Supporting Information

Naked-Eye and Near-Infrared Fluorescence Probe for Hydrazine and Its Applications in *In Vitro* and *In Vivo* Bioimaging

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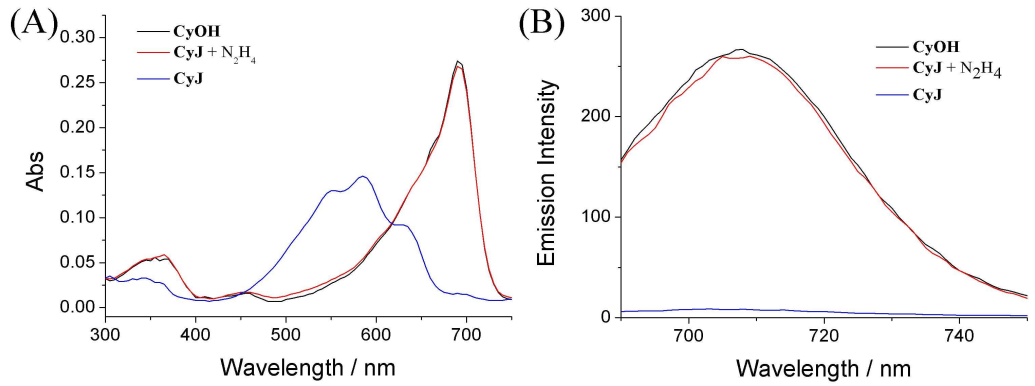


Figure S1. (A) Absorption and (B) fluorescence emission spectra of **CyOH** (5 μM, black line) and **CyJ** (5 μM)**,** before (blue line) and after reacting with N_2H_4 (150 μM, red line) in H_2O/DMSO solution (4: 1, v/v, 10 mM HEPES, pH = 7.4), λ_{ex} = 675 nm.

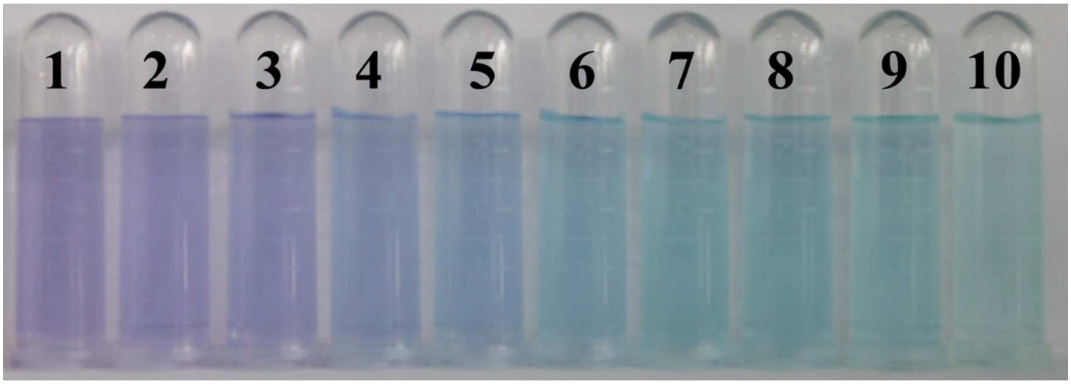


Figure S2. The color change of **CyJ** (5 μM) upon addition of N_2H_4 in HEPES (10 mM, pH = 7.4) containing 20% DMSO. Each picture was recorded at 10 min after the addition of N_2H_4 (**1-10** : 0, 0.5, 1.5, 2.0, 5.0, 15.0, 25.0, 50.0, 100.0, 250.0 μM).

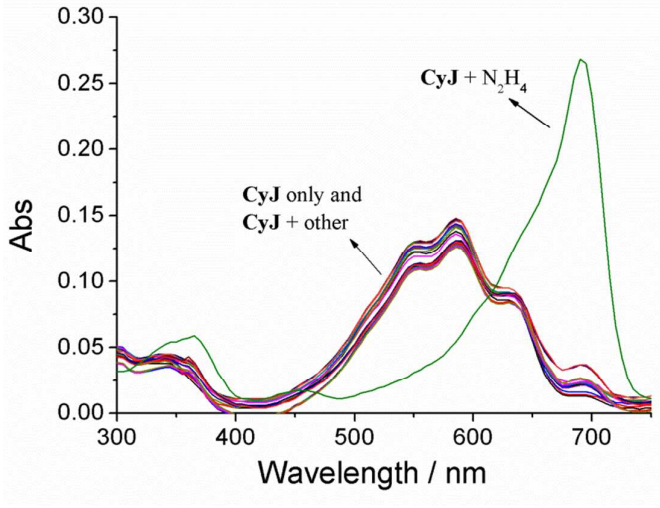


Figure S3. UV response of **CyJ** (5 μM) to various analytes (150 μM), each spectrum was recorded at 10 min after addition of the analytes (N_2H_4, Cys, GSH, Glu, NH_2OH, urea, aniline, thiourea, K^+, Na^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Fe^{3+}, Fe^{2+}, Hg^{2+}, F^-, Br^-, Cl^-, I^-, ClO_4^-, SO_4^{2-}, AcO^-, CO_3^{2-}, $H_2PO_4^-$, N_3^-, BF_4^-);

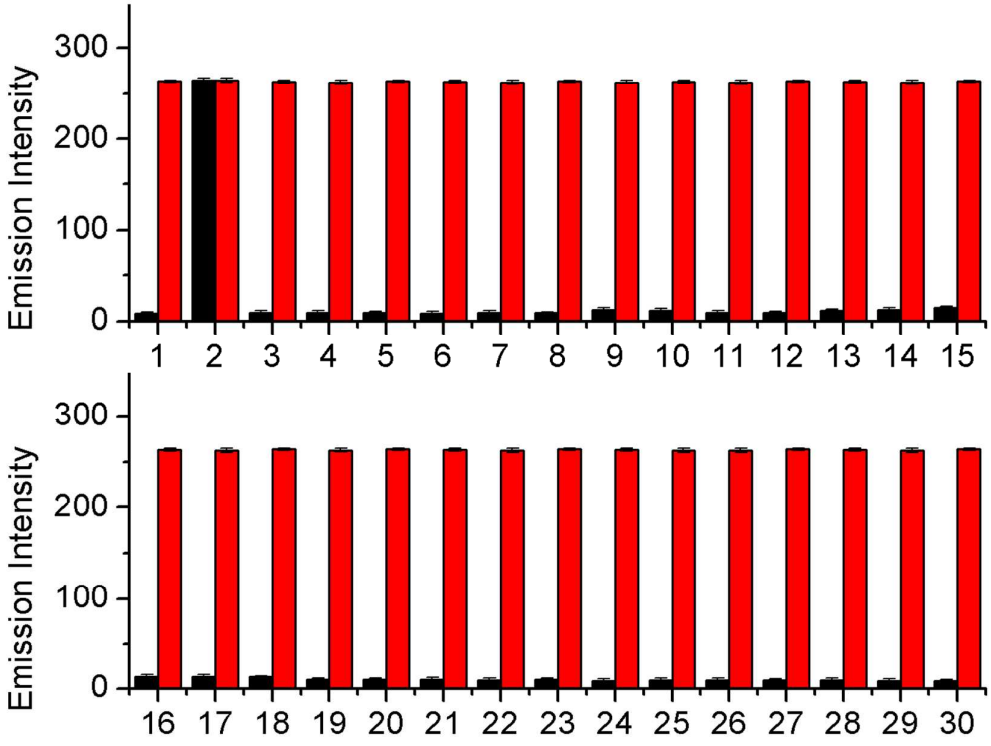


Figure S4. Fluorescence intensity of **CyJ** (5 μM) in the presence of various analytes (150 μM) (N_2H_4, Cys, GSH, Glu, NH_2OH, urea, aniline, thiourea, K^+, Na^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Fe^{3+}, Fe^{2+}, Hg^{2+}, F^-, Br^-, Cl^-, I^-, ClO_4^-, SO_4^{2-}, AcO^-, CO_3^{2-}, $H_2PO_4^-$, N_3^-, BF_4^-) in HEPES buffer (10 mM, pH = 7.40) containing 20% DMSO at 10 min, λ_{ex} = 675 nm. Black bars represent the addition of 30 equiv of the appropriate analytes (1 : Blank, 2 : N_2H_4, 3 : Cys, 4 : GSH, 5 : Glu, 6 :

NH_2OH, 7 : urea, 8 : aniline, 9 : thiourea, 10 : K^+, 11 : Na^+, 12 : Ca^{2+}, 13 : Mg^{2+}, 14 : Cu^{2+}, 15 : Zn^{2+}, 16 : Al^{3+}, 17 : Fe^{3+}, 18 : Fe^{2+}, 19 : Hg^{2+}, 20 : F^-, 21 : Br^-, 22: Cl^-, 23 : I^-, 24 : ClO_4^-, 25 : SO_4^{2-}, 26 : AcO^-, 27 : CO_3^{2-}, 28 : $H_2PO_4^-$, 29 : N_3^-, 30 : BF_4^-) to a 5 μM solution of **CyJ**. Red bars represent the addition of **CyJ** (5 μM) to the mixture solution of 150 μM the appropriate analytes and 150 μM N_2H_4. The data were reported as the mean ± standard deviation of triplicate experiments.

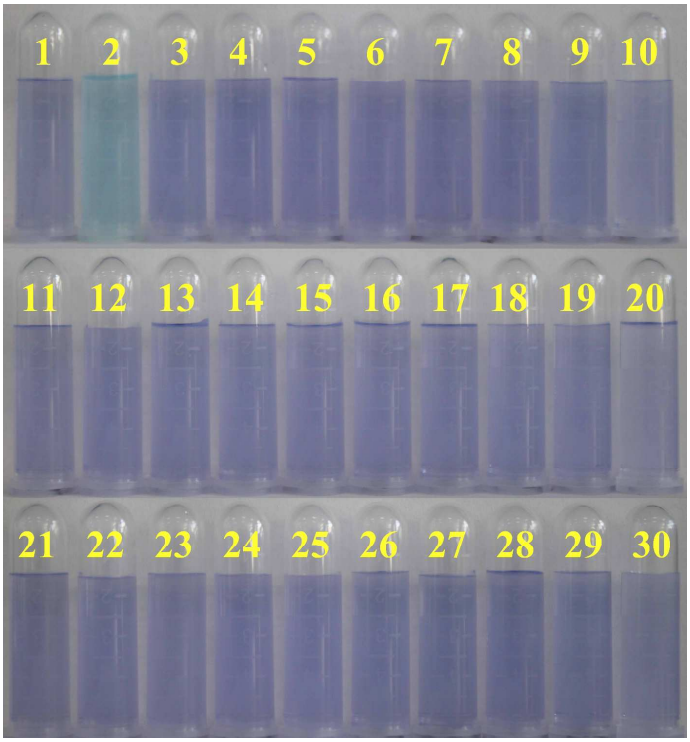


Figure S5. The color change of **CyJ** (5 μM) with various analytes (150 μM). The pictures were recorded at 10 min after addition of the analytes (1 : Blank, 2 : N_2H_4, 3 : Cys, 4 : GSH, 5 : Glu, 6 : NH_2OH, 7 : urea, 8 : aniline, 9 : thiourea, 10 : K^+, 11 : Na^+, 12 : Ca^{2+}, 13 : Mg^{2+}, 14 : Cu^{2+}, 15 : Zn^{2+}, 16 : Al^{3+}, 17 : Fe^{3+}, 18 : Fe^{2+}, 19 : Hg^{2+}, 20 : F^-, 21 : Br^-, 22: Cl^-, 23 : I^-, 24 : ClO_4^-, 25 : SO_4^{2-}, 26 : AcO^-, 27 : CO_3^{2-}, 28 : $H_2PO_4^-$, 29 : N_3^-, 30 : BF_4^-).

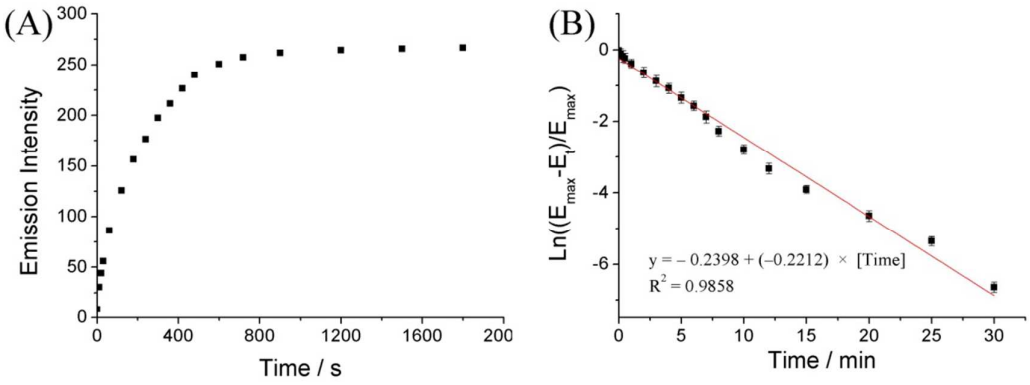


Figure S6. (A) Time-dependent fluorescence changes of **CyJ** (5 μM) upon addition of N_2H_4 (150 μM) in HEPES (10 mM, pH 7.4) containing 20% DMSO. λ_{ex} = 675 nm, λ_{em} = 706 nm. (B) Pseudo first-order kinetic plot of reaction of probe **CyJ** (5 μM) with N_2H_4 (150 μM), for k = 24.6 $M^{-1}s^{-1}$.

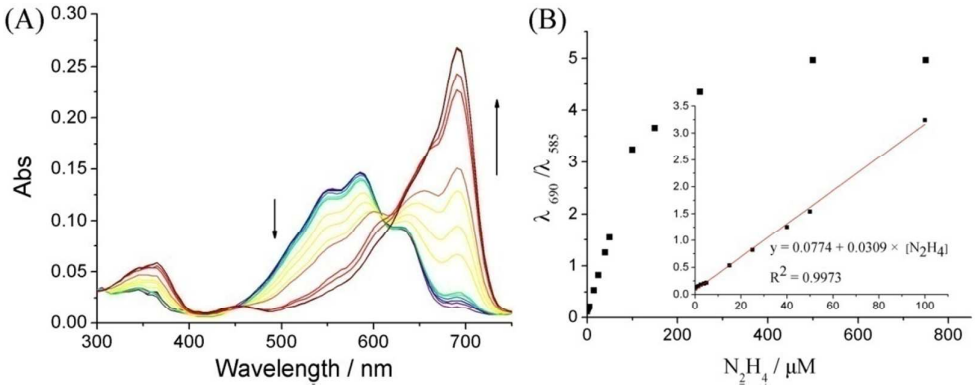


Figure S7. (A) UV titration of **CyJ** (5 μM) upon addition of N_2H_4 in HEPES (10 mM, pH = 7.4) containing 20% DMSO. Each spectrum was recorded at 10 min after the addition of N_2H_4 (0, 0.5, 1.5, 2.5, 4, 5, 15, 25, 40, 50, 100, 150, 250, 500, 750 μM). (B) Absorbance ratio R ($\lambda_{690}/\lambda_{585}$) changes of **CyJ** upon addition of N_2H_4. Inset: Absorbance ratio R ($\lambda_{690}/\lambda_{585}$) changes of **CyJ** as a function of concentrations of N_2H_4 (0–100 μM).

HPLC was carried out using a C18 column (Hedera·ODS-2, 5 μm, 250 mm × 4.6 mm) with a Varian 210 HPLC system. The mobile phase was a mixture of acetonitrile and ammonium acetate buffer (0.025 *M,* pH = 6.0) (8:2, v/v). The flow rate was 1 mL/min and detection at 613 nm. The reaction solutions of **CyJ** (5 μM) and N_2H_4 (25 μM, 150 μM) in HEPES buffer (10 mM, pH = 7.4) was as the samples.

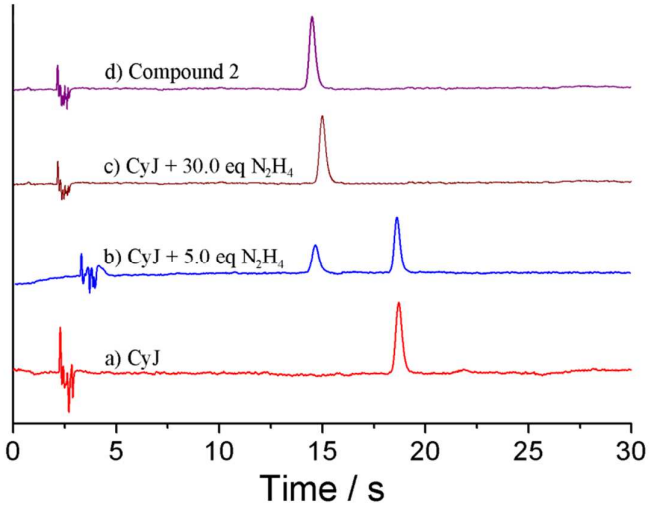


Figure S8. HPLC chromatograms of **CyJ** (a), the reaction solutions of **CyJ** with different amount of N_2H_4 (b, c), and **CyOH** (d). Conditions: incubation for 10 min at room temperature in HEPES (10 mM, pH = 7.4) containing 20% DMSO.

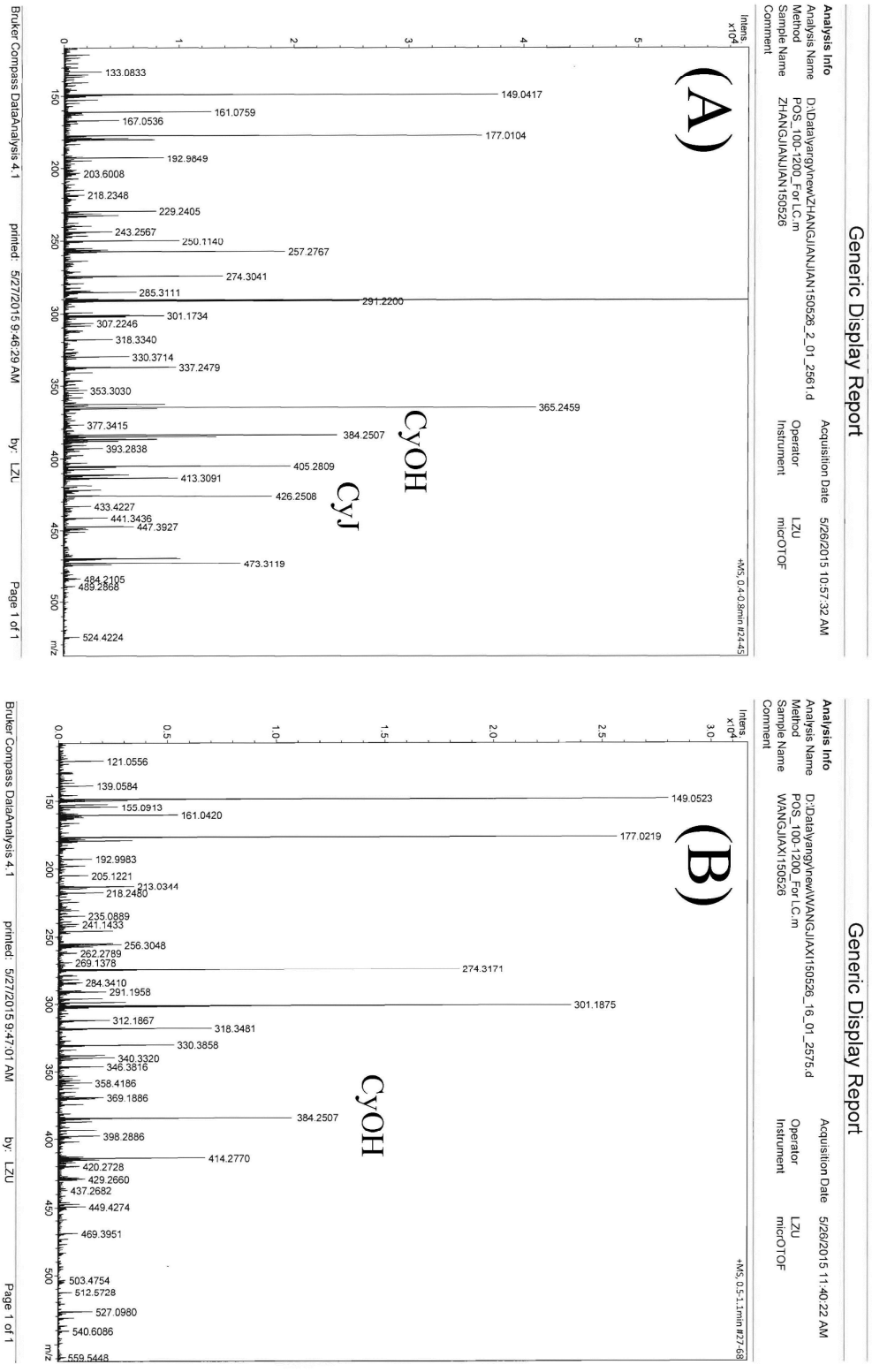


Figure S9. The micrOTOF mass spectra of **CyJ** treating with N_2H_4. (A) **CyJ** + 5.0 equiv N_2H_4; (B) **CyJ** + 30.0 equiv N_2H_4.

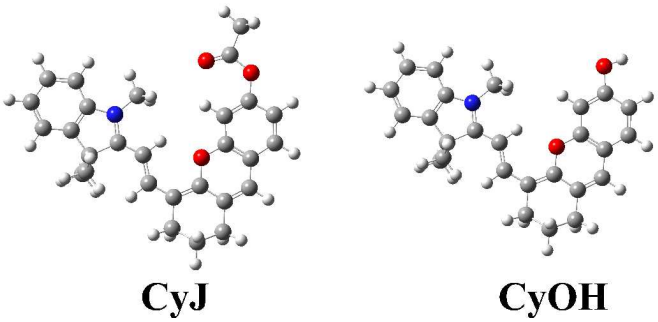


Figure S10. The optimized conformation of **CyJ** and **CyOH**. In the ball-and-stick model, carbon, oxygen and nitrogen atoms are colored in gray, red and blue, respectively.

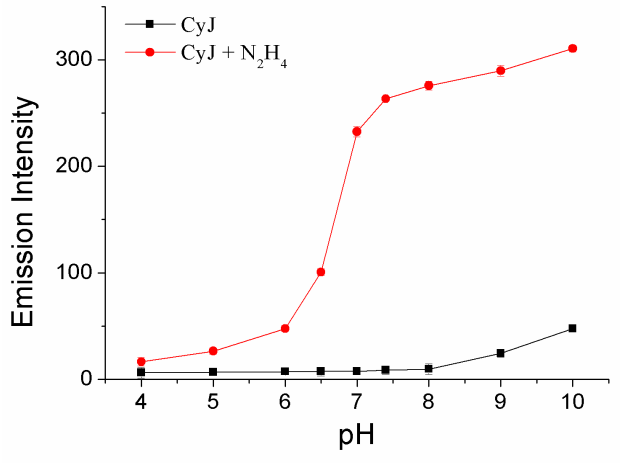


Figure S11. Fluorescence intensity of **CyJ** (■) and **CyJ** (5 μM) with N_2H_4 (150 μM, ●) in 10 min at various pH values, respectively. The data were reported as the mean ± standard deviation of triplicate experiments.

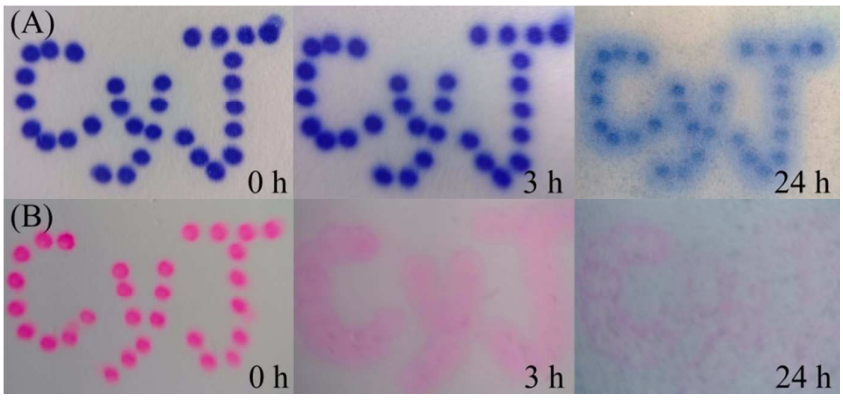


Figure S12. Diffusion-resistance experiments for **CyJ** (A) and Rhodamine **B** (B). The compounds were spotted on silica-gel plates, immersed in water.

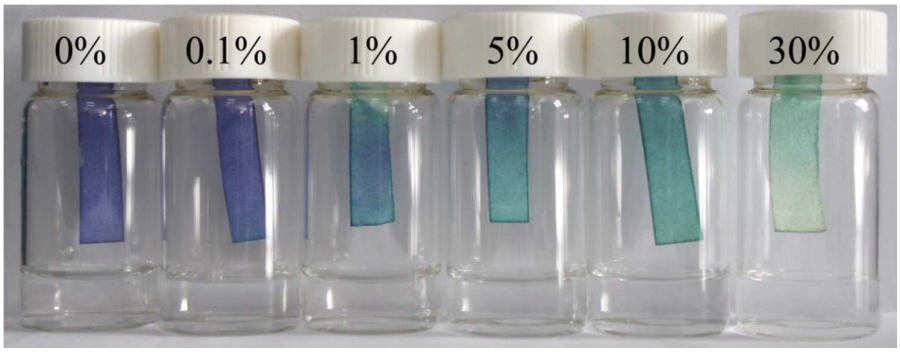


Figure S13. Color changes of **CyJ** (1.0 mM) coated filter paper after exposure to different concentrations of hydrazine aqueous solution.

Table S1. Determination of hydrazine in water samples [a]

Sample	Hydrazine added (μM)	Hydrazine found (μM)	Recovery (%)	R.S.D. (%)[b]
Tap water	5.0	5.21	104	1.7
	30.0	29.75	99	2.1
Yellow River water	5.0	5.24	105	2.2
	30.0	30.95	103	2.5
Diluted Human Serum (10%)	5.0	5.12	102	2.5
	30.0	31.80	106	2.1
Sample 1[c] (hydrazine 5.0 μM)	5.0	9.76	98	1.7
	30.0	35.89	103	1.7
Sample 2[c] (hydrazine 10.0 μM)	5.0	15.26	102	2.1
	30.0	41.98	105	2.4

[a] Conditions: $\lambda_{ex}/\lambda_{em}$ = 675/706 nm, DMSO/HEPES = 20:80, 10 min reaction at 25 ^{o}C.

[b] Each condition was measured three times.

[c] Synthetic samples containing other metal ions (common metal ions in serum: K^{+}, Na^{+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Fe^{3+}, Fe^{2+}) in HEPES buffer containing 20% DMSO.

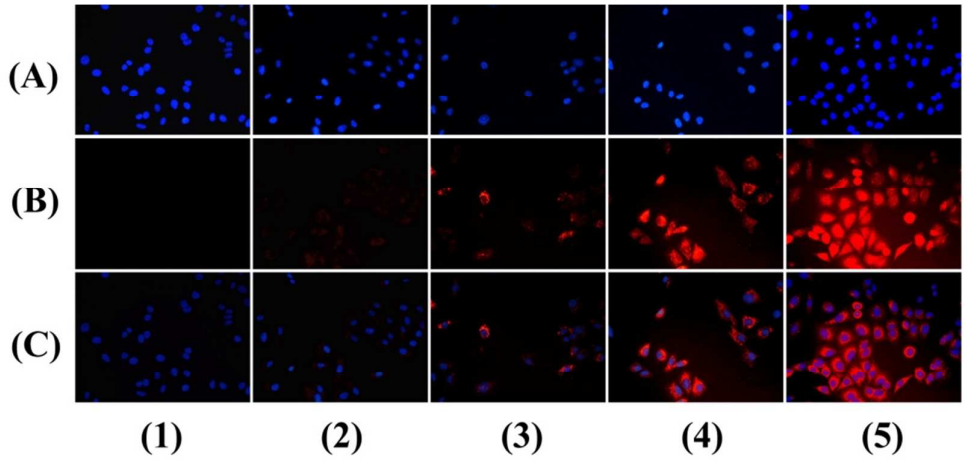


Figure S14. Confocal microscope images of pretreated *HeLa* cells with different concentration of N_2H_4 (**2-5**: 0.0, 5.0, 30.0, 150.0 μM) for 15 min, then incubated with **CyJ** (10 μM) for another 15 min; **1**: control. (A) Fluorescence images for cells co-stained with 4′, 6-diamidino-2-phenylindole (DAPI) to identify cell nuclei (blue dots); (B) Fluorescence images from **CyJ**; (C) Merge.

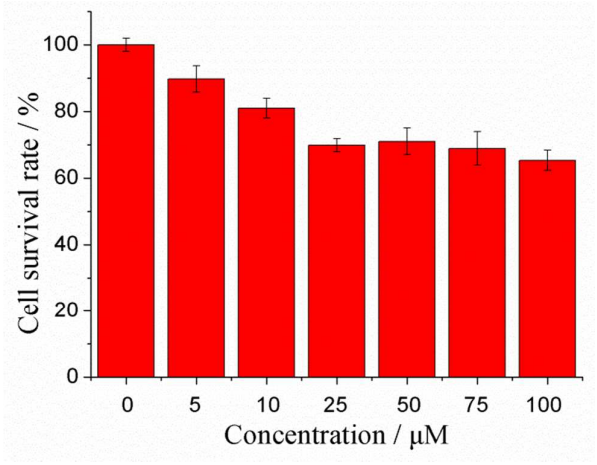


Figure S15. MTT assay for the survival rate of *HeLa* cells treated with various concentrations of **CyJ** for 24 h. Error bars represent the standard deviations of 6 trials.

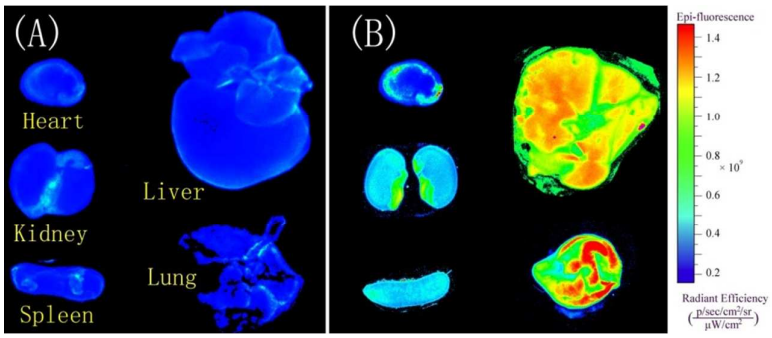
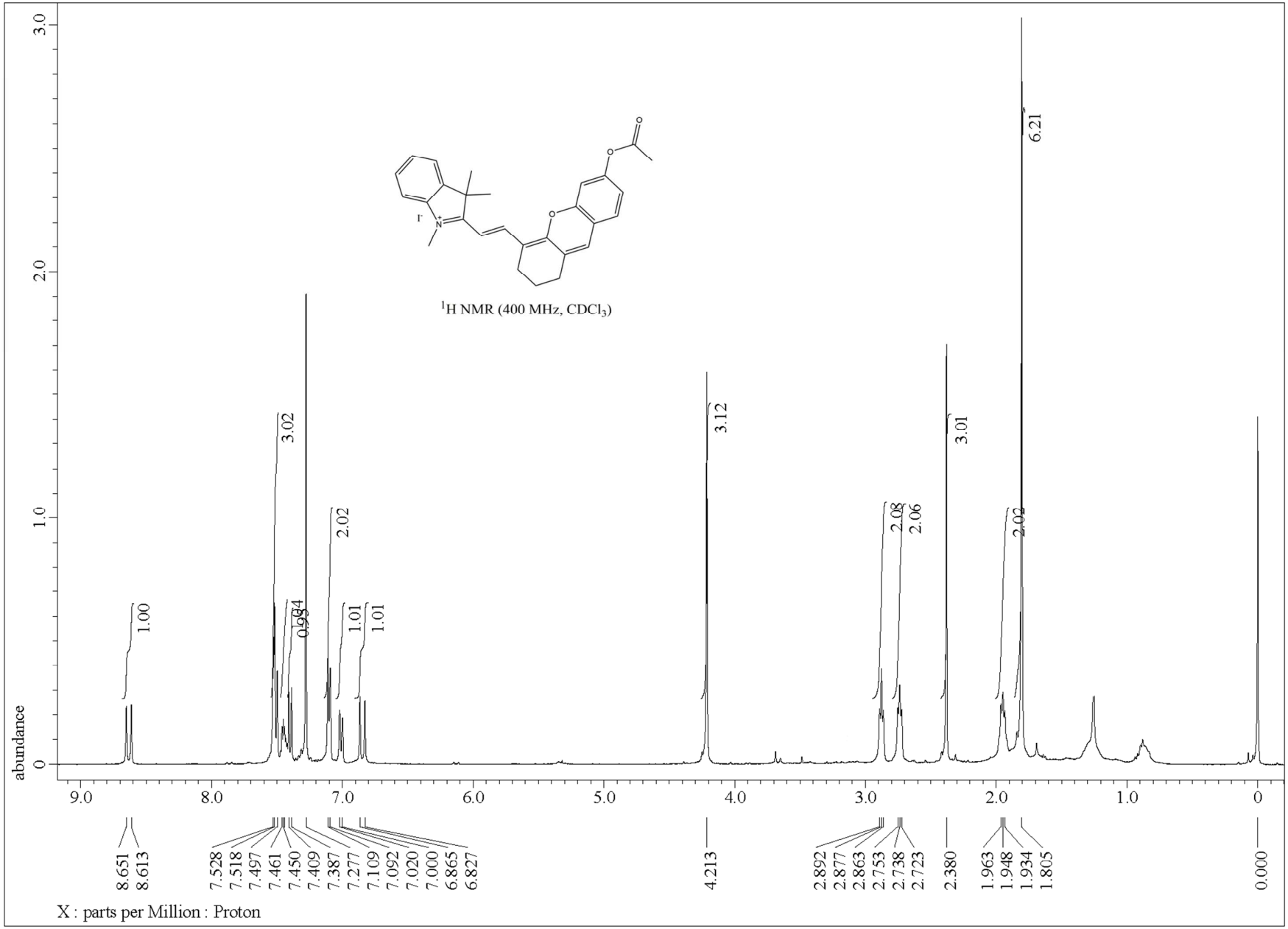


Figure S16. *In vivo* images of tissues from mouse incubated with **CyJ** (50 μM) or N_2H_4 (300 μM). Fluorescence images of heart, kidney, spleen, liver, and lung. (A) The tissues not incubated with anything; (B) The tissues directly treated with **CyJ** (50 μM) and followed by incubated with N_2H_4 (300 μM), each for 20 min.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0.93
2.02
1.01
1.01
3.12
2.08
2.06
3.01
2.02
6.21
8.651
8.613
7.528
7.518
7.497
7.461
7.450
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7.387
7.277
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6.865
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2.892
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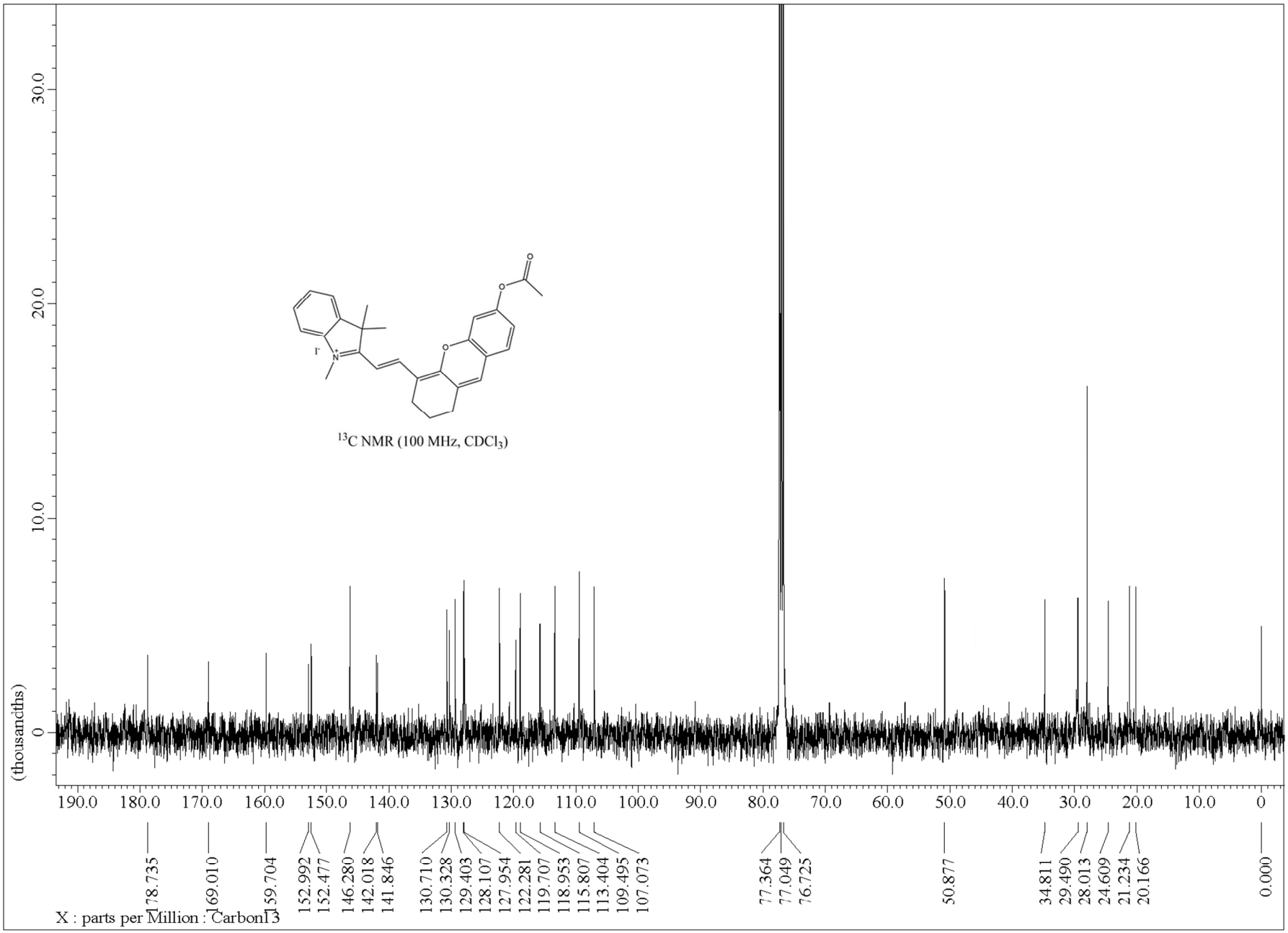
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(thousandths)
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10.0
0
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169.010
159.704
152.992
152.477
146.280
142.018
141.846
130.710
130.328
129.403
128.107
127.954
122.281
119.707
118.953
115.807
113.404
109.495
107.073
77.364
77.049
76.725
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28.013
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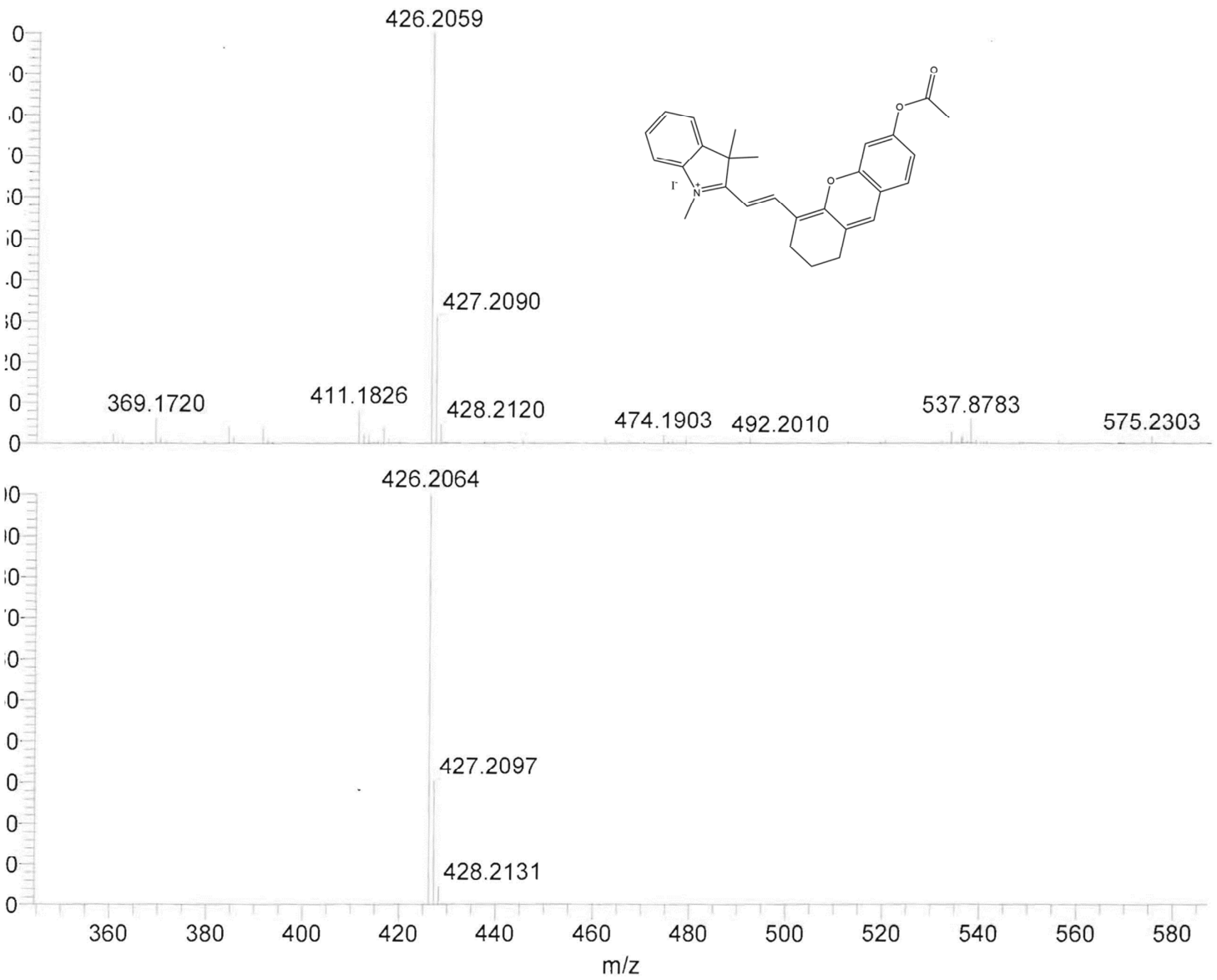

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