

Electronic Supplementary Information

A Hydrogen Fuel Cell for Rapid, Enzyme-Catalysed Organic Synthesis with Continuous Monitoring

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Experimental methods

Fabrication of FNR@ITO/Ti cathodes

To make the electrodes for synthesis, a layer of indium tin oxide (ITO) was deposited on sheets of Ti foil (thickness: 0.127 mm, Aldrich) using electrophoresis. Electrophoretic deposition (EPD) was also used to make electrodes for cyclic voltammetry, where ITO glass was the substrate. The Ti foil was first sonicated in acetone, ethanol, and MQ water for 15 min successively, and then dried in an oven at 80 °C. The ITO nanopowder (0.02 g, Aldrich) was mixed with iodine (0.01 g, Alfa Aesar) dissolved in acetone (20 ml, Aldrich) and sonicated for at least 45 min. The counter electrode used for EPD was a second piece of Ti foil which was held in parallel with its counterpart. The distance between the two pieces of Ti was 1–2 cm and the applied voltage was 10 V. A deposition time of 3–4 minutes was used for each side of the Ti foil to ensure that the ITO film was as uniform as possible. Typically, 8–10 double-side electrodes were prepared in a single session by suspending them from a wire (like a ‘washing line’).

Purification of ferredoxin-NADP⁺-reductase

Purification of the His-tagged FNR used in this paper was carried out as described previously.¹

Electrochemical measurements

All electrochemical measurements were made using an Ivium potentiostat (Ivium CompactStat, Ivium Technologies). For cyclic voltammetry, the reference and counter electrodes were SCE

and Pt wire respectively. Potentials were adjusted to the standard hydrogen electrode (SHE) scale using $E_{\text{SHE}} = E_{\text{SCE}} + 0.242 \text{ V}$ at 25 °C. To scale against the reversible hydrogen electrode, the zero-current potential for the Pt/carbon electrode under 1 atm H_2 was used as the reference point for each experiment. For the electrosynthesis experiments, the FNR@ITO/Ti electrode was connected to the Pt/carbon electrode via the potentiostat and the current was monitored at zero applied voltage. The total charge passed for the electrosynthesis was determined by integration after correcting for steady background current. The H_2 flow rate was 12 scc/min, controlled by a mass flow controller (MFC, Sierra Instruments).

Electrodeposition of Platinum

Toray carbon paper (PTFE treated) was obtained from Alfa Aesar and cleaned in the same way as the Ti foil. The electrolyte for Pt deposition was a mixture of 5 mM H_2ClPt_6 (Aldrich) and 0.5 M H_2SO_4 (Fisher). A saturated calomel electrode (SCE) and a Pt wire were employed as the reference and counter electrodes, respectively. The carbon paper was cut into a long strip and Pt was deposited using two sequential potential applications. The first stage involved applying a potential of -0.32 V vs SCE for 40 s, and the second stage involved applying a potential of -0.25 V vs. SCE for 360 s.

Reagents and NMR analysis

All solutions for electrochemistry were prepared with MQ water (18.2 M Ω /cm, Millipore). Stock solutions of 2-oxoglutarate (Aldrich) and NH_4^+ (either NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$, Aldrich) of certain concentrations were prepared for several experiments. The L-glutamate dehydrogenase (GLDH) was obtained from Sigma (G2501) as a suspension in 2M $(\text{NH}_4)_2\text{SO}_4$: the same batch was used for all experiments, where in each case the final concentration of GLDH was 0.209 mg ml⁻¹. A supply of NADP⁺ (monosodium salt) was obtained from Melford. The cell solutions for ¹H NMR analysis were supplemented with 10 % D_2O , and spectra were recorded using a Bruker AVIIIHD 400 spectrometer. Calibration curves were constructed using known concentrations of L-glutamate (Aldrich).

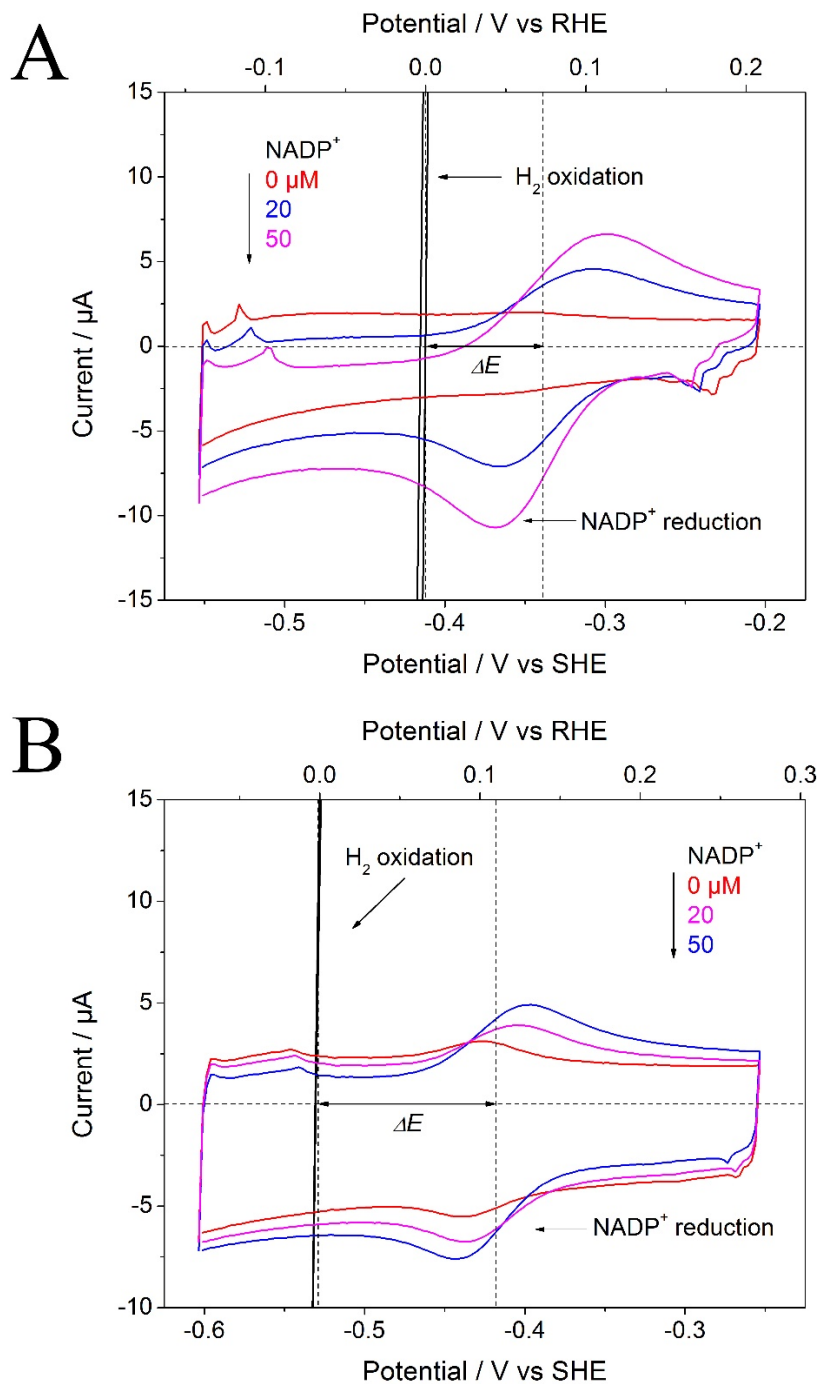


Figure S1. Cyclic voltammograms of $\text{NADP}^+/\text{NADPH}$ interconversion at a FNR@ITO/ITO glass working electrode (area 1.2 cm^2) with different concentrations of NADP^+ , recorded at $25 \text{ }^\circ\text{C}$ (scan rate: 1 mV s^{-1}) under Ar. Each panel includes the reversible electrochemistry of H_2 recorded at a platinised carbon paper (PCP, 1.8 cm^2) electrode, by cycling at 5 mV s^{-1} under a flow of 100% H_2 . Solution conditions are: A) 50 mM MES buffer + 50 mM NaCl (pH 7.0), and B) 50 mM borate buffer (pH = 9.0).

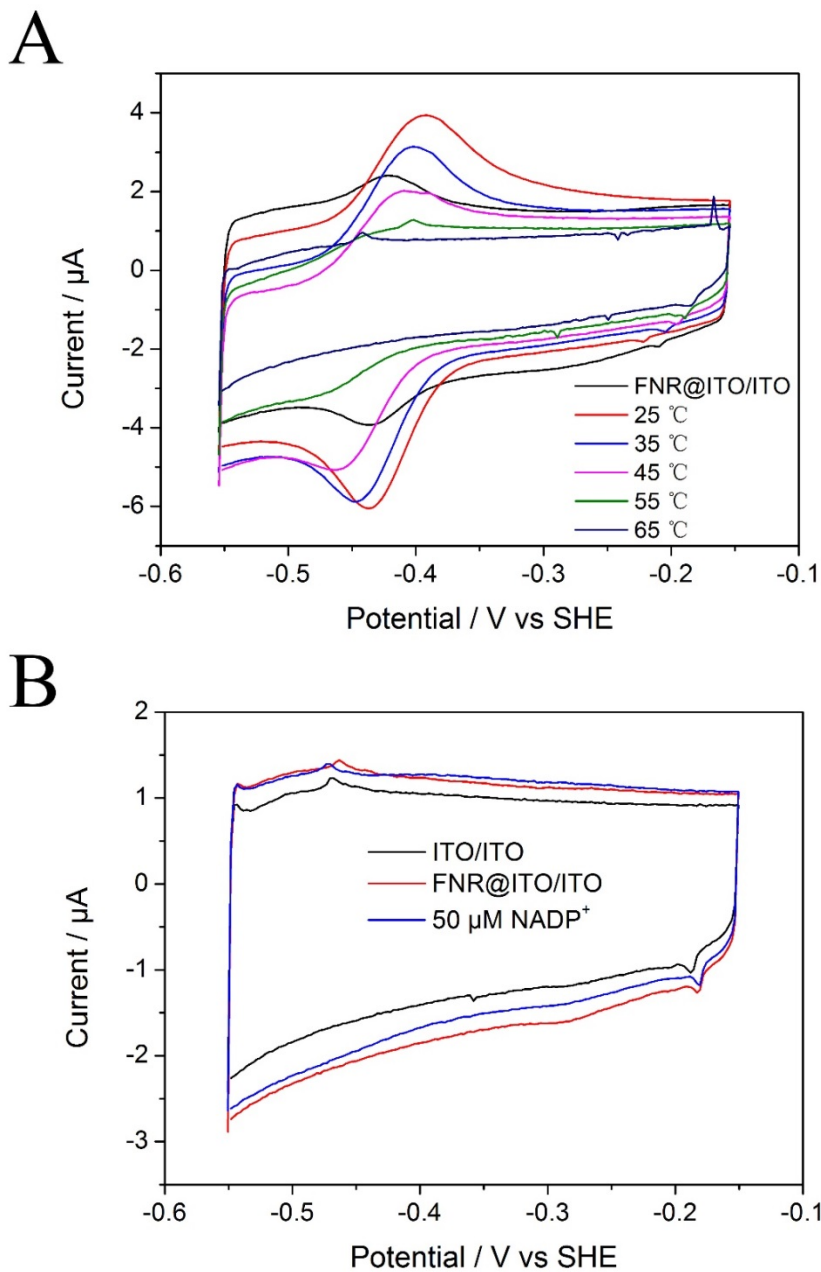


Figure S2. Cyclic voltammograms (CVs) showing the effect of elevated temperature on the activity of a FNR@ITO/ITO glass working electrode (area 1 cm^2) under an Ar atmosphere. In both cases, 50 mM borate buffer was used as the electrolyte at pH = 9.0 and the scan rate was 1 mV s^{-1} . In (A) the non-turnover peak due to FNR was first recorded at 25 °C, then NADP⁺ was added to give a concentration of 50 μM . The temperature was increased to 65 °C, at which point electrochemical activity was no longer detectable. In (B) a background CV of the ITO/ITO glass was first recorded at 25 °C before denatured FNR (incubated at 70 °C for 30 min) was applied to the electrode surface. The CVs were then recorded with and without 50 μM NADP⁺.

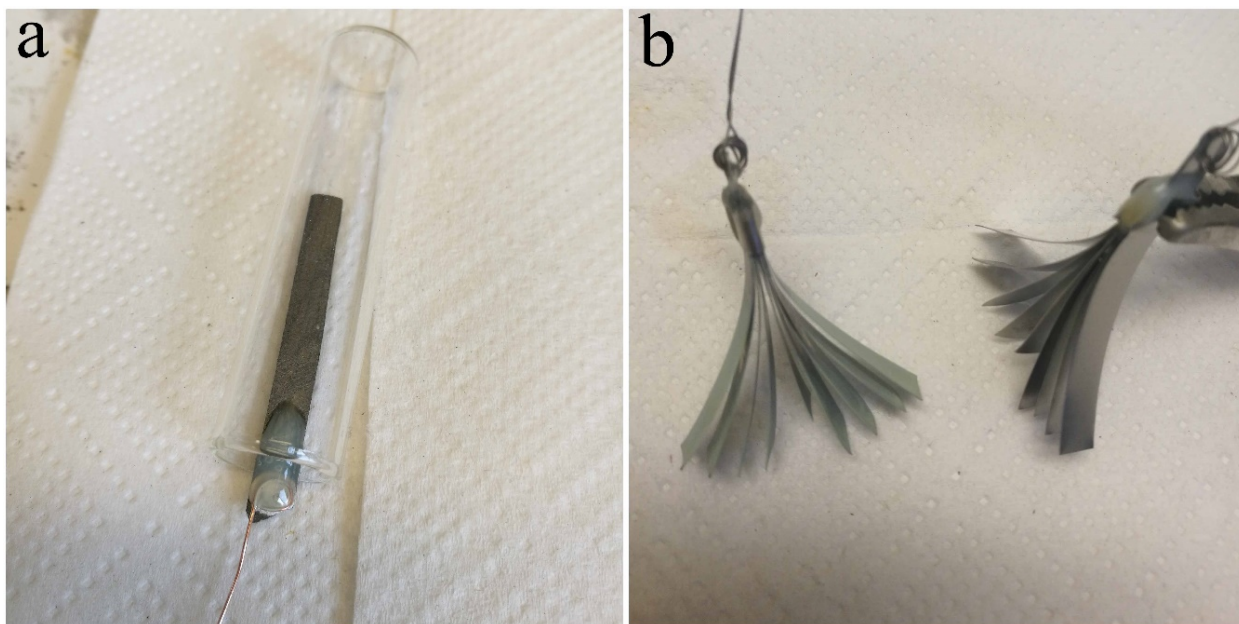


Figure S3. a) The PCP anode (1.6 cm^2) shown inside a typical cell that was used for the fuel cell reaction; b) The 'book' of cathodes. Shown on the left is the complete FNR@ITO/Ti electrode; shown on the right is the bare Ti electrode before depositing ITO.

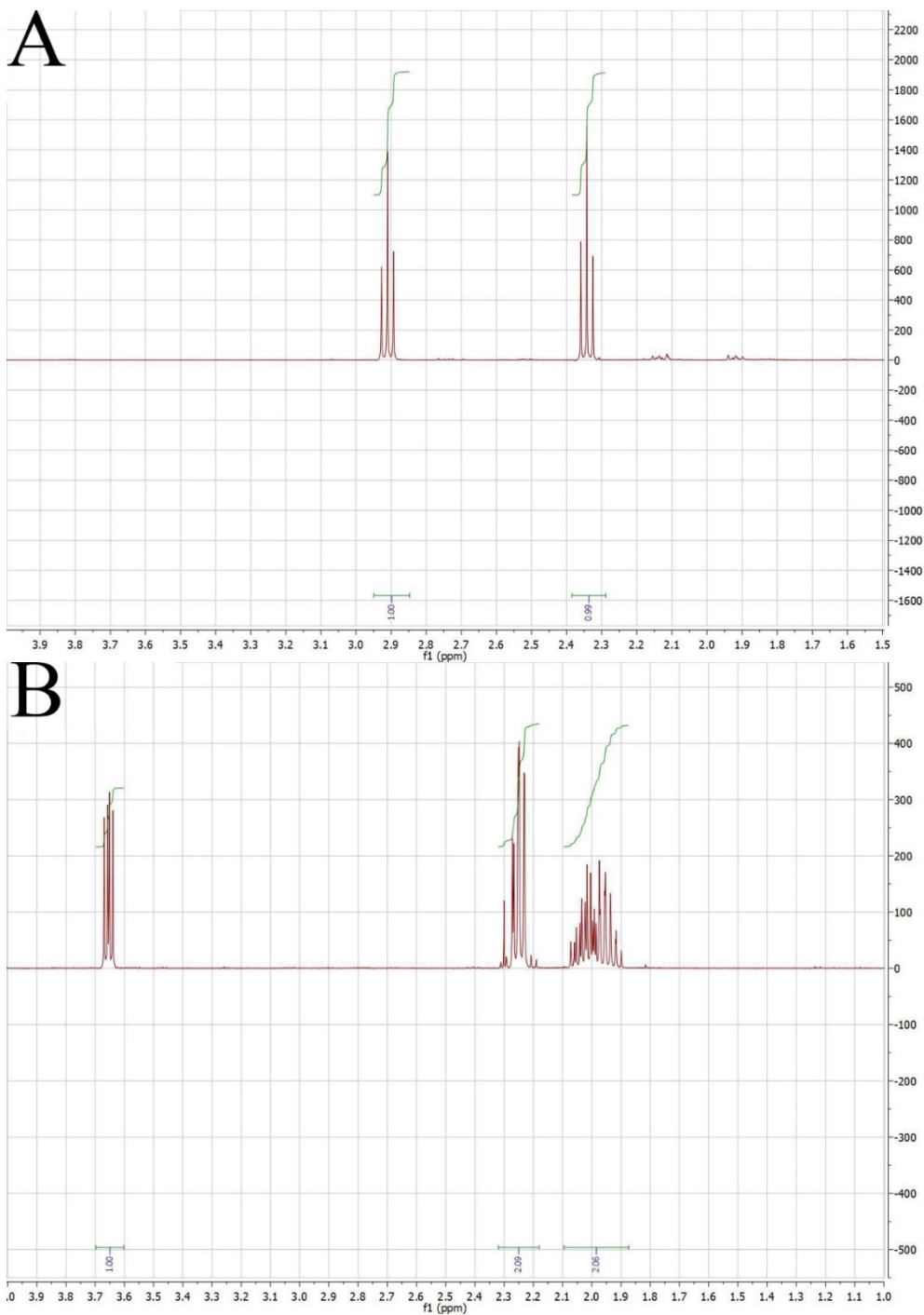


Figure S4. Typical NMR spectra of cell solutions. The example shown corresponds to entry C of Table 1 . **A)** Initial cell solution, showing integrals for 2-oxoglutarate (δ 2.34 (t, 2H), 2.91 (t, 2H)). **B)** After chronoamperometry for 19.3 h, showing integrals for L-glutamate (δ 1.99 (m, 2H), 2.25 (m, 2H), 3.65 (q, 1H)): note the absence of signals due to 2-oxoglutarate.

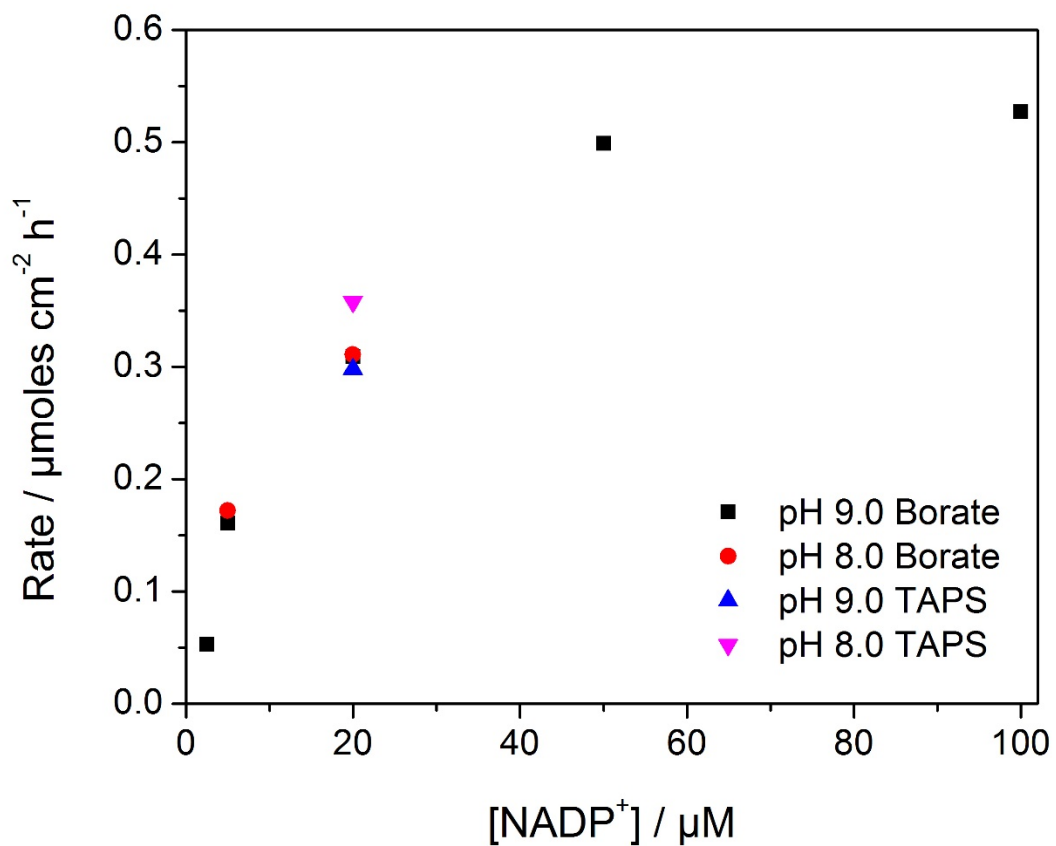


Figure S5. Compilation of results displayed as ‘average rate’ (see main text) vs [NADP⁺]. The rates were obtained by dividing the amount of L-Glu product by the active electrode area and total reaction time (from the point at which GLDH was injected until the moment before full depletion of the reactant).

Notes and References

1. B. Siritanaratkul, C. F. Megarity, T. G. Roberts, T. O. Samuels, M. Winkler, J. H. Warner, T. Happe and F. A. Armstrong, *Chem. Sci.*, 2017, **8**, 4579-4586.