Supporting Information

A Near-infrared Fluorescent Probe Based on Hemicyanine Dye with Oxazolidine Switch for Mitochondrial pH Detection

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1 NMR Spectra for Probe AH+

1.1 ^{1}H NMR Spectra for probe AH+

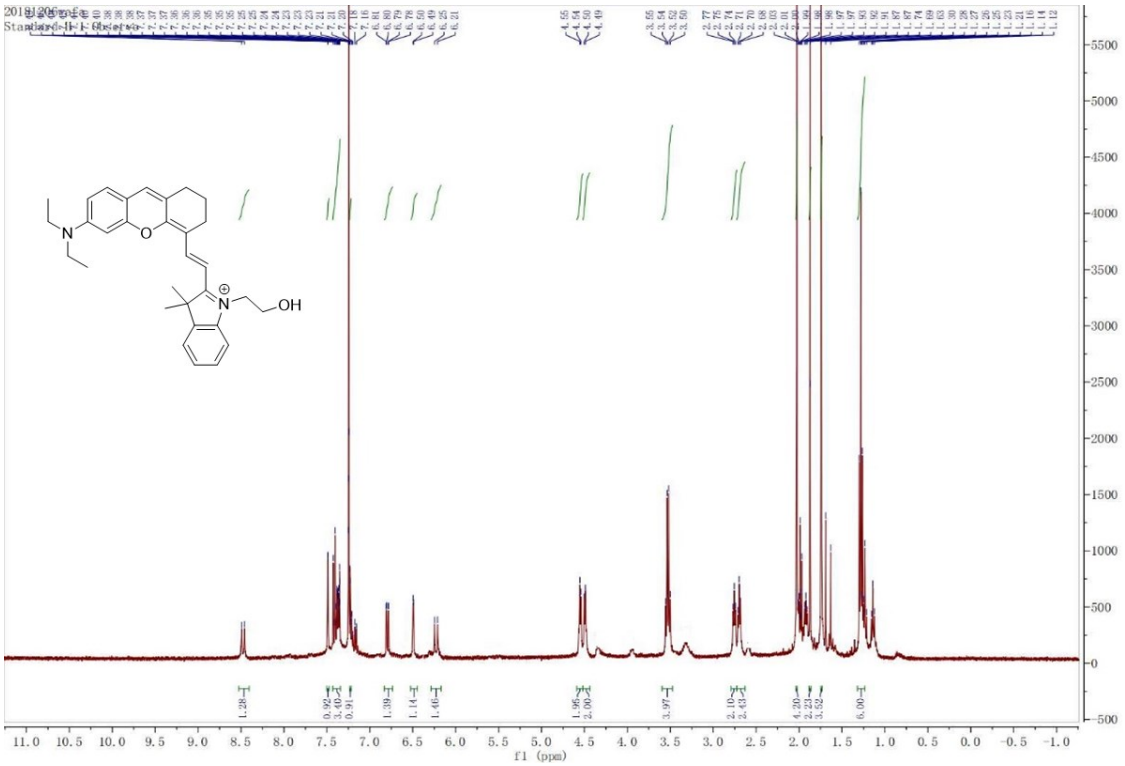


Figure S1: ^{1}H NMR spectrum of probe **AH+** based on rhodamine dye in chloroform-d solution.

1.2 ^{13}C NMR Spectra for probe AH+

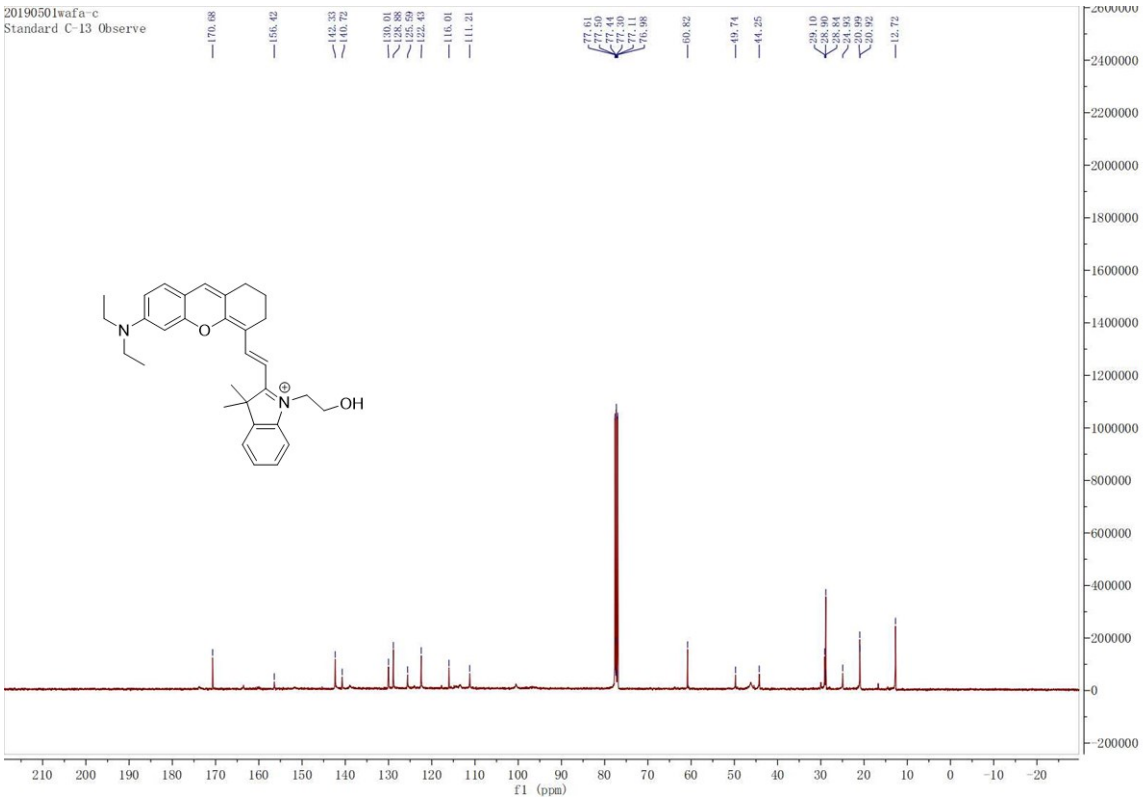


Figure S2: ^{13}C NMR spectrum of probe **AH+** based on rhodamine dye in chloroform-d solution.

1.3 ESI-MS spectra for probe AH+

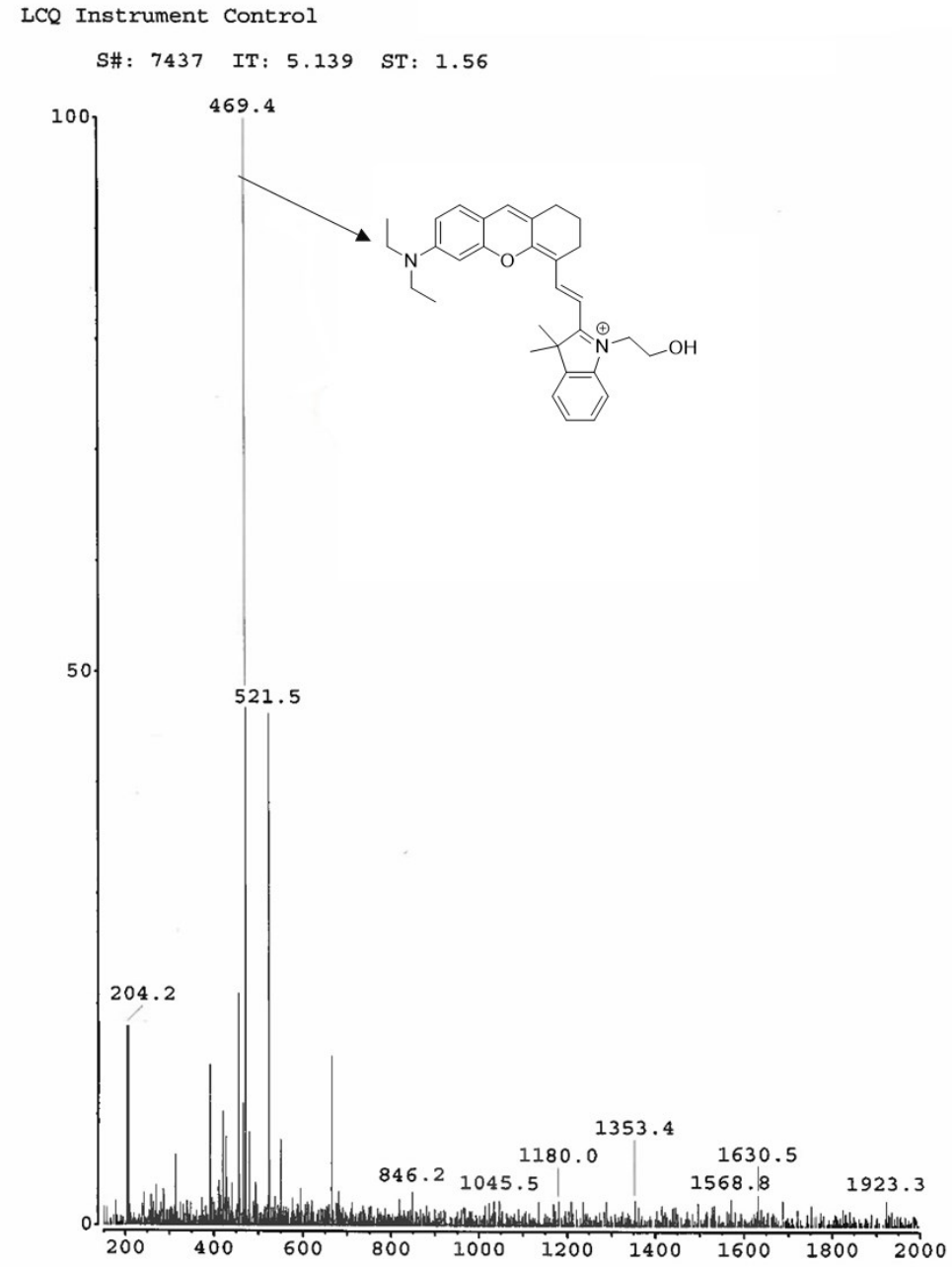


Figure S3. Electrospray Ionization mass spectrum of probe **AH+.**

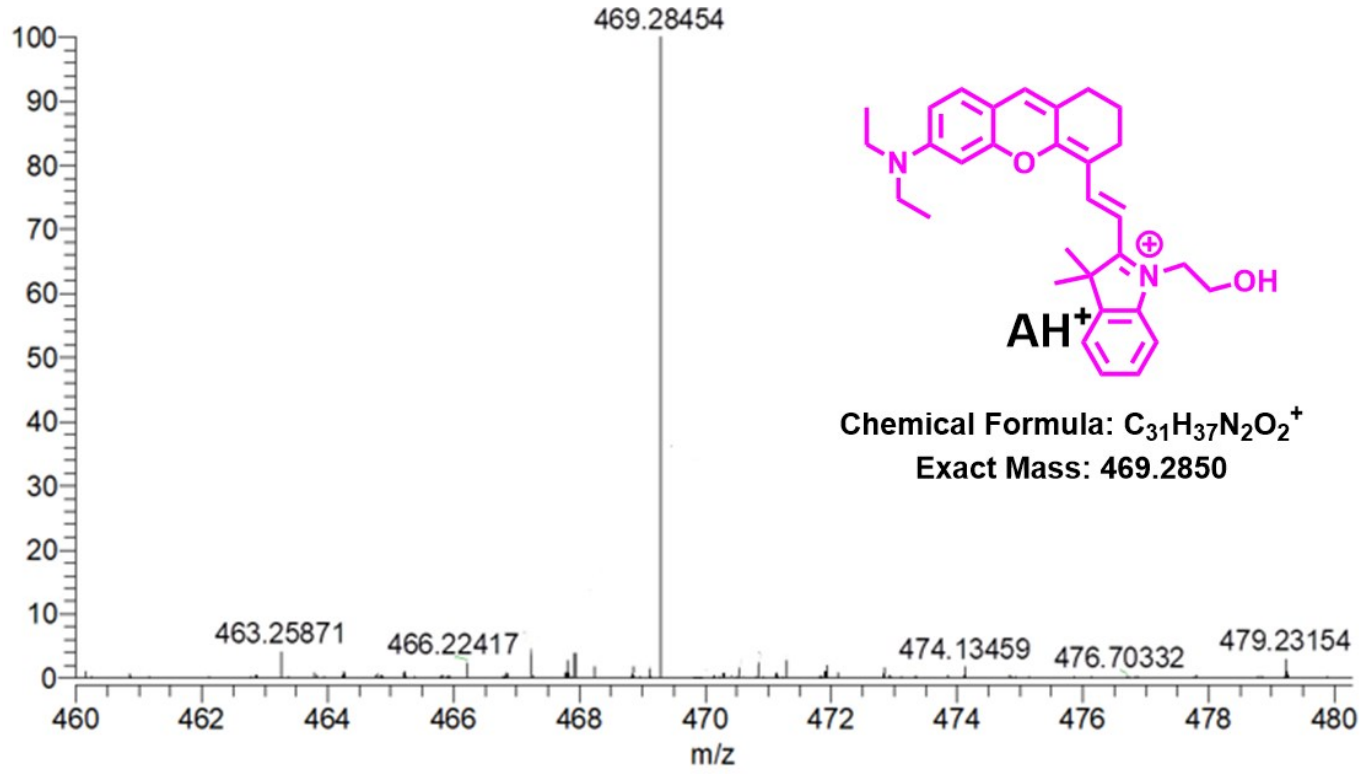


Figure S4. High-resolution electrospray Ionization mass spectrum of probe **AH+**.

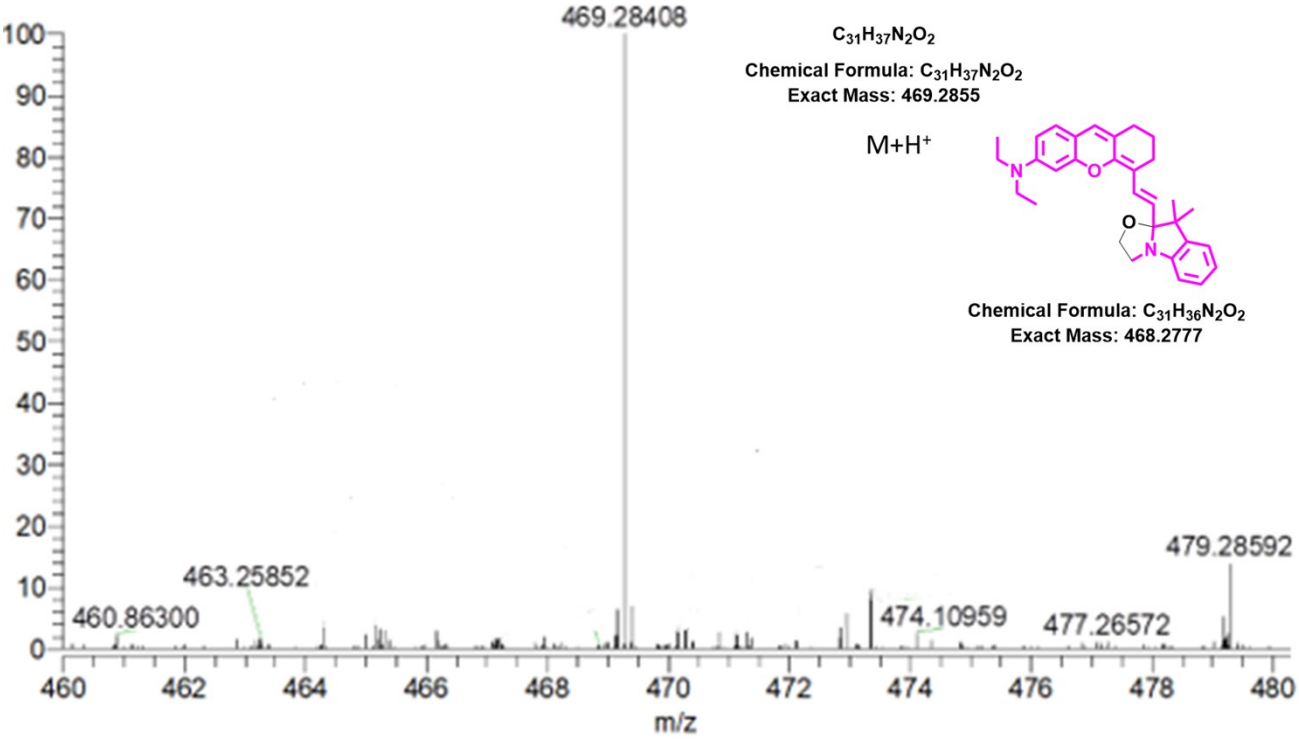


Figure S5. High-resolution electrospray Ionization mass spectrum of probe **A** under a basic condition.

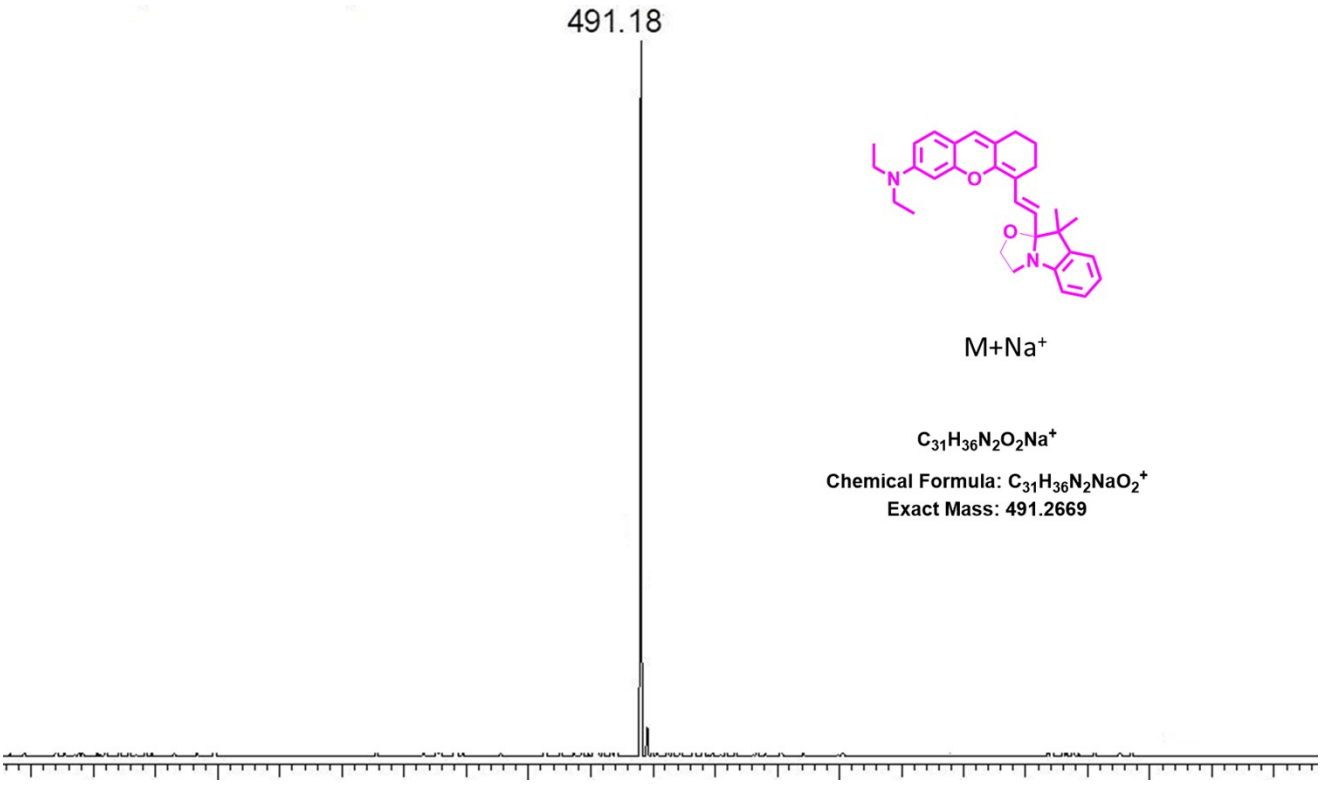
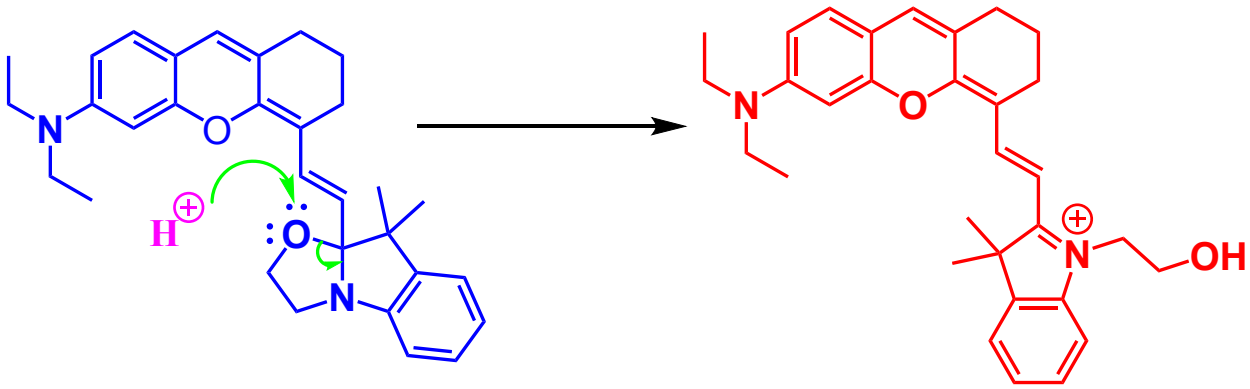


Figure S6. Matrix-assisted Laser Desorption Ionization-time of flight mass spectrum of probe **A** under a basic condition.

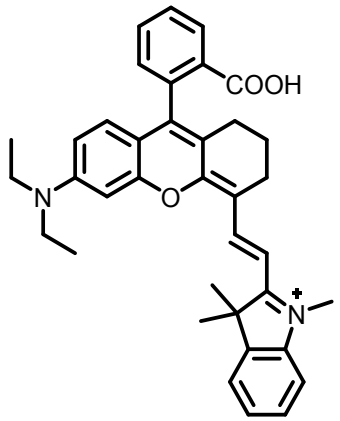


Scheme S1. Ring opening of an oxazolidine switch of the probe is triggered by acidic pH condition.

2 Calculation

2.1 Calculation of Quantum Yield

We chose the near-infrared hemicyanine dye shown in Figure S3 as fluorescent standard dye[1] to calculate fluorescence quantum yields of probe **AH+**.



Hemicyanine dye (Φ = 0.41 in EtOH)

The structure of near- infrared hemicyanine[1]

The UV-Vis absorption spectrum of probe **AH+** was collected in the range from 300 to 800 nm with increments of 1 nm. The UV-Vis absorption spectra measured in freshly prepared buffer. Citrate-phosphate buffer was used for acidic pH 5.0, and phosphate buffer for pH 10.7. The fluorescence spectra of probe **AH+** was collected under the excitation wavelength of 670 nm. The excitation and emission slit widths were set to 5 nm. NIR hemicyanine was chosen as reference standards to calculate the fluorescence quantum yield of probe **AH+**, in ethanol and buffer solutions. The absorption and fluorescence spectra of the standard dye was measured in pH 7.4 PBS buffer with 5% ethanol. The absorbance and fluorescence spectra of the probe A was measured in pH 5.0 citrate-phosphate buffer and in pH 7.4 PBS buffer containing 5% EtOH. The absorbance was kept between 0.05 and 0.1 in order to obtain optimized data. The probe samples and reference were freshly prepared under identical conditions. The fluorescence quantum yields were calculated according to literature[4] using t equation 1 below[1] :

$$\Phi_{F(X)} = \Phi_{F(S)} (AsFx / AxFs) (nx/ns)^2 \qquad (1)$$

Where ΦF is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts s and x refer to the standard and to the unknown, respectively.

2.2 Calculation of probe pK_a value by fluorometric titration

The fluorometric titration as a function of pH was obtained fluorescence spectra. The equation (2) below was used to calculate the pKa value of probe **AH+**.

$$F = \frac{F_{\min}[H^+]^n + F_{\max}K_a}{K_a + [H^+]^n} \qquad (2)$$

The expression of the steady-state fluorescence intensity F as a function of the proton concentration has been extended for the case of n: complex between H^+ and a fluorescent dye. Where $F_{\min}$ and $F_{\max}$ are the fluorescence intensities at maximal and minimal H^+ concentrations, respectively.

n is apparent stoichiometry of H^+ binding to the probe A. Nonlinear fitting of equation expressed above to the fluorescence titration data was plotted as a function of H^+ concentration[2-3].

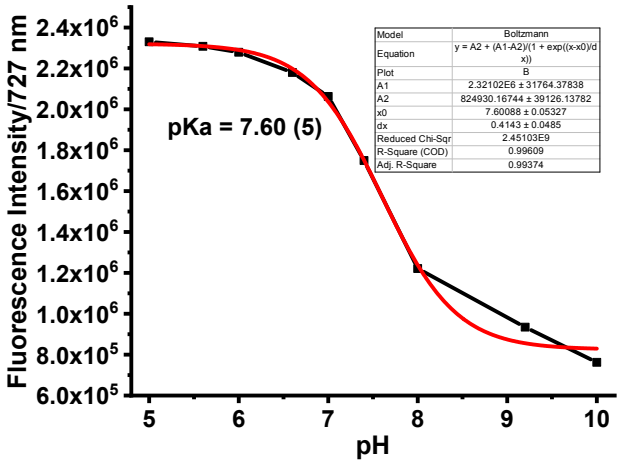


Figure S7. Plot curve of fluorescence intensity of 5 μM probe **AH+** versus pH

3 Solvent Effect

The effect of ethanol percentage was investigated in water-ethanol mixed solution on dye fluorescence intensity (Figure S5). Increase of the percentages of ethanol from 10% to 90% resulted in enhancement of fluorescence intensity of the dyes because water increase percentages can effectively prevent fluorescence quenching due to dye aggregation in aqueous solutions.

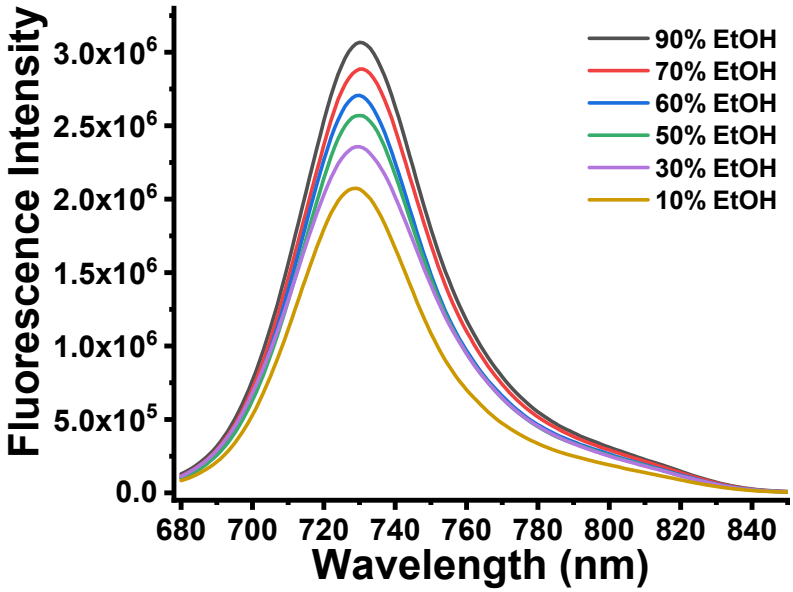
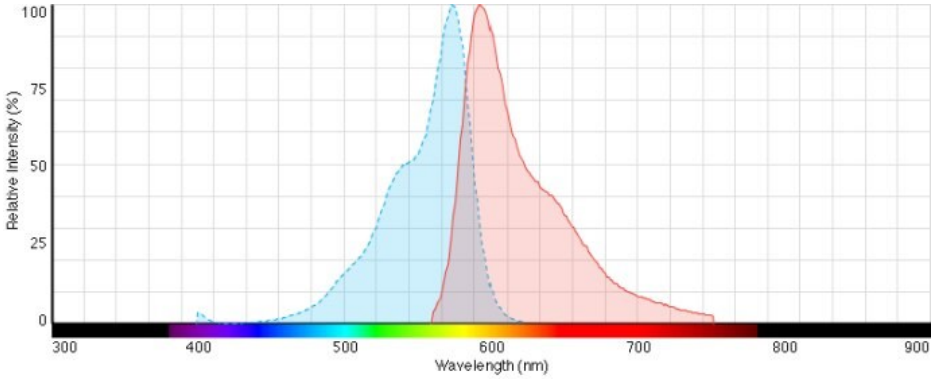


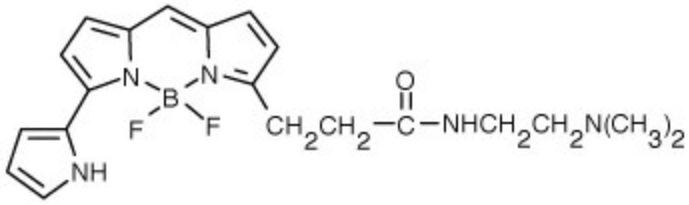
Figure S8. Fluorescence spectra of 5 μM probe **AH+** in pH 7.0 buffers with different percentages of ethanol under excitation at 670 nm.

4. Commercial Lysotracker Red DND-99 emission spectra from Thermo Fisher Scientific

https://www.thermofisher.com/order/catalog/product/L7528#/L7528



Absorption and fluorescence spectra of Lysotracker Red DND-99 which was obtained from the link above.

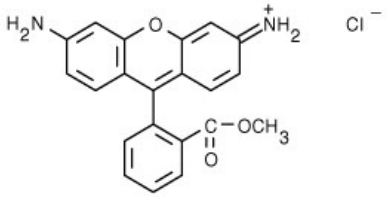


Chemical structure of Lysotracker Red DND-99.

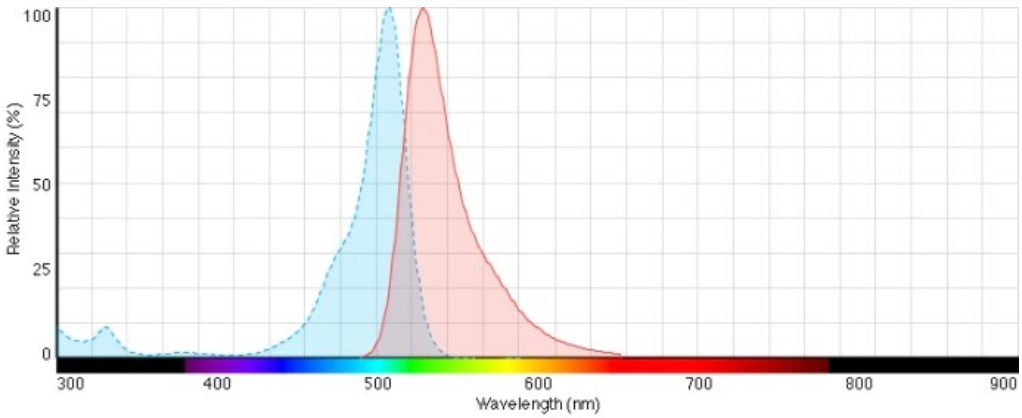
In the cellular fluorescence imaging experiments, we excited Lysotracker Red DND-99 at 559 nm and collected fluorescence from 600 nm to 650 nm while we excited the probe at 635 nm and collected fluorescence from 725 nm to 775 nm. The absorption of Lysotracker Red DND-99 at 635 nm is extremely small according to its absorption peak above which was obtained from the website link above. The collected fluorescence of Lysotracker Red DND-99 from 600 nm to 650 nm is not overlapped with that of the probe from 725 nm to 775 nm (Figure 7).

5. Mitochondria-specific Rhodamine 123.

Rhoadmine dye 123 has been commercialized as a mitochondria-targeting probe by ThermoFisher Scientific. The detailed information is available from the like below.



https://www.thermofisher.com/order/catalog/product/R302?SID=srch-srp-R302#/R302?SID=srch-srp-R302



Absorption and fluorescence spectra of Rhodamine 123 which was obtained from the link above.

In cellular fluorescence imaging experiments, we excited Rhodamine 123 at 488 nm and collected fluorescence from 500 nm to 550 nm while we excited the probe at 635 nm and collected fluorescence from 725 nm to 775 nm. The absorption of Rhodamine 123 at 635 nm is almost zero according to its absorption peak above which was obtained from the website link above. The collected fluorescence of Rhodamine 123 from 500 nm to 550 nm is not overlapped with that of the probe from 725 nm to 775 nm (Figure 7).

6. Golgi-specific fluorescent probe (Golgi-GFP).

A Golgi-specific fluorescent probe (Golgi-GFP, BacMam) from ThermoFisher Scientific was used for colocalization experiment with the probe.

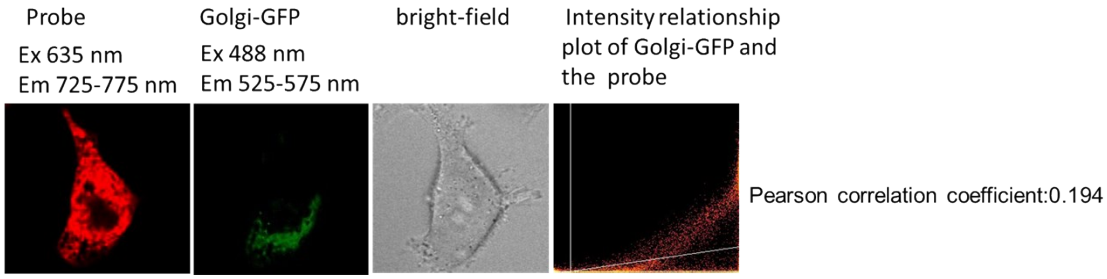


Figure S9. Confocal microscopic fluorescence images and merged images of the probe colocalized with Golgi-GFP, BacMam in HeLa cells. Colocalization scatterplot of the probe with Golgi-GFP, BacMam. Pearson correlation coefficient: 0.194

7. ER-specific fluorescent probe (ER-Tracker™ Green, glibenclamide BODIPY® FL).

ER-Tracker™ Green dye from TheromoFisher Scientific was employed for colocalization experiment with the probe.

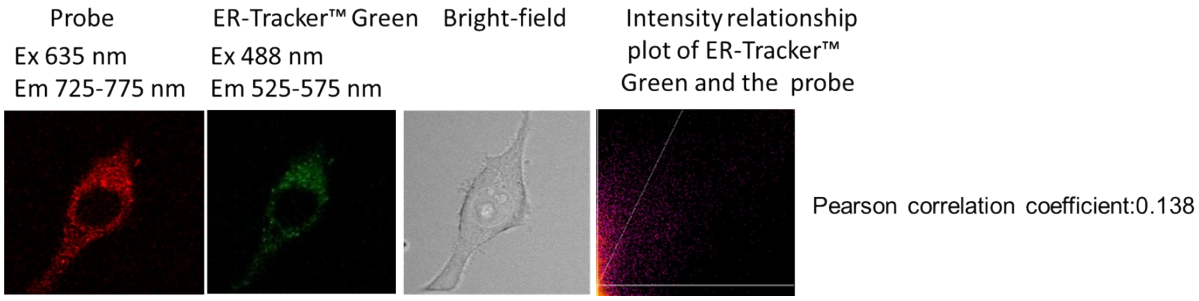


Figure S10. Confocal microscopic fluorescence images and merged images of the probe colocalized with Golgi-GFP, BacMam in HeLa cells. Colocalization scatterplot of the probe with ER-Tracker™ Green (glibenclamide BODIPY® FL). Pearson correlation coefficient: 0.138

8. Computationally derived structures for probe A and AH^+.

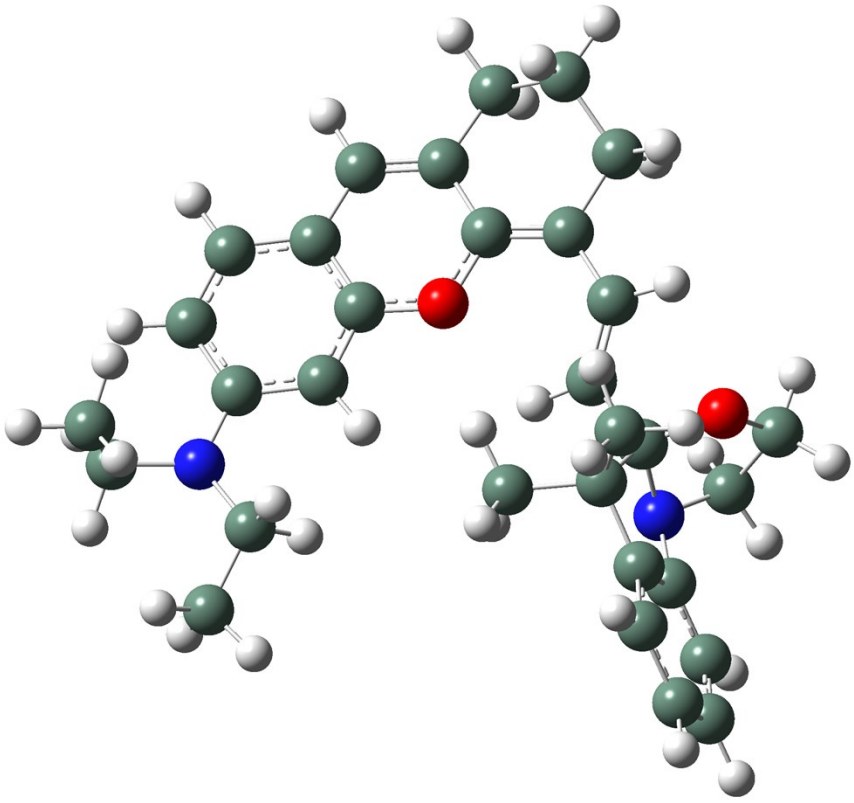


Figure S11. Drawing of probe **A** with atoms represented as spheres of arbitrary size (H-white, C-grey, N-blue and O-red) using the GaussView program.

Table S1. Atomic coordinates for probe **A**.

Row	Symbol	X	Y	Z
1	C	5.065562	-0.30195	0.231392
2	C	3.918238	-1.1465	0.093227
3	C	2.655842	-0.51606	0.026288
4	C	2.570472	0.857508	0.108113
5	C	3.691188	1.695034	0.248157
6	C	4.948799	1.060704	0.30816
7	O	1.328055	1.402961	0.032912
8	C	1.107847	2.734944	0.080026
9	C	2.208399	3.618407	0.21323
10	C	3.47284	3.086059	0.292677
11	C	-0.22955	3.161402	-0.01656
12	C	-0.4802	4.657831	-0.00213
13	C	0.703405	5.460642	-0.52496
14	C	1.956349	5.098755	0.261899
15	C	-1.3436	2.329105	-0.09771
16	C	-1.44635	0.936341	-0.05956
17	N	4.038025	-2.50149	0.029835
18	C	5.348935	-3.1135	-0.15602
19	C	2.849696	-3.2905	-0.31294
20	C	2.999117	-4.79088	-0.13598
21	C	5.839974	-3.04814	-1.59754
22	C	-2.64245	0.23876	-0.12328
23	C	-4.06793	0.769153	-0.23452
24	C	-4.86679	-0.51027	-0.22335
25	C	-3.99988	-1.59284	-0.11677
26	N	-2.68327	-1.11415	-0.07904
27	C	-6.23192	-0.71838	-0.28953
28	C	-6.71414	-2.02998	-0.24242
29	C	-5.83195	-3.10373	-0.12632
30	C	-4.45217	-2.9037	-0.05959
31	C	-4.45013	1.626452	0.982585
32	C	-4.29159	1.521784	-1.55506
33	C	-1.5289	-1.9787	0.048135
34	C	-1.10646	-2.12267	1.502644
35	O	0.054533	-2.92997	1.504442
36	H	6.057687	-0.73129	0.266281
37	H	1.741027	-1.07871	-0.09343
38	H	5.840598	1.672691	0.408444
39	H	4.328261	3.750475	0.389404

Row	Symbol	X	Y	Z
40	H	-1.37601	4.87539	-0.59212
41	H	-0.70675	4.979214	1.024217
42	H	0.499696	6.532647	-0.44503
43	H	0.860348	5.240429	-1.58812
44	H	2.835042	5.629837	-0.11595
45	H	1.832084	5.40953	1.308209
46	H	-2.27694	2.876612	-0.18611
47	H	-0.5422	0.357509	0.04278
48	H	6.059746	-2.64612	0.526935
49	H	5.282479	-4.14878	0.175449
50	H	2.030745	-2.96412	0.328549
51	H	2.552468	-3.06533	-1.34825
52	H	2.028251	-5.25478	-0.32872
53	H	3.714325	-5.24028	-0.82917
54	H	3.290911	-5.05344	0.884998
55	H	6.820545	-3.52354	-1.69084
56	H	5.149922	-3.56559	-2.27037
57	H	5.930477	-2.01398	-1.94166
58	H	-6.92092	0.117394	-0.37411
59	H	-7.78302	-2.21377	-0.2923
60	H	-6.22068	-4.11683	-0.08422
61	H	-3.77821	-3.74774	0.040142
62	H	-3.88934	2.561422	1.020171
63	H	-5.51325	1.874639	0.932159
64	H	-4.27199	1.083253	1.913689
65	H	-3.71512	2.446922	-1.60169
66	H	-4.0121	0.901822	-2.41011
67	H	-5.34914	1.779607	-1.65286
68	H	-1.7752	-2.95251	-0.3764
69	H	-0.71222	-1.57685	-0.55485
70	H	-0.90405	-1.13474	1.935272
71	H	-1.91692	-2.5848	2.080605
72	H	0.349497	-3.06125	2.410781

Table S2. Excitation energies and oscillator strengths for probe A.

Excited State	Nature	E (eV)	λ (nm)	*f*	Orbital transitions	Normalized coefficient
1	Singlet-A	2.9705	417.39	0.5427	126 ->127	0.70254
2	Singlet-A	3.8205	324.52	0.0945	124 ->127 125 ->127 126 ->128 126 ->129	0.12029 -0.16699 0.49384 -0.44206
3	Singlet-A	3.9032	317.65	0.0432	124 ->127 125 ->127 126 ->128 126 ->130	-0.27488 0.45655 0.10080 -0.42340
4	Singlet-A	4.0144	308.85	0.0071	124 ->127 125 ->127	0.55750 0.41595
5	Singlet-A	4.1347	299.86	0.0063	126 ->128 126 ->129	0.48025 0.51226
6	Singlet-A	4.2653	290.68	0.3107	124 ->127 125 ->127 126 ->129 126 ->130 126 ->132	-0.28800 0.27224 -0.11983 0.50848 -0.10521

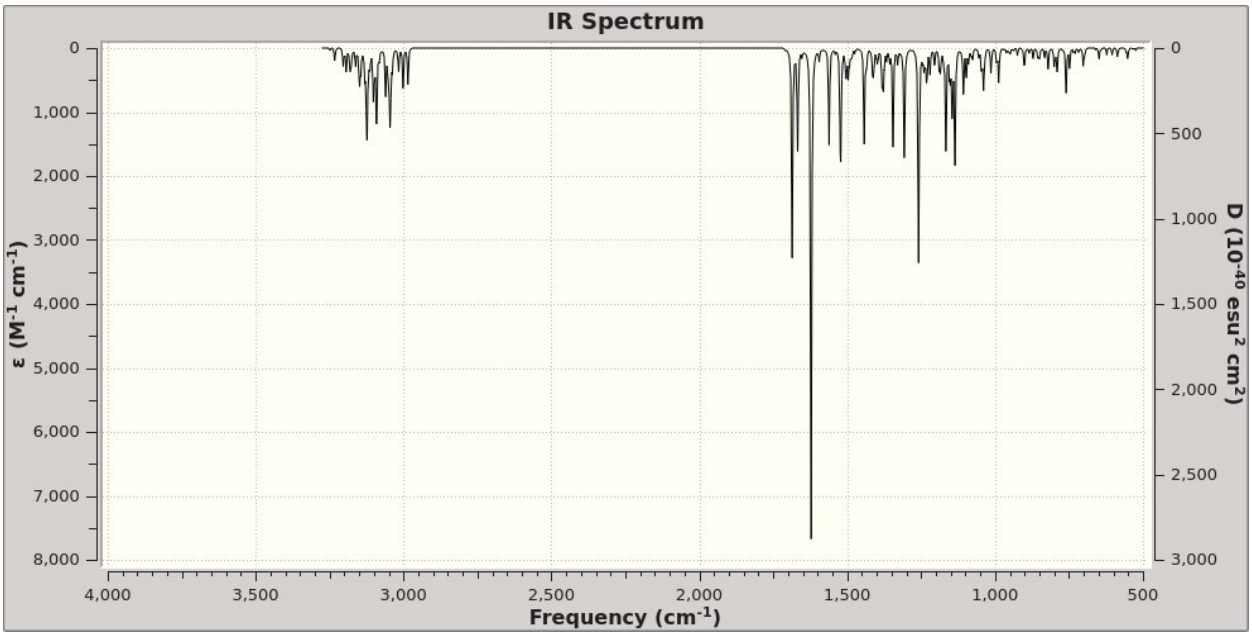


Figure S12. Calculated (top) FTIR spectrum of probe **A.**

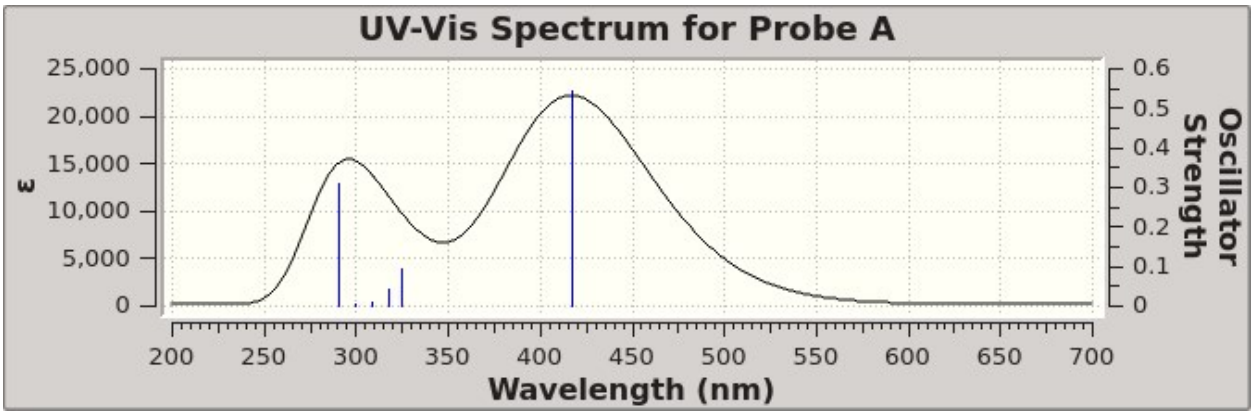


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Figure S13. Calculated UV-Vis spectrum for probe **A** in water.

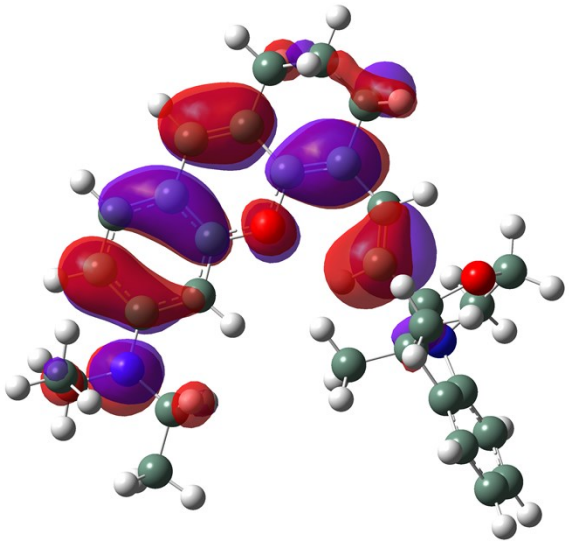

Figure S14. LCAO for level 126 for probe **A** involved with the transition noted as Excited State 1 in Table S2.

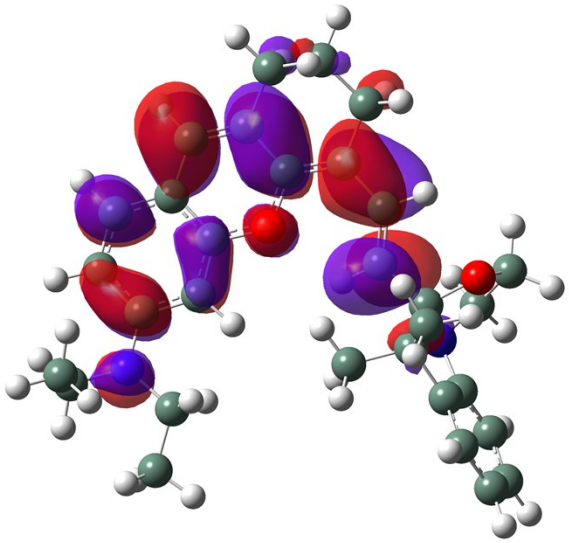

Figure S15. LCAO for level 127 for probe **A** involved with the transition noted as Excited State 1 in Table S2.

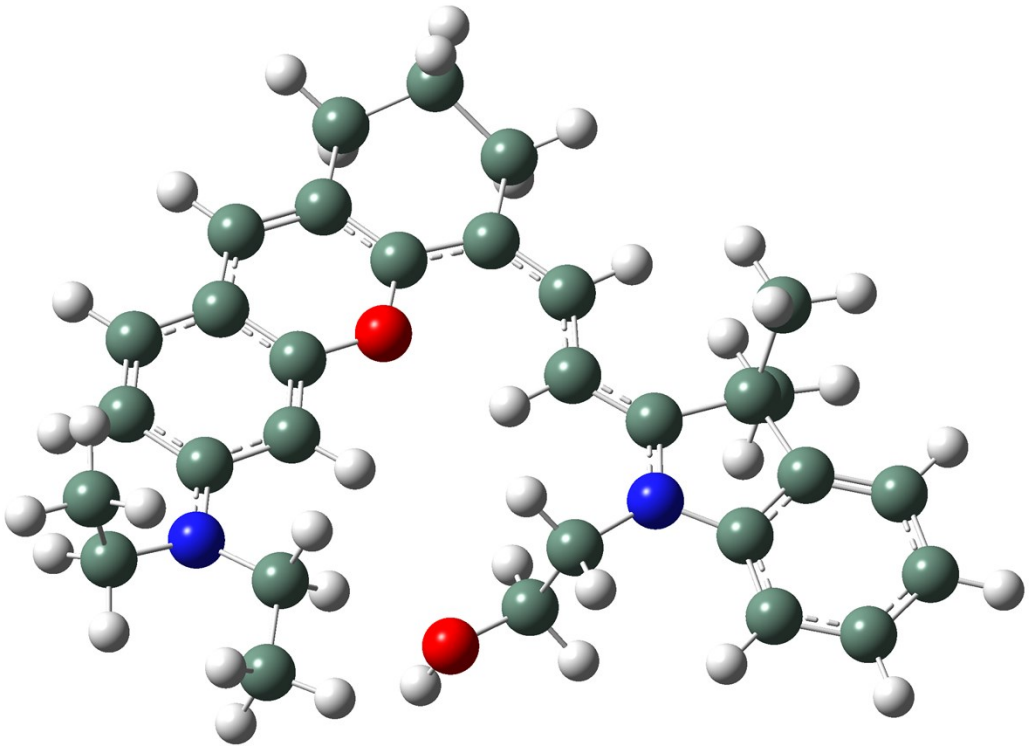

Figure S16. Drawing of probe **AH**$^{+}$ with atoms represented as spheres of arbitrary size (H-white, C-grey, N-blue and O-red) using the GaussView program.

Table S3. Atomic coordinates for probe $\mathbf{AH^+}$.

Row	Symbol	X	Y	Z
1	C	5.065562	-0.30195	0.231392
2	C	3.918238	-1.1465	0.093227
3	C	2.655842	-0.51606	0.026288
4	C	2.570472	0.857508	0.108113
5	C	3.691188	1.695034	0.248157
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11	C	-0.22955	3.161402	-0.01656
12	C	-0.4802	4.657831	-0.00213
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34	C	-1.10646	-2.12267	1.502644
35	O	0.054533	-2.92997	1.504442
36	H	6.057687	-0.73129	0.266281
37	H	1.741027	-1.07871	-0.09343
38	H	5.840598	1.672691	0.408444
39	H	4.328261	3.750475	0.389404

Row	Symbol	X	Y	Z
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42	H	0.499696	6.532647	-0.44503
43	H	0.860348	5.240429	-1.58812
44	H	2.835042	5.629837	-0.11595
45	H	1.832084	5.40953	1.308209
46	H	-2.27694	2.876612	-0.18611
47	H	-0.5422	0.357509	0.04278
48	H	6.059746	-2.64612	0.526935
49	H	5.282479	-4.14878	0.175449
50	H	2.030745	-2.96412	0.328549
51	H	2.552468	-3.06533	-1.34825
52	H	2.028251	-5.25478	-0.32872
53	H	3.714325	-5.24028	-0.82917
54	H	3.290911	-5.05344	0.884998
55	H	6.820545	-3.52354	-1.69084
56	H	5.149922	-3.56559	-2.27037
57	H	5.930477	-2.01398	-1.94166
58	H	-6.92092	0.117394	-0.37411
59	H	-7.78302	-2.21377	-0.2923
60	H	-6.22068	-4.11683	-0.08422
61	H	-3.77821	-3.74774	0.040142
62	H	-3.88934	2.561422	1.020171
63	H	-5.51325	1.874639	0.932159
64	H	-4.27199	1.083253	1.913689
65	H	-3.71512	2.446922	-1.60169
66	H	-4.0121	0.901822	-2.41011
67	H	-5.34914	1.779607	-1.65286
68	H	-1.7752	-2.95251	-0.3764
69	H	-0.71222	-1.57685	-0.55485
70	H	-0.90405	-1.13474	1.935272
71	H	-1.91692	-2.5848	2.080605
72	H	0.349497	-3.06125	2.410781

Table S4. Excitation energies and oscillator strengths for probe **AH**$^{+}$.

Excited State	Nature	E (eV)	λ (nm)	*f*	Orbital transitions	Normalized coefficient
1	Singlet-A	2.0899	593.26	0.7993	126 ->127	0.70631
2	Singlet-A	2.8527	434.62	0.2023	125 ->127 126 ->128	0.68787 0.12101
3	Singlet-A	3.6147	343.00	0.1318	122 ->127 124 ->127 126 ->128	-0.24296 -0.42811 0.49106
4	Singlet-A	3.7149	333.75	0.3055	124 ->127 125 ->127 126 ->128	0.53865 -0.10258 0.41981
5	Singlet-A	3.9046	317.54	0.0383	123 ->127 126 ->129	0.66762 -0.16028
6	Singlet-A	3.9614	312.98	0.1464	122 ->127 123 ->127 125 ->128 126 ->128 126 ->130	0.58588 0.11095 -0.22459 0.19025 -0.14800

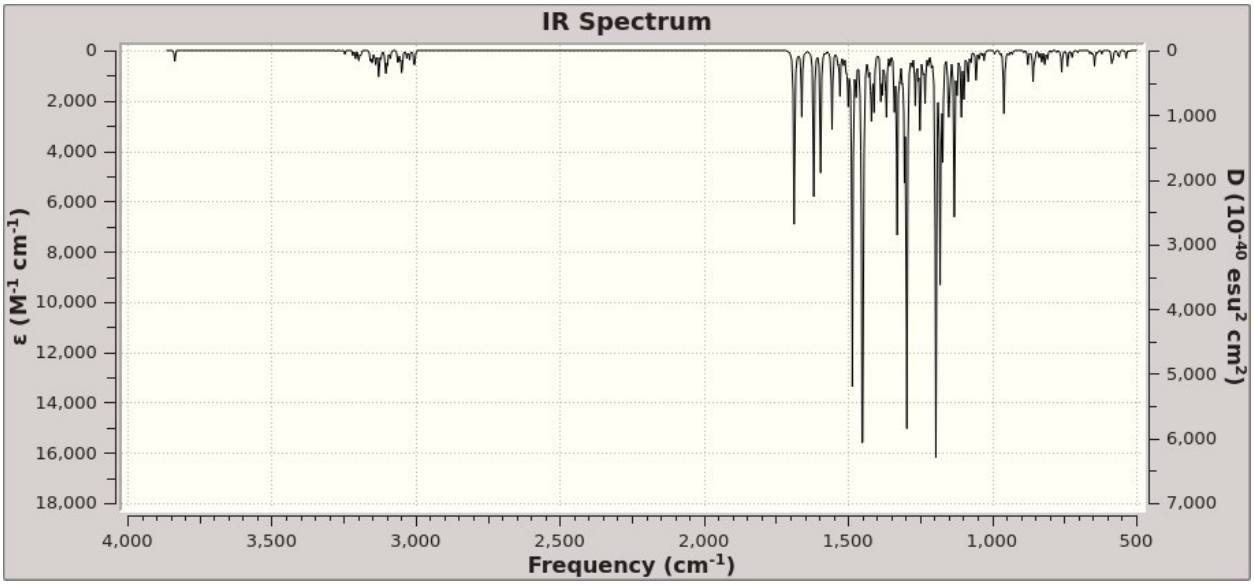

Figure S17. Calculated (top) FTIR spectrum of probe **AH**$^{+}$.

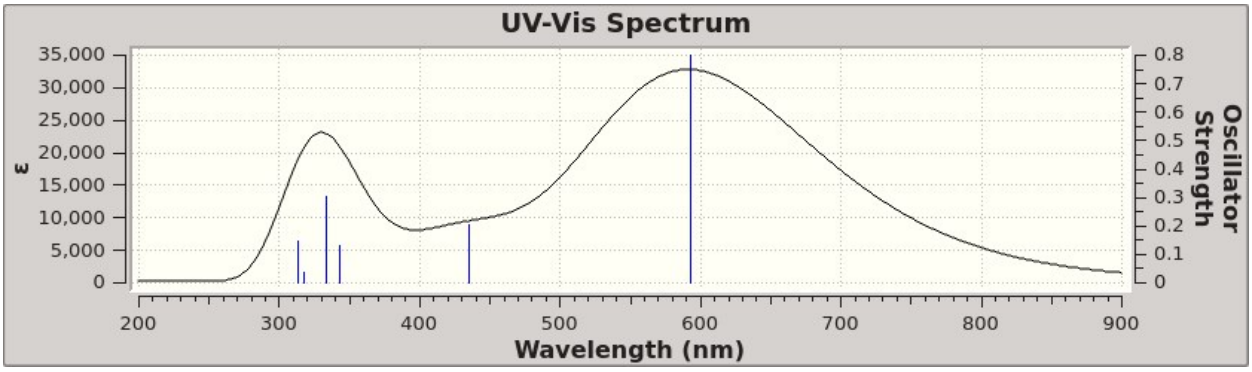

Figure S18. Calculated UV-Vis spectrum for probe **AH**$^+$ in water.

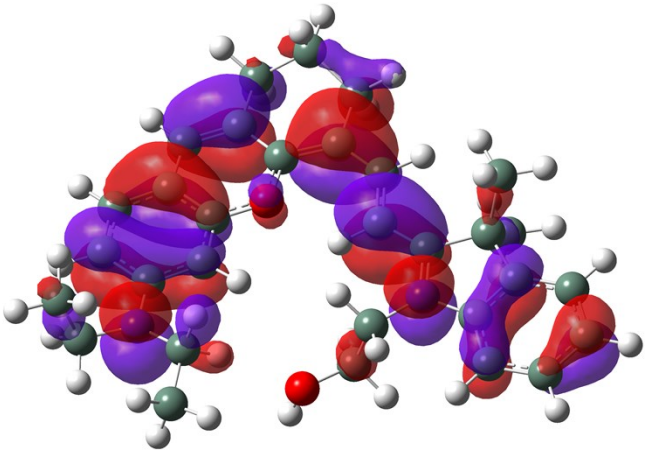

Figure S19. LCAO for level 126 for probe **AH**$^+$ involved with the transition noted as Excited State 1 in Table S4.

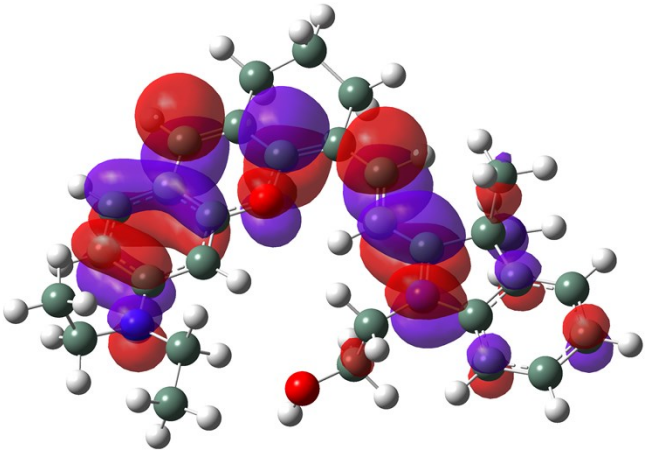

Figure S20. LCAO for level 127 for probe $\mathbf{AH^+}$ involved with the transition noted as Excited State 1 in Table S4.

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