Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Dual mimic enzyme properties of Fe nanoparticles embedded in two-dimensional carbon nanosheets for colorimetric detection of biomolecules

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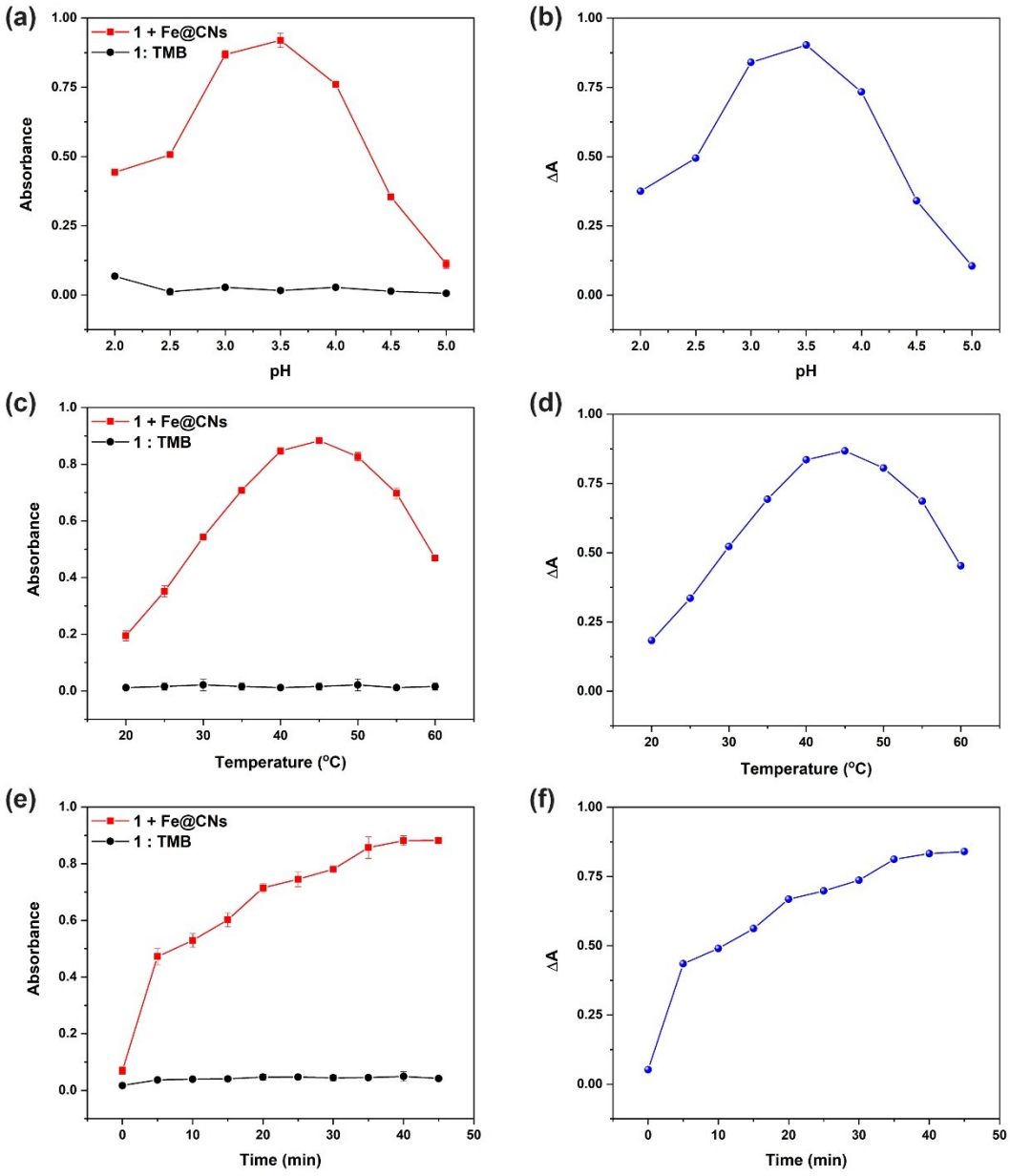

Fig. S1 The effect of pH (a, b), reaction temperature (c, d), and incubation time (e, f) on the oxidase-like activity of Fe@CNs (0.2 g L^{-1}). ΔA is the enhancement of absorbance intensity at 652 nm of the catalytic system ($A-A_0$). A and A_0 are the absorbance intensity at 652 nm of TMB (0.2 mM.) with and without Fe@CNs, respectively.

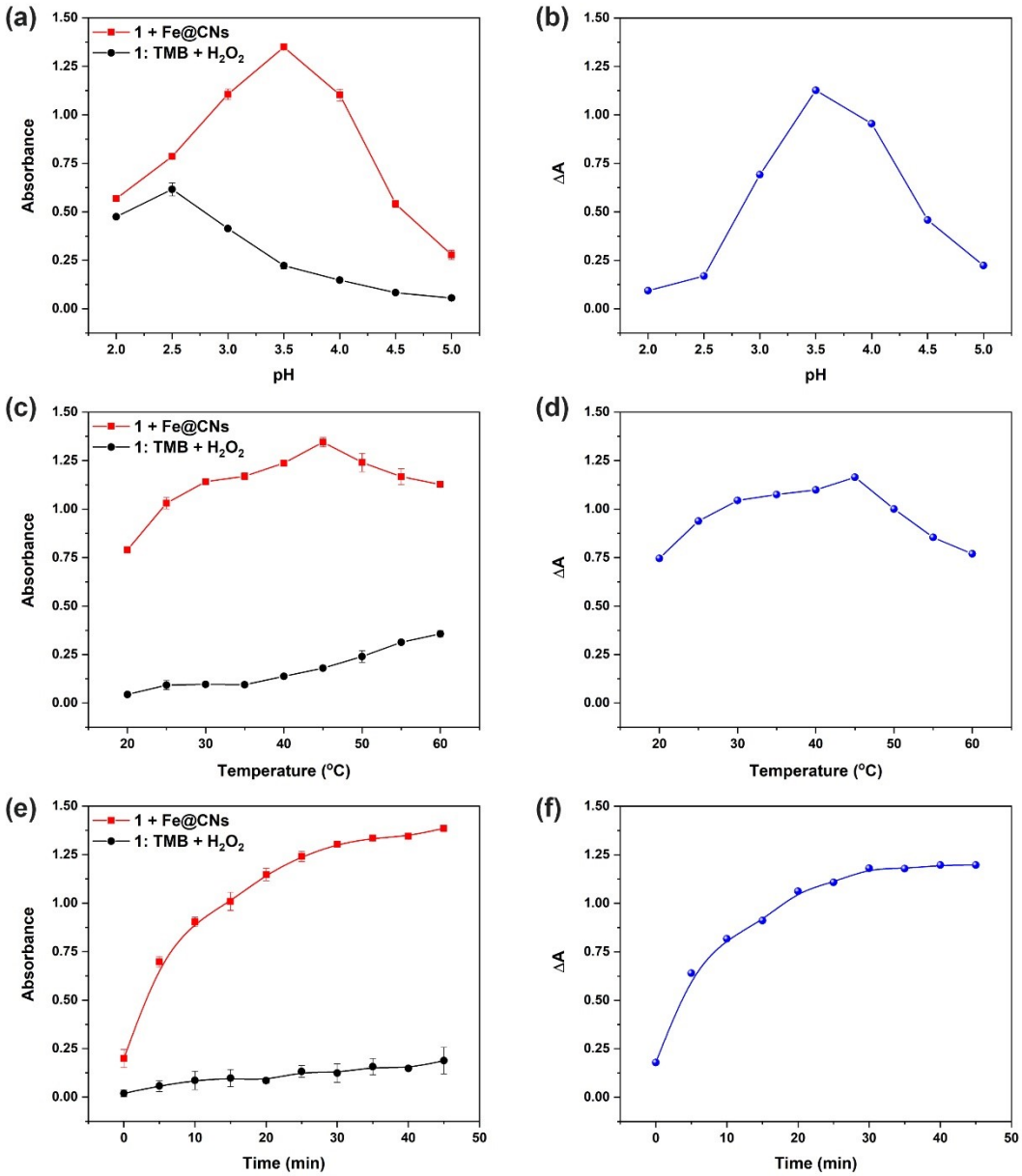

Fig. S2 The effect of pH (a, b), reaction temperature (c, d), and incubation time (e, f) on the peroxidase-like activity of Fe@CNs (0.2 g L^{-1}). ΔA is the enhancement of absorbance intensity at 652 nm of the catalytic system ($A-A_0$). A and A_0 are the absorbance intensity at 652 nm of TMB (0.2 mM) and H_2O_2 (0.1 mM) with and without Fe@CNs, respectively.

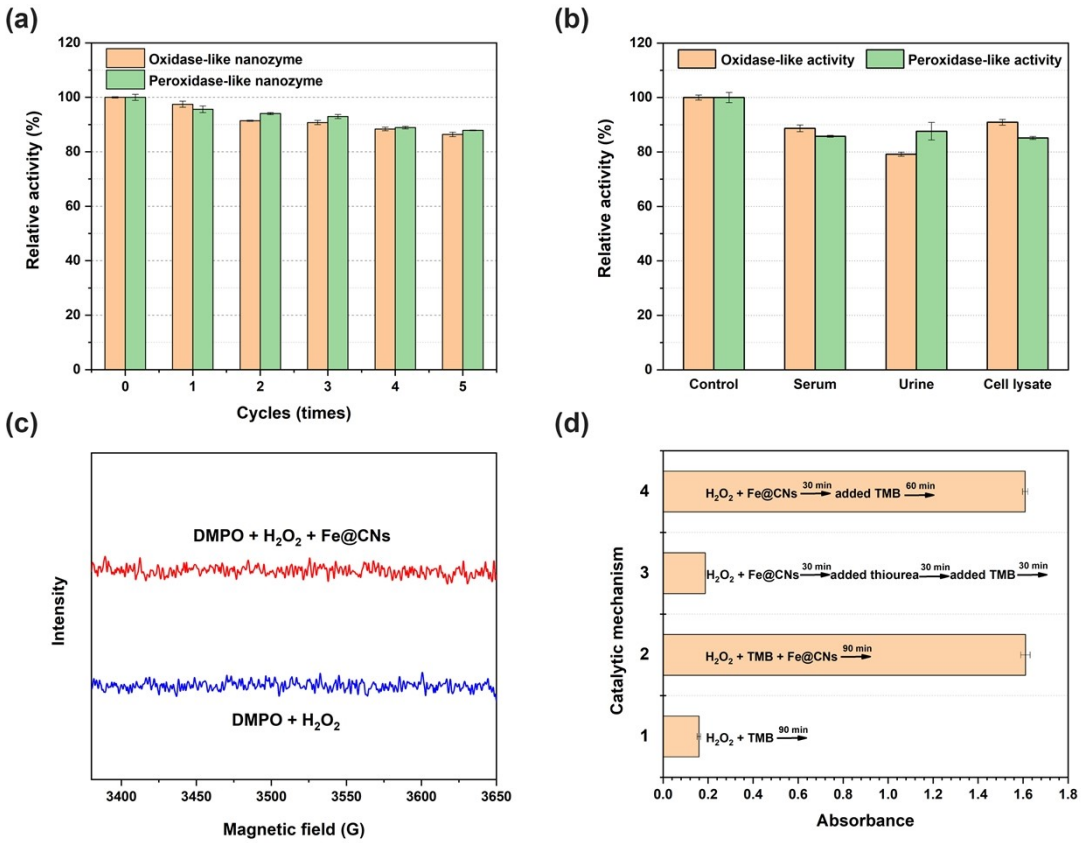

Fig. S3 (a) The reusability of Fe@CNs as the oxidase-like nanozyme and peroxidase-like nanozyme in the catalytic system. (b) The catalytic activity of Fe@CNs in the catalytic system with the presence of 1% serum sample, 1% urine sample, and 1% cell lysate, respectively. (c) DMPO (100 mM) and H_2O_2 (100 mM) with and without Fe@CNs (50 mg L^{-1}) by using methanol as solvent. The sample was conducted in the condition without oxygen. (d) The absorbance intensity at 652 nm of the samples without oxygen under different experimental condition. Inset: schematic representations of the different reaction routes and ingredients. The total volume of samples was 1.5 mL.

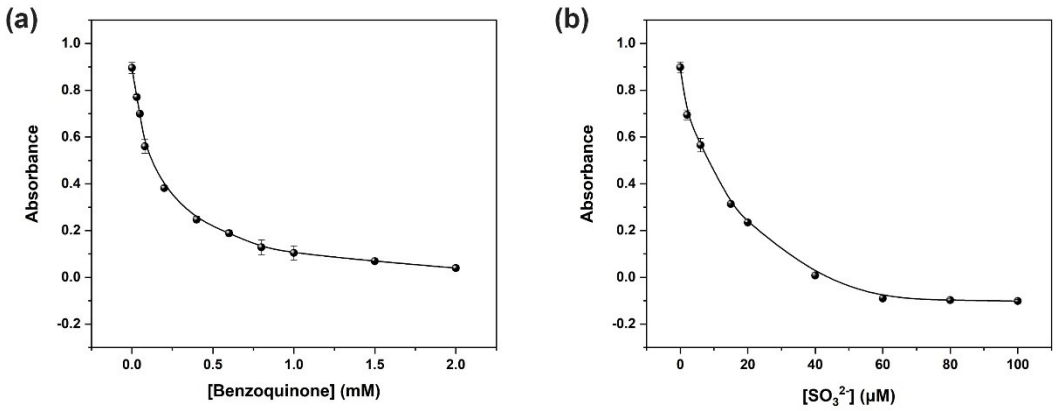

Fig. S4 The effect of benzoquinone (a) and SO_3^{2-} (b) on the oxidase-like activity of Fe@CNs.

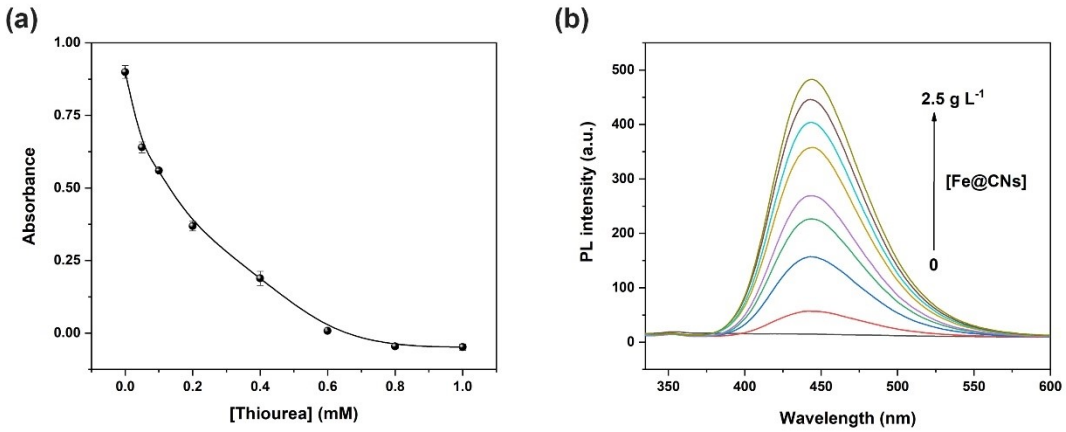

Fig. S5 (a) The effect of thiourea with different concentration (0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM) on the peroxidase-like activity of Fe@CNs. [Fe@CNs] = 0.2 g L^{-1}, [TMB] = 0.2 mM, [H_2O_2] = 0.1 mM. (b) Phosphorescent intensity of the sample containing TA (0.625 mM), H_2O_2 (50 mM) and different concentration of Fe@CNs (0, 0.1, 0.25, 0.4, 0.5, 1.0, 1.5, 2.0 and 2.5 g L^{-1}) under pH 3.5 with an excitation wavelength of 314 nm.

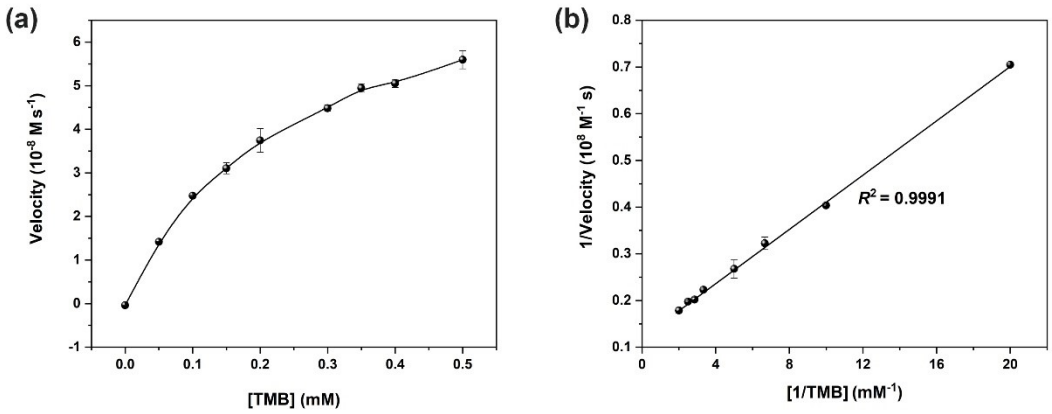

Fig. S6 Steady-state kinetic analysis by using the Michaelis-Menten model (a) and Lineweaver-Burk double-reciprocal model (b) for the oxidase-like activity of Fe@CNs (0.2 g L^{-1}) at pH 3.5.

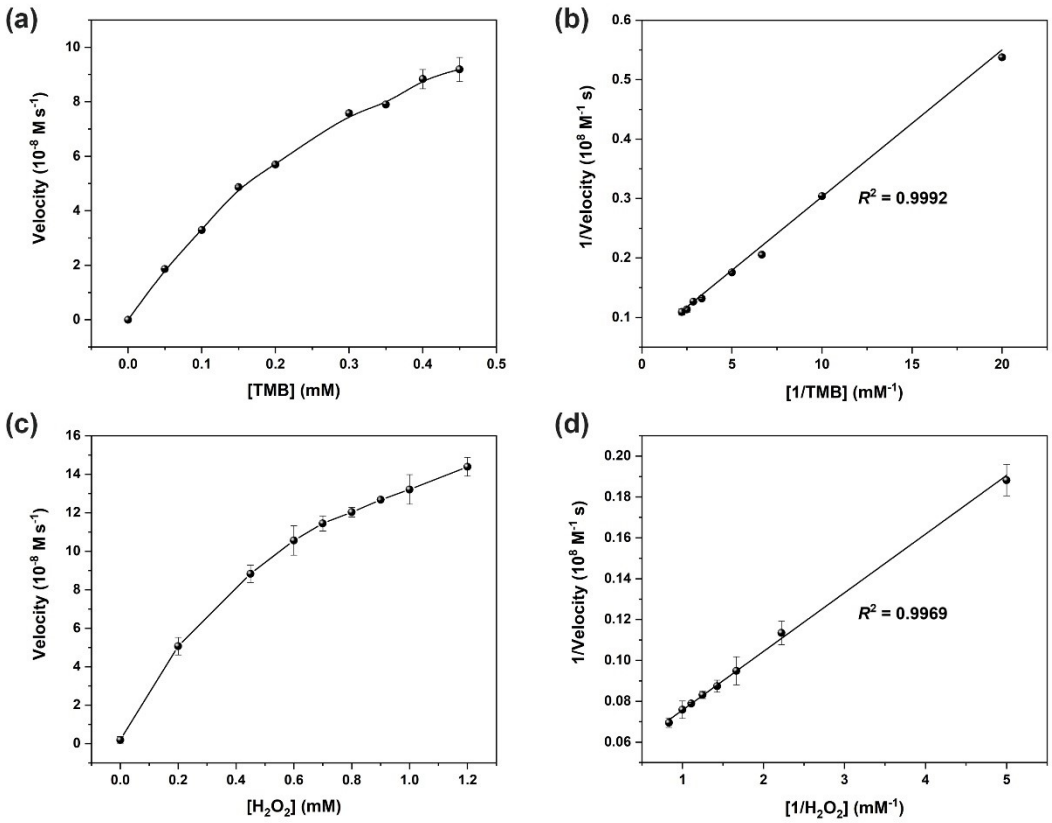

Fig. S7 Steady-state kinetic analysis by using the Michaelis-Menten model (a and c) and Lineweaver-Burk double-reciprocal model (b and d) for the peroxidase-like activity of Fe@CNs (0.2 g L^{-1}) at pH 3.5. (a) Reaction velocity plot with a fixed H_2O_2 concentration (0.1 mM) and various TMB amount. (c) Reaction velocity plot with a fixed TMB concentration (0.2 mM) and various H_2O_2 amount. (b) and (d) Double reciprocal plots for Fe@CNs with the amount of one substrate (TMB or H_2O_2) varied.

Table S1. The Michaelis-Menten constant (K_m) of Fe@CNs and other nanomaterial-based oxidase-like nanozymes to TMB.

Catalyst	K_m (mM)	Reference
PCN-222 (Fe)	1.63	1
Fe-Co-LDH	0.34	2
Fe(III)-L3	2.03	3
CS/Cu/Fe composite	0.27	4
Dex-FeMnzyme	0.33	5
Fe@CNs	**0.24**	**this work**

Table S2. The Michaelis-Menten constant (K_m) and maximum reaction velocity (V_{max}) of HRP, Fe@CNs and other nanomaterial-based peroxidase-like nanozymes to H_2O_2 and TMB.

Catalysts	Substrate	K_m (mM)	V_m (10^{-8} M s^{-1})	Reference
HRP	H_2O_2	3.70	8.71	6
	TMB	0.43	10.00	
$ZnFe_2O_4$ MNPs	H_2O_2	1.66	7.74	7
	TMB	0.85	13.31	
Fe-NDs	H_2O_2	0.87	3.76	8
	TMB	0.76	2.27	
Fe-MOF	H_2O_2	1.3	2.5	9
	TMB	2.6	5.6	
Fe@CNs	**H_2O_2**	**0.50**	**21.22**	**this work**
	TMB	**0.45**	**18.07**	

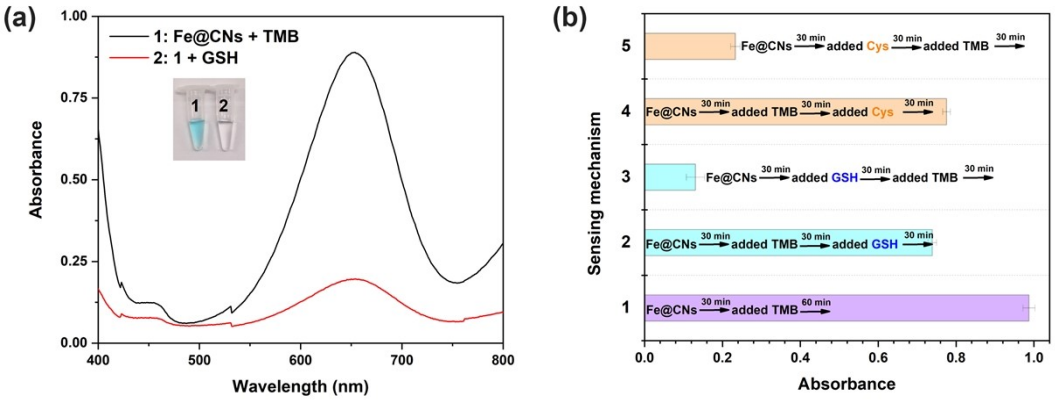

Fig. S8 (a) UV-vis absorption curves of Fe@CNs (0.2 g L^{-1}) and TMB (0.2 mM) without and with GSH (100 μM), respectively. (b) The GSH/Cys sensing mechanism based on the oxidase-like catalytic activity of Fe@CNs. Inset: schematic representations of the different reaction routes and ingredients.

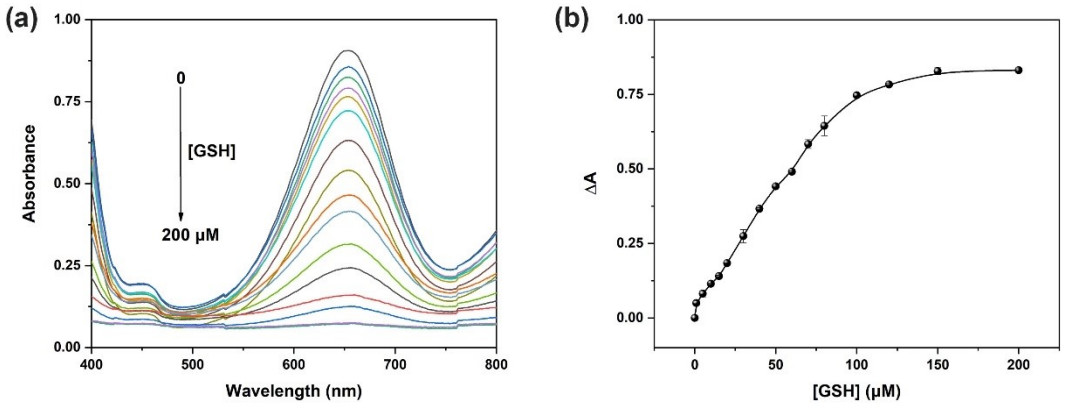

Fig. S9 (a) UV-vis absorption curves of Fe@CNs (0.2 g L^{-1}) with TMB (0.2 mM) and different amounts of GSH. (b) The absorbance changes with increasing concentration of GSH. ΔA is the enhancement of absorbance intensity at 652 nm of the catalytic system (A_0-A). A_0 and A are the absorbance intensity at 652 nm of Fe@CNs and TMB without and with GSH, respectively.

Table S3. Comparison of different nanomaterial-based nanozymes as the sensing platform for GSH detection by colorimetric method.

Nanozyme	Linear range (μM)	Detection limit (μM)	Reference
$Cu_{1.8}S$ NPs	500-10000	60	10
CuS-PDA-Au	0.5-100	0.42	11
CBT-Cys(SEt)	0-87	1	12
Au NCs	2-25	0.42	13
Por-$ZnFe_2O_4$/rGO	2-40	0.76	14
Fe_3O_4 NPs	3-30	3.0	15
MnO_2 NSs	1-25	0.3	16
PS MOF	0-20	0.68	17
Fe@CNs	**1-80**	**0.22**	**this work**

Table S4. Recoveries of GSH, H_2O_2 and glucose in 1% human serum sample.

Target	Added (μM)	Recovered (μM)			Recovery (%)
GSH	20	19.0	19.9	21.3	100.55 ±5.79
	40	36.5	41.4	40.9	98.96 ±6.66
	60	65.6	58.3	65.2	105.09±6.82
H_2O_2	50	51.8	47.3	48.2	98.22±4.75
	100	100.1	102.0	102.3	101.48 ±1.17
	150	157.5	165.3	152.3	105.51±4.45
glucose	50	49.9	46.6	50.8	98.22±4.47
	100	107.6	101.7	95.0	101.43 ±6.27
	150	155.1	151.6	158.6	103.40±2.36

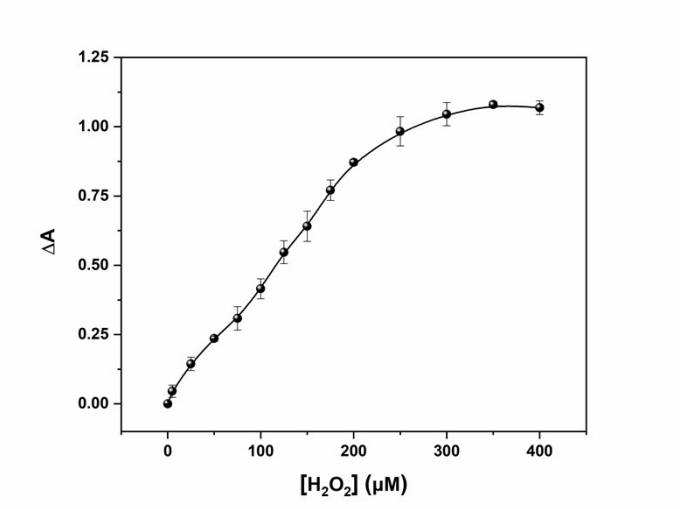

Fig. S10 The absorbance changes with increasing concentration of H_2O_2. ΔA is the enhancement of absorbance intensity at 652 nm of the catalytic system ($A-A_0$). A and A_0 are the absorbance intensity at 652 nm of Fe@CNs (0.2 g L^{-1}) and TMB (0.2 mM) with and without H_2O_2, respectively.

Table S5. Comparison of different nanomaterial-based peroxidase-like nanozyme as the sensing platform for H_2O_2 detection by colorimetric method.

Nanozyme	Linear range (μM)	Detection limit (μM)	Reference
Cys-MoS_2 NFs	0-300	4.103	18
$CePO_4$-CeO_2	5-150	2.9	19
$FeMnO_3$@PPy NTs	0-100	3.2	20
FePPOP-1	20-1000	6.5	21
CuZnFeS NCs	10-55	3	22
M-CQDs	20-200	15	23
ZIF-67	1×10^5-1×10^6	110	24
CDs@ZIF-8-a	100-1000	3.6	25
Fe@CNs	**5-200**	**2.04**	**this work**

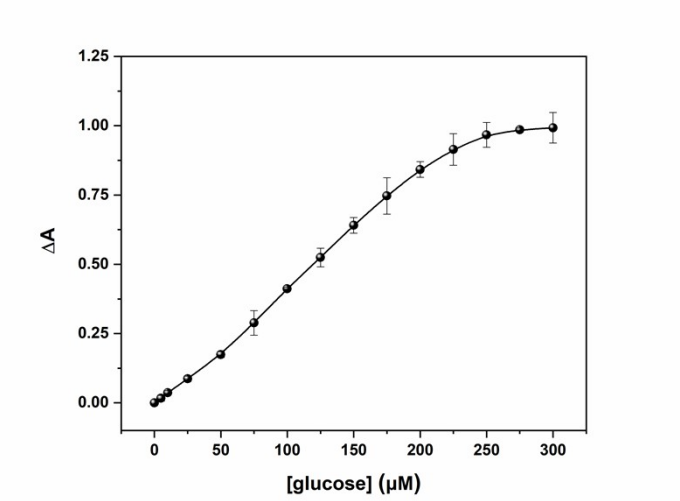

Fig. S11 The absorbance changes with increasing concentration of glucose. ΔA is the enhancement of absorbance intensity at 652 nm of the catalytic system ($A-A_0$). A and A_0 are the absorbance intensity at 652 nm of Fe@CNs (0.2 g L^{-1}) and TMB (0.2 mM) with and without glucose, respectively.

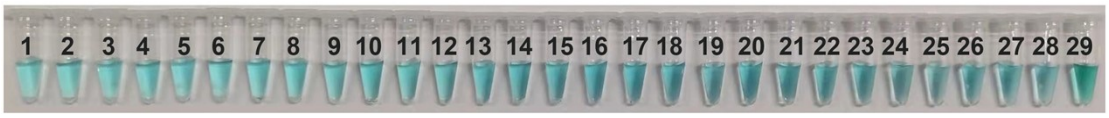

Fig. S12 The photographs of the specificity analysis of the glucose detection. The concentration of the interfering species, and glucose was 150 μM.

Table S6. Comparison of different nanomaterial-based peroxidase-like nanozyme as the sensing platform for glucose detection by colorimetric method.

Nanozyme	Linear range (μM)	Detection limit (μM)	Reference
FePPOPs-SO_3H	200-1500	16.38	26
CeO_2/Zeolite Y	0-340	35.4	27
Fe_3O_4 MNPs	50-1000	30	28
6Fe/CeO_2 NRs	1-100	3.41	29
Fe@PCN-224 NPs	30-800	22	30
WSe_2 NSs	10-60	10	31
$CoPW_{11}O_{39}$	33-500	23.4	32
Co_3O_4 NPs	20-200	5	33
Fe@CNs	**5-225**	**2.28**	**this work**

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