Supplementary Material (ESI) for Chemical Communications

Supporting Information for

Amphiphilic Porphyrin Assembly as Highly Selective Chemosensors for Organic Mercury in Water

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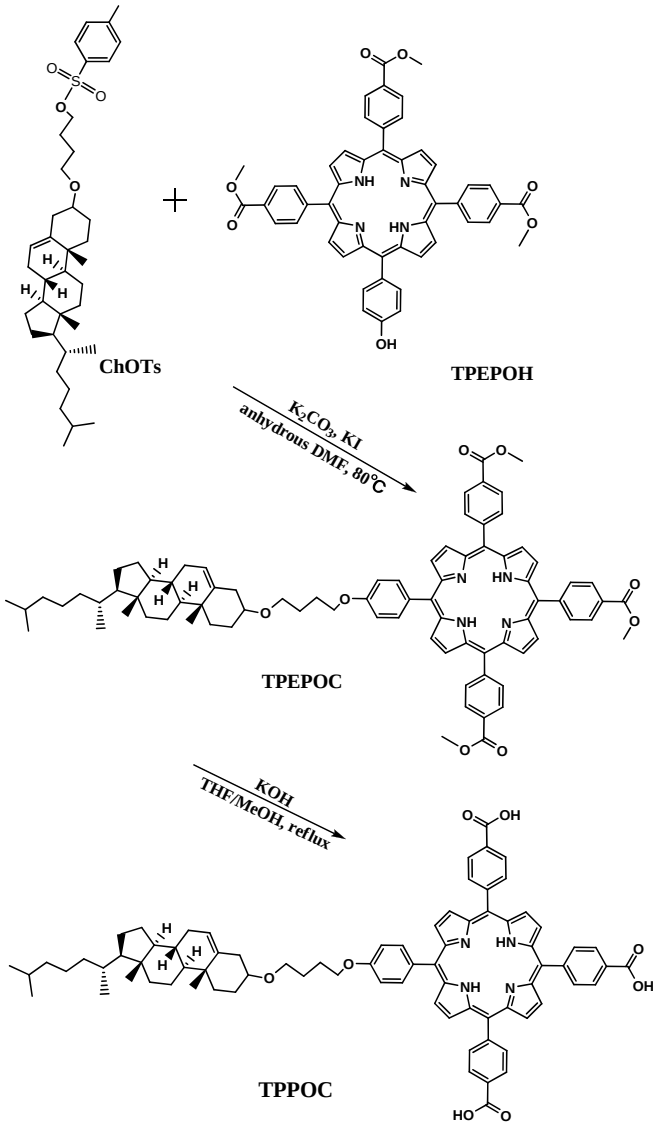
Chemicals and characterization

Organic mercuries, metal Cations and dipenylzinc were commercially available and used without further purification. DMF was stirring in CaH_2 for three days and then distilled under reduced pressure prior to use. DMSO (HPLC grade) was used after distillation. **TPPOC** and **TCAP** were synthesized and identified by ^1H NMR spectrum on a Varian 400 spectrometer, elemental analysis on a Perkin-Elmer-2400C instrument, IonSpec QFT-ESI MS.

Synthetic route



Synthesis of **TPEPOC**. **TPEPOH**[1] (400 mg, 0.497 mmol) and K_2CO_3 (103 mg, 0.746 mmol) were added into 25 mL anhydrous DMF with stirring. The solution was heated at 80 °C for 30 min, then cooled to room temperature. To this solution was carefully added **CHOTs**[2] (370 mg,

0.6 mmol) over 1 h. The reaction was stirred for 48 h. After this time, DMF was removed in vacuo, and the residue was dissolved in methylene chloride and washed thoroughly with distilled water. The organic layer was concentrated and further purified by column chromatography using n-hexane/ethyl acetate (5:1, v/v) as eluent. **TPEPOC** was obtained as purple powder in a 87% yield (R_f = 0.36). ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ = 8.94 (s, 2H, β-pyrrole); 8.82 (s, 6H, β-pyrrole); 8.46 (d, *J* = 6.0 Hz, 6H, ArH); 8.31 (d, *J* = 6.0 Hz, 6H, ArH); 8.11 (d, *J* = 7.2 Hz, 2H, ArH); 7.30 (d, *J* = 7.2 Hz, 2H, ArH); 5.40 (s, 1H, cholesterol); 4.30 (br s, 2H, butylene); 4.13 (s, 9H, methyl); 3.68 (br s, 2H, butylene); 3.25 (m, 1H, cholesterol); 2.50-0.83 (m, 44H, cholesterol + butylene); 0.69 (s, 3H, cholesterol), -2.76 (s, 2H, pyrrole). Anal. Calcd. for $C_{81}H_{88}N_4O_8$: C, 78.11; H, 7.12; N 4.50. Found: C, 78.36; H, 7.01; N, 4.28. MALDI-MS: *m*/*z* 1246 $[M + H]^+$.

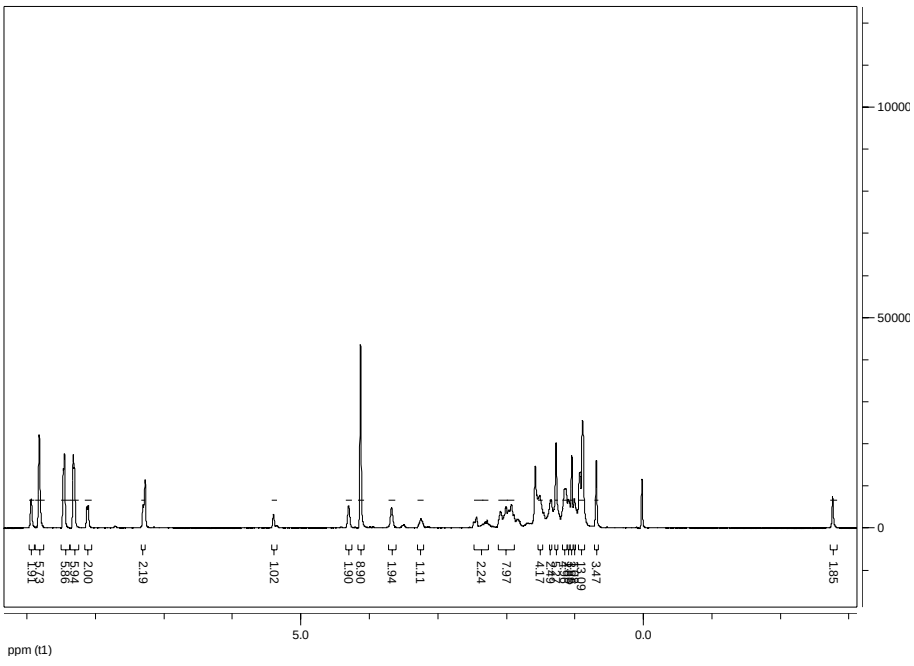


Figure S1 ^{1}H NMR spectrum of **TPEPOC**.

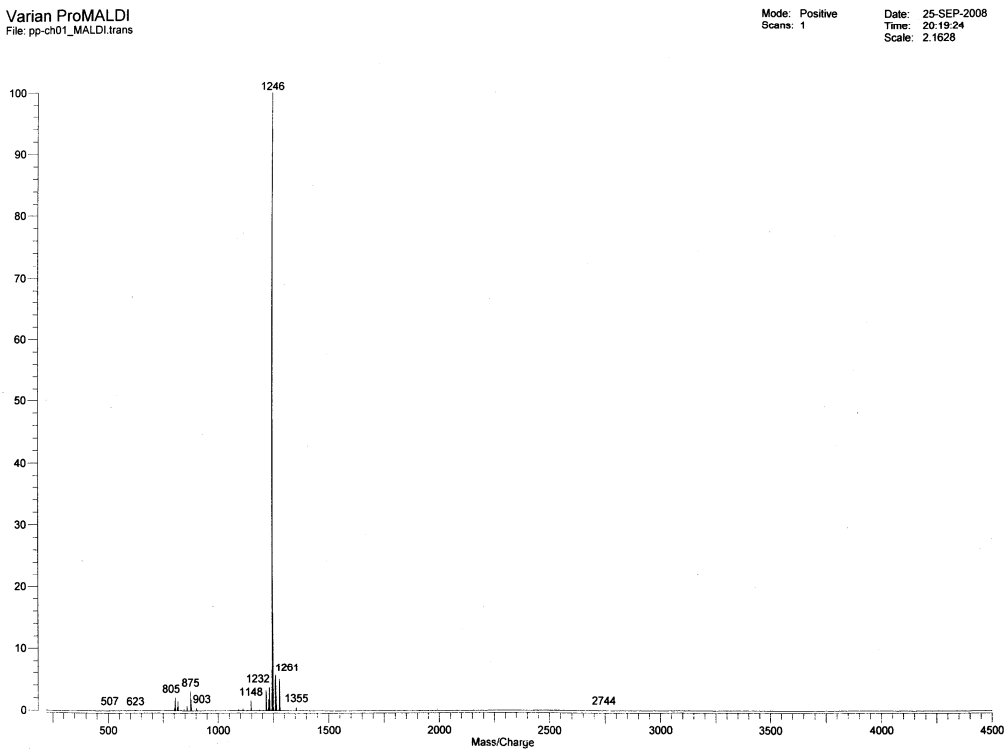


Figure S2 MALDI-Ms spectrum of **TPEPOC**.

Synthesis of **TPPOC**. The hydrolysis of **TPEPOC** was performed using literature methods.[3] The porphyrin ester (200 mg, 0.16 mmol) was dissolved in 200 mL mixed solvent of MeOH/THF (v/v, 1:10), to which grinded KOH (1g, 17.8 mmol) powder was added. The mixture was stirred at room temperature for 3 days, after which 20 mL distilled water was added. The solution was refluxed for 5 hours and then the organic solvents were evaporated under reduced pressure. The residue was diluted by further addition of 30 mL distilled water and then filtered through a 800 nm membrane filter. While the pH of filtrate was adjuted to 5 by 2 N HCl(aq), purple flocky precipitate was forming. The precipitate was filtered and successively washed with 4 × 50 mL water and 4 × 50 mL diethyl ether, to give analytically pure **TPPOC** as purple solid in 92% yield. ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ = 13.44 (br s, 3H, acid proton); 9.27-7.30 (m, 22H, β-pyrrole + ArH); 6.87 (s, 2H, ArH); 4.74 (s, 1H, cholesterol); 3.44-2.91 (m, 4H, butylene); 2.68 (m, 1H, cholesterol); 2.27-0.03 (m, 47H,

cholesterol + butylene); -3.10 (s, 2H, pyrrole). Anal. Calcd. for $C_{78}H_{82}N_4O_8 \cdot 2H_2O$: C, 75.58; H, 6.99; N 4.52. Found: C, 75.27; H, 6.91; N, 4.24. ESI-MS: *m*/*z* 1203 $[M]^+$, 1236 $[M + MeOH]^+$.

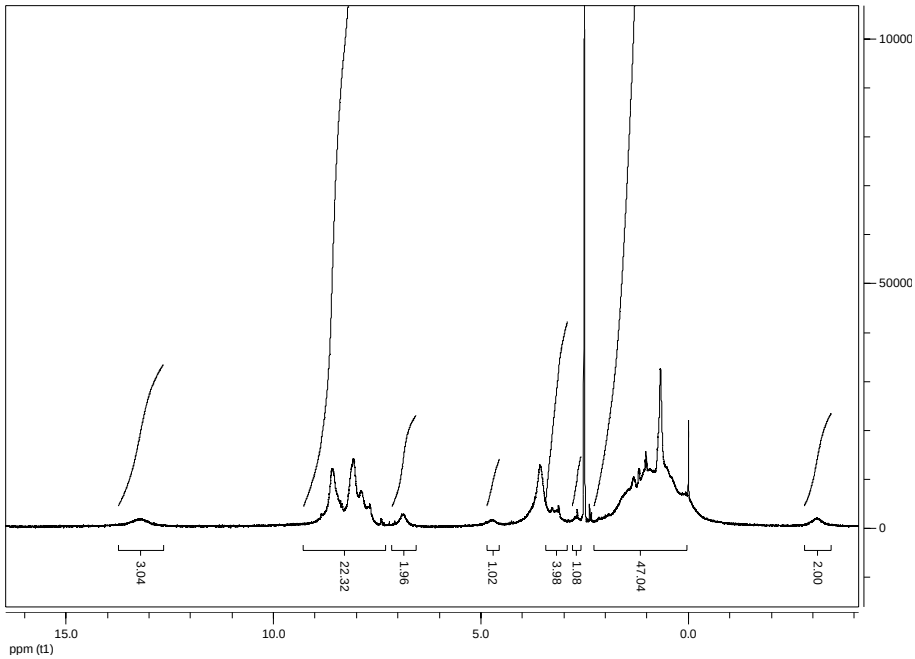


Figure S3 ^{1}H NMR spectrum of **TPPOC**.

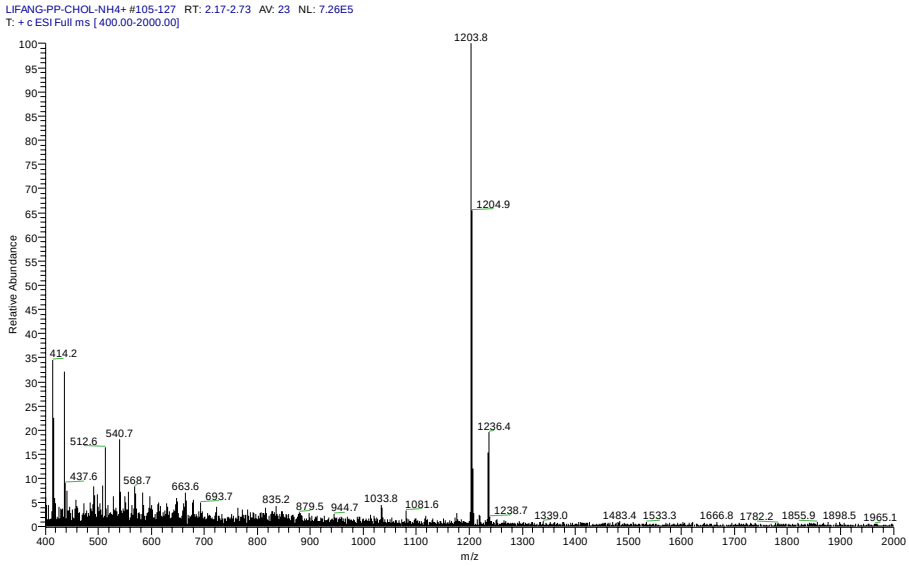


Figure S4 ESI-Ms spectrum of **TPPOC**.

Synthesis of **TCAP**. The synthesis of reference compound **TCAP** was obtained by methylation of **TPEPOH** with iodomethane and then hydrolysis in same procedures as **TPPOC**. The only difference lies in the pH to precipitate **TCAP** should be adjusted to 4.5 for

complete precipitation. **TCAP** was obtaind as purple solid in 86% total yield. ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ = 13.26 (br s, 3H, acid proton); 8.90 (s, 2H, β-pyrrole); 8.85 (s, 6H, β-pyrrole); 8.39 (d, *J* = 8.0 Hz, 6H, ArH); 8.34 (d, *J* = 8.0 Hz, 6H, ArH); 8.15 (d, *J* = 8.0 Hz, 2H, ArH); 7.40 (d, *J* = 8.0 Hz, 2H, ArH); 4.06 (s, 3H, methyl); -2.92 (s, 2H, pyrrole). Anal. Calcd. for $C_{48}H_{32}N_4O_7 \cdot H_2O$: C, 72.54; H, 4.31; N 7.05. Found: C, 72.63; H, 4.19; N, 7.25. ESI-MS: *m*/*z* 777 $[M+H]^+$.

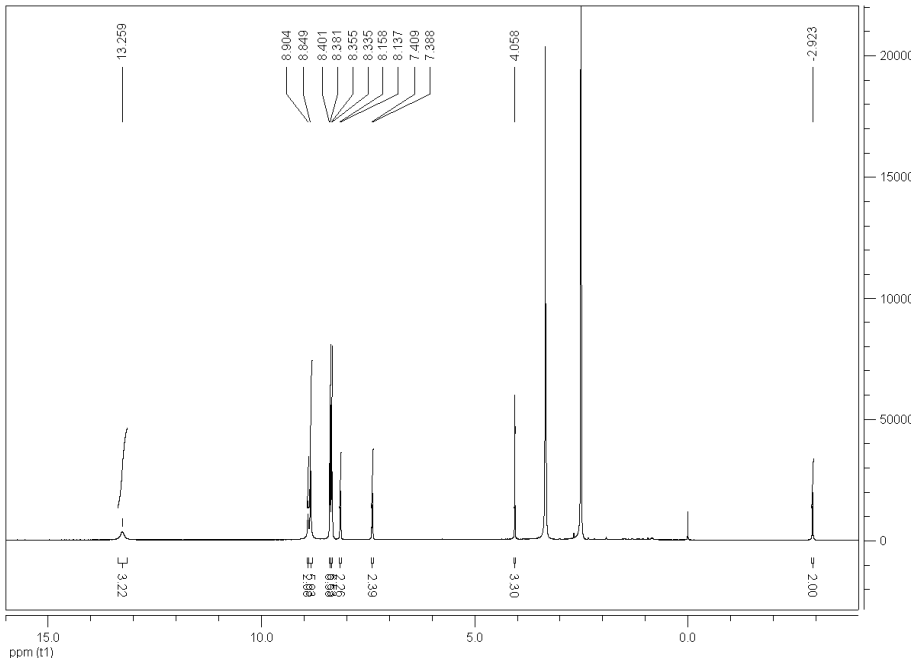


Figure S5 ^{1}H NMR spectrum of **TCAP**.

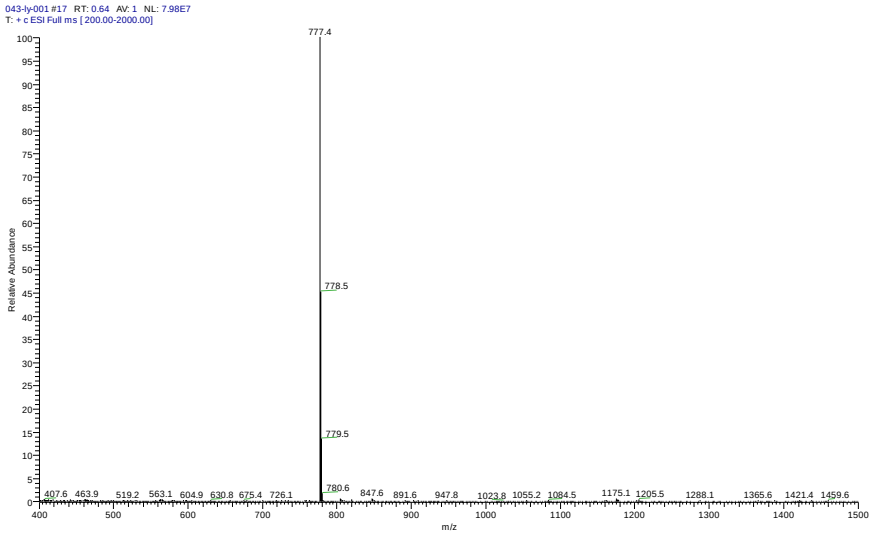


Figure S6 ESI-Ms spectrum of **TCAP**.

Measurements

Transmission Electron Microscopy (TEM): TEM images were recorded on FEI Tecnai G2 T20 microscope operating at an accelerating voltage of 100 keV. The sample was prepared on FEI Vitrobot MK IV.

Atomic Force Microscope (AFM): A small drop of sample solution was dropped onto newly clipped mica and washed with 2 × 1 mL of double-distilled water and then air-dried. The samples were examined using an AFM (Veeco Company, Multimode, Nano IIIa) in tapping mode in the air at room temperature.

Dynamic Light Scattering measurements (DLS): The sample solution for DLS measurements was prepared by filtering solution through a 450 nm Millipore filter into a clean scintillation vial. The sample was examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (Turbo Corr.) at 532 nm at room temperature.

UV-vis absorption and fluorescence spectra: UV-vis spectra were recorded in a quartz cell (light path 10 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller. Steady-state fluorescence spectra were recorded in a conventional quartz cell (light path 10 mm) on a VARIAN CARY Eclipse equipped with a VARIAN CARY single cell peltier accessory to control temperature.

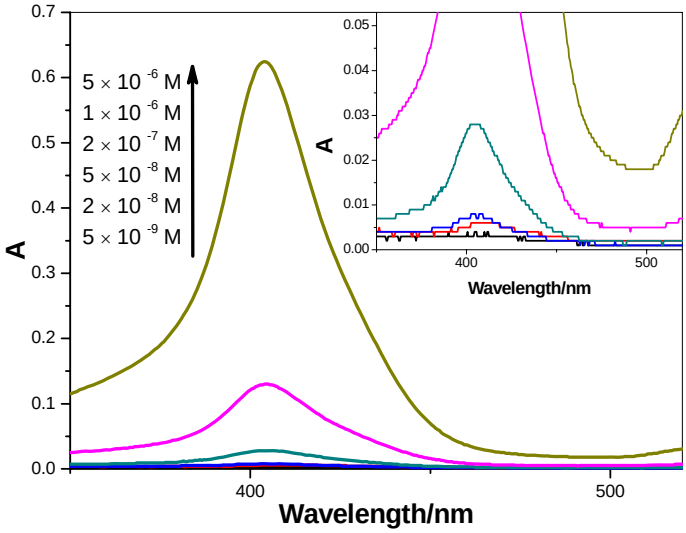


Figure S7 Soret spectra of various concentrations of **TPPOC** in 3% DMSO-CHES buffer (pH = 9.8, 10 mM). The Soret band gradually moved from 413 nm (5×10^{-9} M and 2×10^{-8} M) to 407 nm (5×10^{-8} M), to 405 nm (2×10^{-7} M), and to 404 nm (1×10^{-6} M and 5×10^{-6} M).

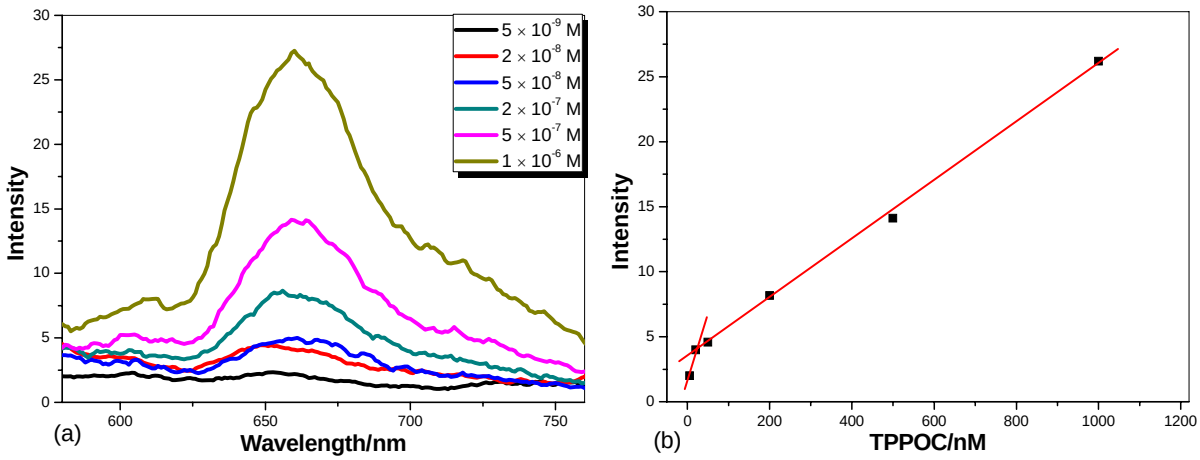


Figure S8 (a) Fluorescence spectra of various concentrations of **TPPOC** in 3% DMSO-CHES buffer (pH = 9.8, 10 mM). The emission peak of **TPPOC** moved from 648 nm (5×10^{-9} M, 2×10^{-8} M) to 664 nm (5×10^{-8} M, 2×10^{-7} M, 5×10^{-7} M, 1×10^{-6} M). Excitation slit: 20 nm, emission slit: 20 nm; λ_{ex} = 404 nm. (b) Plot of emission intensity at 664 nm versus [**TPPOC**]. The turning point appeared at ca. 30 nM.

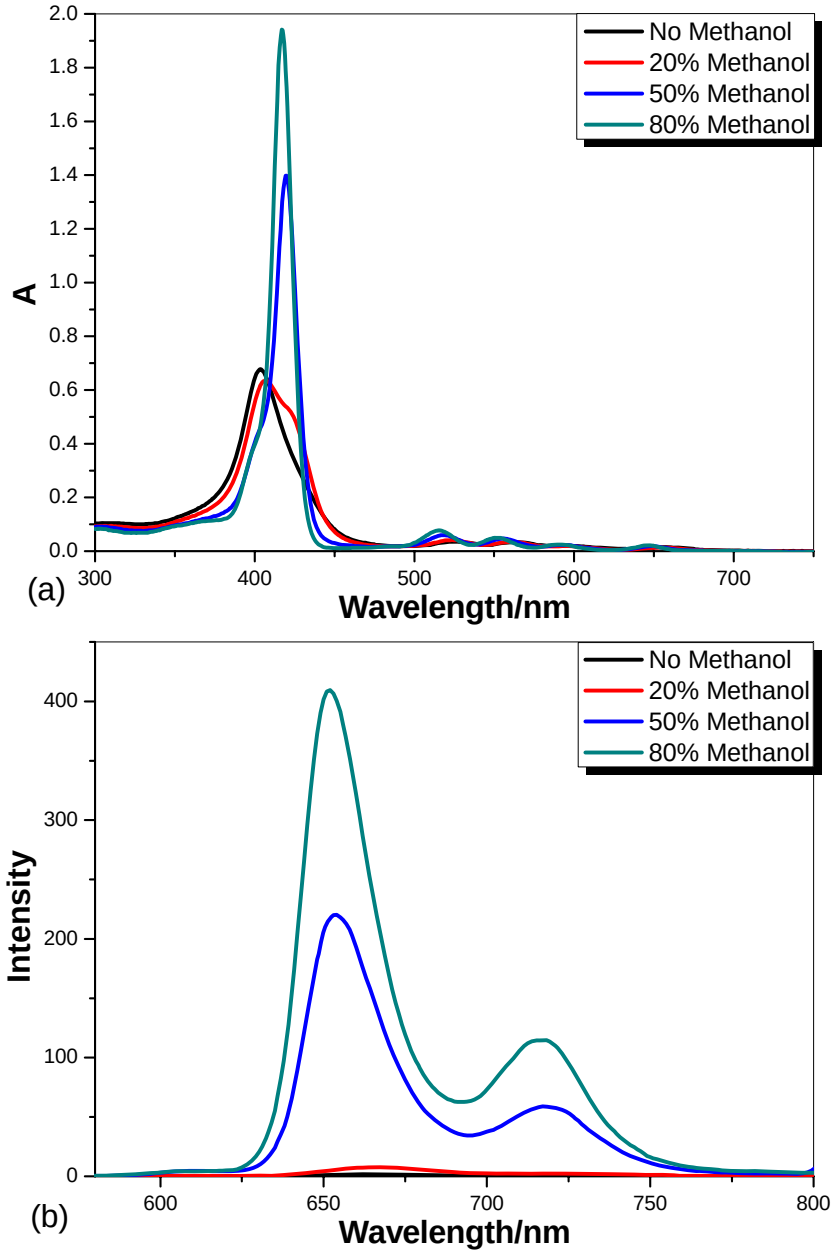


Figure S9 Absorption spectra (a) and fluorescence spectra (b) of **TPPOC** (5 μM) in 3% DMSO-CHES buffer (pH = 9.8, 10 mM) upon addition of incremental volumes of methanol. Excitation slit: 5 nm, emission slit: 5 nm; λ_{ex} = 404 nm. The fluorescence spectra suggest the electronically excited states decayed in non-radioactive ways upon forming aggregates in the absence of methanol.[4]

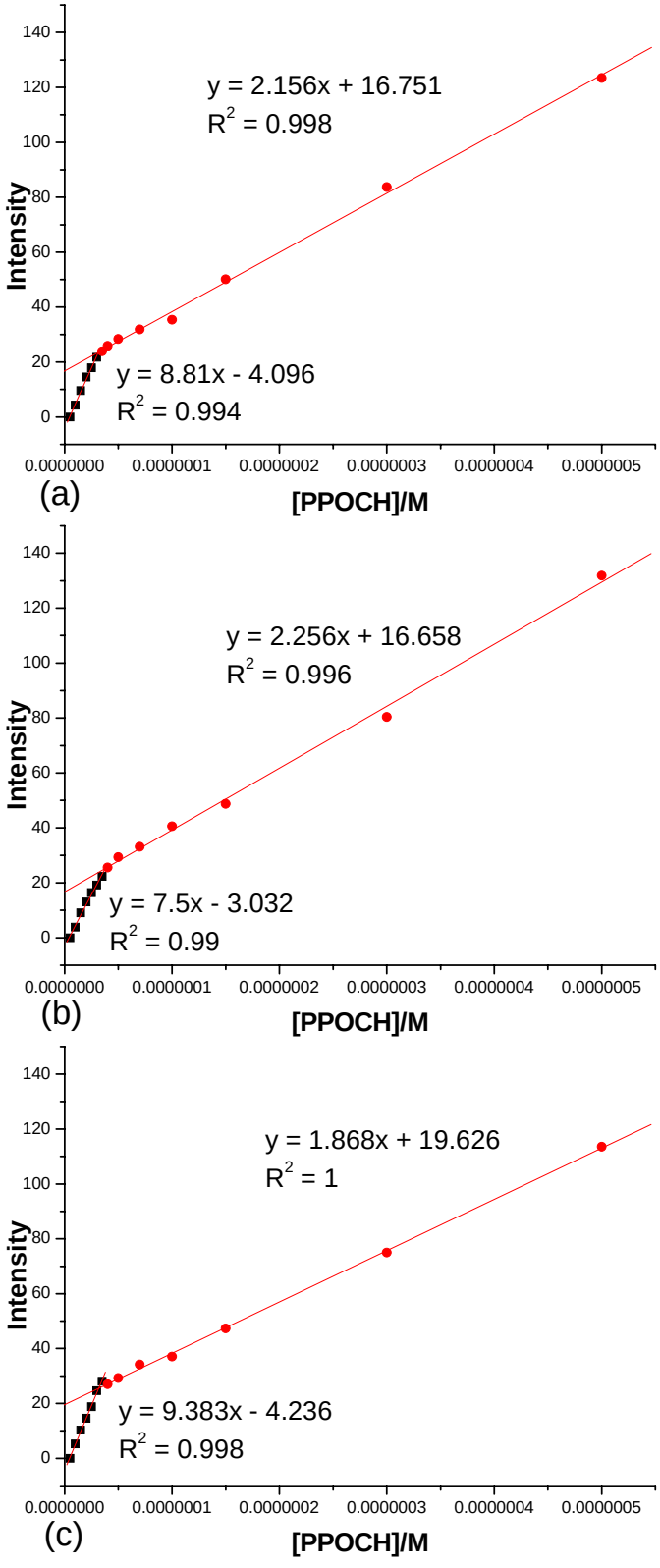


Figure S10 Variation of the fluorescence excitation intensity with the concentration of **TPPOC** in 3% DMSO-CHES buffer solution (pH = 9.8, 10 mM). Excitation slit: 20 nm, emission slit: 20 nm; λ_{em} = 664 nm.

Table S1 The measured CAC values of **TPPOC** in 3% DMSO-CHES (pH = 9.8, 10 mM).

	Figure S10a	Figure S10b	Figure S10c
CAC	37.53 nM	31.35 nM	31.74 nM

In a typical experiment, we prepared 13 diluted solutions of **TPPOC** in 3% DMSO-CHES with concentrations ranging from 5 nM up to 500 nM from the stock solution (5 μM). The samples were left stand for 2 hours to equilibrate and the fluorescence excitation peaks at 404 nm in the Soret region (emission 648 nm) were then recorded. The inflexion point in fluorescence excitation intensity with the concentration was used to determine the CAC value.

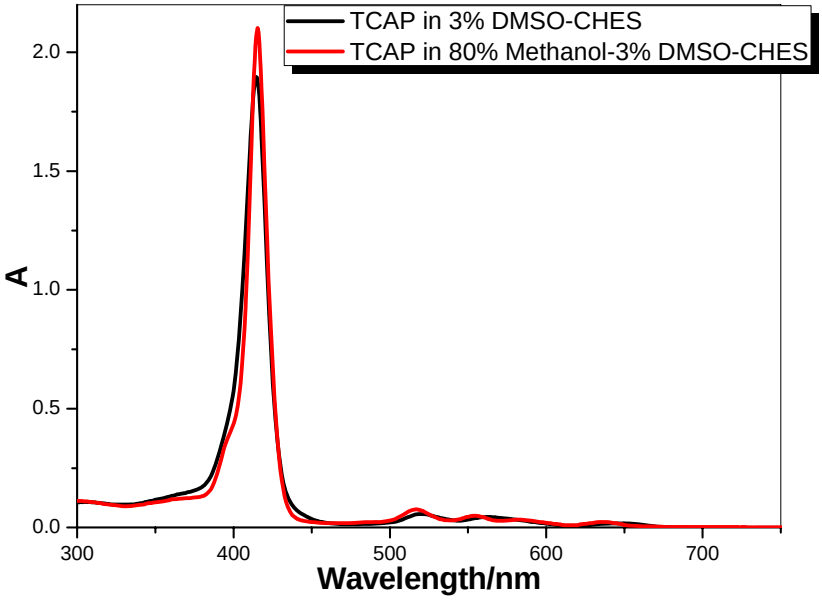


Figure S11 Absorption spectra of **TCAP** (5 μM) in 3% DMSO-CHES buffer (pH = 9.8, 10 mM) and in the presence of 80% methanol.

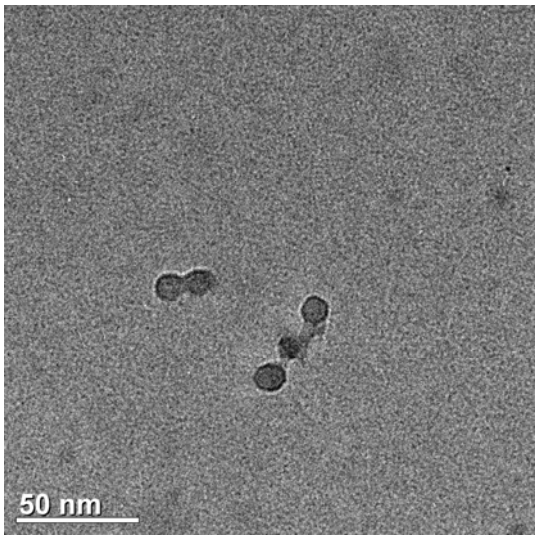


Figure S12 The magnified cryo-TEM image of **TPPOC** aggregates.

STOPPED		Ref Index:	1.334
First Delay:	2.0 μsec	Samples:	1.4991e+08
Last Delay:	1.0 msec	Atot:	4.1809e+06
Elapsed Time:	00:14:04	A CR (avg.):	5.6 kcps
Wavelength:	532.0 nm	M. Base:	1.1638e+05
Angle:	90.0	C. Base:	1.1660e+05
Temp:	24.9 deg C	Base diff:	-0.185%
Liquid:	Water	Eff Dia:	10.6 nm
Viscosity:	0.893 cP	Poly:	0.296

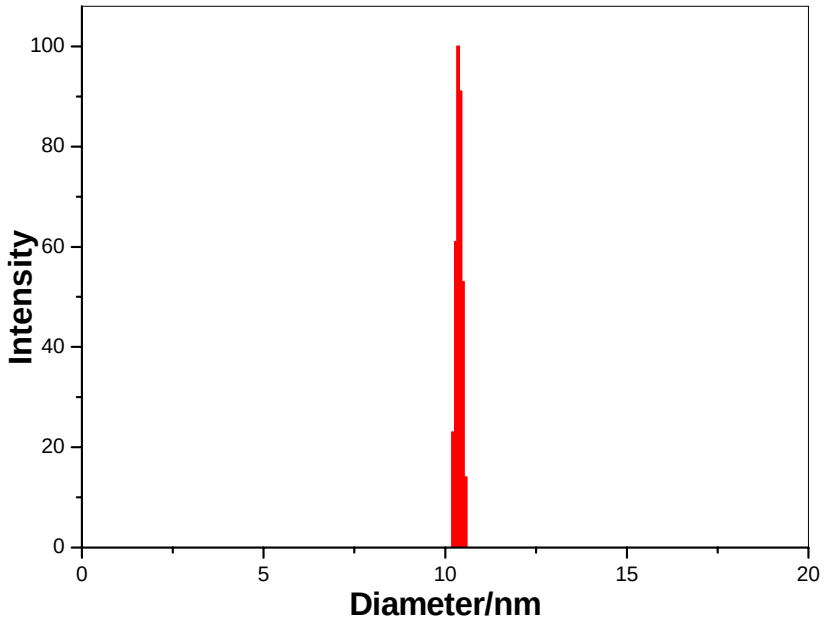


Figure S13 DLS data and size distribution of **TPPOC** aggregates.

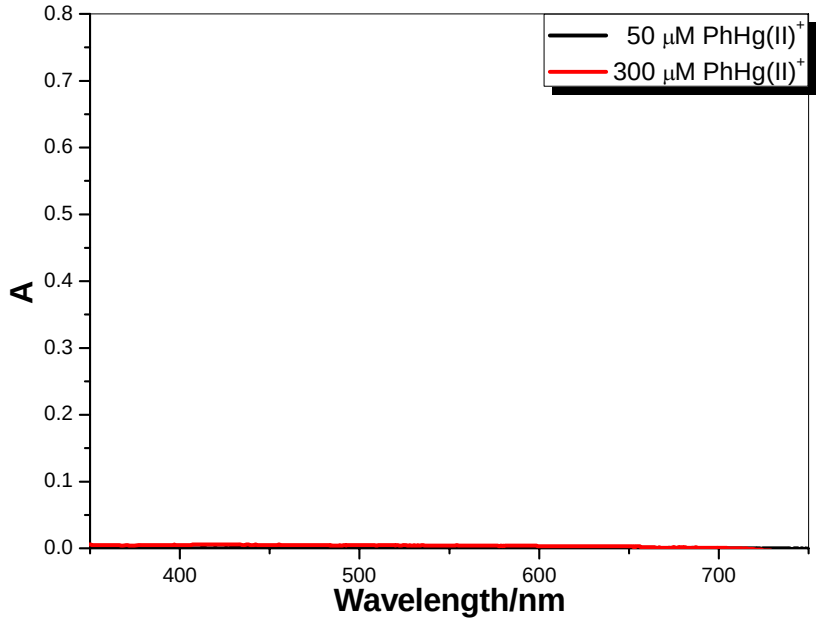


Figure S14 Absorption spectra of $PhHg(II)^+$ in 3% DMSO-CHES (pH = 9.8, 10 mM) solution.

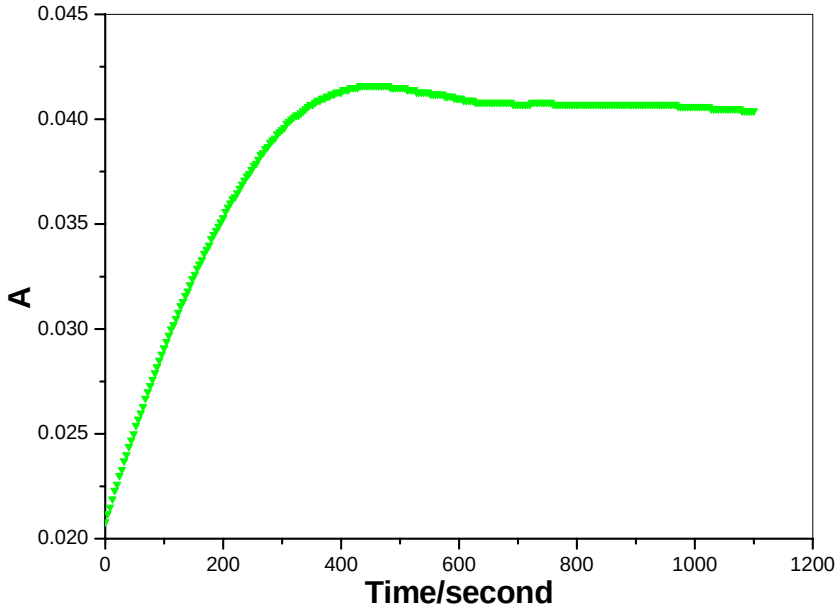


Figure S15 The time-dependent absorption change at 465 nm of 5 μM **TPPOC** upon addition of 1 equiv. $PhHg(II)^+$ in 3% DMSO-CHES.

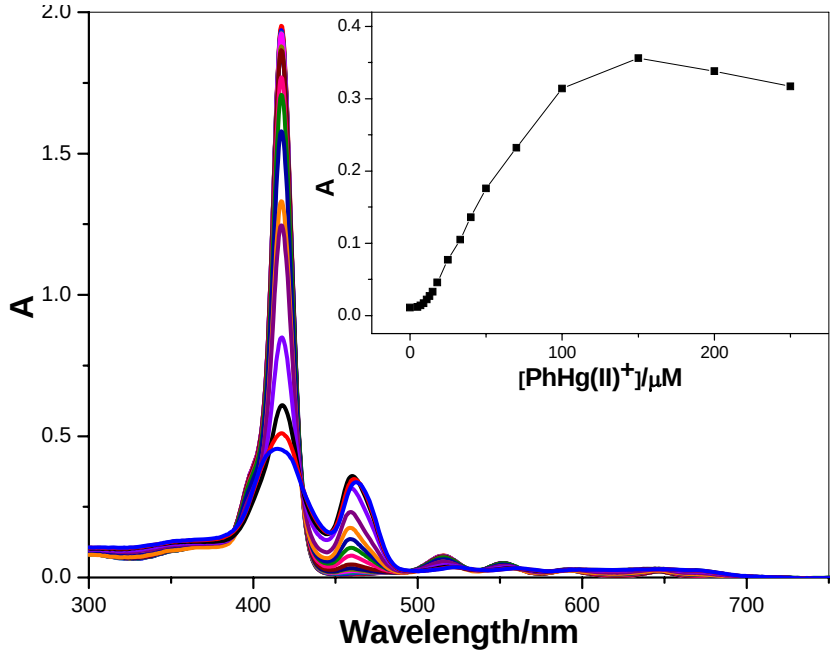


Figure S16 UV-vis titration of **TPPOC** (5 μM) upon addition of $PhHg(II)^+$ (0-250 μM) in 80% methanol-3% DMSO-CHES (pH = 9.8, 10 mM). The new absorption band at 460 nm rose up within the concentration of $PhHg(II)^+$ increasing from 7 μM to 150 μM. The inset shows: absorbance intensity at 460 nm versus [$PhHg(II)^+$].

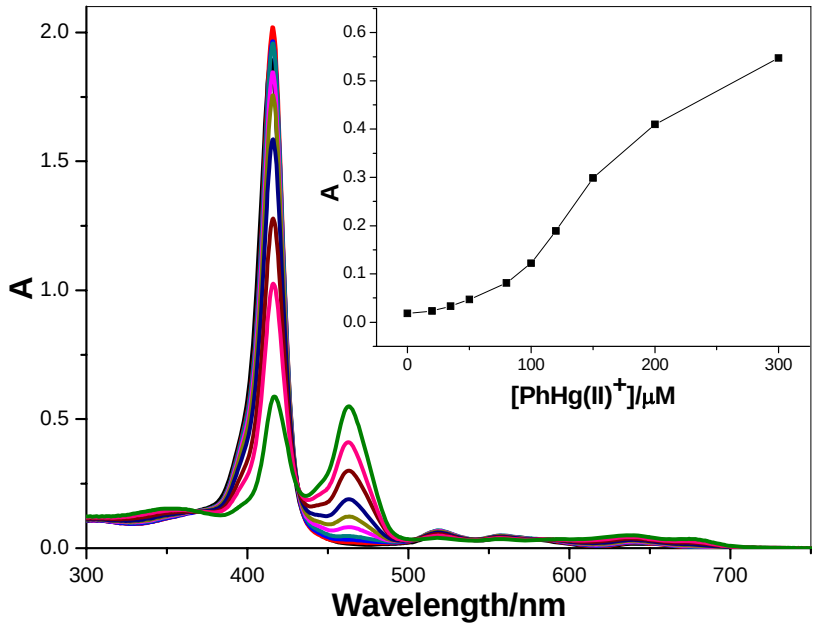


Figure S17 UV-vis titration spectra of **TCAP** (5 μM) upon addition of $PhHg(II)^+$ (0-300 μM) in 3% DMSO-CHES (pH = 9.8, 10 mM). The inset shows: absorbance intensity at 462 nm versus [$PhHg(II)^+$]. **TCAP** gave similar but rather weaker spectral changes that no visible

change below 20 μM $PhHg(II)^+$ added.

The limit of detection (LOD) values were calculated by multiplying the standard derivation of 11 blank measurements by 3 and dividing by the slope of the linear calibration curve.[5]

$$LOD = \frac{3\sigma}{K}$$

The improvement of LOD value of **TPPOC** in 80% methanol- 3% DMSO-CHES compared with that of **TCAP** in 3% DMSO-CHES may stem from the presence of a great amount of methanol which reduced the solvation effect from water.

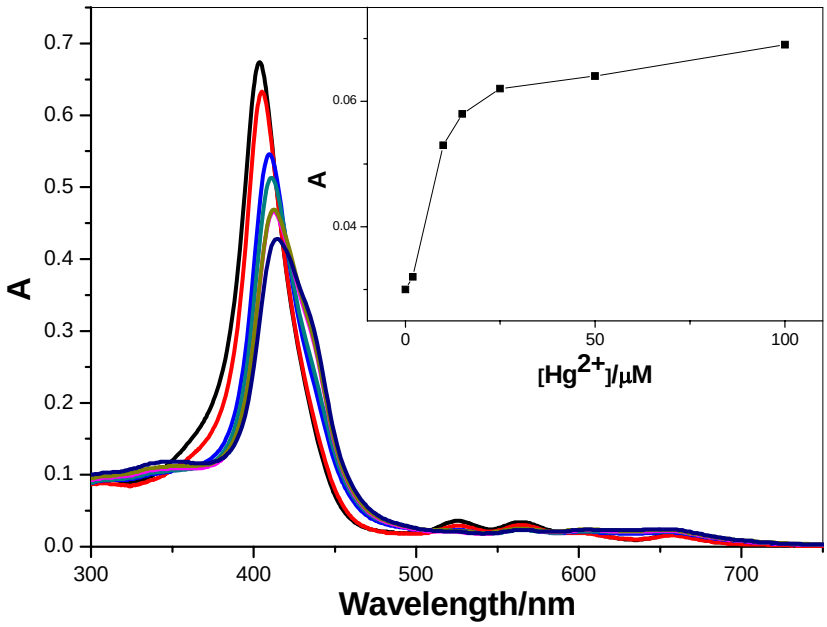


Figure S18 Absorption spectra of **TPPOC** (5 μM) upon addition of Hg^{2+} (0-100 μM) in 3% DMSO-CHES (pH = 9.8, 10mM). The inset shows: absorbance intensity at 430 nm versus $[Hg^{2+}]$. Porphyrin devrivatives have been used for mercury ion detection with high selectivity and sensitivity.[6] Upon coordination to Hg^{2+} by their four pyrrolic nitrogen atoms, porphyrins

display photometric changes in the Soret band (~400 nm, $S_0 \rightarrow S_2$).

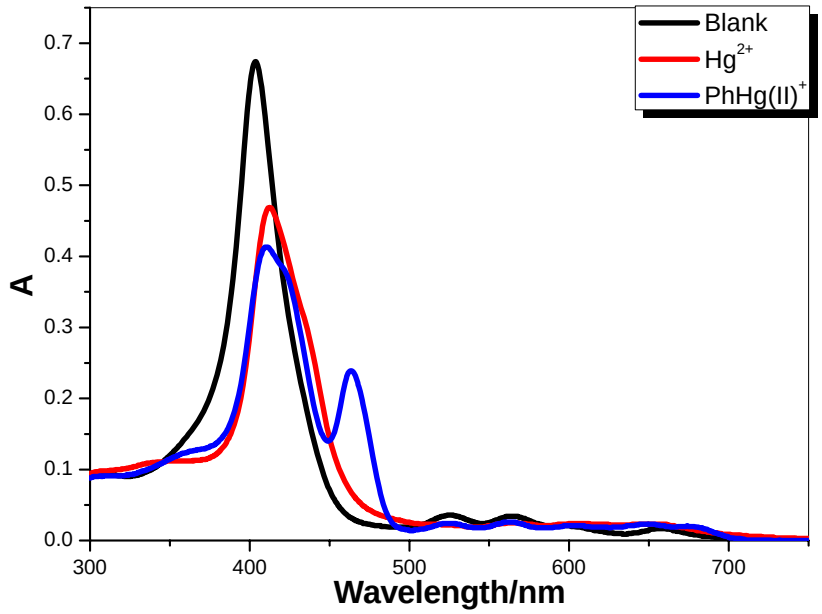


Figure S19 Absorption spectra of **TPPOC** (5 μM) and upon addition of 10 equiv. Hg^{2+} or $PhHg(II)^+$ in 3% DMSO-CHES (pH = 9.8, 10 mM), respectively.

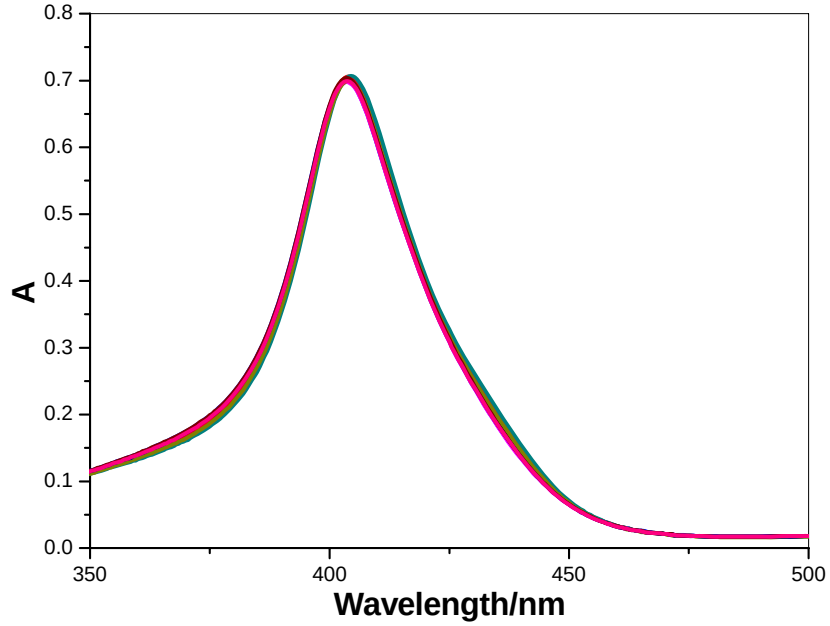


Figure S20 Soret spectra of **TPPOC** (5 μM) upon addition of 50 μM benzene, toluene, nitrobenzene, chlorobenzene, fluorobenzene, phenol, aniline, benzoic acid and

N,N,N-trimethyl-1-phenylmethanaminium bromide in 3% DMSO-CHES (pH = 9.8, 10 mM), respectively.

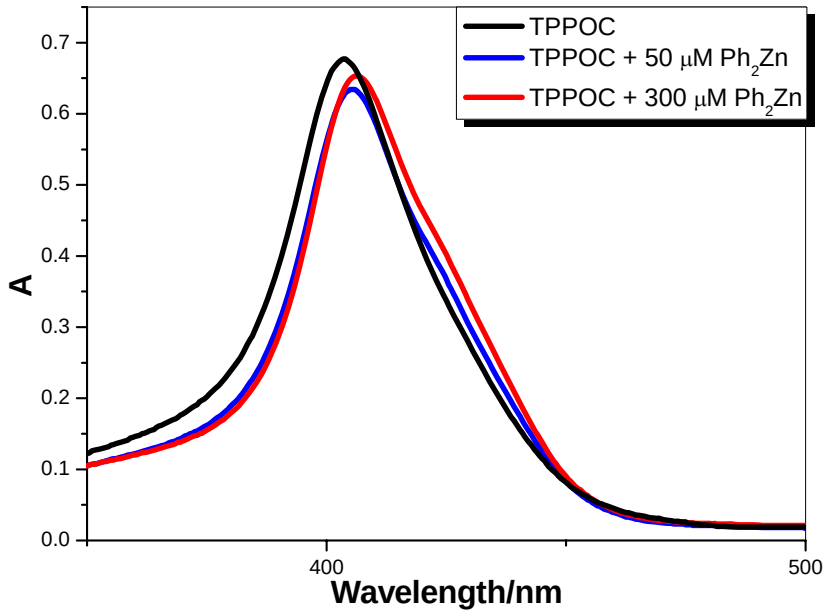


Figure S21 Soret spectra of **TPPOC** (5 μM) upon addition of 50 μM and 300 μM Ph_2Zn in 3% DMSO-CHES (pH = 9.8, 10 mM), respectively.

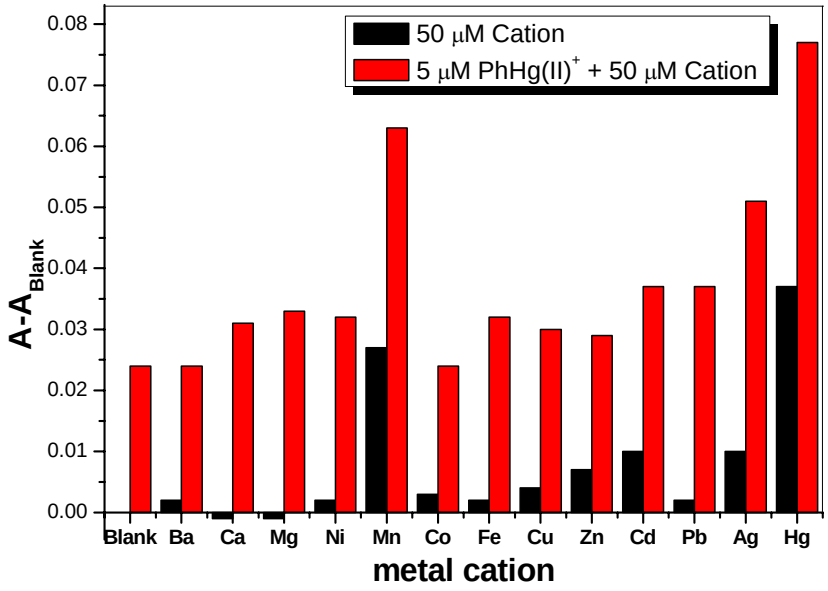


Figure S22 Absorbance intensity of **TPPOC** (5 μM) at 465 nm to various cations and $PhHg(II)^+$. A_{Blank} represents the absorbance intensity of **TPPOC** in the absence of cations.

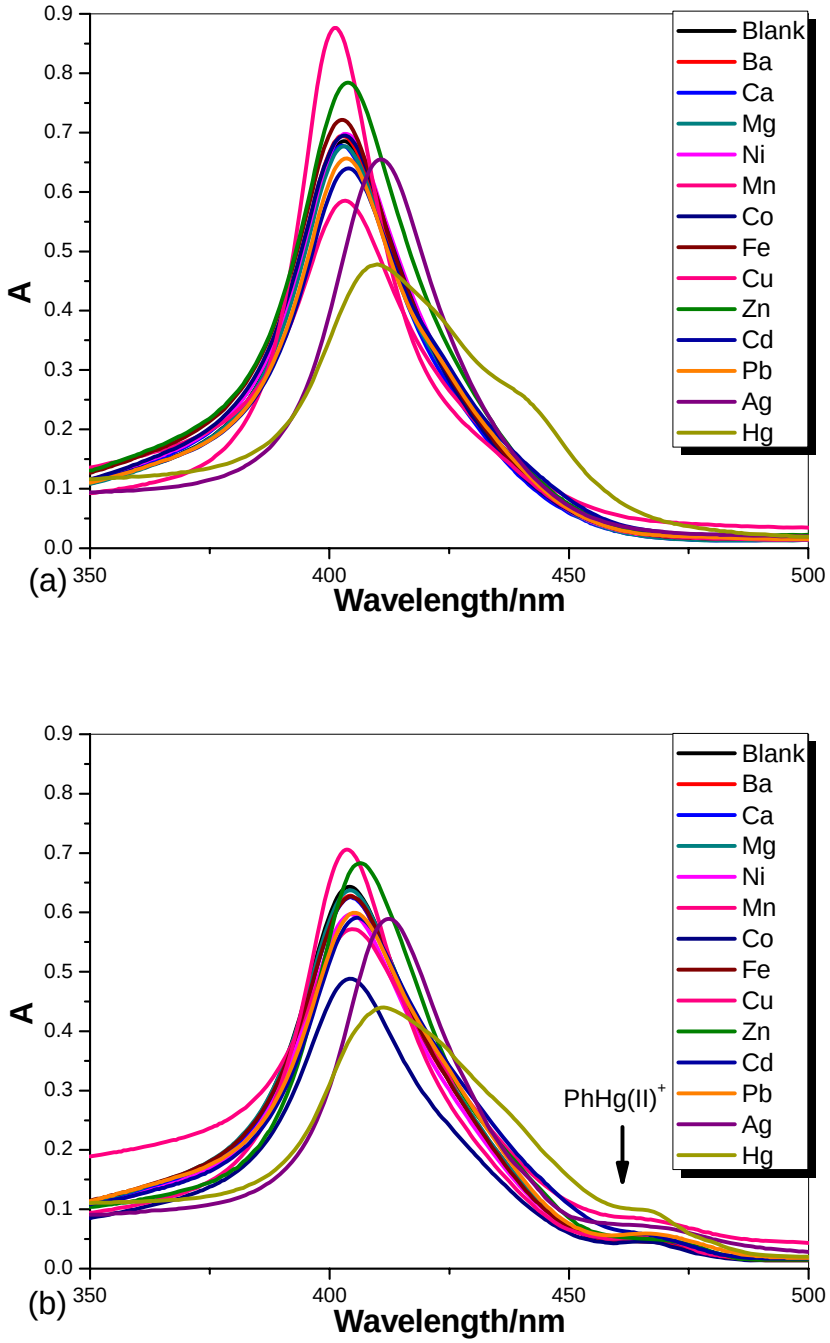


Figure S23 Soret spectra of **TPPOC** (5 μM) upon addition of 50 μM various metal cations (a), and upon addition of 50 μM various metal cations along with 5 μM $PhHg(II)^+$ (b) in 3% DMSO-CHES buffer (pH = 9.8, 10 mM).

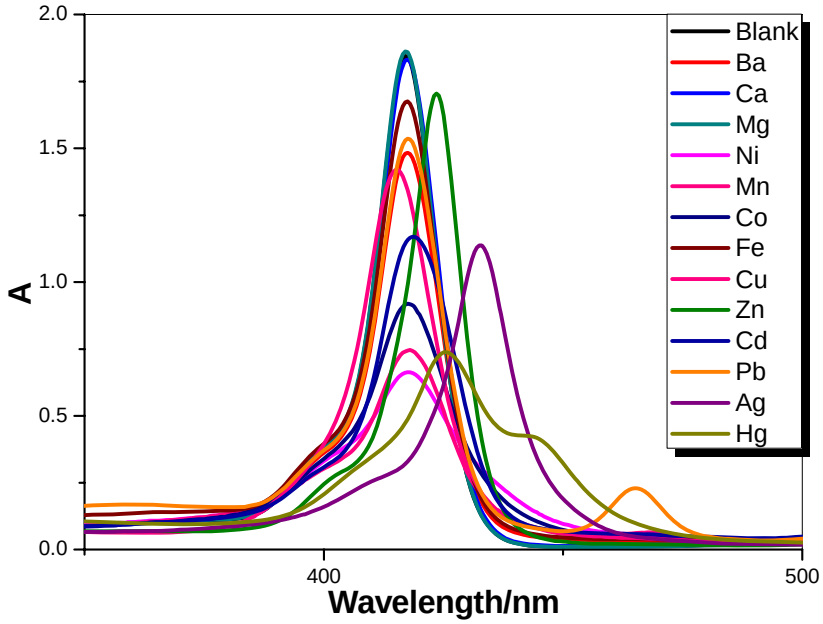


Figure S24 Soret spectra of **TPPOC** (5 μM) upon addition of 50 μM various metal cations in 80% methanol-3% DMSO-CHES (pH = 9.8, 10 mM), respectively.

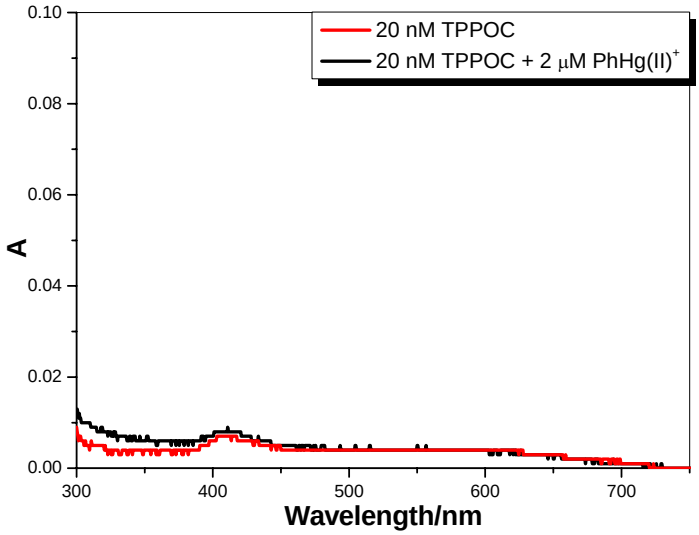


Figure S25 Absorption spectra of **TPPOC** (20 nM) with or without addition of 2 μM $PhHg(II)^+$ (100 equiv.) in 3% DMSO-CHES (pH = 9.8, 10 mM).

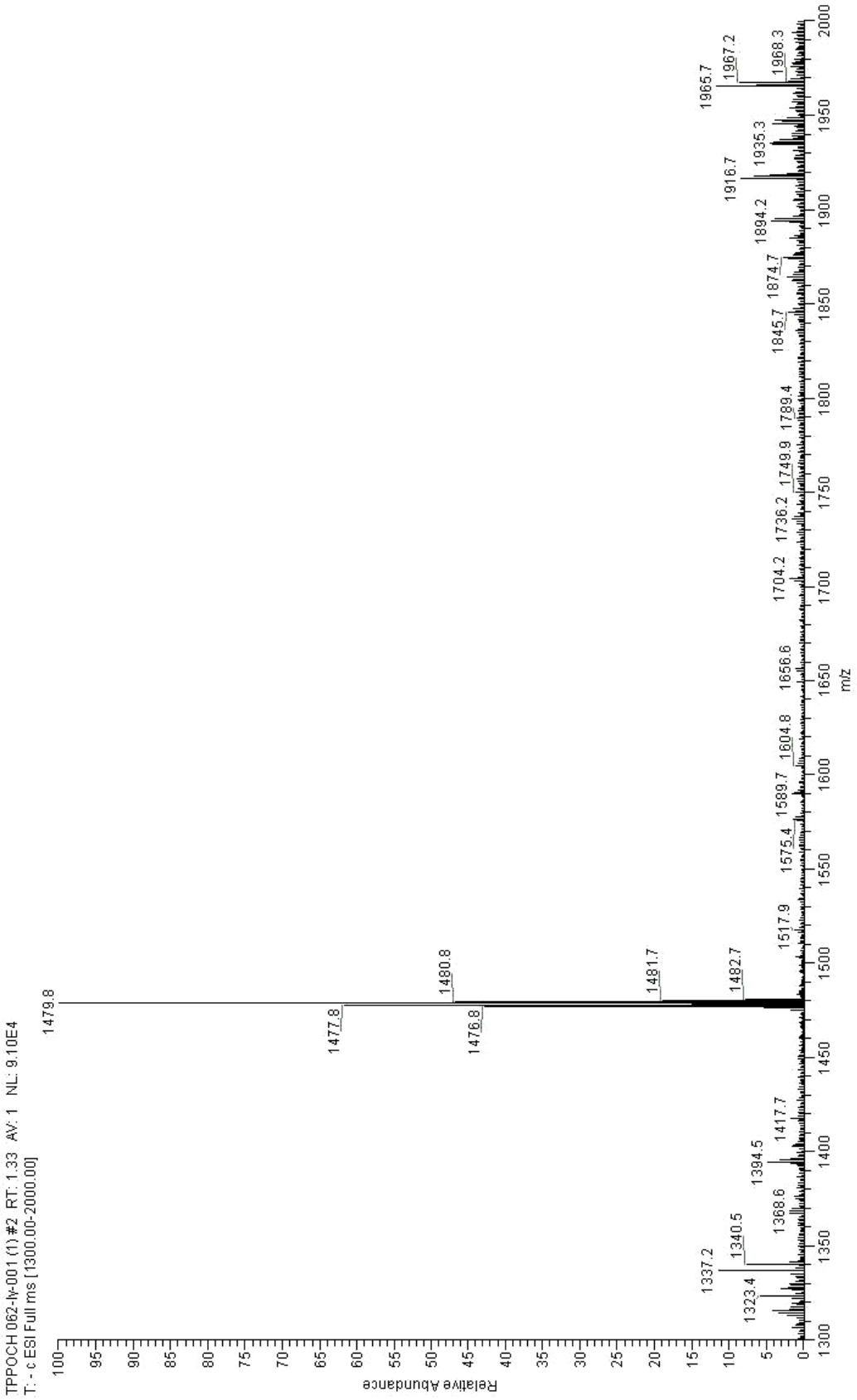


Figure S26 ESI-MS spectrum of the **TPPOC**/PhHg(II)$^{+}$ complex in a dilute solution.

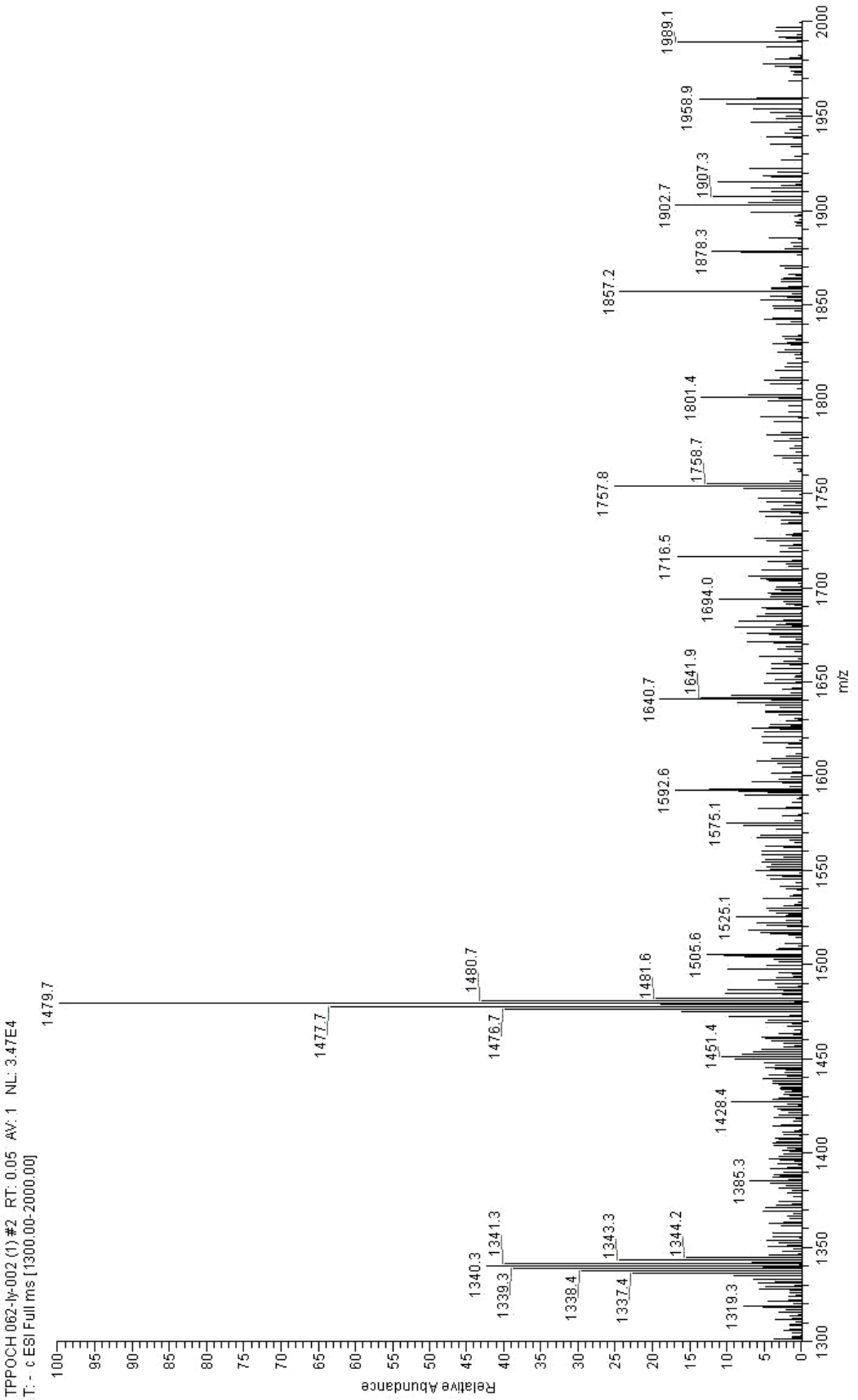


Figure S27 ESI-MS spectrum of the **TPPOC**/PhHg(II)$^+$ complex at high concentration.

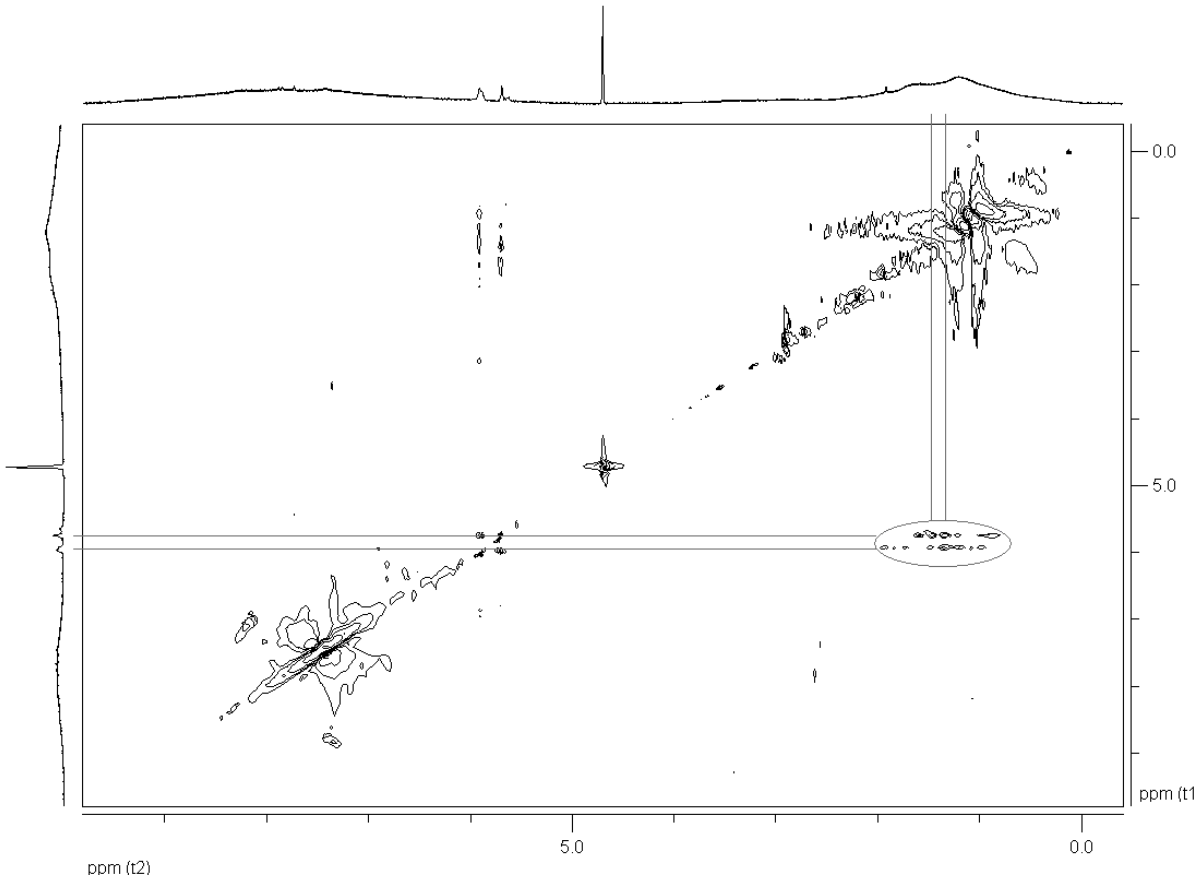


Figure S28 ROESY spectrum of **TPPOC** and PhHg(II)$^+$ in D_2O (1% NaOD) with a mixing time of 250 ms at 298.1 K.

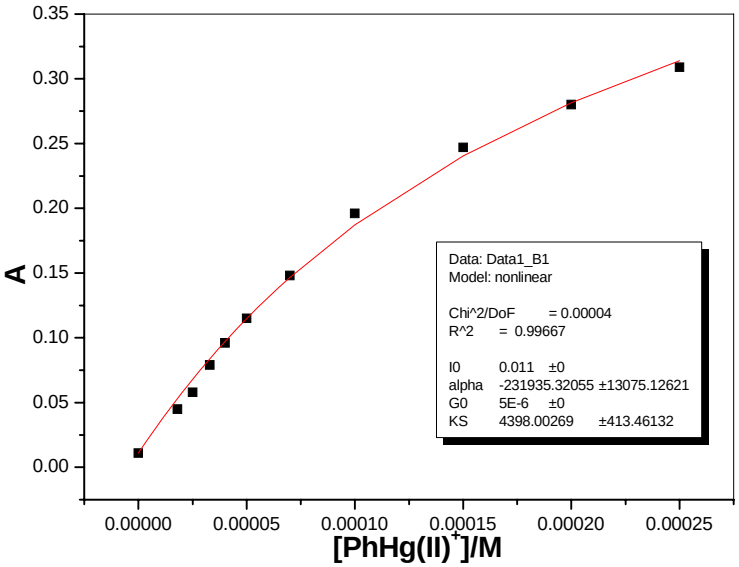


Figure S29 Fitting plot through the nonlinear least-squares analysis of the differential intensity to calculate the binding constant (K_b) for free-state **TPPOC** (in 80% methanol-3%

DMSO-CHES) with $PhHg(II)^+$.

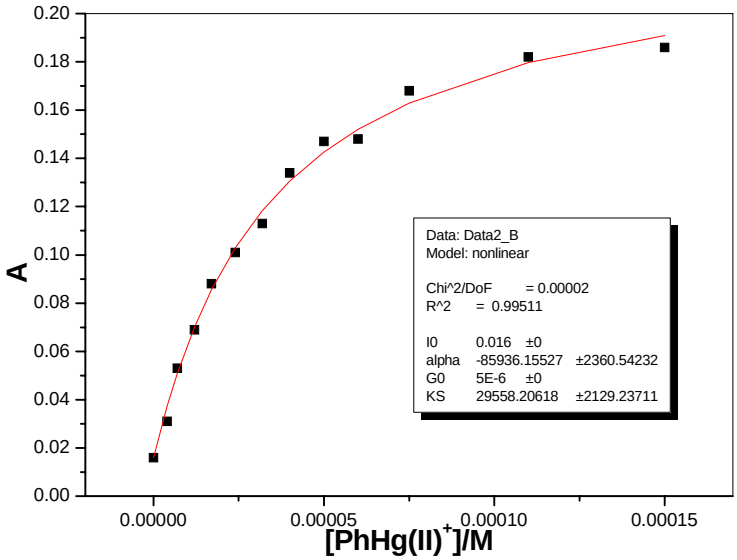
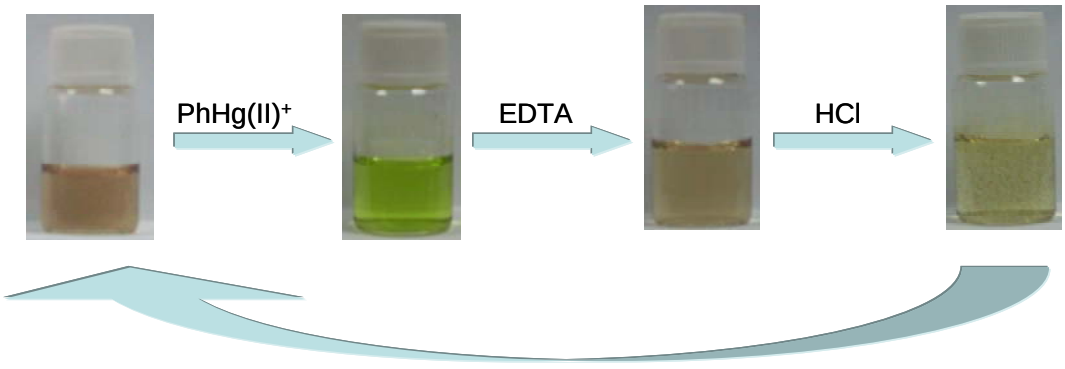


Figure S30 Fitting plot through the nonlinear least-squares analysis of the differential intensity to calculate the apparent binding constant ($K_{b,\,app}$) for aggregated-state **TPPOC** (in 3% DMSO-CHES) with $PhHg(II)^+$.



Scheme S1 The recycling process of self-assembled nanoparticles.

An important advantage of the detection method based on complexation over the traditional chemical-reaction way, especially when coupled with the "bottom-up" nanotechnology, is the low-carbon recycling yield and facile regeneration of these self-assembled particles. The recycling rate is beyond 90%, which could be easily

achieved through addition of EDTA, adjustment of pH to 5 to precipitate the anionic amphiphile, in tandem. The free **TPPOC** was obtained by centrifugation.

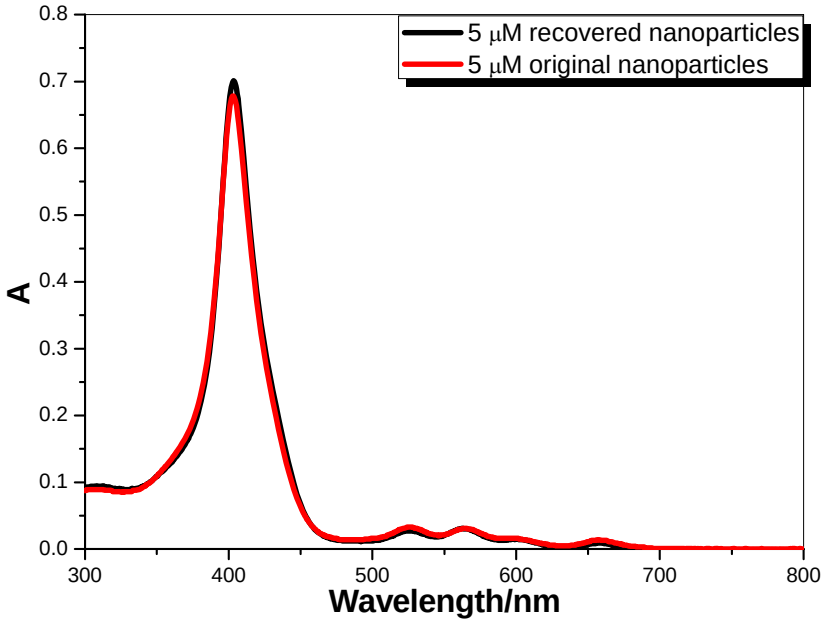


Figure S31 UV-vis spectrum of recycled **TPPOC**.

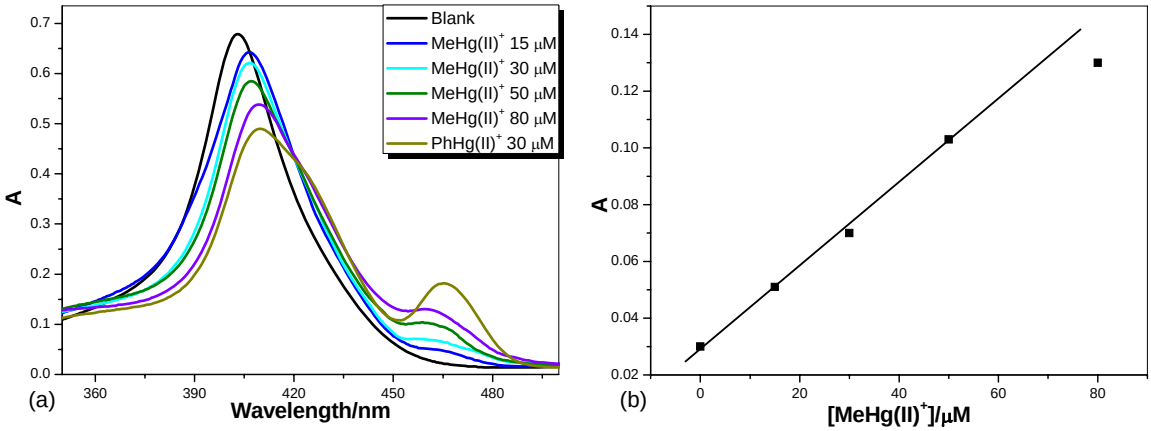


Figure S32 (a) Soret spectra of **TPPOC** (5 μM) solution upon addition of $MeHg(II)^+$ or $PhHg(II)^+$ in CHES buffer(pH = 9.8, 10 mM). (b) The absorbance intensity at 460 nm versus $[MeHg(II)^+]$.

The sensing ability towards $MeHg(II)^+$ was tested in pure CHES buffer, in terms of the

higher water solubility of it. Meanwhile, 30 μM $PhHg(II)^+$ added as solid powder was also tested in pure CHES buffer for comparison. Intriguingly, the structure difference between $MeHg(II)^+$ and $PhHg(II)^+$, resulted in the absorption maximums due to the complex of **TPPOC** with the former arising at 460 nm, with a 5 nm blue shift to that of the latter, suggesting that the **TPPOC** aggregates also has the potential for discrimination between different organic mercury species.

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