

1. Experimental Section

Reagents and materials

The following chemicals were used as received: Tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl, 99.8%, Sigma-Aldrich); β -Nicotinamide adenine dinucleotide hydrate (NAD^+ , Sigma-Aldrich); β -nicotinamide adenine dinucleotide, reduced disodium salt hydrate (β -NADH, $\geq 97\%$, Sigma-Aldrich); L-Lactic Dehydrogenase from rabbit muscle (L-LDH, 600-1200 units/mg, Sigma-Aldrich); sodium pyruvate (98%, Sigma-Aldrich); sodium lactate (98%, Sigma-Aldrich); 4,4',4''-(Pyrene-1,3,6,8-tetrayl)tetrabenzoic acid (97%, Bldpharm); biphenyl-4-carboxylic acid (98%, Bldpharm); zirconium(IV) oxychloride octahydrate (99%, Sigma-Aldrich); (pentamethylcyclopentadienyl) rhodium(III) dichloride dimer ($[\text{Cp}^*\text{RhCl}_2]_2$, 99%, Alfa); 2,2'-bipyridine-4-carboxylic acid (98%, Sigma-Aldrich); methanol (MeOH, analytical reagent, Sigma-Aldrich); acetone (analytical reagent, Sigma-Aldrich); N,N-dimethylformamide (DMF, analytical reagent, Sigma-Aldrich); hydrochloric acid (HCl, 98%, Sigma-Aldrich); 4-aminobenzoic acid (99%, Sigma-Aldrich); potassium ferricyanide (99.5%, Sigma-Aldrich); ferrocenemethanol (97%, Sigma-Aldrich); NaNO_2 (97%, Sigma-Aldrich); Nafion membrane (N-212, Alfa), graphite felt (GFD4.65EA, SGL, Germany) and gas diffusion electrode (GDE, Paxitech France).

Synthesis of Rhodium complex

$[\text{Cp}^*\text{Rh}(\text{bpy})\text{Cl}]^+$ complex (Rh complex) was prepared following the reported literature^[3]. 49.9 mg $[\text{Cp}^*\text{RhCl}_2]_2$ and 40 mg 2,2'-bipyridine-5,5'-dicarboxylic acid was dissolved in 15 mL of MeOH in a 50 mL flask. The mixture was stirred at room temperature overnight to get a deep orange color solution. The yellow precipitate was formed by slowly adding an excess amount of diethyl ether. Then the yellow precipitate was filtrated, washed by 2x20 mL diethyl ether, and dried under vacuum for 2 h.

Preparation of Rh-NU-1000-GF

The preparation process of Rh-NU-1000-GF is derived from a modified method described in a previous report^[2]. Specifically, graphite felt (GF) was immersed into 20 mL of ethanol, and then distilled water was used to wash away the ethanol adsorbed on GF. Subsequently, the GF was grafted with benzoic acid groups by diazonium electrochemical reduction. The solution consist of 1 mM 4-aminobenzoic acid and 2 mM NaNO_2 in 0.5 M HCl solution. The electrochemical reduction process was carried out through 15 cycles of cyclic voltammetry ranging from 0.5 to -0.6 V under N_2 atmosphere. Then, the GF-COOH electrode was thoroughly rinsed with water and acetone, followed by drying, before being stored for subsequent use. In a 25 mL glass vial, 73.5 mg (0.225 mmol) $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ and 2.43 g (12.3 mmol) biphenyl-4-carboxylic acid were dissolved in 6 mL DMF and sonicated until a clear solution was obtained. The GF-COOH electrode was placed at the bottom of the vial and heated at 80 °C for 1 h to grow Zr-nodes on the surface of GF-COOH electrode. The in-situ growing of NU-1000 was carried out following a literature protocol. 0.39 g of $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ and biphenyl-4-carboxylic acid (17.57 g) were dissolved in 32 mL of DMF in a 100 mL flask. The flask was heated to

80 °C for 1 h to form Zr-nodes, and then the solution was allowed to cool down to room temperature. In another flask, 160 mg 4,4',4''-(Pyrene-1,3,6,8-tetrayl)tetrabenzic acid was dissolved in 9.6 mL DMF. The above 2 solutions were combined for further use. The Zr -GF-COOH electrode was placed into 10 mL of combined solution at 100 °C for 24 h in the oven to facilitate the in-situ growth NU-1000 on GF. Finally, biphenyl-4-carboxylic acid was removed by the solution containing 0.5 mL of 8 M HCl and 12 mL of DMF at 100 °C for 48 h. The residual solvent within the electrode was removed by immersing it into acetone overnight. This electrode was named NU-1000-GF. Then, 10 mg Rh complex was dissolved in 10 mL DMF in a vial. Subsequently, NU-1000-GF was introduced into the above solution. Then the vial was sealed and heated at 60 °C for 24 h in the oven. After the vial cool down to room temperature, the electrode was rinsed with fresh DMF and placed in 20 mL acetone to remove any adsorbed Rh complex on GF under stirring overnight. The obtained electrode was named Rh-NU-1000-GF.

Characterizations

The morphology and energy-dispersive X-ray spectroscopy (EDX) of the sample were characterized by a JEOL JSM-IT500HR equipped with an EDX OXFORD Ultim Max 170. Ultraviolet-visible light (UV-vis) absorption spectra was recorded on a Cary 60 Scan UV-vis spectrophotometer. Powder X-ray diffraction (PXRD) was carried out with a Panalytical X'Pert Pro MPD diffractometer. High-performance liquid chromatography (HPLC) was carried out with an LC-20ADVP (Shimadzu). The column was HPX-87H HPLC (300 mm x 7.8 mm, Phenomenex, 45 °C). All electrochemical experiments in bulk solution were carried out using an Auto lab PGSTAT-12. Ag/AgCl (3.0 M/KCl) was used as reference electrode and a Pt wire was used as counter electrode. All electrochemical experiments in flow bioreactor were carried out with a BioLogic potentiostat VMP3e and the flow bioreactor setup employed was similar to the one utilized in a previous study ^[3].

Preparation of carbon paper with LDH

The preparation of carbon paper with LDH followed the methodology outlined in a referenced article ^[3]. Specifically, a piece of Spectra Carb carbon paper measuring 1.5 cm x 1.5 cm was chosen as the enzyme support for L-lactate dehydrogenase. Initially, 10 mg of multi-walled carbon nanotubes (MWCNTs) was dispersed in 50 mL of ethanol through ultrasonication for 5 hours. The suspension was then decanted and filtered under vacuum using carbon paper as the filter medium. The filtered material was dried overnight at 60°C. This process of dispersion, filtration, and drying was repeated three times to achieve the desired number of MWCNT layers. Finally, LDH enzyme was adsorbed onto the MWCNT surface by depositing a 50 µL suspension containing 1600 units (equivalent to 2 mg of protein) and allowing it to adsorb for 2 hours at room temperature, resulting in an immobilized enzyme density of 0.89 mg/cm².

The Monitoring of NADH Concentration

To detect NADH regeneration using UV-visible spectrophotometry, a series of standard solutions with different concentrations of NADH are prepared. The absorbance at 340 nm is measured for each standard solution. Using NADH concentration as the x-axis and absorbance at 340 nm as the y-axis, a linear fit is performed to obtain the standard curve for NADH, as shown in figure S7.

Bioconversion in flow bioreactor

The working principle of redox flow bioreactor in this work is indicated in Scheme 1. A GDE was used as the anode and H₂ was injected at a flow rate of 20 mL/min into anode. A modified graphite felt electrode Rh-NU-1000-GF (1×2×0.5 cm³) was used as cathode. A Nafion membrane (N-212) was placed between the two electrodes for separation and for allowing H⁺ to diffuse from the anode side to the cathode side. A porous carbon layer composed of carbon paper modified by a layer of MWCNT was used to immobilize Lactate dehydrogenase (LDH-MWCNT-CP). This functional layer allowing conversion of pyruvate to lactate was positioned between the cathode and the Nafion membrane. During bioconversion, the solution containing pyruvate and NAD⁺ was continuously introduced into flow bioreactor to generate NADH and lactic acid.

The Monitoring of lactate productions

To analyze the by-products of enzymatic electrosynthesis, electrochemical products were detected using high-performance liquid chromatography (HPLC) with an Aminex HPX-87H column (300 mm x 7.8 mm, Phenomenex). The testing conditions were as follows: the mobile phase consisted of 5 mmol/L H₂SO₄, with a flow rate of 0.7 mL/min. The column temperature was maintained at 45 °C, and the detector wavelength was set at 210 nm. 200 µL aliquots were taken at different intervals from the reaction mixture. These aliquots were further analyzed by HPLC to determine the pyruvate and lactate concentrations.

2. Figures

2.1. SEM image of NU-1000-GF without the carboxyphenyl layer

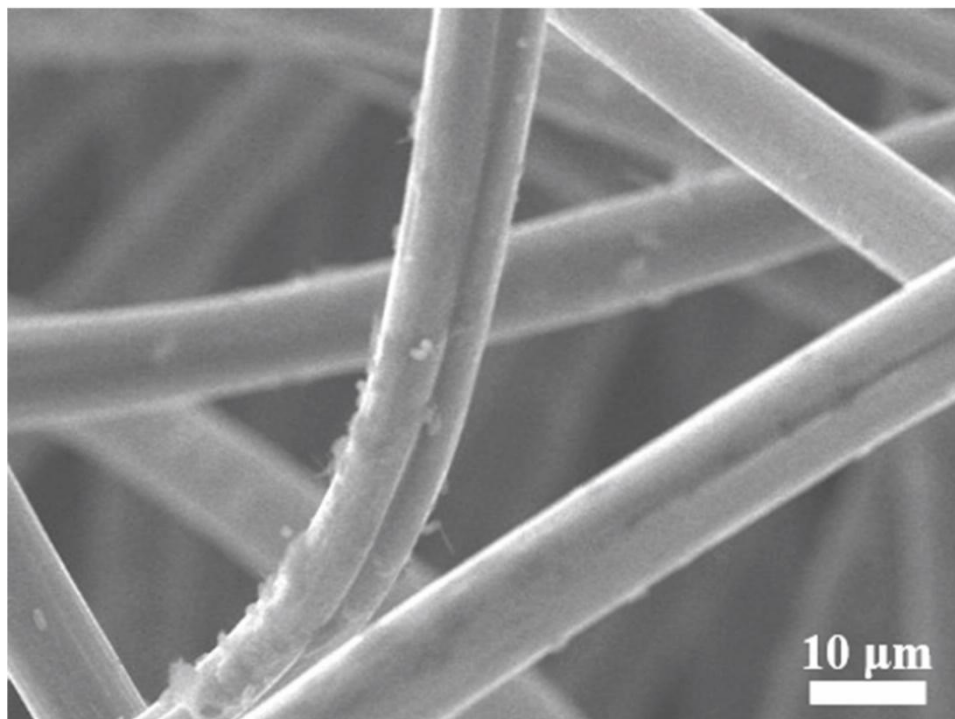


Figure S1. SEM images of a tentative synthesis of NU-1000 on graphite felt without prior electrografting of a layer of carboxyphenyl groups.

2.2. SEM image of NU-1000-GF with the carboxyphenyl layer

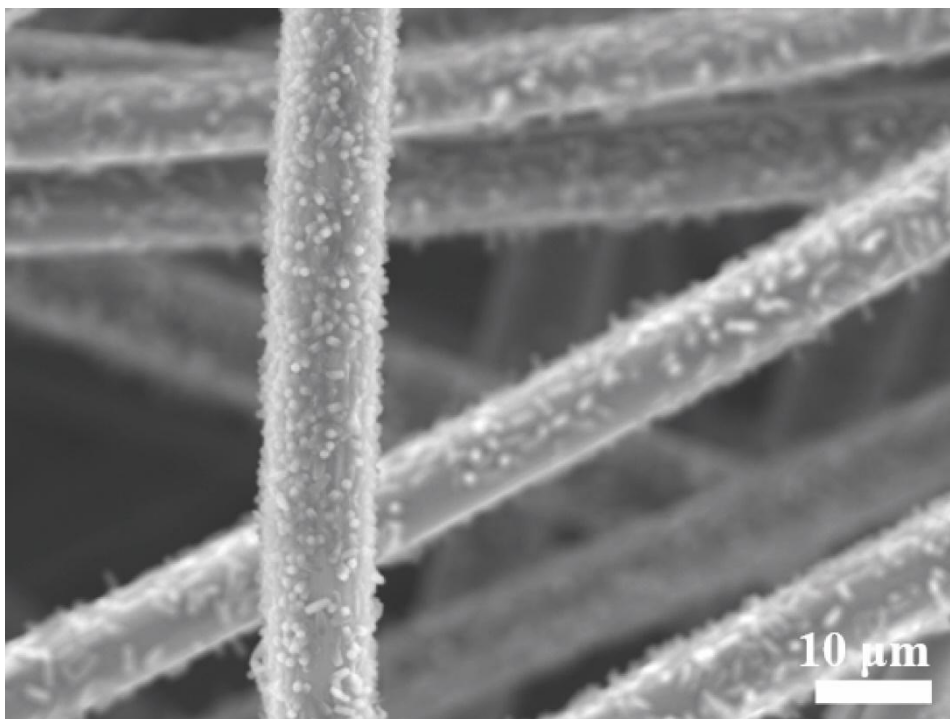


Figure S2. SEM image of the synthesis of NU-1000 on graphite felt with prior electrografting of a layer of carboxyphenyl groups (sample NU-1000-GF).

2.3. SEM image of the middle part of NU-1000-GF

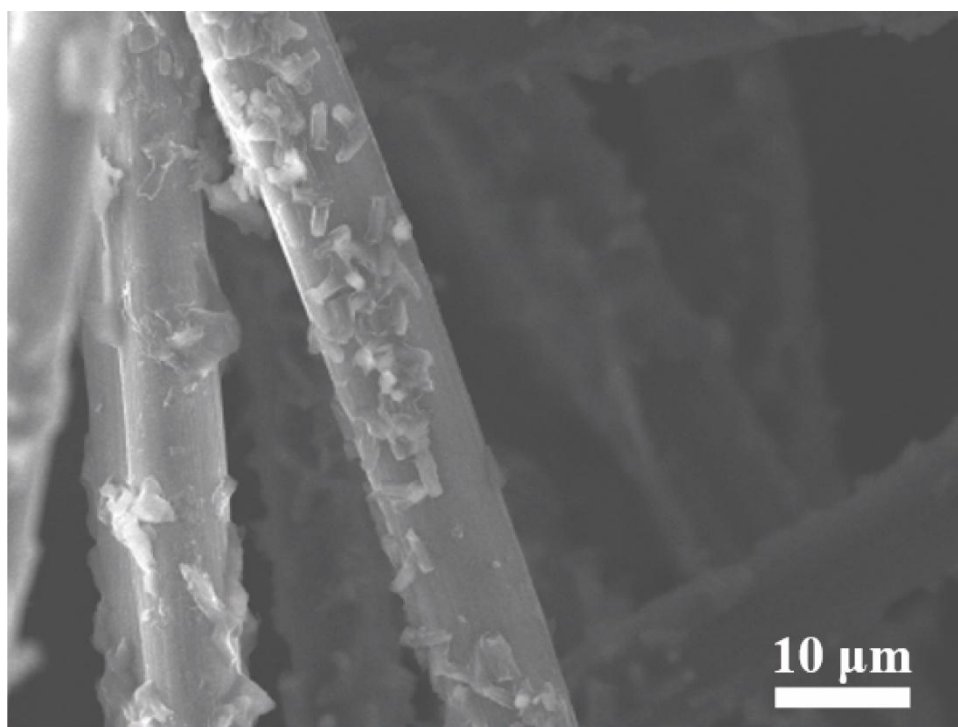


Figure S3. SEM image of the middle part of the sample Rh-NU-1000-GF.

2.4 XPS of Rh-NU-1000-GF and NU-1000-GF.

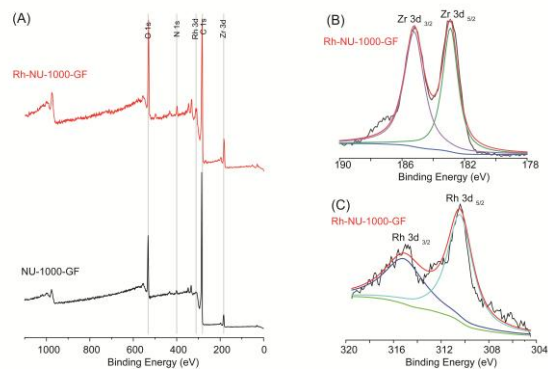


Figure S4. (A) The XPS spectra of NU-1000-GF and Rh-NU-1000-GF, (B) The XPS spectra of Zr 3d and (C) The XPS spectra of Rh 3d from Rh-NU-1000-GF.

2.5 EDS mapping and elemental Contents of Rh-NU-1000-GF

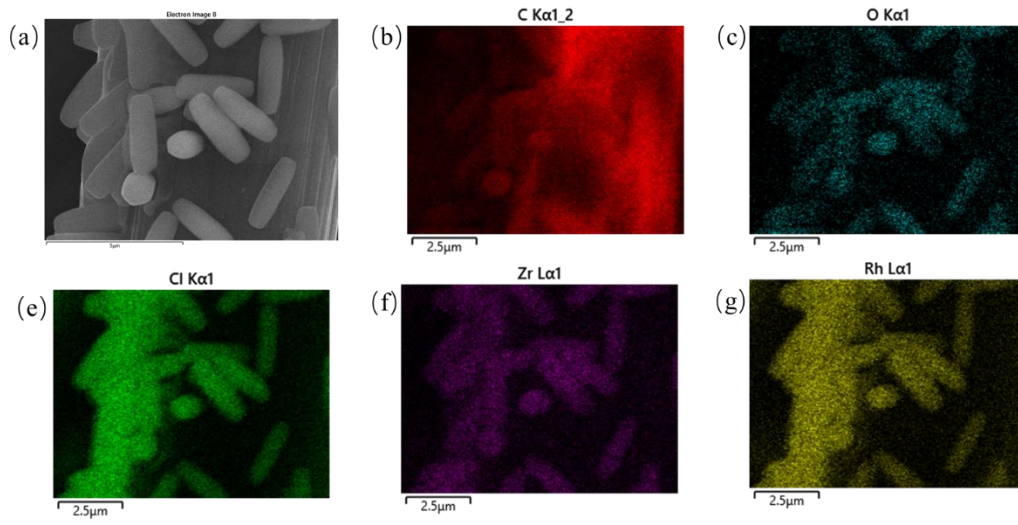


Figure S5. EDX mapping of Rh-NU-1000-GF.

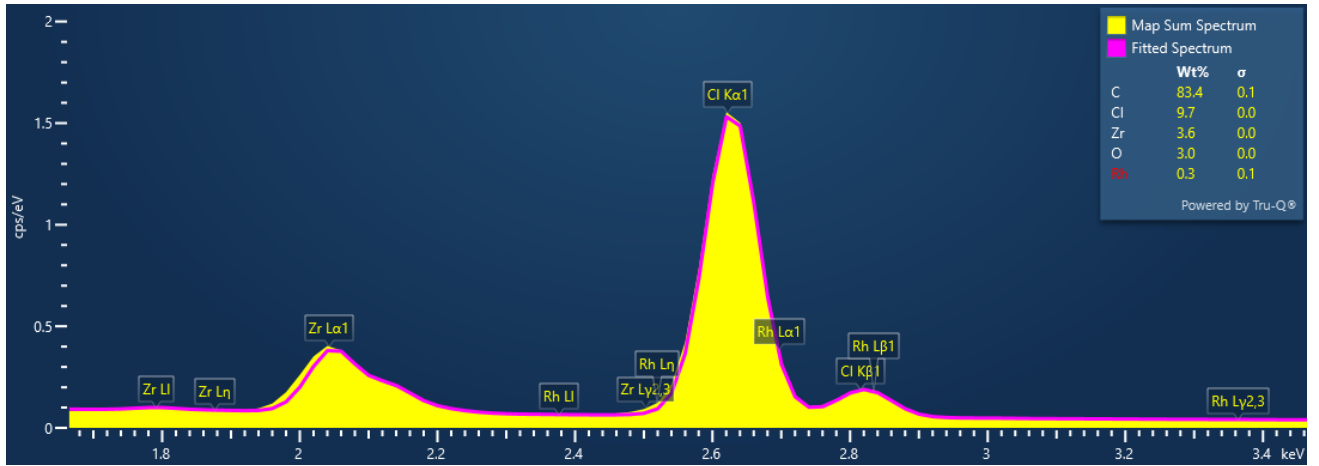


Figure S6. The elemental contents obtained from EDX.

2.7 UV-vis spectroscopy of NADH and standard curve (0-0.3 mM).

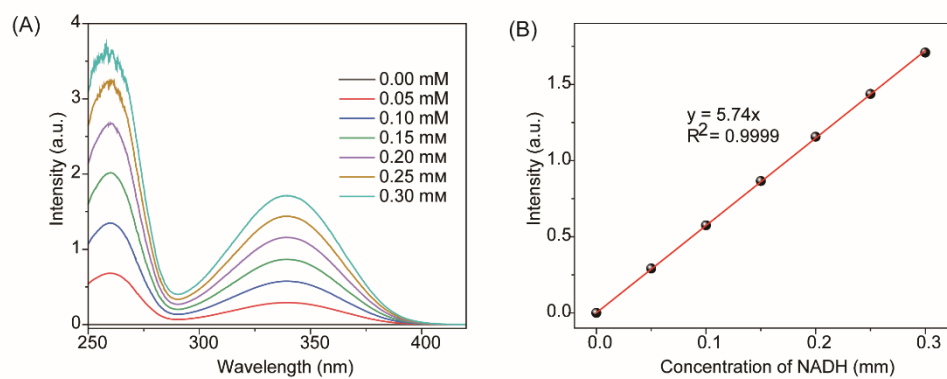


Figure S7. (A) UV-visible absorbance of different concentration of 1,4-NADH in Tris-HCl solution (1M, pH=7.0). (B) Calibration curve of absorption of 1,4-

NADH at 340 nm.

2.7. Cyclic voltammograms of Rh-NU-1000-GF after immersing into Tris-HCl buffer for 6 days

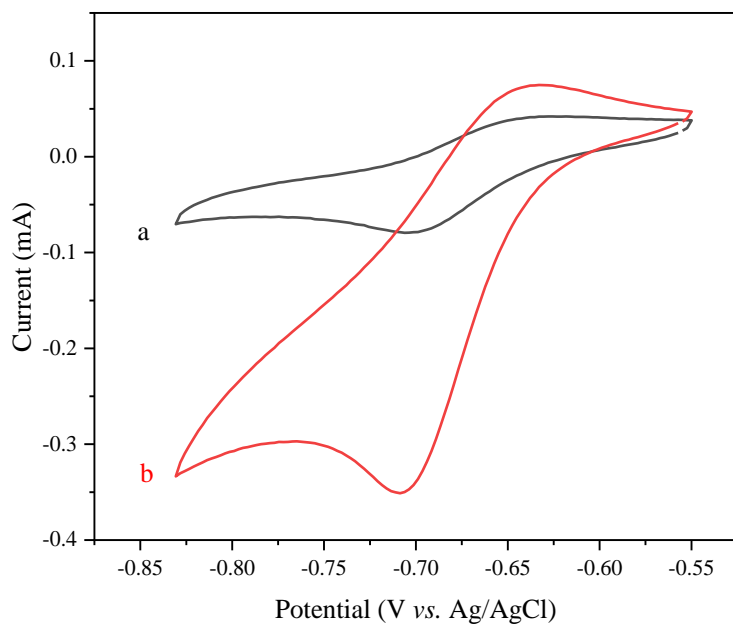


Figure S8. Cyclic voltammograms recorded with Rh-NU-1000-GF (a) without and (b) with 5 mM NAD⁺ after it was immersed into 10 mL Tris-HCl solution (pH=7.0 and 1 M) for 6 days. The experiment was carried out under N₂ with a scan rate of 5 mV/s, and the size of electrode was 1×2×0.5 cm³.

2.8 The UV-vis spectroscopy of products obtained at different time with Rh-NU-1000-GF.

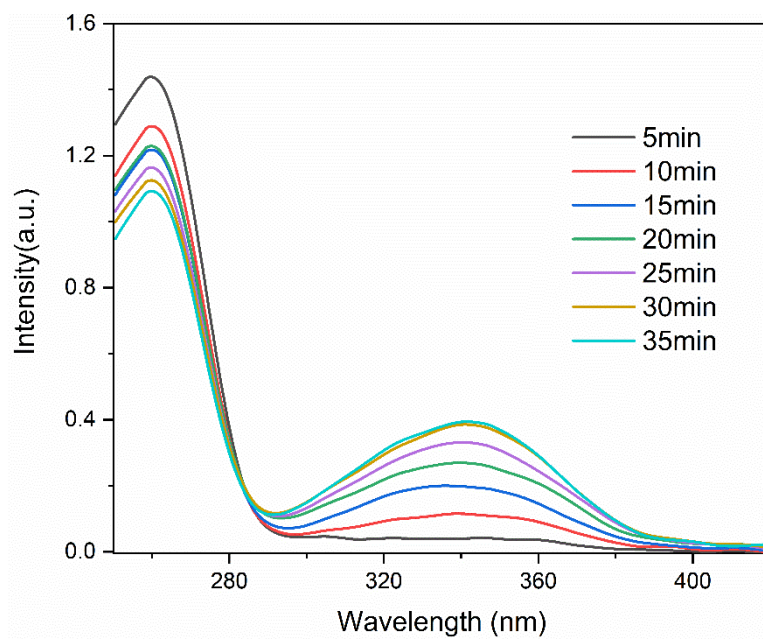


Figure S9. UV-Vis spectrophotometry of Rh-NU-1000-GF at different times. Each sample was prepared by diluting 0.4 mL of the solution with 2.6 mL of Tris-HCl buffer.

2.9 The effect of pyruvate to NADH regeneration.

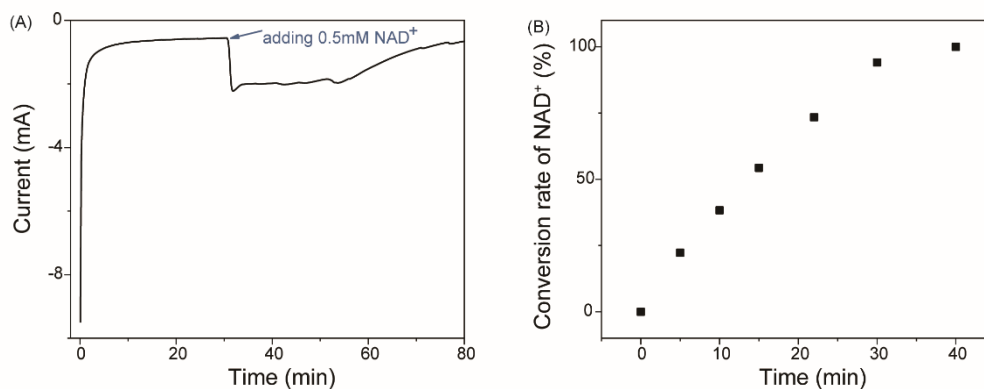


Figure S10. (A) Amperometric response recorded at an applied potential of -0.5 V vs. H_2 with Rh-NU-1000-GF electrode after addition of 0.5 mM NAD^+ in 20 mM pyruvate of 40 mL Tris-HCl buffer (1 M, pH=7.0). (B) Conversion of NADH as a function of time. The electrochemical experiments were carried out under N_2 and the size of GF electrode was $1 \times 2 \times 0.5 \text{ cm}^3$, and H_2 and solution flowrates were at 20 mL/min.

2.10 UV-vis spectroscopy of NADH and standard curve (0-10 μM) and the conversion of 10 μM NAD^+ .

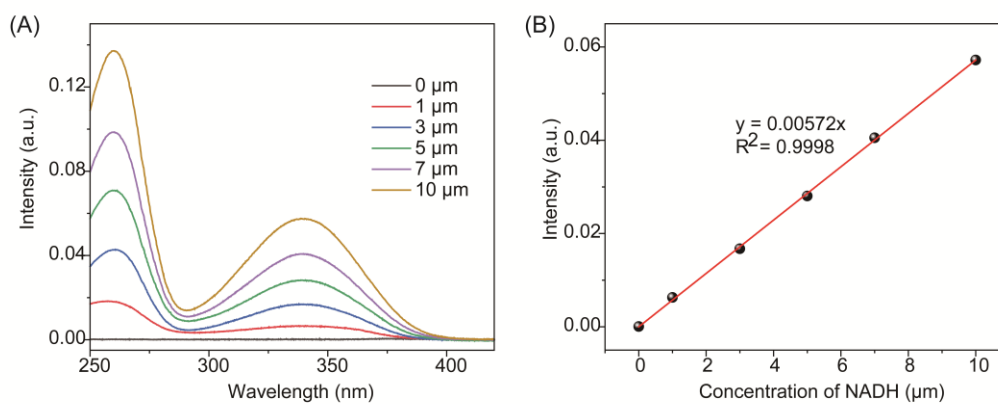


Figure 11. (A) UV-visible absorbance of different concentration of 1,4-NADH in Tris-HCl solution (1M, pH=7.0). (B) Calibration curve of absorption of 1,4-NADH at 340 nm.

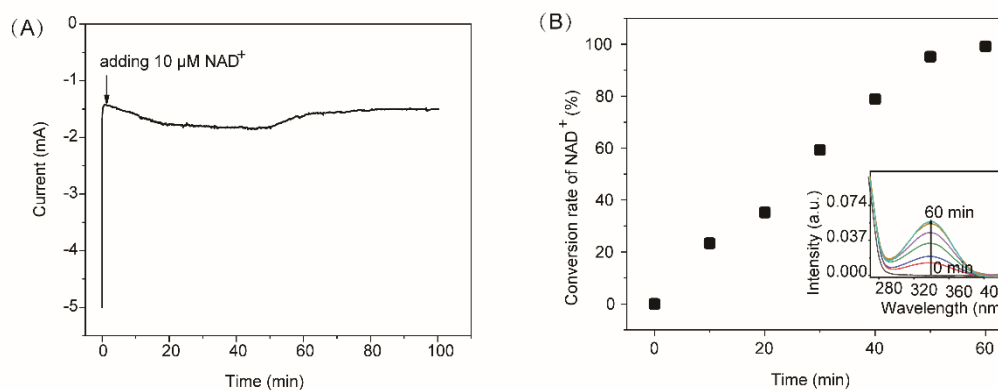


Figure 12. (A) Amperometric response recorded at an applied potential of -0.5 V vs. H_2 with Rh-NU-1000-GF electrode after addition of 10 μM NAD^+ in 40 mL Tris-HCl buffer (1 M, pH=7.0). (B) Conversion of NADH as a function of time and the insert is the corresponding UV-vis absorption spectra. The electrochemical experiments were carried out under N_2 and the size of GF electrode was $1 \times 2 \times 0.5 \text{ cm}^3$, and H_2 and solution flowrates were at 20 mL/min.

2.11 HPLC data of bioconversion.

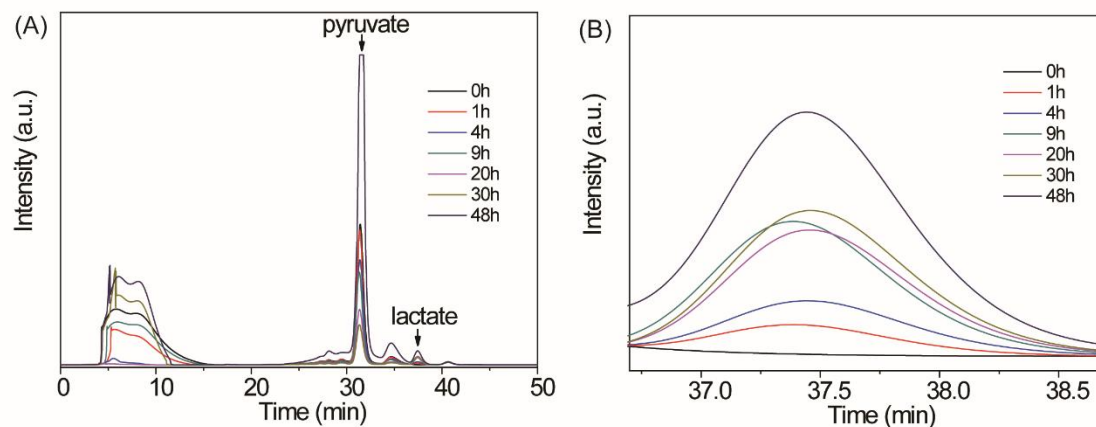


Figure S13. (A) HPLC chromatograms of production versus time. (B) Zoom of the HPLC chromatograms of production versus time. The bioconversion experiment was carried out at an applied potential of -0.5 V vs. H_2 in Tris-HCl buffer (1M, pH=7.0, 40 mL) under N_2 with a flow rate of 20 mL/min and hydrogen flow rate of 20 mL/min. The size of the electrode was $1 \times 2 \times 0.5$ cm³.

3. Calculation part

3.1 Calculation of Faradaic efficiency

A Faradaic efficiency could be calibrated by the formula:

$$\eta_F = \frac{2nF}{\int_0^t I dt} \times 100\%$$

Where F is the Faraday constant (96485 C/mol), n is the moles of generated of NADH, I is the current in A, and t is the reaction time in s.

As for bioconversion in bulk solution, the concentration of generated of NADH was 5 mM, the volume of the solution was 10 mL, so the mole of NADH was:

$$n_{NADH} = C_{NADH} \times V_{solution} = 5 \text{ mM} \times 10 \text{ mL} = 5 \times 10^{-5} \text{ mol}$$

The charge in bioconversion was:

$$Q = 11.91 \text{ C}$$

Therefore, the faradaic efficiency for the bioconversion in bulk solution was:

$$\eta_{bulk \ solution} = 81\%$$

In the same way, the faradaic efficiency for the bioconversion in flow bioreactor was:

$$\eta_{flow \ bioreactor} = 87\%$$

3.2 Calculation of total turnover number (TTN) ,turnover frequency (TOF) and Faradaic effieccy

The amount of Rh complex within the electrode could be calculated by the area from cyclic voltametric characterization.

$$Area_{CV} = 2nF = 0.0069 \text{ C}$$

Where F is the Faraday constant (96485 C/mol), n is number of moles of Rh complex, I is the current in A, and t is the time in s. So, the moles of Rh complex should be:

$$n_{Rh} = \frac{Area_{CV}}{2F} = 3.58 \times 10^{-8} \text{ mol}$$

A TTN of Rh cold be calculated by the formula:

$$TTN_{Rh} = \frac{C_{lactate}V}{n_{Rh}}$$

Where $C_{lactate}$ is the concentration of lactate (17.5 mM), V is the volume of the solution (40 mL). So, TTN_{Rh} is 1.96×10^4 .

A TOF of Rh could be calculated by the formula:

$$TOF_{Rh} = \frac{TTN_{Rh}}{t}$$

Where t is the initial first hour reaction time. So, TOF_{Rh} is $2.35 \times 10^3 \text{ h}^{-1}$. The choice of 1 h reaction allows to determine the rate of this regeneration at the beginning of the synthesis, in conditions that are less limited by the availability of the substrate. In other words, TTN was calculated over the whole reaction time, providing information about the robustness of the system and TOF was calculated at the beginning of the synthesis, at the moment the rate is the highest.

The TTN and TOF of NADH can be calculated in the same way. Therefore:

$$TTN_{NAD^+} = 1750$$

$$TOF_{NAD^+} = 210 \text{ h}^{-1}$$

3.3 Calculation of Faradaic efficiency in bioconversion

A Faradaic efficiency could be calibrated by the formula

$$\eta_F = \frac{2nF}{Q} \times 100\%$$

Where F is the Faraday constant (96485 C/mol), n is the moles of generated of Lactate, Q is total charge during the whole reaction.

The mole of generated of Lactate is:

$$n_{Lac} = C_{Lac} \times V_{solution} = 17.5 \times 10^{-3} M/L \times 40 \times 10^{-3} L = 7 \times 10^{-4} mol$$

Q could be determined from the charge measured by the electrochemical workstation. Before adding pyruvate to the solution, the initial charge was 6.98 C. After the reaction was complete, the final charge measured 143.59 C. Therefore:

$$Q = 143.59 - 6.98 = 136.61 C$$

Therefore:

$$\eta_F = \frac{2nF}{Q} \times 100\% = \frac{2 \times 7 \times 10^{-4} mol \times 96485}{136.61 C} \times 100\% = 98.9\%$$

Reference

- [1] Y. Chen, P. Li, J. Zhou, C. T. Buru, L. Đorđević, P. Li, X. Zhang, M. M. Cetin, J. F. Stoddart, S. I. Stupp, M. R. Wasielewski, O. K. Farha, J. Am. Chem. Soc. **2020**, 142, 1768–1773.
- [2] W. Li, C. Zhang, Z. Zheng, X. Zhang, L. Zhang, A. Kuhn, ACS Appl. Mater. Interfaces **2022**, 14, 46673–46681.
- [3] W. E. Housseini, F. Lopicque, S. Pontvianne, N. Vilà, I. Mazurenko, A. Walcarius, M. Etienne, ChemElectroChem **2022**, 9, e202200463.