

Electronic Supplementary Information (ESI)

A non-peptide NIR fluorescent probe for detection of chymotrypsin and its imaging application

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Section 1. Non-peptide fluorescent probes for detection of chymotrypsin

Table S1 Comparison of the non-peptide fluorescent probes for detection of chymotrypsin

	$\lambda_{\text{ex}}/\lambda_{\text{em}}(\text{nm})$	Near-Infrared	pH	Temperature	Vitro and vivo imaging
Probe(2) ¹	450/515	No	8.0	30°C	No
NI ²	385/450 and 550	No	8.0	30°C	No
CyB	670/695	Yes	7.4	37°C	Yes

Section 2. Characterization

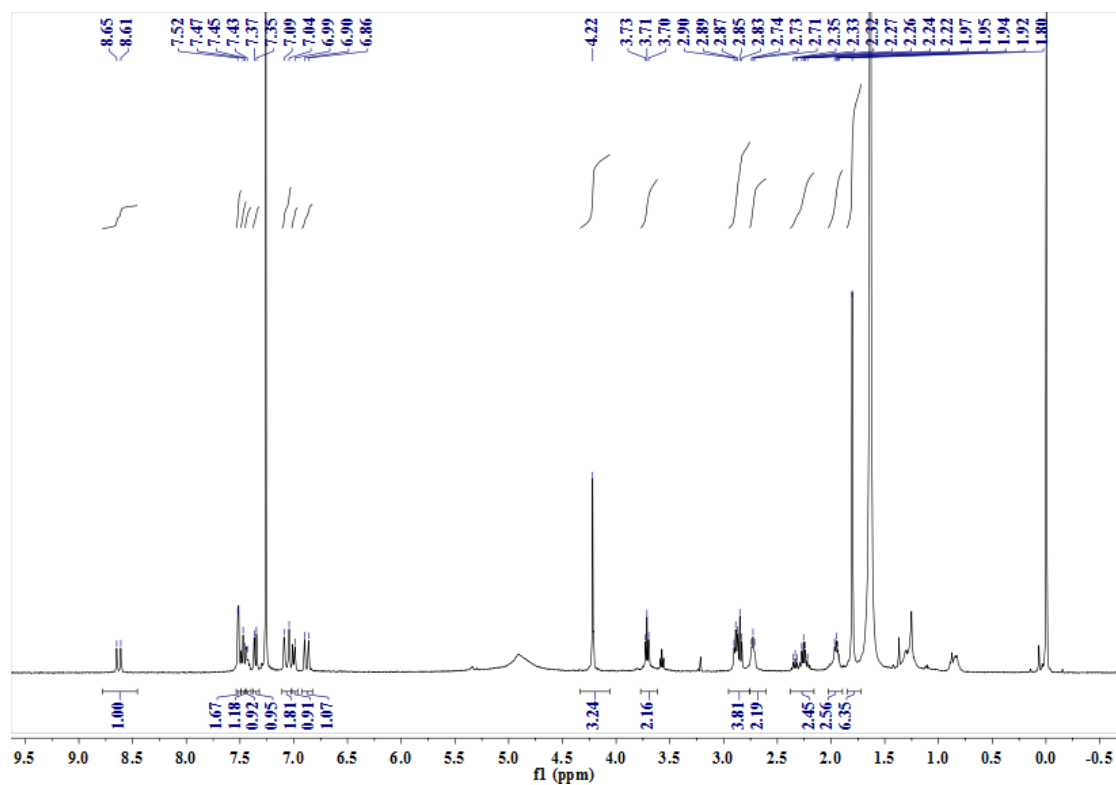


Figure S1. ¹H NMR of CyB.

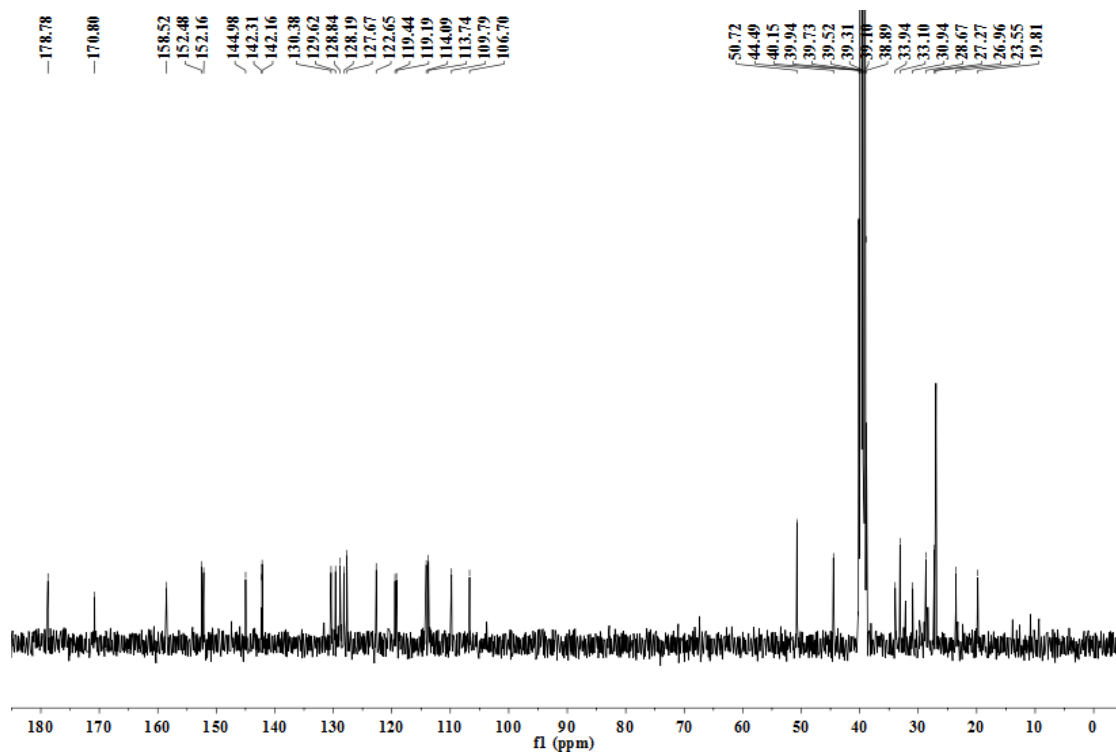


Figure S2. ¹³C NMR of CyB.

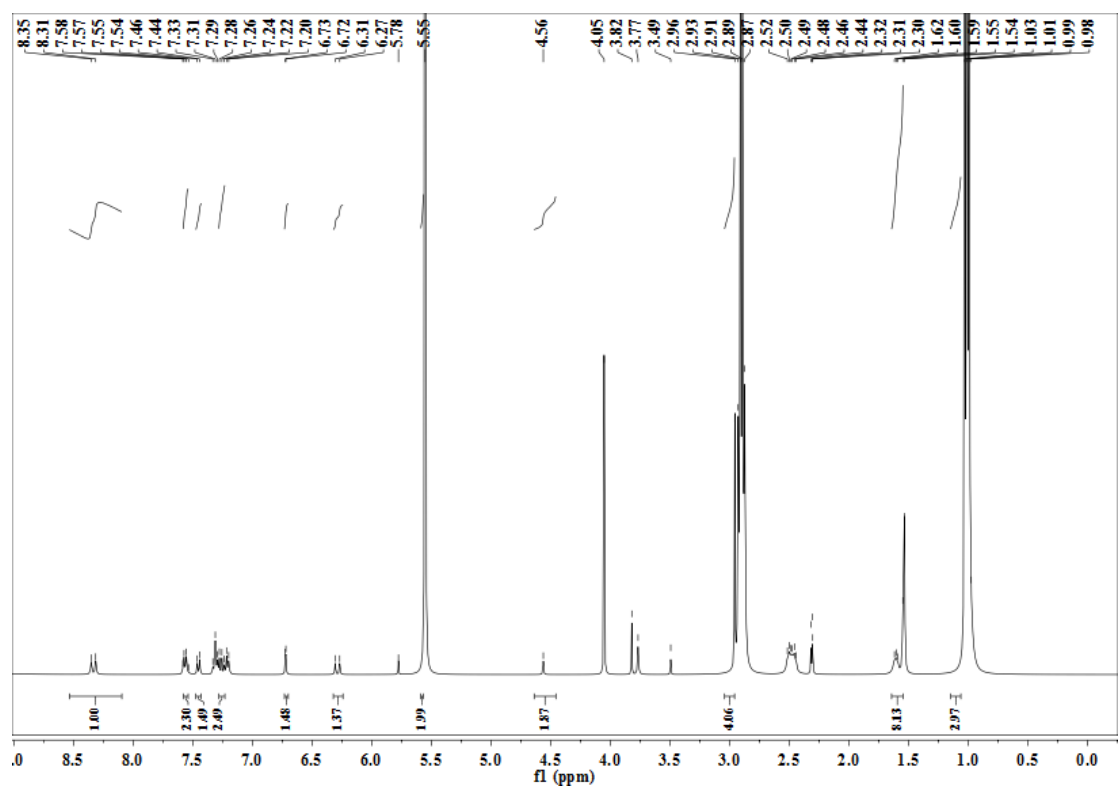


Figure S3. ¹H NMR of CyD

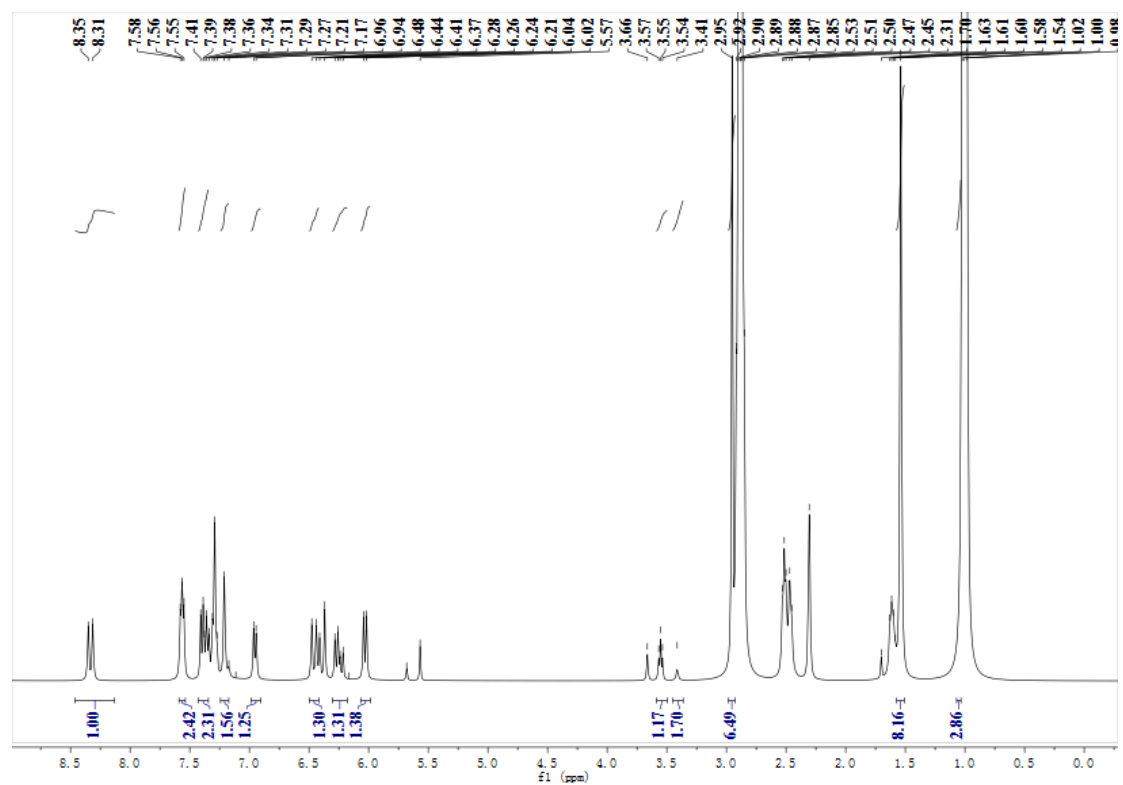


Figure S4. ¹H NMR of CyE

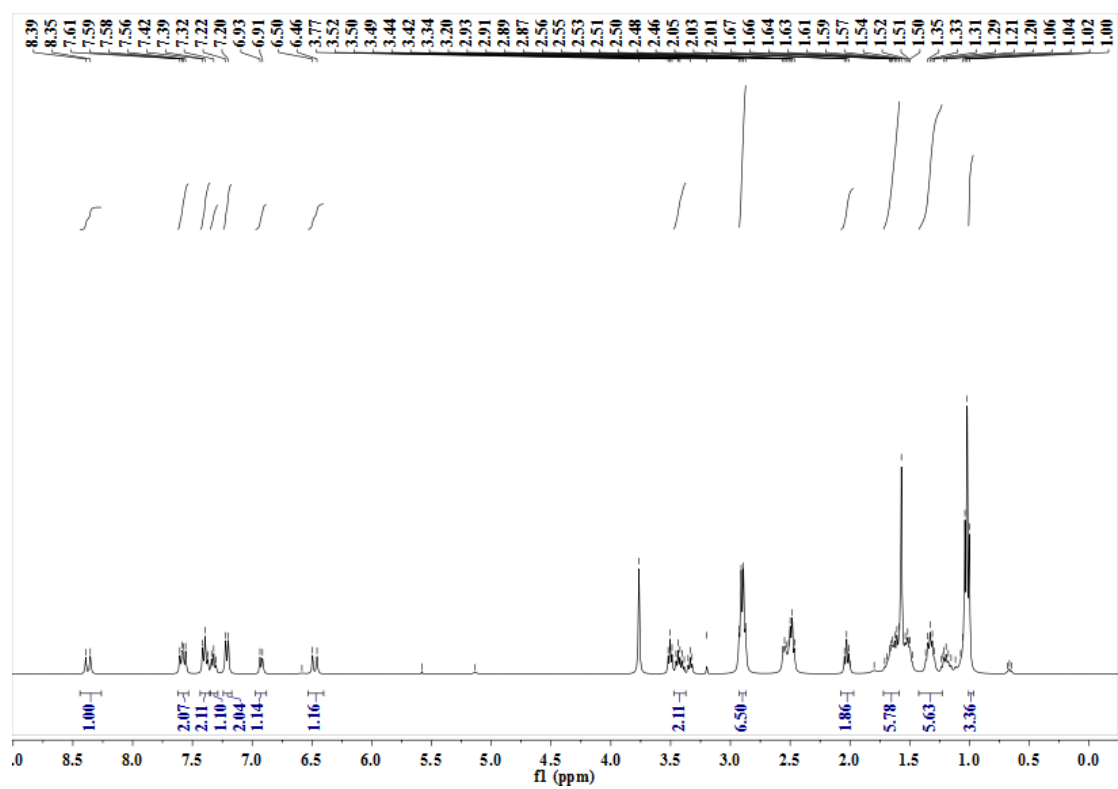


Figure S5. ^1H NMR of CyF

Section 3. Optimization of the recognition group of the probe

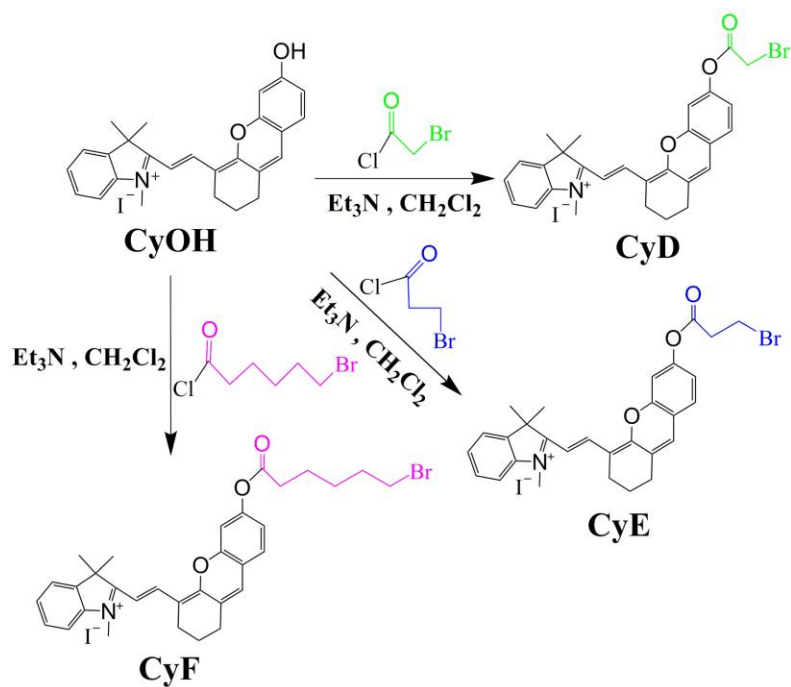


Figure S6. Synthesis protocol of CyD, CyE and CyF

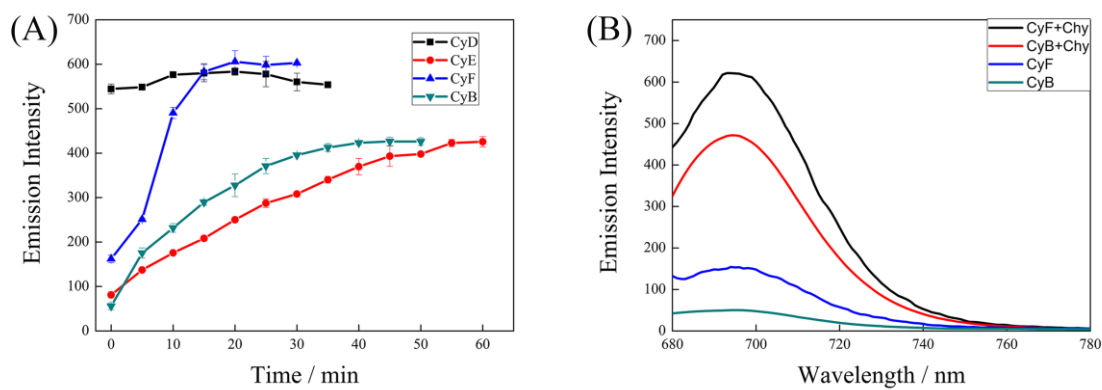


Figure S7. (A) Time response of different probes ($5.0 \mu\text{M}$) to chymotrypsin ($10 \mu\text{g mL}^{-1}$).

(B) Fluorescence emission spectra of CyB and CyF ($5.0 \mu\text{M}$) before and after reacted with Chy ($10 \mu\text{g mL}^{-1}$).

Section 4. The optical property of CyB and its sensing for Chy

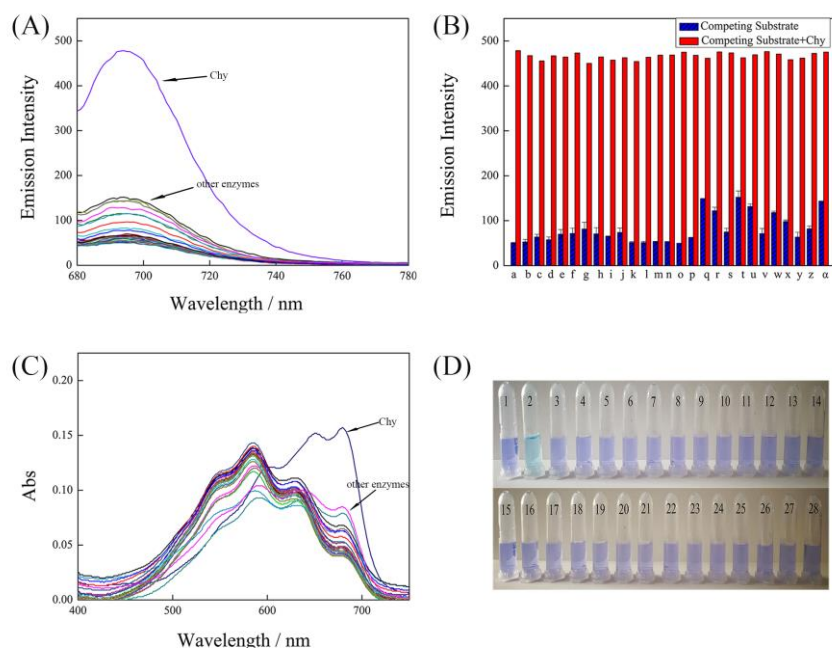


Figure S8. Fluorescence emission spectra (A) and fluorescence emission intensity (B) of CyB (5.0 μM) at 695 nm in the presence of Chy (10 $\mu\text{g mL}^{-1}$) and other competing species (1.0 mM) in HEPES buffer solution (pH = 7.4) at 37 °C. (a) blank, (b) Na^+ , (c) Mg^{2+} , (d) Zn^{2+} , (e) Ca^{2+} , (f) Br^- , (g) I^- , (h) NO_2^- , (i) ClO_4^- , (j) NO_3^- , (k) Phe, (l) Ala, (m) Arg, (n) Lys, (o) Leu, (p) His, (q) GSH, (r) BSA, (s) Lysozyme, (t) Trypsin, (u) Pepsase, (v) Alkaline protease, (w) Compound proteinase, (x) Tyrosinase, (y) Catalase, (z) Glucose Oxidase, (α) Lipase. (C) UV absorption spectrum of CyB (5.0 μM) in the presence of Chy (10 $\mu\text{g mL}^{-1}$) and other competing species (1.0 mM) in HEPES buffer solution (pH = 7.4) at 37 °C. (D) The color change of probe CyB (5.0 μM) in response to various analytes (1 mM). (1: blank, 2: Chy, 3: Na^+ , 4: Mg^{2+} , 5: Zn^{2+} , 6: Ca^{2+} , 7: Br^- , 8: I^- , 9: NO_2^- , 10: ClO_4^- , 11: NO_3^- , 12: Phe, 13: Ala, 14: Arg, 15: Lys, 16: Leu, 17: His, 18: GSH, 19: BSA, 20: Lysozyme, 21: Trypsin, 22: Pepsase, 23: Alkaline protease, 24: Compound proteinase, 25: Tyrosinase, 26: Catalase, 27: Glucose Oxidase, 28: Lipase.)

Section 5. The mechanism study on CyB for sensing of Chy

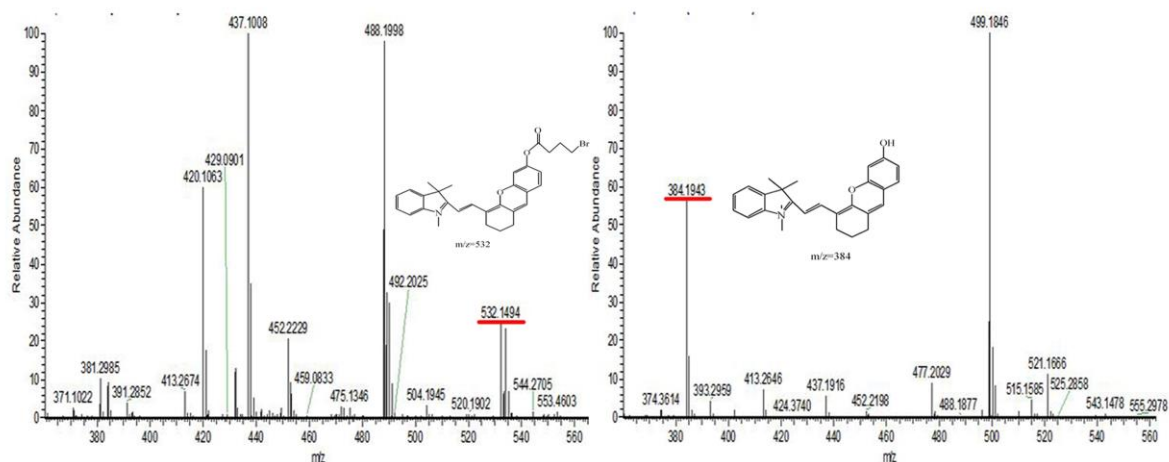


Figure S9. HRMS spectrum of CyB (5.0 μM) without (left) and with (right) the addition of Chy

(10 μg mL⁻¹) incubated for 35 min in HEPES buffer (100 mM, pH 7.4, 0.5% DMSO) at 37 °C.

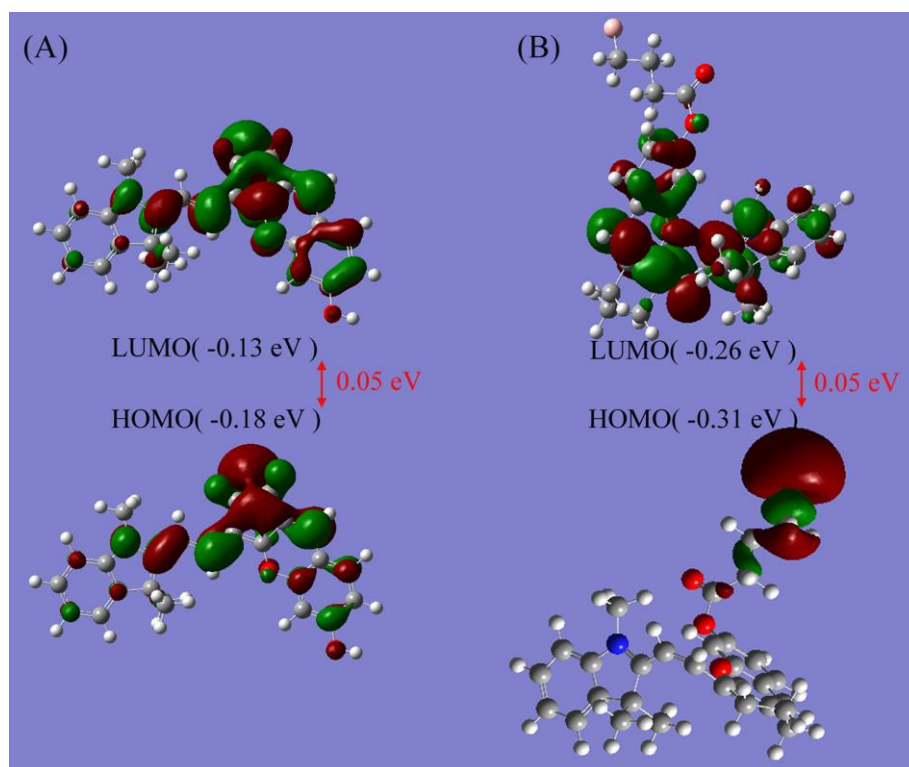


Figure S10. (A) CyOH and (B) CyB density functional theory calculation (DFT) frontier orbital

theory (MOs), (B3LYP/6-311G (d, p)/level using Gaussian 09).

Section 6. Optimization of conditions

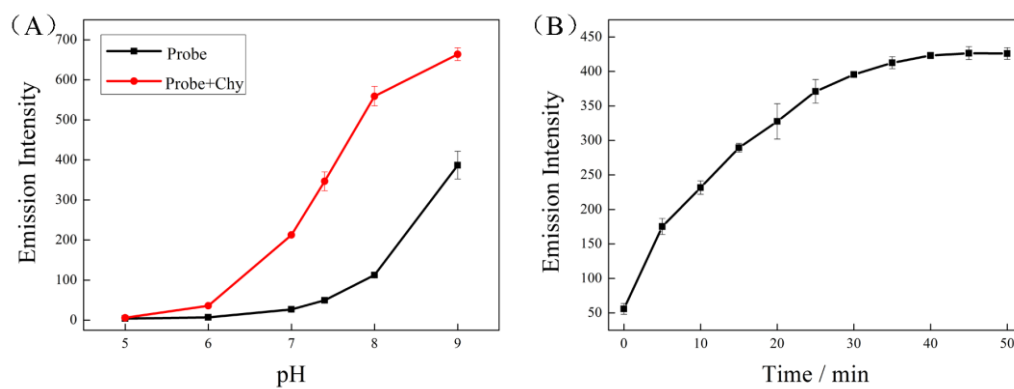


Figure S11. The effect of pH (A) and time (B) on the fluorescence response of CyB (5.0 μM) in the presence and absence of Chy (10 $\mu\text{g mL}^{-1}$). The CyB was incubated with Chy for 35 min.

Section 7. Enzyme kinetics parameters of CyB

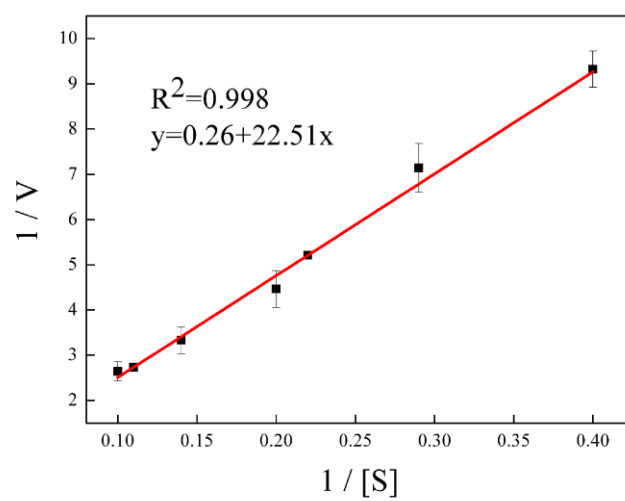


Figure S12. Line weaver-Burke plot for the reaction between CyB and Chy ($10 \mu\text{g mL}^{-1}$) in HEPES buffer solution (100 mM, pH 7.4) at 37°C .

Section 8. Cytotoxicity of CyB

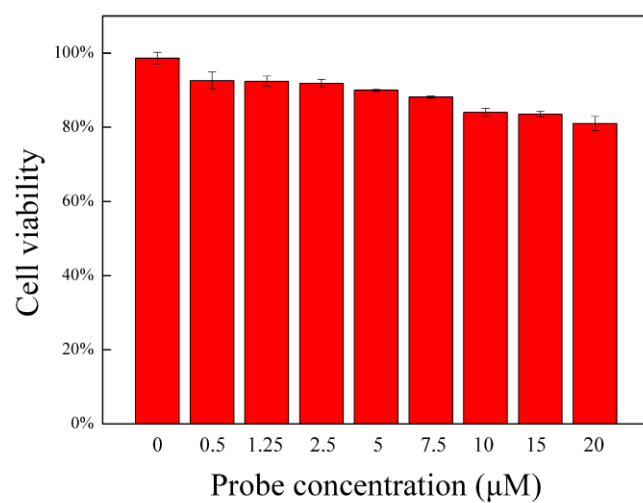


Figure S13. MTT assay for estimating cell viability (%) of P815 cells treated with various concentrations of CyB (0-20.0 μM).

Section 9. References

- 1 L. Wu, S. H. Yang, H. Xiong, J.Q. Yang, J. Guo, W. C. Yang, G. F. Yang, *Anal. Chem*, 2017, **89**, 3687-3693.
- 2 Y. P. Chen, J. Cao, X. X. Jiang, Z. Z. Pan, N. Y. Fu, *Sensors and Actuators B*, 2018, **273**, 204-210.