

Electronic Supplementary Information (ESI) for

Iron Phosphate Microflowers as Peroxidase Mimic and

Superoxide Dismutase Mimic for Biocatalysis and Biosensing

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1. Materials and Methods

(1) Materials

Iron (II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), sodium hypophosphite ($\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$), polyvinylpyrrolidone (PVP), glycol, 30% H_2O_2 solution were obtained from Shanghai Chemical Corporation (Shanghai, China). 3,3',5,5'-tetramethylbenzidine dihydrochloride hydrate ($\text{TMB} \cdot 2\text{HCl} \cdot \text{H}_2\text{O}$) and pyrogallol supplied by Sigma were used without further treatment.

(2) Methods

Preparation of iron phosphates microflowers

In a typical process, 7 mmol of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and 14 mmol of $\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$ were dissolved in 5 ml of distilled water, and then mixed with 1 g of PVP dissolved in glycol (35 ml) under vigorous stirring to obtain a homogeneous solution. Afterwards the mixture was transferred into a 50 ml Teflon-lined autoclave, sealed and maintained at 180 °C for 24 h, then allowed to cool to the room temperature naturally. The resulting product was collected by centrifugation, washed with distilled water and ethanol for several times and finally dried in vacuum for 6 h.

The steady-state kinetic assays

The steady-state kinetic assays were carried out at room temperature in 2.5 mL of $\text{TMB-H}_2\text{O}_2\text{-FePOs}$ reaction system. The kinetic analysis of iron phosphates (FePOs) microflowers with TMB as the substrate was performed with $\text{TMB-H}_2\text{O}_2\text{-FePOs}$ system with constant H_2O_2 concentration of 0.1 mM and 0.4 mg/mL FePOs microflowers but varied TMB concentration (100~1000 μM). The kinetic analysis with H_2O_2 as the substrate was performed with constant TMB concentration of 1 mM and 0.4 mg/mL FePOs microflowers but varied H_2O_2 concentration (10~80 μM).

All the reactions were monitored in timescan mode at 650 nm using the UV-VIS-NIR spectrophotometer. Catalytic parameters were determined by fitting the absorbance data to the Michaelis-Menten equation (eqs. 3) as denoted in the manuscript which describes the relationship between the rates of substrate conversion by an enzyme and the concentration of the substrate.

In eqs. 3, v is the rate of conversion, v_{max} is the maximum rate of conversion, $[S]$ is the substrate concentration, and K_{m} is the Michaelis constant. The Michaelis

constant is equivalent to the substrate concentration at which the rate of conversion is half of v_{\max} and K_m approximates the affinity of the enzyme for the substrate.

Absorption values obtained by timescan mode at 650 nm were converted to the concentration of TMB derived oxidation products by the Beer-Lambert Law (eqs. S1), in which A is the absorption at 650 nm, b is the optical length of 1.0 cm, ε and c are the molar absorption coefficient of $39000\text{ M}^{-1}\text{cm}^{-1}$ and concentration for TMB derived oxidation products¹, respectively.

$$A = \varepsilon bc \quad (\text{S1})$$

Charaterization

XRD studies were conducted on a Rigaku D/max-2500 X-ray powder diffractometer using Cu K α radiation ($\lambda=1.5406\text{ \AA}$). The morphological investigations were carried out with field-emission scanning electron microscopy (FESEM, JEOL JSM-6700F). Dynamic light scattering (Zetasizer Nano, Malvern Instruments) was used to determine the hydrodynamic size and Z-average diameter of the products. UV-Vis-NIR spectrophotometer (Cary500, Varian) was utilized to monitor the reactions happened in TMB-H₂O₂-FePOs and pyrogallol-FePOs systems.

2. Figure S1-S6

Figure S1. XRD pattern of FePOs microflowers. The peaks marked by rectangles are ascribed to $\text{Na}_{4.55}\text{Fe}(\text{PO}_4)_2\text{H}_{0.45}\text{O}$ (JCPDS card No. 52-1393) and those by asterisks to $\text{FeH}_3\text{P}_2\text{O}_8 \cdot \text{H}_2\text{O}$ (JCPDS card No. 46-0197). The inset shows the color of the products.

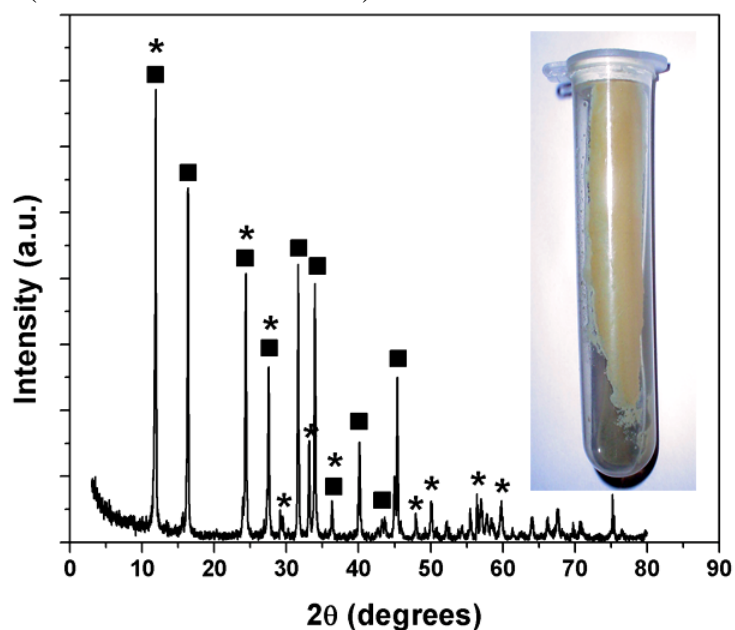


Figure S2. (A) SEM image of FePOs microflowers with lower concentration. (B) Size distribution histogram of FePOs microflowers.

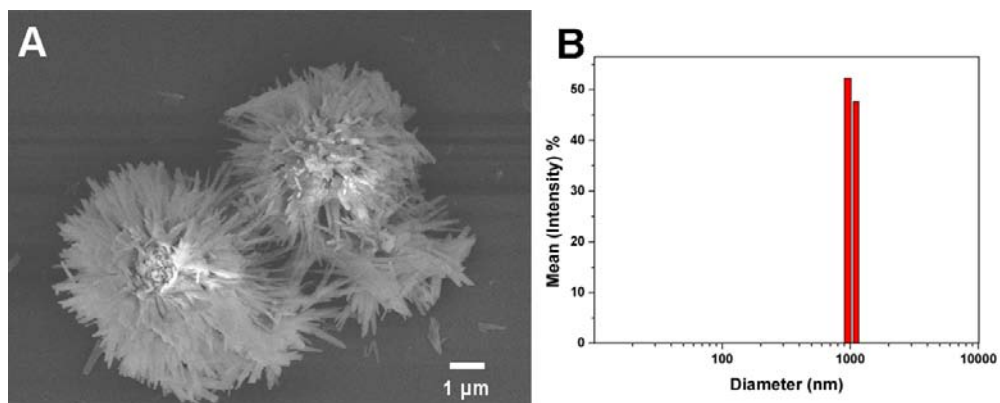


Figure S3. Typical time-dependent absorbance changes at 650 nm demonstrating Michaelis-Menten kinetics of TMB- H_2O_2 reactions catalyzed by the FePOs microflowers (1 mL of 2.5 mM TMB, 25 μL of 10 mM H_2O_2 , 50 μL of 400 $\mu\text{g}/\text{mL}$ FePOs, 1425 μL of ultrapure water).

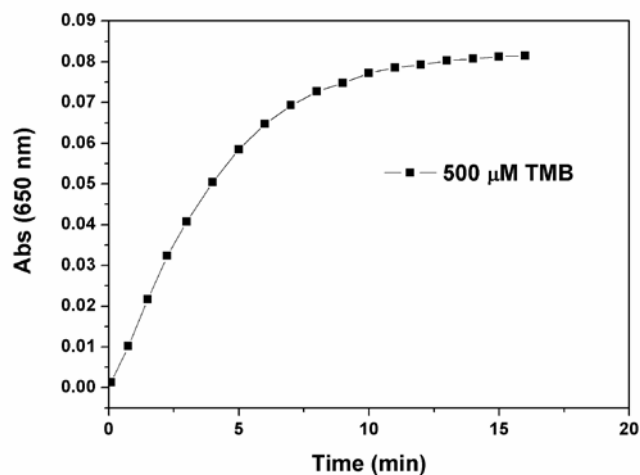


Figure S4. SEM images of FePOs microflowers after TMB- H_2O_2 reaction.



Figure S5. Time-dependent absorbance changes at 650 nm of TMB- H_2O_2 reaction system catalyzed by FePOs microflowers at different (A) pH values and (B) temperatures. (C) and (D) are photos demonstrating the color change varied with pH values and temperatures, respectively. The pH value was adjusted by 0.1 mM HCl or 0.1 mM NaOH solution.

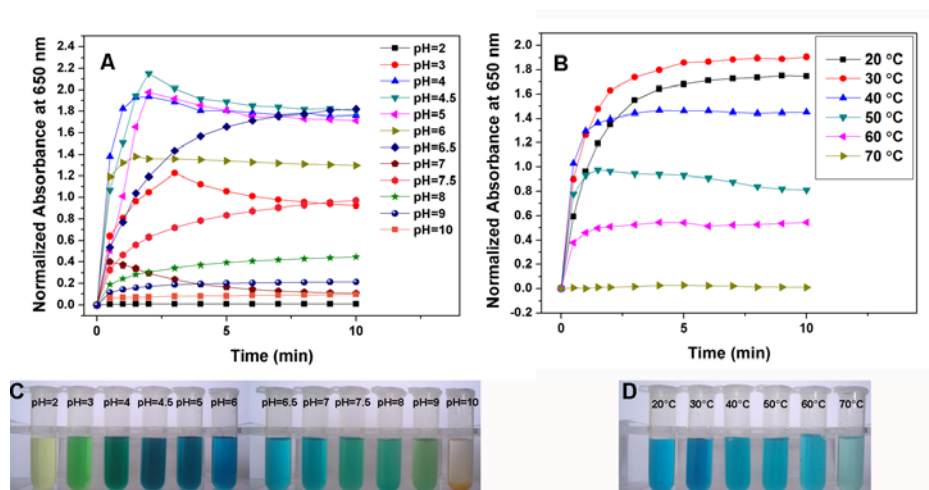
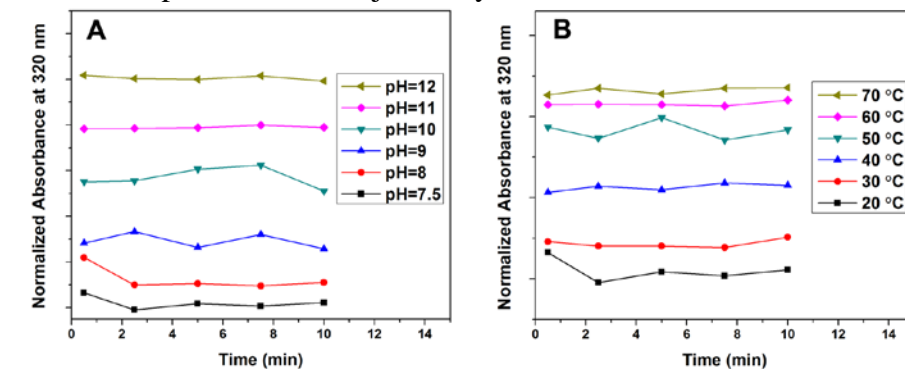


Figure S6. Time-dependent absorbance changes at 320 nm of pyrogallol-oxidation reaction system inhibited by FePOs microflowers at different (A) pH values and (B) temperatures. The pH value was adjusted by 0.1 mM HCl or 0.1 mM NaOH solution.



3. Table S1-S2

Table S1. Comparison of kinetic parameters of FePOs and HRP. $[E]$ is FePOs and HRP concentration, K_m is the Michaelis constant, v_{\max} is the maximal reaction velocity and K_{cat} is the catalytic constant, where $K_{\text{cat}} = v_{\max}/[E]$. (These data is the average of three repetitive measurements.) The values of HRP were taken from the previous standard reports.¹

	$[E]$ (M)	Substrate	K_m (mM)	v_{\max} (Ms ⁻¹)	K_{cat} (s ⁻¹)	K_{cat}/K_m (M ⁻¹ s ⁻¹)
Na _{4.55} Fe(PO ₄) ₂ H _{0.45} O	1.1×10 ⁻³	TMB	1.46	2.252×10 ⁻⁸	2.047×10 ⁻⁵	1.402×10 ⁻⁴
Na _{4.55} Fe(PO ₄) ₂ H _{0.45} O	1.1×10 ⁻³	H ₂ O ₂	0.317	4.316	3.924×10 ³	1.24×10 ⁷
FeH ₃ P ₂ O ₈ •H ₂ O	1.498×10 ⁻³	TMB	1.46	2.252×10 ⁻⁸	1.503×10 ⁻⁵	1.029×10 ⁻⁴
FeH ₃ P ₂ O ₈ •H ₂ O	1.498×10 ⁻³	H ₂ O ₂	0.317	4.316	2.881×10 ³	9.09×10 ⁶
HRP	2.5×10 ⁻¹¹	TMB	0.434	10×10 ⁻⁸	4×10 ³	9.2×10 ⁶
HRP	2.5×10 ⁻¹¹	H ₂ O ₂	3.7	8.71×10 ⁻⁸	3.48×10 ³	9.4×10 ⁵

Table S2. Comparison of detection limit of H₂O₂ and K_m value with H₂O₂ as the substrate measured by FePOs and other current existing nanomaterials-based enzyme mimics.

	FePOs	Fe ₃ O ₄ nanoparticles ²	FeS nanosheets ³	Au nanoparticles ⁴	CoFe ₂ O ₄ nanoparticles ⁵	Carbon nanodots ⁶	Graphene oxide ⁷	BiFeO ₃ nanoparticles ⁸
Detection limit of H ₂ O ₂	10 nM	Not reported	92 nM	500 nM	10 nM	0.2 μM	50 nM	4.5 nM
K_m for H ₂ O ₂	0.317 mM	154 mM	0.0543 mM	Not reported	0.0752 mM	26.77 mM	3.99 mM	Not reported

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