



## A Novel Rare Earth Ion Fluorescent Probe towards the Trace Detection of 2,4,6-Trinitrotoluene Based on Fluorescence Resonance Energy Transfer†

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This paper reports a resonance energy transfer-fluorescence quenching of the core-shell structure of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2$  modified by amino group on the surface for the ultrasensitive and ultratrace detection of 2,4,6-trinitrotoluene (TNT) in solution environments. Organic amine was covalently modified onto the surface of silica shell to form a hybrid monolayer of amino group. The particle can specifically bind TNT species by the charge-transfer complexing or acid-base pairing interactions between electron-rich amine ligands and electron-deficient aromatic rings. The resultant TNT-amine complexes bound at the silica surface can strongly suppress the fluorescence emission of the chosen dye by the fluorescence resonance energy transfer (FRET) from  $\text{CaMoO}_4:\text{Tb}^{3+}$  fluorescence donor to the irradiative TNT-amine acceptor through intermolecular polar-polar interactions at spatial proximity. The nanoparticle can sensitively detect down to 1 nM TNT with the use of only 10  $\mu\text{L}$  of solution (2 pg TNT). The simple FRET-based nanoparticle sensors reported here exhibit a high and stable fluorescence brightness, strong analyte affinity and good assembly flexibility and can thus find many applications in the detection of ultratrace analytes.

**Keywords:** Rare earth ion, Fluorescence quenching, 2,4,6-Trinitrotoluene, FRET, Ultratrace detection.

### INTRODUCTION

The detrimental pollution and powerful explosivity of aromatic nitro compounds have concurrently raised worldwide concerns of public security and environmental problem, due to their general production and application in the field of military and engineering<sup>1</sup>. The presently applied detections of aromatic nitro compounds are usually time-consuming and inconvenient with the employment of ponderous and expensive gas chromatography and high-performance liquid-chromatography mass-spectrometry. Therefore, the development of ultrasensitive chemosensors for the real-time detection of aromatic nitro compounds has attracted considerable research efforts in recent years<sup>1-5</sup>. Since the large overlap between the fluorescence emission spectrum of rare earth ions ( $\text{Tb}^{3+}$ ) and the ultraviolet-visible absorbance spectrum of the complex forming between 2,4,6-trinitrotoluene (TNT) and 3-aminopropyl triethoxysilane (APTS), we have tactfully designed a strategy for the detection of TNT using  $\text{CaMoO}_4$  doped  $\text{Tb}^{3+}$  encapsulated by  $\text{SiO}_2$  covalently anchoring APTS onto the surface, forming the functionalized nanoparticle fluorescence probe.

### EXPERIMENTAL

**Preparation of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2$  particles:** According to the one-step sol-gel process<sup>6</sup>, the molybdate doped  $\text{Tb}^{3+}$  luminescent systems were synthesized by the reaction of calcium carbonate (0.0825 g), ammonium molybdate (0.1532 g) and terbium oxide (0.0081 g) in nitric acid (2 mL) solution. Then the  $\text{CaMoO}_4:\text{Tb}^{3+}$  nanoparticles encapsulated with silica were prepared by the hydrolysis of tetraethyl orthosilicate (TEOS) in  $\text{CaMoO}_4:\text{Tb}^{3+}$  sol in aqueous ammonia solution. The resultant  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2$  particles were calcined for 2 h at 900 °C and obtained the green fluorescence probe.

**Synthesis of surface functionalized  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2\text{-NH}_2$  particles:** Then APTS were covalently anchored onto the shell surface of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2$  particles by the reaction of hydrolysis tetraethyl orthosilicate (TEOS) according to the reported Stöber method<sup>6</sup>, leading to the fluorescence probe of the functionalized  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2\text{-NH}_2$  particles. Typically, The  $\text{CaMoO}_4$  particles doped with rare earth ions ( $\text{Tb}^{3+}$ ) fluorophores and the surface modified amino group were prepared with a functional monomer 3-aminopropyltriethoxy-

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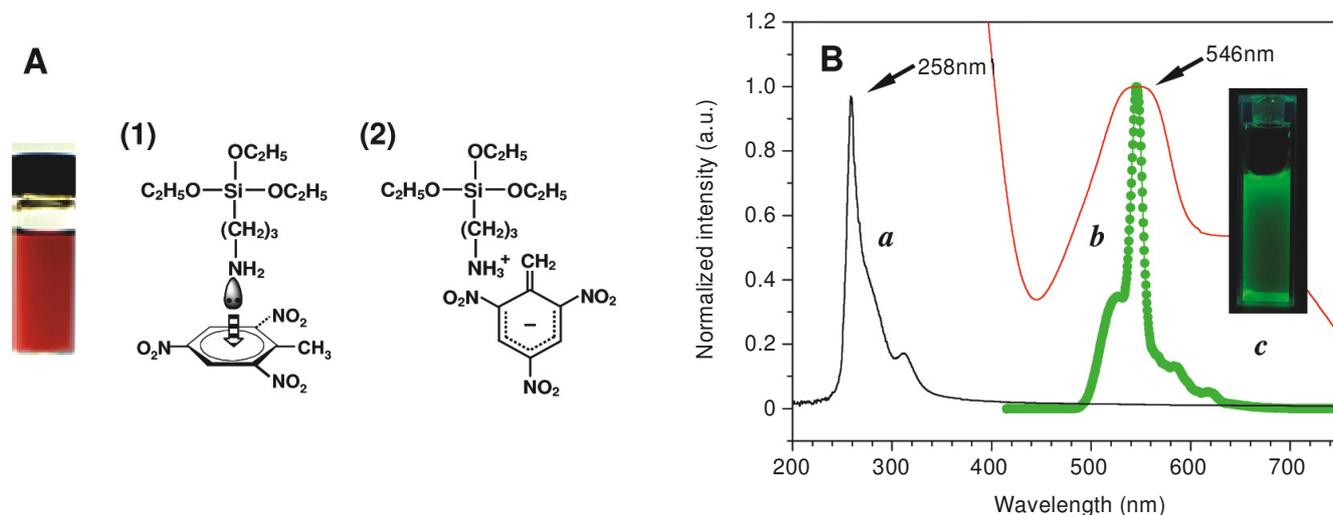


Fig. 1. (A) Interactions between TNT and APTS amine through (1) charge transfer and (2) acid-base pairing mechanisms, leading to the formation of red TNT-amine complexes in solution (inset image, solvent: ethanol/acetonitrile, 8:2). (B) Normalized absorption spectrum of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2\text{-NH}_2$  dispersed in aqueous solution (a) and TNT-APTS solution (b, red line) plotted together with normalized emission spectra of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2\text{-NH}_2$  (c, green dot line) (inset image, solvent: water)

silane (APTS) anchored onto the shell surface of silica in absolute alcohol solution through the covalent coupling of APTS to  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2$ , leading to forming the core-shell nanoparticles. Typically, proper amount of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2$  fluorescence probe and APTS were added into 10 mL anhydrous alcohol solvent.  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2\text{-NH}_2$  particles were carried out to react by hydrolysis at 50 °C in nitrogen atmosphere for 2 h in incubator (NBS), then the resultant product was centrifuged and dispersed for three times. Finally the functionalized fluorescence probe of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2\text{-NH}_2$  particles with the core-shell structure was obtained.

**Characterizations:** The morphology of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2$  particles was examined by FEI Sirion-200 field-emission scanning electron microscope (SEM) and JEOL 2010 transmission electron microscope (TEM). UV-Visible absorbance spectra were obtained by Shimadzu 2550 with diode array spectrometer. Fluorescence emission and excitation spectra were recorded using Perkin-Elmer Luminescence Spectrometer LS-55.

## RESULTS AND DISCUSSION

### FRET Mechanism between APTS and TNT derivative:

TNT-APTS derivative is deep red colour in aqueous solution and does absorb green part of visible light. A strong charge-transfer interaction, as a predominant process, occurs between the electron-deficient TNT and the electron-rich amino group of APTS. The electron transfer from amino groups to TNT leads to the formation of amino complex between TNT and primary amino group (Fig. 1A). The complex can strongly absorb the green part of visible light. The absorbance spectrum of TNT-APTS solution shows a strong visible absorption with  $\lambda_{\text{max}}$  at 546 nm, as plotted with red dot line in Fig. 1B. We thus chose the  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2\text{-NH}_2$  nanoparticles with a green fluorescence emission at ca. 546 nm as an energy donor, which has a spectral overlapping with the absorption peak of TNT-amine complexes at 546 nm. Fig. 1B shows the normalized emission spectra of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2\text{-NH}_2$  nanoparticles, plotted together with the normalized absorption spectrum of

TNT-APTS solution. The green dot line in Fig. 1B shows that the main absorption band of TNT-amine complexes has a whole spectral overlapping with the emission band of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2\text{-NH}_2$  nanoparticles. The fluorescence emission of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2$  fluorescence probe is strongly absorbed by the TNT derivatives, resulting in the non-emissive resonance transfer-based fluorescence quenching. The efficiency of the energy transfer is mainly dependent on the degree of spectral overlapping between the emission of dye donor and the absorption of analyte acceptor.

**FRET-based silica nanoparticle fluorescence probe for TNT detection:** Fig. 2 shows the schematic drawing of  $\text{CaMoO}_4:\text{Tb}^{3+}@\text{SiO}_2\text{-NH}_2$  probe for TNT detection. 3-Aminopropyltriethoxysilane amine were covalently linked onto the surface of silica nanoparticles by the reaction of alkoxy silane derivatives with silica surfaces. The primary amine ligands at the surface of silica particles play a role of recognition receptor of TNT by the strong specific interactions. 2,4,6-Trinitrotoluene analyte is thus attached onto the surface of silica particles in the form of TNT-amine complex. The analyte binding will lead to the quenching of dye fluorescence through the resonance energy transfer from dye donor to TNT-amine complex. The exciting band gap of the chosen dyes is about equal to the absorption band gap of TNT-amine complex and they are of spatial proximity at the surface of nanoparticle.

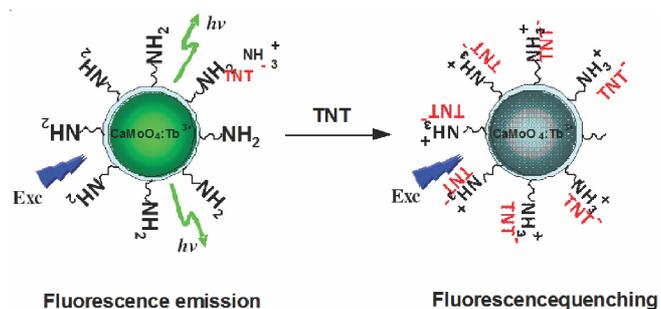


Fig. 2. Schematic illustration of the FRET-based silica nanoparticle probe for TNT detection

**Fluorescence response of dye-silica-NH<sub>2</sub> particles toward TNT:** Fig. 3 shows the fluorescence evolution of CaMoO<sub>4</sub>:Tb<sup>3+</sup>@SiO<sub>2</sub>-NH<sub>2</sub> with the increase of TNT concentration. We can clearly verify that the fluorescence intensities of CaMoO<sub>4</sub>:Tb<sup>3+</sup>@SiO<sub>2</sub>-NH<sub>2</sub> particles decrease rapidly with increasing successive aliquots of TNT concentrations. The Stern-Volmer equation was used to evaluate the quenching efficiency for dye-silica-(NH<sub>2</sub>) systems, as follow:  $(I_0/I)^{-1} = K_{SV} [TNT]$ , where  $I_0$  is the initial fluorescence intensity in the absence of analyte,  $I$  is the fluorescence intensity in the presence of TNT and  $K_{SV}$  is quenching constant with TNT (Fig. 3B).

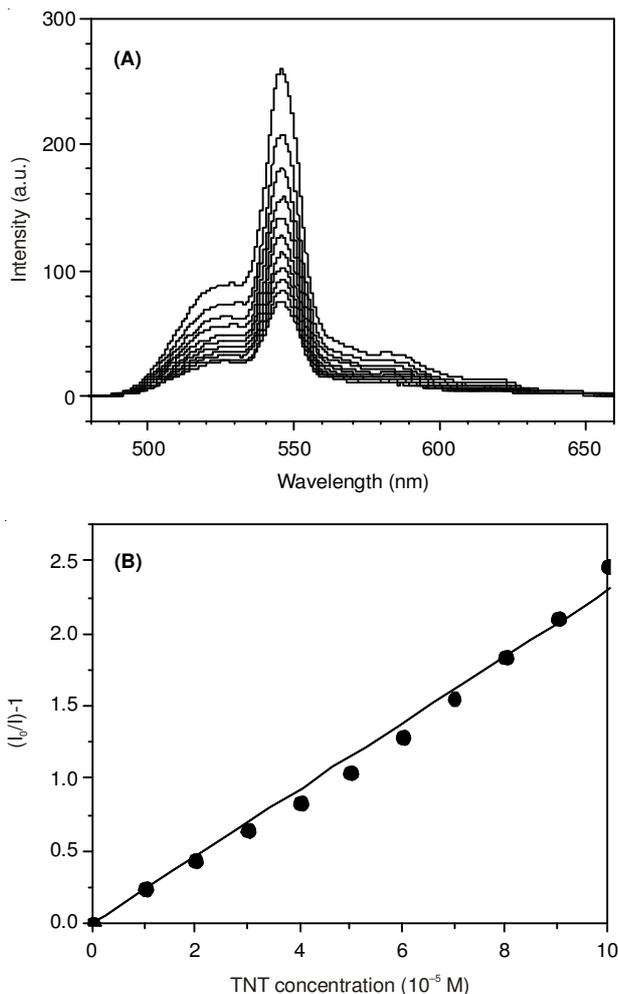


Fig. 3. (A) Evolution of fluorescence spectra of CaMoO<sub>4</sub>:Tb<sup>3+</sup>@SiO<sub>2</sub>-NH<sub>2</sub> nanoparticle with increasing TNT concentration. The concentrations of TNT from top to bottom are 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μM, respectively. The amount of CaMoO<sub>4</sub>:Tb<sup>3+</sup>@SiO<sub>2</sub>-NH<sub>2</sub> particles is 25 μg/mL in solution. (B) Stern-Volmer plots from CaMoO<sub>4</sub>:Tb<sup>3+</sup>@SiO<sub>2</sub>-NH<sub>2</sub> nanoparticle with TNT in solution

## Conclusion

This paper demonstrates the resonance energy transfer amplifying fluorescence quenching toward the ultratrace detection of TNT by CaMoO<sub>4</sub>:Tb<sup>3+</sup>@SiO<sub>2</sub>-NH<sub>2</sub> nanoparticle. The primary amine ligands at the surface of silica particles can specifically bind TNT species from environment by the formation of TNT-amine complex. In addition, the non-emissive fluorescence resonance energy transfer between the CaMoO<sub>4</sub>:Tb<sup>3+</sup>@SiO<sub>2</sub>-NH<sub>2</sub> nanoparticle and TNT in the spatial-approaching leads to a quenching response.

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