Supporting information *for*

Spectroscopic Analysis and in Vitro Imaging Application of a pH Responsive AIE Sensor with the Two-input Inhibit Function

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Experimental

Materials and characterizations.

Cetyltrimethylammonium bromide (CTAB, A.R.), 4-methylbenzaldehyde (99%), and methyl picolinate (97%) were purchased from J&K Scientific. CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS) was purchased from Promega (America). All the other reagents were purchased from Guangzhou Chemical Reagent Factory and used without further purification. ^{1}H-NMR spectra were recorded at 293 K using a Bruker Avance spectrometer (500 MHz) with TMS as an internal standard, all chemical shifts are reported in the standard notation of parts per million. Fluorescence emission, excitation spectra was measured using an Edinburgh FLS920 spectrometer (UK). Ultra-violet absorption spectra were acquired by UV-2550 provided by Shimadzu corporation. The morphologies and microstructures of as-prepared samples were examined with Olympus BX50 inverted fluorescence microscope system (Japan), equipped with a 50 W mercury lamp. X-ray powder diffraction patterns were measured on a Bruker D8 Advance diffractometer at 40 kV and 40 mA with a Cu target tube and a graphite monochromator. Cells were washed three times with PBS and imaged on a Leica confocal laser scanning microscope (TCS SP2 AOBS) equipped with a UV laser (405 nm).

All single-point calculations and geometry optimizations were performed at the B3LYP/ 6-311++G(d,p) level with the Gaussian 09 software. Optimized geometries of TTP have no negative frequencies indicating that they are true minima. According to the reported results,[1,2] the molecular structure is more planar in aggregates than in solution. Thus, we simulate the effect in aggregates by fixing the TTP structure as a plane (TTP-P). The UV absorption

spectrum had been obtained for each structure by a single-point TD-DFT calculation at the same level of theory. The polarizable continuum model (PCM) was also applied to account for solvent effects in geometry and electronic transitions. (The solvent for TTP and TTP-P is DMSO and water, respectively).

1. Q. Y. Wu, Q. Peng, Y. L. Niu, X. Gao, Z. G. Shuai, J. Phys. Chem. A, 2012, 116, 3881

2. Q. Y. Wu, C. M. Deng, Q. Peng, Y. L. Niu, Z. G. Shuai, J. Comput. Chem. 2012, 33, 1862

*Synthesis of compound **TTP**.*

The synthetic scheme for TTP was shown following Scheme S1. Briefly, a mixture of 4-methylbenzaldehyde (1.84 ml, 0.019 mol), methyl picolinate (4.2 ml, 0.037 mol), sodium hydroxide (2.5 ml, 10 mol/L), and anhydrous ethanol (10 ml) was stirred at room temperature for 3 h. Then the red solution was poured into 50 mL water, and the precipitation was filtered and washed with ether to obtain pure compound 1,5-di(pyridin-2-yl)-3-(p-tolyl)pentane-1,5-dione (**DIO**) as white solid. Then, **DIO** (0.5g) was dissolved in 8 ml anhydrous ethanol, and 1 g of sodium hydroxied solid was added into the solution. The mixture was heated to 100 ℃, then ammonia (25％, 10 ml) was added dropwise. After 1 h, the solution was cooled down to room temperature, collected on a filter, and recrystallized with ethanol to obtain the compound 4'-(p-tolyl)-2,2':6',2''-terpyridine (**TTP**). As shown in Scheme S2, ^{1}H NMR ($CDCl_3$, ppm): 2.46 (s, 3H), 7.36 (d, J = 6.4 Hz, 2H), 7.38 (m, 2H), 7.85 (d, J = 10.8 Hz, 2H), 7.91 (m, 2H), 8.70 (d, J = 6.4 Hz, 2H), 8.76 (m, 4H); ESI-MS m/z: 324.2 (M + H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.02, 155.40, 149.74, 149.703, 139.62, 137.85, 134.94, 130.41, 127.12, 124.92, 121.34, 118.03, 21.26 (Scheme S3). HRMS (ESI-ion trap) m/z: $[M + H]^-$ calcd for $C_{22}H_{17}N_3$, 324.1495; found 324.1500 (Scheme S3).

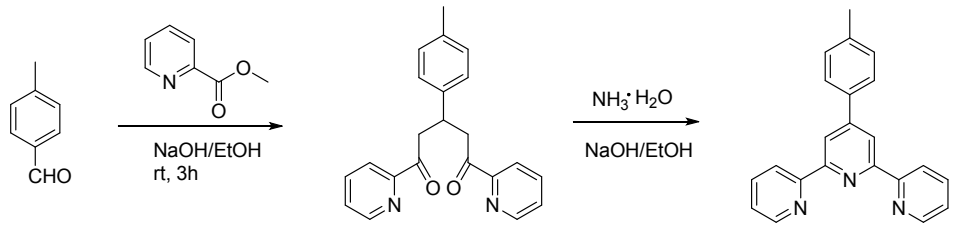
Self-assembly of TTP in the presence of CTAB.

50 μl of different concentration of TTP (0.5, 1, 2, 5 mM) was poured into 0.1 mM of CTAB aqueous solution (5 ml) under stirring at room temperature. After 3 h, the above solution was filtered with microfilm (0.22 μm), then the filtrate was centrifuged at 1,4000 rpm twice with 10K centrifugal filter to remove excessive CTAB.

Cells culture and MTT assay and cells imaging.

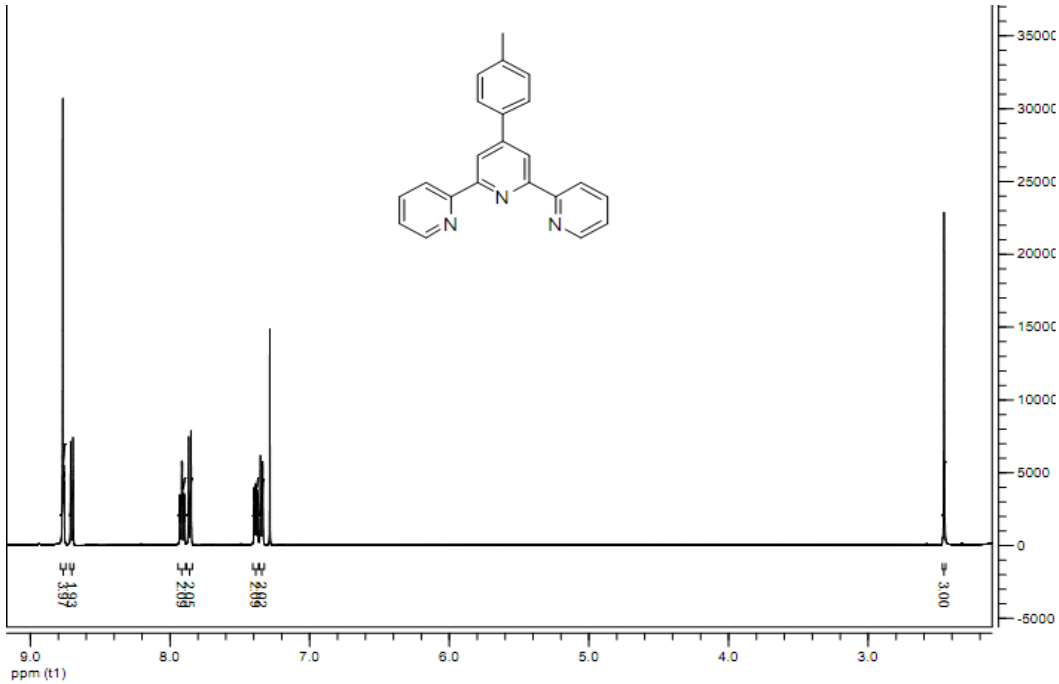
HeLa cells (human embryonic kidney cells) were cultured in Dulbecco's modified medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin. Cells were maintained at 37 °C in 5% CO_2. For the comparison purpose, two cell lines such as bone marrow cells from mice and human umbilical vein endothelial cells were also used. As for the bone marrow cells, the bone marrow were collected from a newly killed mice and dispersed in 5 ml media (RPMI 1640 medium containing 10% fetal bovine serum and 1% antibiotics penicillin). 10 ml of red blood cell lysis buffer was added and the incubation time was 20 mins at room temperature. After centrifugation for 5 mins, we decanted supernatant and resuspended pellet in 5 ml media. Purified normal bone marrow cells were obtained by centrifugation again. For the cell imaging, the normal bone marrow cells were suspended in 1 ml fresh medium containing TTP-CTAB (50 μM) for incubating 10 min and 60 min. After washing three times with PBS, the cells were re-suspended in PBS and observed using a Leica confocal laser scanning microscope. Human umbilical vein endothelial cells (abbreviated as HUVEC) were cultured in endothelial growth medium (EGM, Lonza, USA). For the cell imaging, HUVEC were washed with medium, then incubated in medium containing TTP-CTAB (50 μM) for 10 min and 60 min. After washing three times with PBS, the cells were observed using a Leica confocal laser scanning microscope.

To investigate cytotoxicity, cells were cultured in wells of 96-well plates for one day. Medium was then replaced with PBS buffer solution containing **TTP-CTAB** (5, 10, 20, 50 μM), and cells were cultured for another 24 h. An aliquot of 20 μL MTT solution was added to each well. After incubated with 4 h, the absorbance at 490 nm was measured using a Polarstar microplate reader. For the cell imaging, Hela cells were washed with medium, then incubated in medium containing **TTP-CTAB** (50 μM) for 10 min and 60 min. After washing three times with PBS, the cells were observed using a Leica confocal laser scanning microscope.

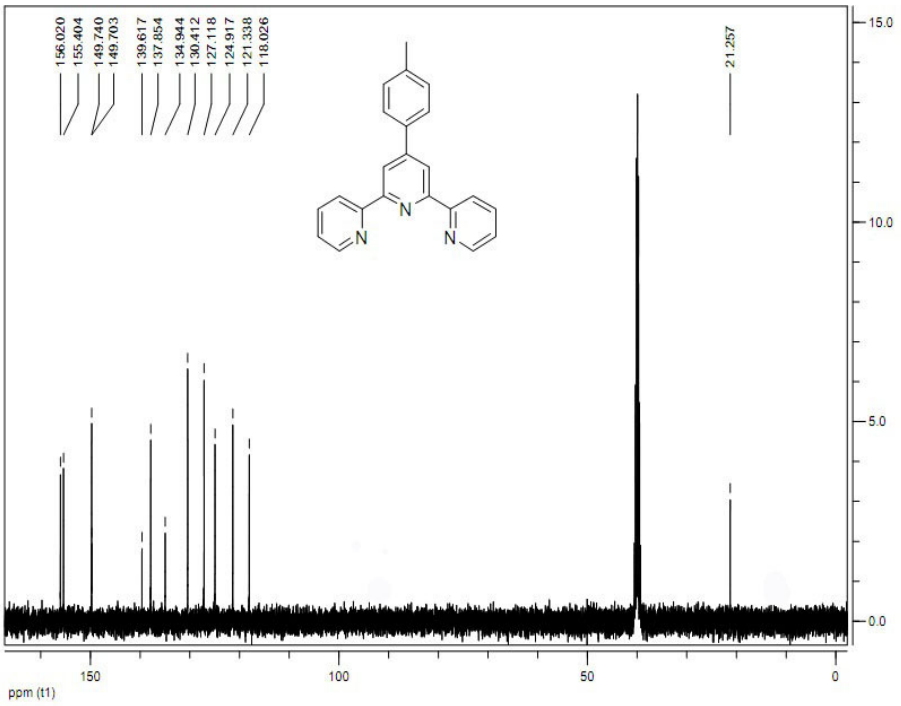


DIO **TTP**

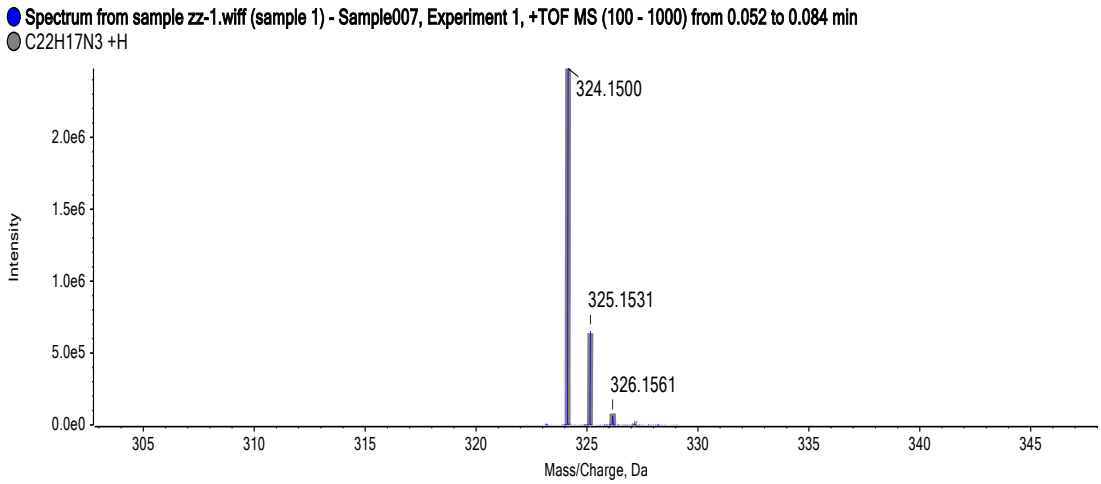
Scheme S1 Synthesis steps for **TTP**.



Scheme S2 ^{1}H NMR spectrum of **TTP**.



Scheme S3 ^{13}C NMR spectrum of **TTP** in DMSO (d_6).



Scheme S4 HRMS (ESI) spectrum of **TTP**.

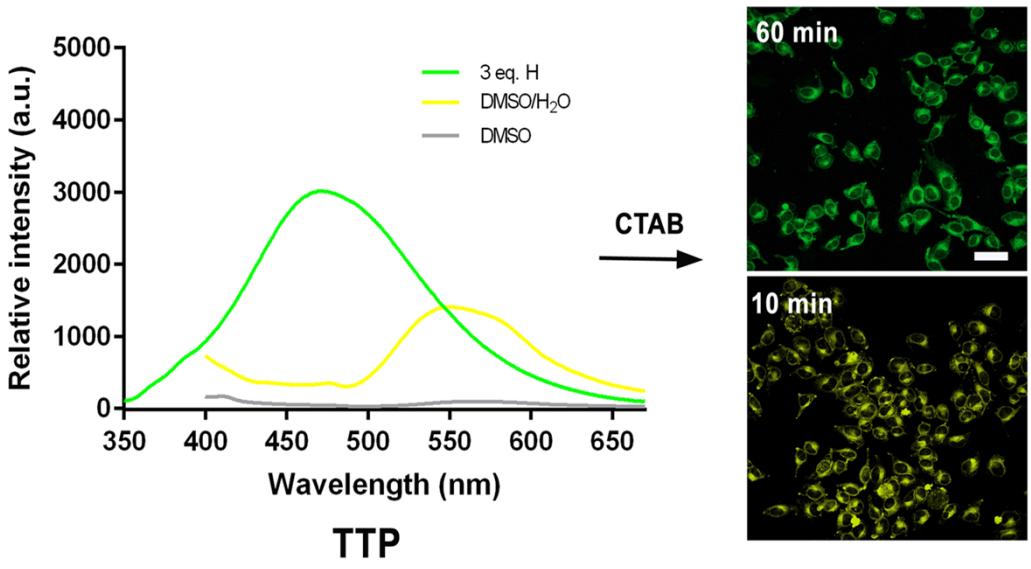


Fig.S1 Fluorescence spectra of **TTP** (50 μM) in DMSO and DMSO/water (1/99) mixture with or without 3 eq. H^+. Photo: HeLa cells staining with the aggregation of **TTP**.

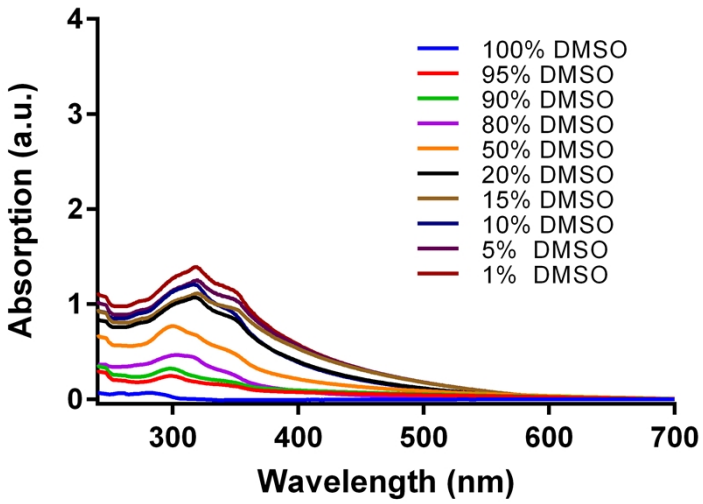


Fig. S2 UV absorption of **TTP** (50 μM) in DMSO/water mixtures with different fractions of water.

TTP (HOMO) TTP (LUMO)

TTP-P(HOMO) TTP-P(LUMO)

Fig. S3 Molecular orbitals of TTP and its planar structure (TTP-P). HOMO: the highest occupied molecular orbital; LUMO: the lowest unoccupied molecular orbital.

Table S1 Calculated Vertical Transition Energies for the Model Complexes from TD-DFT/PCM Calculations

complex	Experimental λ/nm	Excitation energy/eV	λ/nm	f	Composition of the excited-state wave functions
TTP	283	4.304	288	0.4736	$-0.1442\Psi_{H-1->L+0}+0.6719\Psi_{H-0->L+0}$
TTP-P	319	4.065	305	0.4963	$0.6973\Psi_{H+0->L-0}$

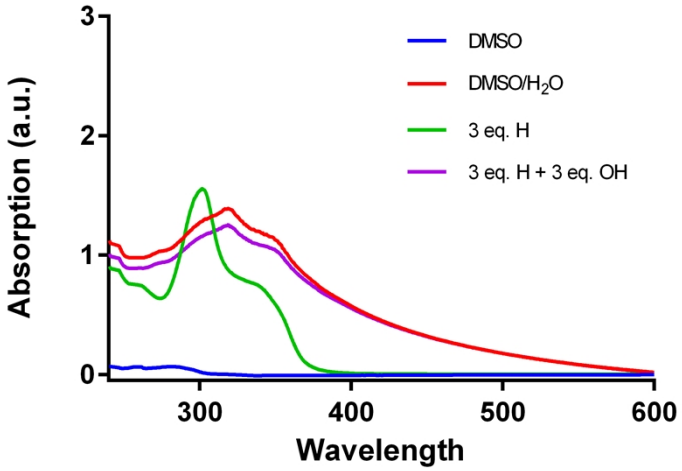


Fig. S4 UV absorption of TTP (50 μM) in DMSO (blue) and DMSO/water (1/99) (red); TTP (50 μM) in DMSO/water (1/99) in the presence of 3 eq. H^+ (green) or in the presence of 3 eq. H^+ and 3eq. OH^- (pink).

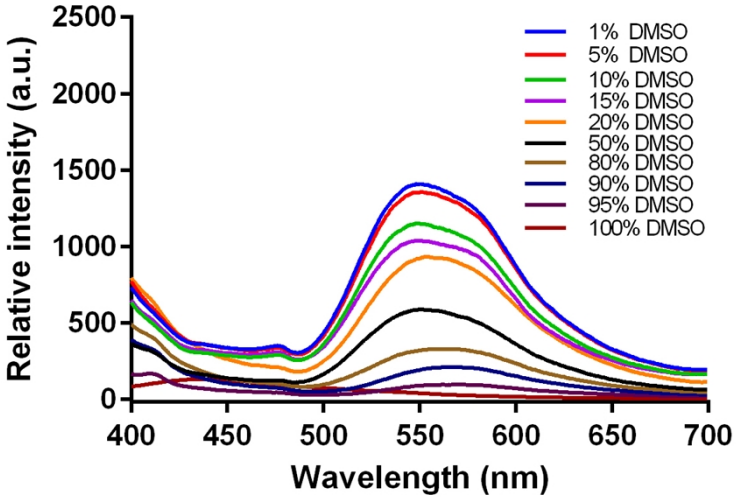


Fig. S5 Fluorescence spectra of **TTP** (50 μM) in DMSO/water mixture with different fractions of water.

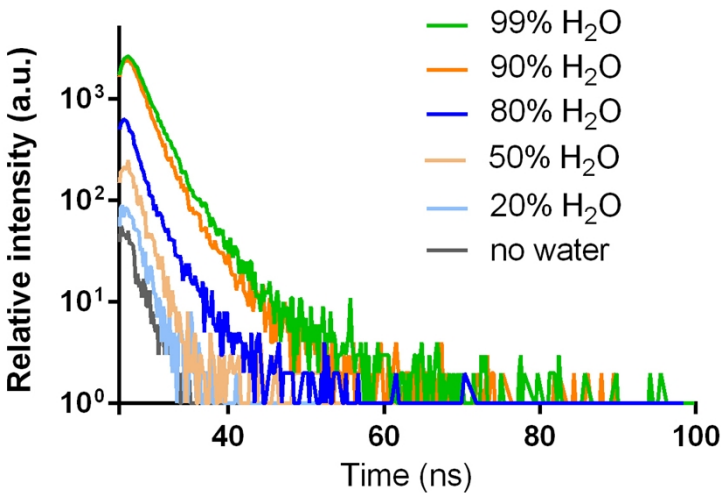


Fig. S6 Time-resolved fluorescence of **TTP** in solution with various volume fractions of water and DMSO.

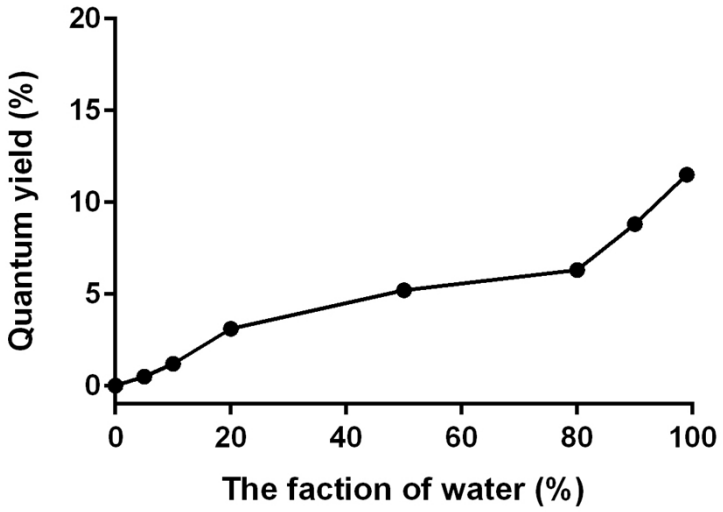


Fig. S7 Changes of the quantum yields (Φ) of **TTP** in DMSO/water mixture with various fractions of water (0, 5, 10, 20, 50, 80, 90, and 99%). The Φ values were estimated by using quinine sulfate in 0.1 N H_2SO_4 (Φ = 54.6%) as a standard.

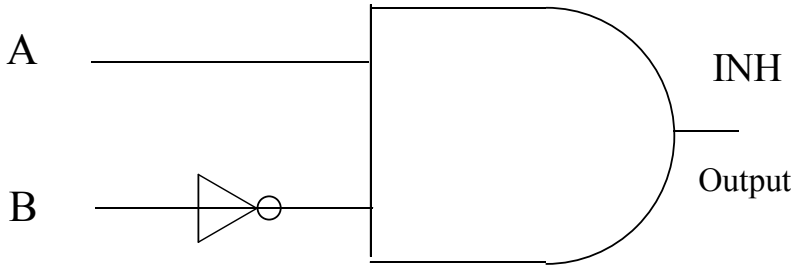


Fig. S8 Representation of logic circuit for the inhibit gate (A and B mean the three equivalents of H^+ and OH^- respectively).

Table S2 A truth table for the INH logic gate (Green emission was achieved when H^+ = 1 and OH^- = 0). The absence of input or output value less than a predefined threshold level are

represented as binary “0”; the presence of any input or output value higher than the threshold level are translated into binary “1”. X= 1 represented the emergence of emission at 475 nm) when A = 1 and B = 0

Input ($\lambda_{emission}$)		X = Output
H^+ (A)	OH^- (B)	INH
0	0	0
1	0	1
0	1	0
1	1	0

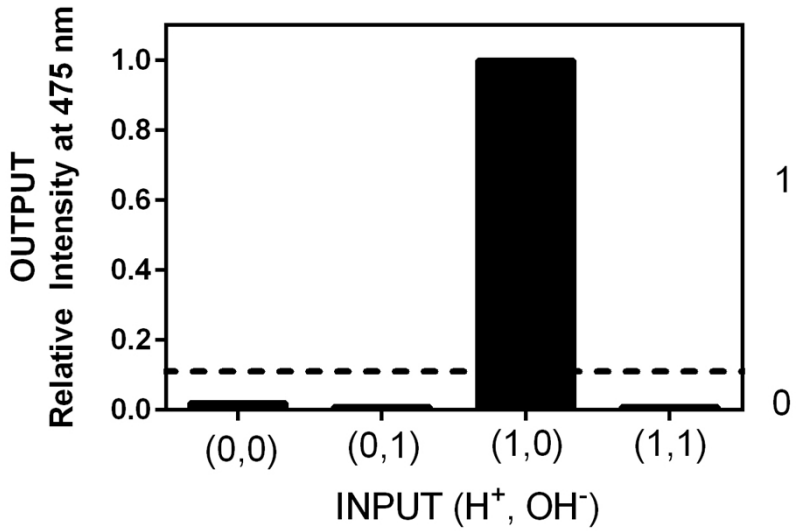


Fig. S9 Performance of aggregated TTP as an INHIBIT gate. Fluorescence responses of TTP in DMSO/H_2O (1/99) with various input combinations. The experimental conditions for the inputs were described in the text and Table S2. The dashed line means the threshold level for readout purpose.

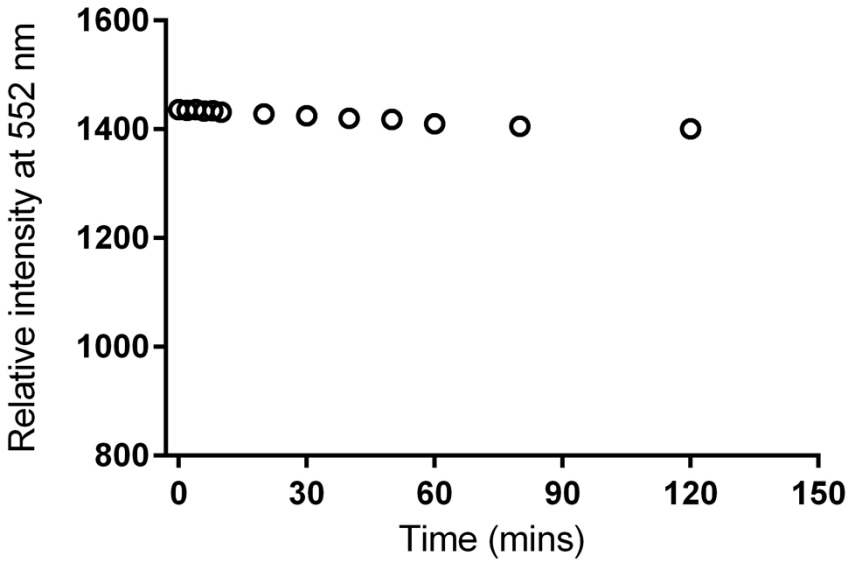


Fig. S10 Emission peak intensity of **TTP** (50 μM) in aqueous solution containing 1 % DMSO at 552 nm under UV light-irradiation.

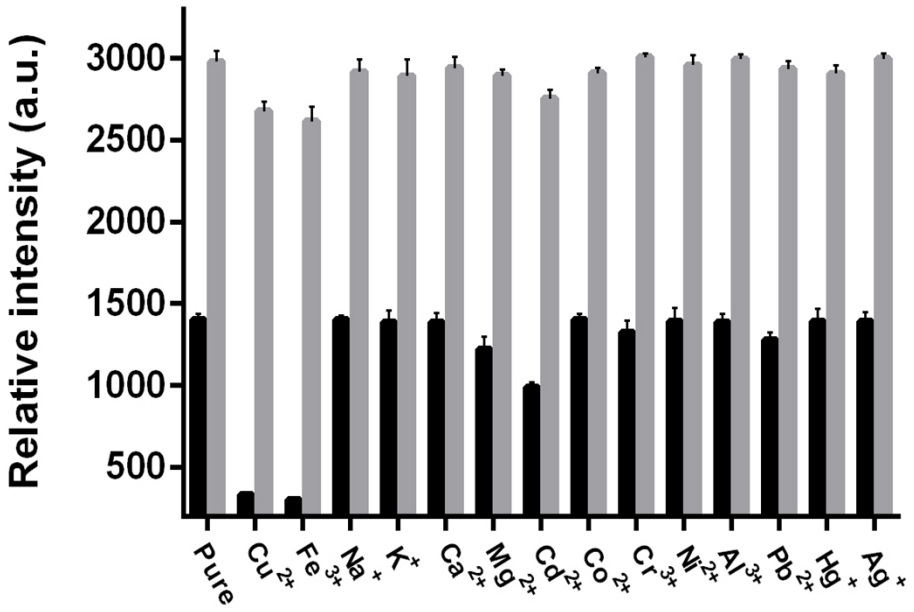
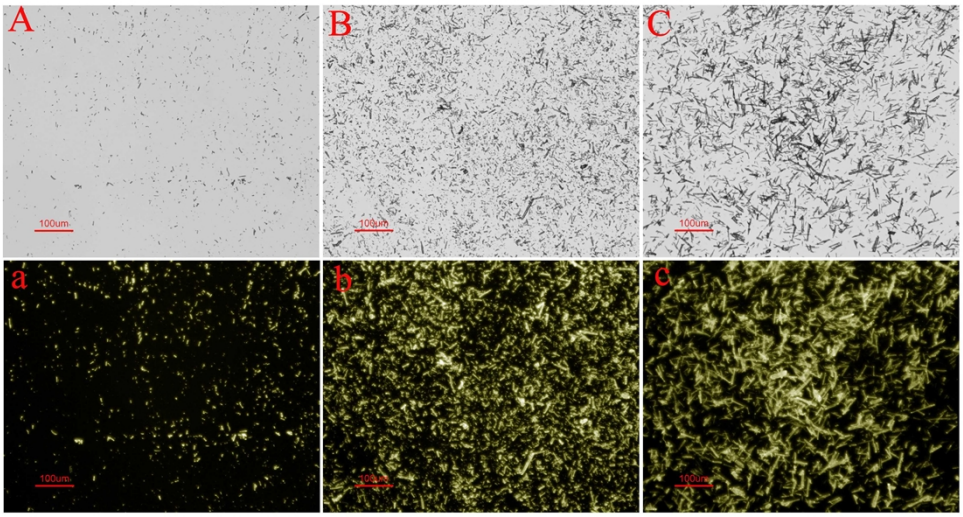


Fig. S11 Fluorescence response of TTP (50 μM) towards various interference metal ions (100

μM) with (gray, λem = 472 nm) and without (black, λem = 552 nm) H^+. Each bar represents the means of three independent measurements with standard deviation.



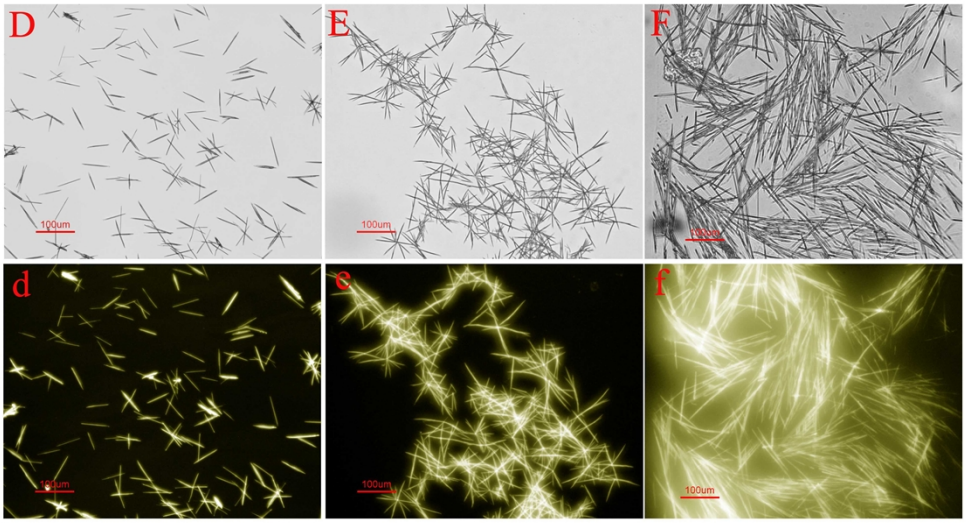


Fig. S12 Fluorescence microscope images of **TTP** (50 μM) in DMSO/water mixtures (a 50%, b 20%, c 15%, d 10%, e 5%, f 1%). A, B, C, D, E, and F represent the corresponding bright field images.

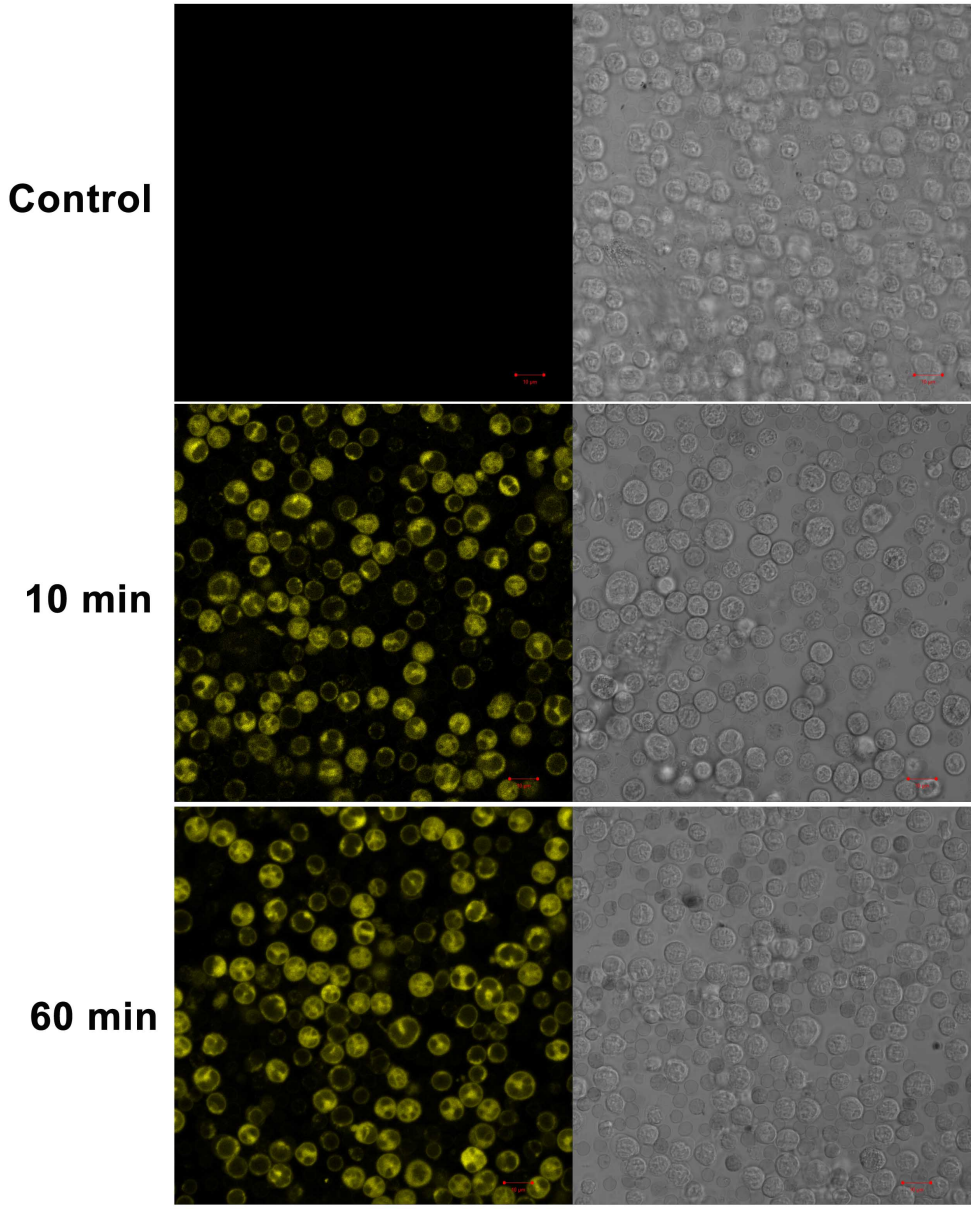


Fig. S13 Confocal images of normal mice bone marrow cells incubated without (control) and with TTP-CTAB (50 μM) for 10 min and 60 min (left: fluorescence field, right: bright field). The scale bar is 10 μm.

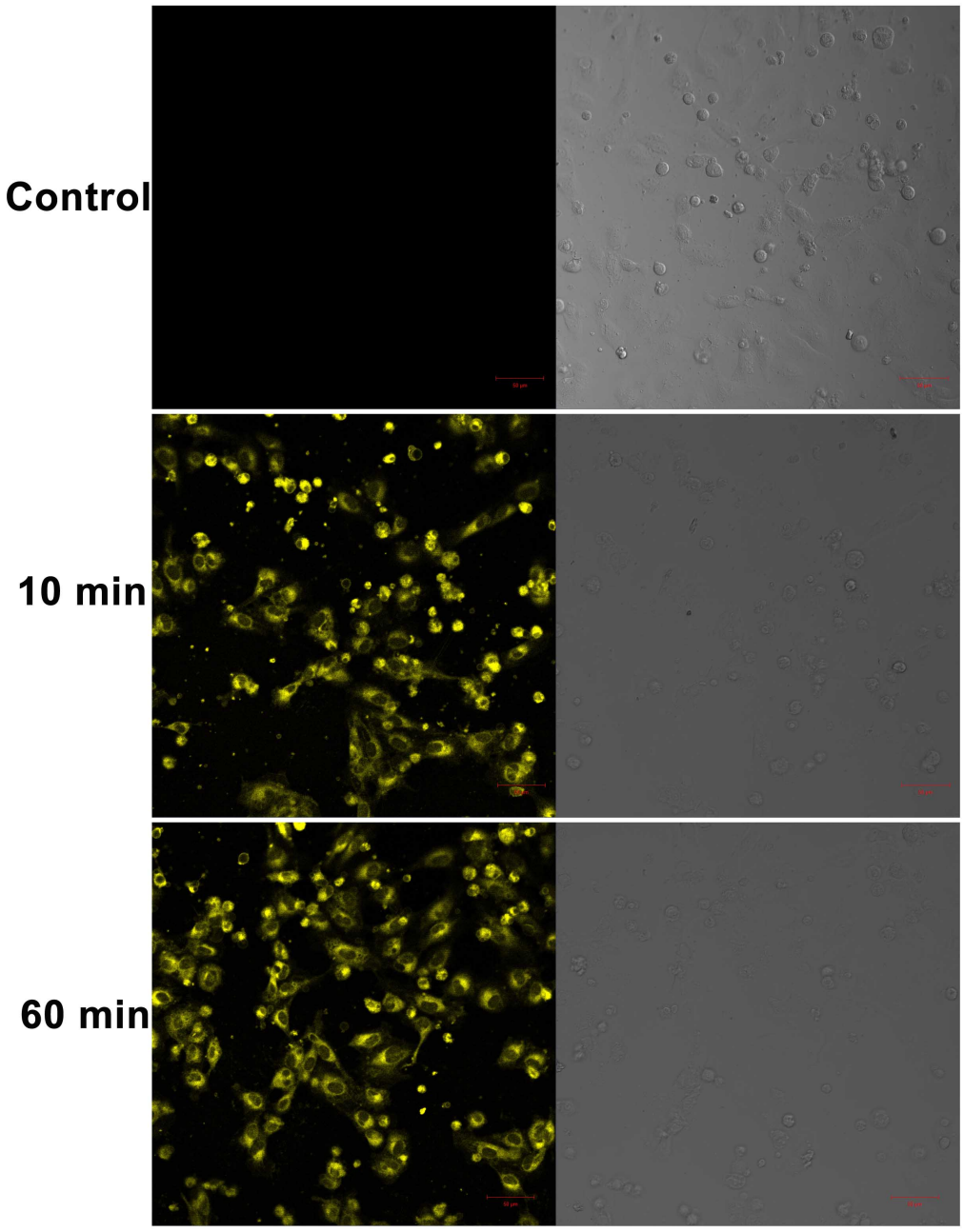


Fig. S14 Confocal images of human umbilical vein endothelial cells (HUVEC) incubated without (control) and with TTP-CTAB (50 μM) for 10 min and 60 min (left: fluorescence field, right: bright field). The scale bar is 50 μm.

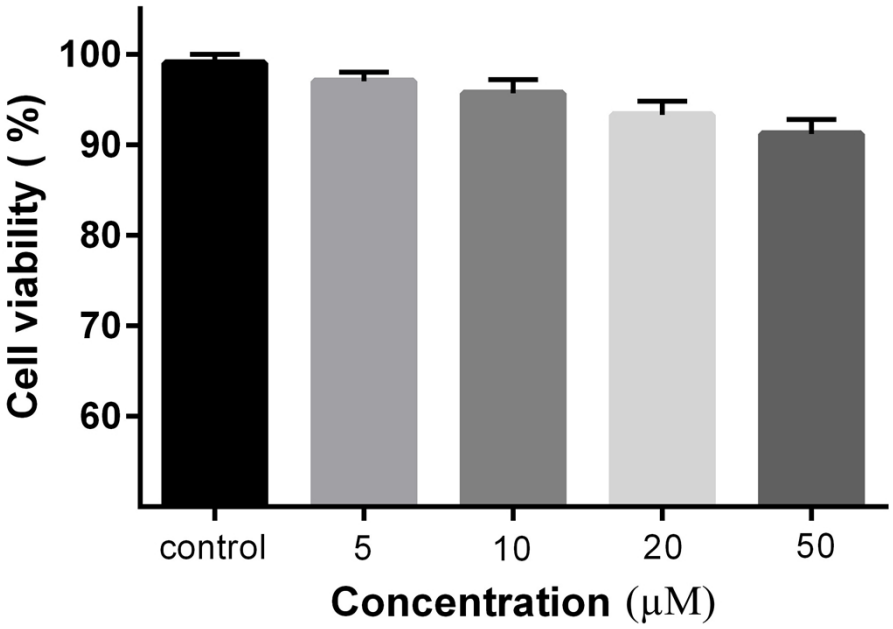


Fig. S15 Hela cells viability at different concentrations of **TTP-CTAB** for 24 h at 37 ^{o}C.