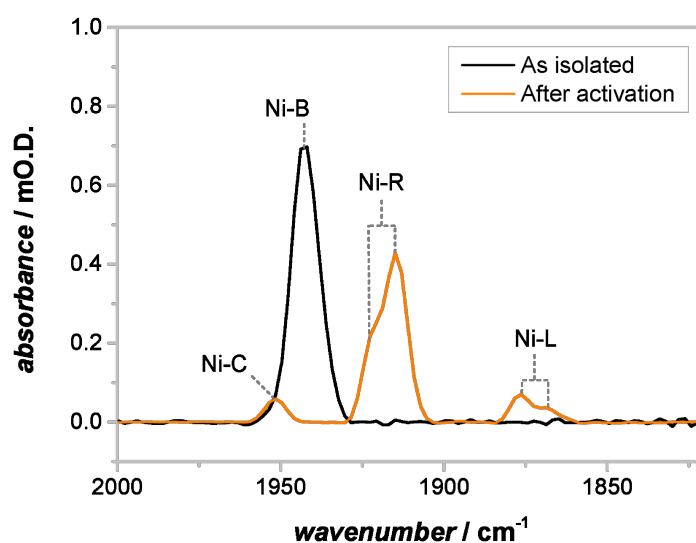


# Discovery of dark pH-dependent H<sup>+</sup> migration in a [NiFe]-hydrogenase and its mechanistic relevance: mobilizing the hydrido ligand of the Ni-C intermediate.

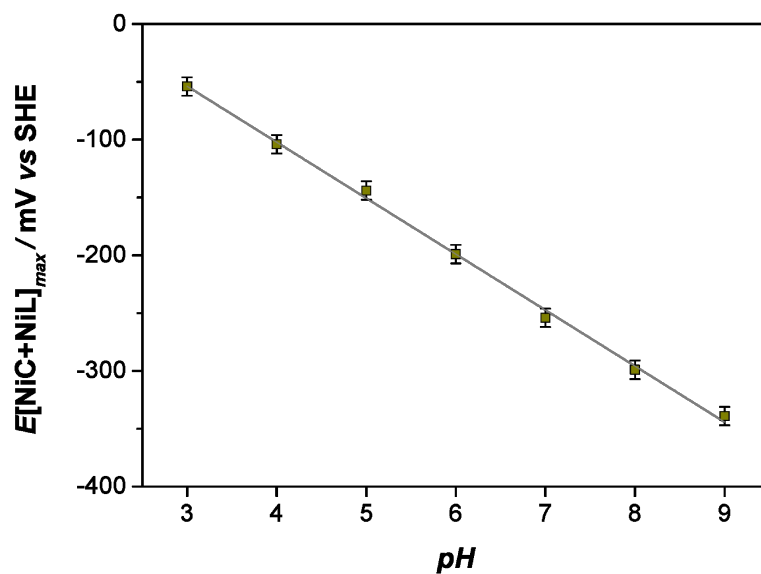
Bonnie J. Murphy<sup>a</sup>, Ricardo Hidalgo<sup>a</sup>, Maxie M. Roessler<sup>a,1</sup>, Rhiannon M. Evans<sup>a</sup>, Philip A. Ash<sup>a</sup>

William K. Myers,<sup>a,b</sup> Kylie A. Vincent<sup>a\*</sup> and Fraser A. Armstrong<sup>a\*</sup>

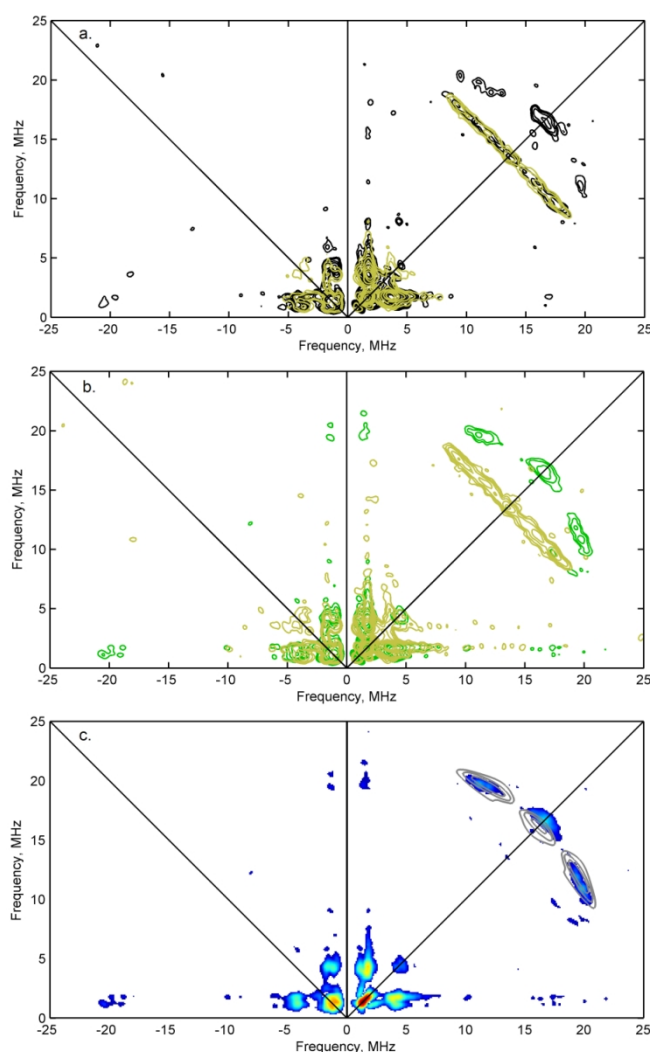
## Supporting Information



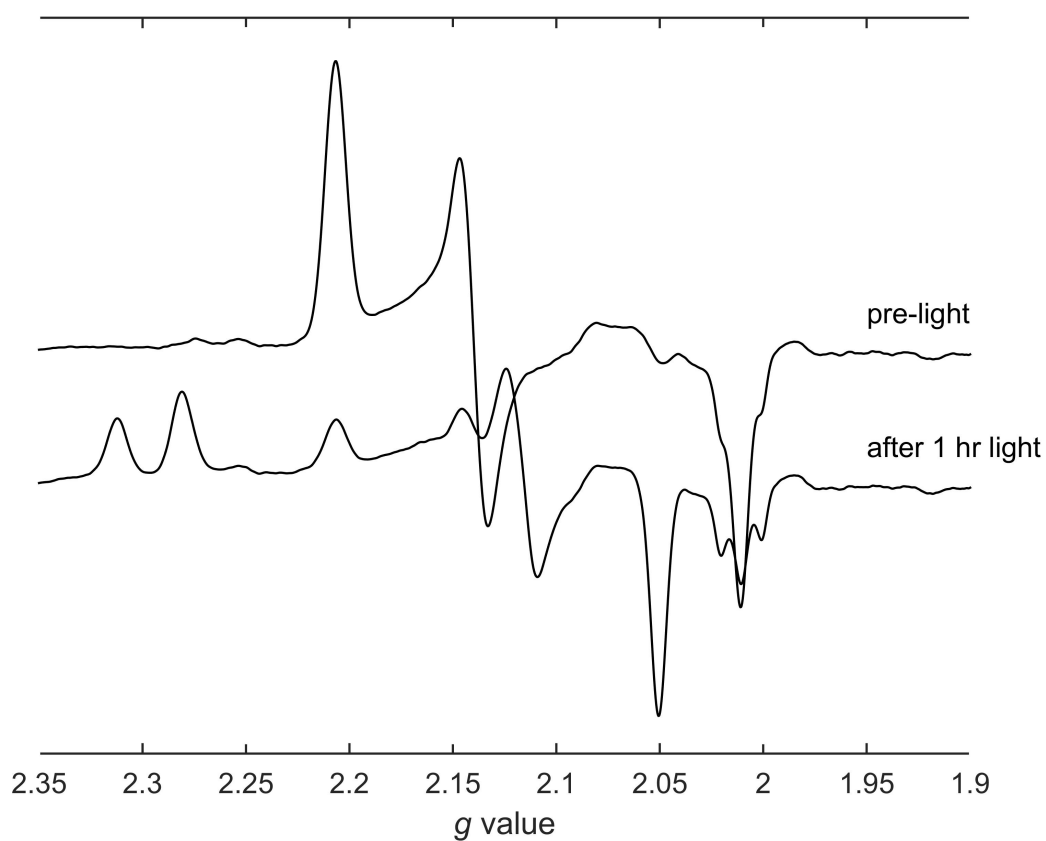
**Figure S1.** Infrared spectra of Hyd-1 adsorbed on carbon black particles before (black) and after (orange) activation under 1 bar H<sub>2</sub> in pH 6.0 buffered electrolyte. Spectra were recorded at 4 cm<sup>-1</sup> resolution with an acquisition time of 345 s. Spectra were baseline corrected only, using Origin 9.1.



**Figure S2.** Graph showing how the potential at which the Ni-C and Ni-L peaks have maximum combined intensity varies as a function of solution pH. The linear fit has a slope of  $-0.048\text{V/pH}$  unit, signifying coupling between a one-electron transfer and multiple ( $>1$ ) single protonation sites having a spread of  $pK$  values.



**Figure S3.** X-band HSCORE of wild-type Hyd-1 sample, pH 4.0, -126 mV vs. SHE, at  $g = 2.18$ . In Panel a., the sample equilibrated in the dark (black) with some mixture of Ni-L, is overlaid with that of an illuminated sample (dark yellow), prepared by 30 min. of laser pulses at 1 mJ pulse energy, 550 nm wavelength, and repetition rate of 20 Hz. In panel b., the spectrum after annealing for 90 min. at 200 K (green) is plotted against the spectrum of the illuminated sample (dark yellow). In panel c., the annealed sample is plotted in color, underlying a simulation (gray lines) that uses hyperfine values  $A(^1\text{H}) = [18.4 -10.8 -18.0]$  MHz, and Euler Angles  $(\alpha, \beta, \gamma) = (135, 129, 163)$  deg., from proton H3, identified by Lubitz and co-workers (Brecht, M.; van Gastel, M.; Buhrke, T.; Friedrich, B.; Lubitz, W. *J. Am. Chem. Soc.* 2003, *125*, 13075) as the bridging hydride. Data details are: 200 x 200 points with a time step,  $dt = 12$  ns,  $\tau = 140$  ns,  $t_1 = t_2 = 100$  ns, all pulses of 16 ns, at a temperature of 2.5K with a repetition time of 30 msec, and 7 shots per point.



**Figure S4.** X-band CW EPR spectra of Hyd-1 at pH 3.0, -60mV vs SHE showing the effect of illumination as described in the text. Conditions for measuring spectra: T = 130 K, microwave power = 2.0 mW, modulation amplitude 5.0 G.