Electronic Supplementary Information

5-Carboxyfluorescein: Intrinsic peroxidase-like catalytic activity and its application to biomimetic synthesis of polyaniline nanoplatelets

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Experimental

Reagents and chemicals

5-Carboxyfluorescein (5-FAM) was purchased from TCI (Shanghai) Development Co., Ltd. (Shanghai, China). Thiourea (TU) and *N*-methyl-2-pyrrolidinone (NMP) were purchased from J&K Scientific Ltd. (Shanghai, China). Horseradish peroxidase (HRP, 250~330 U mg^{-1}), superoxide dismutase (SOD, 3000 U mg^{-1}), 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), 3,3',5,5'-tetramethylbenzidine (TMB), ascorbic acid (AA), and aniline (purity 99.5%) were purchased from Sigma-Aldrich (St. Louis, MO). Hydrogen peroxide (H_2O_2, 30%), *N,N*-dimethylformamide (DMF), and concentrated hydrochloric acid (HCl, 38%) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium azide (NaN_3) was purchased from Xiya Reagent Co., Ltd (Chengdu, China). All other chemicals were of analytical grade and used as supplied. Ultrapure water (≥ 18.25 MΩ) purified by Millipore Milli-Q water purification system was used in all experiments.

Apparatus

Absorption spectra and the absorbance at 654 nm (A_{654}) were recorded on a Shimadzu UV-3600 spectrophotometer. Inductively coupled plasma mass spectrometry (ICP-MS) was collected using an iCAP Q spectrometer (Thermo Scientific, USA). Electron paramagnetic resonance (EPR) was recorded on a Bruker EMX 10/12 EPR spectrometer (center field, 3480 G; sweep width, 200 G; microwave frequency, 10.034 GHz; microwave power, 20.410 mW; modulation frequency, 100 KHz; modulation amplitude, 1.0 Gauss; time constant, 40.960 ms; sweep time, 83.886 s). Fourier transform infrared (FTIR) spectroscopy was performed with a Nicolet iS10 spectrometer (Thermo Scientific, USA) and the spectra were collected after 32 scans between 4000 and 600 cm^{-1}. X-ray diffraction (XRD) patterns were acquired using Bruker AXS diffractometer (D8 advance, 40 kV, 40 mA) with Cu Kα radiation (λ = 1.5418 Å); the scan range (2θ) was 10–50°. Cyclic voltammetry (CV) was carried out in 1.0 M H_2SO_4 (nitrogen saturated) at room temperature with a RST5200F electrochemical workstation (Suzhou Risetest Electronic Co. Ltd., China). A conventional three-electrode system was used with a glassy carbon electrode (GCE, Φ = 3 mm) covered with a film of the biomimetically synthesized PANI as the working electrode, platinum wire as the auxiliary electrode, and saturated calomel electrode (SCE) as the reference electrode, respectively. A scan rate of 100 mV s^{-1} was applied and the potential of the GCE was swept between -0.2 and 0.8 V vs SCE. All potentials mentioned in this work were referred to SCE. Scanning electron microscopy (SEM) images were collected on a JEOL JSM-7800F field emission scanning electron microscope (FESEM). Transmission electron microscopy (TEM) images were obtained from FEI Tecnai G20 operated at 200 kV.

Peroxidase-like catalytic activity of 5-FAM

The peroxidase-like catalytic activity of 5-FAM was explored using TMB as the chromogenic substrate in the presence of H_2O_2. Briefly, 10 μL of 5-FAM (6.0 mM in DMF), 10 μL of TMB (60 mM in DMF), and 10 μL of 0.6 M H_2O_2 were added into 570 μL of 0.2 M HAc/NaAc buffer solution (pH 3.0); after an incubation of 30 min at 25 °C, the absorption spectrum of the resulting solution was recorded.

Biomimetic synthesis of PANI

The biomimetic synthesis of PANI was carried out in 1.0 M HCl, containing 0.2 M NMP. In the reaction solution, the final concentrations of 5-FAM, aniline, and H_2O_2 were 0.2 mM, 0.1 M, and 25 mM, respectively. The solution was incubated at 37 °C for 15 h, without any stirring or mechanical agitation, and then the resulting dark-green product was collected and purified by dialysis (dialysis tube, 12,000-14,000 MW cutoff) with ultrapure water, and dried under dynamic vacuum. For comparisons, the chemically synthesized PANI was obtained under similar conditions but in the absence of 5-FAM.

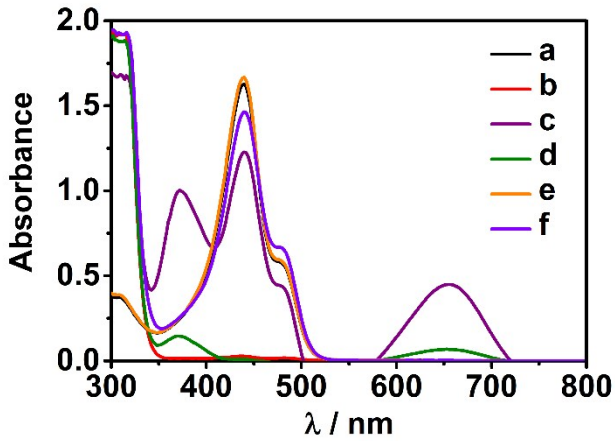

Fig. S1 Absorption spectra in the presence of (a) 0.1 mM 5-FAM, (b) 1.0 mM TMB, (c) 0.1 mM 5-FAM + 1.0 mM TMB + 10 mM H_2O_2, (d) 1.0 mM TMB + 10 mM H_2O_2, (e) 0.1 mM 5-FAM + 10 mM H_2O_2, or (f) 0.1 mM 5-FAM + 1.0 mM TMB. Experiments were carried out in 0.2 M HAc/NaAc (pH 3.0). The incubation time was 30 min.

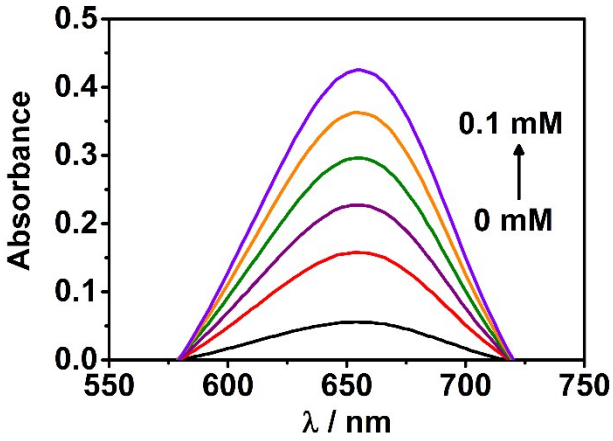

Fig. S2 Absorption spectra of the resulting solutions in the presence different concentrations of 5-FAM. Experiments were carried out in 0.2 M HAc/NaAc (pH 3.0) in the presence of 1.0 mM TMB and 10 mM H_2O_2. The incubation time was 30 min.

Table S1 IPC-MS analysis of residual metals in 5-FAM

Element	Content	Element	Content	Element	Content	Element	Content
Mg	0.016‰ (24.95 nM)	Cr	0.003‰	Cu	<0.001‰	Sb	0.001‰
Ca	0.119‰ (112.09 nM)	Mn	0.009‰ (5.83 nM)	Zn	<0.001‰	Ba	0.003‰
Al	<0.001‰	Fe	0.010‰ (6.59 nM)	Mo	0.010‰	Tl	<0.001‰
Ti	0.003‰	Co	<0.001‰	Ag	0.002‰	Pb	0.003‰
V	0.006‰	Ni	<0.001‰	Cd	0.002‰		

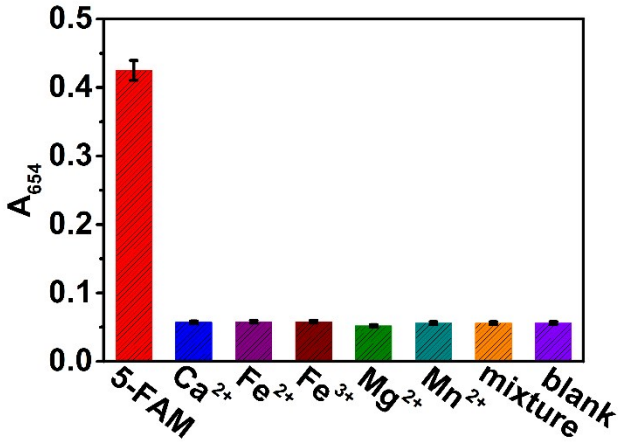

Fig. S3 A_{654} in the presence of 5-FAM (6.0 mM, 10 μL), Ca^{2+} (120 nM, 10 μL), Fe^{2+} (7.0 nM, 10 μL), Fe^{3+} (7.0 nM, 10 μL), Mg^{2+} (30 nM, 10 μL), Mn^{2+} (6.0 nM, 10 μL), their mixture, or the blank control (i.e., without the addition of 5-FAM or any kind of metal ions). Experiments were carried out in 0.2 M HAc/NaAc (pH 3.0) in the presence of 1.0 mM TMB and 10 mM H_2O_2. The incubation time was 30 min. Error bars represent the standard deviations of three repeated measurements.

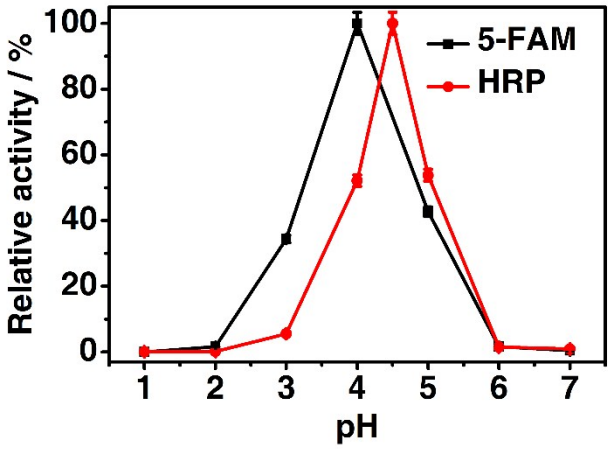

Fig. S4 Effects of pH on the catalytic activity of 5-FAM and HRP. In a typical assay, 10 μL of 6.0 mM 5-FAM (dissolved in DMF) or 10 μL of 0.3 ng mL^{-1} HRP, followed by 10 μL of 60 mM TMB (dissolved in DMF) and 10 μL of 0.6 M H_2O_2 were added into 570 μL of 0.2 M HAc/NaAc buffer solution with the pH ranging from 1.0 to 7.0; after an incubation of 30 min at 25 °C, the A_{654} was recorded. The maximum point in each curve is set as 100% and the relative activity for others is calculated accordingly. Error bars represent the standard deviations of three repeated measurements.

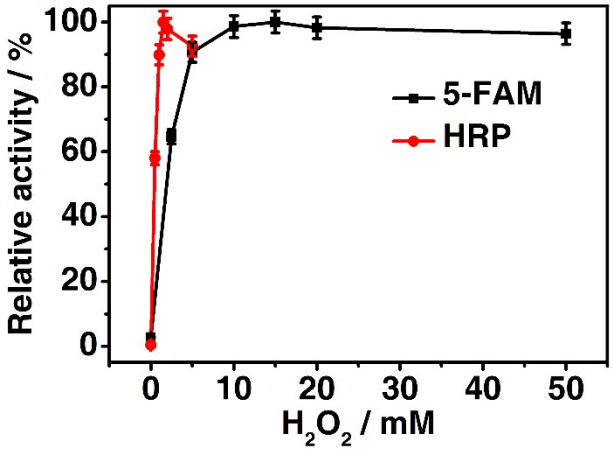

Fig. S5 Effects of H_2O_2 concentration on the catalytic activity of 5-FAM and HRP. For 5-FAM, 10 μL of 6.0 mM 5-FAM (dissolved in DMF), 10 μL of 60 mM TMB (dissolved in DMF), and 10 μL of H_2O_2 were added into 570 μL of 0.2 M HAc/NaAc buffer solution (pH 4.0), with the final concentration of H_2O_2 ranging from 0 to 50 mM; after an incubation of 30 min at 25 °C, the A_{654} was recorded. For HRP, 10 μL of 0.3 ng mL^{-1} HRP, 10 μL of 60 mM TMB (dissolved in DMF), and 10 μL of H_2O_2 were added into 570 μL of 0.2 M HAc/NaAc buffer solution (pH 4.5), with the final concentration of H_2O_2 ranging from 0 to 5.0 mM; after an incubation of 30 min at 25 °C, the A_{654} was recorded. The maximum point in each curve is set as 100% and the relative activity for others is calculated accordingly. Error bars represent the standard deviations of three repeated measurements.

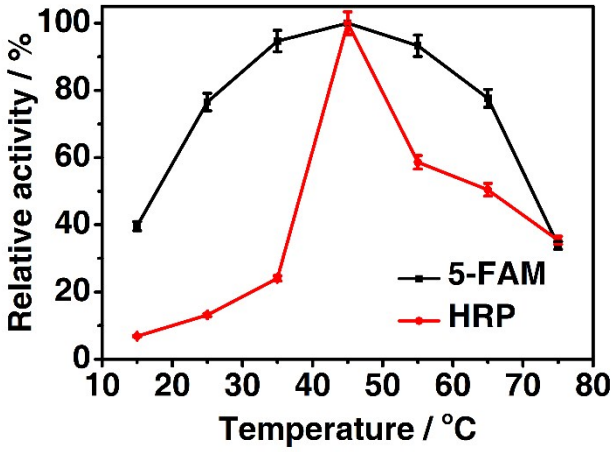

Fig. S6 Effects of reaction temperature on the catalytic activity of 5-FAM and HRP. For 5-FAM, 10 μL of 6.0 mM 5-FAM (dissolved in DMF), 10 μL of 60 mM TMB (dissolved in DMF), and 15 μL of 0.6 M H_2O_2 were added into 565 μL of 0.2 M HAc/NaAc buffer solution (pH 4.0); after an incubation of 30 min at different temperatures, the A_{654} was recorded. For HRP, 10 μL of 0.3 ng mL^{-1} HRP, 10 μL of 60 mM TMB (dissolved in DMF), and 1.5 μL of 0.6 M H_2O_2 were added into 578.5 μL of 0.2 M HAc/NaAc buffer solution (pH 4.5); after an incubation of 30 min at different temperatures, the A_{654} was recorded. The maximum point in each curve is set as 100% and the relative activity for others is calculated accordingly. Error bars represent the standard deviations of three repeated measurements.

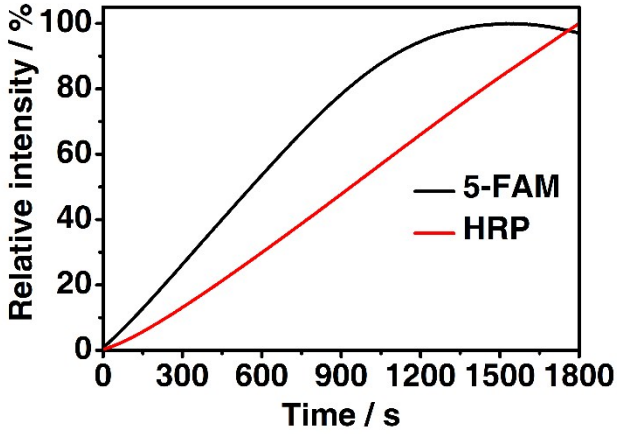

Fig. S7 Kinetic curves at 654 nm. For 5-FAM, experiments were carried out at 45 °C in 0.2 M HAc/NaAc (pH 4.0) in the presence of 0.1 mM 5-FAM, 1.0 mM TMB and 15 mM H_2O_2; for HRP, experiments were carried out at 45 °C in 0.2 M HAc/NaAc (pH 4.5) in the presence of 5.0 pg mL^{-1} HRP, 1.0 mM TMB and 1.5 mM H_2O_2. The maximum point in each curve is set as 100% and the relative intensity for others is calculated accordingly.

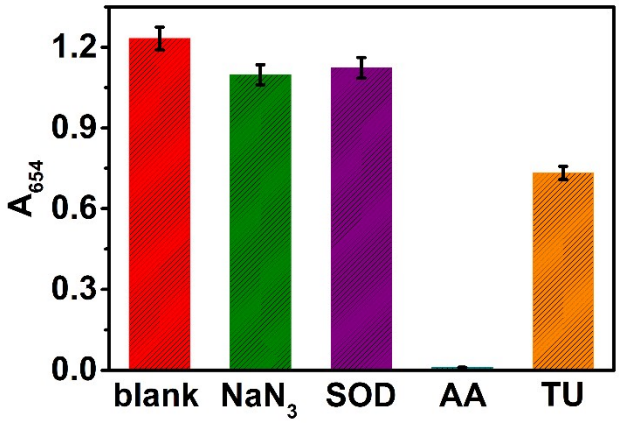

Fig. S8 A_{654} in the presence of NaN_3 (1.0 mM), SOD (30 U mL^{-1}), AA (1.0 mM), TU (1.0 mM), or the blank control (i.e., without the addition of any scavenger). Experiments were carried out in 0.2 M HAc/NaAc (pH 4.0) in the presence of 0.1 mM 5-FAM, 1.0 mM TMB, and 15 mM H_2O_2. The incubation time was 30 min. Error bars represent the standard deviations of three repeated measurements.

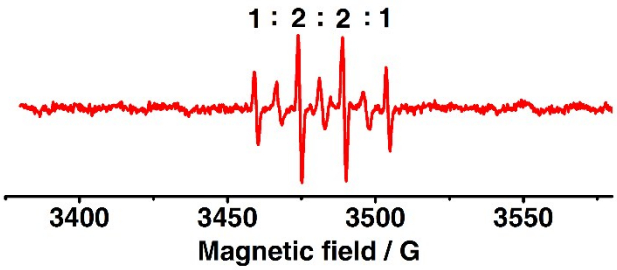

Fig. S9 EPR spectrum with DMPO as the spin trap. It was acquired in 0.2 M HAc/NaAc (pH 4.0) in the presence of 50 μM 5-FAM, 15 mM H_2O_2, and 0.1 M DMPO (dissolved in 0.1 M PBS, pH 7.4). The appearance of other peaks, apart from the characteristic peaks of the typical DMPO-$^{\bullet}$OH adduct, is due to the partial oxidation of the DMPO spin trap during the transport and storage.

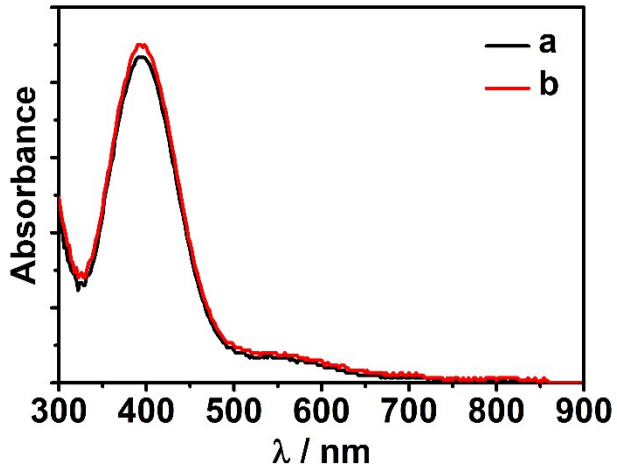

Fig. S10 Absorption spectra of PANI synthesized in the presence (a) or absence (b) of 5-FAM. The solvent was NMP. The intense absorption band at 395 nm is due to the polaron transition.[1]

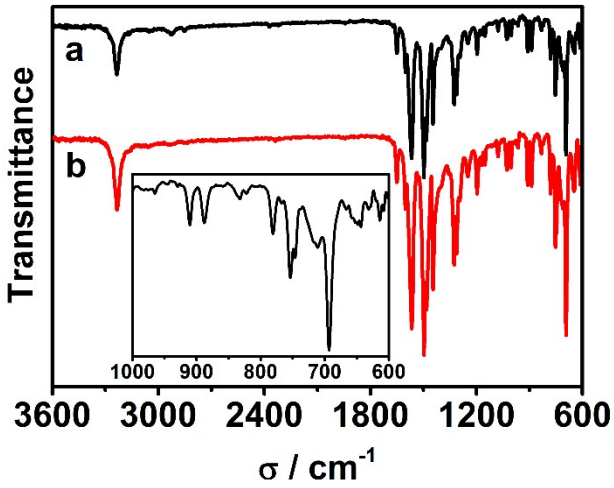

Fig. S11 FTIR spectra of PANI synthesized in the presence (a) or absence (b) of 5-FAM. The characteristic bands at 3234 and 1650 cm^{-1} arise from the anti-symmetric stretching vibration and the in-plane bending vibration of N-H bonds, respectively.[2] The bands at 1602 and 1567 cm^{-1} are due to the stretching of quinoid ring.[3] The bands at 1497, 1484, and 1446 cm^{-1} derive from the stretching of benzenoid ring.[3,4] Those at 1326, 1312, and 1295 cm^{-1} are originated from the stretching of the C-N bonds of the secondary aromatic amines.[3a,5] The characteristic bands at 1249, 1196, 1077, 1027, and 1004 cm^{-1} arise from the C-H in-plane bending vibrations, while those at 910, 888, 833, 781, 754, 746, and 693 cm^{-1} from the C-H out-of-plane bending vibrations.[6]

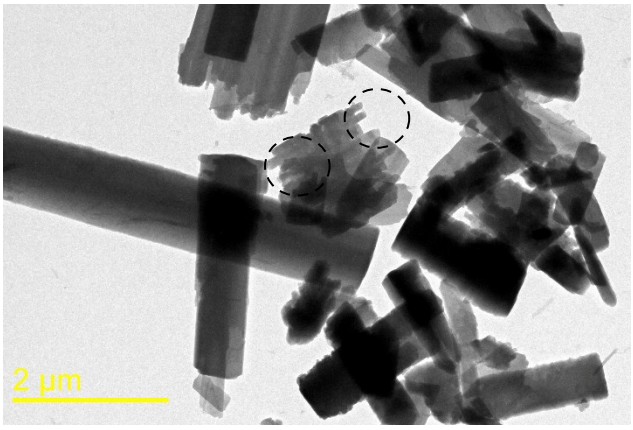

Fig. S12 TEM image of the biomimetically synthesized PANI nanoplatelets.

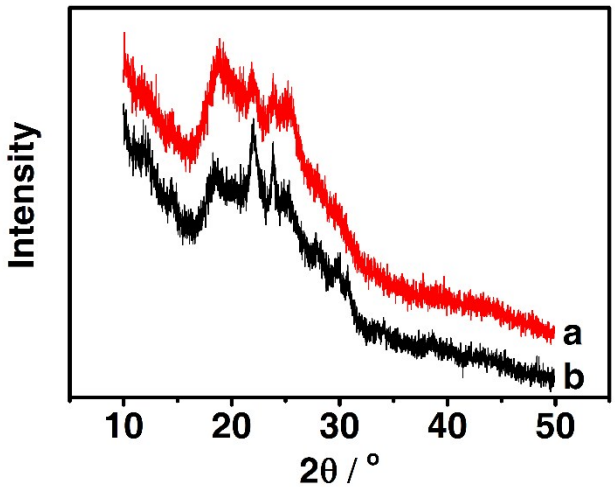

Fig. S13 XRD patterns of PANI synthesized in the presence (a) or absence (b) of 5-FAM. The diffraction peaks at $2\theta \sim 19^{o}$ and 25^{o} correspond to the amorphous peak and the crystalline peak, respectively.[7]

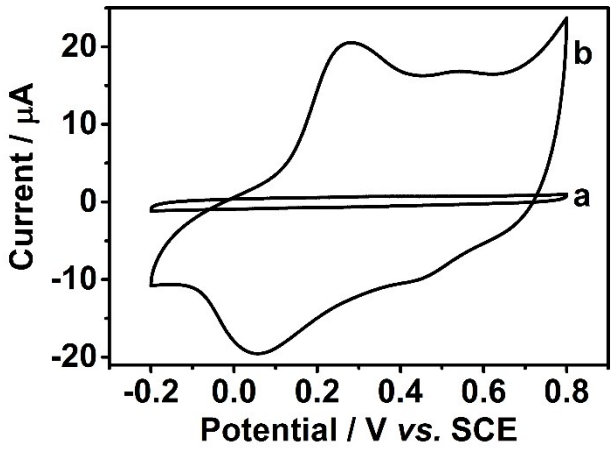

Fig. S14 Cyclic voltammograms of (a) the bare GCE or (b) in the presence of a PANI film.

Notes and references

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