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Supporting information for

Polydopamine tethered enzyme/metal-organic framework composites with high stability and reusability

Xiaoling Wu, [a] Cheng Yang, [a] Jun Ge *,[a], Zheng Liu[a]

[a] Key Lab for Industrial Biocatalysis, Ministry of Education, Department of Chemical Engineering, Tsinghua University, Beijing 100084, China

To whom correspondence should be addressed.

E-mail: junge@mail.tsinghua.edu.cn

Materials

Glucose oxidase (GOx) from *Aspergillus niger*, horseradish peroxidase (HRP) (reagent grade), 2-methylimidazole, dopamine hydrochloride, glucose, phosphate buffer saline (1x) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich. Zinc nitrate hexahydrate (99.998%) were purchased from Alfa Aesar. Other reagents are of analytical grade.

Synthesis of the GOx/ZIF-8 composite

GOx water solution (10 mg/mL, 2 mL) and $Zn(NO_3)_2$ water solution (310 mM, 4 mL) were added into 2-methylimidazole water solution (4.1 g dissolved in DI water), followed by stirring at room temperature. The mixture turned milky almost instantly after mixing. After stirring for about 30 min, the product was collected by centrifuging at 6 000 rpm for 10 min, washed with DI water for three times and dried.

Synthesis of polydopamine tethered GOx/ZIF-8

GOx/ZIF-8 powder (120 mg) was added to 10 mL of Tris-HCl buffer solution (10 mM, pH 8.5) containing dopamine hydrochloride (2 mg/mL), followed by stirring at room temperature for 24h.[1] The solution was then centrifuged at 6000 rpm for 5 min, followed by three washing and centrifugation cycles to remove unreacted reagents. The product was lyophilized and applied to TGA for protein qualification and SEM for morphology measurement.

Transmission electron microscopy (TEM)

Samples were dispersed in methanol (HPLC grade) and sonicated for seconds. 2 μL of the solution was first placed on the carbon-coated grid. The sample was dried at room temperature for 24h before TEM measurement conducted on a JEOL JEM-2010 high-resolution TEM with an accelerating voltage of 120 kV.

XRD and XPS analysis of ZIF-8, GOx/ZIF-8 and PDA@GOx/ZIF-8 composites

Powder X-ray diffraction (XRD) patterns were recorded using a Bruker D8 Advance X-Ray diffractometer with a Cu Kα anode (λ= 0.15406 nm) at 40 kV and 40 mA. X-ray photoelectron spectroscopy (XPS) measurement with Al-K alpha radiation was acquired on Thermo Scientific ESCALAB 250Xi for surface analysis.[2]

Thermogravimetric analysis of ZIF-8, GOx/ZIF-8 and PDA@GOx/ZIF-8 composites

Thermal gravimetric analyses (TGA) in air were performed on a TA Instruments TGA 2050 Thermogravimetric Analyzer. The sample was heated from room temperature to 600 ^{o}C at a rate of 5 ^{o}C/min under air atmosphere

Enzymatic activity assay

To determine the enzymatic kinetics of free GOx, GOx/ZIF-8 and PDA@GOx/ZIF-8, free GOx (0.15 μg of protein), GOx/ZIF-8 (3.75 μg of protein),

PDA@GOx/ZIF-8 (2 μg of protein) was added to 1 mL of phosphate-buffered saline (PBS, 10 mM, pH 7.4) containing 0.05 mg/mL of HRP, 274 μg/mL ABTS and various concentrations of glucose. The increase of absorbance at 415 nm was recorded on a UV/Vis spectrophotometer. The values of K_m and V_{max} can be calculated using the Lineweaver-Bruke plot:

$$\frac{1}{\vartheta} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

Enzyme stability in polar organic solvents

PDA@GOx/ZIF-8, GOx/ZIF-8 and free GOx were incubated in aqueous solutions containing 50% methanol, 50% ethanol and 50% acetone for 0.5 h, respectively. Tiny amount of the enzyme solution was taken out and diluted by phosphate buffer saline to appropriate concentration and subjected to the above enzymatic assays.

Enzyme resistance to proteolysis in the presence of trypsin

PDA@GOx/ZIF-8, GOx/ZIF-8 and free GOx were incubated in phosphate buffer saline containing 1 mg/mL of trypsin for 2 h at 50 ^{o}C. Tiny amount of the enzyme solution was taken out and diluted by phosphate buffer saline to appropriate concentration and immediately subjected to the above enzymatic assays.

Reuse of enzyme catalysts

The recycling use of enzyme catalysts was performed by adding GOx/ZIF-8 and PDA@GOx/ZIF-8 with equivalent amount of protein in PBS solution containing 80 mM of glucose, 0.274 mg/mL of ABTS and 0.05 mg/mL HRP. After reaction for 1

min, the mixture was centrifuged at 12000 rpm for 2 min. The supernatant was separated and the absorbance was determined at 415 nm on a UV/Vis spectrophotometer. The precipitates were washed for 3 times with PBS and centrifuged at 12000 rpm for 2 min. The recovered enzyme composite was used for the next batch of the enzymatic reaction. The procedure was repeated for 10 times to determine the reusability of enzyme catalysts.

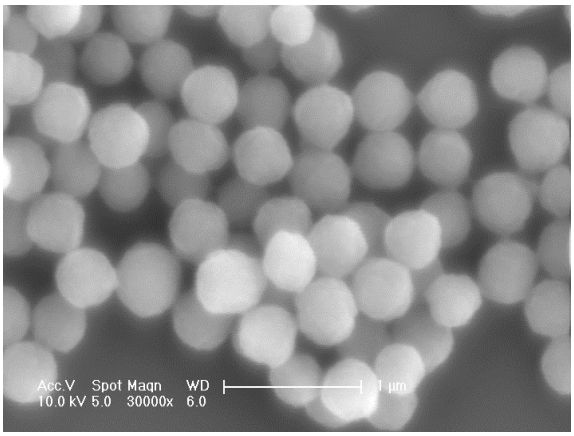

Figure S1. SEM image of ZIF-8.

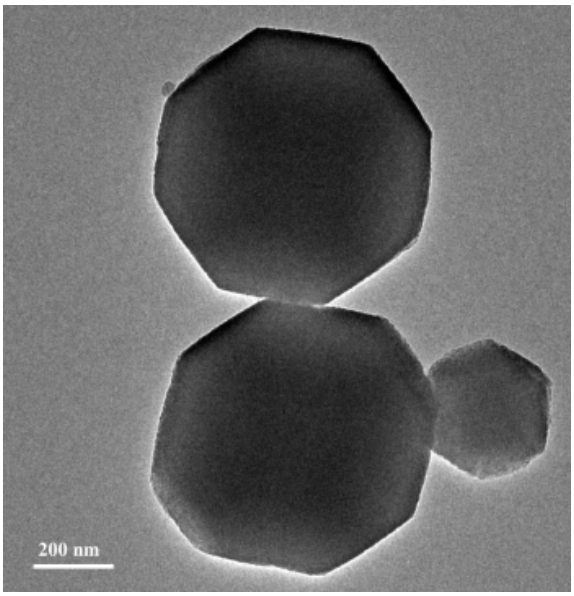

Figure S2. TEM image of ZIF-8.

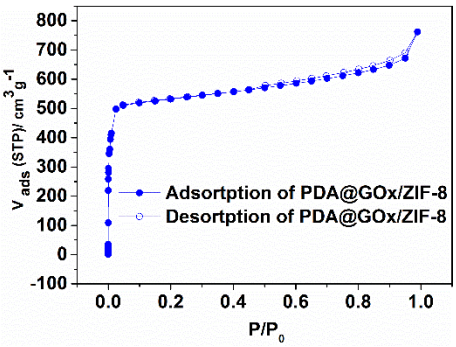

Figure S3. N_2 adsorption isotherm of PDA@GOx/ZIF-8

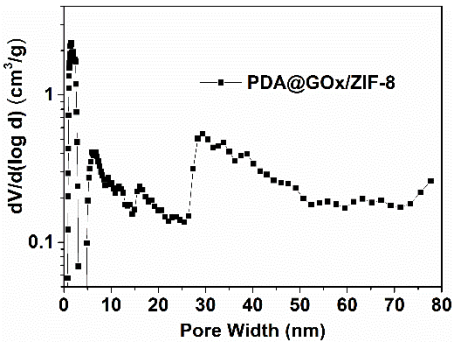

Figure S4. DFT pore size distribution of PDA@GOx/ZIF-8 obtained from the N_2 isotherm measured at 77 K.

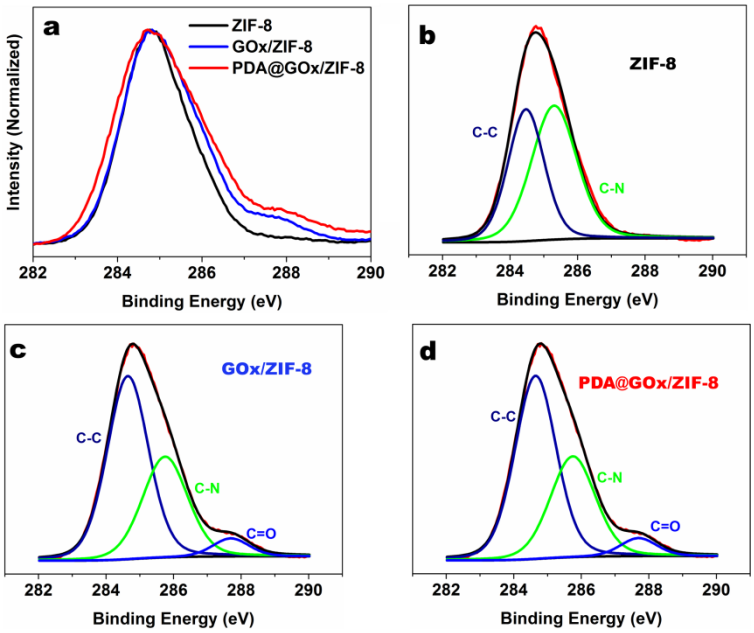

Figure S5. (a) C1s XPS spectra (normalized intensity) of ZIF-8, GOx/ZIF-8 and PDA@GOx/ZIF-8; narrow scan for C1 peaks for ZIF-8 (b), GOx/ZIF-8 (c) and PDA@GOx/ZIF-8 (d).

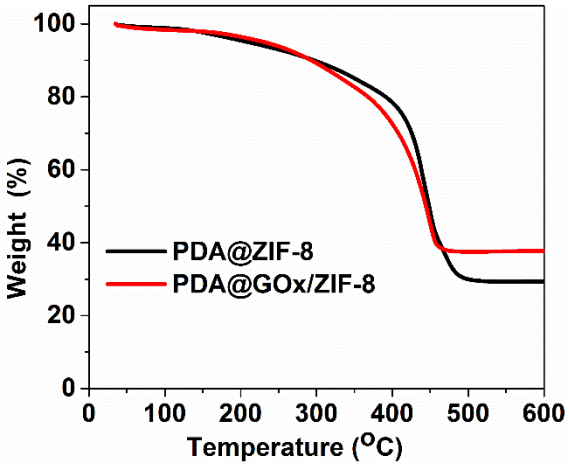

Figure S6. TGA analysis of PDA@GOx/ZIF-8 and PDA@ZIF-8.

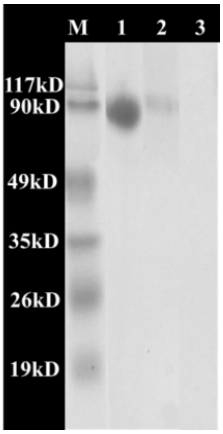

Figure S7. SDS-PAGE analysis (M: protein marker, lane 1: free GOx, lane 2: washed and digested GOx/ZIF-8; lane 3: washed and digested GOx@ZIF-8).

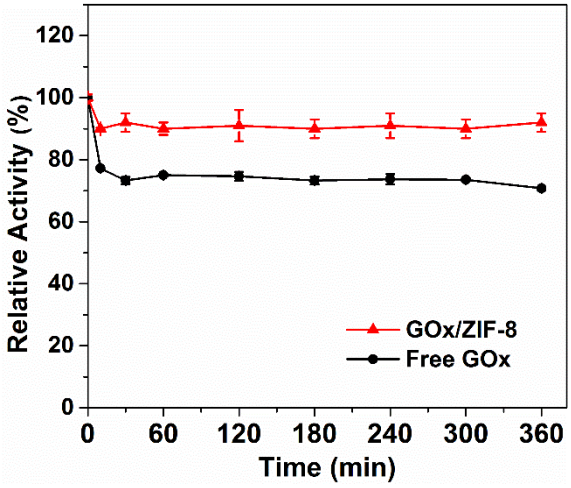

Figure S8. Thermal stability of GOx in the framework and free GOx incubated at 40 ^{o}C for 6 hours.

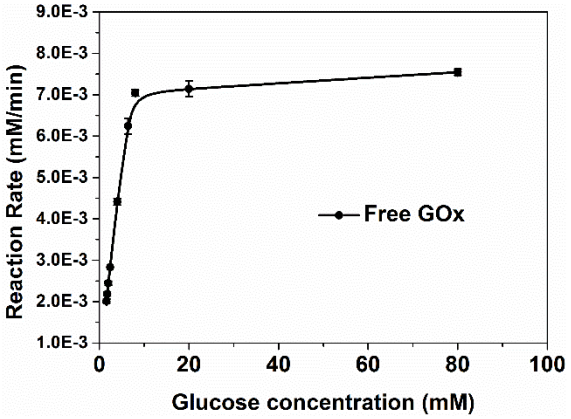

Figure S9. Michaelis-Menten curve for glucose oxidation catalyzed by free GOx.

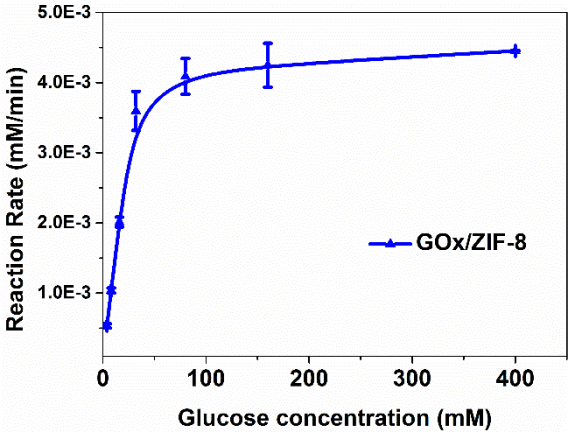

Figure S10. Michaelis-Menten curve for glucose oxidation catalyzed by GOx/ZIF-8.

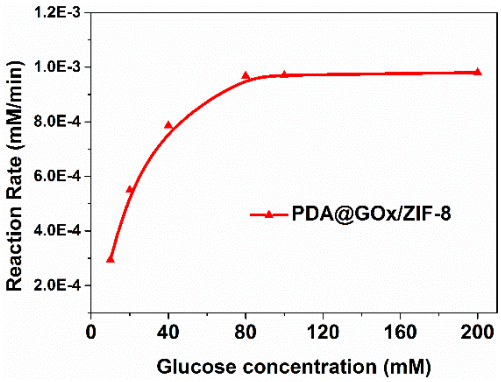

Figure S11. Michaelis-Menten curve for glucose oxidation catalyzed by PDA@GOx/ZIF-8.

Table S1. Kinetic parameters of free GOx, GOx/ZIF-8 and PDA@GOx/ZIF-8.

Enzyme	**K_m (mM)**	**k_{cat} (s^{-1})**	**k_{cat}/K_m (s^{-1} mM^{-1})**
Free GOx	14	314	22
GOx/ZIF-8	45	4.7	0.10
PDA@GOx/ZIF-8	42	2.2	0.05

Supplementary Reference

S1. Q. Liu, N. Wang, J. r. Caro and A. Huang, *J. Am. Chem. Soc.*, 2013, **135**, 17679.

S2. Luanwuthi, A. Krittayavathananon, P. Srimuk and M. Sawangphruk, *RSC Adv.*, 2015, **5**, 46617.