Supporting for

A catalytic triplex DNAzyme for porphyrin metalation

Xiong Zheng, Mujing Yang, Tong Yang, Yun Chang, Shuzhen Peng, Qiuda Xu, Dandan Wang, Xiaoshun Zhou and Yong Shao*

Materials and methods

Materials. 5,10,15,20-Tetrakis-(3,5-dihydroxyphenyl)porphyrin (POH_2) and 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (POH_1) were obtained from J&K Scientific Ltd. (Shanghai, China) and used as received. Chelerythrine (CHE) (Huicheng Biological Technology Co., Shanghai, China) was purchased in their highest commercially available purities (>98%). DNAs were synthesized by TaKaRa Biotechnology Co., Ltd and purified by HPLC (Dalian, China). They were first dissolved in pure water for concentration measurement using the 260 nm extinction coefficients calculated by nearest neighbor analysis. All other chemicals were analytical-reagent grade (Sigma Chemical Co., St. Louis, USA) and used without further purification. For pH-changing experiments, KOH, HAc, KCl, and H_3PO_4 were used to prepare buffer solutions within pH 2.0-9.2 (20 mM phosphate, 100 mM K^+). If not specified, the metalation experiments were carried out in 20 mM Tris-HCl (pH 7.2, 100 mM K^+). Milli-Q water (18.2 MΩ; Millipore Co., Billerica, USA) was used throughout experiments.

Fluorescence measurements. Fluorescence spectra were acquired with an FLSP920 spectrofluorometer (Edinburgh Instruments Ltd., Livingston, U.K.) at 20±1 °C, which was equipped with a temperature-controlled circulator (Julabo Labortechnik GmbH, Seelbach, Germany). Fluorescence was measured in a quartz cell with a path length of 1 cm. To prepare the nucleic acid solutions with thermodynamically stable conformations, the nucleic acid strands were annealed in 100 mM K^+ at specified pH in a thermocycler (first at 92 °C, then slowly cooled to room temperature) and stored at 4 ^oC overnight. Then, POH_3 at the specified concentration was added into the

nucleic acid or 50% DMSO solution and in the case of further addition of metal ions, the resulting solution also allowed incubation for another 60 min before fluorescence measurements.

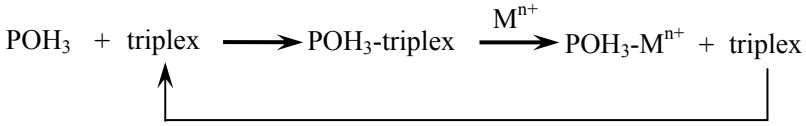
To measure the POH_3 binding constant with triplex, the fluorescence intensity at 659 nm was plotted as a function of the triplex concentration under 0.5 μM POH_3, and the data were fitted by KaleidaGraph (Synergy Software, Reading, PA) according to a 2:1 binding mode. Namely, each triplex molecule had two binding sites for POH_3. Since the free POH_3 was non-fluorescent in comparison to the triplex binding state, according to the previous method (K. Yoshimoto, S. Nishizawa, M. Minagawa and N. Teramae, *J. Am. Chem. Soc.*, 2003, **125**, 8982-8983), we used the following simplified equation to describe the triplex dependent fluorescence by assuming that the two binding sites in triplex were independent:

$$F = \mathrm{F}_{\infty} \frac{1 + \mathrm{K}C + \mathrm{K}\mathrm{C}_0 - \sqrt{(1 + \mathrm{K}C + \mathrm{K}\mathrm{C}_0)^2 - 4\mathrm{K}^2\mathrm{C}_0 C}}{2\mathrm{K}\mathrm{C}_0}$$

where F was the fluorescence response in the presence of triplex. F_{∞} was the extreme fluorescence response with excess of triplex for POH_3 at the concentration of C_0 (0.5 μM in this work). C was the binding site concentration that was two times the added triplex. K was the apparent binding constant based on the binding site.

The binding stoichiometry between porphyrin and DNA was determined by Job's plot analysis. The total concentration of porphyrin and DNA was maintained at 2 μM, and the porphyrin-to-DNA concentration ratio was sequentially varied.

Fluorescence kinetics of metal ion insertion into POH_3. Metalation reaction kinetics was fluorescently monitored (F2700 spectrofluorometer, Japan) with immediate addition of metal ions into the POH_3 (5 μM) solution that was pre-incubated with 0.5 μM triplex in 20 mM Tris-HCl (pH 7.2) containing 100 mM K^+. The control experiments without triplex were carried at pH 4.7 or 7.2 and the solution contained 50% DMSO to disperse POH_3 as the triplex binding occurred. The dilution effect on fluorescence was not considered in the repeated reaction kinetics shown in Fig. 2E. The catalytic pathway of metalation by triplex was described as follows:



UV-Vis absorption spectra and DNA melting temperature (T_m) measurements. UV-Vis absorption spectra were acquired with a UV2550 spectrophotometer (Shimadzu Corp., Kyoto, Japan) using a quartz cuvette with a path length of 1 cm. The melting temperatures (T_m) of DNA in the absence and presence of porphyrin were determined using the same spectrophotometer but equipped with a TMSPC-8 T_m analysis accessory. A micro-multicell with eight chambers was used to eliminate the temperature discrepancy between the samples. The DNA absorbance at 260 nm as a function of the solution temperature between 5 and 100 °C was collected in 0.5 °C increments, with a 30 s equilibration time applied after each temperature increment. We found that the possible changes in the porphyrin aggregation state upon increasing the solution temperature had no effect on the DNA T_m measurements. The raw data were first sigmoidally fitted and the 1st derivative method was used to accurately calculate the T_m values.

Circular dichroism (CD) spectra measurements. The CD spectra were measured on MOS-500 CD spectrometer (Bio-Logic Science Instruments, France) using a 2-mm path-length quartz cell. Scans were performed at a temperature-controlled holder (20 °C) over a wavelength range of 230-500 nm with a scanning speed of 200 nm/min and 5 nm pitch. The CD spectra were performed in 20 mM Tris-HCl (pH 7.2). Solution without DNA was subtracted from the collected data for blank correction. The given CD spectra were three scans averaged and zero-corrected at 500 nm.

Synthesis and characterization of POH_3

5, 10, 15, 20-Tetrakis(3, 4, 5-trihydroxyphenyl)porphyrin (POH_3) was synthesized from 5, 10, 15, 20-tetrakis(3, 4, 5-trimethoxyphenyl)porphyrin (POM_3) according to our previous procedure (ref. 32 in text). Briefly, in a three-neck round-bottom flask, 24.3 mL of pyridine and 28.5 mL of hydrochloric acid (37% in water) were heated

under nitrogen atmosphere with the mixture temperature increasing up to 170 °C. The POM_3 (500.0 mg, 0.513 mmol) was added, and the mixture was kept at 170 °C overnight. The reaction mixture was cooled, and 20 mL of methanol was added. The mixture was poured slowly into 600 mL of stirring dichloromethane. The precipitate was filtered and washed with dichloromethane and heptane. The porphyrin was dried at 120 °C under vacuum overnight to yield a purple solid (150.0 mg, 36%). The ^1H NMR spectrum for POH_3 was recorded on a Bruker DRX-300 AVANCE spectrometer. Chemical shifts for the ^1H NMR spectrum was measured in DMSO-d_6. The mass spectrum was obtained on a Bruker Daltonics Ultraflex II spectrometer in the MALDI/TOF reflectron mode using dithranol as a matrix. The spectra were presented in Fig. S1. ^1H NMR (300 MHz, DMSO-d_6) (ppm): -2.94 (s, 2H, NH); 7.12 (s, 8H, $H_{o\text{-}Ph}$); 8.56 (s, 4H, $OH_{p\text{-}Ph}$); 8.93 (s, 8H, $H_{\beta\text{-}pyrr}$); 9.28 (s, 8H, $OH_{m\text{-}Ph}$). MS (MALDI/TOF): m/z = 807.1347 for $[M+H]^+$ (807.1938 calculated for $C_{44}H_{31}N_4O_{12}^+$). UV-vis (ethanol): λ_{max} (nm) = 424 (100120); 515 (9821); 555 (4583); 594 (3884); 654 (4967) (values in brackets are extinction coefficients at M^{-1} cm^{-1}).

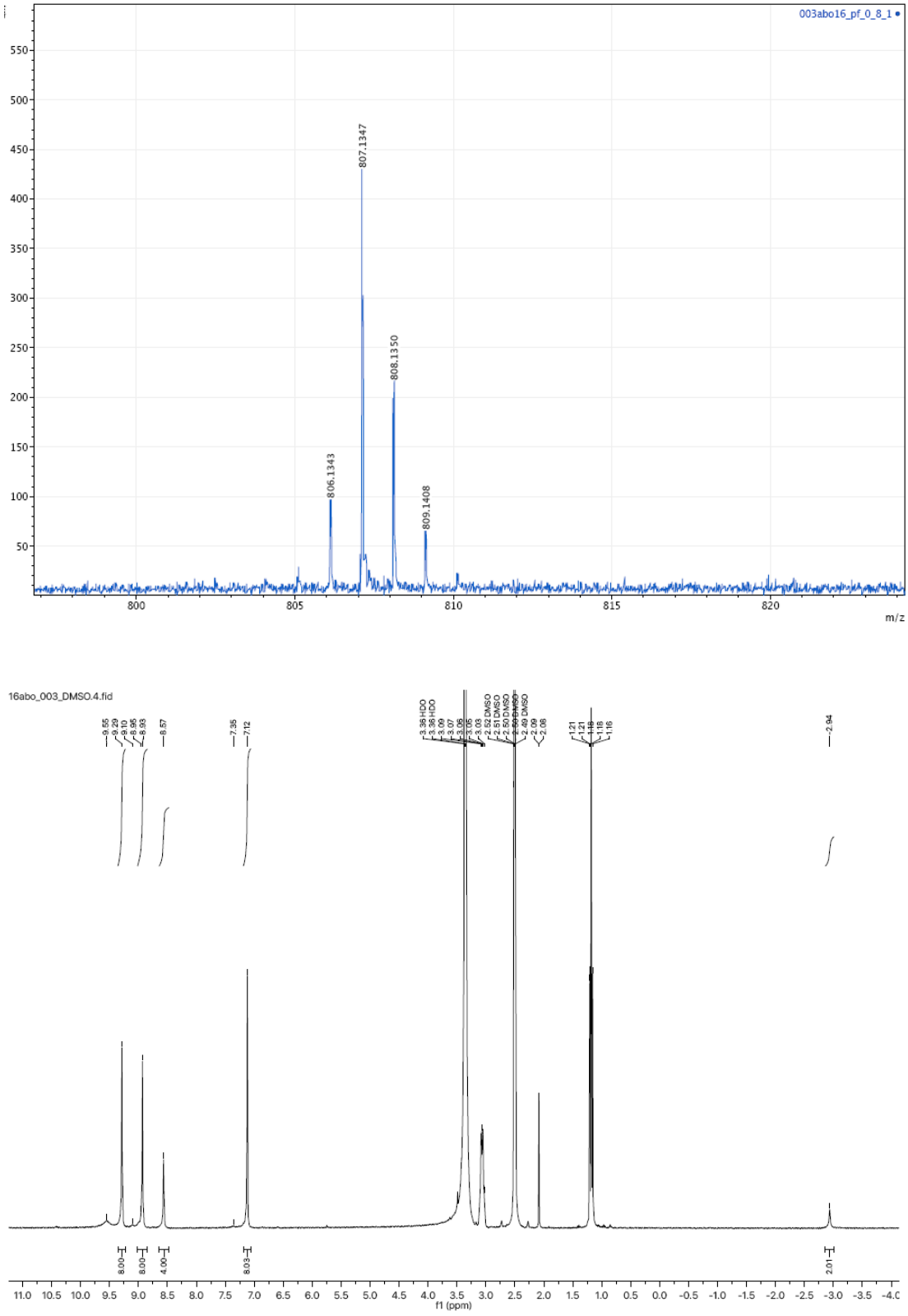


Fig. S1. MS and ^{1}H NMR spectra of POH_3.

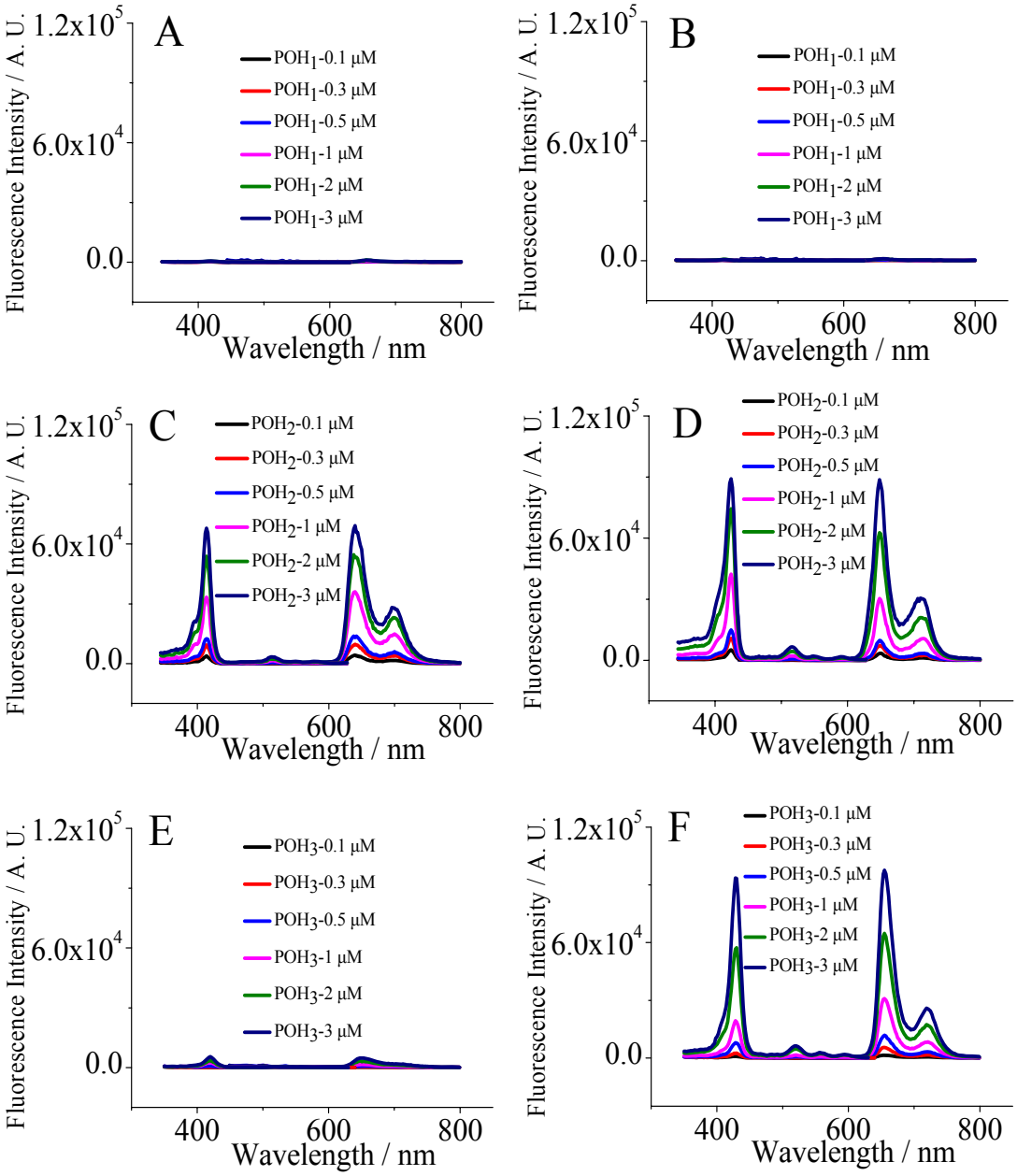


Fig. S2. Fluorescence excitation and emission spectra of POH_n in the (A, C, E) absence and (B, D, F) presence of 0.5 μM TP-AT at pH 7.2.

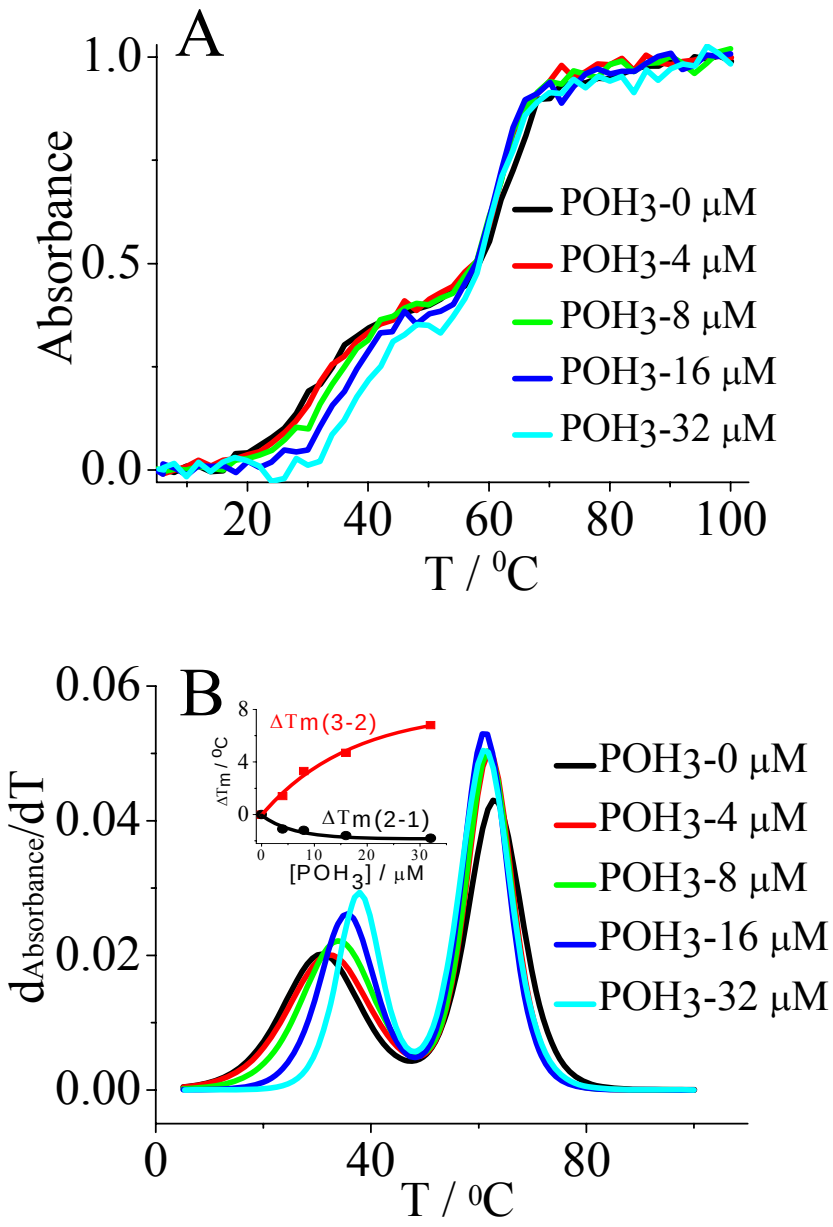


Fig. S3. (A) Raw melting curves of TP-AT (2 μM) in variant concentrations of POH_3 at pH 7.2. The curves were normalized in order to avoid the effect of the POH_3 absorption on the triplex DNA absorption at 260 nm. (B) The 1st derivative plots of dAbsorbance/dT as a function of T for a clear comparison. Inset: Changes of melting temperatures ($\Delta T_{m(3-2)}$ and $\Delta T_{m(2-1)}$) of TP-AT (2 μM) for triplex and duplex melting upon increasing the POH_3 concentration.

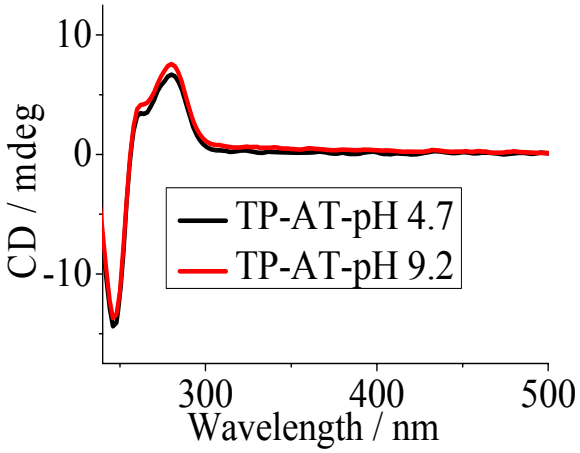


Fig. S4. CD spectra of TP-AT at pH 4.7 and 9.2.

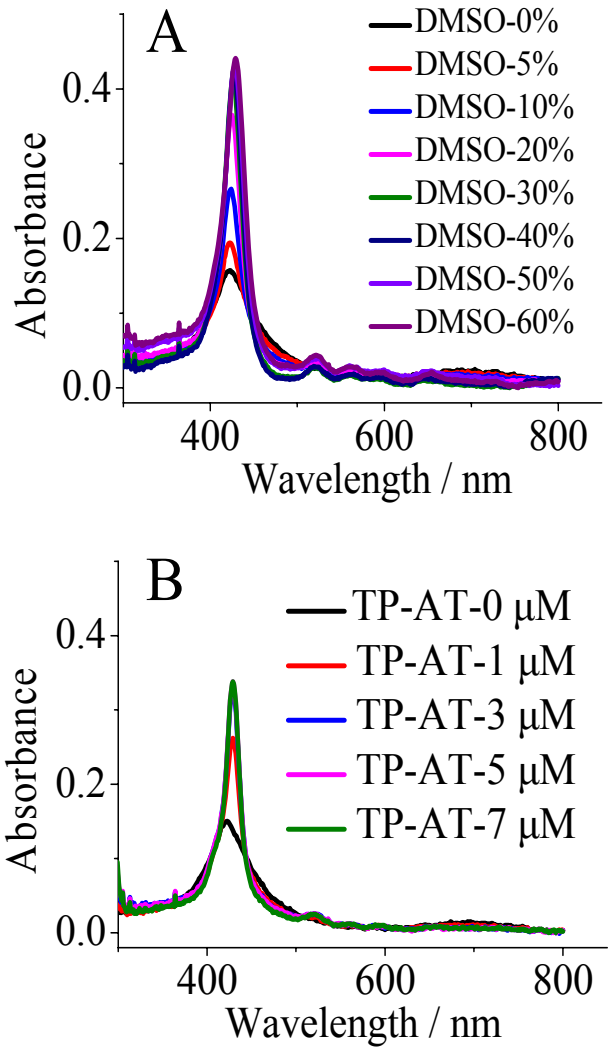


Fig. S5. (A) Absorption spectra of POH_3 (3 μM) upon gradual addition of DMSO until up to 60% at pH 7.2. (B) Absorption spectra of POH_3 (3 μM) with increasing the concentration of triplex at pH 7.2.

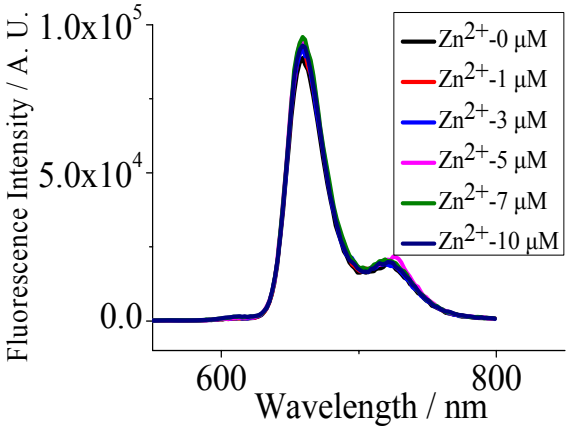


Fig. S6. (A) Emission spectra of POH_3 (5 μM) at pH 7.2 (50% DMSO) with addition of Zn^{2+}.

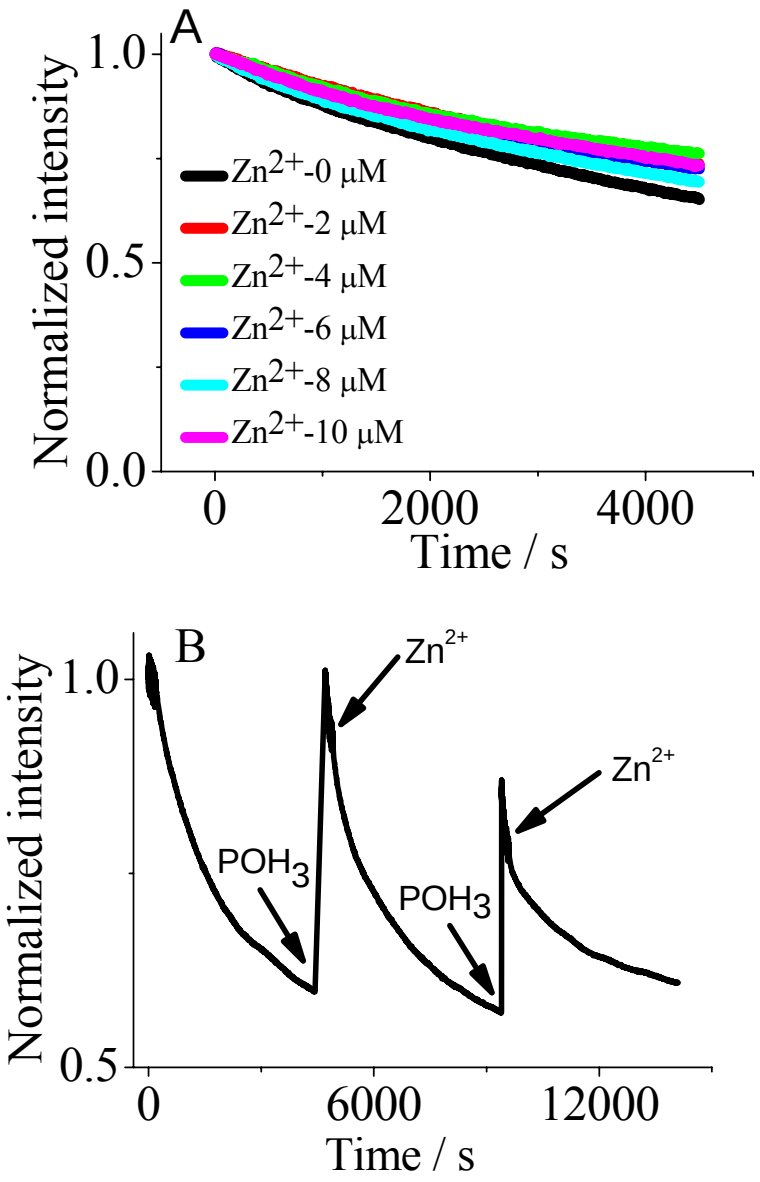


Fig. S7. (A) Fluorescence kinetics of POH_3 (5 μM) in reacting with Zn^{2+} at pH 7.2 (50 % DMSO). (B) Repeated fluorescence kinetics of POH_3 (5 μM) and TP-AT (0.5 μM) on reaction with Zn^{2+} (5 μM) at pH 7.2. Arrows indicated that POH_3 (5 μM) and Zn^{2+} (5 μM) were further added after the preceding catalysis. Note that the decreased responses after each round catalysis were caused by the solution diluting (finally diluted 40%). Excitation: 429 nm; emission: 659 nm.

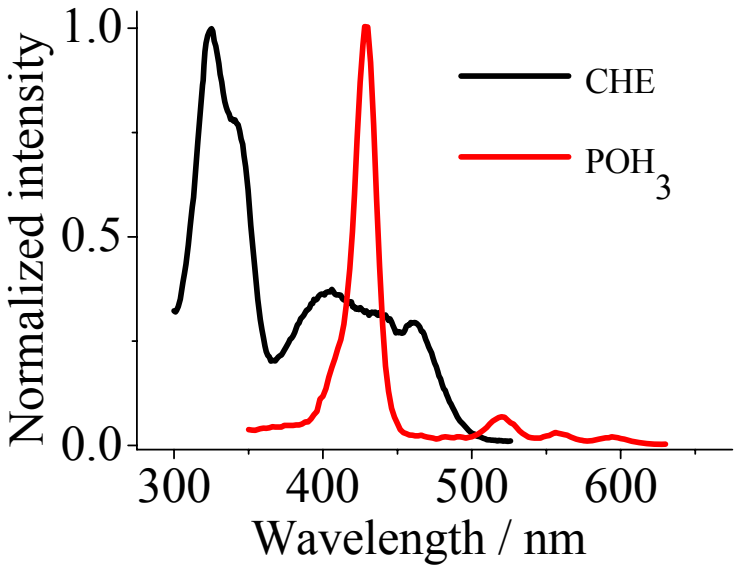


Fig. S8. Normalized fluorescence excitation spectra of CHE and POH_3 (0.5 μM) in the presence of TP-AT (0.5 μM). These spectra were measured with emission at 560 and 659 nm for CHE and POH_3, respectively.

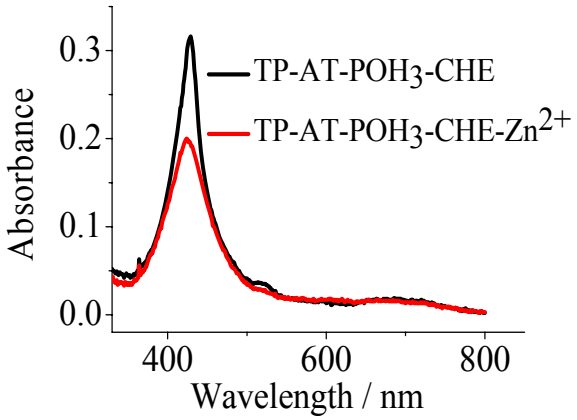


Fig. S9. Absorption spectra of the TP-AT (0.5 μM) solution containing POH_3 (5 μM) and CHE (0.5 μM) in the absence and presence of Zn^{2+} (10 μM), respectively.

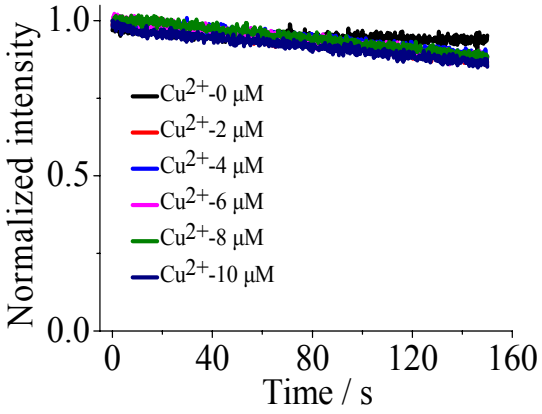


Fig. S10. Fluorescence reaction kinetics for POH_3 (5 μM) reacting with variant concentration of Cu^{2+} at pH 7.2 (50% DMSO).

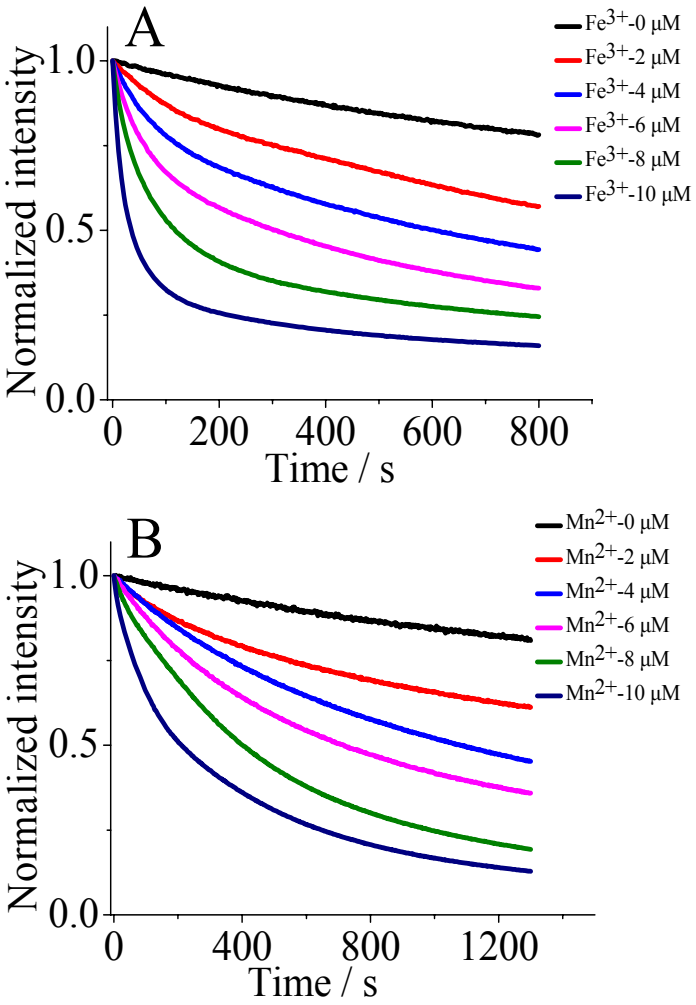


Fig. S11. Fluorescence kinetics of POH_3 (5 μM) and TP-AT (0.5 μM) in reacting with (A) Fe^{3+} and (B) Mn^{2+} at pH 7.2. Excitation: 429 nm; Emission: 659 nm.

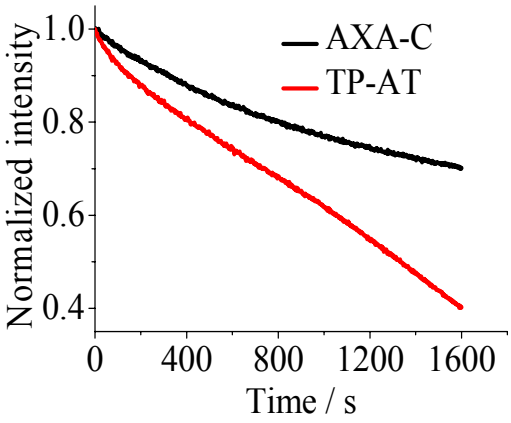


Fig. S12. Time-dependent fluorescence responses of POH_3 (5 μM) in reacting with Zn^{2+} (5 μM) in the presence of 0.5 μM AXA-C and TP-AT at pH 7.2. Excitation/emission: 429/659 nm.