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Supporting Information

A Simple Route to CoFe₂O₄ Nanoparticles with Shape and Size Control and their Tunable Peroxidase-Like Activity

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Scheme S1. Synthesis of PEG-3, 4-dihydroxybenzylamine (DIB-PEG-NH₂), CoFe₂O₄-DIB-PEG-NH₂ (1a) and CoFe₂O₄-DIB-PEG-NH₂-FA (1b).



Figure S1. TEM image of CoFe₂O₄ nanoparticles. (A) ~ 13.8 \pm 4.6 nm, (B) ~24.5 \pm 5.3 nm, (C) ~ 32.1 \pm 4.2 nm, (D) ~ 45.2 \pm 15.1 nm and (E) ~ 4.1 \pm 0.3 nm.



Figure S2. The catalytic activity of the $CoFe_2O_4$ NPs is dependent on pH, temperature and the concentration of substrate. In order to investigate the best experimental condition, we measured the peroxidase-like activity of $CoFe_2O_4$ NPs while (A) varying the pH from 2.5 to 6.0, (C) the temperature from 20 $^{\circ}C$ to 50 $^{\circ}C$ and compared the results over the same concentration of subsequent (B and D).



Figure S3. (A) The time-dependent UV absorbance curve of the HAc-NaAc solution (pH = 4.0) containing different concentration of H_2O_2 and 1.5 mM TMB in the presence of an equal number of CoFe₂O₄ NPs. (B) The time-dependent UV absorbance curve of the HAc-NaAc solution (pH = 4.0) containing different concentration of TMB and 10 mM H_2O_2 in the presence of an equal number of CoFe₂O₄ NPs.



Figure S4. UV-vis spectra of the HAc-NaAc solution (pH = 4.0) containing 10 mM H₂O₂ and 1.5 mM TMB while (A) at different time, and (B) in the presence of different nanoparticles in 10 min(a. 4.2 ± 0.2 nm Fe₃O₄, b. 4.1 ± 0.3 nm CoFe₂O₄, c. 13.8 ± 4.6 nm CoFe₂O₄, d. 24.5 ± 5.3 nm CoFe₂O₄, e. 32.1 ± 4.2 nm CoFe₂O₄, f. ~ 45.2 ± 15.1 nm CoFe₂O₄, g. without H₂O₂ and h. without NPs).



Figure S5. The time-dependent UV absorbance curve of the HAc-NaAc solution (pH = 4.0) containing 10.0 mM H₂O₂ and 1.5 mM TMB in the presence of an equal number of CoFe₂O₄ NPs (a. 4.1 ± 0.3 nm CoFe₂O₄, b. 4.2 ± 0.2 nm Fe₃O₄) and iron ions leaching into solution (c. iron leaching from 4.2 ± 0.2 nm Fe₃O₄ and d. iron leaching from 4.1 ± 0.3 nm CoFe₂O₄). It demonstrated that CoFe₂O₄ activity does not result from iron leaching.

CoFe ₂ O ₄	K _m [mM]	V _{max} [M/min]
4.1±0.3 nm	0.00645	0.08258
$13.8 \pm 4.6 \text{ nm}$	0.05537	0.01586
$24.5 \pm 5.3 \text{ nm}$	0.01725	0.06163
$32.1 \pm 4.2 \text{ nm}$	0.02427	0.04052
45.2±15.1 nm	0.03476	0.03881

Table S1. Comparison of the Kinetic Parameters of Different NPs when changing the concentrations of H_2O_2 (TMB as a substrate). K_m is the Michaelis constant, V_{max} is the maximal reaction rate.

Table S2. Comparison of the Kinetic Parameters of Different NPs when changing the concentrations of TMB. K_m is the Michaelis constant, V_{max} is the maximal reaction rate.

CoFe ₂ O ₄	K _m [mM]	V _{max} [M/min]
4.1±0.3 nm	0.03551	0. 50178
$13.8 \pm 4.6 \text{ nm}$	0.22769	0.02629
24.5±5.3 nm	0.03934	0.07688
$32.1 \pm 4.2 \text{ nm}$	0.06607	0.05522
45.2±15.1 nm	0.11073	0.06253

Table S3. Comparison of the Kinetic Parameters of HRP, Fe_3O_4 (4 nm) and $CoFe_2O_4$ (4 nm) when changing the concentrations of H_2O_2 (TMB as a substrate). K_m is the Michaelis constant.

Nanoparticles	K _m [mM]
HRP	0.062
Fe ₃ O ₄	0.0096
CoFe ₂ O ₄	0.0065