†Electronic Supporting Information

A ratiometric fluorescent and colorimetric probe for selective detection of hydrazine

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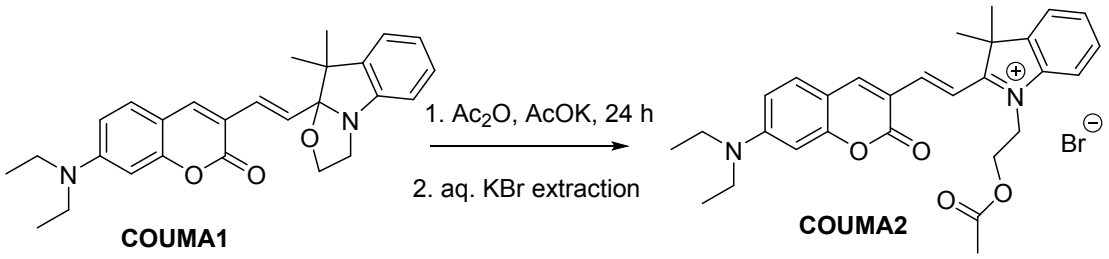
Content

Experimental – Preparation of COUMA2



(*E*)-1-(2-acetoxyethyl)-2-(2-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)vinyl)-3,3-dimethyl-3H-indol-1-ium bromide

A mixture of **COUMA1** (87 mg, 0.2 mmol)[1] and **AcOK** (100 mg, 1 mmol) in acetic anhydride (1 mL) was stirred at room temperature for 24 h under N_2 atmosphere. Then, the mixture was diluted with ethyl acetate (EA, 5 mL) and saturated potassium bromide solution (5 mL), organic layer was separated and aqueous layer was extracted (3 x 25 mL). The organic layers was combined, re-washed with saturated potassium bromide solution for three times and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (EA to EA/MeOH=10/1) to afford **COUMA 2** as a dark blue solid (90 mg, 82% yield). m.p.: > 250 °C decomposed.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.32 (1H, s), 8.62 (1H, d, *J* = 16.0 Hz), 8.21 (1H, d, *J* = 16.0 Hz), 8.08 (1H, d, *J* = 9.2 Hz), 7.50-7.42 (4H, m), 6.66 (1H, dd, *J* = 9.2 Hz, *J'* = 2.4 Hz), 6.45 (1H, d, *J* = 2.4 Hz), 5.28 (2H, t, *J* = 4.8 Hz), 4.79 (2H, t, *J* = 4.8 Hz), 3.51 (4H, q, *J* = 7.2 Hz), 1.81 (6H, s),), 1.73 (3H, s), 1.28 (6H, t, *J* = 7.2 Hz) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6, 170.5, 161.3, 159.0, 154.8, 151.2, 149.5, 142.9, 141.6, 134.9, 128.9, 128.4, 122.6, 113.3, 112.8, 111.4, 111.0, 109.1, 96.9, 61.9, 51.6,45.6, 30.9, 27.6, 20.4, 12.6 ppm.

HRMS (MALDI-TOF): m/z calcd for $C_{29}H_{33}N_2O_4^+$ [M^+] 473.2435, found, 473.2429.

Notes and references

1. Q. You, Y. M. Lee, W. H. Chan, N. K. Mak, A. W. M. Lee, S. C. K. Hau, T. C. W. Mak, "A colorimetric and ratiometric fluorescent pH probe based on ring opening/closing approa change",*RSC Advances*,

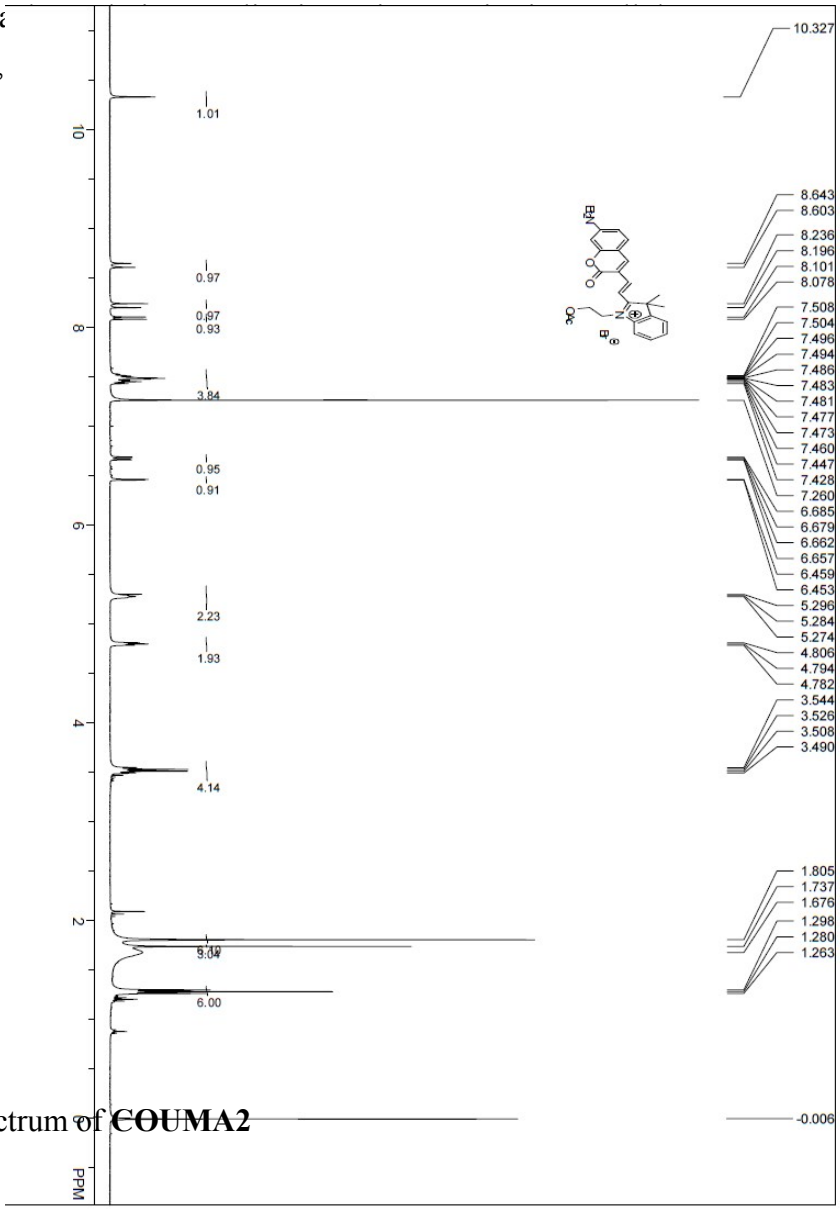


Fig. S1 [1]H NMR spectrum of **COUMA2**

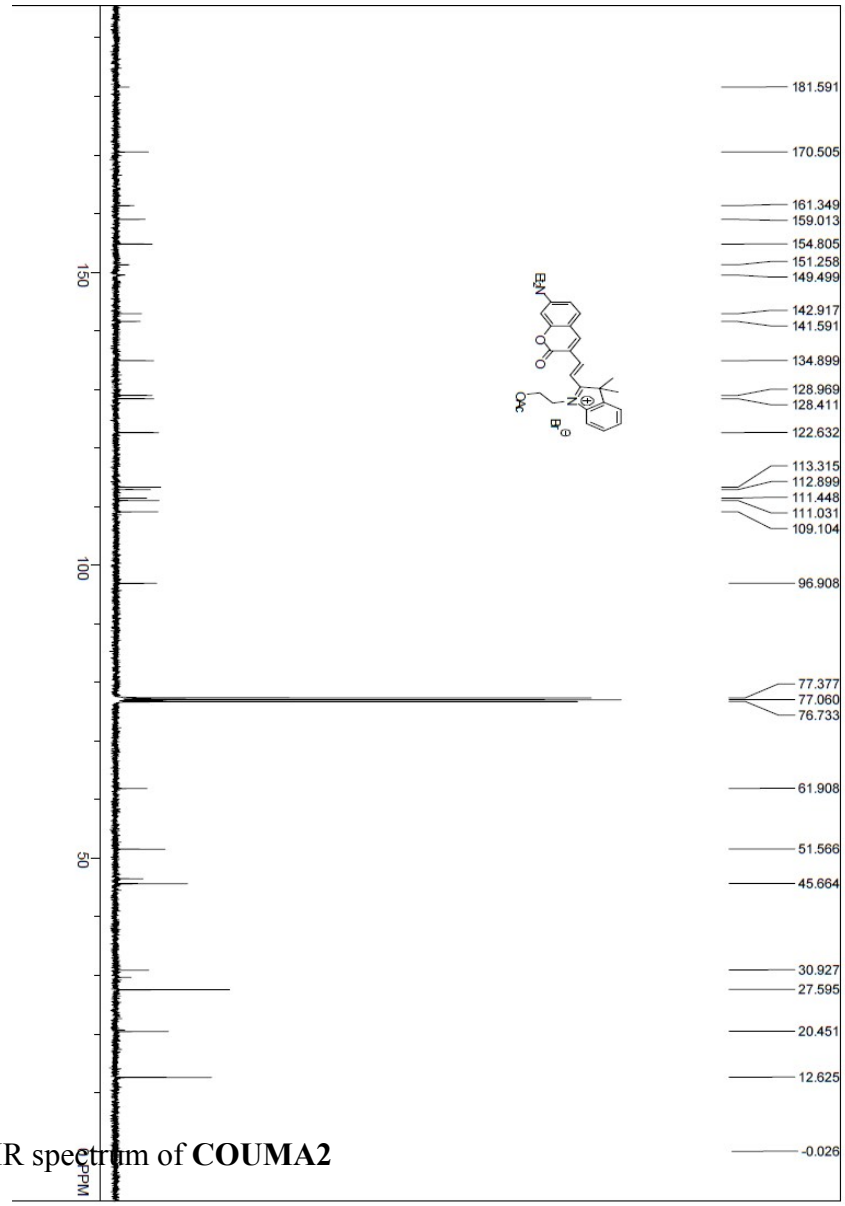


Fig. S2 ^{13}C NMR spectrum of **COUMA2**

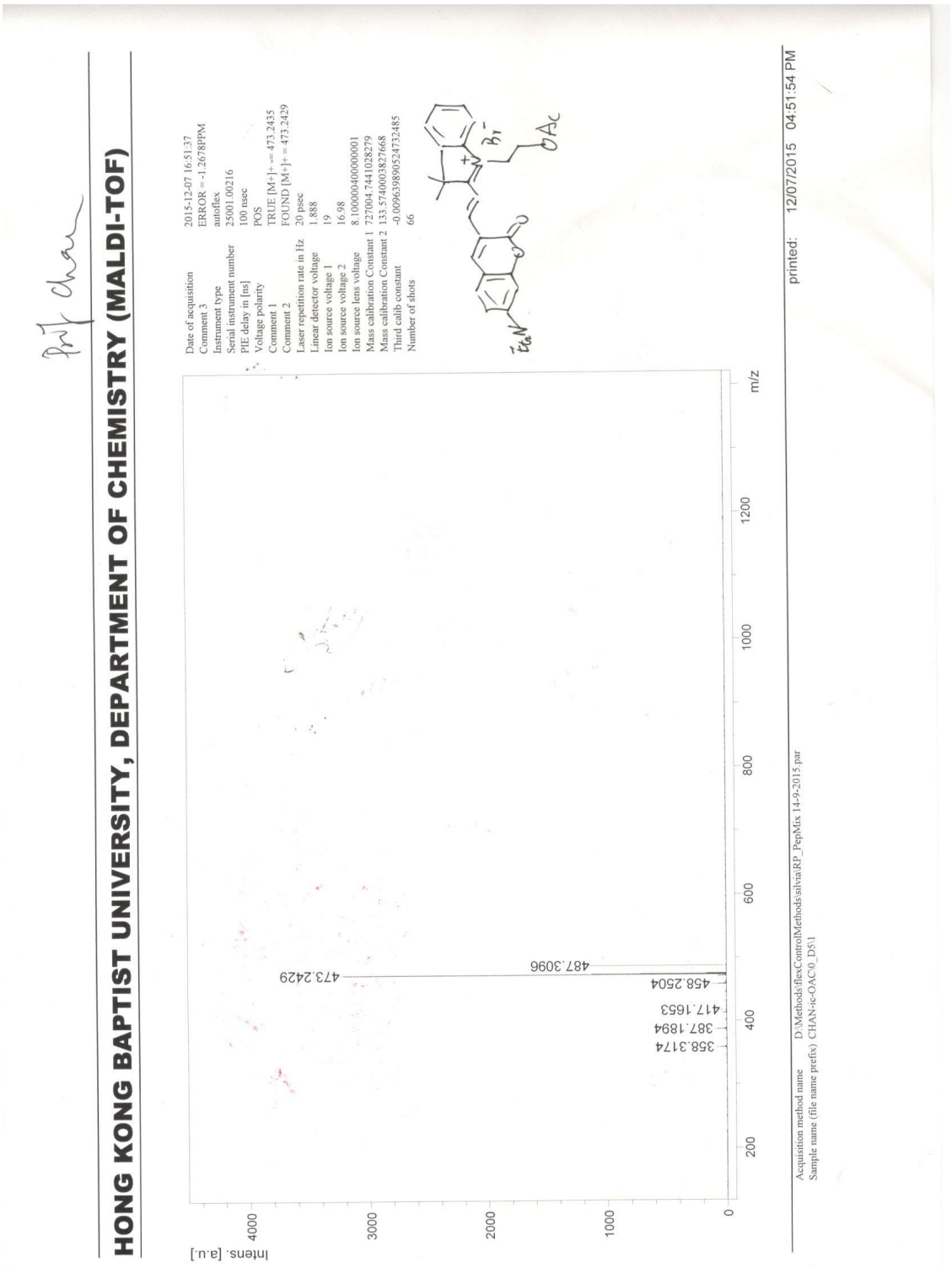


Fig. S3 HRMS MALDI-TOF spectrum of **COUMA2**

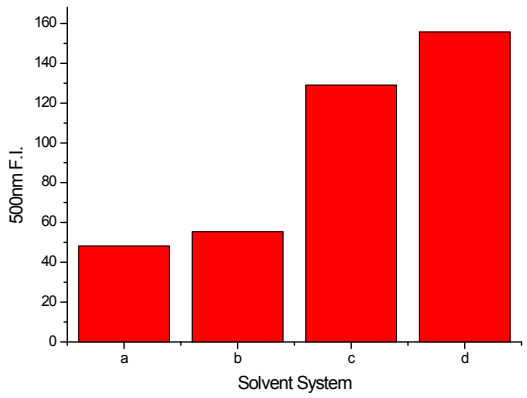
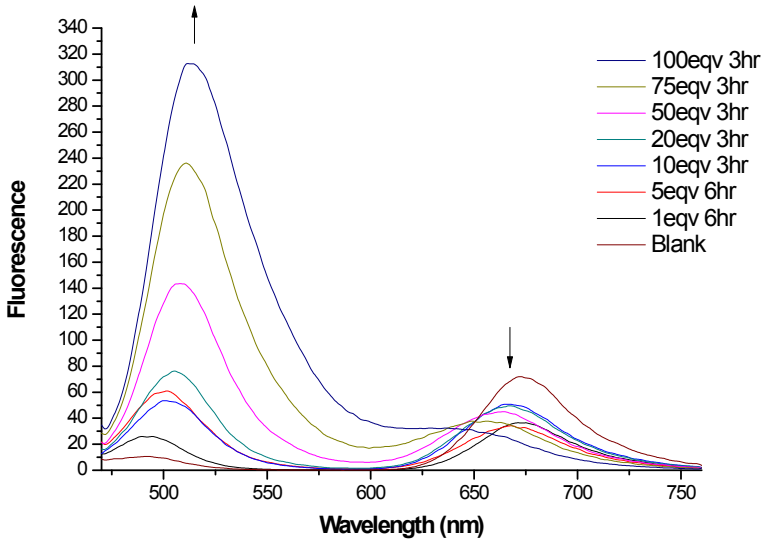


Fig. S4 Emission of the probe **COUMA2** (50μM) interacting with 100 equiv. of NH_2NH_2 in different solvent systems (50μM) at 17°C for 180min: Left: a. 100% H_2O; b. 2:8, v/v, ACN-H_2O; c. 2:8, v/v, EtOH-H_2O; d. 2:8, v/v, DMSO-H_2O in 40 mM Britton-Robinson buffer solution, pH=7.0.



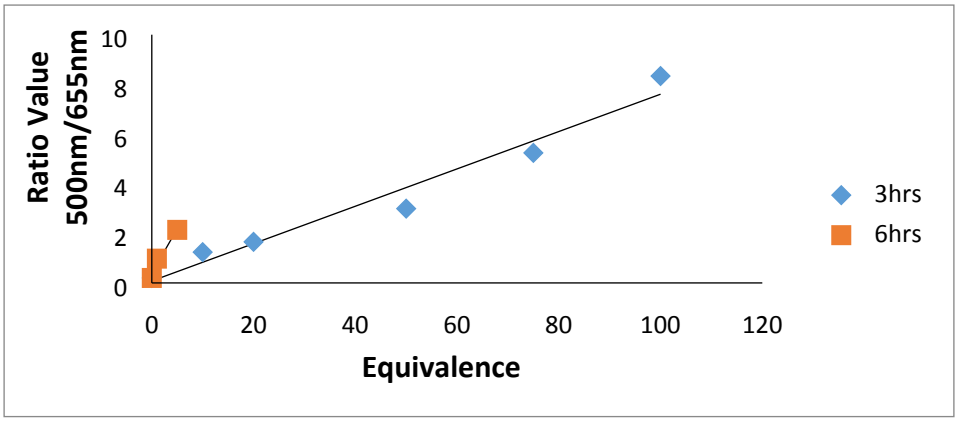


Fig. S5 Change of fluorescence spectra of **COUMA2** (50μM) in response to various concentration of NH_2NH_2 covering the concentration range from 50 μM to 5 mM (2:8, v/v, DMSO-H_2O, 40 mM Britton-Robinson buffer solution, pH=7.0).

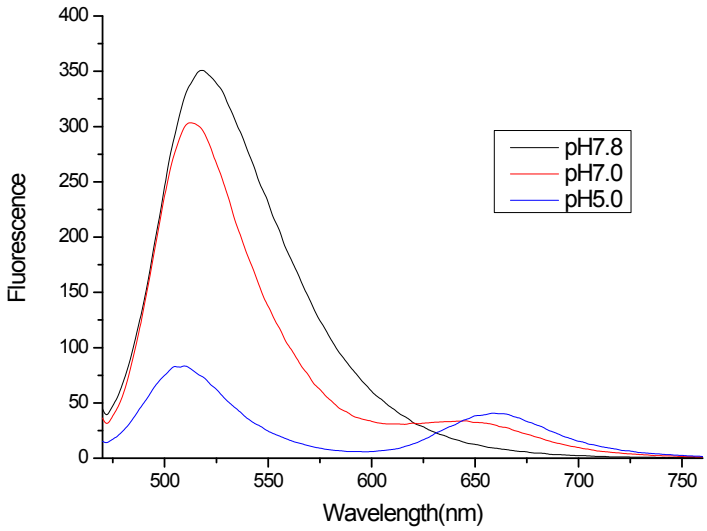


Fig. S6 Change of fluorescence spectra of **COUMA2** (50μM) in response to 100 equiv. NH_2NH_2 at different pH buffer solution (2:8, v/v, DMSO-H_2O, 40 mM Britton-Robinson buffer solution).

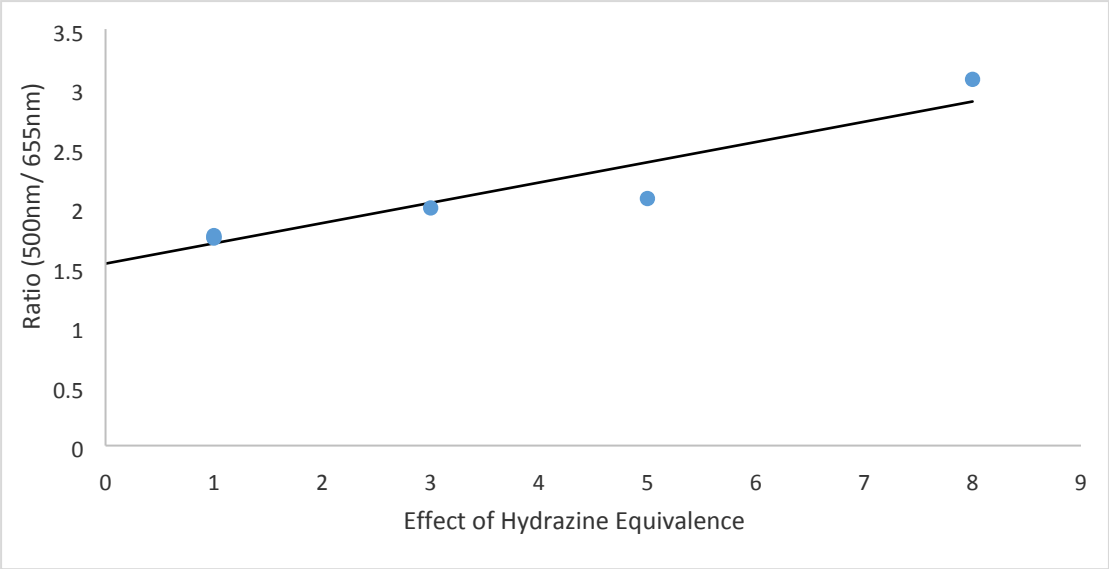


Fig. S7 Fluorescent titration of the probe (50 μM) versus low equivalent of hydrazine in 2:8, v/v, DMSO-H_2O in 40 mM Britton-Robinson buffer solution, pH=7.0 with reaction time of 15 hours.

Table S1 Determination of the LOD of the probe

For 15 hours reaction time	
50 μM COUMA2 vs 50 μM hydrazine (1.0 equivalent of hydrazine)	**Fluorescence Ratio Value (500nm/ 655nm)**
1st trial	1.7646
2nd trail	1.7438
3rd trial	1.7510

Estimation of the LOD (Level of Detection) of the probe:

3 x Standard Deviation of Fluorescence ratio at Low Concentration/ Slope of the Calibration Line found in **Fig. S7**

= 3 x (0.010565)/ 0.1703

= 0.186 μM