# **Electronic Supplementary Information:**

Insights into the effects of graphene oxide sheet on the conformation and activity of glucose oxidase: towards developing a nanomaterial-

based protein conformation assay

Qian Shao, Ping Wu, Xiaoqing Xu, Hui Zhang and Chenxin Cai\*

Jiangsu Key Laboratory of New Power Batteries, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210097, China.

\* Corresponding author, E-mail: <u>cxcai@njnu.edu.cn</u> (C. Cai); Tel: 86-25-85891780; Fax: 86-25-85891767.

## 1. XPS spectrum of GO



**Fig. S1** (A) XPS spectrum of the prepared GO sheets; (B) is XPS spectra of C1s in GO and their related curve-fitted components.

#### 2. FAD fluorescence emission spectra



**Fig. S2** The FAD fluorescence emission spectra of native GOx (150  $\mu$ g mL<sup>-1</sup>, a) and the GOx–GO bioconjugate system with GOx concentration fixed at 150  $\mu$ g mL<sup>-1</sup> and GO concentration of 2.5 (b), 5 (c), 10 (d), 20 (e), and 25  $\mu$ g mL<sup>-1</sup> (f) in 0.1 M PBS (pH 7.0). The excitation wavelength is 373 nm.

### 3. Fluorescence emission spectra of GO



**Fig. S3** The fluorescence emission spectra of GO in PBS at a concentration of 1.25, 2.5, 5, 7.5, 10, 12.5, and 15  $\mu$ g mL<sup>-1</sup>, respectively. The excitation wavelength is 279 nm.

### 4. CD spectra of the GOx–GO bioconjugate system at different interaction time



**Fig. S4** Far–UV CD spectra of native GOx (300  $\mu$ g mL<sup>-1</sup>, a) and GOx–GO bioconjugate system with GOx concentration at 300  $\mu$ g mL<sup>-1</sup> and GO concentration at 25  $\mu$ g mL<sup>-1</sup> in PBS (0.1 M, pH 7.0) for the interaction time of 4 (b), 12 (c), 24 (d), and 48 h (e), respectively.

5. CD spectra of native GOx and GOx–GO bioconjugate system at different solution pH



**Fig. S5** Far–UV CD spectra of native GOx (300  $\mu$ g mL<sup>-1</sup>, a) and GOx–GO bioconjugate system with the GOx concentration at 300  $\mu$ g mL<sup>-1</sup> and GO concentration at 25  $\mu$ g mL<sup>-1</sup> in PBS (0.1 M) at pH of 6.0 (A), 7.0 (B), and 8.0 (C), respectively.

#### 6. CD spectra of native GOx at different ionic strength



**Fig. S6** Far–UV CD spectra of native GOx (300  $\mu$ g mL<sup>-1</sup>) in PBS (0.1 M, pH 7.0) under the presence of 0 (a), 0.5 (b), 1.0 (c), and 2.0 M NaCl (d), respectively.



**Fig. S7** Relative amount of  $\alpha$ -helix (A),  $\beta$ -sheet (B),  $\beta$ -turn (C), and random coil (D) of native GOx in PBS (0.1 M, pH 7.0) under the presence of 0 (a), 0.5 (b), 1.0 (c), and 2.0 M NaCl (d), respectively. The data were obtained from CD spectra presented in Fig. S6 using a CDNN program. The data represented here are obtained by averaging the five independent measurements (n = 5). The error bar represents the standard deviation.

## 7. Effects of ionic strength on the disperse ability of GO and the GOx–GO bioconjugates



**Fig. S8** Pictures of GO (a–d) and GOx–GO bioconjugates (e–h) in PBS (0.1 M, pH 7.0) containing different concentration of NaCl. The concentration of NaCl is 0 (a, e), 0.5 (b, f), 1.0 (c, g), and 2.0 M (d, h). The concentration of GO in PBS is 25  $\mu$ g mL<sup>-1</sup>. The GOx–GO bioconjugates were prepared with GOx at 300  $\mu$ g mL<sup>-1</sup> and GO at 25  $\mu$ g mL<sup>-1</sup>.

#### 8. Dependent of absorbance of the enzymatic system on reaction time



**Fig. S9** Dependent of absorbance of oxidized form of *o*-dianisidine generating in the catalytic system at 436 nm on reaction time for native GOx (0.6  $\mu$ g mL<sup>-1</sup>, a) and GOx–GO bioconjugate system with the concentrations of GO of 2.5 (b), 5 (c), and 25  $\mu$ g mL<sup>-1</sup> (d) in PBS (0.1 M, pH 7.0). The catalytic system contains 16.7 mg mL<sup>-1</sup> $\beta$ –D–glucose, 8  $\mu$ g mL<sup>-1</sup> HRP, and 53  $\mu$ g mL<sup>-1</sup>*o*-dianisidine. The volume of the system is 3.1 mL. Curve (e) displays the absorbance of the system with native GOx being replaced by GO (25  $\mu$ g mL<sup>-1</sup>). The data represented here are obtained by averaging the five independent measurements (*n* = 5). The error bar represents the standard deviation.