Supporting Information

FRET Spectral Unmixing: A Ratiometric Fluorescent Nanoprobe For Hypochlorite

Gengwen Chen , Fengling Song,* Jingyun Wang, Zhigang Yang, Shiguo Sun, Jiangli Fan, Xinxin Qiang, Xu Wang, Bairui Dou, Xiaojun Peng *

State Key Laboratory of Fine Chemicals Dalian University of Technology, 2 Linggong Road, Hi-tech Zone, Dalian 116024, P.R. China

songfl@dlut.edu.cn; pengxj@dlut.edu.cn

Contents

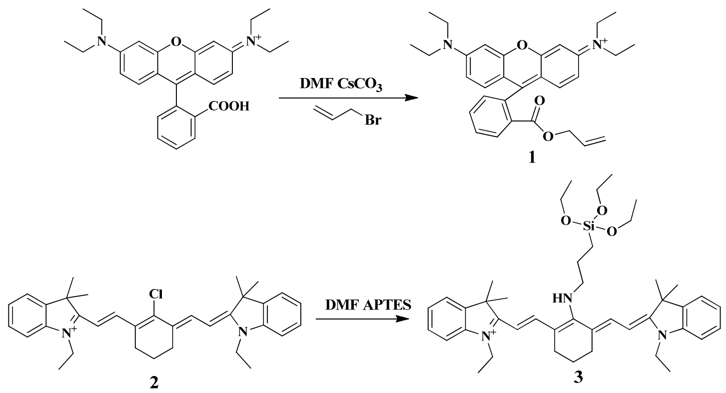
General information

All the reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Silica gel (100-200 mesh) was used for flash column chromatography.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a VARIAN INOVA-400 spectrometer with chemical shifts reported as ppm (in $CDCl_3$, TMS as internal standard). Mass spectrometric data were obtained on a Q-TOF Micro mass spectrometry. Fluorescence measurements were performed on a PTI-700 Felix and Time-Master system, visible absorption spectra were determined using an HP-8453 spectrophotometer. The size and shape of nanoparticles were characterized by transmission electron microscope, TEM (JEM-1200EX). The following abbreviations are used to indicate the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

Experimental Section

Scheme 1. Synthetic routes of the dyes



Compound 1: A mixture of rhodamine B (0.48 g, 1 mmol), allyl bromide (1.46 g, 12.0 mmol), $CsCO_3$ (2.65 g, 25.0 mmol), and dry DMF (50 mL) was heated and stirred at 80 ℃ under N_2 for 25 h. Vacuum evaporation of the solvent yielded the crude product. The title compound was isolated by column chromatography on silica (2.00 g, 77.5%) (methanol/dichloromethane = 1 : 25).[1]

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (t, CH_3, J = 8.0 Hz, 12H), 3.67 (q, CH_2, J = 7.0 Hz, 8H), 4.52 (d, CH_2, J = 8.0 Hz, 2H), 5.1 (m, CH_2, 2H), 5.70 (m, CH, 1H), 6.81 (d, Ar, J = 4 Hz, 2H), 6.93 (dd, Ar, J = 8.0, 2.5 Hz, 2H), 7.08 (d, Ar, J = 8.0 Hz, 2H), 7.33 (d, Ar, J = 8.0 Hz, 1H), 7.76 (t, Ar, J = 8.0 Hz, 1H), 7.84 (t, Ar, J = 8.0 Hz, 1H), 8.32 (d, Ar, J = 8.0 Hz, 1H).

HRMS (ESI+Tof) m/z Found 483.2647 M+, calculated 483.2642 for $C_{31}H_{35}N_2O_3^+$.

Compound 2: Compound **2** was facilely synthesized in high yield by the procedure as published in literature.[2]

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (t, CH, *J* = 8.0 Hz, 1H), 7.38~7.43(m, Ar, 2H), 7.27 ~ 7.26 (m, Ar, 1H), 7.18 (d, Ar, *J* = 4.0 Hz, 1H), 6.25 (t, CH, *J* = 6.5 Hz, 1H), 4.28 (m, CH_2, *J* = 8.0 Hz, 2H), 2.77 (t, CH_2, *J* = 8.0 Hz, 2H), 1.99 (m, CH_2, *J* = 4.0 Hz, 2H), 1.72 (s, CH_3, 6H), 1.47 (t, CH_2, *J* = 8.0 Hz, 2H)
HRMS (ESI+Tof) m/z Found 511.2867 M^+, calculated 511.2875 for $C_{34}H_{40}ClN_2^+$.

Compound 3:
A solution of compound **2** (104 mg, 0.2 mmol) and 3-aminopropyltriethoxysilane (APTES; 187 μL, 0.8 mmol) in anhydrous DMF (50 mL) was stirred at 120 ℃ for 30 min under a dry nitrogen atmosphere. The solvent was evaporated and the crude product was purified by flash chromatography on silica gel (DCM/MeOH = 10:1), affording 30 mg (21%) of compound 3 as a blue powder.
^{1}H-NMR (400 MHz, $CDCl_3$) : δ 0.69 (t, 2H, $SiCH_2$, *J* = 8.0 Hz), 1.21 (t, 9H, OCH_2CH_3, *J* = 8.0 Hz), 1.35 (m, 4H), 1.69 (s, 12H, indole-$(CH_3)_2$), 1.82 (m, 2H, $CH_2CH_2CH_2$ cyclohexene), 2.04 (m, 2H, $SiCH_2CH_2CH_2$), 2.49 (t, 4H, $CH_2CH_2CH_2$ cyclohexene, *J* = 8.0 Hz), 3.80 (m, 8H, OCH_2CH_3, NCH_2CH_3, *J* = 8 Hz), 5.60 (d, 2H, indole-CHCH, *J* = 4 Hz), 6.85 (d, 2H, indole-CHCH, *J* = 4 Hz), 7.05 (t, 2H, ArH, *J* = 8.0 Hz), 7.27 (m, 2H, ArH), 7.71 (d, ArH, 2H), 8.27 (br.s, 1H, NH). HRMS: *m/z* calcd M^+ for $C_{43}H_{62}N_3O_3Si$ 696.4555; found, 696.4553.

Preparation of NP sensor

NP-1: Pure tetraethyl orthosilicate (TEOS, 2 mL) and 3-methacryloxypropyltrimethoxysilane (**PMTMS,** 0.1 mL) was dissolved in 20 mL dried ethanol, then 1 mL ammonia and 1 mL de-ionized water were added with stirring. After 12 hours of stirring, nanoparticles were isolated by centrifugation at the speed of 14,000 rpm, and the isolated products were re-dispersion in ethanol. After repeating 3 times of washing and re-dispersion, the solution was centrifuged at the speed of 2,000 rpm to remove any aggregated particles. The product was pure SiO_2 core which was modified with **PMTMS**. The obtained nanoparticles were dispersed in dried ethanol, followed by addition of Allyl Rhodamine B (0.05 μM) under nitrogen. Next the mixture was stirred at 70 □ for 1 hour. 15 mg of KPS was added to initiate the reaction and the mixture was stirred for another 4 hours. The product was isolated by centrifugation at the speed of 14,000 rpm.
NP–2: The prepared NP-1 was dissolved in 40 mL dried ethanol, then 0.2 mL NH_4OH(aq) (2.8 wt %) and 1 mL de-ionized water were added under magnetic stirring. After 1 hour of stirring, a shell of compound **3** (15 mg) in ethanol solution was added dropwise. (Note: a stepwise addition of compound **3** is necessary to keep the total amount of compound **3** and hydrolyzed compound 3 in the system at any time below the critical nucleation concentration). After 24 hours of stirring, nanoparticles were isolated by centrifugation at the speed of 14,000 rpm.

Determination of the concentration of Allyl rhodamine B and compound 3

The concentration of compound **3** was determined based on the Beer-Lambert law. The value of ε of compound **3** was determined in a mixed water/ethanol solution (4:1, v/v). The ε value was calculated to be 5810 $M^{-1}cm^{-1}$. And the concentration of compound 3 in NP-2 was calculated to be 0.04 μM/mg. During the copolymerization of Allyl rhodamine B and **PMTMS**, the unreacted Allyl rhodamine B was also determined based on Beer-Lambert law, and the concentration was calculated to be 2.43×10^{-3}μM/mg in NP-1.

Preparation of stock solutions or generation of ROS[2-5]

(a) H_2O_2
H_2O_2 was diluted appropriately in water. The concentration of H_2O_2 was determined based on the molar extinction coefficient at 240 nm (43.6 M^{-1} cm^{-1}). Then, a H_2O_2 stock solution in water was prepared.
(b) •OH
$Fe^{2+}$was dissolved in water. To a solution of H_2O_2 in 100 μM sodium phosphate buffer at pH 7.4 as a cosolvent, the $FeSO_4$ solution (10 μM) was added at room temperature. Then, •OH was generated from Fe^{2+} and H_2O_2 (Fenton reaction).
(c) OCl^-
NaOCl solution was diluted appropriately in 0.1 M NaOHaq. The concentration of OCl^- was determined based on the molar extinction coefficient at 292 nm (350 M^{-1} cm^{-1}). Then, a OCl^- stock solution in 0.1 M NaOHaq. was prepared.
(d) Generation of $•O_2^-$
Superoxide ($•O_2^{-)}$ was added as solid KO_2.
(e) $^1O_2$
A solution of $NaMoO_4$ was added to a solution of H_2O_2 in 0.1 M sodium phosphate buffer at pH 7.4 as a cosolvent at room temperature.
(f) NO•
Nitric oxide was generated from SNP (Sodium Nitroferricyanide (III) Dihydrate). SNP (final 10 μM) in deionizer water was added then stirred for 30 min at 25 □.
(g) TBHP
Tert-butyl hydroperoxide (TBHP) was delivered from 5% aqueous solutions.
(h) TBO•

tert-butoxy radical (TBO•) were generated by Fenton reaction of 100μM TBHP with 10 Fe^{2+}

Selectivity studies

A solution of NP-2 (10 μg/mL) was made in water/ethanol (4 : 1, v/v). Various reactive oxygen species were added to the solution (final concentration: 10 μM), and the fluorescence spectra was recorded with excitation at 550 nm after 5 min of incubation at 25□.

NP-2 was also treated with a wide variety of cations, anions, and oxidants to examine the selectivity. Solutions of different analytes (final concentration: 100 mM) represented by $CuCl_2$, $FeSO_4$, $NaHSO_3$, $NaClO_4$, $MgCl_2$, CH_3COONa, Na_2SO_4, $NaHSO_4$, Na_2SO_3, $FeCl_3$, $NaClO_3$ and HSA were added to the solution of NP-2 (10 μg/mL).

FRET efficiency Analysis.

Experimentally, FRET efficiency En (where n is the ratio of CY7 / RhB) was determined using

$$E_n = \frac{F_D - F_{DA}}{F_D}$$

where F_D and F_{DA} designate the integrated fluorescence intensities of donor alone and donor in the presence of acceptor, respectively.

Preparation and Staining of Cell Cultures.

Hela cells were maintained in minimum essential medium (DMEM) supplemented with 10% fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin. The cells were incubated at 37□ in 5% CO_2. Two days before imaging, cells were plated on tissue culture plates. Cells were then incubated in fresh media at 37 °C, 5% CO_2. Before the experiments, the media was exchanged for 500 μL fresh media with NP-2 (10 μg/mL) and NP-1 (10 μg/mL). The cells were incubated with NP-1 and NP-2 for 2 hours, with or without NaOCl for 5 min. Luminescence imaging was performed after washing the cells three times with PBS buffer.

Fluorescence Imaging Experiments.

Fluorescence imaging studies were performed with a Leica TCS-SP2 confocal fluorescence microscope, confocal fluorescence image 100 × objective lens. The microscope settings (brightness, contrast, and exposure time) were held constant before and after pretreatment of cells with NP-1 and NP-2 to compare the relative intensity of fluorescence. Image analysis was performed in Adobe Photoshop and Image Pro-plus 6.0.

Measurement of cell viability

Cell viability was evaluated by the reduction of MTT (3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide) to formazan crystals by mitochondrial dehydrogenases (Mosmann, 1983). Briefly, Hela cells were seeded in 96-well microplates (Nunc, Denmark) at a density of $1*10^5$ cells/mL in 100ul medium containing 10% FBS. After 24 h of cell attachment, plates were washed with 100 uL/well phosphate buffered saline (PBS) and then cells were cultured in medium with various concentrations (0–90 ug/mL) of NP-2 for 24 h. Cells in culture medium without NP-2 were used as the control. Six replicate wells were used for each control and test concentration. 10 ul of MTT (5 mg/mL) prepared in PBS was added to each well and the plates were incubated at 37 ℃ for 4 h in a 5% CO_2 humidified incubator. The medium was then carefully removed, and the purple products were lysed in 200 uL dimethyl sulfoxide (DMSO). The plate was shaken for 10 min and the absorbance was measured at 570 nm and 630 nm using a microplate reader

(Thermo Fisher Scientific). Cell viability was expressed as a percent of the control culturevalue.

Absorption and Fluorescence Quantum Yields Measurements.

Absorption spectra were measured on a Lamda LS35 spectrophotometer. Fluorescence spectra were obtained with a FP-6500 spectrophotometer (Jasco, Japan). The relative fluorescence quantum yields were determined with Rhodamine B[6] as a standard and calculated using the following equation:

$$\Phi_x = \Phi_s (F_x/F_s)(A_x/A_s)(\lambda ex_s/\lambda ex_x)(n_x/n_s)^2$$

where Φ_x represents quantum yield; F stands for integrated area under the corrected emission spectrum; A is absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; n is the refractive index of the solution (because of the low concentrations of the solutions (10^{-7}-10^{-8} mol/L), the refractive indices of the solutions were replaced with those of the solvents); and the subscripts x and s refer to the unknown and the standard, respectively.

Figures and tables

Table S1. Properties of dyes and nanoparticles

	NP-1	NP-2	1	2	3
E_x (nm)	557	555	555	781	628
E_m (nm)	576	575/750	576	805	749
Φ^a	0.48		0.37	0.085	0.096
Zeta potential (mV)	-1.52	1.32			

[a] Measured in anhydrous ethyl alcohol. Rhodamine B was used as a standard reference with a fluorescence quantum yield of 0.97 in ethanol

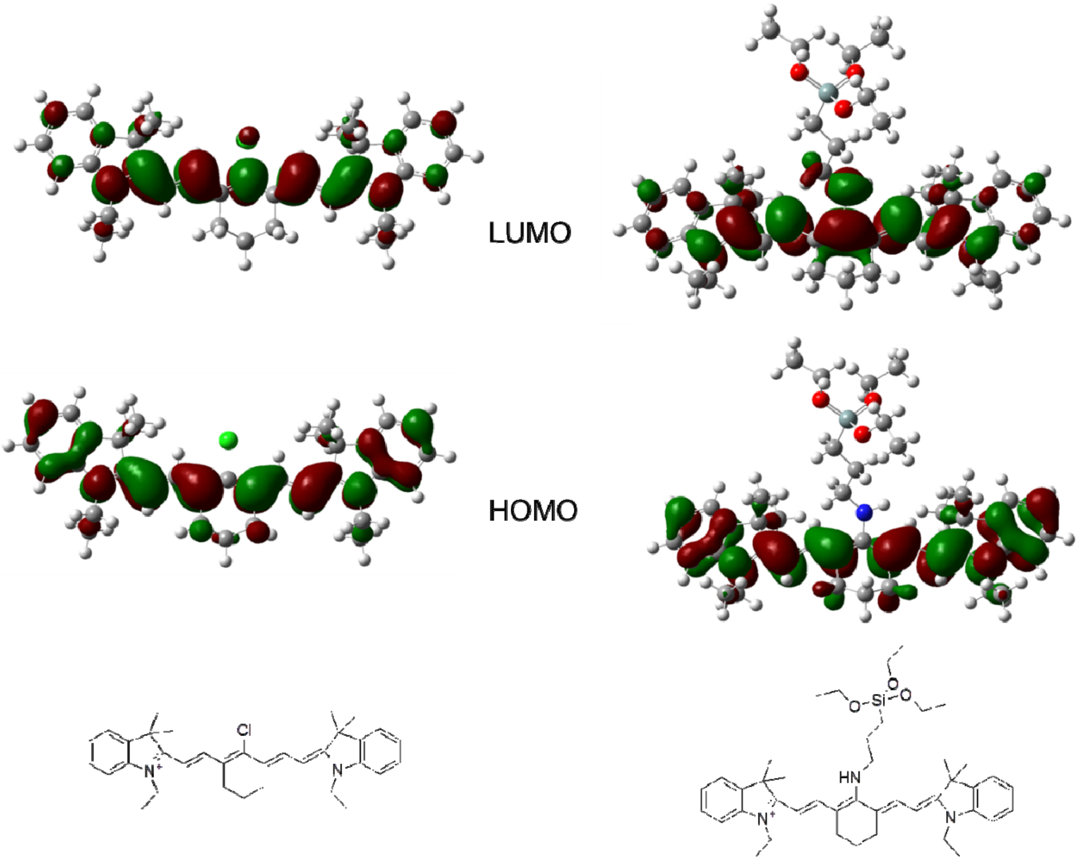


Figure S1. The ground state geometry optimization of **2** and **3** calculated with DFT at the B3LYP/6-31G (d, p) level.

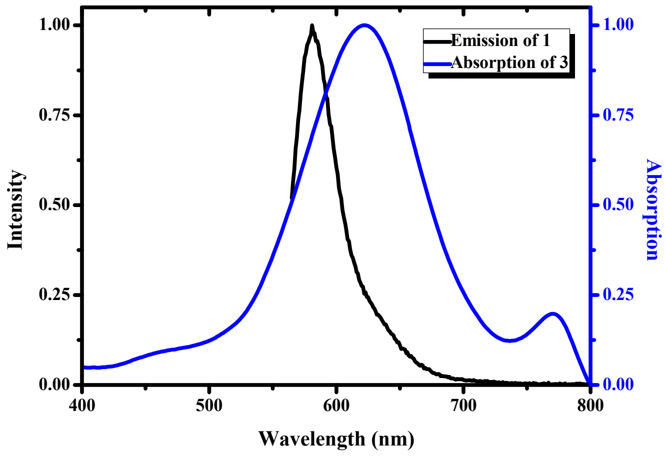


Figure S2. Spectral overlap profiles for FRET donor and acceptor species used in this study. Normalized absorption and emission profiles of the RhB (donor 1) and Cy7-N (acceptor 3).

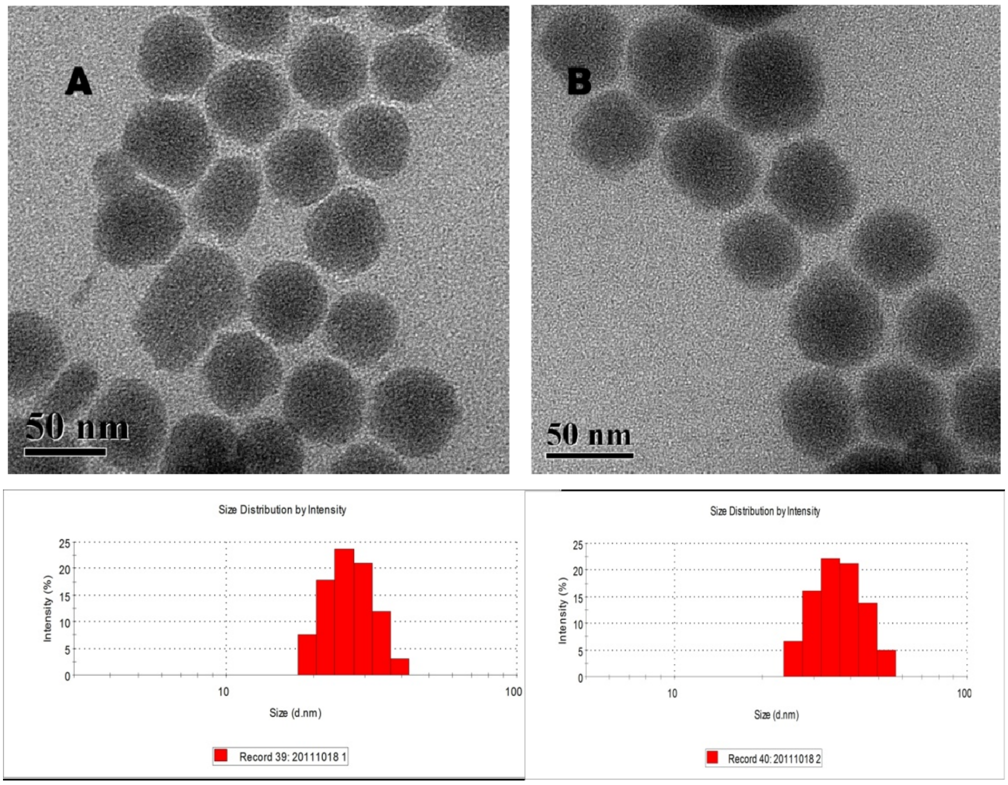


Figure S3. TEM and DLS images of **NP-1** (A) and **NP-2** (B).

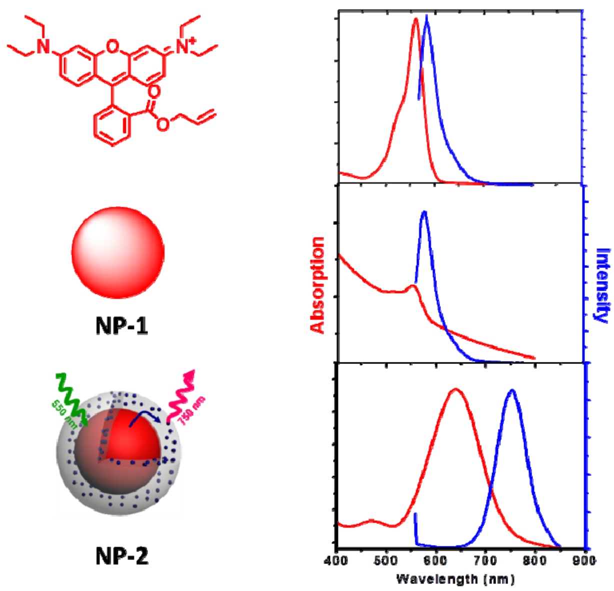


Figure S4. Absorption spectra (red lines) and emission spectra (blue lines) of compound 1, and two nanoparticles NP-1(15.6 μg/mL), NP-2(5μg/mL) in H_2O/ethanol (4 : 1, v/v), respectively. The left shows the chemical structures. The dye and nanoparticles were excited at 550 nm.

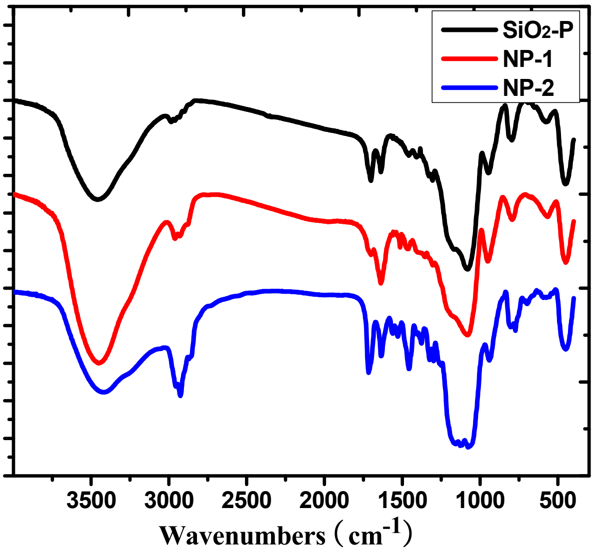


Figure S5 IR spectra of SiO_2-P, NP-1 and NP-2.

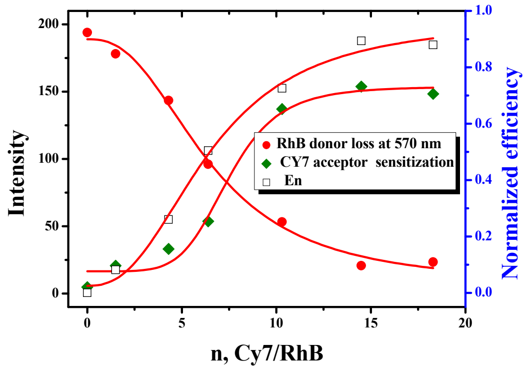


Figure S6. The RhB donor PL intensity loss at 570 nm (red dots) is plotted as a function of the number of Cy7/RhB, along with the Cy7 acceptor emission (green diamond) at 750 nm. En (square) is FRET efficiency. The ratio of Cy7-N to Rhodamine B is set at 16.3 for NP-2

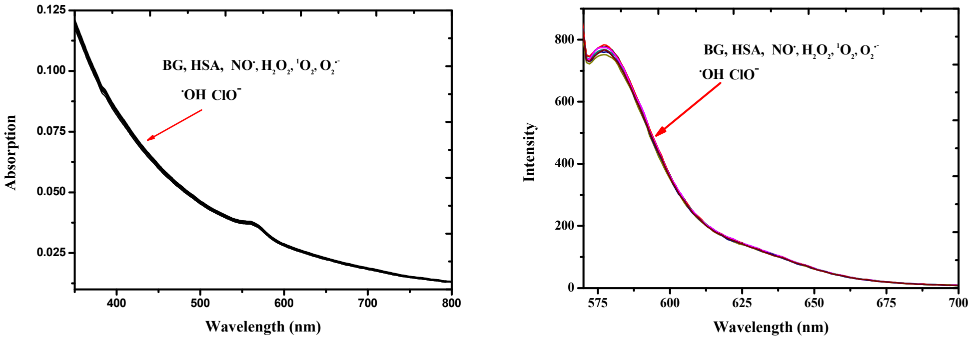


Figure S7. Absorption and fluorescence spectra of NP-1 (10 μg/mL) in the present of hydrogen peroxide (H_2O_2), nitric oxide (NO·), hydroxyl radical (·OH), superoxide ($\cdot O_2^-$), bovine serum albumin (BSA), singlet oxygen ($^1O_2$) and hypochlorous acid (100 equiv respectively) in H_2O-ethanol (4:1, v/v) (25℃). BG = background.

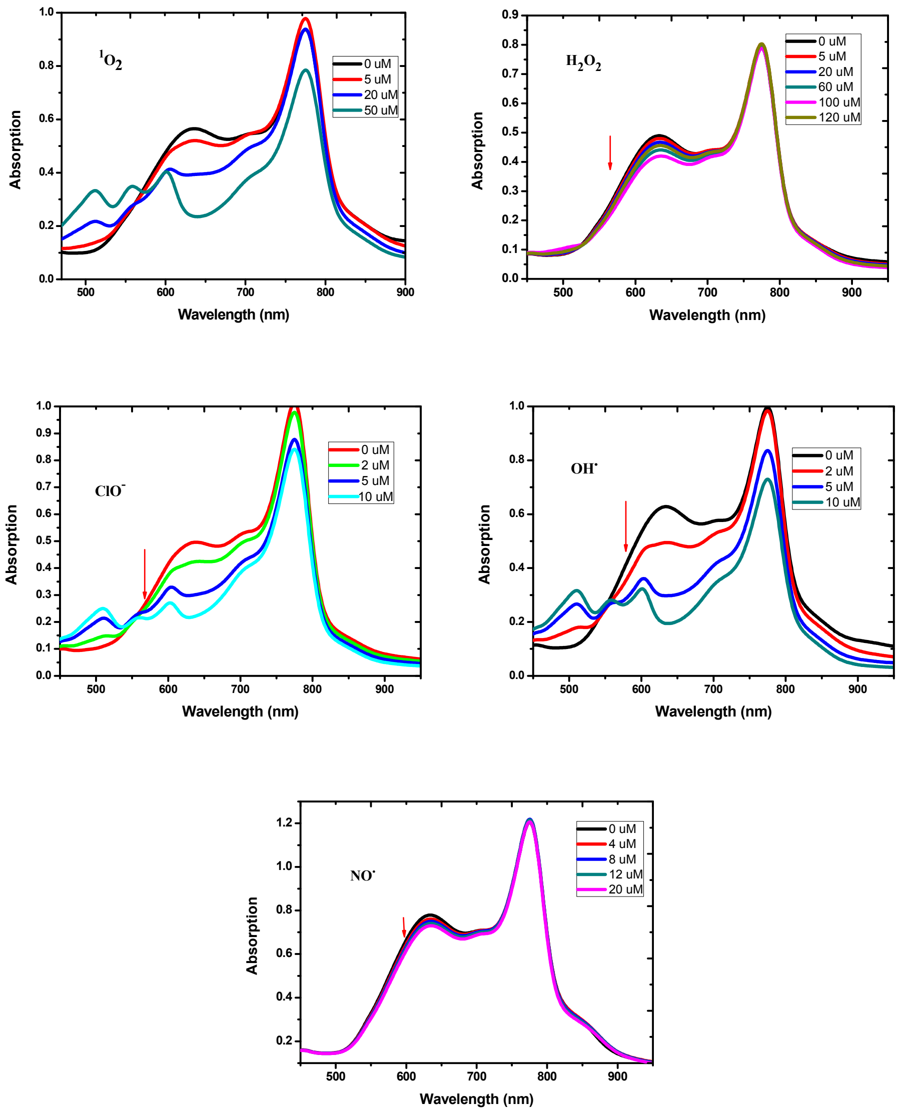


Figure S8. The absorption spectra of 5 μM solution of compound **2** and **3** after reaction with various ROS in phosphate buffer (pH = 7.4, 25 ℃) .

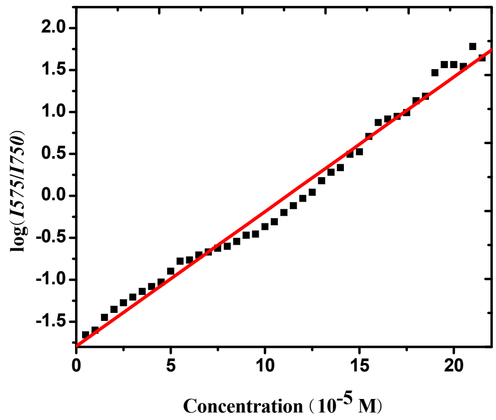


Figure S9. Linear dependence of log(I575/I750) with increasing NaOCl concentrations

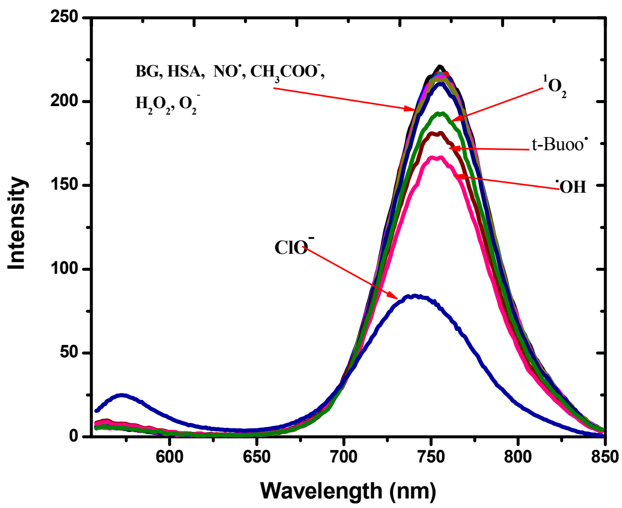


Figure S10. Specificity: fluorescence spectra (λex = 550 nm) of NP-2 (10 μg/mL) in the presence of various species in H_2O/ethanol (4 : 1, v/v). HOCl = NaOCl (final 5 μM) was added and the mixture was stirred at 25℃. •OH = ferrous perchlorate (5 μM) and H_2O_2 (100 μM) were added at room temperature. •O_2^- = KO_2 (5 μM) was added and the mixture was stirred at 25 ℃ for 30 min. H_2O_2 = H_2O_2 (10 μM) was added and the mixture was stirred at 25 ℃ for 30min. •NO = SNP (5 μM) was added and the mixture was stirred at 25 ℃ for 30 min. t-Buoo. was generated by Fenton reaction of 100μM TBHP with Fe^{2+}. $^1O_2$ = 10 μM $NaMoO_4$ was added to a solution of H_2O_2 in 100 μM sodium phosphate buffer at pH 7.4 . BG = background.

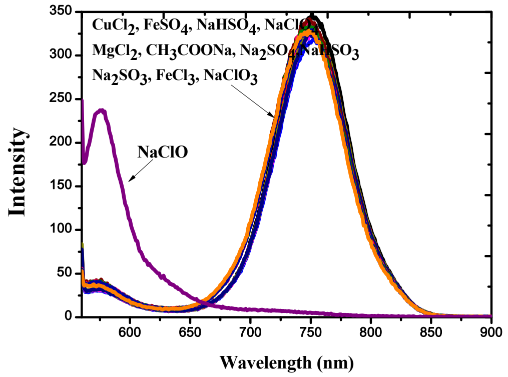


Figure S11. Fluorescence response of NP-2 (10 μg/mL) to a wide variety of cations and anions (final concentration: 100 mM) in H_2O/ethanol (4 : 1, v/v), Excitation wavelength was 550 nm.

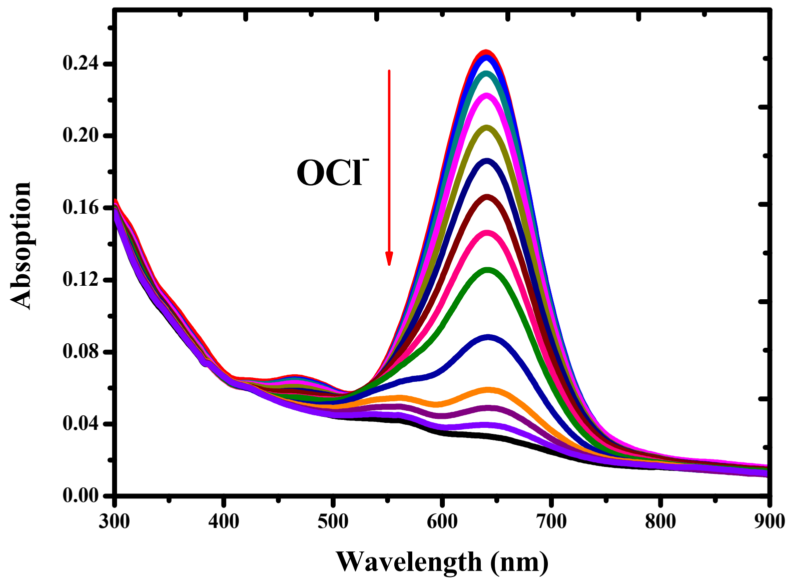


Figure S12. Absorption titration of NP-2 (10 μg/mL) with OCl- in H_2O/ethanol (4:1, v/v) (25℃).

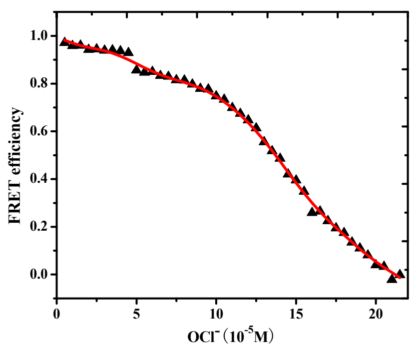


Figure S13. FRET efficiency of NP-2 (10 μg/mL) in the present of different concentration of OCl^- in H_2O/ethanol (4:1, v/v).

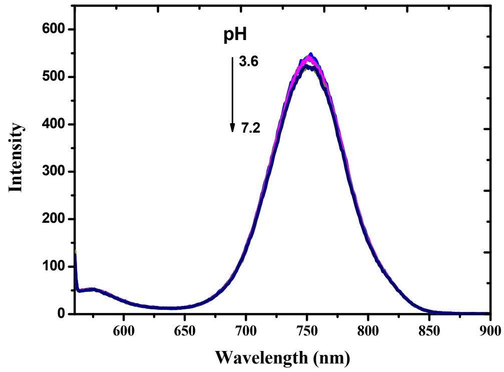


Figure S14. Fluorescent intensity of 10 μg/mL NP-2 in PBS under various pH.

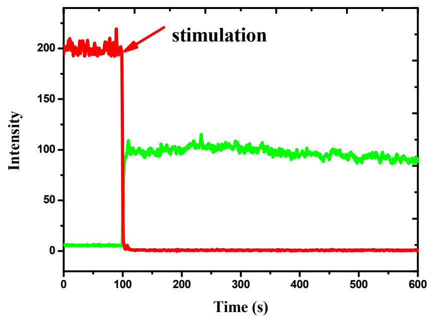


Figure S15. Time dependent fluorescence intensity changes of NP-2 (10 μg/mL) with 5 equiv of NaOCl in H_2O/ethanol (4:1, v/v) (25 ℃, Ex = 550 nm).

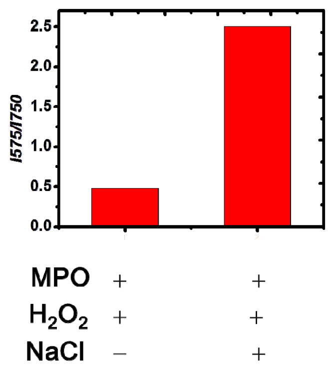


Figure S16. The fluorescence response of NP-2 to HOCl generated by MPO. The fluorescence intensity were measured in 0.1 M Na_2HPO_4 containing 0.01 Unit of purified MPO (Sigma) and H_2O_2 (100 μM) in the presence or absence of NaCl (100 mM).

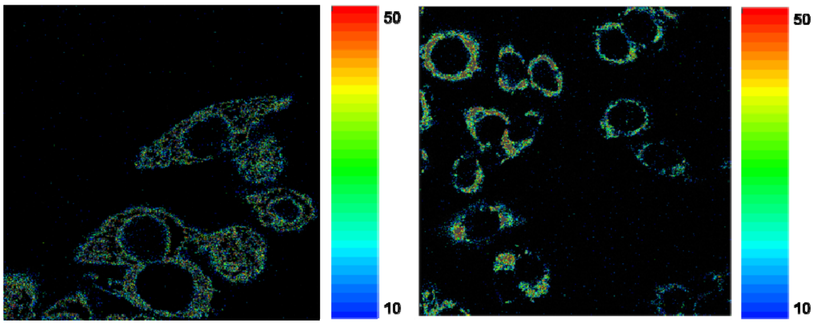


Figure S17. The fluorescence ratio images of Hela cells stained with NP-2 and HOCl. (left) Image of cells after treatment with NP-2 (10 μg/mL) for 2 hours and subsequent treatment of the cells with 5 μM NaOCl. (right) Image of cells after treatment with NP-2 (10 μg/mL) for 2 hours and subsequent treatment of the cells with 10 μM NaOCl. The ratiometric images were obtained by the image analysis software of Image Pro-plus 6.0.

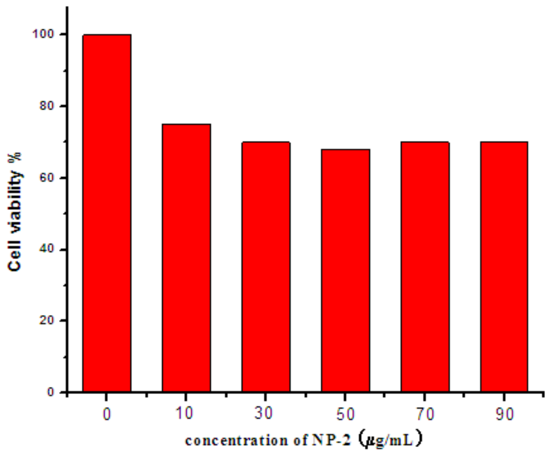


Figure S18. Cytotoxicity assay of NP-2. After Hela cells were incubated with different concentrations of NP-2 in FBS buffer for 12 h, cell viabilities were examined using Thermo Fisher Scientific.

References

(1) Liu, Q. H.; Liu, J.; Guo, J. C.; Yan, X. L.; Wang, D. H.; Chen, L.; Yan, F. Y.; Chen, L. G. *J Mater Chem* **2009**, *19*, 2018.

(2) Oushiki, D.; Kojima, H.; Terai, T.; Arita, M.; Hanaoka, K.; Urano, Y.; Nagano, T. *J Am Chem Soc* **2010**, *132*, 2795.

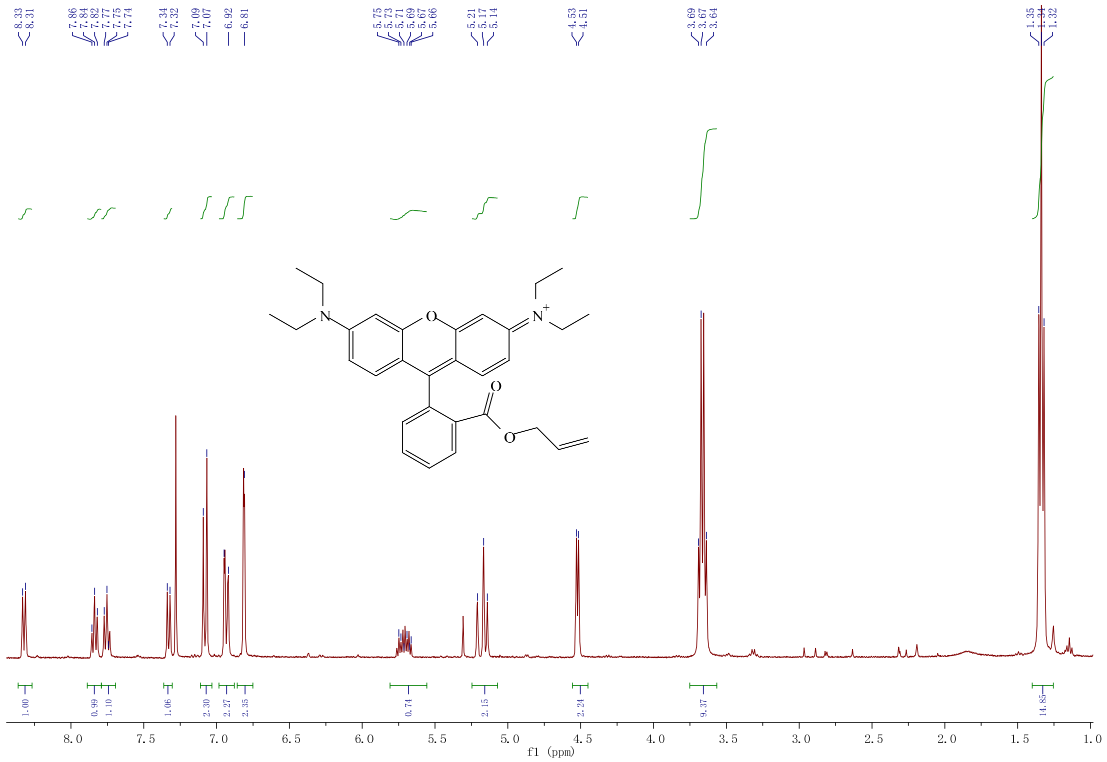
(3) Yuan, L.; Lin, W.; Song, J. *Chem Commun (Camb)* **2010**, *46*, 7930.

(4) Chen, S. M.; Lu, J. X.; Sun, C. D.; Ma, H. M. *Analyst* **2010**, *135*, 577.

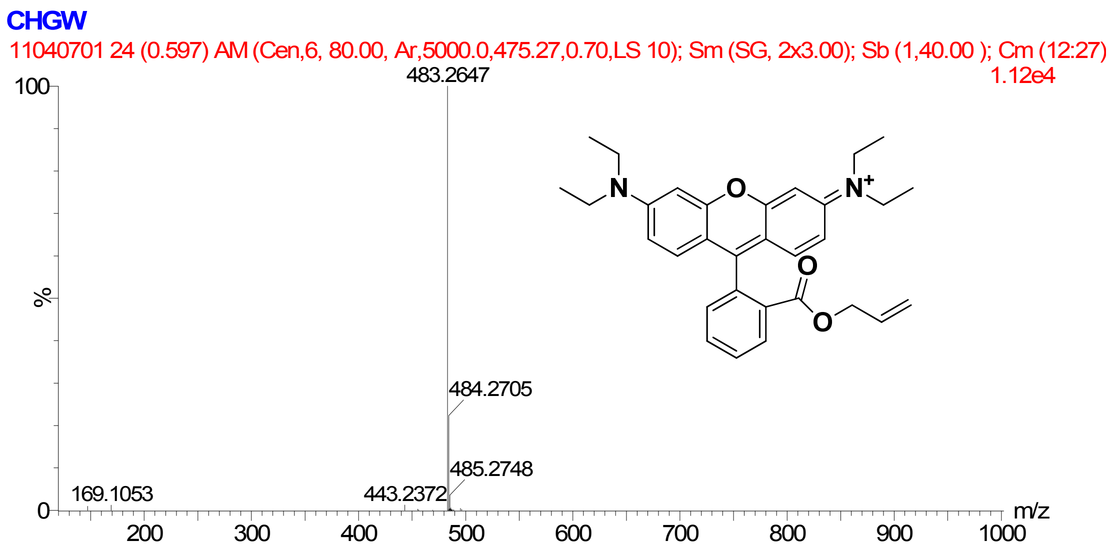
(5) Kundu, K.; Knight, S. F.; Willett, N.; Lee, S.; Taylor, W. R.; Murthy, N. *Angew Chem Int Edit* **2009**, *48*, 299.

(6) Velapoldi, R. A.; Tonnesen, H. H. *J Fluoresc* **2004**, *14*, 465.

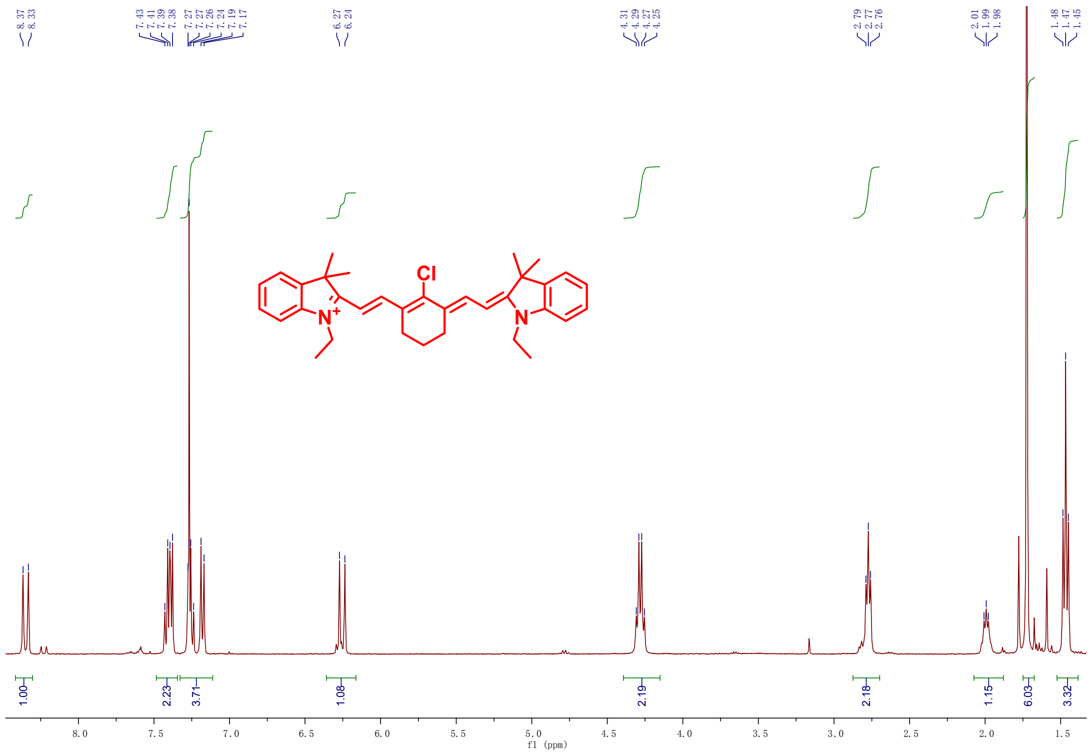
NMR of compounds



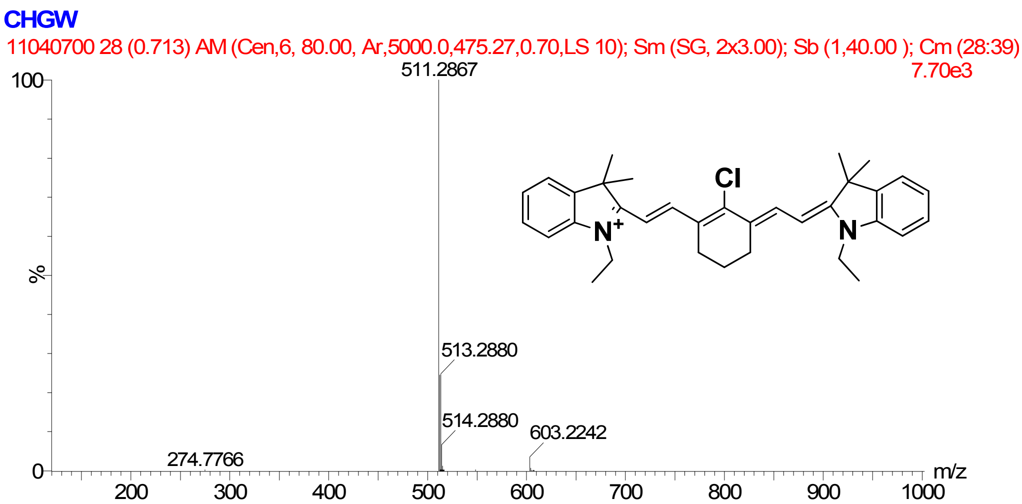
[1]H-NMR spectrum of compound **1**



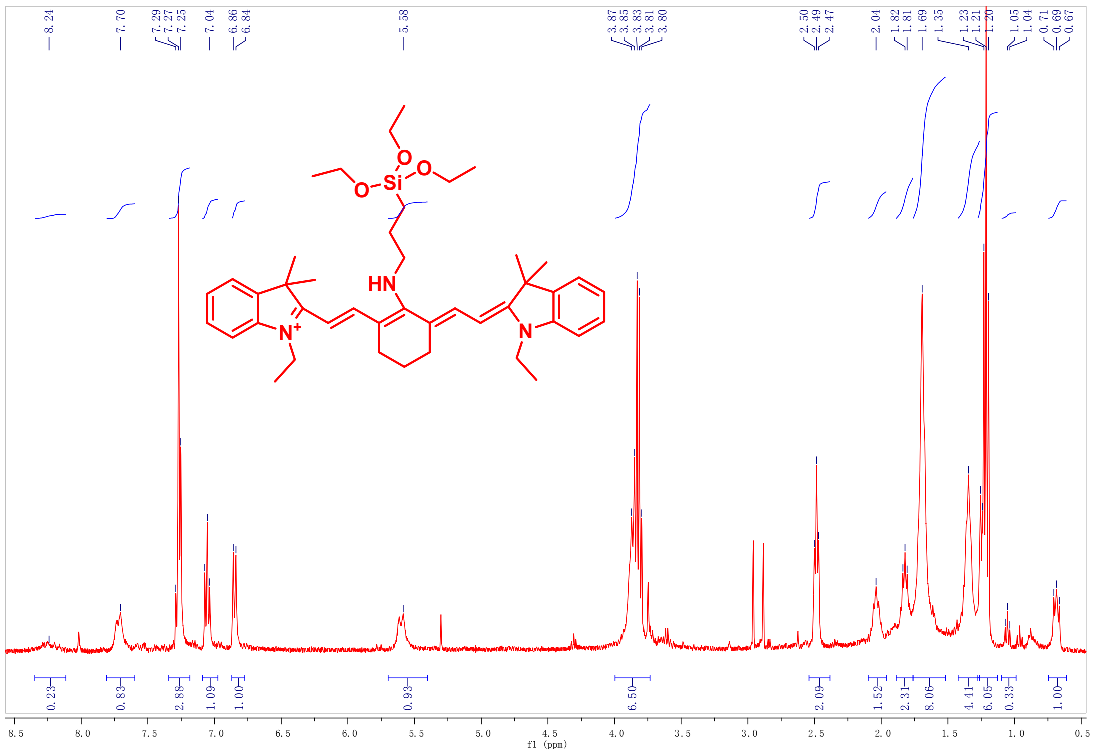
TOF ESI+MS of **1**



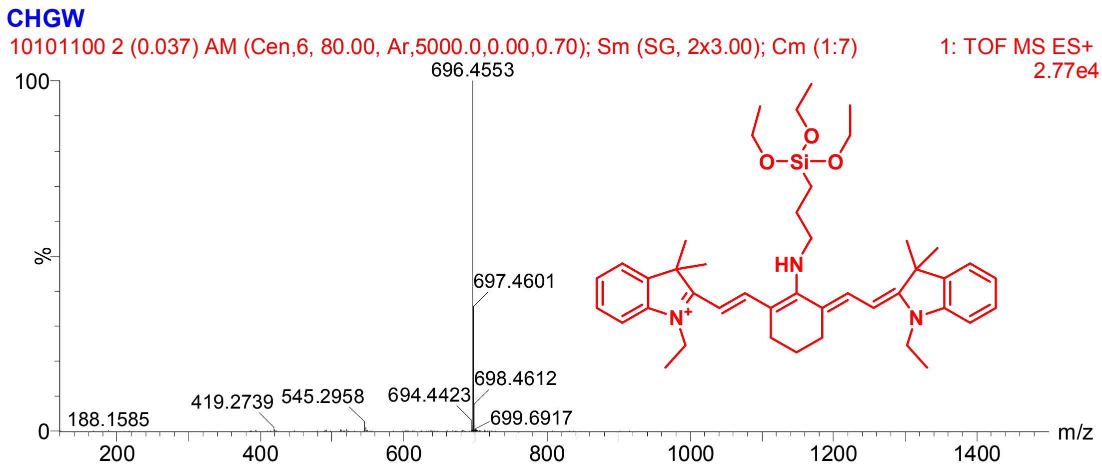
^{1}H-NMR spectrum of compound **2**



TOF ESI+MS of **2**.



^{1}H-NMR spectrum of compound **3**



TOF ESI-MS of **3**.

The ground state geometry optimization of 2

Standard orientation:

Center Number	Atomic Number	Atomic Type	X	Y	Z
1	6	0	7.228694	-0.119815	-0.201490
2	6	0	6.688199	-1.396056	0.008866

3	6	0	8.602663	0.103843	-0.285318
4	6	0	6.474366	3.043869	0.799653
5	6	0	4.945350	0.212068	-0.149037
6	6	0	7.531130	-2.494480	0.134617
7	6	0	5.165862	-1.303965	0.058736
8	6	0	3.746184	0.929582	-0.178621
9	6	0	8.920124	-2.294846	0.050463
10	6	0	9.444336	-1.011298	-0.156025
11	6	0	2.467053	0.374361	-0.055088
12	6	0	1.258775	1.091267	-0.072108
13	6	0	-0.000002	0.466040	0.004142
14	6	0	-1.258778	1.091276	-0.071954
15	6	0	1.263754	2.612467	-0.207068
16	6	0	-1.263828	2.612504	-0.206634
17	6	0	-2.467056	0.374358	-0.055048
18	6	0	-3.746186	0.929619	-0.178390
19	6	0	-4.945355	0.212100	-0.148959
20	6	0	-5.165856	-1.303993	0.058388
21	6	0	-6.688196	-1.396068	0.008569
22	6	0	-7.228700	-0.119768	-0.201407
23	6	0	-7.531126	-2.494523	0.134058
24	6	0	-4.645629	-1.780584	1.443491
25	6	0	-8.602671	0.103921	-0.285103
26	6	0	-9.444342	-1.011251	-0.156079
27	6	0	-8.920124	-2.294858	0.050028
28	6	0	0.000070	3.251899	0.381378
29	6	0	6.392265	2.257974	-0.516060
30	6	0	-4.540272	-2.142162	-1.092312
31	6	0	-6.392279	2.258108	-0.515365
32	6	0	4.645713	-1.780144	1.444015
33	6	0	4.540212	-2.142486	-1.091668
34	6	0	-6.474339	3.043646	0.800563
35	17	0	0.000000	-1.390471	0.200280
36	1	0	7.131554	-3.490358	0.295875
37	1	0	3.822788	2.001640	-0.309814
38	1	0	-6.656877	4.101409	0.585982
39	1	0	-5.541648	2.963846	1.367462
40	1	0	-7.291670	2.674232	1.427647
41	1	0	6.656909	4.101572	0.584780
42	1	0	7.291706	2.674620	1.426822
43	1	0	5.541685	2.964232	1.366590
44	1	0	-3.556461	-1.722511	1.509026
45	1	0	-4.940065	-2.823180	1.600147
46	1	0	-5.073957	-1.177589	2.249694

47	1	0	-3.448679	-2.089532	-1.084646
48	1	0	-4.897297	-1.796037	-2.066878
49	1	0	-4.830918	-3.190682	-0.972404
50	1	0	3.448619	-2.089864	-1.083950
51	1	0	4.830873	-3.190968	-0.971456
52	1	0	4.897172	-1.796659	-2.066363
53	1	0	3.556550	-1.722040	1.509590
54	1	0	5.074093	-1.176912	2.250014
55	1	0	4.940151	-2.822695	1.600962
56	1	0	-7.319564	2.357798	-1.083780
57	1	0	-5.592236	2.639858	-1.154005
58	1	0	5.592201	2.639554	-1.154775
59	1	0	7.319529	2.357511	-1.084536
60	1	0	0.000046	4.329057	0.183985
61	1	0	0.000264	3.122521	1.471432
62	1	0	-9.591261	-3.140982	0.147433
63	1	0	-10.518218	-0.871970	-0.215590
64	1	0	-9.019809	1.091594	-0.438722
65	1	0	-7.131552	-3.490448	0.295027
66	1	0	-3.822788	2.001713	-0.309289
67	1	0	-2.388915	-0.697979	0.050885
68	1	0	-2.143584	3.020205	0.301584
69	1	0	-1.358488	2.886141	-1.268257
70	1	0	1.357935	2.885925	-1.268780
71	1	0	2.143706	3.020293	0.300698
72	1	0	2.388915	-0.697951	0.051105
73	1	0	10.518209	-0.872037	-0.215626
74	1	0	9.591264	-3.140945	0.148070
75	1	0	9.019801	1.091470	-0.439236
76	7	0	6.164424	0.815571	-0.304495
77	7	0	-6.164432	0.815646	-0.304201

The ground state geometry optimization of 3

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-6.021564	-3.243392	-0.433480
2	6	0	-5.834539	-1.878480	-0.275991
3	6	0	-7.263332	-3.861859	-0.307032
4	6	0	-1.887422	5.196396	3.874282
5	6	0	-3.742315	-2.923547	-0.349257

6	6	0	-6.922617	-1.086502	0.071245
7	6	0	-4.385776	-1.465355	-0.533635
8	6	0	-2.415989	-3.252227	-0.293442
9	6	0	-8.188660	-1.677790	0.224473
10	6	0	-8.358457	-3.050984	0.013971
11	6	0	-1.380481	-2.329815	-0.271551
12	6	0	-0.013050	-2.571803	-0.151587
13	6	0	1.026311	-1.578010	-0.035634
14	6	0	2.451646	-1.999833	0.055538
15	6	0	0.406078	-4.039184	-0.118897
16	6	0	2.782508	-3.472352	0.244410
17	6	0	3.539904	-1.161903	-0.035592
18	6	0	4.862567	-1.591415	0.031346
19	6	0	5.953769	-0.808008	-0.068870
20	6	0	6.053783	0.714334	-0.255245
21	6	0	7.565719	0.884822	-0.283504
22	6	0	8.168880	-0.379462	-0.129844
23	6	0	8.349779	2.023262	-0.444126
24	6	0	-0.306612	6.120773	-2.199588
25	6	0	9.565752	-0.529455	-0.147940
26	6	0	10.339453	0.628810	-0.303347
27	6	0	9.747431	1.889994	-0.457958
28	6	0	1.620994	-4.290711	0.763455
29	6	0	-4.650191	-5.293638	-0.182919
30	6	0	8.118268	-3.137967	1.549319
31	6	0	7.440322	-2.763251	0.213927
32	6	0	-3.955133	-0.405287	0.505547
33	6	0	-4.164216	-1.177971	-2.032064
34	6	0	5.429682	1.169081	-1.596774
35	6	0	5.439530	1.487490	0.936861
36	6	0	-0.596112	0.288457	-0.115164
37	6	0	-0.784494	1.799202	0.010762
38	6	0	-2.299859	2.070617	-0.207816
39	6	0	-5.272990	-5.800161	1.120118
40	6	0	-1.442109	5.109662	-2.054270
41	6	0	-5.292729	4.411809	-0.852992
42	6	0	-2.374479	5.364092	2.441146
43	6	0	-6.569936	4.865789	-0.147192
44	1	0	-1.612775	-1.304441	-0.290235
45	1	0	-9.340017	-3.500116	0.106788
46	1	0	-9.036955	-1.062933	0.502050
47	1	0	-2.170929	-4.299069	-0.247732
48	1	0	-6.806366	-0.017764	0.209874
49	1	0	-0.419013	-4.638678	0.256006

50	1	0	0.626096	-4.383449	-1.136963
51	1	0	1.493401	0.377829	0.128535
52	1	0	-7.385100	4.958965	-0.867882
53	1	0	-6.419111	5.835003	0.330487
54	1	0	-6.868178	4.149014	0.619293
55	1	0	-1.968915	6.141176	4.414217
56	1	0	-0.843851	4.878578	3.894478
57	1	0	-2.482525	4.447829	4.399623
58	1	0	-0.115993	6.325878	-3.254890
59	1	0	0.613502	5.742692	-1.751937
60	1	0	-0.566366	7.058818	-1.707204
61	1	0	8.228216	-4.221055	1.606301
62	1	0	7.505926	-2.810606	2.390244
63	1	0	9.100244	-2.704977	1.676196
64	1	0	-5.129309	-6.879577	1.162454
65	1	0	-6.342454	-5.594302	1.197698
66	1	0	-4.779862	-5.351356	1.983393
67	1	0	-2.379455	5.504750	-2.496192
68	1	0	-1.175633	4.165892	-2.532192
69	1	0	-5.444516	3.416818	-1.324619
70	1	0	-4.985919	5.154527	-1.618302
71	1	0	-3.421248	5.665438	2.422077
72	1	0	-1.768631	6.099548	1.911414
73	1	0	-2.962899	1.267528	0.386943
74	1	0	-2.564023	1.784792	-1.262267
75	1	0	-0.191629	2.361081	-0.708785
76	1	0	-0.462143	2.094837	1.010572
77	1	0	-1.176448	-0.102463	0.717062
78	1	0	-0.955688	0.026055	-1.116553
79	1	0	4.351507	1.371609	0.992066
80	1	0	5.660960	2.550844	0.841812
81	1	0	5.865455	1.133931	1.875941
82	1	0	4.342563	1.041410	-1.609405
83	1	0	5.848774	0.594224	-2.422501
84	1	0	5.647706	2.223243	-1.770215
85	1	0	-3.130199	-0.880124	-2.253098
86	1	0	-4.843637	-0.376447	-2.383672
87	1	0	-4.383383	-2.071545	-2.611384
88	1	0	-2.990025	0.038571	0.371770
89	1	0	-4.023213	-0.886888	1.474208
90	1	0	-4.680816	0.405315	0.475213
91	1	0	8.026717	-3.113082	-0.639160
92	1	0	6.452907	-3.253960	0.190478
93	1	0	-3.602133	-5.534592	-0.174677

94	1	0	-5.113729	-5.744398	-1.059995
95	1	0	1.883111	-5.351026	0.768142
96	1	0	1.395511	-4.017672	1.799116
97	1	0	10.375618	2.759793	-0.608231
98	1	0	11.419158	0.531458	-0.302380
99	1	0	10.042987	-1.492199	-0.064379
100	1	0	7.893184	3.000294	-0.564385
101	1	0	5.022040	-2.667938	0.159997
102	1	0	3.422668	-0.096207	-0.192627
103	1	0	3.614264	-3.553073	0.947632
104	1	0	3.109217	-3.896299	-0.711934
105	1	0	-7.394839	-4.923097	-0.490505
106	7	0	0.741633	-0.268500	0.009579
107	7	0	7.185587	-1.339353	0.008577
108	7	0	-4.759788	-3.823396	-0.320092
109	8	0	-4.250723	4.303307	0.129353
110	8	0	-1.654763	4.870945	-0.657338
111	8	0	-2.243258	4.083918	1.768872
112	14	0	-2.672984	3.860684	0.179609
