Electronic Supplementary Information for

Dual Enzymatic Formation of Hybrid Hydrogels with Supramolecular-Polymeric Networks

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Contents

Index **Page**

1. Materials S2

2. Pre-treatments and Characterizations S2

3. Test of catalytic activity S3

4. Test of the reusability.....S4

5. Figures S5

1. Materials

Fmoc-Tyr(PO_3H_2)-OH was purchased from GL biochem (shanghai) Ltd. Acid phosphatase from potato (AP, MW = 69 kDa, 3-10 U mg^{-1} solid) and glucose oxidase from Aspergillus niger (GOx, MW = 160 kDa, EC 1.1.3.4) were purchased from Sigma-Aldrich. N-Hydroxyphthalimide (NHPI) was obtained from Shanghai Energy Chemical Co., Ltd. N, N-dimethylacrylamide (DMAA) and glucose were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All materials were used without further purification.

2. Pre-treatments and Characterizations

Electron Paramagnetic Resonance (EPR) assay: The EPR spectra were recorded on a Bruker EMX-8/2.7 Spectrometer operating at 9.873 GHz (microwave power: 20 mW; modulation frequency: 100 kHz; modulation amplitude: 0.5 G; receiver gain: 4×10^5; 25 °C). As for the signal of propagating radicals, the aqueous solution of 15×10^{-3} M glucose, 3.125×10^{-6} M GOx, 0.2% NHPI and 5% DMAA was rapidly transferred to a standard quartz capillary (1 mm in diameter) and placed into the EPR spectrometer.

Morphological measurements: After frozen the silicon wafer coated the sample in liquid nitrogen, the gel samples were dried in vacuum at least 12 hours. The sample on the silicon wafer was sputter-coated with a thin layer of gold (about 5 nm) before observing with a with a field emission scanning electron microscope (Hitachi S-4800) at a 1 KV voltage. For the TEM test of hydrogels, the samples coating on the carbon-coated copper grid were frozen dried as the previous SEM test. TEM pictures of hydrogels were acquired by JEM-2010 transmission electron microscopy (TEM) at an 80 KV accelerating voltage.

Rheological characterization: The rheological properties of hydrogels were measured using an RS6000 rheometer (Thermo Scientific, Karlsruhe, Germany) with a parallel plate geometry (25 mm diameter, 0.3 mm gap) at 25 °C. The frequency amplitude sweep of gels was performed as a function of angular frequency at a fixed

strain of 1%. The strain amplitude sweep of gels was carried out at a fixed frequency of 1 Hz. The recovering process of the hydrogel in response to applied shear forces was investigated using continuous step strain sweep test with alternate small oscillation force (γ = 0.01%) and large one (γ = 300%). The temperature dependence of Gel II was explored by using a temperature control program: 25 °C→95 °C→25 °C with 0.05 °C s^{-1} heating rate and 0.01 °C s^{-1} cooling rate.

Mechanical analysis: The compressive performance of the hydrogels was performed on a FR-108B testing machine (Farui Co., China) at a crosshead speed of 1 mm min^{-1}. The shape of gel is about 15 mm in diameter and 7–8 mm in thickness. The compressive stress (σ) was approximately calculated as $\sigma = Load/\pi R^2$, where R is the original radius of the specimen. The compressive strain (ε) is defined as the change in the thickness relative to the original thickness. The stress and strain between ε = 5 and 10% were used to calculate the Young's compressive modulus. At least three specimens were tested for each hydrogel.

3. Test of catalytic activity

We firstly tested the activity of AP in hydrogels by monitoring the absorbance of p-nitrophenol at 405 nm, which is the production of the hydrolysis p-nitrophenylphosphate catalyzed by AP. The molar coefficient of p-nitrophenol at 405 nm is about 1800 M^{-1} cm^{-1}. According to the standard Sigma procedure (Acid phosphatase Assay Kit, CS0740) in water, we measured the activity of two forms AP (hydrogel and native AP) with same concentration (20 μg/L). The selected concentration of substrate pNPP is 12.5mM (about 10 times of Km value of AP). Therefore, the reaction rate in the first 5 minutes was defined as the activity of AP.

We characterized the catalytic activity of Gox by detecting the H_2O_2 formed by the GOx-catalyzed reaction of glucose and oxygen. The excessive HRP and o-phenylenediamine (OPD) were employed to measure the H_2O_2 via the colorful model reaction. The mixture of OPD (10 mM), HRP (4 U/mL) and glucose (300 mM) in 100 mL phosphate buffer (pH 7.0, 50 mM) were catalyzed by Gox (0.05 U/mL) in

different forms with slightly stirring at room temperature (298 K). We employed UV-Vis spectrometer (UV-2700, Shimadzu) to monitor the absorbance at 450 nm (phenazine-2, 3-diamine, the molar extinction coefficients is 16300 $M^{-1}cm^{-1}$ in water) with 0.2-min intervals, and, the concentration of substrate glucose is 300 mM (about 10 times of Km of GOx). Meanwhile, the excessive OPD and HRP can immediately consume the fresh H_2O_2 from the reaction of glucose and GOx. Therefore, the reaction rate in the first 1 minute is defined as the activity of GOx in the reaction.

4. Test of the reusability

In order to measure the reusability of AP and GOx immobilized in Gel II, we utilized the Gel II particles to catalyze corresponding reaction in 10 minutes. The initial reaction rate was selected to assay the remained activity during reusability. The recovered enzyme in Gel II particles were separated from the reaction system by centrifuging and the 2 times washing with excessive water to remove the product.

5. Figures

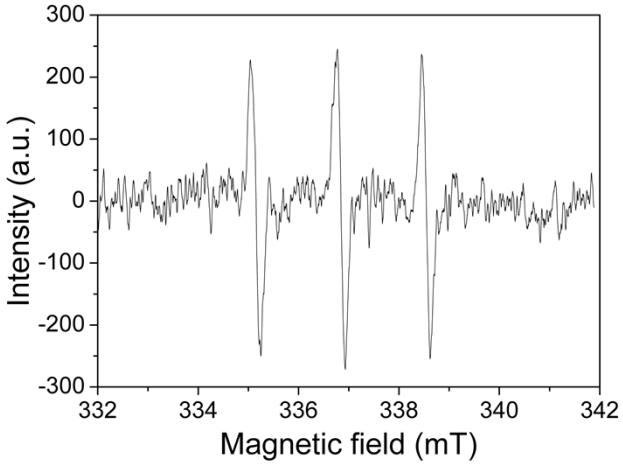

Figure S1. The EPR spectrum of the precursor solution of the NHPI-GOx-glucose aqueous solution and DMAA.

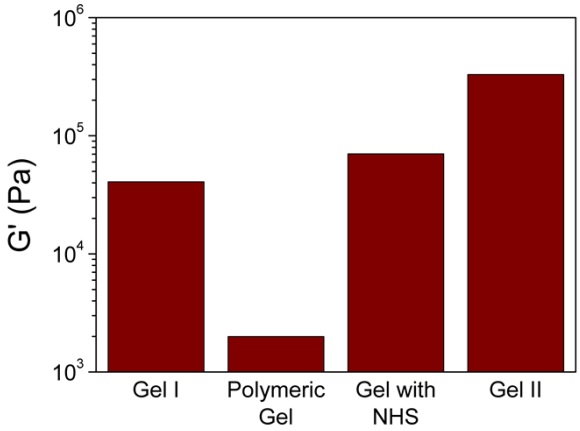

Figure S2. The G' of Gel I, the polymeric gel with 7.5% DMAA monomer and 0.15% N,N'-Methylenebisacrylamide, the gel with NHS as initiators, and Gel II.

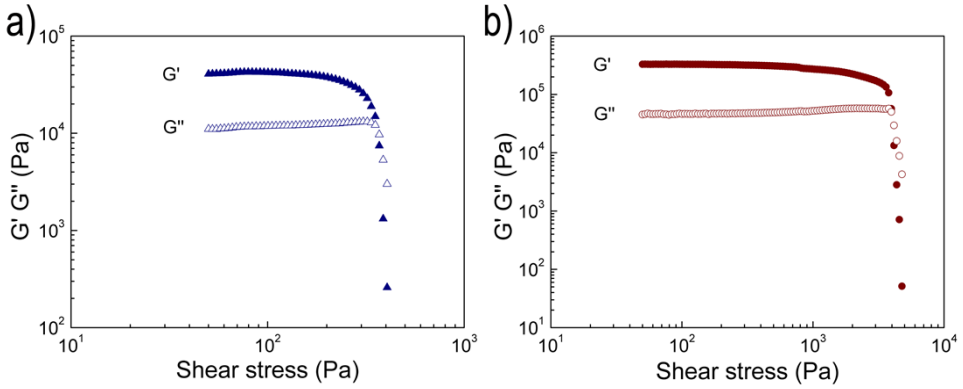

Figure S3. The oscillatory stress sweep experiments for the Gel I (left) and Gel II (right) at a constant frequency of 1 Hz.

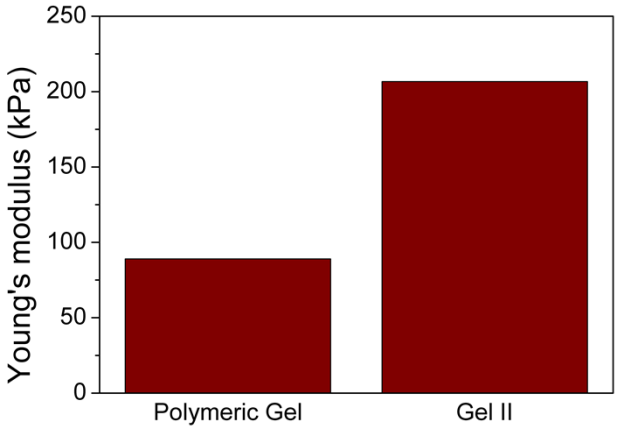

Figure S4. The compressive Young's modulus of polymeric gel with 7.5% DMAA monomer (0.15% N,N'-Methylenebisacrylamide) and Gel II.

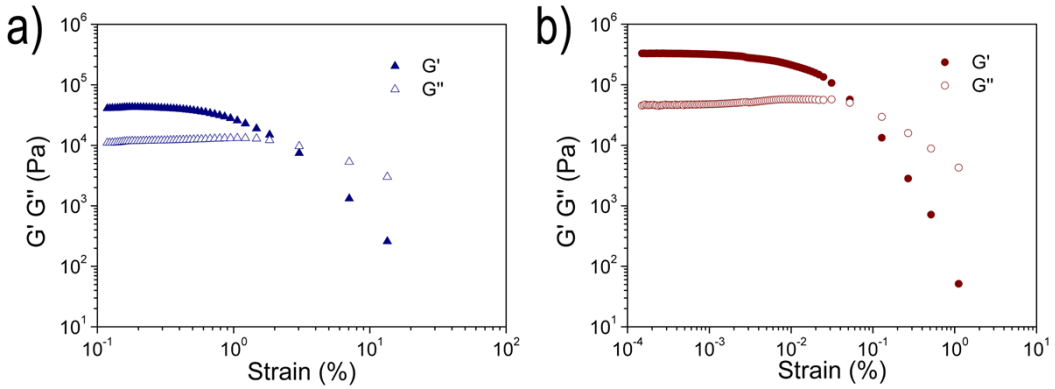

Figure S5. The oscillatory strain sweep experiments for the Gel I (a) and Gel II (b) at a constant frequency of 1 Hz.