## **Supporting Information**

## A facile route for constructing Cu-N-C peroxidase mimics

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**Figure S1** X-ray photoelectron spectra of Cu-N-C SAzymes: (A) full survey spectrum, high-resolution (B) C 1s, (C) N 1s, (D) O 1s, (E) Cu 2p, and (F) Na 1s.



**Figure S2** The k<sup>2</sup>-weighted EXAFS fitting curves in k-space and their Fourier transforms in the R domain of (A-B) Cu-N-C SAzymes, (C-D) Cu foil, and (E-F) CuCh.



**Figure S3** Photographs and absorption spectra of common chromogenic peroxidase substrates catalytically oxidized by Cu-N-C SAzymes in the presence of  $H_2O_2$ : (A) Cu-N-C SAzymes + $H_2O_2$ +OPD, (B) Cu-N-C SAzymes+ $H_2O_2$ +ABTS, (C) Cu-N-C SAzymes + $H_2O_2$ +4-AAP/TOPS, (D) Cu-N-C SAzymes+ $H_2O_2$ +MBTH/TOOS.



**Figure S4** Typical absorption spectra of TMB in the presence of Cu-N-C SAzymes (black line), ACM and  $H_2O_2$  (red line),  $H_2O_2$  (blue line), Cu-N-C SAzymes and  $H_2O_2$  (pink line), Mg-ACM and  $H_2O_2$  (green line), Cu@Cu<sub>2</sub>O aerogels and  $H_2O_2$  (navy line). The concentration of copper in Cu-N-C SAzymes and Cu@Cu<sub>2</sub>O aerogels are the same, which are determined by ICP MS.



**Figure S5** Relative activity of Cu-N-C SAzymes after incubation for 2 h at various (A) temperatures, (B) pH values, (C) NaCl concentrations and short- and long- term stabilities of Cu-N-C SAzymes at room temperature.



**Figure S6** The effects of Cu-N-C SAzymes prepared from different (A) amount of sodium copper chlorophyll, (B) reaction pH, (C) reaction temperature, and (D) TMB concentration on the peroxidase-mimiking activity of Cu-N-C SAzymes in the presence of  $H_2O_2$ .



**Figure S7** The effect of the time on the catalysis oxidation of TMB by Cu-N-C SAzymes.



Figure S8 (A) ESR spectra of Cu-N-C SAzymes solution and Cu-N-C SAzymes- $H_2O_2$  solution. (B) Time-dependent ESR spectra of Cu-N-C SAzymes- $H_2O_2$  solution.



Figure S9 Selectivity of glucose detection by monitoring the absorbance at 450 nm with the colorimetric method using GOx and the as-prepared Cu-N-C SAzymes. The concentration of glucose was 20  $\mu$ M. And all the interferents with a concentration of 100  $\mu$ M were used.

Sample	Path	Coordination Number	Bond length R (Å)	Bond disorder σ <sup>2</sup> ×10 <sup>-3</sup> (Å <sup>2</sup> )	ΔE (eV)	R factor
Cu foil	Cu-Cu	12*	2.54±0.01	8.6±0.6	-6.5±0.9	0.002
Cu-N-C	Cu-N	4.2±0.5	1.90±0.01	4.8±1.5	-8.9±1.7	0.012
SAzymes	Cu-N/O-Cu	1.8±1.0	3.17±0.03	9.0±5.5	9.7±3.0	0.012
CuCh	Cu-N	4.5±0.6	2.00±0.01	5.2±1.2	1.4±1.6	0.008
	Cu-C	5.1±1.4	3.00±0.02	3.2±2.4	0.8±2.3	

Table S1. The K-edge EXAFS curves fitting parameters.

Catalyst	Substrate	K <sub>m</sub> (mM)	V <sub>max</sub> (10 <sup>-8</sup> M s <sup>-1</sup> )	References
Cu-N-C	TMB	0.1010	1.6025	This work
SAzymes				
	$H_2O_2$	0.1715	1.2981	
M-CQDs	TMB	0.2189	0.8819	[1]
	$H_2O_2$	0.4305	0.4611	
N-CQDs	TMB	11.19	0.38	[2]
	$H_2O_2$	0.1	0.14	
C-Dots	TMB	0.039	3.61	[3]
	$H_2O_2$	26.77	30.61	
CuNPs@C	TMB	1.65	12.05	[4]
	$H_2O_2$	1.89	5.3	
Cu@Cu <sub>2</sub> O	TMB	0.94	571	[5]
Cu-N-C	TMB	3.76	75.05	[6]
	$H_2O_2$	19.94	20.7	
CuO	TMB	0.013	/	[7]
	$H_2O_2$	85.6	/	
HRP	TMB	0.434	10.00	[8]
	$H_2O_2$	3.7	8.71	

**Table S2.** Michaelis–Menten constant  $(K_m)$  and maximum velocity  $(V_{max})$  obtained from the double reciprocal plots which have been compared with other nanozymes

System	Method	Linear range	Detection	References
		(µM)	limit (µM)	
Cu-N-C SAzymes	Colorimetry	0.1-20	0.05	this work
E-GQDs	Colorimetry	10-100	6.0	[9]
CuNPs@C	Colorimetry	50-350	0.32	[10]
r-CDs	Colorimetry	10-400	2	[11]
CuNPs-gC <sub>3</sub> N <sub>4</sub>	Colorimetry	1-100	0.37	[12]
Fe <sub>3</sub> O <sub>4</sub> @C	Colorimetry	6-100	2	[13]
CNT/FeNC SAN	Colorimetry	10000-100000	20	[14]
Rosette-GCN	Fluorescence	5-275	1.2	[15]
m-CeO <sub>2</sub>	Colorimetry	20-1000	10	[16]

 Table S3. Comparison of various nanoparticles-based methods for detection of glucose.

Serum sample	Spiked (mM)	Found (mM)	Recovery (%)	RSD (%)	Glucose
					assay
					kit(mM)
1	0	2.09		4.57	2.03
	2	4.05	98.0	1.73	
	5	7.23	102.8	0.57	
2	0	2.15		0.80	2.12
	2	4.07	96.0	4.99	
	5	7.29	102.8	0.66	
	0	1.73		1.54	1.68
3	2	3.76	101.5	3.22	
	5	6.96	104.6	3.67	
	0	2.01		1.73	1.97
4	2	3.98	98.5	1.14	
	5	7.11	102.0	3.04	
5	0	2.43		4.50	2.4
	2	4.44	100.5	1.45	
	5	7.36	98.6	1.73	
6	0	2.53		3.60	2.55
	2	4.50	98.5	0.41	
	5	7.66	102.6	0.75	

Table S4. Determination of glucose in human serum samples (n=3).

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