Supporting Information

Cell Membrane-Specific Fluorescent Probe Featuring Dual and Aggregation-Induced Emissions

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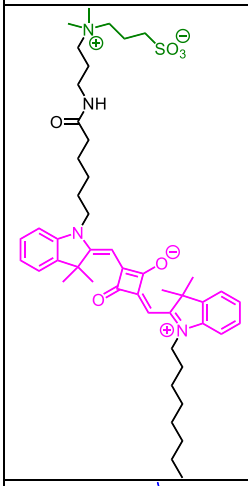
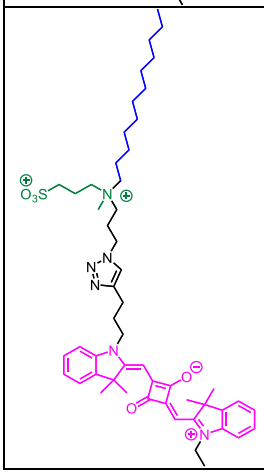
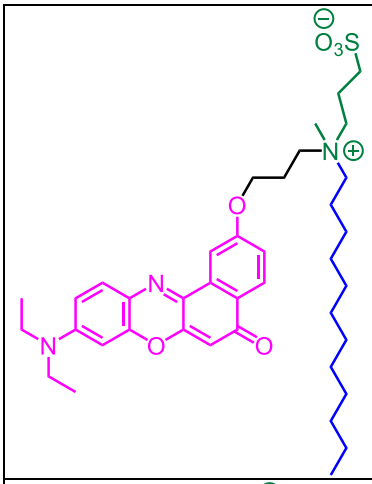
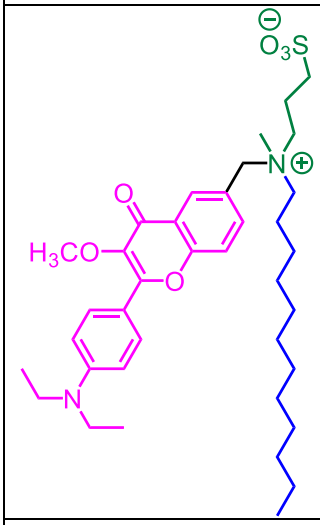
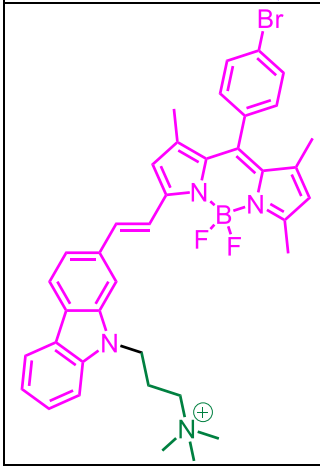
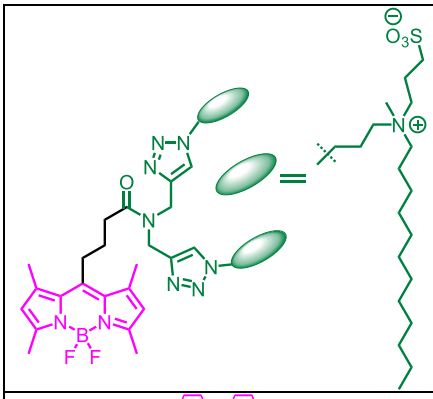
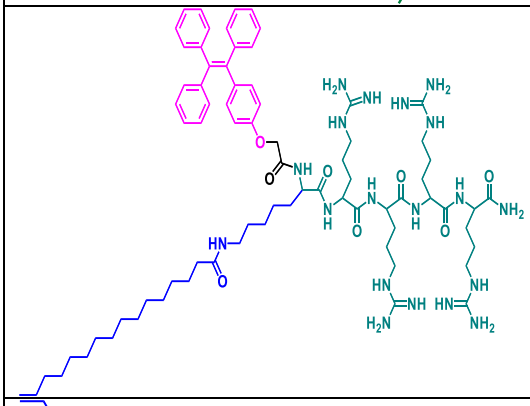
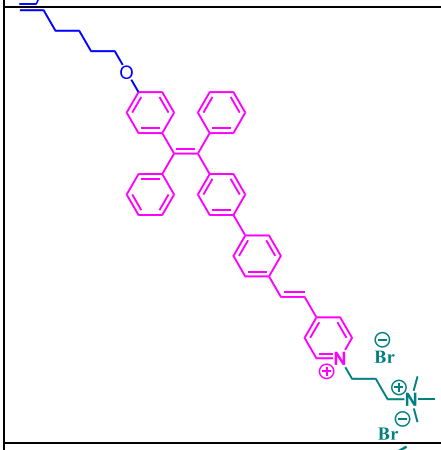
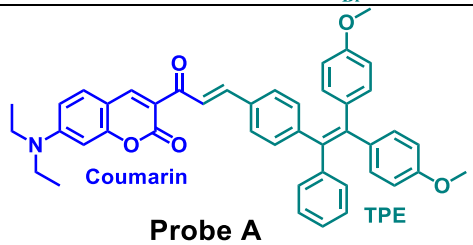
Contents

Table S1. Summary of cell membrane-specific fluorescent probes

Probe	Solvent	λ_{ab} (nm)	λ_{em} (nm)	QY (%)
	pH 7.4 buffer	628	636	2.2[1]
	pH 7.4 buffer	626	634	1.4[1]

	pH 7.4 Buffer	521	657	0.2[2-4]
	water	412	537	<0.5[5]
	water	594	615	1.0[6]

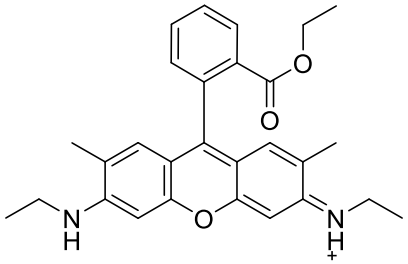
	pH 7.4 Buffer	501	505	3.0[7]
	water	330	460	unknown[8]
	THF/DMSO	365	650	0.5[9] water/DMSO (99% water fraction)
 **Probe A** This work	THF/H_2O (90% water fraction)	457	482 and 591	22.7 under 430 nm excitation

Calculation of fluorescence quantum yield of the probe

All absorbance spectra and emission spectra were obtained at room temperature using a standard 1 cm path length quartz fluorescence cuvette. The concentration of probes **A** or **B** was 5 μM for each measurement and the solutions of the measurement were 10 % ethanol in different pH buffers. For the selectivity experiment, all metal ion solutions were freshly made, and the concentrations of all the metal ions in testing samples were controlled at 100 μM. The slit widths of excitation and emission of fluorescence measurements were both set to 5 nm. The quantum yields were calculated according to the reference as reported.[10] Fluorescence quantum yields were calculated by measuring fluorescence of fluorophores of the known quantum yield with the same experimental parameters (excitation wavelength and slit width). The samples and the reference solutions were freshly prepared under identical conditions. The fluorescence quantum yields were calculated using the following equation:

$$\Phi_X = \Phi_{st}\left(\frac{Grad_X}{Grad_{st}}\right)\left(\frac{\eta^2{}_X}{\eta^2{}_{st}}\right)$$

Where the subscripts 'st' and 'X' stand for standard and test, respectively, Φ is the fluorescence quantum yield, "Grad" represents the gradient from the plot of integrated fluorescence intensity versus absorbance, and η is the refractive index of the solvent. Rhodamine 6G with fluorescence quantum yield of 0.95 in H_2O was used as a standard reference[11] to calculate the quantum yield of the flurescent probe.



rhodamine 6G

Fluorescent quantum yield of probe **A** is 22.7% in a mixed water and THF solution containing 95% water under excitation at 430 nm.

Theoretical calculation on probe A.

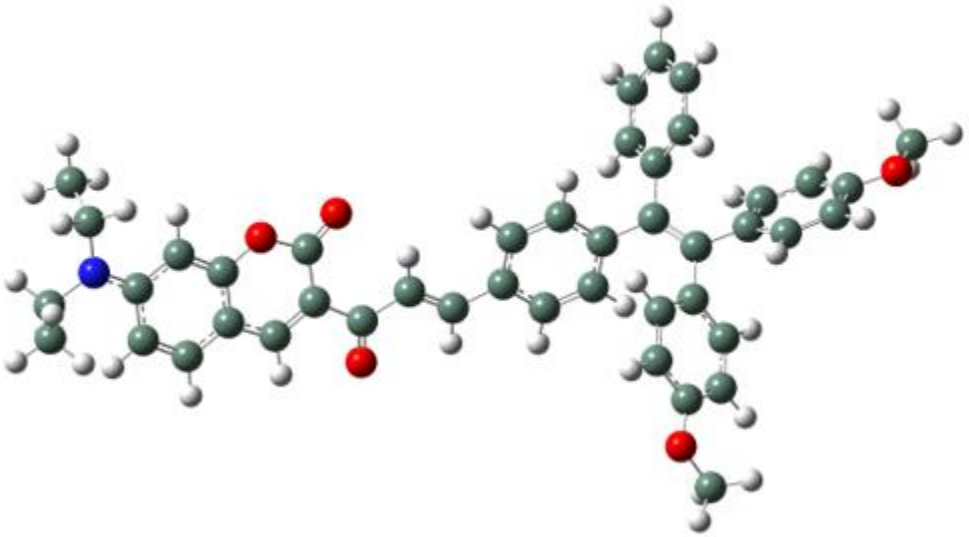


Figure S1. GaussView representation of probe **A**.

Table S2. Computational results for probe **A**.

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Charge	0	
Spin	Singlet	
Solvation	scrf=solvent=tetrahydrofuran	
E(RAPFD)	-2129.796108	Hartree
RMS Gradient Norm	0.000003	Hartree/Bohr
Imaginary Freq		
Dipole Moment	7.155786	Debye
Point Group	C1	
Job cpu time:	**5 days 14 hours 30 minutes 15.1 seconds.**	

Table S3. Calculated atomic coordinates for probe **A** in tetrahydrofuran.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	9.637915	1.083696	-0.33827	40	O	-3.92443	5.572479	1.031836
2	C	9.729741	-0.1597	0.362031	41	O	-10.6064	-1.2522	0.720013
3	C	8.548821	-0.92384	0.506678	42	N	10.90939	-0.58833	0.871693
4	C	7.363482	-0.46234	-0.02085	43	C	12.16012	0.123383	0.644202
5	C	7.264198	0.760399	-0.71326	44	C	12.42401	1.207681	1.681705
6	C	8.445784	1.518545	-0.85209	45	C	11.00326	-1.79629	1.679797
7	O	6.266766	-1.24378	0.139075	46	C	11.19732	-3.05632	0.844613
8	C	5.00276	-0.91447	-0.34242	47	O	4.146219	-1.74959	-0.15444
9	C	4.878315	0.365671	-1.02233	48	O	3.625147	1.818189	-2.36898
10	C	6.003131	1.136145	-1.20079	49	C	-11.2535	-2.10741	-0.20424
11	C	-0.17581	0.349252	-1.17752	50	C	-4.6681	6.60867	0.417659
12	C	-1.28699	0.966462	-1.77376	51	H	10.51347	1.709142	-0.45395
13	C	-2.57892	0.608397	-1.42597	52	H	8.543111	-1.88422	1.004996
14	C	-2.81347	-0.38602	-0.46904	53	H	8.398304	2.47062	-1.37345
15	C	-1.70462	-1.03022	0.102016	54	H	5.892578	2.075673	-1.73612
16	C	-0.41401	-0.66358	-0.23258	55	H	-1.12712	1.738166	-2.52271
17	C	-5.06346	-2.82227	1.075017	56	H	-3.4238	1.102662	-1.895
18	C	-5.28053	-4.19363	1.133697	57	H	-1.86861	-1.82127	0.828265
19	C	-4.86617	-5.01402	0.085525	58	H	0.420078	-1.17168	0.241953
20	C	-4.2207	-4.45227	-1.0141	59	H	-5.38939	-2.18343	1.890405
21	C	-3.99607	-3.08065	-1.06601	60	H	-5.77063	-4.6253	2.002304
22	C	-4.42998	-2.24444	-0.03131	61	H	-5.03745	-6.08609	0.12982
23	C	-4.85774	1.561558	0.394425	62	H	-3.8889	-5.08496	-1.83301
24	C	-3.79957	1.968673	1.220206	63	H	-3.48618	-2.64633	-1.92189
25	C	-3.51643	3.307327	1.41622	64	H	-3.19259	1.21622	1.71451
26	C	-4.28758	4.289975	0.784701	65	H	-2.69998	3.617597	2.061568
27	C	-5.35882	3.90637	-0.02712	66	H	-5.98062	4.643541	-0.52241
28	C	-5.63715	2.55391	-0.2014	67	H	-6.4758	2.26518	-0.82911
29	C	-8.5344	-1.43342	-0.5563	68	H	-8.96821	-2.07266	-1.31689
30	C	-7.19449	-1.07413	-0.65448	69	H	-6.60793	-1.44878	-1.48779
31	C	-6.58491	-0.24713	0.290127	70	H	-6.93088	0.901378	2.078782
32	C	-7.37595	0.240149	1.340416	71	H	-9.31136	0.24767	2.287181
33	C	-8.70654	-0.11814	1.46264	72	H	2.372563	-0.45235	-0.33103
34	C	-9.29772	-0.96203	0.51576	73	H	1.202502	1.552032	-2.33231
35	C	3.595955	0.887936	-1.56345	74	H	12.96063	-0.6213	0.663015
36	C	2.327493	0.325317	-1.08136	75	H	12.16724	0.532851	-0.3685
37	C	1.15521	0.778233	-1.56684	76	H	11.63997	1.969343	1.672584
38	C	-4.18718	-0.7831	-0.0943	77	H	13.3801	1.701601	1.485672
39	C	-5.15978	0.130017	0.179406	78	H	12.46545	0.784106	2.689258

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
79	H	11.84576	-1.66182	2.36367	85	H	-11.2635	-1.67288	-1.20905
80	H	10.11852	-1.87432	2.316562	86	H	-10.7765	-3.09228	-0.23575
81	H	11.27187	-3.93615	1.490083	87	H	-4.20869	7.54024	0.742675
82	H	10.36433	-3.21206	0.154276	88	H	-4.61696	6.541791	-0.67385
83	H	12.11464	-2.99565	0.252045	89	H	-5.71497	6.5908	0.737424
84	H	-12.2765	-2.21378	0.151511					

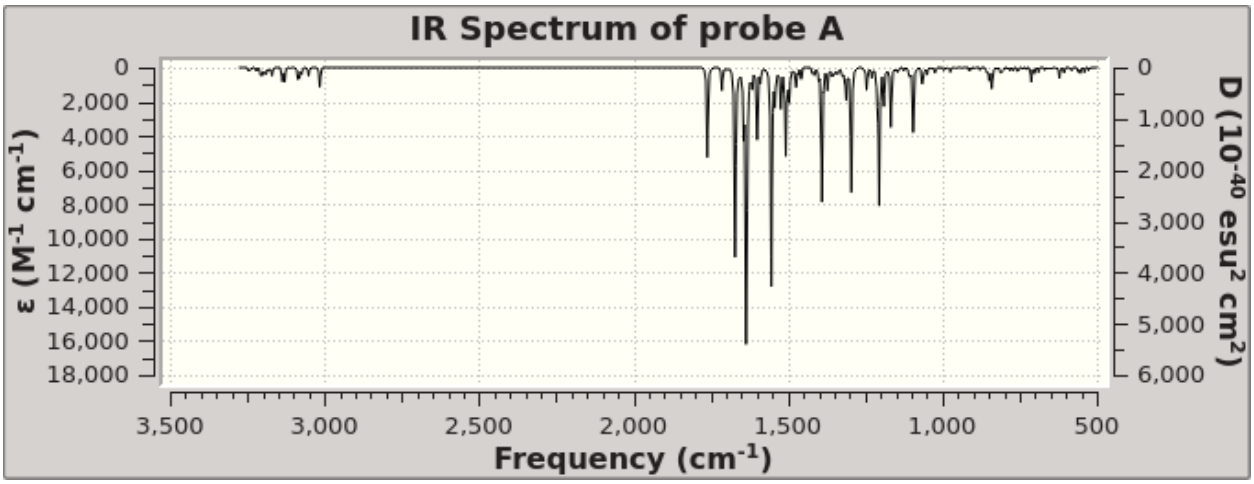

Figure S2. Calculated IR spectrum for probe **A**.

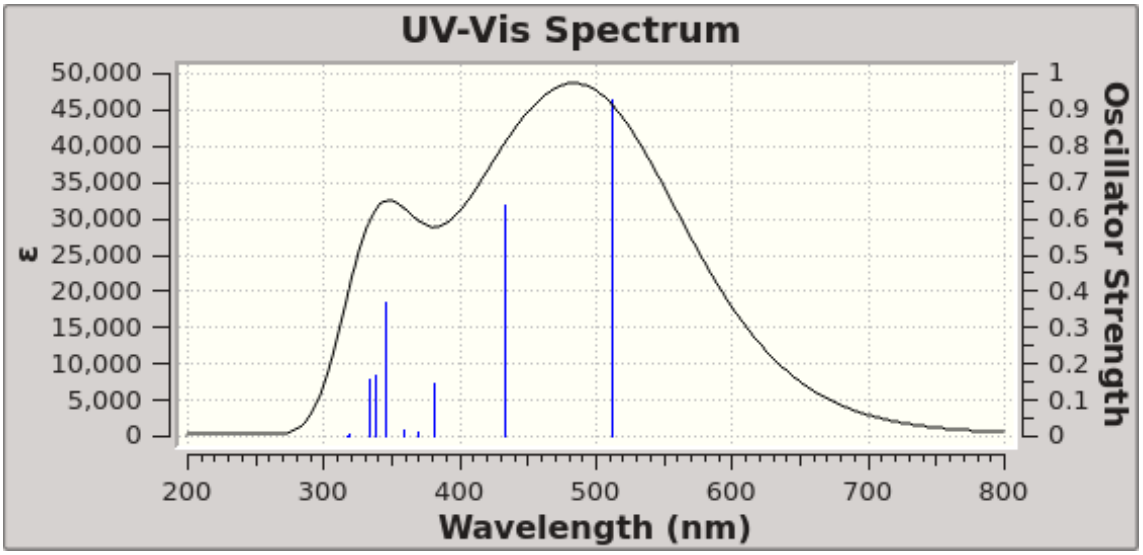

Figure S3. Calculated UV-Vis spectrum for probe **A** in tetrahydrofuran.

Table S4. Excitation energies and oscillator strengths listing for probe **A** in tetrahydrofuran.

Excited State	Nature	E (eV)	λ (nm)	*f*	Orbital transitions	Normalized coefficient
1:	A	2.4204	512.24	0.9302	174 -> 176	-0.10748
					175 -> 176	0.68973
2:	A	2.8589	433.68	0.6367	174 -> 176	0.68774
					175 -> 176	0.11599
3:	A	3.2556	380.83	0.1468	175 -> 177	0.68346
					175 -> 178	0.13196
4:	A	3.3521	369.87	0.0126	167 -> 176	0.67770
5:	A	3.4498	359.40	0.0155	173 -> 176	0.69364
					173 -> 177	0.10867
6:	A	3.5852	345.82	0.3701	172 -> 176	0.56836
					174 -> 177	-0.24306
					175 -> 178	-0.31289
7:	A	3.6737	337.50	0.1680	174 -> 177	0.55866
					174 -> 178	-0.11353
					175 -> 178	-0.39479
8:	A	3.7174	333.52	0.1581	172 -> 176	0.39362
					174 -> 177	0.31157
					175 -> 177	-0.10926
					175 -> 178	0.46157
9:	A	3.8835	319.26	0.0029	169 -> 176	0.68039
					169 -> 177	-0.12875
10:	A	3.9163	316.58	0.0024	165 -> 176	0.12347
					166 -> 176	0.11069
					168 -> 176	-0.12941
					170 -> 176	0.20947
					171 -> 176	0.26772
					175 -> 179	0.55456

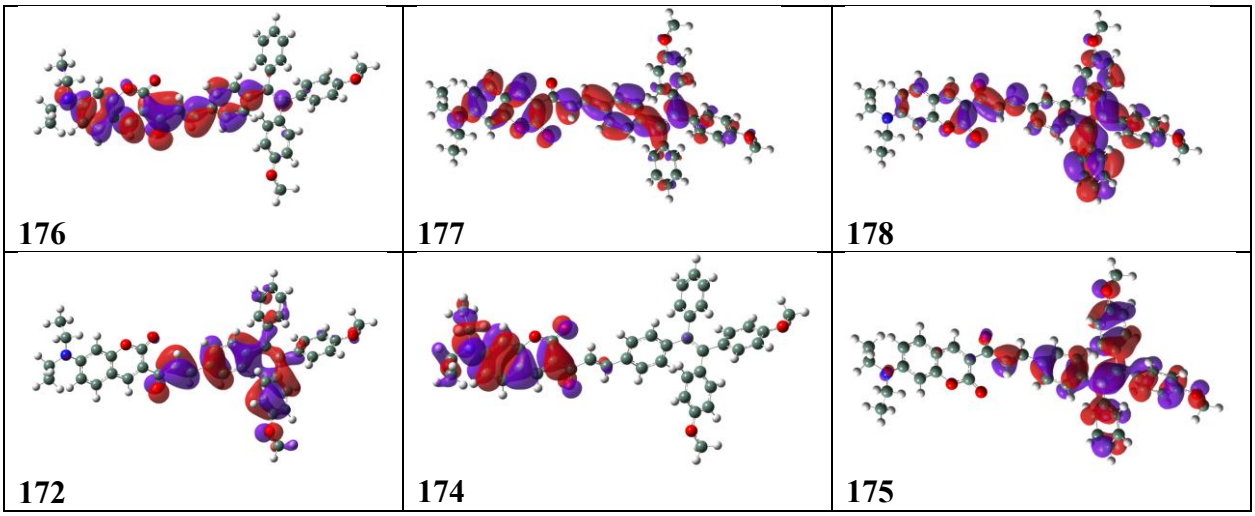

Figure S4. Drawings of selected molecular orbitals listed in Table S3.

Table S5. Calculated atomic coordinates for probe **A** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	9.637915	1.083696	-0.33827	19	C	-4.86617	-5.01402	0.085525
2	C	9.729742	-0.1597	0.362031	20	C	-4.2207	-4.45227	-1.0141
3	C	8.548822	-0.92384	0.506678	21	C	-3.99607	-3.08065	-1.06601
4	C	7.363482	-0.46234	-0.02085	22	C	-4.42998	-2.24444	-0.03131
5	C	7.264199	0.760399	-0.71326	23	C	-4.85774	1.561559	0.394425
6	C	8.445784	1.518545	-0.85209	24	C	-3.79957	1.968674	1.220206
7	O	6.266767	-1.24378	0.139075	25	C	-3.51643	3.307327	1.41622
8	C	5.00276	-0.91447	-0.34242	26	C	-4.28758	4.289975	0.784701
9	C	4.878315	0.365671	-1.02233	27	C	-5.35882	3.906371	-0.02712
10	C	6.003131	1.136145	-1.20079	28	C	-5.63715	2.55391	-0.2014
11	C	-0.17581	0.349252	-1.17752	29	C	-8.5344	-1.43342	-0.5563
12	C	-1.28699	0.966462	-1.77376	30	C	-7.19449	-1.07413	-0.65448
13	C	-2.57892	0.608397	-1.42597	31	C	-6.58491	-0.24713	0.290127
14	C	-2.81347	-0.38602	-0.46904	32	C	-7.37595	0.240149	1.340416
15	C	-1.70462	-1.03021	0.102016	33	C	-8.70654	-0.11814	1.46264
16	C	-0.41401	-0.66358	-0.23258	34	C	-9.29772	-0.96203	0.51576
17	C	-5.06346	-2.82227	1.075017	35	C	3.595955	0.887936	-1.56345
18	C	-5.28053	-4.19363	1.133698	36	C	2.327493	0.325318	-1.08135

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
37	C	1.15521	0.778233	-1.56684	64	H	-3.19259	1.21622	1.71451
38	C	-4.18718	-0.7831	-0.0943	65	H	-2.69998	3.617597	2.061568
39	C	-5.15978	0.130017	0.179406	66	H	-5.98062	4.643541	-0.52241
40	O	-3.92443	5.57248	1.031836	67	H	-6.4758	2.26518	-0.82911
41	O	-10.6064	-1.2522	0.720013	68	H	-8.96821	-2.07266	-1.31689
42	N	10.90939	-0.58833	0.871693	69	H	-6.60793	-1.44878	-1.48779
43	C	12.16012	0.123383	0.644202	70	H	-6.93088	0.901378	2.078782
44	C	12.42401	1.207682	1.681706	71	H	-9.31136	0.24767	2.287182
45	C	11.00326	-1.79629	1.679797	72	H	2.372563	-0.45235	-0.33103
46	C	11.19732	-3.05632	0.844614	73	H	1.202502	1.552032	-2.33231
47	O	4.146219	-1.74959	-0.15444	74	H	12.96063	-0.6213	0.663015
48	O	3.625148	1.81819	-2.36898	75	H	12.16724	0.532851	-0.3685
49	C	-11.2535	-2.10741	-0.20424	76	H	11.63997	1.969343	1.672584
50	C	-4.6681	6.608671	0.417659	77	H	13.3801	1.701601	1.485672
51	H	10.51347	1.709142	-0.45395	78	H	12.46545	0.784106	2.689259
52	H	8.543112	-1.88422	1.004997	79	H	11.84576	-1.66182	2.36367
53	H	8.398305	2.47062	-1.37345	80	H	10.11852	-1.87432	2.316563
54	H	5.892579	2.075673	-1.73611	81	H	11.27187	-3.93615	1.490083
55	H	-1.12712	1.738166	-2.52271	82	H	10.36433	-3.21206	0.154276
56	H	-3.4238	1.102662	-1.895	83	H	12.11464	-2.99565	0.252045
57	H	-1.86861	-1.82127	0.828265	84	H	-12.2765	-2.21378	0.151511
58	H	0.420078	-1.17168	0.241953	85	H	-11.2635	-1.67288	-1.20905
59	H	-5.38939	-2.18343	1.890405	86	H	-10.7765	-3.09228	-0.23575
60	H	-5.77063	-4.6253	2.002304	87	H	-4.20869	7.540241	0.742675
61	H	-5.03745	-6.08609	0.12982	88	H	-4.61696	6.541792	-0.67385
62	H	-3.8889	-5.08496	-1.83301	89	H	-5.71497	6.590801	0.737424
63	H	-3.48618	-2.64633	-1.92189					

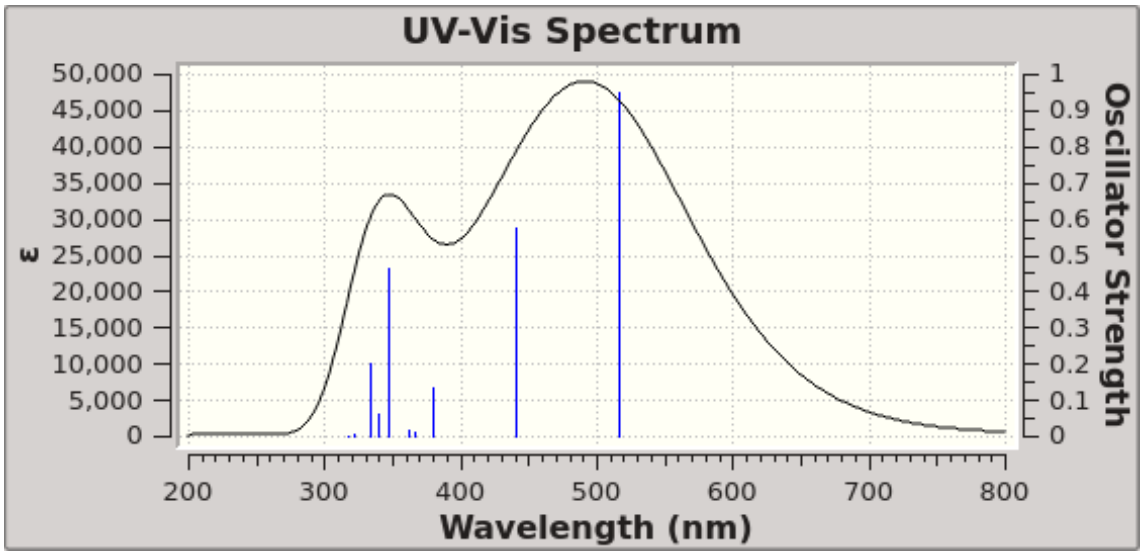

Figure S5. Calculated UV-Vis spectrum for probe **A** in water.

Table S6. Excitation energies and oscillator strengths listing for probe **A** in water.

Excited State	Nature	E (eV)	λ (nm)	*f*	Orbital transitions	Normalized coefficient
1:	A	2.40391	515.76	0.9474	174 -> 176	-0.10633
					175 -> 176	0.69044
2:	A	2.8186	439.87	0.5774	174 -> 176	0.68943
					175 -> 176	0.11337
3:	A	3.2635	379.91	0.1333	175 -> 177	0.68382
					175 -> 178	0.13178
4:	A	3.3897	365.77	0.0111	167 -> 176	0.67897
5:	A	3.4220	362.32	0.0162	173 -> 176	0.69372
					173 -> 177	0.10447
6:	A	3.5762	346.69	0.4654	172 -> 176	0.46998
					174 -> 177	-0.43870
					175 -> 178	-0.25404
7:	A	3.6478	339.88	0.0640	172 -> 176	0.23794
					174 -> 177	0.48947
					174 -> 178	-0.10860
					175 -> 178	-0.41533
8:	A	3.7193	333.35	0.2030	172 -> 176	0.44788
					174 -> 177	0.19053
					175 -> 177	-0.10867
					175 -> 178	0.47858
9:	A	3.8458	322.39	0.0029	169 -> 176	0.68315
					169 -> 177	-0.13039
10:	A	3.9132	316.83	0.0018	165 -> 176	0.13700
					166 -> 176	0.11616
					168 -> 176	-0.12287
					170 -> 176	0.23998
					171 -> 176	0.27266
					175 -> 179	0.53706

Instruments and materials

Unless specifically indicated, all reagents and solvents were obtained from commercial suppliers and used without further purification. Compounds **1** and **6** were prepared according to the reported procedures.[12-13] ^{1}H NMR and ^{13}C NMR spectra were performed with a Varian Unity Inova NMR spectrophotometer at 400 MHz and 100 MHz, respectively. Standard chemical shifts (δ) of intermediates and probes **A** and **B** were determined by using internal standards in ppm from solvent residual peaks (1H: δ 7.26 for $CDCl_3$, 13C: δ 77.3 for $CDCl_3$). High-resolution mass spectrometer data (HRMS) were obtained by using a double focusing magnetic mass spectrometer and a fast atom bombardment (FAB) ionization mass spectrometer. Absorption and fluorescence spectra were collected by using a Cary 60 UV-Vis spectrometer and Jobin Yvon Fluoromax-4 spectrofluorometer, respectively.

Synthesis of intermediates

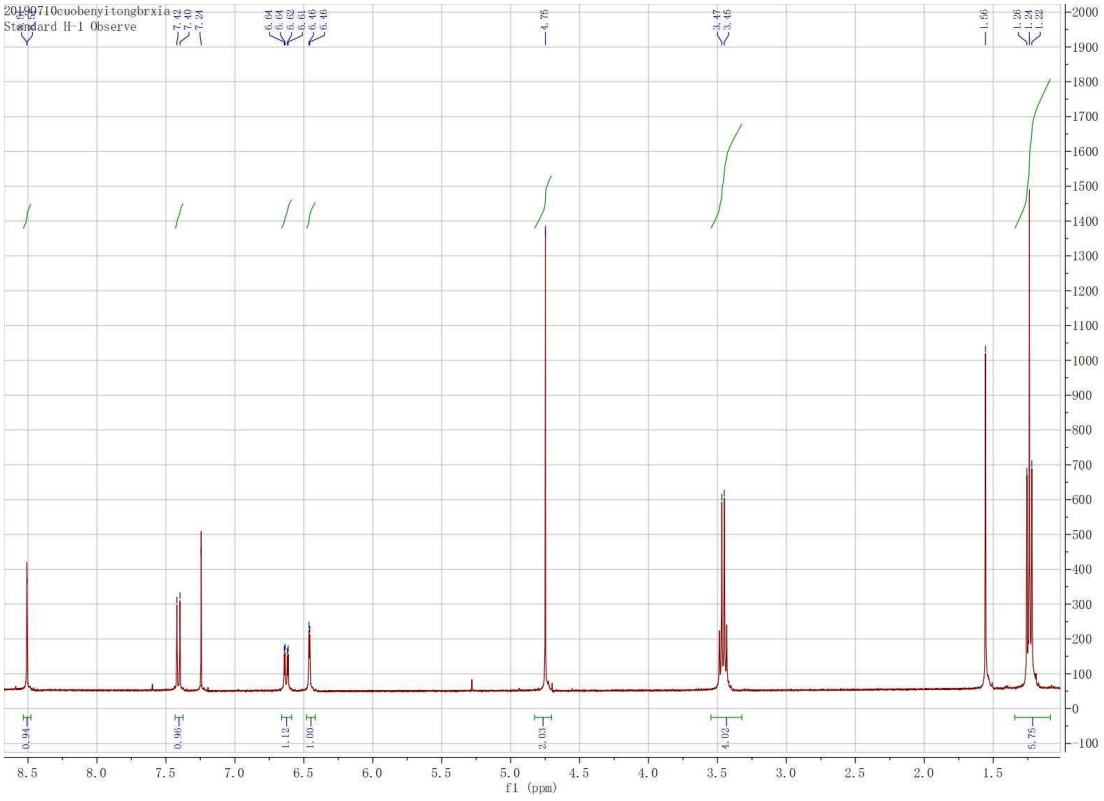

Figure S6. ^{1}H NMR spectrum of compound **7** in $CDCl_3$ solution.

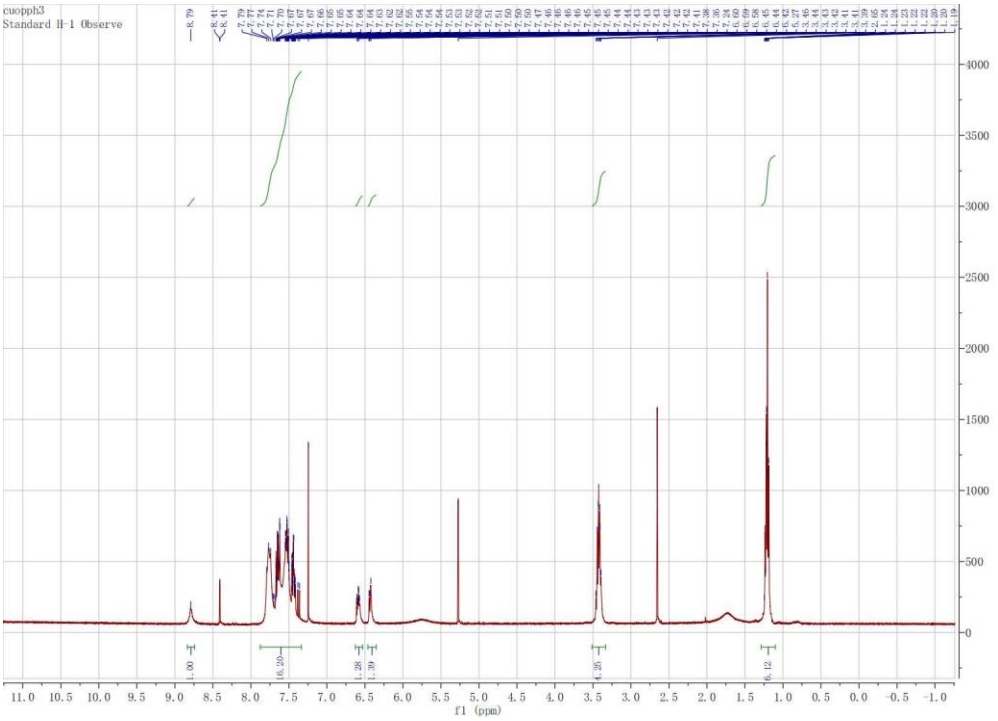

Figure S7. ^{1}H NMR spectrum of compound **8** in $CDCl_3$ solution.

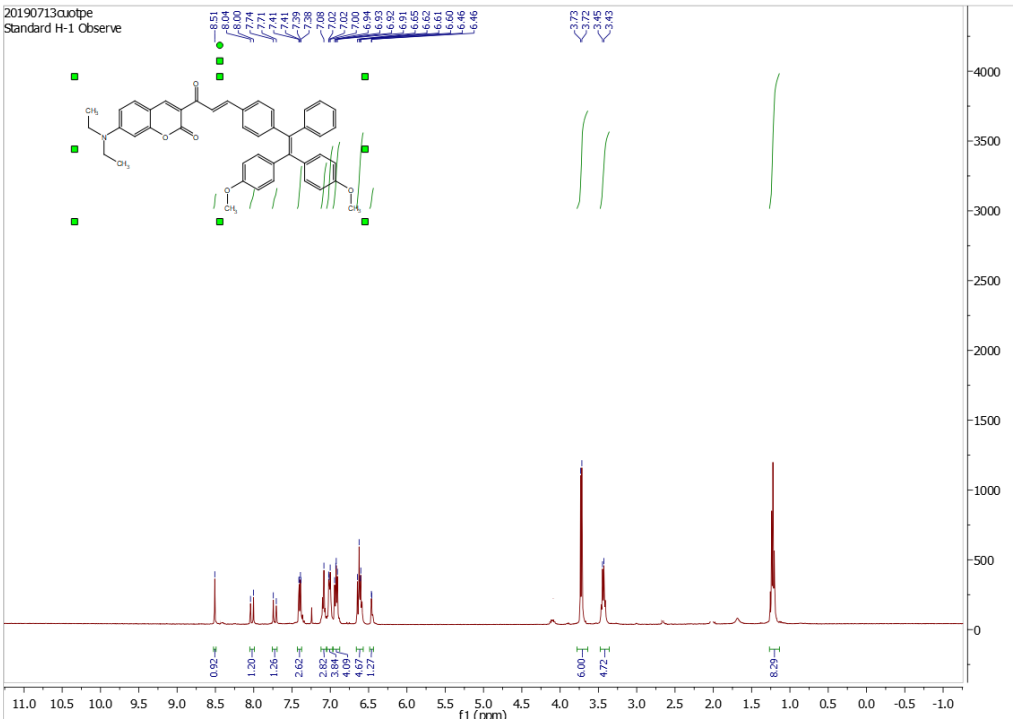

Figure S8. ^{1}H NMR spectrum of probe **A** in $CDCl_3$ solution.

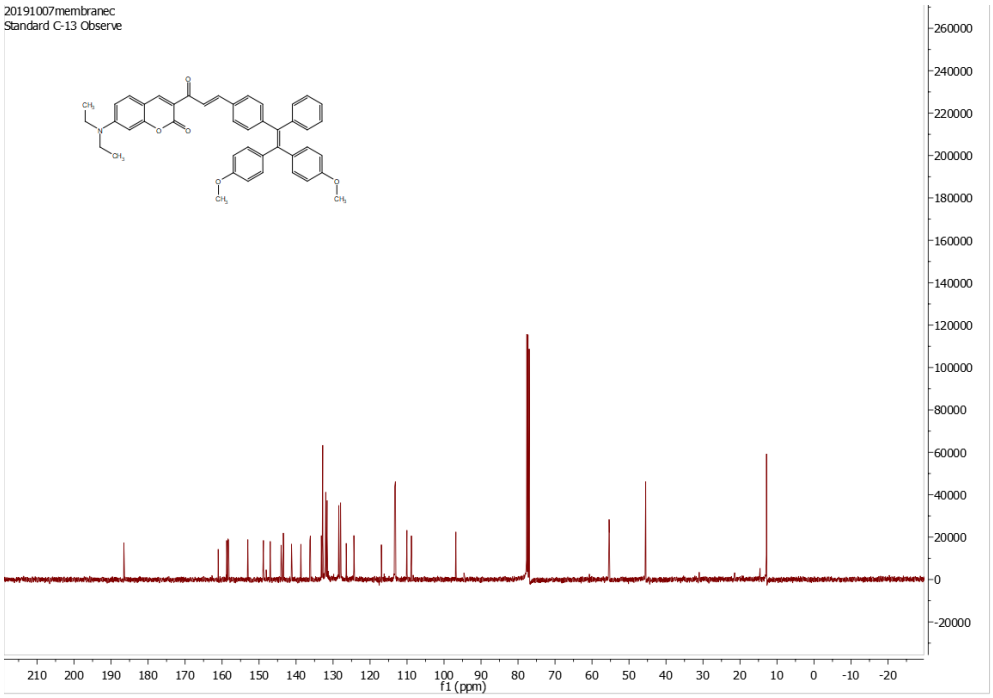

Figure S9. ^{13}C NMR spectrum of probe **A** in $CDCl_3$ solution.

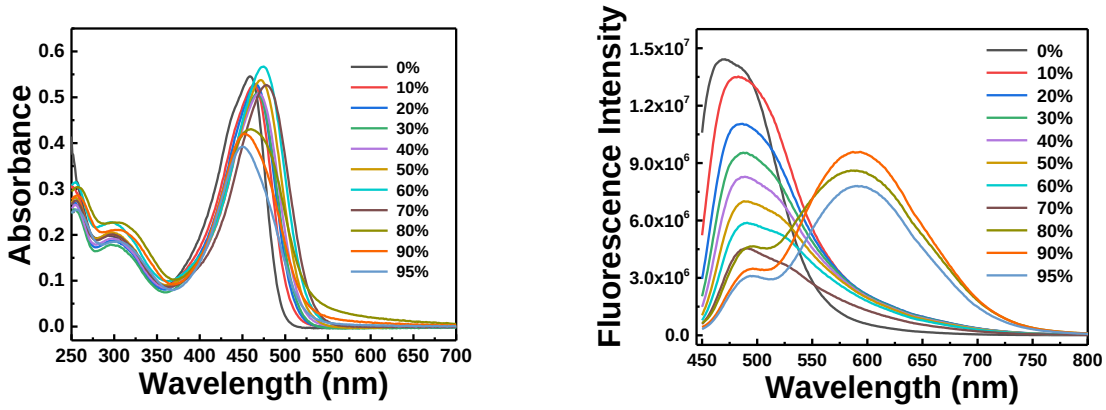

Figure S10. Absorption and fluorescence spectra of probe **A** in the mixture solutions of THF and water with various water percentages. Concentration of probe **A**: 10 μM; excitation wavelength: 430 nm.

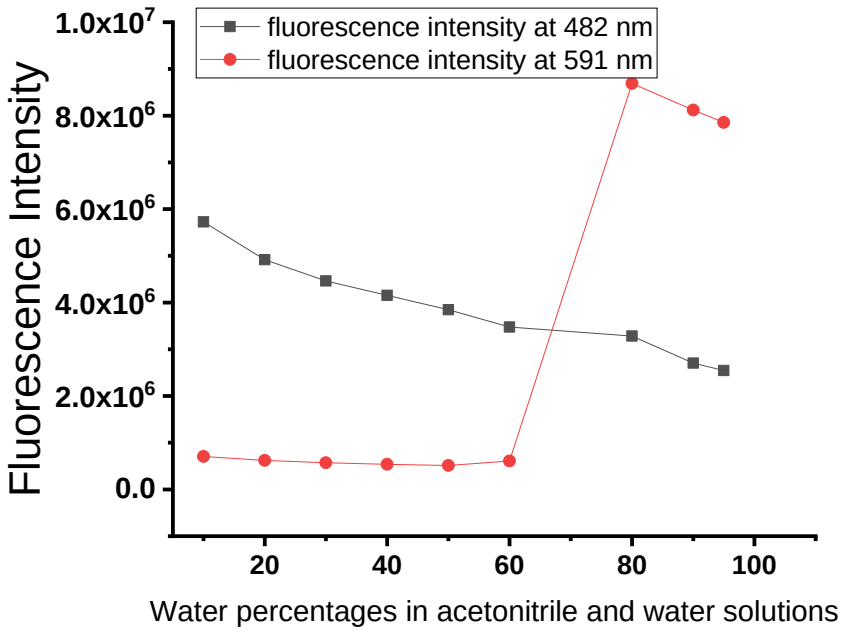

Figure S11. Fluorescence intensity of probe **A** (10 μM) in mixed water and acetonitrile solutions with various water percentages under excitation of 430 nm.

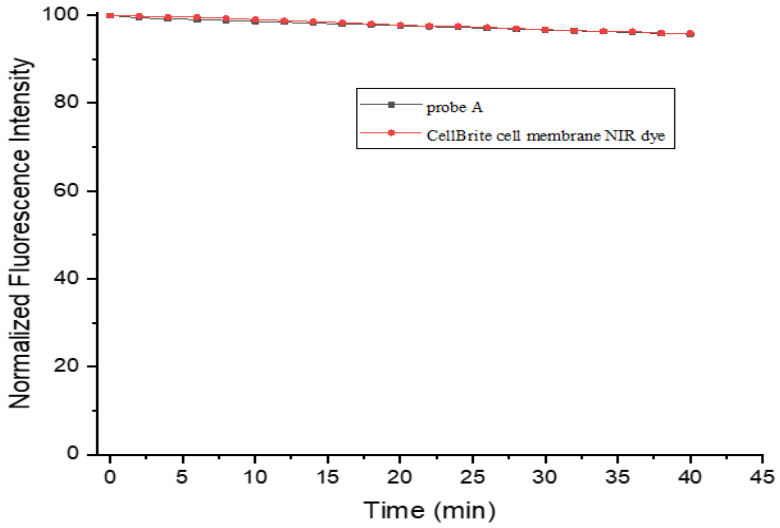

Figure S12. Fluorescence intensities at 591 nm for 10 μM probe **A** in mixed THF and water solution with 95% water under excitation at 488 nm versus time. Fluorescence intensities at 720 nm for 10 μM CellBrite cell membrane NIR dye in the same solvent condition under excitation at 630 nm versus time. Jobin Yvon Fluoromax-4 spectrofluorometer equipped with 150 W CW Ozone-free xenon arc lamp was used for photostability study.

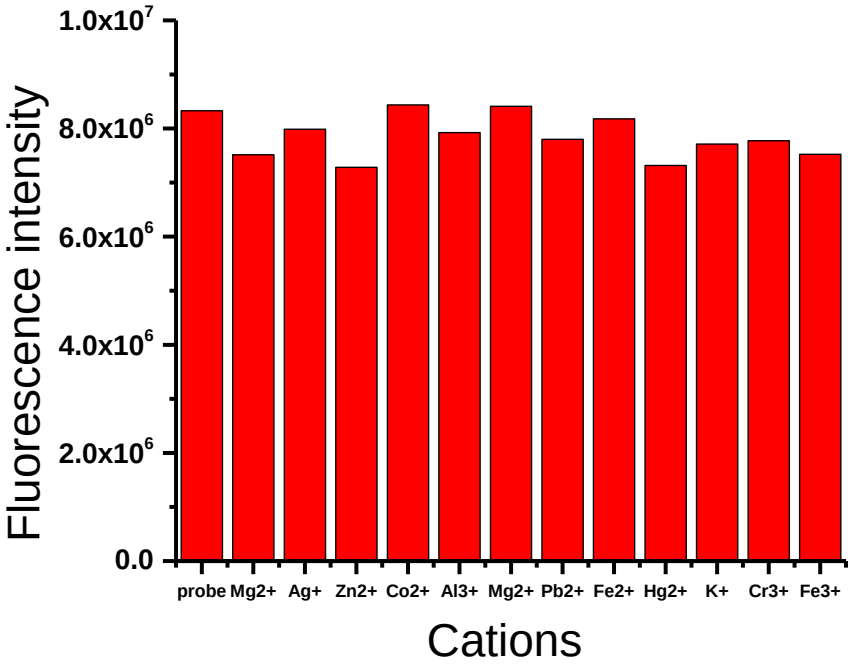

Figure S13. Fluorescence intensity of 5 µM probe **A** at 595 nm in the presence of 200 µM cations, such as Mg^{2+}, Ag^{+}, Zn^{2+}, Co^{2+}, Al^{3+}, Mg^{2+}, Pb^{2+}, Fe^{2+}, Hg^{2+}, K^{+}, Cr^{3+} and Fe^{3+} at 488 nm excitation.

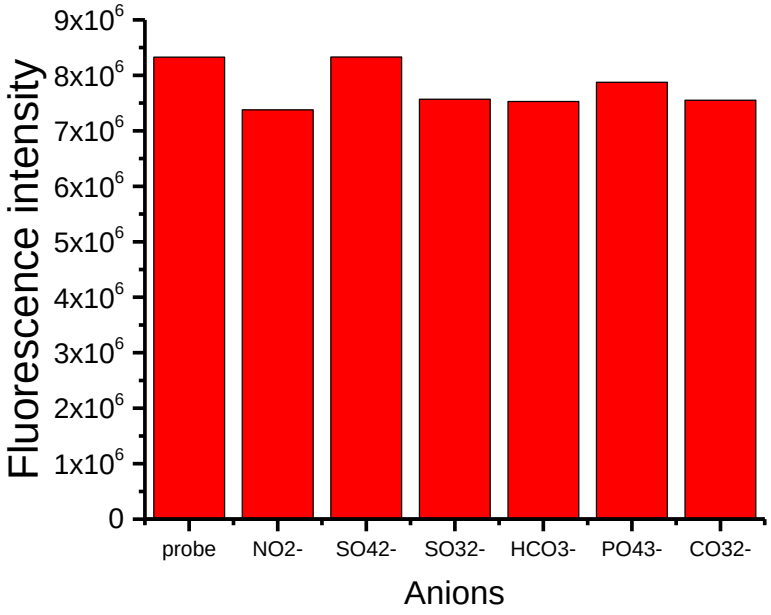

Figure S14. Fluorescence intensity of 5 µM probe **A** at 595 nm in the presence of 200 µM anions, such as NO_3^-, SO_4^{2-}, SO_3^{2-}, HCO_3^-, PO_4^{3-}, and CO_3^{2-} at 488 nm excitation.

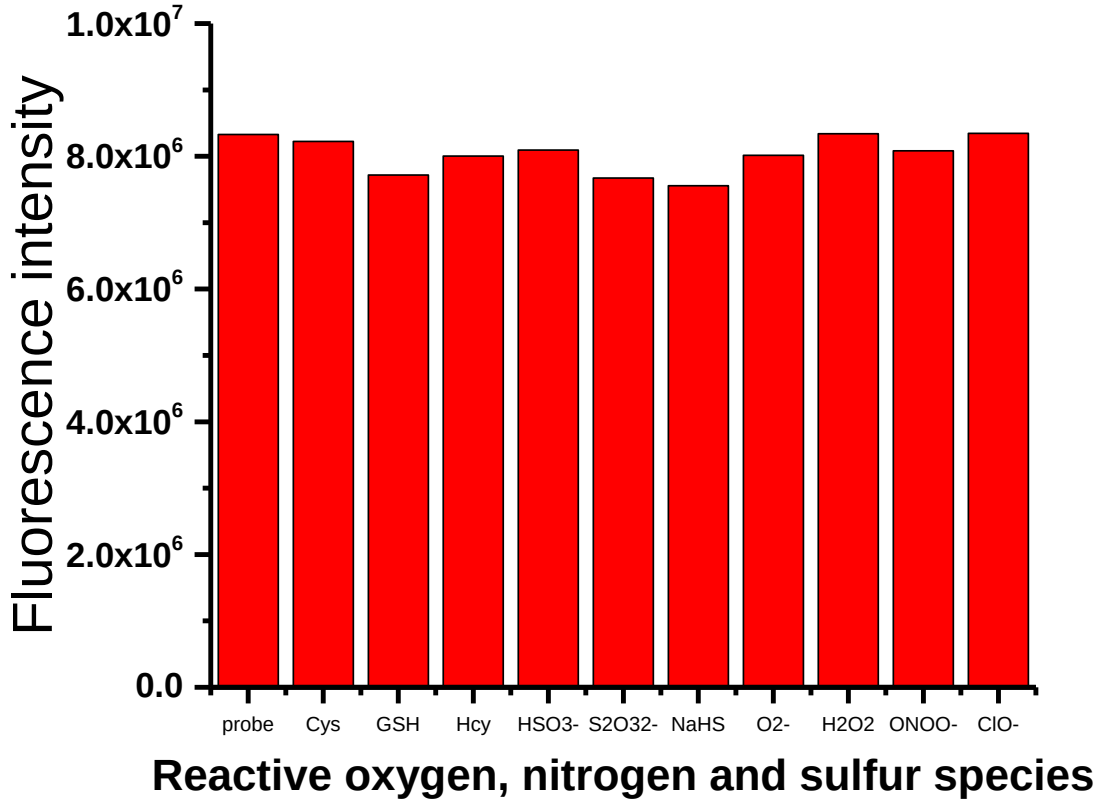

Figure S15. Fluorescence intensity of 5 μM probe at 595 nm in the presence of 200 μM reactive oxygen, nitrogen and sulfur species, such as cysteine (Cys), homocysteine (Hcy), glutathione (GSH), HSO_3^-, $S_2O_3^{2-}$, H_2S, O_2^-, H_2O_2, $ONOO^-$, and ClO^- at 488 nm excitation.

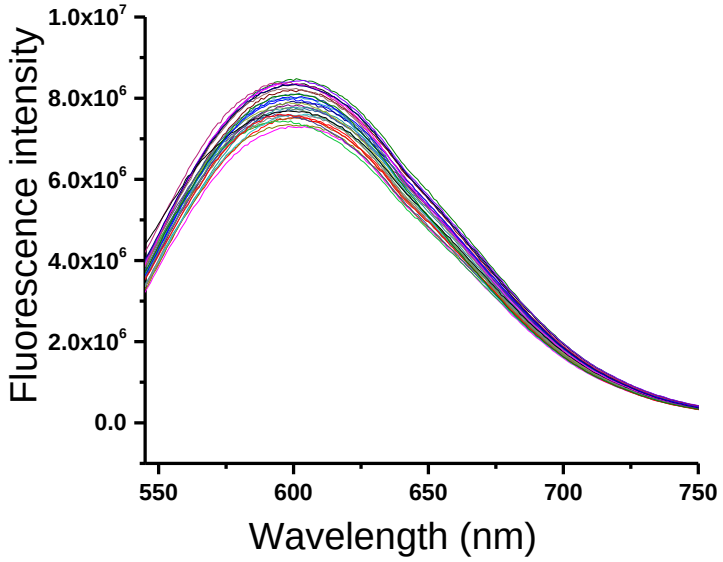

Figure S16. Fluorescence intensity of 5 μM probe at 595 nm in the presence of 200 μM anions, cations, biothiols and reactive oxygen, nitrogen and sulfur species, such as Mg^{2+}, Ag^{+}, Zn^{2+}, Co^{2+}, Al^{3+}, Mg^{2+}, Pb^{2+}, Fe^{2+}, Hg^{2+}, K^{+}, Cr^{3+}, Fe^{3+}, NO_3^{-}, SO_4^{2-}, SO_3^{2-}, HCO_3^{-}, PO_4^{3-}, CO_3^{2-}, Cys, GSH, Hcy, HSO_3^{-}, $S_2O_3^{2-}$, H_2S, O_2^{-}, H_2O_2, $ONOO^{-}$, and ClO^{-} at 488 nm excitation.

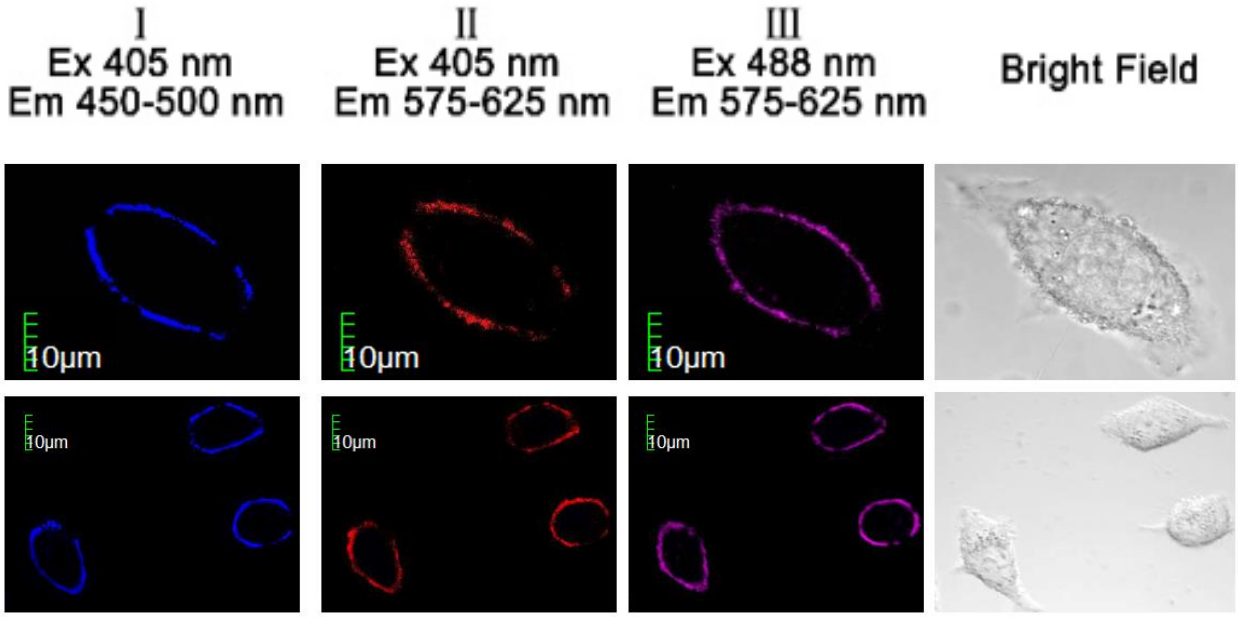

Figure S17. Fluorescence images of HeLa cells incubated with 10 μM probe **A** for 15 minutes under excitation of 405 nm and 488 nm, respectively. Scale bars: 10 μM

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