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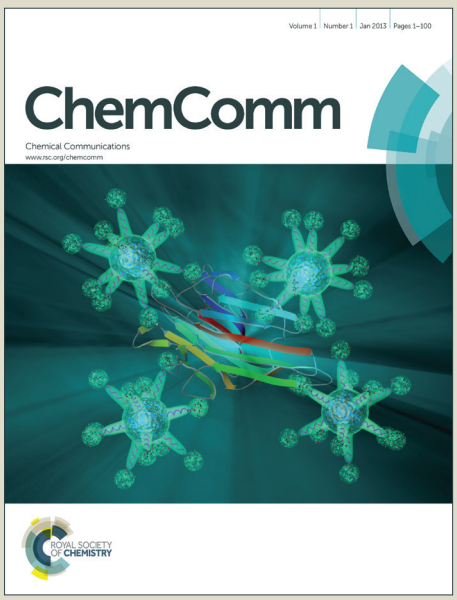

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Luminescent lanthanide graphene for detection of bacterial spores and cysteine

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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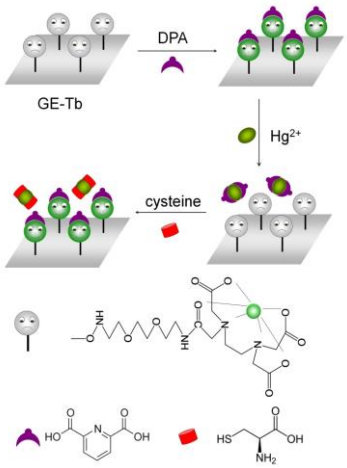
Here, we describe a new approach for preparation of luminescent lanthanide graphene in the presence of dipicolinic acid (DPA). Hg^{2+} can competitively bind with DPA which greatly quenches the fluorescence and the resultant complex is able to selectively and sensitively detect cysteine with the detection limit of 5 nM.

As a rising star in material sciences, two-dimensional graphene has attracted tremendous attention in the past few years.[1-4] Potential applications ranging from novel drug delivery, biosensing and clinical diagnosis have been actively explored.[5-8] To meet this end, water-soluble, visible fluorescent graphene is a suitable candidate because they can be easily tracked in situ diagnosis or drug delivery path way in vivo. Due to the good water solubility and easy functionalization, graphene oxide (GO) was widely used in biological application.[3, 9] However, GO only exhibits a weak infrared fluorescence, which limits its use in vitro and in vivo.[10] Much effort has been devoted for developing fluorescent graphene. Previous studies have demonstrated that most organic compounds have short fluorescence lifetime, and more importantly, their fluorescence are strongly quenched due to photoinduced electron transfer or energy transfer to graphene when organic fluorophores are bound to the graphene sheets.[11] It has been reported that rare-earth compounds have been widely used as laser materials, optoelectronic devices and as fluorescence probes in immunoassays.[12, 13] This is attributed to the 4f orbitals of rare-earth elements are shielded by 5s5p6s orbitals, rare-earth compounds exhibit unique spectroscopic characteristics, such as long luminescence lifetime, large Stoke's shift and sharp line-like atomic emission.[13-15] As these properties can overcome autofluorescence and light scattering, rare-earth compounds are commonly used as fluorophores in chemical biology.[13]



Scheme 1. Schematic illustration of GE-Tb-DPA based sensor for detection of DPA and cysteine.

In this work, we design and synthesize europium (Eu^{3+}) or terbium (Tb^{3+}) complex covalently modified graphene (GE-Eu /GE-Tb) (Fig. S1, Scheme 1). In the presence of dipicolinic acid (DPA), a biomarker and major constituent of B. anthracis spores, the complex exhibits strong red or green luminescence under UV excitation which attributes to the formation of the GE-Eu-DPA or GE-Tb-DPA complex. Due to the threats in biological attack, B. anthracis spores have aroused particular concern throughout the world in the past decades.[16-20] Development of methods for rapid and ultrasensitive detection of B. anthracis spores is greatly important for prevention and control of anthrax disease. Both of the luminescence complexes provide a rapid and simple method for DPA detection. The GE-Tb-DPA complex shows higher sensitivity than GE-Eu-DPA for detection of DPA. We found that mercury ion can competitively bind to DPA and thereby decrease the fluorescence intensity of GE-Eu-DPA, while the intensity was recovered in the presence of cysteine. As an essential amino acid in proteins and biologically activity, cysteine plays an important role in the human body, such as protein synthesis, detoxification, and

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

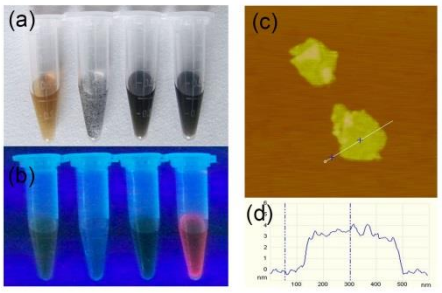

Figure 1. (a, b) Photographs of GO, GA, GE-Eu and GE-Eu-DPA (from left to right) in ddH_2O taken under daylight lamp (a) and 365 nm UV light (b). (c) AFM image of GE-Eu-DPA; (d) Height profile taken across the white line in (c).

metabolism.[21-25] The concentration of cysteine is highly correlated to the physiological functions and useful in diagnosing the underlying disease.[26] Therefore, a graphene-based biosensor is also developed to detect cysteine.

Syntheses of GE-Eu and GE-Tb are illustrated in Fig. S1 and detailed in the Experimental section. We firstly obtained amino functionalized graphene (GA) by covalently grafting 1,2-Bis(2-aminoethoxy)ethane onto graphene through the reaction of epoxy groups on graphene oxides and amino groups on 1,2-Bis(2-aminoethoxy)ethane in the presence of KOH. Subsequently, $NaBH_4$ was added to reduce the product. Then ethylenediamine tetraacetic acid dianhydride (EDTAD) was covalently grafted onto the surface of graphene by the reaction of the amino group presenting on the graphene surface with the anhydride group of EDTAD molecules.[27] The EDTA ligand on graphene is then readily converted into a complex coordinating with Eu^{3+} or Tb^{3+}(GE-Eu or GE-Tb). Upon exposure of GE-Eu or GE-Tb to DPA, DPA will coordinate with EDTA-Eu or EDTA-Tb group to form luminescent GE-Eu-DPA or GE-Tb-DPA.

The covalently grafted 1,2-bis(2-aminoethoxy)ethane onto graphene was confirmed by FTIR spectra of GA (Fig. S2). The strong CH_2 (2925 cm^{-1}, 2852 cm^{-1}) vibrations and a characteristic C–N stretch mode (1320 cm^{-1}, ν C–N binding with an aromatic ring) confirm that the 1,2-bis(2-aminoethoxy)ethane has been covalently grafted to the graphene sheet successfully.[28] Due to the reduction of $NaBH_4$, GA shows a maximum absorption at 269 nm. Conjugation of EDTA ligands to GA (GE) was confirmed by TGA and FTIR measurements. The new peaks at 1726 cm^{-1} and 1650 cm^{-1} in the FTIR spectrum indicate the presence of -COOH and CO–NH groups in the GE. The amount of EDTA grafted on GA has been evaluated by thermogravimetric analysis (TGA) under N_2 atmosphere and the content of EDTA in GE was 21.8 % (Fig. S3a). After the graft of EDTA on GA, the solution was redispersed, which attribute to the enhancement of electrostatic repulsion in the presence of EDTA. The formation of GE-Eu-DPA complex on graphene was evaluated by Energy-dispersive X-ray (EDX) spectra (Fig. S3b). The signals in the EDX spectrum indicate that Eu^{3+}/Tb^{3+} are introduced onto graphene surface. Furthermore, the XPS images showed that Eu^{3+}/Tb^{3+} are averagely distributed on the graphene sheets (Fig. S4). After DPA was introduced in EDTA-Eu, an intense narrow band in UV-Vis spectra at 274 nm was observed (Fig. S3c). The solution of GE-Eu-DPA exposed in UV light exhibits strong red florescence, also

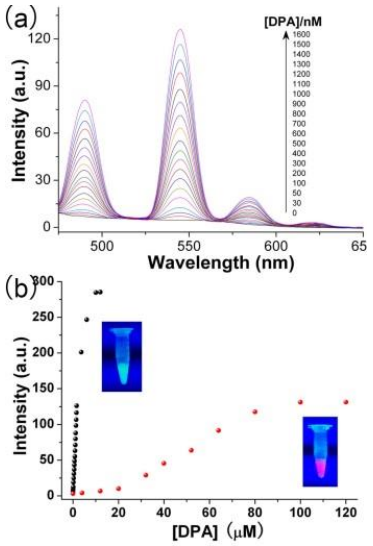

Figure 2. (a) Fluorescence response of 4 μgml^{-1} GE-Tb upon addition of different concentrations of DPA. (b) The fluorescence intensity of GE-Tb (545 nm, black) or GE-Eu (6[illegible] nm, red) as a function of DPA concentration. Inset: Photographs of GE-Tb-DPA (green) or GE-Eu-DPA (red) taken under 365 nm UV light.

suggesting that EDTA-Eu-DPA complex was successfully grafted on the graphene surface (Fig. 1a, b). Our atomic force microscopy (AFM) images show that the height of EDTA-Eu-DPA is 3.8 nm, which is higher than GO (~1 nm) (Fig. 1c, d).

In our graphene based system, we found Tb^{3+} is more sensitive than Eu^{3+} for the detection of B. anthracis spore. In the absence of DPA, the solution of GE-Tb was essentially nonemissive upon excitation at 270 nm (Fig. 2). Due to nonradiative deactivation from vibronic coupling of the OH oscillators with the excited lanthanide, coordinated water molecules can quench Tb^{3+} luminescence.[17] However, addition of DPA will make the solution exhibit sharp emission bands at 492, 546, 586 and 621 nm, respectively, corresponding to the deactivation of the Tb_{III} excited states $^5D_4$-^7Fn (n = 6, 5, 4 and 3). The DPA detection limit for this system was estimated to be 30 nM. The result is comparable to that previous reported by Lin et al, suggesting that graphene almost has no impact on the sensitivity. [29] The luminescent Tb^{3+}-complex tag was linked by a diamine linker, which not only covalently immobilized Tb^{3+}-complex on the graphene sheets but also efficiently prevented graphene luminescence quenching effect on Tb^{3+} complex. We compared both of the complexes for detection of DPA, GE-Tb shows higher sensitivity than GE-Eu for detection of DPA.

With the addition of Hg^{2+}, the fluorescent intensity of GE-Tb-DPA at 546 nm decreased gradually. Due to the $\pi \rightarrow \pi^*$ transition of bound DPA, the UV absorption spectrum of EDTA-Tb-DPA exhibits two well observed peaks at 271 and 278 nm (Fig. S5). However, in the presence Hg^{2+}, the peak at 271 nm was shifted to 273 nm and the peak at 278 nm was diminished. This resulted spectrum is very similar to DPA-Hg complex, suggesting that Hg^{2+} was competitively bound to DPA which inhibited EDTA-Tb binding to DPA. Therefore, the formation of DPA-Hg complex leads to the decrease of fluorescent intensity of GE-Tb-DPA.

It has been reported that cysteine can react with Hg^{2+} to form

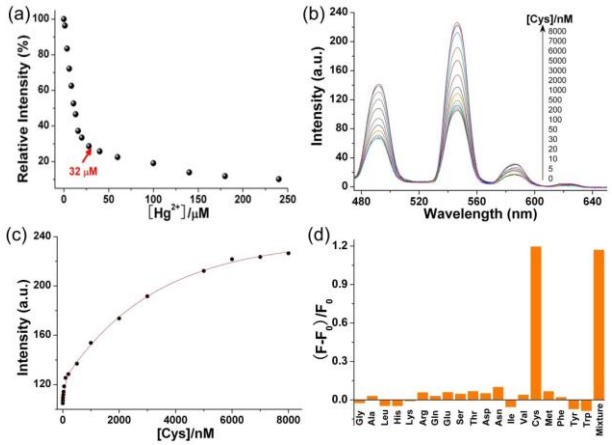

Figure 3. (a) Fluorescence intensity response of GE-Tb-DPA (4 μgml^{-1} GE-Tb, 6 μM DPA) upon addition of different concentrations of Hg^{2+}. (b) Fluorescence response of GE-Tb-DPA/Hg (4 μgml^{-1} GE-Tb, 6 μM DPA, 32 μM Hg^{2+}) upon addition of different concentrations of cysteine. (c) Fluorescence emission intensity (545 nm) of GE-Tb-DPA containing 32 μM Hg^{2+} with increasing amounts of cysteine. (d) Fluorescence emission response profiles of GE-Tb-DPA/Hg (4 μgml^{-1} GE-Tb, 6 μM DPA, 32 μM Hg^{2+}) toward amino acids (1 × 10^{-4} M). The sensor response to Cys in the presence of a mixture of other amino acids is also presented.

stable complex, in which Hg^{2+} is coordinated to both the carboxyl oxygen and sulfur atoms of cysteine.[30] Upon the addition of cysteine to the solution of the GE-Tb-DPA/Hg^{2+} ensemble, the fluorescence of the solution at 540 nm turned on immediately. The dynamic range of the sensor can be tuned by adjusting the concentration of mercury ions. At low concentration of Hg^{2+}, the detection system exhibited high sensitivity for cysteine. However, at high concentration of Hg^{2+}, the added cysteine would react first with free Hg^{2+} in solution, which decreases the enhancement effect (Fig. S6). To keep the sensor with low background as well as high sensitivity, the turning point at 32 μM Hg^{2+} was used to detect cysteine (Fig. 3a). Under this condition, we can detect as low as 5 nM cysteine, which is comparable to or even better than previous reports for cysteine detection (Fig. 3b, c).[31, 32] In addition, this sensor shows selectivity for detection of cysteine. Various amino acids were examined and only cysteine exhibited a significant enhancement. Moreover, the coexistence of cysteine and other amino acids almost has no influence on the fluorescence signal (Fig. 3d). This high selectivity was attributed to the high binding affinity of Hg^{2+} to the thiol group in cysteine.

In conclusion, this work describes a new approach for preparation of luminescent lanthanide graphene. Eu^{3+} or Tb^{3+} was covalently and averagely grafted on the graphene sheets. In the presence of DPA, the graphene complex exhibits strong red or green luminescence under UV excitation. The GE-Tb complex was used to sensitively detect DPA with a detection limit of 30 nM. Hg^{2+} can competitively bind to DPA and thereby decrease the fluorescence intensity of GE-Tb-DPA, while the intensity was recovered in the presence of cysteine. Therefore, a biosensor was also developed for selectively detection of cysteine with a detection limit as low as 5 nM. Our work will facilitate the utilization of unique luminescence properties of rare-earth complex functionalized graphene in medical diagnostics, bioimaging and environmental monitoring.

This work was financially supported by the National Natural Science Foundation of China (Projects 81160076) and the Foster... Talents Project for Young Academic and Technology leaders i... Yunnan Province (Project 2012HB029).

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