Electronic Supplementary Material (ESI) for Chemical Communications.

Oxidative inactivation of NiFeSe hydrogenase. Supplementary Information

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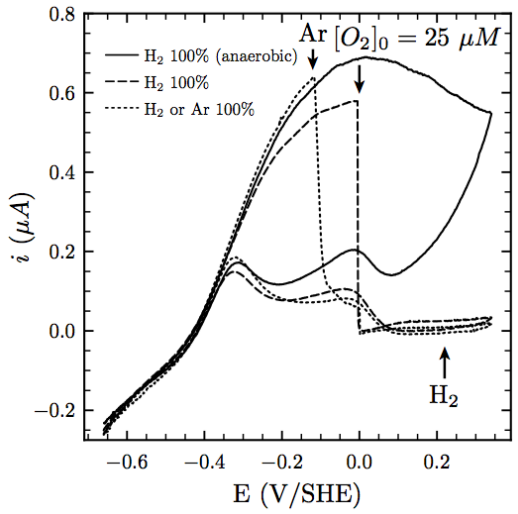

Fig. S1 Effect of O_2 on the formation of the two inactive states of DvH NiFeSe hydrogenase. The three experiments exemplify the effect of anaerobic inactivation under 1 atm. H_2 (plain line), aerobic inactivation under 1 atm. H_2 (dashed line), and aerobic inactivation in the absence of H_2 (dotted line). The three downward scan show the same reactivation pattern, demonstrating that aerobic and anaerobic inactivation produce the same inactive states. CVs recorded at 1mV/s, 50°C, 3.5 krpm, starting from −650mV, then scanning up to +350mV, and returning. The plain line shows an anaerobic experiment. In the experiments shown as dashed and dotted lines, an aliquot of O_2-saturated buffer was injected at 0mV on the forward scan, while the solution was either saturated with H_2 (dashed line), or H_2 had been removed by a stream of Ar (dotted line). In the latter case, H_2 was reintroduced at 0.2V on the return scan, so that all three downard scans are recorded under an atmosphere of 100% H_2.

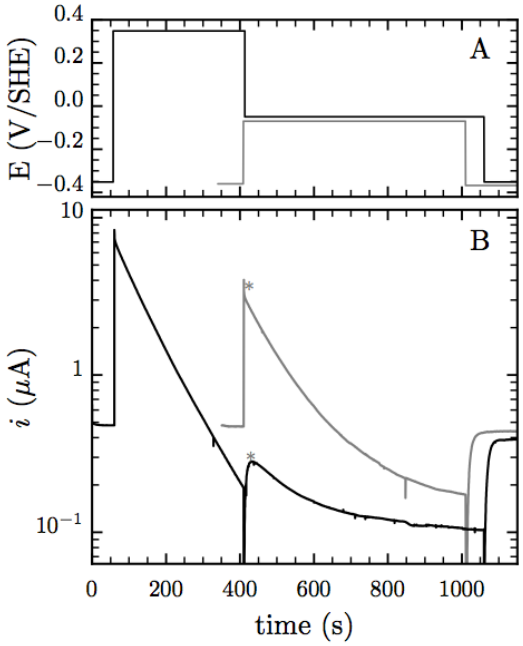

Fig. S2 Control experiment for main text fig 3. The black line is the same as in main text fig 3. The grey trace is a control experiment where we stepped the potential in the sequence −380/ −60/ −380mV, showing the inactivation at −60mV/SHE of an initially fully active enzyme. The grey trace was normalized so that the currents on the first step are the same for the two experiments. At about 420s (stars), the current in black is about 10% of that seen in the control experiment, with the fully active enzyme, showing that the transient faction of active enzyme is about 10%.

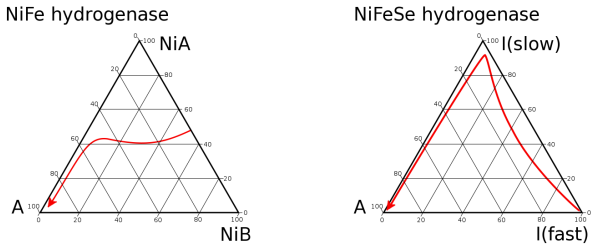

Fig. S3 Ternary diagram showing the reactivation"pathway", for an inactive state of hydrogenase. Left: reactivation of the Ni-B state of standard NiFe hydrogenase. Right: reactivation of the high potential ("fast") inactive state of NiFeSe hydrogenase, the two arrow showing the two transformations that occur in the CA experiments in main text fig 3 (right).
NB: These two-dimensional diagrams can be used to depict the composition of a system that consists of three components when one is not independent of the two others. Here, we consider that the hydrogenase is in a mixture of one active and two inactive forms (the three species add up to 100%), and every point on the ternary plot represents a different composition of the three species. The concentration of each species is 100% in each corner of the triangle and 0% at the line opposite it.