Supporting information for

Electrodeposition of Hierarchical MnO_2 Spheres for Enzyme Immobilization and Mediatorless Glucose Sensing

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Experimental section

Glucose, glucose oxidase from *Aspergillus niger* (GOx, 100,000~250,000 units/g) and manganese acetate were purchased from Sigma-Aldrich. All other chemicals were of analytical grade and all solutions were prepared by double distilled water.

Electrochemical deposition of MnO_2 was conducted in a conventional three-electrode system. ITO glass with the dimension of 1.5 cm × 1 cm was used as the working electrode; platinum foil and Ag/AgCl were used as the counter electrode and reference electrode, respectively. 10 mM manganese acetate aqueous solution was used as the electrolyte throughout the experiment. After the electrodeposition at 1.5 V for 300 s, the MnO_2 film on ITO was scraped by scalpel and dispersed in double distilled water by ultrasonication. The MnO_2 powder was collected by centrifugation and dried at 80 ºC.

The enzyme immobilization was performed by mixing 10 mg MnO_2 with 1 mL PBS solution (pH 7.4) containing 10 mg GOx. The mixture was vigorously shaken at room temperature for 30 min, following by storage at 4 ºC overnight to allow the adsorption of GOx on MnO_2. The bioconjugate was then collected by centrifugation, and washed by enzyme-free PBS for three

times. Finally, the composite material was resuspended in PBS solution by continuously staking for 20 min.

The GOx activity was measured at 420 nm by a standard enzymatic assay with 2,2′-azino-di-[3-ethylbenzthiazoline-6-sulfonate] diammonium salt (ABTS) as chromogenic substrate. Assays were performed under oxygen saturation in 0.1 M sodium acetate buffer, pH 6, at 25°C with 0.1 M glucose as substrate.[1] One unit of GOx is defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of glucose to gluconolactone and H_2O_2 in 1 min at 25°C.

Glassy carbon electrodes (GCE, 3 mm in diameter) were polished to mirror-like surfaces with alumina powder followed by sonication with acetone, ethanol and water respectively. The electrode surface was then washed with double distilled water and dried with ultrapure nitrogen. After that, 5 μL GOx/MnO_2 was dropped on center of GCE, which was allowed to dry at 4°C for 24 h. Finally, 10 μL Nafion was casted on the entire electrode surface to fix the modified layer.

The surface morphology of the synthesized materials was characterized by field-emission scanning electron microscopy (FESEM; JEOL, JSM-6700F, 5 kV). The element composition of the sample was investigated by energy-dispersive X-ray spectrometer (EDX) attached to the FESEM equipment. The surface area and pore dimension of the sample was measured using a BET analyzer (Quantachrome Instruments Autosorb AS-6B instrument). The protein immobilization was studied by Fourier transform infrared spectrometer (FTIR, Bruker EQUINOX 55 Duroscope^{TM}). All electrochemical measurements were conducted on CHI 760D (CH instrument, USA) with a three-electrode system, where GCE was used as working electrode, Pt foil/wire was used as counter electrode and Ag/AgCl was used as reference electrode throughout the experiment. All the cyclic voltammetry (CV) measurements were

carried out in N_2-satureated 0.1 M PBS solution. Electrochemical impedance spectroscopy (EIS) study was performed in the solution 10 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl.

The IR spectrum of pure GOx (Figure S2a) exhibits two peaks at 1657 and 1539 cm^{-1}, ascribing to the characteristic amid I and amide II vibrational bands of the GOx molecules.[2] Another peak observed at 1103 cm^{-1} is attributed to the stretching vibration of C-O bond in GOx.[3] MnO_2 displays two peaks at 560 cm^{-1} and 730 cm^{-1} (Figure S2c), corresponding to the stretching vibration of the Mn-O and Mn-O-Mn bonds, respectively, while the peaks presented at 1600 cm^{-1} and 3400 cm^{-1} could be assigned to the vibration of hydroxyl group in the MnO_2 nanostructure.[4, 5] All the above characteristic peaks are apparently observed in the spectrum of GOx immobilized on MnO_2 (Figure 2b), indicating that the immobilized GOx is not denatured but well maintained its conformation upon the adsorption on the mesopores of MnO_2.

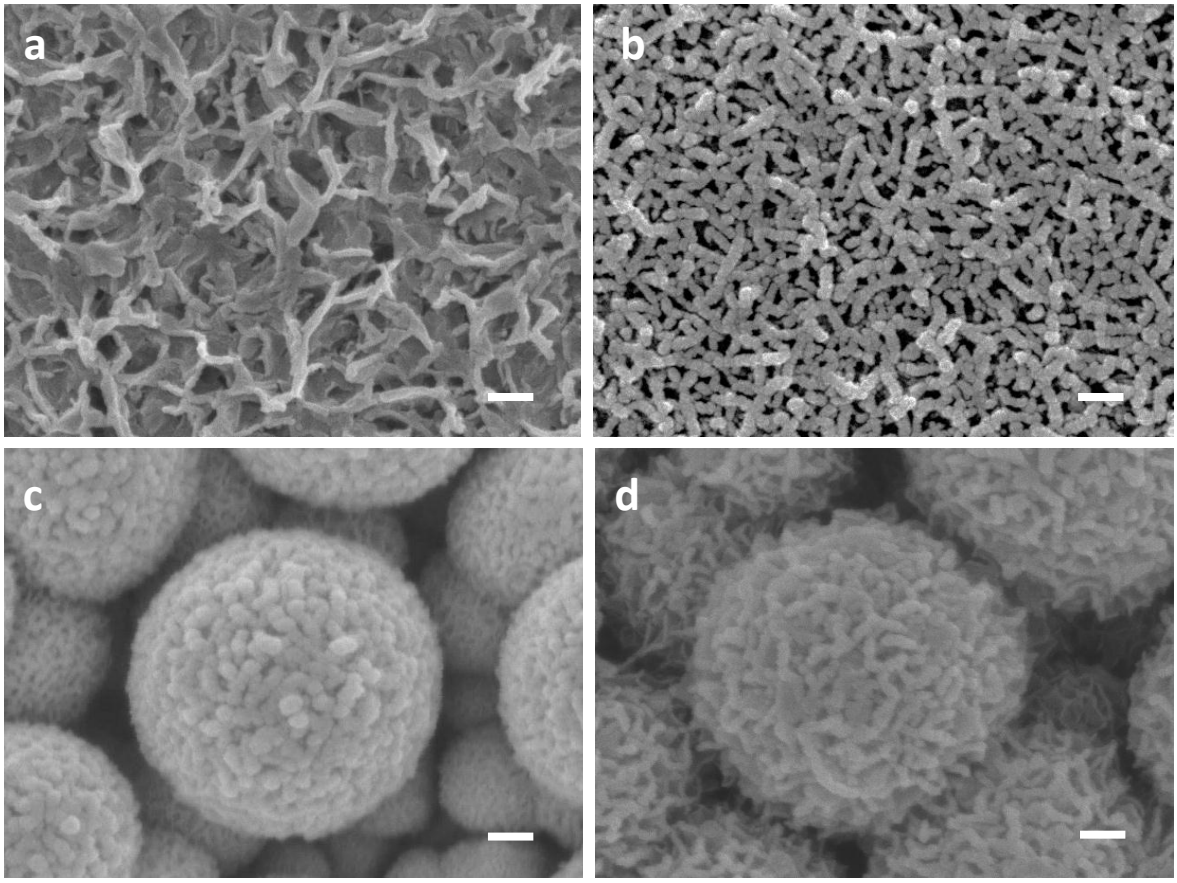

Figure S1. SEM images showing the architecture evolution of MnO_2 synthesized in the solution of 10 mM manganese acetate with different applied deposition potentials: (a) 0.5 V, (b) 0.8 V, (c) 1.2 V and (d) 1.5 V. The growth time is 300 s. The scale bar represents 100 nm.

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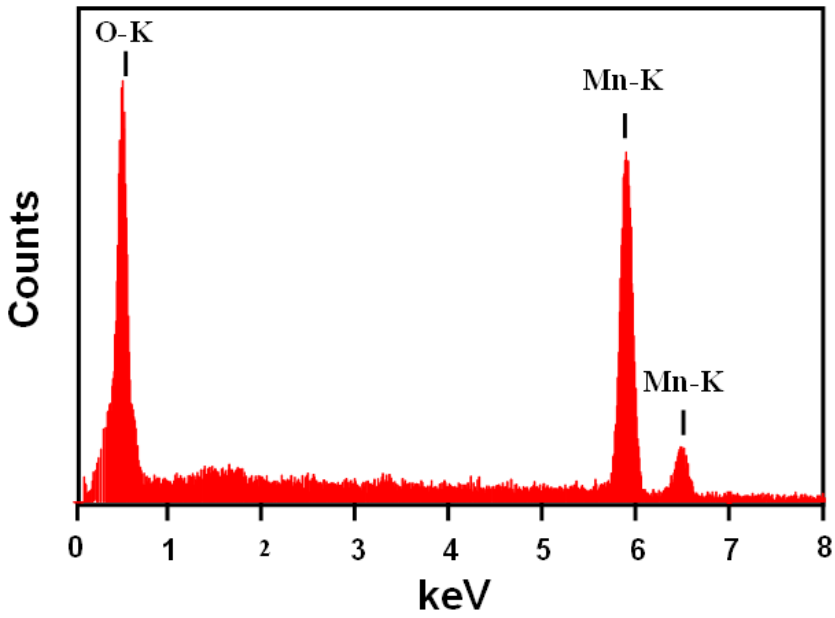

Figure 2. EDX spectrum of the as synthesized MnO_2 NRHS.

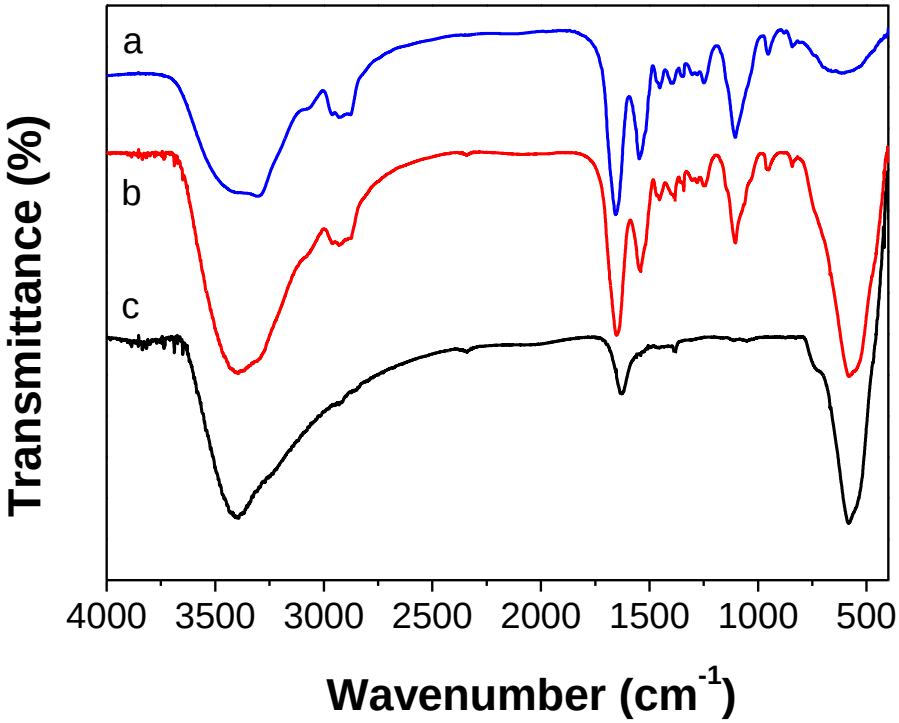

Figure S3. FT-IR spectra of (a) pure GOx, (b) GOx/MnO_2 NRHS composite and (c) MnO_2 NRHS.

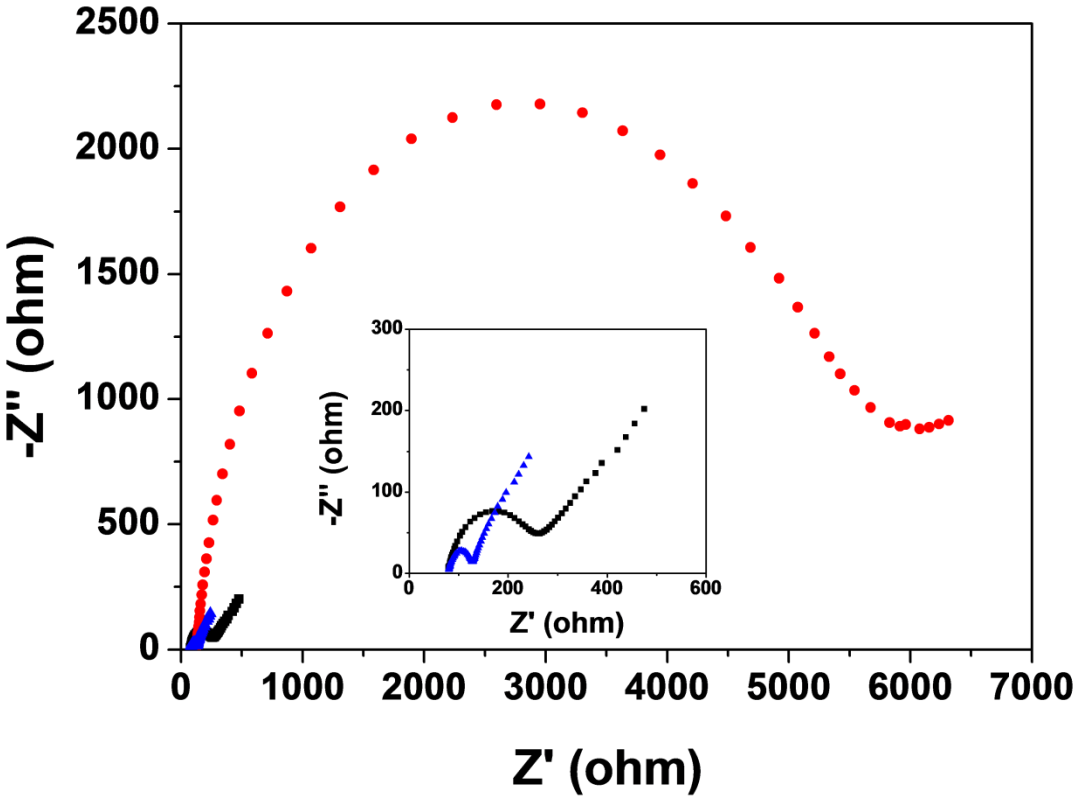

Figure S4. Nyquist plots of a bare GCE (black), MnO_2 NRHS-modified GCE (blue) and GOx/MnO_2 NRHS-modified GCE (red) measured in 0.1 M KCl solution containing 10 mM $Fe(CN)_6^{3-/4}$.

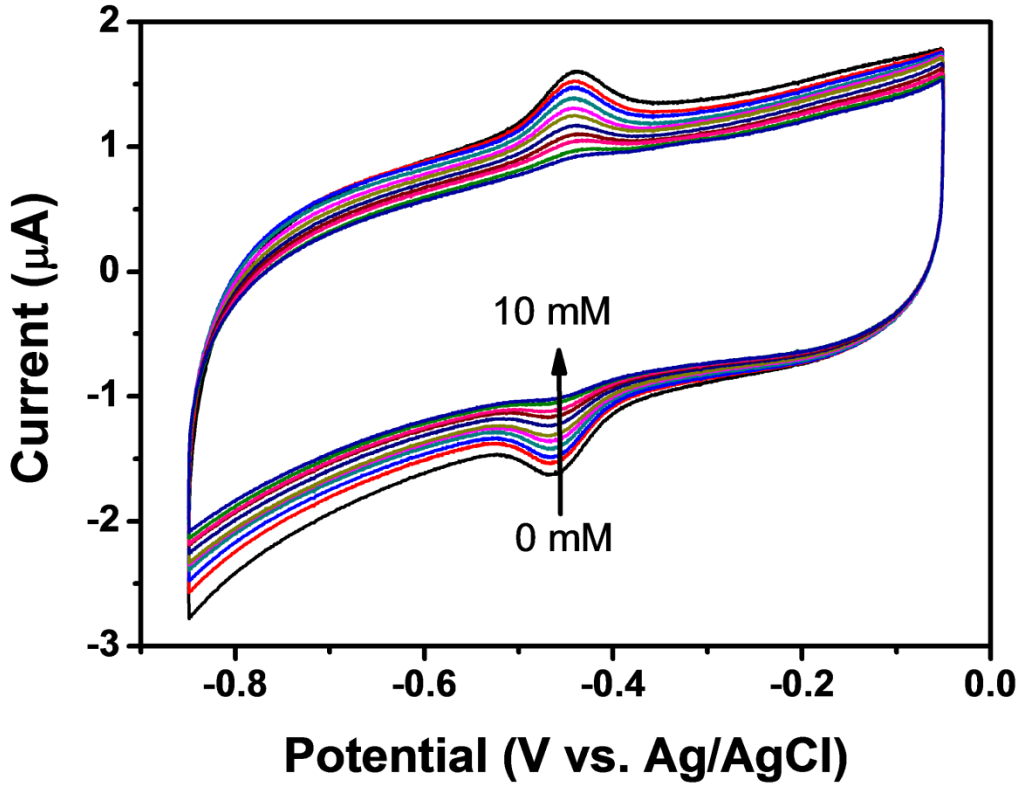

Figure S5. CVs of GOx/MnO_2 NRHS/Nafion-GCE measured in N_2-saturated 0.1 M PBS with different glucose concentrations: (from outside to inside) 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mM.

References

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