Supporting Information

A one-pot and modular self-assembly strategy for high-performance organized enzyme cascade bioplatform based on dual-functionalized protein-PtNP@ mesoporous iron oxide hybrid

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Fig. S1. XRD patterns of CM-PtNP/ m-Fe₃O₄ nanohybrid (A) and m-Fe₃O₄ (B). The nether lines in A and B correspond to the standard position and the relative intensities for the crystals of Pt (JCPDS 04-0802) and Fe₃O₄ (JCPDS 19-0629), respectively. (C) The EDX spectroscopy of CM-PtNP/ m-Fe₃O₄ nanohybrid.



Fig. S2. SEM images of CM-PtNP/ $m\text{--}Fe_3O_4$ nanohybrid.



Fig. S3. TEM images of m-Fe₃O₄ NPs.



Fig. S4. (A) XPS spectra of survey scan of CM-PtNP/ m-Fe₃O₄ nanohybrid. High-resolution Fe 2p (B) and Pt 4f_{7/2} (C) XPS spectra of the hybrid.



Fig. S5. (A) FTIR spectra of β -casein, m–Fe₃O₄ and CM-PtNP/ m–Fe₃O₄ nanohybrid. (B) FTIR spectra of CM / m–Fe₃O₄ and CM-PtNP.



Fig. S6. (A) N_2 adsorption-desorption isotherm of CM-PtNP/ m–Fe₃O₄ nanohybrid and (B) the corresponding pore size distribution.



Fig. S7. Effect of temperature (A), pH (B), TMB concentration (C) and H_2O_2 concentration (D) on the peroxidase-like activity of the CM-PtNP/ m–Fe₃O₄ nanohybrid.



Fig. S8. (A, C) Correlation of initial reaction velocity with the concentration of one substrate (H_2O_2 or TMB) fixed and the other varied. (B, D) The double-reciprocal plots of (A) and (C).



Fig. S9. Relative activity of CM-PtNP/m–Fe₃O₄ nanohybrid after stored in refrigerator at 4 $\,^{\circ}$ C for one month (A) and recycling five times (B).



Fig. S10. SEM images of CM-PtNP/ $m{-}{\rm Fe_3O_4}$ nanohybrid after repeated uses.



Fig. S11. (A) XPS spectra of survey scan of ChOx/CM-PtNP/ m-Fe₃O₄ nanohybrid. High-resolution Pt 4f_{7/2} (B) and Fe 2p (C) XPS spectra of the hybrid.



Fig. S12. SEM image of ChOx/CM-PtNP/ $m\text{--}Fe_3O_4$ nanohybrid.



Fig. S13. UV-vis absorbance spectra of ChOx before (black) and after blending with CM-PtNP/m-Fe₃O₄ (pink), CEH-Pt/m-Fe₃O₄ (red) and Pt/m-Fe₃O₄ nanohybrids (blue).



Fig. S14. Effect of pH (A) and temperature (B) on the ChOx/CM-PtNP/Fe₃O₄ nanohybrid cascade platform. [TMB]=0.8 mM, [cholesterol]=0.5 mM

Enzyme	$K_{\rm m}({\rm mM})$		$V_{\rm max}(10^{-8}{ m M})$		Deferrere
	TMB	H_2O_2	TMB	H_2O_2	Kelerence
HRP	0.434	3.7	10.0	8.71	1
CM-Pt NPs	0.052	63.86	16.4	29.0	2
m-Fe ₃ O ₄	-	458.9	-	0.31	3
CM-PtNP@m-Fe ₃ O ₄	0.257	0.036	19.5	31.5	This work

Table S1 Comparison of the apparent Michaelis-Menton constant (K_m) and maximum reaction rate (V_{max}) of CM-PtNP@m-Fe₃O₄ nanohybrid.

H ₂ O ₂ sensing systems	Liner range (µM)	Detection limit (µM)	Reference
Co ₄ N NWs	0.5-30	0.024	4
3D graphene/Fe ₃ O ₄ -Au NPs	20-190	12	5
Cu(HBTC)-1/Fe ₃ O ₄ -Au NPs	2.86-71.43	1.10	6
FeHPO	57.4-525.8	1.0	7
Au@TiO ₂	5-100	4	8
Ce(OH)CO ₃	0-80	0.3	9
N-GQDs	2-1170	5.3	10
CM-PtNP/Fe ₃ O ₄	0.01-1000	0.001	This work

Table S2 Comparison of different nanozymes for H_2O_2 detection by colorimetric method.

Nanozyme	Liner range (µM)	Detection limit (µM)	Steps	Reference
Au@Ag NPs	0.3–300	0.15	Two	11
Pt/PCNs	25-500	8.3	Two	12
MoS ₂ NRs/Au NPs	40-1000	15	Two	13
Graphene QDs	20-600	6.0	Two	14
PB/MWCNT	4 - 100	3.01	Two	15
MWCNT@rGONR	20-1000	10	Two	16
BNNS@CuS	10–100	2.9	Two	17
MoS ₂ nanosheet	2–200	0.76	Two	18
DNAzymes	1-30	0.1	Two	19
Fe ₃ O ₄ @MIL-100(Fe)	2-50	0.8	Two	20
Fe ₃ O ₄ / ChOx / silica	10-250	5	One	21
ChOx/CM-PtNP /Fe ₃ O ₄	0.1-400	0.05	One	This work

Table S3 Comparison of different nanozymes for cholesterol detection.

	Cholesterol concentration	Cholesterol concentration	
Sample	determined by our method	obtained by the hospital	RSD (n=5, %)
	(mM)	(mM)	
1	4.39	4.36	2.01
2	3.05	3.07	1.84
3	4.79	4.80	1.59

 Table S4 Detection of cholesterol in serum samples using the proposed method.

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