Supporting Information

Tetraphenylethene-pyridine salts as the first self-assembling chemosensor for pyrophosphate

Hao-Ran Xu, Kun Li,* Shu-Yan Jiao, Sheng-Lin Pan, Jun-Ru Zeng and Xiao-Qi Yu*

College of Chemistry, Key Laboratory of Green Chemistry and Technology (Ministry of Education) Sichuan University No.29 Wangjiang Road, Chengdu, P. R. of China 610064 Fax: (+86 28 85415886)
E-mail: kli@scu.edu.cn; xqyu@scu.edu.cn

Content:

1. Experimental Section S2
2. Figure S1. Photographs of powdered solid of sensors S4
3. Figure S2. Bright-field and fluorescent images of **TPM** crystal S4
4. Figure S3. PH effect of **TPM** S5
5. Figure S4. PH effect of **TPH** S5
6. Figure S5. Fluorescence titration of **TPM** with PPi S6
7. Figure S6. UV-vis titration of **TPM** with PPi S6
8. Figure S7. Fluorescence kinetics of **TPM** S7
9. Figure S8. Fluorescence titration of **TPH** with PPi S7
10. Figure S9. UV-vis titration of **TPM** with PPi S8
11. Figure S10. Fluorescence kinetics of **TPH** S8
12. Figure S11. DLS profile of **TPM**, **TPH** and **APM** S9
13. Figure S12. SEM image of **TPH** with PPi S9
14. Figure S13. Fluorescence confocal laser scanning images of **TPH** S10
15. Figure S14. Fluorescence titration of **APM** with PPi S10
16. Figure S15. Fluorescence responses of **TPH** with various anions S11
17. Figure S16. Fluorescence responses of **TPH** to various anions together with PPi S11
18. Figure S17. Fluorescence responses of **TPM** with various metal ions S12
19. Figure S18. Fluorescence responses of **TPH** with various metal ions S12
20. Figure S19. Fluorescence titration of **TPH** with Hg^{2+} S13
21. Figure S20. Fluorescence titration of **TPM** with Hg^{2+} S13
22. Figure S21. Cytotoxicity of **TPM** on Hela cells. S14
23. ^{1}H-NMR, ^{13}C-NMR and ESI-MS spectra of **TPM**, **TPH** and **APM**. S15

EXPERIMENTAL SECTION

2.1 Details for Fluorescence Measurements and UV−Vis.

Stock solutions of, **TPM** and **TPH** (10 M) was prepared in distilled water as stock solutions for each measurement. Chloride (Hg^{2+}, Ba^{2+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}), nitrate (Ca^{2+}, Ni^{2+}, Cr^{3+}, Cu^{2+}, Al^{3+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Ag^{+}) and perchlorate (K^{+}, Na^{+}, Li^{+}, Mg^{2+}) (50mM) were prepared in distilled water. Stock solutions of anions as sodium salts ($P_2O_7^{4-}$, $H_2PO_4^{-}$, HPO_4^{2-}, PO_4^{3-}, Cl^{-}, Br^{-}, F^{-}, I^{-}, NO_3^{-}, Ci^{3-}, AcO^{-}, N_3^{-}, CN^{-}, SO_4^{2-}, SO_3^{2}-, S^{2-}, HCO_3^{-}, CO_3^{2-}, HSO_3^{-}, HSO_4^{-}, ClO_2^{-}) (10mM) were prepared in distilled water. The fluorescence studies performed in pure water. Each time a 3 mL of receptor solution (10 μM) was filled in a quartz cell of 1 cm of optical path length and the stock solution of metal ion or anion was dropped into a quartz cell using a microsyringe. The excitation and emission slits of fluorescence spectra were set at 5.0 nm if not specified. For absorption studies, the final concentrations of receptors were kept constant at 10μM, and the procedure used for the titrations was the same as that used for fluorescence titrations.

2.2 Determination of the fluorescence quantum yield

The fluorescence quantum yield (Q) is defined as the ratio of the number of photons emitted and the number of photons of the excitation light absorbed while the fluorescent substance absorbed photons. People usually use ratio method for the determination of the fluorescence quantum yield.

$$Q_x = Q_r \left(\frac{A_r(\lambda_r)}{A_x(\lambda_x)} \right) \left(\frac{I(\lambda_r)}{I(\lambda_x)} \right) \left(\frac{n_x^{\,2}}{n_r^{\,2}} \right) \left(\frac{D_x}{D_r} \right)$$

In this equations ***I (a)*** is the relative intensity of the exciting light at wavelength A, ***n*** is the average refractive index of the solution to the luminescence, ***D*** is the integrated area under the corrected emission spectrum, and ***A(a)*** is the absorbance of the solution at the exciting wavelength ***a***. Subscripts x and r refer to the sample and reference solutions, respectively[1].

In this paper, we use the same wavelength to excite the sample and the reference solution. They have same solvent (H_2O), the middle two formulas of the equations are 1. That means we only need to determine the absorbance and the emission spectra of peak area integration. We use fluorescein as reference, Qr = 0.85. The result of the calculation is the fluorescence quantum yield increase from 0.136% to 3.283% after **TPM** complex with the PPi and from 0.127% to 4.143% after **TPH** complex with the PPi.

2.3 Calculations for detection limit

The detection limit was calculated on the basis of the fluorescence titration using the following equation[2]:

$$DL = \frac{3\sigma}{k}$$

Here, σ is the standard deviation of blank measurement, k is the slop between the ratio of emission intensity versus [PPi]. The fluorescence emission of **TPM** and **TPH** were measured 10 times to obtain the standard deviation of blank measurement.

2.4 Cytotoxicity experiment

Toxicity toward Hela cells were determined by Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) following literature procedures[3]. Briefly, about 9000 cells per well were seeded in 96-well plates and cultured for 24 h. After removing the old medium, Hela cells were incubated with 0.5 μM, 1 μM, 2 μM and 4 μM **TPM** for another 24 h. The mediumwas replaced by 100 mL fresh medium containing 10 mL CCK-8 and the plates were incubated at 37 ℃ for further 2 h. Then, the absorbance of each sample was measured using an ELISA plate reader (BioRad, imark) at a wavelength of 450 nm. The cell ability (%) was obtained according to the manufacturer's instruction.

2.5 Imaging of living cells

Hela cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic–antimycotic at 37 ℃ in a 5% CO_2/95% air incubator. For fluorescence imaging, cells (4×10^3 per well) were passed on confocal dishes and incubated for 24 h. Immediately before the staining experiment, cells were washed twice with PBS (10 mM). The dish incubated with 2 μM **TPM** for 30min at 37 ℃, and then washed with PBS (10 mM) 3 times, and analyzed with a fluorescent inverted microscope using an excitation wavelength of 405 nm.

Reference

[1] G.A. Crosby, J.N. Demas, J. Phys. Chem., 75 (1971) 991-1024.

[2] B.K. Datta, S. Mukherjee, C. Kar, A. Ramesh, G. Das, Anal. Chem., 85 (2013) 8369-8375.

[3] K. Luo, C. Li, L. Li, W. She, G. Wang, Z. Gu, Biomaterials, 33 (2012) 4917-4927.

Figure S1. Photographs of powdered solid of sensors under A) daylight and B) 365nm UV illumination (From left to right: **TPM**, **TPH, TPM-C** and **APM**).

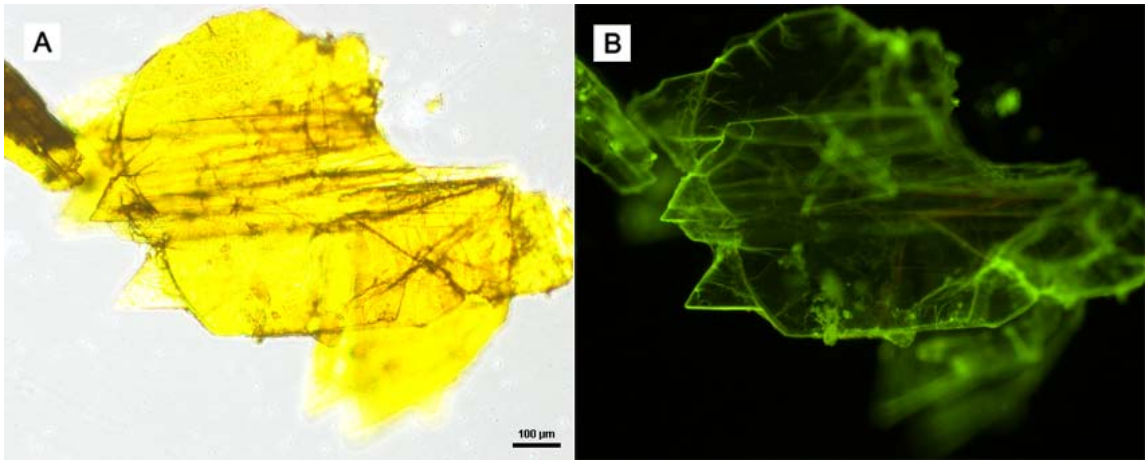


Figure S2. A) Bright-field and B) fluorescent images of **TPM** crystal. Scale bar: 100 µm.

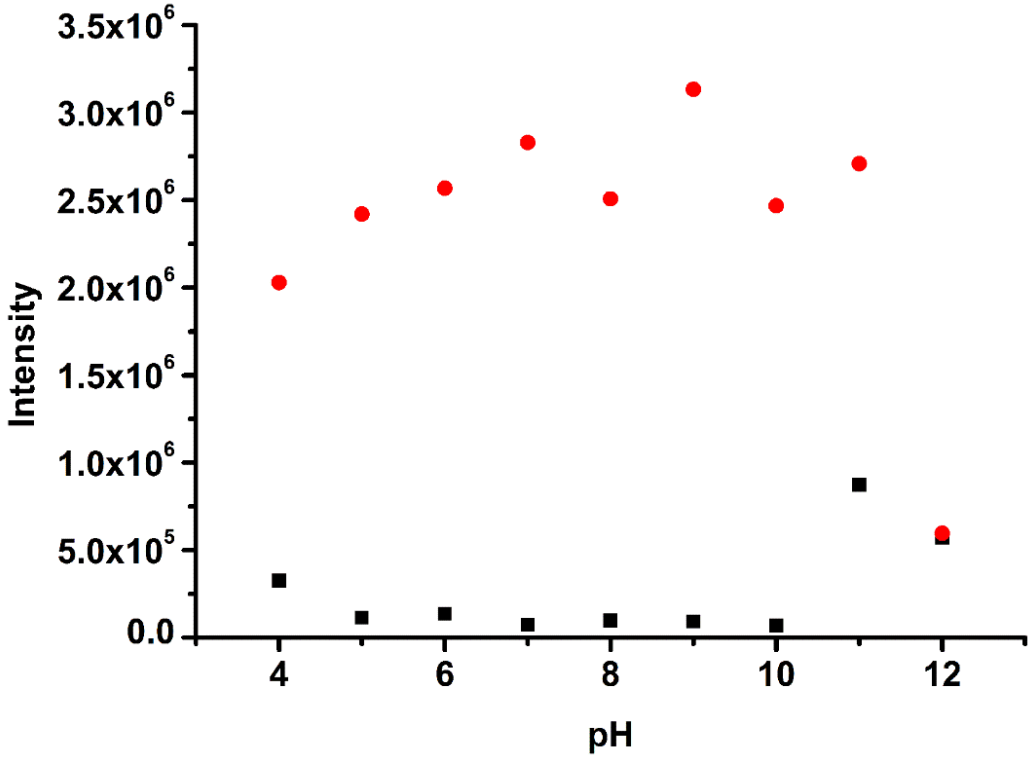


Figure S3. Effect of pH on the fluorescence intensity at 535nm of **TPM** (black, ■) and **TPM** + 2eq. PPi (red, ●) in aqueous solution. The pH of solution was adjusted by NaOH (1M) and HCl (1M).

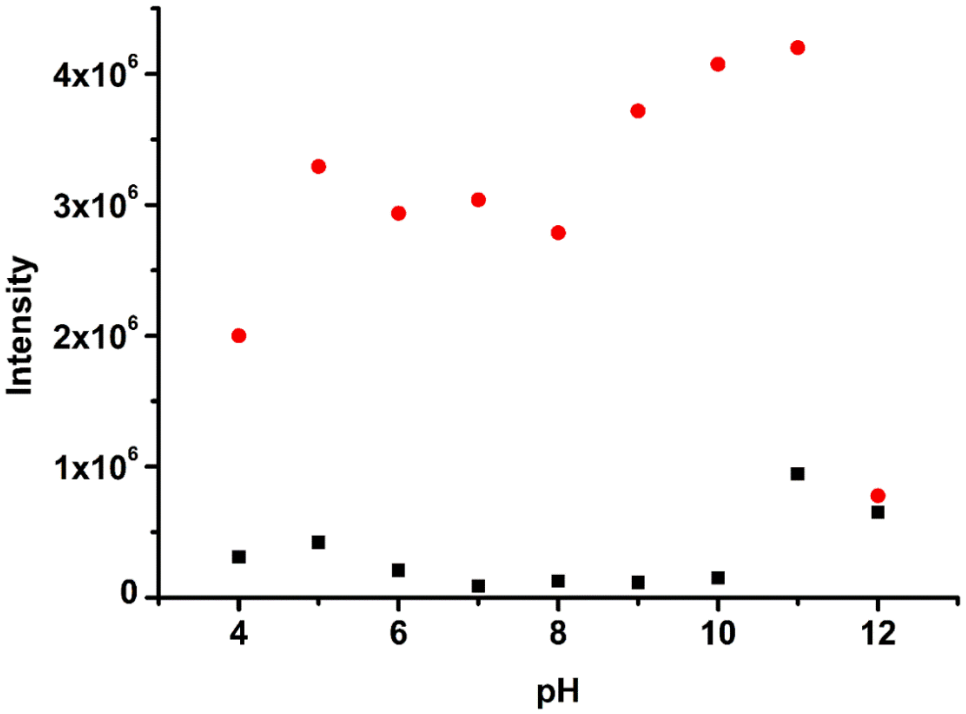


Figure S4. Effect of pH on the fluorescence intensity at 535nm of **TPH** (black, ■) and **TPH** + 2eq. PPi (red, ●) in aqueous solution. The pH of solution was adjusted by NaOH (1M) and HCl (1M).

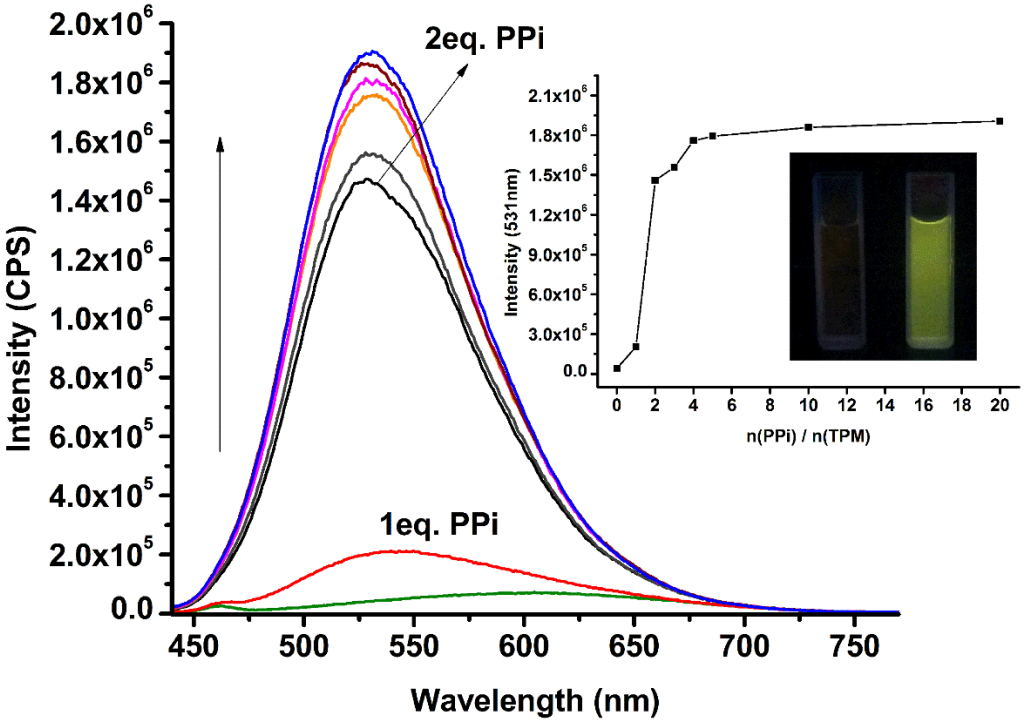


Figure S5. Fluorescence titration of **TPM** (10 μM) with PPi (0 - 20 eq.) in water. Inset shows the fluorescence change at 531nm as a function of the amount of PPi (λex = 400 nm). Photographs of **TPM** (10 μM) in water with PPi of 0 (left) and 50μM (right) taken under 365nm UV illumination.

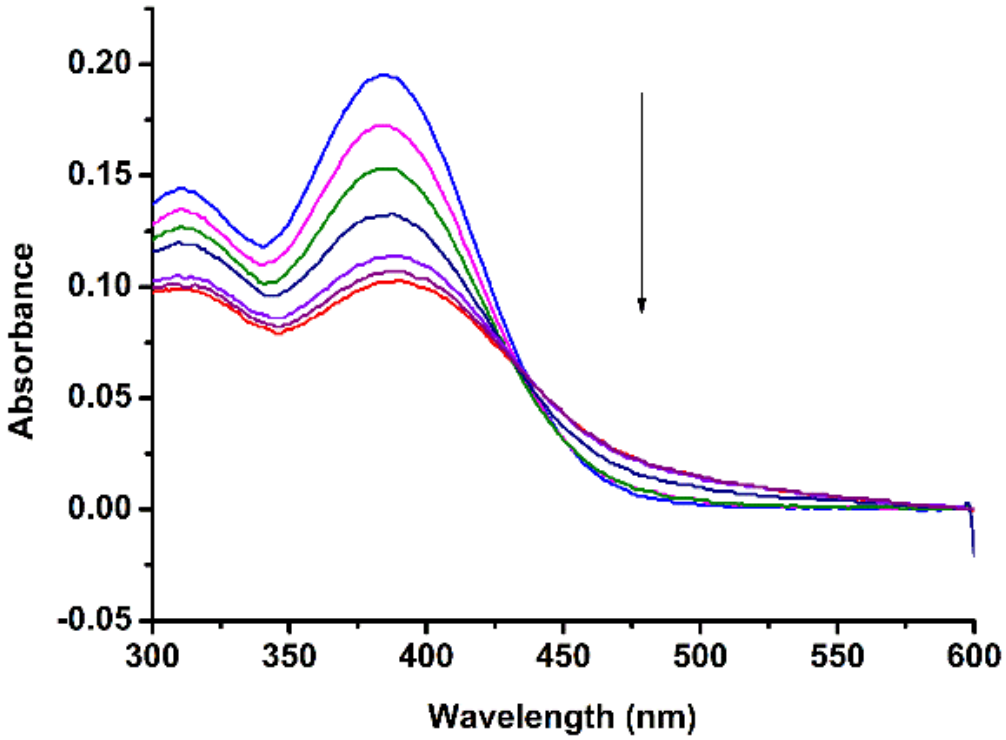


Figure S6. UV-vis titration of **TPM** (10μM) with PPi (0-20 equiv.) in water.

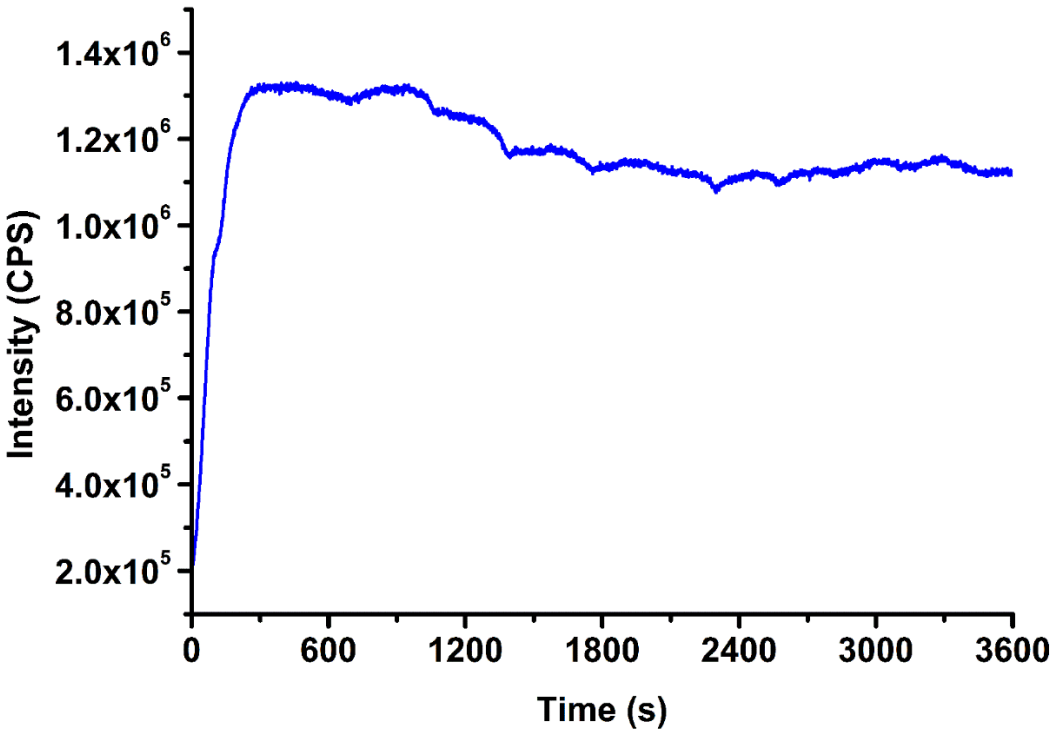


Figure S7. Fluorescence response of **TPM** (10μM) in the presence of PPi (20μM), λex=400 nm, λem=531 nm.

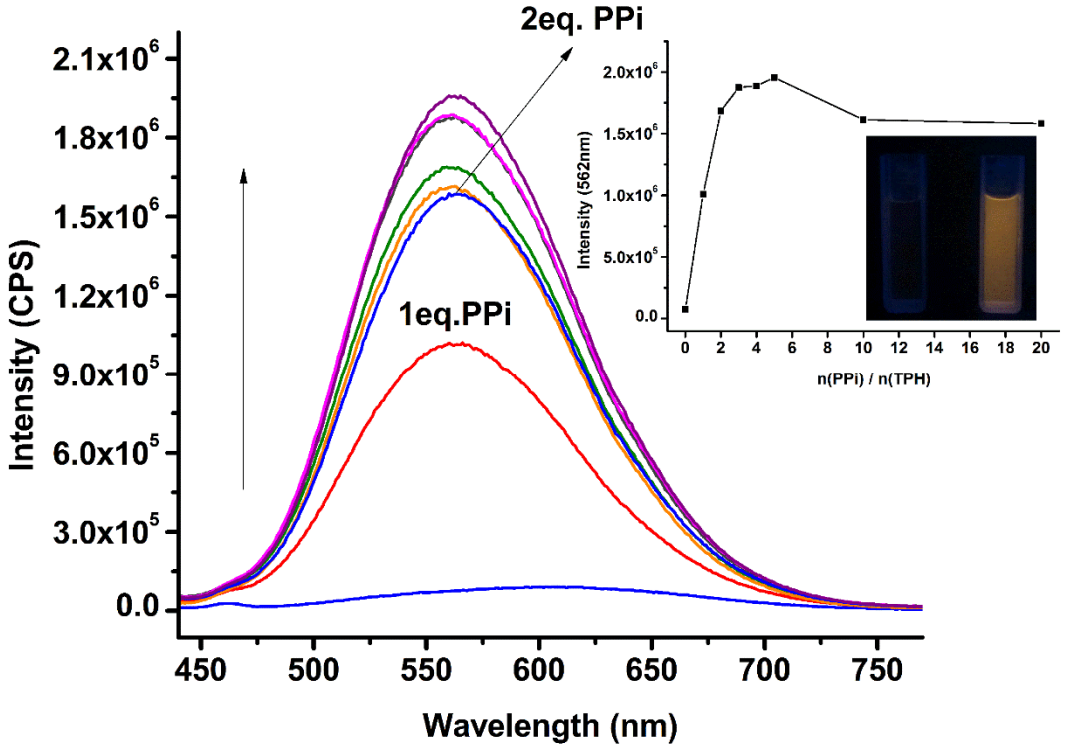


Figure S8. Fluorescence titration of **TPH** (10 μM) with PPi (0 - 20 eq.) in water. Inset shows the fluorescence change at 562nm as a function of the amout of PPi (λex = 400 nm). Photographs of **TPH** (10 μM) in water with PPi of 0 (left) and 50μM (right) taken under 365nm UV illumination.

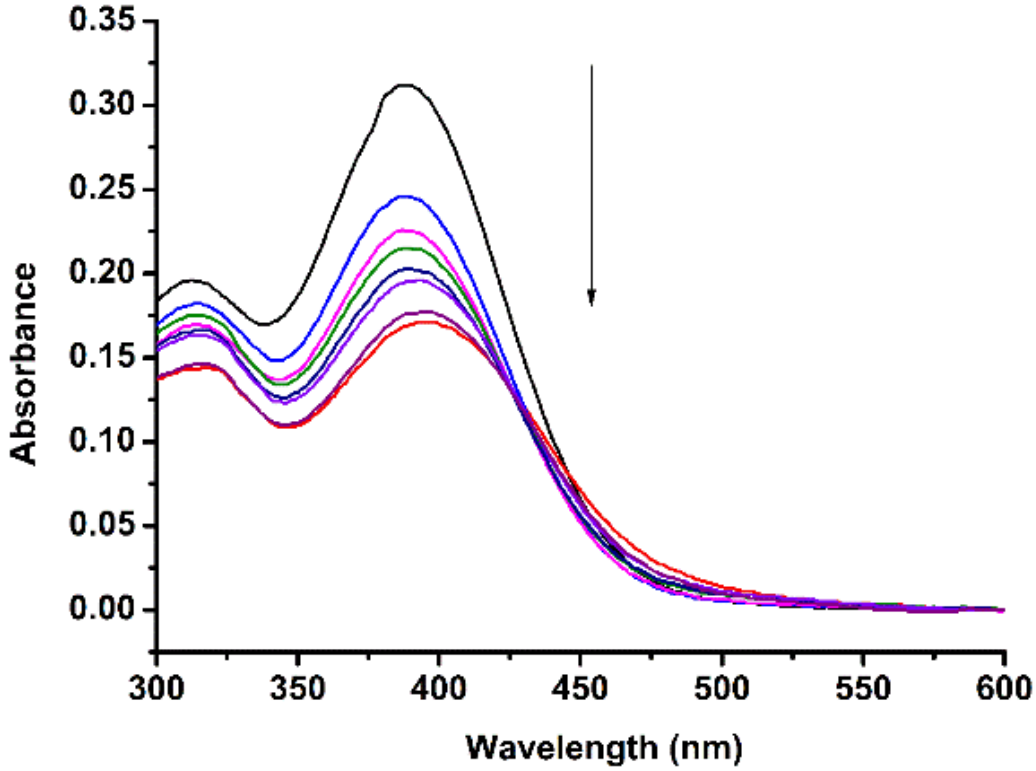


Figure S9. UV-vis titration of **TPM** (10μM) with PPi (0-20 equiv.) in water.

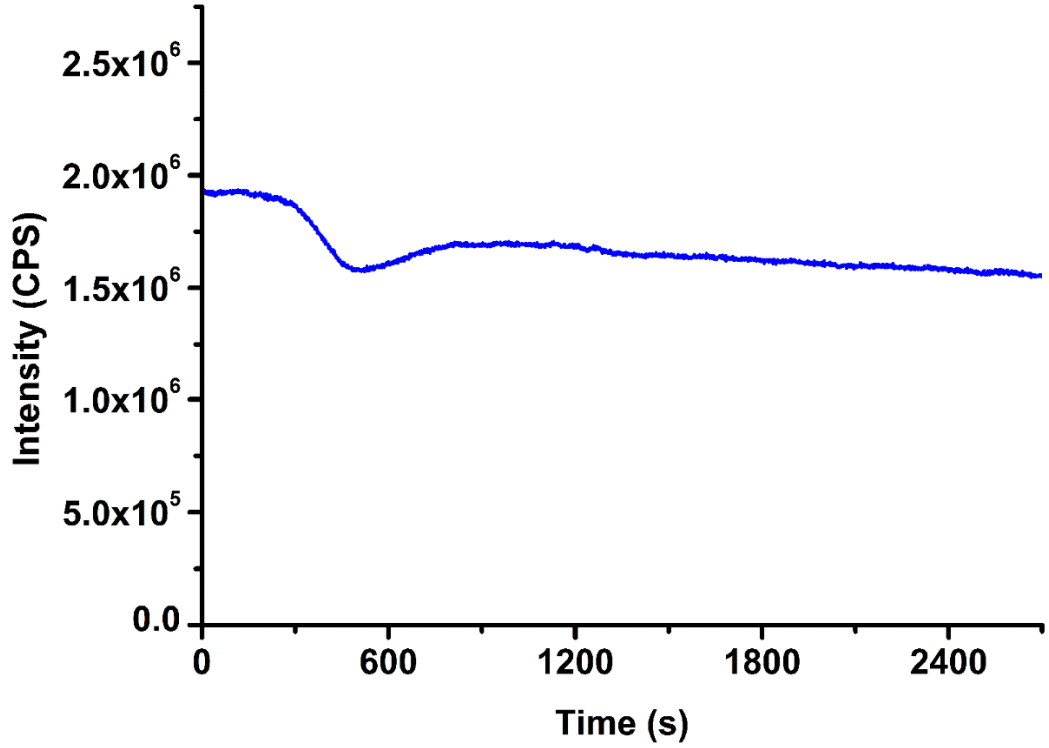


Figure S10. Fluorescence response of **TPH** (10μM) in the presence of PPi (20μM), λex=400 nm, λem=562 nm.

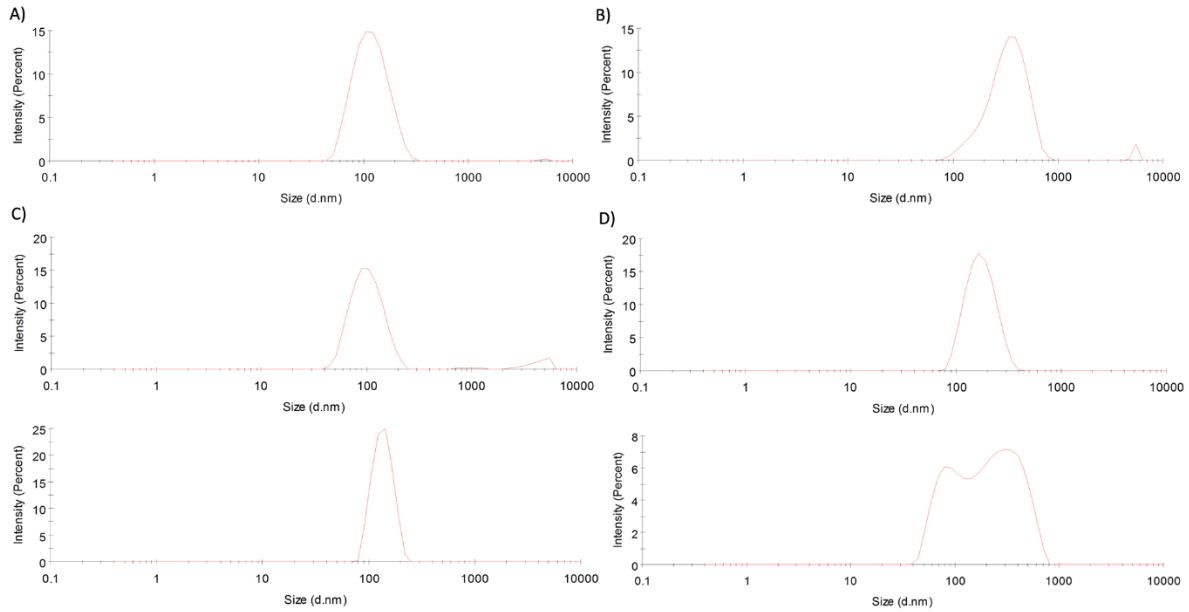


Figure S11. (A)(C) DLS profile of **TPM**, **TPH** and **APM** (10μM) in water; (B) DLS profile of **TPM**, **TPH** and **APM**(10μM) in water after the addition of PPi (5 equiv).

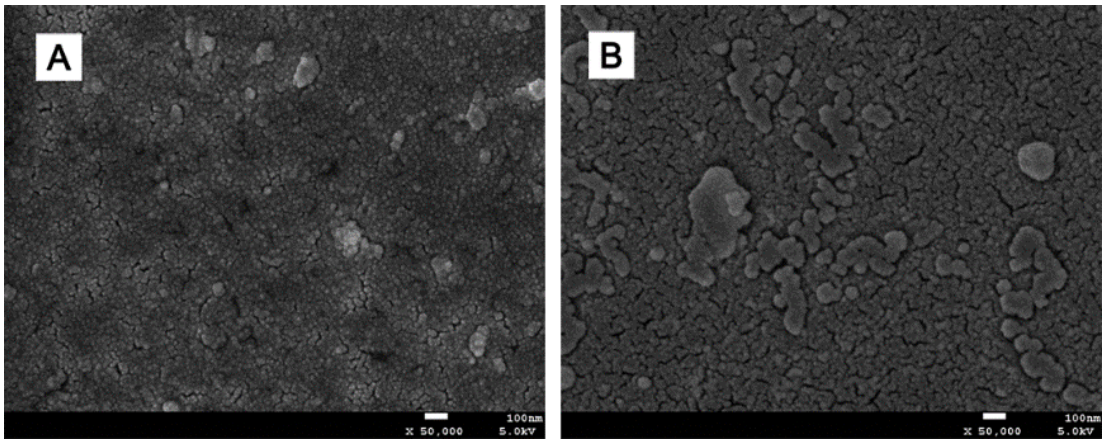


Figure S12. SEM image of (A) **TPH** and (B) **TPH** + 3eq. PPi formed in pure water. c [**TPH**] = 10μM.

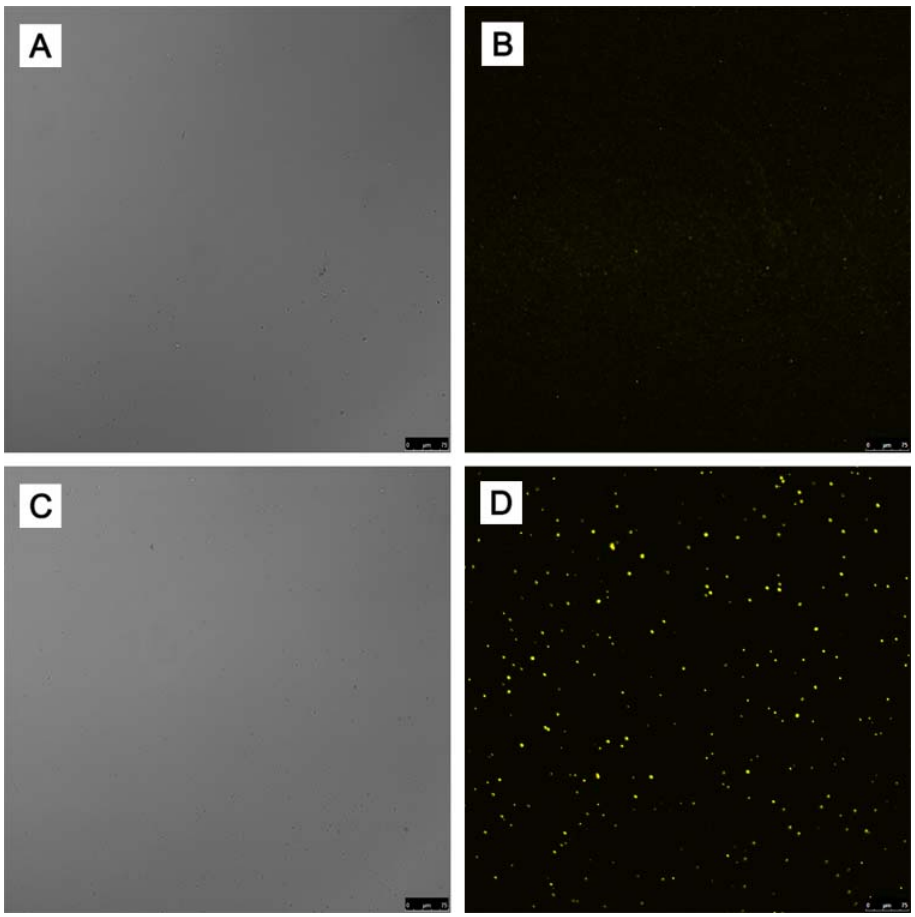


Figure S13. A,C) Bright-field and B,D) fluorescent images of **TPH** (50μM) in water with A,B) 0 and 200μM C,D) of PPi. Scale bar: 75μm.

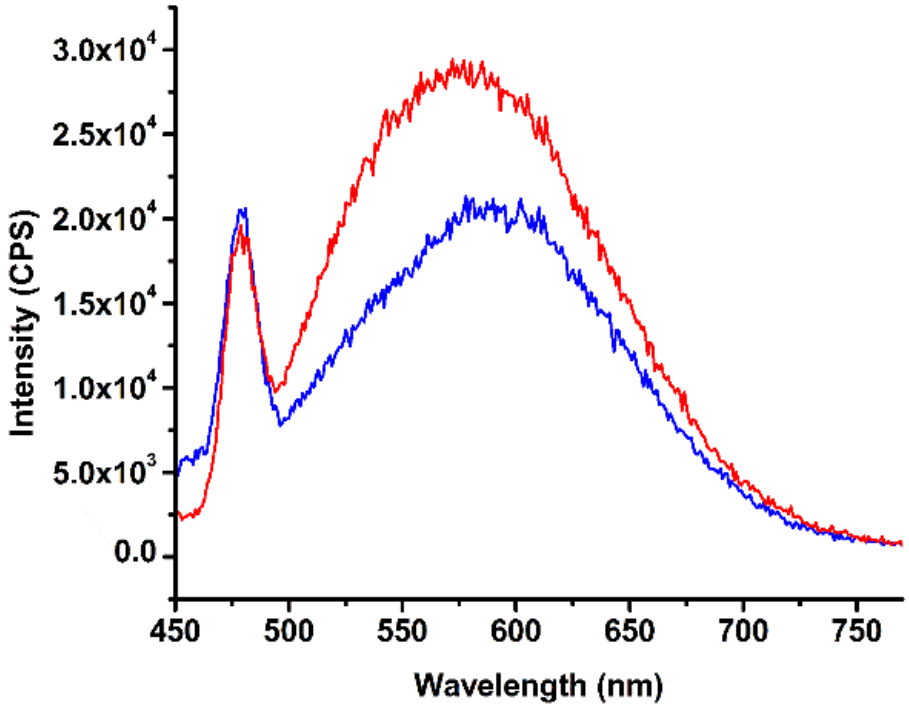


Figure S14. Fluorescence titration of **APM** (10 μM) with PPi (20 eq.) in water.

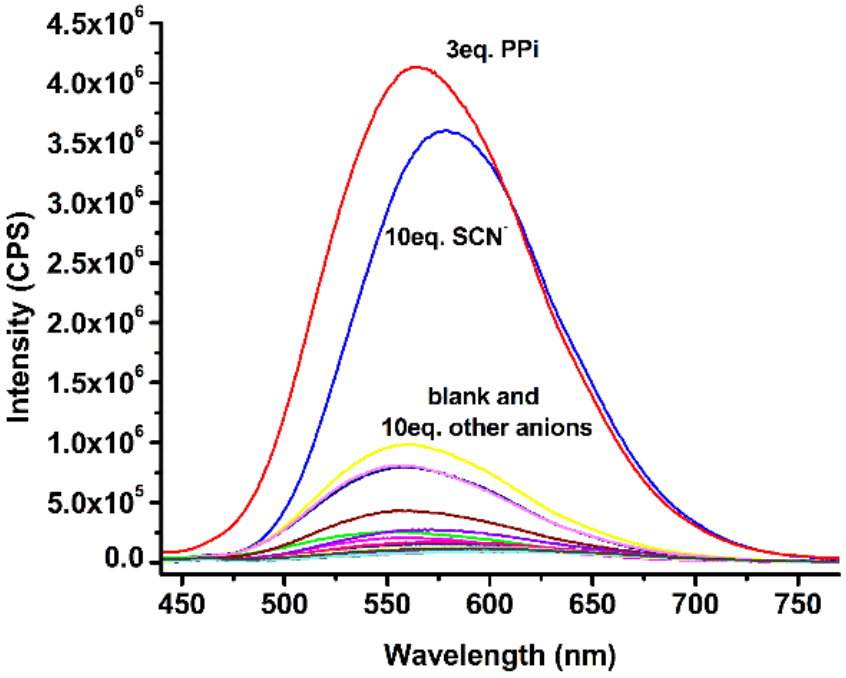


Figure S15. Fluorescence responses of **TPH** (10 μM) in water with PPi (30 μM) and various anions (100 μM) including PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, HCO_3^-, SO_4^{2-}, Ci^{3-}, Br^-, N_3^-, F^-, AcO^-, I^-, Cl^-, SCN^-, NO_3^-, S^{2-}, SO_3^{2-}, CO_3^{2-}, HSO_3^-, HSO_4^-, ClO_2^-.

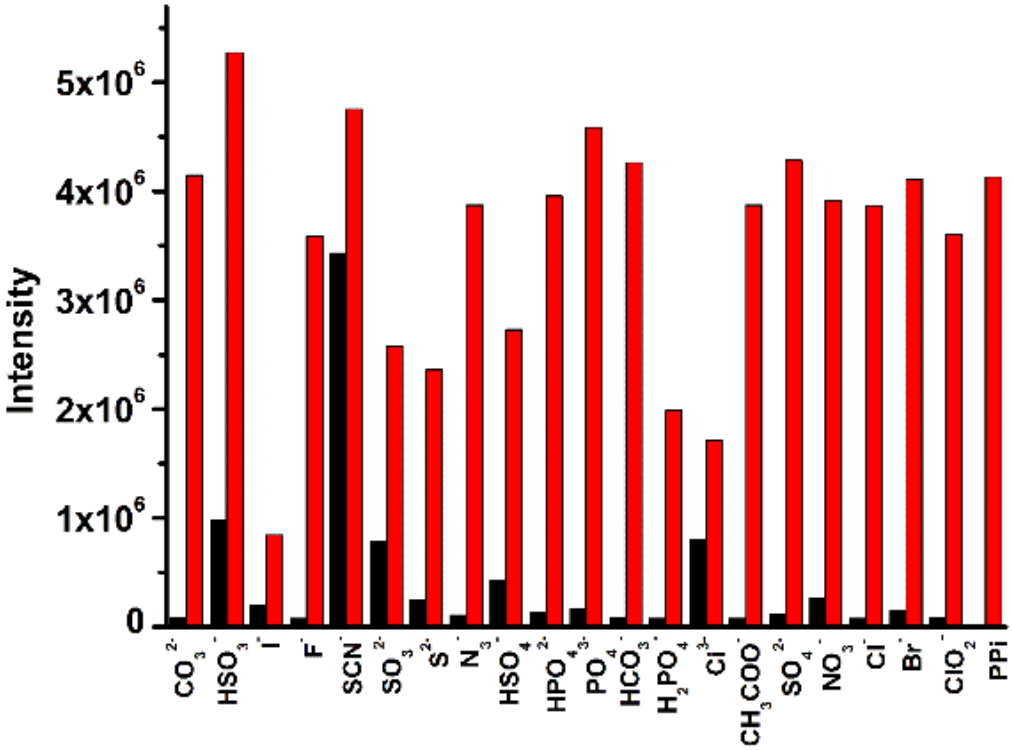


Figure S16. Fluorescence responses of **TPH** (10 μM) in water to various anions (100 μM) together with PPi (30 μM). Black bars represent selectivity of **TPH** upon addition of different anions. Red bars represent competitive selectivity of **TPH** towards PPi in the presence of other anions.

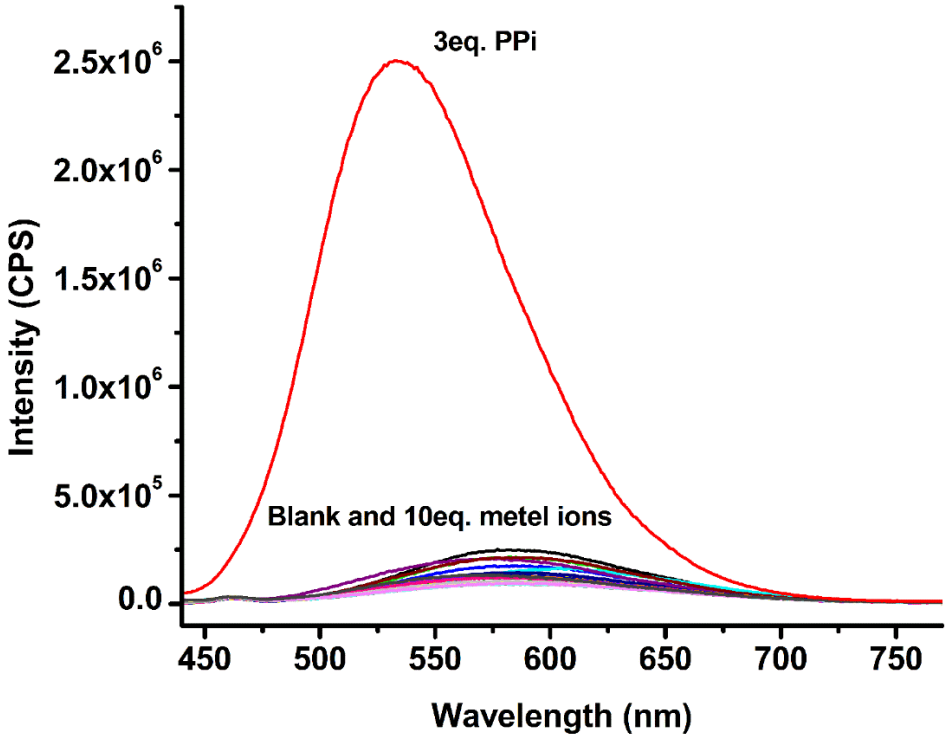


Figure S17. Fluorescence responses of **TPM** (10 μM) in water with PPi (30 μM) and various metal ions (100 μM) including Pb^{2+}, Ni^{+}, Al^{3+}, Cd^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, Cr^{3+}, Co^{2+}, Ag^{+}, Hg^{2+}, Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Li^{+}, Ba^{2+}, Zn^{2+}.

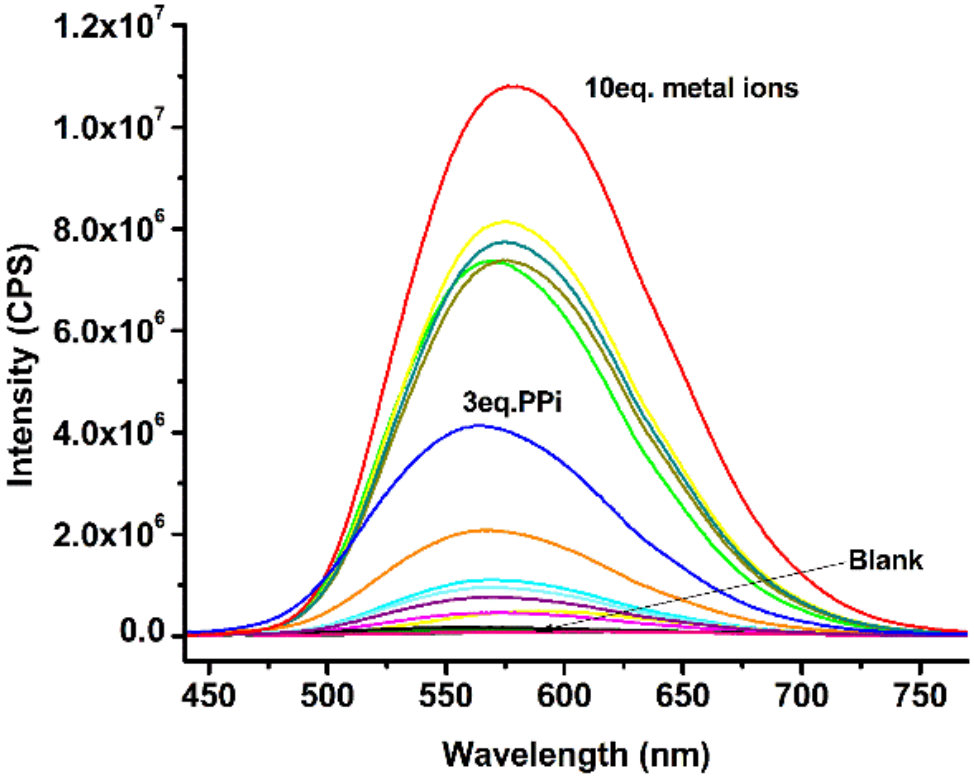


Figure S18. Fluorescence responses of **TPH** (10 μM) in water with PPi (30 μM) and various metal ions (100 μM) including Pb^{2+}, Ni^{+}, Al^{3+}, Cd^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, Cr^{3+}, Co^{2+}, Ag^{+}, Hg^{2+}, Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Li^{+}, Ba^{2+}, Zn^{2+}.

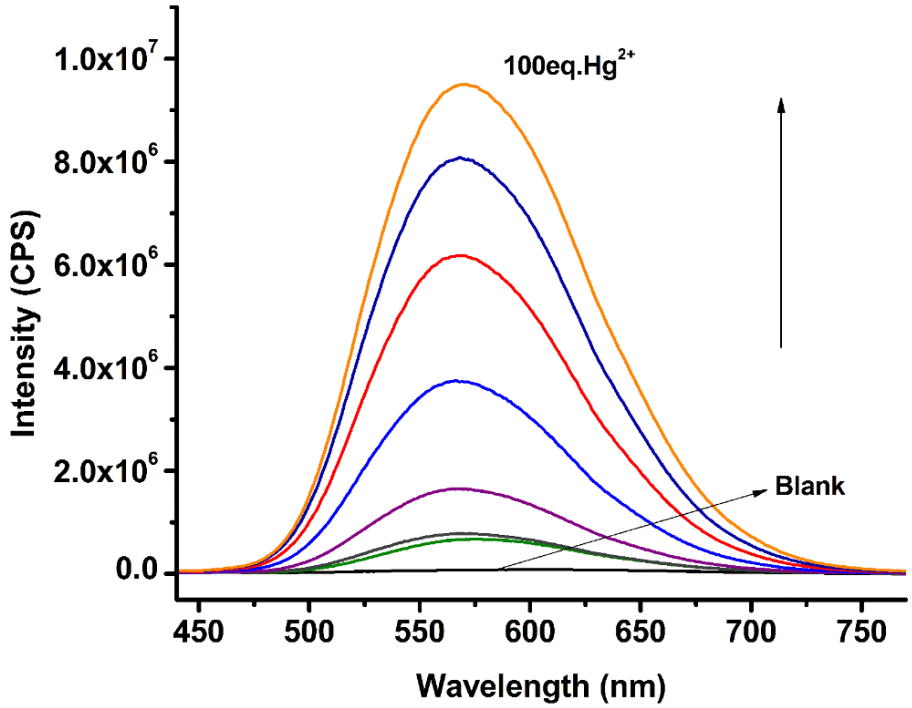


Figure S19. Fluorescence titration of **TPH** (10 μM) with Hg^{2+} (0 - 100 eq.) in water. (λex = 400 nm).

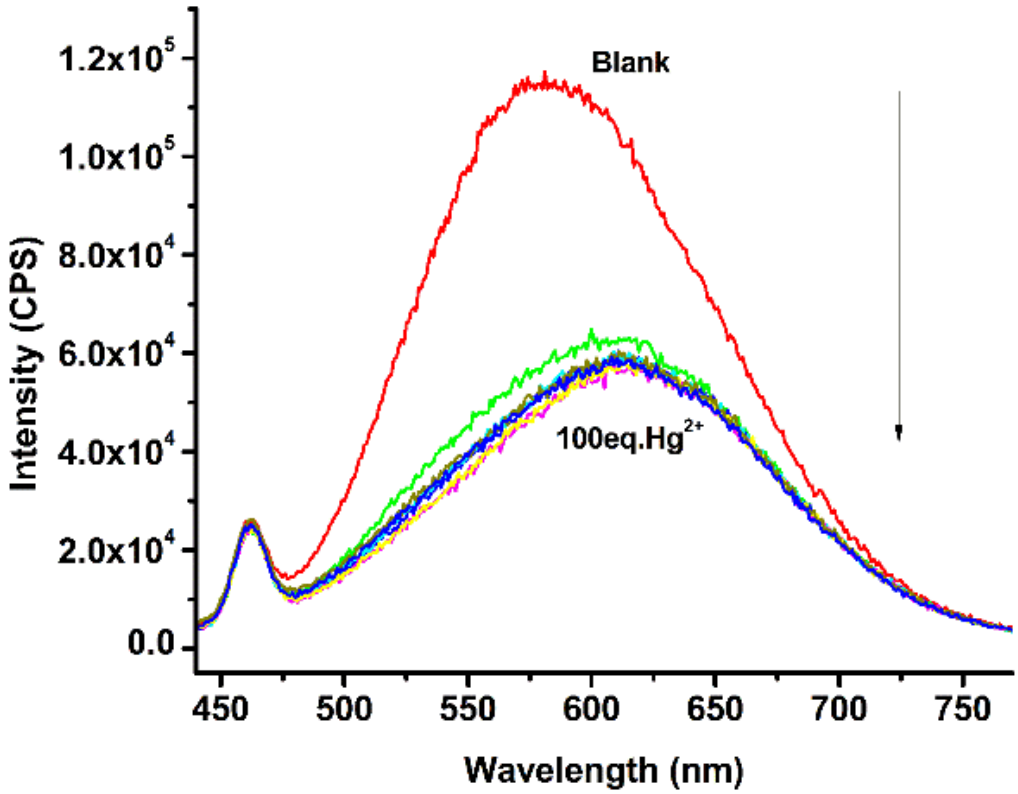


Figure S20. Fluorescence titration of **TPM** (10 μM) with Hg^{2+} (0 - 100 eq.) in water. (λex = 400 nm).

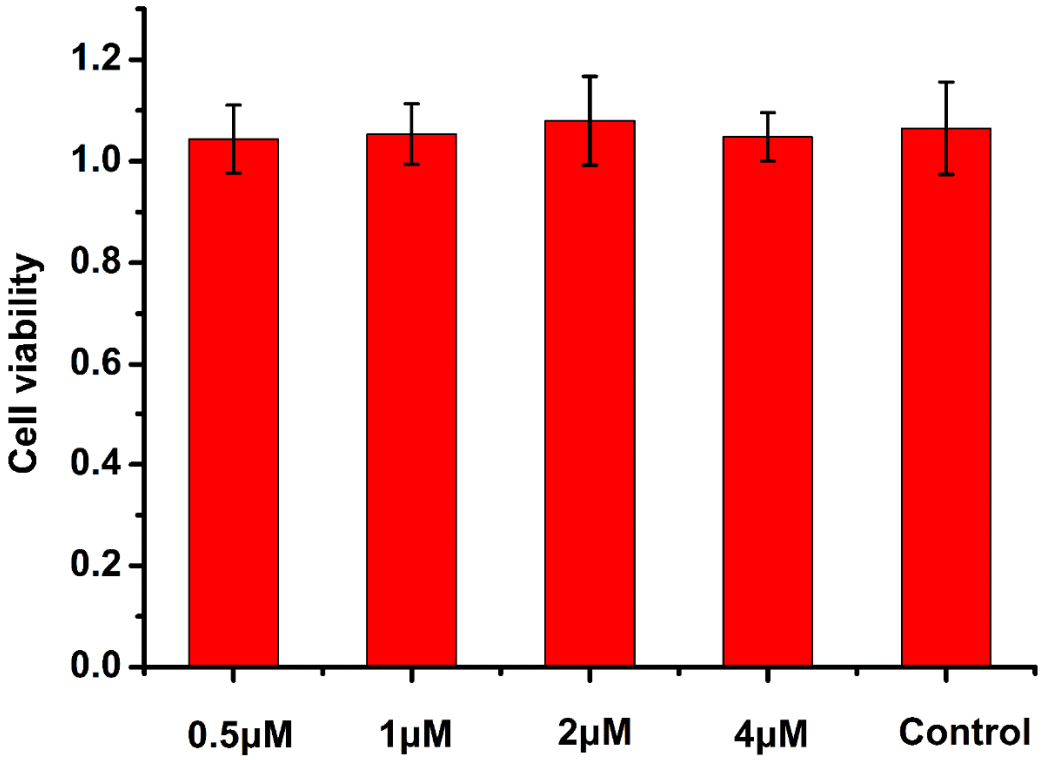
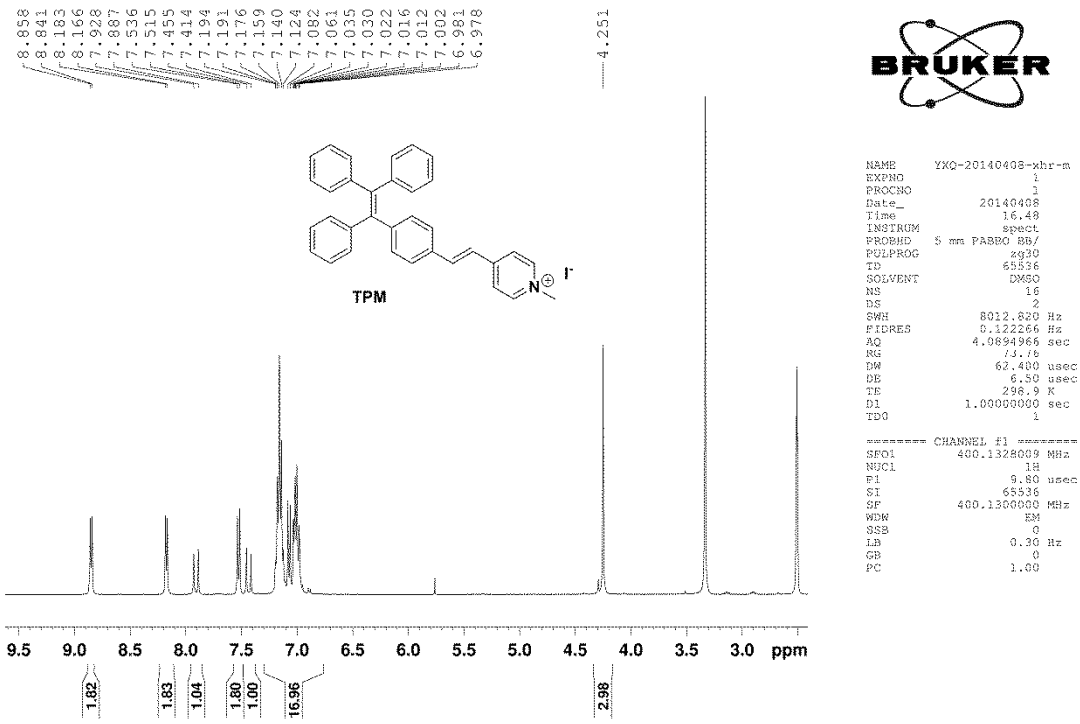
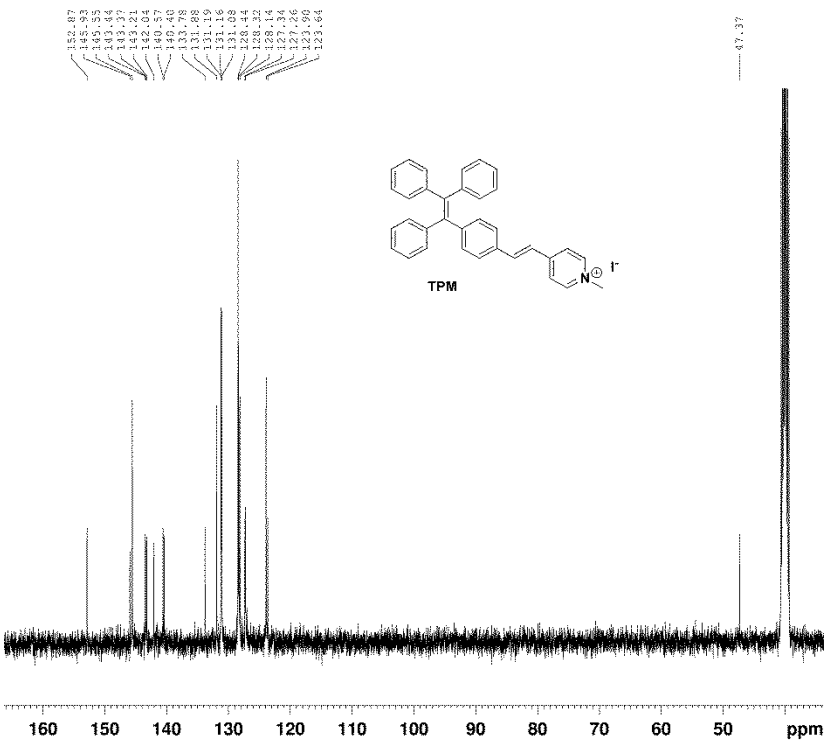
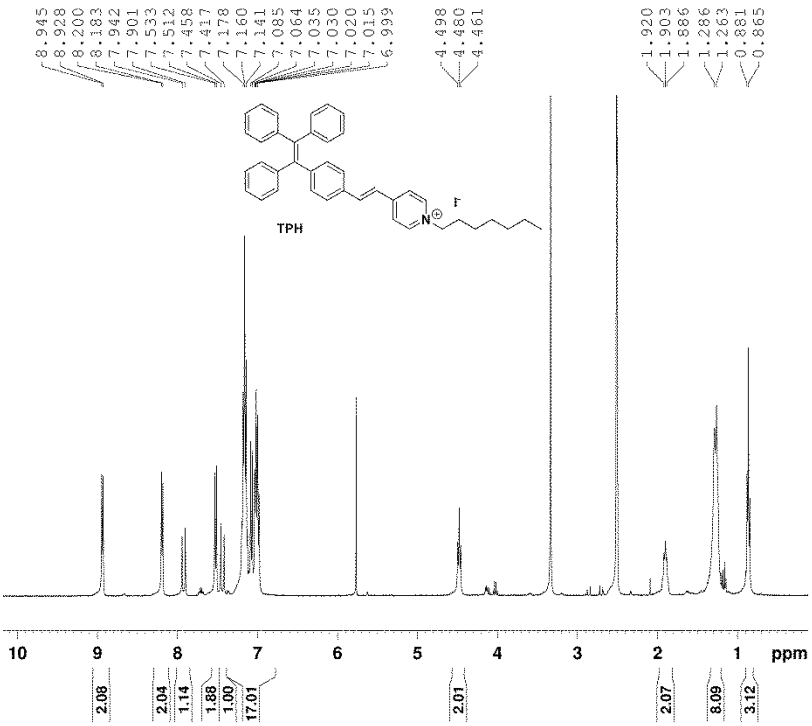


Figure S21. Cytotoxicity of **TPM** on Hela cells.

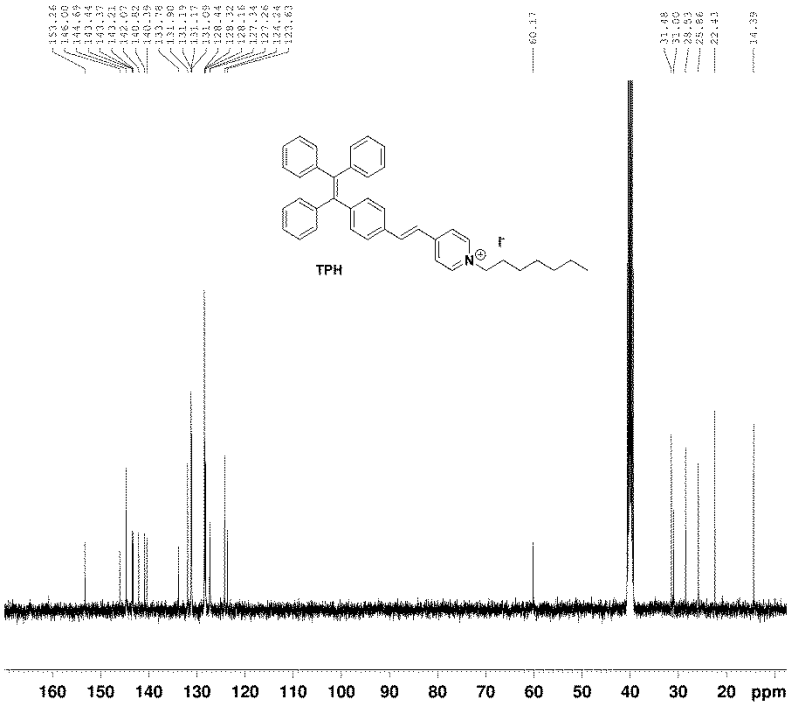





```
NAME          yxq-20140918-xhr-mc
EXPNO                           1
PROCNO                          1
Date_                    20140918
Time                        19.16
INSTRUM                      spect
PROBHD       5 mm PABBO BB/
PULPROG                   zgpg30
TD                         65536
SOLVENT                     DMSO
NS                           512
DS                             4
SWH                    24038.461 Hz
FIDRES                  0.366798 Hz
AQ                     1.3631988 sec
RG                        203.91
DW                        20.800 usec
DE                         6.50 usec
TE                        297.0 K
D1                   2.00000000 sec
D11                  0.03000000 sec
TD0                            1

======== CHANNEL f1 ========
SFO1           100.6228293 MHz
NUC1                       13C
P1                        9.80 usec
SI                        32768
SF                 100.6127690 MHz
WDW                          EM
SSB                           0
LB                         1.00 Hz
GB                            0
PC                         1.40
```

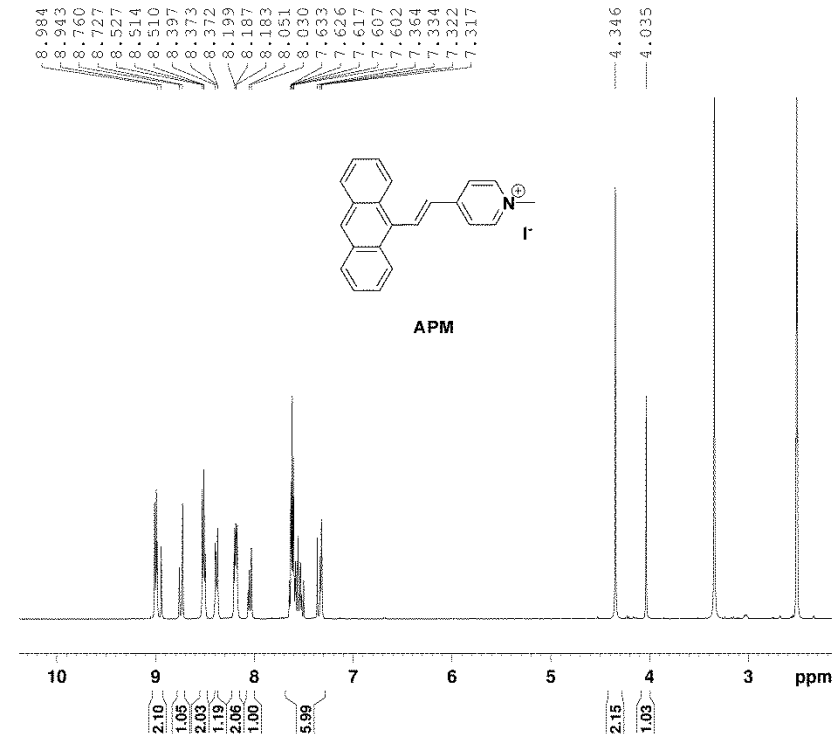



```
NAME          yxq-20140327-xhr-tpelbl
EXPNO                           1
PROCNO                          1
Date_                    20140328
Time                        0.47
INSTRUM                      spect
PROBHD       5 mm PABBO BB/
PULPROG                     zg30
TD                         65536
SOLVENT                     DMSO
NS                            16
DS                             2
SWH                    8012.820 Hz
FIDRES                 0.122266 Hz
AQ                     4.0894966 sec
RG                         91.97
DW                        62.400 usec
DE                         6.50 usec
TE                        296.3 K
D1                   1.00000000 sec
TD0                            1

======== CHANNEL f1 ========
SFO1           400.1328009 MHz
NUC1                        1H
P1                        9.80 usec
SI                        65536
SF                 400.1300000 MHz
WDW                          EM
SSB                           0
LB                         0.30 Hz
GB                            0
PC                         1.00
```



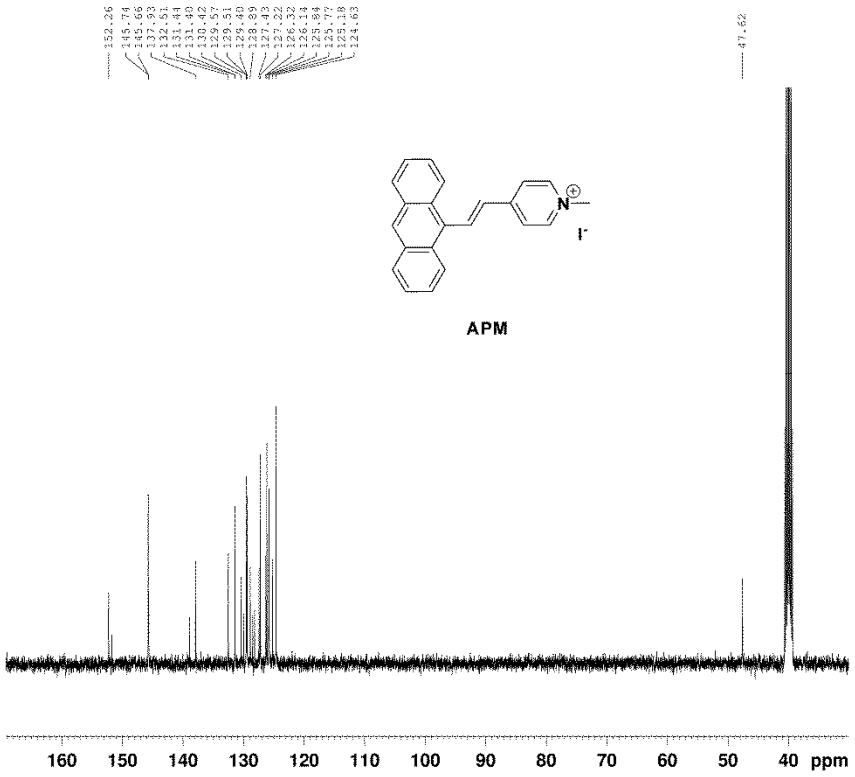
BRUKER

```
NAME          yxq-20140918-xhr-hc
EXPNO                          1
PROCNO                         1
Date_                   20140918
Time                       19.47
INSTRUM                    spect
PROBHD   5 mm PABBO BB/
PULPROG                   zgpg30
TD                         65536
SOLVENT                     DMSO
NS                           512
DS                             4
SWH                    24038.461 Hz
FIDRES                 0.366798 Hz
AQ                     1.3631988 sec
RG                        203.91
DW                        20.800 usec
DE                         6.50 usec
TE                       297.2 K
D1                     2.00000000 sec
D11                    0.03000000 sec
TD0                            1

========== CHANNEL f1 ==========
SFO1                 100.6228293 MHz
NUC1                         13C
P1                          9.80 usec
SI                          32768
SF                   100.6127690 MHz
WDW                           EM
SSB                            0
LB                          1.00 Hz
GB                             0
PC                          1.40
```



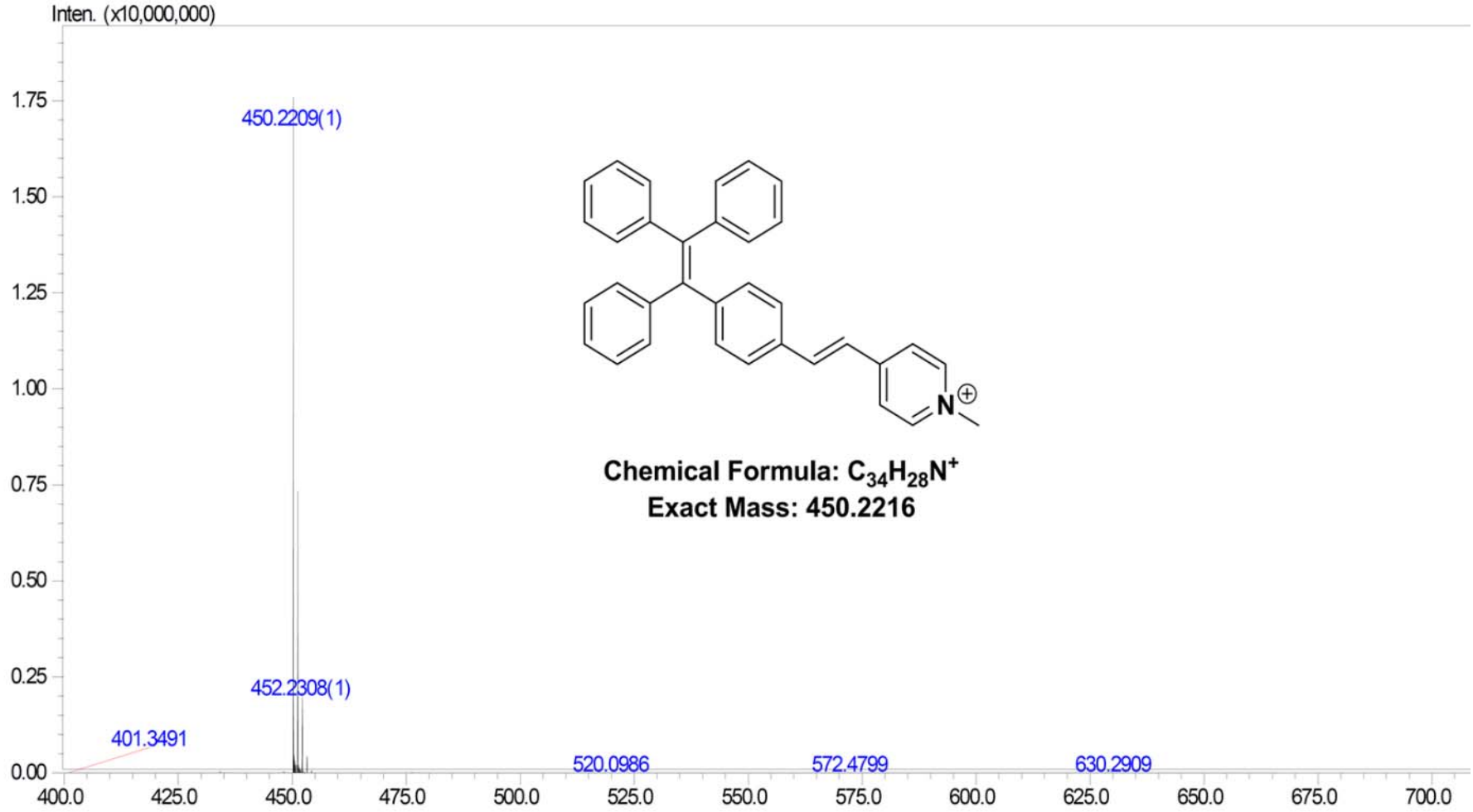
BRUKER

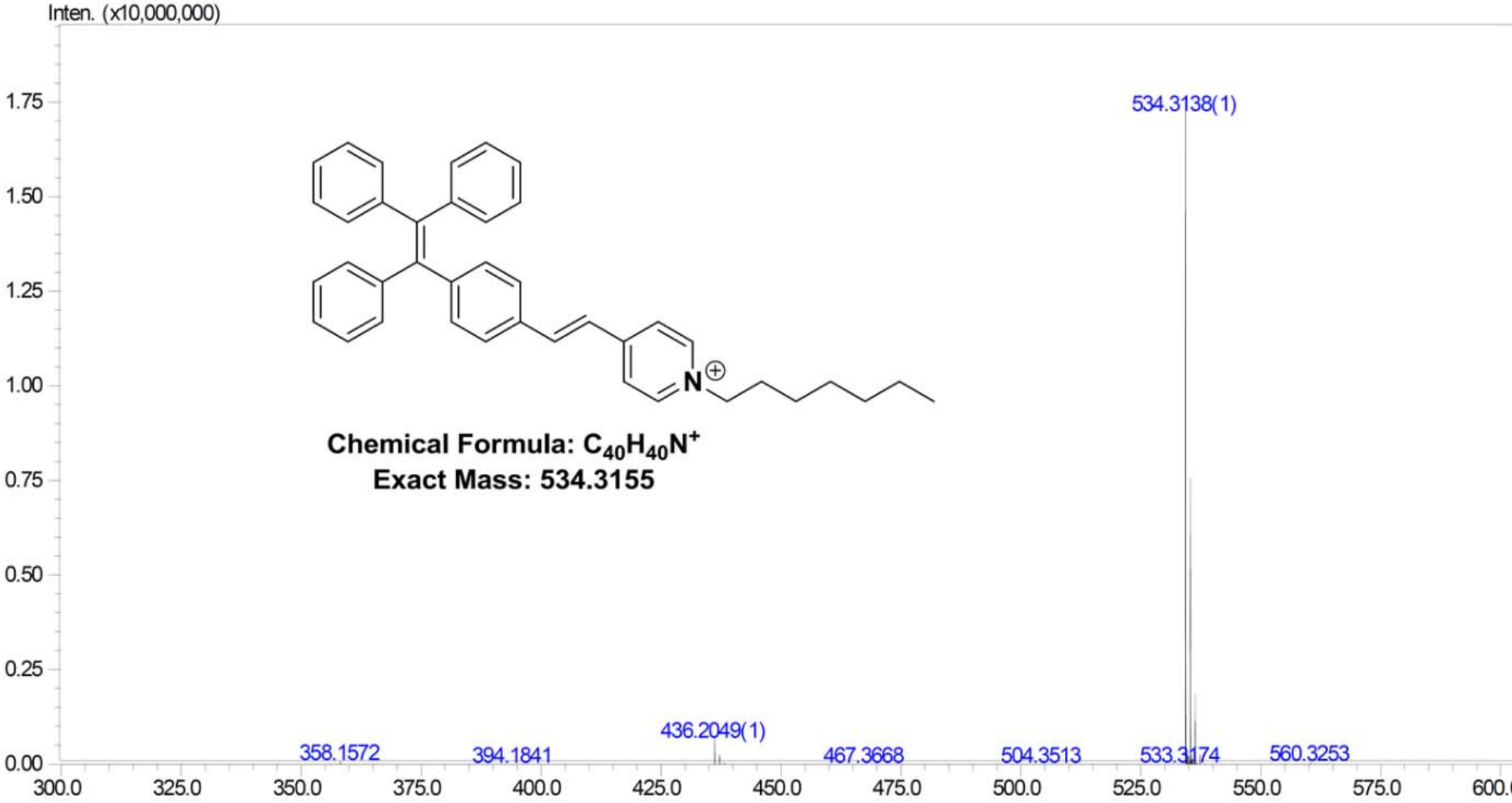
```
NAME          yxq-20141009-LLL-6
EXPNO                          1
PROCNO                         1
Date_                   20141009
Time                       16.54
INSTRUM                    spect
PROBHD   5 mm PABBO BB/
PULPROG                     zg30
TD                         65536
SOLVENT                     DMSO
NS                           16
DS                            2
SWH                    8012.820 Hz
FIDRES                 0.122266 Hz
AQ                     4.0894966 sec
RG                        91.97
DW                        62.400 usec
DE                         6.50 usec
TE                       296.1 K
D1                     1.00000000 sec
TD0                            1

========== CHANNEL f1 ==========
SFO1                 400.1328009 MHz
NUC1                          1H
P1                          9.75 usec
SI                          65536
SF                   400.1300000 MHz
WDW                           EM
SSB                            0
LB                          0.30 Hz
GB                             0
PC                          1.00
```

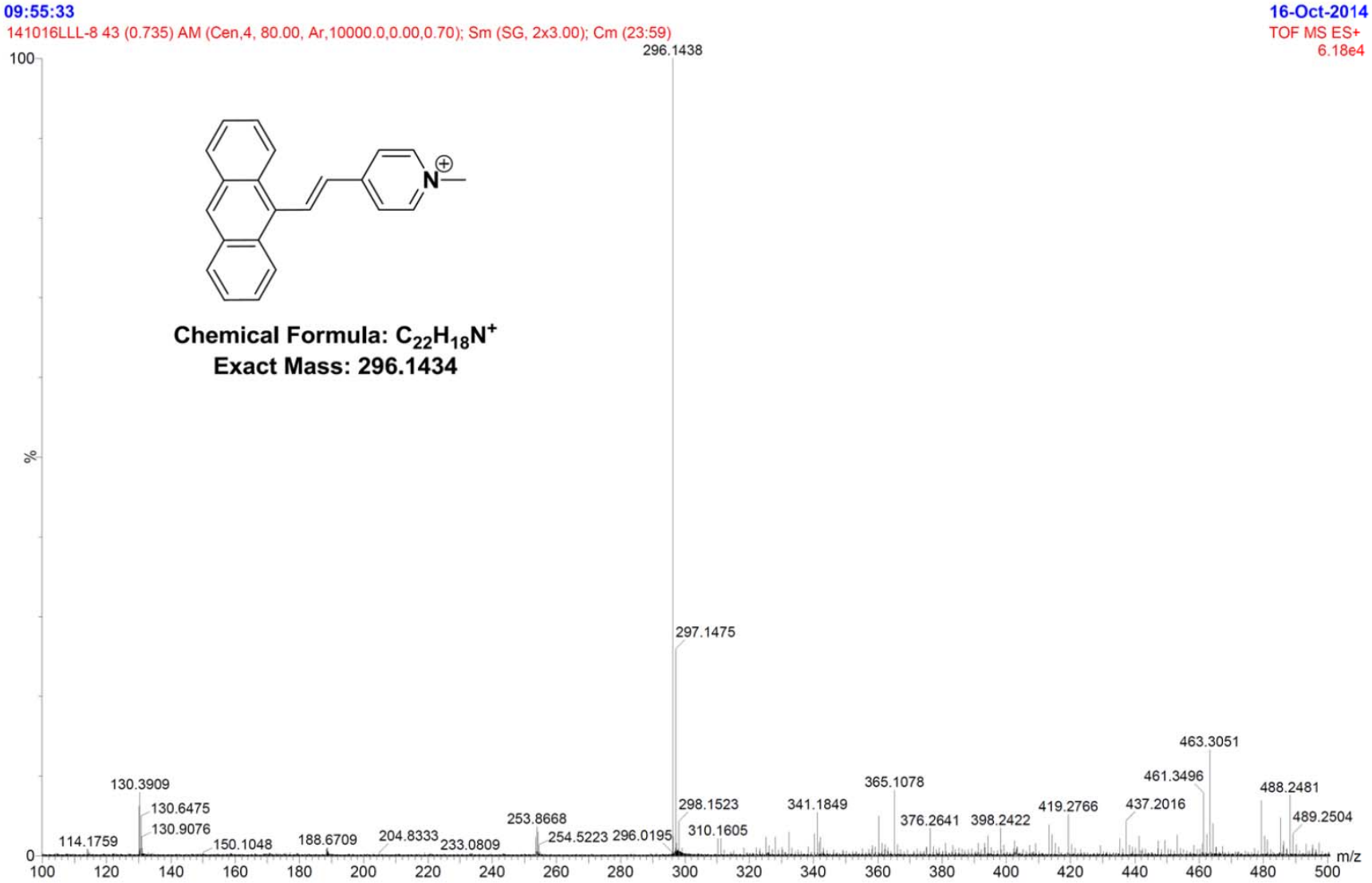


BRUKER

NAME	yxq-20141017-111-9
EXPNO	1
PROCNO	1
Date_	20141017
Time	22.07
INSTRUM	spect
PROBHD	5 mm PABBO BB/
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	256
DS	4
SWH	24038.461 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	203.91
DW	20.800 usec
DE	6.50 usec
TE	296.6 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
SFO1	100.6228293 MHz
NUC1	13C
P1	9.80 usec
SI	32768
SF	100.6127690 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40


Inten. (x10,000,000)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
450.2209(1)
452.2308(1)
401.3491
520.0986
572.4799
630.2909
400.0
425.0
450.0
475.0
500.0
525.0
550.0
575.0
600.0
625.0
650.0
675.0
700.0
N
Chemical Formula: C34H28N+
Exact Mass: 450.2216


Inten. (x10,000,000)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
534.3138(1)
436.2049(1)
358.1572
394.1841
467.3668
504.3513
533.3174
560.3253
300.0
325.0
350.0
375.0
400.0
425.0
450.0
475.0
500.0
525.0
550.0
575.0
600.0
N⊕
Chemical Formula: C40H40N+
Exact Mass: 534.3155


09:55:33
141016LLL-8 43 (0.735) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (23:59)
16-Oct-2014
TOF MS ES+
6.18e4
296.1438
100
%
0
N
Chemical Formula: C22H18N+
Exact Mass: 296.1434
297.1475
463.3051
461.3496
488.2481
365.1078
130.3909
298.1523
341.1849
419.2766
437.2016
489.2504
130.6475
253.8668
376.2641
398.2422
130.9076
310.1605
114.1759
150.1048
188.6709
204.8333
233.0809
254.5223
296.0195
m/z
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500