Supporting Information

Multifunctional Binding Strategy on Nonconjugated Polymer Nanoparticles for Ratiometric Detection and Effective Removal of Mercury Ions

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This supporting information contains 18-page document, including EXPERIMENTAL SECTION, 6 tables, 12 figures, references and this cover page.

EXPERIMENTAL SECTION

1. Chemicals. Mercaptosuccinic acid (MSA) and thiosemicarbazide (TSC) were supplied by Adamas Reagent, Ltd., China. $Hg(NO)_3$, HNO_3 (wt. 68%), Na_2HPO_4, KH_2PO_4, NaF, NaCl, NaBr, KI, Na_2S, $Cu(NO_3)_2$, $Al(NO_3)_3$, $CdCl_2$, $FeCl_3$, $FeCl_2$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $CrCl_3$, $KCrO_4$, $Zn(NO_3)_2$, $HAuCl_3$, $AgNO_3$, and EDTA were obtained from Aladdin Reagent Co., Ltd., Shanghai, China. Phosphate buffers (PB, 1/15 M) with different pH values were prepared according to a standard protocol. All chemicals used were of analytical reagent grade. Ultrapure water (18 MΩ cm) was used in all experiments.

2. Instruments. Transmission electron microscopy (TEM) analysis was obtained with a Talo F200X transmission electron microscope (FEI, Czech Republic). X-ray photoelectron spectra (XPS) were taken on a Thermo ESCALAB 250XI surface analysis system. A Bruker IFS 113v spectrometer (Bruker, Germany) was utilized for recording Fourier transform infrared (FTIR) spectrum. An F-4500 spectrofluorometer (Hitachi, Japan) was applied for collecting fluorescence (FL) and second-order scattering (SOS) spectra. Photomultiplier tube (PMT) voltage was fixed at 700 V, and the slits were set at 5 nm for both excitation and emission. Ultraviolet visible (UV-vis) absorption spectra were collected on a UV-vis 2450 spectrophotometer (Shimadzu, Japan). Fluorescence lifetimes were measured with a Fluorolog-3 fluorescence spectrophotometer (Horiba, USA). The dynamic light scattering (DLS) and zeta potential were using a NanoBrook Omni particle size and zeta potential analyzer (Brook, China). A pH meter (FE28, Mettler-Toledo, Switzerland) was used for the measurement of solution pH values.

3. Preparation of MT-PNPs. The nonconjugated PNPs were synthesized by one-pot hydrothermal treatment. Typically, 2.00 g of MSA and 1.20 g of TSC were dissolved with 80 mL of ultrapure water (18 MΩ cm). The mixed solution was then moved into a Teflon-lined autoclave, where it was heat-treated for 6 h at 200 ℃. After the autoclave was cooled naturally to room temperature, the resultant brown

solution was dialyzed with a filter bag (molecular weight cut off = 500 Da) for 24 h to obtain the pure MT-PNPs. The purified MT-PNPs solution was concentrated to 5 mL by rotary evaporation as a stock solution for analytical experiments. In addition, the product was freeze-dried (−70 °C) to obtain the powder for characterization.

4. Hg^{2+} Detection Procedure with MT-PNPs.

Ratiometric Detection Procedure of Hg^{2+}. At room temperature, 10 μL of MT-PNPs stock solution, 200 μL of phosphate buffer (PB, 1/15 M, pH = 5.0), and 300 μL of ultrapure water were mixed. Then, various concentrations of Hg^{2+} standard solution (10 μL) was added to the mixture. The total volume of sensing system was 520 μL. After the reaction for 30 min, the fluorescence and second-order scattering spectra were collected at 365 nm excitation.

Ratiometric Detection Procedure of Hg^{2+} in Natural Water. The real water sample was collected in the Beibei reservoir (Chongqing, China). Firstly, the water was centrifugated (10000 r/min, 10 min) and filtrated (0.22 μM membrane filter) to remove the precipitable and suspended particles, respectively. Then, HNO_3 (0.1 M, 100 μL) was added to the water sample (5.0 mL) until the pH reaches about 2.5 to prevent metal ions hydrolysis and precipitation. Before measurement, 0.1 M NaOH (80 μL) was added to the water sample to recover the pH approximately 5.0. 300 μL of real water, 200 μL of phosphate buffer, and 10 μL of MT-PNPs stock solution were mixed. After reaction for 30 min, the FL and SOS spectra were collected under excitation at 365 nm. Then, various concentrations of Hg^{2+} standard solution (10 μL) was added to the mixture and the FL and SOS spectra were recorded with the same procedure.

5. Hg^{2+} Removal Procedure with MT-PNPs.

Procedure for Hg^{2+} Removal. The removal experiment was carried out at room temperature. In detail, Hg^{2+} aqueous solution (20 μM, 5 mL, pH 6.0 regulated with 0.1 M NaOH and 0.1 M HCl) was mixed with MT-PNPs (0.4576 mg). After reaction for 50 min, the precipitate was separated from the mother liquid via centrifugation at 10000 r/min for 10 min. Finally, the supernatant was taken for ICP-MS analysis of the

residual Hg^{2+}. The ICP-MS technique offers the advantages including high sensitivity, accuracy, and good selectivity. Each result in the work is obtained in three determinations in parallel.

Procedure for Hg^{2+} Sorption Isotherm. Hg^{2+} aqueous solution (5 mL, pH 6.0) with different concentrations was mixed with MT-PNPs (0.4576 mg). The mixtures were stirred at room temperature for 50 min, and then suffered centrifugation (10000 r/min, 10 min). The remaining Hg^{2+} in supernatant was analyzed by ICP-MS.

Procedure for Hg^{2+} Removal at pH 2.0-6.0. 5 mL of Hg^{2+} solution (20 μM) at different pH (2.0-6.0) was mixed with MT-PNPs (0.4576 mg). The mixtures stirred at room temperature for 50 min, and then suffered centrifugation (10000 r/min, 10 min). The remaining Hg^{2+} in supernatant was analyzed by ICP-MS.

Procedure for Hg^{2+} Sorption Kinetics. MT-PNPs (0.4576 mg) were added to the Hg^{2+} aqueous solution (20 μM, 5 mL, pH 6.0). The mixtures were stirred at room temperature for different contact times (10–110 min) followed by centrifugation (10000 r/min, 10 min). Finally, the supernatant was collected and analyzed by ICP-MS to determine the remaining Hg^{2+} content.

Procedure for Hg^{2+} Removal with Coexisting Metal Ions. Firstly, 5 mL of aqueous solution (pH 6.0) containing Hg^{2+} (20 μM) and coexisting metal ions (20 μM) were prepared. Then, MT-PNPs (0.4576 mg) were added to the solution and reacted for 50 min. After centrifugation (10000 r/min) for 10 min, the supernatant was taken for ICP-MS analysis of the metal concentrations.

Procedure for Hg^{2+} Removal from Real Sample. The water sample was collected from Beibei reservoir (Chongqing, China), and then was filtrated with 0.22 μM membrane filter to remove the suspended particles. Then, $Hg(NO_3)_2$ standard solution were spiked in the 5 mL of water sample to obtained 20 and 40 μM spiking level, respectively. After the pH value of the spiking water sample was adjusted to 6.0, it was treated with MT-PNPs (0.4576 mg) for 50 min. Upon equilibrium, samples were centrifugated and analyzed for Hg^{2+}.

6. Cell Viability Assay. To examine the cytotoxicity of the MT-PNPs in living

cells, LO2 cells were incubated in 96-well plates and allowed to grow for 24 h at 37 ℃ with 5% CO_2. Then, different concentrations of MT-PNPs (0, 0.88, 2.20, 4.40, 6.60, 8.80, and 11.00 mg/mL) were added to the wells followed by incubation for 24 h at 37 ℃ under 5% CO_2. The cell viability was assessed colorimetrically using cell counting kit-8 (CCK-8). The optical density (OD) was measured at 450 nm. The following equation was used to calculate the cell viability:[1]

$$\text{cell viability (\%)} = ([OD]_{treated}/[OD]_{control})\times 100\%$$

where $OD_{control}$ and $OD_{treated}$ were obtained in the absence and presence of MT-PNPs, respectively. The results are the mean standard deviation of four separate measurements.

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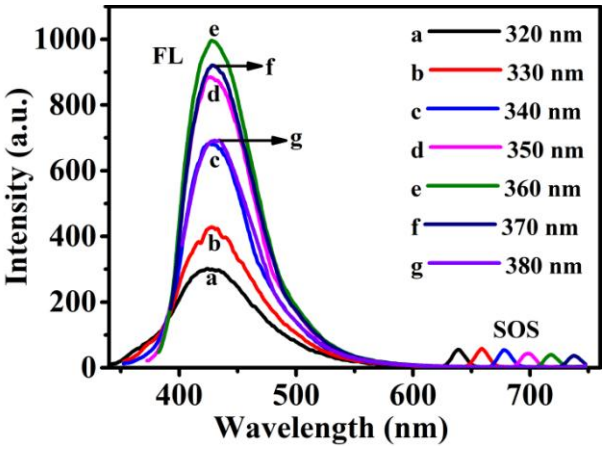

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126 **Figure S1.** FL emission spectra of MT-PNPs with different excitation wavelengths.

127 [MT-PNPs] = 2% v/v.

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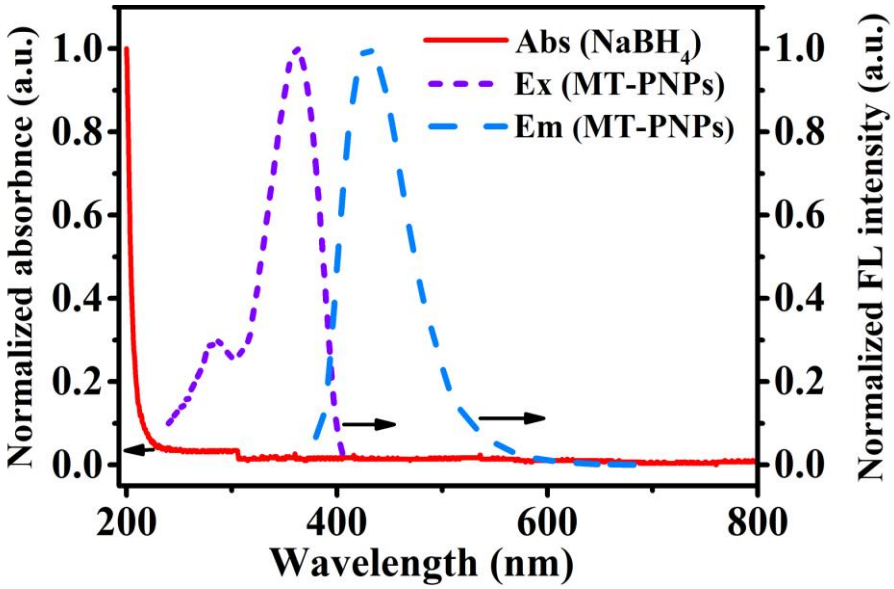

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131 **Figure S2.** Normalized absorption spectrum (Abs) of $NaBH_4$ solution (1.0 mM) and

132 the normalized excitation (Ex) and emission (Em) spectra of MT-PNPs (2% v/v).

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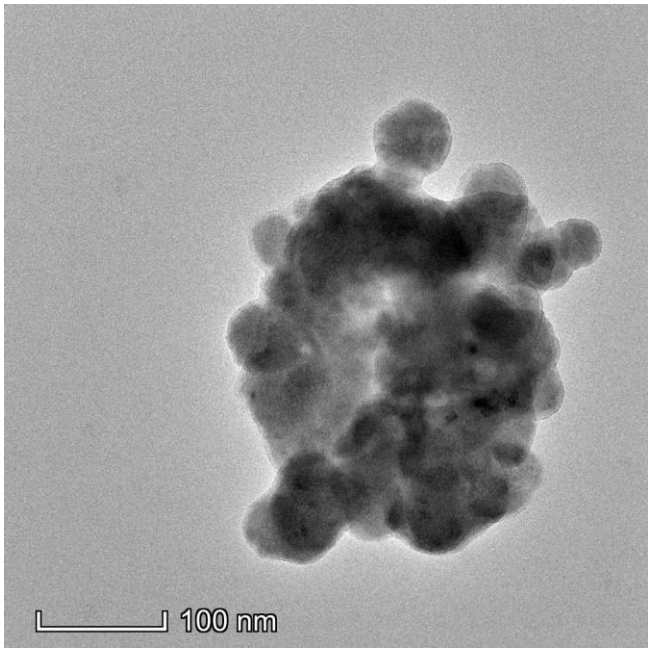


Figure S3. High-resolution TEM image of MT-PNP-Hg^{2+} complex. [MT-PNPs] = 2% v/v, [Hg^{2+}] = 192 μM, PB (pH = 5.0).

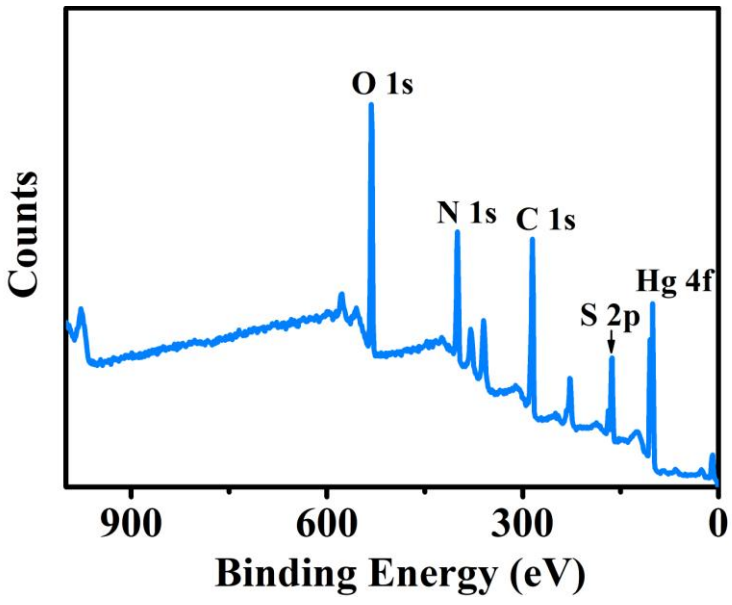


Figure S4. XPS spectrum of MT-PNPs in the presence of Hg^{2+}.

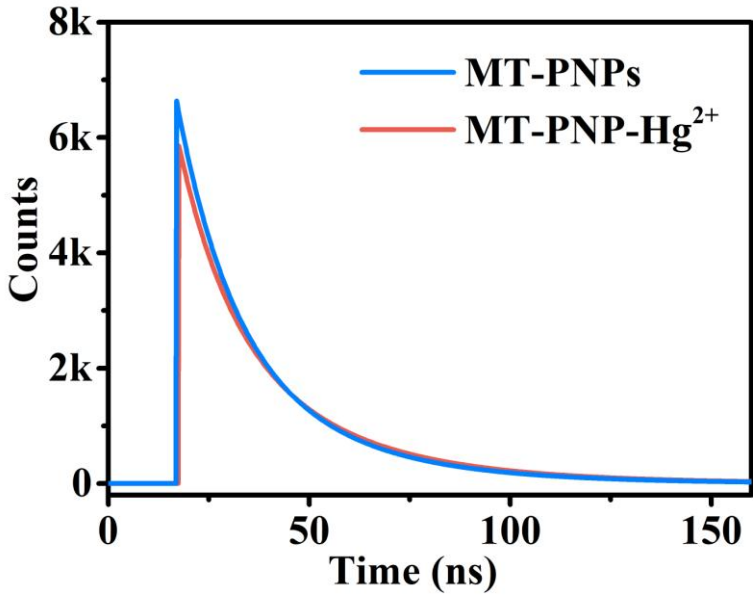


Figure S5. Fluorescence lifetimes of MT-PNPs in the absence and presence of Hg^{2+}. [MT-PNPs] = 2% v/v, [Hg^{2+}] = 192 μM, PB (pH = 5.0), λ_{Ex} = 365 nm; λ_{Em} = 430 nm.

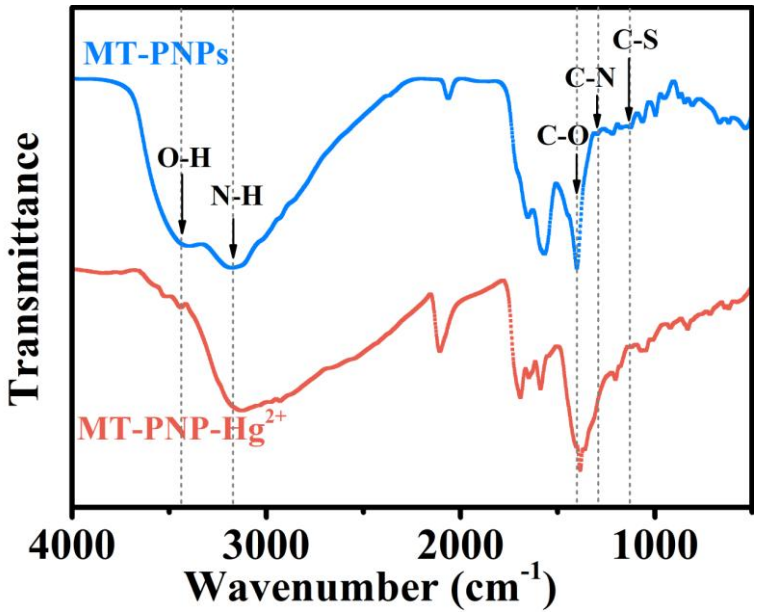
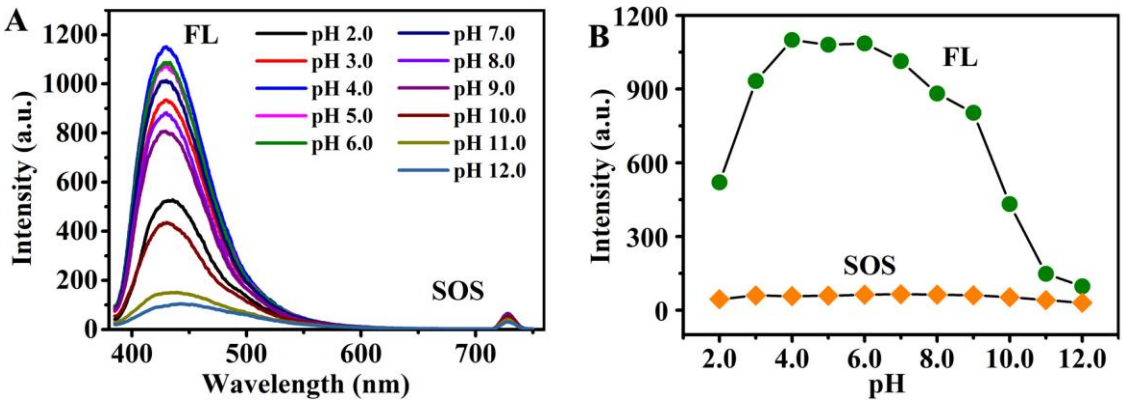


Figure S6. FTIR spectra of MT-PNPs and MT-PNP-Hg^{2+}.



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147 **Figure S7.** Effect of pH value on the FL and SOS intensity of MT-PNPs. [MT-PNPs]

148 = 2% v/v, PB.

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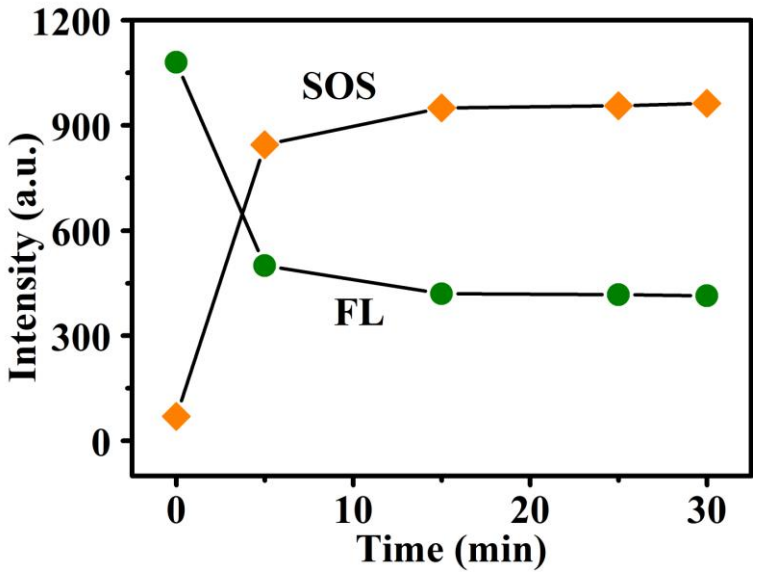

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152 **Figure S8.** FL and SOS intensity of PNPs containing Hg^{2+} as a function of time.

153 [MT-PNPs] = 2% v/v, [Hg^{2+}] = 192 μM, PB (pH = 5.0), λ_{Ex} = 365 nm; λ_{Em} = 430 nm,

154 λ_{SOS} = 730 nm.

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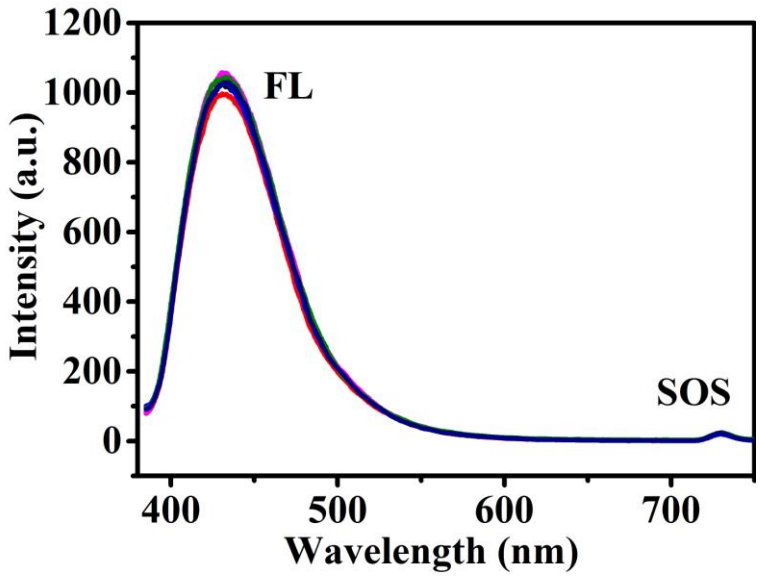

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157 **Figure S9.** Effect of standing time (0-10 monthes) on the FL and SOS spectra of

158 MT-PNPs. [MT-PNPs] = 2% v/v.

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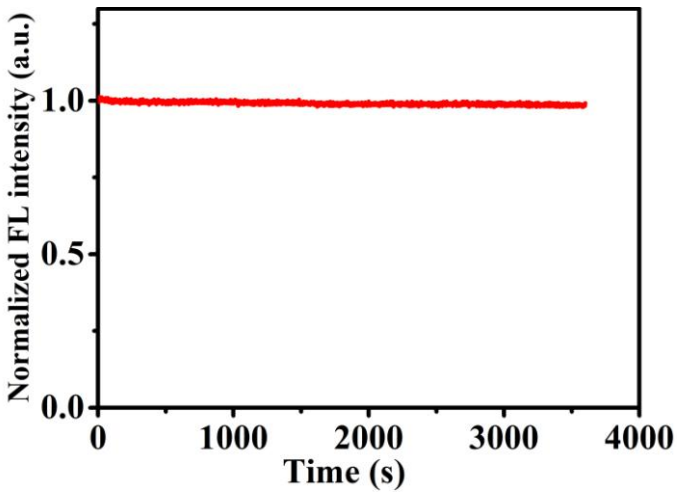

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162 **Figure S10.** Normalized FL intensity (430 nm) of MT-PNPs (2% v/v) irradiated by a

163 700 W xenon lamp (365 nm) for 60 min.

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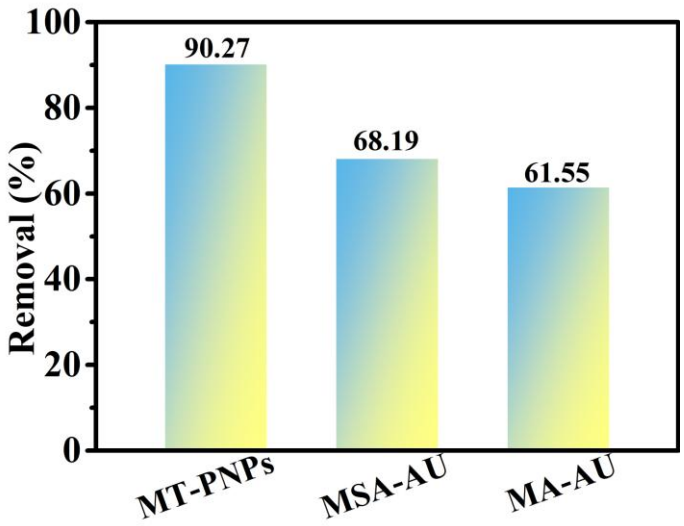

Figure S11. Mercury removal efficiency of MT-PNPs and other polymer materials after reaction with Hg^{2+} (20 μM) for 50 min.

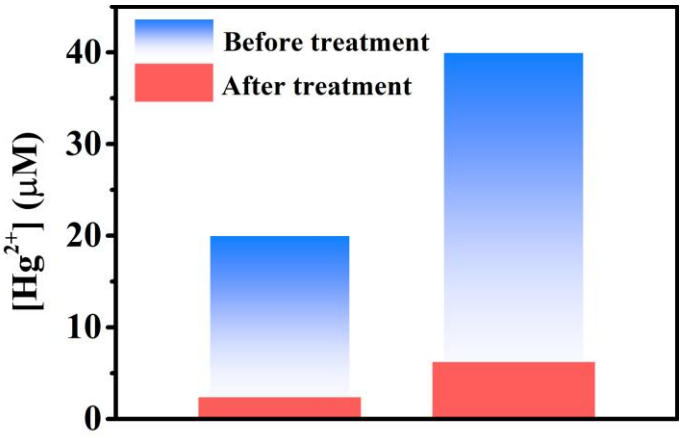

Figure S12. Application of MT-PNPs for Hg^{2+} removal from reservoir water at 20 and 40 μM spiked levels.

List of Tables

Table S1. XPS analysis of MT-PNPs before and after $NaBH_4$ treatment.

Sample	C (%)	N (%)	O (%)	S (%)
MT-PNPs	60.60	15.59	17.36	6.45
MT-PNPs with $NaBH_4$	39.79	4.92	51.79	3.49

Table S2. Binding energy of N and O of MT-PNPs in the absence and presence of Hg^{2+}.

Sample	$C–NH_2$ (eV)	C–NH–C (eV)	C=N–N (eV)	C–OH (eV)	C=O (eV)
MT-PNPs	397.25	399.43	400.65	531.21	532.77
MT-PNP-Hg^{2+}	397.86	399.11	400.56	531.30	532.73

194 **Table S3.** Photostability comparison of MT-PNPs with the commercially available
195 organic dyes and quantum dots.

Material	Light source	Bleaching[1]	References
BODIPY FLC5-Ceramide	500 W iodine-tungsten lamp (>400 nm)	~5 % (60 min)	2
NBD C6-Ceramide	500 W iodine-tungsten lamp (>400 nm)	~7.5% (60 min)	2
FITC[a]	450 W xenon lamp (488 nm)	100% (10 min)	3
3,3'-diethyl-2,2'thiadicarbocyanine ethyl sulfate	150 W Hg(Xe) lamp (>550 nm)	32% (2 min)	4
SYTO RNA select	Xe lamp (620 nm)	~ 50% (60 min)	5
CdTe QDs[b]	450 W xenon lamp (365 nm)	>90% (20 min)	3
CdTe/CdS/ZnS QDs[c]	450 W xenon lamp (365 nm)	>90% (30 min)	3
MT-PNPs	700 W xenon lamp (365 nm)	<0.8% (60 min)	**This work**

196 [1] Percentage of fluorescence lost after continuous irradiation.
197 [a] fluorescein isothiocyanate
198 [b] CdTe quantum dots
199 [c] CdTe/CdS/ZnS quantum dots
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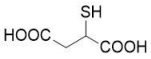
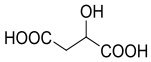
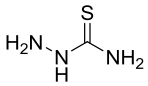
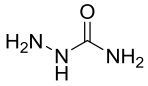
201

Table S4. Determination of Hg^{2+} in real water samples (n = 3).

Sample taken	Spiked (μM)	Found (μM)	Recovery (%)	RSD (%)
1	0	ND[a]		
2	10	10.49	104.93	5.29
3	100	101.79	101.79	1.05

[a] ND: Not detected.

Table S5. Detailed information of reactants involved in the experiments.

Number	Reactants (abbreviation)	Molecular formula	Molecular weight	Structure
1	Mercaptosuccinic acid (MSA)	$C_8H_{10}O_8S$	266.22	
2	Malic acid (MA)	$C_4H_6O_5$	134.09	
3	Thiosemicarbazide (TSC)	CH_5N_3S	91.14	
4	Aminourea (AU)	CH_5N_3O	75.07	

211 **Table S6.** XPS analysis of MT-PNPs, MSA-AU, and MA-AU.

Samples	C (%)	N (%)	O (%)	S (%)
MT-PNPs	60.6	15.59	17.36	6.45
MSA-AU	72.64	3.47	22.58	1.31
MA-AU	50.61	14.7	34.69	0

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