Supporting information

Highly active metal-free Peroxidase Mimics based on Oxygen-doped carbon nitride by promoting electron transfer capacity

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Reagents and chemicals

Melamine, H_2O_2, NaCl, KCl, L(+)-Ascorbic acid, L-Cysteine, H_2SO_4 and HNO_3 were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Glucose oxidase (GOx), glucose were obtained from Sigma-Aldrich Chemical Co. 3,3′5,5′-Tetramethylbenzidine (TMB), D-Fructose, D-Lactose monohydrate and Maltose were obtained from Aladdin. Formulating phosphate-buffered saline (PBS, Na_2HPO_4-NaH_2PO_4, 0.1 M) in the laboratory. Doubly distilled water (18.2 MΩ) was used throughout the experiment. All chemical reagents were of analytical grade and directly used as received without further purification.

Apparatus

Transmission electron microscopy (TEM) was conducted using a JEOL 100 instrument (JEOL, Japan) with an accelerating voltage of 200 kV. Fourier transform infrared (FTIR) spectrum was received on a Fourier transform spectrometer (Tensor 27, Bruker). UV–vis absorption spectra were measured by UV-2450 spectrophotometer (Shimadzu, Japan). X-ray photoelectron spectroscopy (XPS) was carried out using Thermo ESCALAB250Xi. X-ray diffraction (XRD) spectra were obtained by a Bruker instrument with Cu Kα radiation (40 kV, 40 mA). All the photographs were taken by a Canon digital camera.

Preparation of bulk g-C_3N_4

The bulk g-C_3N_4 was prepared based on a previously reported literature with minor modification[1]. In a typical synthesis, 8.0 g of the melamine was ground in the quartz mortar, heated to 550 ℃ in a muffle furnace for 3 h and maintained for another 4 h. Then take out g-C_3N_4 and ground, repeat the above heating step again.

Preparation of oxygen doped carbon nitride

OCN (oxygen-doped carbon nitride) was prepared by oxidizing the bulk g-C_3N_4

with HNO_3 and H_2SO_4 at the ratio of 1:1 according to the previous report[2]. In brief, 0.5 g of the bulk g-C_3N_4 powder was thoroughly mixed with 20 ml of HNO_3 and 20 ml of H_2SO_4 in the flask, followed by vigorous magnetic stirring at room temperature for 0.25h, 0.5 h, 1h, 1.5h, 2 h, 4 h, 6 h, 8 h respectively to obtain OCN with different oxidation states. Afterward, the mixture was collected by centrifugation at 12000 rpm for 5 minutes to get rid of the residual acids and the resulting solid sample was washed at least 5 times with deionized water until the solution became neutral. Finally, the obtained sample was ultrasonically dispersed for use.

Detection of H_2O_2 and glucose using OCN

The detection method of H_2O_2 was as followed: Add 20 μL OCN (1 mg/mL) and 30 μL TMB (8 mM) into 2 ml centrifuge tube with 400 μL phosphate buffer (0.1 M, pH 4.0) vortex for 30s. Then add different concentrations of 50 μL H_2O_2 solution to the centrifuge tube, the centrifuge tube was incubated in thermostat water bath shaker at 40℃ for 15 min with slight shaking (200 rpm). After 15 min, add 20 μL H_2SO_4 (2 M) to terminate the catalytic reaction and the color of the solution immediately turned from blue to yellow due to the TMB blue oxidation product was converted to the yellow form of diamine. Finally, centrifuge the tube at 13000 rpm for 10 min, the supernatant was removed and the absorption peak at 450 nm was recorded by ultraviolet absorption spectroscopy.

The detection method of glucose was as followed: 10 μL glucose oxidase (2mg/mL) was added into 2 ml centrifuge tube with 40 μL phosphate buffer (0.1 M, pH 7.4) containing different concentrations of glucose, then vortex 1 min at room temperature. The centrifuge tube was incubated in a thermostat water bath shaker at 37℃ for one hour with slight shaking (200 rpm). After one hour, 20 μL OCN (2 mg/mL), 30 μL TMB (8 mM), 400 μL phosphate buffer (0.1 M, pH 4.0) was added into

the centrifuge tube, incubating for 15 min at 40℃, then added 20 μL H_2SO_4 (2 M) to terminate the catalytic reaction. Finally, centrifuge the tube at 13000 rpm for 10 min, the supernatant was removed and the absorption peak at 450 nm was recorded by ultraviolet absorption spectroscopy.

Before testing the milk sample, the sample needed to be treated before use[3], the method was as followed: Firstly, milk was centrifuged at 13,000 rpm to remove organic substances such as protein, fat. Then the resulting supernatant was further centrifuged until there was no precipitate. The final solution was diluted three-fold for the colorimetric determination of H_2O_2 in the milk sample.

Characterization of OCN

The phase structure, molecular structure and the chemical states of the OCN and g-C_3N_4 were explored by XRD patterns and FTIR. The XRD diffraction peaks around 27.3° (0 0 2) and the peaks around 13.11° (1 0 0) were related to the interlayer superposition reflection of aromatic system[4] and superposition reflection of in-plane units[5] respectively(Fig. S2A). The XRD pattern of OCN had a similar peak structure to g-C_3N_4, which indicated that the phase structure of OCN had not changed. Compared with g-C_3N_4, the characteristic peak (002) and the (100) peak were significantly decreased. This phenomenon indicated that OCN had two-dimensional structural features[2].

The FTIR spectra revealed a typical molecular structure of g-C_3N_4 (Fig. S2B). The characteristic peaks at 810 cm^{-1} belonging to the s-triazine ring system are corresponding to the stretching modes of CN heterocycles. The peaks at ~1240, 1319, 1406, 1555, and 1632 cm^{-1} for nitrogen-containing groups (C=N and C-N). However, C=O and C-O bonds are difficult to distinguish in OCN by FT-IR. Because according

to the work of Chen and Larkin, C-N, C-C, and C-O bonds had very similar force constants[6]. In addition, the broad absorption band between 3000 and 3600 cm-1 was attributed to the stretches of N-H and O-H.

Optimization of the reaction conditions

The effect of reaction conditions on the catalytic activity of OCN peroxidase-like activity is significant. Therefore, optimization of various conditions, such as reaction time, reaction temperature, the concentration of TMB, and pH value is very necessary. As shown in Fig. S4, the pH of 4.0 displays the maximum value of the absorption peak intensity. In the range of 20 ℃ to 70 ℃, the catalytic activity reached a peak at 40 ℃. Through the relationship between reaction time and absorption peak, the reaction speed slowed down after 15 min. So 15 min was chosen in the following experiment. The effect of TMB concentration was also explored and the optimal concentration was chosen as 8 mM.

Figure S1. Suspension of (A) g-C_3N_4 and (B) OCN

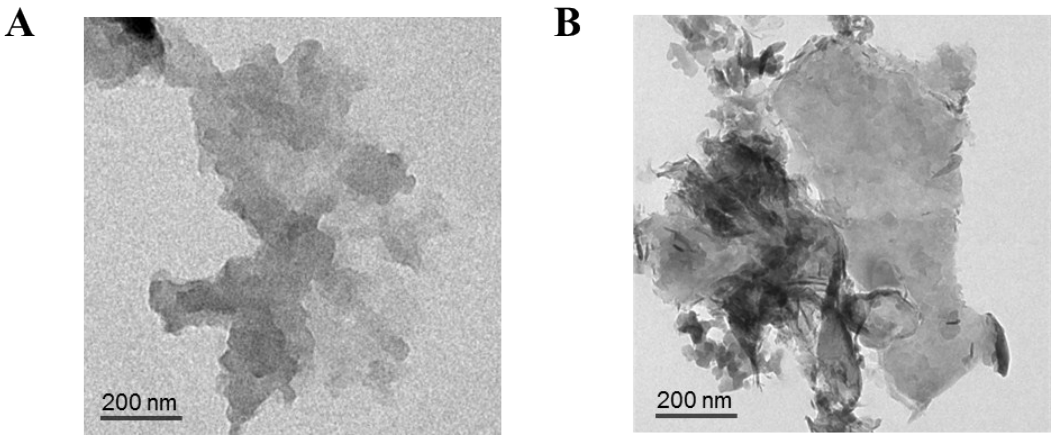

Fig. S2. TEM images of the (A) OCN and (B) g-C_3N_4

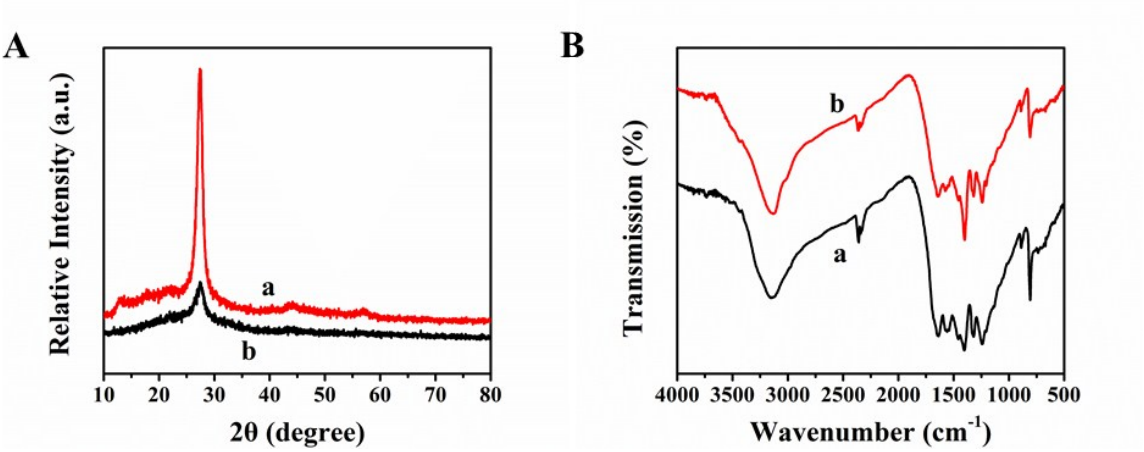

Figure S3. (A) XRD patterns and (B) FT-IR spectra of the (a) g-C_3N_4 and (b) OCN.

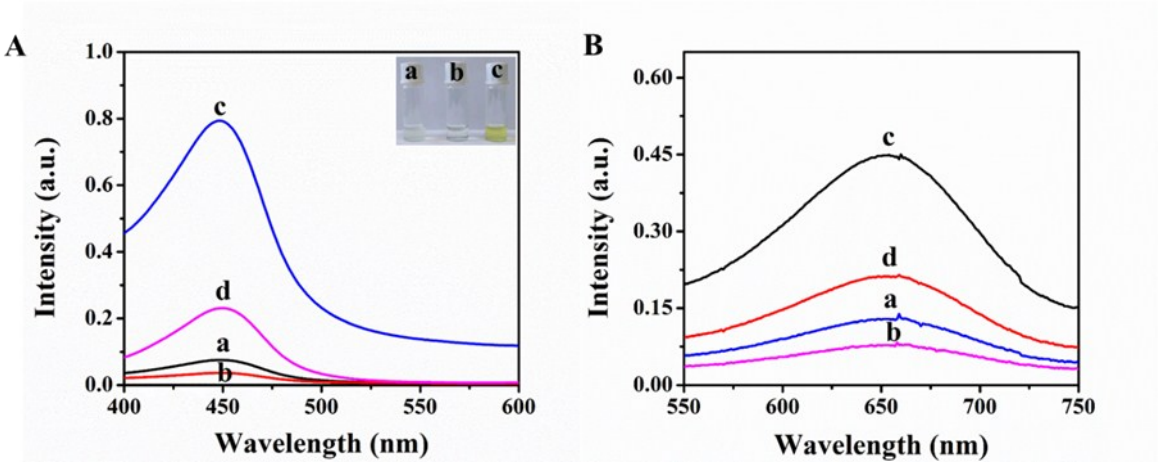

Fig. S4. UV absorption curve of TMB mixing with (a) OCN. (b) H_2O_2. (c) OCN and H_2O_2. (d) g-C_3N_4 and H_2O_2. Adding H_2SO_4 to terminate the reaction (A) and not adding H_2SO_4 to terminate the reaction (B). Inset: photographs of different reaction solutions to a-c. Conditions: 20 μL OCN (1mg/ml) or 20 μL g-C_3N_4 (1mg/ml), 30 μL TMB (8mM) and 400 μL PBS (0.1M pH 4.0) were vortex for 30 s. Then add 50 μL H_2O_2 (500 μM) and the mixture was placed in the thermostat water bath shaker incubated at 40℃ for 15 min with slight shaking (200 rpm). Afterward, added 20 μL H_2SO_4 (2M) to terminate the catalytic reaction.

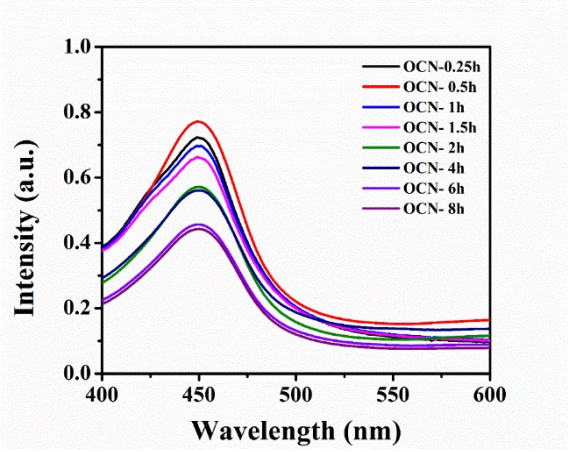

Figure S5. UV absorption curve of TMB catalyzed by OCN under different oxidation times.

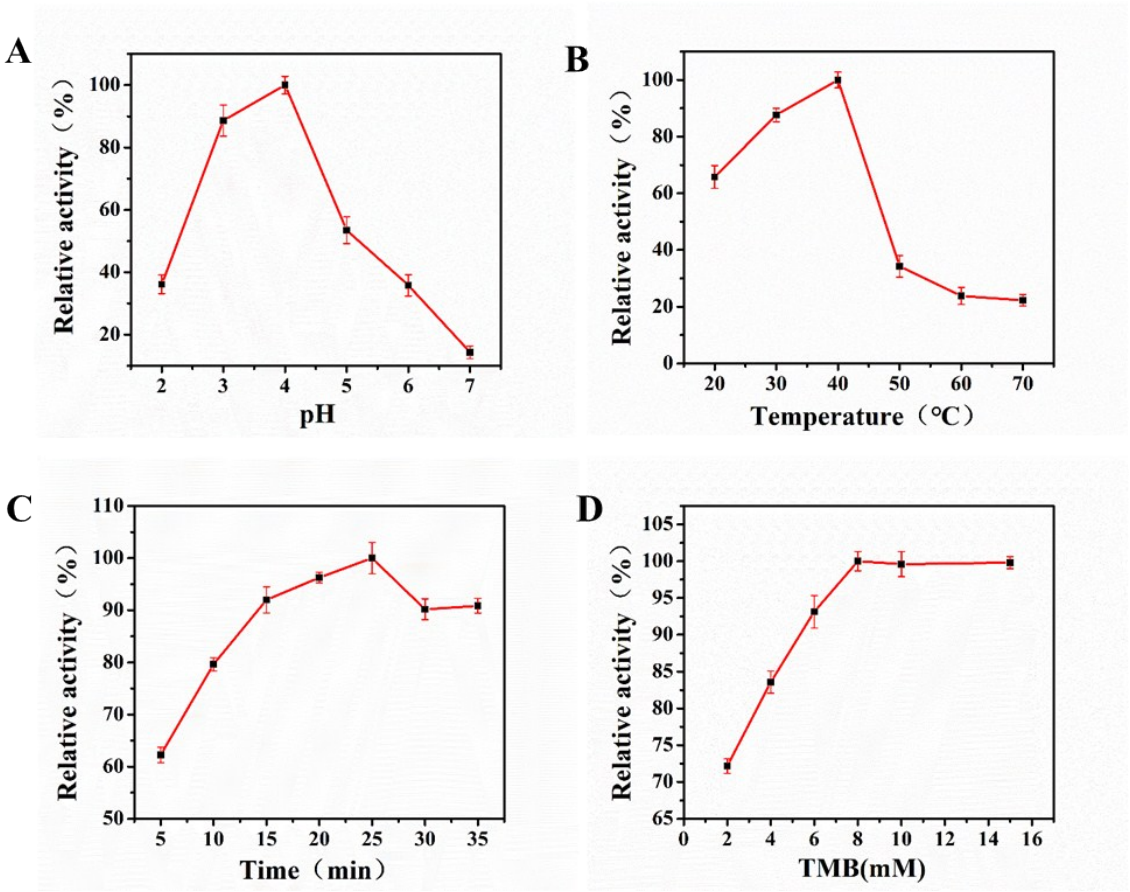

Figure S6. Dependence of the OCN peroxidase-like activity on (A) pH, (B) temperature, (C) reaction time, and (D) TMB concentration. The maximum point in each curve was set as 100 %.

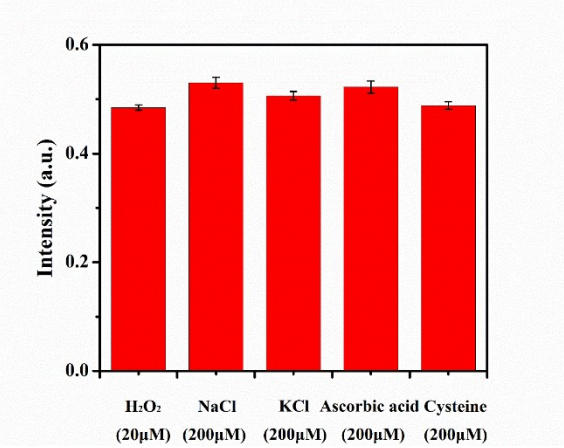

Figure S7. Selectivity analysis for H_2O_2 detection by monitoring the relative absorbance. The error bars represent the standard deviation of three measurements.

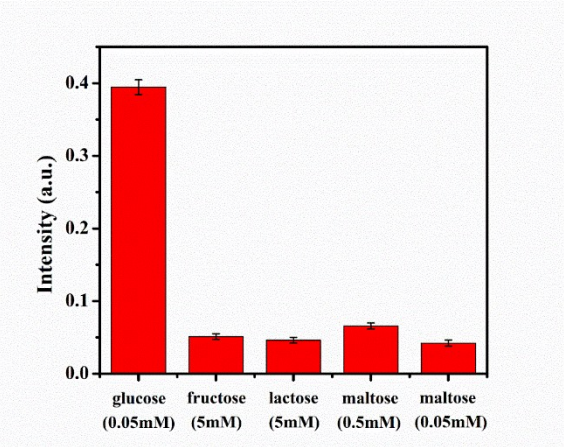

Figure S8. Selectivity analysis for glucose detection by monitoring the relative absorbance. The error bars represent the standard deviation of three measurements.

Table S1. Element analysis of g-C_3N_4 and OCN.

Materials	Element		Content(wt%)
	C	N	O
g-C_3N_4	33.5	64.72	1.78
OCN-0.5h	29.2	57.91	12.89
OCN-2h	29.78	56.34	13.88
OCN-4h	29.44	55.97	14.59
OCN-6h	29.81	55.07	15.12
OCN-8h	30.24	53.78	15.98

Table S2. A list of a series of H_2O_2 sensors based on nanomaterials owning peroxidase-like activity.

Catalyst	Methods	Linear range (μM)	LOD (μM)	Refs.
WS_2	Colorimetric	10-100	1.2	7
GO-COOH	Colorimetric	0.05-1	0.05	8
g-C_3N_4	Colorimetric	5-30	0.9	9
g-C_3N_4–Fe_3O_4	Colorimetric	1-40	0.3	10
Carbon nitride dots	Colorimetric	1-100	0.4	11
OCN	Colorimetric	0.1-50	0.03	This work

Table S3. A list of a series of glucose sensors based on nanomaterials owning peroxidase-like activity

Materials	Methods	Linear range (μM)	LOD (μM)	Refs.
WS_2	Colorimetric	5-300	2.9	7
GO-COOH	Colorimetric	1-20	1	8
g-C_3N_4	Colorimetric	5-100	1	9
g-C_3N_4-Fe_3O_4	Colorimetric	1-140	0.25	10
Carbon nitride dots	Colorimetric	1-5	0.5	11
OCN	Colorimetric	0.1-100	0.03	This work

Table S4. The determination of H_2O_2 content in milk samples.

Table S5. Comparison of Michaelis–Menten constant for H_2O_2 and TMB with different nanomaterials as catalyst.

Sample	Found	Added (μM)	Experimental result (μM±SD, n=3)	Recovery (%)	RSD (%)
1	Not found	1	1.06±0.07	106.87	7.20
		20	20.56±0.71	102.81	3.48
		40	40.76±0.93	101.91	2.28
2	Not found	1	1.04±0.06	104.46	6.32
		20	19.52±0.74	97.64	2.38
		40	40.34±0.37	100.86	3.81
3	Not found	1	0.98±0.03	98.95	3.13
		20	19.37±0.84	96.89	4.34
		40	39.14±0.97	97.87	2.49

Catalysts	K_m[TMB]/mM	K_m[H_2O_2]/mM	Refs
MnSe	0.786	0.069	12
HRP	0.434	3.7	13
MnSe-g-C_3N_4	0.137	0.623	14
TiO_2 NTA	0.127	5.26	15
OCN	0.098	0.44	This work
β -$AgVO_3$ nanorods	0.04118	5.291	16
BSA-MnO_2	0.04	0.12	17

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