

Supporting Information (SI)

Colorimetric and near infrared ratiometric fluorescent probe for endogenous tyrosinase activity determination based on cyanine aggregation

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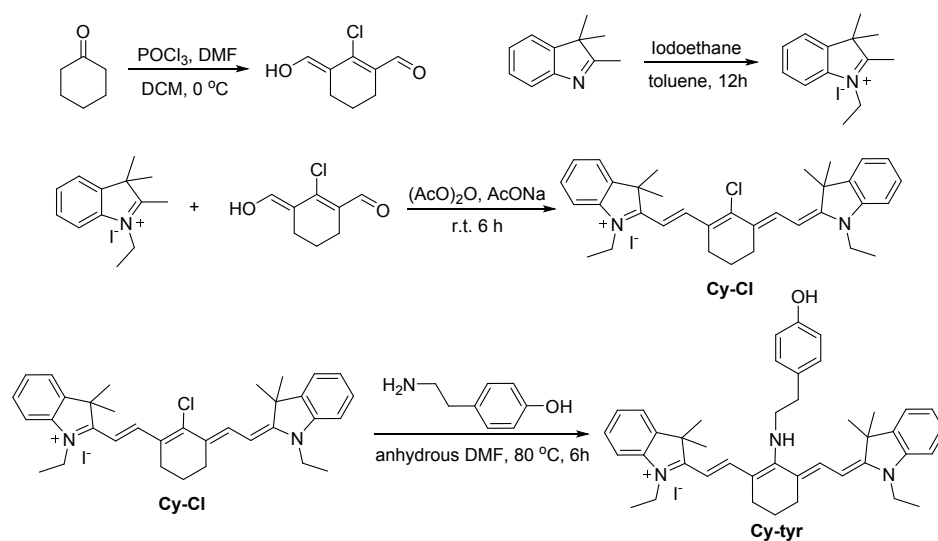
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1. Synthesis of Cy-tyr



Scheme 1. Synthesis route of compound Cy-tyr.

Synthesis of Cy-Cl

Cy-Cl was synthesized according to our previous work. ^[1]

Synthesis of Cy-tyr

64 mg (0.1 mmol) of Cy-Cl and 55 mg (0.4 mmol) of tyramine were dissolved in 5 ml anhydrous DMF in a 25 ml round bottom flask. The mixture was stirred at $80\text{ }^\circ\text{C}$ under argon for two hours. Then it was cooled and added to 80 ml of ether with violent stirring. The obtained blue solid was filtered and dried under vacuum, then purified on a silica gel column (CH_2Cl_2 : MeOH = 20 : 1) with a final yield of 52%. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 9.24 (s, 1H), 8.60 (s, 1H), 7.53 (d, $J = 12.9$ Hz, 2H), 7.43 (d, $J = 7.3$ Hz, 2H), 7.29 (t, $J = 7.6$ Hz, 2H), 7.12 (d, $J = 8.0$ Hz, 2H), 7.05 (t, $J = 7.5$ Hz, 2H), 7.00 (d, $J = 8.4$ Hz, 2H), 6.67 (d, $J = 8.3$ Hz, 2H), 5.72 (d, $J = 12.4$ Hz, 2H), 3.97 (d, $J = 7.1$ Hz, 4H), 3.92 (d, $J = 6.1$ Hz, 2H), 2.94 (s, 2H), 2.44 (t, $J = 5.8$ Hz, 4H), 1.62 (s, 2H), 1.54 (s, 12H), 1.21 (t, $J = 7.0$ Hz, 6H), the proton signals designation were shown in Figure S1; ^{13}C NMR (214 MHz, $\text{DMSO}-d_6$) δ 169.74, 166.47, 140.29, 137.83, 130.06, 129.87, 128.62, 128.36, 122.76, 122.53, 115.81, 109.23, 94.20, 51.28, 47.53, 35.84, 28.68, 25.32, 21.50, 11.73 (Figure S2). ESI-MS m/z : M^+ Calcd for $\text{C}_{42}\text{H}_{50}\text{N}_3\text{O}^+$ 612.3948; Found 612.3931 (Figure S3).

2. ^1H , ^{13}C NMR and HRMS spectra

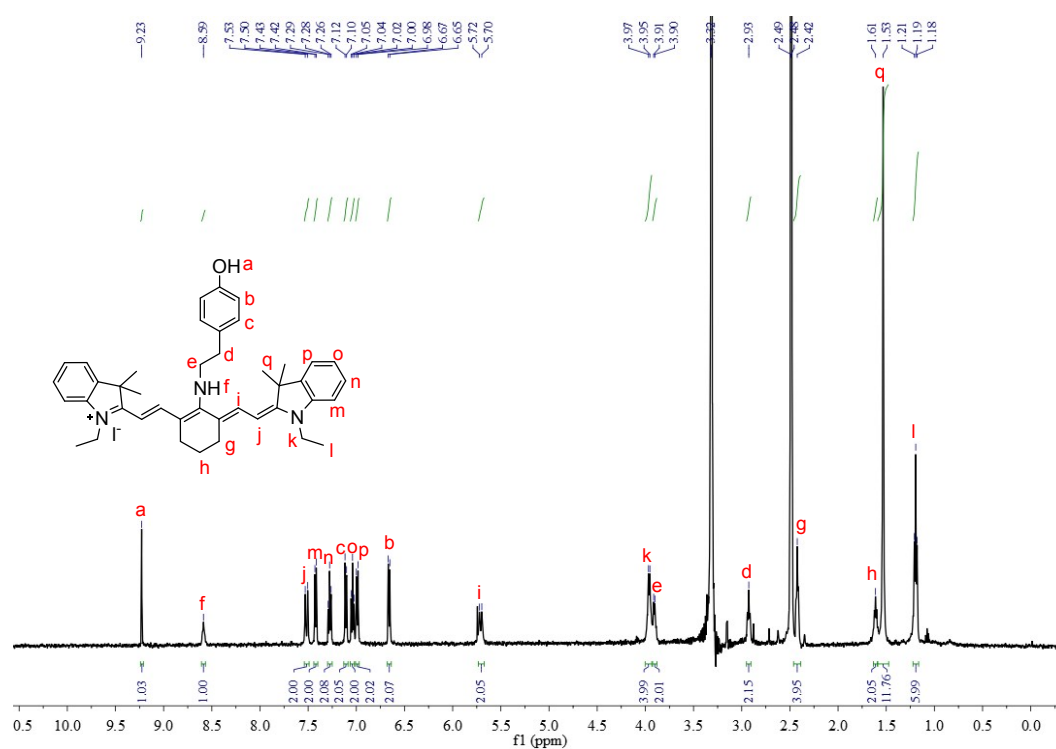


Figure S1. ^1H NMR spectrum (500 MHz) of Cy-tyr in $\text{DMSO-}d_6$.

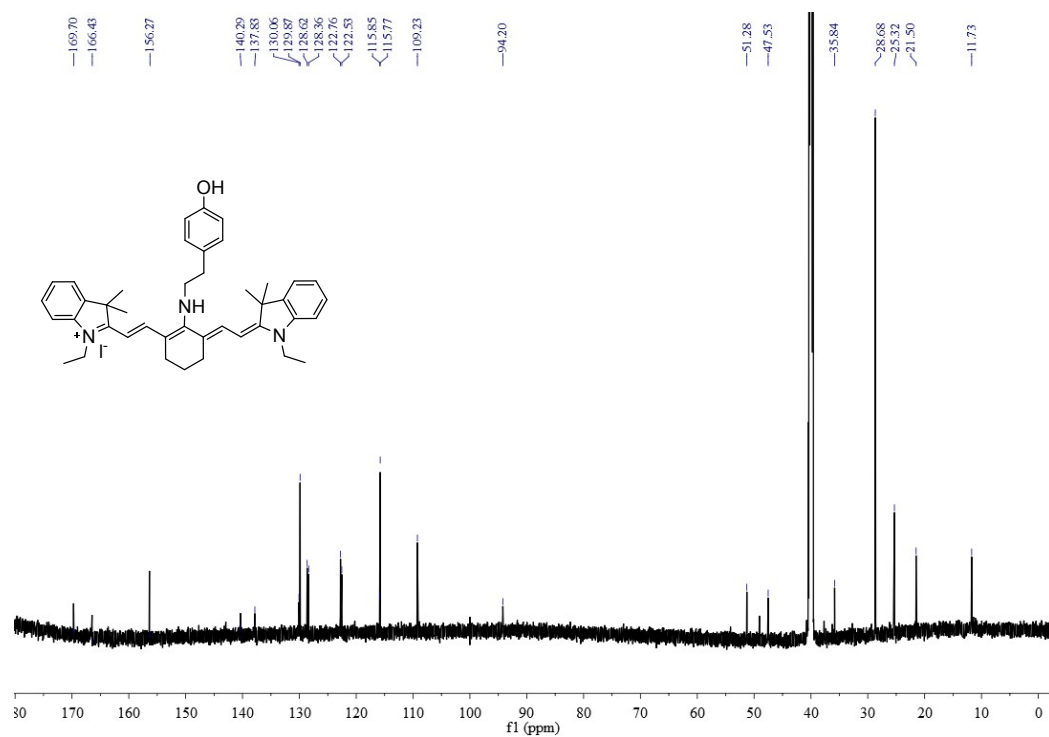


Figure S2. ^{13}C NMR spectrum (850 MHz) of Cy-tyr in $\text{DMSO-}d_6$.

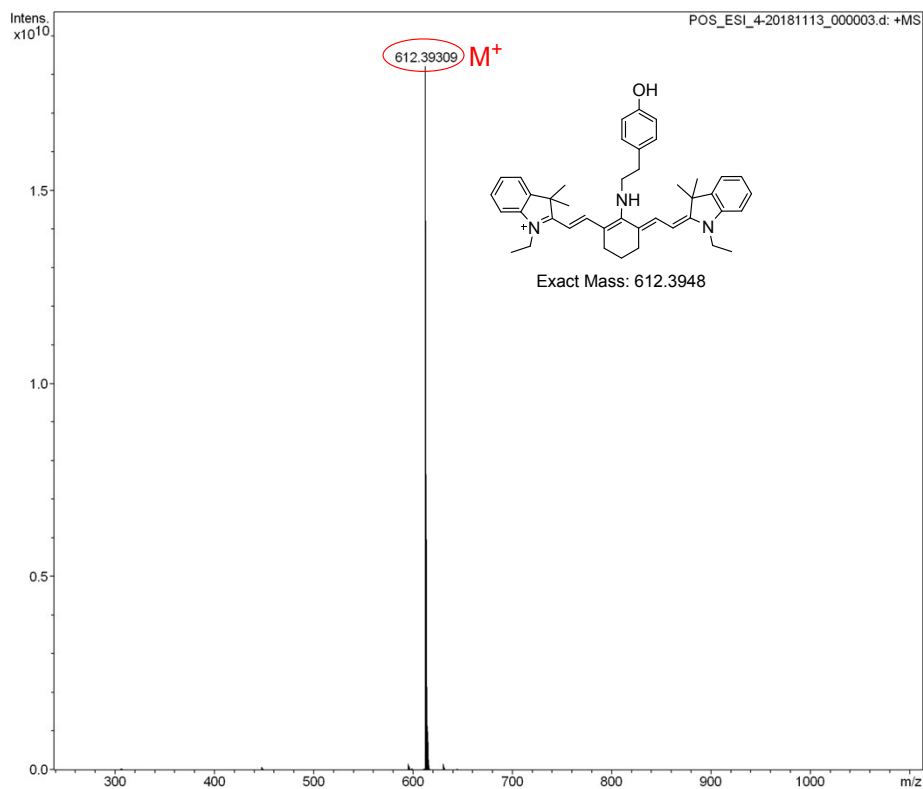


Figure S3. HRMS spectrum of Cy-tyr in MeOH.

3. Table S1

Table S1. Properties of ratiometric fluorescent probes for TYR.

| Probe | Linear range (U/mL) | LOD (U/mL) | λ_{ex} (nm) | λ_{em} (nm) | Reaction time | Application | Ref. |
|-----------|---------------------|------------|----------------------------|----------------------------|---------------|-------------|-----------|
| Naph-L3 | 0-160 | 0.2 | 467 | 535/467 | 140 min | Living cell | [2] |
| S-Try-CDs | 0-200 | 1.2 | 340 | 440/540 | 120 min | Living cell | [3] |
| DCM-1 | 0.5-100 | 0.6 | 450 | 548/660 | 180 min | Living cell | [4] |
| Cy-tyr | 0-5 | 0.02 | 516 | 556/760 | 120 min | Living cell | This work |

4. Spectral profiles

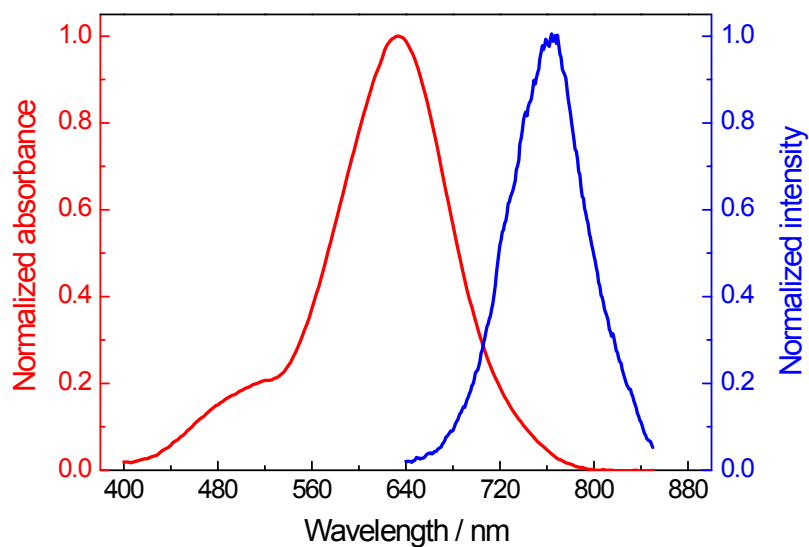


Figure S4. Normalized absorbance (red line) and fluorescence (blue line) spectra of Cy-tyr in 10 mM PBS buffer.

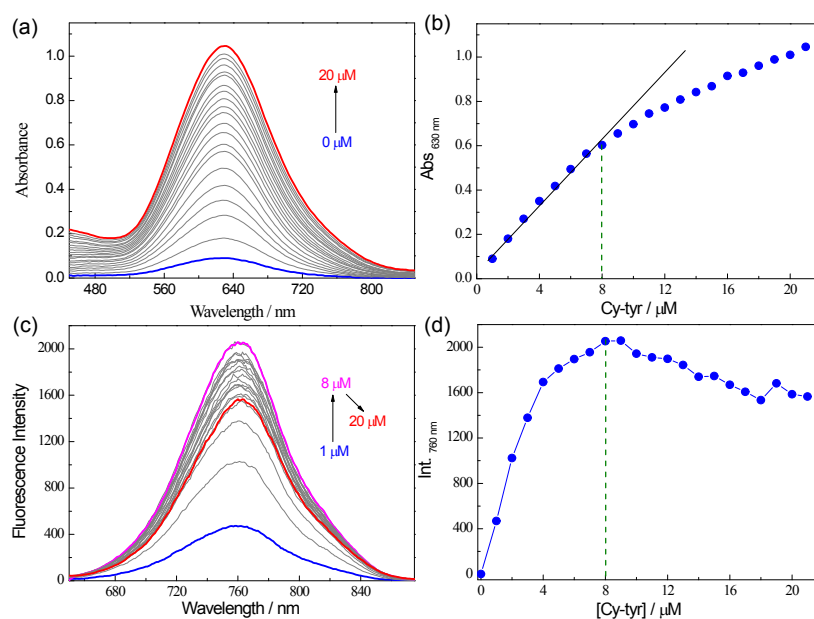


Figure S5. Absorption (a) and fluorescence (c) spectra of Cy-tyr of increasing concentrations from 0 to 20 μM in 10 mM PBS buffer of pH 4.0; Plots of absorbance at 630 nm (b) and fluorescence intensity at 760 nm (d) versus Cy-tyr concentration. $\lambda_{\text{ex}} = 630 \text{ nm}$.

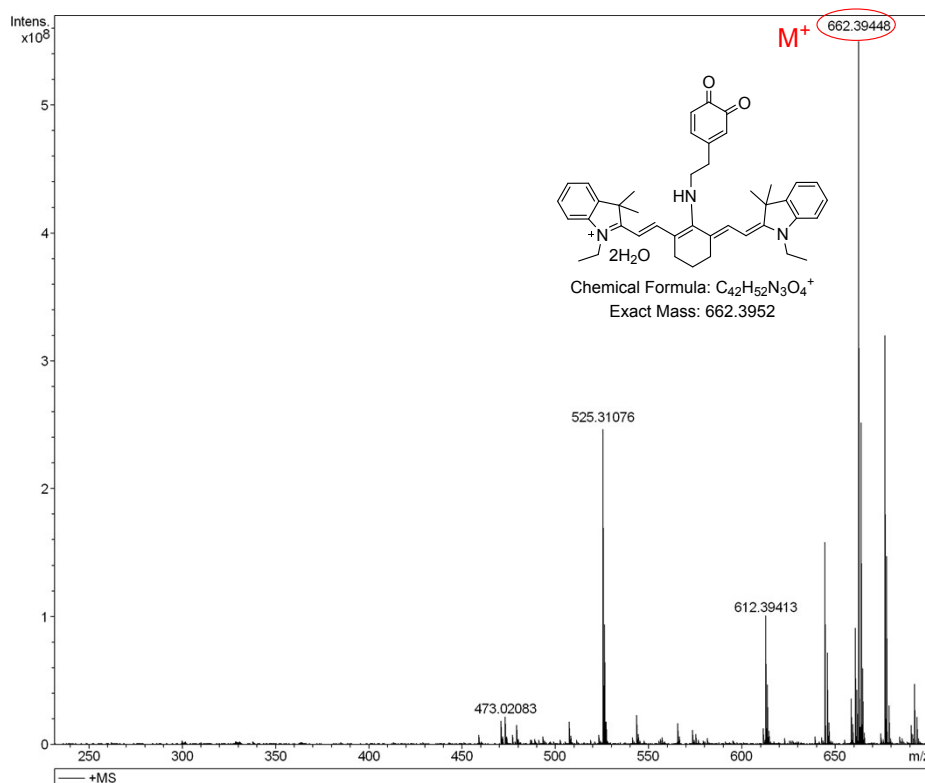


Figure S6. HRMS spectrum of Cy-tyr (8 μ M) incubated with 5.0 U/mL TYR for 120 min in 10 mM PBS buffer of pH 4.0.

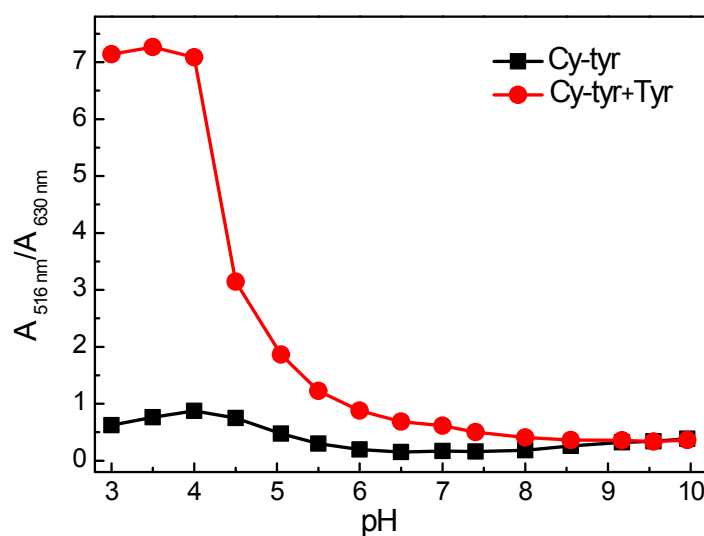


Figure S7. Plots of $A_{516\text{ nm}}/A_{630\text{ nm}}$ of Cy-tyr without (black squares) or with (red dots) 5.0 U/mL of TYR versus solution pH between 3.0 and 10.0. Solution pH was tuned by 10 mM PBS buffer. [Cy-tyr] = 8 μ M.

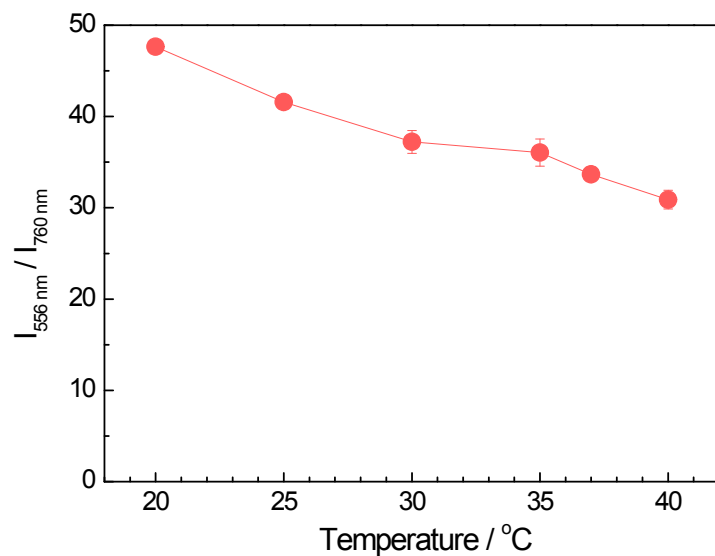


Figure S8. Temperature-dependent $I_{556 \text{ nm}}/I_{760 \text{ nm}}$ of Cy-tyr upon treated with 5.0 U/mL TYR for 120 min in PBS buffer of pH 4.0. [Cy-tyr] = 8 μM , λ_{ex} = 516 nm.

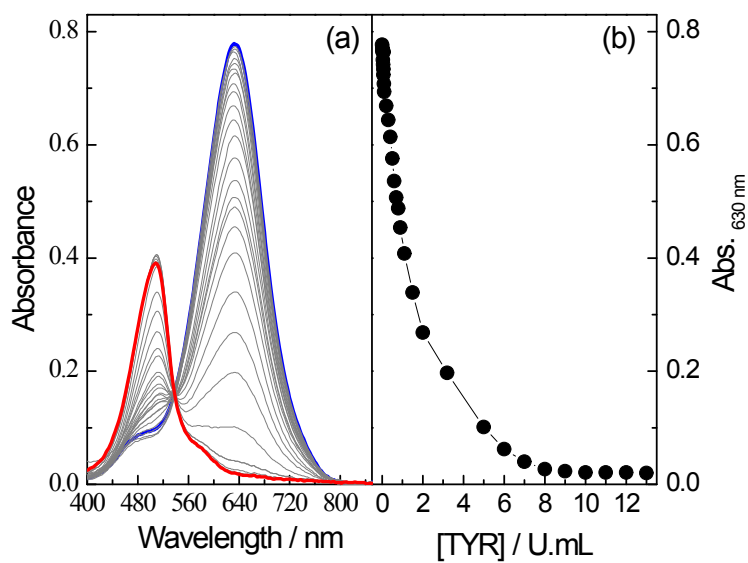


Figure S9. (a) Absorption spectra of Cy-tyr incubated with increasing TYR concentrations from 0 to 14 U mL⁻¹ for 120 min in 10 mM PBS buffer of pH 4.0. (b) Plots of absorption at 516 nm versus TYR concentration. [Cy-tyr] = 8 μM .

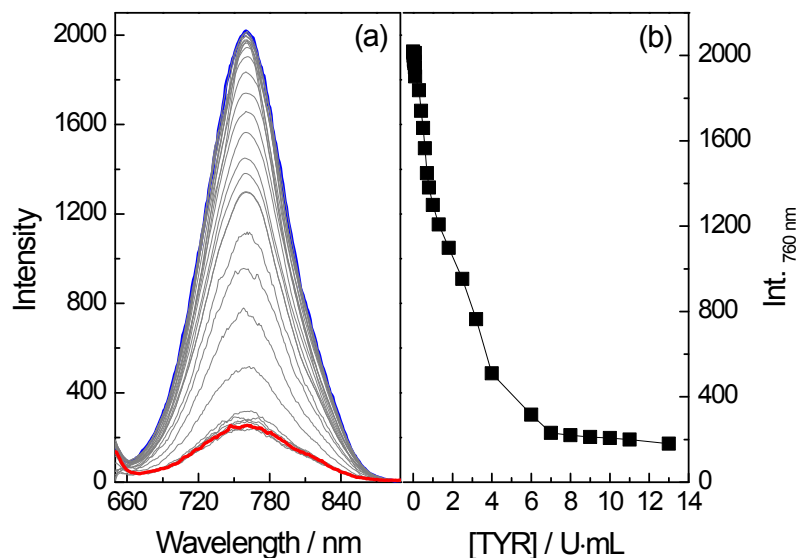


Figure S10. (a) Fluorescence spectra of Cy-tyr incubated with increasing TYR concentrations from 0 to 14 U mL⁻¹ for 120 min in 10 mM PBS buffer of pH 4.0. (b) Plots of intensity at 760 nm versus TYR concentration. [Cy-tyr] = 8 μM, λ_{ex} = 630 nm.

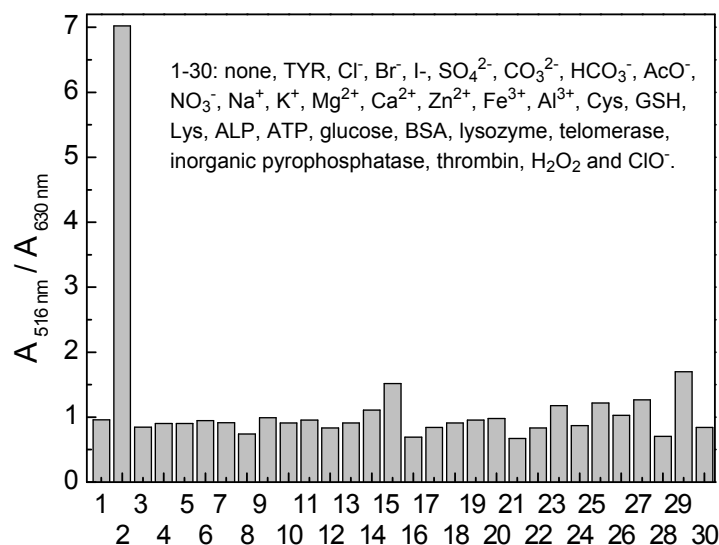


Figure S11. $A_{516 \text{ nm}}/A_{630 \text{ nm}}$ of Cy-tyr in 10 mM PBS buffer pH 4.0 upon addition of 100 μM of competition species 1-30: none, TYR, Cl⁻, Br⁻, I⁻, SO₄²⁻, CO₃²⁻, HCO₃⁻, AcO⁻, NO₃⁻, Na⁺, K⁺, Mg²⁺, Ca²⁺, Zn²⁺, Fe³⁺, Al³⁺, Cys, GSH, Lys, ALP, ATP, glucose, BSA, lysozyme, telomerase, inorganic pyrophosphatase, thrombin, H₂O₂ and ClO⁻. [Cy-tyr] = 8 μM.

5. References

- [1] Zhang, P.; Zhu, M. S.; Luo, H.; Zhang, Q.; Guo, L. E.; Li, Z.; Jiang, Y. B. *Anal. Chem.* **2017**, *89*, 6210-6215.
- [2] Q. Li, C. Yan, J. Zhang, Z. Guo and W. H. Zhu, *Dyes Pigments*, 2019, **162**, 802-807.
- [3] J. S. Sidhu, A. Singh, N. Garg, N. Kaur and N. Singh, *Analyst*, 2018, **143**, 4476-4483.
- [4] J. S. Sidhu and N. Singh, *J. Mater. Chem. B*, 2018, **6**, 4139-4145.