

Supporting information:

N-dots as photoluminescent probe for rapid and selective detection
of Hg²⁺ and Ag⁺ in aqueous solution.

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Materials and Apparatus

All chemicals were obtained from commercial suppliers and were used without further purification. The probe stock solutions were prepared in distilled water at a concentration of 2 mg mL⁻¹. The stock solutions of various testing species were prepared from CdCl₂, CrCl₃, MgCl₂, CaCl₂, FeCl₃ 6H₂O, NaCl, CoCl₂ 6H₂O, KCl, Hg(ClO₄)₂ 3H₂O, AgNO₃ at 10 mM in distilled water. PBS buffer was prepared using distilled water. Infrared spectra were obtained on a NEXUS-470 Fourier transform infrared (FTIR) spectrometer ranged from 4000 to 500 cm⁻¹. The measurement of C, H, and N elements was performed on Varo EL III elemental analyser. Atomic force microscope (AFM) image was recorded on a Bruker Multimode 8 atomic force microscope. The fluorescence (FL) spectra were measured on a fluorimeter (Cary Eclipse) equipped with an integrating sphere.

Synthesis of N-dots

Raw N-dots were prepared by heating 2-azidoimidazole (0.2g) in methanol (5 mL) at 50 °C for 60 hours. After cooling, methanol was removed under reduced pressure. The obtained residue was washed with ethyl acetate for 5 times. The supernatant was discarded and the obtained solid was dispersed in water (5 mL). After filtration with 0.22 µm filter, the solution was concentrated and vacuum-frozen-dried, yielding 22.3 mg solid, 11 % yield.

Photoluminescence measurement

All the fluorescence measurements were performed at room temperature using PBS buffer (pH 7.4) solution. In a typical assay, 10 µL N-dots stock solution (2 mg/mL) was diluted in PBS buffer at a final concentration of 20 µg/mL for photoluminescence spectra. For cation selectivity study, photoluminescence emission spectra were recorded after mixing with various metal ions (Cd²⁺, Cr³⁺, Mg²⁺, Ca²⁺, Fe³⁺, Na⁺, Co²⁺, K⁺, Hg²⁺, Ag⁺). In kinetic studies, photoluminescence intensity at 515 nm (excitation at 460 nm) against time was monitored in the presence and the absence of analytes (Hg²⁺ or Ag⁺) every 10 seconds. In sensitivity studies, different concentrations of Hg²⁺ or Ag⁺ (final concentration: 0~100 µM) were added to N-dots solutions (20

μg/mL), followed by photoluminescence measurement. To distinguish Hg²⁺ from Ag⁺, EDTA (30 μM) was added to N-dots-Hg or N-dots-Ag aqueous solutions and mix well for 1 min, followed by photoluminescence spectral measurement. In competitive binding experiments with biothiols, Cys, Hcy, GSH and Na₂S at a final concentration 1 mM were added into Hg-N-dots or Ag-N-dots solutions, and the resulting solutions were shaken well and incubated for 1 min, followed by photoluminescence measurement.

Limit of detection (LOD) and Stern-Volmer quenching constant:

The detection limit was calculated using equation: LOD=3σ/K. (σ is the standard deviation of the control N-dots solutions, K is the slop of obtained linear curve in Fig. 3).

The quenching was also described by a modified Stern-Volmer equation:

$$F_0/(F_0-F) = 1/(f_a K_a [Q]) + 1/f_a$$

Where Q is the concentration of the quencher (Hg²⁺ or Ag⁺), F₀ is the photoluminescence intensity in the absence of the quencher, F is the observed intensity in the presence of the quencher, f_a is the fraction of initial photoluminescence that is accessible to the quencher and K_a is the Stern–Volmer quenching constant.

Quantum Yield

The quantum yield measurements were determined in PBS buffer using quinine sulfate (0.10 M H₂SO₄, Φ = 0.54) as a standard according to the following equation:

$$\Phi_u = \Phi_s (Y_u/Y_s)(A_s/A_u)(n_u/n_s)$$

Φ is quantum yield; Y is the measured integrated fluorescence emission intensity; A is the optical density measured at the excitation wavelength; n is the refractive index. The subscript “s” refers to the standard quantum yield of reference quinine sulfate. The subscript “u” refers to the unknown quantum yield of as-prepared N-dots. In order to minimize re-absorption effects, absorbance in the 1cm fluorescence cuvette were kept under 0.05 at the excitation wavelength.

Table S1. Elemental composition percentage of N-dots prepared at 50 °C.

Elemental analysis	Composition percentage (%)
C	36.47
H	5.14
N	36.27

Fig. S1 Fluorescence images (top) and digital images (bottom) of reaction solutions after heating different time at 50 °C.

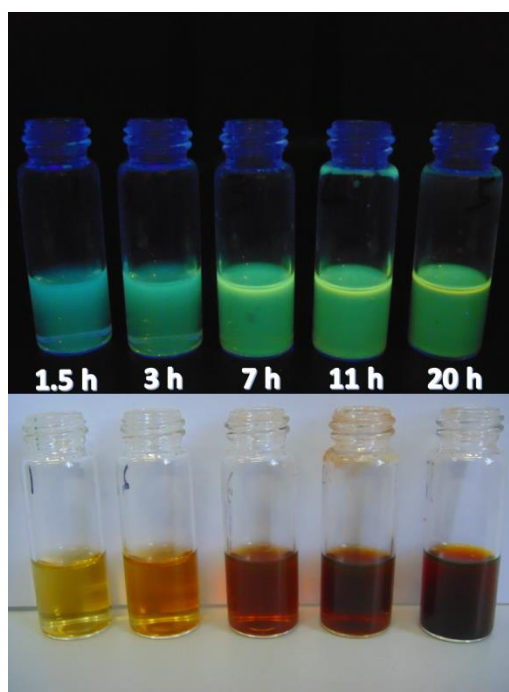


Fig. S2 Atomic force microscopy (AFM) image and size profile of as-prepared N-dots.

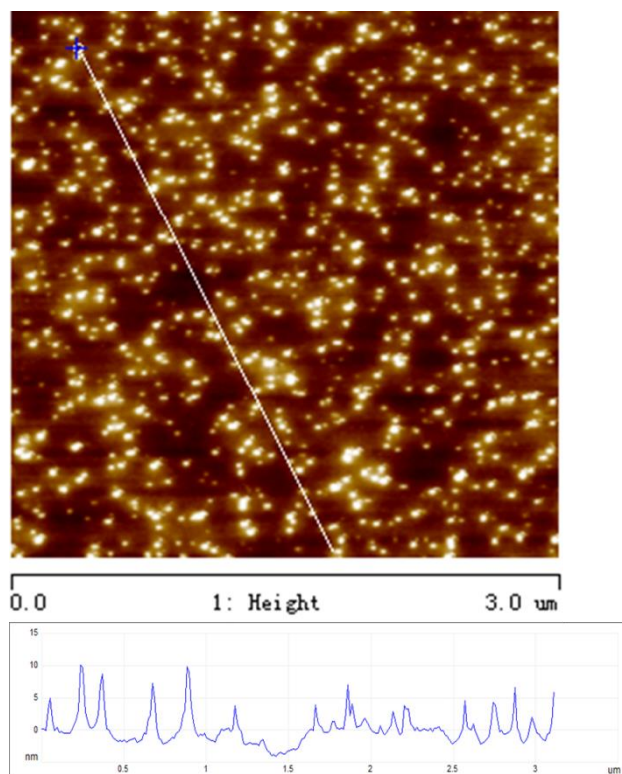


Fig. S3 FTIR spectra of N-dots prepared at 50 °C.

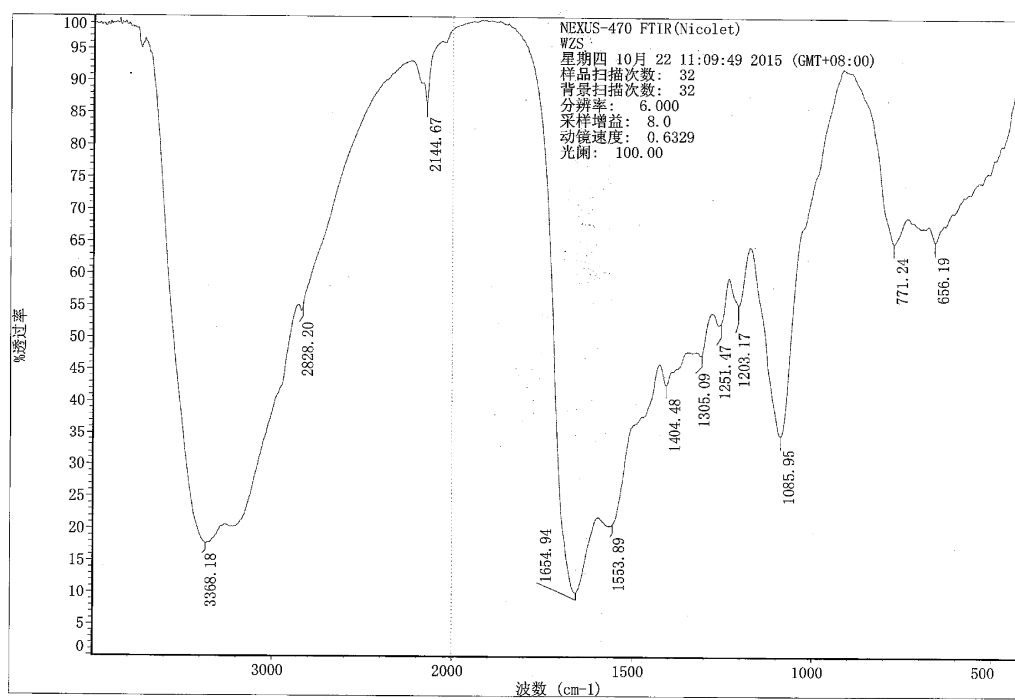


Fig. S4 Time-dependent photoluminescence intensity (515 nm) change upon the addition of analytes (100 μM) to N-dots solutions (20 $\mu\text{g mL}^{-1}$). F_0 is the initial intensity. $\lambda_{\text{ex}} = 460 \text{ nm}$.

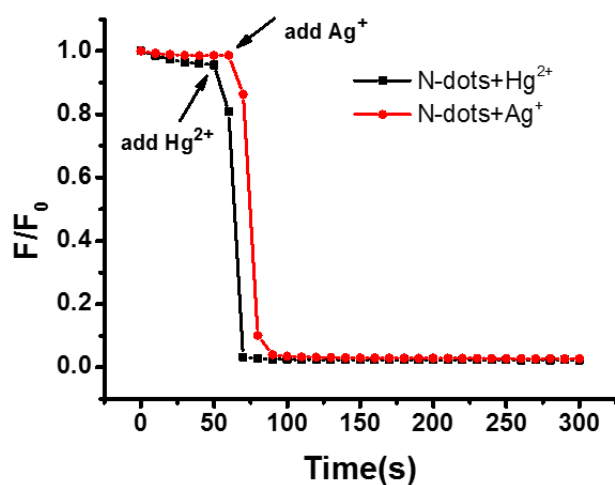


Fig. S5 A linear plot of $F_0/(F_0-F)$ versus $1/[\text{Hg}^{2+}]$ and $1/[\text{Ag}^+]$.

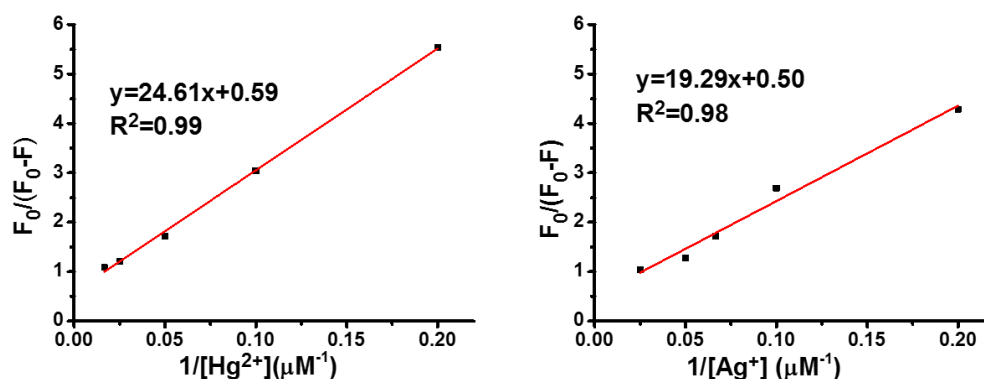


Fig. S6 Photoluminescence emission of N-dots in the presence of analytes (100 μM), followed by the addition of biothiols (1 mM) in 20 mM PBS buffer (pH=7.4). $\lambda_{\text{ex}} = 460 \text{ nm}$.

