Electronic Supplementary Information

Anatase TiO_2 Nanoparticles-Graphene Nanocomposites: One-Step Preparation and their Enhanced Direct Electrochemistry of Hemoglobin

Qingming Shen, Shiwei Zhou, Xiaomei Zhao, Li-Ping Jiang*, Wenhua Hou, and Jun-Jie Zhu

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, P. R. China.

E-mail: jianglp@nju.edu.cn

Experimental Detail

1. Reagent and apparatus

$TiCl_3$, KNO_3, graphite powder (KS-10), and Hb were purchased from Sinopharm Chemical Reagent Co., Ltd. and Sigma-Adrich, respectively. TiO_2 nanoparticles (P25) were obtained from the Degussa Co., Germany. All the other chemicals were of analytical grade and used without further purification. The solutions were prepared with Millipore water.

Electrochemical measurements were performed on a CHI 660D workstation (Shanghai Chenhua, China) with a conventional three electrode system comprised of a platinum wire auxiliary, a saturated calomel reference electrode (SCE) as a reference electrode and glassy carbon electrode (GCE) with 3 mm in diameter as a working electrode. Characterizations were performed via high resolution transmission electron microscopy (HRTEM, JEOL 2010), Fourier-transform infrared (FT-IR Bruker Vector 22), atomic force microscope (AFM, Agilent 5500), X-ray powder diffraction (XRD, Shimadzu XD-3A), Raman spectra (Renishaw Micro-Raman spectroscope) and zeta potential measurement (Nano-Z zeta potential analyzer).

2. Synthesis of ATG nanocomposites

First, 10 mg of GO, prepared by modified Hummers method[1] were exfoliated in distilled water (20 mL) with ultrasonic treatment to form a colloidal suspension. Subsequently, 0.64 mL of $TiCl_3$ and 0.20 g of KNO_3 were added under vigorous stirring, and then the mixture was continually stirred at 25 °C for 5 h. The product was rinsed completely with water and ethanol, and then dried at room temperature.

3. Assembly of Hb and fabrication of the Hb-ATG/GCE

For the immobilization of Hb, 5 mg ATG nanocomposites were dispersed into Hb solution (2 mL, 2.5 mg mL^{-1} in pH 8.0 PBS) by continuously stirring at 4 ^{o}C for 2 h. As the isoelectric point of Hb is 7.4, Hb is negatively charged in pH 8.0 PBS, and it could be assembled on the surface of the positively-charged ATG nanocomposites via electrostatic interaction. After that, the mixture was centrifuged and the Hb-ATG nanohybrids were collected by removing the supernatant. The nanohybrids were thoroughly washed with PBS to remove the loosely assembled protein molecules. The loading amount of Hb can be calculated from difference of the UV-vis absorption of the Hb solution before and after the assembly with ATG, and the loading amount Hb in the Hb-ATG were calculated to be $1.29 \bullet 10^{-2}$ mg.

To fabricate the Hb-ATG/GCE, the Hb-ATG nanohybrids obtained above were redispersed into 2 mL of water to form a homogeneous suspension. Then, the suspension (10 μL) was casted onto the surface of the GCE, and the solvent was allowed to evaporate at room temperature before use. With similar procedures, the Hb/GCE and Hb-GR/GCE were also prepared. The similar amount of Hb molecular was assembled on the Hb-ATG/GCE, Hb-GR/GCE and Hb /GCE ($1.29 \bullet 10^{-2}$ mg).

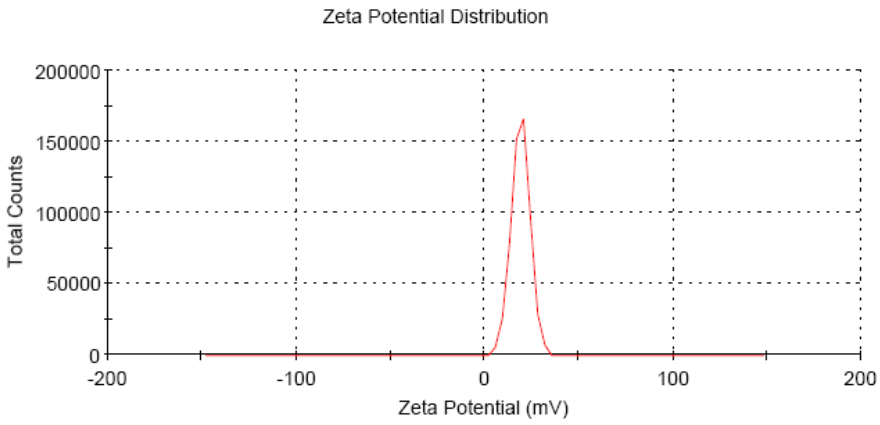


Fig. S1 Zeta potential distribution of ATG nanocomposites.

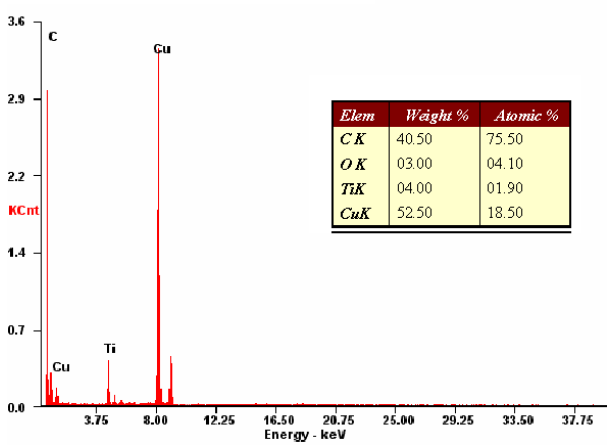


Fig. S2 EDS spectrum of ATG.

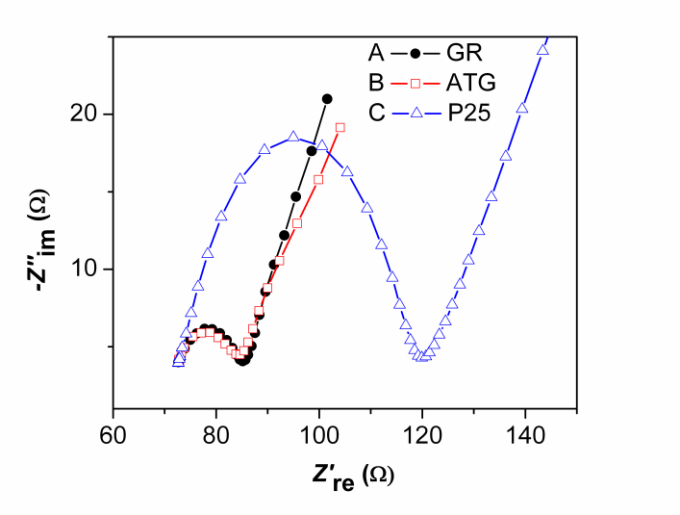
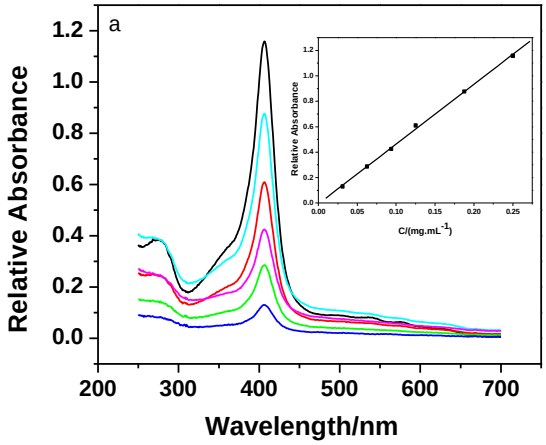


Fig. S3 Nyquist plots of (A) GR, (B) ATG and (C) P25 film electrode.



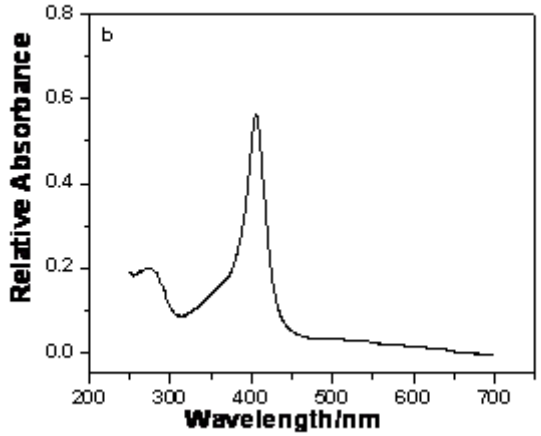


Fig. S4 (a) The UV-vis spectra of different concentration of Hb, the inset is the linear plot of absorption intensity vs. Hb concentration. (b) The UV-vis spectra of the supernatant of Hb-ATG solution.

The loading amount of Hb can be calculated from difference of the UV-vis absorption of the Hb solution before and after the assembly with ATG. From the UV-vis spectra curves of different concentration of Hb and the linear plot of absorption intensity vs. Hb concentration (Fig. S4a), the loading amount Hb in the Hb-ATG/GCE can be calculated to be $1.29 \bullet 10^{-2}$ mg.

Reference

1 W. S. Hummers and R.E. Offeman, *J. Am. Chem. Soc.*, 1958, **6**, 1339.