Supplementary Information (SI) for Chemical Science.
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Supporting Information

Hydrogen-Bond-Assisted Meta-Nitrogen-Doped Graphyne Enables Real-Time Electrocatalytic NADH Tracking in Single Cells

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1. Experimental Section

1.1 Reagents and Instrumentation

Sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), nitric acid (HNO_3) were all purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Potassium dihydrogen phosphate (KH_2PO_4) was purchased from Xilong Scientific Co., Ltd. (China). N, N-Dimethylformamide (DMF) was purchased from Aladdin Co., Ltd. (Shanghai, China). Nicotinamide adenine dinucleotide (NADH) was purchased from InnoChem Science & Technology Co., Ltd. (Beijing, China). Adenosine Diphosphate (ADP), Adenosine Triphosphate (ATP), uric acid (UA), glucose (Glu) were purchased from Macklin Biochemical Technology Co., Ltd. (Beijing, China). Glutathione (GSH) and nicotinamide adenine dinucleotide phosphate – reduced (NADPH), 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) were purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). Glucose dehydrogenase (GDH) was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Potassium ferricyanide ($K_3[Fe(CN)_6]$) and potassium ferrocyanide ($K_4[Fe(CN)_6]$) were purchased from Guangfu Science and Technology Development Co., Ltd. (Tianjin, China). Potassium chloride (KCl) and other chemicals were purchased from Institute of Chemical Reagents Co., Ltd. (Beijing, China). All reagents were of analytical reagent grade and used as received. The deionized (DI) water was produced by a Milli-Q system (Millipore, Bedford, MA, USA, 18.2 MΩ•cm). The buffer solution is prepared by dissolving NaCl (8.776 g), KCl (0.3728 g), $MgCl_2$ (0.2440 g), Glu (0.9 g), Hepes (2.383 g), and $CaCl_2$ (0.2220 g) in 1 L of deionized water, followed by adjusting the pH to 7.4 with 1 M NaOH.

The transmission electron microscopy (TEM) images were obtained by a Hitachi 7650 microscope operating at 80 kV. Scanning Electron Microscopy (SEM) images were obtained using a Hitachi SU8010. X-ray Powder diffraction (XRD) was carried out on a Bruker D8 Advance powder diffractometer. Atomic force microscope (AFM) images were carried out using the Bruker Multimode 8 Atomic Force Microscope. X-ray photoelectron spectra (XPS) measurements were performed on an ESCA Lab 250 X-ray photoelectron spectrometer. Raman spectra were performed with Renishaw in Via Qontor Raman spectrometer. The excitation laser beam (λ=532 nm, continuous wave) was focused on the samples through an objective (50×, NA=0.5). Flourier transform infrared (FT-IR) spectra were accomplished by Bruker Equinox55 spectrophotometer. The UV-Visible (UV-Vis) absorption spectra were gained by using the Shimadzu UV-2500 UV-Vis spectrophotometer. The electrochemical signals were recorded on a CHI 1030c electrochemical workstation (CH Instruments, Shanghai, China). Nanoelectrodes were prepared using a gravity-drawn apparatus (PC-100, Narishige, Japan). The inverted microscope (IX73, OLYMPUS, Japan) and micromanipulator (PS-7000 C, Scientifica, UK) was

used to observe and control the movement of electrodes in and out of the cells.

1.2 Preparation of xNGY (x=1, 2, 3)

The xNGY (x=1, 2, 3) materials were synthesized by the research group of Professor Ye Daixin at Shanghai University. The synthesis used calcium carbide (CaC_2) and pyridine as the initial reactants, with ball milling serving as the high-energy driving force for the mechanochemical reaction. Initially, calcium carbide, pyridine, and 5 mm ZrO_2 balls were mixed in a ZrO_2 vial. The mixture was then ball-milled on a planetary ball mill at 600 rpm under an argon atmosphere for 16 hours. Subsequently, the resulting solid sample was dispersed in water, washed with 1 M nitric acid and deionized water to remove residual CaC_2, and washed with anhydrous ethanol to remove residual organics. The sample was then subjected to continuous ultrasonication in anhydrous ethanol for 1 hour to obtain a monolayer material. Finally, the sample was centrifuged again, and the resulting solid was labeled as 1NGY and dried thoroughly at 70°C for 12 hours. The final product was a black material. The 2NGY and 3NGY materials were synthesized by replacing pyridine with pyrazine or 2,4,6-trichloro-1,3,5-triazine, respectively.

1.3 Preparation of 3NGYO

The 3NGY powder (5 mg) was carefully mixed with concentrated nitric acid (16 M, 2 mL) in a centrifuge tube. The mixture was subsequently subjected to ultrasonication to ensure complete reaction over a period of 24 hours. The product was collected via centrifugation, washed three times with deionized water, and ultimately dried overnight at 60°C in a vacuum drying oven, yielding the 3NGYO powder.

1.4 Electrochemical performance testing of electrocatalysts

All electrochemical measurements were performed on a computer-controlled electrochemical analyzer (CHI 1030C, Shanghai, China) using a three-electrode system. The electrochemical experiments were conducted with a glassy carbon working electrode modified with the electrocatalyst, an Ag/AgCl reference electrode, and a platinum counter electrode. The electrolyte solution used was a 0.05 M phosphate-buffered saline (PBS) solution at pH 7.4.

1.5 Preparation of Nanoelectrodes

A 7 μm carbon fiber was drawn into a borosilicate glass capillary (1.5 mm outer diameter, 0.84 mm inner diameter). Using a gravity puller, the glass capillary was pulled into two separate electrodes. Under a microscope, the protruding fiber was trimmed to a length of 100-150 μm using a surgical blade. Subsequently, the carbon fiber was etched by flame. The electrode was held at the edge of the blue part of a butane flame for less than 2 seconds. When the tip of the fiber turned red, the electrode was rotated to ensure uniform etching. The fiber, with an axial

length of about 25-75 μm, was then sealed with epoxy resin.

1.6 Preparation of Functionalized Nanoelectrodes

A solution of 3NGYO material at a concentration of 2 mg/mL was prepared in a 1:1 mixture of water and DMF, with 0.1 M KCl added as the supporting electrolyte. A clean carbon fiber nanoelectrode (CFNE) and an Ag/AgCl reference electrode were immersed in the aforementioned solution. Electrochemical deposition was performed using the chronoamperometry method at a constant potential of +1.5 V for 10 minutes. This process resulted in the electrochemical plating of a 3NGYO-modified layer onto the surface of the CFNE, yielding the 3NGYO/CFNE composite.

1.7 Cell culture

SH-SY5Y cells were cultured in RPMI with 10% fetal bovine serum and 1% penicillin–streptomycin at 37 ℃ in a 5% CO_2 humidified atmosphere. Throughout the entire lifecycle of the cultures, the medium was refreshed every 2-3 days, and the cells were passaged every 4-5 days. Before further electrochemical experiments, the SH-SY5Y cells were seeded on small circular glass slides and cultured for 12 hours to obtain essentially isolated single cells.

1.8 Amperometric measurements

The cells cultured in Buffer solution were observed under an inverted microscope placed within a Faraday cage. An Ag/AgCl electrode was used as the reference electrode. A potentiostat was employed to maintain the working electrode at a constant potential of +0.2 V relative to a self-made Ag/AgCl reference electrode. The 3NGYO/CFNE, mounted on a micromanipulator, was carefully inserted into SH-SY5Y cells. After achieving a stable current baseline, Buffer solution and MPP^+ (3 mM) solution were delivered in real-time, respectively, and the resulting currents were recorded.

1.9 Cell experiments

1.9.1 Intracellular α-syn assay

ThT fluorescence assay was used to quantify the amount of α-syn. The cultured cells were inoculated in a 6-well plate. Cells were incubated for 24 hours and then pretreated with H_2S (0, 0, 200 μM) for 12 hours. Subsequently, the cells were treated with MPP^+ (0, 1, 1 mM) for an additional 12 hours. After incubation, the cells were rinsed three times with phosphate-buffered saline (PBS), stained with ThT probe (50 μM) for 15 minutes, and then imaged using a fluorescence microscope.

1.9.2 Intracellular Ca^{2+} imaging

The cultured cells were inoculated in a 6-well plate. Cells were incubated for 24 hours and then pretreated with H_2S (0, 0, 200 μM) for 12 hours. Subsequently, the cells were treated with

MPP^+ (0, 1, 1 mM) for an additional 12 hours. Subsequently, all cells were rinsed twice with PBS, followed by the addition of Fluo-4 AM (0.5 μM in culture medium). The mixture was incubated in a 37 ℃ incubator for 20 min. The cells were washed three times with PBS to remove the Fluo-4 AM that did not enter the cells, followed by imaging under a fluorescence microscope.

1.9.3 Intracellular ROS imaging

The cultured cells were inoculated in a 6-well plate. Cells were incubated for 24 hours and then pretreated with H_2S (0, 0, 200 μM) for 12 hours. Subsequently, the cells were treated with MPP^+ (0, 1, 1 mM) for an additional 12 hours. After incubation, the cells were rinsed three times with phosphate-buffered saline (PBS), stained with DCFH-DA (10 μM) for 30 min, and then imaged using a fluorescence microscope.

2. Supplementary Figures

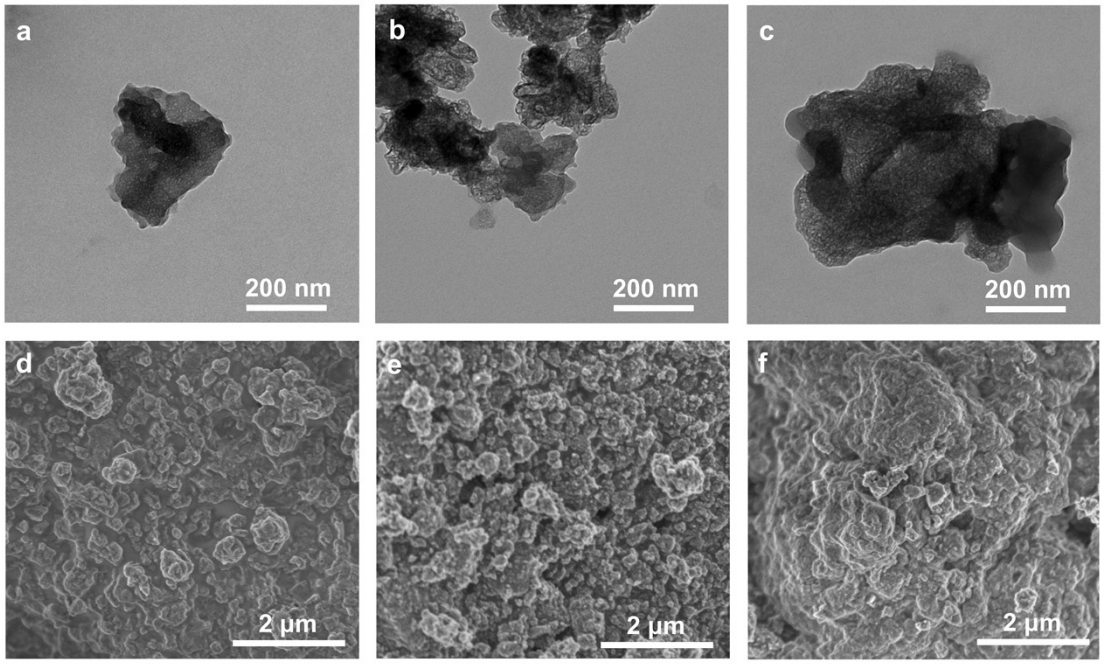

Fig. S1 TEM images of (a) GY, (b) 1NGY and (c) 2NGY. SEM images of (d) GY, (e) 1NGY and (f) 2NGY.

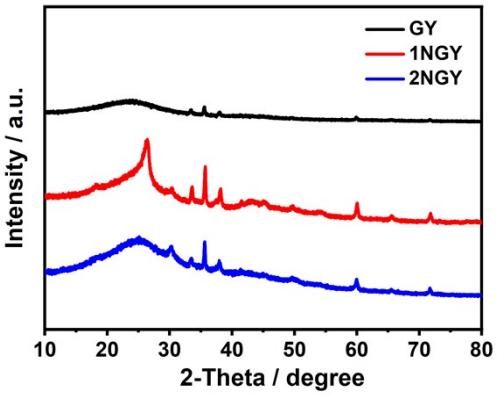

Fig. S2 XRD patterns of GY, 1NGY, 2NGY.

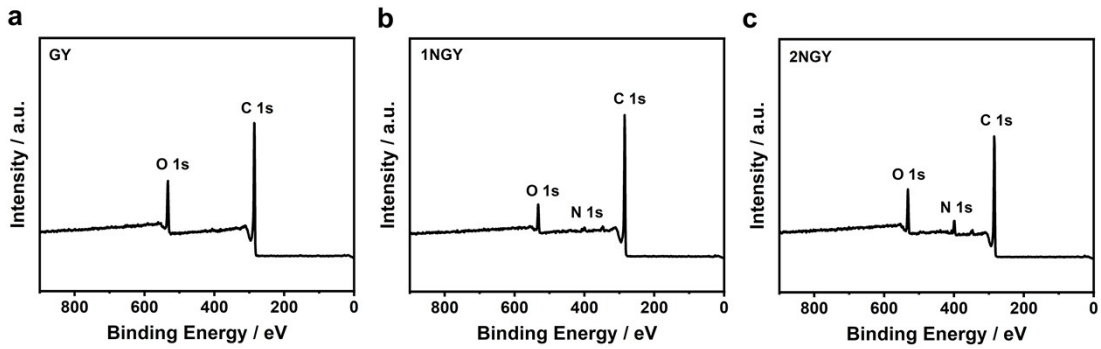

Fig. S3 XPS survey spectra of (a) GY, (b) 1NGY and (c) 2NGY.

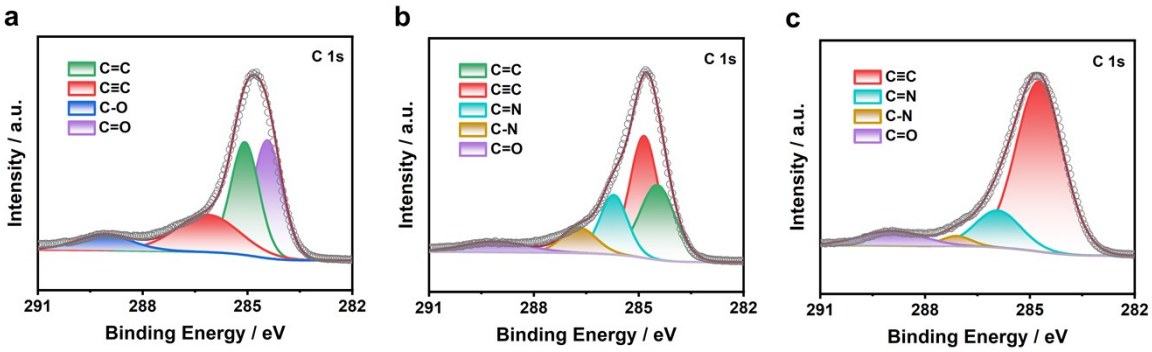

Fig. S4 XPS spectra of C 1s for (a) GY, (b) 1NGY and (c) 2NGY.

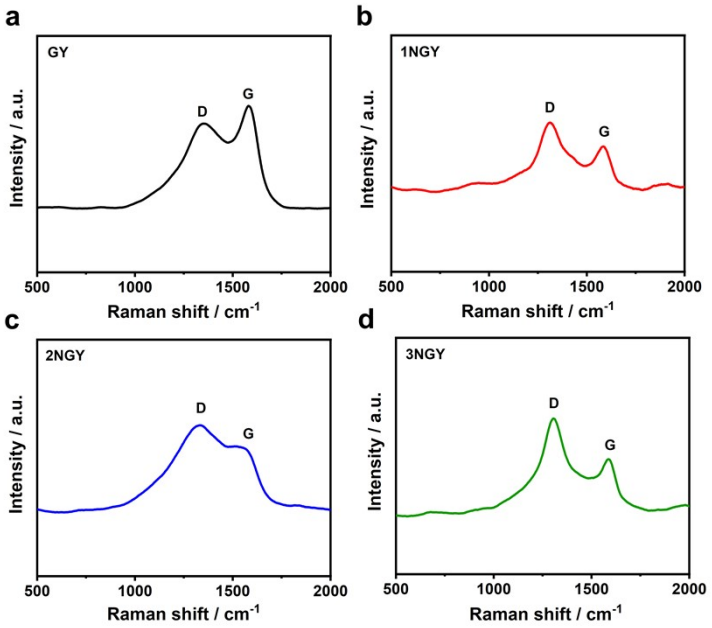

Fig. S5 Raman spectra of (a) GY, (b) 1NGY, (c) 2NGY and (d) 3NGY.

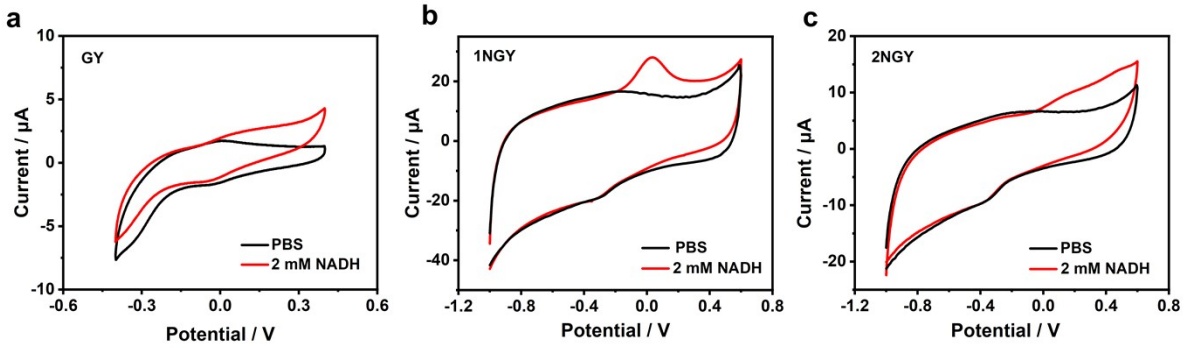

Fig. S6 Cyclic voltammograms obtained at glassy carbon electrodes modified with (a) GY, (b) 1NGY and (c) 2NGY in blank PBS and PBS containing 2 mM NADH.

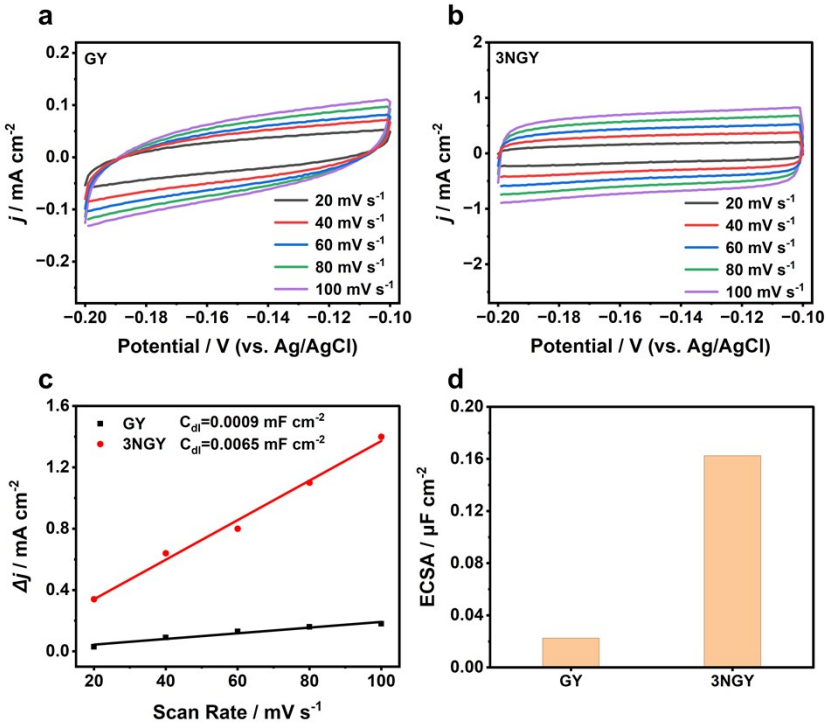

Fig. S7 Cyclic voltammograms of (a) GY and (b) 3NGY for double-layer capacitance characterization. (c) C_{dl} values and (d) ECSA results of GY and 3NGY.

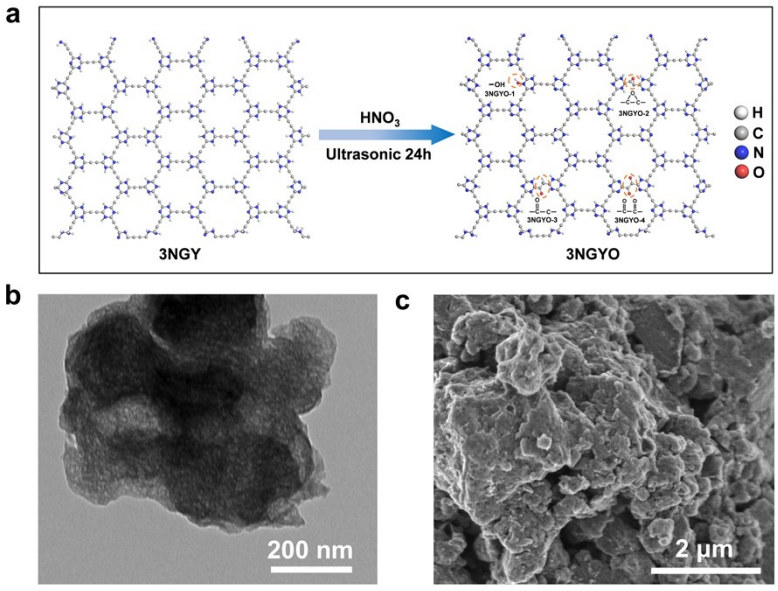

Fig. S8 (a) The schematic diagram of 3NGYO preparation. (b) TEM and (c) SEM images of 3NGYO.

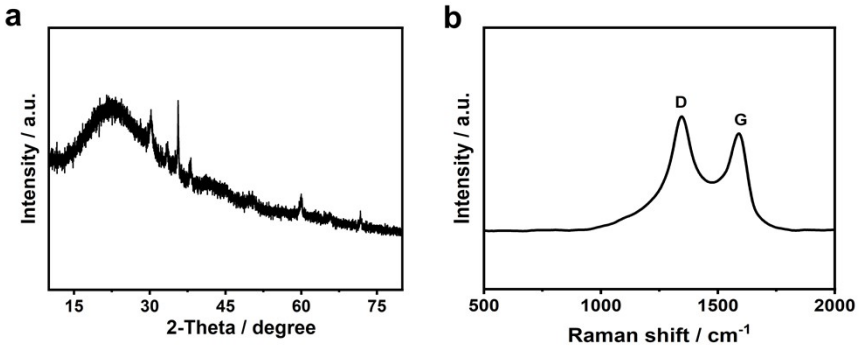

Fig. S9 (a) XRD pattern and (b) Raman spectrum of 3NGYO.

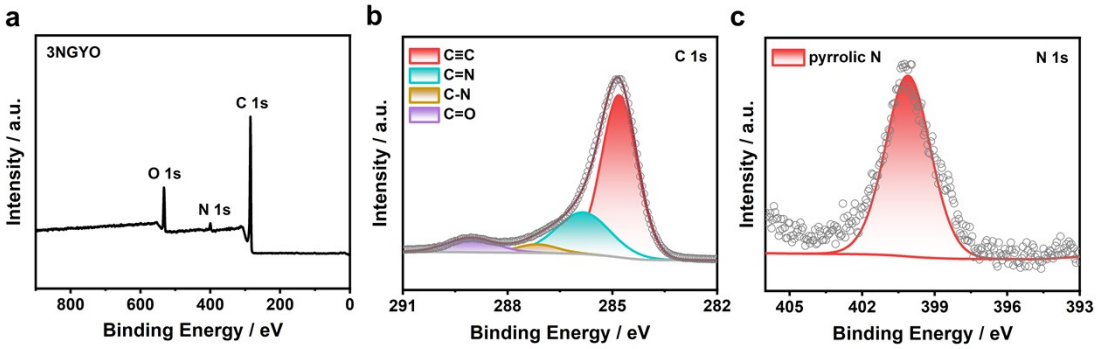

Fig. S10 (a) XPS survey spectrum of 3NGYO. (b) XPS spectrum of C 1s for 3NGYO. (c) XPS spectrum of N 1s for 3NGYO.

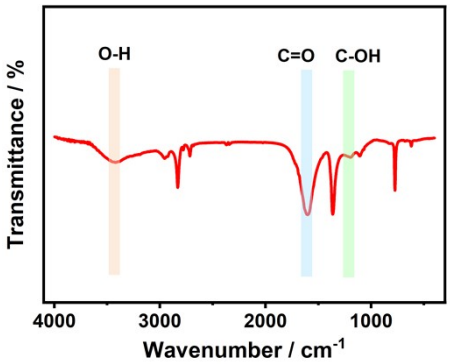

Fig. S11 FT-IR spectra of 3NGY and 3NGYO.

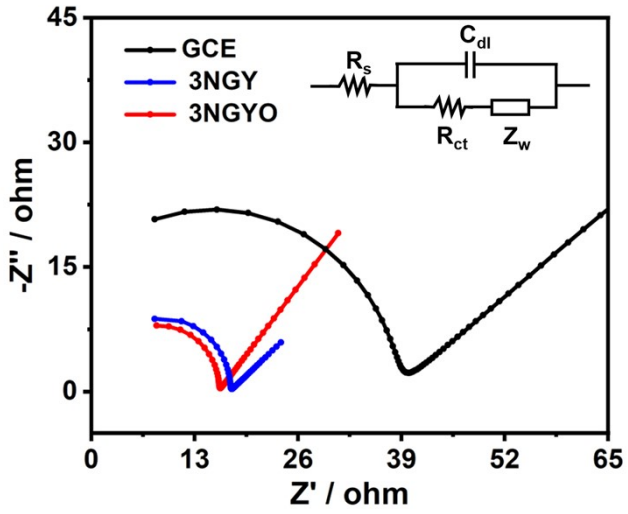

Fig. S12 Electrochemical impedance spectra of 3NGY and 3NGYO.

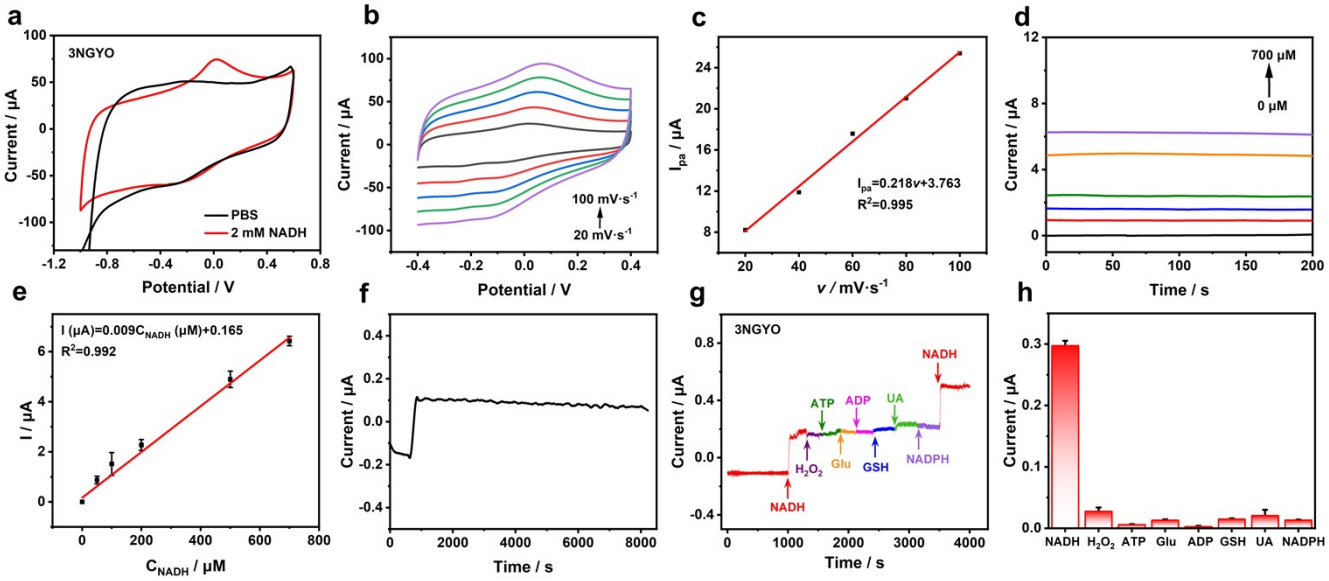

Fig. S13 (a) Cyclic voltammograms obtained at glassy carbon electrodes modified with 3NGYO in blank PBS and PBS containing 2 mM NADH. (b) Cyclic voltammograms of 3NGYO/GCE in PBS solution containing 1 mM NADH at different scan rates (20 to 100 mV/s). (c) The relationship between Ip and v. (d-e) The amperometry response curve and calibration curve of 3NGYO/GCE to continuously adding different concentrations of NADH at 0.05 V (mean ± SD, n=3). (f) The stability of 3NGYO/GCE in PBS solution (pH 7.4) upon the addition of 40 μM NADH. (g) Selective testing of 3NGYO/GCE with NADH (40 μM), H_2O_2 (20 μM), ATP (40 μM), Glu (40 μM), ADP (40 μM), GSH (20 μM), UA (20 μM), and NADPH (40 μM) was carried out at 0.05 V constant voltage. (h) Statistical histogram of the current response of each interference at 3NGYO/GCE (mean ± SD, n=3).

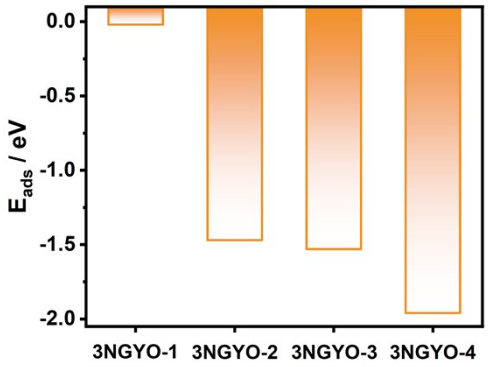

Fig. S14 Adsorption energy between NADH and 3NGYO.

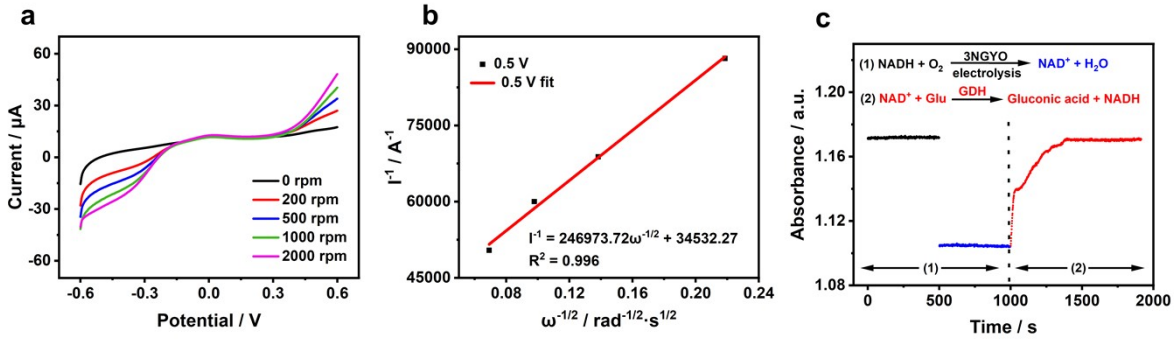

Fig. S15 (a) Steady-state voltammograms obtained at the 3NGYO rotating disk electrode in 0.05 M PBS (pH 7.4) containing 1 mM NADH at a scan rate of 50 $mV{\cdot}s^{-1}$ at the various rotation rates. (b) The Koutecky–Levich plot derived at 0.5 V from the obtained voltammograms. (c) Electrocatalytic oxidation of 0.25 mM NADH was carried out with a 3NGYO-modified electrode at a constant potential of + 0.05 V. Record the changes in absorbance at 340 nm over time for the system before and after 60 min of electrolysis, as well as the curve of absorbance changes after the addition of 10 mM glucose and glucose dehydrogenase to the solution following 60 min of electrolysis.

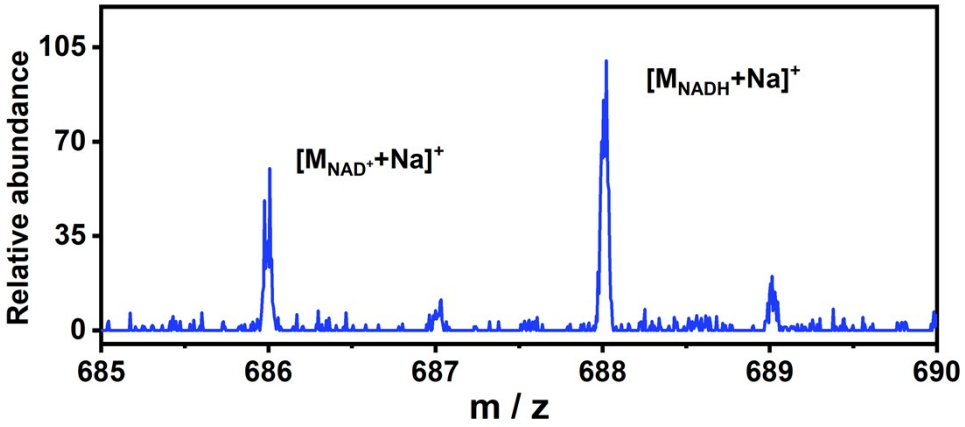

Fig. S16 Mass spectrometry characterization of the solution system after electrocatalytic oxidation of 0.25 mM NADH at a constant potential of + 0.05 V using a 3NGYO-modified electrode for 60 minutes.

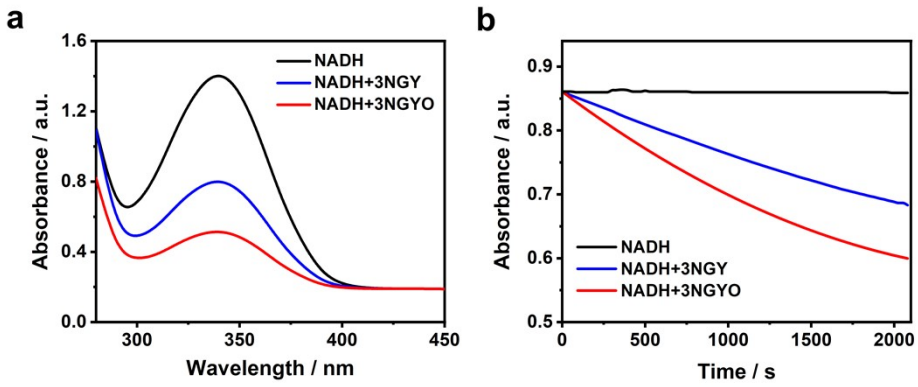

Fig. S17 (a) UV-Vis absorption of NADH catalyzed by 3NGY and 3NGYO. (b) Kinetic spectra of NADH catalyzed by 3NGY and 3NGYO.

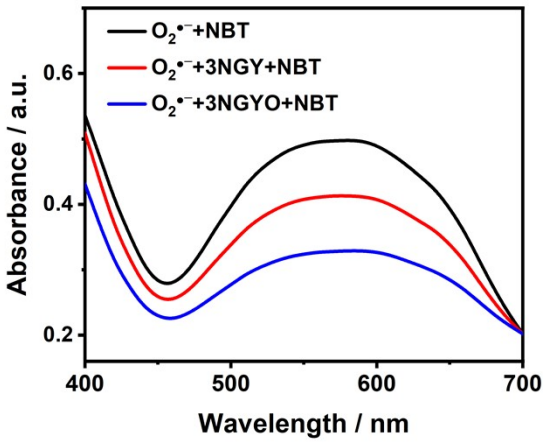

Fig. S18 Comparison of SOD-like activities between 3NGY and 3NGYO.

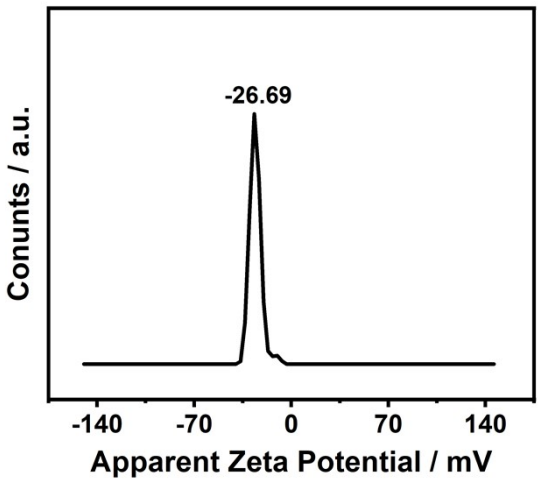

Fig. S19 Zeta potential of 3NGYO.

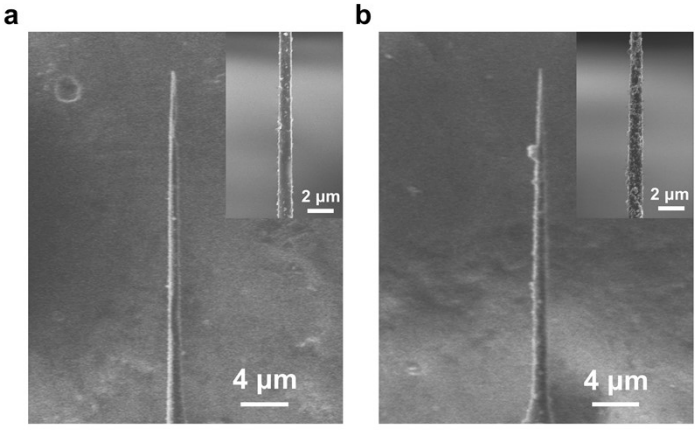

Fig. S20 SEM images of CFNE (a) and 3NGYO/CFNE (b).

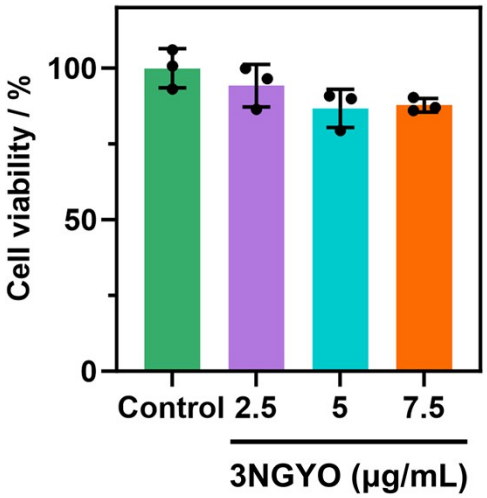

Fig. S21 The cytocompatibility of 3NGYO with SH-SY5Y cells (mean ± SD, n=3).