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BSA-rGO nanocomposite hydrogel formed by UV polymerization and in-situ reduction applies as biosensor electrode

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Materials:

N,N-Dimethylacrylamide (DMAA, 99%), N-Succinimidyl Acrylate (NAS, >98%) and 2,2-Diethoxyacetophenone (DEAP) were bought from TCI Shanghai Development Co. Ltd. Bovine serum albumin (BSA) was purchased from Baoman Biological technology. Hemin chloride (98%) was bought from Alfa-Aesar. Graphite powder (SP), $NaNO_3$ (AR), H_2SO_4 (AR), $KMnO_4$ (AR) and H_2O_2 (30%) was purchased from Sinopharm Chemical Reagent Co. Ltd. Deionized water was used throughout the experiments.

Preparation of GO:

A modified Hummers method is used for the preparation of graphite oxide. A solid mixture of 1.0 g graphite powder and 0.5 g sodium nitrate were put into cold (0 °C) concentrated H_2SO_4 (23 mL).Then, $KMnO_4$ (6 g) was added gradually under stirring and the temperature of the mixture was kept to be below 20 °C by cooling. Successively, the mixture was stirred at 35 °C for 2 h, and then diluted with DI water (46 mL). Because the addition of water in concentrated sulfuric acid medium released a large amount of heat, the addition of water was carried out in an ice bath to keep the temperature below 50 °C. After adding all of the 46 mL of DI water, the mixture was stirred for 2 h, and then additional 140 mL of DI water was added. Shortly after the dilution with 140 mL of water, 3 mL of 30% $H_2O_2$was added to the mixture, and the color of mixture changed into brilliant yellow along with bubbling. The mixture was washed with 1:10 HCl aqueous solution (200 mL) to remove metal ions followed by 200 mL of DI water to remove the acid. The resulting solid was dried in air and diluted to make a GO dispersion of 0.5 mg/mL.

Characterizations:

For SEM observations, the hydrogel was cut to a cube shape of about 2 × 2 × 2 mm^3. Then, the small piece of sample was soaped successively in 50% ethanol (15 min), 70% ethanol (15 min), 90% ethanol (15 min), 100% ethanol (15 min), a mixture of 1:1 ethanol and isoamyl acetate (30 min) and last in pure isoamyl acetate (30 min) to replace the water in hydrogel with isoamyl acetate. Finally, the treated sample was dried by Critical point drying (Critical Point Dryer CPD, K850, Quorum) and observed by SEM (Hitachi S-4800) at a voltage of 3kV. For TEM observations, the hydrogel sample was cut to a cube shape of about 1 × 1 × 1 mm^3. The small piece of sample is soaped successively in 50% ethanol (15 min), 70% ethanol (15 min), 90% ethanol (15 min), a mixture of 1:1 90% ethanol and 90% acetone (30 min), a mixture of 1:1 90%

acetone and entrapped liquid (12 h), and last in pure entrapped liquid (3 h) at room temperature, to replace the water in hydrogel with entrapped liquid. The treated sample is placed in an oven of 37 °C for 12 h, 45 °C for 12 h and 60 °C for 48 h. Then several pieces with thickness of about 70 nm, are obtained using an ultramicrotome (Leica, German) and placed onto copper grid. The sample was stained with 3% uranyl acetate and observed by TEM (JEM-2010, JEOL) at an accelerating voltage of 120 KV. The high dispersed GO solution was drop to copper grid and directly observed by TEM after drying.

All proton NMR spectra were obtained using a Bruker 400 MHz NMR spectrometer. In our conversion calculation experiments, dioxane is used as internal standard material to calculate the remaining content of DMAA, because 1,4-dioxane has no change in the light-induced reaction and its only peak at 3.65 ppm separates from other peaks of DMAA and the products. In a typical measurement, DMAA (500 mg, 5.043 mmol), 1,4-dioxane (62.5 mg, 0.704 mmol), BSA (300 mg), GO (20mg) and D_2O (9.4 g) were mixed under vigorous stirring to get a homogenous solution. Five NMR tubes, each containing 0.6 mL of the above solution were irradiated by the Xenon lamp (2.0 mW cm^{-2} intensity at 365 nm) for different time：0 min, 10min, 20 min, 30 min and 40 min. In the control experiment without GO, a precursor solution with DMAA (5.043 mmol), 1,4-dioxane (0.704 mmol), BSA (300 mg) and D_2O (9.4 g), was placed at the same condition the same time. Raman spectra were recorded from 200 to 2000 cm^{-1} on a Renishaw 2000 Confocal Raman Microprobe (Rhenishaw Instruments, England) using a 514.5-nm argon ion laser.

Preparation of electrodes and electrochemical measurements:

Prior to use, the glass carbon (GC) electrode was first polished with sand paper and Chamois followed by 1.0 and 0.05 mm alumina slurry, then ultrasonically cleaned with double distilled water and ethanol, each for 10 min, and finally rinsed with doubly distilled water and dried in N_2. The cleaned GC electrode was continually scanned in 0.5 M sulfuric acid until steady voltammograms were established, and then rinsed with water. A slice of BSA-GH about 25.7mg (about 2 mm thickness) was form on the surface of GB by photopolymerization. The BSA-H modified electrode was prepared by the same way.

Cyclic voltammetry (CV) and AC impedance technique measurements were carried out on a CHI 660D electrochemical workstation. Platinum sheet and saturated calomel electrode (SCE) were used as the counter and reference electrode, respectively.

Figures:

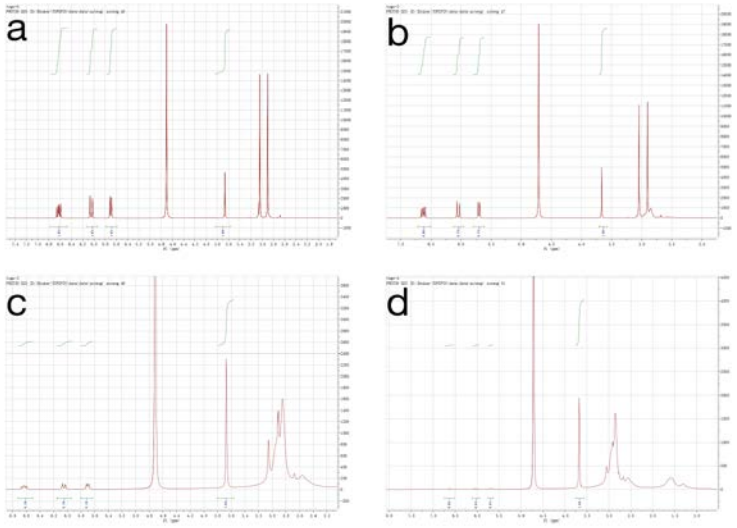

Figure S1. The NMR spectra of BSA-GH samples under Xenon lamp. The precursor was consisted of 5% DMAA, 3% clay ,94% D_2O and 0.02% GO under different reaction time:0 min (a); 10 min (b); 20 min (c); 30 min (d).

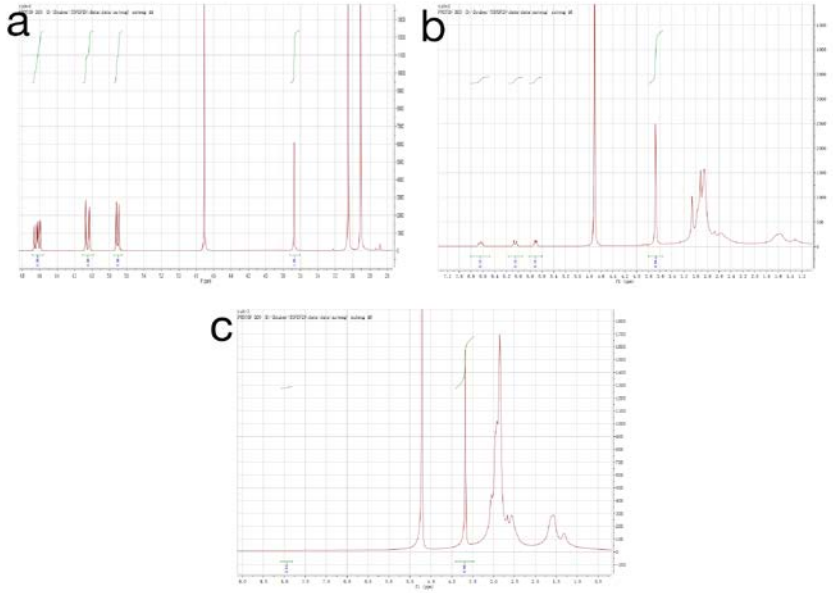

Figure S2. The NMR spectra of BSA-H samples under Xenon lamp. The precursor was consisted of 5% DMAA, 3% BSA, and 92% D_2O under different reaction time:0 min (a); 10 min (b); 20 min (c).

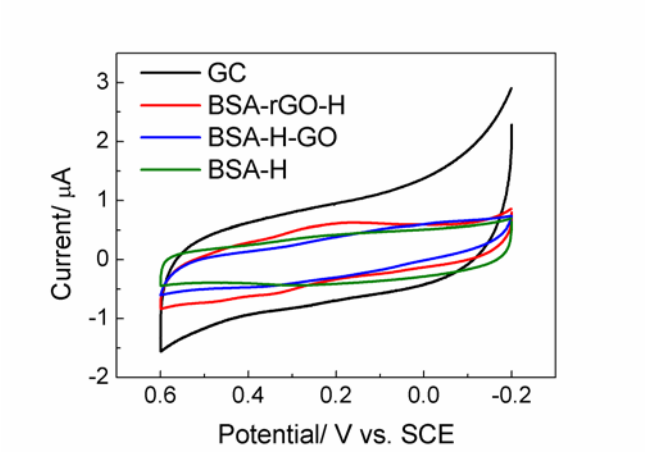

Figure S3. The CV experiments of BSA-rGO-H modified electrode, BSA-H modified electrode, BSA-H modified electrode after soaking with 1mg/ml GO, and GC electrode in 0.5 M H_2SO_4 solution, scan rate: 100 mV s^{-1}.

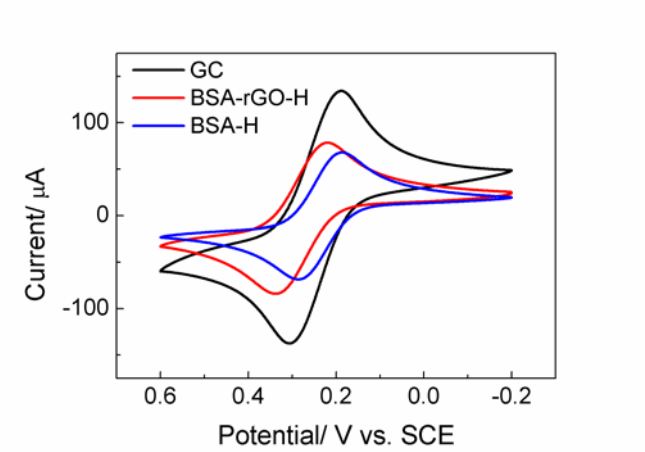

Figure S4. The CV experiments of BSA-rGO-H modified GC electrode, BSA-H electrode modified GC and GC electrode in 5 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl, scan rate: 100 mV s^{-1}. The peak current of BSA-rGO-H modified electrode is 1.3 times than BSA-H electrode modified electrode.

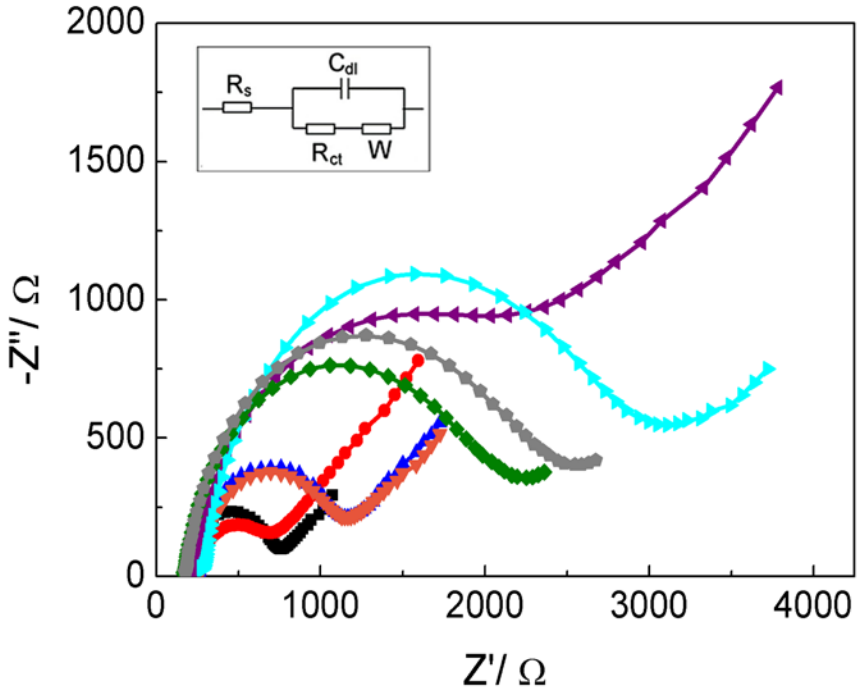

Figure S5. The AC impedance experiments of BSA-rGO-H modified electrode, BSA-H modified electrode, BSA-H modified electrode after soaking with 1mg/ml GO, and GC electrode. The frequency range is from 1Hz to 10 kHz. Inset is the equivalent circuit. By fitting the data, the sequence of the values of charge-transfer resistance (*R*ct) for different electrodes is Hemin/BSA-H modified electrode (purple, 1523.9 Ω) > BSA-H modified electrode (cyan, 1437.6 Ω) > BSA-H-GO modified electrode (gray, 1296.7 Ω) > Hemin/BSA-H-GO- modified electrode (green, 1167.1 Ω) > Hemin/BSA-rGO-H modified electrode (blue, 690.8 Ω) > BSA-rGO-H modified electrode (orange, 681.4 Ω) > Hemin/GC electrode (red, 412.3 Ω) > GC electrode (black, 402.1 Ω).

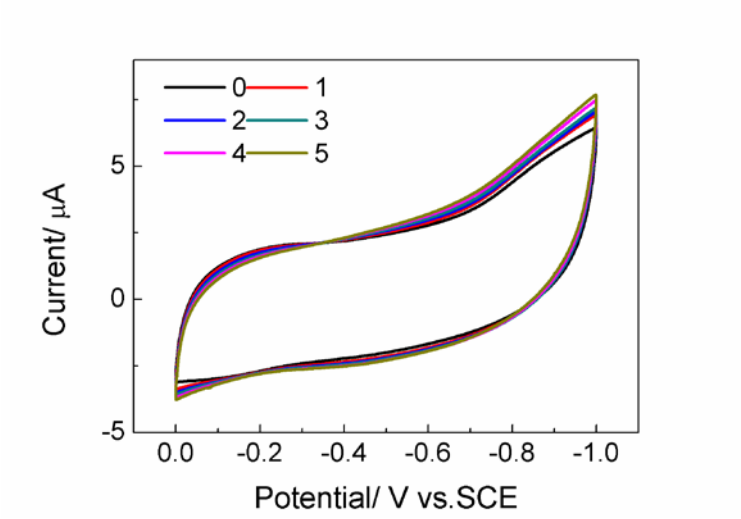

Figure S6. Cyclic voltammograms of BSA-rGO-H modified electrode in 0.1M PBS buffer before and after the addition of different amounts of H_2O_2 at a scan rate of 100 mV s^{-1}. The concentration increment of H_2O_2 for each step is 100 μmol L^{-1}.

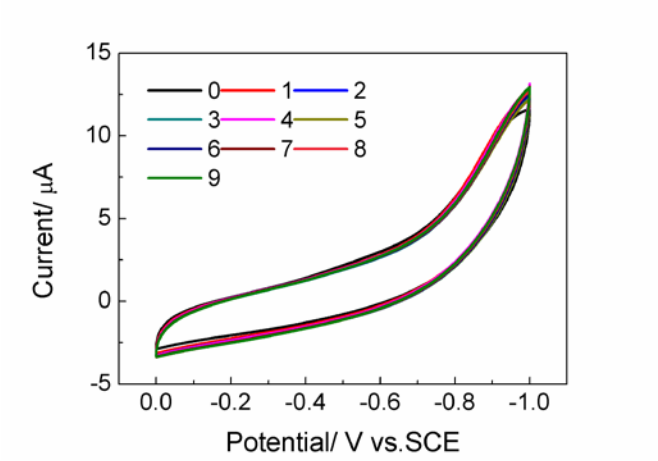

Figure S7. Cyclic voltammograms of bare electrode in 0.1M PBS buffer before and after the addition of different amounts of H_2O_2 at a scan rate of 100 mV s^{-1}. The concentration increment of H_2O_2 for each step is 100 μmol L^{-1}.

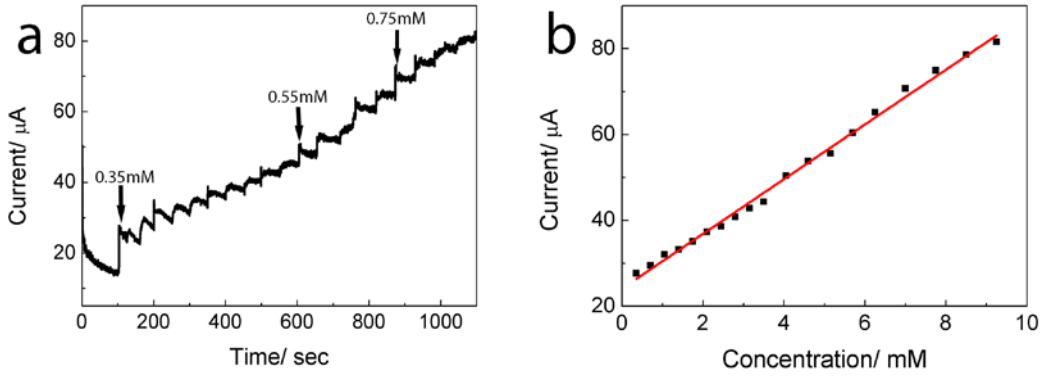

Figure S8. (a) Current-time curves for Hemin/BSA-rGO-H modified electrode (at -0.35 V), with successive addition of 0.35 mM, 0.55mM and 0.75mM H_2O_2. (b) Calibration curves for H_2O_2 at Hemin/BSA-rGO-H modified electrode. The selected Electrolyte was 0.1 M pH 7.0 PBS with stirring.

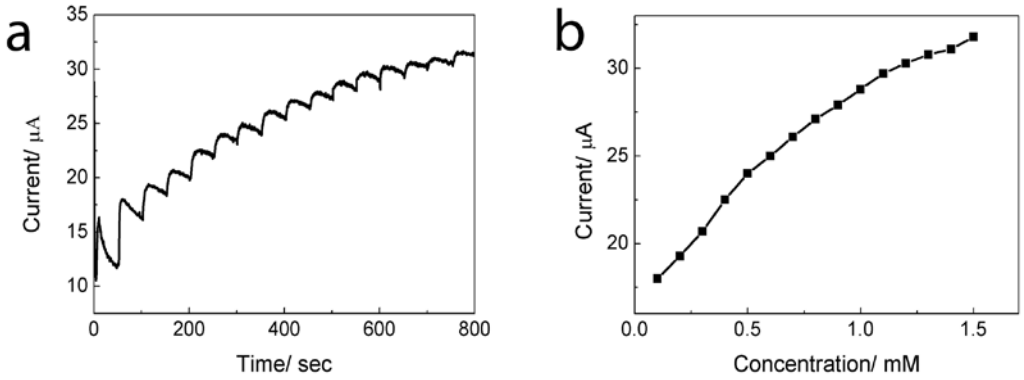

Figure S9. (a) Current-time curves for Hemin/BSA-rGO-H modified electrode (at -0.35 V), with successive addition of 0.1 mM H_2O_2. (b) Calibration curves for H_2O_2 at Hemin/BSA-rGO-H modified electrode. The selected Electrolyte was 0.1 M pH 7.0 PBS with stirring.