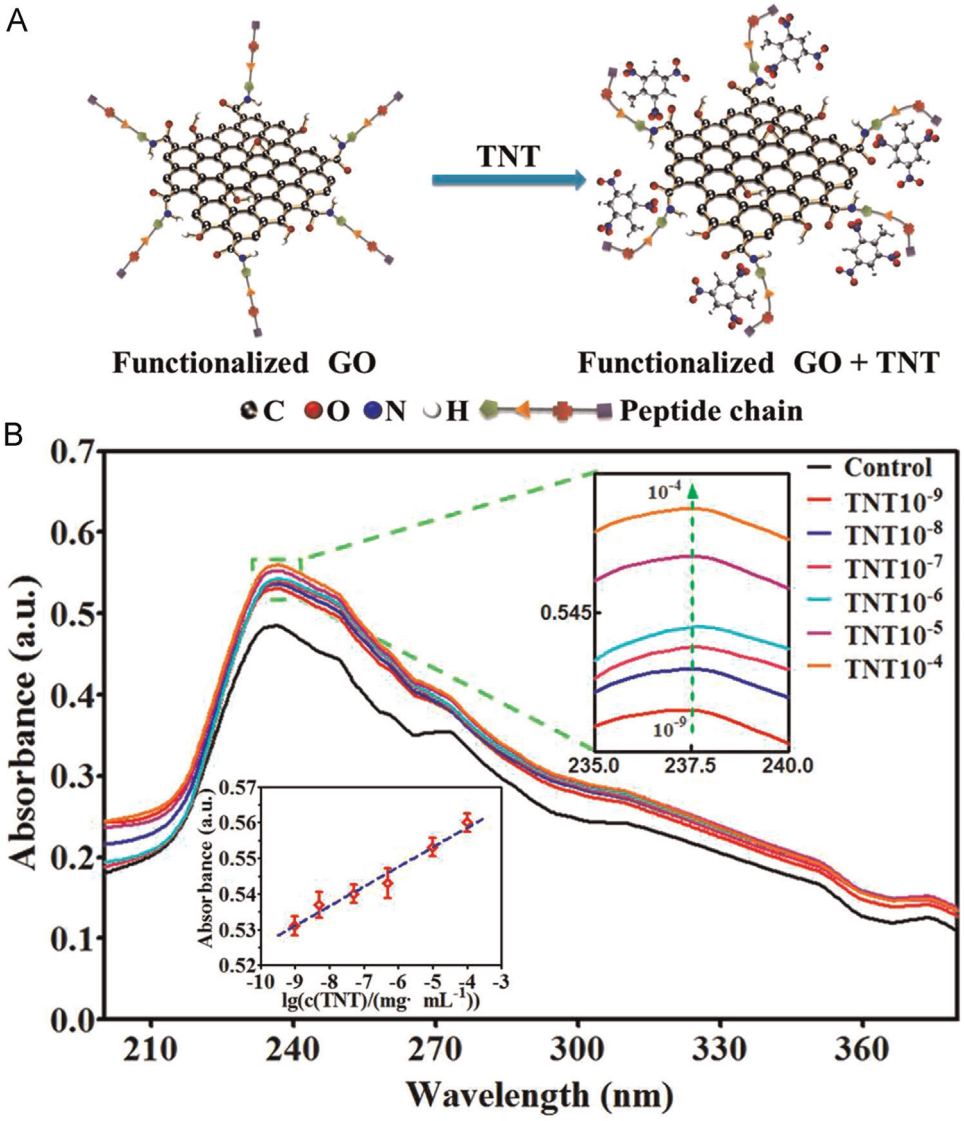

Fig. 2. Schematic illustration (A) and UV absorption spectra (B) of TNT detection. The inset is the linear plot of statistical results of the absorption peak values (n=4).

and absorption increases at ~238 nm were plotted against the mole fraction of TNT. As shown in Fig. S2 (Supplementary Material). The absorption increase changed with the mole fraction of TNT altering, which would be attributed to the formation of different concentrations of complexes of TNT and biofunctionalized GO. The interaction seemed to be a 1:1-complex stoichiometry, because the largest absorption response was obtain at mole fraction of 0.5. A platform appeared when the concentration ratio of TNT and peptides was smaller than 1, which might indicate that more than one peptide chain could interact with one TNT molecule as peptides were excessive. Moreover, the results we obtained above showed that the absorption increases of TNT were less than the increase induced by interactions between TNT and the biofunctionalized GO. All these indicated that the optical responses of the biosensor to TNT were not caused by free TNT in the solution but due to the recognition events between peptides and TNT molecules.

It has been known that GO is an electronically hybrid material in which a large fraction of carbon is sp^3 hybridized binding with oxygen in form of epoxy and hydroxyl groups, and the other carbon is sp^2 hybridized binding with neighboring carbon atoms or with oxygen in the form of carboxyl and carbonyl groups (Eda et al., 2010). The strongly heterogeneous atomic and electronic structures give GO unique optoelectronic and chemical properties. Researchers have verified that tuning the ratio of the sp^2 and sp^3 fractions can tune the bandgap of GO and therefore contribute to the possibilities for tailoring the electrical, optical and/or chemical properties of GO (Loh et al., 2010), such as enhancement of the conductivity and shift of the absorption peak. In this research, when carboxyl groups on the GO were replaced by the tridecapeptides, a red shift of the absorption peak was observed. Based on these, we speculated that the ratio of the sp^2 and sp^3 fractions might be changed through this chemical modification, which led to smaller energy gap and less absorption of photons, resulting in red shift of the absorption peak. On the other hand, when this biofunctionalized GO interacted with TNT molecules, the results showed only absorption magnitude increases without peak shift. Jaworski et al. suggested that specific binding ability of peptides with TNT molecules might due to the donor–acceptor interactions between tryptophan and TNT, π–π interactions between histidine and TNT, and partial charge–charge interactions or hydrogen bonding between imidazole side chains in peptides and nitro groups in TNT molecules (Jaworski et al., 2008). Therefore, it could be inferred that charge exchange did exist between TNT molecules and peptide chains on GO, but this charge exchange might be too small to change the absorption peak of the biosensor, which only

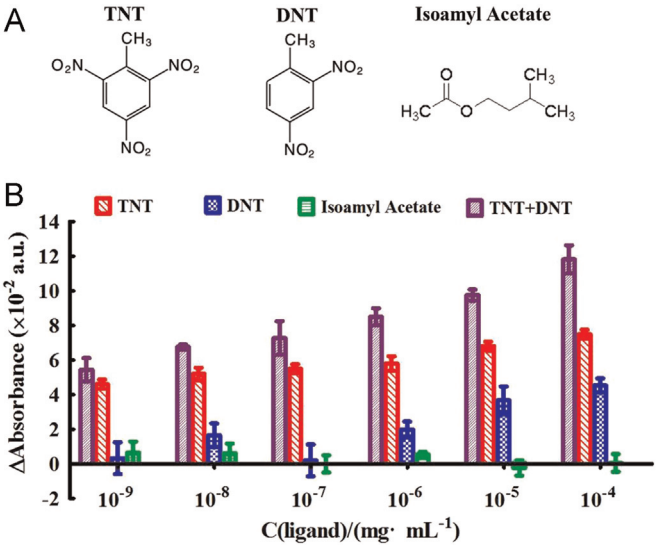

Fig. 3. Selectivity analysis for TNT detection by monitoring the relative absorption peak value changes at ~238 nm compared with DNT and isoamyl acetate and mixture of TNT and DNT. The concentration of each substance is ranged from 10^{-9} to 10^{-4} mM (n=4).

affected the local electronic distribution, and the external manifestation of this local electronic distribution change was the increase of the absorption magnitude, owing to the increasing TNT molecules interacted with peptide chains on the biofunctionalized GO nanosheets.

GO is a remarkable platform for chemical modification and optoelectronic detection. With advantages of UV/vis optical detection such as easy-to-operate, non-contact, pollution-free and high-throughput (Eda and Chhowalla, 2010), the optical biosensor constructed in this research provided an easy and stable approach to detect TNT in high-throughput, allowing for further fabrication of miniaturized, integrated and portable devices. The biosensor showed great specificity to TNT versus DNT and isoamyl acetate with optical measurement, which might owe to sequence-controlled peptides that replaced many uncontrollable natural substances as biosensing components in our study. The peptides can be designed to have various artificial sensitivities, prepared by synthetic chemistry and used for different biodetections of virus, proteins or odor molecules (Yadav et al., 2011). Meanwhile, more and more amino acid sequence in natural proteins have been reported to show great affinities to chemical volatiles and biomarkers of diseases, providing abundant source of biosensitivities to design versatile peptides (Lutz et al., 2013; Seker et al., 2014). Therefore, combining with the high throughput biosensors based on biofuctionalized GO, universal biosensing assay platforms can be developed and used in environment monitoring, food safety and healthcare diagnosis.

5. Conclusion

The optical biosensor constructed based on peptide-functionalized GO is used for specific detection of TNT, as low as 4.40×10^{-9} mM. Stable and specific responses are observed through UV absorption of the detection. Without additional label and complex detection approaches, this biosensor is a promising tool for explosive detection.

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Appendix A. Suplementary Information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.01.040.

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