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Electronic Supplementary Information for

Hydrogel-coated Enzyme Electrodes Formed by the GOx-mediated Polymerization for Glucose Detecting

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1. Materials

N, N-Dimethylacrylamide (DMAA, 99%) and N-Succinimidyl Acrylate (NAS, >98%) were obtained from TCI Shanghai Development Co. Ltd（China）. Bovine serum albumin (BSA), Horseradish peroxidase (HRP) and glucose oxidase (GOx) were purchased from Baoman Biological technology（China）. Glucose (Glu) was acquired from Sinopharm Chemical Reagent Co. Ltd（China）. All the other reagents were of analytical purity and used without further purification. Deionized water was used throughout the experiments.

2. Pre-treatments and characterizations

2.1 *Preparation of hydrogels via the mild GOx-mediated polymerization*

The progress to prepare the BSA-GOx hydrogels is simple. To modified the BSA, 600 mg BSA and 45 mg NAS were dissolved in 15.4 ml phosphate buffer (pH 7.0) and stirred 2h at 25 °C. In addition, 0.05g GOx and 0.059g Glu were all dissolved in 5ml phosphate buffer and the Glu solution was stored at 4°C overnight. In a typical example of hydrogelation, 50μl DMAA, 80μl Glu, and 770μl acryloyted BSA solution were added into a sealed glass vial. After the addition of 100 μL GOx solution and rapidly stirred, the mixed solution will be gelation about 9mins later at the room temperature (approx. 25℃). Both of the precursor and the BSA-GOx hydrogels are light yellow.

2.2 *Modification of electrode*

We use the gold electrode as the work electrode. First, the gold electrode was polished sequentially by the particle size of 0.3μm, 0.05μm α-Al_2O_3 powder, and then ultrasonically cleaned with double distilled water and ethanol, each for 10 min. Finally, the gold electrode caries out the cyclic voltammetry in the 0.5mol/L sulfuric acid solution until the voltammograms become stable. 35μl BSA-GOx-H precursor

solution coating on the electrode formed the GOx-Hydrogel electrode at the room temperature (approx. 25℃).

2.3 *Measurement of kinetics mechanism and glucose detection*

Three-electrode cell was employed for the kinetics mechanism and series glucose sensing test, which employed platinum electrode and saturated calomel electrode (SCE) as the counter electrode and reference electrode, respectively. The electrochemical curves of the glucose sensor were measured in the 0.1 M phosphate buffer solution (PBS) with the increment glucose concentration of 60 μmol L^{-1}. The scanning rate is 100 mV s^{-1} and the PBS solution was removed oxygen before measurement. All the modified work electrodes were dipped in the electrolyte at least 1 hour. The electrochemical detection was carried out on the CHI660 electrochemical workstation.

2.4 *Preparation of scanning electron microscope samples*

The scanning electron microscope (SEM) analysis was performed on the Hitachi S4800 field emission SEM. The BSA-GOx-H was cut to a cube shape of about 2 × 2 × 2 mm^3. Then the sample was soaked sequentially 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 (30min) and last in pure isoamyl acetate (30 min) to replace the water in hydrogel with isoamylacetate. Finally, freeze-dried the sample for 48h and observed by SEM at a voltage of 3kV.

2.5 *Mechanical measurement*

The mechanical analysis was performed on a FR-108B testing machine (Farui Co., China). Removed the hydrogel from the sealed bottle and keep the cylindrical shape of the hydrogel. Put the hydrogel on the platform of the FR-108B, the crosshead speed is 1 mm min^{-1} to carry out the strain-stress measurement. The compressive stress (σ) was approximately calculated as $\sigma = Load/\pi R^2$, where R is the original radius of the

specimen. The strain (ε) under compression is defined as the change in the thickness relative to the original thickness. In order to insurance the accuracy of the measurement, we maintained the integrity of the original shape and used the same batch to detect the property.

2.6 *Test of rheological properties*

The rheological properties analysis was performed on the RS6000 rheometer (Thermo Scientific, Germany). We dropped 600μl precursor solution on the parallel plate geometry (35 mm diameter) and the gap between the plates is 0.45mm. Dynamic time sweep measurements were carried out to measure the storage modulus (G') and loss modulus (G'') as a function of time at a constant frequency of 1 rad s^{-1}. For the dynamic frequency sweep, the stress value is 1Pa and the scan range between 0.01Hz to 1Hz.

2.7 *Catalytic activity measurement*

The activity of the GOx within BSA-GOx hydrogels were measured via the model reaction of o-phenylenediamine (OPD). In a typical measurement, 100 μg GOx (or the BSA-GOx hydrogel containing 100 μg GOx) was added into the substrate (OPD, 10 mM) ， HRP (100 μL, 10mg/μL) and glucose (800 μL, 1.25M) mixed solution for catalyzing the reaction. Then the mixture was slightly stirred at room temperature (approx. 25 °C). The increase of the absorbance at 450 nm in the first minute with 0.1 min intervals was measured by the UV-Vis spectrometer (UV-2700, Shimadzu).

3. Figures

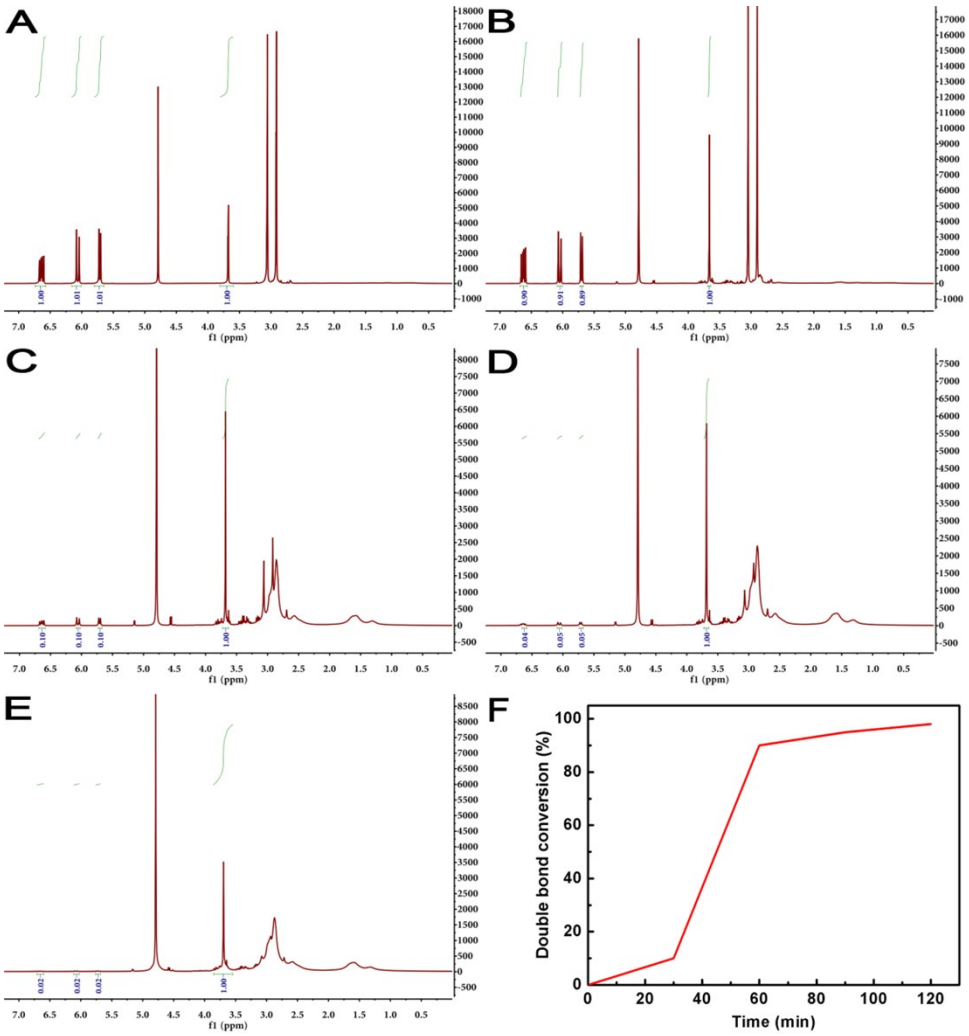

Figure S1. The conversion of monomers in the BSA-GOx hydrogels. The NMR spectra of the D_2O-substituted BSA-GOx hydrogels precursor under different reaaction time : 0 min (A); 30 min (B);60 min (C); 90 min (D); 120 min (E). The monomer conversion in the hydrogel is calculated using dioxane as internal standard material (F).

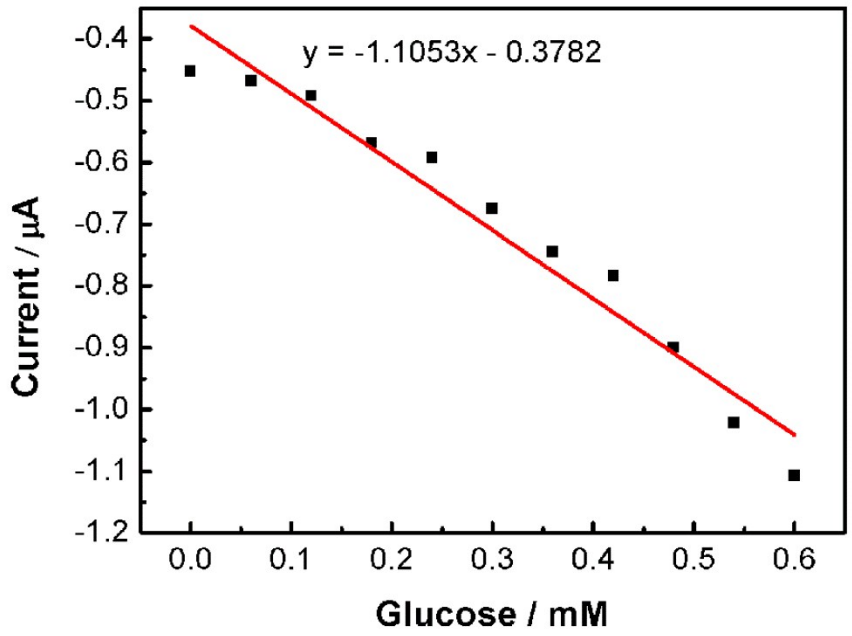

Figure S2. Peak current linear relationship of GOx-Hydrogel electrode at different glucose concentration

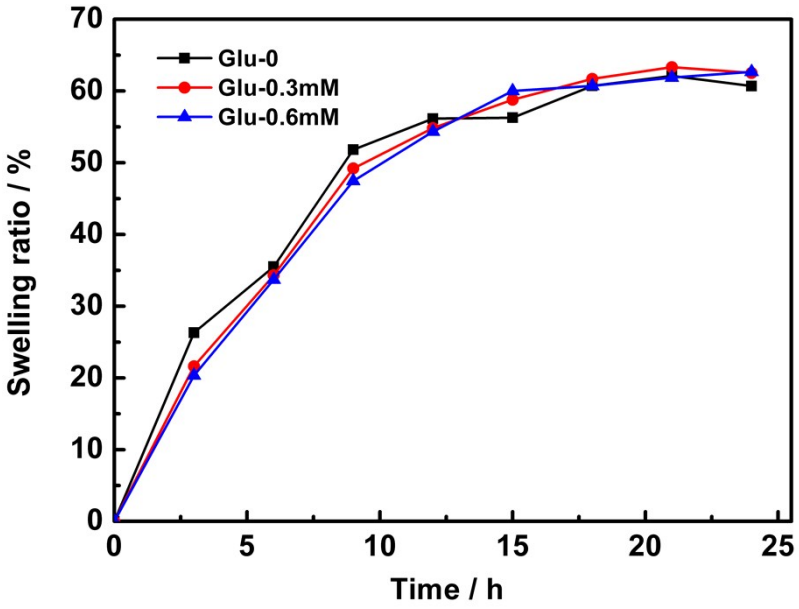

Figure S3. BSA-GOx hydrogel swelling test under different glucose concentration.

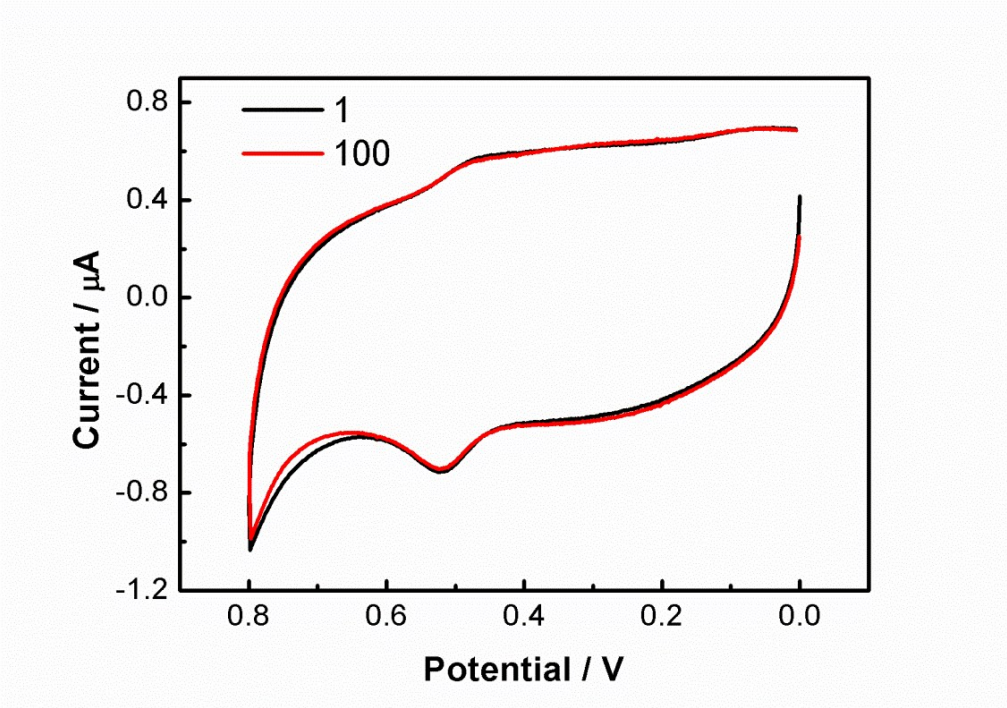

Figure S4. The first and 100th cycles of cyclic voltammograms of BSA-GOx-H modified electrode in 0.1 M PBS buffer saturated with N_2, scan rate 120 mV/s.

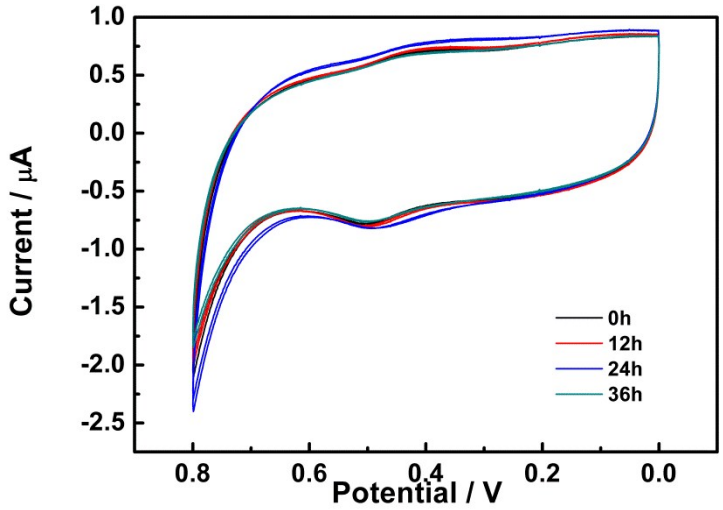

Figure S5. Recyclability of BSA-GOx-H modified electrode

Table S1. Mechanical properties of GOx-BSA-H with various compositions

No	GOx/ DMA/ BSA（wt%）	Compressive strength（kPa）	Young，s modulus（kPa）	Broken strain（%）
1	0.1/5/1	>241	3.44	-
2	0.1/5/3	130	16.10	79
3	0.1/5/5	90	30.09	66
4	0.1/5/7	99	50.18	59