SUPPORTING INFORMATION

Leveraging Electrochemistry to Uncover the Role of Nitrogen in the Biological Reactivity of Nitrogen-Doped Graphene

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Characterization of N-doped graphene samples

X-ray photoelectron spectroscopy (XPS). Surface elemental composition of all samples were analyzed by XPS operating in a Thermo Scientific ESCALAB 250Xi instrument with a monochromatic Al K α X-ray source (1486.7 eV) and a 650 μ m spot size. The survey spectra were obtained at 150 eV of pass energy and 1.0 eV of step size and were used to determine the elemental composition. The high resolution C1s and N1s spectra were collected at 50 eV of pass energy and 0.1 eV of step size. Triplicate measurements in different positions of each sample were conducted and the data were presented as averages with standard deviations. Peak deconvolution of N1s spectra was carried out using Thermo Scientific Avantage software. A Shirley-derived background was used for spectral substation and C-C at 284.8 eV was used for energy scale correction. Two N-containing polymers, poly(3,5 pyridine) and polypyrrole were used as the reference standards to obtain the peak fitting constraints for binding energies of pyridinic-N (398.7 eV) and pyrrolic-N (399.8 eV), respectively. The peak fitting constraint for binding energy of graphitic-N (401.7 eV) was adopted from a previous publication where a nearly pure graphitic-N doped graphene was fabricated.¹ The remaining tail region toward high binding energy was assigned to N-oxide (centered at ~404 eV). Peak positions were constrained to shift within ± 0.3 eV from the assigned binding energies, and values of full width at half maximum (FWHM) for all four components were kept at the same level between components with ± 0.2 eV deviation and at least as large as the values measured from two reference polymers.

Raman spectroscopy. Raman spectra were collected by a Horiba Scientific XplorA Raman-AFM/TERS system with an excitation wavelength of 638 nm. Three measurements at different locations of each sample were performed. The intensities and positions of D and G peaks were determined by fitting D peak with a Lorentzian character and G peak with a Breit-Wigner-Fano (BWF) function due to its asymmetry shape^{2, 3} after a polynomial baseline subtraction.

Transmission electron microscopy (TEM). Electron microscopy imaging was conducted on a JEOL JEM-2100F transmission electron microscope operated at 200 kV and equipped with an Oxford Aztec energy dispersive X-ray spectroscopy (EDS) with windowless solid-state silicon drift detector for elemental analysis. The morphology of all graphene samples was studied at TEM mode, while the mapping of elemental distribution within selective areas of the graphene sheets was carried out at scanning transmission electron microscopy (STEM) mode. TEM specimens

were prepared by applying 10-20 μ L suspensions of graphene (100 μ g mL⁻¹) in ethanol onto holey carbon films supported on Cu TEM grids.

Brunauer-Emmett-Teller (BET) analysis for surface area measurement. Surface areas of samples in powder form were measured on a Micromeritics ASAP 2020 surface area analyzer using nitrogen adsorption at liquid nitrogen temperature (-196 °C). A relative pressure (P/P₀) range of 0.06 to 0.20 was used to determine the BET surface areas.

Surface area in suspension measured by methylene blue (MB) adsorption. A sample suspension in water (0.1 mg mL⁻¹) after 1 h bath sonication was mixed with a MB solution (0.05 mg mL⁻¹) in a 1:1 volume ratio. The mixture vials in triplicate were shaken for 24 h in a rotator at room temperature to achieve the adsorption equilibrium. Then the free MB was isolated from the solution by centrifugation at 12,000 rpm for 15 min and its concentration was determined by measuring its light absorbance at 663 nm using a UV-vis spectrophotometer (Thermo Scientific Evolution 201). The literature value of 2.54 m² of surface area covered by 1 mg of adsorbed MB⁴, ⁵ was used to calculate the surface area of all samples in deionized water, as shown in the following equation. M_{MB} is the mass change of MB after the equilibrium incubation, and M_{Sample} is the mass of the sample.

Surface area $(m^2 g^{-1}) = 2.54(m^2 g^{-1}) \times M_{MB}(mg)/M_{Sample}(mg)$



Figure S1. Deconvoluted XPS N1s spectra of NG samples. Peak positions for four N components were constrained to shift within ± 0.3 eV from the assigned binding energies, and values of full width at half maximum (FWHM) were kept at the same level between components with ± 0.2 eV deviation and at least as large as the values measured from two reference polymers (poly(3,5 pyridine) and polypyrrole).



Figure S2. Raman spectra of rGO and NG samples with the 2D region magnified on the right.



Figure S3. STEM-EDS elemental maps of selected areas (a, d, g) indicating the distribution of C (b, e, h; red dots) and O (c, f, i; green dots) in rGO, NG-U, NG-UA, respectively.



Figure S4. Plots of natural logarithm of the GSH concentration (ln [GSH], mM) versus time by applying the first-order kinetic model to the GSH oxidation mediated by rGO and NG samples. The kinetic rate constants (k, mL mg⁻¹ h⁻¹) follow the order: NG-U-650 (2.30) \leq rGO (2.84) \leq NG-U-950 (7.33) < NG-U (7.97) < NG-UA (13.14) < NG-UA-650 (94.40) < NG-UA-800 (208.27). k is significantly different between all samples (P < 0.05), except for NRG-U versus NRG-U-950 (P = 0.12). Three replicates were measured at each time point. Geometric symbols represent the mean values of ln [GSH] at each sampling time over the mean concentration of the control (no rGO/NG). Error bars indicate the standard deviation (n 3).



Figure S5. Electrochemical indicators for the ORR performance of rGO and NG samples, including (a) the electron transfer numbers *n* and (b) the yield of H_2O_2 , H_2O_2 %. *n* and H_2O_2 % were determined by RRDE.



Figure S6. Nitrogen adsorption-desorption isotherms for NG-U-950 (circles) and NG-UA-800 (triangles) measured at liquid-nitrogen temperature (-196°C).



Figure S7. Two representative photographs on urea (left) and uric acid (right) samples dispersed in deionized water (0.05 mg mL⁻¹).

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