Electronic Supplementary Material (ESI) for RSC Advances.

Peroxidase-Like Activity of Ferric Ions and Its Application to Cysteine Detection

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Supporting Information

1. Materials and Instruments

Materials

3, 3', 5, 5'-tetramethyl benzidine (TMB, 99%) and amino acids (L-form) were purchased from Sigma-Aldrich. $FeCl_2.4H_2O$ (99%, AR) was obtained from Gracia (China). $Fe(NO_3)_3.9H_2O$ (98.5%, AR), H_2O_2 (30%, GR), NaOAc (99%, AR), HOAc (99.5%, AR), hydroxymethyl aminomethane (Tris, BR) and HCl (36%-38%, AR) were purchased from GuoYao (Shanghai, China). All chemicals were used in the experiments without further purification.

Instruments

UV-vis absorption spectra were recorded by employing Thermo Evolution 300 UV-vis absorption spectrophotometer equipped with a temperature controller. Transmission electron microscopy (TEM) experiments were done on HITACHI H-7650 system. The Fe contents of the Fe_3O_4 MNPs were analyzed by using Thermo ELECTRON CORPORATION M6.

2. Preparation of Fe_3O_4 MNPs and measurement of the total Fe concentration.

Preparation of Fe_3O_4 MNPs of size ~400 nm:

4.05 g $FeCl_3.6H_2O$, 10.8 g sodium oleate and 3.0 g poly ethylene

glycol (PEG) was dissolved in 120 mL ethylene under magnetic stirring. The resulting solution was transferred to a Teflon-lined stainless-steel autoclave and heated at 200 ^{o}C for 20 h. The resulting black MNPs were washed three times with ethanol and dried in vacuum at 50 ^{o}C for 8 h. The diameter of the MNPs was estimated to be ~400 nm according to the TEM results.

Preparation of Fe_3O_4 MNPs of size ~6 nm:

1.99 g $FeCl_2.4H_2O$ and 5.14g $FeCl_3.6H_2O$ were dissolved in 50 mL water, and then 10 mL 20 % PEG was added to the mixture to obtain Precursor solution I. 10 mL aqueous ammonium hydroxide solution was mixed with 2 mL 20% PEG to obtain Precursor solution II. Precursor solution I was added into Precursor solution II dropwise with strong stirring under the protection of dry nitrogen at room temperature for 60 min. The precipitates of Fe_3O_4 MNPs were washed by repeated cycles of centrifugation and redispersion in distilled water for further use. The diameter of the MNPs was estimated to be ~6 nm according to the TEM results.

Measurement of the total Fe concentration

The total Fe contents of Fe_3O_4 MNPs were analyzed by flame atomic absorption spectrometry. The Fe_3O_4 MNPs stock solution was prepared by adding certain volumes of HCl solution (1.2 M) for completely

converting Fe_3O_4 into Fe ions. The resulting solution turned in colorless transparent from original light brown. The Fe standard solution with five different concentrations was employed to obtain the calibration curve, and then the Fe contents of the MNPs were calculated by measuring three parallel acidified MNPs solution.

3. Measurements of peroxidase-like Activity

To investigate the peroxidase-like activity of Fe^{3+} ions, the catalytic oxidation of the peroxidase substrate TMB in acetate buffer solution was carried out in the presence of H_2O_2. In a typical experiment, 100 μL 40 mM H_2O_2 and 100 μL 5 mM TMB were added in 775 μL 0.2 M NaOAc-HOAc buffer solution of pH 4.2, and 25 μL 0.2 mM $Fe(NO_3)_3$ was then added in the mixing solution. The resulting solution was instantly monitored by employing Thermo Evolution 300 UV-vis absorption spectrophotometer equipped with a temperature controller at 30 ^oC, and UV-vis absorption spectra data were collected at a time interval at 652 nm wavelength. To investigate the effects of concentration of H_2O_2 and Fe^{3+} ions, pH, and temperature, the peroxidase-like activity of Fe^{3+} ions were measured when H_2O_2 concentration was varied from 0.4 μM to 0.4 M and Fe^{3+} ions concentration was changed from 0.5 to 20 μM, pH was adjusted from 2.0 to 9.0, and the temperature was varied from 25 to 60 ^oC, respectively. The kinetic analysis of Fe^{3+} ions with H_2O_2 as the substrate was performed by employing 25 μM 0.2 mM $Fe(NO_3)_3$ solution

containing 0.5 mM TMB and varying concentration of H_2O_2 (4×10^{-7}, 4×10^{-6}, 4×10^{-5}, 4×10^{-4}, 4×10^{-3}, 4×10^{-2}, 0.1, 0.2, 0.3 and 0.4 M). Likewise, the kinetic analysis with TMB as the substrate was carried out with 4 mM H_2O_2 and changing TMB concentration (0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mM). The apparent kinetic parameters were calculated based on the Michaelis-Menten equation $v = V_{max} \times [S] / (K_m + [S])$, where v is the initial catalytic rate, V_{max} is the maximal reaction velocity, $[S]$ is the substrate concentration and K_m is the apparent Michaelis-Menten constant. To investigate the catalytic mechanism, experiments were carried out under the conditions followed by varying H_2O_2 concentration (0.05, 0.07, 0.1 and 0.2 mM) at three different fixed TMB concentrations (0.1, 0.3 and 0.5 mM), respectively, or by varying TMB concentration (0.1, 0.2, 0.3 and 0.5 mM) at three different fixed H_2O_2 concentrations (0.1, 1.0 and 4.0 mM), respectively.

4. Sensing L-Cys

For sensing L-Cys, 0.01 M L-Cys solution with various volumes was mixed with 0.2 M NaOAc-HOAc buffer of pH 4.2 containing H_2O_2, TMB and Fe^{3+} ions to afford varied L-Cys concentration. When Fe^{3+} ions concentration was fixed to 5, 2, and 0.8 μM, L-Cys concentration was varied from 0 to 0.5 mM, from 0 to 0.2 mM, and from 0 to 0.1 mM, respectively. After quickly stirring, the resulting solution was immediately monitored by employing a UV-vis absorption spectrometer,

temperature was fixed at 30 ^{o}C and wavelength was set at 652 nm. Similar procedures were operated in the case of S^{2-}, which was employed to probe the mechanism of this sensing system. To investigate the sensing selectivity, other 19 natural amino acids employed their L-form were also tested in this sensing system and the concentration of these amino acids was 15-fold higher than that of L-Cys.

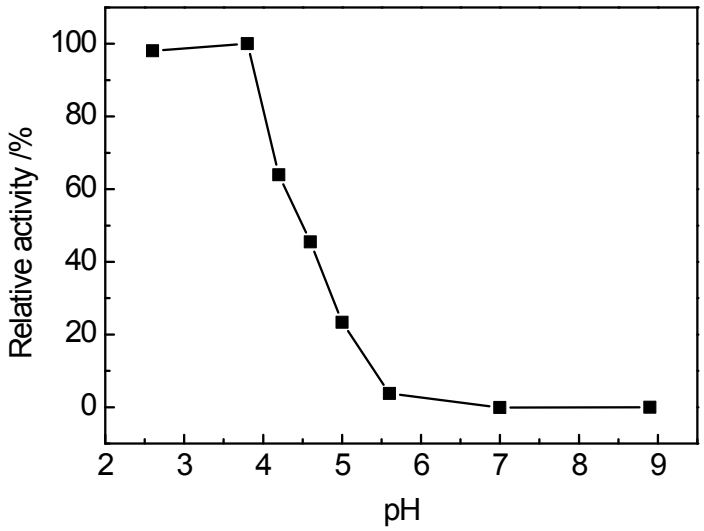

Fig. S1 pH-dependent peroxidase-like activity of Fe^{3+} ions towards the TMB-H_2O_2 system. Experimental conditions: [TMB] = 0.5 mM; [H_2O_2] = 4 mM; [Fe^{3+}] = 5 μM; temperature of 30 ^{o}C.

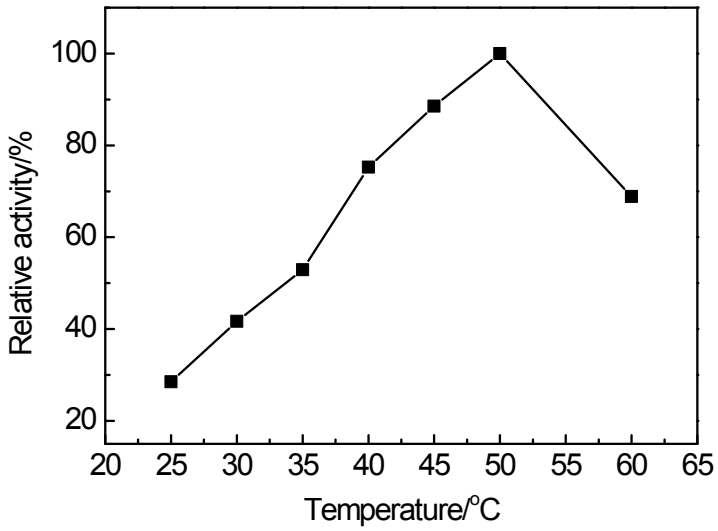

Fig. S2 Temperature-dependent peroxidase-like activity of Fe^{3+} ions towards the TMB-H_2O_2 system. Experimental conditions: [TMB] = 0.5 mM; [H_2O_2] = 4 mM; [Fe^{3+}] = 5 μM; 0.2 M NaOAc-HOAc buffer solution of pH 4.2.

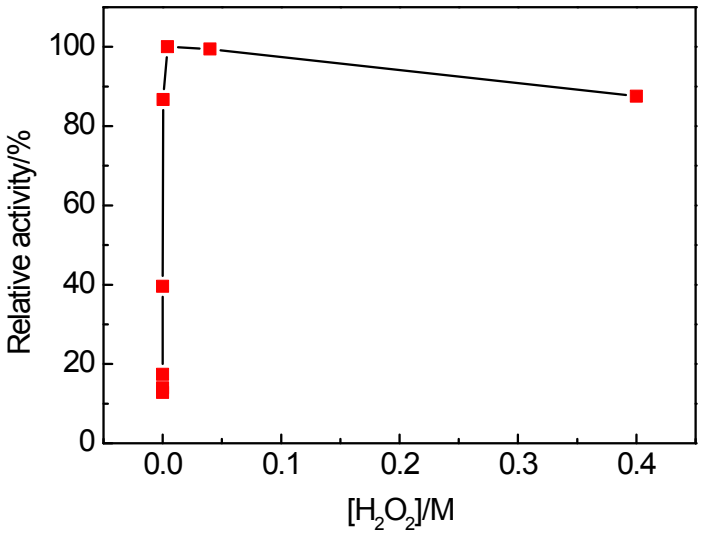

Fig. S3 H_2O_2 concentration-dependent peroxidase-like activity of Fe^{3+} ions towards the TMB-H_2O_2 system. Experimental conditions: [TMB] = 0.5 mM; [Fe^{3+}] = 5 μM; temperature of 30 °C; 0.2 M NaOAc-HOAc buffer solution of pH 4.2.

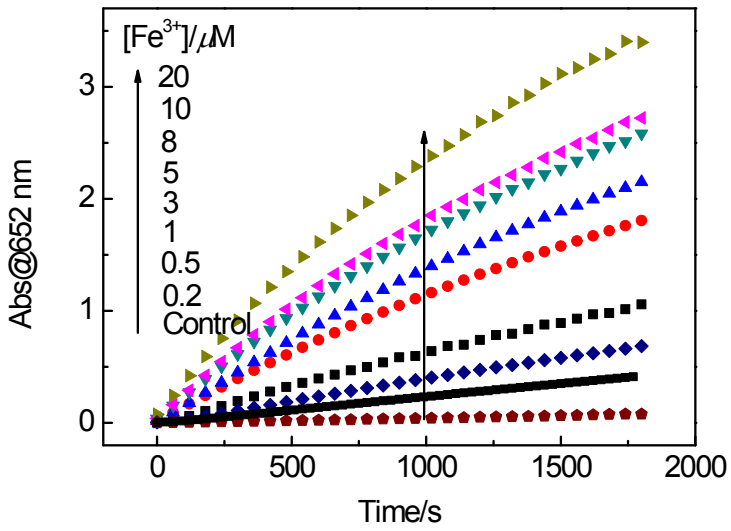

Fig. S4 Fe^{3+} ions concentration-dependent peroxidase-like activity of Fe^{3+} ions towards the TMB-H_2O_2 system. Experimental conditions: [TMB] = 0.5 mM; [H_2O_2] = 4 mM; temperature of 30 °C; 0.2 M NaOAc-HOAc buffer solution of pH 4.2.

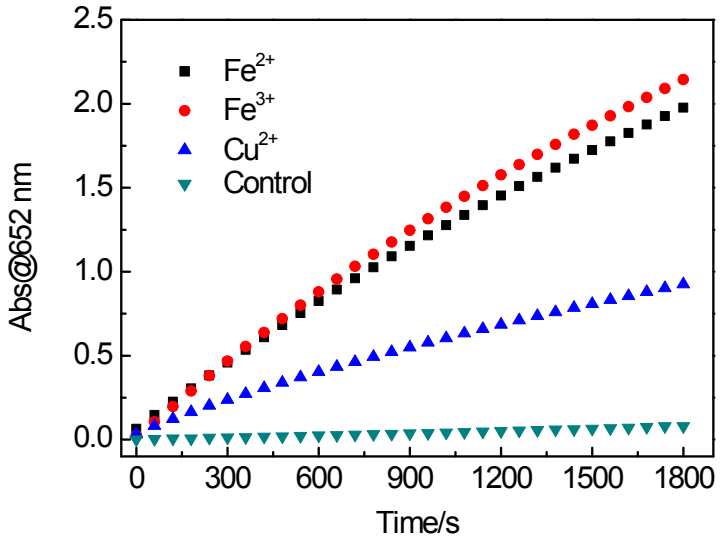

Fig. S5 Comparison of the peroxidase-like activity of Fe^{3+}, Fe^{2+} and Cu^{2+} ions towards the TMB-H_2O_2 system. Experimental conditions: Experimental conditions: [Fe3+] = 5 μM; [Fe2+] = 5 μM; [Cu2+] = 700 μM; [TMB] = 0.5 mM; [H_2O_2] = 4 mM; temperature of 30 °C; 0.2 M NaOAc-HOAc buffer solution of pH 4.2 for Fe^{3+} and Fe^{2+} ions cases; 0.2 M NaOAc-HOAc buffer solution of pH 5.6 for Cu^{2+} ions case.

Table S1: Kinetic parameters of the Fe^{3+}-TMB-H_2O_2 system.

[E] (M)	Substrate	K_m (mM)	V_{max} (Ms^{-1})	k_{cat} (s^{-1})
5×10^{-6}	TMB	4.8	4.03×10^{-7}	8.06×10^{-2}
5×10^{-6}	H_2O_2	0.062	3.55×10^{-8}	7.1×10^{-3}

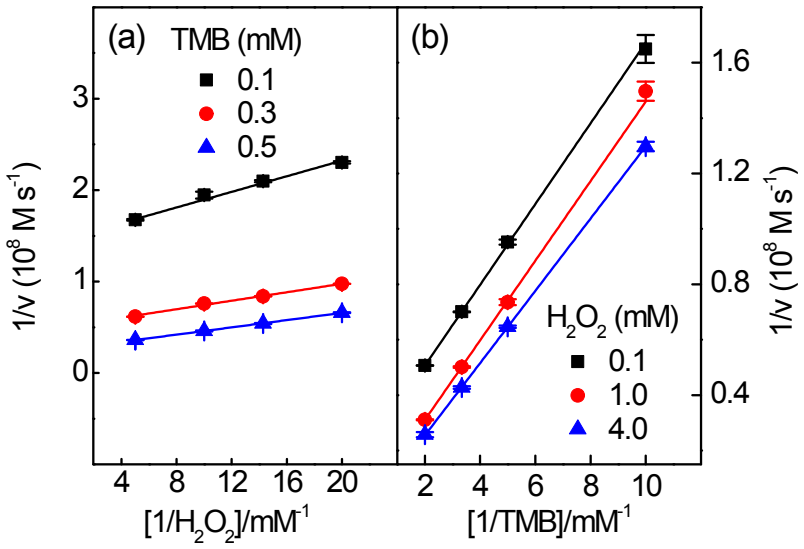

Fig. S6 Double-reciprocal plots of activity of Fe^{3+} ions towards the TMB-H_2O_2 system at a fixed concentration of one substrate versus varying concentration of the second substrate for TMB (a) and H_2O_2 (b).

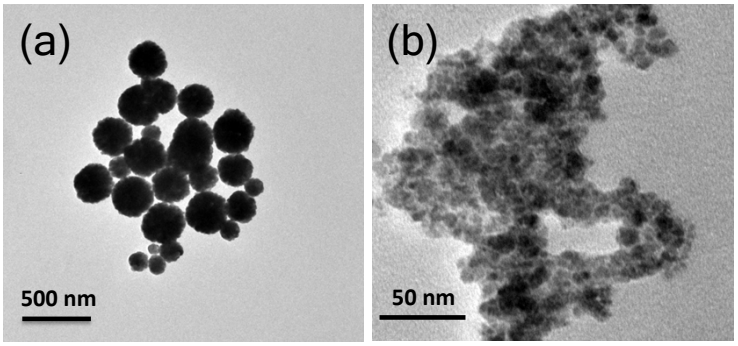

Fig. S7 TEM images of ~400 nm (a) and ~6 nm (b) Fe_3O_4 MNPs.

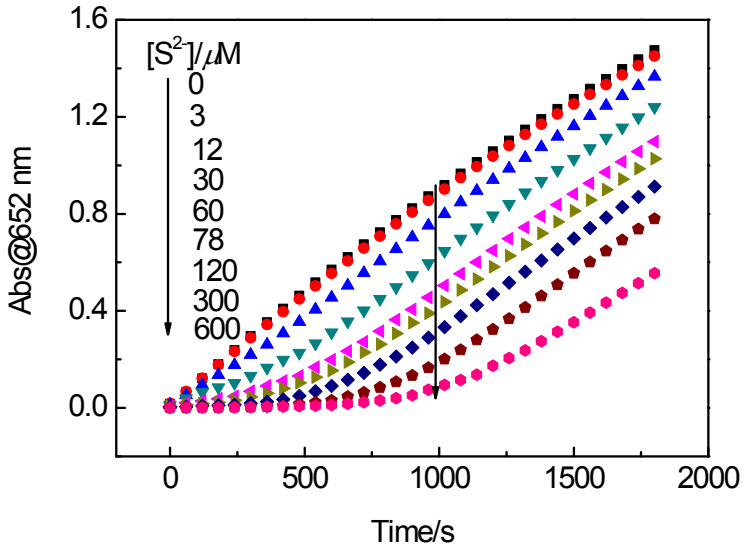

Fig. S8 Time-dependent absorbance changes at 652 nm of the Fe^{3+}-TMB-H_2O_2 system in the presence of various concentration of S^{2-}. Experimental conditions: [TMB] = 0.5 mM; [H_2O_2] = 4 mM; [Fe^{3+}] = 5 μM; temperature of 30 ^{o}C; 0.2 M NaOAc-HOAc buffer solution of pH 4.2.