

Electronic Supporting Information (ESI)

Ratiometric fluorescence and mesoporous structure dual signal amplification for sensitive and selective detection of TNT based on MIPs@QDs fluorescence sensor

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1. Experimental Section

Reagents. Tellurium powder, Cd(NO₃)₂·4H₂O, thioglycolic acid (TGA), sodium borohydride, tetraethyl orthosilicate (TEOS), trinitrophenol (TNP), cyclohexane, n-hexanol, and Triton X-100 (TX-100) were purchased from Tianjin Reagent Plant (Tianjin, China). 3-aminopropyl triethoxy silane (APTES), cetyltrimethylammonium bromide (CTAB) and TNT standard solution (1.00 mg/mL in methanol) were purchased from J&K Technology Ltd. (Beijing, China). All chemicals were of at least

analytical grade and used without any purification. Double deionized water (DDW) was used as solvent.

Characterization. Fluorescence measurements were performed with a Fluoromax-4 Spectrofluorometer (Horiba Scientific) equipped with 1 cm quartz cell at 25 °C, with excitation and emission slit widths of 5 and 3 nm, respectively, and the excitation wavelength at 365 nm. The morphological evaluation was examined with a scanning electron microscope (SEM, Hitachi S-4800 FE-SEM, operating at 5 kV) and a transmission electron microscope (TEM, JEM-2100F). Brunauer-Emmett-Teller (BET) surface area was determined by nitrogen adsorption/desorption at 77 K using a Micromeritics ASAP 2020 Sorptometer (Micromeritics, ASAP 2020, USA).

Synthesis mesoporous structured Ratiometric fluorescence TNT imprinted QD@SiO₂@mSiO₂

The green and red emissive CdTe QDs were synthesized in the aqueous phase by a previous method ^[1]. The red QDs embedded silica nanoparticles with diameter about 55 nm was prepared by reverse micro-emulsion method according to our previous work ^[2]. Then mesoporous structured TNT imprinted QD@SiO₂@mSiO₂ were prepared on the surface of red QDs@SiO₂ by dummy molecular imprinting method using TNP as dummy template as reported in our previous work ^[2]. Typically, 10 mg red QD@SiO₂ dispersed into 17 mL DDW, then 2 mL green QDs solution, 0.8 mL CTAB solution (0.2 M), 0.1 mL NaOH (0.2 M) was added. After stirring 30 min, 100 µL of TEOS, 20 µL of APTES and 5 mg of TNP in 0.2 mL anhydrous ethanol were added. The mixture was stirred for 24 h in darkness and the obtained materials were centrifuged. Typically, acid methanol and calcining were the commonly used method to remove template for molecular recognition and template CTAB for mesoporous structure ^[3, 4]. In the present work, considering that the fluorescence intensity of QDs could be quenched obviously after washing with acid ethanol or calcining, so the templates TNP for molecular recognition and template CTAB for mesoporous structure was washed by a mixture solvent of ethanol/acetonitrile (8:2), including immersion over a period of 3 h on fresh ethanol/acetonitrile and ultrasound assistance washing repeatedly ^[5,6]. UV, IR and fluorescence spectrums were used to confirm the

completely removal of TNP and CTAB. TNP has UV absorbance at 245 nm, the washing solvent was detect by UV until no absorbance was detected at 245 nm. In the meantime, the fluorescence intensity of as-prepared QD@SiO₂@mSiO₂ restored to the level of those NIPs. CTAB has typical bands observed in the region 2800–3000 cm⁻¹, which is attributed to the vibrations of -CH₂ of CTAB templates. The imprinted QD@SiO₂@mSiO₂ powder was detected by FT-IR until no adsorption peaks were observed in the range of 2800–3000 cm⁻¹.

The control TNT imprinted ratiometric fluorescence QD@SiO₂@SiO₂ without mesoporous structure was prepared in the same manner but without adding template CTAB. The control TNT imprinted single fluorescence QD@SiO₂@mSiO₂ was prepared in the same manner but only green QDs were used in the whole procedure. The control non-imprinted ratiometric fluorescence QD@SiO₂@mSiO₂ was prepared in the same manner but without adding template TNT.

Detection of TNT

The disperse systems were optimized by monitoring the fluorescence stability and sensitivity in different media, including water, water/ethanol mixture and ethanol. The quenching amount, defined as $(F_0 - F)/F_0$, increased with the increase of ethanol. Therefore, ethanol was selected as the dispersion medium. The amounts of TNT imprinted QD@SiO₂@mSiO₂ had an obvious effect on the quenching efficiency. After optimization using quenching amount as the index of quenching capacity, the concentration of TNT imprinted QD@SiO₂@mSiO₂ was fixed at 40 mg/L. TNT were added into 5 mL of the probe solution one by one with the final concentrations are 50 nM to 900 nM. The fluorescence spectra were collected 10 min after each addition because the fluorescence spectra became stable 5 min after the addition of TNT ion into the probe solution.

Reusability of the MIPs

The recovery and reusability are an important characteristic of the MIPs based sensor, and which is likely to be a key factor in improving the economic efficiency. The removal–rebinding cycle was repeated ten times using the same MIPs@QDs or NIPs@QDs. During the process, the MIPs@QDs could be recovered by centrifuging

and washing to remove the rebinding TNT with an ethanol/ACN solvent.

Selectivity and Interference Experiments.

In order to test of selectivity of the as-prepared TNT imprinted QD@SiO₂@mSiO₂, the fluorescent responses to the other template analogues (DNP, 4-NP, phenol, and DNT) and metal ions (Cd²⁺, Pb²⁺, Cu²⁺, Hg²⁺) were examined by a similar procedure mentioned above. The concentration of metal ions fixed as 10 μm while the value was 500 nM for template analogues. The solution of Hg²⁺ was prepared in 0.1 M HNO₃ while the solutions of other metal ions were prepared in DDW for the experiments.

Real sample detection

Soil sample was first treated with acetone to retrieve TNT from the soil samples, then acetone was evaporated by treatment with nitrogen followed by dissolution in ethanol. The tap water sample was acquired in the laboratory and filtered through 0.45 μm pore size membrane to remove the suspended particles. With the addition of TNT in the real samples, the fluorescence intensity of the ratiometric probe decreased. The relative standard deviation (RSD) was obtained by repeating the experiment 5 times under the same condition.

1. J. Yao, K. Zhang, H. Zhu, F. Ma, M. Sun, H. Yu, J. Sun and S. Wang, *Anal. Chem.*, 2013, **85**, 6461–6468.
2. S. F. Xu, H. Lu, J. Li, X. Song, A. Wang, L. Chen and S. Han, *ACS App. Mater. Interfaces*, 2013, **5**, 8146–8154.
3. D. Gao, Z. Zhang, M. Wu, C. Xie, G. Guan and D. Wang, *J. Am. Chem. Soc.* 2007, **129**, 7859-7866.
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2. N₂ sorption isotherms and the pore size distribution

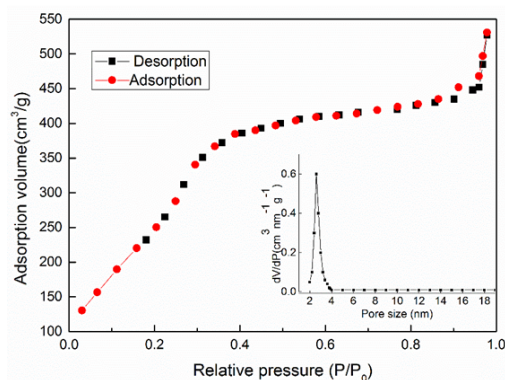


Figure S1 N₂ sorption isotherms and the pore size distribution (inset) of the TNT imprinted QD@SiO₂@mSiO₂.

3. Fluorescence emission spectra of as-prepared ratiometric MIP@QDs

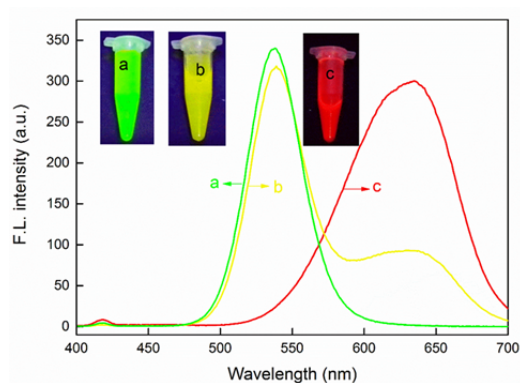


Figure S2 Fluorescence emission spectra of (a) green emissive QDs, (b) the ratiometric MIP@QDs probe, and (c) red emissive QDs@SiO₂. The inset photos show the corresponding fluorescence colors under a 365 nm UV lamp, respectively.

4. The effect of disperse medium, amount of MIPs@QDs and Fluorescence response time

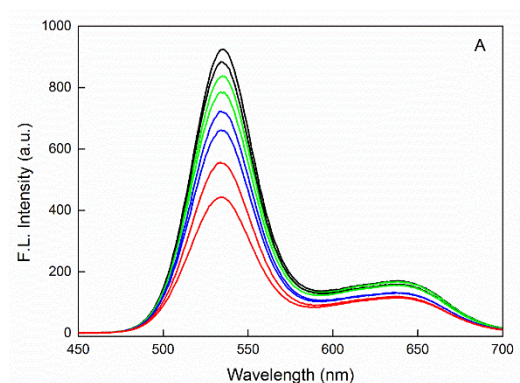


Figure 3A The effect of disperse medium on quenching efficiency. During the experiment, the concentration of TNT was fixed as 100 nM, changing the disperse medium for water (black line), water/ethanol (8:2) (green line), water/ethanol (5:5) (blue line), and ethanol (red line).

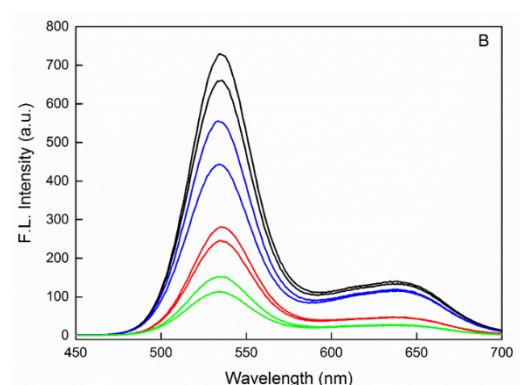


Figure 3B The effect of amount of MIPs@QDs on quenching efficiency. During the experiment, the concentration of TNT was fixed as 100 nM, changing the amount of MIPs@QDs from 20 mg/L (green line), 30 mg/L (red line), 40 mg/L (blue line) and 50 mg/L (black line).

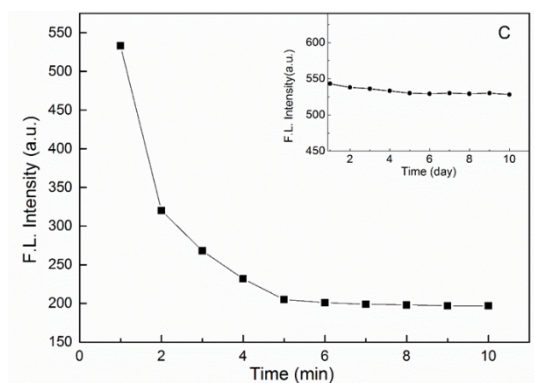


Figure 3 C: Fluorescence response time of TNT imprinted QD@SiO₂@mSiO₂ for TNT. Inset was fluorescence intensity change of DMIP@QDs within 10 days. The fluorescence intensity was

recorded at $\lambda_{540\text{nm}}$.

5. The fluorescence colors and the corresponding fluorescence spectra of the signal fluorescence probe

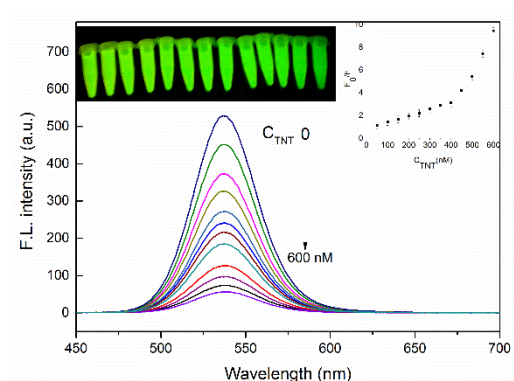


Figure S4 The fluorescence colors and the corresponding fluorescence spectra ($\lambda_{\text{exc}} = 365 \text{ nm}$) of the signal fluorescence probe upon the exposure to different concentrations of TNT. The experimental conditions were MIPs@QDs, 40 mg/L; excited light, 365 nm; slit widths of excitation and emission, 5 and 3 nm, respectively.

6. The reusability of MIPs@QDs for detection of TNT

It was found that the sensor could retain its fluorescence intensity and detection sensitivity during those ten recycles. This indicated that the MIPs had excellent stability during the fluorescence sensor process.

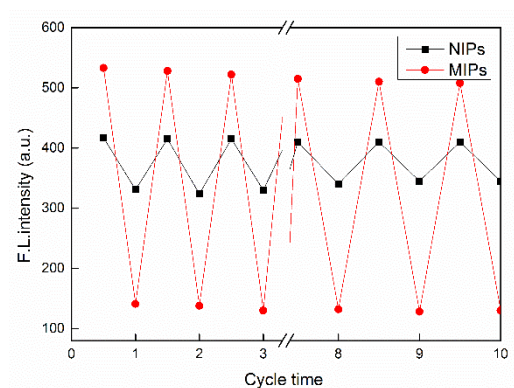


Figure S5 Recovery of FL intensity of MIPs@QDs after removal of TNT. The experimental conditions were MIPs@QDs:40 mg, C_{TNT} : 400 nM, excited light, 365 nm; slit widths of excitation and emission, 5 and 3 nm, respectively.

7. Analysis of real samples

Table S1 Spiked recoveries and relative standard deviations for detection of TNT in TNT-Spiked soil and tap water samples by TNT imprinted QD@SiO₂@ mSiO₂ ratiometric probe

Sample	Added TNT (nM)	TNT imprinted QD@SiO ₂ @ mSiO ₂ ratiometric probe		HPLC	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Soil	20	95.7	3.4	97.4	3.2
	50	98.2	2.9	95.7	2.5
	100	96.3	4.2	98.3	1.9
Tap water	20	94.2	4.3	96.8	2.6
	50	95.7	3.2	97.3	3.0
	100	97.4	3.5	99.4	2.9