Supporting Information

Greatly Improved Catalytic Activity and Direct Electron Transfer Rate of Cytochrome C Due to the Confinement Effect in a Layered Self-Assembly Structure

Bo-Yang Hua, Jiong Wang, Kang Wang, Xiang Li, Xue-Jun Zhu, Xing-Hua Xia*

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China. Fax: +86-25-83685947; Tel: +86-25-83597436; E-mail: xhxia@nju.edu.cn (X.H. Xia)

Experimental section

Materials:

All reagents used in this communication were of analytical grade and used as received. Deionized (DI) water was from a Millipore system (18.2 MΩ).

Apparatus:

Attenuated total reflection Fourier transfer infrared spectrum (ATR-FTIR) characterizations were performed on a Thermo Nicolet iS10 FTIR Spectrometer with Smart iTR accessory. UV-visible spectra were characterized on Thermo NanoDrop 2000c. Atomic force microscopic (AFM) images were obtained on an Agilent 5500 AFM/SPM system with Picoscan v5.3.3 software. AFM imaging was performed in the tapping mode under ambient conditions. X-ray powder diffractometer (XRD, Philips PANalytical X′PERT, Cu Kα radiation) was used to determine the interlayer d spacing in both the sulfonated graphene (G-SO_3H) sample and the G-SO_3H/cytochrome C (cyt C) self-assembly. Zeta potential was measured on a Malvern Zetasizer Nano Z measurement system. Raman scattering measurements were performed on a JY-HR 800 Raman spectrometer using a 488-nm laser source. X-ray photoelectron spectroscopy (XPS) data were obtained on an ULVAC-PHI PHI 5000 VersaProbe X-ray photoelectron spectrometer. Circular dichroism (CD) spectra were collected on a JASCO J-810 spectrometer. Transmission electron microscopic (TEM) images were collected on a JEOL JEM 2100 electron microscopy and high resolution TEM (HRTEM) images were collected on the same instrument with 200 kV accelerating voltage. Scanning electron microscopic (SEM) images were collected on a Hitachi S-4800 electron microscopy with 15 kV accelerating voltage. All electrochemical experiments were performed on a CHI 840 electrochemical workstation (CH Instrument Co., USA). The three-electrode system was consisted of modified glassy carbon electrodes (GCE) as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl (3M KCl) as the reference electrode.

Synthesis of the graphene oxide (GO):

GO was synthesized by using graphite flakes as raw material. 400 mL mixture of concentrated H_2SO_4/H_3PO_4 (9:1 V/V) was added into the mixture of 3.0 g graphite flakes and 18.0 g $KMnO_4$. Heat the reaction to 50 ^{o}C and keep it under stirring for 12 h. After the oxidation, the product was cooled to room temperature. Then, 3 mL 30% H_2O_2 was slowly added into the mixture to obtain a bright yellow dispersion with ice bath to keep the temperature low. The dispersion was centrifuged and rinsed with 300 mL 1:10 (V/V) HCl and subsequently 300 mL DI water, and then dialyzed for a week to get rid of the residual metallic ions thoroughly. The resultant dispersion was dried in the vacuum centrifugation system (LNG-T98, Taichang Huamei Biochemical Instruments, China) to get the final solid product of GO.

Synthesis of the sulfonated graphene (G-SO_3H):

There are altogether three steps to synthesize the G-SO_3H from GO. First, 75 mg dried GO was dispersed in 75 mL water through sonication for 30 min. The dispersion was centrifuged at 3000 rpm for 10 min to remove the unexfoliated GO before use. Then, 5% wt sodium carbonate solution was used to adjust the pH of the GO dispersion to 9-10. 600 mg sodium borohydride was added into the dispersion with 15 mL water. The mixture was thereafter heated to 80 ^{o}C and kept for 1 h under mechanic stirring. This step of pre-reduction could lead to agglomeration of graphene. After rinsing the sediment with water and centrifuging, pre-reduced GO was obtained. In the second step, 18 mg sodium nitrite was dissolved in 5 mL water and the resulting solution was added into 5 mL water containing 46 mg sulfanilic acid and 0.5 mL 1 M HCl in the ice bath to keep the temperature below 5 ^{o}C. After the aryl diazonium salt was prepared, it was added into the dispersion of pre-reduced GO with temperature strictly controlled below 5 ^{o}C. This mixture was kept for 2 h to allow complete reaction. In the last step, 150 μL hydrazine and 1 mL ammonia solution were added into the sample dispersion. The dispersion was kept at 80 ^{o}C for 1 h under stirring. After the reduction was completed, repeated centrifuging and rinsing with water were used to purify the G-SO_3H sample. The final G-SO_3H solid was obtained by drying the dispersion in the vacuum centrifugation system (LNG-T98, Taichang Huamei Biochemical Instruments, China).

Preparation of G-SO_3H/cyt C self-assembly:

The self-assembled G-SO_3H/cyt C layered nanostructure was synthesized by mixing 2 mL of 0.2 mg/mL G-SO_3H with 0.8 mL of 1 mg/mL cyt C (from equine heart, sigma aldrich) and then storing at 4 ^{o}C for 24 h. Aggregation could be observed after the mixture of these two kinds of solutions and the color of the supernatant became almost colorless after assembling. By measuring the UV-vis absorbance of the supernatant at the wavelength of 410 nm after assembling, the amount of cyt C that is intercalated between graphene sheets can be estimated. The sediment was collected by centrifuging and washed with water for three times. The purified sediment was either dried for the measurements of XRD, ATR-FTIR and XPS, or re-suspended with certain amount of water to get the aqueous dispersion.

Measurement of catalytic activity:

We employed the oxidation of o-phenylenediamine(OPD) as our model reaction to test the catalytic activity of free cyt C and G-SO_3H/cyt C assembly. Typically, 60.0 mM H_2O_2 and 5.0 mM OPD was mixed together. Then 4.0 μM free cyt C or the assembly containing same amount of cyt C was added into the mixture. Once the catalyst was added, the UV-vis absorbance at 450 nm was recorded along with the reaction time. The initial reaction rate was calculated from the data obtained in the firtst half minute. To measure the catalytic kinetic constants, H_2O_2 concentration was varied, i.e. 6.0 mM, 12.0 mM, 15.0 mM, 24.0 mM, 30.0 mM and 60.0 mM.

Preparation of modified electrodes:

The assembly modified electrode was prepared by drip coating. Before coating, GCE was carefully polished by 1, 0.3 and 0.05 μm alumina powder and cleaned by sonication in ethanol and water, respectively. The pre-treated GCE was blown to dry by pure N_2 gas. 10 μL of ~ 2 mg/mL assembly dispersion was dropped onto the GCE and dried at 4 ^{o}C. In comparison, we dropped 1 mg/mL G-SO_3H dispersion onto the GCE and dry the electrode under ambient condition. This G-SO_3H modified GCE was then immersed in 10 mg/mL cyt C solution for 6 h at 4 ^{o}C to adsorb cyt C on the surface of electrode. Electrodes prepared following these methods were used for further electrochemical measurements.

Supplementary results:

Table S1. Zeta potential of GO and G-SO_3H.

	GO	G-SO_3H
Zeta potential (mV)	-48.3	-30.8

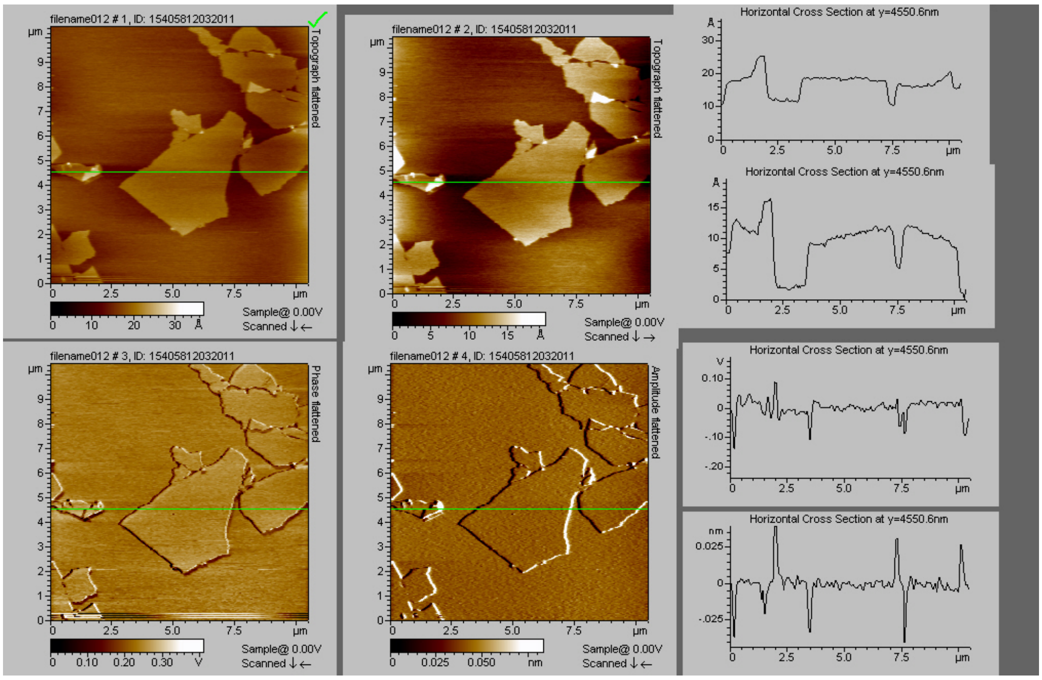

Figure S1. AFM images of GO.

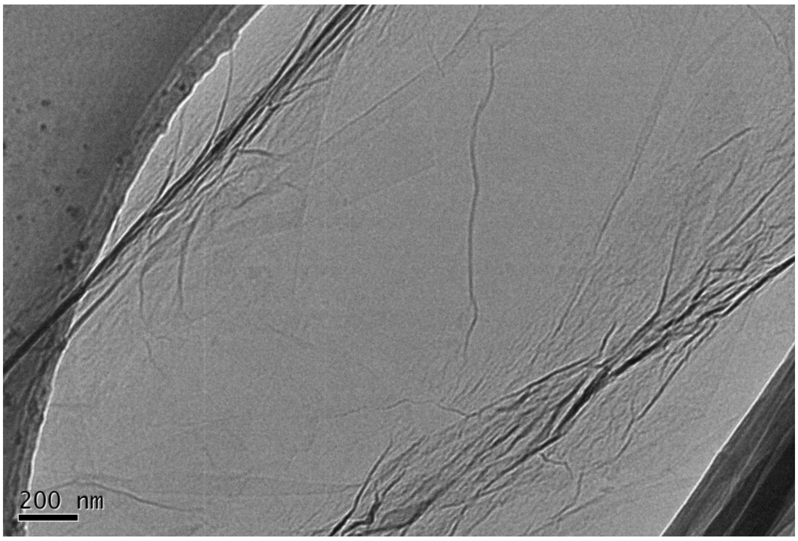

Figure S2. TEM image of the sulfonated graphene (G-SO_3H).

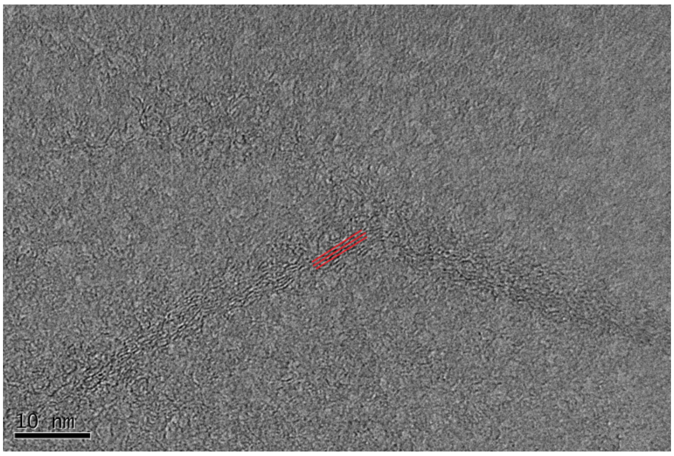

Figure S3. HRTEM image of the ripples on the sulfonated graphene (G-SO_3H) sheets.

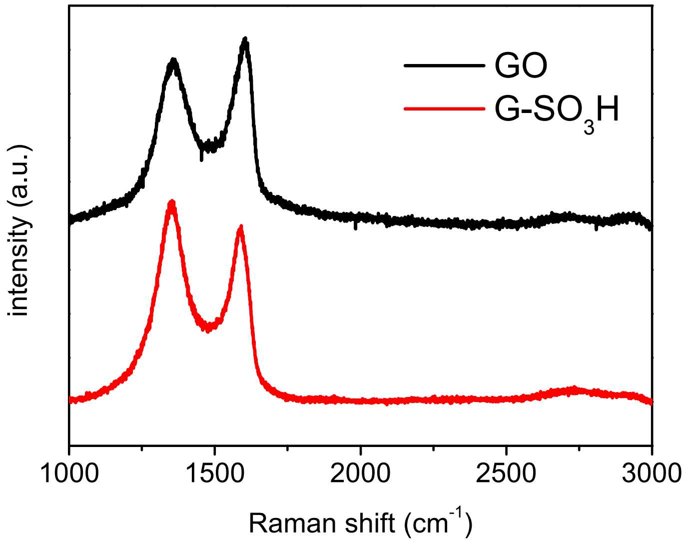

Figure S4. Raman scattering spectra of GO and the sulfonated graphene (G-SO_3H).

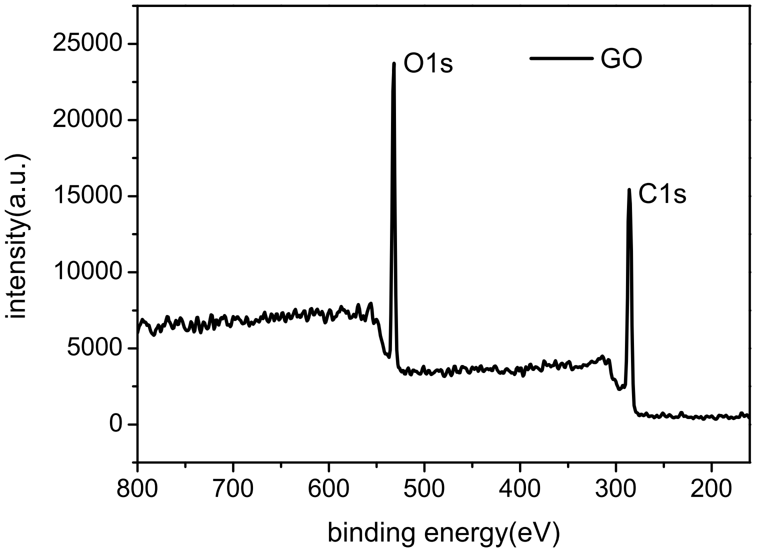

Figure S5. Survey XPS spectrum of GO.

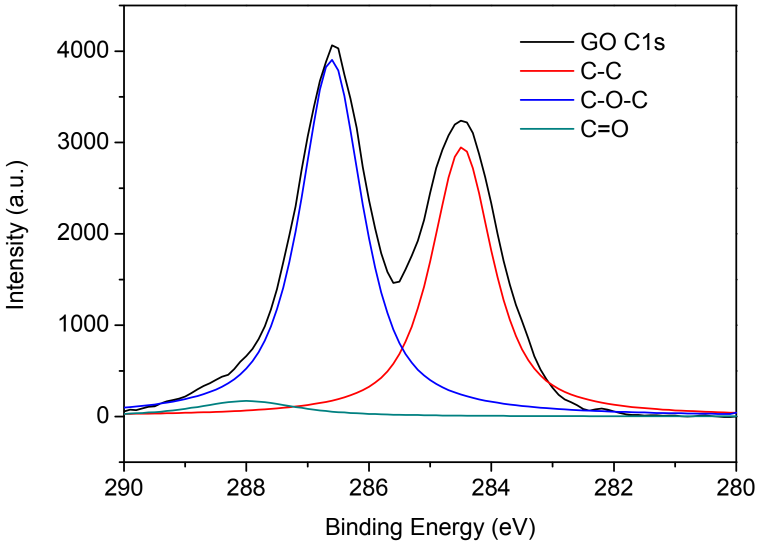

Figure S6. High resolution XPS spectrum and corresponding fittings for GO.

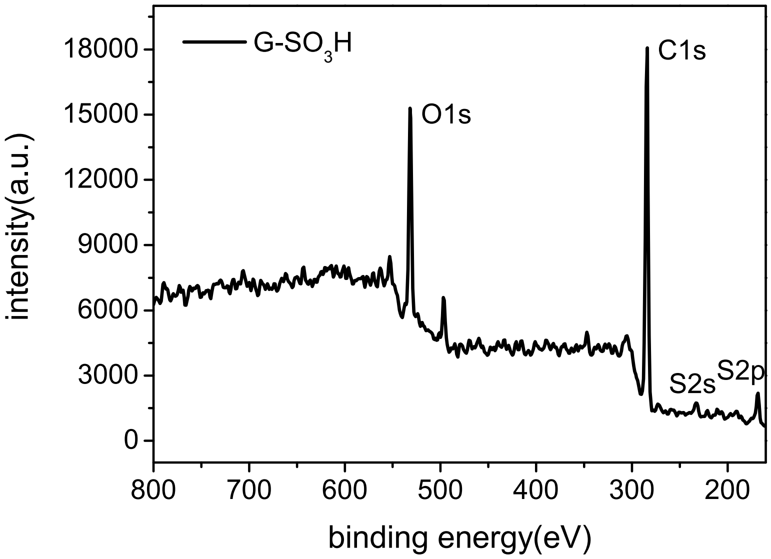

Figure S7. Survey XPS spectrum of the sulfonated graphene (G-SO$_3$H).

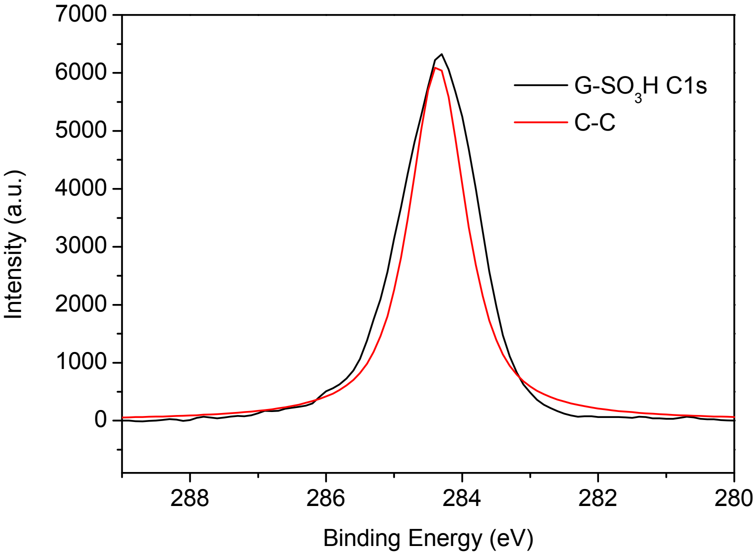

Figure S8. High resolution XPS spectrum and corresponding fitting for the sulfonated graphene (G-SO$_3$H).

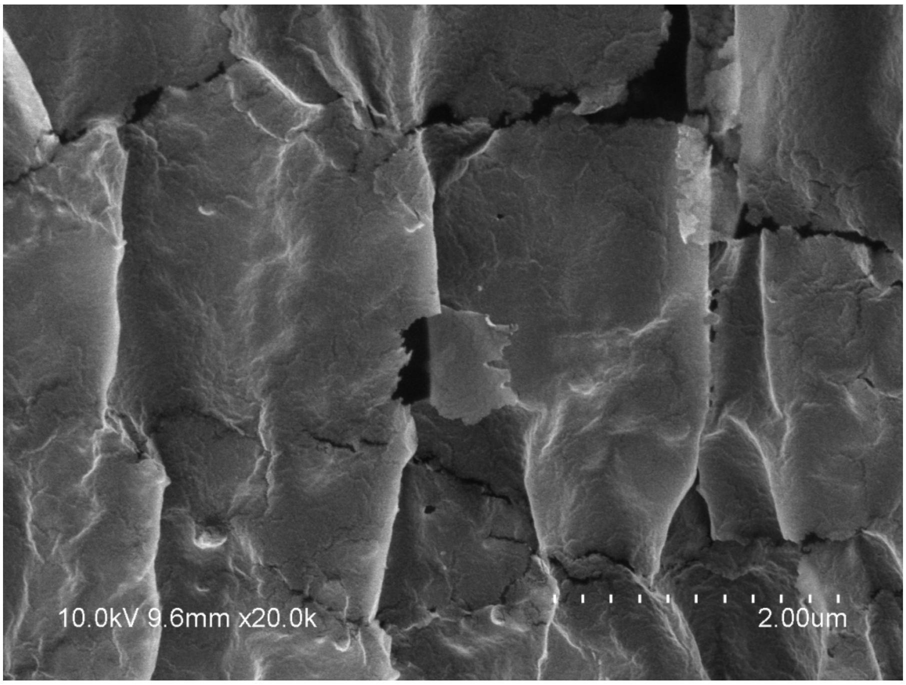

Figure S9. SEM image of the G-SO_3H/cyt C assembly.

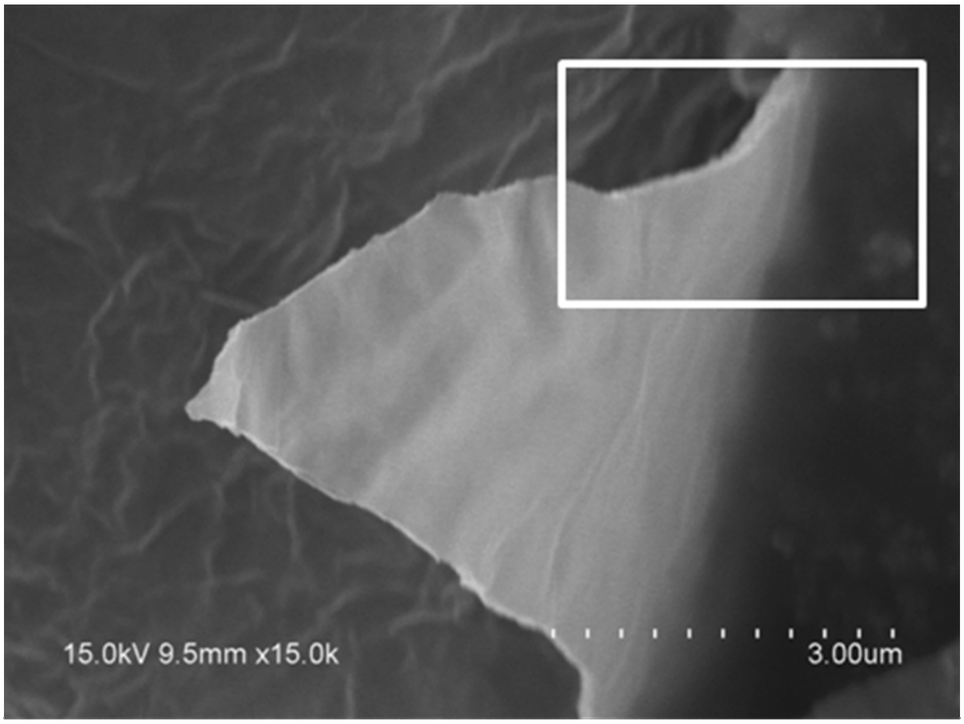

Figure S10. SEM image of a tilted G-SO_3H/cyt C assembly.

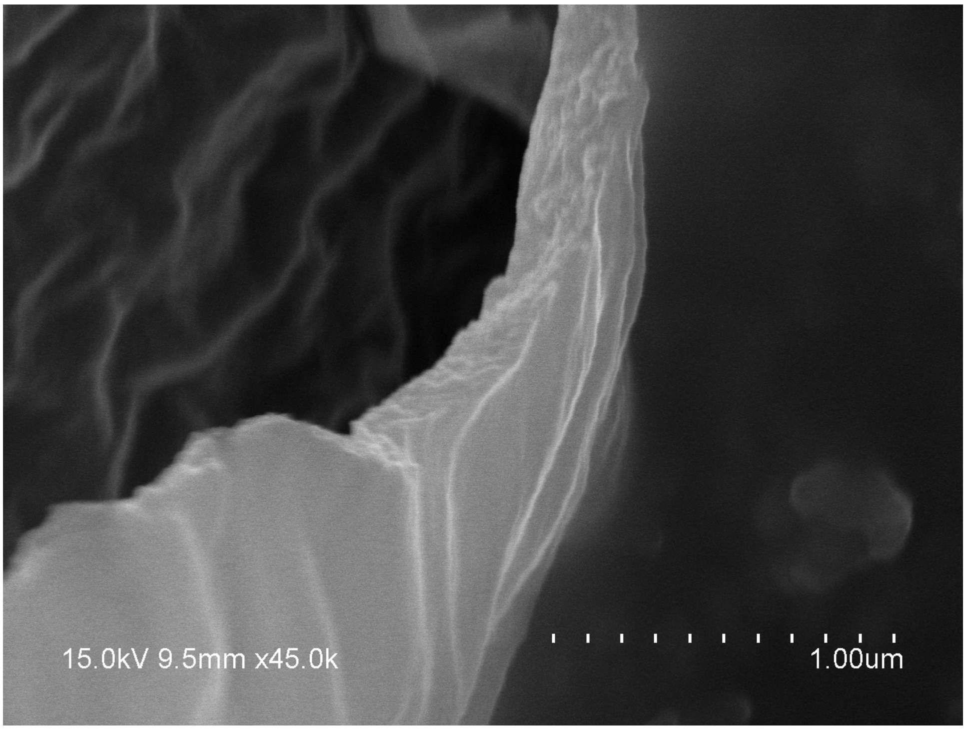

Figure S11. Magnified SEM image of Figure S10.

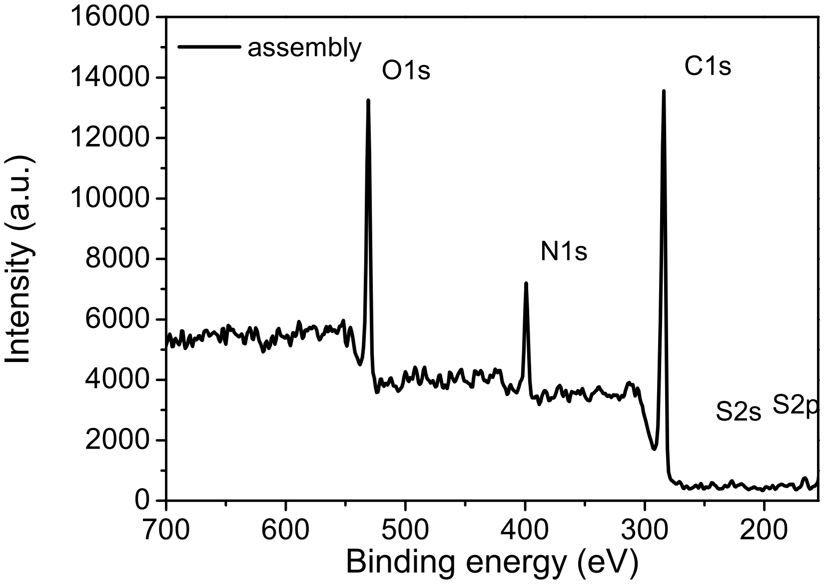

Figure S12. Survey XPS spectrum of the G-SO_3H/cyt C assembly.

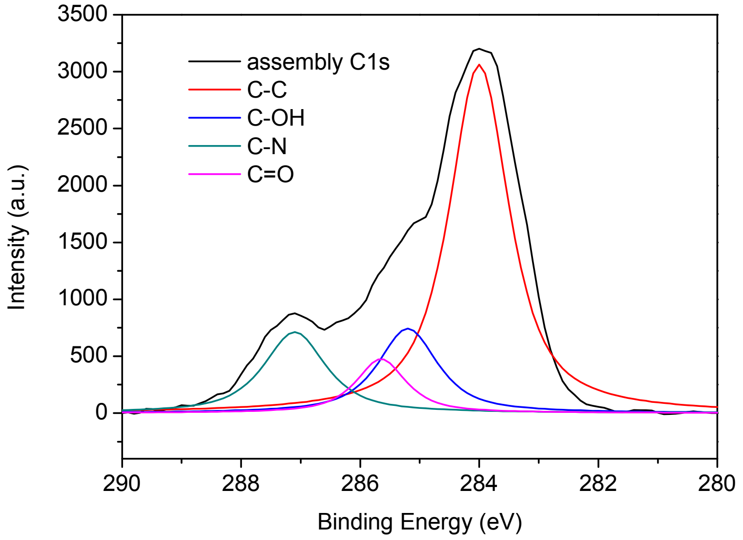

Figure S13. High resolution XPS spectrum and corresponding fittings of the G-SO_3H/cyt C assembly.

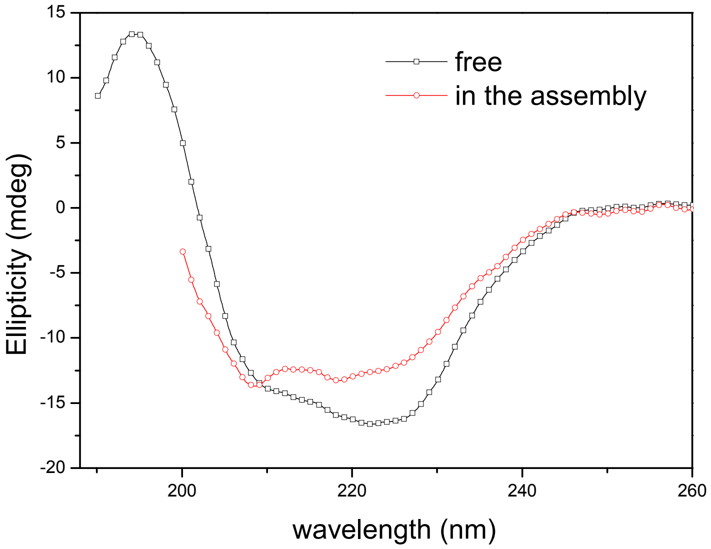

Figure S14. CD spectra of free cyt C and cyt C in the assembly.

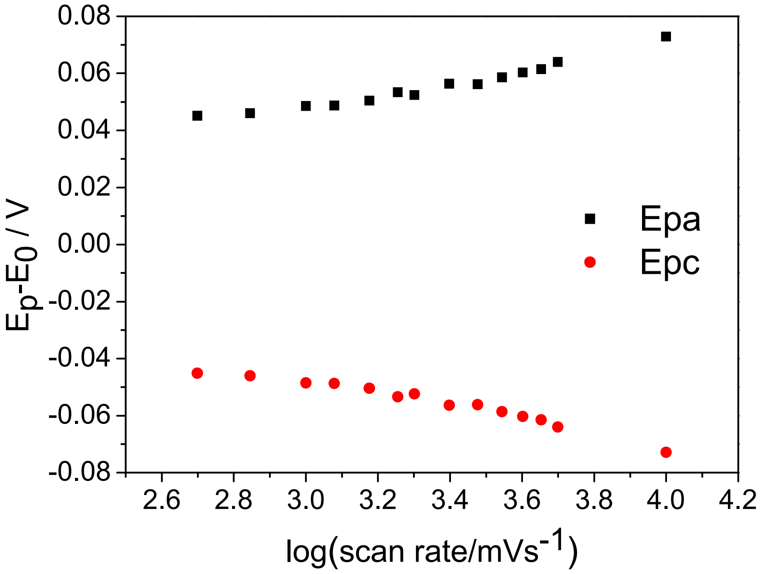

Figure S15. Laviron plot of peak potential versus the logarithm of scan rate of G-SO_3H modified GCE adsorbed with cyt C.