Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Diverse applications of TMB-based sensing probes

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Table S1 Crystallographic Data of TMBS ($C_{30}H_{28}N_2O_2$)

Identification code	exp_4214
Empirical formula	$C_{30}H_{28}N_2O_2$
Formula weight	448.54
Temperature/K	298.9(4)
Crystal system	Monoclinic
Wavelength/Å	0.71073
Space group	*P12$_1$/c1*
a/Å	10.7465(6) alpha = 90 deg.
b/Å	13.0800 (8) beta = 91.407(5) deg.
c/Å	17.6313 (8) gamma = 90 deg.
	2477.6 (2)
V/Å^3	4
Z	1.203
ρ_{calc} mg/mm^3	0.075
Absorption coefficient/mm	952
F(000)	0.09×0.08×0.07
Crystal size/mm^3	2.79 to 26.37°
2Θ range for data collection	10778/5066 [R_{int} = 0.0224]
Reflections collected/unique	99.9 %
Completeness to theta = 26.37	

Table S2 Crystallographic Data of TMBB ($C_{30}H_{28}N_2$)

Identification code	a
Empirical formula	$C_{30}\ H_{28}\ F_0\ N_2\ O_0$
Formula weight	416.54
Temperature/K	273(2)
Wavelength	0.71073A
Crystal system	Monoclinic
Space group	$P2_1/c$
a/Å	15.770(3) A alpha = 90 deg.
b/Å	16.302(3) A beta = 114.213(3) deg.
c/Å	14.959(3) A gamma = 90 deg.
V/Å^3	3507.4(12)
Z	6
ρ_{calc} mg/mm^3	1.183
Absorption coefficient/mm	0.069
F(000)	1332
Crystal size/mm^3	0.090 × 0.080 × 0.070
2Θ range for data collection	1.416 to 26.998°
Reflections collected / unique	25900 / 7624 [R(int) = 0.0356]
Completeness to theta = 25.242	99.8 %

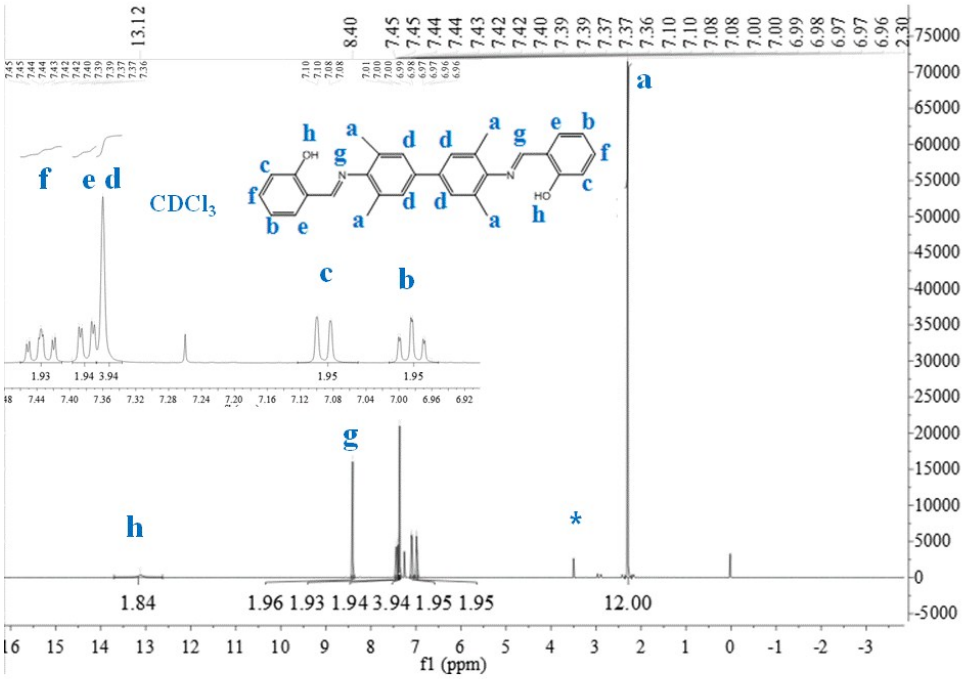

Fig. S1 ^{1}H NMR spectrum (500 MHz) of TMBS in $CDCl_3$.

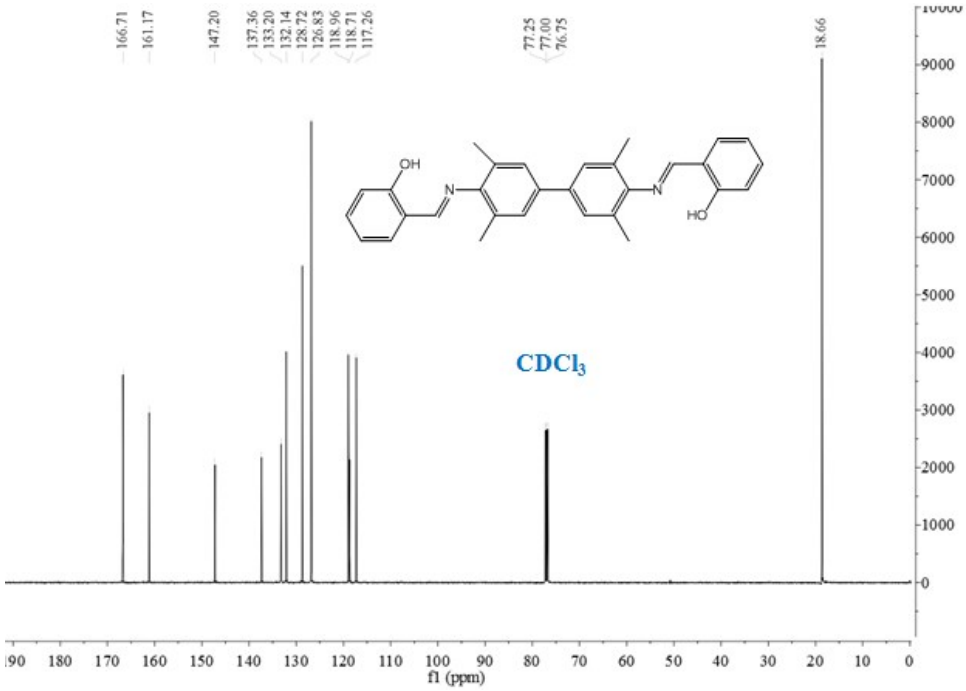

Fig. S2 ^{13}C NMR spectrum (500 MHz) of TMBS in $CDCl_3$.

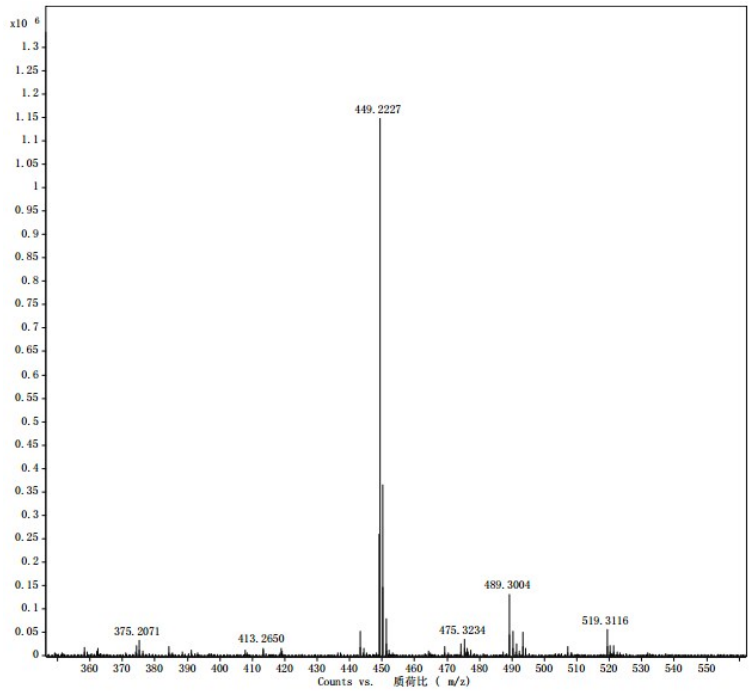

Fig. S3 The ESI-MS result of TMBS.

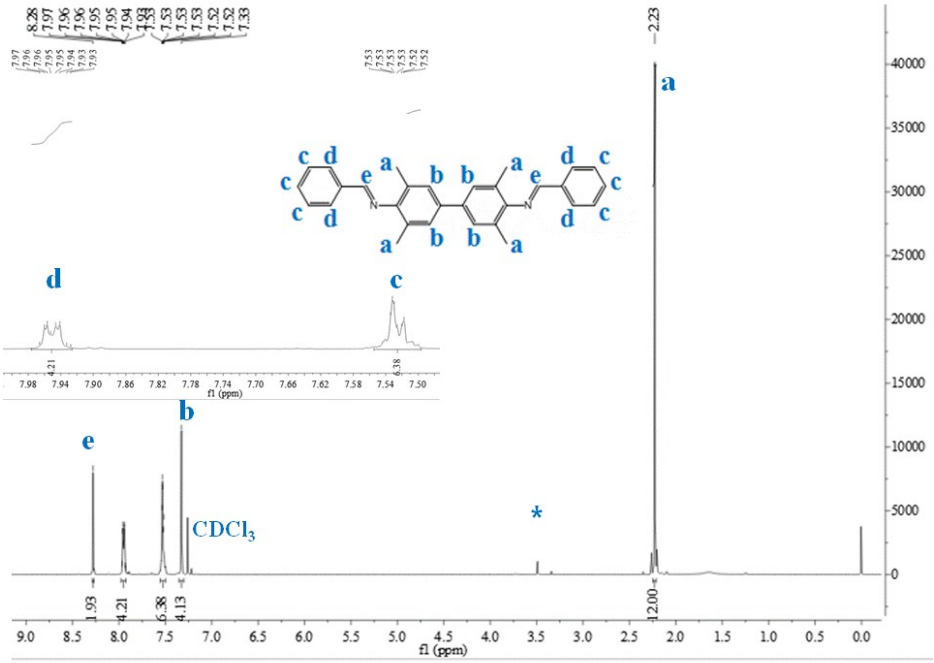

Fig. S4 ^{1}H NMR spectrum (500 MHz) of TMBB in $CDCl_3$.

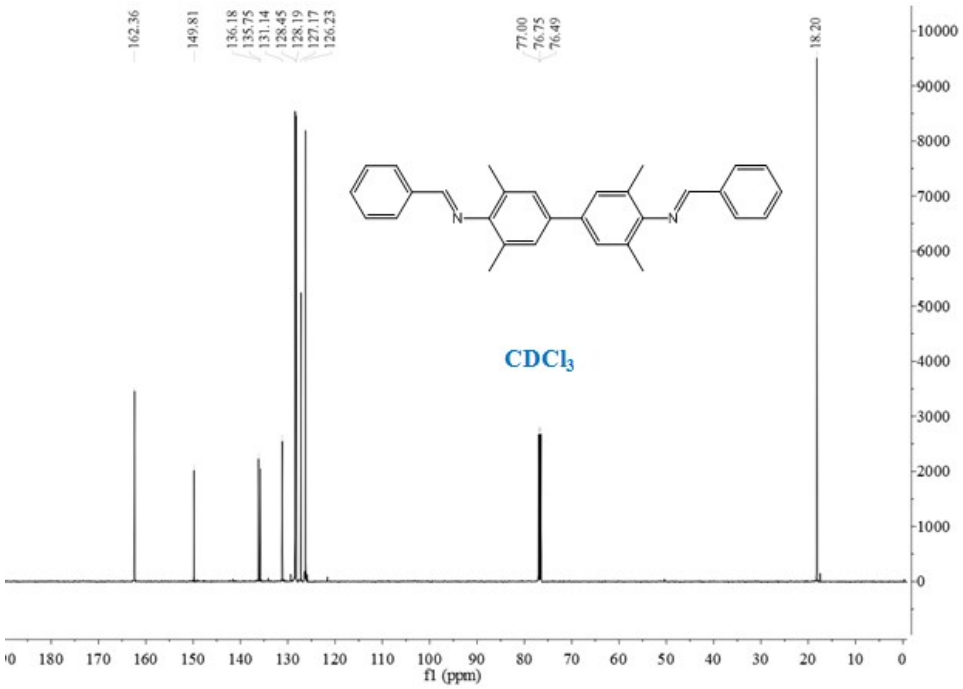

Fig. S5 ^{13}C NMR spectrum (500 MHz) of TMBB in $CDCl_3$.

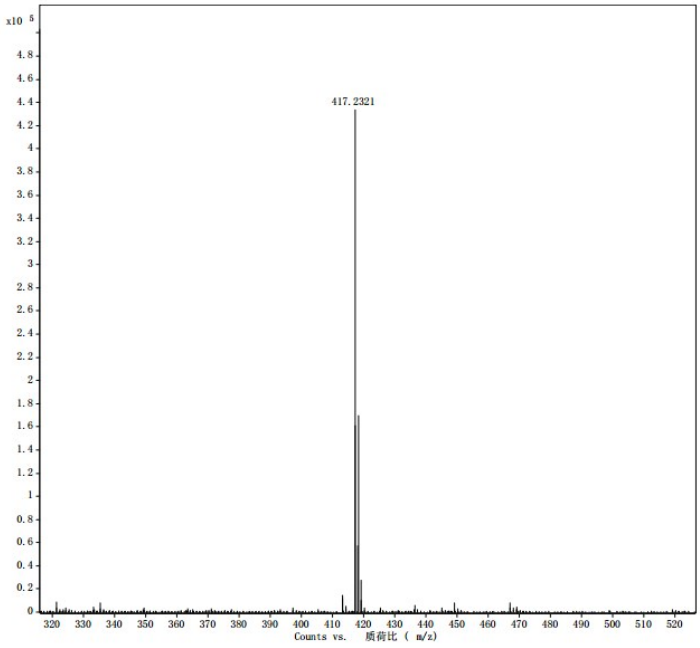

Fig. S6 The ESI-MS result of TMBB.

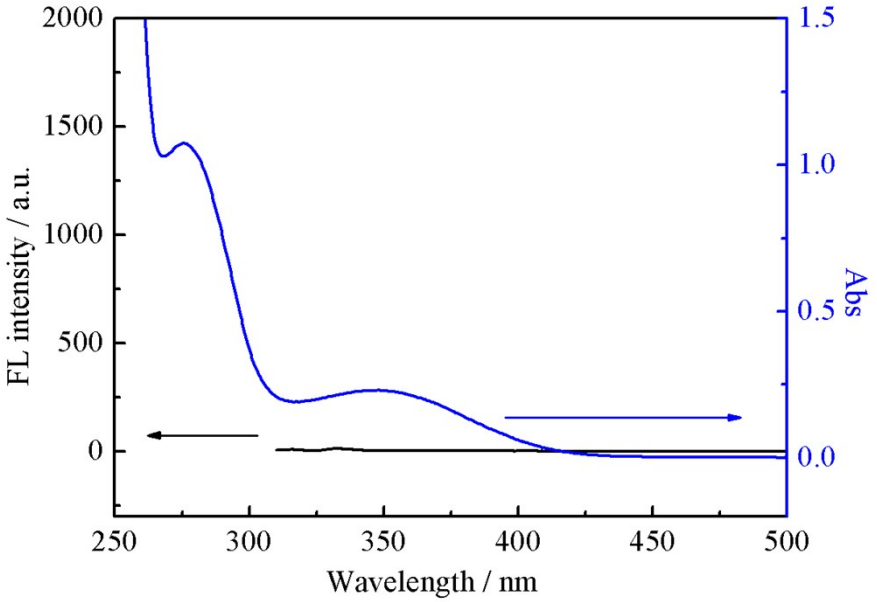

Fig. S7 The UV-vis absorption (blue line) and fluorescence emission (black line) spectra of TMBS.

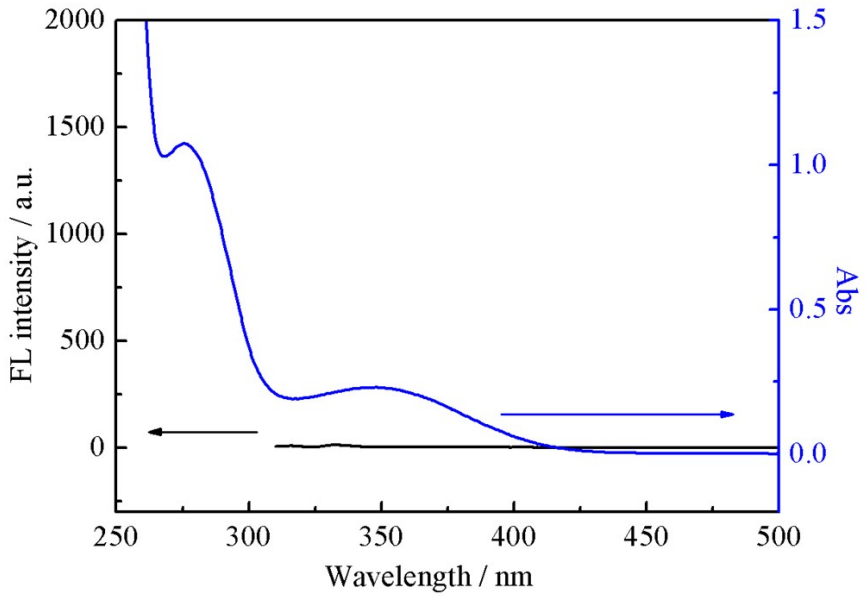

Fig. S8 The UV-vis absorption (blue line) and fluorescence emission (black line) spectra of TMBB.

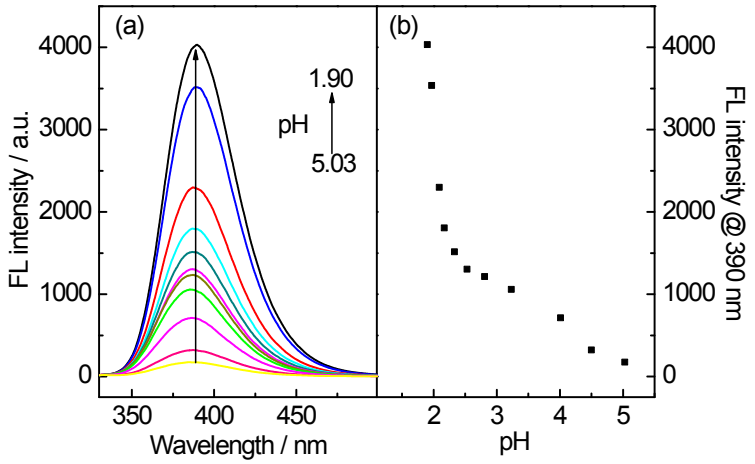

Fig. S9 (a) pH-dependent fluorescence spectral change of TMBB solution with pH ranging from 1.90 to 5.03. (b) The relationship between the fluorescence intensity @ 390 nm wavelength and pH in TMBB solution. [TMBB] = 20 μM. The excitation wavelength is 300 nm.

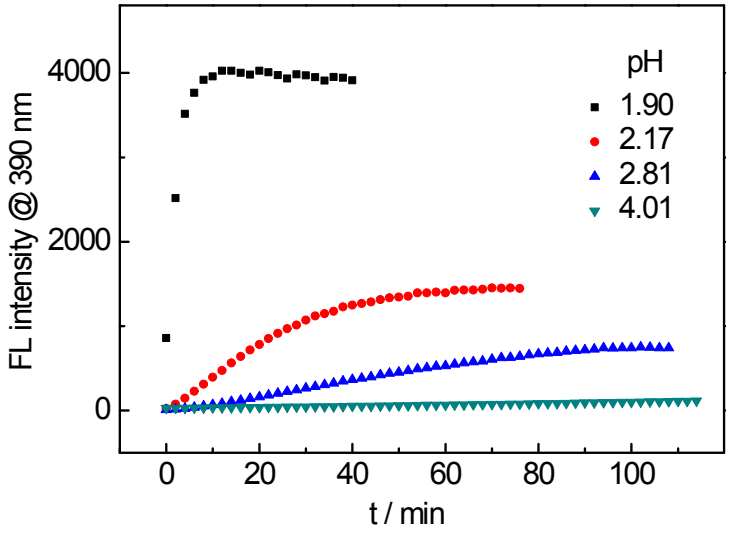

Fig. S10 The time-dependent change of fluorescence intensity at 390 nm wavelength under different pH in TMBS solution. [TMBS] = 20 μM.

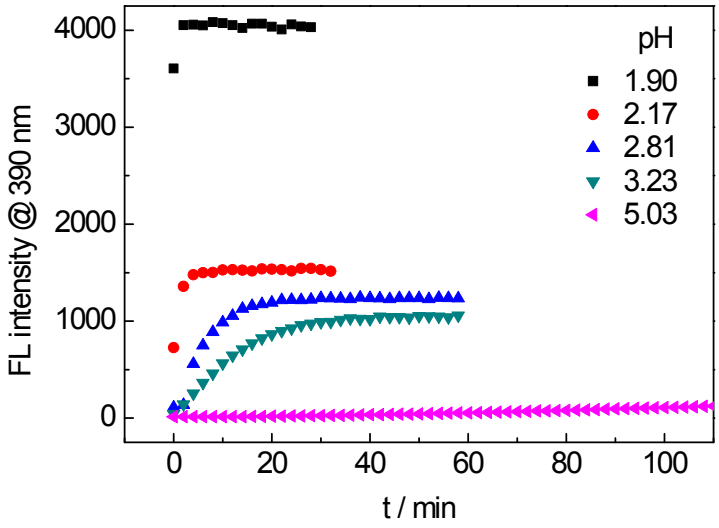

Fig. S11 The time-dependent change of the fluorescence intensity at 390 nm wavelength under different pH in TMBB solution. [TMBB] = 20 μM.

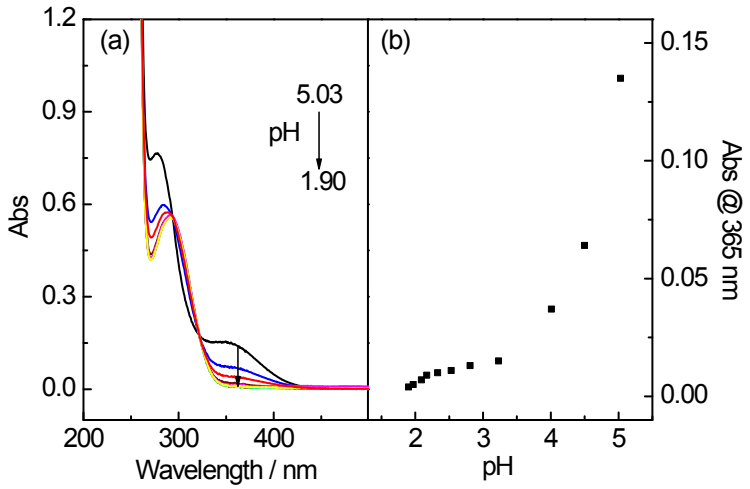

Fig. S12 (a) pH-dependent UV-vis absorption spectral change of TMBB solution with pH ranging from 1.90 to 5.03. (b) The relationship between the absorbance @ 365 nm wavelength and pH in TMBB solution. [TMBB] = 20 μM.

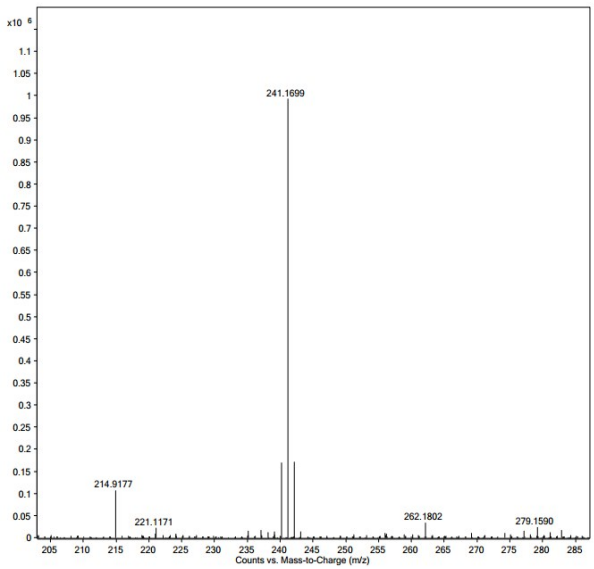

Fig. S13 ESI-MS experimental result of the hydrolyzed product of TMBS in pH of 1.8. The main peak of m/z of 241.1699 can be ascribed to [TMB + H^+].

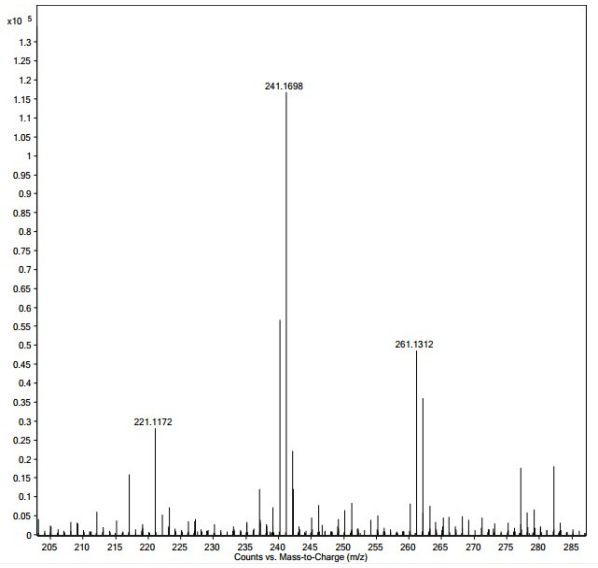

Fig. S14 ESI-MS experimental result of the hydrolyzed product of TMBB in pH of 1.8. The main peak of m/z of 241.1698 can be ascribed to [TMB + H^+].

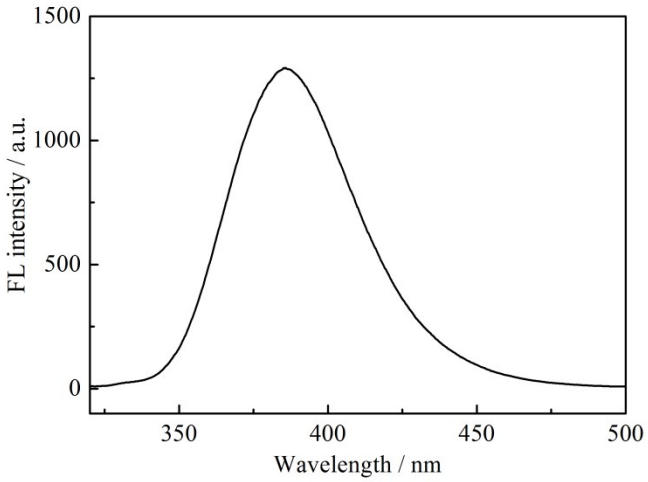

Fig. S15 The fluorescence emission spectrum of TMB. The excitation wavelength is 300 nm.

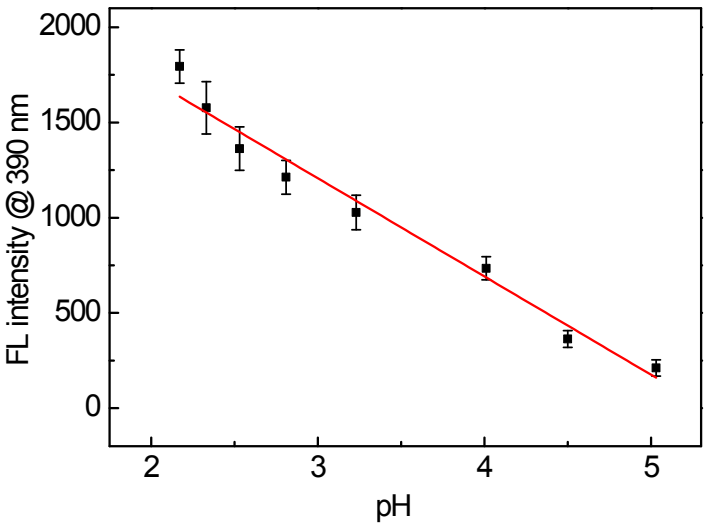

Fig. S16 The linear response curve of the fluorescence intensity at 390 nm wavelength towards different pHs in TMBB solution. [TMBB] = 20 μM. The linear regression equation was fitted as F = 2755.27 - 516.02 pH with a good linear coefficient of 0.975.

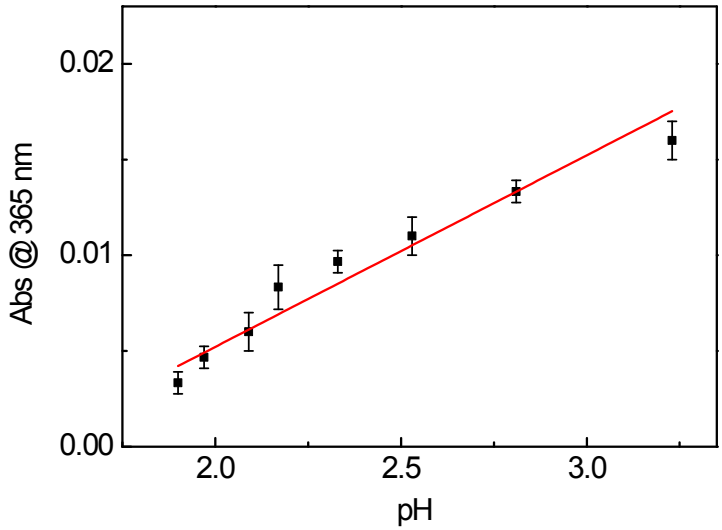

Fig. S17 The linear response curve of the absorbance at 365 nm wavelength towards different pHs in TMBB solution. [TMBB] = 20 μM. The linear regression equation was fitted as Abs = -0.015 + 0.01 pH with a good linear coefficient of 0.954.

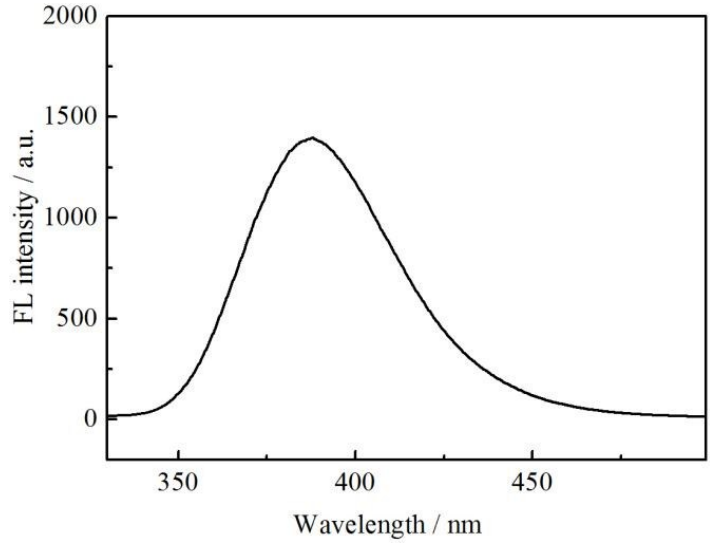

Fig. S18 The fluorescence response of TMBS towards Chinese rice vinegar. The excitation wavelength is 300 nm. 1 mL Chinese rice vinegar sample was added to the tube containing 1 mL TMBS solution and 1 mL DMF, resulting in that the final TMBS concentration was 20 μM. The fluorescence intensity at 390 nm

wavelength was introduced into the linear regression equation of Fig. 4 (F = 2997.94 - 715.03 pH) to calculate the pH of Chinese rice vinegar to be 2.24. The pH value of the corresponding sample was measured by a pH meter with a standard method to be 2.26.

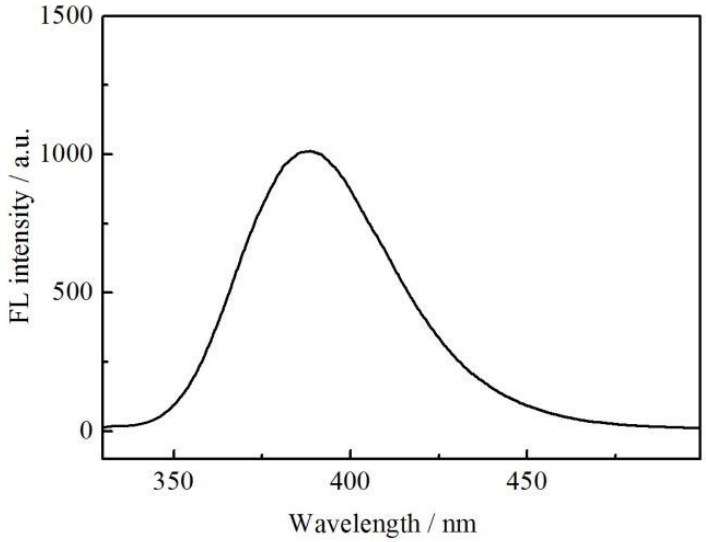

Fig. S19 The fluorescence response of TMBS towards lemon juice. The excitation wavelength is 300 nm. Lemon juice was filtered and the filtrate was directly diluted 10 times by ultra-pure water. 1 mL diluted sample was added to the tube containing 1 mL TMBS solution and 1 mL DMF, resulting in that the final TMBS concentration was 20 μM. The fluorescence intensity at 390 nm wavelength was introduced into the linear regression equation of Fig. 4 (F = 2997.94 - 715.03 pH) to calculate the pH of lemon juice sample to be 2.78. The pH value of the diluted sample was measured by a pH meter with a standard method to be 2.76.

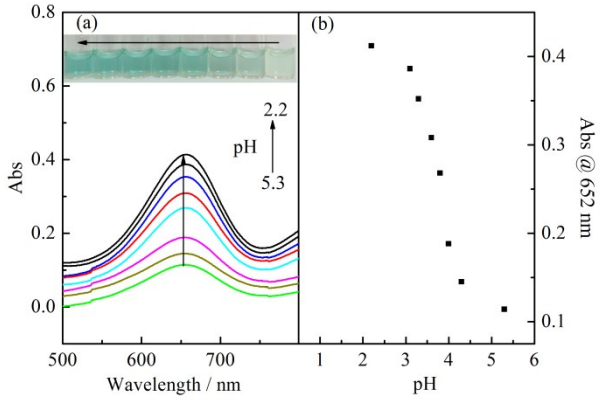

Fig. S20 (a) The UV-vis absorption spectral change of the Fe^{3+} - H_2O_2 catalytic system in 0.2 M HAc - NaAc buffer solution of pH 4.2 with changing pH of TMBB solution which could release TMB as the catalytic substrate. (b) The relationship between the absorbance at 652 nm wavelength and pH of TMBB solution. Insect of (a) is the photos of the color change of the catalytic chromogenic reaction with lowering pH.

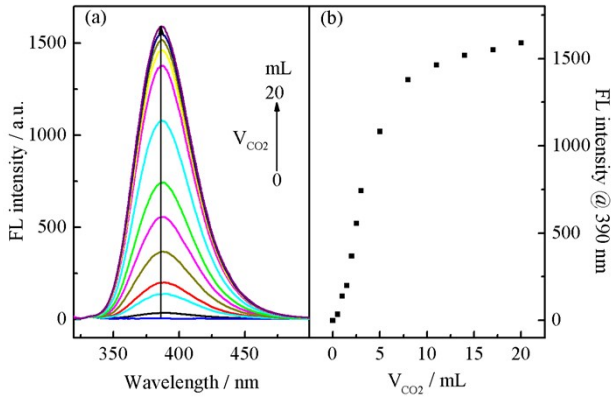

Fig. S21 CO_2-dependent fluorescence spectral change of TMBB solution with CO_2 volume changing from 0 to 20 mL. (b) The relationship between the fluorescence intensity @ 390 nm wavelength and CO_2 volume bubbled through TMBB solution. [TMBB] = 20 μM. The excitation wavelength is 300 nm.

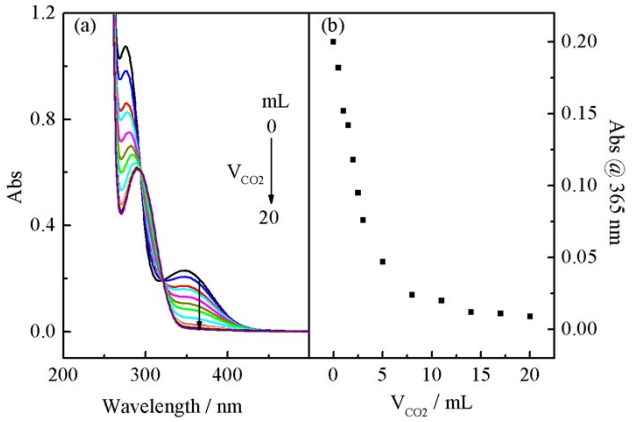

Fig. S22 (a) CO_2-dependent UV-vis absorption spectral change of TMBB solution with CO_2 volume changing from 0 to 20 mL. (b) The relationship between the absorbance @ 365 nm wavelength and CO_2 volume bubbled through TMBB solution. [TMBB] = 20 μM. The linear regression equation could be obtained as Abs @ 365 nm = 0.2 - 0.04 V_{CO2} (R^2=0.995, the CO_2 volume ranges from 0 to 3.0 mL).

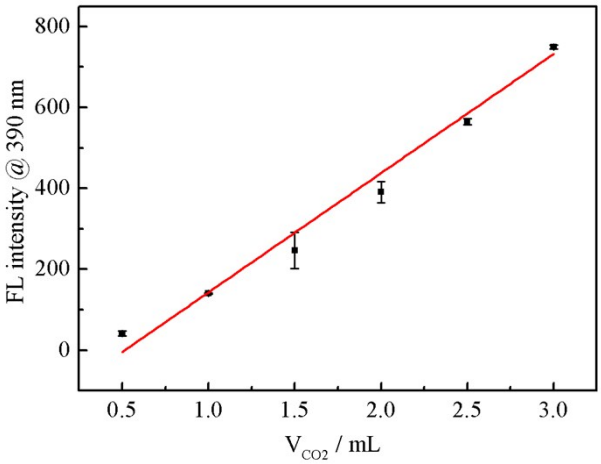

Fig. S23 The linear response curve of the fluorescence intensity at 390 nm wavelength towards different CO_2 volumes bubbled through TMBB solution. [TMBB] = 20 μM. The linear regression equation was fitted as F = -151.5 + 294.75 V_{CO2} with a good linear coefficient of 0.994.

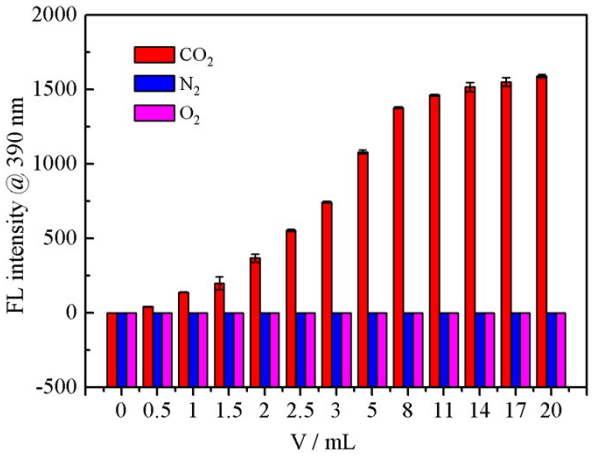

Fig. S24 The response of the fluorescence intensity at 390 nm wavelength of TMBB solution under different volumes of CO_2, N_2, and O_2 gas. [TMBB] = 20 μM.

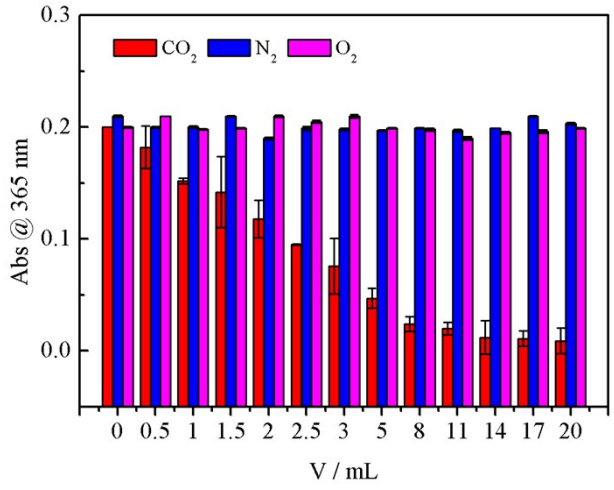

Fig. S25 The response of the UV-vis absorbance at 365 nm wavelength of TMBB solution under different volumes of CO_2, N_2, and O_2 gas. [TMBB] = 20 μM.

The calculation of LOD of CO_2 (by molar concentration):

For TMBS:

Because the aqueous solubility and the gas density of CO_2 are 1.45 $g.L^{-1}$ (that is, 1.45 g CO_2 gas can be dissolved in 1 L water) and 1.96 $g.L^{-1}$, respectively, the aqueous solubility of CO_2 can be changed as w=1.45/1.96 = 0.74 L/L (that is, 0.74 L CO_2 gas can be dissolved in 1 L water).

On the other hand, the LOD of CO_2 (by volume) $V_t = 3s/k = 3\times1.14 / 340.48 = 0.01$ mL.

The total volume in the reaction V_0 is 3 mL. Therefore, the LOD of CO_2 (by molar concentration) is $c = \frac{(V_t \times w)\times \rho}{Mr \times V_o} = \frac{(0.01\times10^{-3}\times0.74)\times1.96}{44\times3\times10^{-3}} = 110\times10^{-6} M$.

Where, Mr is the relative molecular mass of CO_2 ($g.mol^{-1}$). ρ is the density of CO_2 gas ($g.L^{-1}$).

For TMBB:

The LOD of CO_2 (by volume) $V_t = 3s/k = 3\times1.20 / 294.75 = 0.012$ mL.

The total volume in the reaction V_0 is 3 mL. Therefore, the LOD of CO_2 (by molar concentration) is $c = \frac{(V_0 \times w)\times \rho}{Mr \times V_t} = \frac{(0.012\times10^{-3}\times0.74)\times1.96}{44\times3\times10^{-3}} = 132\times10^{-6} M$

Where, Mr is the relative molecular mass of CO_2 ($g.mol^{-1}$). ρ is the density of CO_2 gas ($g.L^{-1}$).

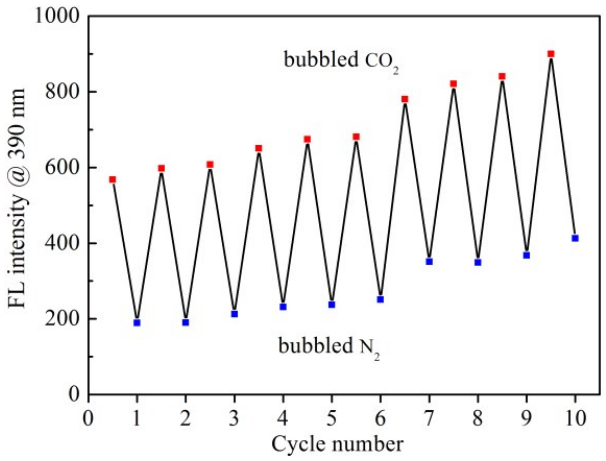

Fig. S26 The fluorescence intensity at 390 nm wavelength reversible change of TMBB solution bubbled by CO_2 and N_2 gas in turn for 10 cycles. [TMBB] = 20 μM.

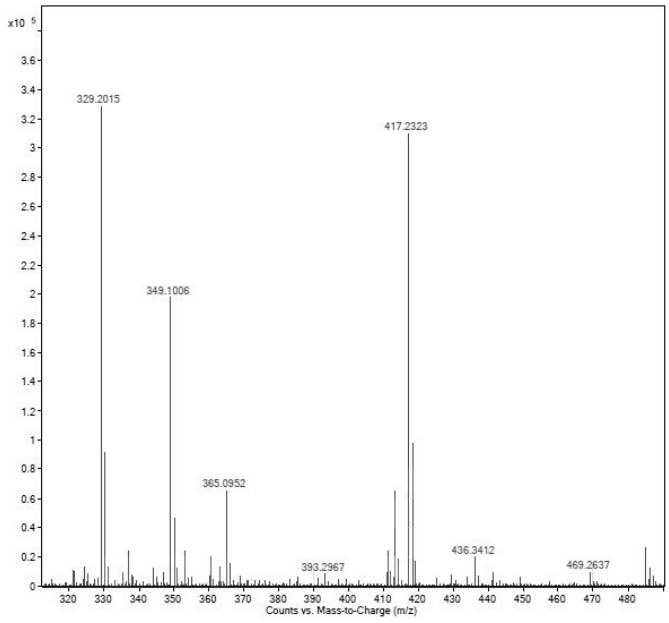

Fig. S27 ESI-MS experimental result of TMBB after the CO_2 gas-bubbled solution was bubbled by N_2 gas.

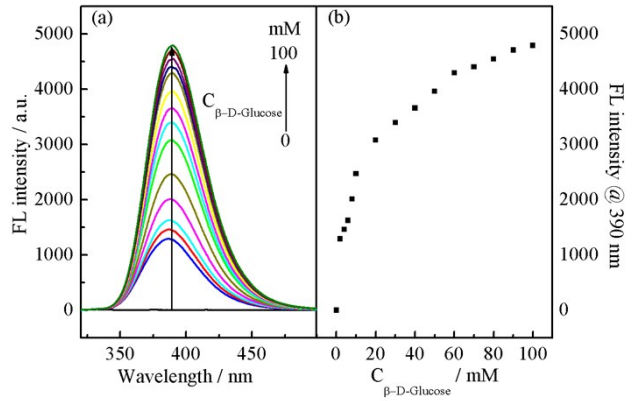

Fig. S28 (a) The change of the fluorescence spectra of TMBB under different *β*-D-glucose concentrations. (b) The relationship between the fluorescence intensity @ 390 nm wavelength and *β*-D-glucose concentration. [TMBB] = 20 μM. The excitation wavelength is 300 nm.

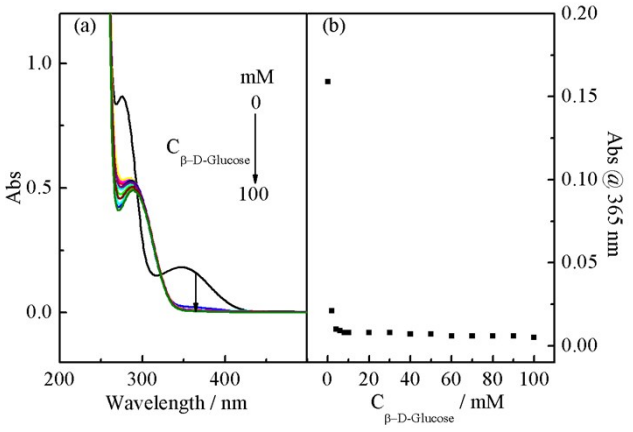

Fig. S29 (a) The change of the UV-vis absorption spectra of TMBB under different *β*-D-glucose concentrations. (b) The relationship between the absorbance @ 365 nm wavelength and *β*-D-glucose concentration. [TMBB] = 20 μM.

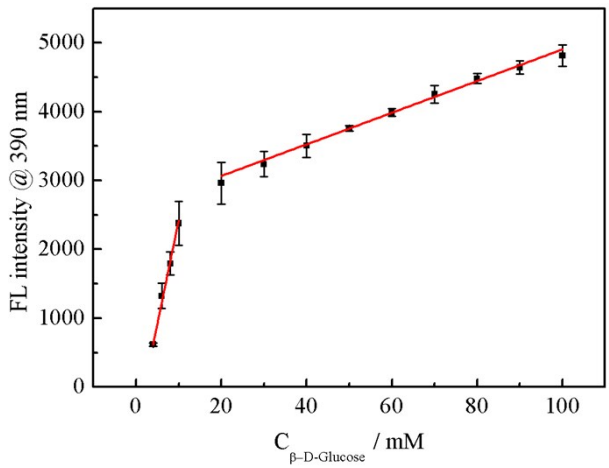

Fig. S30 The linear relationship between fluorescence intensity @ 390 nm wavelength and *β*-D-glucose concentration in TMBS case. [TMBS] = 20 μM. The linear regression equations are F = -582.54 + 300.47 $C_{\beta\text{-D-glucose}}$ (R^2 = 0.994, the concentration range from was 4 mM to 10 mM) and F = 2606.58 + 22.99 $C_{\beta\text{-D-glucose}}$ (R^2 = 0.994, the concentration range was from 20 mM to 100 mM), respectively.

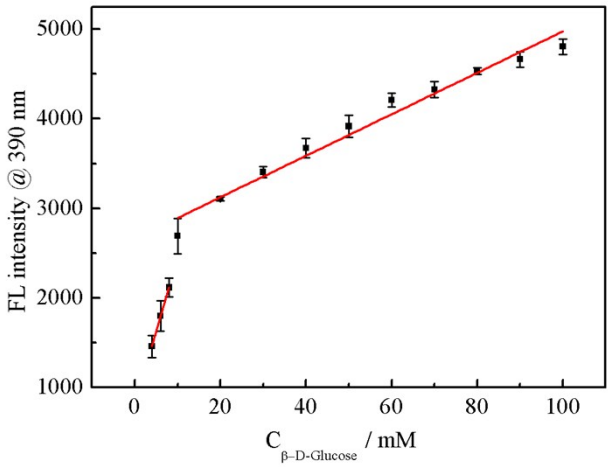

Fig. S31 The linear relationship between fluorescence intensity @ 390 nm wavelength and *β*-D-glucose concentration in TMBB case. [TMBB] = 20 μM. The linear regression equations are F = 802.9 + 164.42 $C_{\beta\text{-D-glucose}}$ (R^2 = 0.999, the concentration range was from 4 mM to 8 mM) and F = 2661.2 + 23.16 $C_{\beta\text{-D-glucose}}$ (R^2 = 0.991, the concentration range was from 10 mM to 100 mM), respectively.