Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Near-infrared Rhodol Dyes Bearing Salicylaldehyde Moieties for Ratiometric pH Sensing in Live Cells During Mitophagy and Under Hypoxia Conditions

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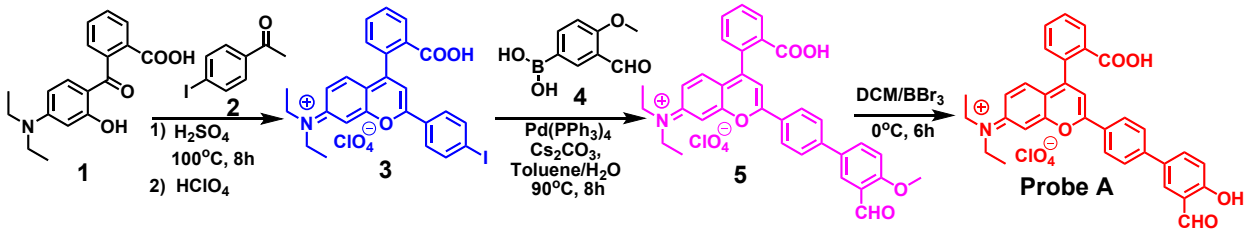
Scheme S1:

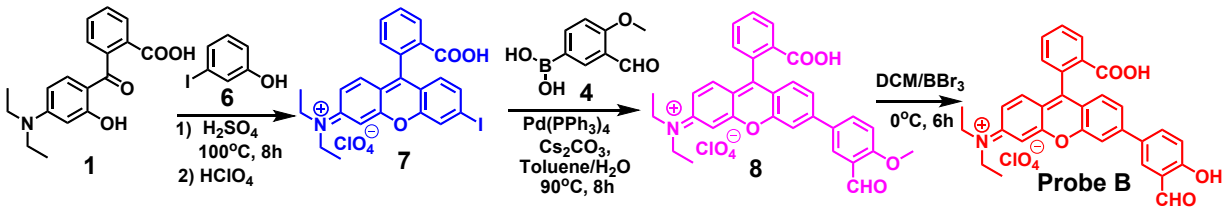


Synthesis of compound 3: 2-(4-(diethylamino)-2-hydroxybenzoyl) benzoic acid (1.0 g, 3.19 mmol) and 1-(4-iodophenyl) ethan-1-one (785.22 mg, 3.19 mmol) were put in concentrated sulphuric acid (20.0 mL) and stirred the reaction mixture at 100°C for overnight. After completion of reaction (monitored on TLC), the reaction mixture was cooled down to room temperature, and perchloric acid was added to the mixture to precipitate the produce. The dark purple color precipitate was collected, dried it on air and purified by column chromatography using EtOAc:Hexane (6:4, v/v) as eluent to get compound **3** with a yield 72%. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.10 (6H, t, J = 6.0 Hz), 3.26 (4H, m), 5.51 (1H, d, J = 4.0 Hz), 6.35 (1H, m), 6.43 (1H, d, J= 4.0), 6.52 (1H, d, J = 8.0 Hz), 7.33 (5H, m) 7.51(3H, m), 7.80 (1H, m).

Synthesis of compound 5: Compound **3** (1.0 g, 1.60 mmol) and (3-formyl-4-methoxyphenyl)boronic acid (346.17 mg, 1.92 mmol) were put in toluene/H_2O (9:1), 20.0 mL followed by the addition of tetrakis(triphenylphosphine)palladium(0) (5.0 mol %) and cesium carbonate (1.30 g, 4.0 mmol). the reaction mixture was stirred for overnight at 90°C under nitrogen atmosphere. After completion of reaction (monitored on TLC), the reaction mixture was cooled down to room temperature, and the solvent was evaporated under reduced pressure. After ice cold water was added to the mixture, extracted with dichloromethane and washed with brine, the organic layer was dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure to get the crude. The crude was used for the next step without further purification.

Synthesis of probe A: Compound **5** (632.0 mg, 1.0 mmol) was put in anhydrous dichloromethane (10.0 mL) at 0°C and added boron tribromide (750.0 mg, 3.0 mmol) dropwise in the duration of 20 min. After 2-h warm the reaction mixture at room temperature, and further stirred for 6 hours. After completion of the reaction (monitored on TLC), the reaction mixture was neutralized with saturated sodium bicarbonate (pH 7.0), extracted the reaction mixture with dichloromethane and washed with water. After the organic layer was collected and dried over anhydrous sodium sulfate, the solvent was evaporated under reduced pressure to get the crude product. The crude product was purified by column chromatography using EtOAc:Hexane (7:3, v/v) as eluent to obtain probe **A** with a reaction yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.11 (6H, t, J = 8.0 Hz), 3.32 (4H, m), 5.57 (1H, d, J = 4.0 Hz), 6.36 (1H, m), 6.48 (1H, s), 6.54 (1H, s), 7.05 (1H, t, J = 6.0 Hz), 7.22 (2H, m), 7.52 (4H, m), 7.60 (4H, m), 7.92 (1H, d, J = 8.0 Hz), 9.96 (1H, s), 11.01 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$), δ (ppm): 12.62, 44.50, 84.34, 96.98, 97.64, 104.82, 109.24, 118.24, 120.66, 123.79, 124.87, 126.03, 126.49, 126.56, 128.34, 129.29, 131.75, 131.85, 132.03, 132.19, 132.26, 134.37, 135.50, 140.29, 149.25, 152.09, 152.82, 153.68, 161.13, 169.52, 196.43. MS/Z = 518.50

Scheme S2:



Synthesis of compound 7: 2-(4-(diethylamino)-2-hydroxybenzoyl) benzoic acid (1.0 g, 3.19 mmol) and 3-iodophenol (701.83 mg, 3.19 mmol) were taken in concentrated sulphuric acid (20.0 mL) and stirred the reaction mixture for overnight at 100°C. After completion of the reaction (monitored on TLC), the reaction mixture was cooled down to room temperature and perchloric acid was added the mixture to precipitate the product. The dark purple color precipitate was dried on air and purified by column chromatography using dichloromethane as an eluent to

obtain compound **7** with a reaction yield 74%. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): ^{1}H R (400 MHz, $CDCl_3$), δ (ppm): 1.12 (6H, t, *J* = 6.0 Hz), 3.30 (4H, m), 5.26 (1H, s), 6.31 (1H, m), 6.45 (2H, m), 6.51(1H, d, *J* = 6.0 Hz), 7.12 (1H, m), 7.22 (1H, m), 7.55 (3H, m), 7.96 (1H, m).

Synthesis of compound 8: Compound **7** (1.0 g, 1.67 mmol) and (3-formyl-4-methoxyphenyl)boronic acid (361.25 mg, 2.00 mmol) were put in toluene/H_2O (9:1), 20 mL followed by the addition of tetrakis(triphenylphosphine)palladium(0) (5.0 mol %) and cesium carbonate (1.36 g, 4.17 mmol). The reaction mixture was stirred for overnight at 90^{o}C under nitrogen atmosphere. After completion of the reaction (monitored on TLC), the reaction mixture was cooled down to room temperature and the solvent of the mixture was evaporated under reduced pressure. After ice cold water was added to the mixture, extracted with dichloromethane and washed with brine. After the organic layer was collected, dried over anhydrous sodium sulfate, and filtered, the filtrate was evaporated under reduced pressure to get crude. The crude was used for the next step without further purification.

Synthesis of probe B: Compound **8** (606.0 mg, 1.0 mmol) was put in anhydrous dichloromethane (10.0 mL) at 0 ^{o}C and added boron tribromide (750.00 mg, 3.0 mmol) dropwise in the duration of 20 min. After 2-h warm the reaction at room temperature, the mixture was further stirred for 6 hours. After completion of the reaction (monitored on TLC), the reaction mixture was neutralized with saturated sodium bicarbonate (pH 7.0), extracted the reaction mixture with dichloromethane and washed with water. The organic layer was collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to get the crude product. The crude product was purified by column chromatography using EtOAc:Hexane (6:4, v/v) as an eluent to afford probe B with a reaction yield 65%. ^{1}HNMR (400 MHz, $CDCl_3$), δ (ppm): 1.13 (6H, t, *J* = 8.0 Hz), 3.31 (4H, m), 6.33 (1H, m), 6.44 (1H, d, *J* = 4.0 Hz), 6.56 (1H, d, *J* = 8.0 Hz), 6.80 (1H, d, *J* = 8.0 Hz), 7.04 (1H, m), 7.13 (3H, m), 7.40 (1H, d, *J* = 4.0 Hz), 7.57 (2H, m), 7.63 (2H, t, *J* = 6.0 Hz), 7.99 (1H, d, *J* = 8.0 Hz), 9.94 (1H, s), 11.01 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$), δ (ppm): 12.59, 44.54, 83.76, 97.48, 104.80, 108.45, 114.68, 118.28, 118.39, 120.63, 121.37, 123.92, 124.89, 127.02,

128.70, 128.89, 134.75, 135.48, 141.39, 149.56 152.01, 152.70, 153.00, 161.29, 169.42, 196.35.

MS/Z = 492.50

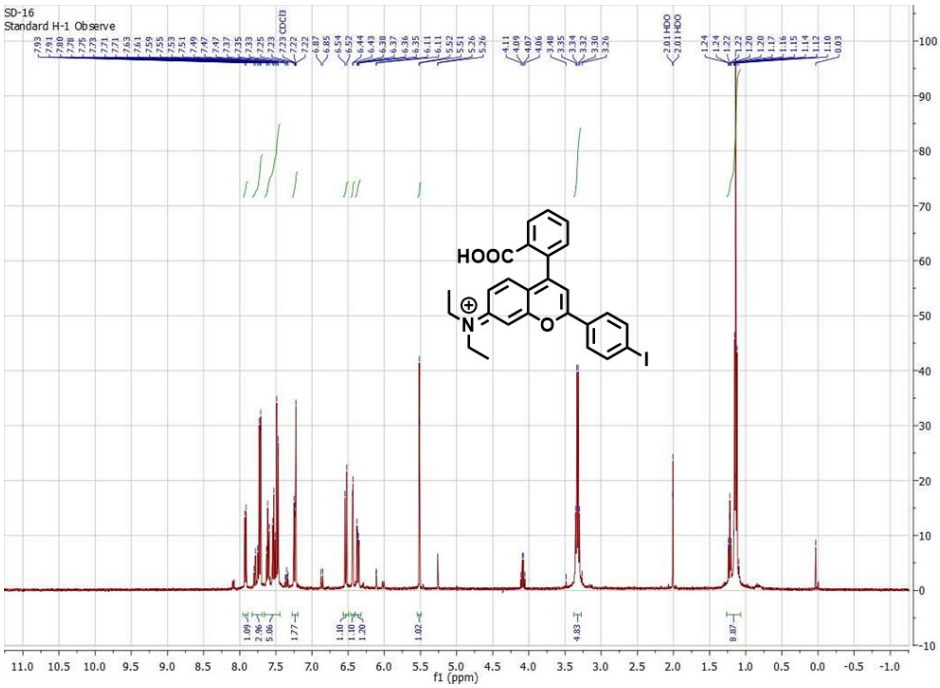


Figure S1: ^{1}H NMR spectrum of compound **3** in $CDCl_3$.

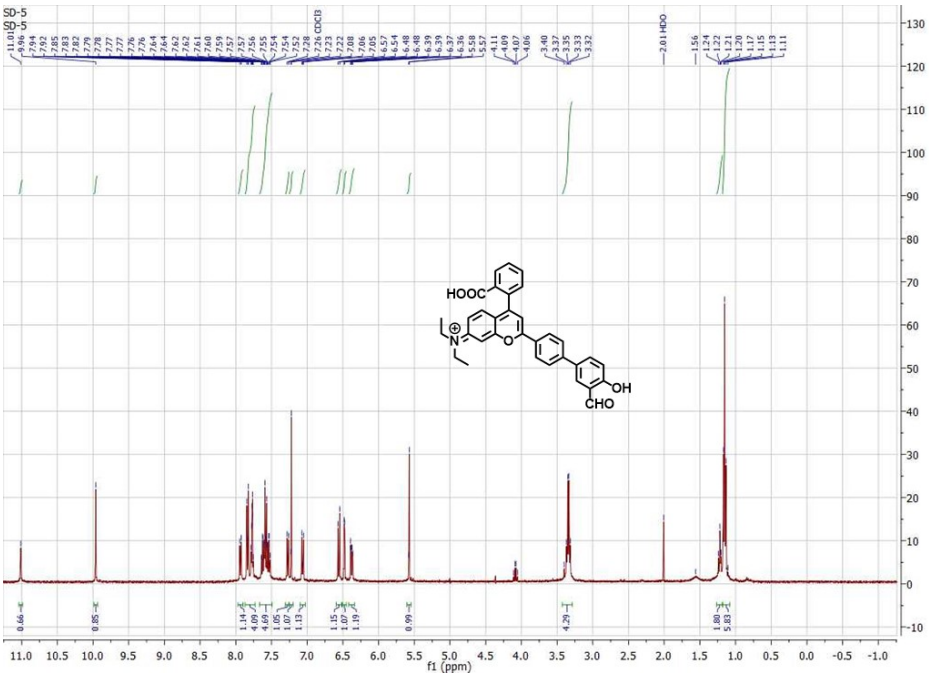


Figure S2: ^{1}H NMR spectrum of probe **A** in $CDCl_3$.

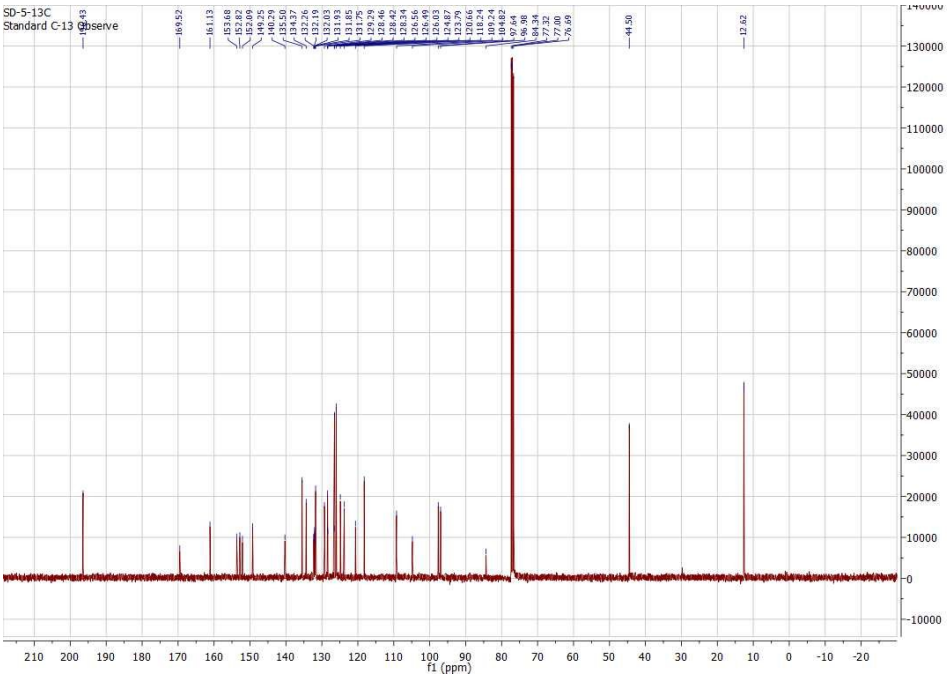


Figure S3: ^{13}C NMR spectrum of probe **A** in $CDCl_3$.

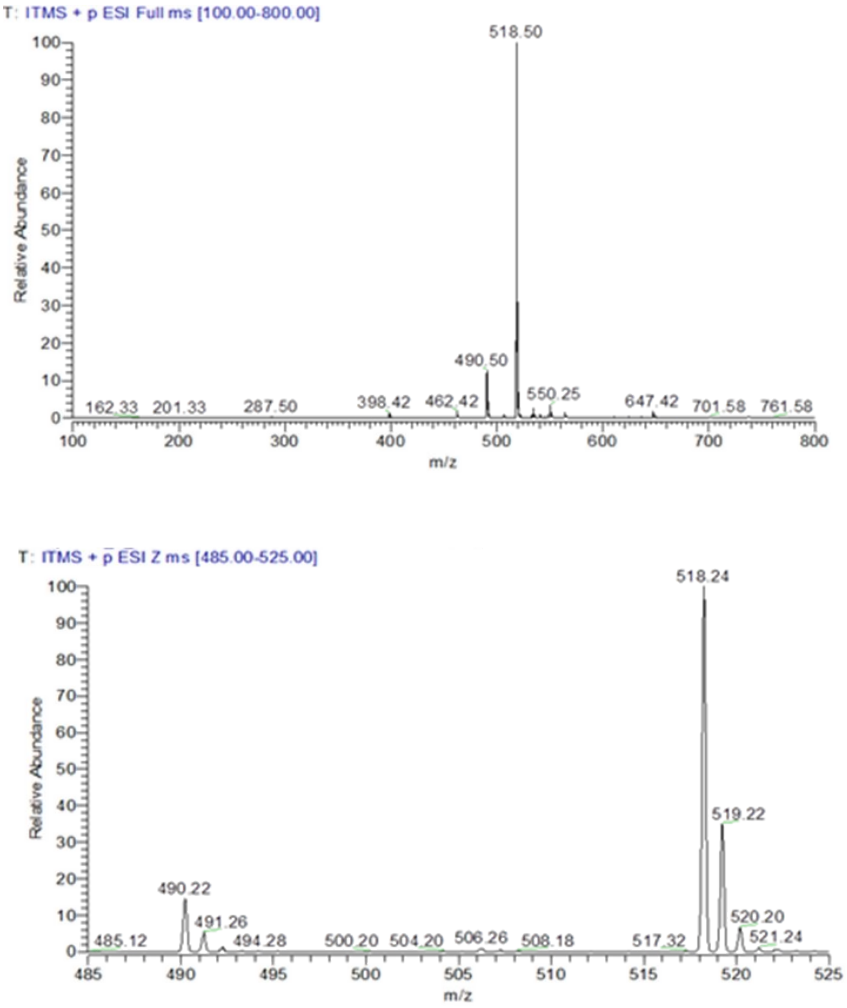


Figure S4: LC-MS spectrum of probe **A**.

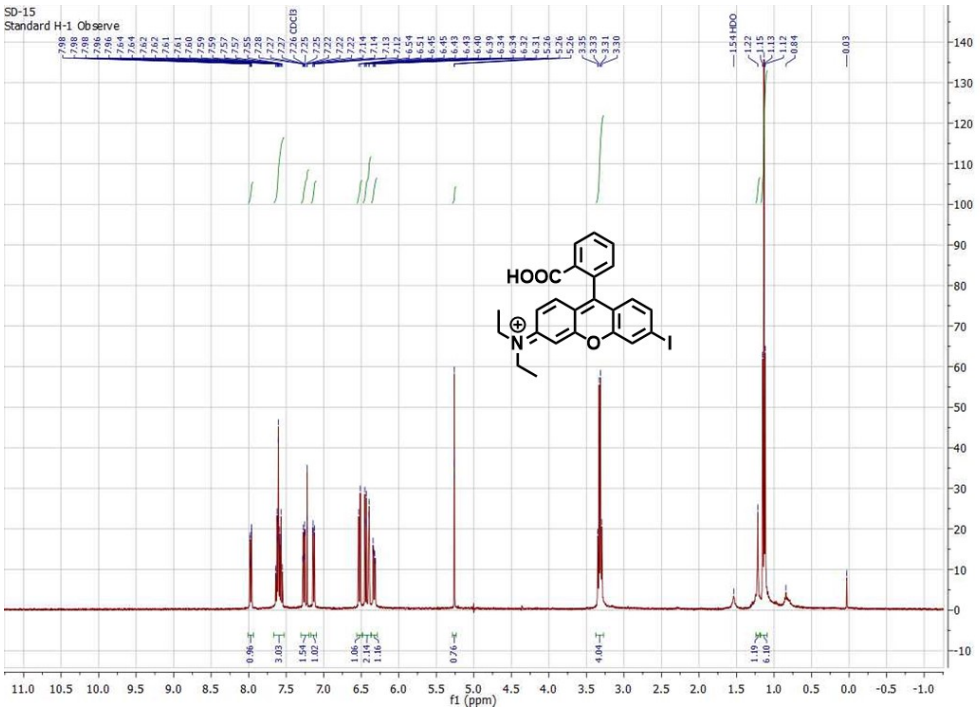


Figure S5: ^{1}H NMR spectrum of compound **7** in $CDCl_3$.

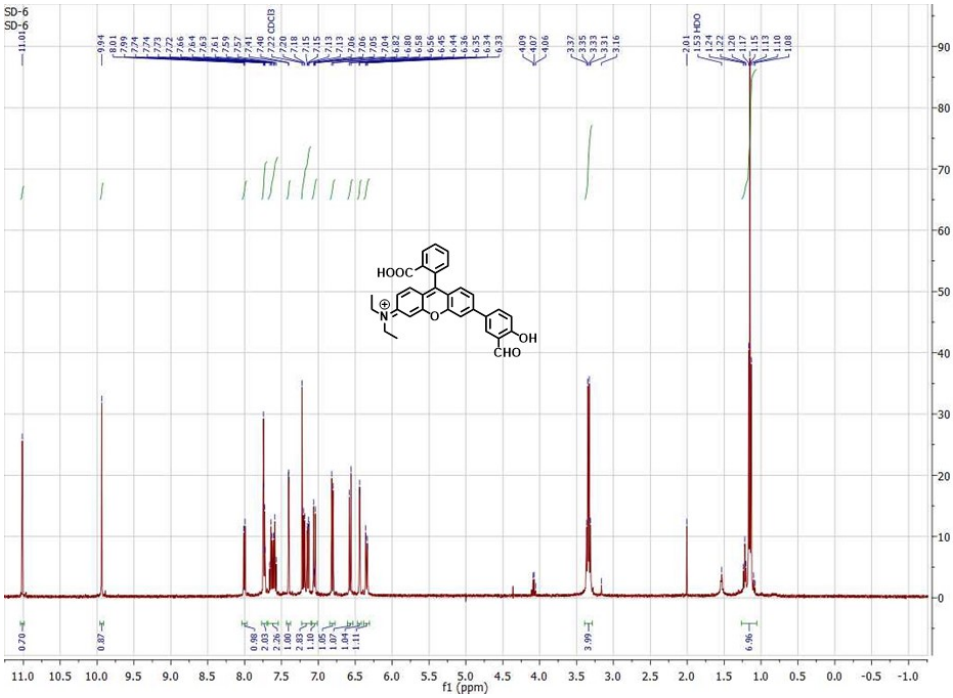


Figure S6: ^{1}H NMR spectrum of probe **B** in $CDCl_3$.

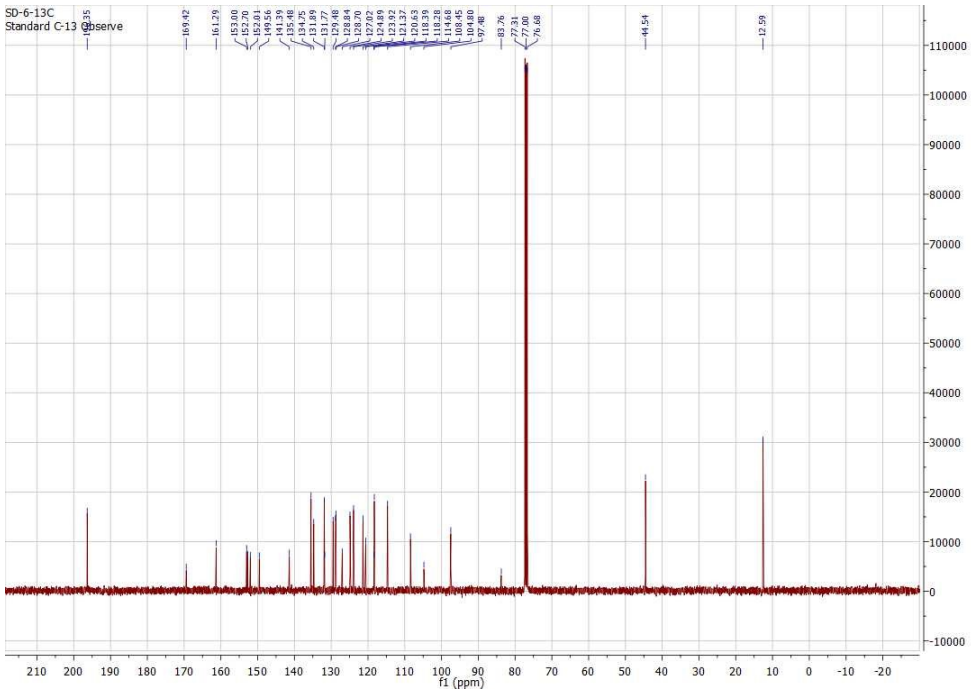


Figure S7: ^{13}C NMR spectrum of probe **B** in $CDCl_3$.

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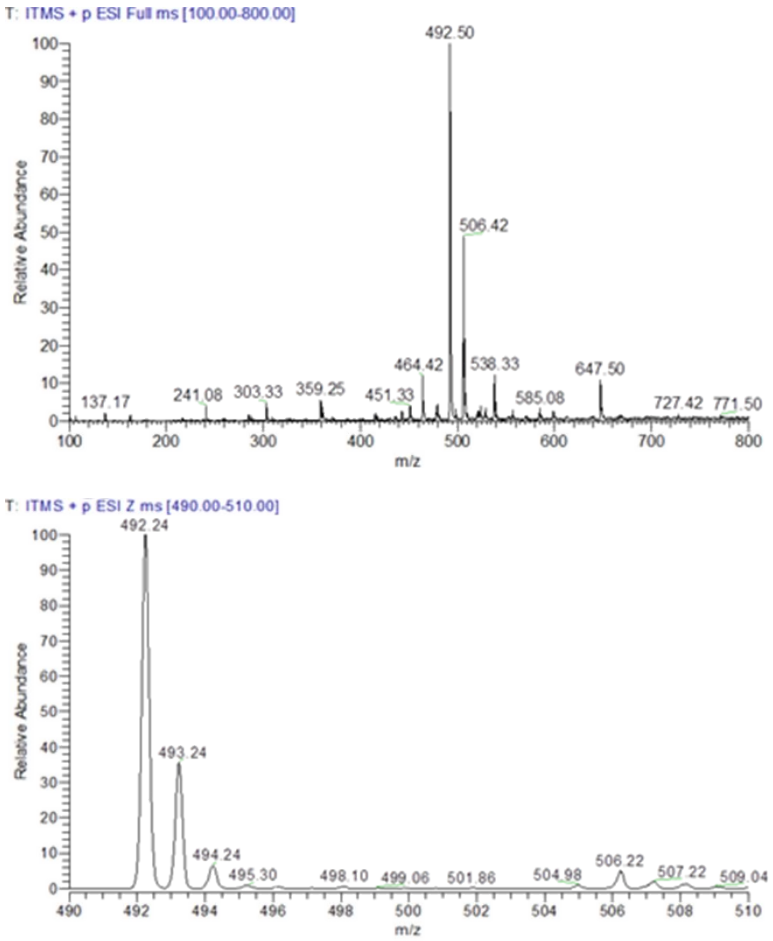


Figure S8: LC-MS spectrum of probe **B**.

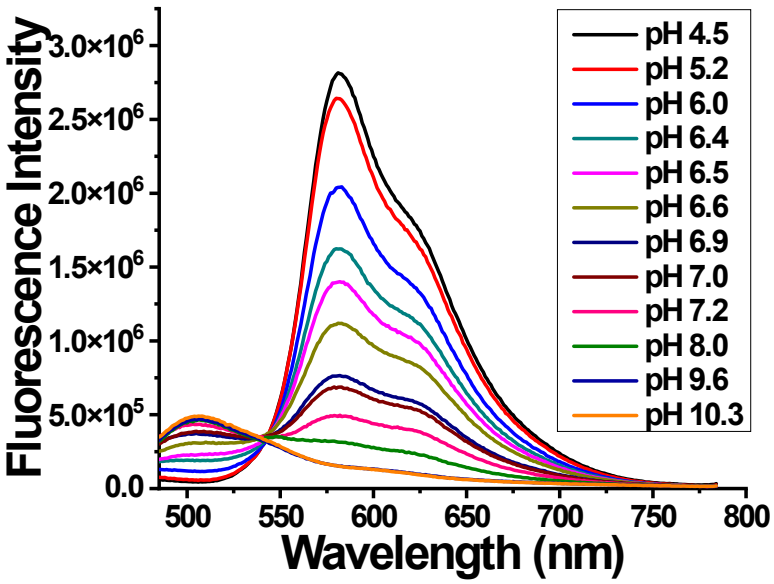


Figure S9: Fluorescence spectra of probes **B** (10 µM) in different pH buffers containing 10% EtOH.

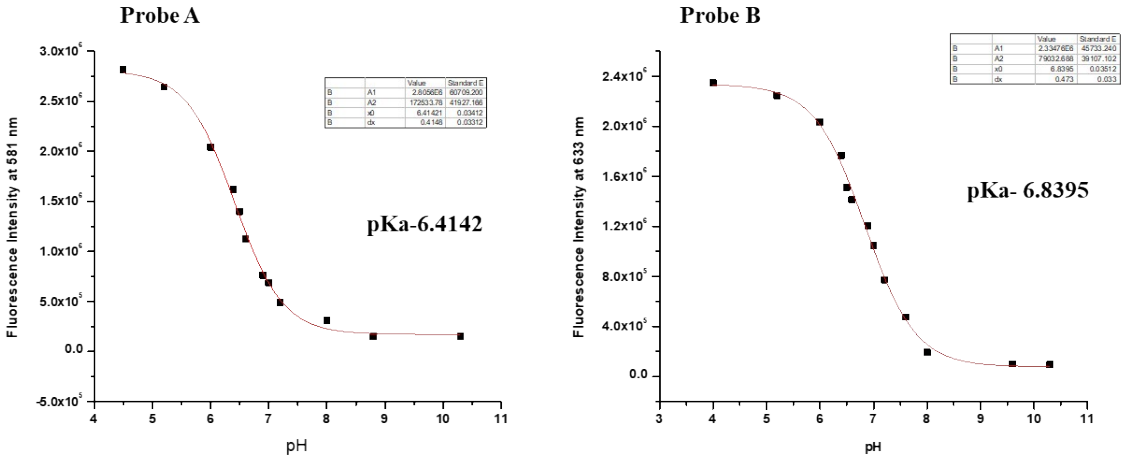


Figure S10: Plot curve of fluorescence intensity of 10 µM probes **AH⁺** (left) and **BH⁺** (right) versus pH.

Theoretical Calculations

Methods.

Models of all probes were generated as described previously[1]. Convergence of the atomic positions in the models were obtained using Gaussian 16 [2] and density functional theory (DFT), using the APFD functional [3] and electron basis sets at the 6-311+qg(d,p) level in a Polarizable Continuum Model (PCM) of water [4]. Imaginary frequencies were not obtained. s were assessed, using TD-DFT optimizations in a PCM in water with the 6-311+g(d,p) basis set. Results were interpreted using GaussView 6 [5] for all data and figures.

Results of the theoretical calculations.

Theoretical calculations for probe **A**.

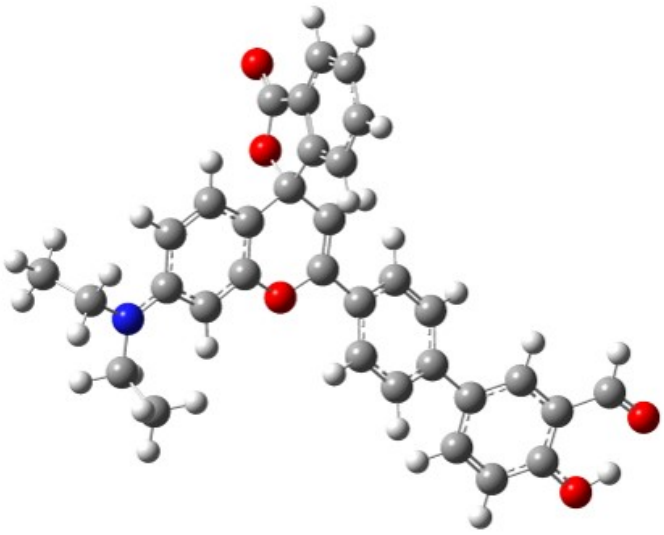


Figure S11: GaussView representation of probe **A**.

Table S1: Excitation energies and oscillator strengths listing for Probe **A** in water.

Calculated atomic coordinates for Probe **A** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-0.83278	1.453805	-0.29003	34	C	8.243755	-1.09335	-0.0542
2	C	-0.24026	0.250006	-0.31612	35	C	7.369191	-2.13305	-0.37677
3	C	1.210746	0.022179	-0.29564	36	C	6.00669	-1.89936	-0.42104
4	C	-2.30725	1.62496	-0.35413	37	O	9.558016	-1.33629	-0.01591
5	C	-3.00837	0.320341	-0.17884	38	C	8.594703	1.281404	0.57299
6	C	-2.29992	-0.87771	-0.20608	39	O	9.817512	1.173935	0.639688
7	O	-0.93668	-0.91662	-0.32804	40	H	-0.22476	2.34704	-0.22744
8	C	-4.39545	0.227086	-0.02302	41	H	-4.98259	1.140994	0.000865
9	C	-5.04196	-0.98457	0.087029	42	H	-6.11974	-0.99199	0.183847
10	C	-4.31355	-2.20444	0.054769	43	H	-2.28298	-2.9916	-0.09224
11	C	-2.91682	-2.11467	-0.09006	44	H	-4.91928	-5.40679	-0.36101
12	N	-4.94219	-3.41179	0.164589	45	H	-3.44708	-4.55286	-0.74115
13	C	-4.21714	-4.66354	0.027993	46	H	-2.89913	-4.42804	1.743537
14	C	-3.61009	-5.15388	1.339664	47	H	-4.38967	-5.3153	2.090038
15	C	-7.22031	-3.49562	-0.8322	48	H	-3.0831	-6.10086	1.189569
16	C	-6.36726	-3.51498	0.432779	49	H	-8.28123	-3.59223	-0.58276
17	C	-2.80796	2.714494	0.55619	50	H	-7.08336	-2.56363	-1.38734
18	C	-2.77621	2.775701	1.940924	51	H	-6.9509	-4.32449	-1.49352
19	C	-3.3136	3.907764	2.549692	52	H	-6.52835	-4.44894	0.979282
20	C	-3.86825	4.948442	1.792533	53	H	-6.66722	-2.71711	1.117851
21	C	-3.89542	4.87751	0.404678	54	H	-2.35095	1.969207	2.52944
22	C	-3.35509	3.742452	-0.18946	55	H	-3.30476	3.986705	3.632366
23	C	-3.24691	3.389581	-1.61518	56	H	-4.27962	5.815752	2.298462
24	O	-3.60389	4.01485	-2.58804	57	H	-4.32149	5.672666	-0.19815
25	O	-2.65405	2.183172	-1.70974	58	H	1.055999	-1.93568	0.583777
26	C	1.730883	-1.17432	0.2095	59	H	3.482152	-2.30719	0.682353
27	C	3.101635	-1.38372	0.256943	60	H	4.137746	1.531466	-1.12853
28	C	3.995931	-0.4113	-0.20485	61	H	1.720891	1.907556	-1.2131
29	C	3.468676	0.778101	-0.72459	62	H	5.945057	1.385375	0.406453
30	C	2.100071	0.991633	-0.77228	63	H	7.775874	-3.11392	-0.59857
31	C	5.453007	-0.63263	-0.15126	64	H	5.346605	-2.71718	-0.69366
32	C	6.327819	0.39633	0.169134	65	H	9.999718	-0.49009	0.228426
33	C	7.712097	0.188466	0.222423	66	H	8.116451	2.252466	0.786393

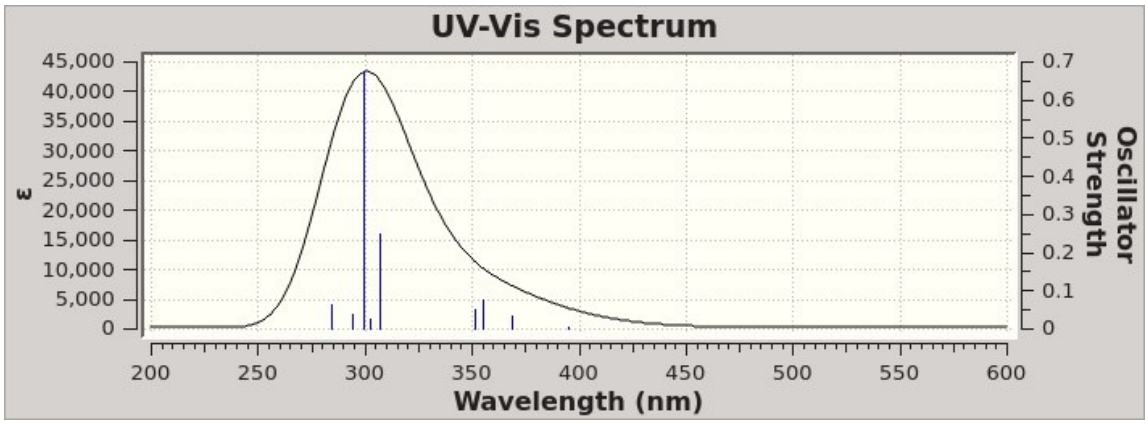


Figure S12: Calculated UV-Vis spectrum for probe **A** in water.

Table S2: Excitation energies and oscillator strengths listing for Probe **A** in water.

Excited State	**Nature**	**E (eV)**	**□□(nm)**	***f***	**Orbital transitions**	**Normalized coefficient**
1:	A	3.1373	395.20	0.0026	136 -> 137	0.70320
2:	A	3.3643	368.53	0.0320	136 -> 138	0.67582
					136 -> 139	-0.18896
3:	A	3.4929	354.96	0.0754	136 -> 138	0.18385
					136 -> 139	0.67433
4:	A	3.5312	351.11	0.0488	134 -> 137	0.15617
					135 -> 137	0.67415
5:	A	4.0397	306.92	0.0408	128 -> 137	0.63339
					135 -> 138	0.23733
6:	A	4.0414	306.78	0.2501	128 -> 137	-0.26008
					134 -> 137	-0.10852
					134 -> 138	-0.15113
					134 -> 139	0.13632
					135 -> 138	0.58835
					135 -> 139	-0.11128
7:	A	4.0975	302.58	0.0267	133 -> 137	0.12568
					134 -> 137	0.61000
					135 -> 137	-0.16502
					135 -> 139	-0.24767
8:	A	4.1448	299.13	0.6704	134 -> 137	0.23859
					134 -> 138	0.12387
					134 -> 139	-0.10132
					135 -> 138	0.21090
					135 -> 139	0.59564

9:	A	4.2148	294.16	0.0383	136 -> 140	0.69316
10:	A	4.3617	284.26	0.0619	136 -> 141	-0.31539
					136 -> 142	0.52231
					136 -> 143	-0.22865
					136 -> 144	0.19200

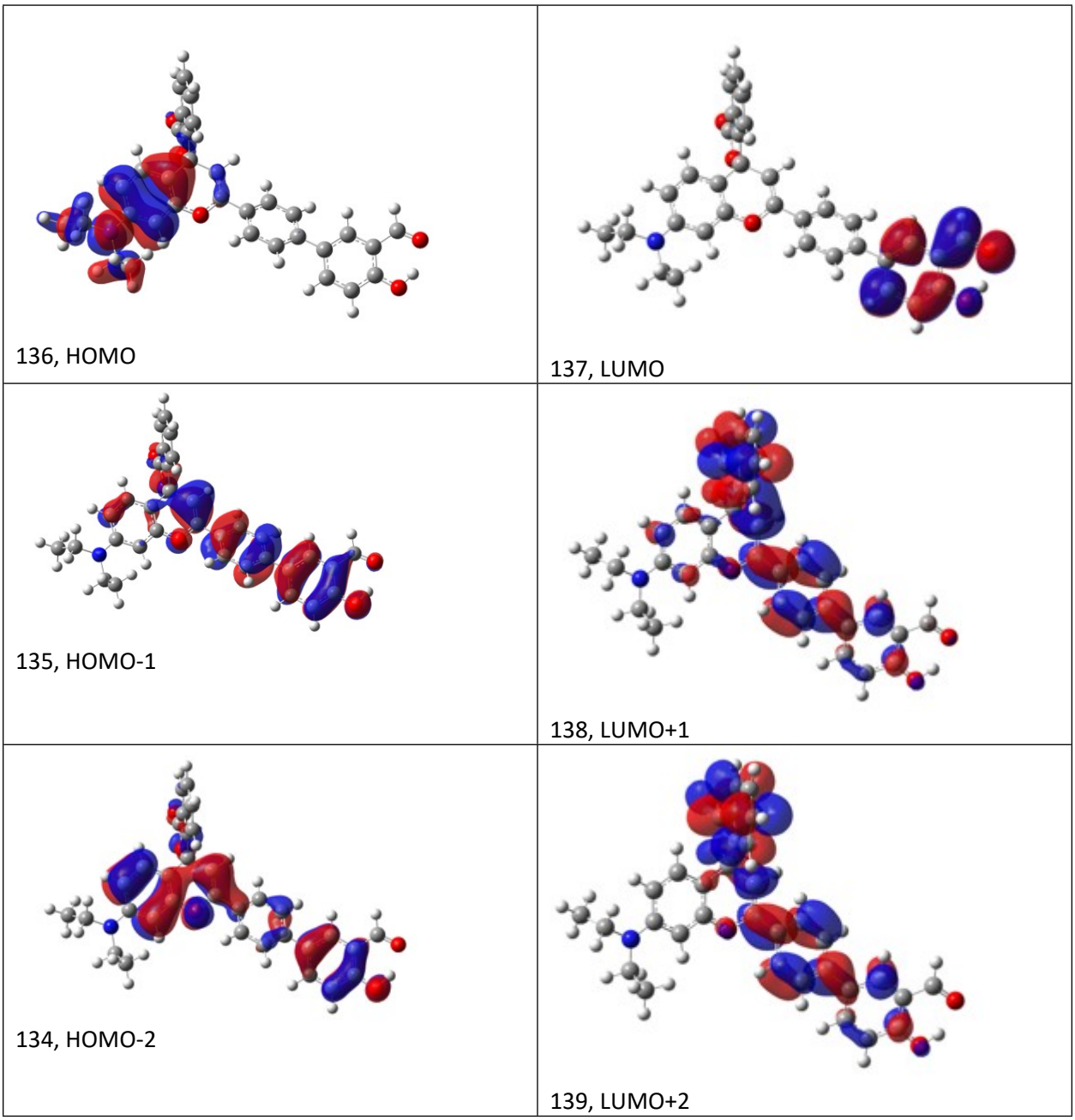


Figure S13: Drawings of selected molecular orbitals listed in Table S2.

Theoretical calculations for probe **AH**$^{+}$.

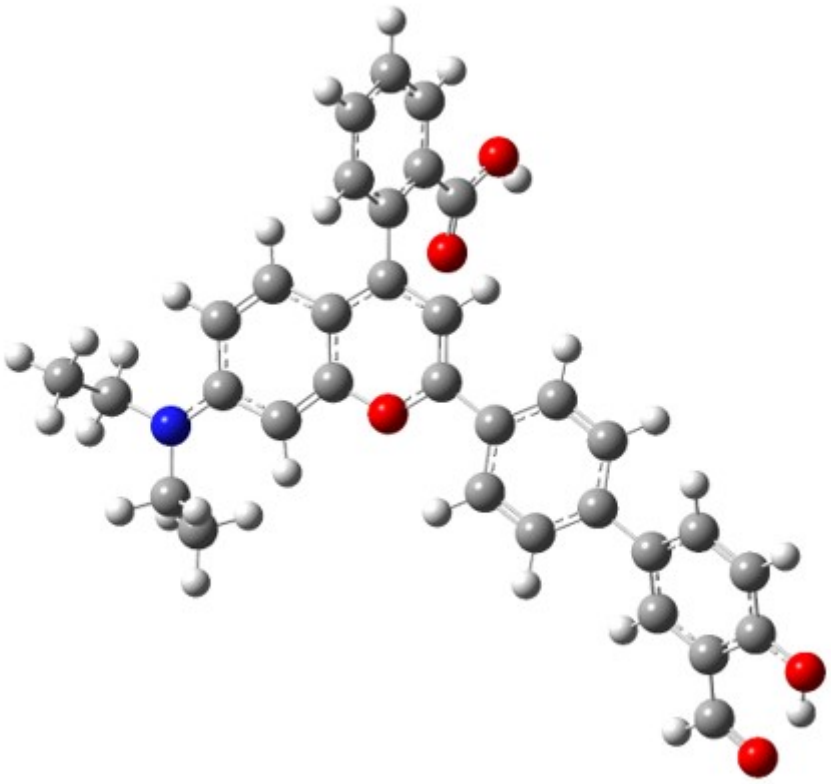


Figure S14: GaussView representation of probe **AH**$^{+}$.

Table S3: Calculated atomic coordinates for Probe **AH$^+$** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-1.05789	-1.48511	-0.33529	35	C	7.600368	-1.21982	-0.99993
2	C	-0.26717	-0.36193	-0.26987	36	C	6.218721	-1.23226	-0.97235
3	C	1.184503	-0.32983	-0.28644	37	O	9.634663	-0.18793	-0.41189
4	C	-2.45145	-1.36886	-0.30706	38	C	8.274282	1.924186	0.933856
5	C	-3.0313	-0.10137	-0.23258	39	O	9.49894	2.019616	0.965125
6	C	-2.17129	1.026011	-0.17667	40	H	-0.60182	-2.46086	-0.41967
7	O	-0.82724	0.850253	-0.19202	41	H	-5.11479	-0.67366	-0.21033
8	C	-4.42406	0.161457	-0.17837	42	H	-5.96989	1.586412	-0.06447
9	C	-4.89961	1.433696	-0.09089	43	H	-1.89322	3.112922	-0.03681
10	C	-4.01532	2.567073	-0.04985	44	H	-4.21017	5.801321	-0.4557
11	C	-2.62383	2.318206	-0.0918	45	H	-2.80775	4.795813	-0.71154
12	N	-4.50215	3.817035	0.025751	46	H	-3.94214	5.613293	2.035486
13	C	-3.62894	4.98668	-0.01732	47	H	-2.5226	4.580481	1.811528
14	C	-3.11285	5.382679	1.361199	48	H	-2.48052	6.271079	1.284255
15	C	-6.63497	4.20393	-1.18656	49	H	-7.6909	4.446181	-1.04015
16	C	-5.92992	4.099565	0.160152	50	H	-6.18812	4.990362	-1.80096
17	C	-3.28419	-2.58429	-0.45178	51	H	-6.5729	3.263959	-1.7412
18	C	-3.90388	-2.80045	-1.68143	52	H	-6.39217	3.342258	0.795826
19	C	-4.65042	-3.95334	-1.90306	53	H	-6.0135	5.04061	0.709018
20	C	-4.78449	-4.90205	-0.89486	54	H	-3.78658	-2.06528	-2.47082
21	C	-4.17432	-4.69308	0.335101	55	H	-5.12543	-4.10752	-2.86641
22	C	-3.42654	-3.53718	0.569667	56	H	-5.36803	-5.80086	-1.06314
23	C	-2.84189	-3.27656	1.908481	57	H	-4.28763	-5.41955	1.130886
24	O	-2.4321	-2.19686	2.275102	58	H	-2.46048	-4.11332	3.547947
25	O	-2.82332	-4.36598	2.687071	59	H	1.438207	-2.47285	-0.08736
26	C	1.931484	-1.51395	-0.19557	60	H	3.86832	-2.40131	-0.109
27	C	3.313421	-1.47485	-0.2117	61	H	3.755502	1.875705	-0.52733
28	C	4.001354	-0.25758	-0.32134	62	H	1.309284	1.816533	-0.47508
29	C	3.250049	0.922503	-0.4128	63	H	5.642181	1.597892	0.803641
30	C	1.867353	0.891664	-0.39172	64	H	8.156697	-2.0012	-1.50618
31	C	5.472651	-0.22043	-0.33559	65	H	5.696028	-2.03717	-1.47967
32	C	6.173967	0.808772	0.279262	66	H	9.938834	0.612823	0.075971
33	C	7.572849	0.843801	0.270733	67	H	7.650524	2.688261	1.427815
34	C	8.300104	-0.18301	-0.37764					

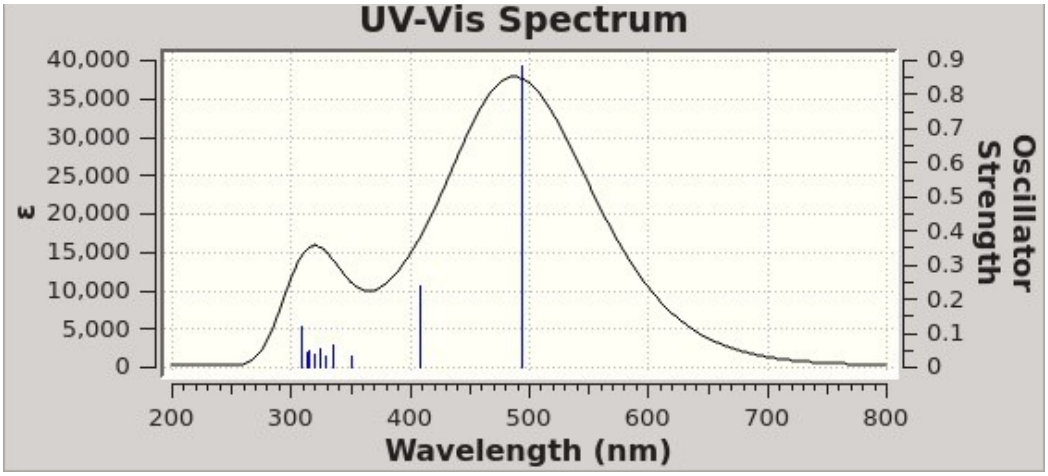


Figure S15: Calculated UV-Vis spectrum for probe **AH**$^{+}$ in water.

Table S4: Excitation energies and oscillator strengths listing for Probe **AH**$^{+}$ in water.

Excited State	Nature	E ()	()	*f*	Orbital	Normalized
1:	A	2.5128	493.42	0.8843	136 -> 137	0.70250
2:	A	3.0404	407.79	0.2370	135 -> 137	0.69508
3:	A	3.5367	350.56	0.0304	135 -> 138	-0.26617
					136 -> 138	0.64585
4:	A	3.7036	334.76	0.0631	134 -> 137	0.67470
					136 -> 140	-0.13382
5:	A	3.7614	329.62	0.0315	136 -> 139	0.69421
6:	A	3.8306	323.67	0.0553	128 -> 137	-0.16883
					131 -> 137	0.23189
					132 -> 137	0.17189
					133 -> 137	0.57487
					136 -> 140	0.16203
7:	A	3.8803	319.52	0.0353	128 -> 137	0.15648
					131 -> 137	-0.22250
					132 -> 137	0.62142
8:	A	3.9335	315.20	0.0504	135 -> 138	0.61880
					136 -> 138	0.28003

9:	A	3.9499	313.89	0.0437	128 -> 137	0.11485
					129 -> 137	0.10427
					130 -> 137	-0.16404
					131 -> 137	0.60815
					132 -> 137	0.13951
					133 -> 137	-0.20421
10:	A	4.0139	308.89	0.1200	128 -> 137	-0.30977
					129 -> 137	-0.10021
					132 -> 137	0.16321
					133 -> 137	-0.28934
					134 -> 137	0.10725
					136 -> 140	0.48483

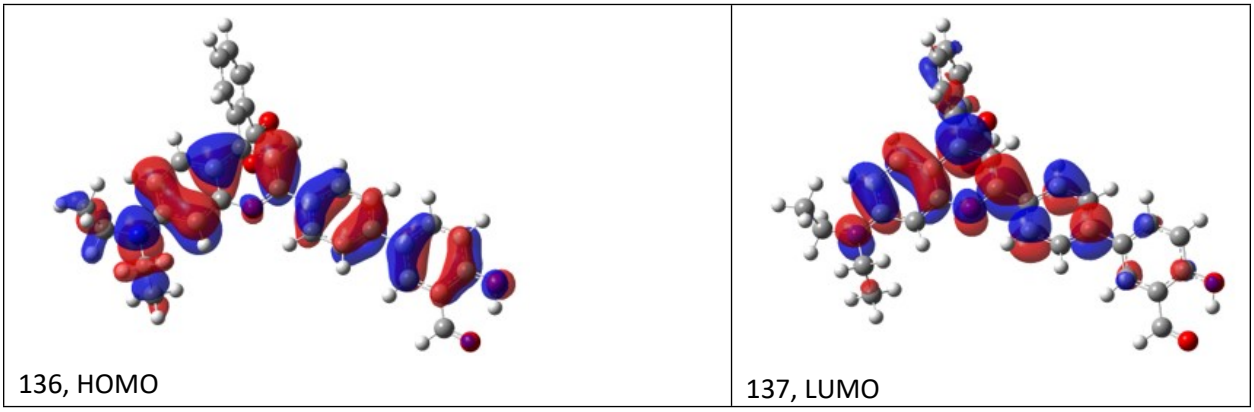


Figure S16: Drawings of selected molecular orbitals listed in Table S4.

Theoretical calculations for probe **B**.

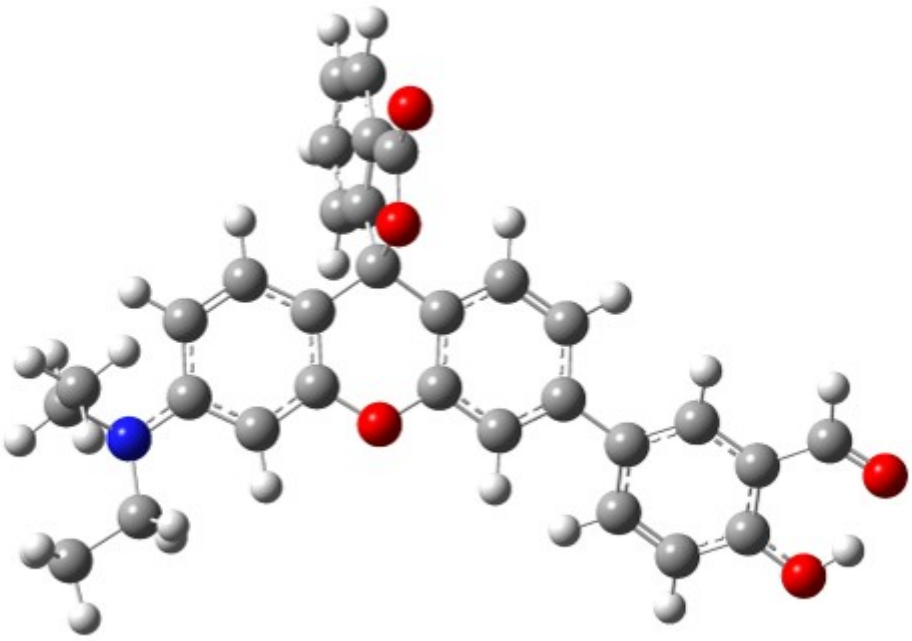


Figure S17: GaussView representation of probe **B**.

Table S5: Calculated atomic coordinates for Probe **B** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-0.2574	0.8701	-0.08973	15	N	5.130788	-2.62847	0.258636
2	C	-0.46565	-0.50488	-0.00939	16	C	4.701478	-4.02487	0.195099
3	C	-1.75021	-1.0349	0.086218	17	C	5.770027	-5.03699	0.575244
4	C	-2.85881	-0.19496	0.105698	18	C	6.733184	-2.27242	-1.62167
5	C	-2.65655	1.193258	0.028886	19	C	6.507462	-2.32399	-0.11328
6	C	-1.37699	1.706068	-0.06667	20	C	1.369787	2.643475	0.621847
7	C	1.126576	1.434635	-0.24323	21	C	1.396184	2.739034	2.004387
8	C	2.170156	0.37634	-0.08437	22	C	1.650672	3.991242	2.558252
9	C	1.838255	-0.97175	-0.01162	23	C	1.87371	5.114924	1.750439
10	O	0.544939	-1.4169	-0.02082	24	C	1.846473	5.007982	0.365408
11	C	3.534223	0.688631	-0.06455	25	C	1.59008	3.752054	-0.17416
12	C	4.510117	-0.27841	0.024715	26	C	1.50023	3.335221	-1.58286
13	C	4.167649	-1.65408	0.11564	27	O	1.628391	3.990956	-2.58987
14	C	2.799058	-1.97178	0.078491	28	O	1.233485	2.010575	-1.61554

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
29	C	-4.22043	-0.75428	0.210121	46	H	5.310793	-6.02821	0.614543
30	C	-5.27915	-0.20722	-0.50072	47	H	6.586471	-5.08568	-0.14939
31	C	-6.57243	-0.73664	-0.40589	48	H	6.191626	-4.82464	1.562084
32	C	-6.81857	-1.85117	0.430442	49	H	7.778002	-2.03911	-1.84656
33	C	-5.75718	-2.40546	1.14891	50	H	6.494278	-3.23426	-2.08544
34	C	-4.48992	-1.86334	1.034762	51	H	6.105293	-1.50719	-2.08708
35	O	-8.03737	-2.3867	0.552807	52	H	6.800656	-1.38356	0.356533
36	C	-7.652	-0.14868	-1.17098	53	H	7.150411	-3.07838	0.33968
37	O	-8.8107	-0.55786	-1.13768	54	H	1.225852	1.870712	2.632334
38	H	-1.86037	-2.11354	0.121733	55	H	1.67805	4.101426	3.637824
39	H	-3.50548	1.867175	0.07098	56	H	2.069775	6.075466	2.21544
40	H	-1.23003	2.780985	-0.11478	57	H	2.017643	5.865926	-0.2762
41	H	3.832941	1.731361	-0.12687	58	H	-5.11433	0.636564	-1.16529
42	H	5.545283	0.034642	0.025445	59	H	-5.94606	-3.25354	1.798283
43	H	2.441987	-2.99162	0.11419	60	H	-3.68291	-2.29705	1.617545
44	H	3.86747	-4.14188	0.892531	61	H	-8.64061	-1.864	-0.0249
45	H	4.309237	-4.25827	-0.80744	62	H	-7.39169	0.712534	-1.80952

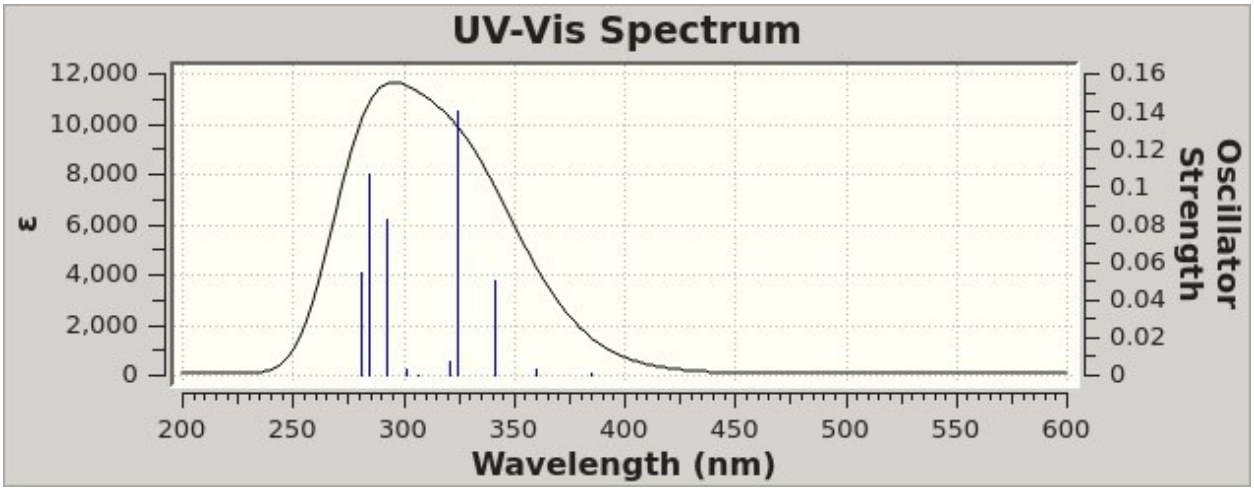
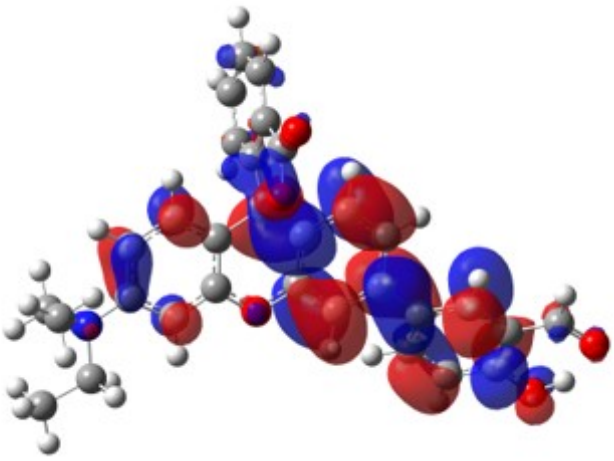


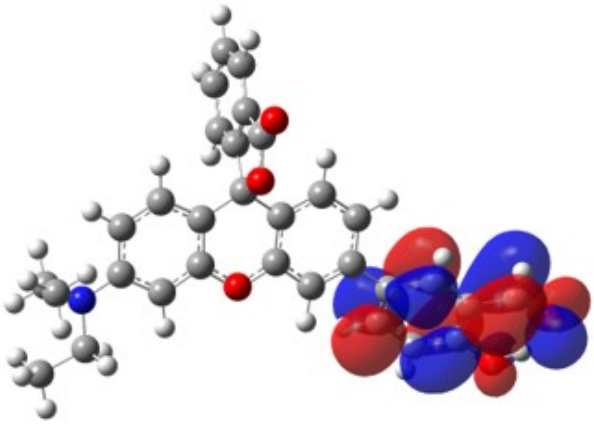
Figure S18: Calculated UV-Vis spectrum for probe **B** in water.

Table S6: Excitation energies and oscillator strengths listing for Probe **B** in water.

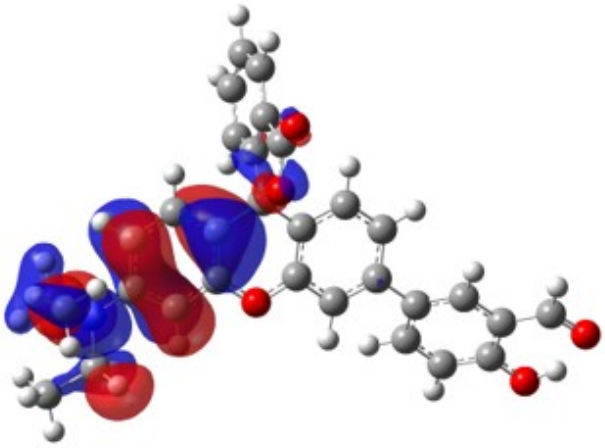
Excited State	Nature	E (eV)	□□(nm)	*f*	Orbital transitions	Normalized coefficient
1:	A	3.2177	385.31	0.0013	129 ->130	0.70508
2:	A	3.4458	359.81	0.0027	129 ->131	0.70176
3:	A	3.6361	340.98	0.0504	127 ->130	-0.30252
					128 ->130	0.62516
4:	A	3.8220	324.39	0.1403	129 ->132	0.69523
5:	A	3.8635	320.91	0.0071	127 ->130	0.62649
					128 ->130	0.31145
6:	A	4.0385	307.01	0.0005	121 ->130	0.54383
					122 ->130	0.41795
7:	A	4.1122	301.51	0.0035	127 ->131	0.17461
					128 ->131	0.67472
8:	A	4.2395	292.45	0.0825	128 ->132	0.18224
					129 ->133	0.67119
9:	A	4.3547	284.71	0.1063	127 ->131	-0.18553
					127 ->132	0.18025
					128 ->132	0.53779
					129 ->133	-0.18384
					129 ->134	0.22249
					129 ->135	0.13617
10:	A	4.4141	280.88	0.0549	127 ->131	0.64692
					128 ->131	-0.16308
					128 ->132	0.15380
					129 ->135	0.10235



132, LUMO+2



130, LUMO



129, HOMO

Figure S19: Drawings of selected molecular orbitals listed in Table S6.

Theoretical calculations for probe **BH⁺**.

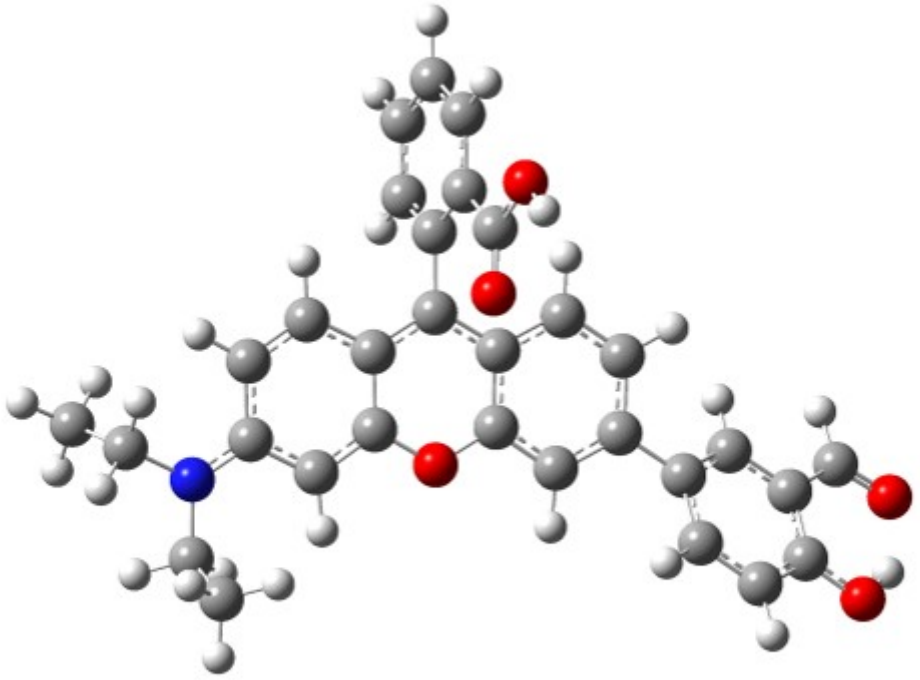


Figure S20: GaussView representation of probe **BH⁺**.

Table S7: Calculated atomic coordinates for Probe **BH⁺** in water.

Calculated atomic coordinates for Probe **BH⁺** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	0.182047	0.754693	-0.20208	12	C	-4.52219	-0.40746	-0.29323
2	C	0.441353	-0.62292	-0.06845	13	C	-4.1693	-1.80071	-0.18313
3	C	1.731365	-1.12047	0.029321	14	C	-2.79461	-2.13219	-0.10633
4	C	2.811901	-0.24285	-0.00072	15	N	-5.11694	-2.74551	-0.15683
5	C	2.568529	1.142834	-0.13862	16	C	-6.54639	-2.43224	-0.14553
6	C	1.287679	1.628025	-0.23825	17	C	-7.12193	-2.292	-1.54903
7	C	-1.17102	1.186486	-0.27663	18	C	-4.58764	-4.69779	1.281439
8	C	-2.18252	0.242178	-0.26602	19	C	-4.7875	-4.16983	-0.13413
9	C	-1.84886	-1.14229	-0.14294	20	C	-1.47683	2.625018	-0.45752
10	O	-0.56099	-1.53173	-0.04163	21	C	-1.71327	3.075114	-1.75443
11	C	-3.57109	0.55807	-0.33351	22	C	-1.97751	4.419578	-1.99589

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
23	C	-2.00842	5.32547	-0.94083	44	H	-6.71624	-1.53394	0.449802
24	C	-1.77714	4.884361	0.355163	45	H	-7.03999	-3.24556	0.391329
25	C	-1.51072	3.537108	0.609182	46	H	-8.19591	-2.09637	-1.49409
26	C	-1.28969	3.051195	1.992907	47	H	-6.65047	-1.46836	-2.09136
27	O	-1.14204	1.884981	2.287813	48	H	-6.97259	-3.20839	-2.12632
28	O	-1.27141	4.03868	2.896186	49	H	-4.36749	-5.76797	1.252424
29	C	4.187424	-0.75127	0.108953	50	H	-3.75941	-4.19091	1.782959
30	C	5.229201	-0.16508	-0.59878	51	H	-5.48918	-4.55243	1.882536
31	C	6.536203	-0.65373	-0.50528	52	H	-5.61176	-4.69232	-0.62481
32	C	6.817513	-1.76033	0.33162	53	H	-3.9066	-4.3431	-0.75519
33	C	5.774114	-2.35022	1.050467	54	H	-1.68358	2.366763	-2.57571
34	C	4.490619	-1.85372	0.933031	55	H	-2.15935	4.756037	-3.01135
35	O	8.050141	-2.25455	0.454572	56	H	-2.21577	6.37395	-1.12606
36	C	7.598013	-0.03241	-1.27153	57	H	-1.80782	5.582443	1.183047
37	O	8.767232	-0.40731	-1.23611	58	H	-1.13269	3.645218	3.769474
38	H	1.871844	-2.19285	0.101426	59	H	5.039704	0.672745	-1.26387
39	H	3.403425	1.834463	-0.13885	60	H	5.991546	-3.19017	1.701144
40	H	1.111335	2.693677	-0.3345	61	H	3.699492	-2.31433	1.516438
41	H	-3.86079	1.599042	-0.4233	62	H	8.638152	-1.71342	-0.123
42	H	-5.56198	-0.11898	-0.36165	63	H	7.311982	0.819726	-1.91086
43	H	-2.45674	-3.15268	0.006135					

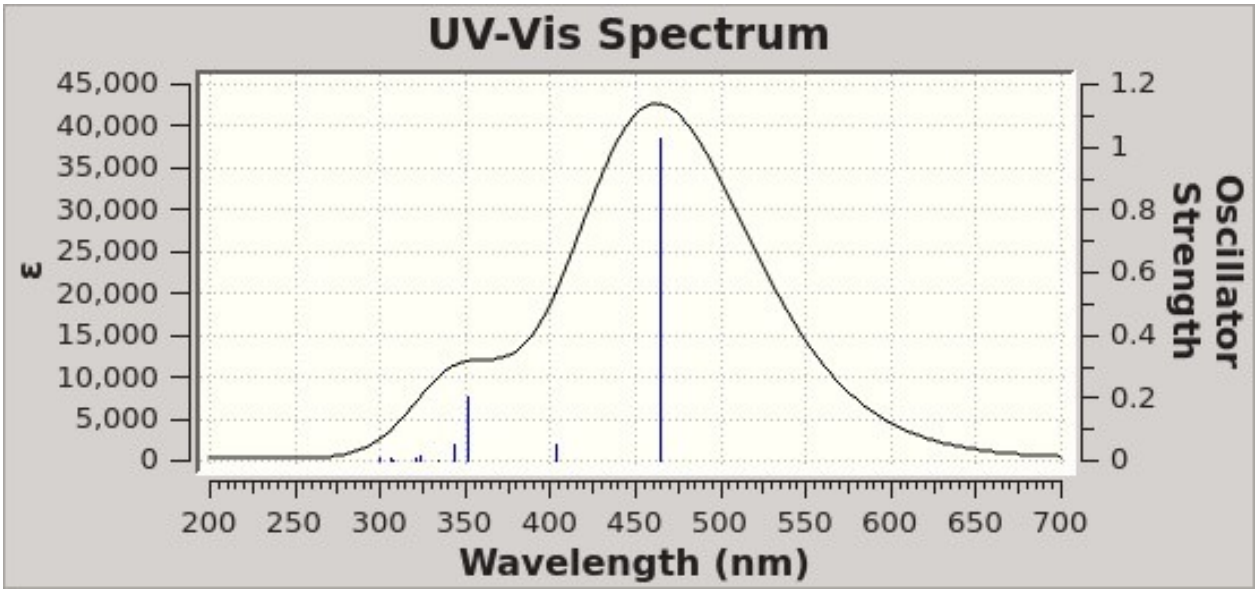


Figure S21: Calculated UV-Vis spectrum for probe **BH**$^{+}$ in water.

Table S8: Excitation energies and oscillator strengths listing for Probe **BH**$^{+}$ in water.

Excitation energies and oscillator strengths listing for Probe **BH**$^{+}$ in water.

Excited State	Nature	E (eV)	□□(nm)	*f*	Orbital transitions	Normalized coefficient
1:	A	2.6685	464.62	1.0257	129 ->130	0.69906
2:	A	3.0722	403.57	0.0536	127 ->130	0.21388
					128 ->130	0.66851
3:	A	3.5223	352.00	0.2036	127 ->130	0.64723
					128 ->130	-0.20214
					129 ->133	-0.10511
4:	A	3.6058	343.85	0.0496	128 ->131	-0.20402
					129 ->131	0.65947
5:	A	3.7013	334.97	0.0001	125 ->130	0.11965
					126 ->130	0.68119
6:	A	3.8234	324.28	0.0124	129 ->132	0.69725
7:	A	3.8649	320.79	0.0055	125 ->130	0.69189
					126 ->130	-0.12520
8:	A	4.0315	307.54	0.0016	123 ->131	0.66219
					128 ->131	0.17973
9:	A	4.0501	306.12	0.0088	123 ->131	-0.19639
					124 ->130	-0.23315
					128 ->131	0.58134
					129 ->131	0.22777
10:	A	4.1449	299.12	0.0081	121 ->130	0.13193
					122 ->130	0.64718
					124 ->130	0.19670

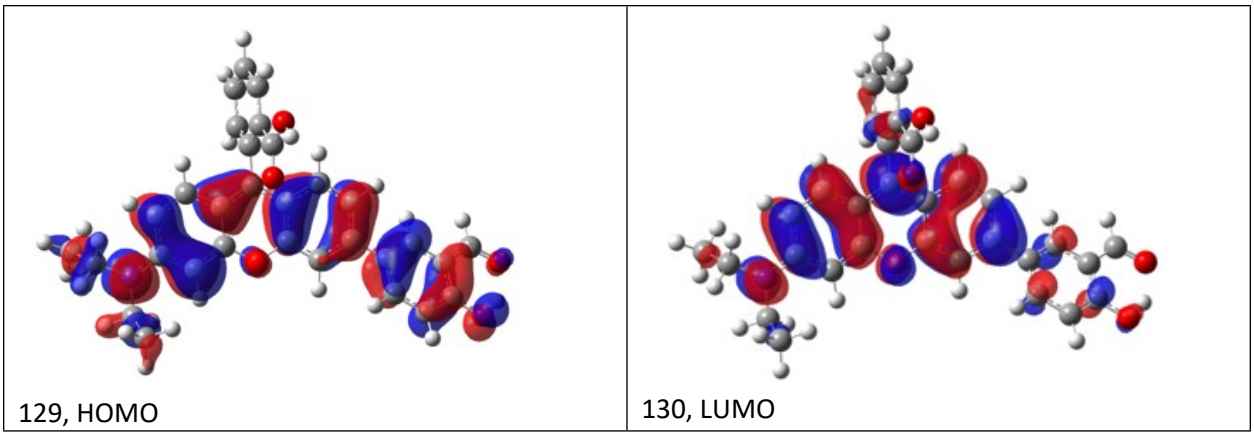


Figure S22: Drawings of selected molecular orbitals listed in Table S8.

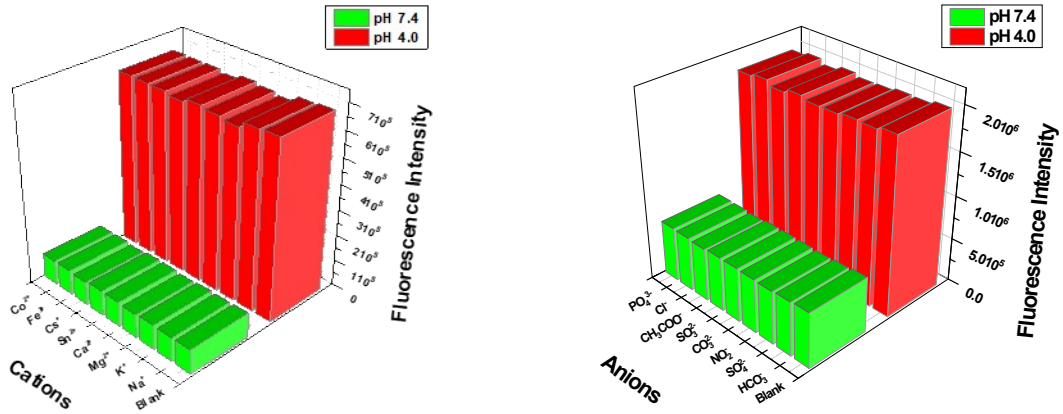


Figure S23: Emission intensities of probe **A** (10 µM) in the absence and presence of different cations (50 µM) and anions (50 µM) in pH 4.0 and 7.4 buffers under excitation at 465 nm.

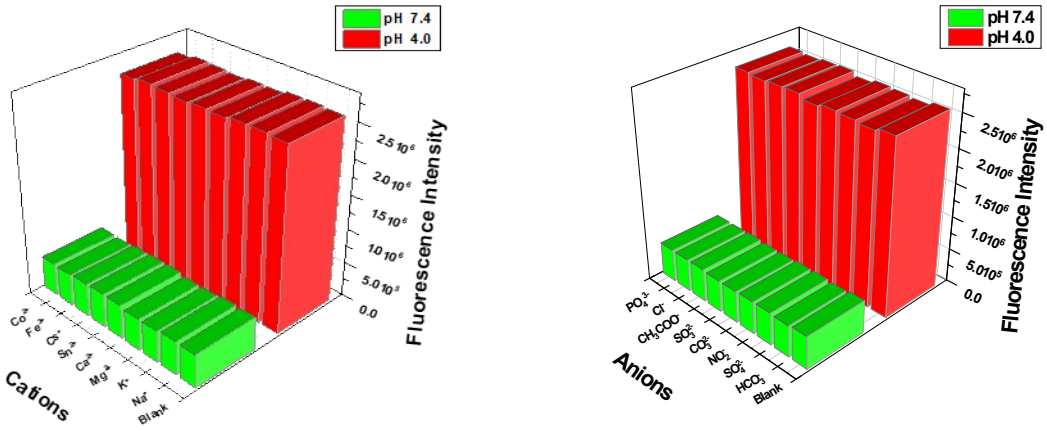


Figure S24: Emission intensities of probe **B** (10 µM) in the absence and presence of different cations (50 µM) and anions (50 µM) in pH 4.0 and 7.4 buffers under excitation at 465 nm.

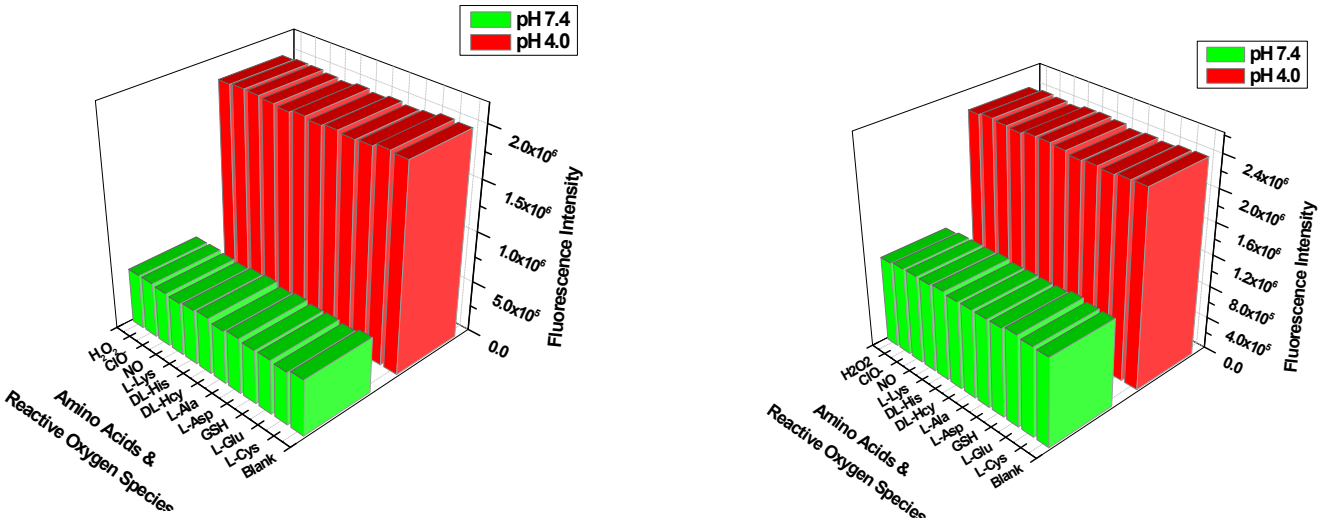


Figure S25: Emission intensities of 10 µM probes **A** (left) and **B** (right) in the absence and presence of different amino acids and reactive oxygen and nitrogen species (50 µM) in pH 4.0, and 7.4 buffers under excitation at 465 nm.

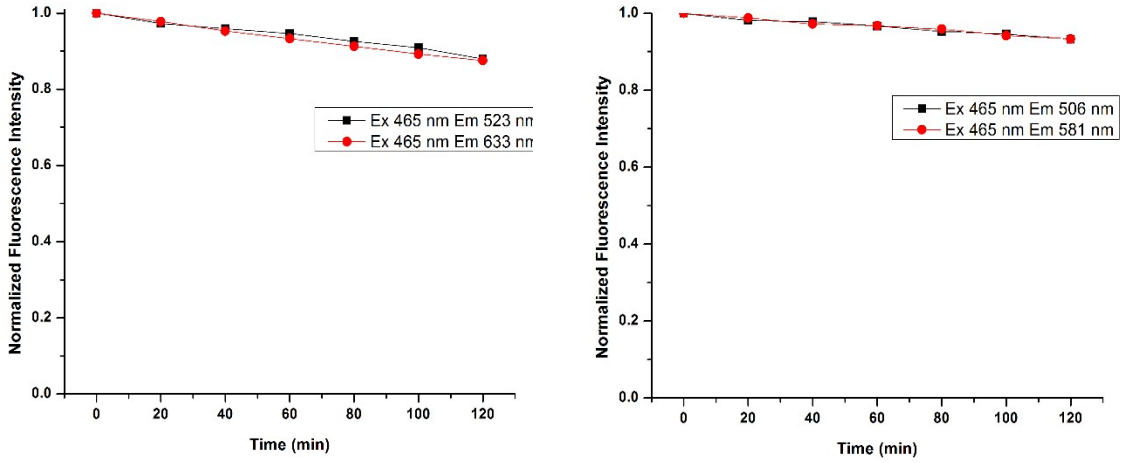


Figure S26: Photostability of 10 µM probe **A** (Left) and probe **B** (right) in pH 7.4 buffer under excitation at 465 nm.

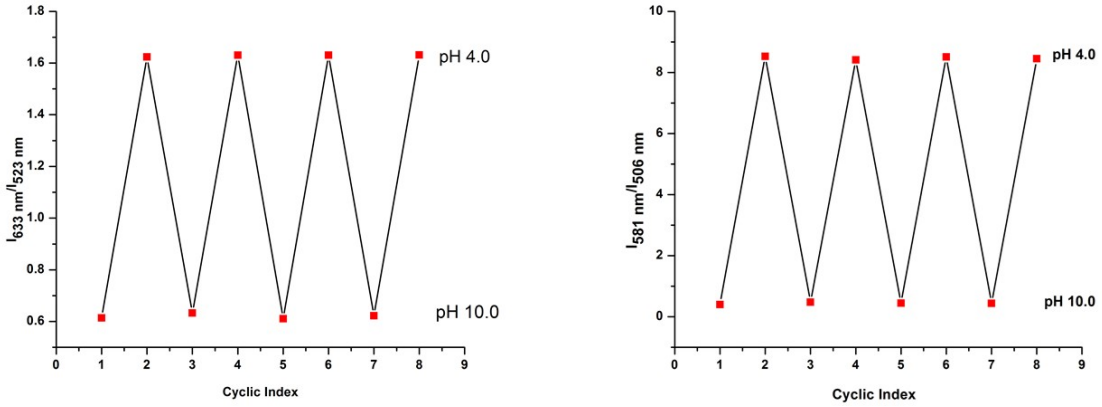


Figure S27: Fluorescence responses of 10 µM probe **A** (left) and probe **B** (right) in 10 % EtOH under pH changes between 4.0 and 10.0 under excitation at 465 nm.

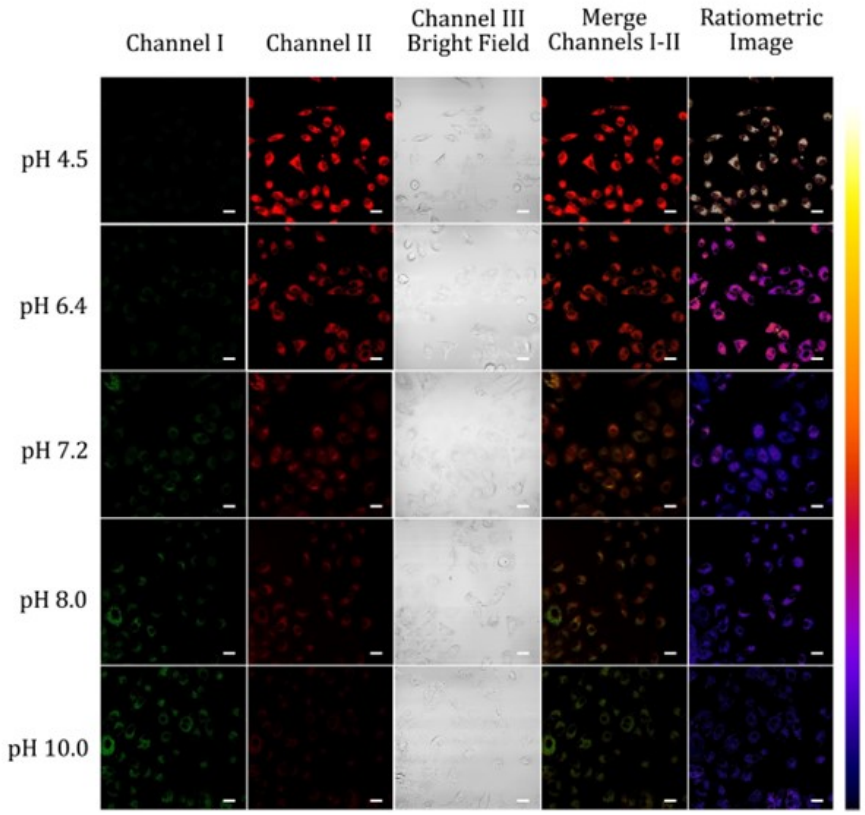


Figure S28: Fluorescence images of A549 cells incubated with 10 µM of probe **B** in different pH buffers in the presence of 10 µM nigericin. The green channel I was used to collect visible fluorescence of the probe from 500 nm to 550 nm while red channel II was utilized to collect red fluorescence from 575 nm to 650 nm of probe **B** at 488 nm excitation. Scale bars of all images above are at 50 µm.

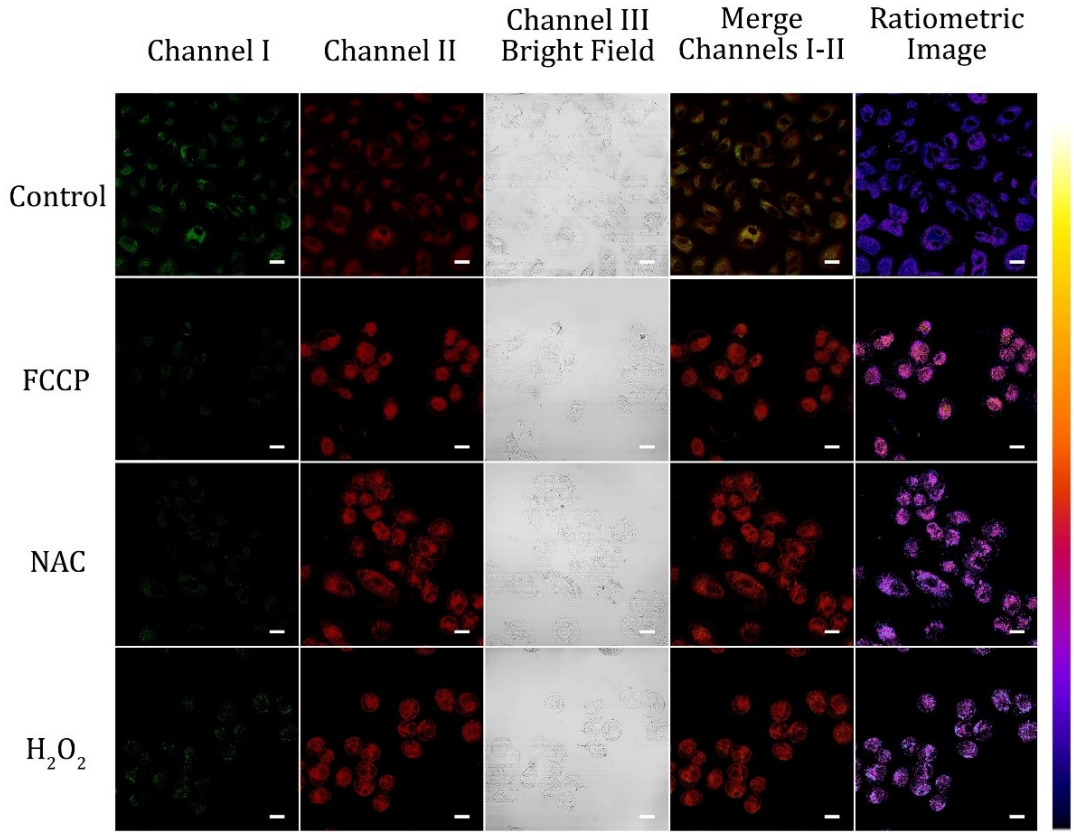


Figure 29: Fluorescence imaging of A549 cells incubated with 10 μM of probes **B** in normal medium with 20-min treatment of FCCP (10 μM), NAC (1 mM) or H_2O_2 (500 μM). The green channel I was used to collect visible fluorescence of probe **B** from 500 nm to 550 nm while red channel II was utilized to collect red fluorescence from 575 nm to 650 nm of probe **B** at 488 nm excitation. Scale bars of all images above are at 50 μm.

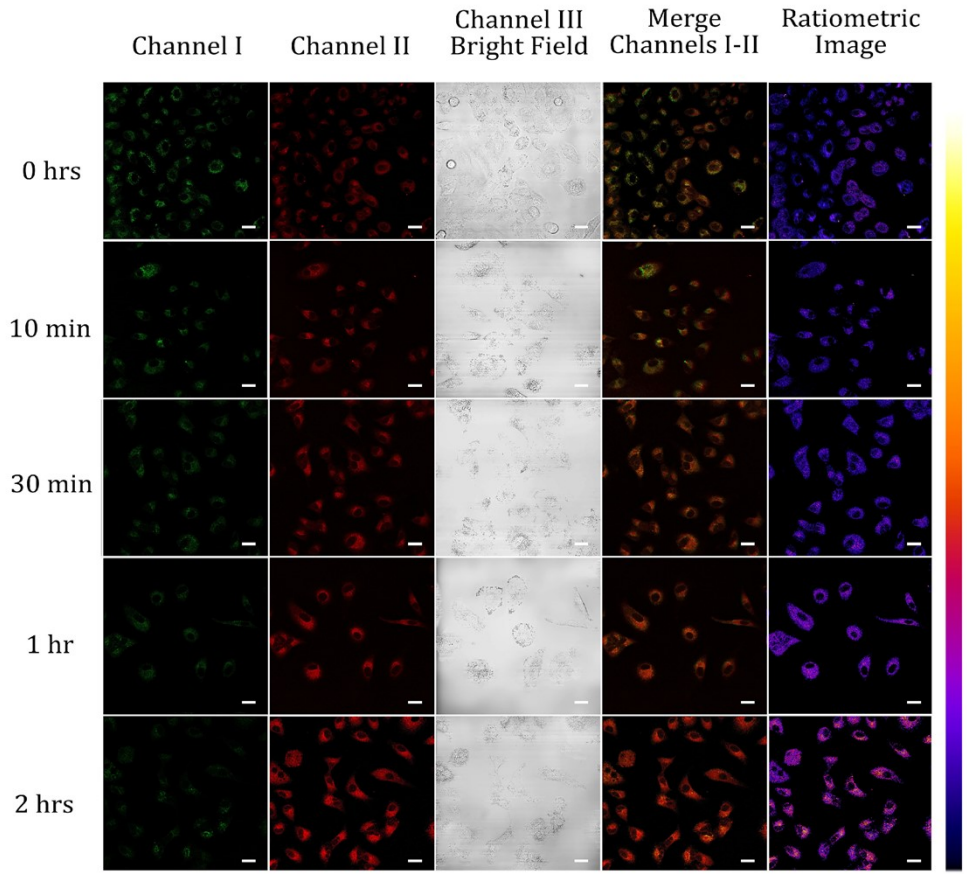


Figure 30: Fluorescence imaging of A549 cells incubated with 10 µM of probe **B** in serum-free medium for different times. The green channel I was used to collect the probe visible fluorescence of from 500 nm to 550 nm while red channel II was utilized to collect the probe fluorescence from 575 nm to 650 nm at 488 nm excitation. Scale bars of all images above are at 50 µm.

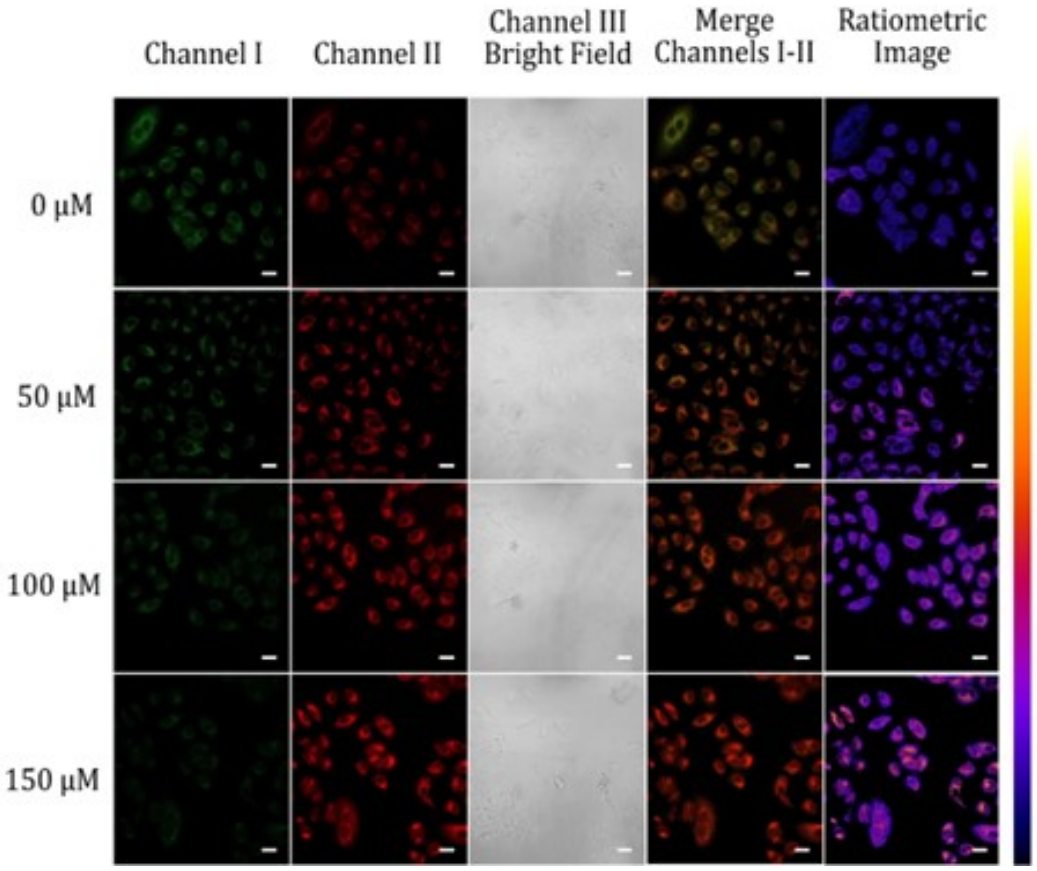


Figure 31. Confocal fluorescence microscopy images of A549 cells grown with 10 µM probe **B** under different hypoxia conditions in the absence and presence of 50, 100, 150 µM $CoCl_2$. The green channel I was used to collect the probe visible fluorescence from 500 nm to 550 nm while red channel II was utilized to the probe red fluorescence from 575 nm to 650 nm at 488 nm excitation. Scale bars of all images above are at 50 µm.

References:

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