Supporting Information

Electron Hopping by Interfacing Semiconducting Graphdiyne Nanosheets and Redox Molecules for Selective Electrocatalysis

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Supplementary methods:

Reagents and materials. Sodium ascorbate (AA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), uric acid (UA), 5-hydroxytryotamine (5-HT), dihydronicotiamide adenine dinucleotide (NADH), nicotinamide adenine (NAD⁺), glucose, and glucose dehydrogenase (GDH, EC 1.1.1.47, from Pseudomonas sp.) were all purchased from Sigma and used as supplied. Methylene green (MG) was purchased from Beijing Chemical Company (Beijing, China). Multi-walled carbon nanotubes (MWNTs, < 2 nm in diameter and $0.5 - 50 \mu$ m in length) were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). Potassium phosphate buffer (0.5 mM, pH 7.4) containing 126 mM NaCl was used as the electrolyte for electrochemical experiments.

GDY preparation. Bulk GDY nanosheets were synthesized by using hexaethynylbenzene as a precursor and copper foil as both the catalyst and substrate according to previously published method¹. Briefly, hexaethynylbenzene monomer was synthesized by adding tetrabutylammonium fluoride (TBAF) into a THF solution of hexakis-[(trimethylsilyl)ethynyl]benzene and stirring the mixture for 10 min at 8 °C. GDY was grown on the surface of copper foil by the cross-coupling reaction of hexaethynylbenzene monomer under nitrogen atmosphere at 60 °C for 72 h in the presence of pyridine. To get the GDY powder, the black film grown on the copper foils was washed with acetone, peeled off by sonication in hot DMF (80 °C) for 1 h and refluxed at 100 °C successively in NaOH (4 M), HCI (6 M) and NaOH (4 M), each for 2 h to remove the residual copper and other impurities. The product was collected by centrifugation, washed with hot DMF (80 °C) and hot ethanol (70 °C), and finally let drying in the vacuum oven overnight to give the powder of GDY nanosheets. Structural characterization of GDY nanosheets by Raman spectroscopy and electron microscopy has been published in our previous work². Single-layered and few-layered GDY nanosheets (eGDY) were prepared by exfoliating bulk GDY nanosheets with Li₂SiF₆ according to our lately published method³ and kept isopropanol before MG modification.

MG/GDY preparation. GDY nanosheets was dispersed into an aqueous solution of 1 mM MG to the final concentration of 1 mg⋅mL⁻¹. The suspension was agitated with an ultrasonication probe for 5 h before centrifugation at 1500 rpm for 5 min. Precipitates were washed with deionized water to remove free MG and then dried under ambient atmosphere to get MG/GDY composites. Multiple washes were conducted until the UV-Vis absorbance of supernatant did not change. Unbound MG in the supernatant was collected for estimating MG loading in GDY nanosheets.

MG/eGDY preparation. MG powder was dissolved in the suspension of eGDY (1 mg·mL⁻¹) to 1 mM. The mixture was continuously stirred at room temperature for 5 h and then collected and washed by ultracentrifugation at 20000 rpm. This process also restacked MG-decorated eGDY into bulk nanosheets. The resultant precipitates were dried under ambient atmosphere to get MG/eGDY composites.

MG-GDY preparation. A suspension of MG nanosheets $(1 \text{ mg} \cdot \text{mL}^{-1})$ and MG (1 mM) was continuously stirred at room temperature for 5 h before centrifugation at 1500 rpm for 5 min. Precipitates were washed with deionized water by centrifugation multiple times to remove free MG and then dried under ambient atmosphere to get MG-GDY composites.

GDH-MG/GDY preparation. The GDH-MG/GDY nanosheets were obtained by mixing equal volumes of GDH solution ($10 \text{ mg} \cdot \text{mg}^{-1}$) and the MG/GDY dispersion ($2 \text{ mg} \cdot \text{mg}^{-1}$) on vortex for 5 min. After centrifugation at 1000 rpm for 5 min, the composites were dried and stored at 4 °C before experiments.

Contact angle measurement. A drop of GDY or CNT dispersion in deionized water was deposited and dried on a glass slide. Surface wettability was examined by a contact angle measuring analyzer (JC2000D, Powereach, China) at five different points at ambient temperature.

FT-IR spectroscopy. MG/GDY composites were dried in a vacuum oven overnight and pressed into a KBr pellet by a pelletizer for transmittance spectra recording on a Bruker Tensor-27 FT-IR spectrometer.

UV-Vis spectroscopy. MG/GDY or MG-GDY composites were dispersed in deionized water in 1 cm quartz cuvette for UV-vis spectroscopic measurements with a TU-1900 spectrometer. MG loading in GDY was estimated by comparing the absorbance decrease at 650 nm of free MG before and after modification, which was about 8.70×10⁻⁷ mole of MG monomers per 1 mg of GDY.

X-ray photoelectron spectroscopy. Aqueous dispersions of GDY or CNTs were spin-coated on silicon wafers cleaned by piranha solution and then measured by an ESCALab220i-XL electron spectrometer (VG Scientific) under excitation by 300W Al Kα radiation.

Electrochemistry. GCEs (diameter is 3 mm) were polished with aqueous slurries of fine alumina powders (0.3 and 0.05 μ m) on a polishing cloth and washed with deionized water in an ultrasonic bath for 3 min before surface modification. MG/GDY, MG/eGDY, MG-GDY or GDH-MG/GDY nanosheets were re-dispersed in deionized water and sonicated to form a homogeneous suspension (1 mg·mg⁻¹), 5 μ L of which was coated onto each GCE by drop-casting and dried under a hot incandescent lamp (for non-enzymatic electrodes) or at ambient atmosphere (for enzymatic electrodes). Cyclic voltammetry was performed with a CHI660E potentiostat in a three-electrode electrolytic cell using bare or GCE as the working electrode, platinum wire as the counter electrode and Ag/AgCl (KClsaturated) as the reference electrode.

Assuming charge transport by electron hopping in MG/GDY as an infinite diffusion process, we estimated the corresponding apparent diffusion coefficient for charge transport (D_{CT}) to by the Randles-Sevcik equation (1)

$$i_{\rm p} = 0.4463 n FAc \left(\frac{n F v D_{\rm CT}}{RT}\right)^{1/2} \tag{1}$$

where *n* is the number of electrons transferred in half-reaction for the redox couple (n = 2 for MG), *F* is the Faraday's constant (96485 C·mol⁻¹), *A* is the electroactive area of GCE (0.071 cm²), *v* is the scan rate (V·s⁻¹), *R* is the universal gas constant (8.314 J·mol⁻¹·K⁻¹), *T* is the absolute temperature (298 K) and *c* is the volume concentration of immobilized MG estimated by UV-Vis absorbance measurement (6.13×10^{-5} mol·cm⁻³).

For electrocatalytic oxidation of AA by electron tunneling with MG/GDY, the rate-determining step was the first proton-coupled electron transfer reaction. The rate constant (k_1^*) for the rate-determining step was then calculated by equation (2)

$$j_{o} = Fk_{1}^{*}C_{R}^{*}exp[(1-\alpha)F\Delta E_{1}/RT]$$
⁽²⁾

where $C_{\rm R}^*$ is the bulk concentration of AA (1 mM in reduced form), α is the transfer coefficient (0.5) and $\Delta E_1 = E_{\rm eq} - E_1^{0'}$ (formal potential for the first electron-transfer step is 0.06 V vs. NHE)⁴. The apparent rate constant for the overall reaction was determined by equation (3)⁵:

$$k^{0'} = k_1^* exp(0.5F\Delta E_1/RT)$$
(3)

Flow cell test. Split-disk plastic carbon film electrode (SPCF, 3 mm diameter for each disk electrode) was modified with the GDH-MG/GDY nanosheets in the same way for GCE modification and used as the working electrode in an electrochemical flow cell consisting of a thin-layer radial flow block, a stainless steel as the counter electrode and Ag/AgCl (KCl-saturated) as the reference electrode, connected to a CHI potentiostat. NAD⁺ (5 mM) in phosphate buffer (pH 7.4) was continuously perfused through the flow cell by a syringe pump at a flow rate of 2 μ L·min⁻¹ while the test solution containing glucose, AA, DA, 5-HT, DOPAC or UA simultaneously perfused by another syringe pump.

DFT simulation. The monomer and dimer of MG and the corresponding complexes with singlelayered GDY nanosheet were structurally optimized by using the B3LYP function with the 6-31G(d) basis set for C, H, O atoms and the pseudopotential LANL2DZ basis for MG_{mono} and MG_{di}. All the calculations were implemented in the Gaussian 09 program. Based on the optimized molecular geometries and the assumption that MG_{di} is an *H* aggregate, the binding energies of the MG_{mono}/GDY and MG_{di}/GDY complexes were corrected by the basis set superposition error (BSSE).



Figure S1. Contact angle measurement of GDY (a) and CNT (b).



Figure S2. FT-IR spectra of MG, GDY and MG/GDY. In the spectrum of MG, the sharp peak at 1597 cm⁻¹ belongs to the ring stretching. Peak at 1500 cm⁻¹ belongs to asymmetric stretching of O=N=O. Peak at 1403 cm⁻¹ with a shoulder belongs to symmetric stretching of O=N=O or aromatic C=N stretching. Peak at 1364 cm⁻¹ belongs to symmetric bending of C-H. Peak at 1331 cm⁻¹ belongs to C-N stretching in aromatic tertiary amine. Peaks at 1194 and 1126 cm⁻¹ belong to heterocycle vibration. Peak at 900 cm⁻¹ belongs to out-of-plane C-H bending of the aromatic ring. In the spectrum of GDY, the broad band centered at 2150 cm⁻¹ belongs to C=C stretching. Peak at 1629 cm⁻¹ belongs to C=C stretching of the benzene ring. Peak at 1439 cm⁻¹ belongs to surface residual C=O stretching. The broad band covering 1250~800 cm⁻¹ is originated from C-O vibrations in surface oxygen-containing moieties. In the spectrum of MG/GDY, all above mentioned peaks of GDY (indicated by \blacktriangle) and MG (indicated by \bigstar) are observed. The two peaks of MG having significant blue shift on MG/GDY are indicated by \bigstar . Notably, peak at 1629 cm⁻¹ becomes sharper and larger in MG/GDY as compared to that in GDY, indicating of existence of intercalated water.



Figure S3. DFT simulated UV-Vis spectra of MG/GDY. DFT simulations of MG_{mono} and MG_{di} were conducted with optimized molecular geometry in aqueous phase (water). Free MG_{mono} and MG_{di} have their respective maximum oscillator strength at 545 nm (black line) and 500 nm (blue line). Interaction of MG and GDY was simplified to an electrostatic model since MG is positively charged while GDY is rich in electrons. Simulated peaks of MG_{mono}/GDY (red line) and MG_{di}/GDY (green line) have perfect overlap with those of free MG, indicating that binding interactions do not change the chemical structures of MG_{mono} or MG_{di} .



Figure S4. Calculated binding energy of the monomeric and dimeric MG interacting with GDY nanosheets. Top view and side view of optimized molecular orientations are presented. Grey, white, red, blue and yellow spheres represent C, H, O, N and S atoms, respectively.



Figure S5. Schematic illustration of surface adsorption of MG monolayers on GDY nanosheets by mild stirring.



Figure S6. UV-Vis spectra of MG-GDY nanosheets (2 μ g·mL⁻¹) with respect to 2 mM free MG in water (**a**) and pristine MG nanosheets (1 mg·mL⁻¹) dispersed in water (**b**).



Figure S7. Preparation and electrochemical characterization of MG/eGDY nanocomposites. (**a**) Schematic illustration of bulk GDY exfoliation, MG adsorption and restacking of MG/eGDY nanosheets on GCE. (**b**) CV of MG/eGDY-modified GCE in potassium phosphate buffer (pH 7.4). Scan rate is 50 mV·s⁻¹. (**c**) CVs of MG/eGDY-modified GCE at varying scan rates. (**d**) Scan rate dependence of peak currents of MG_{di} in MG/eGDY. Peak currents of MG_{mono} are even smaller and not taken for linear plot.



Figure S8. CVs pristine GDY-modified GCE (a) and CNT-modified GCE (b) in the absence (dashed lines) and presence of 5 mM AA (solid lines) in potassium phosphate buffer (pH 7.4). Scan rate is 50 $\text{mV}\cdot\text{s}^{-1}$.



Figure S9. Electrooxidation of AA with MG/graphene nanosheets. (**a**) CVs of MG/graphene-modified GCE in the absence (dashed line) and presence of 1 mM AA (solid line) in potassium phosphate buffer at pH 7.4. Scan rate is 10 mV·s⁻¹. (**b**) Amperometric monitoring of current response to AA injection with MG/graphene and MG/GDY-modified GCEs poised at 0 V *vs*. Ag/AgCl in a continuous-flow system. Flow rate is 2 μ L·min⁻¹. Arrows indicate additions of AA at 10, 20, 30 and 40 μ M.



Figure S10. FT-IR spectra of GDH, GDY and GDY/GDH. In the spectra of GDH and GDY/GDH, the sharp peaks at 1700~1600 cm⁻¹ and 1600~1500 cm⁻¹ (indicated by \blacktriangle) belongs to the C=O stretching (Amide I band) and N-H in-plane bending.



Figure S11. Schematic setup of flow-cell test. In a typical continuous-flow analysis, two streams of electrolyte solutions (phosphate buffer, pH 7.4) carrying analytes and NAD⁺ were respectively perfused at a flow rate of 2 μ L·min⁻¹ by pump 1 and 2 and joint before entering the test cell.



Figure S12. Amperometric responses to glucose and interferents. The solution of glucose (500 μ M), AA (30 μ M), DA (20 μ M), 5-HT (10 μ M), DOPAC (15 μ M) or UA (30 μ M) in potassium phosphate buffer (pH 7.4) was successively injected through the flow cell with the GDY-MG/GDH-modified carbon film electrode as the working probe at 0.0 V *vs.* Ag/AgCl. NAD⁺ (5 mM) was perfused simultaneously from another syringe pump. Each analyte was continuously perfused for 500 s at a flow rate of 2 μ L min⁻¹ and tested at the biologically relevant concentration.



Figure S13. CVs of MG/eGDY-modified GCE in the absence (dashed line) and presence of 1 mM AA (solid line) in potassium phosphate buffer (pH 7.4). Scan rate is $10 \text{ mV} \cdot \text{s}^{-1}$.

	NADH	AA
pKa ₁	1.85	4.36
pKa ₂	4.01	11

Table S1. pKa values of NADH and AA obtained from The Human Metabolome Database.

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