Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supplementary Information

Attenuated total reflectance infrared spectroelectrochemistry at a carbon particle electrode; unmediated redox control of a [NiFe]-hydrogenase solution

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Curve fitting used to determine peak positions of active site bands

Spectral curve fitting was applied to equilibrium spectra (reported in Figure 3 A – D of the main text) in order to determine peak positions of the CO and CN bands in the active site of the regulatory [NiFe]-hydrogenase from *Ralstonia eutropha* (RH) in the Ni-S (oxidised) and Ni-C (reduced) states. The spectral lineshapes were fitted to Voigt profiles using OriginPro 8.5.1 (OriginLab Corporation), and a 3rd-order polynomial baseline correction was applied simultaneously. Figure S1 A and B show the result of this peak fitting for the Ni-S and Ni-C states, respectively. The measured peak positions agree well with previously reported spectra of the Ni-S and Ni-C states of RH by the groups of De Lacey[18] and Hildebrandt,[19] measured using as-isolated and H_2-reduced enzyme. Figure S1C shows a comparison of Ni-C *minus* Ni-S difference spectra calculated from both the experimental and fitted equilibrium spectra. Clear difference peaks can be seen corresponding to shifts in the CO band and both CN bands. It should be noted that the time-resolved difference spectra shown in Figure 3E of the main text do not contain an obvious feature arising from the peak shift of the lower wavenumber, less intense CN band at *ca* 2070 cm^{-1}, although this feature is clearly visible in both Figure S1C and the H_2-reduced difference spectrum in Figure 5 of the main text. This can be attributed to a combination of the shorter acquisition time (100 s rather than 250 s) and hence lower signal-to-noise ratio of the time-resolved spectra, and the inherently low intensity of difference features derived from weak, closely-separated bands.

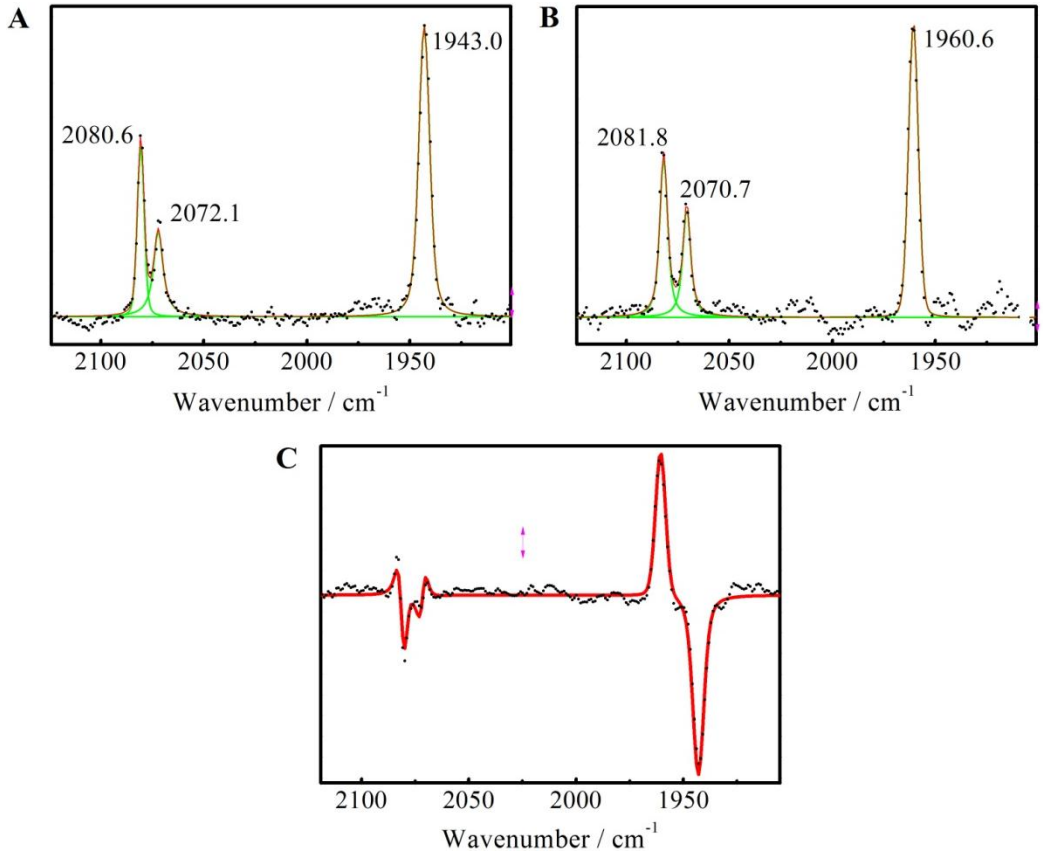

Figure S1. Infrared spectra of RH. Panels A and B: equilibrium experimental spectra (black points) and the results of peak fitting (individual peaks in green, sum in red) to determine positions of the CO and CN active site bands in the Ni-S (Panel A) and Ni-C (Panel B) states. Panel C: comparison of fitted (red line) and experimental (black points) Ni-C *minus* Ni-S difference spectra, calculated from data shown in Panels A and B.

Current response during spectroelectrochemical titrations

Figure S2A shows the current response at, and potential applied to, the working electrode as a function of time during recording of the spectroelectrochemical titration shown in Figure 4 of the main text. After each potential step there is a capacitive spike due to charging at the electrode-solution interface that decays over *ca* 15 seconds. The titration was carried out in both reductive (Figure S2A) and oxidative (Figure S2B) directions. Figure S2C shows the spectroelectrochemical titration derived from the oxidative sweep. Both titrations were measured using the same enzyme-loaded particle network electrode in a continuous experiment lasting *ca* 9 hours. The long-term stability of RH within this electrode is demonstrated in Figure S2D which compares spectra recorded at 0 V before (red) and after (black) the spectroelectrochemical titrations were recorded, at times indicated by the red arrows in Figure S2 A and B.

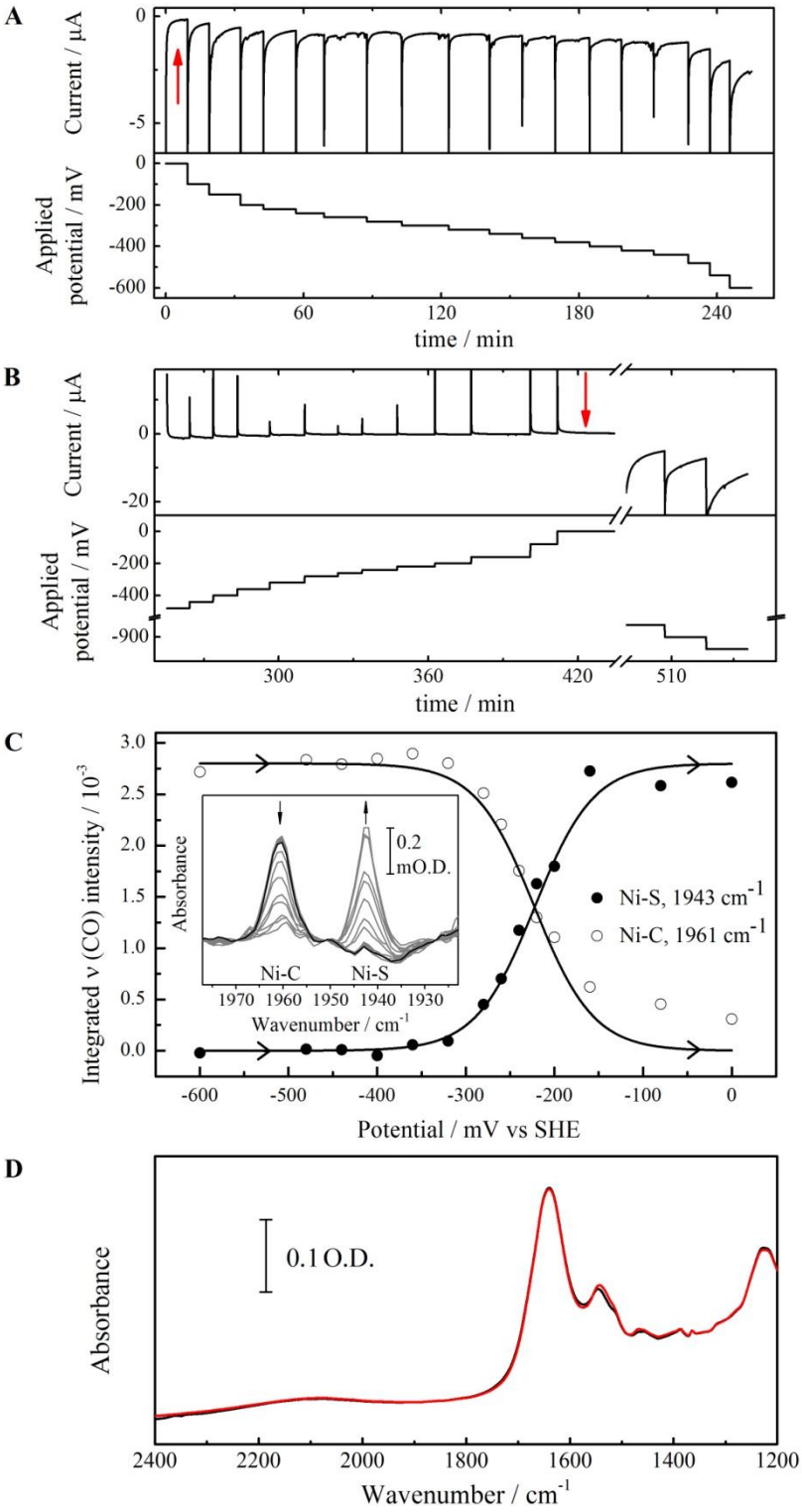

Figure S2. Panel A: Current-time and accompanying potential-time traces for potential steps during the reductive redox titration shown in Figure 4 of the main text, and additional steps at 0 V, -100 mV and -150 mV (no spectral changes were observed in this potential window). Panel B: Current-time and accompanying potential-time traces for potential steps during the subsequent titration in the oxidative direction. Potential steps between -800 and -1000 mV (shown in Figure 4 of the main text) are also shown. Panel C: Infrared spectroelectrochemical titration of RH in the oxidative direction (corresponding to the potential steps shown in Panel B), based upon the integrated intensity of ν(CO) stretching bands as a function of applied potential (see inset). Panel D: spectra in the amide region recorded at an applied potential of 0 V at the beginning (red) and end (black) of the reductive and oxidative titrations. All data in this figure were recorded using the same enzyme-loaded particle network electrode.