Supporting Information

A novel fluorescent off-on probe based on 4-methylumbelliferone for highly sensitive determination of tyrosinase

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1. NMR and HRMS Spectra

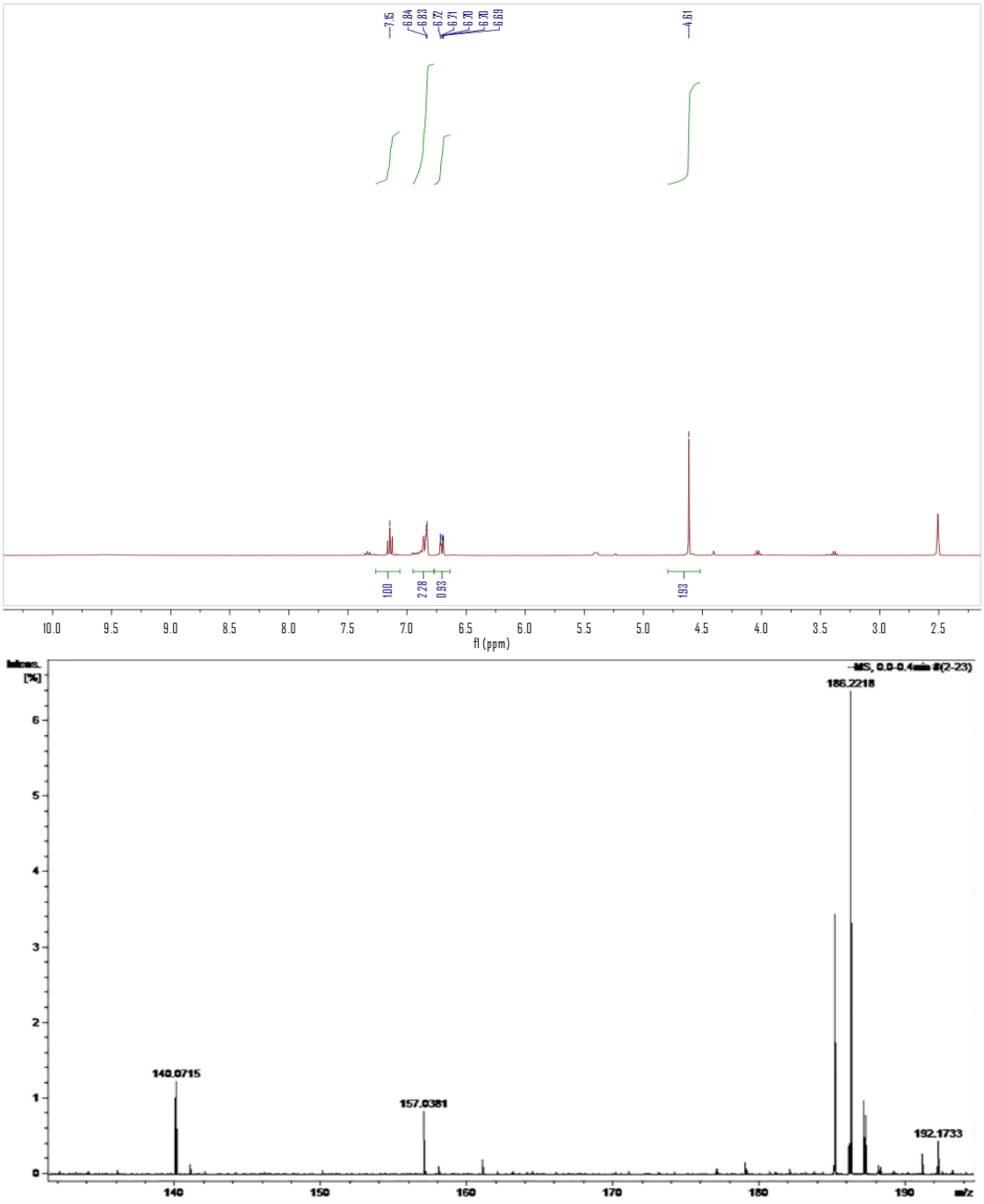


Fig. S1 [1]HNMR and HRMS Spectra of 3-bromomethylphenol.

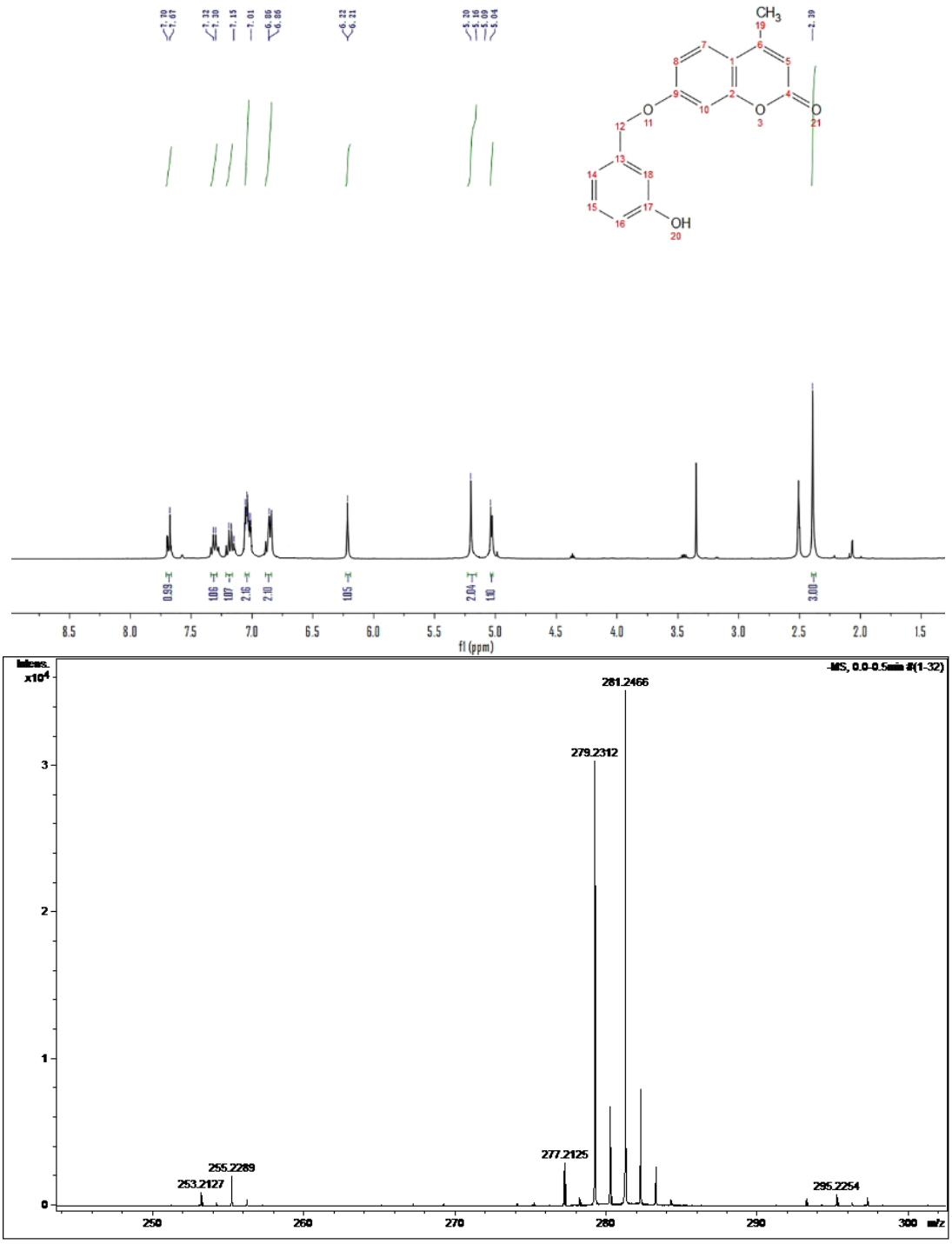


Fig. S2 ^{1}HNMR and HRMS Spectra of probe MU.

2.Determination of fluorescence quantum yield

Fluorescence quantum yield (Φ) was determined by using quinine sulfate (Φ = 0.54 in 0.1 M H_2SO_4 solution) as a standard[1].

$$\phi = \frac{\phi_r I A_r \eta}{I_r A \eta_r}$$

ϕ is the quantum yield; I is the integrated area under the emission spectrum; A is the absorbance at the absorption peak; η is the refractive index of the solvent used; subscript r is the standard.

3. HPLC and HRMS spectra of MU + Tyr

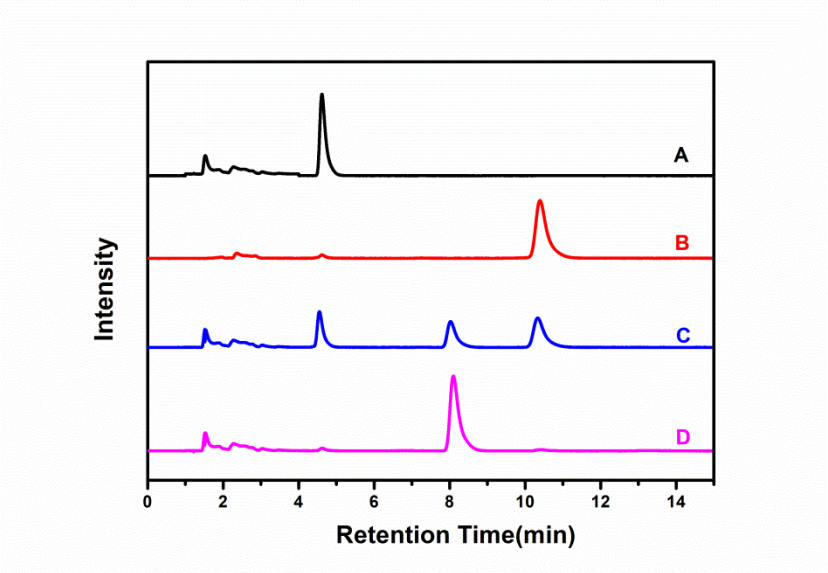


Fig. S3 Chromatograms of different species. 4-methylumbelliferone (200 μM) (A), probe MU (200 μM) (B), probe MU (200 μM) + TYR (500 $U \cdot mL^{-1}$) (C), 3-hydroxybenzyl alcohol (200 μM) (D). Chromatographic conditions: the detection wavelength was set at 320 nm, the flow rate was set at 1.0 $mL \cdot min^{-1}$, the mobile phase is acetonitrile: ammonium formate (25 mM) (1:1), and all the chromatographic runs were carried out at 25 °C.

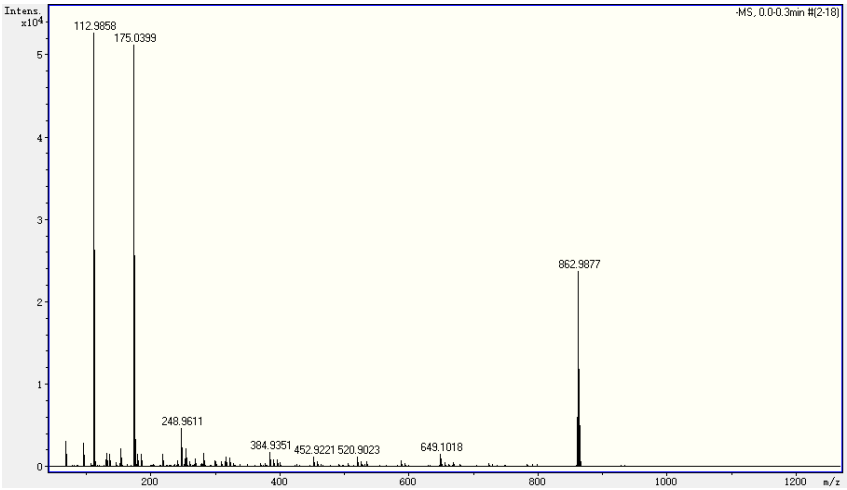


Fig. S4 HRMS Spectra of the reaction solution of the probe MU and TYR.

4.Optimization of the experimental conditions

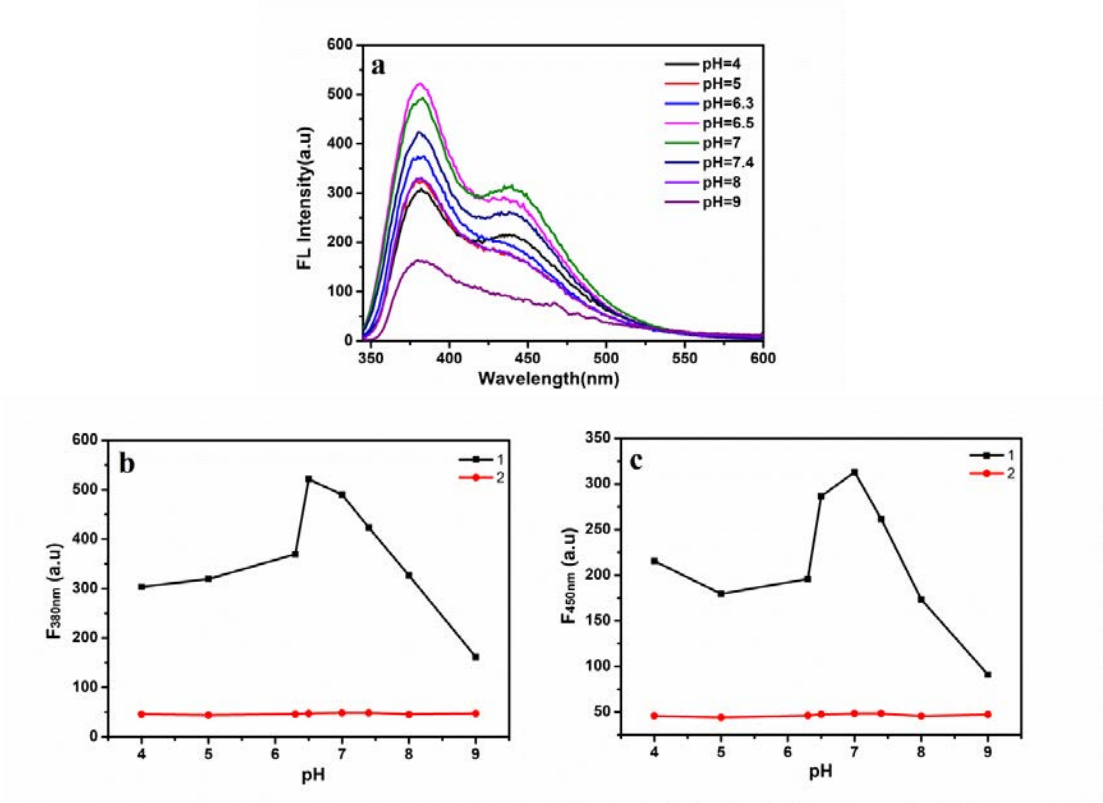


Fig. S5 Effect of pH, (a) fluorescence spectra of the probe MU response to TYR at different pH, (b) the relationship between the fluorescence intensity at 380 nm and pH, (c) the relationship between the fluorescence intensity at 450 nm and pH. Curve 1 is the fluorescent probe MU and enzyme response, curve 2 is the fluorescent probe MU.

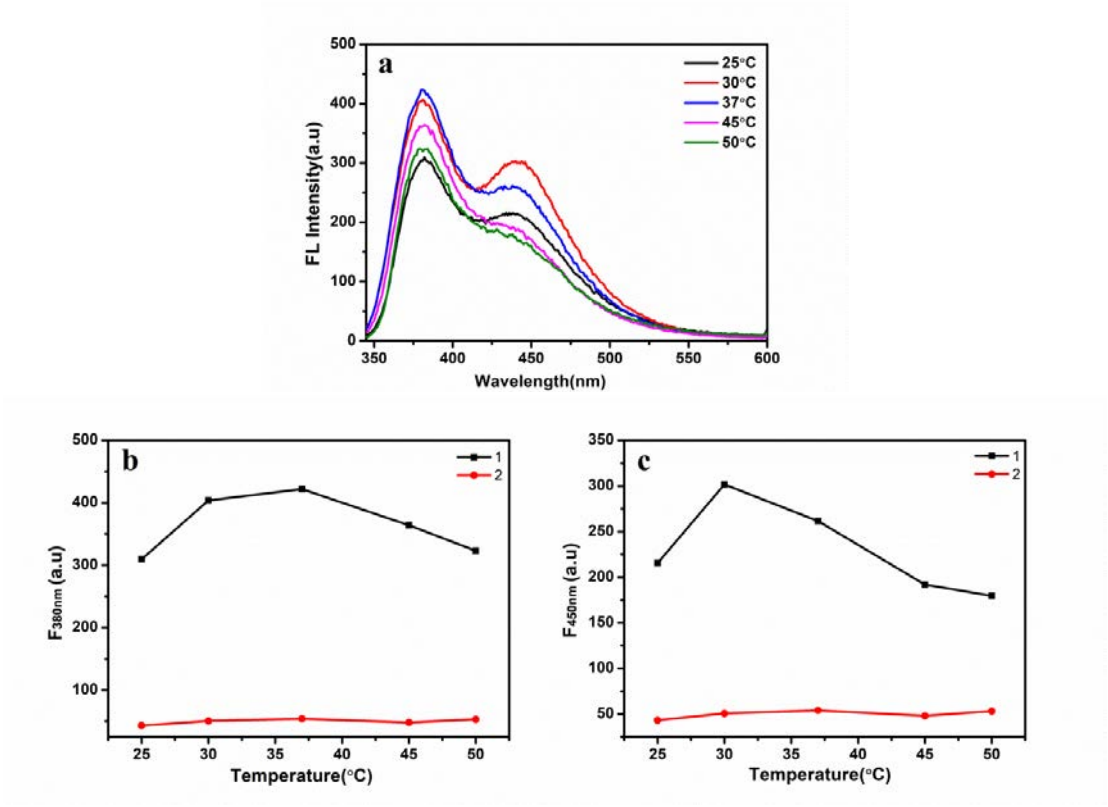


Fig. S6 The influence of incubation temperature on the fluorescence detection system, (a) Fluorescence spectra of the probe MU response to TYR at different incubation temperatures, (b) the relationship between the fluorescence intensity at 380 nm and the incubation temperature, (c) the relationship between the fluorescence intensity at 450 nm and the incubation temperature, Curve 1 is the fluorescent probe MU and enzyme response, curve 2 is the fluorescent probe MU.

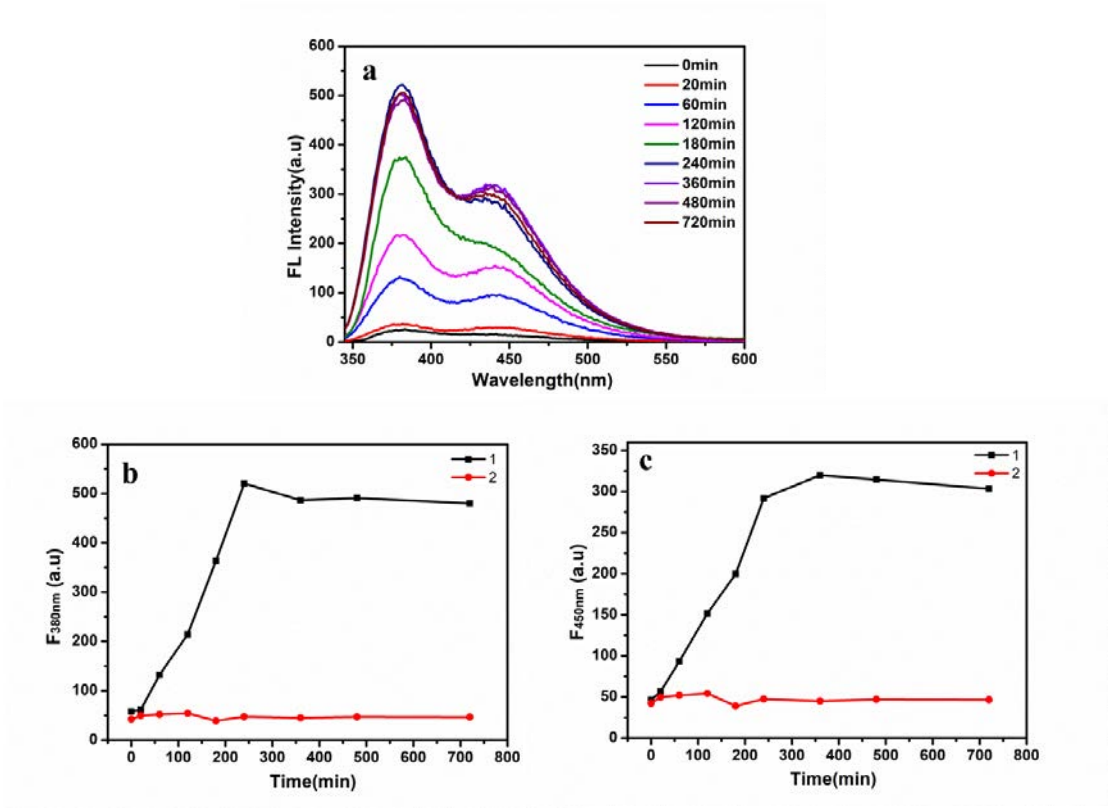
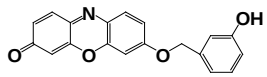
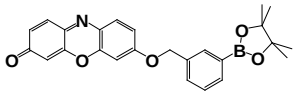
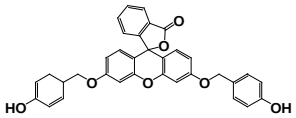
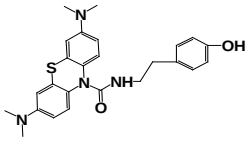
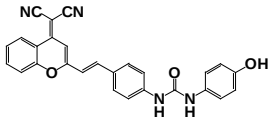
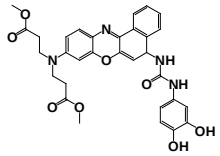
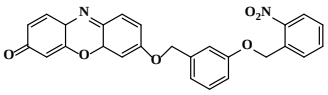
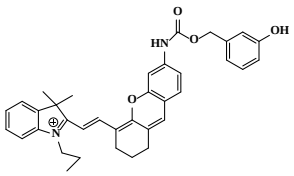
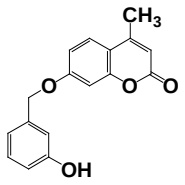


Fig. S7 The effect of incubation time on the fluorescence intensity of the reaction system, (a) Fluorescence spectra of the probe response to TYR under different incubation times, (b) the relationship between the fluorescence intensity at 380 nm and the incubation time, and (c) the relationship between the fluorescence intensity at 450 nm and the incubation time. Curve 1 is the fluorescent probe MU and enzyme response, 2 is the fluorescent probe MU.

5. Comparison of the performance of TYR probes

Table S1 Recent reports about TYR probes.

Fluorescent probe	Experimental conditions	Linear range ($U \cdot mL^{-1}$)	LOD ($U \cdot mL^{-1}$)	References
	37 °C, 3 h, pH=7.4	1-90	2.76	[2]
	37 °C, 5 h, pH=7.4	1-100	0.5	[3]
	37 °C, 5 h, pH=7.4	0-45	0.127	[4]
	37 °C, 0.5 h, pH=7.4	0-1	-	[5]
	37 °C, 3 h, pH=7.4	0.5-100	0.6	[6]
	37 °C, 4 h, pH=7.4	-	-	[1]
	37 °C, 3 h, pH=7.4	0-190	0.06	[7]
	37 °C, 2.5 h, pH=7.4	2-8	0.36	[8]
	37 °C, 4 h, pH=6.5	0.4-80	0.2	This work

6. Kinetic experiments

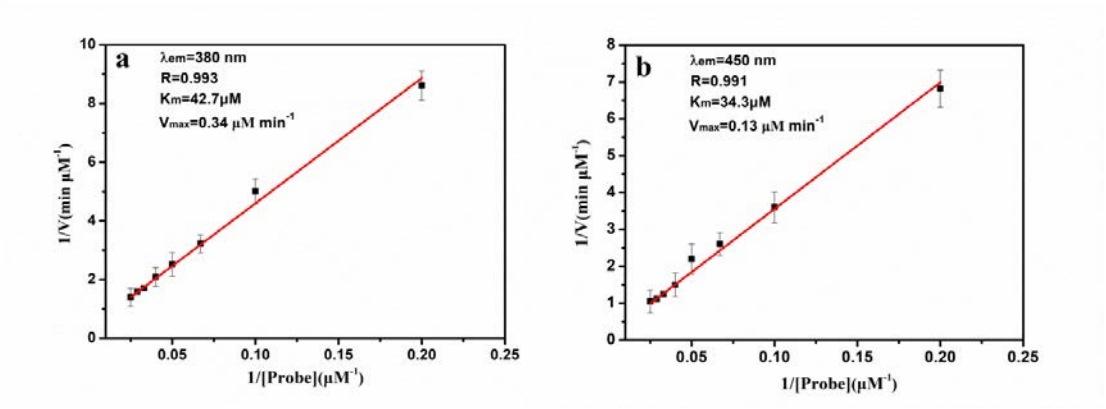


Fig. S8 Lineweaver-Burk diagram of the enzymatic reaction at the emission peaks of 380 nm (a) and 450 nm (b), in the equation: $V = V_{max}$ [probe]/ (K_m + [probe]), where V is the reaction rate, [probe] is the probe concentration, K_m is the Michaelis constant. Measurement conditions: TYR concentration: 20 $U \cdot mL^{-1}$, the probe MU concentration: 5-40 μM, the reaction of each probe concentration is repeated three times, and the error bar represents the standard deviation.

References

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