

## Electronic supplementary information

### **Enhancing the Peroxidase-Like Activity and Stability of Gold Nanoparticle Nanozymes by Coating a Partial Iron Phosphate Shell**

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## Materials and Methods

**Chemicals.** Gold (III) chloride trihydrate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ), 6-mercapto-1-hexanol (mercaptohexanol or MCH),  $\text{H}_2\text{O}_2$  (30 wt%), 3,3',5,5'-tetramethylbenzidine (TMB), and all the metal chloride salts were purchased from Sigma-Aldrich (St Louis, MO). Sodium acetate, sodium phosphate monobasic, and 4-(2-hydroxyethyl) piperazine-1-ethane sulfonate (HEPES) were from Mandel Scientific (Guelph, ON, Canada). Silica microspheres were from Polyscience, Inc (Warrington, PA, USA). 50 nm citrate-capped AuNPs were from Cytodiagnosics (Burlington, ON, Canada). Milli-Q water was used for preparing buffers and solutions.

**Synthesis of citrate-capped AuNP and HEPES-capped AuNS.** The 13 nm citrate-capped Au NPs were synthesized as previously reported.<sup>1</sup> Based on an extinction coefficient of  $2.7 \times 10^8 \text{ M}^{-1} \cdot \text{cm}^{-1}$  at 520 nm, the stock concentration of the 13 nm AuNP was 9.8 nM.<sup>2</sup> To synthesize HEPES-capped gold nanostars (AuNSs), 200  $\mu\text{L}$  20  $\mu\text{M}$   $\text{HAuCl}_4$  was added into freshly prepared 20 mL HEPES solution (pH 7.4, 50 mM).<sup>3</sup> Then, this mixture was incubated in a dark room. After 1 h, the solution started to become blue. Both the citrate-capped AuNPs and HEPES-capped AuNSs were store at 4°C for further use.

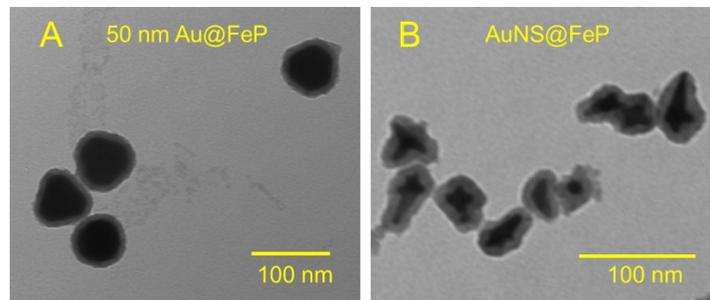
**Preparation of Au@FeP.** To form Au@FeP of various thickness, different concentrations of  $\text{Fe}^{2+}$  were respectively added into AuNPs dispersed in phosphate buffer. Taking Au@1 mM FeP as an example, first, 1 nM AuNPs were stirred in phosphate buffer (10 mL, 1 mM, pH 7.1) for 3 min at room temperature. Then, 200  $\mu\text{L}$  of 50 mM  $\text{Fe}^{2+}$  was added into the solution under high-speed stirring in five additions. The time between each addition was 3 min. Finally, the  $\text{Fe}^{2+}$  concentration reached 1 mM and the sample was further reacted for 15 min. Finally, all the nanoparticles were washed with Milli-Q water by centrifugation for 3 times (10000 rpm, 20 min). The growth of FeP on HEPES-capped AuNS was performed in the same way.

**The growth of transition metal phosphate on AuNPs.** First, 1 nM AuNPs were stirred in 1 mM phosphate buffer (pH 7.1) for 3 min at room temperature. Then, 5 mM transition metal ion was added into the mixture solution under high-speed stirring in one step. The final concentration of transition metal ion was 0.05 mM. All the nanoparticles were washed with Milli-Q water by centrifugation for 3 times (10000 rpm, 20 min) after preparation.

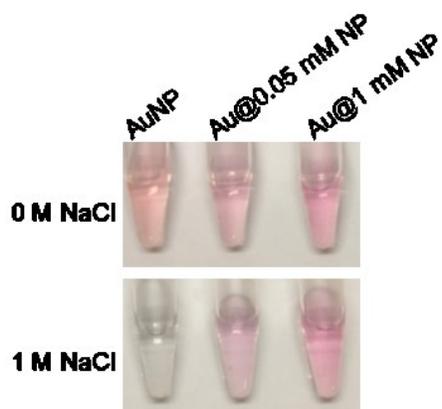
**$\zeta$ -potential.**  $\zeta$ -potentials were measured using dynamic light scattering (Zetasizer Nano 90, Malvern). In a typical experiment, 0.1 nM AuNPs and Au@FeP NPs were respectively dispersed in 1 mL buffer (20 mM acetate buffer, pH 5). The  $\zeta$ -potential values were measured at 25°C.

**TEM and XRD.** TEM images were taken by a Phillips CM10 100 kV transmission electron microscope. XRD measurements were conducted at room temperature on a PANalytical Empyrean diffractometer with Cu  $K\alpha$  radiation equipped with a PIXcel bidimensional detector.

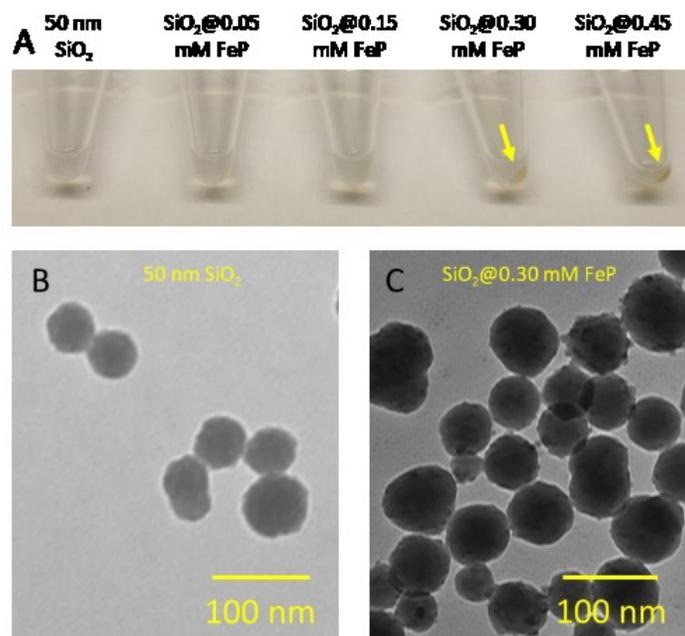
**TMB oxidation kinetics.** For a typical peroxidation reaction, TMB substrate (0.5 mM) was mixed with 0.1 nM NPs in 100  $\mu\text{L}$  20 mM pH 5 acetate buffer. Then, 2.5  $\mu\text{L}$  200 mM  $\text{H}_2\text{O}_2$  was added to initiate the reaction. The final volume of each sample was 100  $\mu\text{L}$ . The absorbance at 652 nm for TMB was monitored by using a microplate reader (SpectraMax M3). The oxidized TMB spectra were measured by a microplate reader (Tecan Spark).



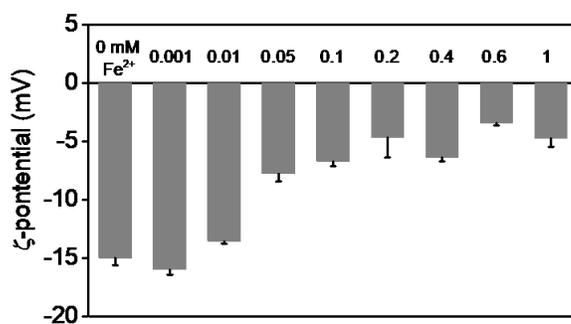
**Fig. S1.** TEM images of (A) 50 nm citrate-capped AuNPs and (B) HEPES-capped AuNSs coated by FeP.



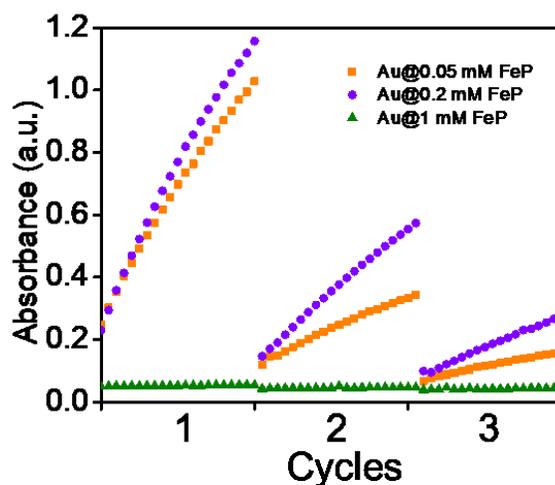
**Fig. S2.** Photographs of AuNPs, Au@0.05 mM FeP NPs, and Au@1 mM FeP NPs before and after adding 1 M NaCl. The AuNP concentration was  $\sim 1$  nM. The retained red color indicated the colloidal stability of the FeP coated AuNPs.



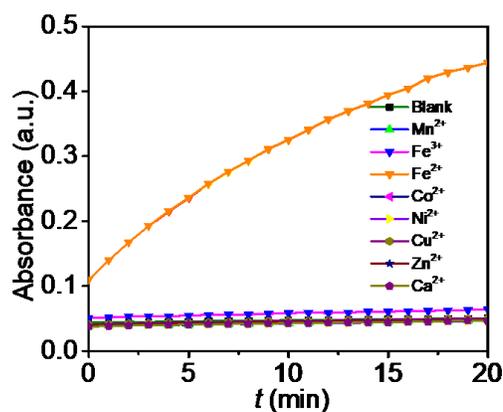
**Fig. S3.** (A) The photographs of SiO<sub>2</sub> nanoparticles and the SiO<sub>2</sub> coated by a FeP shell after centrifugation. The yellow products pointed out by the arrowheads suggest the successful synthesis of FeP on SiO<sub>2</sub>. The TEM images (B) before and (C) after FeP coating also confirmed the growth of FeP on SiO<sub>2</sub>. New features assigned to FeP particles were found on the SiO<sub>2</sub> NPs.



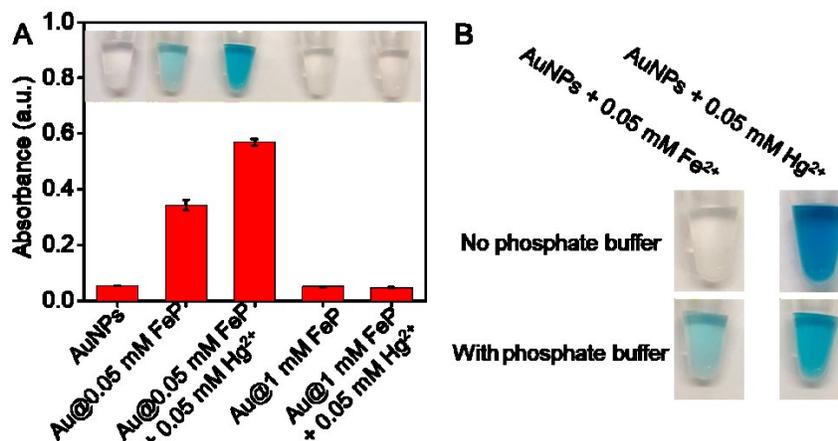
**Fig. S4.** The zeta-potentials of the citrate-capped AuNPs and Au@FeP NPs with different FeP shell thicknesses in 20 mM pH 5 acetate buffer. The concentration of the AuNPs was 0.1 nM.



**Fig. S5.** The recyclability of the peroxidase-like activity of Au@0.05 mM FeP, Au@0.2 mM FeP and Au@1 mM FeP in three catalytic cycles. The kinetics of TMB oxidation were recorded for 10 min in each cycle. Taking washing time into consideration, each cycle was 40 min. 0.5 nM Au@FeP NPs were used. 5 mM H<sub>2</sub>O<sub>2</sub> and 0.5 mM TMB substrate were reacted in 20 mM pH 5 acetate buffer.



**Fig. S6.** The kinetics of TMB oxidation in the presence of AuNPs mixed with various transition metal ions in phosphate buffer. 5 mM H<sub>2</sub>O<sub>2</sub> and 0.5 mM TMB substrate were used. The concentration of the NPs was 0.1 nM.



**Fig. S7.** (A) The absorbance of oxidized TMB at 652 nm catalyzed by AuNPs, Au@0.05 mM FeP NPs, and Au@1 mM FeP NPs without/with 0.05 mM Hg<sup>2+</sup>. Inset: corresponding photographs of TMB oxidized by the NPs. (B) Photographs of TMB oxidized by Au/Fe and Au/Hg complexes, which were respectively formed in H<sub>2</sub>O and phosphate buffer. All the NPs were washed with H<sub>2</sub>O for three times after the synthesis. 0.1 nM NPs, 5 mM H<sub>2</sub>O<sub>2</sub>, 0.5 mM TMB substrate, and 20 mM pH 5 acetate buffer were used. The absorbance intensities and photos were collected after 15 min reaction. Phosphate promoted the activity of the Fe<sup>2+</sup> sample, but inhibited the activity of the Hg<sup>2+</sup> sample, which can be used to tell these two metal ions apart.

## References

1. J. Liu and Y. Lu, *Nat. Protoc.*, 2006, **1**, 246.
2. H. D. Hill and C. A. Mirkin, *Nat. Protoc.*, 2006, **1**, 324-336.
3. P.-J. Jimmy Huang, J. Yang, K. Chong, Q. Ma, M. Li, F. Zhang, W. J. Moon, G. Zhang and J. Liu, *Chem. Sci.*, 2020, **11**, 6795-6804.