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Supporting Information

A Dual-cell Device Designed as Oxidase Mimics and Its Use for Study of Oxidase-like Nanozymes

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Experimental Section

Synthesis of the N-doped Fe_3C-based composite

The N-doped Fe_3C-based composite was prepared according to our previous study.[1] In brief, firstly, Prussian blue (PB) cubes were synthesized using a previously reported method.[2] 38 g polyvinylpyrrolidone (PVP, K30) and 1.15 g $K_4Fe(CN)_6$ were dissolved in 500 mL of HCl solution (0.1 M) under magnetic stirring. When the solution became clear, the bottle was placed into an electric oven and heated at 80 ^oC for 24 h. The obtained blue product was filtered by using 0.45 μm nylon membrane and washed several times with deionized water and absolute ethanol, then dried in a vacuum oven at 60 ^oC for 12 h. Then, the as-prepared blue product was pyrolyzed in a horizontally tubular furnace in Ar atmosphere at 550 ^oC for 6 h. The heating rate was 2 ^oC min^{-1}. The as-prepared black product was treated with 0.50 M H_2SO_4 for 24 h to remove the α-Fe and iron oxides possibly generated during the pyrolysis process, then washed with deionized water for five times and dried at 60 ^oC overnight.

The characterizations of the N-doped Fe_3C-based composite were shown in our previous study[1] and not included in this study.

Preparation of the electrodes coated with the N-doped Fe_3C-based composite

The indium tin oxide (ITO) glasses (1 cm× 2 cm) were cleaned in water, ethanol and acetone, respectively, by sonication, and dried by N_2 blowing. 2 mg of the N-doped Fe_3C-based composite was dispersed in 1 mL of N,N-Dimethylformamide (DMF) by sonication. Then, 50 μL of the resultant solution was dropped onto the cleaned ITO glass and dried with an infrared (IR) light. Finally, 10 μL of 0.05% Nafion solution was used to coat the N-doped Fe_3C-based composite on the ITO surfaces.

Assembly of the dual-cell device

For assembly of the dual-cell device, one conductive electrode (denoted as the electrode A) immersing in the substrate-containing anaerobic buffer solution is used to mimic the active site of oxidases oxidizing substrates and the other conductive

electrode (denoted as the electrode B) immersing the aerobic buffer solution mimics the catalytic site reducing molecular oxygen. The electrons electrochemically extracting from the substrates at the electrode A|solution interface can flow along the conducting wire and be used to electrochemically reduce molecular oxygen at the electrode B|solution interface. The cell-i and the cell-ii were also connected with an agar bridge. All of solutions were prepared with 0.10 M Britton-Robinson (B-R) buffer solution. The solution pH values were adjusted using 1 M NaOH or 1 M HCl solution. The solution in the cell-i was 0.25 mM TMB solution deoxygenated with argon for 15 min. The solution in the cell-ii was 0.10 M buffer solution saturated with O_2 gas. The TMB solution in the cell-i was renewed for each change of the pH value of the cell-i and the cell-ii.

The UV-vis absorption spectra were recorded on a Shimadzu UV-1800 spectrometer.

References:

1. H.K. Yang, J.Y. Xiao, L. Su, T. Feng, Q.Y. Lu, X.J. Zhang, *Chem. Commun.* 2017, 53, 3882.
2. (a) R. Qiang, Y. C. Du, H. T. Zhao, Y. Wang, C. H. Tian, Z. G. Li, X. J. Han and P. Xu, *J. Mater. Chem. A* 2015, 3, 13426; (b) L. Zhang, H. B. Wu, S. Madhavi, H. H. Hng and X. W. Lou, *J. Am. Chem. Soc.* 2012, 134, 17388.

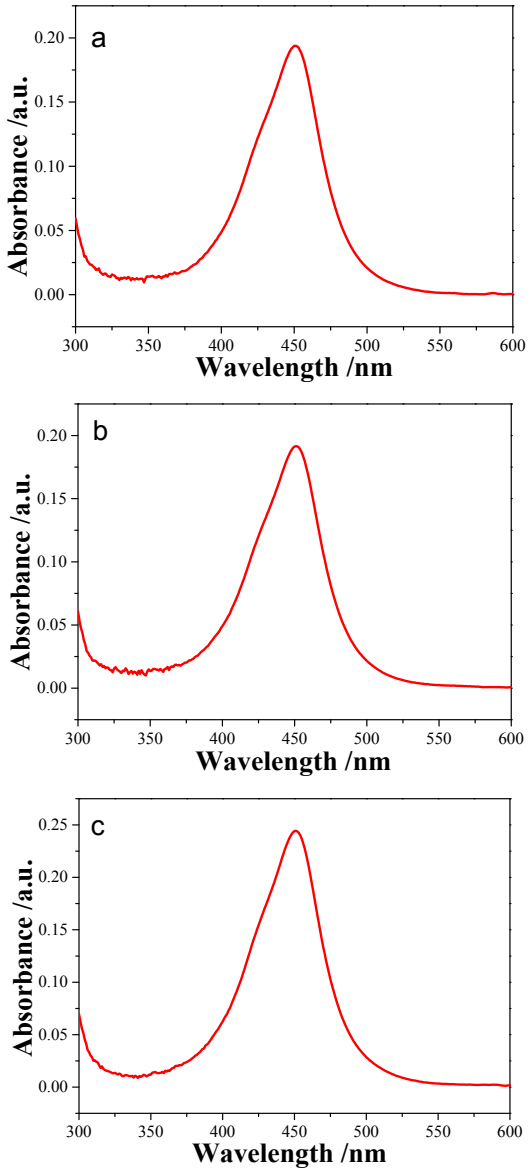

Figure S1. The UV-vis absorption spectra of 0.25 mM TMB solution (pH 3.5) prepared with 0.10 M B-R anaerobic buffer solution in the cell-I with the electrode-A made of different materials, including GC (a), Au (b) and ITO (c). Oxidation of TMB was driven by the oxygen reduction at the electrode B in the cell-ii containing 0.10 M B-R aerobic buffer solution (pH 3.5). Reaction temperature was 30°C and reaction time was 3 min before H_2SO_4 stop solution was added.

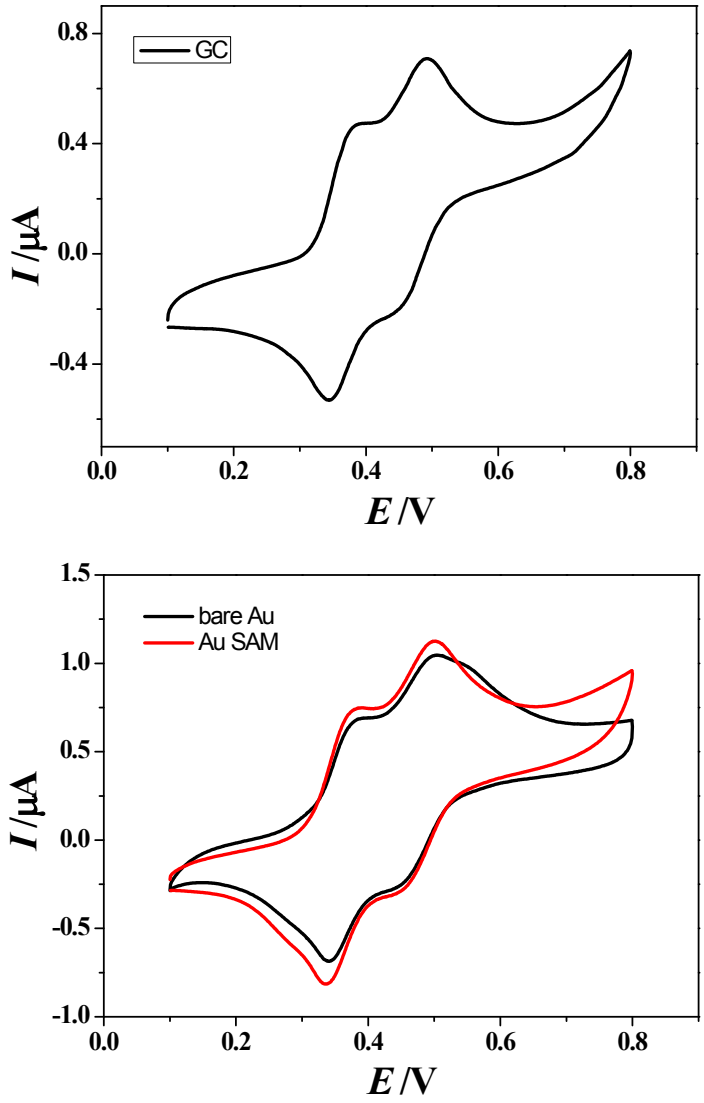

Figure S2. Cyclic voltammetry of 0.25 mM TMB at different electrodes in 0.10 M B-R buffer solution (pH 3.5) at a scan rate of 3 mV/s. Cyclic voltammetric measurements were performed with a CHI 660D electrochemical analyzer (CH Instruments) with a three-electrode configuration. Bare or self-assembled monolayer of cysteine-modified electrodes were used as the working electrode, Ag/AgCl (KCl-saturated) electrode as the reference electrode, and platinum coil as the counter electrode.

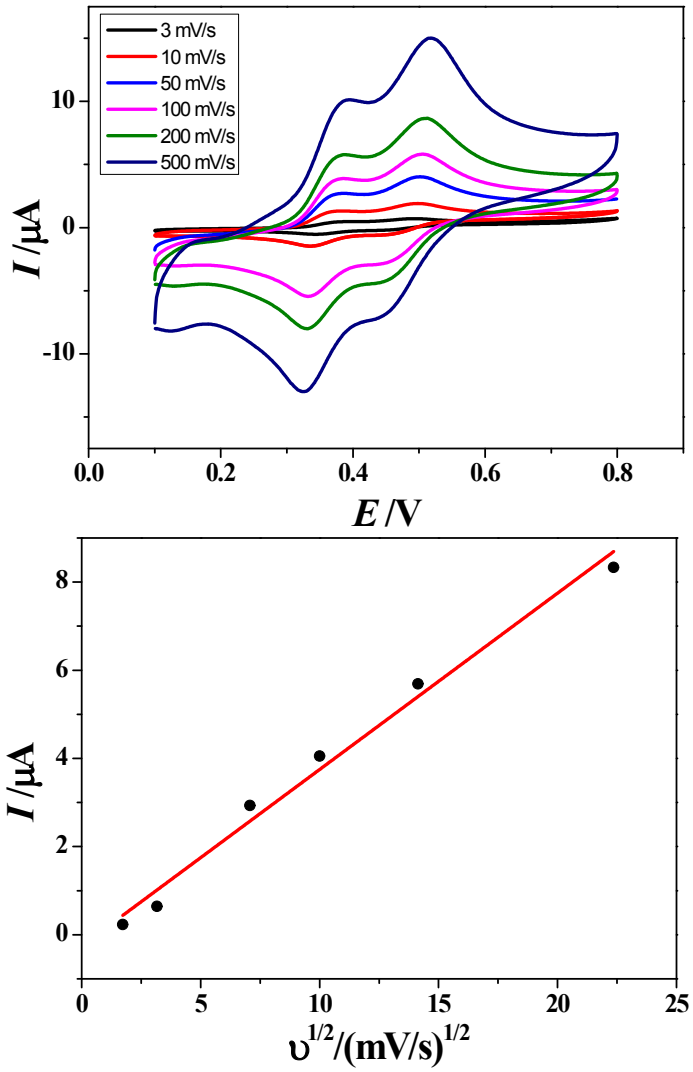

Figure S3. Cyclic voltammetric curves of 0.25 mM TMB at GC electrode in 0.10 M B-R buffer solution (pH 3.5) at various scan rates and the linear relationship between the oxidation peak current and the square root of the scan rate. The ΔE_p is not affected by varying the scan rate, indicating a fast kinetics of TMB oxidation. The observed linear relationship indicates the diffusion-controlled process for TMB electrooxidation.

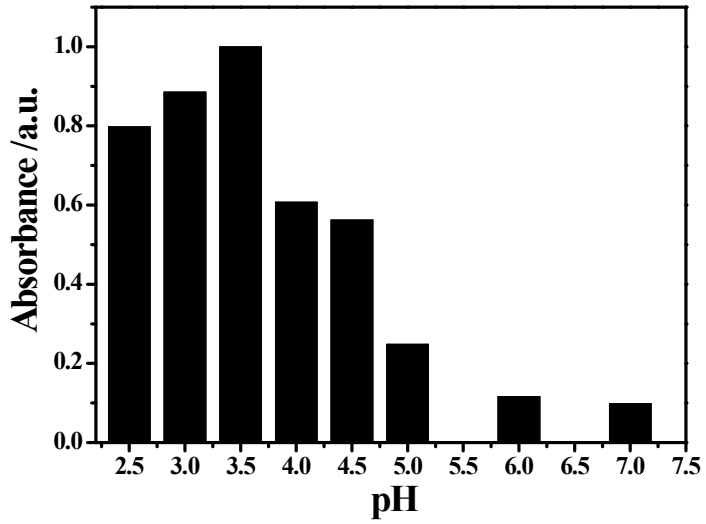

Figure S4. Effect of pH on the oxidation of TMB (in the cell-i) by oxygen catalysed by the N-doped Fe_3C composite in the cell-ii of the dual-cells device.