Supporting Information

Ratiometric Fluorescent Probes Based on Through-Bond Energy Transfer of Cyanine Donors to Near-infrared Hemicyanine Acceptors for Mitochondrial pH Detection and Monitoring of Mitophagy Process

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1. Instruments

^{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.

2. Materials and Methods

2.1 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.[1, 2]

Synthesis of compound 3: A mixture of compound **1** (0.75 g, 2.0 mmol) and **2a** (0.45 g, 2.2 mmol) in anhydrous Ac_2O was heated at 50°C for 1 h. After the reaction had completed, the solvent was evaporated under reduced pressure. Water (25 mL) was added, and the mixture was extracted by dichloromethane. The organic layer was combined, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography using dichloromethane and MeOH (v/v 20:1) to obtain the compound **3** as red solid (0.57 g, 50%). ^{1}H NMR (400M, $CDCl_3$) δ 8.36 (t, J = 13.6 Hz, 1H), 7.46 (dd, J_1 =8.4 Hz, J_2 =1.6 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.40 - 7.35 (m, 2H), 7.28 (d, J = 13.6 Hz, 1H), 7.24 - 7.21 (m, 1H), 7.19 (d, J = 13.6 Hz, 1H), 7.16 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 3.79 (s, 3H), 3.73 (s, 3H), 1.67 (s, 3H), 1.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 173.3, 150.9, 142.7, 142.4, 142.1, 140.8, 132.0, 129.2, 126.0, 125.6, 122.3, 118.3, 112.3, 111.4, 106.0, 105.0, 49.3, 48.9, 33.2, 33.1, 28.4, 28.3. HRMS (ESI): calculated for $C_{25}H_{28}BrN_2^+$ $[M]^+$, 435.1430; found, 435.1446.

Synthesis of compound 5: A mixture of compound **3** (0.56 g, 1.0 mmol), bis(pinacolato)diboron (0.33 g, 1.3 mmol), $Pd(dppf)Cl_2$ (37 mg, 0.05 mmol), and potassium acetate (0.29 g, 3 mmol) in DMF (10 mL) was heated at 80°C under a N_2-atmosphere overnight. Thin-layer chromatography (TLC) showed the reaction completed. Then, 15 mL of ethyl acetate were added, and the mixture was filtered through a pad of Celite. The solvent was evaporated under reduced pressure. The residue was directly used for the next reaction.

Synthesis of compound 8: Compound **7** (cyclohexanone, 2.75 mmol, 0.27 g) was added dropwise to concentrated H_2SO_4 (10.0 mL) at 0°C. Then, 2-(4-diethylamino-2-hydroxybenzoyl) benzoic acid (compound **6**, 2.75 mmol, 1.00 g) was added in portions with vigorous stirring. The mixture was heated at 90°C for 2.5 h, cooled down, and poured onto ice (50.0 g). Perchloric acid (70%, 1.0 mL) was then added, and the resulting

precipitate was filtered and washed with cold water (50.0 mL). Compound 3 was obtained as a red solid (0.9 g, 63%). HRMS (ESI): calculated for $C_{22}H_{21}BrNO^+$ [M] $^+$, 426.0699; found, 426.0706.

Synthesis of compound 9a: A mixture of compound **8** (0.37 g, 0.7 mmol) and 2-(1,3,3-Trimethylindolin-2-ylidene)-acetaldehyde (compound **2a**, 0.14 g, 0.7 mmol) in anhydrous Ac_2O (8 mL) was heated at 50°C for 1 h. After completed, the mixture was evaporated under reduced pressure, poured into water (25 mL), and extracted with dichloromethane. The organic layer was combined, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography, using dichloromethane and MeOH (v/v 20:1) as eluent to obtain the compound **9a** as a green solid (0.28 g, 65%). ^{1}H NMR (400M, $CDCl_3$) δ 8.46 (d, *J* = 14.0 Hz, 1H), 8.24 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.38 - 7.33 (m, 2H), 7.19 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.68 - 6.64 (m, 1H), 6.51 (s, 2H), 5.98 (d, *J* = 14.0 Hz, 1H), 3.59 (s, 3H), 3.08 (s, 6H), 2.57 – 2.56 (m, 2H), 2.27 – 2.16 (m, 2H), 1.73 (s, 6H), 1.65 – 1.62 (m, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 163.6, 155.8, 154.1, 143.2, 141.6, 140.8, 134.3, 130.3, 129.2, 128.8, 128. 5, 122.4, 116.4, 114.2, 112.5, 110.4, 101.4, 99.1, 96.2, 94.6, 49.2, 40.6, 31.6, 29.9, 28.8, 26.8, 24.5, 20.6. HRMS (ESI): calculated for $C_{35}H_{34}BrN_2O_3^+$ [M] $^+$, 609.1747; found, 609.1752.

Synthesis of compound 9b: According to the synthesis process of compound **9a**, compound **9b** was prepared from **8** (0.37 g, 0.7 mmol) and **2b** (0.18 g, 0.7 mmol). After workup and purification by flash column chromatography using dichloromethane and MeOH as the eluent, **9b** was obtained as green solid (0.27 g, 59%). ^{1}H NMR (400M, $CDCl_3$) δ 8.58 (d, *J* = 13.2 Hz, 1H), 8.27 (s, 1H), 8.21 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 7.2 Hz, 2H), 7.70 (d, *J* = 7.6 Hz, 1H), 7.61 - 7.58 (m, 1H), 7.44 - 7.37 (m, 2H), 6.95 (d, *J* = 8.0 Hz, 1H), 6.61 - 6.55 (m, 1H), 6.56 (s, 1H), 6.47 (d, *J* = 8.0 Hz, 1H), 6.05 (d, *J* = 13.2 Hz, 1H), 3.72 (s, 3H), 3.06 (s, 6H), 2.59 – 2.58 (m, 2H), 2.21 – 2.18 (m, 2H), 2.00 (s, 3H), 1.99 (s, 3H), 1.71 – 1.68 (m, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 162.9, 155.5, 153.9, 142.0, 140.3, 135.5, 133.8, 132.1, 130.8, 130.2, 129.2, 128.3, 128.1, 125.3, 123.2, 122.6, 121.6, 115.9, 113.6, 112.0, 110.8, 99.9, 96.7, 94.6, 51.4, 40.6, 32.4, 29.9, 28.3, 28.2, 26.9, 24.4, 20.6. HRMS (ESI): calculated for $C_{39}H_{36}BrN_2O_3^+$ [M] $^+$, 659.1904; found, 659.1904.

Synthesis of compound 11a: To a solution of compound **9a** (120 mg, 0.2 mmol) in dry dichloromethane (10 mL) was added dicyclohexylcarbodiimide (DCC) (50 mg, 0.25 mmol) and N-hydroxy-succinimide (NHS) (28 mg, 0.25 mmol) under a nitrogen atmosphere at room temperature. After the mixture was stirred for 30 min, 4-(2-aminoethyl)-morpholine (compound **10**, 39 mg, 0.3 mmol) was added, and the reaction was continually stirred overnight. The mixture was washed with water (2 × 20 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography, using ethyl acetate and hexane as the eluent, to afford probe as a gray solid (50 mg, 35%). ^{1}H NMR (400M, $CDCl_3$) δ 7.96 (d, *J* = 1.6 Hz, 1H), 7.55 (dd, J_1 = 8.0 Hz, *J* = 2.0 Hz, 1H),

7.46 (d, *J* = 12.4 Hz, 1H), 7.14 (d, *J* = 7.2 Hz, 2H), 7.01 (d, *J* = 8.0 Hz, 1H), 6.83 (t, *J* = 8.4 Hz, 1H), 6.60 (d, *J* = 7.6 Hz, 1H), 6.38 - 6.29 (m, 3H), 5.35 (d, *J* = 12.4 Hz, 1H), 3.59 (t, *J* = 4.4 Hz, 2H), 3.40-3.36 (m, 1H), 3.28 - 3.23 (m, 1H), 3.14 (s, 3H), 2.96 (s, 6H), 2.61 – 2.57 (m, 1H), 2.44– 2.32 (m, 7H), 2.20 – 2.17 (m, 2H), 1.71 (s, 3H), 1.70 (s, 3H), 1.64 – 1.60 (m, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 158.2, 152.7, 151.6, 150.5, 148.4, 145.5, 139.0, 135.3, 134.2, 128.7, 127.9, 126.2, 125.3, 122.4, 121.7, 120.1, 120.0, 119.6, 109.1, 106.0, 105.8, 103.0, 98.6, 92.1, 67.2, 66.9, 56.5, 53.7, 45.7, 40.6, 37.2, 34.2, 29.3, 28.7, 28.6, 25.5, 23.2, 22.4. HRMS (ESI): calculated for $C_{41}H_{46}BrN_4O_3$ $[M+H]^+$, 721.2753; found, 721.2748.

Synthesis of compound 11b: According to the syntheses process of compound **11a** using **9b** (134 mg, 0.2 mmol) and **10** (39 mg, 0.3 mmol), compound **11b** was obtained as a gray solid after workup and purification by flash column chromatography, using ethyl acetate and hexane as the eluent (65 mg, 43%).^{1}H NMR (400M, $CDCl_3$) δ 8.05 (d, *J* = 8.8 Hz, 1H), 7.97 (d, *J* = 1.6 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.56 (dd, J_1 = 8.0 Hz, *J* = 1.6 Hz, 2H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 2H), 6.39 - 6.37 (m, 2H), 6.33 - 6.31 (m, 1H), 5.38 (d, *J* = 12.8 Hz, 1H), 3.60 (t, *J* = 4.4 Hz, 4H), 3.43-3.38 (m, 1H), 3.29 - 3.27 (m, 1H), 3.23 (s, 3H), 2.99 (s, 6H), 2.64 – 2.60 (m, 1H), 2.48 – 2.46 (m, 1H), 2.36– 2.34 (m, 6H), 2.23 – 2.16 (m, 2H), 2.05 (s, 3H), 2.04 (s, 3H), 1.93 – 1.90 (m, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 160.0, 152.7, 151.6, 150.5, 148.4, 142.9, 135.3, 134.2, 130.1, 129.7, 129.6, 129.4, 128.7, 126.8, 126.2, 125.2, 122.4, 122.0, 121.8, 120.0, 119.9, 109.1, 105.8, 103.0, 98.6, 91.8, 67.2, 66.9, 56.5, 53.7, 47.7, 40.6, 37.2, 34.2, 29.6, 27.6, 27.5, 23.2, 22.4. HRMS (ESI): calculated for $C_{45}H_{48}BrN_4O_3$ $[M+H]^+$, 771.2910; found, 771.2910.

Synthesis of probe A. A mixture of compounds **11a** (50 mg, 0.07 mmol) and **5** (90 mg, 0.15 mmol) in DMF (10 mL) was stirred under argon. Then, $Pd(PPh_3)_4$ (8 mg) and Na_2CO_3 (20 mg) were added. The reaction was heated at 85°C under argon for 15 h. Thin-layer chromatography (TLC) was periodically used during this period to determine the extent of the reaction. The mixture was evaporated under vacuum, and the residue was diluted with dichloromethane. The organic layer was washed with brine, dried over Na_2SO_4, and the resulting mixture filtered. The crude product was purified by flash column chromatography, using dichloromethane and methanol (v/v 10:1) as eluent to result in probe **A** as a red solid (38 mg, 49%). ^{1}H NMR (400M, $CDCl_3$) δ 8.41 (t, *J* = 13.2 Hz, 1H), 8.06 (d, *J* = 1.2 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.69 – 7.61 (m, 2H), 7.43 – 7.34 (m, 5H), 7.23 – 7.18 (m, 2H), 7.16 – 7.13 (m, 3H), 6.85 – 6.82 (m, 1H), 6.60 (d, *J* = 7.6 Hz, 1H), 6.45 – 6.43 (m, 1H), 6.36 (d, *J* = 2.0 Hz, 1H), 6.34 – 6.30 (m, 2H), 5.35 (d, *J* = 12.8 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.61 (t, *J* = 4.4 Hz, 4H), 3.46 – 3.42 (m, 1H), 3.33 - 3.27 (m, 1H), 3.14 (s, 3H), 2.96 (s, 6H), 2.63 – 2.59 (m, 1H), 2.47 – 2.33 (m, 7H), 2.24 – 2.18 (m, 2H), 1.75 – 1.68 (m, 18H), 1.55 – 1.51 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 174.0, 168.3, 158.2, 152.7, 151.6, 150.9, 145.5, 142.9, 141.5, 140.8, 139.0, 137.8, 131.1, 129.1, 128.1, 127.9, 125.6, 121.7, 121.1,

120.2, 119.6, 111.3, 111.1, 109.0, 106.2, 106.0, 103.4, 98.7, 92.1, 67.2, 67.0, 56.6, 53.7, 49.1, 49.0, 45.7, 40.6, 37.2, 32.7, 29.3, 28.7, 28.6, 28.5, 28.3, 25.6, 23.3, 22.4. HRMS (ESI): calculated for $C_{66}H_{73}N_6O_3$ $[M-I]^+$, 997.5744; found, 997.5741. Probe **A** has a solubility of at least 2.5 g/L in aqueous solution.

Synthesis of probe B. Reacting compounds **11b** (55 mg, 0.07 mmol) and **5** (90 mg, 0.15 mmol) in a similar manner to that for probe **A** afforded probe **B** as a gray solid (30 mg, 52%) after workup and purification by flash column chromatography with dichloromethane and methanol (V/v 10:1) as the eluents. ^{1}H NMR (400M, $CDCl_3$) δ 8.41 (t, *J* = 13.2 Hz, 1H), 8.06 (m, 2H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 7.69 – 7.51 (m, 4H), 7.45 (d, *J* = 7.2 Hz, 1H), 7.39 – 7.33 (m, 4H), 7.21 – 7.18 (m, 3H), 7.15 – 7.11 (m, 2H), 7.04 (d, *J* = 8.0 Hz, 1H), 6.45 – 6.41 (m, 2H), 6.35 – 6.33 (m, 1H), 5.38 (d, *J* = 13.3 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.62 (t, *J* = 4.4 Hz, 4H), 3.46 – 3.44 (m, 1H), 3.35 – 3.32 (m, 1H), 3.24 (s, 3H), 3.00 (s, 6H), 2.66 – 2.62 (m, 1H), 2.50 – 2.48 (m, 1H), 2.40 – 2.34 (m, 6H), 2.26 – 2.20 (m, 2H), 2.06 (s, 3H), 2.05 (s, 3H), 1.75 (s, 6H), 1.72 (s, 6H), 1.57 – 1.52 (m, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 174.0, 172.4, 168.3, 160.0, 157.9, 152.8, 151.6, 151.0, 150.8, 148.4, 142.9, 140.7, 130.1, 129.7, 129.6, 129.4, 129.1, 128.3, 128.1, 126.8, 125.7, 122.2, 122.0, 121.8, 121.2, 120.1, 111.3, 111.2, 109.1, 105.6, 98.7, 91.8, 67.1, 56.6, 53.7, 49.8, 49.2, 49.1, 47.7, 40.6, 37.1, 32.7, 29.6, 28.4, 28.3, 28.0, 27.6, 25.6, 23.3, 22.5. HRMS (ESI): calculated for $C_{70}H_{75}N_6O_3$ $[M-I]^+$, 1047.5901; found, 1047.5895. Probe **B** has a solubility of at least 2.0 g/L in aqueous solution.

2.2 Computational calculations

Models suitable for calculations for probes **A** and **B** and their protonated forms, $\mathbf{AH^+}$ and $\mathbf{BH^+}$, were obtained as described previously,[3] using Chem3d software with MM2 minimization of energies,[4] followed by force field (UFF) calculations in Avogadro. [5] The molecular data for probes were initially refined, using density functional theory (DFT) employed with the B3LYP functional[6] and electron basis sets initially at the 6-31g(d) level to convergence in Gaussian 16.[7] In order to determine a suitable functional and basis set for probe **A**, a TD-DFT optimization[8] in a Polarizable Continuum Model (PCM) of water,[9] using the APFD functional[10] and the 6-311+g(d)[11] basis set resulted in two maxima at 380 and 481 nm, as compared to the experimental values of 380 and 558 nm. We then utilized the exchange correlation TPSSH[12] functional with TZVP (triple zeta valence)[13] basis sets and obtained corresponding peaks at 395 and 502 nm, which agreed more closely (i.e., for the visible transition) with the experimental value for probe **A**. This TPSSH/TZVP functional/basis set combination was utilized in all subsequent refinements. Imaginary frequencies were not obtained in any frequency calculations for the probes, see supporting information. In all cases, ten excited states were assessed on the basis of TD-DFT optimizations[8] in a PCM of water[9] with TZVP basis sets. The results were interpreted using GaussView 6[14] for all data and figures. The results of the calculations are outlined in the Supporting Information as below.

2.3 Confocal fluorescence imaging of HeLa cells

HeLa cells were purchased from ATCC. The culturing conditions were as described previously.[15-17] For the co-staining study, the cells were incubated with 10 μM of probe **A** or **B** plus either 10 μM MitoView blue or 10 μM Lysosensor blue. Solution media with or without FBS in the presence of 1% DMSO for various durations were used. The cells were then washed twice in PBS and imaged. In order to observe the ratiometric intracellular changes due to differences in pH levels, the cells were rinsed twice with PBS buffer and nigericin (5 μg/mL) dissolved in different K^+ buffers of pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 was added. The intra- and extracellular pH values were equilibrated for 30 min and then either 10 μM of probe **A** or probe **B** was added**.** After an incubation period of 30 min, the cells were rinsed twice with FBS buffer and imaged. For Lysosensor Blue or MitoView Blue (blue channel) under 405 nm wavelength excitation, emission spectra from 425 nm to 475 nm were recorded. Under 559 nm excitation, the collecting windows of visible fluorescence (green channel) ranged from 625 nm to 675 nm, and for the near-infrared fluorescence (red channel) from 720 nm to 770 nm. The near-infrared fluorescence channel of the probes (magenta channel) under excitation of 535 nm was collected at 720−770 nm. We processed the images with an Olympus FV10-ASW 3.1 viewer, ImageJ, and Photoshop.

3. ^{1}H and ^{13}C NMR spectra of intermediates and the probes

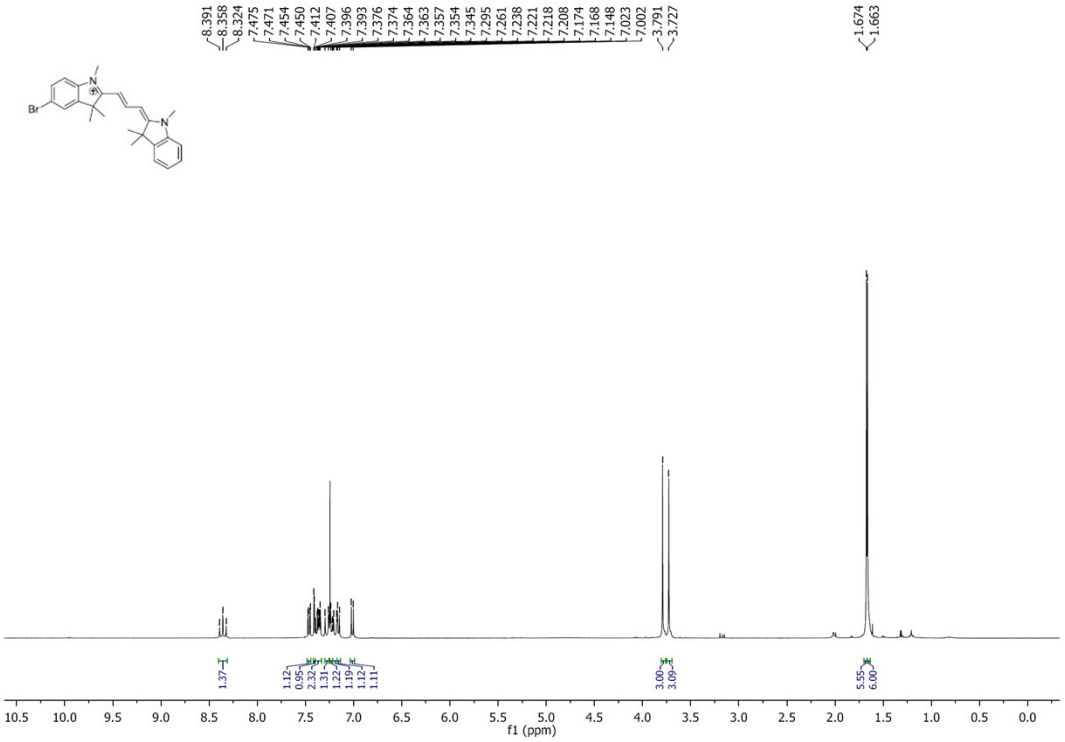


Figure S1. ^{1}H NMR spectrum of compound **3** in $CDCl_3$ solution.

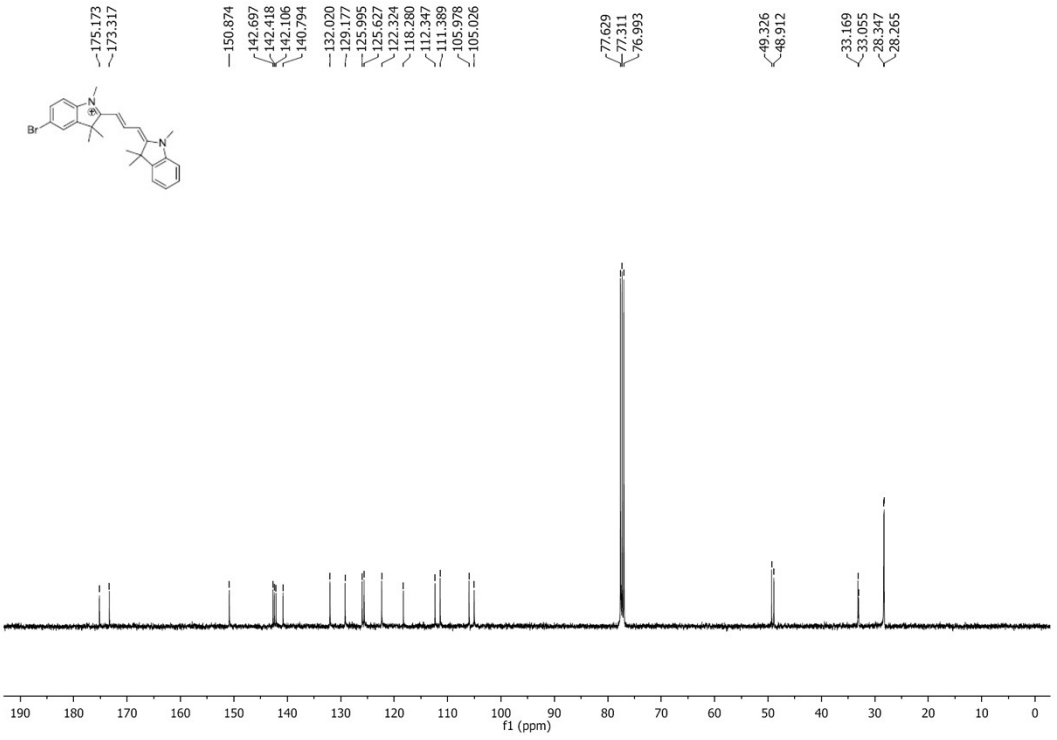


Figure S2. ^{13}C NMR spectrum of compound **3** in $CDCl_3$ solution.

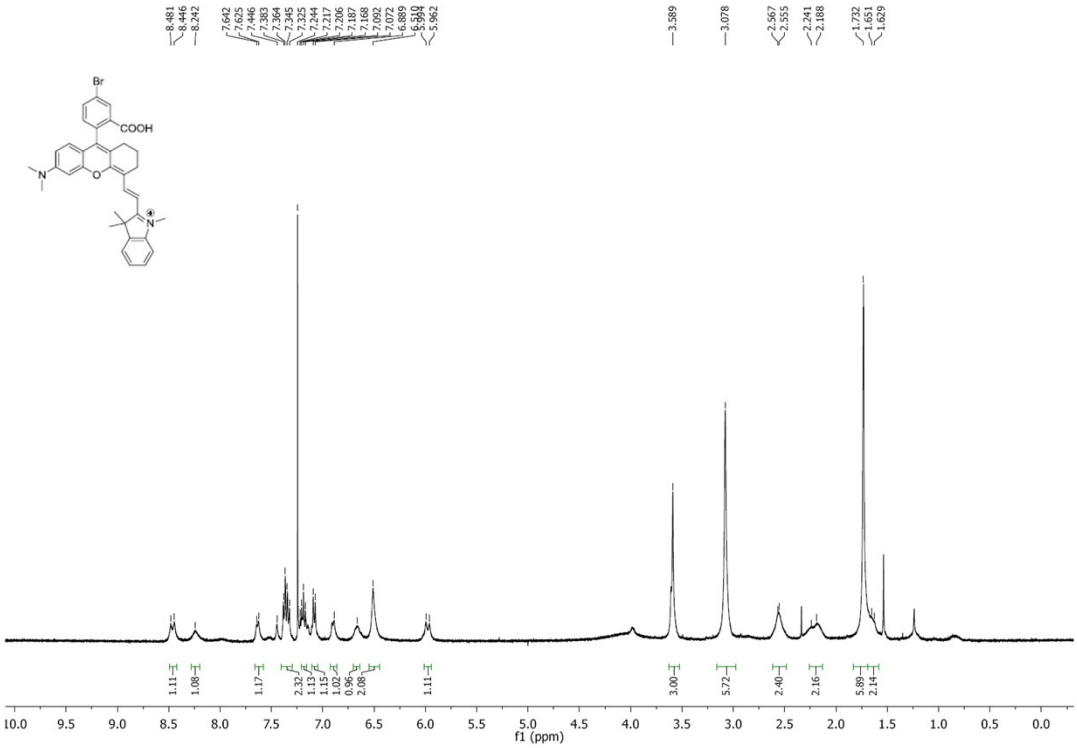
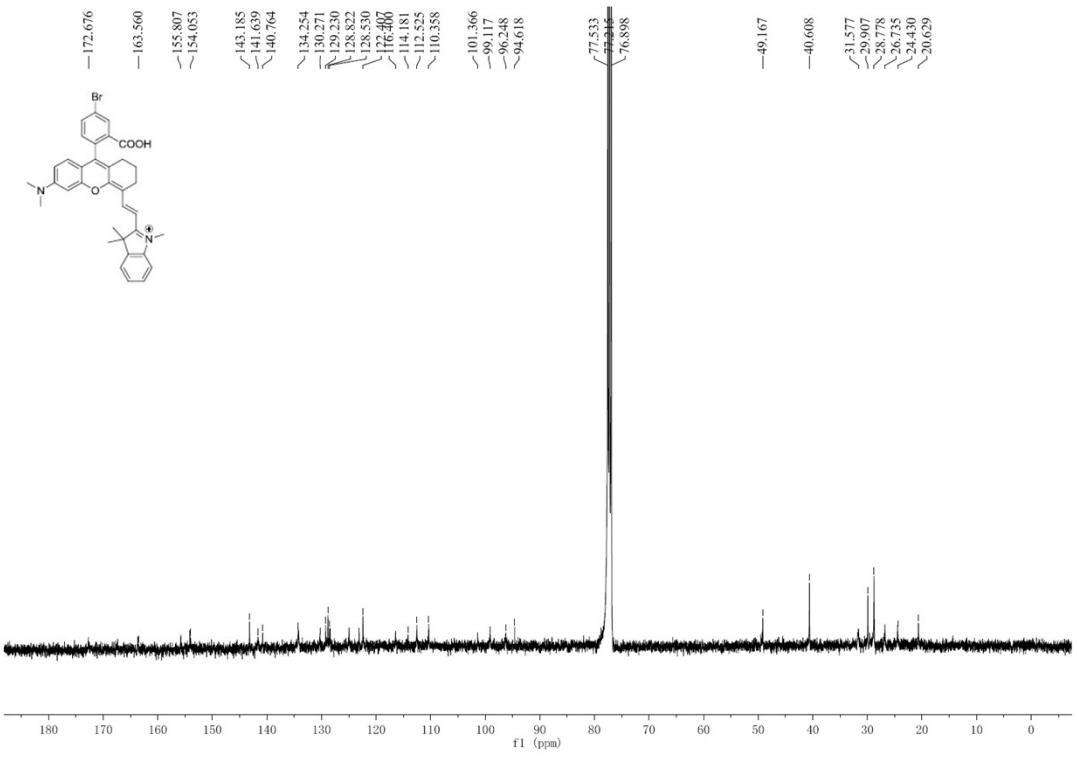


Figure S3. ^{1}H NMR spectrum of compound **9a** in $CDCl_3$ solution.



- **Figure S4**. ^{13}C NMR spectrum of compound **9a** in $CDCl_3$ solution.

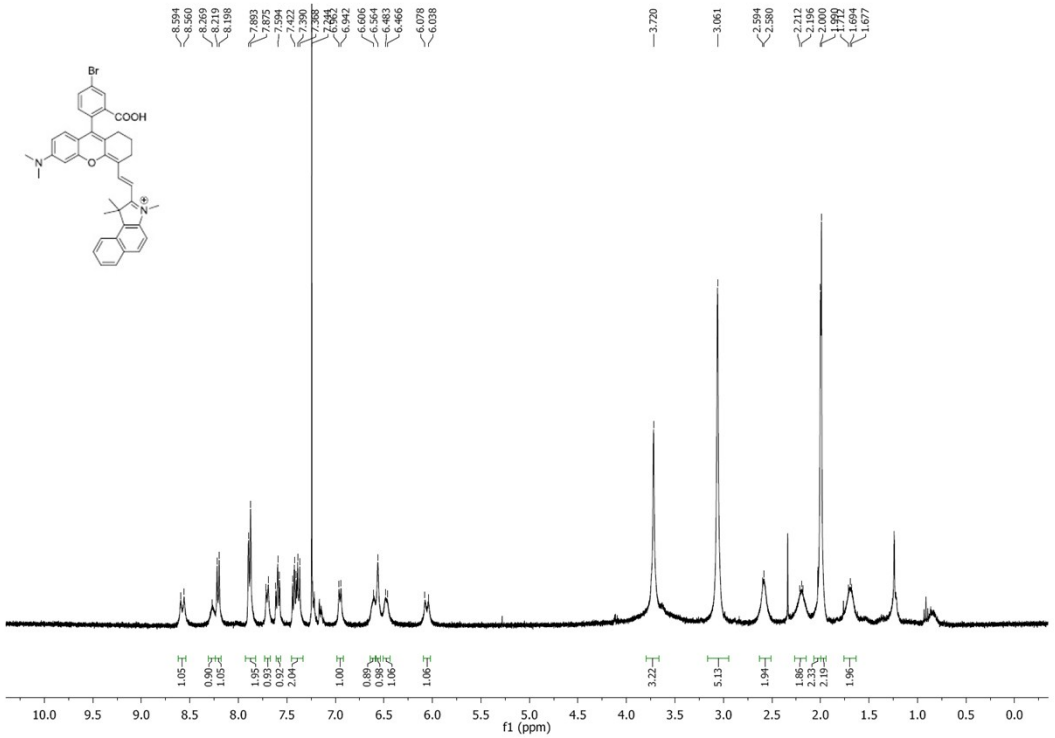


Figure S5. ^{1}H NMR spectrum of compound **9b** in $CDCl_3$ solution.

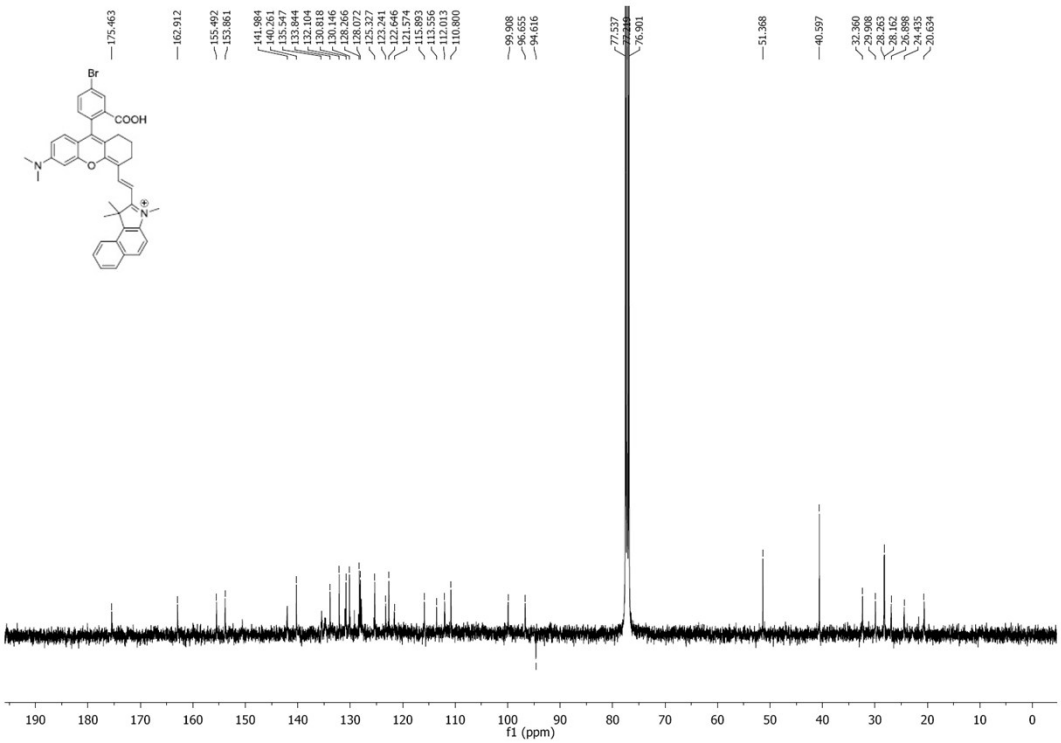


Figure S6. ^{13}C NMR spectrum of compound **9b** in $CDCl_3$ solution.

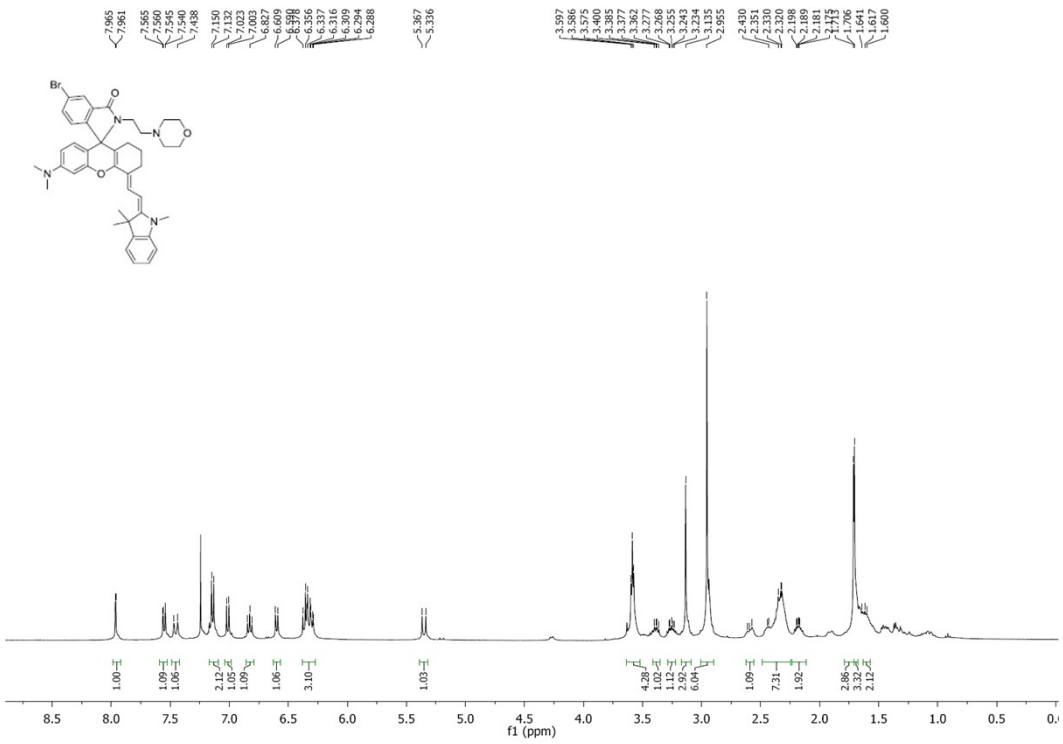


Figure S7. ^{1}H NMR spectrum of compound **11a** in $CDCl_3$ solution.

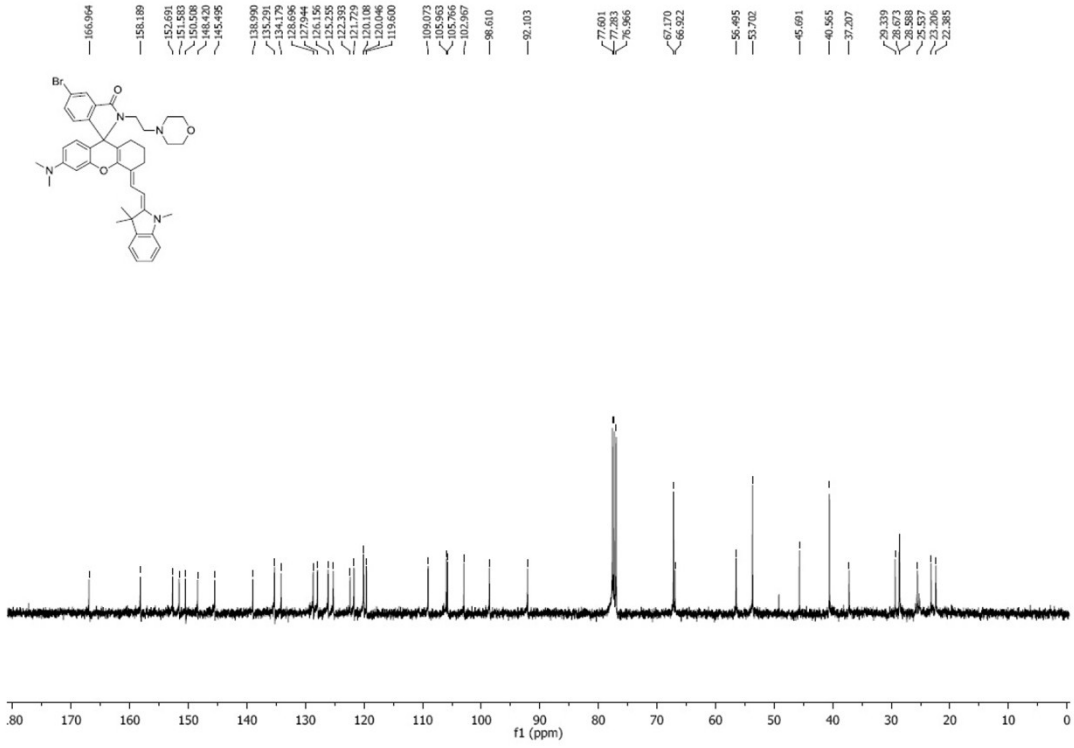


Figure S8. ^{13}C NMR spectrum of compound **11a** in $CDCl_3$ solution.

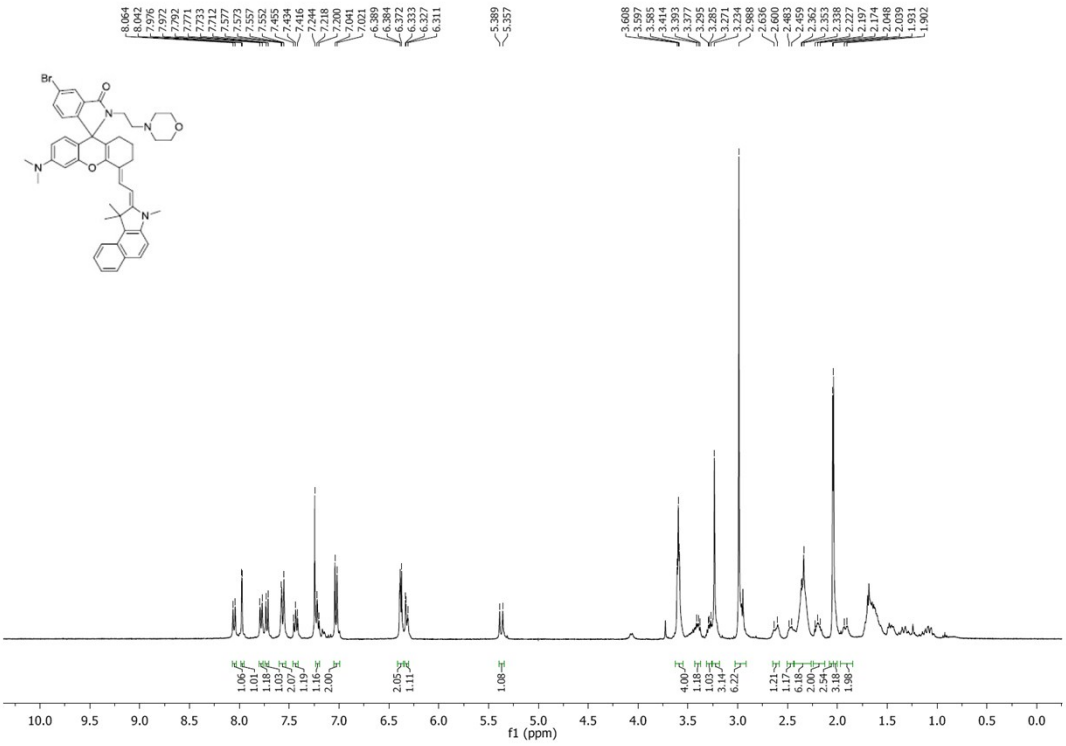


Figure S9. ^{1}H NMR spectrum of probe **11b** in $CDCl_3$ solution.

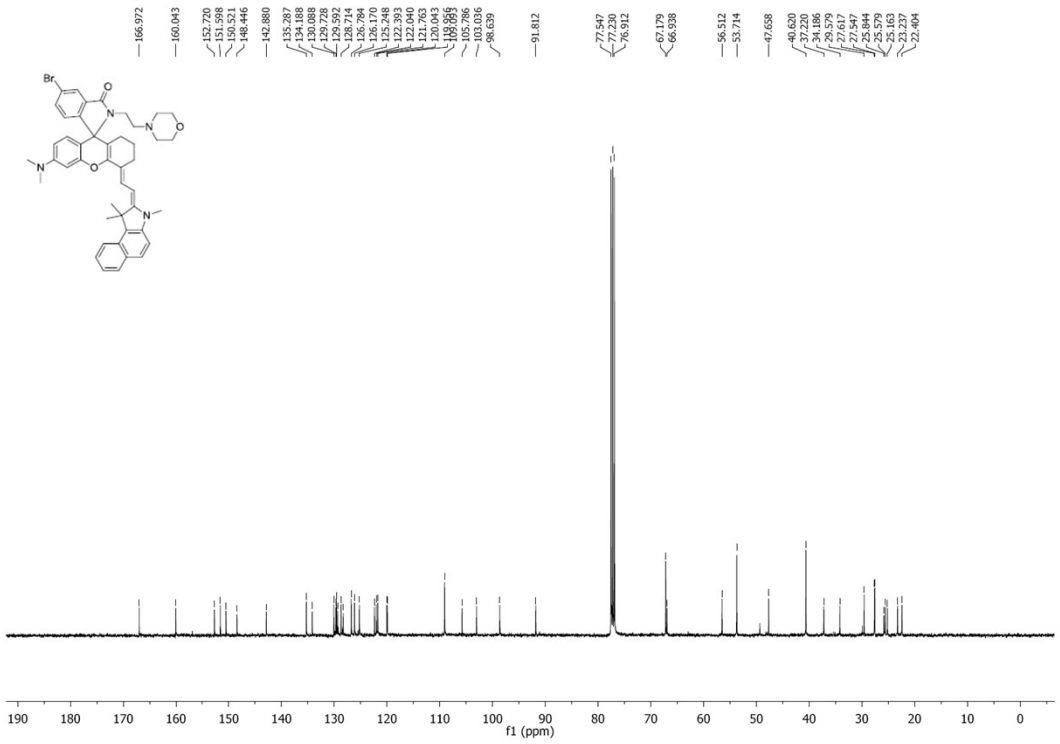


Figure S10. ^{13}C NMR spectrum of probe **11b** in $CDCl_3$ solution.

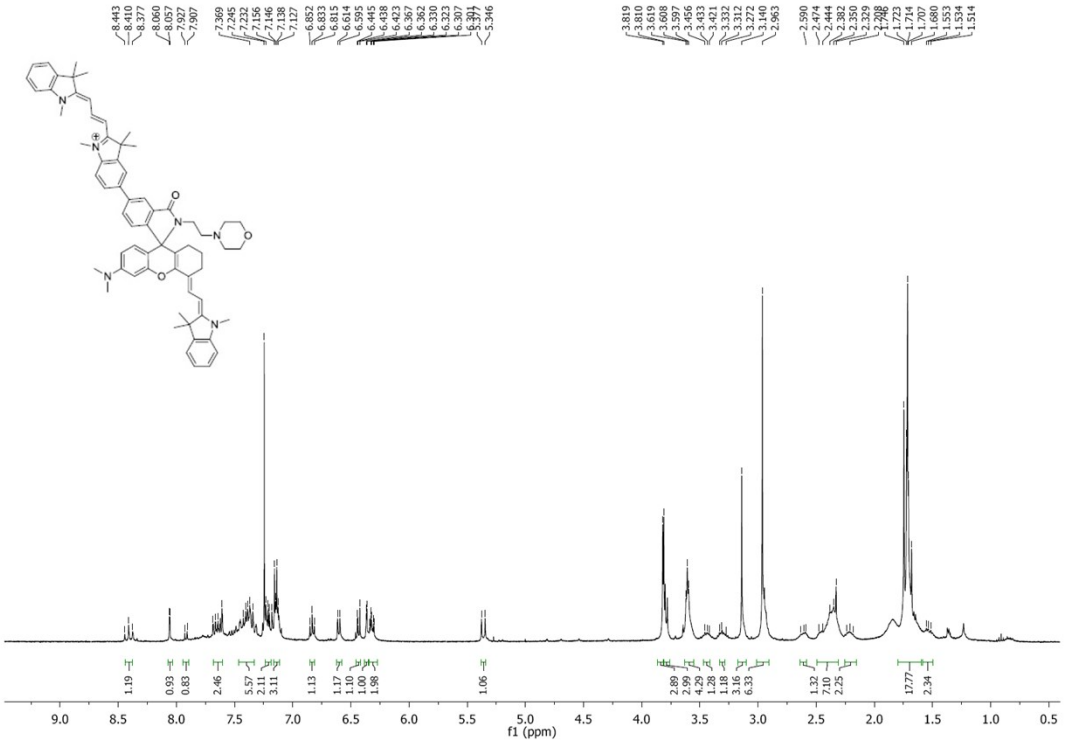


Figure S11. ^{1}H NMR spectrum of probe **A** in $CDCl_3$ solution.

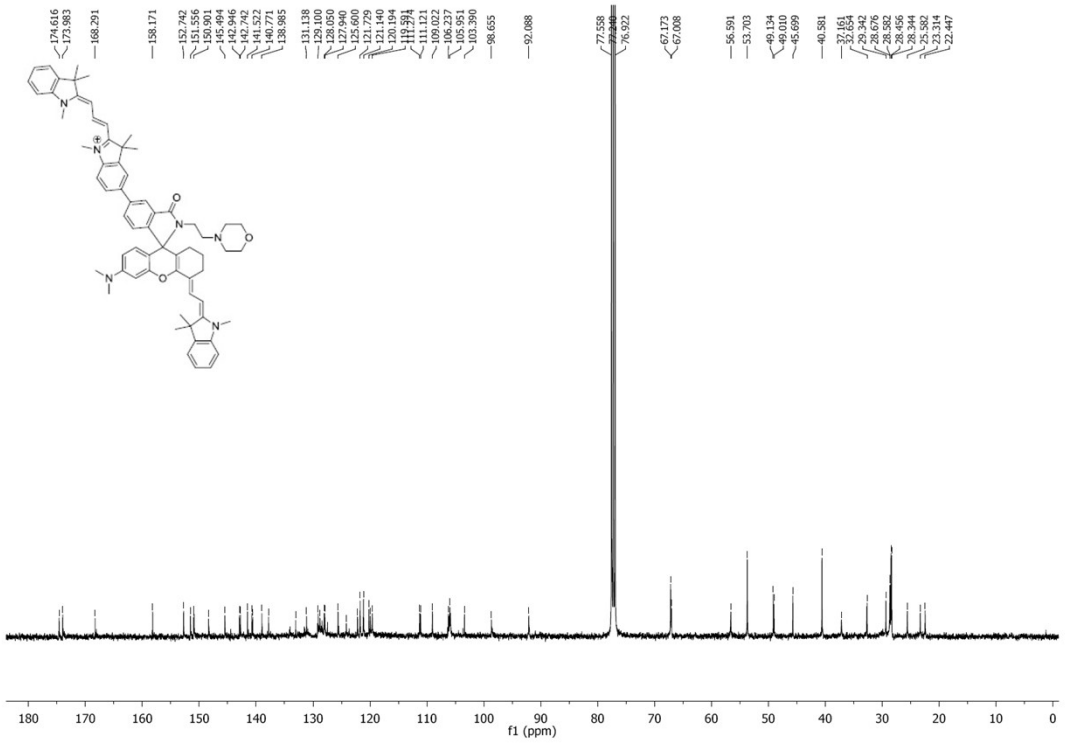


Figure S12. ^{13}C NMR spectrum of probe **A** in $CDCl_3$ solution.

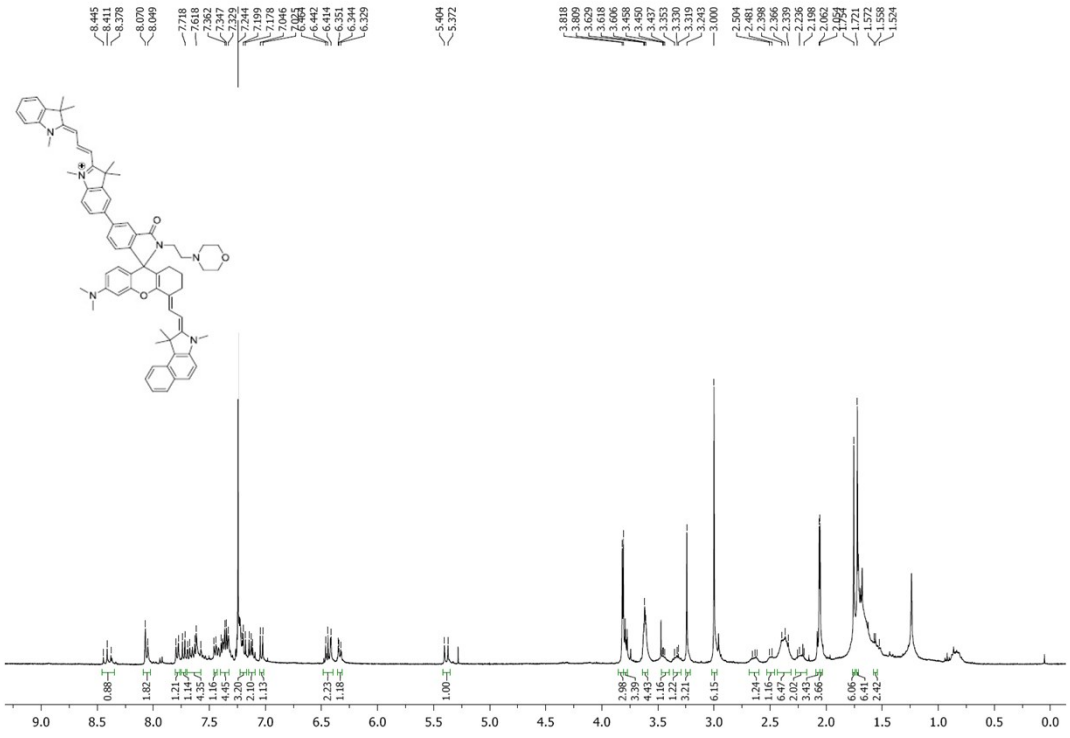


Figure S13. ^{1}H NMR spectrum of probe **B** in $CDCl_3$ solution.

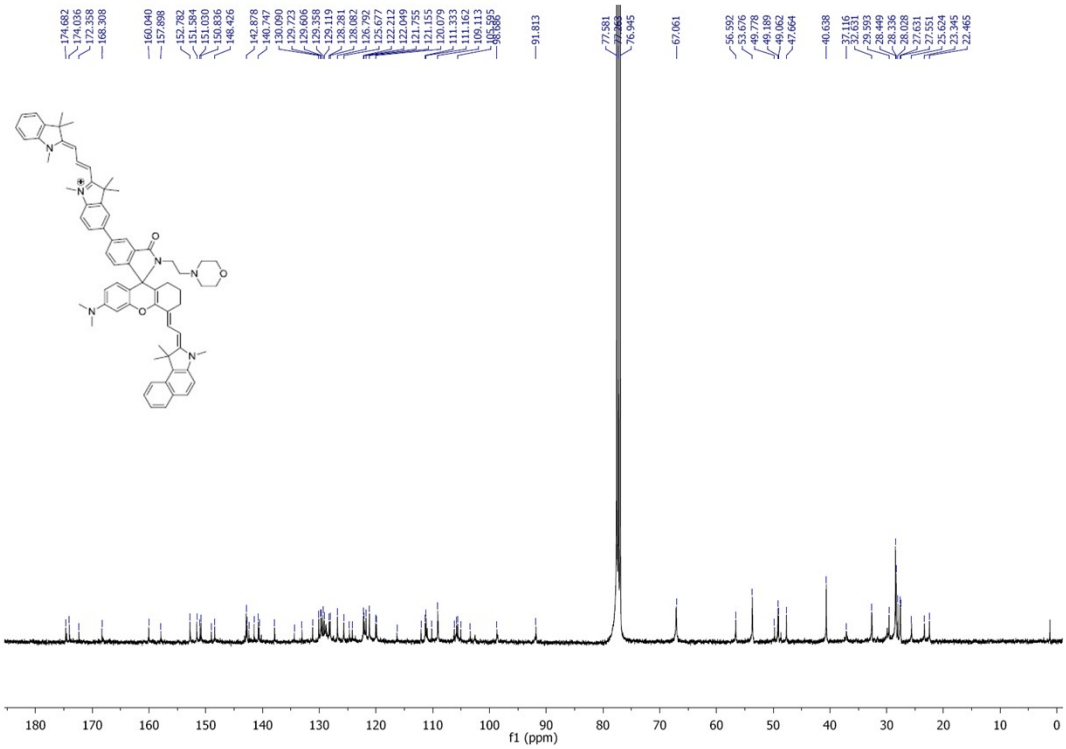


Figure S14. ^{13}C NMR spectrum of probe **B** in $CDCl_3$ solution.

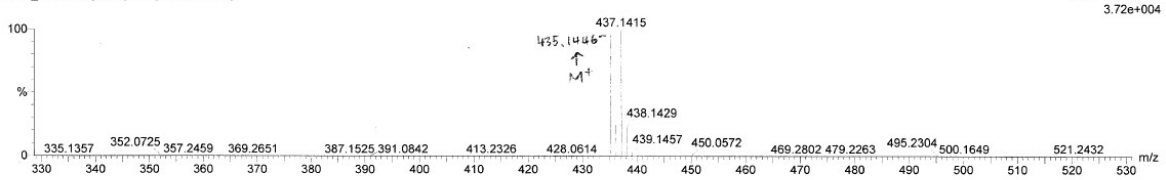
4. HR-MS spectra of intermediates and the probes

Elemental Composition Report **Page 1**

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 10.0 PPM / DBE: min = -1000.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
64 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 20-1000 H: 0-1000 N: 2-2 79Br: 0-1 81Br: 0-1

LFT-2 KE267 29-Jun-2017 16:34:07
0629_LFT-2 58 (2.104) Cm (58-1x100.000) 1: TOF MS ES+ 3.72e+004



Minimum:	90.00				-1000.0			
Maximum:	100.00		5.0	10.0	1000.0			
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
435.1446	96.32	435.1436	1.0	2.3	12.5	54.3	0.0	C25 H28 N2 79Br
437.1415	100.00	437.1415	0.0	0.0	12.5	55.4	0.0	C25 H28 N2 81Br

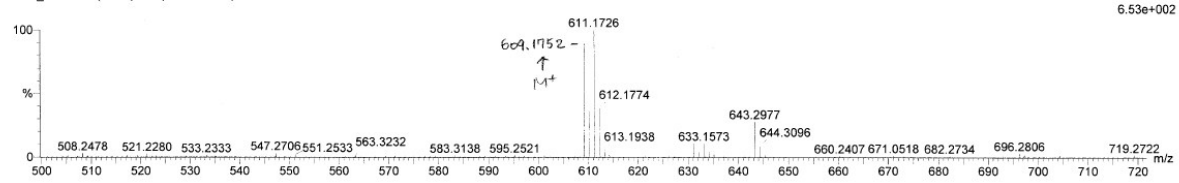
Figure S15. HR-MS spectrum of compound **3**.

Elemental Composition Report **Page 1**

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 1.5 PPM / DBE: min = -1000.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
147 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 20-1000 H: 0-1000 N: 2-2 O: 3-3 79Br: 0-1 81Br: 0-1

LFT-3 KE267 29-Jun-2017 16:42:16
0629_LFT-3 63 (2.280) Cm (63-19x0.010) 1: TOF MS ES+ 6.53e+002



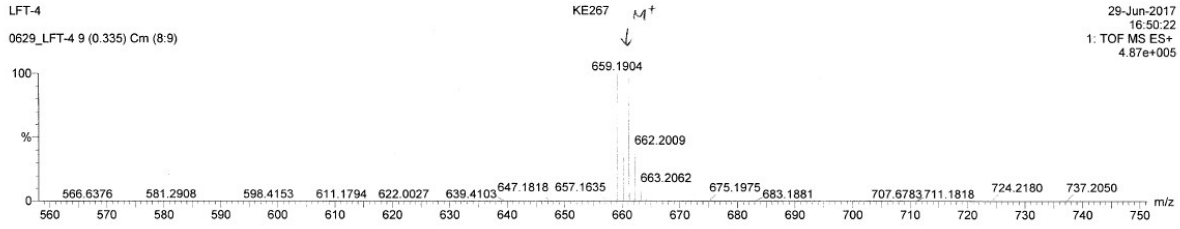
Minimum:	70.00				-1000.0			
Maximum:	100.00		5.0	1.5	1000.0			
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
609.1752	89.41	609.1753	-0.1	-0.2	19.5	17.5	0.0	C35 H34 N2 O3 79Br
611.1726	100.00	611.1732	-0.6	-1.0	19.5	18.2	0.0	C35 H34 N2 O3 81Br

Figure S16. HR-MS spectrum of compound **9a**.

Elemental Composition Report **Page 1**

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 2.0 PPM / DBE: min = -1000.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
101 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 30-1000 H: 0-1000 N: 2-2 O: 3-3 79Br: 0-1 81Br: 0-1



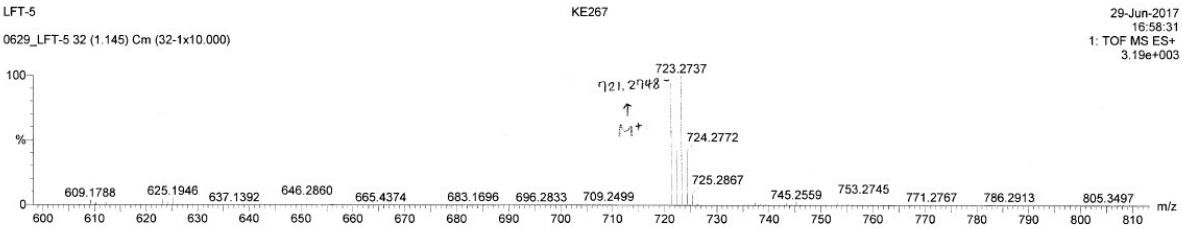
Minimum:	70.00				-1000.0			
Maximum:	100.00		5.0	2.0	1000.0			
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
659.1904	100.00	659.1909	-0.5	-0.8	22.5	127.1	0.0	C39 H36 N2 O3 79Br
661.1898	97.95	661.1889	0.9	1.4	22.5	144.3	0.0	C39 H36 N2 O3 81Br

Figure S17. HR-MS spectrum of compound **9b**.

Elemental Composition Report **Page 1**

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 20.0 PPM / DBE: min = -1000.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
123 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 30-1000 H: 0-1000 N: 4-4 O: 3-3 79Br: 0-1 81Br: 0-1



Minimum:	70.00				-1000.0			
Maximum:	100.00		5.0	20.0	1000.0			
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
721.2748	97.68	721.2753	-0.5	-0.7	20.5	22.2	0.0	C41 H46 N4 O3 79Br
723.2737	100.00	723.2733	0.4	0.6	20.5	18.6	0.0	C41 H46 N4 O3 81Br

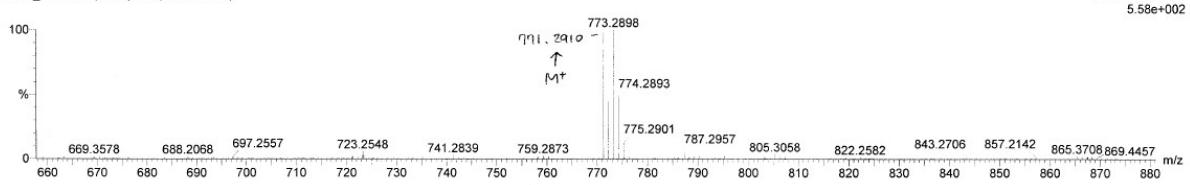
Figure S18. HR-MS spectrum of compound **11a**.

Elemental Composition Report **Page 1**

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 1.5 PPM / DBE: min = -1000.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
397 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-1000 H: 0-1000 N: 4-4 O: 3-3 79Br: 0-1 81Br: 0-1

LFT-6 KE267 29-Jun-2017 16:17:51
0629_LFT-6 42 (1.525) Cm (42-1x10.000) 1: TOF MS ES+ 5.58e+002



Minimum:	90.00				-1000.0			
Maximum:	100.00		5.0	1.5	1000.0			
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
771.2910	97.83	771.2910	0.0	0.0	23.5	20.4	0.0	C45 H48 N4 O3 79Br
773.2898	100.00	773.2889	0.9	1.2	23.5	18.8	0.0	C45 H48 N4 O3 81Br

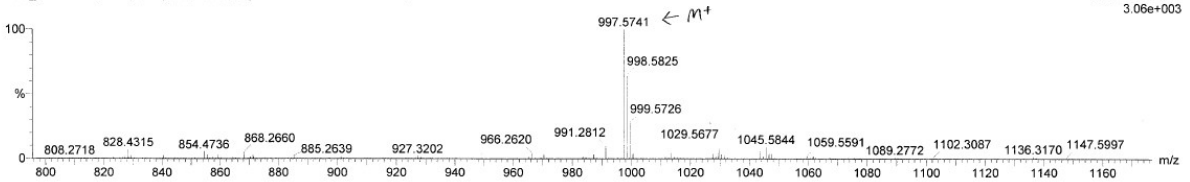
Figure S19. HR-MS spectrum of compound **11b**.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1000.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
73 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-1000 H: 0-1000 N: 6-6 O: 3-3

LFT-10 KE267 29-Jun-2017 14:27:30
0629_LFT-10 41 (1.840) Cm (41:47-1x10.000) 1: TOF MS ES+ 3.06e+003



Minimum:				-1000.0			
Maximum:		5.0	2.0	1000.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
997.5741	997.5744	-0.3	-0.3	33.5	59.9	0.0	C66 H73 N6 O3

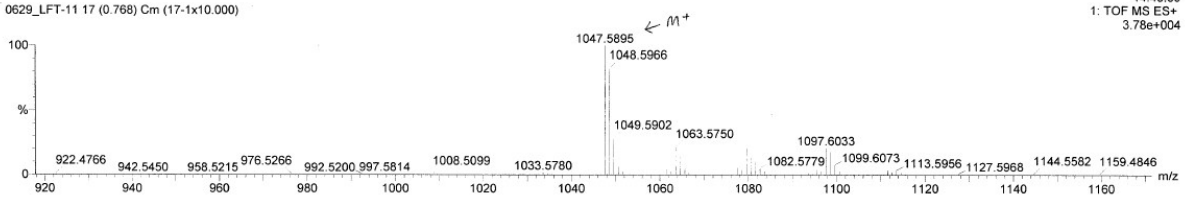
Figure S20. HR-MS spectrum of probe **A**.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1000.0, max = 1000.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
77 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-1000 H: 0-1000 N: 6-6 O: 3-3

LFT-11 KE267 29-Jun-2017 14:43:39
0629_LFT-11 17 (0.768) Cm (17-1x10.000) 1: TOF MS ES+ 3.78e+004



Minimum:				-1000.0			
Maximum:		5.0	2.0	1000.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1047.5895	1047.5901	-0.6	-0.6	36.5	33.9	0.0	C70 H75 N6 O3

Figure S21. HR-MS spectrum of probe **B**.

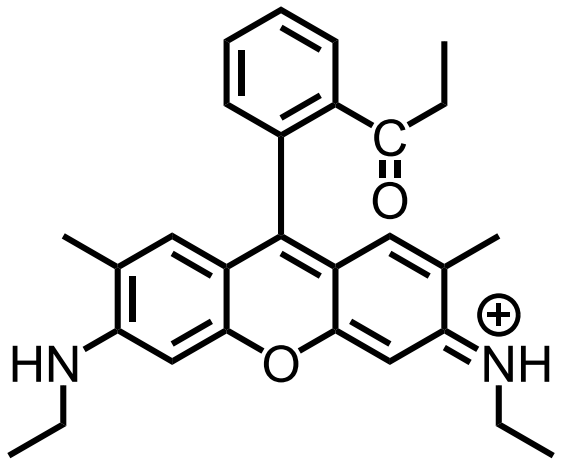
5. Optical measurements of probes A and B

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 contained 10% ethanol in different pH buffers. For the selectivity experiment, all metal ion solutions were freshly made, and the concentrations of all metal ions were 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 Cielen *et al*. (1998).[18] 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:

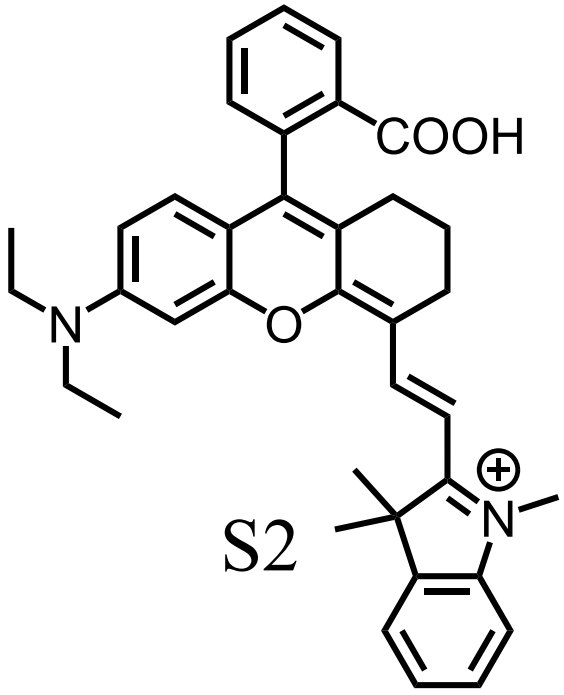
$$\Phi X = \Phi st \left(\frac{Grad_X}{Grad_{st}}\right)\left(\frac{\eta^2{}_X}{\eta^2{}_{st}}\right)$$

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.

According to the literature, rhodamine 6G (**S1**) (0.95 in ethanol)[19] was used as the standard to the calculate quantum yield of cyanine donor, and a typical hemicyanine dye (**S2**) (0.41 in ethanol)[20] was used as standard to calculate the quantum yield of hemicyanine moiety.



S1



S2

6. Determination of pKa of probes A and B by fluorometric titration

The constant K_a of probes were obtained by fluorometric titration as a function of the pH, using the fluorescence spectra. The expression of the steady-state fluorescence intensity F as a function of the proton concentration has been extended for the case of a n: 1 complex between H^+ and a fluorescent probe, which is expressed by Henderson-Hasselbalch equation below:[21]

$$\mathbf{pH = pK_a + \log(F\text{-}F_{min})/(F_{max}\text{-}F)}$$

F_{min} and F_{max} are the minimum and maximum fluorescence intensity values of probe **A** or **B** during the fluorometric titration, respectively. F is the fluorescence intensity value of probe **A** or **B**.

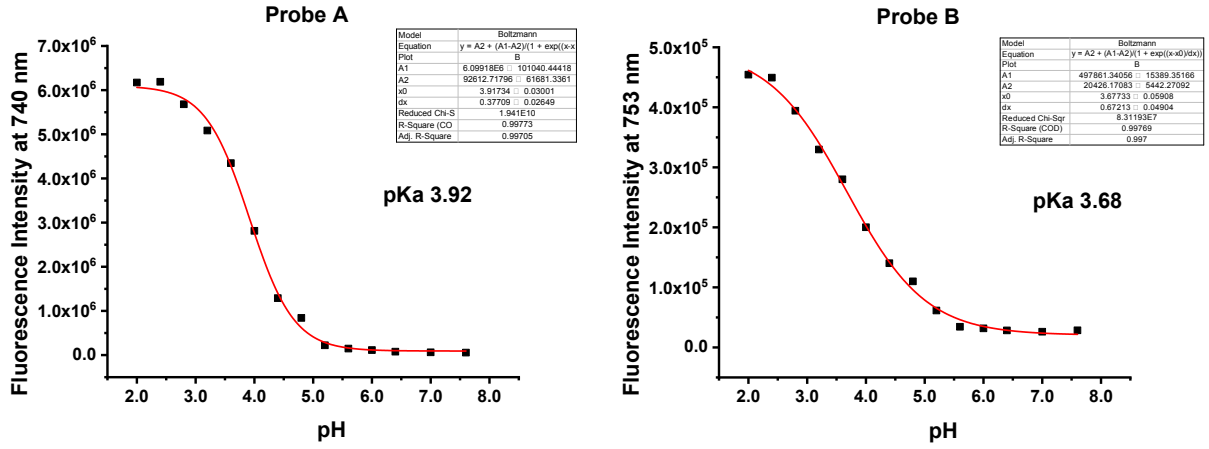


Figure S22. Plots of hemicyanine fluorescence intensity of probes **A** (left) and **B** (right) versus pH values in citrate-phosphate buffers with 10% ethanol under the excitation of 520 nm.

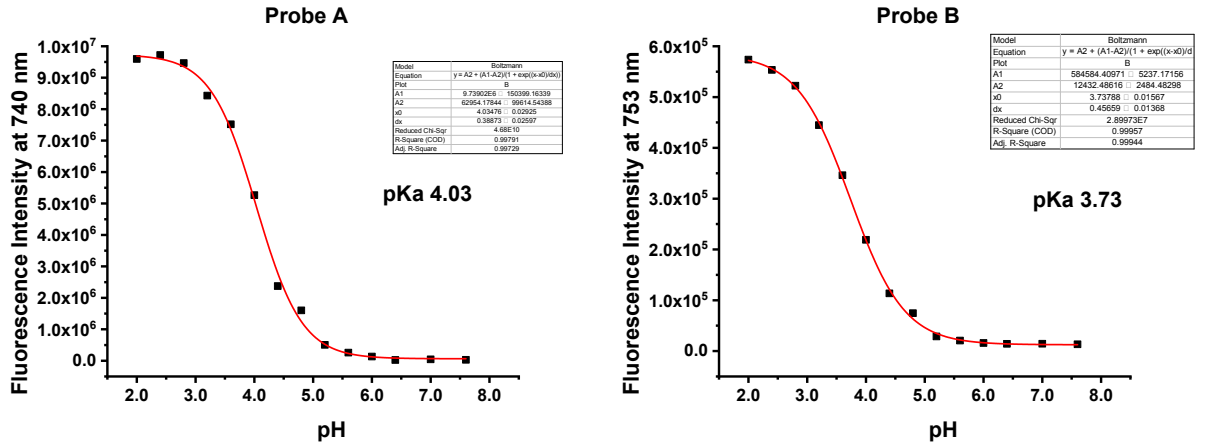


Figure S23. Plots of hemicyanine fluorescence intensity of probes **A** (left) and **B** (right) versus pH values in citrate-phosphate buffers with 1% ethanol with excitation wavelengths of 665 and 680 nm, respectively.

7. Theoretical calculation of the probes A and B

Data for Probe A.

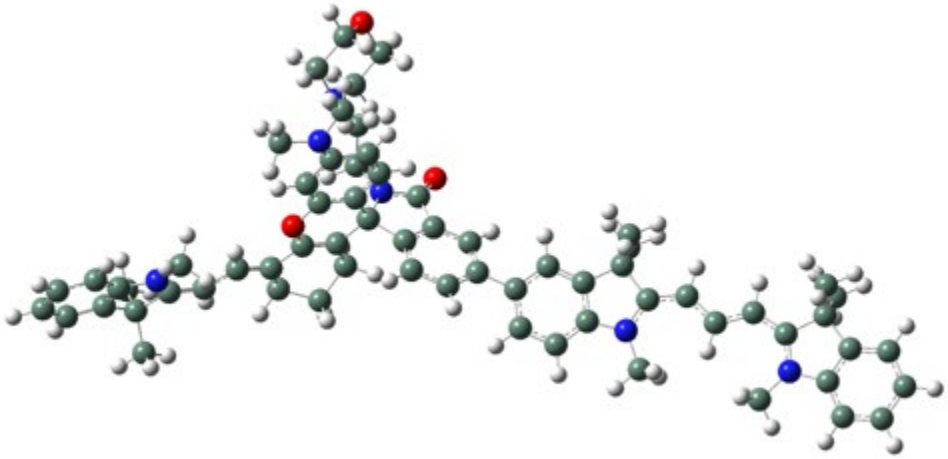


Figure S24. GaussView representation of Probe A.

Table S1. Computational results for Probe A.

rfpa5 (Optimization completed)		
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Calculation Type	FREQ	
Calculation Method	RTPSSh	
Basis Set	TZVP	
Charge	1	
Spin	Singlet	
Solvation	scrf=solvent=water	
E(RTPSSh)	-3114.231763	Hartree
RMS Gradient Norm	0.000002	Hartree/Bohr
Imaginary Freq		
Dipole Moment	44.067281	Debye
Point Group	C1	
Job cpu time:	14 days 23 hours 54 minutes ...	

Table S2. Calculated atomic coordinates for Probe **A**.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-3.22081	2.59149	3.022867	46	O	-4.34579	7.822956	-1.4472
2	C	-4.46296	2.101643	3.50352	47	C	-3.49171	7.015791	-2.27131
3	C	-5.16696	1.205074	2.681071	48	C	-4.23854	5.790073	-2.79293
4	C	-4.63522	0.800711	1.457623	49	C	2.717484	-0.73417	-0.23552
5	C	-3.414	1.267343	0.982778	50	C	3.697443	0.276042	-0.2693
6	C	-2.72606	2.17007	1.803676	51	C	5.035142	-0.06898	-0.26098
7	N	-4.95209	2.477741	4.737082	52	C	5.414173	-1.41012	-0.22428
8	C	-6.32108	2.129907	5.093587	53	C	4.477537	-2.43529	-0.18982
9	C	-4.3418	3.601784	5.437324	54	C	3.131546	-2.07486	-0.19623
10	C	-2.85969	0.816512	-0.35065	55	C	6.276764	0.792232	-0.27301
11	C	-1.4477	0.257582	-0.21008	56	C	7.385393	-0.26358	-0.23579
12	C	-0.54216	1.017862	-0.93986	57	N	6.824846	-1.49389	-0.22397
13	C	0.812833	0.719496	-0.97089	58	C	7.540372	-2.76506	-0.23518
14	C	1.278242	-0.38348	-0.24105	59	C	6.370178	1.637117	-1.56208
15	C	0.349865	-1.14842	0.49346	60	C	6.350663	1.703199	0.970999
16	C	-1.00816	-0.84032	0.516542	61	C	8.727982	0.103962	-0.21669
17	C	-1.27846	2.104586	-1.63301	62	C	9.886017	-0.66784	-0.09815
18	O	-0.82639	2.954981	-2.40483	63	C	11.14858	-0.07481	-0.06063
19	O	-5.40856	-0.08829	0.754583	64	C	12.41466	-0.63903	0.085148
20	C	-4.98061	-0.52818	-0.48543	65	C	13.65765	0.237225	0.261132
21	C	-3.79937	-0.15103	-1.03198	66	C	14.74568	-0.8011	0.404505
22	C	-5.93856	-1.42221	-1.12347	67	C	14.17687	-2.07078	0.311004
23	C	-5.57897	-1.89874	-2.51252	68	N	12.78247	-1.93774	0.102763
24	C	-4.06518	-2.00804	-2.70261	69	C	16.11064	-0.66777	0.599134
25	C	-3.38943	-0.6759	-2.38628	70	C	16.89099	-1.82401	0.704148
26	N	-2.58428	1.981787	-1.25888	71	C	16.30392	-3.0863	0.615406
27	C	-7.1017	-1.76049	-0.49259	72	C	14.92938	-3.23375	0.417416
28	C	-8.14584	-2.56598	-1.05456	73	C	13.87849	1.127632	-0.98118
29	C	-9.32254	-2.99521	-0.50735	74	C	11.90487	-3.08418	-0.10326
30	C	-10.383	-3.74398	-1.3339	75	H	-2.64633	3.297937	3.605848
31	C	-11.4927	-3.93124	-0.3204	76	H	-6.12926	0.804762	2.968633
32	C	-11.0878	-3.39247	0.905664	77	H	-1.7734	2.563226	1.462926
33	N	-9.7944	-2.86762	0.787346	78	H	-6.46429	1.047113	5.059634
34	C	-12.7378	-4.52249	-0.44441	79	H	-6.50867	2.461268	6.113377
35	C	-13.5866	-4.56726	0.670044	80	H	-7.05974	2.59964	4.429591
36	C	-13.1741	-4.0208	1.884031	81	H	-4.43927	4.544811	4.882811
37	C	-11.9187	-3.42104	2.023533	82	H	-4.83326	3.713451	6.402191
38	C	-9.04991	-2.34838	1.922661	83	H	-3.28158	3.414593	5.621268
39	C	-10.8522	-2.89955	-2.53415	84	H	-5.98102	-1.19844	-3.25789
40	C	-9.8394	-5.10189	-1.82466	85	H	-6.04729	-2.86582	-2.70842
41	C	-3.63978	2.822575	-1.80326	86	H	-3.83728	-2.31271	-3.72774
42	C	-3.84097	4.129499	-1.02031	87	H	-3.67006	-2.78114	-2.03475
43	N	-4.82162	4.974182	-1.71312	88	H	-2.30102	-0.78825	-2.4154
44	C	-5.60903	5.839612	-0.822	89	H	-3.64114	0.05776	-3.16446
45	C	-4.83308	7.059478	-0.33341	90	H	-7.24787	-1.34745	0.495265

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
91	H	-8.0228	-2.85988	-2.09178	120	H	8.069092	-2.91349	0.70779
92	H	-13.0588	-4.94634	-1.39049	121	H	8.242041	-2.78267	-1.06959
93	H	-14.5659	-5.02438	0.586669	122	H	6.823685	-3.56989	-0.36728
94	H	-13.8378	-4.05333	2.741128	123	H	7.298205	2.212442	-1.57782
95	H	-11.6223	-2.99187	2.972791	124	H	6.333307	1.00397	-2.45046
96	H	-9.53218	-2.68125	2.83966	125	H	5.532805	2.337151	-1.59681
97	H	-8.03298	-2.74163	1.906175	126	H	7.279825	2.276851	0.971063
98	H	-9.01768	-1.25538	1.919341	127	H	5.514495	2.405361	0.957363
99	H	-11.6482	-3.42246	-3.07065	128	H	6.299514	1.117032	1.890376
100	H	-10.0256	-2.72942	-3.22849	129	H	11.16221	1.009644	-0.11551
101	H	-11.2334	-1.93072	-2.20456	130	C	13.5339	1.109562	1.528816
102	H	-9.00345	-4.9515	-2.51256	131	H	16.57146	0.311169	0.67153
103	H	-9.49468	-5.71072	-0.98611	132	H	17.96024	-1.73882	0.858626
104	H	-10.6246	-5.6495	-2.35246	133	H	16.92066	-3.97315	0.703658
105	H	-3.37842	3.046802	-2.83997	134	H	14.48812	-4.22042	0.358271
106	H	-4.56624	2.244566	-1.80088	135	H	14.80449	1.693386	-0.85936
107	H	-4.23041	3.894498	-0.02853	136	H	13.05673	1.837624	-1.09652
108	H	-2.8655	4.615687	-0.87896	137	H	13.95379	0.525302	-1.88831
109	H	-6.48489	6.185583	-1.38222	138	H	12.51555	-3.96596	-0.27386
110	H	-5.96341	5.249168	0.026658	139	H	11.2764	-3.24991	0.773619
111	H	-5.47602	7.7304	0.239383	140	H	11.28686	-2.91348	-0.9846
112	H	-3.98685	6.756951	0.30019	141	H	13.35902	0.494163	2.413339
113	H	-3.16877	7.654159	-3.09559	142	H	12.71116	1.820357	1.426643
114	H	-2.60557	6.718591	-1.69284	143	H	14.45845	1.672572	1.672388
115	H	-5.05524	6.122918	-3.44224	144	H	9.800348	-1.73811	0.001619
116	H	-3.5669	5.172324	-3.39392	145	H	8.882679	1.176973	-0.27949
117	H	3.398682	1.318364	-0.27814	146	H	0.706414	-1.98958	1.07728
118	H	4.75677	-3.48075	-0.16312	147	H	-1.69614	-1.44321	1.099036
119	H	2.386688	-2.86197	-0.19045	148	H	1.489157	1.320323	-1.56867

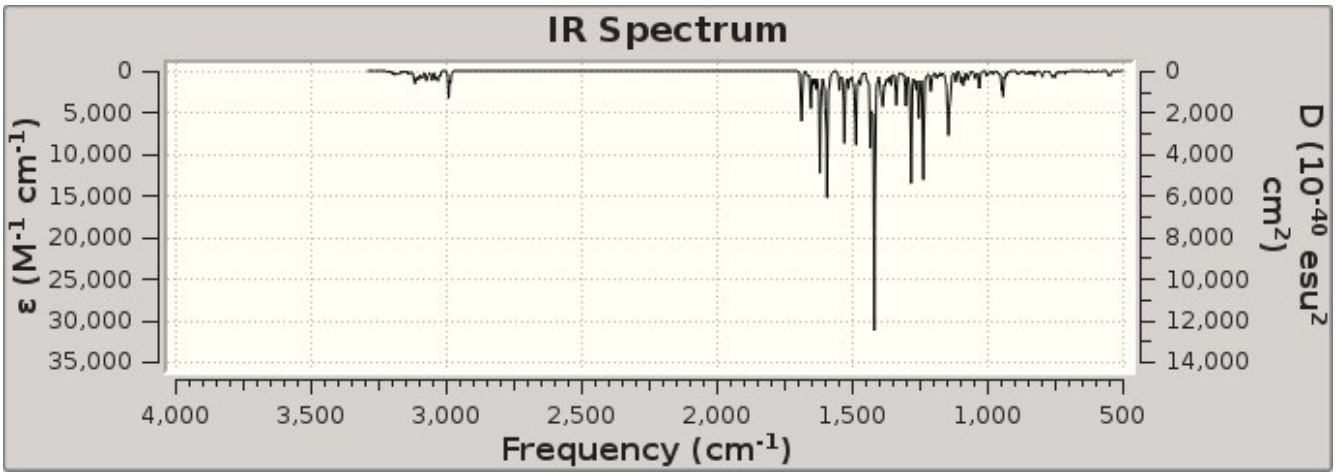

Figure S25. Calculated IR spectrum for Probe **A**.

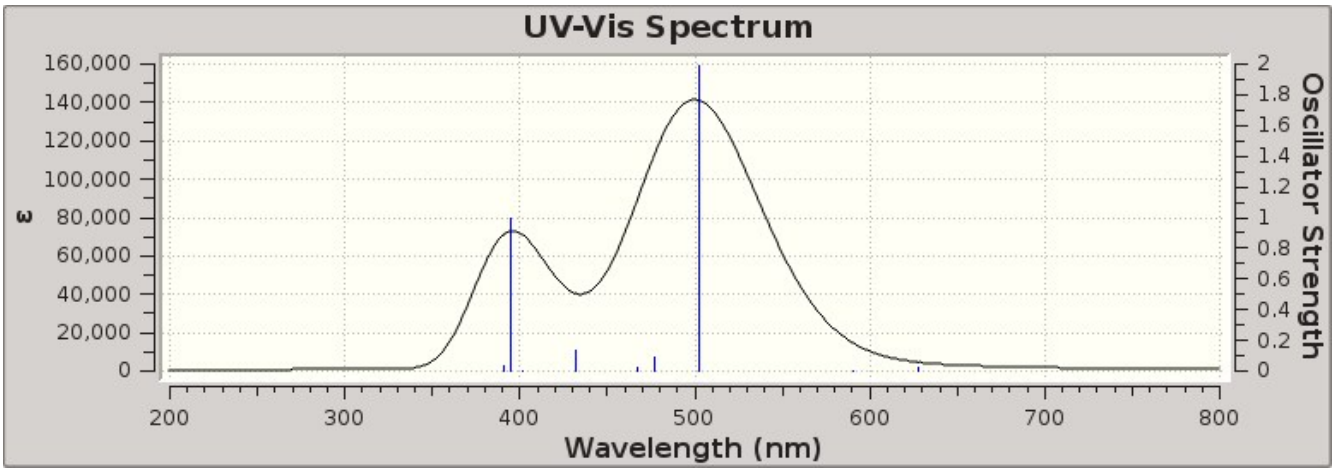

Figure S26. Calculated UV-Vis spectrum for Probe **A**.

Table S3. Excitation energies and oscillator strengths listing for Probe **A**.

Excited State	Nature	E (eV)	λ (nm)	*f*	Orbital transitions	Normalized coefficient
1:	A	1.3966	887.77	0.0130	267 -> 268	0.70702
2:	A	1.9750	627.77	0.0277	266 -> 268	0.70574
3:	A	2.1007	590.20	0.0001	265 -> 268	0.70702
4:	A	2.4699	501.99	1.9923	263 -> 268	0.12127
					264 -> 268	0.68211
5:	A	2.6016	476.56	0.0916	263 -> 268	0.69576
					264 -> 268	-0.11823
6:	A	2.6563	466.76	0.0247	267 -> 269	0.63812
					267 -> 270	-0.29193
7:	A	2.8704	431.94	0.1367	267 -> 269	0.19620
					267 -> 270	0.50486
					267 -> 271	0.44883
8:	A	3.0905	401.18	0.0006	262 -> 268	0.70522
9:	A	3.1393	394.94	0.9927	266 -> 269	-0.17057
					267 -> 269	-0.19664
					267 -> 270	-0.36494
					267 -> 271	0.51306
10:	A	3.1712	390.97	0.0349	261 -> 268	0.53876
					266 -> 269	0.43195

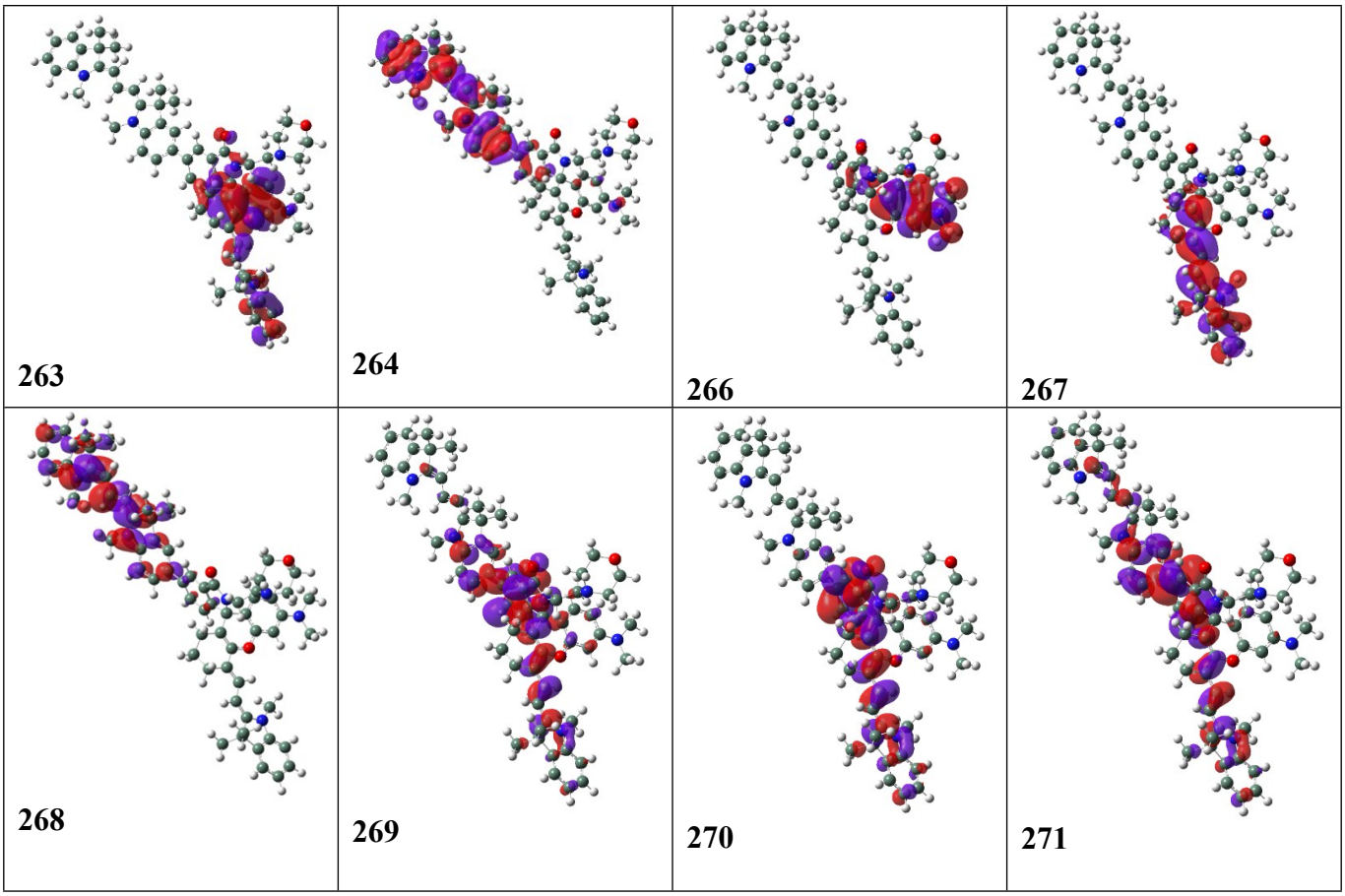

Figure S27. Drawings of selected molecular orbitals listed in Table S3.

Data for Probe AH^+.

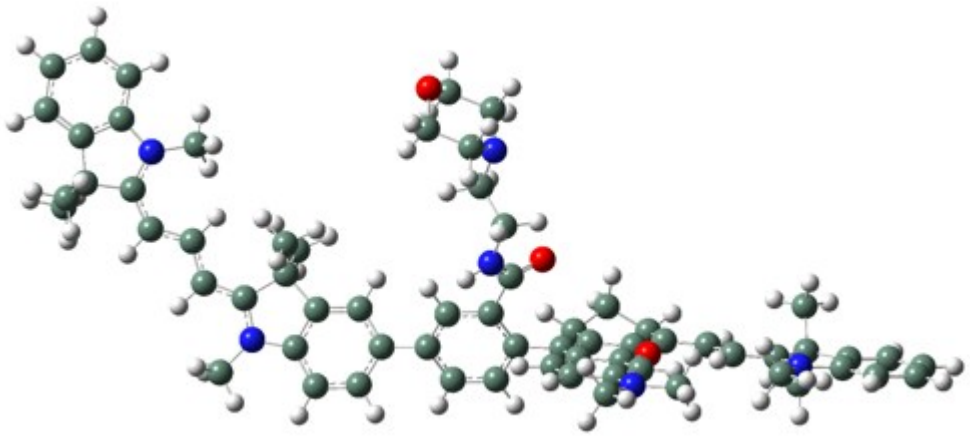

Figure S28. GaussView representation of Probe **AH^+**.

Table S4. Computational results for Probe **AH^+**.

rfppah (Opt step 1 of 24)		
/storage/liu/rfp/tpssh/tpah.log		
File Type	.log	
Calculation Type	FREQ	
Calculation Method	RTPSSh	
Basis Set	TZVP	
Charge	2	
Spin	Singlet	
Solvation	scrf=solvent=water	
E(RTPSSh)	-3114.699665	Hartree
RMS Gradient Norm	0.000003	Hartree/Bohr
Imaginary Freq	0	
Dipole Moment	16.465132	Debye
Polarizability (α)	856.224333	a.u.
Point Group	C1	
Job cpu time:	14 days 7 hours 9 minutes 47...	

Table S5. Calculated atomic coordinates for Probe **AH**$^{+}$.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	4.22112	0.663478	3.833125	42	C	-3.94168	-3.76601	2.550831
2	C	5.604684	0.94633	3.620817	43	C	-2.61398	-3.4118	2.325042
3	C	6.18811	0.517022	2.408423	44	C	-2.25533	-2.39223	1.428896
4	C	5.412386	-0.13709	1.47284	45	C	-3.27301	-1.70711	0.737527
5	C	4.039049	-0.40127	1.651257	46	C	-4.59338	-2.04916	0.954201
6	C	3.476806	0.014803	2.88086	47	C	-4.91714	-3.06731	1.850294
7	N	6.336439	1.599808	4.560088	48	C	-5.86622	-1.49086	0.347709
8	C	5.727535	2.019854	5.820458	49	C	-6.92913	-2.34858	1.041618
9	C	7.753728	1.862997	4.331736	50	N	-6.31498	-3.21779	1.881251
10	C	3.33355	-1.06275	0.60742	51	C	-5.86764	-1.69991	-1.18241
11	C	1.885654	-1.36534	0.786721	52	C	-6.02124	0.00307	0.709703
12	C	0.889302	-0.3729	0.74805	53	C	-8.31382	-2.32134	0.914179
13	C	-0.44514	-0.72197	0.960605	54	C	-9.05152	-1.47403	0.086564
14	C	-0.83251	-2.04298	1.219232	55	C	-10.4452	-1.49536	0.06499
15	C	0.171591	-3.01854	1.267594	56	C	-11.3527	-0.72596	-0.66221
16	C	1.502324	-2.68204	1.056454	57	C	-12.8537	-0.77491	-0.36588
17	C	1.242935	1.049725	0.407845	58	C	-13.3927	0.233982	-1.35294
18	O	2.021338	1.318962	-0.51237	59	C	-12.3339	0.74835	-2.1
19	O	6.044349	-0.5419	0.32789	60	N	-11.1294	0.135503	-1.67641
20	C	5.391528	-1.21419	-0.66252	61	C	-14.6847	0.671468	-1.59409
21	C	4.004342	-1.47465	-0.53544	62	C	-14.8936	1.635099	-2.58644
22	C	6.172577	-1.60124	-1.77196	63	C	-13.8209	2.144809	-3.31822
23	C	5.452501	-2.33968	-2.89056	64	C	-12.5143	1.709456	-3.0866
24	C	3.990635	-1.92237	-3.00605	65	C	-7.01445	-4.1909	2.716134
25	C	3.284427	-2.15939	-1.67422	66	C	-9.84961	0.391734	-2.32651
26	N	0.642279	1.994739	1.167936	67	C	-13.4158	-2.1883	-0.63582
27	C	7.544072	-1.39519	-1.93911	68	C	-13.1367	-0.35653	1.092318
28	C	8.510483	-0.7985	-1.12105	69	C	-0.29494	3.997657	0.035969
29	C	9.85605	-0.69626	-1.46271	70	N	-0.12051	5.446036	-0.11911
30	C	10.59067	-1.17882	-2.72069	71	C	-0.59036	5.975956	-1.40857
31	C	12.01317	-0.74869	-2.41913	72	C	-2.10906	6.103211	-1.4844
32	C	12.05345	-0.11801	-1.17579	73	O	-2.59749	6.925429	-0.41383
33	N	10.75403	-0.10235	-0.63434	74	C	-2.23186	6.364265	0.85533
34	C	13.174	-0.89201	-3.16053	75	C	-0.71497	6.233319	0.975616
35	C	14.37305	-0.3949	-2.63791	76	H	3.746723	0.958893	4.758382
36	C	14.39569	0.233579	-1.39263	77	H	7.232703	0.684594	2.194966
37	C	13.23208	0.383996	-0.63559	78	H	2.431636	-0.19215	3.075424
38	C	10.41883	0.484008	0.657904	79	H	4.894871	2.707524	5.647167
39	C	10.52481	-2.71515	-2.87438	80	H	5.364946	1.161109	6.393597
40	C	10.08311	-0.46579	-3.99456	81	H	6.481216	2.534686	6.410637
41	C	0.809093	3.425735	0.937782	82	H	8.316409	0.930776	4.216648

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
83	H	7.900227	2.475219	3.436452	117	H	-6.78005	-1.31399	-1.63757
84	H	8.149582	2.403	5.187897	118	H	-5.18478	0.563104	0.286941
85	H	-1.20212	0.053296	0.916472	119	H	-6.94672	0.416502	0.307446
86	H	-0.08771	-4.05527	1.448089	120	H	-6.02081	0.141967	1.792291
87	H	2.261384	-3.45482	1.102674	121	H	-8.87818	-3.02176	1.520853
88	H	5.97944	-2.15412	-3.82949	122	H	-8.51858	-0.76444	-0.52961
89	H	5.502041	-3.42126	-2.71005	123	H	-10.9177	-2.18589	0.757025
90	H	3.494738	-2.49147	-3.79594	124	H	-15.5222	0.280199	-1.02704
91	H	3.929026	-0.8622	-3.27303	125	H	-15.8973	1.991376	-2.78665
92	H	2.25417	-1.79979	-1.70772	126	H	-13.9983	2.895219	-4.07984
93	H	3.235017	-3.2376	-1.47963	127	H	-11.6903	2.120333	-3.65592
94	H	0.056777	1.697072	1.935012	128	H	-7.69067	-3.6765	3.400472
95	H	7.906285	-1.78369	-2.88469	129	H	-7.57955	-4.88273	2.089823
96	H	8.210464	-0.38971	-0.16891	130	H	-6.28352	-4.74909	3.292829
97	H	13.16194	-1.37984	-4.12912	131	H	-10.0358	0.852116	-3.29294
98	H	15.29091	-0.49881	-3.20464	132	H	-9.23428	1.062234	-1.72349
99	H	15.33164	0.613961	-0.99991	133	H	-9.3331	-0.55432	-2.48573
100	H	13.26597	0.873402	0.329484	134	H	-14.4973	-2.17938	-0.4853
101	H	10.01795	-0.28113	1.325419	135	H	-12.9802	-2.91447	0.053723
102	H	11.31854	0.901937	1.099114	136	H	-13.2082	-2.50506	-1.65948
103	H	9.684419	1.280689	0.527175	137	H	-12.6945	-1.07186	1.788995
104	H	9.510313	-3.05528	-3.08495	138	H	-12.7311	0.634936	1.301814
105	H	11.16556	-3.01881	-3.70497	139	H	-14.2154	-0.33542	1.260552
106	H	10.87601	-3.21052	-1.96717	140	H	-0.21158	3.534527	-0.94975
107	H	9.057337	-0.74908	-4.23297	141	H	-1.27646	3.714876	0.444074
108	H	10.12512	0.618382	-3.87425	142	H	-0.15024	6.970734	-1.53928
109	H	10.71854	-0.74552	-4.83746	143	H	-0.22093	5.335441	-2.21327
110	H	0.810029	3.922083	1.909554	144	H	-2.41622	6.59238	-2.41058
111	H	1.780126	3.580604	0.468638	145	H	-2.58753	5.114509	-1.43312
112	H	-4.18135	-4.55215	3.255206	146	H	-2.62448	7.042339	1.615068
113	H	-1.8402	-3.93007	2.879007	147	H	-2.72146	5.38721	0.973969
114	H	-3.01665	-0.93283	0.022898	148	H	-0.27051	7.233771	0.949716
115	H	-5.02028	-1.16604	-1.61724	149	H	-0.45386	5.779555	1.934795
116	H	-5.77595	-2.75869	-1.43078					

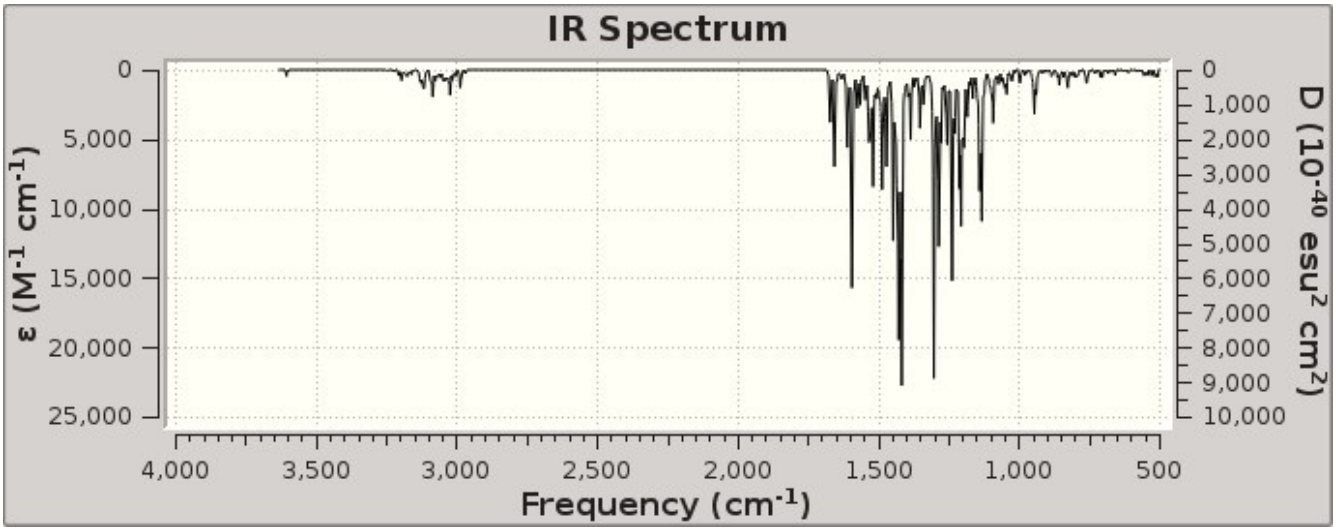

Figure S29. Calculated IR spectrum for Probe **AH**$^{+}$.

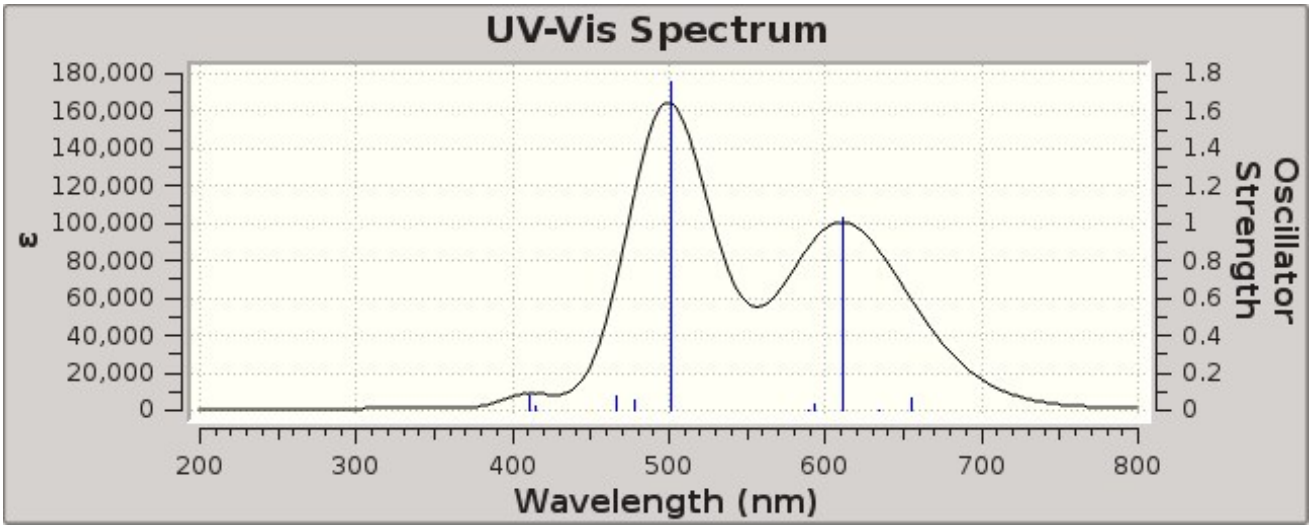

Figure S30. Calculated UV-Vis spectrum for Probe **AH**$^{+}$.

Table S6. Excitation energies and oscillator strengths listing for Probe **AH^+**.

Excited State	Nature	E (eV)	λ (nm)	*f*	Orbital transitions	Normalized coefficient
1:	A	1.8907	655.75	0.0673	267 -> 268	-0.31763
					267 -> 269	0.63155
2:	A	1.9534	634.70	0.0000	266 -> 268	0.70670
3:	A	2.0303	610.66	1.0343	265 -> 268	-0.15726
					267 -> 268	0.61209
					267 -> 269	0.30991
4:	A	2.0923	592.57	0.0362	265 -> 268	0.68191
					265 -> 269	0.10236
					267 -> 268	0.13735
5:	A	2.1046	589.10	0.0001	266 -> 269	0.70669
6:	A	2.4750	500.94	1.7568	264 -> 268	0.20411
					265 -> 269	0.65816
7:	A	2.5903	478.64	0.0517	264 -> 268	0.64021
					265 -> 269	-0.20412
					267 -> 270	0.11306
					267 -> 271	0.11184
8:	A	2.6594	466.21	0.0743	264 -> 269	0.69459
9:	A	2.9896	414.71	0.0164	263 -> 268	0.67239
					267 -> 270	-0.16889
10:	A	3.0187	410.72	0.0720	263 -> 268	0.16154
					267 -> 270	0.65235
					267 -> 271	-0.17954

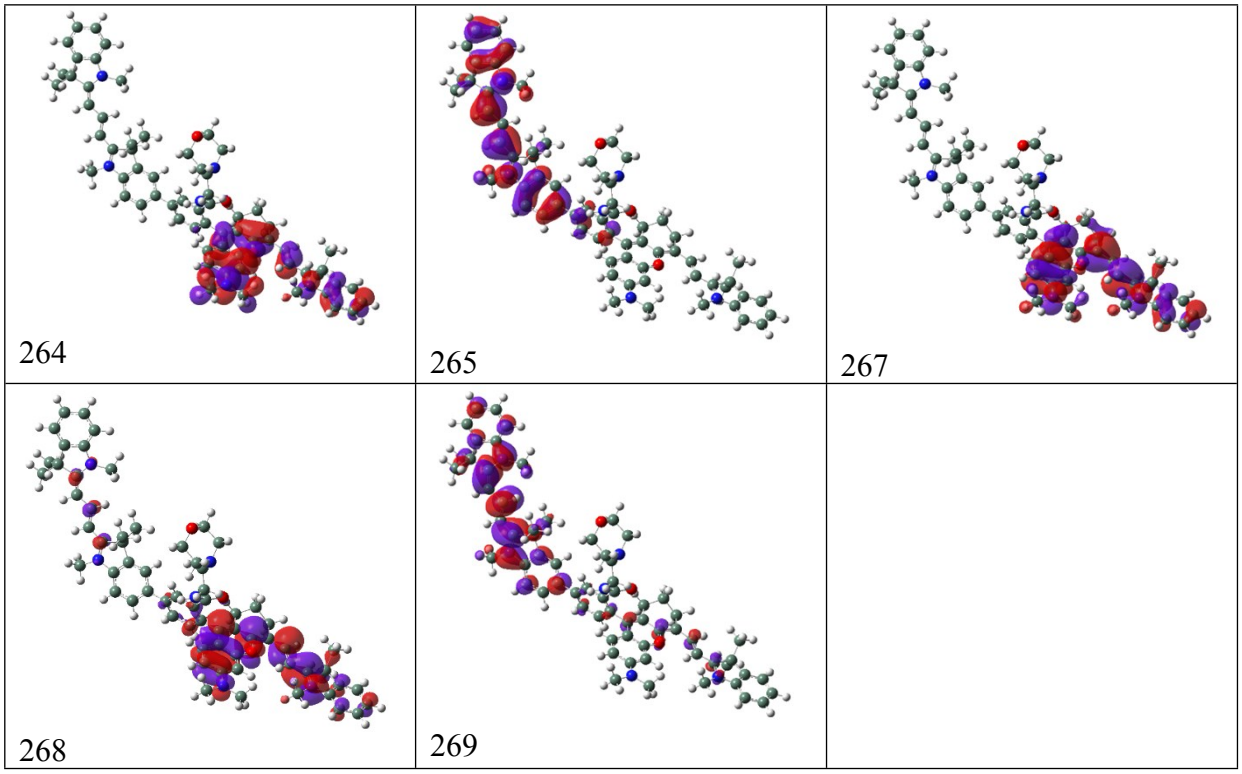

Figure S31. Drawings of selected molecular orbitals listed in Table S6.

Data for Probe B.

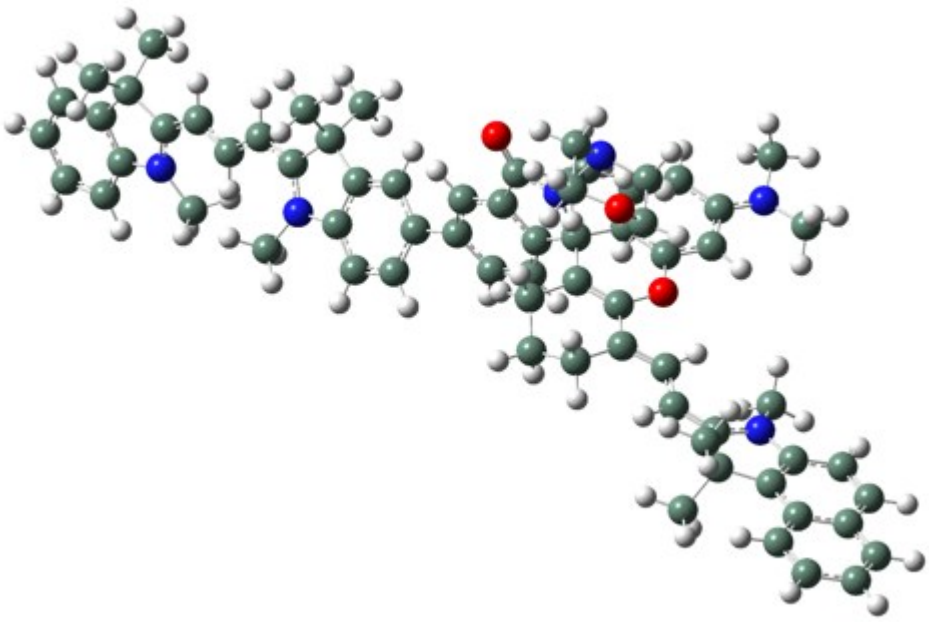

Figure S32. GaussView representation of Probe **B**.

Table S7. Computational results for Probe **B**.

rfpb (Optimization completed)		
/storage/liu/rfp/tpssh/tpb.log		
File Type	.log	
Calculation Type	FREQ	
Calculation Method	RTPSSh	
Basis Set	TZVP	
Charge	1	
Spin	Singlet	
Solvation	scrf=solvent=water	
E(RTPSSh)	-3267.928186	Hartree
RMS Gradient Norm	0.000001	Hartree/Bohr
Imaginary Freq	0	
Dipole Moment	48.247354	Debye
Polarizability (α)	854.291667	a.u.
Point Group	C1	
Job cpu time:	17 days 4 hours 1 minutes 23...	

Table S8. Calculated atomic coordinates for Probe **B**.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	2.117177	-2.56923	3.861903	42	C	3.330365	-3.74528	-1.9464
2	C	3.394682	-2.11344	4.280044	43	N	4.282824	-4.86295	-1.97666
3	C	4.173449	-1.41716	3.339387	44	C	4.495031	-5.43414	-3.31569
4	C	3.680563	-1.16777	2.059839	45	C	5.395606	-4.57382	-4.19751
5	C	2.42287	-1.59495	1.646022	46	O	6.662109	-4.35486	-3.55821
6	C	1.661642	-2.30355	2.584973	47	C	6.477233	-3.70462	-2.29216
7	N	3.847508	-2.33097	5.56381	48	C	5.589386	-4.5427	-1.37456
8	C	5.238899	-2.04288	5.884877	49	C	-3.60828	0.347646	-0.12108
9	C	3.137272	-3.26712	6.427	50	C	12.75577	3.585803	-1.72164
10	C	1.920733	-1.3332	0.244407	51	C	14.05079	4.036248	-1.82281
11	C	0.520521	-0.73107	0.243009	52	C	14.88282	4.113463	-0.68266
12	C	-0.38295	-1.58417	-0.37818	53	C	14.39171	3.735019	0.54398
13	C	-1.72634	-1.26257	-0.51276	54	C	-4.62155	-0.62832	-0.05792
14	C	-2.18285	-0.03865	-0.00309	55	C	-5.94489	-0.24773	-0.16978
15	C	-1.2569	0.816885	0.62754	56	C	-6.27554	1.094892	-0.3467
16	C	0.089962	0.485968	0.752844	57	C	-5.30652	2.087454	-0.41137
17	C	0.335627	-2.80625	-0.81851	58	C	-3.97554	1.691011	-0.29863
18	O	-0.13525	-3.80379	-1.37158	59	C	-7.21626	-1.06468	-0.12761
19	O	4.530724	-0.47762	1.234198	60	C	-8.28499	0.020582	-0.28527
20	C	4.115059	-0.13953	-0.04181	61	N	-7.67976	1.219931	-0.4363
21	C	2.903548	-0.49003	-0.53861	62	C	-8.33641	2.490717	-0.72247
22	C	5.125831	0.611403	-0.77581	63	C	-7.28421	-2.06878	-1.29953
23	C	4.7777	0.972542	-2.20154	64	C	-7.37956	-1.80094	1.218447
24	C	3.273759	1.187757	-2.37596	65	C	-9.643	-0.28581	-0.26283
25	C	2.500234	-0.04946	-1.92601	66	C	-10.7503	0.560471	-0.18214
26	N	1.643159	-2.63071	-0.46943	67	C	-12.0563	0.070999	-0.19174
27	C	6.322538	0.920854	-0.19461	68	C	-13.2664	0.755583	-0.09386
28	C	7.417675	1.573236	-0.85011	69	C	-14.6026	0.064015	-0.37483
29	C	8.636045	1.956209	-0.36276	70	C	-15.5803	1.200522	-0.18466
30	C	9.751558	2.503427	-1.2739	71	C	-14.8761	2.349993	0.171882
31	C	10.88929	2.711672	-0.28524	72	N	-13.4954	2.043348	0.240461
32	C	10.44602	2.356398	0.977329	73	C	-16.9597	1.244803	-0.30326
33	N	9.1118	1.936757	0.934139	74	C	-17.6161	2.457387	-0.06669
34	C	12.20944	3.184569	-0.46953	75	C	-16.8944	3.599156	0.281837
35	C	13.06097	3.267397	0.689581	76	C	-15.5037	3.566771	0.407019
36	C	12.55929	2.877834	1.957378	77	C	-14.6392	-0.48632	-1.81616
37	C	11.27202	2.423689	2.118475	78	C	-14.8498	-1.07425	0.639972
38	C	8.331004	1.642589	2.125094	79	C	-12.5016	3.009258	0.694913
39	C	10.10009	1.46121	-2.35678	80	H	1.485645	-3.12877	4.537994
40	C	9.293152	3.82759	-1.92332	81	H	5.165555	-1.05821	3.57576
41	C	2.628755	-3.69654	-0.58178	82	H	0.680174	-2.66557	2.295197

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
83	H	5.46996	-0.99276	5.689392	119	H	7.472916	-3.58157	-1.86235
84	H	5.395704	-2.22903	6.94578	120	H	6.04364	-2.70815	-2.45684
85	H	5.938817	-2.66584	5.311334	121	H	6.09902	-5.48729	-1.15687
86	H	3.149498	-4.29257	6.034172	122	H	5.436768	-4.02235	-0.42603
87	H	3.615561	-3.26382	7.404844	123	H	12.14376	3.538181	-2.61289
88	H	2.098123	-2.95844	6.561299	124	H	14.43951	4.335905	-2.79011
89	H	-2.39938	-1.94264	-1.02281	125	H	15.90197	4.470086	-0.78018
90	H	-1.6067	1.752966	1.048014	126	H	15.01952	3.78962	1.427977
91	H	0.775873	1.16412	1.248584	127	H	-4.36138	-1.6688	0.101234
92	H	5.103801	0.169977	-2.87773	128	H	-5.55153	3.133295	-0.54492
93	H	5.318439	1.872883	-2.50134	129	H	-3.20441	2.449033	-0.36879
94	H	3.045027	1.414569	-3.42091	130	H	-8.7638	2.921584	0.185196
95	H	2.959524	2.049257	-1.77675	131	H	-9.11614	2.331895	-1.46691
96	H	1.425472	0.155252	-1.94073	132	H	-7.60069	3.178784	-1.13029
97	H	2.661994	-0.86421	-2.64372	133	H	-8.23138	-2.61214	-1.28522
98	H	6.454232	0.601898	0.829432	134	H	-7.1843	-1.55963	-2.25979
99	H	7.30034	1.759395	-1.91245	135	H	-6.47287	-2.79307	-1.20184
100	H	13.2189	2.938561	2.816758	136	H	-8.32871	-2.34012	1.247159
101	H	10.91717	2.120442	3.095477	137	H	-6.57002	-2.52412	1.337372
102	H	8.837046	2.061235	2.992426	138	H	-7.34802	-1.10065	2.055145
103	H	7.349862	2.110658	2.041371	139	H	-9.85358	-1.35102	-0.25733
104	H	8.209222	0.566224	2.273663	140	H	-10.5912	1.624943	-0.10977
105	H	10.90549	1.819608	-3.00027	141	H	-12.1666	-0.99821	-0.34551
106	H	9.226628	1.264904	-2.9832	142	H	-17.5258	0.361027	-0.57596
107	H	10.41548	0.520635	-1.90015	143	H	-18.6946	2.511279	-0.15799
108	H	8.415519	3.653105	-2.55067	144	H	-17.4169	4.532542	0.456417
109	H	9.031704	4.562581	-1.15909	145	H	-14.9554	4.462845	0.668497
110	H	10.0812	4.247679	-2.55105	146	H	-15.6223	-0.91776	-2.01507
111	H	3.354154	-3.55437	0.22243	147	H	-13.8878	-1.2678	-1.94713
112	H	2.117887	-4.64708	-0.41531	148	H	-14.452	0.305986	-2.54339
113	H	2.577415	-3.91899	-2.71805	149	H	-14.1025	-1.8622	0.524008
114	H	3.794142	-2.77204	-2.15425	150	H	-14.8133	-0.70187	1.665402
115	H	4.967049	-6.41471	-3.18823	151	H	-15.8356	-1.50846	0.461007
116	H	3.525232	-5.58663	-3.79598	152	H	-13.0112	3.806573	1.229394
117	H	5.612623	-5.07384	-5.14323	153	H	-11.8096	2.517148	1.377759
118	H	4.922481	-3.60477	-4.41232	154	H	-11.9571	3.436649	-0.14953

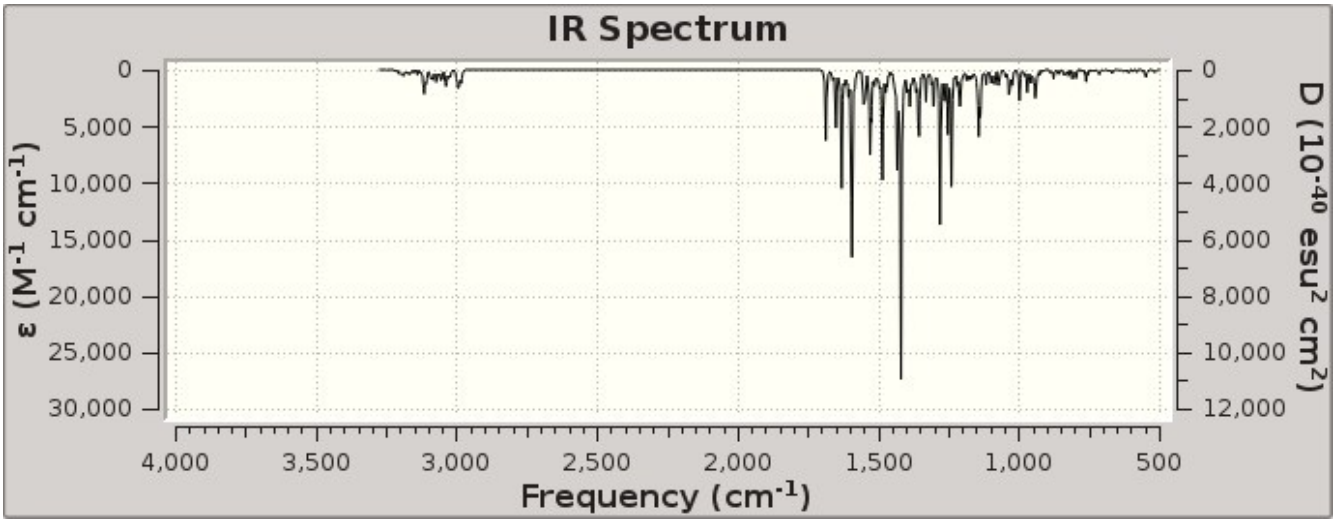

Figure S33. Calculated IR spectrum for Probe **B**.

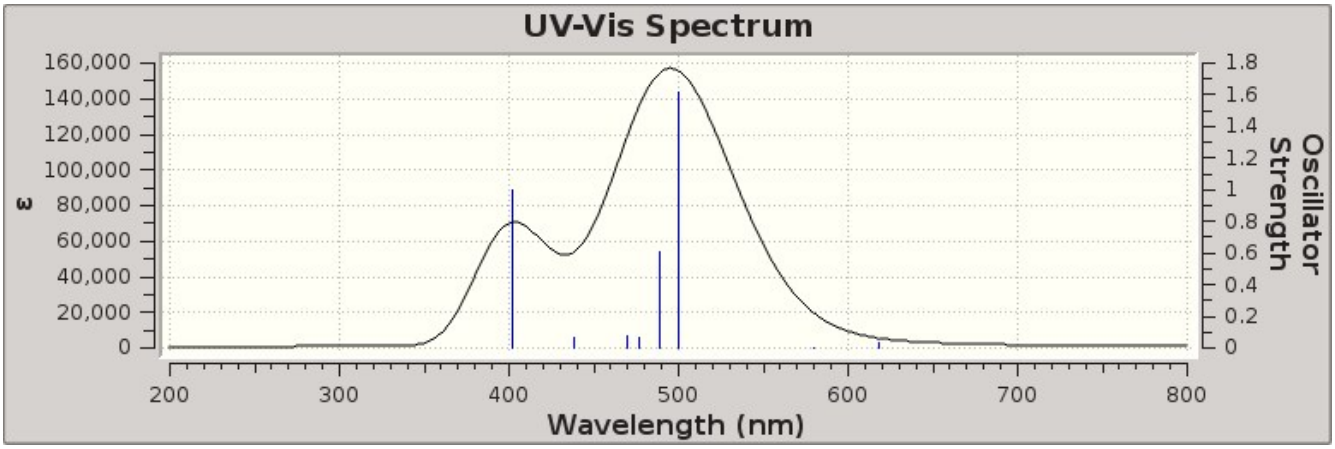

Figure S34. Calculated UV-Vis spectrum for Probe **B**.

Table S9. Excitation energies and oscillator strengths listing for Probe **B**.

Excited State	**Nature**	**E (eV)**	**λ (nm)**	***f***	**Orbital transitions**	**Normalized coefficient**
1:	A	1.3806	898.02	0.0118	280 -> 281	0.70701
2:	A	2.0046	618.50	0.0296	279 -> 281	0.70568
3:	A	2.1372	580.12	0.0003	278 -> 281	0.70693
4:	A	2.4779	500.37	1.6189	276 -> 281	-0.33065
					277 -> 281	0.60361
					280 -> 282	-0.11458
5:	A	2.5342	489.25	0.6029	276 -> 281	0.62335
					277 -> 281	0.31689
6:	A	2.6032	476.27	0.0658	277 -> 281	0.12490
					280 -> 282	0.55186
					280 -> 283	-0.37289
					280 -> 284	-0.14453
					280 -> 285	0.12954
7:	A	2.6369	470.18	0.0728	280 -> 282	0.34420
					280 -> 283	0.59499
					280 -> 285	0.13597
8:	A	2.8311	437.94	0.0020	275 -> 281	0.67643
					280 -> 284	-0.18902
9:	A	2.8313	437.90	0.0659	275 -> 281	0.20060
					280 -> 284	0.64244
					280 -> 285	0.18062
10:	A	3.0837	402.07	0.9996	279 -> 282	-0.10494
					280 -> 282	-0.19913
					280 -> 284	-0.13721
					280 -> 285	0.62842
					280 -> 290	-0.10783

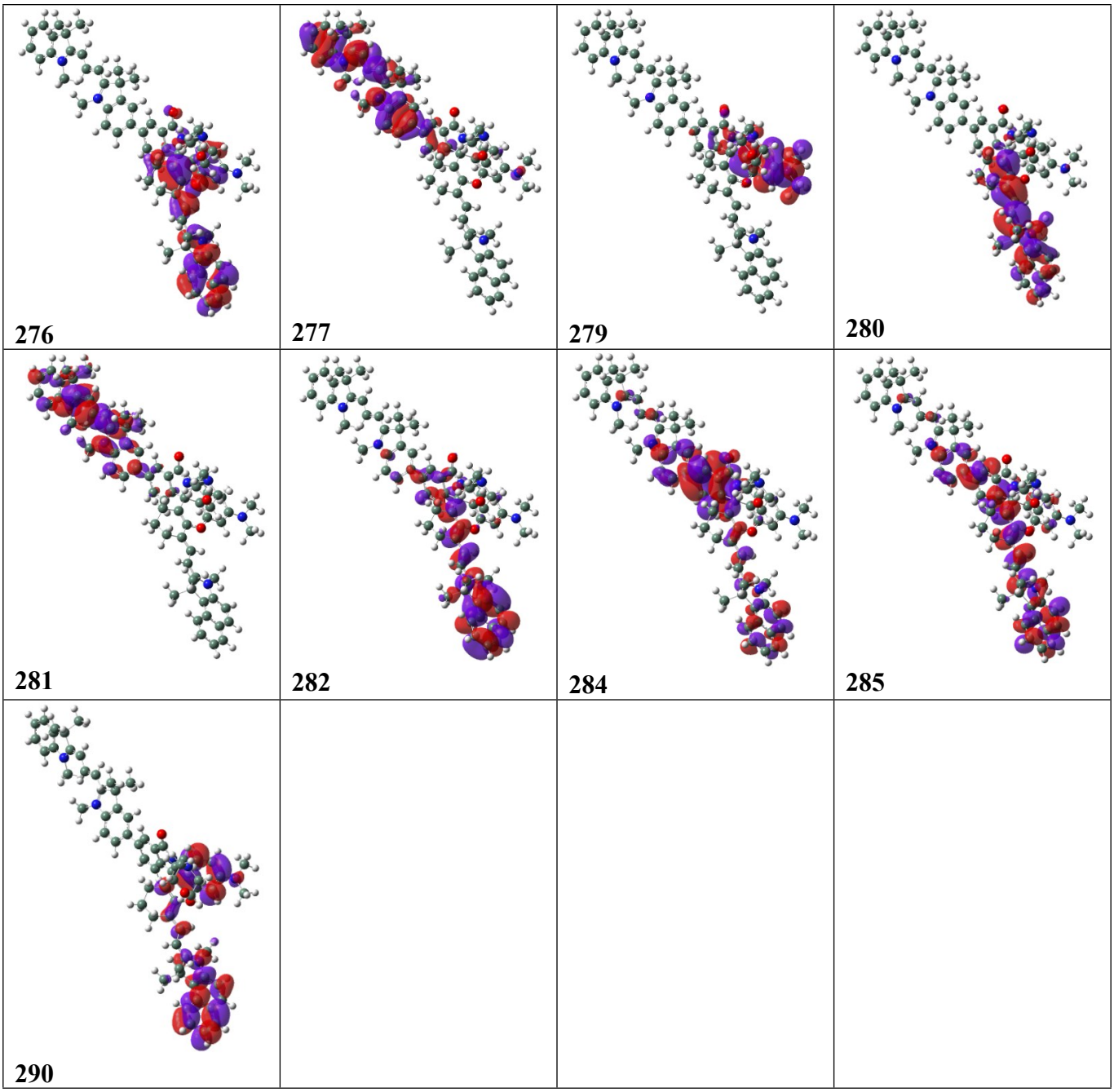

Figure S35. Drawings of selected molecular orbitals listed in Table S9.

Data for Probe BH^+.

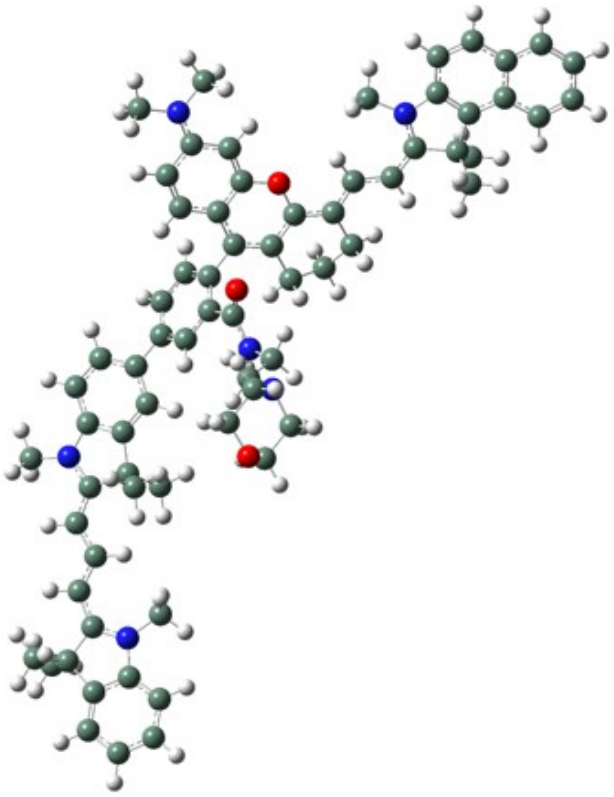

Figure S36. GaussView representation of Probe **BH^+**.

Table S10. Computational results for Probe **BH^+**.

rfpbh (Optimization completed)		
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Calculation Type	FREQ	
Calculation Method	RTPSSh	
Basis Set	TZVP	
Charge	2	
Spin	Singlet	
Solvation	scrf=solvent=water	
E(RTPSSh)	-3268.399682	Hartree
RMS Gradient Norm	0.000001	Hartree/Bohr
Imaginary Freq		
Dipole Moment	22.232411	Debye
Point Group	C1	
Job cpu time:	15 days 18 hours 0 minutes 4...	

Table S11. Calculated atomic coordinates for Probe **BH$^+$**.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	4.950488	5.041284	1.676746	42	C	-6.59006	0.973152	-0.95791
2	C	5.515077	3.90797	1.051371	43	C	-7.78013	1.911137	-1.18386
3	C	4.688878	2.993024	0.43042	44	N	-7.32117	3.178614	-1.32785
4	C	3.286381	3.124176	0.367913	45	C	-6.66578	0.275069	0.41766
5	C	2.535623	2.107889	-0.29065	46	C	-6.4536	-0.05559	-2.10215
6	C	1.058158	2.262335	-0.40909	47	C	-9.13963	1.623101	-1.24409
7	C	0.139892	1.466398	0.29925	48	C	-9.72835	0.364577	-1.1144
8	C	-1.23019	1.662838	0.117554	49	C	-11.1119	0.19507	-1.14771
9	C	-1.73075	2.631023	-0.76232	50	C	-11.8919	-0.95334	-1.01839
10	C	-0.80485	3.408094	-1.46982	51	C	-13.4155	-0.86776	-0.89849
11	C	0.560429	3.226686	-1.29091	52	C	-13.7918	-2.32261	-0.74286
12	C	0.620343	0.468124	1.316994	53	C	-12.6344	-3.09851	-0.79138
13	O	1.557588	0.719576	2.080875	54	N	-11.5166	-2.24877	-0.97747
14	O	5.301003	1.915531	-0.14592	55	C	-15.0252	-2.93109	-0.5768
15	C	4.597442	0.939027	-0.78388	56	C	-15.0764	-4.3236	-0.45392
16	C	3.187519	1.029082	-0.87136	57	C	-13.9074	-5.08345	-0.49795
17	C	5.366985	-0.11835	-1.31567	58	C	-12.6585	-4.48191	-0.66726
18	C	4.651116	-1.18909	-2.11018	59	C	-8.17248	4.342787	-1.55898
19	C	3.333441	-0.68363	-2.69569	60	C	-10.1618	-2.75971	-1.14972
20	C	2.459167	-0.07445	-1.60109	61	C	-13.8216	-0.03661	0.336784
21	N	-0.05056	-0.7076	1.337535	62	C	-14.0271	-0.25721	-2.17905
22	C	6.740777	-0.16138	-1.08206	63	C	-0.63721	-1.64002	3.564707
23	C	7.589221	-1.19319	-1.49853	64	N	-0.35879	-2.75208	4.480411
24	C	8.958463	-1.34454	-1.30548	65	C	-0.53119	-2.41941	5.903105
25	C	9.682776	-2.64671	-1.66201	66	C	-1.99478	-2.36908	6.331601
26	C	11.10642	-2.33588	-1.23968	67	O	-2.64501	-3.61582	6.042292
27	C	11.14592	-1.04768	-0.74479	68	C	-2.5727	-3.90366	4.63832
28	N	9.86065	-0.46491	-0.81225	69	C	-1.12021	-3.97549	4.171593
29	C	12.28825	-3.11407	-1.28593	70	C	12.35745	-4.44407	-1.78336
30	C	13.49872	-2.51159	-0.79355	71	C	13.54583	-5.13554	-1.79417
31	C	13.47452	-1.18659	-0.28603	72	C	14.7322	-4.54155	-1.30895
32	C	12.31855	-0.44504	-0.2524	73	C	14.70407	-3.25746	-0.82014
33	C	9.603521	0.92426	-0.45029	74	H	6.58058	3.728484	1.049201
34	C	9.070903	-3.81616	-0.85896	75	H	-1.92491	1.08465	0.716671
35	C	0.230263	-1.76428	2.30318	76	H	-1.14992	4.150129	-2.18039
36	C	-5.07742	4.338645	-1.30449	77	H	1.257677	3.831174	-1.86014
37	C	-3.71133	4.112451	-1.15602	78	H	4.458679	-2.05976	-1.47018
38	C	-3.18812	2.830411	-0.92474	79	H	5.300754	-1.53389	-2.91851
39	C	-4.07498	1.739618	-0.84312	80	H	2.802119	-1.50486	-3.18212
40	C	-5.43182	1.9517	-0.98938	81	H	3.5389	0.073077	-3.45993
41	C	-5.92059	3.237819	-1.21662	82	H	1.530934	0.319011	-2.01987

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
83	H	2.177161	-0.85815	-0.88715	127	H	-1.69448	-1.56791	3.270113
84	H	-0.77188	-0.86431	0.648691	128	H	-0.02289	-3.19287	6.489438
85	H	7.147709	0.652191	-0.49964	129	H	-0.04106	-1.46436	6.107936
86	H	7.131185	-2.03496	-2.00503	130	H	-2.08346	-2.22052	7.409313
87	C	9.564936	-2.90328	-3.1824	131	H	-2.52464	-1.5532	5.819088
88	H	14.39846	-0.75796	0.086619	132	H	-3.07405	-4.86254	4.495511
89	H	12.32209	0.560111	0.148543	133	H	-3.12699	-3.13456	4.08188
90	H	10.54971	1.456196	-0.40639	134	H	-0.6279	-4.81382	4.675538
91	H	8.97964	1.386101	-1.21522	135	H	-1.0824	-4.16675	3.096367
92	H	9.111198	0.989087	0.522258	136	H	11.46438	-4.92407	-2.16134
93	H	9.597386	-4.74556	-1.07922	137	H	13.57319	-6.14883	-2.17921
94	H	8.020483	-3.94813	-1.12648	138	H	15.66042	-5.10123	-1.32433
95	H	9.136503	-3.62658	0.214086	139	H	15.60968	-2.79155	-0.44533
96	H	0.058713	-2.72129	1.808254	140	H	9.974712	-2.06614	-3.75057
97	H	1.282672	-1.70009	2.578256	141	H	8.518443	-3.0389	-3.46347
98	H	-5.44636	5.342581	-1.47038	142	H	10.10973	-3.80752	-3.45645
99	H	-3.03967	4.961734	-1.19815	143	C	2.738044	4.256227	1.013463
100	H	-3.68875	0.738305	-0.68792	144	H	1.663338	4.389908	1.01163
101	H	-7.50411	-0.42033	0.46707	145	C	3.529933	5.184552	1.64179
102	H	-5.74593	-0.28908	0.584203	146	N	5.73167	5.965829	2.294094
103	H	-6.77421	1.00752	1.21944	147	C	7.180143	5.791313	2.333584
104	H	-5.53785	-0.63261	-1.95882	148	H	7.618761	6.640382	2.851381
105	H	-7.2938	-0.75017	-2.1151	149	H	7.455373	4.876329	2.868391
106	H	-6.40171	0.444691	-3.07072	150	H	7.598029	5.747127	1.323135
107	H	-9.81279	2.459455	-1.40067	151	C	5.134637	7.121616	2.959313
108	H	-9.08778	-0.49144	-0.9587	152	H	4.564984	7.733348	2.253861
109	H	-11.6957	1.105162	-1.2487	153	H	4.473938	6.814406	3.77575
110	H	-15.9368	-2.34501	-0.53976	154	H	5.934394	7.730128	3.373667
111	H	-16.0323	-4.81649	-0.32056	155	H	3.06248	6.032396	2.122742
112	H	-13.9628	-6.16106	-0.39636					
113	H	-11.7594	-5.08426	-0.69131					
114	H	-8.75942	4.199969	-2.46714					
115	H	-8.83929	4.493206	-0.70839					
116	H	-7.54399	5.2199	-1.67809					
117	H	-10.2134	-3.82647	-1.34795					
118	H	-9.69814	-2.269	-2.0052					
119	H	-9.5681	-2.59297	-0.2492					
120	H	-14.9088	-0.04492	0.437429					
121	H	-13.4952	0.999626	0.2262					
122	H	-13.3832	-0.44792	1.2479					
123	H	-13.7034	0.778302	-2.30402					
124	H	-13.7364	-0.8267	-3.06365					
125	H	-15.116	-0.26854	-2.09844					
126	H	-0.38015	-0.70656	4.070248					

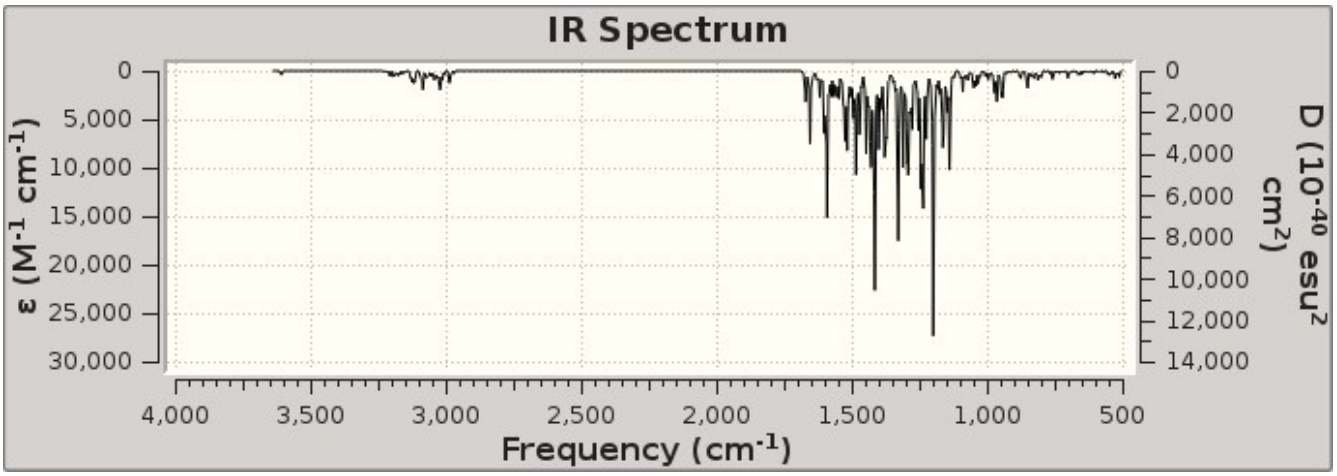


Figure S37. Calculated IR spectrum for Probe **BH**$^{+}$.

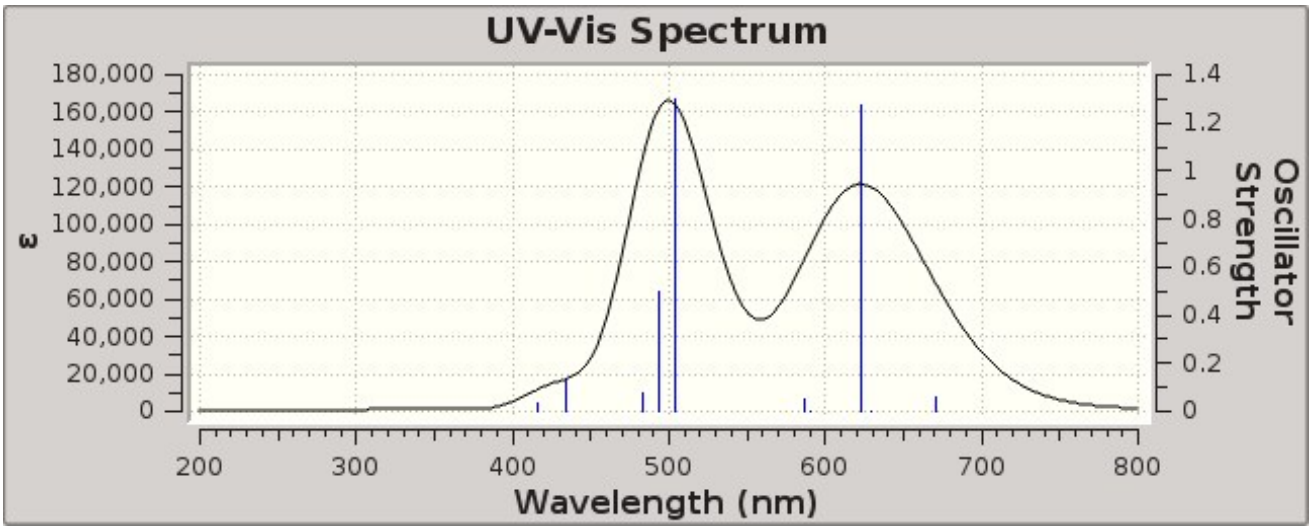


Figure S38. Calculated UV-Vis spectrum for Probe **BH**$^{+}$.

Table S12. Excitation energies and oscillator strengths listing for Probe **BH^+**.

Excited State	Nature	E (eV)	λ (nm)	*f*	Orbital transitions	Normalized coefficient
1:	A	1.8470	671.28	0.0620	280 -> 281	0.30760
					280 -> 282	0.63641
2:	A	1.9703	629.28	0.0033	279 -> 281	0.70516
3:	A	1.9898	623.09	1.2728	280 -> 281	0.62592
					280 -> 282	-0.30408
4:	A	2.0991	590.64	0.0001	279 -> 282	0.70611
5:	A	2.1139	586.51	0.0474	278 -> 281	0.69174
					278 -> 282	-0.11552
6:	A	2.4630	503.40	1.2985	277 -> 281	-0.47667
					278 -> 282	0.49272
7:	A	2.5095	494.05	0.4996	277 -> 281	0.49102
					278 -> 282	0.47305
8:	A	2.5642	483.51	0.0768	277 -> 282	0.69829
9:	A	2.8578	433.85	0.1307	276 -> 281	0.66196
					280 -> 283	0.10836
					280 -> 284	0.12321
10:	A	2.9842	415.47	0.0336	276 -> 281	-0.10986
					276 -> 282	-0.13943
					280 -> 283	0.63176
					280 -> 284	-0.17330
					280 -> 285	-0.11306

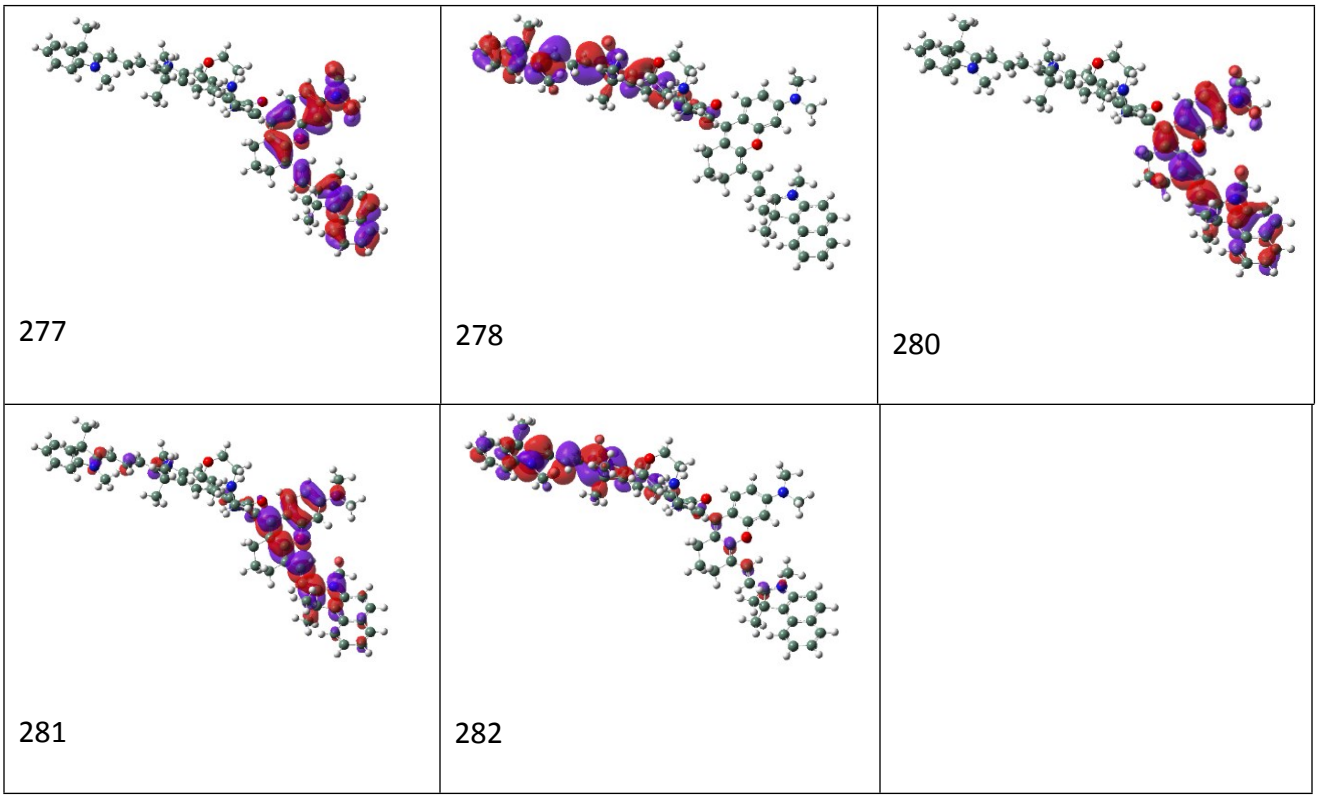


Figure S39. Drawings of selected molecular orbitals listed in Table S12.

8. Photostability of the fluorescent probes A and B

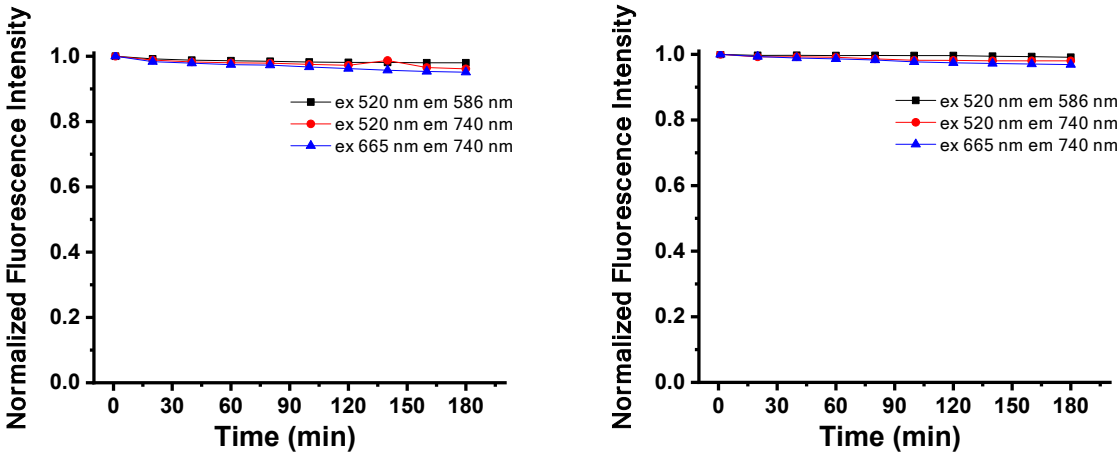


Figure S40. Fluorescence intensity of cyanine and hemicyanine moiety of 5 µM probes **A** under donor excitation (520 nm) and acceptor excitation (665 nm) in pH 2.0 (left) and 7.6 (right) citrate-phosphate buffer containing 10% ethanol versus excitation time.

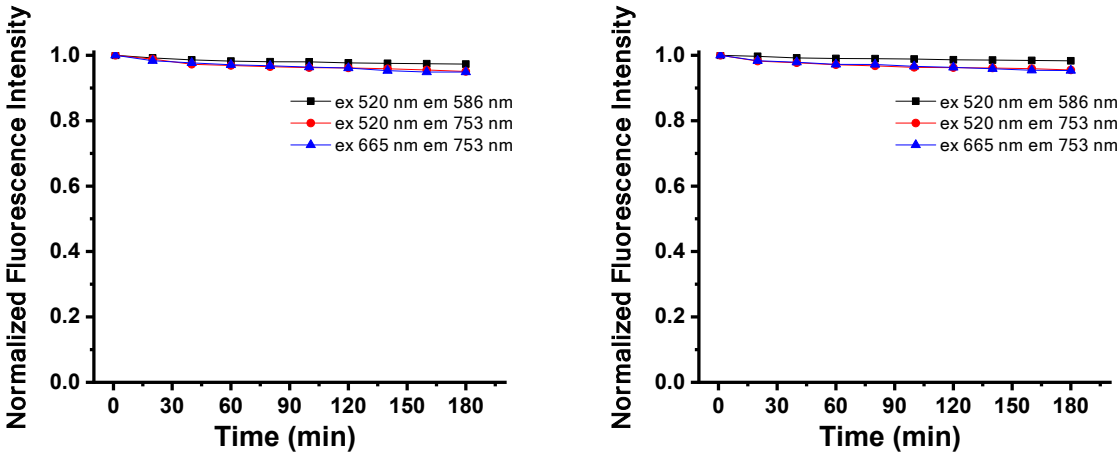


Figure S41. Fluorescence intensity of cyanine and hemicyanine moiety of 5 µM probes **B** under donor excitation (520 nm) and acceptor excitation (680 nm) in pH 2.0 (left) and 7.6 (right) citrate-phosphate buffer containing 10% ethanol versus excitation time.

9. Reversibility fluorescence responses of the fluorescent probes A and B

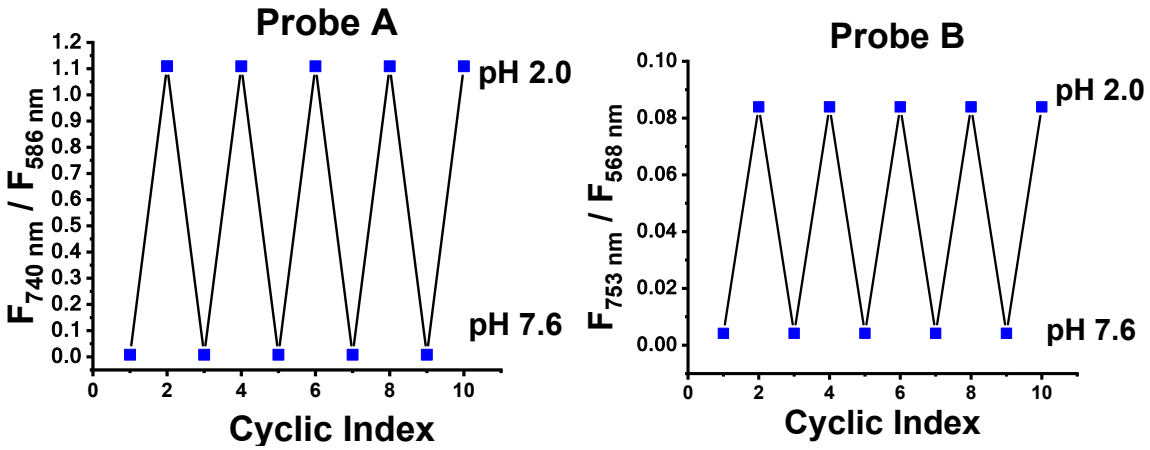


Figure S42. Fluorescence intensity ratio of cyanine and hemicyanine moiety of 5 μM probes **A** and **B** under donor excitation (520 nm) with pH changes in citrate-phosphate buffers.

10. Selectivity of fluorescent probes A and B

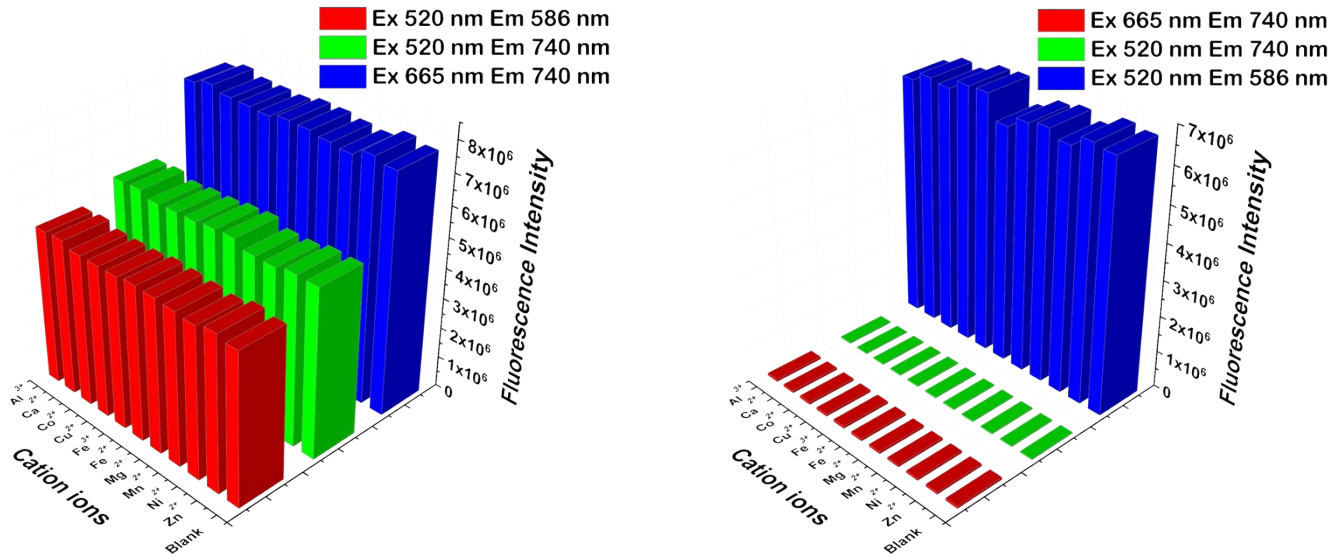


Figure S43. Fluorescence intensity of the cyanine and hemicyanine moieties of 5 μM probes **A** in the absence and presence of 100 μM of different cations under donor and acceptor excitation at pH 2.0 (left) or 7.6 (right) buffer containing 10% ethanol.

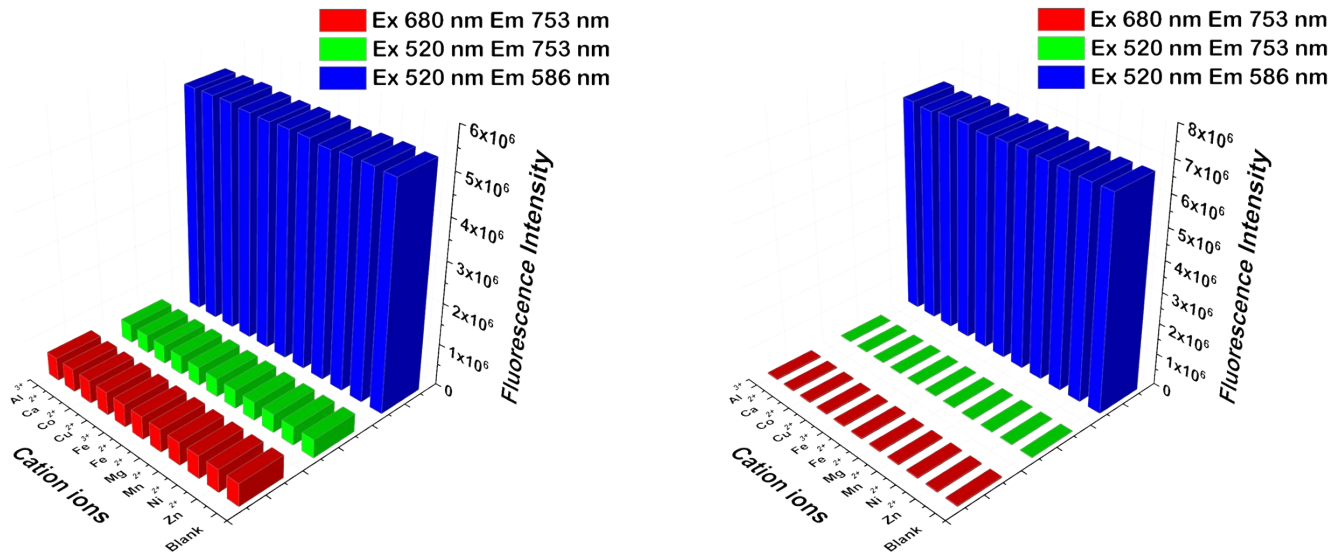


Figure S44. Fluorescence intensity of the cyanine and hemicyanine moieties of 5 µM probes **B** in the absence and presence of 100 µM of different cations under donor and acceptor excitation at pH 2.0 (left) or 7.6 (right) buffer containing 10% ethanol.

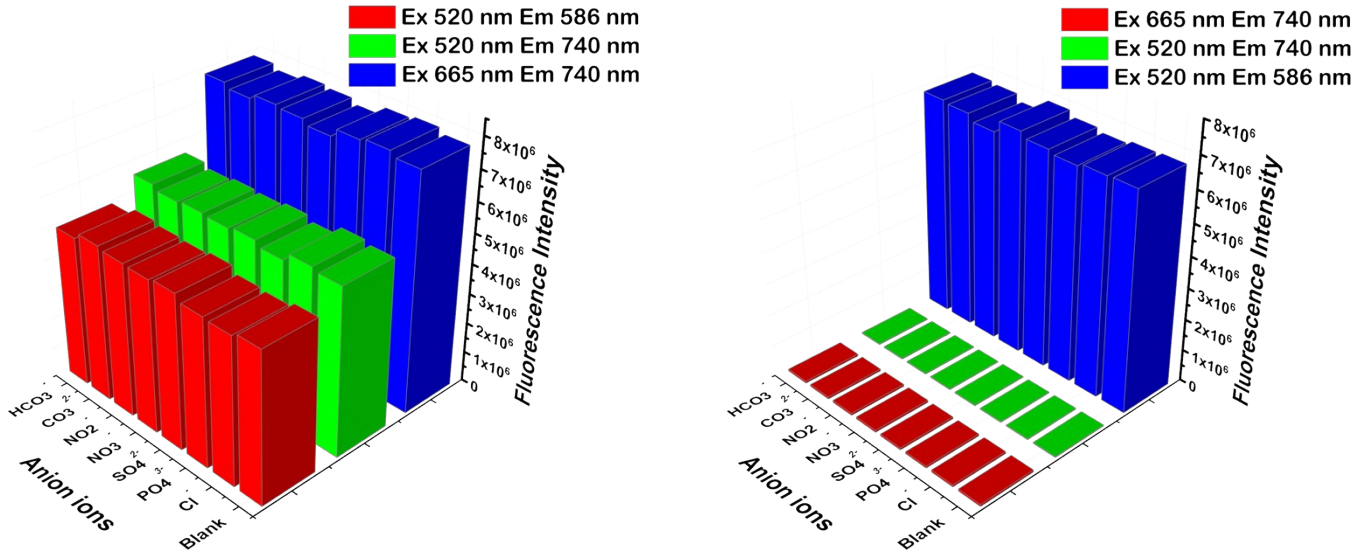


Figure S45. Fluorescence intensity of the cyanine and hemicyanine moieties of 5 µM probes **A** in the absence and presence of 100 µM of different anions under donor and acceptor excitation at pH 2.0 (left) or 7.6 (right) buffer containing 10% ethanol.

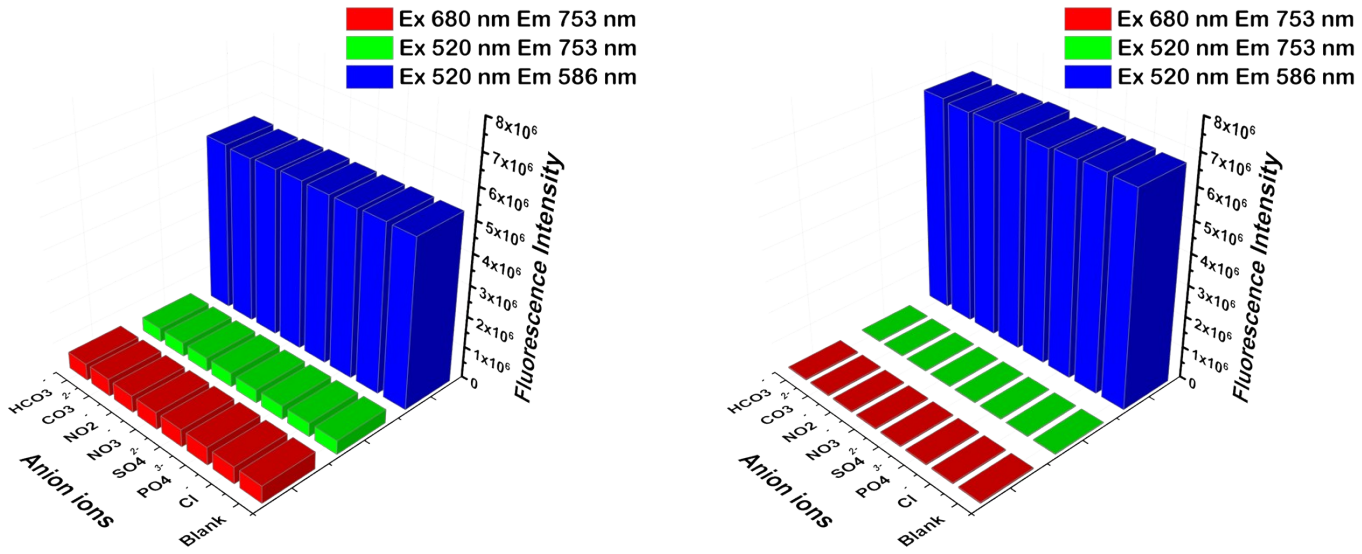


Figure S46. Fluorescence intensity of the cyanine and hemicyanine moieties of 5 μM probes **B** in the absence and presence of 100 μM of different anions under donor and acceptor excitation at pH 2.0 (left) or 7.6 (right) buffer containing 10% ethanol.

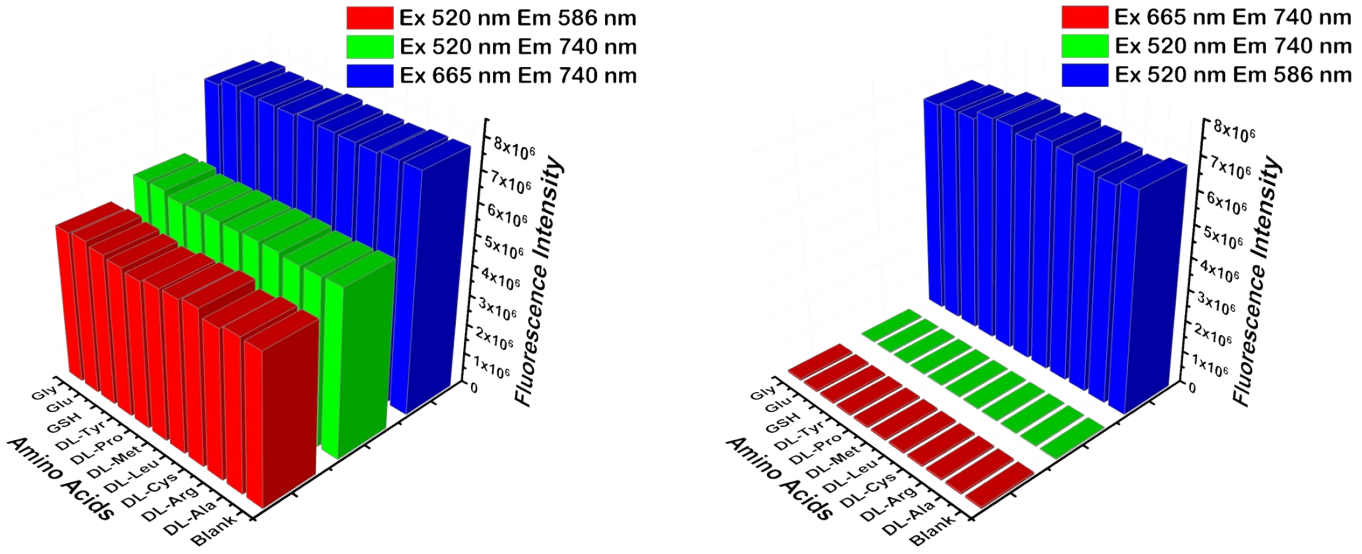


Figure S47. Fluorescence intensity of the cyanine and hemicyanine moieties of 5 μM probes **A** in the absence and presence of 100 μM of different amino acids under donor and acceptor excitation at pH 2.0 (left) or 7.6 (right) buffer containing 10% ethanol.

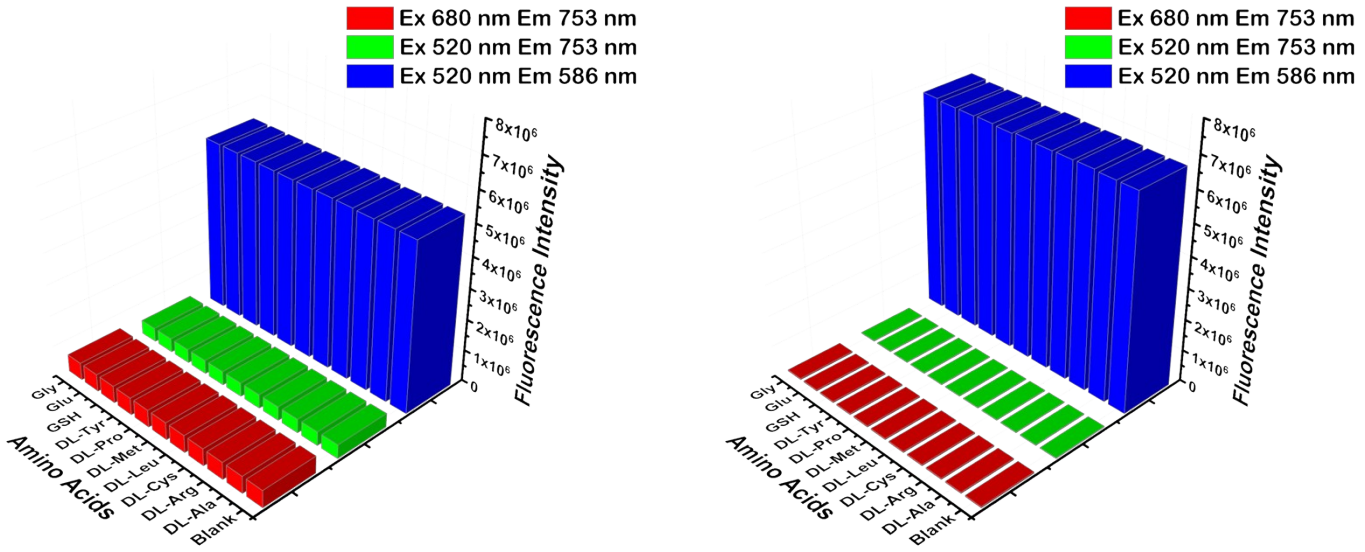
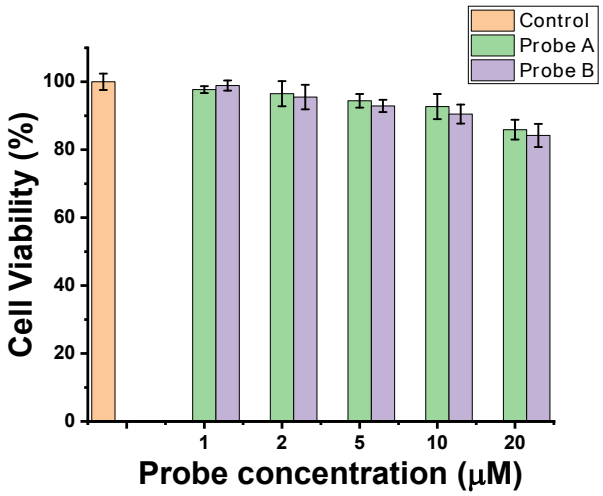


Figure S48. Fluorescence intensity of the cyanine and hemicyanine moieties of 5 μM probes **B** in the absence and presence of 100 μM of different amino acids under donor and acceptor excitation at pH 2.0 (left) or 7.6 (right) buffer containing 10% ethanol.

11. MTT assay



12.

Figure S49. MTT assays were conducted to determine the cytotoxicity of probes **A** and **B** by incubating HeLa cells with 1, 2, 5, 10 or 20 μM of probe **A** or **B** for 24 h. The cell viability was determined by the absorbance obtained at 490 nm and with n = 3 replicates.

12. Fluorescence images of HeLa cells

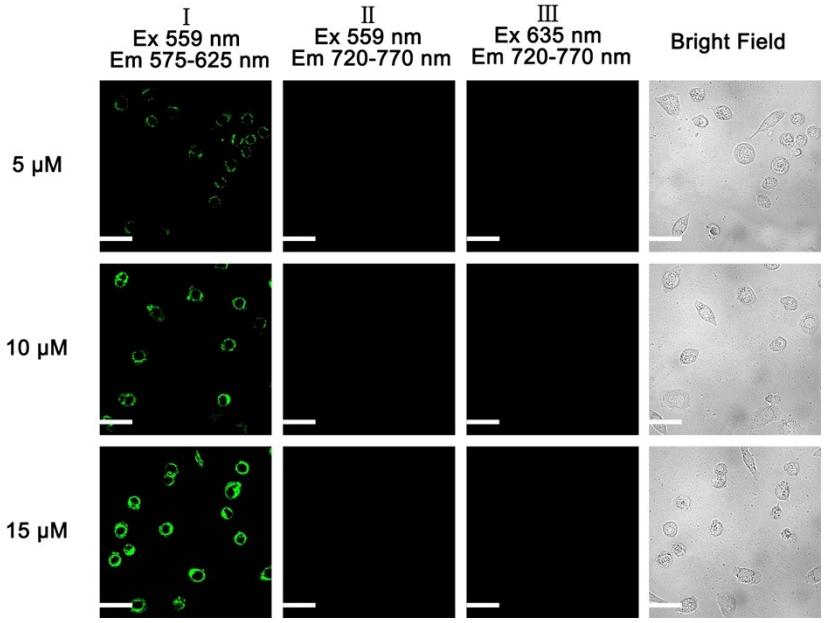


Figure S50. Cellular fluorescence images of HeLa cells incubated with different concentrations of probe **A** for 30 min in normal medium. Green channel, emission collected from 575 nm to 625 nm under donor excitation at 559 nm; NIR channels, emission collected from 720 nm to 770 nm under donor excitation at 559 nm and acceptor excitation at 635 nm, respectively. Scale bar: 50 μm.

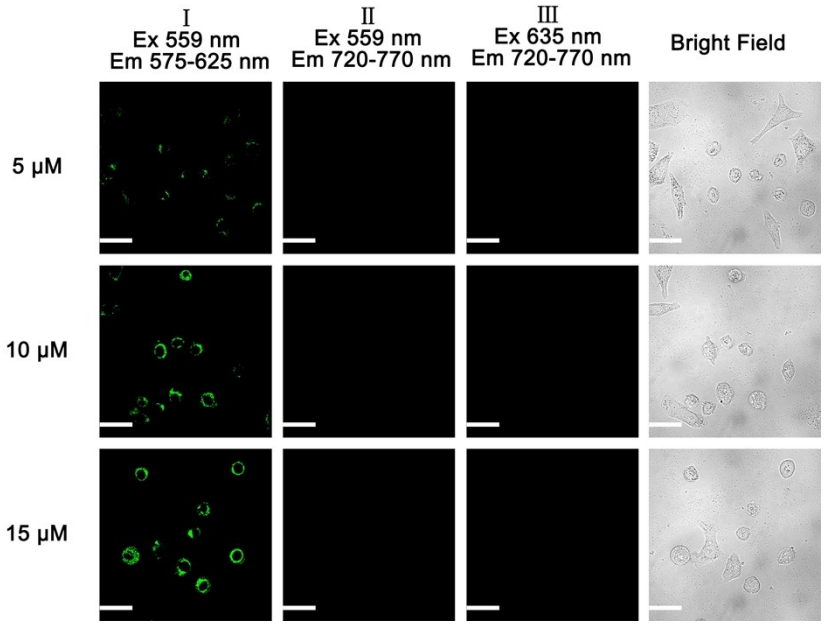


Figure S51. Cellular fluorescence images of HeLa cells incubated with different concentrations of probe **B** for 30 min in normal medium. Green channel, emission collected from 575 nm to 625 nm under donor excitation at 559 nm; NIR channels, emission collected from 720 nm to 770 nm under donor excitation at 559 nm and acceptor excitation at 635 nm, respectively. Scale bar: 50 μm.

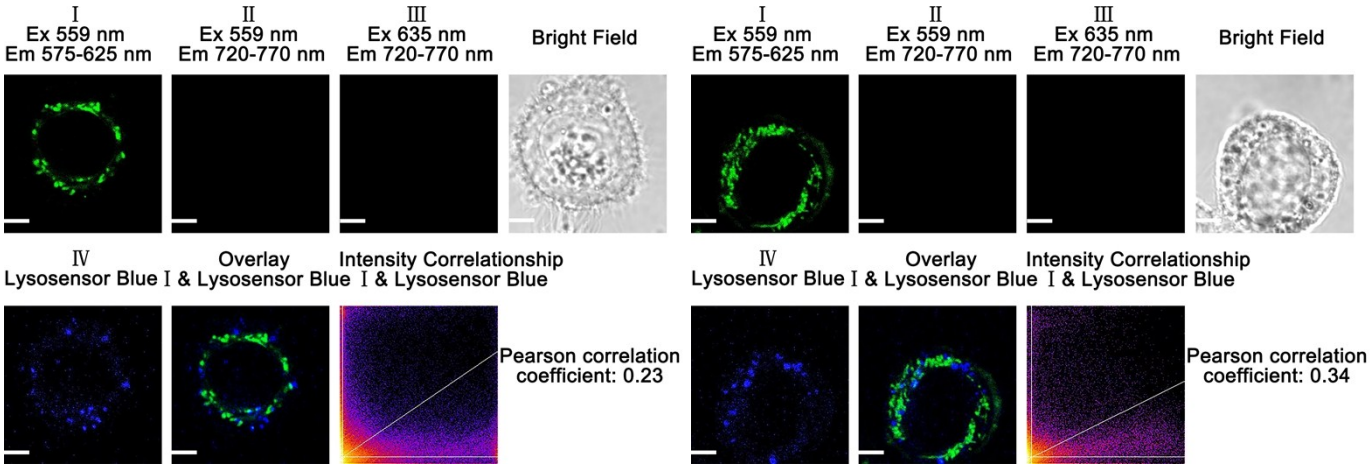


Figure S52. Cellular fluorescence images of HeLa cells incubated with 10 μM of of either probe **A** (left) or **B** (right) and Lysosensor blue after 30 min in normal medium. Scale bar: 10 μm.

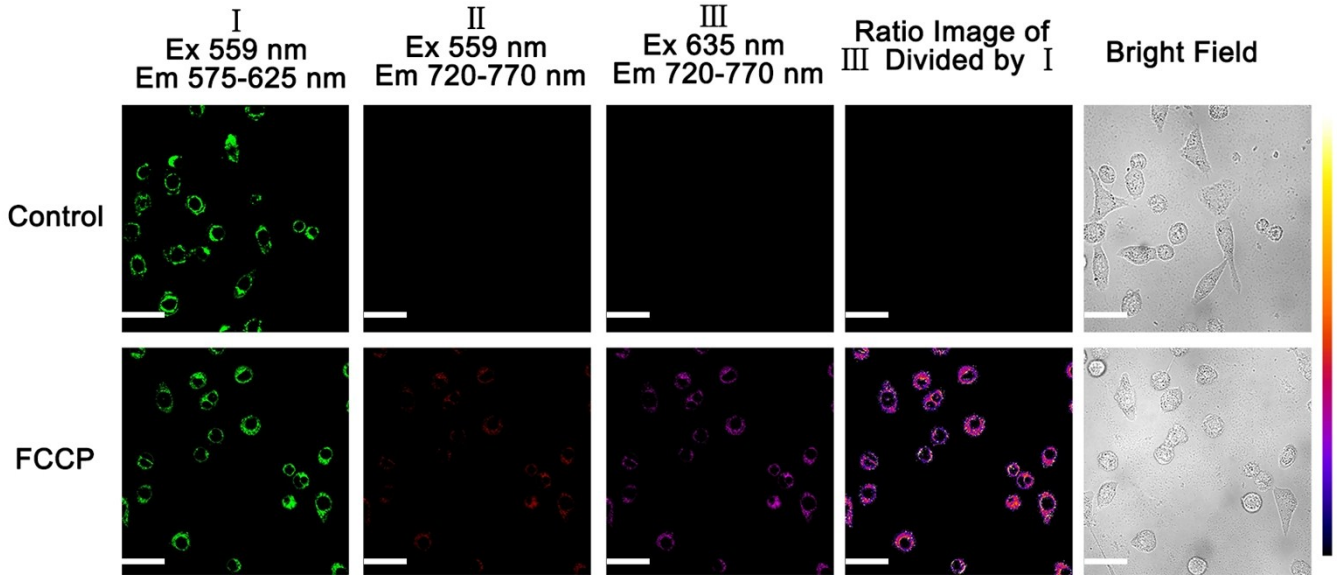


Figure S53. Ratiometric fluorescence imaging of HeLa cells with 7.5 μM probe **A** in normal medium after a 20-min incubation before and after FCCP treatment. The ratio images were obtained by dividing the near-infrared fluorescence in the third channel by the visible fluorescence in the first channel. Scale bar: 50 μm.

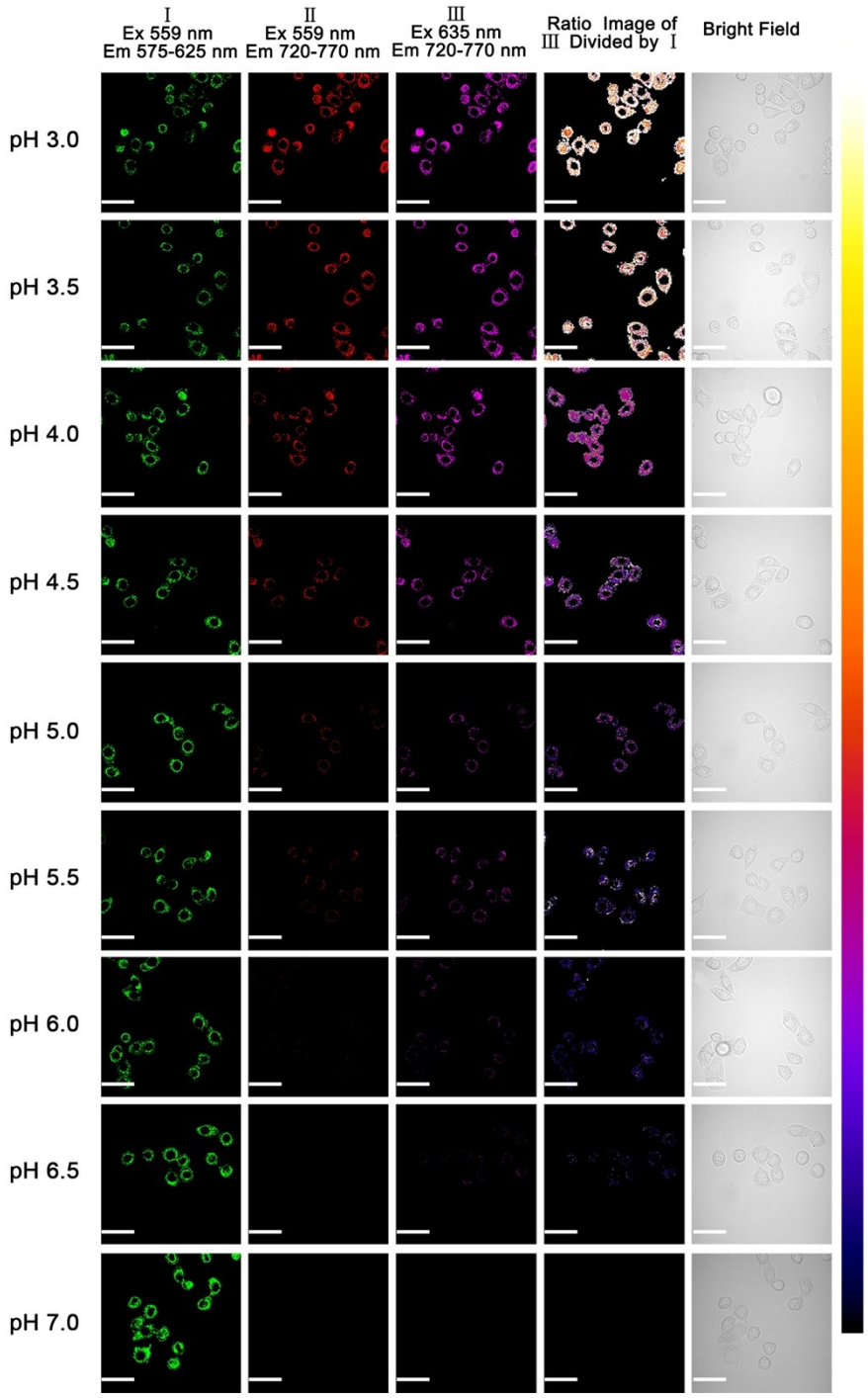


Figure S54. Ratiometric cellular fluorescence images of HeLa cells incubated with 10 μM probe **A** in different pH buffers containing 10 μg/mL nigericin. Green channel, emission collected from 575 nm to 625 nm under donor excitation at 559 nm; NIR channels, emission collected from 720 nm to 770 nm under donor excitation at 559 nm and acceptor excitation at 635 nm, respectively. Scale bar: 50 μm. The ratio images were obtained by dividing the near-infrared fluorescence in the third channel by the visible fluorescence in the first channel.

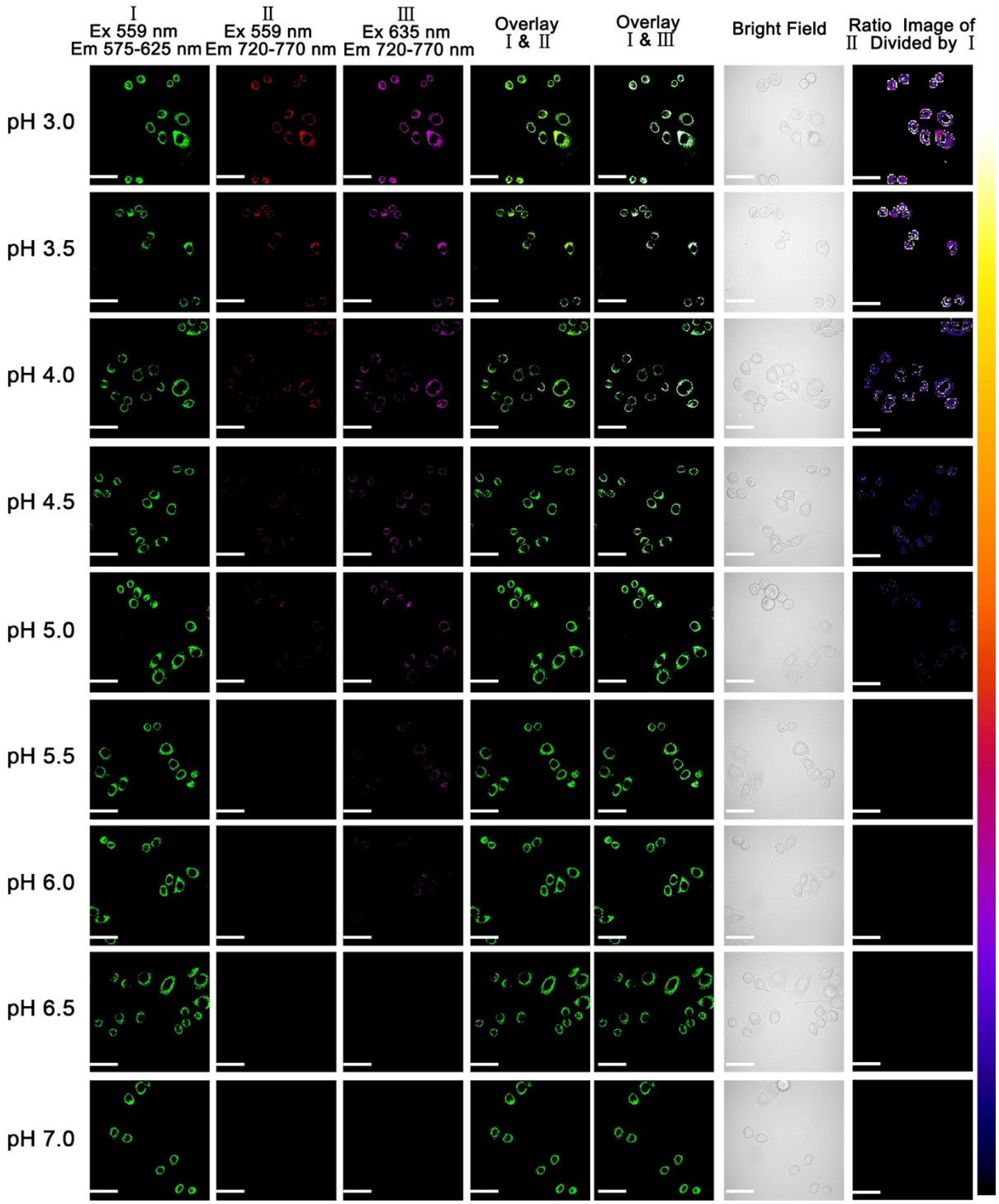


Figure S55. Cellular fluorescence images of HeLa cells incubated with 10 μM probe **B** in different pH buffers containing 10 μg/mL nigericin. Green channel, emission collected from 575 nm to 625 nm under donor excitation at 559 nm; NIR channels, emission collected from 720 nm to 770 nm under donor excitation at 559 nm and acceptor excitation at 635 nm, respectively. Scale bar: 50 μm. The ratio images were obtained by dividing the near-infrared fluorescence in the second channel by the visible fluorescence in the first channel.

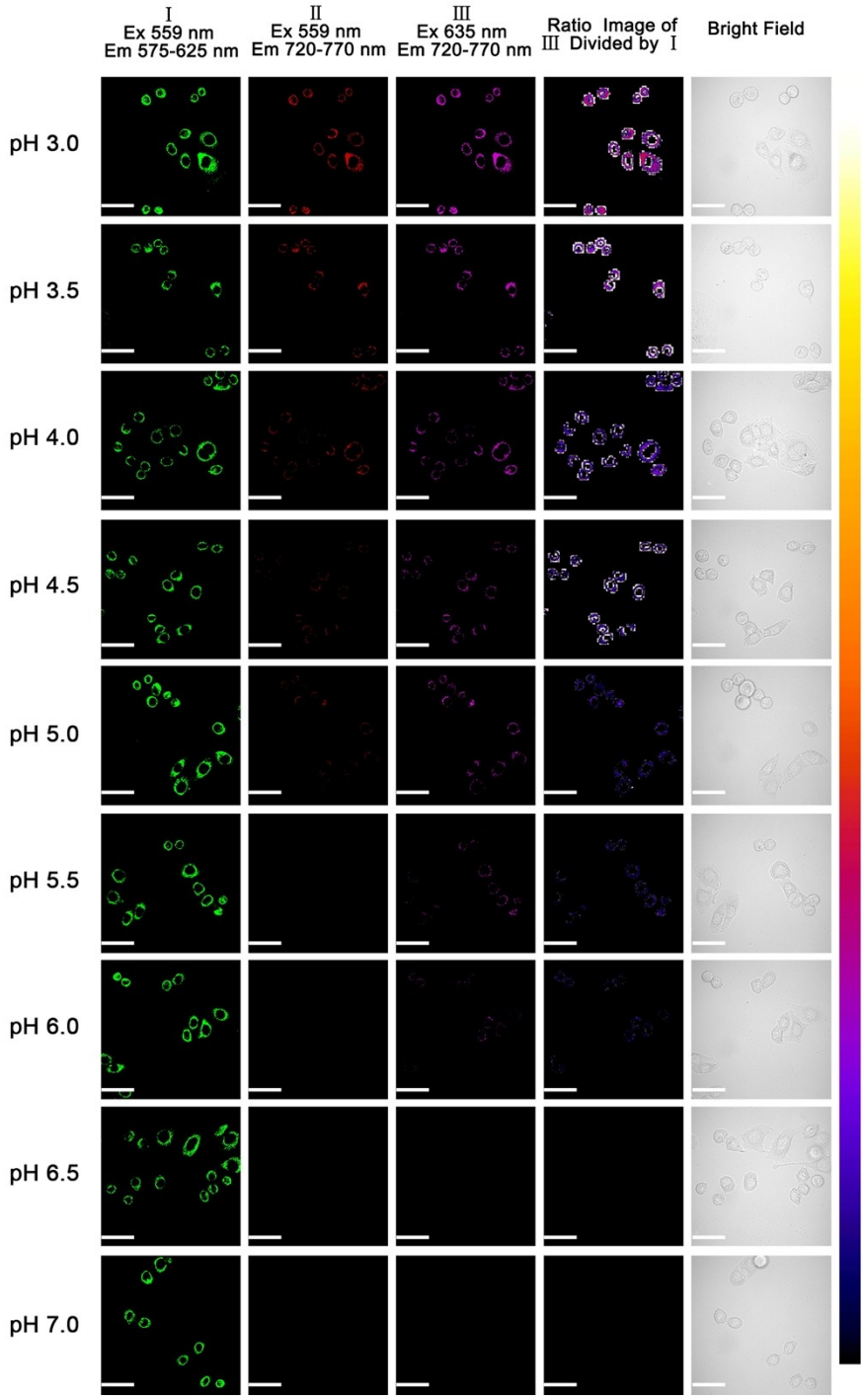


Figure S56. Ratiometric cellular fluorescence images of HeLa cells incubated with 10 μM probe **B** in different pH buffers containing 10 μg/mL nigericin. Green channel, emission collected from 575 nm to 625 nm under donor excitation at 559 nm; NIR channels, emission collected from 720 nm to 770 nm under donor excitation at 559 nm and acceptor excitation at 635 nm, respectively. Scale bar: 50 μm. The ratio images were obtained by dividing the near-infrared fluorescence in the third channel by the visible fluorescence in the first channel.

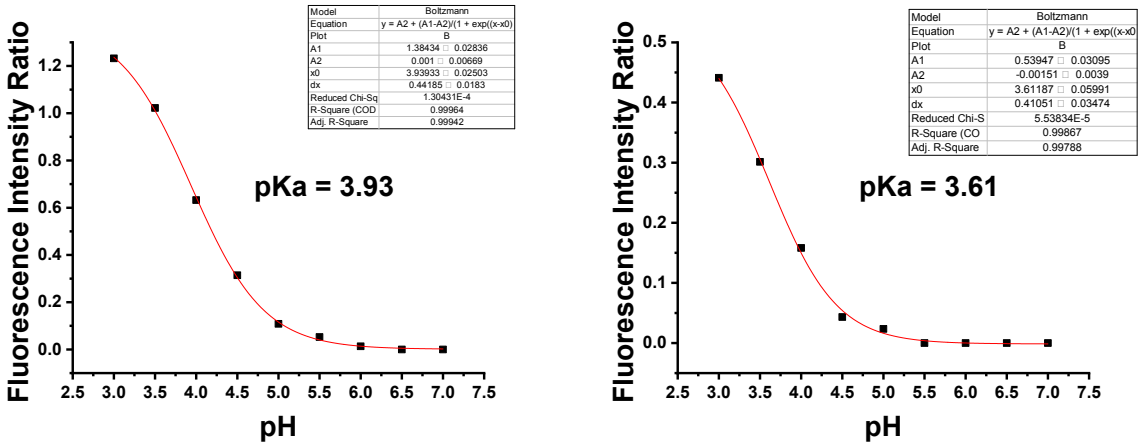


Figure S57. Statistical analysis of the confocal imaging data in Figure 9 (left) and Figure S53 (right) generated from the ratio of fluorescence intensities of Channel III divided by Channel I in live HeLa cells.

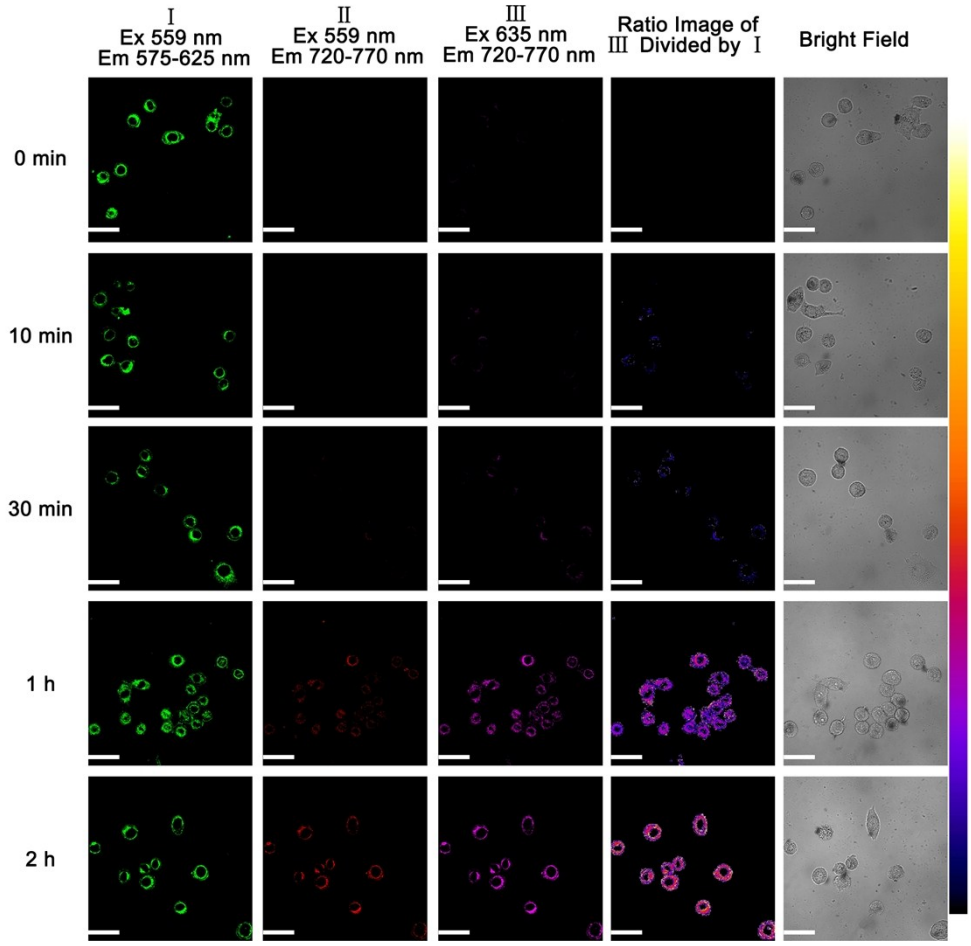


Figure S58. Ratiometric fluorescence imaging of HeLa cells with 10 μM probe **A** in serum-free medium under donor and acceptor excitation at various times with scale bars of 50 μm. The ratio images were obtained by dividing the near-infrared fluorescence in the third channel by the visible fluorescence in the first channel.

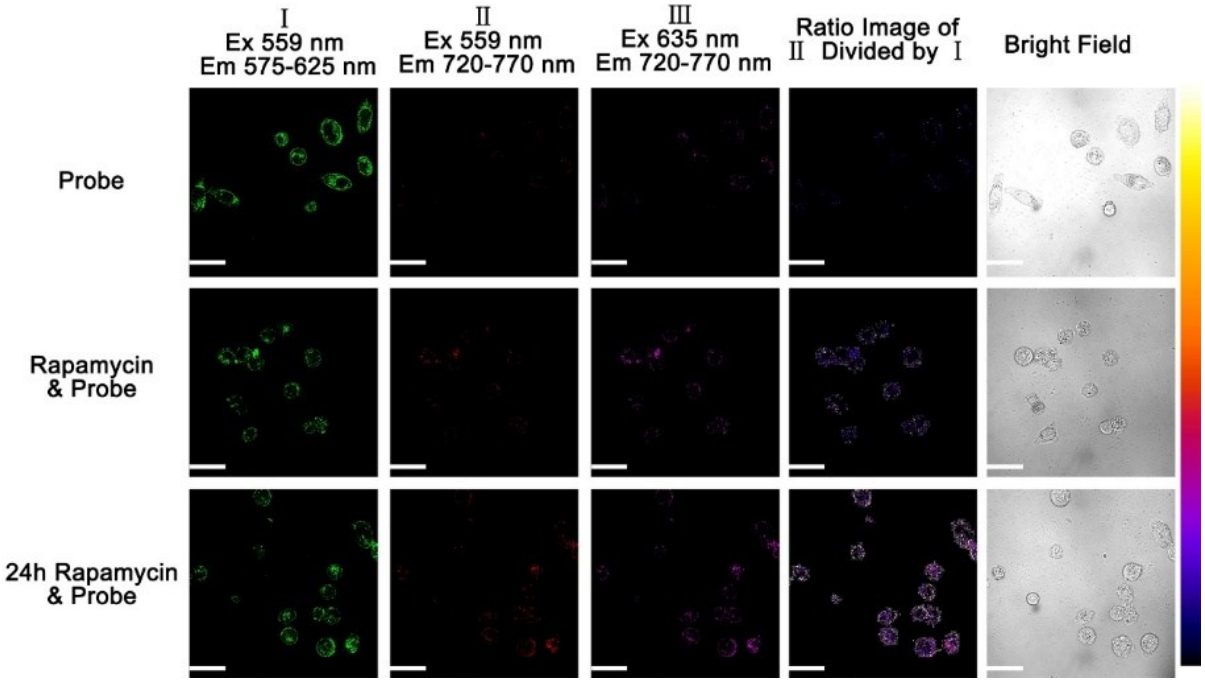


Figure S59. Ratiometric fluorescence images of HeLa cells incubated with 10 μM probe **A** and rapamycin (100 nM) in normal culture medium. The ratio images were obtained by dividing the near-infrared fluorescence in the second channel by the visible fluorescence in the first channel. The Scale bars are 50 μm.

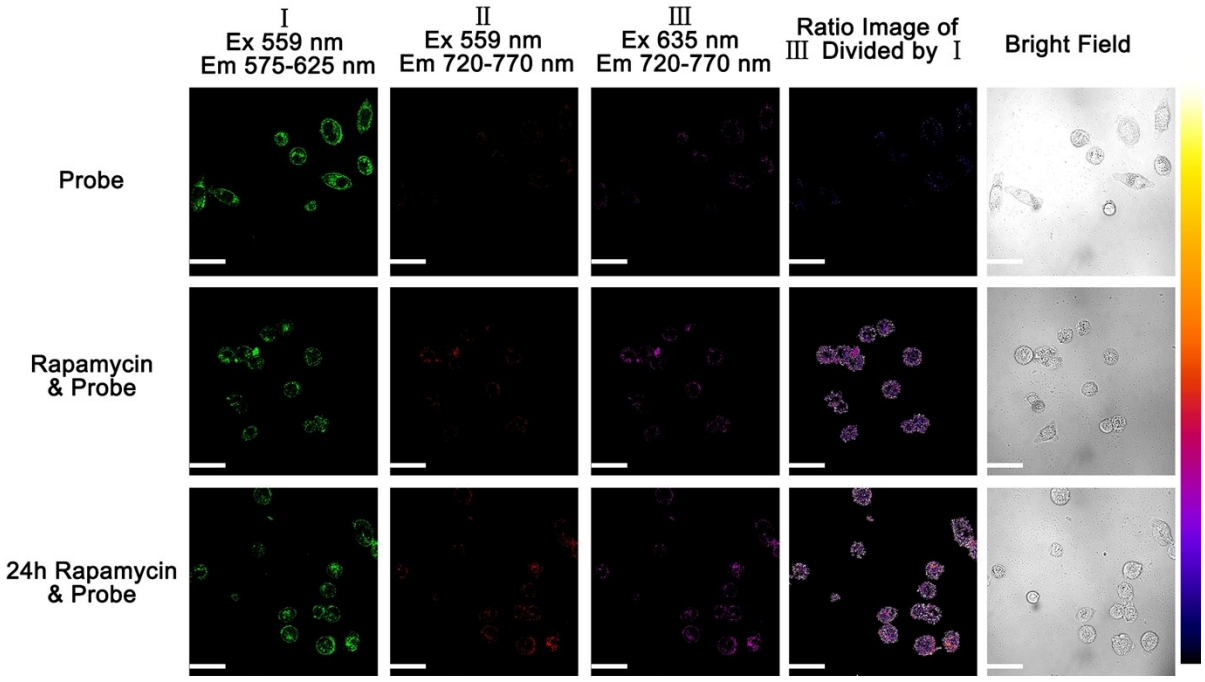


Figure S60. Ratiometric fluorescence images of HeLa cells incubated with 10 μM probe **A** and rapamycin (100 nM) in normal culture medium. The ratio images were obtained by dividing the near-infrared fluorescence in the third channel by the visible fluorescence in the first channel. The Scale bars are 50 μm.

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