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Supporting Information

Enzyme-Modified Particles for Selective Biocatalytic Hydrogenation by Hydrogen-Driven NADH Recycling

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Further details of experimental conditions for experiments described in the main text

Table S1: The quantities of enzyme and carbon in the aliquots of enzyme-modified particles used for each experiment.

Figure / Table Entry	Mole ratio hydrogenase / NAD^+ reductase	Hydrogenase	Mass hydrogenase / µg	Mass NAD^+ reductase / µg	Mass carbon / µg	Mass ADH / µg
Fig. 1 (=Table 1 Entry 1)	0.7	*E. coli*	1.6	6.7	200	-
Table 1 Entry 2	22.7	*D. vulgaris*	15.0	2.5	100	-
Table 1 Entry 3	8.8	*D. vulgaris*	17.5	7.5	60	-
Table 2 Entry 1	2.5	*D. vulgaris*	10.0	15.0	100	-
Table 2 Entry 2	7.6	*D. vulgaris*	10.0	5.0	100	-
Table 2 Entry 3	22.7	*D. vulgaris*	15.0	2.5	100	-
Fig. 2(a)	9.5	*D. vulgaris*	2.7	1.1	70	-
Fig. 2(b and c)	3.9	*E. coli*	2.3	1.9	70	-
Fig. 3	3.9	*E. coli*	4.0	3.2	80	-
Fig. 4, 5, Table 3	2.0	*E. coli*	8.3	13.3	33	340

Error bars indicated on data in the main text are calculated from standard deviations observed when measurements are conducted in triplicate.

Control experiments to confirm the requirement for hydrogenase, NAD^+ reductase and carbon particles

Control experiments as set out in Table S1 were performed to confirm that the hydrogenase and NAD^+ reductase must be co-immobilised on particles for efficient H_2-driven NADH generation. UV-visible spectral traces showing the absence or formation of NADH (peak at 340 nm) from each of these experiments are shown in Figure S1. These experiments confirm that hydrogenase alone, or NAD^+ reductase alone, or carbon alone, or a mixture of the two enzymes without particles, are not able to carry out H_2-driven NADH generation. Only the experiment in which hydrogenase and NAD^+ reductase were exposed to carbon particles before addition to H_2-saturated NAD^+ led to the generation of NADH.

Table S2. Details of the control experiments shown in SI Fig. 1 and the concentration of NADH detected by UV-vis after 30 minutes.

Entry	H	N	C	NADH generated / mM	Experimental notes
1	-	-	-	N.D.	No enzyme added
2	✔	-	-	N.D.	Hydrogenase only
3	-	✔	-	N.D.	NAD^+ reductase only
4	-	-	✔	N.D.	Carbon particles only
5	✔	✔	-	N.D.	Hydrogenase and NAD^+ reductase added successively
6	✔	✔	-	N.D.	Hydrogenase and NAD^+ reductase premixed and injected together
7	✔	✔	✔	0.37 ± 0.2	Hydrogenase and NAD^+ reductase-modified particles

Hydrogenase (H); NAD^+ reductase (N); Carbon (C); Not Detected (N.D.). Other conditions: H_2-saturated Tris-HCl buffer, pH 8.0, 30 ^oC, 1 mM NAD^+.

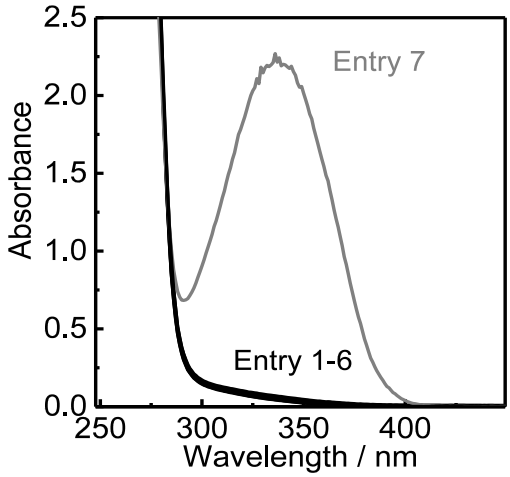

Figure S1. UV-vis spectra recorded 30 minutes after addition of the enzyme or enzyme particle mixture to H_2-saturated NAD^+ (1 mM).

Using other carbon materials as an electronically conducting support for enzyme catalysed H_2-driven NADH generation

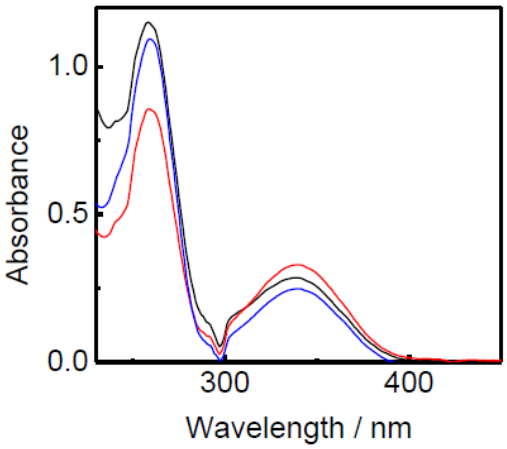

Figure S2: UV-Vis spectra confirming that a range of carbon materials modified with both *D. vulgaris* MF hydrogenase and NAD^+ reductase (HoxFU)[1] are able to catalyse H_2-driven NADH generation. For each experiment the carbon material was first exposed to a mixture of hydrogenase and NAD^+ reductase before addition to H_2 saturated NAD^+ (2 mM in mixed buffer pH 7). Spectra were recorded after 5 hours. Carbon materials used: carbon paper (black), single walled carbon nanotubes (red) and carbon nanopowder (blue). The presence of a peak at 340 nm confirms generation of NADH in each experiment.

Detection of NADH

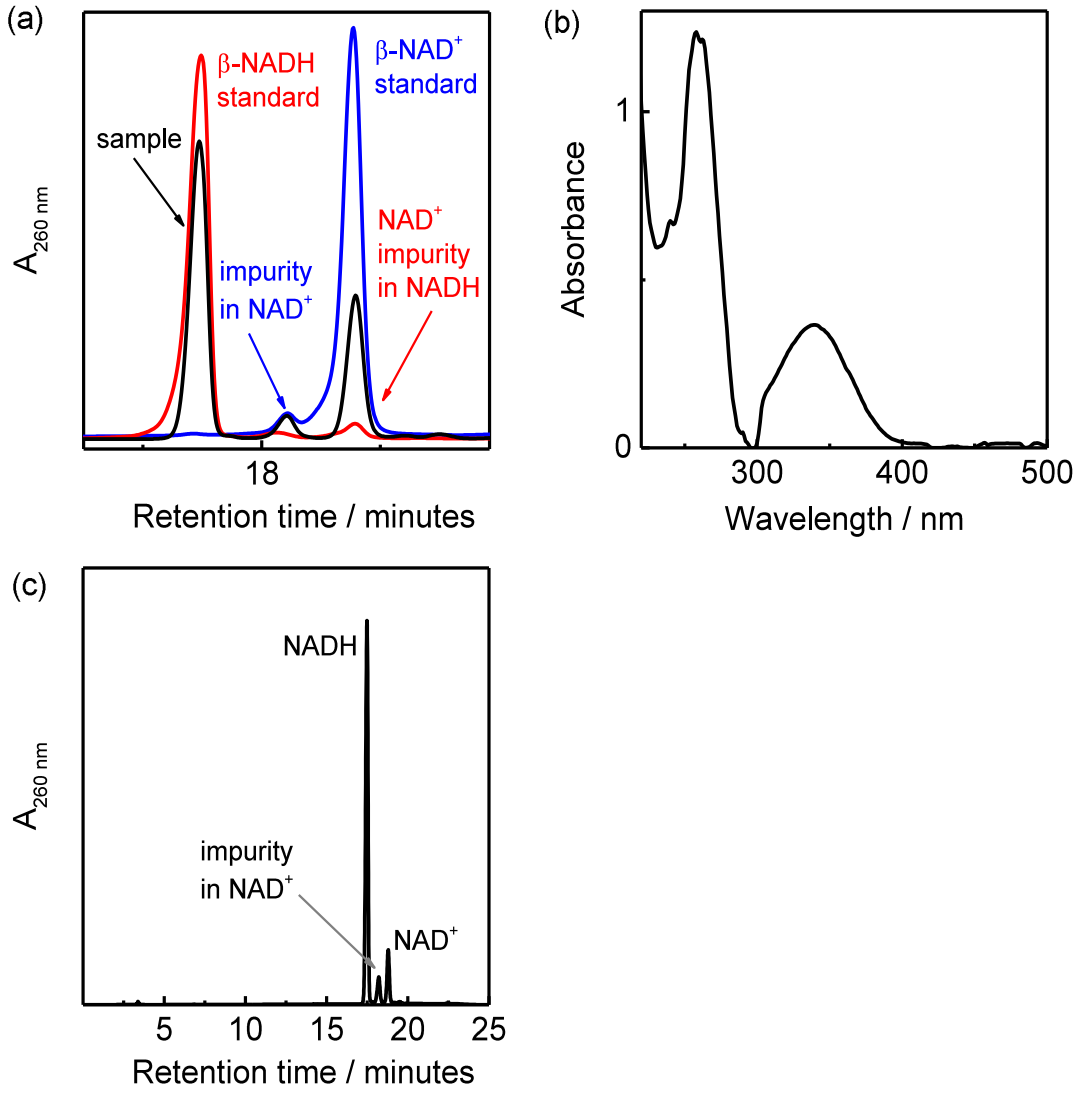

Figure S3. (a) Chromatogram of as-supplied NAD^+ (blue) and NADH (red) and the final reaction solution after addition of H_2 (1 bar) and enzyme-modified particles (black). The as-supplied NAD^+ contains an impurity (18.2 minutes) and the NADH contains a small amount of NAD^+; the chromatograms (blue and black) are normalised to the impurity. (b) Significant conversion (*ca* 75 %) of NAD^+ to NADH was confirmed using UV-vis spectroscopy. (c) The whole chromatogram of the final reaction solution shows only NADH, NAD^+ and the impurity, with no other species detected, demonstrating the high selectivity of the enzyme-modified particle system. Separation achieved using a Zic-Hilic column, a gradient from 20:80 A:B to 80:20 A:B over 30 minutes (A = 20 mM ammonium acetate in water, B = 20 mM ammonium acetate in acetonitrile).

Comparison of electrocatalytic properties of the two NiFe hydrogenases used in the study

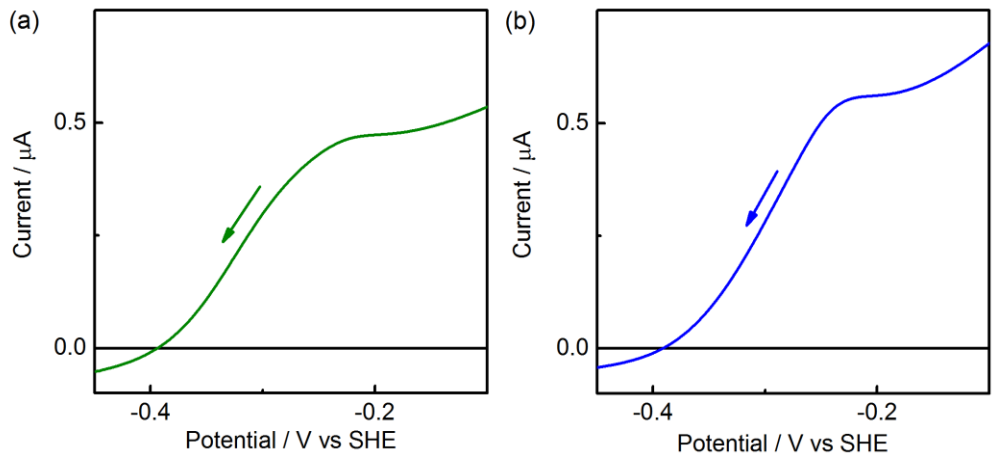

Figure S4. Protein film electrochemistry data comparing the electrocatalytic properties of immobilised (a*) Desulfovibrio vulgaris Miyazaki F* NiFe hydrogenase, and (b) *Escherichia coli* NiFe hydrogenase 2, in the potential range relevant for coupling H_2 oxidation to NAD^+ reduction. The hydrogenases were immobilised by adsorption from dilute solution (comparable to that used for preparing the enzyme-modified particles for experiments described in the main text) on a pyrolytic graphite rotating disk electrode of diameter *ca* 2 mm. Linear sweep electrochemical traces were performed under an atmosphere of 1 bar H_2, in potassium phosphate buffer (pH 7.0) at 25 ^oC, at a scan rate of 10 mV s^{-1} with the electrode rotating at 2000 rpm to provide efficient mass transport. The direction of scan is indicated by an arrow. The electrochemistry was controlled by an Autolab 128N potentiostat (Metrohm), and the electrode rotation was controlled by an EG&G electrode rotator interfaced with a glass electrochemical cell with gas inlet and outlet fittings to control the headspace gas.

In each case, the current crosses the *x*-axis at the thermodynamic potential for the H^+/H_2 couple corrected for the experimental conditions, with H^+ reduction (negative current) occurring at potentials more negative than this value, and H_2 oxidation (positive current) occurring at more positive potentials. In a protein film electrochemistry experiment, the electrocatalytic current for an enzyme film is directly proportional to the activity of the immobilised enzyme sample, and these experiments confirm that the two hydrogenases show very similar activity when adsorbed under comparable conditions onto carbon. Each hydrogenase undergoes slow, reversible oxidative inactivation at high potentials, and this gives rise to the small re-activation peak on the sweeps at *ca* -0.22 V vs SHE.

HPLC detection of acetophenone and phenylethanol

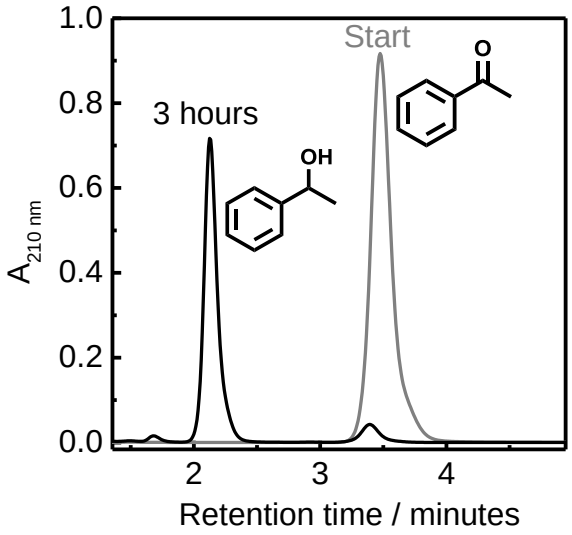

Figure S5. Particles modified with hydrogenase, NAD^+ reductase and ADH sustain H_2-driven acetophenone reduction in H_2-saturated un-buffered MilliQ water containing only NAD^+ (1 mM) and acetophenone (10 mM). HPLC trace at the start (grey) and after 3 hours (black) shows ca 90 % conversion of acetophenone to phenylethanol.

Method for determining NADH concentration

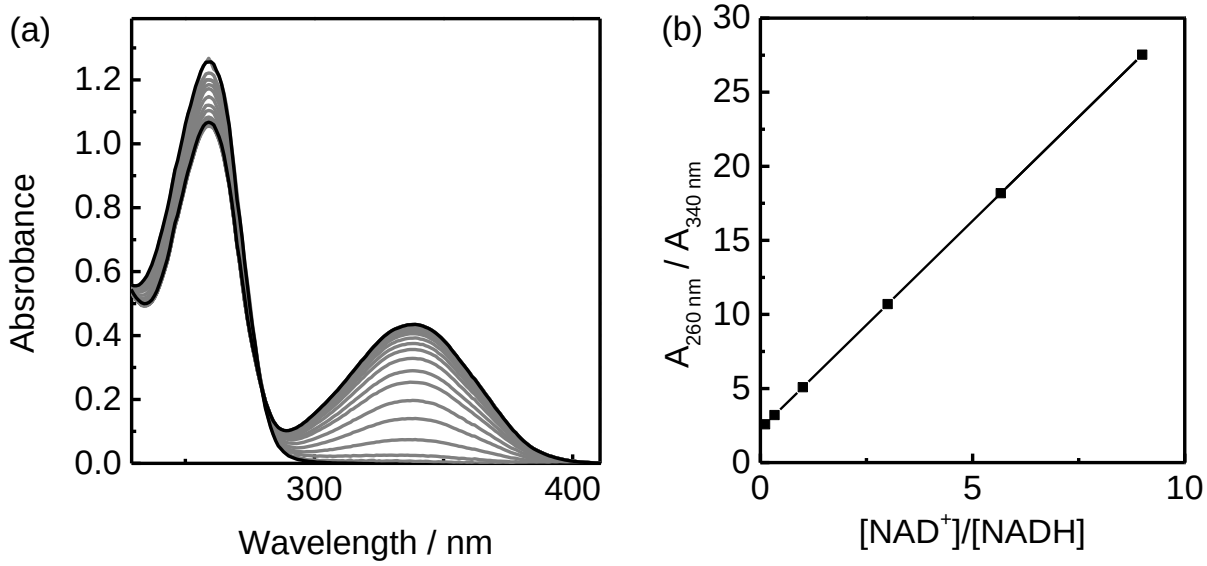

Figure S6. (a) The UV-vis spectra recorded every 1 minute after addition of hydrogenase and NAD^+ reductase modified particles into H_2-sautrated solution containing NAD^+ (70 μM). A simple baseline correction was used to account for the light scattering by particles in solution. This scattering did not vary significantly with wavelength. (b) Using the literature values of the extinction coefficients at 260 nm for NAD^+ (17.4 mM^{-1} cm^{-1}) and NADH (14.1 mM^{-1} cm^{-1}) and at 340 nm for NADH (6.22 mM cm^{-1}) a plot of $A_{260\ nm}/A_{340\ nm}$ *vs* $[NAD^+]/[NADH]$ can be constructed.[2] This approach is a good comparison to using only the absorbance at 340 nm as it is independent of slight changes to the overall concentration of the cofactor (caused by addition of the particle suspension or by evaporation on bubbling H_2 into solution). Furthermore, interaction of the cofactor and the buffer system means that the literature value of the extinction coefficient is higher than that recorded 'in house'.

Control experiments to test for background reaction of acetophone in water and buffer solutions

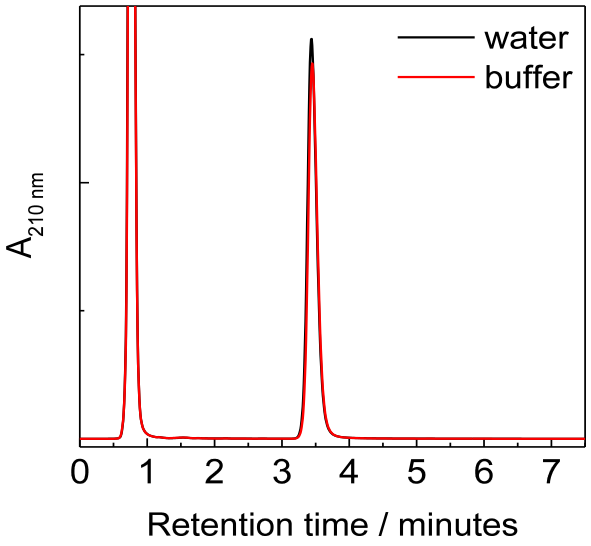

Figure S7. Samples of acetophenone (10 mM) left shaking in water (black) or Tris-HCl buffer (red) in the presence of H_2 and NADH (1 mM) were analysed using HPLC after 20 hours. This experiment demonstrates that there is no background reaction involving acetophenone in the absence of the enzymes.

References for Supporting Information

1. L. Lauterbach, Z. Idris, K. A. Vincent, O. Lenz, *Plos One* **2011**, *6*, e25939

2. E. Haid, P. Lehmann, J. Ziegenhorn, *Clin. Chem.* **1975**, *21*, 884-887.