Electronic Supplementary Information

Fluorescein as an artificial enzyme to mimic peroxidase

Li Liu [1,2], Ying Shi, Yufang Yang[1], Menglu Li[1], Yijuan Long[1], Yuming Huang[1]*, Huzhi Zheng[1]*

[1]Key Laboratory on Luminescent and Real-Time Analytical Chemistry, Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China

[2]College of Chemistry and Chemical Engineering, Qujing Normal University, Qujing, 655011, P. R. China

*Corresponding authors. E-mail addresses: ymhuang@swu.edu.cn (Y. Huang); zhenghz@swu.edu.cn (H. Zheng).

Experimental

Reagents and chemicals

Horseradish peroxidase (HRP, 300 U mg^{-1}), fluorescein (Reagent Ph. Eur. grade) and 3,3′,5,5′-tetramethylbenzidine (TMB) (99%) were purchased from Sigma-Aldrich. H_2O_2 (30%) was purchased from Chongqing Chuandong Chemical Co., Ltd. (Chongqing, China). Acetate (HPLC grade, ≥99.9%) was obtained from Shanghai Mackin biochemical Co. Ltd. (Shanghai, China). Fluorescein chloride (HPLC grade, 95.0%) and Fluorescein diacetate (HPLC grade, 98.0%) were purchased from TCI Chemical Industry (Tokyo, Japan). Rhodamine B (HPLC grade, 95.0%), rhodamine 6G (95%) and sodium acetate anhydrous (99.99% metals basis) were obtained from Aladdin (Shanghai, China). All other reagents were of analytical grade and were used directly with no further purification. Ultrapure water (18.2 MΩ) was prepared with a Milli-Q system (USA) and used in all experiments.

Electron spin resonance (ESR)

DMPO was used to trap the •OH radicals to form the DMPO/•OH spin adduct. 50 mM DMPO, 10 mM H_2O_2 and fluorescein with different concentrations were added in 0.20 M HAc-NaAc buffer solutions (pH 3.0), and then the prepared samples were transferred to a quartz capillary tube and placed in the ESR cavity. The ESR spectra were recorded on an X-band JES FA200 ESR Spectrometer (JEOL, Japan) (sweep time 60 s; modulation width, 0.1 mT; modulation amplitude, 2 Gauss; frequency power, 0.998 mW; modulation frequency, 9.066 GHz).

Peroxidase-like activity of fluorescein

In the presence of H_2O_2, the peroxidase-like activity of fluorescein was evaluated through the catalytic oxidation of peroxidase substrate TMB with major absorbance peaks at 652nm. In a typical experiment, 125 μL of fluorescein (2.0 mM in ethanol anhydrous solution), followed by 125 μL of TMB solution (12 mM) and 250 μL of H_2O_2 (100 mM) were added into 2.0 mL HAc-NaAc buffer solution (0.20 M, pH 3.0).

The absorbance change at 652 nm was recorded using a UV-2450 UV-Vis spectrophotometer (Shimadzu, Japan).

Robustness and stability of the fluorescein as enzyme mimic

To examine its robustness, fluorescein was first incubated at a range of temperatures from 4 to 90 °C, a range of pH values from 1 to 12 and a range of NaCl concentrations from 0 to 500 mM for 2 h, respectively. Then their activities were measured under conditions identical to those mentioned above. Activity of 100% is set where absorption is highest and the relative activities for other absorptions are calculated accordingly. Error bars represent the standard deviation of three independent measurements.

Kinetic Assay

Kinetic measurements were carried out by monitoring the absorbance change at 652 nm with a 1 min interval. Experiments were carried out using 1.0 ng mL^{-1} HRP in 4.0 mL of 0.20 M phosphate buffer (pH 5.0) at 35°C or 100 μM fluorescein in 4.0 mL of 0.20 M NaAc buffer (pH 3.0) at 35°C. To investigate double reciprocal plots of activity of fluorescein, assays were carried out under standard reaction conditions as described above by varying concentrations of TMB at a fixed concentration of H_2O_2 or vice versa. The Michaelis-Menten constant was calculated using the Lineweaver-Burk plot: $1/v = K_m/ (V_{max}[S]) +1/V_{max}$, where v is the initial velocity, V_{max} is the maximal reaction velocity, and [S] corresponds to the substrate concentration.

Ultra performance liquid chromatography-mass spectrum (UPLC-MS) Analysis

A Waters (Milford, MA) Xevo TQ-S mass spectrometer coupled to a Waters ACQUITY H-Class UPLC system was used. Separation was carried out with a Waters BEH C18 column (2.1 × 50 mm, 1.7 m particle size) with column oven temperature at 40 °C. The injection volume was 10 μL, and the UPLC flow rate was 200 μL/min using an isocratic elution mobile phase with 50% water and 50% 0.1% formic acidin (FA, in methanol).

Mass spectra were acquired using electrospray ionization in positive ion mode and multiple reaction monitoring (MRM). Nitrogen was used as desolvation gas at a flow rate of 650 L/h; argon at a pressure around 3.0×10^{-3} mbar was used as collision gas at a flow rate of 0.15 ml/min. The ion source and the desolvation temperature were maintained at 150 and 350 °C, respectively. Collision energy was set at 45, 35 electron volt, respectively, for the above mentioned transitions at a delay time of 0.005 s. All aspects of system operation and data acquisition were controlled using Masslynx v4.1 software with automated data processing using the Quanlynx Application Manager (Waters).

Detection Procedure of H_2O_2

H_2O_2 detection was carried out as follows: 125 μL of 2.0 mM fluorescein, 125 μL of 12 mM TMB and 250 μL of different concentrations of H_2O_2 were added into 2.0 mL of 0.20 M HAc-NaAc buffer solution (pH 3.0). The resulting mixture was incubated at 35 °C for 30 min. Then UV-vis spectra measurements were taken.

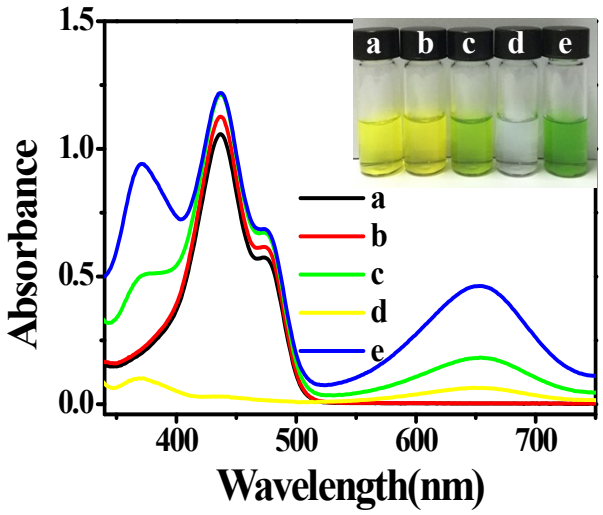


Fig. S1 UV-vis absorbance spectra of different reaction systems: (a) 100 μM fluorescein (black line), (b) 100 μM fluorescein + 10 mM H_2O_2 (red line), (c) 100 μM fluorescein + 0.60 mM TMB (green line), (d) 0.60 mM TMB + 10 mM H_2O_2 (yellow line), (e) 0.6 mM TMB + 10 mM H_2O_2 + 100 μM fluorescein (blue line) in NaAc buffer (pH 3.0) after incubation for 30 min at 35 °C. The insert shows the corresponding optical photographs.

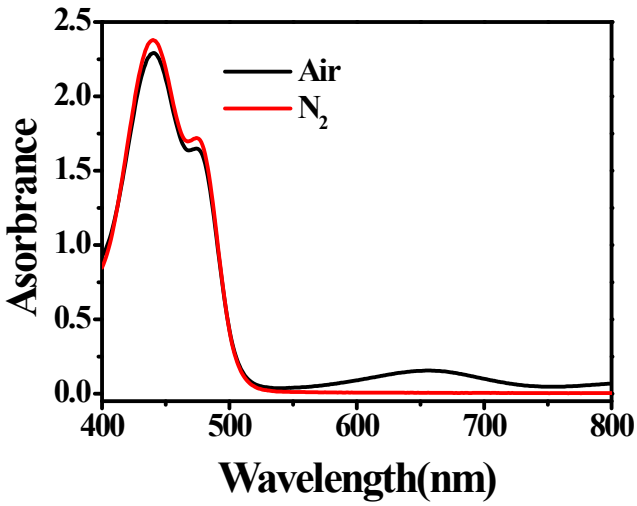


Fig. S2 The absorbance spectra of TMB in the presence of fluorescein in an air (black line) or N_2 atmosphere for 30 min (red line). Reaction condition: 0.60 mM TMB and 50 μM fluorescein in NaAc buffer (pH 3.0) for 30 min.

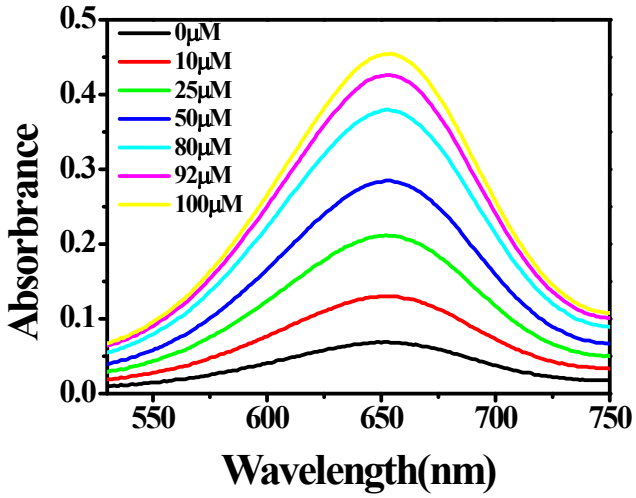


Fig. S3 The absorbance spectra of the TMB in the presence of fluorescein with different concentrations. Reaction conditions: 0.60 mM TMB, 10 mM H_2O_2, 0.20 M acetate buffer (pH 3.0), 35°C for 30min.

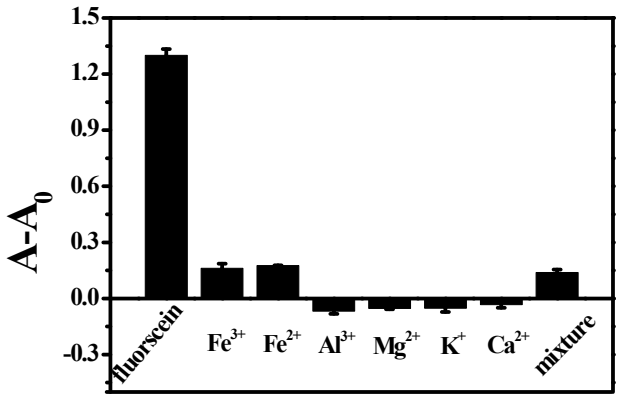


Fig. S4 Catalytic activity of metal ions. 0.60 mM TMB and 10 mM H_2O_2 in 0.20 M NaAc buffer (pH 3.0, 35°C) in the presence of a kind of metal ion or a mixture of five metal ions (Fe^{3+} , Al^{3+} , Mg^{2+} , K^+ and Ca^{2+}) were used to test the catalytic activity. The final concentrations of fluorescein, Fe^{3+}, Fe^{2+}, Al^{3+}, Mg^{2+} , Ca^{2+} and K^+ were 100 μM, 30, 30, 50, 50, 50 and 50 nM, respectively. A_0 and A is the absorbance in the absence and presence of fluorescein.

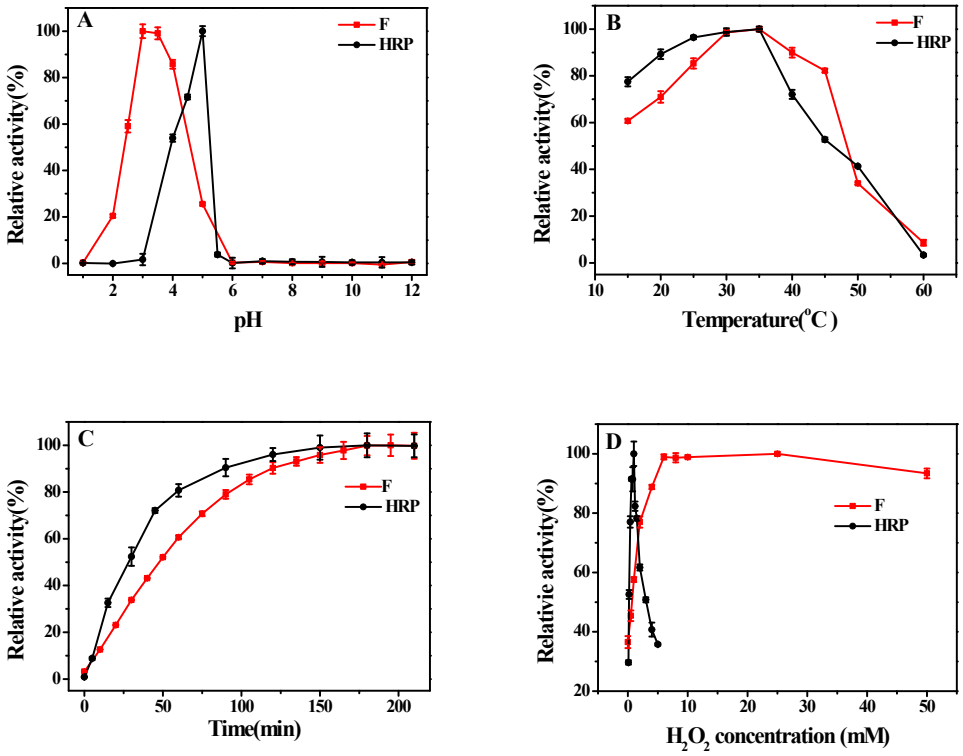


Fig. S5 Effects of pH (A), temperature (B), reaction time (C), H_2O_2 concentration (D) on the peroxidase-like activity of fluorescein (red line) and HRP (black line). Experiments were carried out using 100 μM fluorescein or 1.0 ng mL^{-1} HRP. Details were showed in the Experimental Section. For each curve, the maximal point was set as 100%, and the relative activities for other absorptions were calculated accordingly. Error bars indicate the standard deviations of three independent measurements.

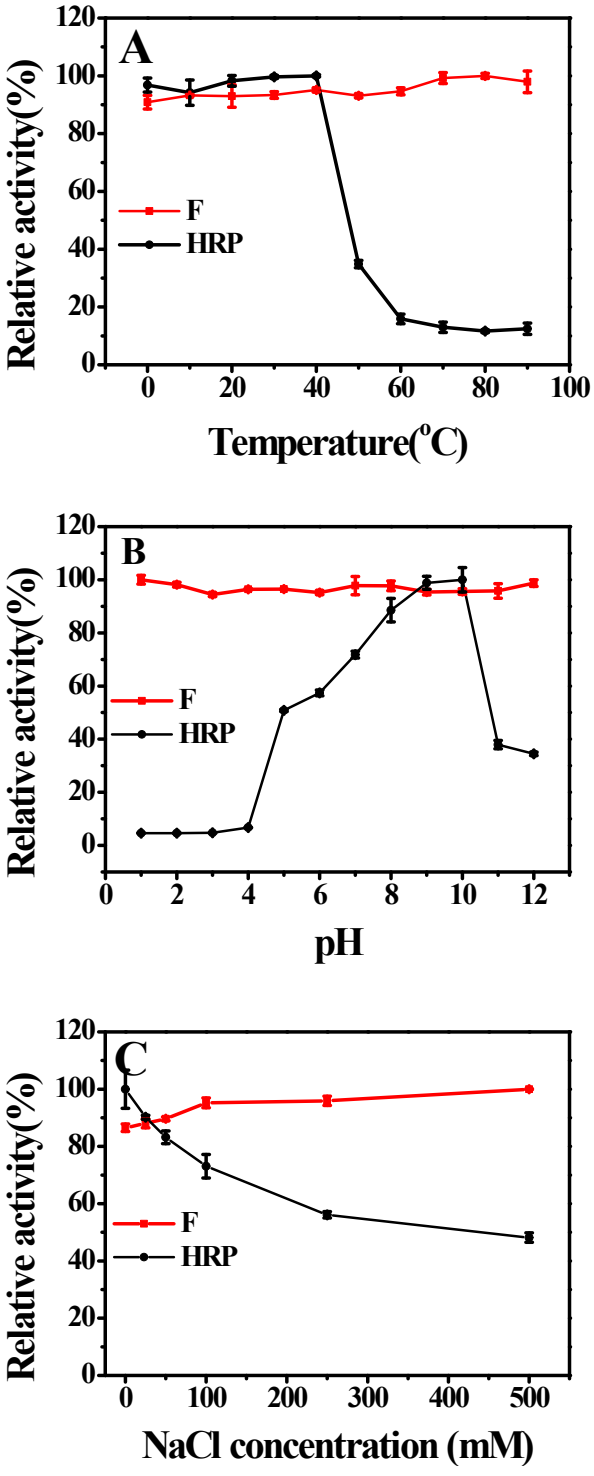


Fig. S6 Comparison of stability of fluorescein (red line) and HRP (black line) towards the relative catalytic activity after incubation at different temperatures (A), at a range of pH values (B), at a range concentrations of NaCl (C) for 2 h, and the activity was measured under optimum conditions. For each curve, the maximum point was set as 100% and the relative activities for other absorptions were calculated accordingly. Error bars indicate the standard deviations of three independent measurements.

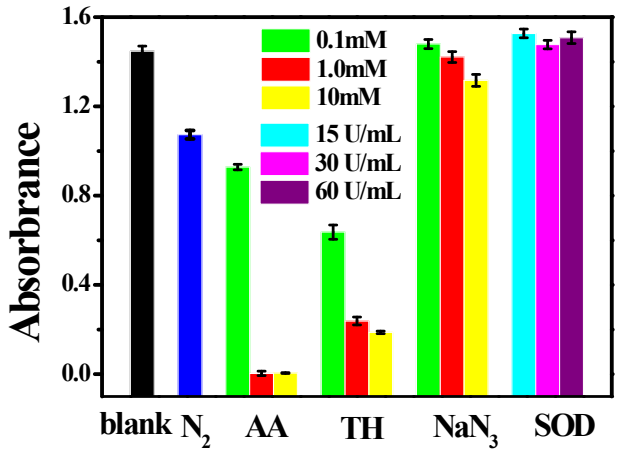


Fig. S7 Effects of scavengers on the TMB-H_2O_2-fluorescein chromogenic reaction. Absorbance of oxTMB at 652 nm have been monitored in presence of different radical scavengers: blank (without any scavenger), AA (ascorbic acids), TH (thiourea), NaN_3 and SOD. Procedures: 0.60 mM TMB, 10 mM H_2O_2, 100μM fluorescein and different concentration scavenger were mixed in 0.20 M NaAc (pH 3.0), at 35°C for 180 min incubation.

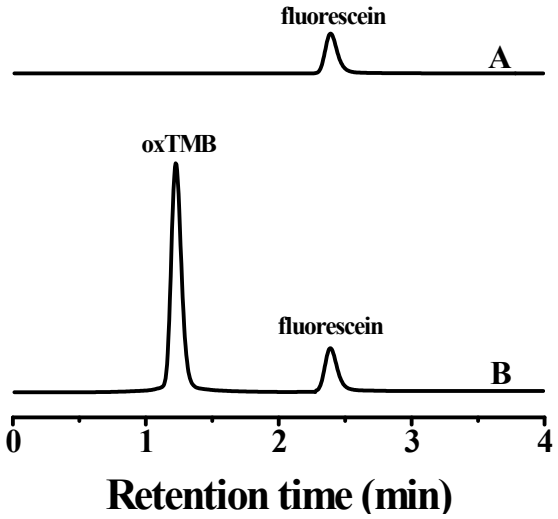
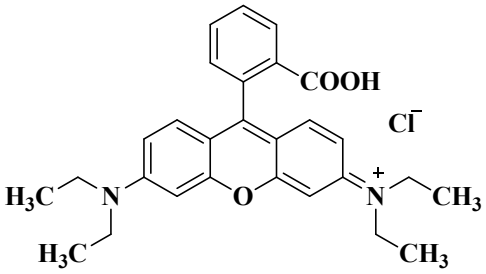
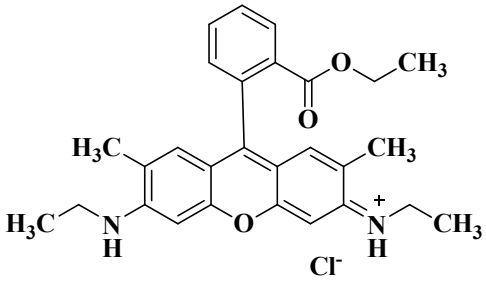


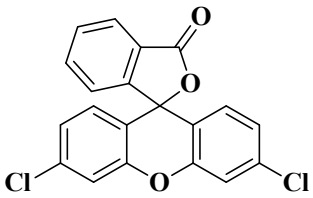
Fig. S8 Representative chromatogram of (A) 100μM fluorescein and (B) 0.60 mM TMB + 10 mM H_2O_2 + 100μM fluorescein were incubated under optimal conditions.



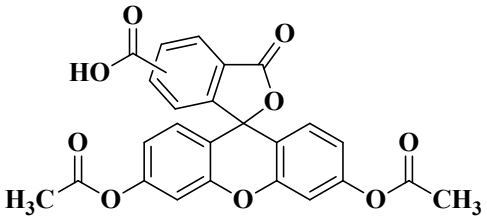
Rhodamine B (inactive)



Rhodamine 6G (inactive)



Fluorescein Chloride (inactive)



Fluorescein diacetate (inactive)

Fig. S9 Structures of four kinds of xanthene dyes

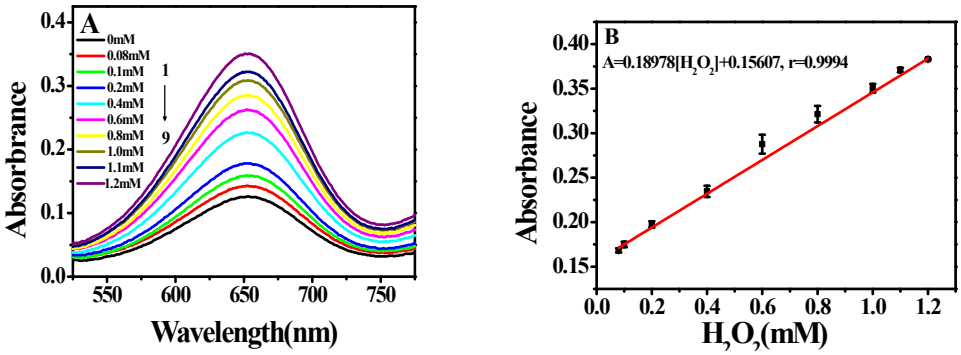


Fig. S10 (A) UV-visible spectra of fluorescein system with different concentration of H_2O_2. (B) A calibration curve for H_2O_2 detection at 652 nm under the optimum conditions.

Table S1 IPC-MS analysis of element content in fluorescein

Element	Content(%)	Element	Content(%)	Element	Content(%)	Element	Content(%)
Al	0.0015% (18 nM)	W	<0.0001	Co	<0.0001	Sb	<0.0001
Ca	0.0056% (47 nM)	Li	<0.0001	Ni	<0.0001	Sn	<0.0001
Fe	0.0051% (30 nM)	Be	<0.0001	As	<0.0001	Pb	<0.0001
Mg	0.0006% (8.3 nM)	Ti	<0.0001	Cu	<0.0001	Hg	<0.0001
K	0.0015% (13 nM)	V	<0.0001	Zn	<0.0001	Cr	<0.0001
Na	0.024	Mn	<0.0001	Mo	<0.0001	P	0.0005

Table S2. Waters (Milford, MA) Xevo TQ-S mass spectrometry parameters used to analyze the catalytic productions

Compound	Migration time (min)	MRM (*m/z*)	Cone (v)	Collision energy (v)	Dwell(s)
fluorescein	2.39	333.27>202.11	35	45	0.1
TMB	1.22	240.22>195.11	35	35	0.1