

Electronic Supplementary Information for *chemical communications*

High biocurrent generation in *Shewanella*-inoculated microbial fuel cells using ionic liquid functionalized graphene nanosheets as anode

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Section A: Preparation and characterization of the Ionic liquid functionalized graphene nanosheets (IL-GNS)

Chemicals Graphite powder, 3-bromopropylamine hydrobromide and 1-methylimidazole were purchased from Sigma-Aldrich and used without further purification. KOH ($\geq 82\%$) was obtained from Shanghai Reagent Company, China. All reagents were of analytical reagent grade and used as received. The ultra-pure water ($18.2 \text{ M}\Omega\cdot\text{cm}$ at 25°C) prepared on a Milli-Q (MQ) water system was used throughout all experiments. Fluorescence measurements were performed using a Bruker RF-5301PC fluorescence spectrometer. Excitation (emission measured at 515 nm) and emission (excited at 450 nm) spectra showing the presence of riboflavin molecules.

Preparation of graphene oxide (GO)

Graphene oxide (GO) was prepared and purified by using natural graphite powder according to the modified Hummers method.^{1,2} The obtained GO was dispersed in water by ultra-sonication (100W, 40 kHz) to create a 0.5% w/w dispersion, which was used for reduction.

Preparation of IL-NH₂

The amine-terminated ionic liquid 1-(3-aminopropyl)-3-methylimidazolium bromide (IL-NH₂) was prepared according to the method reported in the literature.³ In the experiment, 3-bromopropylamine hydrobromide (2.200 g, 10 mmol) and 1-methylimidazole (0.790 mL, 10 mmol) were added to 25.0 mL ethanol which was refluxed for 24 h under nitrogen atmosphere. The resulting mixture was re-crystallization with ethanol, and then precipitated in ethyl acetate. The resulting solid was then dried under vacuum at 60°C overnight. ¹H NMR (300 MHz, D₂O): δ 8.85 ppm (1 H, s), 7.59 ppm (1 H, s), 7.52 ppm (1 H, s), 4.39 ppm (2 H, t, J 7.3), 3.95 ppm (3 H, s), 3.20 – 3.04 ppm (2 H, m), 2.34 ppm (2 H, dt, J 15.1, 7.5).

Preparation of unfunctionalized graphene nanosheets (GNS) and Ionic liquid functionalized graphene nanosheets (IL-GNS)

GNS was obtained through a facile microwave-assisted solvothermal (MW-ST) process reduction of exfoliated GO. Briefly, 40 mL 0.5 mg/ml exfoliated GO was ultrasonicated and then transferred to the reaction tube and sealed inside the quartz vessels of CEM Instruments (CEM Discover synthesis system, USA). Under microwave irradiation (200W), the reaction was maintained at 180°C and 160 psi for 30 min, and then the reactor was cooled

to room temperature. The black precipitate of formed graphene was centrifuged, washed with water, and air-dried.

IL-GNS was obtained by an epoxide ring-opening reaction between GO and IL-NH₂ according to the literature.⁴ Briefly, 40 mg IL-NH₂ was added into 40 mL of 0.5 mg mL⁻¹ exfoliated GO (the weight ratio of IL-NH₂ to GO was 2:1), and 40 mg KOH was added into the above mixture, and then the mixture was subjected to ultrasonication for 30 minutes. After ultrasonication, the mixture solution was for the MW-ST process. The experimental condition is identical to the preparation of the GNS. The final product IL-GNS was then centrifuged, washed with ethanol and water, and air-dried.

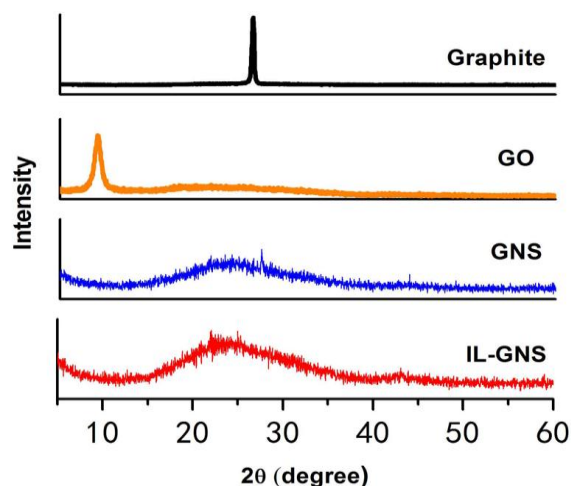


Fig S1 The XRD patterns of pristine graphite, GO, GNS and IL-GNS.

X-ray diffraction (XRD) measurements were performed on a Japan Shimadzu XRD-6000 powder X-ray diffractometer, using Cu K α ($\lambda = 1.5405\text{\AA}$) as the incident radiation. The XRD patterns (Figure S1) show that the (002) diffraction peak of pristine graphite appears at ca. 26.6°. After the oxidation of graphite to GO, the peak center shifts to low angle (10.2°), and then shifts back to higher angle (23.5°, 23.4°) with a significant broadening after the MW-ST reduction of GO to GNS and IL-GNS.

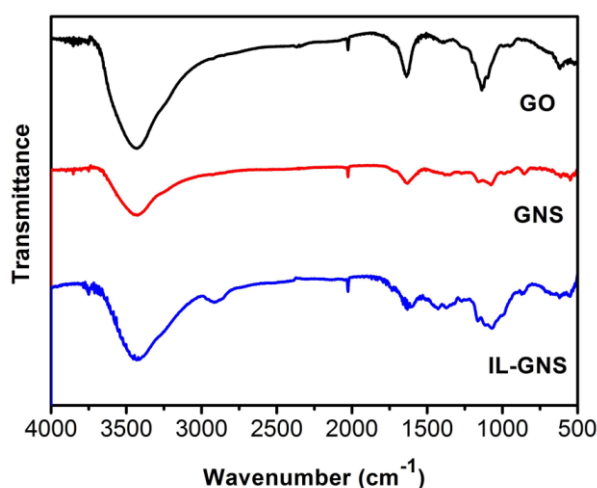


Fig S2 FT-IR spectra of GO, GNS and IL-GNS.

Fourier-transform Infrared Radiation (FTIR) spectroscopic measurements were performed on a Bruker model VECTOR22 FTIR spectrometer using KBr pressed disks. Figure S2 shows the FTIR spectra of GO and the

reduction products obtained by the MW-ST process. It is found that the FTIR spectra of GO with a strong absorption band around 1720 cm^{-1} due to the C=O stretching. The bands around 1420 cm^{-1} , 1260 cm^{-1} and 1090 cm^{-1} could be assigned to carboxy C-O stretching, epoxy C-O stretching and alkoxy C-O stretching, respectively.⁵ After MW-ST process, the intensity of C=O conjugation (1720 cm^{-1}) and C-O band (1420 cm^{-1}) of GNS or IL-GNS were weak, suggesting that most of the oxygen functional groups had been removed. In addition, the FTIR of IL-GNS exhibits typical IL-NH₂ absorption features at 2920 cm^{-1} corresponds to symmetric $\nu_s(\text{CH}_2)$ of imidazolium ring, and ring in-plane asymmetric stretching at 1169 cm^{-1} . The bend at 1290 cm^{-1} is assigned to the C-N stretching, confirming the successful covalent functionalization reaction between IL-NH₂ and the epoxy groups on GO sheets.

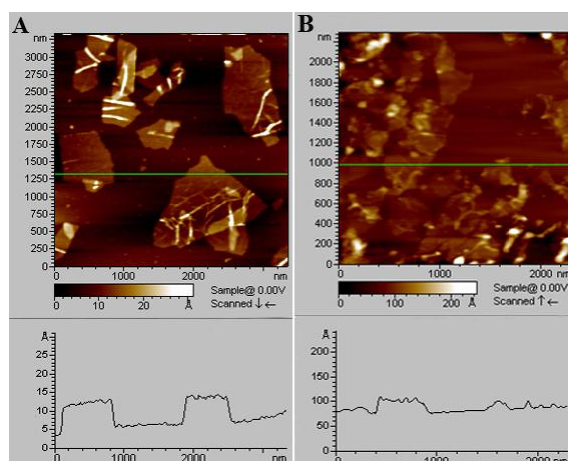


Fig S3 AFM images of (A) GNS, and (B) IL-GNS.

The tapping mode Atomic force microscope (AFM) images of GNS and IL-GNS were performed on SPA-300 HV with a SPI 3800 controller (Seiko). The samples for AFM measurement were obtained by ultrasonic treatment of GNS and IL-GNS dispersion in water. From Figure S3, we could see that the exfoliated GNS with a measured thickness of 0.8~0.9 nm, and the interlayer spacing for exfoliated IL-GNS was about 1.5 nm~2.0 nm, which is a typical characteristic of functional-molecule-protected single-layer graphene.⁶ The distance between IL-GNS is thicker than that of GNS, indicating the successful attachment of IL-NH₂ to GO nanosheets.

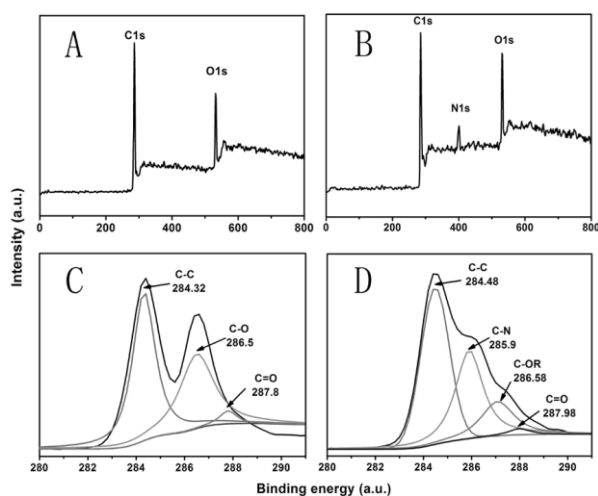


Fig .S4 XPS survey spectra of (A) GNS and (B) IL-GNS, and higher resolution C1s spectrum of (C) GNS and (D) IL-GNS.

The X-ray photoelectron spectra (XPS) were taken on a Thermo Scientific K-alpha electron energy spectrometer using Al K α (1486.6eV) as the X-ray excitation source. As shown in Fig S4A and B, compared to the spectrum of GNS, there is an additional peak of N1s in the XPS spectrum of IL-GNS. Moreover, the corresponding C1s deconvolution spectrum of GNS revealed the presence of three types of carbon bonds (Fig S4C): C-C (284.32 eV), C-O (286.5eV) and C=O (287.8 eV),² while an additional C-N group at 285.9 eV was observed for the IL-GNS (Fig S4D), which accompanies the dramatic decrease in epoxy groups, and the new component can be assigned to the combination of the C bound to nitrogen which newly appears after reaction and the C-N groups from the imidazolium ring of the ionic liquid.

Zeta potential measurement

Zeta potential analysis was performed with a PALS Zeta Potential Analyzer Ver. 3.43 (Brookhaven Instruments Corp.). The zeta-potential of GO and GNS aqueous solution was measured to be -41.2 mV and -20.3 mV, respectively. After functionalization with IL-NH₂, a significant positive shift to +36.8 mV was observed, indicating that sufficient positive charge on the surface of IL-GNS to ensure attachment of bacterial cells. The zeta potential of bacteria in the cultivated condition was -10.2 mV.

Section B: ECs and MFCs operation

Electrode preparation

The IL-GNS modified carbon paper (CP/IL-GNS) and GNS modified carbon paper (CP/GNS) electrodes were fabricated through a simple dipping-drying process⁷: a piece of carbon paper (2 cm²) was dipped in IL-GNS or GNS inks, then dried at 60°C for 12 h, respectively. The concentration of IL-GNS or GNS was 0.5 mg/mL. The dipping-drying process was repeated for several times to increase the loading of IL-GNS or GNS on carbon paper electrodes. The consumption of the IL-GNS or GNS ink solution was 2.0 mL after several times dipping-drying process. Therefore, the loading of IL-GNS onto the carbon paper was estimated to be 0.5 mg cm⁻².

ECs and MFCs construction and operation

ECs (50 ml volume) made of glass with conventional three electrode system were used. The carbon paper (CP), CP/GNS and CP/IL-GNS were used as working electrode (anode, 1cm × 1cm, projected area of 2 cm²), respectively. A platinum wire and a saturated calomel electrode (SCE) were used as counter and reference electrodes. *I-t* curves were measured with a CHI 660D workstation (Shanghai Chenhua, China), with anodes poised at 0.2 V (*vs.* SCE). A 50 mL M9 buffer solution containing microbial suspension with lactate (18 mM) as electron donor was used as an electrolyte solution after bubbling for 30 minutes with oxygen-free N₂.

H-shaped MFCs were constructed from two glass bottles joined by a 30-mm-diameter glass tube. The anode was the bare CP, the CP/GNS and the CP/IL-GNS electrode (1cm×2cm, 4 cm²) respectively, and the cathode was the CP electrode. The anode and the cathode were connected to a 1 k Ω external resistor circuit with titanium wire, and separated by a proton exchange membrane (PEM, Nafion 211, Dupon). The *Shewanella onesidensis* MR-1 cells harvested at late stationary phase were inoculated into the MFCs anode chamber containing 100 mL of M9 buffer solution with 5% LB broth and 18 mM lactate as the sole electron donor. The catholyte was a 50 mM

$K_3Fe(CN)_6$ solution with 100 mM phosphate buffer (PBS). *S. onosidensis* MR-1 cells were cultivated at 30°C in Luria-Bertani broth medium and harvested at late stationary phase by centrifugation (6000 r.p.m, 5min), washed three times with M9 buffer that was composed of 22 mM KH_2PO_4 , 42 mM Na_2HPO_4 , 85.5 mM NaCl, and 1.0 mM $MgSO_4$. The MFC was conducted at 25 °C under nitrogen atmosphere.

Electrochemical measurements

Electrochemical impedance spectroscopy (EIS) tests were performed with an Autolab PGSTAT12 (Eco chemie, BV, The Netherlands) in an anaerobic culture of *Shewanella* cell suspension solutions before and after ECs test, respectively. The frequency range is between 10^{-2} Hz and 10^5 Hz. Cyclic voltammetry (CV) analysis of CP, CP/GNS and CP/IL-GNS-based biofilm bioanodes from MFCs were performed in fresh mineral medium using a CHI 660D workstation with a conventional three electrode system comprised of a platinum counter electrode, a SCE reference electrode and the working electrode.

The polarization and power density curves were obtained by varying the external resistance (50–10,000 Ω) applied to the circuit after power output stabilization using a multimeter. Current was calculated using $I=E/R$, where I is the current, E the measured voltage, and R the applied resistance. Power were calculated using $P=I E$. The current and power densities calculations were then normalized to the surface area of the anode.

Scanning electron microscopy (SEM)

The morphology of the electrode surface was characterized with field-emission scanning electron microscopy (FE-SEM, HITACHI S4800). Small pieces of anodes from MFCs were fixed for 2 h in 2.5% glutaraldehyde, washed three times with M9 buffer solution, dehydrated in increasing concentrations of ethanol solution (25%, 50%, 75%, 85%, 95%, and 100%), and then vacuum dried. Samples were coated with Au prior to the SEM observation.

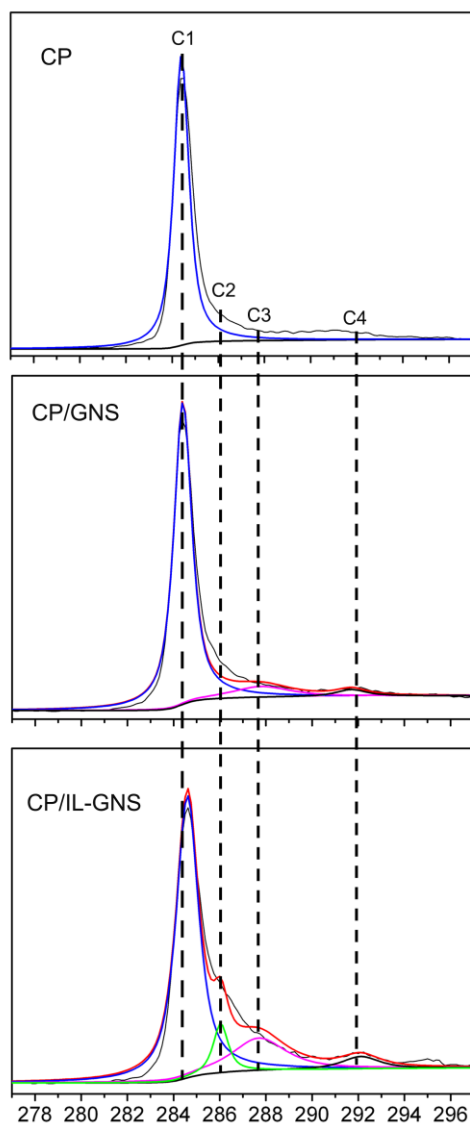


Fig S5 XPS C1s spectra of (a) CP, (b) CP/GNS and (c) CP/IL-GNS.

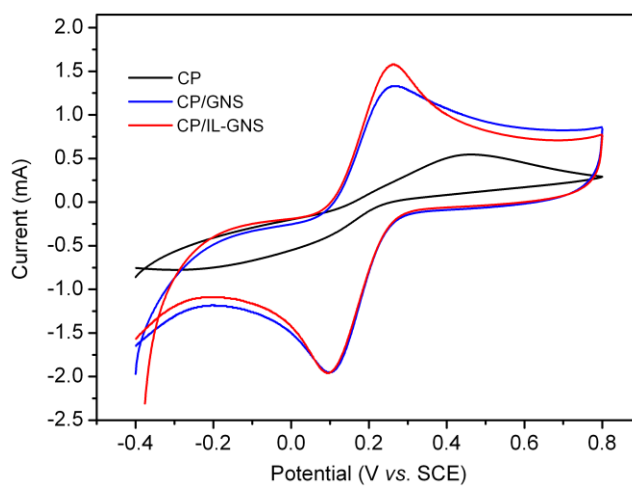


Fig. S6 Cyclic voltammograms of different electrodes in 5.0 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ and 0.1 M Na_2SO_4 .

In order to investigate the electrochemical active surface area of different electrode, CV was carried out using a ferrocyanide solution as the probe. As shown in Fig S6, the CP electrode displayed no obvious redox peaks, while the other two electrodes (CP/GNS and CP/IL-GNS) both exhibited a couple of well-defined redox peaks. In addition, the CP/GNS and CP/IL-GNS electrodes exhibited much higher peak current than that of the CP electrode. Based on Cottrell equation, it indicated that the active surface area of the CP/GNS and CP/IL-GNS was larger than that of the CP electrode.

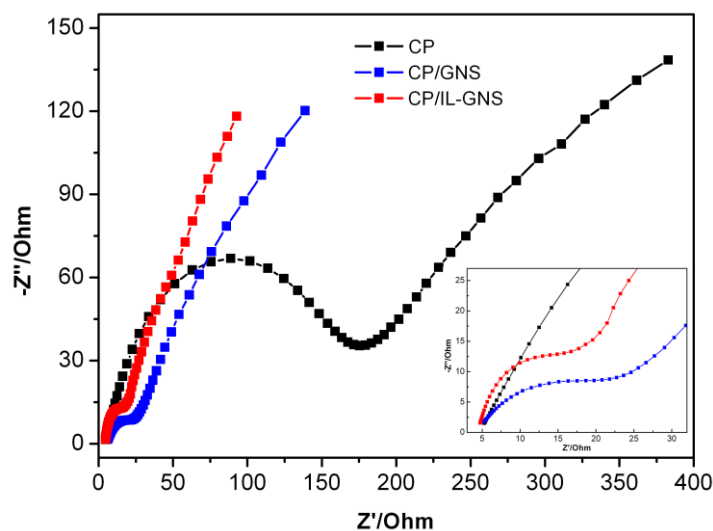


Fig .S7 EIS Nyquist plots of three different anodes after *Shewanella* biofilm formation. The inset illustrates the high-frequency part of the results.

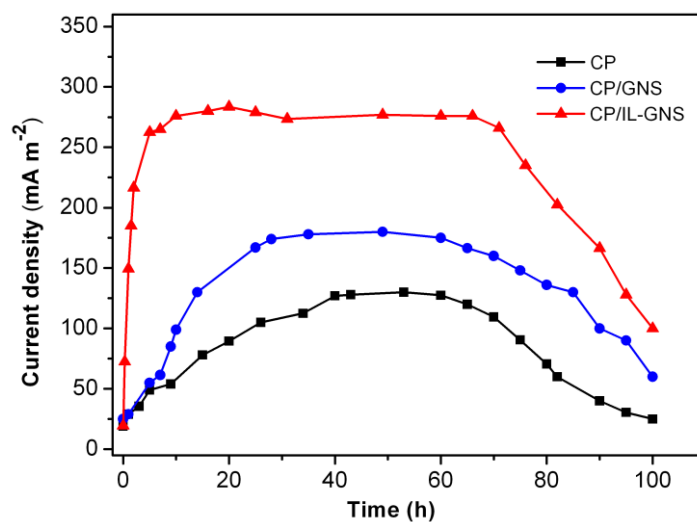


Fig .S8 Time courses of the current density in dual-chambered MFCs equipped with three different anodes with 1000 Ω loading.

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