Supplementary Information

The Mechanism of Nitrogenase H_2 Formation by Metal-Hydride Protonation Probed by Mediated Electrocatalysis and H/D Isotope Effects

Nimesh Khadka, Ross D. Milton, Sudipta Shaw, Dmitriy Lukoyanov, Dennis R. Dean, Shelley D. Minteer, Simone Raugei, Brian M. Hoffman, Lance C. Seefeldt

Figures

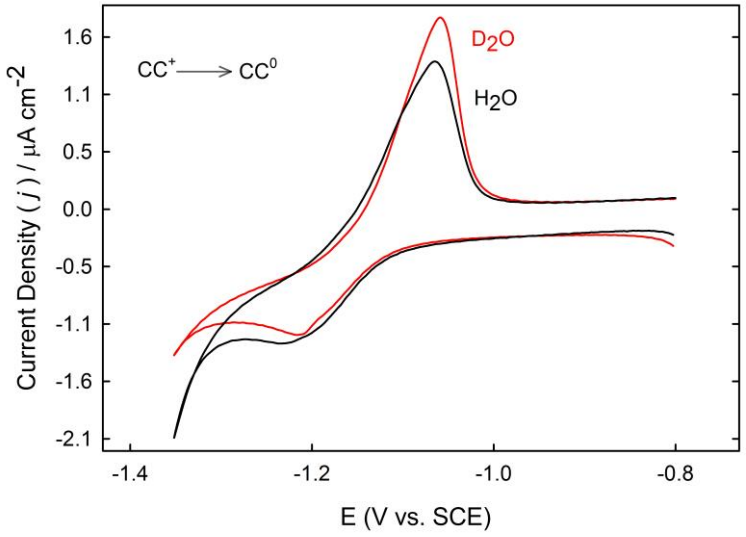

Figure S1: Representative cyclic voltammogram (CV) for cobaltocene in H_2O (black) and D_2O (red). Bovine serum albumin (stock 20 mg/mL) was encapsulated in the matrix, instead of the MoFe protein, as described in the **Material and Methods**. Conditions: 250 mM HEPES pH 7.2 (pD 6.8) with 50 µM cobaltocene. The scan was from positive to negative potential at a rate of 2 mV/s.

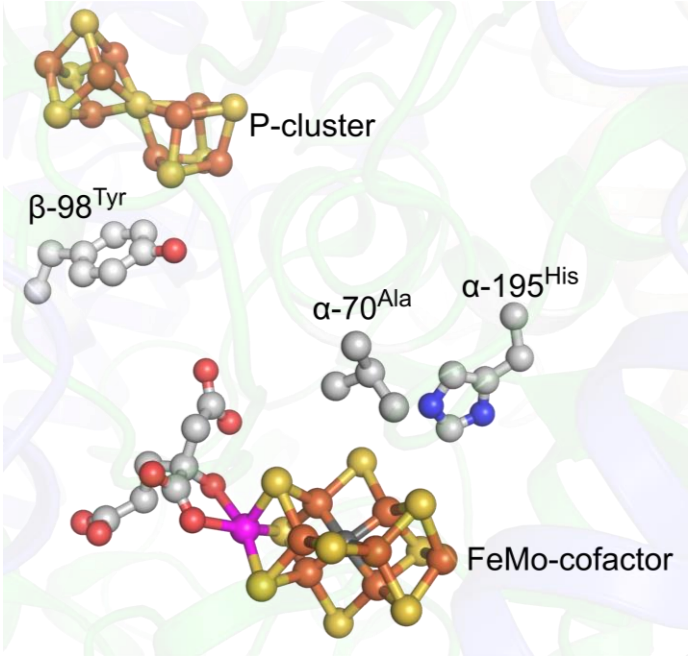

Figure S2: FeMo-co and key residues. Shown is a ball and stick representation of FeMo-cofactor along with the side chains of a few MoFe protein amino acids. Also shown is the P cluster for orientation. Element colors are Fe in rust, S in yellow, Mo in magenta, C in gray, O in red, and N in blue. Taken from PDB 2AFI.

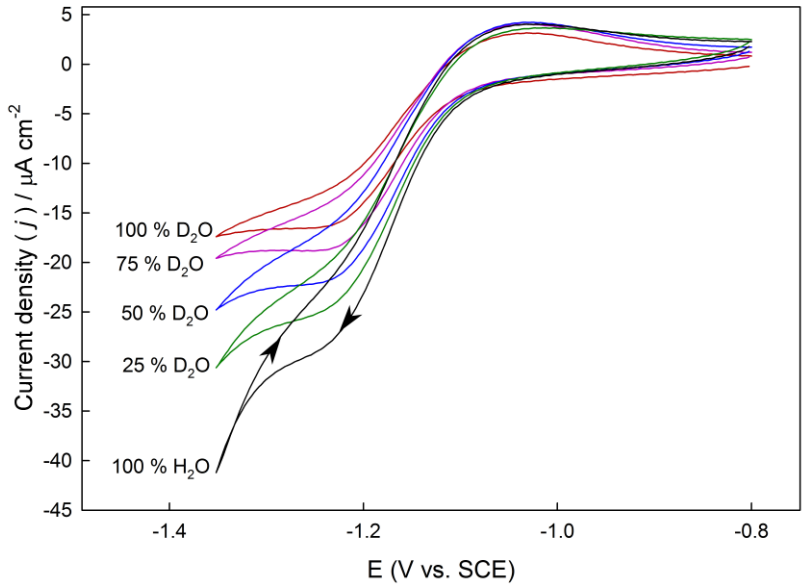

Figure S3. Cyclic voltammogram for α-70$^{Val \rightarrow Ile}$ MoFe protein under the same condition mentioned in **Figure 2**.

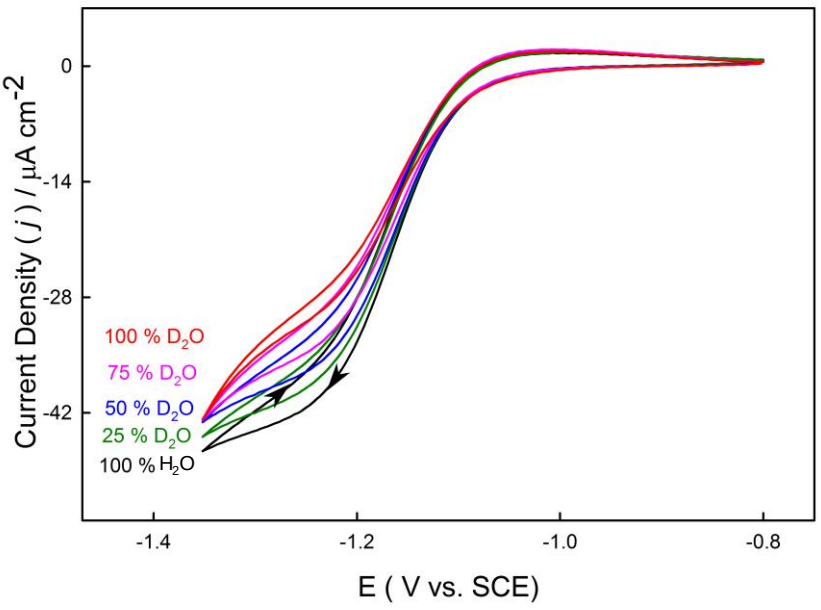

Figure S4. Cyclic voltammogram for α-70$^{Val→Ala}$ /α-195$^{His→Gln}$ MoFe protein under the same conditions as in **Figure 2**.

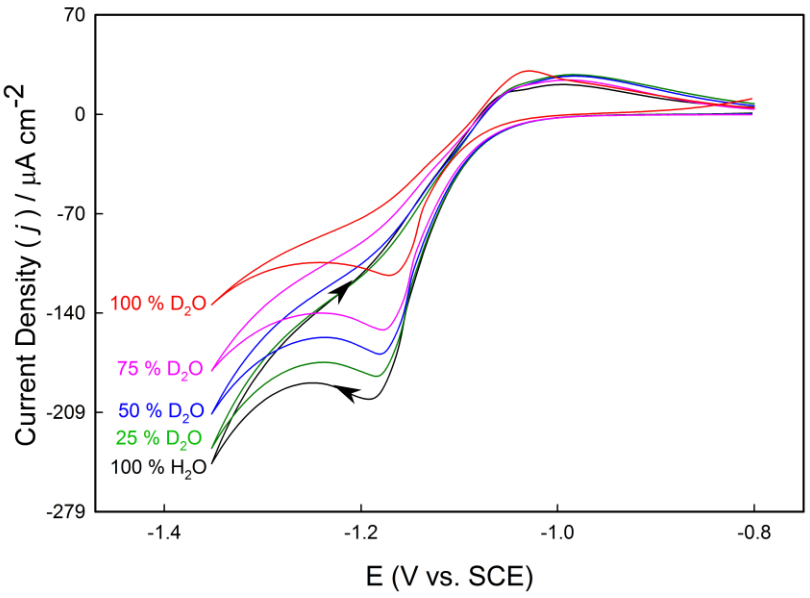

Figure S5. Cyclic voltammogram for β-98$^{Tyr\rightarrow His}$ MoFe protein under the same conditions as in **Figure 2**.

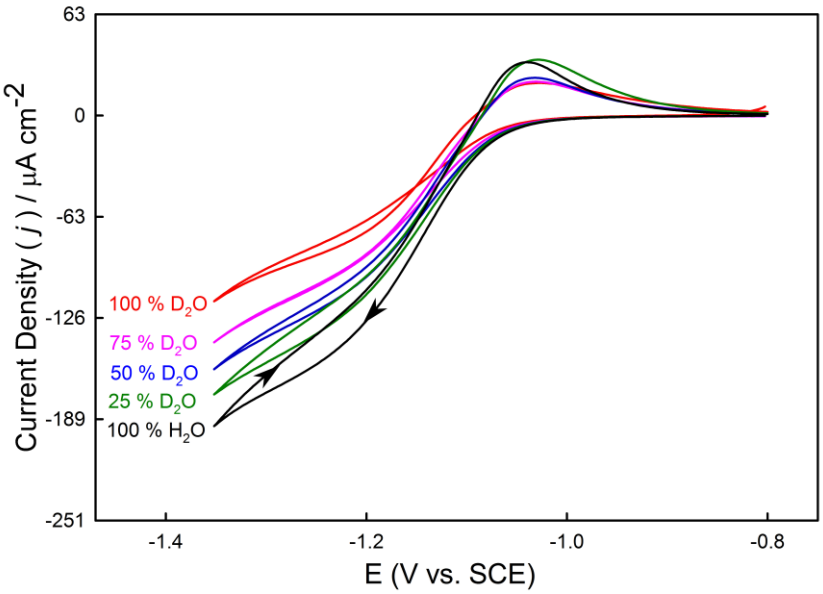

Figure S6. Cyclic voltammogram for nifX-FeMo-co under the same condition as in **Figure 2**.

Web Enhanced Objects:

Animation S1: Animation of the H_2 formation transition state. Shown is the oscillation from the $E_2(2H)$ state to the transition state $E_2(2H)^{‡}$ with the breaking S-H bond and the forming H-H bond as red dots. Atom colors are H in gray, Fe in rust, S in yellow, C in dark gray, and Mo is light blue.

Animation S2: Animation of the H_2 formation transition state. Shown is the progression from the $E_2(2H)$ state to the transition state $E_2(2H)^{‡}$ with the breaking S-H bond and the forming H-H bond as red dots. Atom colors are H in gray, Fe in rust, S in yellow, C in dark gray, and Mo is light blue.