# Supporting Information (SI)

# Promoted fluorescent sensing strategy for hypochlorous acid by using serum albumin as a signal amplifier

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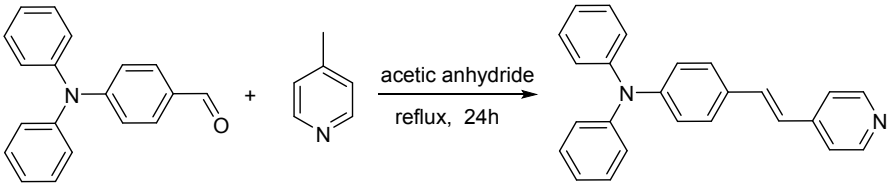
## Contents

**1. Synthesis of compound TPP.**




**Scheme 1.** Structure and synthesis of TPP.

To the solution of 4-(N, N-diphenylamino)benzaldehyde (0.54 g, 2.0 mmol) in acetic anhydride (10 mL) was added 4-methylpyridine (0.23 g, 2.5 mmol) at r.t.. The mixture was refluxed for 24 h., after completion of the reaction the reaction mixture was cooled to r.t. and the solvent was removed by rotary evaporation under vacuum. The resulting crude product was then subjected to column purification (silica gel, 40-50 % EtOAc in hexanes) to afford the pure TPP (yield: 30 %). $^{1}$H NMR (500 MHz, DMSO-*d*6) $\delta$ (ppm) 8.51 (d, $J$ = 4.7 Hz, 2H), 7.56 (d, $J$ = 8.6 Hz, 2H), 7.52 (d, $J$ = 5.7 Hz, 2H), 7.47 (d, $J$ = 11.6 Hz, 1H), 7.33 (m, 4H), 7.10 (d, $J$ = 4.9 Hz, 2H), 7.07 (m, 5H), 6.96 (d, $J$ = 8.6 Hz, 2H); $^{13}$C NMR (126 MHz, DMSO-*d*6) $\delta$ (ppm) 150.34, 148.10, 147.16, 144.97, 132.92, 130.37, 130.06, 129.83, 128.70, 124.94, 124.38, 124.04, 123.66, 122.67, 121.05. ESI-MS *m/z*: $[M+H]^+$ Calcd for $C_{25}H_{21}N_2^+$ 349.1699; Found 349.1703.

**2. Generation of various ROSs**

(1) $ClO^-$ was prepared by dilution of commercial NaClO solution in deionized water and the concentration of the $ClO^-$ stock solution was determined by measuring the absorbance at 209 nm with a molar extinction coefficient of 350 $M^{-1}cm^{-1}$.

(2) $OONO^-$ stock solution was prepared by mixing the following three kinds of solutions simultaneously, the mixture of hydrogen peroxide (0.7 M, 1.5 mL) and hydrochloric acid (0.6 M, 1.5 mL), solution of sodium nitrite (0.6 M, 3 mL) and solution of sodium hydroxide (1.5 M, 3 mL).[1] The concentration of the $OONO^-$ stock solution was determined in 0.1 M NaOH by measuring the absorbance at 302 nm with a molar extinction coefficient of 1670 $M^{-1}$ $cm^{-1}$.

(3) •OH was generated in the Fenton system from ferrous ammonium sulfate and

hydrogen peroxide. [2]

(4) $H_2O_2$ solution was purchased from Aladdin reagent. The concentration of the $H_2O_2$ stock solution was determined by measuring the absorbance at 240 nm with a molar extinction coefficient of 43.6 $M^{-1}cm^{-1}$.

(5) $O_2^-$was obtained by the water solution of potassium superoxide.

(6) t-$BuOO^-$ was prepared by dilution of commercial t-BuOOK in water.

(7) $^1O_2$was obtained by adding NaClO solution (1.0 M, 2 mL) to $H_2O_2$ solution (1.0 M, 2 mL).

## 3. Spectral titration profiles

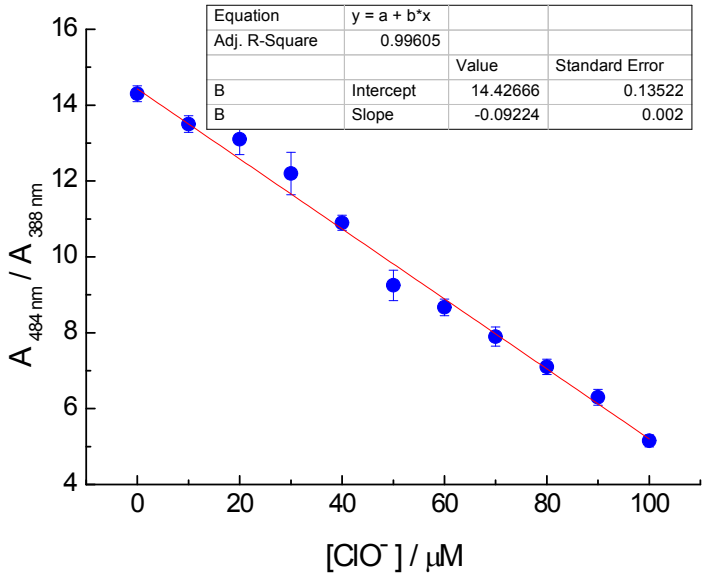



**Figure S1** The linear relationship between ratio of $A_{484\ nm}/A_{388\ nm}$ and $ClO^-$ concentrations with in 0–100 μM in ethanol.[3]

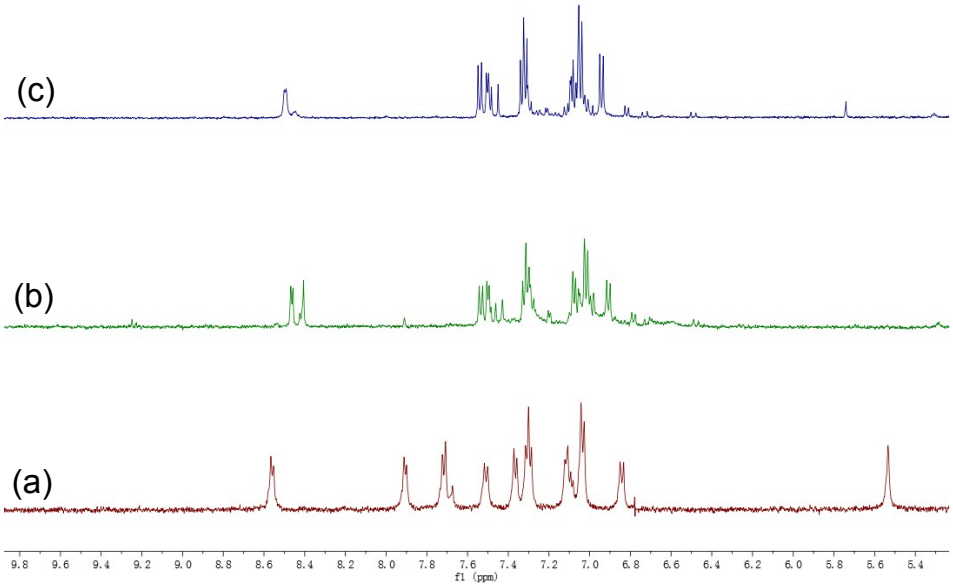



**Figure S2** Partial $^{1}$H NMR (500 MHz) spectra of TPM (a), TPM+$ClO^-$ (b) in MeOD and TPP in DMSO-$d_6$ (c). [TPM]=[TPP]=2.0 mM.

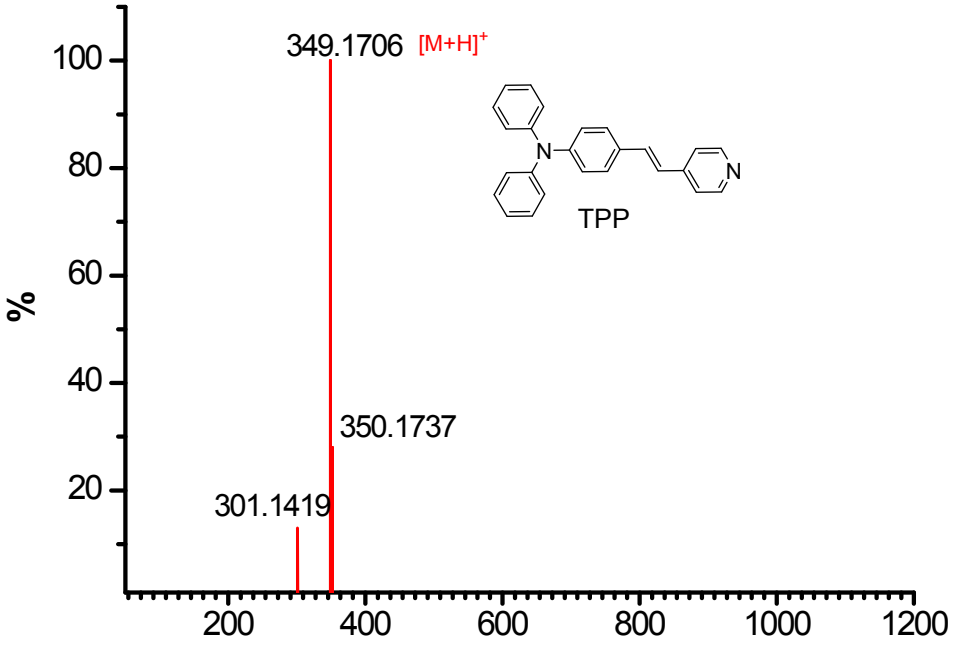



**Figure S3** HRMS spectrum of TPM with 1 equivalent $ClO^-$ in EtOH. [TPM]=50 μM. Signal at m/z 349.1706 (calcd. 349.1705) for the oxidation product $[TPP+H]^+$ ($[C_{25}H_{21}N_2]^+$) in the mixture of TPM/$ClO^-$ in EtOH matched well with the theoretical simulation.

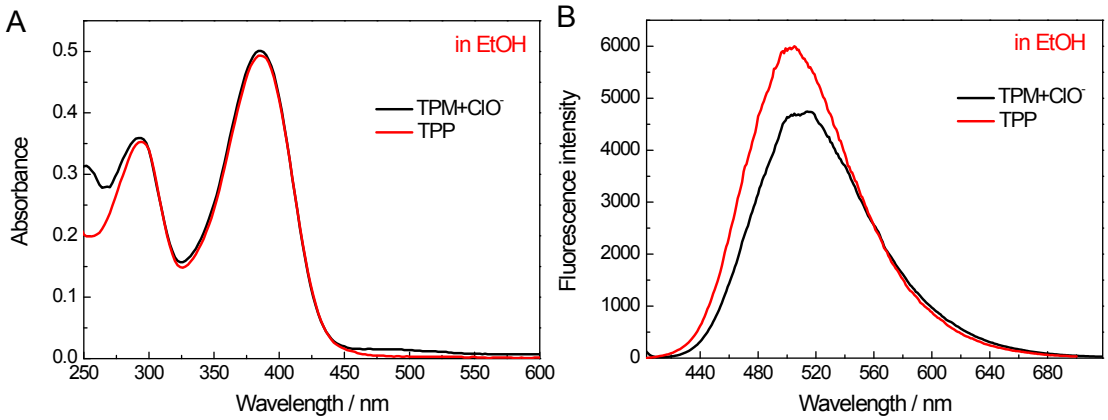



**Figure S4** (A) Absorption and (B) fluorescence spectra of 50 μM TPM in the presence of 15 equivalents of $ClO^-$ and 50 μM TPP in ethanol.

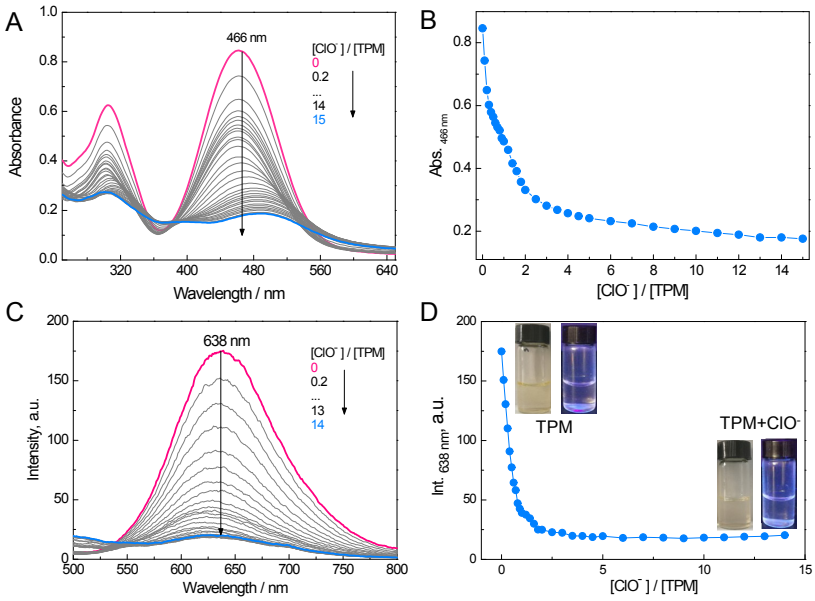



**Figure S5** (A) Absorption and (C) fluorescence spectra of 50 μM TPM in the presence of $ClO^-$ of increasing concentration in PBS buffer (10 mM, pH 7.4). Excitation wavelength: 462 nm. Plot of (B) absorption ($A_{466\ nm}$) and (D) fluorescence intensity (Int. $_{638\ nm}$) changes as a function of ratio [$ClO^-$]/[TPM]. Inset shows the color changes upon the addition of 2.0 eq. $ClO^-$ under ambient light or 365 nm UV light.

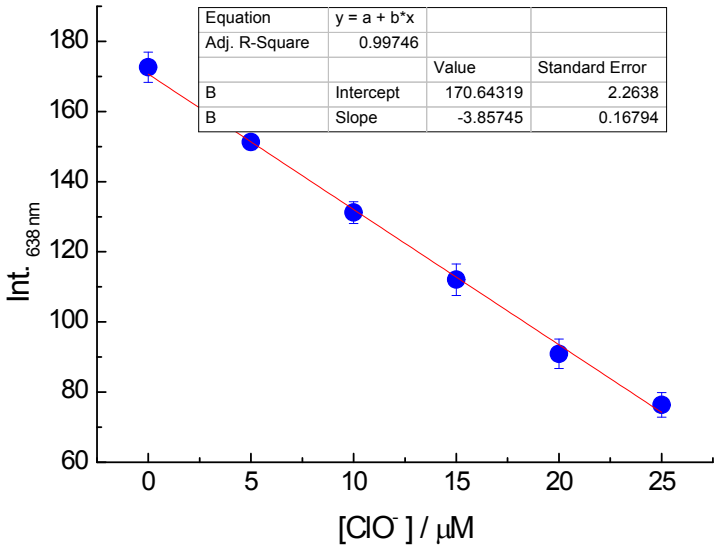



**Figure S6** The linear relationship between fluorescence intensity and $ClO^-$ concentrations within 0–25 μM in PBS buffer solution.

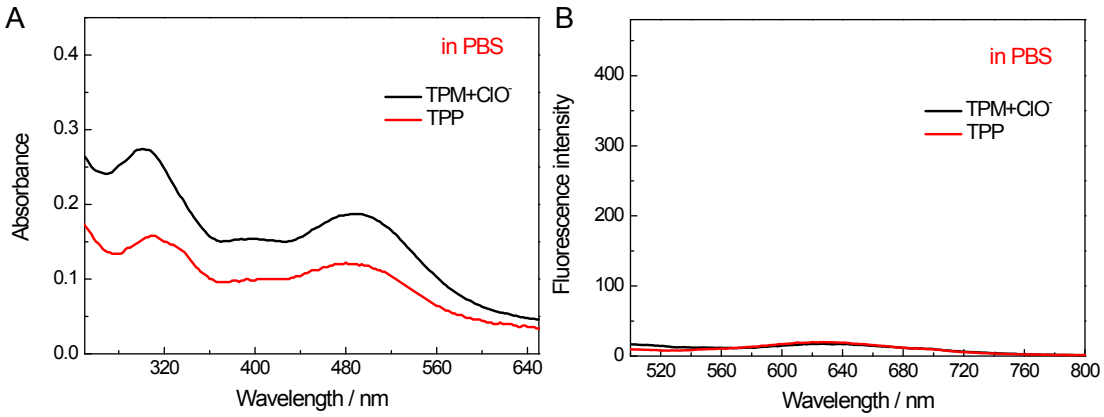



**Figure S7** (A) Absorption and (B) fluorescence spectra of 50 μM TPM in the presence of 1.0 equivalents of $ClO^-$ and 50 μM TPP in PBS buffer.

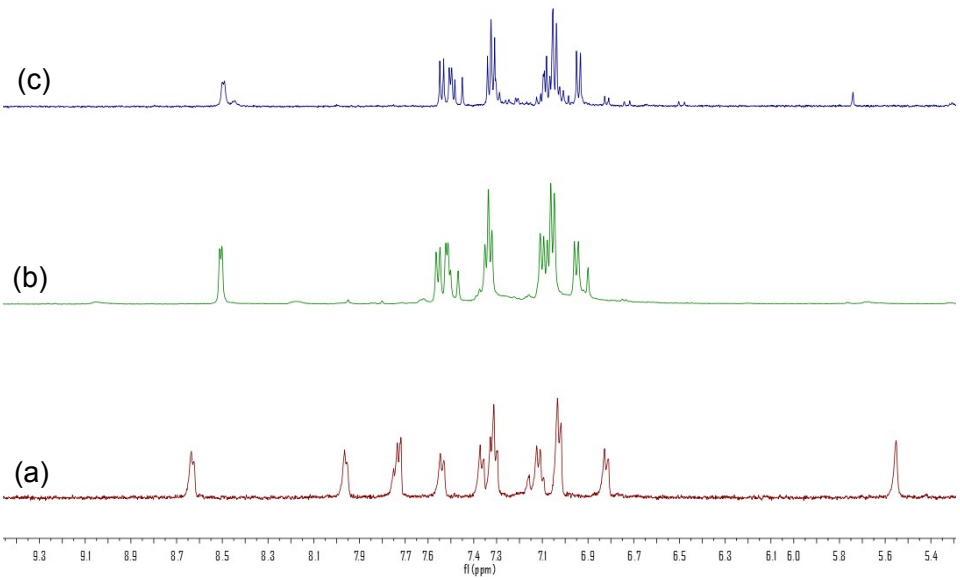



**Figure S8** Partial $^{1}$H NMR (500 MHz) spectra of TPM (a), TPM+$ClO^-$ (b) in the mixture of DMSO-$d_6$ and $D_2O$ (v/v: 4/6) and TPP in DMSO-$d_6$ (c). [TPM]=[TPP]=2.0 mM.

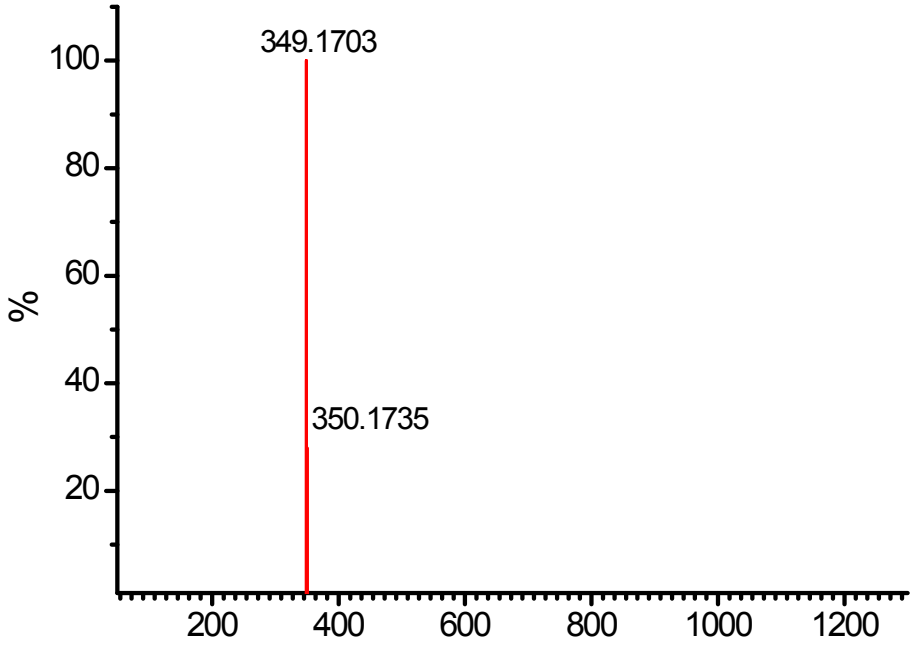



**Figure S9** HRMS spectrum of TPM with 1 equivalent $ClO^-$ in PBS buffer. [TPM]=50 μM.

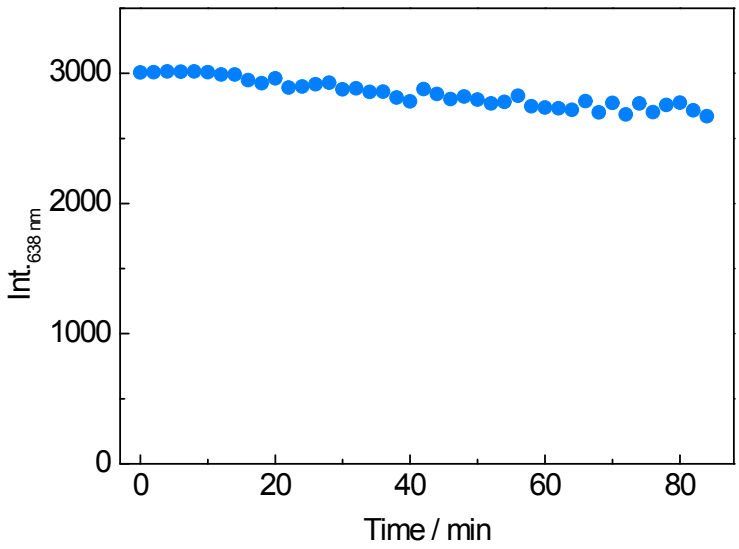



**Figure S10** Time-dependent fluorescence intensity at 638 nm upon the addition of 20 μM BSA to 50 μM TPM in PBS buffer solution (10 mM, pH 7.4).

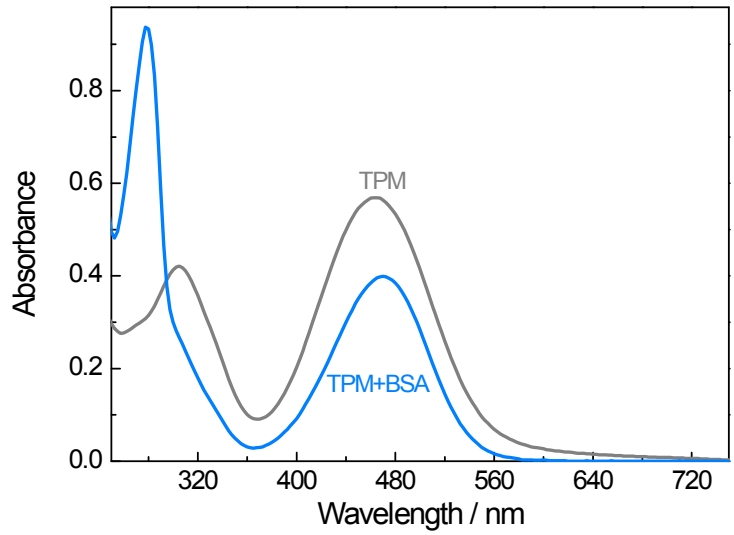



**Figure S11**.Absorption of 50 μM TPM in the presence and absence of 20 μM BSA in 10 mM PBS solution at pH=7.4.

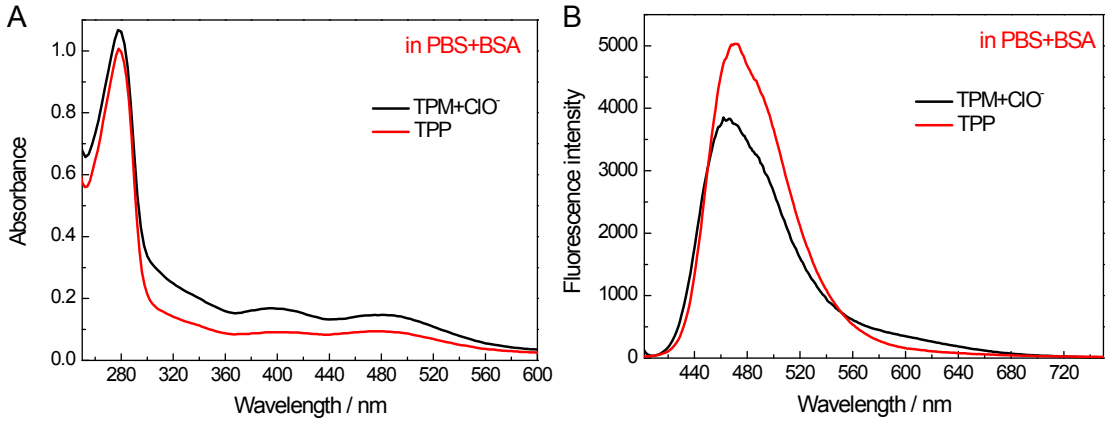



**Figure S12** (A) Absorption and (B) fluorescence spectra of 50 μM TPM in the presence of 1.0 equivalents of $ClO^-$ and 50 μM TPP in PBS buffer with 20 μM BSA.

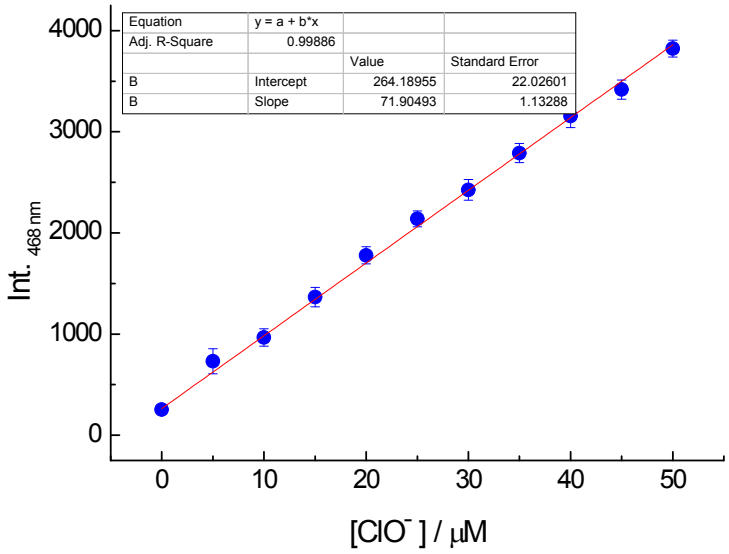



**Figure S13** The linear relationship between fluorescence intensity and $ClO^-$ concentrations within 0–50 μM in the presence of 20 μM BSA in PBS buffer solution.

**Table S1.** Photophysical properties of TPM without or with $ClO^-$ in different solvents.

| Solution | Entry | Ex/Em (nm) | Stokes shift (nm) | ε/$10^4$(L·mol$^{-1}$·cm$^{-1}$) | QY/% | LOD for $ClO^-$ |
|---|---|---|---|---|---|---|
| EtOH | TPM | -- | -- | 1.32 | -- | -- |
| | TPM+$ClO^-$ | 416/514 | 98 | 1.01 | 45.1 | 22 nM |
| PBS | TPM | 462/638 | 176 | 1.62 | 0.27 | -- |
| | TPM+$ClO^-$ | -- | -- | 0.36 | -- | 680 nM |
| PBS+BSA | TPM | 390/600 | 210 | 0.83 | 9.9 | -- |
| | TPM+$ClO^-$ | 390/468 | 178 | 0.37 | 37.8 | 1.5 nM |

**Table S2.** Properties of respresentive fluorescent HOCl probes

| Entry | Probe | $\lambda_{abs}/\lambda_{em}$ nm | LOD nM | Target Imaging | Reaction Time |
|---|---|---|---|---|---|
| **1**[4] | BClO | 500/505 | 0.56 | - | within 1 s |
| **2**[5] | SeCy7 | --/786 | 310 | - | within dozens of seconds |
| **3**[6] | PZ–Py | 400/562 | 17.9 | mitochondria | within seconds |
| **4**[7] | Ir2 | 405/565 | - | mitochondria | within seconds |
| **5**[8] | Rh-Py | 544/577 | 24 | mitochondria | Within seconds |
| **6**[9] | MITO-TP | 375/500 | 17.2 | mitochondria | within seconds |
| **7**[9] | LYSO-TP | 375/500 | 19.6 | lysosome | within seconds |
| **8**[10] | HBP | 480/508 | 2.4 | - | within 30 min |
| **9**[11] | BRT | 525/580&540 | 38 | - | within 15 seconds |
| **10**[12] | HES-BODIPY | 480/532&562 | 430 | - | within seconds |
| **11**[13] | Lyso-1 | 500/563 | 60 | lysosome | ca. 5 min |
| **12**[14] | PNIS | 325/447 | 210 | mitochondrial | - |
| **13**[15] | YDN | 485/516 | 8.7 | - | 2 min |
| **14**[16] | HKOCl-3 | 490/527 | 0.33 | - | within 1 min |
| **15**[17] | meso-(4-pyridinyl)-substituted BODIPY | 495/515 | 600 | mitochondria | within 5 min |
| **16** **this wok** | TPM | 390/468&600 | 1.5 | mitochondria | within seconds |

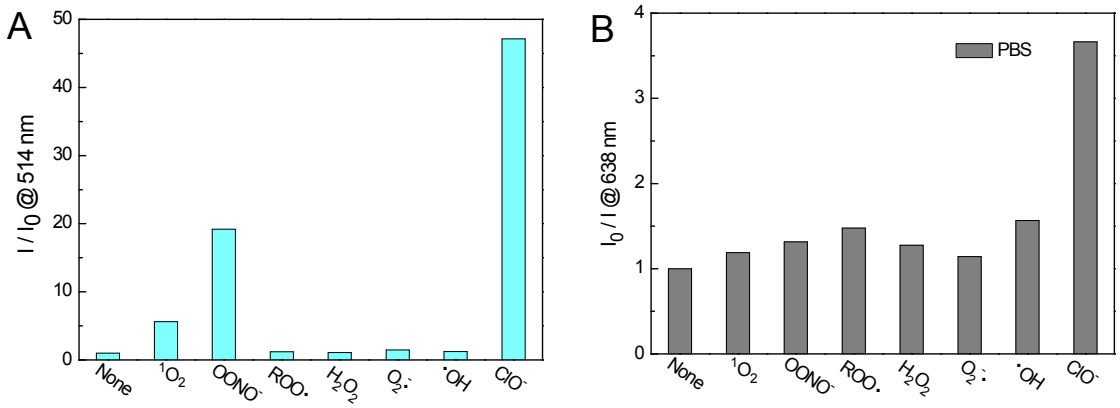



**Figure S14** Ratiometric fluorescence responses of 50 μM TPM toward various analytes in (A) ethanol and (B) PBS buffer solution respectively.

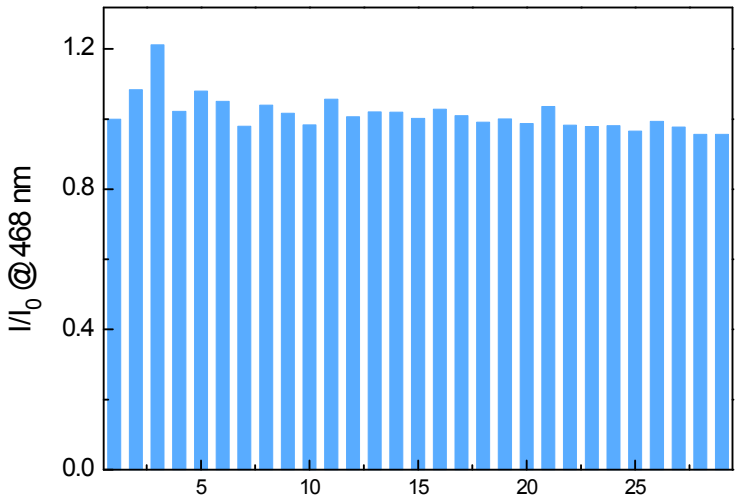



**Figure S15** $I/I_0$ at 468 nm of TPM (50 μM) in the present of BSA (20 μM) in response to $ClO^-$ in the presence of various ROSs (2-6: $^1O_2$, $H_2O_2$, *t*-$BuOO^-$, $O_2^-$, and •OH), RNSs (7-8:$NO_2^-$ and $ONOO^-$), RSSs (9-14: $S^{2-}$, $HSO_3^-$, $SO_3^{2-}$, Cys, Hcy and GSH), anions (15-21: $F^-$, $Cl^-$, $I^-$, $AcO^-$, $SO_4^{2-}$, $NO_3^-$ and $CO_3^{2-}$) and cations (22-29: $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Ni^{2+}$, $Fe^{3+}$, $Al^{3+}$ and $Cu^{2+}$).

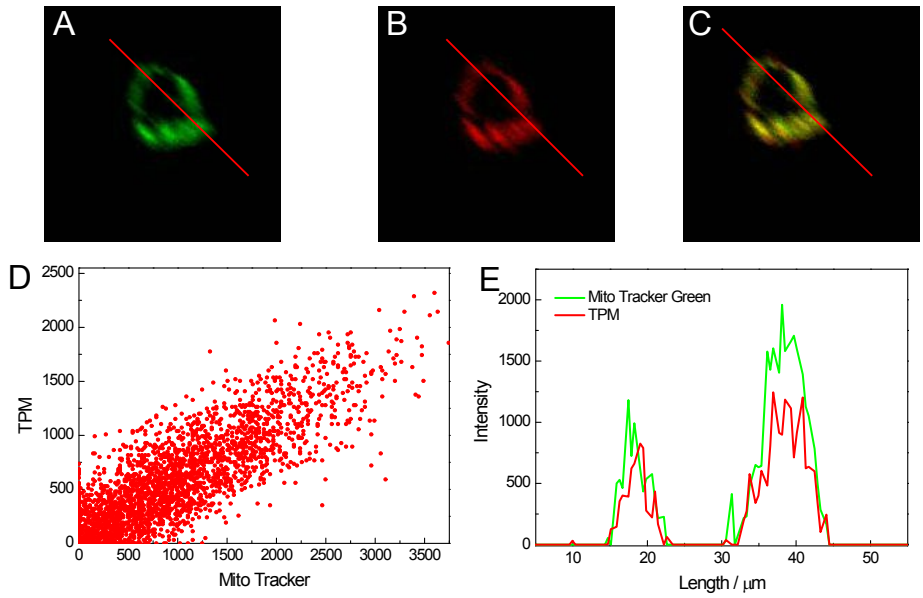



**Figure S16** Fluorescence images of mitochondria in HeLa cells. HeLa cells were incubated with Mito Tracker Green (100 nM) and TPM (10 μM) for 30 min respectively. (A) Emission from the green channel (Mito Tracker Green, $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500−540 nm), (B) emission from the red channel (TPM, $\lambda_{ex}$ = 458 nm, $\lambda_{em}$ = 580–680 nm), (C) merged image of images (A) and (B), (D) intensity correlation plot of TPM and Mito Tracker Green, and (E) intensity profile of ROIs across HeLa cells.

Scale bar = 20 μm.

## 4. $^{1}H$, $^{13}C$ NMR and HRMS spectra

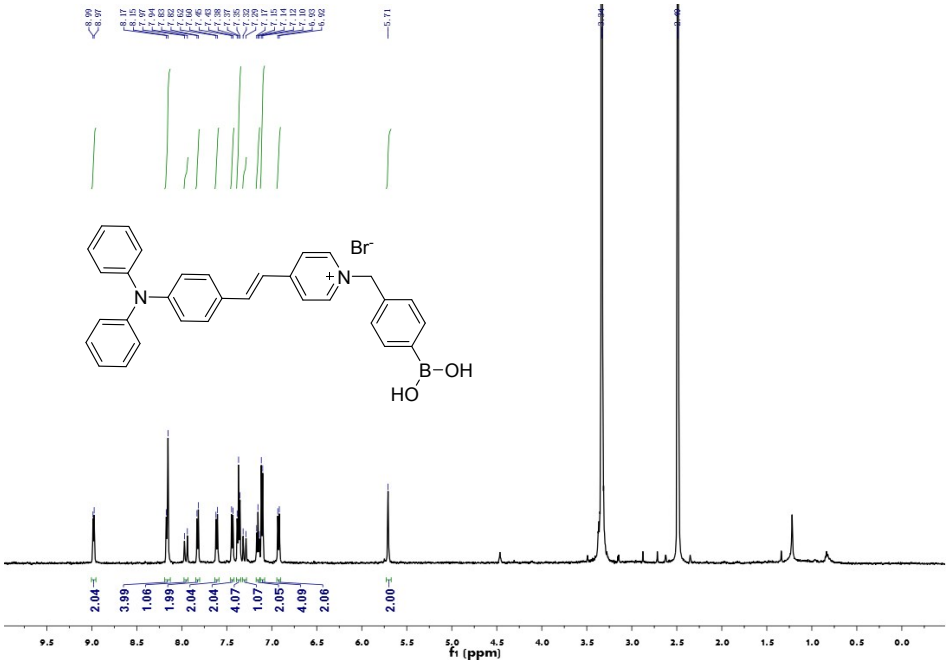



**Figure S17** $^{1}H$ NMR spectrum of TPM in DMSO-$d_6$.

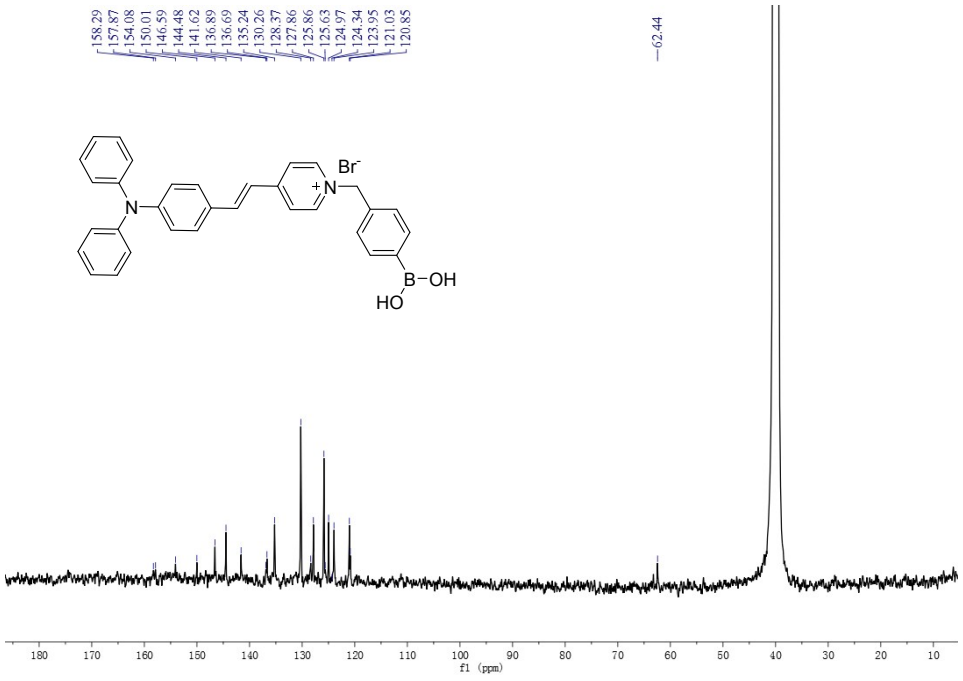



**Figure S18** $^{13}C$ NMR spectrum of TPM in DMSO-$d_6$.

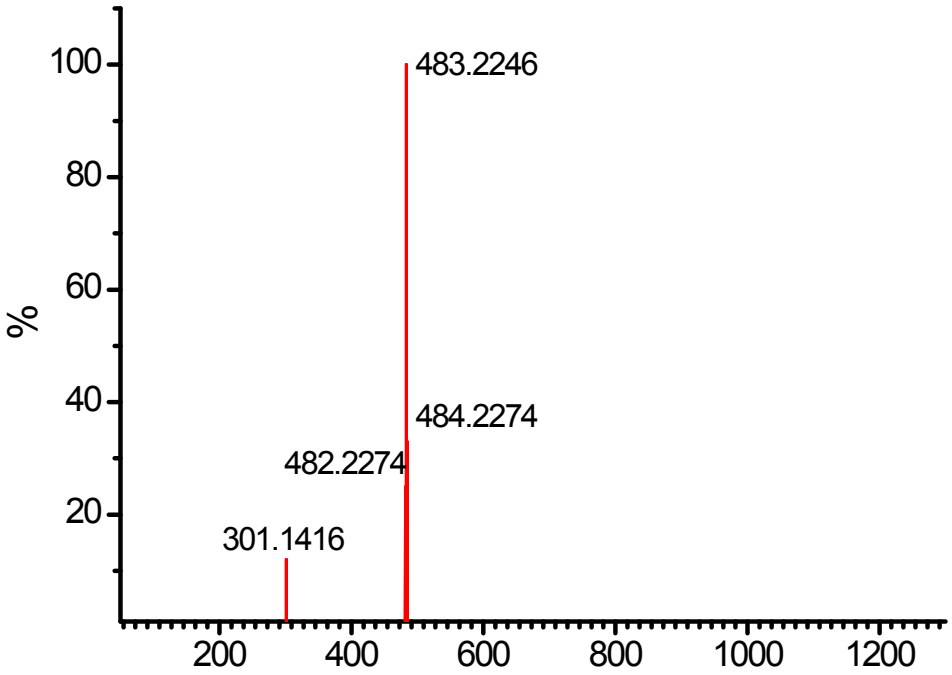



**Figure S19** HRMS spectrum of TPM in MeOH.

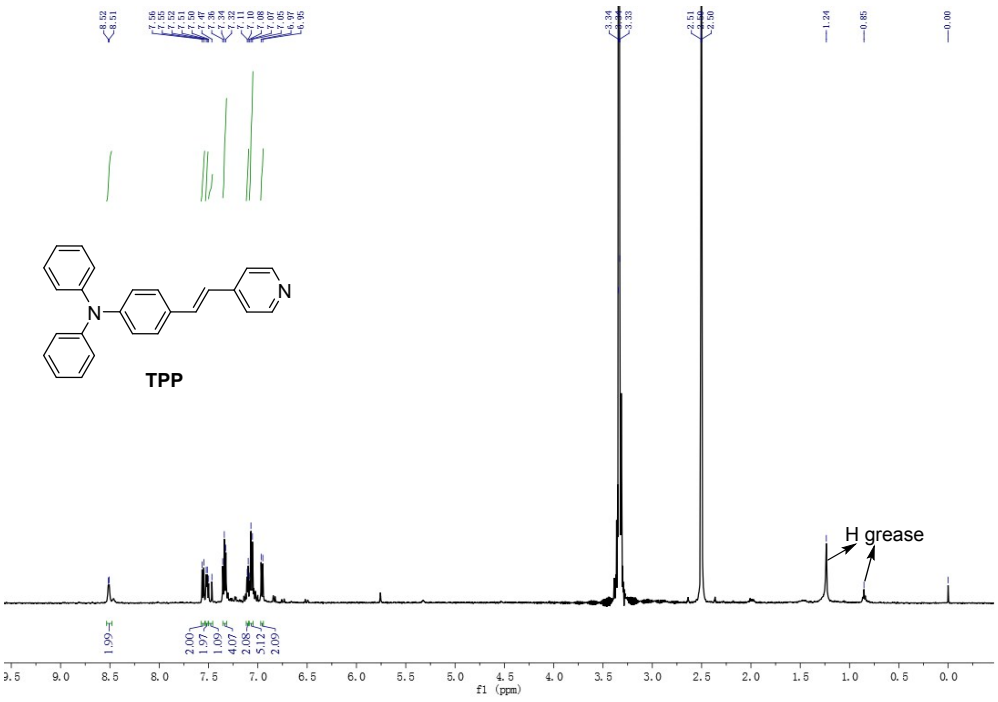



**Figure S20** $^{1}$H NMR spectrum of TPP in DMSO-$d_6$.

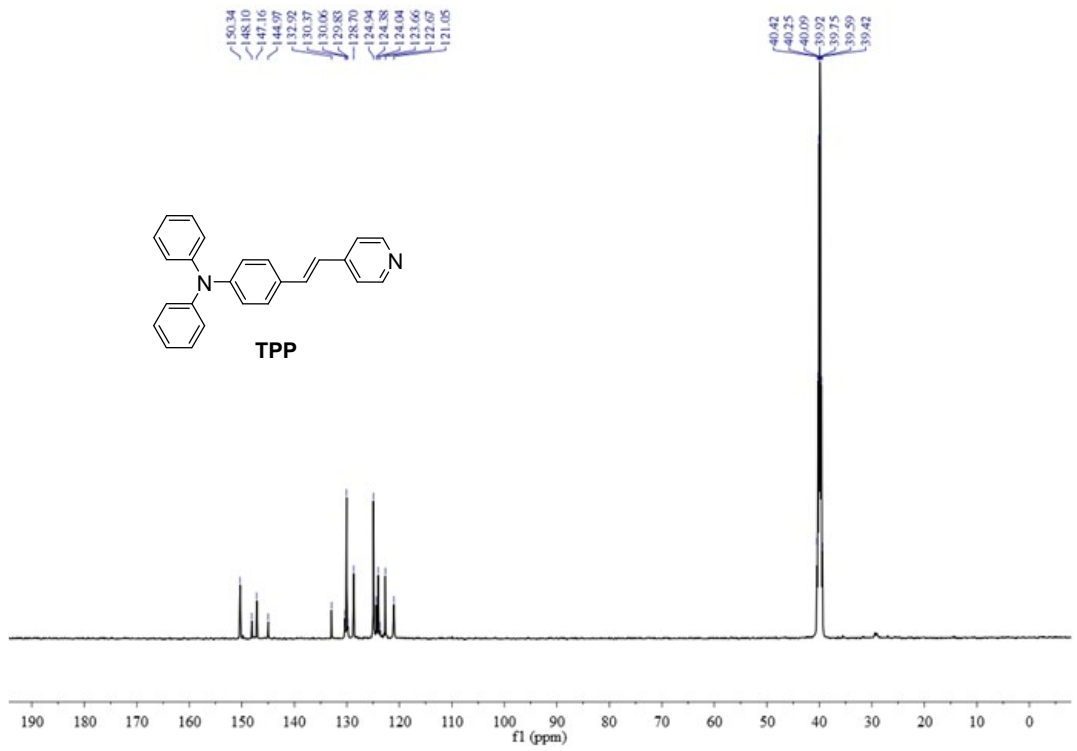



**Figure S21** $^{13}$C NMR spectrum of TPP in DMSO-$d_6$.

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