Supporting Information for

Graphene oxide/hemoglobin composite hydrogel for enzymatic catalysis in organic solvents

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1. Materials

Natural graphite powder (325 mesh) was purchased from Qingdao Huatai lubricant sealing S&T Co. Ltd (Qingdao, China). Hemoglobin (Sigma H2500) made from bovine blood was bought from Sigma-Aldrich. Methylene dichloride, toluene and chloroform are chromatographically pure and they were purchased from Beijing Chem. Reagent Co. (Beijing, China). All the chemicals were used as received.

2. Characterizations

The rheological behaviors of GO/Hb hydrogels and GO solution were investigated by a MCR 300 (Paar Physica) Rheometer using a 25-mm parallel-plate geometry at 25 °C. The gap distance between two plates was fixed to be 1 mm. Dynamic frequency sweep experiments were measured from 1 to 100 rad/s at a fixed oscillatory strain of 0.5%. Atomic force microscopic (AFM) images were performed by the use of a Nanoscope III MultiMode SPM (Digital Instruments). SEM images were recorded on a scanning electron microscope (Hitachi S-4500). The freeze dried hydrogels and GO solution were broken to small pieces with a
tweezer to expose their interior surfaces. The UV/Vis spectra were tested on a U-3010 UV/Vis spectrometer (Hitachi).

3. Synthesis and purification of GO

GO was prepared by oxidation of natural graphite powder according to the modified Hummers’ method. The details are described as follows. Graphite (3.0 g) was added to concentrated sulfuric acid (70 mL) under stirring at room temperature, then sodium nitrate (1.5 g) was added, and the mixture was cooled to 0 °C. Under vigorous agitation, potassium permanganate (9.0 g) was added slowly to keep the temperature of the suspension to be lower than 20 °C. Successively, the reaction system was transferred to a 35–40 °C water bath for about 0.5 h, forming a thick paste. Then, 140 mL water was added, and the solution was stirred for another 15 min. Additional 500 mL water was added and followed by a slow addition of 20 mL H$_2$O$_2$ (30%), turning the color of the solution from brown to yellow. The mixture was filtered and washed with 1:10 HCl aqueous solution (250 mL) to remove metal ions followed by repeated washing with water and centrifugation to remove the acid. The resulting solid was dispersed in water by ultrasonication for 1 h to make a GO aqueous dispersion (1 wt. %). The obtained brown dispersion was then subjected to 30 min of centrifugation at 4000 r.p.m to remove any aggregates. Finally, it was purified by dialysis for one week to remove the remaining salt impurities for the following experiments.

4. Catalyzed oxidation of pyrogallol by H$_2$O$_2$

The oxidation of pyrogallol by H$_2$O$_2$ was selected to be the model reaction to characterize the activity of the catalysts (GO/Hb hydrogel, Hb and GO). Typically, 4 mg Hb (or a piece of hydrogel containing 4 mg Hb or 0.8 mL GO solution with a concentration of 9 mg/mL) was added into a 20 mL organic solution containing 2 mM pyrogallol and 6 mM H$_2$O$_2$ for catalyzing
the reaction. The absorbance change at 425 nm of the reaction system was detected by using a UV-Vis spectrometer.

5. Calculation of the kinetic parameters.

The kinetic constant values, maximum initial velocity \((V_{\text{max}})\) and Michaelis-Menten constant \((K_m)\) were estimated by Lineweaver-Burk plot, \(1/V = K_m/V_{\text{max}}[S] + 1/V_{\text{max}}\); where \(V\) is the initial reaction rate and \([S]\) is the concentration of the substrate. To get the Lineweaver-Burk plot, we changed the substrate concentration from 2 mM to 1.5, 1.0, 0.4, or 0.2 mM and the corresponding initial reaction rate was measured from its reaction course in the first five minutes.

6. Supplementary figures (Figs. S1–7)

![AFM image of GO sheets on a mica surface.]

**Fig. S1** AFM image of GO sheets on a mica surface.
**Fig. S2** Rheological test of a GO solution (9 mg/mL).

**Fig. S3** Viscosities of GO/Hb composite hydrogel (A) and GO solution (9 mg/mL) (B) at different shear rates.

**Fig. S4** UV-vis spectroscopy of purpurogallin
Fig. S5 The extended 170 min reaction course in methylene dichloride with GO/Hb hydrogel.

Fig. S6 Reaction courses in toluene (A and C), and chloroform (B and D)
**Fig. S7** Reaction course in toluene catalyzed by the GO/Hb composite hydrogel which had been stored at room temperature for over two weeks.