Electronic Supplementary Material (ESI) for Analyst.

Nitrogen-doped graphene/gold nanoparticles/formate dehydrogenase bioanode for high power output membrane-less formic acid/O_2 biofuel cell

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Section 1 Experimental

Regeats. Formate dehydrogenase (FDH) from *Candida boidinii* (EC 1.2.1.2, 15 U/mg solid), laccase from *Trametes versicolor* (EC 1.10.3.2, > 20 units mg^{-1}), poly (diallyldimethylammonium chloride) (PDDA, 20%, w/w in water, MW=200 000-350 000) and 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich. Both of the enzymes were used as received without further purification. Dihydronicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide (NAD^+) were received from Nanjing Aoduofuni Biological Technology Co. Ltd. (Nanjing, China). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and formic acid were obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). 0.1 M pH=6.0 phosphate buffer solutions (PBS) consisting of Na_2HPO_4 and NaH_2PO_4 were employed as the supporting electrolyte. Graphene (G) was synthesized according to a previously published method.[1] AuNPs were prepared according to the literature by adding a sodium citrate solution to a boiling $HAuCl_4$ solution.[2] All the other reagents were of analytical grade and used without further purification. Ultrapure fresh water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used throughout the whole experiment.

Apparatus. The field emission scanning electron microscopy (FESEM) images were obtained with a Hitachi S4800 scanning electron microscope. Transmission electron micrographs (TEM) were measured on a JEOLJEM 200CX transmission electron microscope using an accelerating voltage of 200 kV. X-ray photoelectron spectroscopy (XPS, KR) analysis was carried out on a Thermo Fisher X-ray photoelectron spectrometer system. Electrochemical impedance spectroscopy (EIS) was performed with an Autolab electrochemical analyzer (Eco Chemie, The Netherlands) in 2.5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ aqueous solution with 0.5 M KNO_3 as the supporting electrolyte, within the frequency range of 0.01 Hz to 100 kHz. Electrochemical measurements were performed on a CHI 660B workstation (Shanghai Chenhua Apparatus Corporation, China). The three-electrode system were composed of a platinum wire as the auxiliary, a saturated calomel electrode (SCE) as the reference, and a NG/AuNPs/FDH, G/AuNPs/FDH and NG/AuNPs/laccase modified Au sheets as the working electrode, respectively. The open circuit potential of the electrodes were tested with a two-electrode configuration (SCE as the reference electrode). After a stable E^{ocp} was observed, the variable external load ranged from 100 Ω to 100 kΩ was connected in series between anode and cathode. Then the power outputs were obtained with a precision digital multimeter.

Synthesis of the NG. The NG was synthesis according to a previously published procedure[3] with some modification: 100 mg of GO solids were put in a quartz boat,

and then placed it into the center of a tube furnace. The furnace was heated up to 500ºC after a mixed gas of NH_3 (20 sccm) and Ar (180 sccm) passed through the furnace for 30 min then cooled down to room temperature at a constant flow rate of 200 sccm (Ar + NH_3).

Preparation of NG/AuNPs and G/AuNPs. 5.0 mg of NG was dispersed in 1 % PDDA salt solution (0.02 M NaCl, 5.0 mL), which was introduced to sonicate for 30 min. Residual PDDA polymer was removed by centrifugation (15000 rpm, 10 min), and the obtained precipitate was washed with water for at least three times to form a homogeneous suspension of positively charged NG/PDDA. Subsequently, the purified NG/PDDA was dispersed in the prepared negatively charged AuNPs solution, and stirred at room temperature for 2 h. After that excessive AuNPs were removed by centrifugation (8000 rpm, 10 min) and the NG/AuNPs hybrid precipitate was recovered and dissolved with water to a concentration of 1 mg/mL. G/AuNPs was also prepared with the similar method except that G was used as the support instead of NG.

Preparation of bioanode and biocathode

The Au substrates (1 cm × 0.5 cm) were provided by the 55th Institute of China Electronic Group (Nanjing, China). The Au substrates were prepared by sputtering 200 nm Au onto the quartz wafers with a few nanometers of Ti adhesion layer in vacuum.[4] Before using, the Au substrates were carefully scraped to a mirror finish by pledget, and then they were rinsed and sonicated by ethanol and ultrapure water, respectively, and dried under nitrogen flow.

The NG/AuNPs/FDH bioanode and NG/AuNPs/laccase biocathode of the formic acid EBFC were fabricated as follows, 50 μL of as-prepared NG/AuNPs suspension was dropped on the surface of the pretreated Au substrate electrode. Then, the NG/AuNPs hybrid electrode was dried at 37°C for 2 h and immersed in a solution containing 1 mg/mL 1-ethyl-3-(3-dimethyl-aminopropyl) carbodimiide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) for 30 min to activate the carboxyl group. After rinsing with ultrapure water to eliminate excess EDC and NHS, the activated electrodes were immersed in 50 μL of FDH (20 mg/mL, dissolved in ice water) solution for 24 h at 4°C. Fourier transform infrared spectroscopy (FT-IR) was used to ascertain FDH was bound to the AuNPs by means of a condensation reaction between terminal amino groups on lysine residues of FDH and carboxyl groups on the AuNPs (Fig. S1). The NG/AuNPs/laccase biocathode of the EBFC was fabricated similar to the bioanode, in which 50 μL of laccase solution (60 mg mL^{-1}, dissolved in 0.05 M pH 7.0 PBS solution) was instead of FDH. Before the fabrication of the EBFC, both the prepared NG/AuNPs/FDH bioanode and NG/AuNPs/laccase biocathode were purged with ultrapure water to wipe off unbound enzymes.

Biofuel cell assembly.

We constructed a membrane-less EBFC in which the bioanode and biocathode were prepared according to the above method. The supporting electrolyte was oxygen-saturated 0.1 M PBS (pH=6.0) containing 50 mM of formic acid, 5 mM of NAD^+ and 0.5 mM of ABTS.

Section 2 FT-IR spectra of the NG/AuNPs, NG/AuNPs/FDH and NG/AuNPs/laccase

FT–IR was utilized to further confirm FDH and laccase were bound to the AuNPs through condensation reaction. Fig. S1 shows the typical FT–IR spectra obtained for the NG/AuNPs, NG/AuNPs/FDH and NG/AuNPs/laccase. As for the NG/AuNPs, the peak at 3440 cm^{-1}, 1392 cm^{-1} and 1725 cm^{-1} were ascribed to the characteristic stretching vibration and deformation of O–H group and C=O stretching vibrations of carboxyl group, respectively, which indicated that the surface of the NG/AuNPs was functionalized with carboxyl groups. In the case of NG/AuNPs/FDH and NG/AuNPs/laccase, the spectra revealed the characteristic absorption bands of amide I (1640 cm^{-1}) and amide II (1530 cm^{-1}). The formation of amide indicated the enzymes were covalently bound to AuNPs through a condensation reaction between terminal amino groups on lysine residues of enzyme and carboxyl groups on the AuNPs.

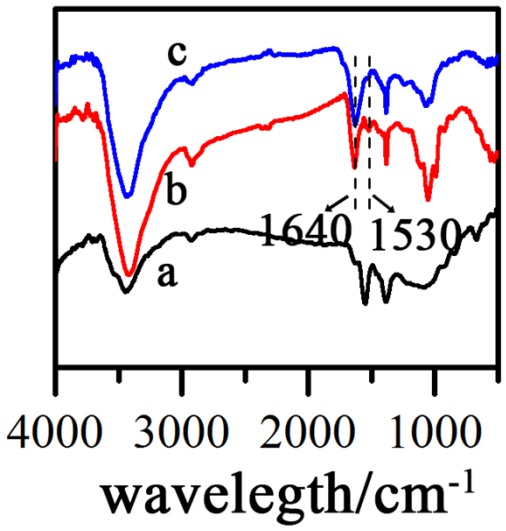

Fig. S1 FT-IR spectra of the NG/AuNPs (a), NG/AuNPs/FDH (b) and NG/AuNPs/laccase (c).

Section 3 Control experiments at different electrode.

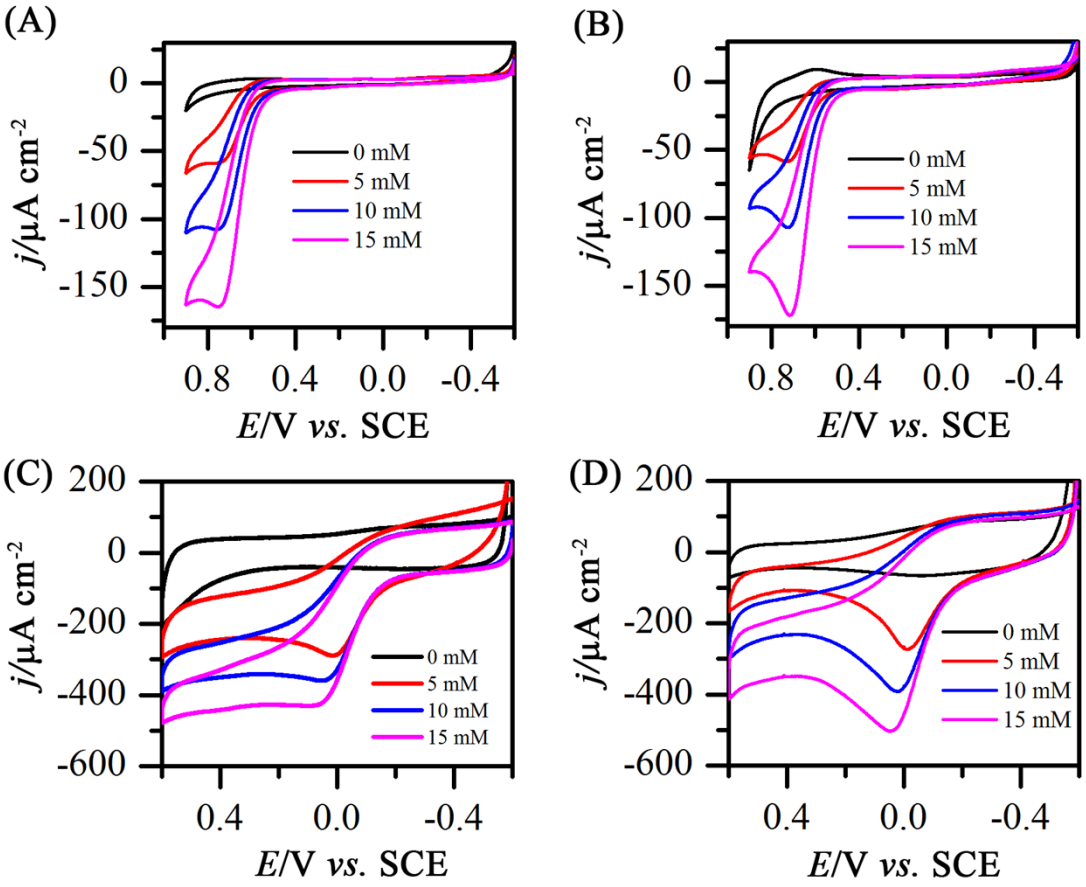

Fig. S2 CVs of NADH with different concentrations at the bare electrode (A), AuNPs-modified electrode (B), NG-modified electrode (C) and NG/AuNPs-modified electrode (D) in 0.1 M PBS (pH=6.0). Scan rate: 5.0 mV s^{-1}.

Section 4 CV of the formic acid oxidation

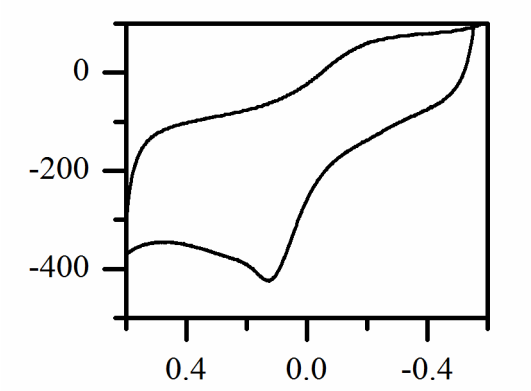

Fig. S3 CV of the NG/AuNPs/FDH electrode in 0.1 M PBS (pH=6.0) containing 50 mM formic acid with 5 mM NAD^+. υ= 5 mV s^{-1}.

Section 5 Polarization curves of the NG/AuNPs/FDH electrode and G/AuNPs/FDH electrode

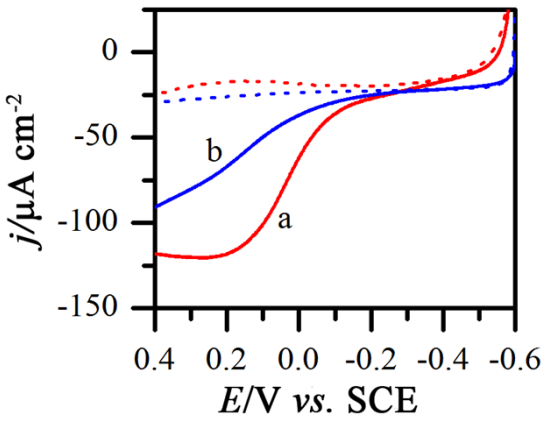

Fig. S4 Polarization curves of the NG/AuNPs/FDH electrode (a) and G/AuNPs/FDH electrode (b) in 0.1 M PBS (pH=6.0) containing 50 mM formic acid. (Dotted and solid curves denote measurements obtained without and with both 5 mM NADH and 5 mM NAD^+, respectively). υ= 1 mV s^{-1}.

Section 6 Crossover between the bioanode and biocathode

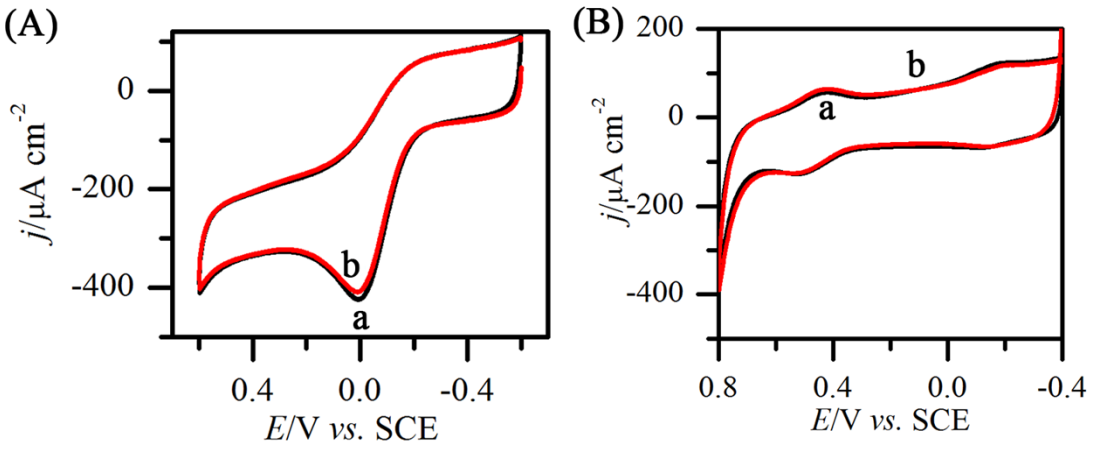

Fig. S5 (A) CVs recorded at the NG/AuNPs/FDH bioanode in 0.10 M PBS (pH=6.0) containing 5 mM NAD^+, 5 mM NADH and 50 mM formic acid saturated with N_2 (curve a) or O_2 (curve b). (B) CVs recorded at the NG/AuNPs/laccase biocathode in 0.10 M N_2-saturated PBS in the absence (curve a) and presence (curve b) of 50 mM formic acid. υ=5 mV s^{-1}.

Section 7 Dependence of power density on the formic acid concentration for EBFC

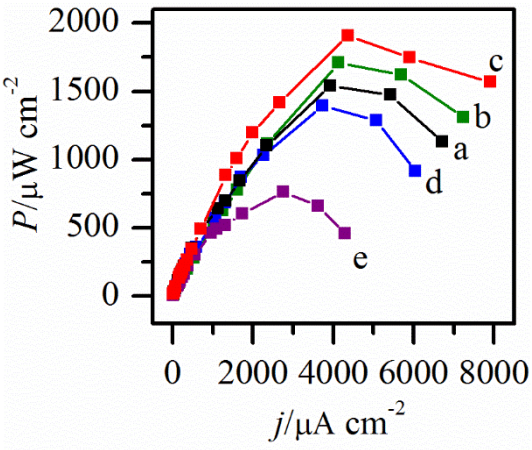

Fig. S6 Dependence of power density on the formic acid concentration for EBFC. a, 5 mM, b, 25 mM, c, 50 mM, d, 75 mM, e, 100 mM.

Section 8 The blank and control experiments of formic acid EBFC

Under the optimal conditions and in the absence of formic acid or O_2 (Fig. S6A and Fig. S6B), the blank experimental results showed that the maximal power output of the EBFC was only 123.08 ± 3.23 μW cm^{-2} or 126.56 ± 2.72 μW cm^{-2}, respectively; In the absence of FDH in bioanode or laccase in biocathode (Fig. S6C and Fig. S6D), the control experimental results displayed that the maximal power output of the EBFC was only 309.25 ± 10.65 μW cm^{-2} or 322.58 ± 6.13 μW cm^{-2}, respectively.

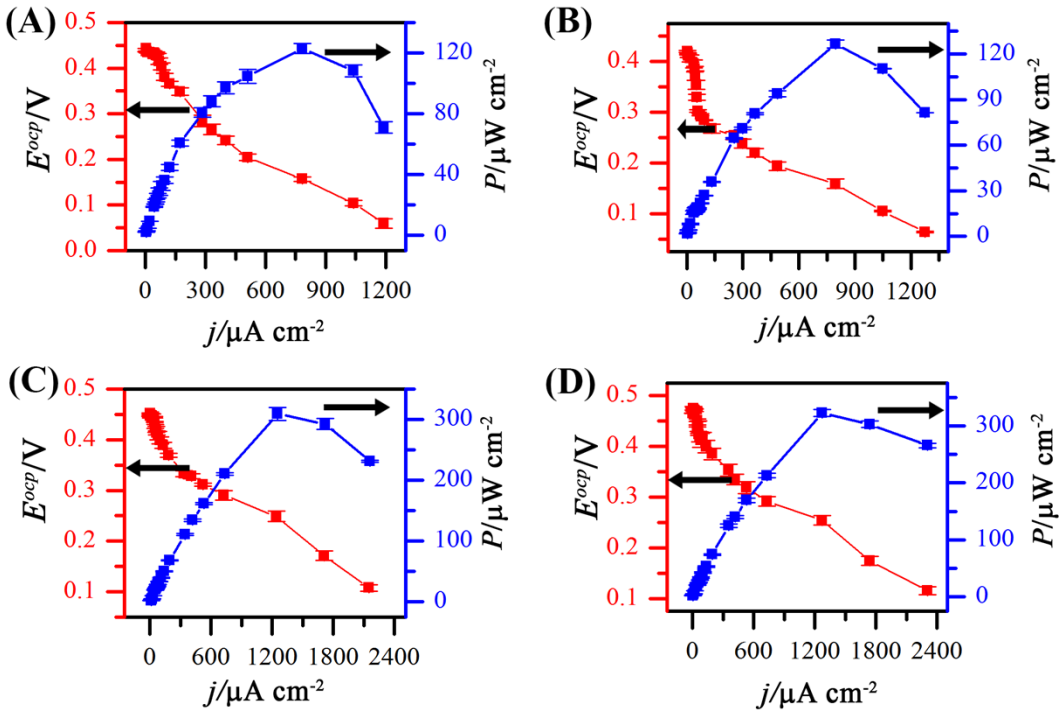

Fig. S7 Polarization curve and power density curve of the EBFC under the optimal conditions, (A) without formic acid and (B) without O_2 in EBFC, and (C) without FDH in bioanode and (D) without laccase in the biocathode. The supporting electrolyte was oxygen-saturated 0.1 M PBS (pH=6.0) containing 50 mM of formic acid, 5 mM of NAD^+ and 0.5 mM of ABTS. Every point corresponds to the average value of three independent measurements.

Section 9 References

1. D. Li, M. B. Muller, S. Gilje, R. B. Kaner and G. G. Wallace, *Nat Nano*, 2008, **3**, 101.
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