# Supporting Information for

# Ultrahigh Electron-Donating Quaternary-N-Doped Reduced Graphene

### **Oxide@Carbon Nanotubes Framework: A Covalently Coupled Catalyst**

### **Support for Enzymatic Bioelectrodes**

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#### **Experimental Section**

*Materials.* Absolute ethyl alcohol (≥99.7%, AR grade), urea (≥99.0%, AR grade), boric acid (≥99.5%, AR grade), phosphoric acid (85%, AR grade), hydrogen peroxide (30%, AR grade), sulfuric acid (98%, AR grade), acetic acid (≥99.5%, AR grade) and hydrochloric acid (36.0%~38.0%, AR grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. Ni foam was purchased from Lizhiyuan Company. Before using, Ni foam was sequentially ultrasonicated in acetone, 3 M diluted hydrochloric acid and doubly distilled water for 30 min each to remove the impurities and then dried at 50 °C in vacuum oven. GO (2 mg mL<sup>-1</sup>, dispersion in water), GOx (~ 200 U mg<sup>-1</sup> solid, from Aspergillus niger), Lac ( $\geq$ 4.0 U mg<sup>-1</sup> solid, from *Pleurotus ostreatus*), Nafion 117 solution (5%, in a mixture of lower aliphatic alcohols and water) and Nafion 117 membrane were purchased from Alfa-Aesar. Before using, Nafion 117 membrane was sequentially heated in hydrogen peroxide (5%), doubly distilled water, 1 M sulfuric acid, doubly distilled water at 80 °C for 1 h each, and then immersed in doubly distilled water. GOx solution (10 mg mL<sup>-1</sup>) was prepared by dissolving the powder in 0.1 M phosphate buffered saline (PBS) buffer (pH 4.0). Lac solution (10 mg mL<sup>-1</sup>) was prepared by dissolving the powder in 0.04 M Britton-Robinson (B-R) buffer (pH 5.0). Amino-modified CNTs (>95%, 8~15 nm in diameter and ~50 µm in length) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 98%) were purchased from Aladdin Ind., Corp. Alpha-D-(+)-Glucose anhydrous (99+%) was purchased from Acros Organics. Doubly distilled water was used in all experiments and all the chemicals were used without further purification.

*Preparation of GO*. GO was prepared from graphite flakes (99.8%, Alfa Aesar) using a modified Hummers' method.<sup>[1]</sup> Typically, 1 g of graphite flakes were mixed with 25 ml concentrated H<sub>2</sub>SO<sub>4</sub> in a 250 mL round-bottom flask with ice-water bath. 3 g of KMnO<sub>4</sub> was then slowly added into the mixture and stirred for 2 h. The mixture was kept stirring at 35 °C for 30 min. Afterward, 100 mL of deionized water was slowly added into the mixture and vigorously stirred at 90 °C for 30 min. After that, H<sub>2</sub>O<sub>2</sub> aqueous solution (30 %) was dropwise added into the mixture until the color turned into bright yellow. The resulting mixture was centrifuged and washed thoroughly with 1 M HCl and deionized water. After the washing process, the resultant GO hydrogel (2 mg mL<sup>-1</sup>) was obtained.

*Preparation of N-rGO@CNTs network.* To obtain N-rGO@CNTs interlinked network with less aggregation, an electrostatic interaction was firstly utilized to prepared GO@CNTs precursor which

was then thermally treated with urea. It is well known that among the three different N configurations in N-doped carbon materials, N-Q was the most stable bond type. Therefore, the annealing temperature was very critical for the synthesis of ultrahigh N-Q-doped carbon material. Specific steps are as follows: 1 mL of GO hydrogel was dispersed in 50 mL of deionized water under ultrasonication for 30 min to obtain a uniform dispersion. 10 mg of amino-modified CNTs was then added in the GO suspension with ultrasonication for 30 min. The pH of the mixture was adjusted to 2~3 using 1 M HCl. The mixture was incubated at ambient temperature for several hours till the GO@CNTs precursor was completely precipitated from the solution. The hybrid was filtered under vacuum and dried at 50 °C for 12 h. To synthesize ultrahigh N-Q contained product, the obtained GO@CNTs precursor and urea with a mass ratio of 1:10 were heated at 1600 °C for 2 h in an Ar-filled tubular furnace (denoted as QN-rGO@CNTs). To investigate the influence of annealing temperature on the chemical and structural properties of the final products, the GO@CNTs and urea were annealed at various temperatures (800, 1000, 1200 and 1400 °C). Note that GO can be simultaneously reduced and doped with N during the thermal treatment. The corresponding products were denoted as N-rGO@CNTs-t (t represents the annealing temperature).

GO and amino-modified CNTs were annealed with urea at 1600 °C using a similar preparation process, respectively, the corresponding sample was denoted as N-rGO-1600 and CNTs-1600. As a comparison, commercial CNTs and graphene were mechanically mixed, and the hybrid was denoted as CNTs@GN.

*Preparation of working electrode.* Before modification, bare glassy carbon electrode (GCE, 3 mm in diameter) was polished, respectively, with alumina slurries (1.0, 0.3 and 0.05  $\mu$ m) on a polishing cloth and ultrasonicated successively in doubly distilled water, 1 M H<sub>2</sub>SO<sub>4</sub>, doubly distilled water and absolute ethyl alcohol for 3 min, separately, and then dried at room temperature. The working electrodes were prepared using a simple drop cast method. Typically, 5  $\mu$ L of 5 mg mL<sup>-1</sup> N-rGO@CNTs-t suspension (dispersed in doubly distilled water) was casted on the surface of the pretreated GCE and dried in air, and the electrodes were denoted as N-rGO@CNTs-t/GCE. Then, 5  $\mu$ L of GOx solution was casted on the surface of N-rGO@CNTs-t/GCE and the electrode was stored in refrigerator at 4 °C until the water evaporated completely (denoted as GOx/N-rGO@CNTs-t/GCE). Finally, 5  $\mu$ L of diluted Nafion solution (1%) was dropped on the surface of GOx/N-rGO@CNTs-t/GCE).

t/GCE and the electrode was stored in refrigerator at 4 °C for 1 h (denoted as GOx/N-rGO@CNTst/GCE for brevity in the main text). For control experiments, GOx/CNTs-1600/GCE were prepared using the similar procedure.

Preparation of bioanode and biocathode. The pretreated Ni foam was compressed at 5 MPa to form a sheet (0.25 mm in thickness) and was served as current collector for the fabrication of bioanode and biocathode. To prepare bioanode, 50 µL of QN-rGO@CNTs suspension was casted onto the surface of Ni foam and dried in air (denoted as QN-rGO@CNTs/Ni foam). Then, 20 µL of GOx solution was coasted on N-rGO@CNTs-1600/Ni foam surface and the electrode was stored in refrigerator at 4 °C until the water evaporated completely (denoted as GOx/QN-rGO@CNTs/Ni foam). Finally, 50 µL of Nafion solution was dropped on GOx/QN-rGO@CNTs/Ni foam surface and the electrode was stored in refrigerator at 4 °C for 1 h (denoted as Nafion/GOx/QN-rGO@CNTs/Ni foam). The amount of GOx immobilized on the Ni foam was ~1 mg cm<sup>-2</sup>. The fabrication procedure of biocathode was similar to bioanode. Typically, 50 µL of QN-rGO@CNTs suspension was casted onto the surface of Ni foam and dried in air (denoted as QN-rGO@CNTs/Ni foam). Then, 100 µL of Lac solution was coasted on QN-rGO@CNTs/Ni foam surface and the electrode was stored in refrigerator at 4 °C until the water evaporated completely (denoted as Lac/QN-rGO@CNTs/Ni foam). Finally, 50 µL of Nafion solution was dropped on Lac/QN-rGO@CNTs/Ni foam surface and the electrode was stored in refrigerator at 4 °C for 1 h (denoted as Nafion/Lac/QN-rGO@CNTs/Ni foam). The amount of Lac immobilized on the Ni foam was  $\sim 5 \text{ mg cm}^{-2}$ .

*Electrochemical test of working electrode.* The electrochemical measurements were performed on a CHI 660E electrochemical workstation in a three-electrode system that uses a GOx/N-rGO@CNTs-t modified GCE as the working electrode, an Ag/AgCl in 3 M KCl as the reference electrode and a Pt foil (surface area: 1 cm<sup>2</sup>) as the counter electrode. The electrolyte was 0.1 M PBS (pH 7.2) and saturated with high purity Ar gas for at least 20 min before using in the cells. All experiments were operated at ambient temperature.

*Glucose/O*<sub>2</sub> *biofuel cell design and test.* GBFCs were assembled using Nafion/GOx/QN-rGO@CNTs/Ni foam as bioanode and Nafion/Lac/QN-rGO@CNTs/Ni foam as biocathode in a two-chamber separated by a Nafion 117 membrane. Ar-saturated 0.1 M PBS (pH 5.0) containing 100 mM glucose was used as anolyte in anode chamber, and O<sub>2</sub>-saturated 0.04 M B-R (pH 5.0) containing 0.5

mM ABTS was used as catholyte in cathode chamber.

The open circuit potential (OCP) and stability of the as-assembled GBFCs were measured using CHI 660E electrochemical workstation. At steady state OCP, an external resistance varying from 10  $\Omega \sim 100 \text{ k}\Omega$  was connected in series between the anode and cathode of the GBFC, the potential and current were recorded using two digital multimeters to determine the polarization and power density output. The operation of the GBFC was at ambient temperature.

*Characterization Techniques.* X-ray diffraction (XRD) was performed on a PANalytical X'pert Pro X-ray diffractometer. Raman spectra were recorded on a WITecCRM200 instrument using a 532-nm laser. Field emission scanning electron microscopy (FESEM) was operated on a JSM-6701 field emission SEM instrument at an accelerating voltage of 10 kV. Transmission electron microscopy (TEM) was carried out on a JEOL JEM-2100 microscope, which was operated at an acceleration voltage of 200 kV. X-ray photoelectron spectroscopy (XPS) analysis was conducted on a Thermo Scientific Escalab 250 spectrometer using an Al Kα X-ray source. N<sub>2</sub> sorption measurements were performed at 77 K on a Micromeritics ASAP 2020 HD analyzer. Raman spectra were performed on a WITecCRM200 instrument using a 532-nm laser. Fourier Transform Infrared Spectroscopy (FT-IR) was recorded on a Spectrum 100 FT-IR. Atomic force microscopy (AFM) was performed on Bioscope Catalyst.

*Computational Methods and Models.* We performed the density functional theory (DFT) in the generalized gradient approximation (GGA) with Perdew-Burke-Ernzerhof (PBE) functional for exchange and correlation effects of the electrons, as implemented in the DMol<sup>3</sup> package.<sup>[2,3]</sup> For the best accuracy, we adopted a double numerical basis set plus a d-type polarization function (DNP) to expand wave functions. The Brillouin-zone integration was sampled by  $5\times5\times1$  special k-points for atomic structure relaxation and a total of  $15\times15\times1$  k-points for electronic structure and diffusion performance, respectively, generated by the Monkhorst-Pack scheme.<sup>[4,5]</sup> The global orbital cutoff energy for each element was set to 5.1 Å. Geometry optimization was carried out before the single point energy calculations with the self-consistent-field (SCF) tolerance of  $1.0\times10^{-6}$  Ha, the maximum force of 0.002 Ha·Å<sup>-1</sup> and the maximum displacement of 0.005 Å. The Ab Initio Molecular Dynamic (AIMD) Simulation was performed using the thermodynamic ensemble with fixed volume and constant energy. AIMD simulations are performed at 300 and 1873 K with a time step of 1 fs for 3 ps

time steps. Graphene and N-doped graphene in this research were modeled with a  $3\times3$  supercell for both electrical properties and AIMD calculations. Periodic boundary conditions in the x-y plane were applied to simulate the two-dimensional phosphorus infinite monolayer throughout the study. A vacuum space of 30 Å in the perpendicular direction to the layers was applied to eliminate the spurious interactions between adjacent layers, and simultaneously ensure the convergence of the total energy.



Fig. S1 (A) SEM and (C) TEM images of N-rGO-1600. (B) SEM and (D) TEM image of CNTs-1600.



Fig. S2 3D AFM image of QN-rGO@CNTs.



Fig. S3 FT-IR spectrum of QN-rGO@CNTs.



Fig. S4 (A) XRD patterns and (B) Raman spectra of N-rGO@CNTs-t samples.



**Fig. S5** High-resolution XPS spectra of C 1s for N-rGO@CNTs-t samples, in which C1, C2, C3, and C4 represent C=C, C=O, C-O or C-N, and C-C=O, respectively.



Fig. S6 High-resolution XPS spectra of O 1s for N-rGO@CNTs-t samples.



**Fig. S7** The corresponding structural information (A-D), the energy band diagram (E-H), and the PDOS (I-L) of pristine graphene and the three kinds of N-doped graphene. The Fermi level was set to zero and denoted by the red dashed line.



**Fig. S8** Molecular dynamics simulation analysis for N-5-doped graphene (A), N-6-doped graphene (B), N-Q-doped graphene (C). The stability can be evaluated using the standard deviation (SD). The smaller the SD is, the more stable the structure is.



Fig. S9 CV curves of (A) GOx/CNTs-1600/GCE, (B) GOx/CNTs@GN/GCE, and (C) GOx/N-rGO/GCE in Ar-saturated 0.1 M PBS (pH = 7.2) at 50 mV s<sup>-1</sup>.



**Fig. S10** (A) CV curves of bare GCE and QN-rGO@CNTs/GCE in 5 mM [Fe(CN)<sub>6</sub>]<sup>3-/4</sup> containing 0.1 M KCl at a scan rate of 50 mV s<sup>-1</sup>. B) Nyquist plots of bare GCE and QN-rGO@CNTs/GCE in 5 mM [Fe(CN)<sub>6</sub>]<sup>3-/4</sup> containing 0.1 M KCl.



**Fig. S11** (A) CV curves of GOx/N-rGO@CNTs-t/GCE in Ar-saturated 0.1 M PBS (pH = 7.2) at 50 mV s<sup>-1</sup>. (B) Catalytic current *vs*. temperature. (C) Peak potential separation *vs*. temperature. (D) Formal potential *vs*. temperature. Error bar represents standard deviation of three parallel experiments.



Fig. S12 Peak potential separation ( $\Delta E_p$ ) vs. scan rate for GOx/CNTs-1600/GCE and GOx/QN-rGO@CNTs/GCE.



**Fig. S13** (A) CV curves of GOx/QN-rGO@CNTs/GCE in Ar-saturated 0.1 M PBS (pH 7.2) at 50 mV s<sup>-1</sup> for 100 cycles. (B) CV curves of GOx/QN-rGO@CNTs/GCE in Ar-saturated 0.1 M PBS (pH 7.2) at 50 mV s<sup>-1</sup> for 10 days measured by once a day. Inset shows cathodic current retention  $(I_p/I_{p1})$  for 10 days.



Fig. S14 (A) CV curves of five GOx/QN-rGO@CNTs/GCE electrodes in Ar-saturated 0.1 M PBS (pH

7.2) at a scan rate of 50 mV s<sup>-1</sup>.



**Fig. S15** (A) LSV curves of glucose oxidation obtained at the GOx/QN-rGO@CNTs/GCE bioanode in Ar-saturated 0.1 M PBS (pH 7.2) containing 0, 10 and 20 mM glucose. Scan rate is 10 mV s<sup>-1</sup>. (B) CV curves of Lac/GCE (a) and Lac/QN-rGO@CNTs/GCE (b) in Ar-saturated B-R buffer without ABTS. (c) CV curve of Lac/QN-rGO@CNTs/GCE in Ar-saturated B-R buffer with 0.5 mM ABTS. (d) CV curve of Lac/QN-rGO@CNTs/GCE in O<sub>2</sub>-saturated B-R buffer with 0.5 mM ABTS.

#### Equations

$$E_{pc} = E^{'0} - \left[\frac{RT}{\alpha nF}\right] In \left[\frac{\alpha Fn}{RTk_s}v\right]$$
(S1)

$$E_{pa} = E^{'0} + \left[\frac{RT}{(1-\alpha)nF}\right] In \left[\frac{(1-\alpha)Fn}{RTk_s}v\right]$$
(S2)

where  $E^{0}$  is the formal potential (given by the average of anodic and cathodic peak potentials),  $\alpha$  is the charge transfer coefficient of the system, v is the scan rate, n is the electron transfer number in the reaction,  $k_s$  is the heterogeneous electron transfer rate constant, T, R, and F are temperature, the ideal gas constant and Faraday's constant, respectively.

$$Logk_{s} = \alpha Log(1 - \alpha) + (1 - \alpha)Log\alpha - Log\left(\frac{RT}{nFv}\right) - \alpha(1 - \alpha)\left(\frac{nF\Delta E_{p}}{2.3RT}\right)$$
(S3)  
$$i_{pc} = \frac{n^{2}F^{2}A\Gamma^{*}}{4RT}v$$
(S4)

Where  $i_{pc}$  is the cathodic peak current, v is the scan rate, n is the number of electron transfer,  $\Gamma^*$  is the surface coverage of enzyme, T, R, and F are temperature, the ideal gas constant and Faraday's constant, respectively

Samples	SSA $(m^2 g^{-1})$	Pore volume (cm <sup>3</sup> g <sup>-1</sup> )
N-rGO-1600	63.30	0.85
CNTs-1600	165.25	0.80
QN-rGO@CNTs	154.80	1.30

**Table S1** Comparison of SSA and pore volume of N-rGO-1600, CNTs-1600 and QN-rGO@CNTs.

Anode	Cathode	OCP	P <sub>max</sub>	Ref.
		(V)	(mW cm <sup>-2</sup> )	
GOx/OMCs/GCE	Pt	1.20	0.110	6
GOx/3D-GNs/Fc/GCE	Lac/3D-GNs-PTCA-	0.38	0.112	7
	DA/GCE			/ 
GOx/C-MEMS	Lac/C-MEMS	0.91	0.136	8
GOx/GN/SWCNTs	BOD/ GN/SWCNTs	0.61	0.190	9
aerogel	aerogel			
GOx/CP/LPEI-C <sub>6</sub> /Fc	Lac/CP/MWCNTs/TBA	0.55	0.024	10
	В			
	BOD/Ag			
GOH/Ag	NPs/GN/nanoporous	0.61	0.143	11
NPS/GN/GEA/Au	PtNi			
GOx/Pd aerogel/β-	DOD/Dd Dt aaragal	0.40	0.020	10
cyclodextrin/Fc		0.40	0.020	12
GOx/PANI1600@GO/GC		0.76	0 756	12
Е	PI-C/GCE	0.70	0./30	15
GOx/PANI1600@GO/CP	Lac/PANI <sub>1600</sub> @GO/CP	0.78	1.120	14
GOx/PCP/GCE	Pt-C/GCE	0.68	0.548	15
GOx/CNCs/GCE	Pt	0.59	0.055	16
GDH/SWNHs/CFME	BOD/SWNHs/CFME	0.72	0.140	17
GOx/QN-	Lac/QN-	0.89	0.900	this
rGO@CNTs/Ni foam	rGO@CNTs/Ni foam			work

**Table S2** Performance of 3D structures as bioelectrodes applied in EBFC application.

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