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Electronic Supplementary Information

Graphdiyne Oxide: A New Carbon Nanozyme

Wenjie Ma,[a,b] Yifei Xue,[a,b] Shuyue Guo,[a,b] Yanan Jiang,[a,b] Fei Wu,[a,b] Ping Yu,[a,b] Lanqun Mao,[*,a,b]

[a] *Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, The Chinese Academy of Sciences (CAS), CAS Research/Education Center for Excellence in Molecule Science, Beijing 100190, P. R. China.*

[b] *University of Chinese Academy of Sciences, Beijing 100049, P. R. China.*

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S1- Experimental Section

1 Chemical Reagent

3,3,5,5-Tetramethylbenzidine (TMB, Alfa Aesar), 30% H_2O_2 (Beijing Chemical Reagents), 98% H_2SO_4 (Beijing Chemical Reagents), glucose oxidase (GOx, Sigma-Aldrich), glucose (Sigma-Aldrich) were used without any further purification. Britton-Robinson buffer (B-R buffer) was prepared by mixing 0.04 M H_3PO_4, HNO_3, H_3BO_3, and the pH values of the solutions were adjusted by 0.2 M NaOH. Aqueous solutions were all prepared with pure water obtained from a Milli-Q water system (Millipore, 18.2 MΩ cm). Unless stated otherwise, all experiments were carried out at room temperature.

2 Experimental Details

2.1 Materials Synthesis

Preparation of GDYO

Graphdiyne (GDY) was prepared by using a cross-coupling reaction using hexaethynylbenzene precursors on the surface of copper according to the method reported previously. GDYO was synthesized by using concentrated HNO_3 as the solvent and oxidizing agent. Briefly, 20 mg GDY was dispersed in 5 mL concentrated HNO_3, and the dispersion was heated at 80 °C for 24 h. The reaction mixture was poured into ice water and the precipitation was collected by centrifuge, washed with water for several times and re-dispersed in water for further experiments.

2.2 Instrument and Characterization. High-resolution TEM images were obtained by using a FEI Tecnai G2 F20 S-Twin high-resolution transmission electron microscope operated at 200 kV. X-Ray photoelectron spectra (XPS) were collected on VG Scientific ESCALab220i-XL X-Ray photoelectron spectrometer, using Al Ka radiation as the excitation sources. Binding energies obtained in the XPS analysis were corrected with reference to C1s (284.8 eV). Raman spectra were recorded on a LabRAM ARAMIS Raman spectrometer at resolution of 2 cm^{-1} by using the 532 nm line of an Argon ion laser as the excitation source.

2.3 Peroxidase-like activity of GDYO. The peroxidase-like activity was verified by determining the absorbance of ox-TMB at 652 nm using a Synergy H1M microplate reader (BioTek, USA). In a typical test, 50 μg/mL GDYO was added to a solution of 0.5 mM TMB and 50 mM H_2O_2 in B-R buffer (pH 5.0), and the absorbance was recorded as a function of reaction time. The kinetic analysis of the GDYO with TMB as the substrate was performed by varying the concentration of TMB (0-1 mM) with 50 mM H_2O_2 or H_2O_2 (0-30 mM) with 0.5 mM TMB in the presence of GDYO at fixed concentration of 50 μg/mL. The corresponding Lineweaver-Burk plots were obtained from Michaelis-Menten curves.

2.4 H_2O_2 and glucose detection. The sensing platform for H_2O_2 was developed by determining the oxidation of TMB. In typical tests, 50 μg/mL GDYO was added to solutions of 0.5 mM TMB in

BR buffer (pH 5.0) with different concentrations of H_2O_2, and the absorbance of ox-TMB was recorded at different reaction times. The absorbance at 20 min was chosen to acquire the calibration plot for H_2O_2. Glucose detection was realized as follows: a) 5 μL of 40 mg/mL GOx and 100 μL of glucose of different concentrations in B-R buffer (pH 7.0) were incubated at 37 °C for 1 h; b) 100 μL B-R buffer (pH 5.0) containing TMB and GYDO were added into the above 105 μL glucose reaction solution; the mixed solution was used to perform the time course measurement. The absorbance changes at 20 min were used to acquire the linear response toward glucose.

S2- XPS spectra of GDY and GDYO

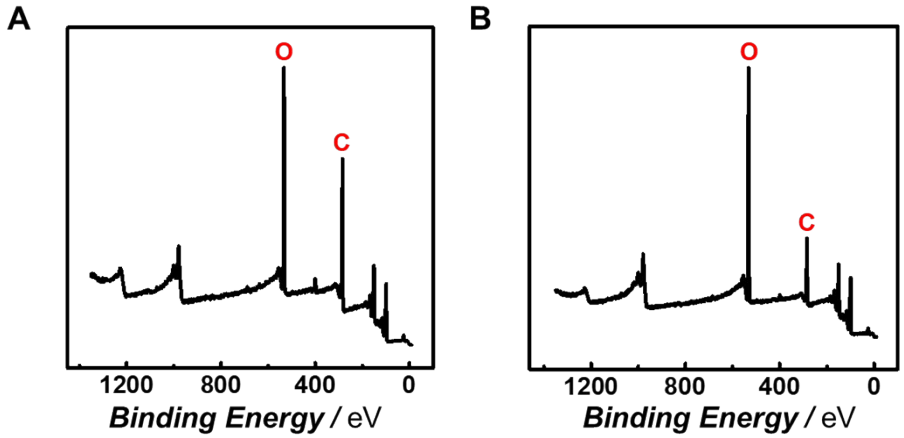

Figure S1. XPS survey scan of GDY (A) and GDYO (B).

S3- Peroxidase-activity of GDYO

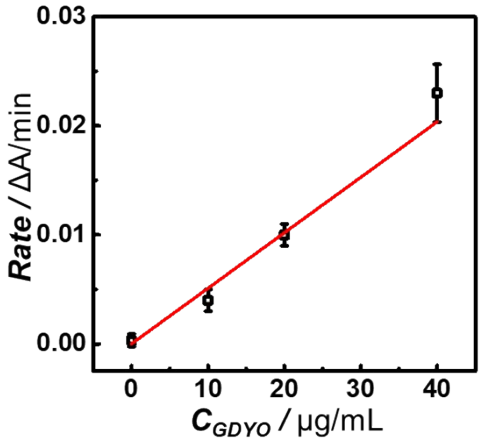

Figure S2. Effect of variable concentration of GDYO catalysts on the peroxidase-like activity.

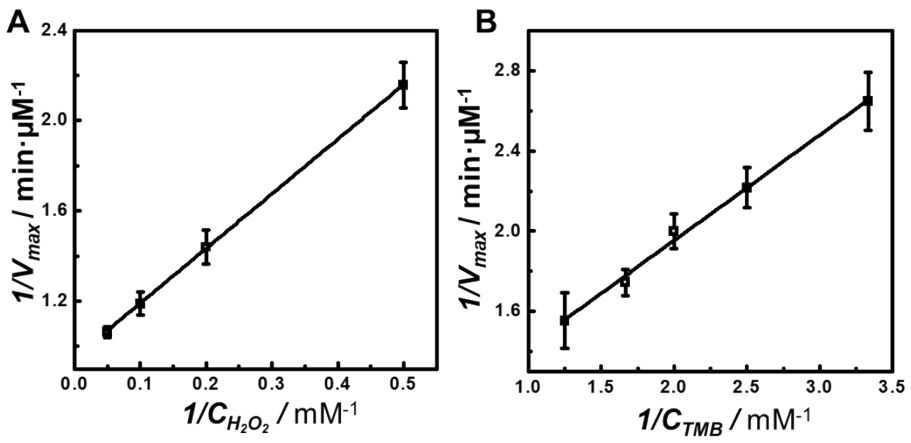

Figure S3. Lineweaver-Burk plots for peroxidase-like activity obtained with H_2O_2 (A) and TMB (B) as the substrates.

Table S1. Comparison of the apparent Michaelis-Menten constant (K_M) and maximum reaction rate (V_{max}) of carbon nanozymes.

Catalysts	Substrate	K_M (mM)	V_{max} (μM/min)	Reference
GDYO	TMB	0.62	1.15	This work
GDYO	H_2O_2	2.59	1.06	
$C_{60}[C(COOH)_2]_2$	TMB	0.23	0.21	*Biosens. Bioelectron*. 2013, 47, 502-507
$C_{60}[C(COOH)_2]_2$	H_2O_2	24.58	0.24	
GO	TMB	0.02	2.07	*Adv. Mater.* 2010, 22, 2206-2210
GO	H_2O_2	3.99	2.31	

S4-H_2O_2 and glucose detection

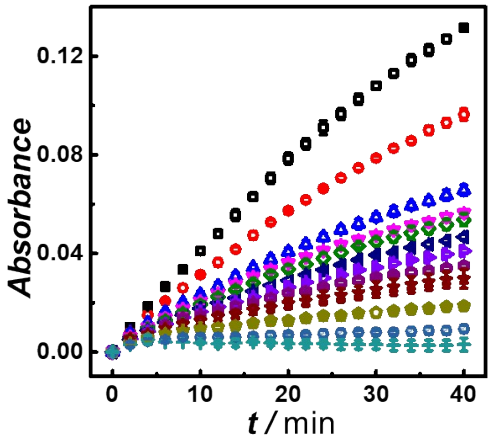

Figure S4. Time-dependent absorbance of ox-TMB monitored at 652 nm for the catalytic oxidation by 50 μg/mL GDYO in B-R buffer (pH 5.0) with different concentrations of H_2O_2.

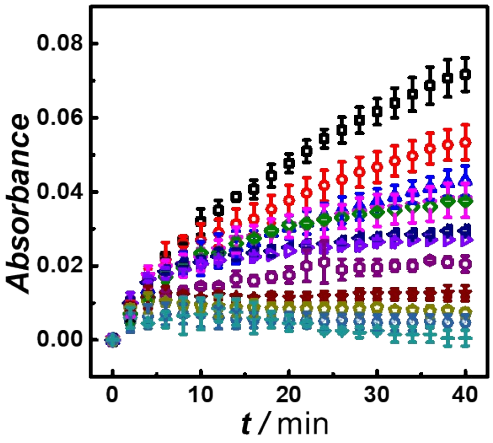

Figure S5. Time-dependent absorbance of ox-TMB monitored at 652 nm for the catalytic oxidation by 50 μg/mL GDYO in B-R buffer (pH 5.0) with different concentrations of glucose.

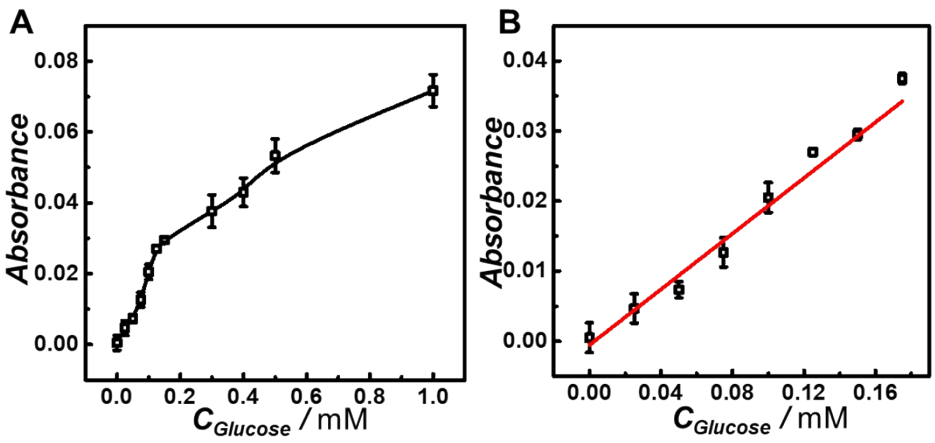

Figure S6. (A) The absorbance of ox-TMB monitored at 652 nm for the catalytic oxidation by 50 μg/mL GDYO catalysts in BR buffer (pH = 5.0) with different concentrations of glucose, the initial TMB concentration was 0.5 mM. (B) Calibration curve for glucose detection.