A "two-step" chronoamperometric method for studying the anaerobic inactivation of an oxygen tolerant NiFe hydrogenase Supplementary material

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S1 Influence of the high-potential slope of $i(E)$ on the value of E_{switch} in the limit of zero scan rate

We have derived the value of $E_{\text{switch}}^{v \to 0}$ in the stationnary limit, by assuming that $i(E)$ is independent on potential in the potential range of potential around $E_{\text{switch}}^{v \to 0}$ (eq. 18). However, the data in figure 5 suggest that $E_{\text{switch}}^{v \to 0}$ may fall in the region where $i(E)$ depends linearly on potential, as predicted by the model in reference 34. Let us now estimate how this shifts the position of the inflection point of $i(E) = i_A(E) \times A_\infty(E)$.

We are aiming at calculating the position of the inflection point of $i(E)$ defined by:

$$i(E) = \left[1 + \epsilon\left(E - E_{\text{switch}}^{v \to 0}\right)\right] A_\infty(E) \tag{S1}$$

where $A_\infty(E)$ is a function that displays an inflection point at $E_{\text{switch}}^{v \to 0}$. The second derivative of $i(E)$ is:

$$i''(E) = \left[1 + \epsilon\left(E - E_{\text{switch}}^{v \to 0}\right)\right] A_\infty''(E) + 2\,\epsilon\,A_\infty'(E) \tag{S2}$$

The inflection point of $i(E)$ is shifted with respect to that of $A_\infty(E)$ by a quantity δE, defined by:

$$i''(E_{\text{switch}}^{v \to 0} + \delta E) = 0 \tag{S3}$$

This translates into:

$$[1 + \epsilon\,\delta E]\,A_\infty''(E_{\text{switch}}^{v \to 0} + \delta E) + 2\,\epsilon\,A_\infty'(E_{\text{switch}}^{v \to 0} + \delta E) = 0 \tag{S4}$$

We estimate the values of $A_\infty''(E_{\text{switch}}^{v \to 0} + \delta E)$ and $A_\infty'(E_{\text{switch}}^{v \to 0} + \delta E)$ to the first order in δE:

$$A_\infty''(E_{\text{switch}}^{v \to 0} + \delta E) = A_\infty''(E_{\text{switch}}^{v \to 0}) + \delta E A_\infty'''(E_{\text{switch}}^{v \to 0}) = \delta E A_\infty'''(E_{\text{switch}}^{v \to 0}) \tag{S5}$$

$$A_\infty'(E_{\text{switch}}^{v \to 0} + \delta E) = A_\infty'(E_{\text{switch}}^{v \to 0}) + \delta E A_\infty''(E_{\text{switch}}^{v \to 0}) = A_\infty'(E_{\text{switch}}^{v \to 0}) \tag{S6}$$

Keeping only the first-order terms in δE gives:

$$\delta E = -\frac{2\,\epsilon\,A_\infty'(E_{\text{switch}}^{v \to 0})}{A_\infty'''(E_{\text{switch}}^{v \to 0})} \tag{S7}$$

Now, we equate $A_\infty(E)$ and $A_\infty(E)$, hence

$$\frac{A'_\infty(E^{v\to0}_{\text{switch}})}{A'''_\infty(E^{v\to0}_{\text{switch}})} = -\frac{2}{(\alpha\,f)^2} = 2.10^{-3} \text{ V}^2 \tag{S8}$$

with $\alpha = 0.66$. We identify $i(E)$ and $1 + \epsilon(E - E^{v\to0}_{\text{switch}})$, and a linear fit of the green trace of figure 5 returns a value of ϵ of about 2 V^{-1}. This gives:

$$\delta E = +13 \text{ mV} \tag{S9}$$

Under steady-state conditions, E_{switch} is therefore 10 mV to 15 mV greater than the position of the inflection point of $A_\infty(E)$, given by eq. 18 in the main text.

S2 The EC model

In the framework of scheme 1, the equations giving the evolution of the fractions of enzyme in the NiB, I and A forms read:

$$\frac{d[\text{NiB}]}{dt} = -k_r[\text{NiB}] + k_o[\text{I}] \tag{S10}$$

$$\frac{d[\text{A}]}{dt} = -k_{-1}[\text{A}] + k_1[\text{I}] \tag{S11}$$

$$1 = [\text{A}] + [\text{NiB}] + [\text{I}] \tag{S12}$$

We are interested in the evolution of the active fraction $A(t)$. The quantity $A' = [\text{A}] - [\text{A}]_\infty$, where $[\text{A}]_\infty$ is the asymtotic value for $[\text{A}]_\infty$, is governed by:

$$\frac{d^2A'}{dt^2} + (k_r + k_o + k_1 + k_{-1})\frac{dA'}{dt} + (k_r\,k_1 + k_r\,k_{-1} + k_o\,k_{-1})\,A' = 0 \tag{S13}$$

The general solution is:

$$A'(t) = A_+ \exp(-k_+\,t) + A_- \exp(-k_-\,t) \tag{S14}$$

with

$$k_\pm = \frac{k_r + k_o + k_1 + k_{-1} \pm \sqrt{(k_r + k_o + k_1 + k_{-1})^2 - 4\,(k_r\,k_1 + k_r\,k_{-1} + k_o\,k_{-1})}}{2} \tag{S15}$$

on condition that $k_+ \neq k_-$. A_+ and A_- are determined by the initial conditions. In general, the reaction scheme 1, leads to a bi-exponential relaxation. However, a mono-exponential relaxation can be observed in particular cases: (1) after a rapid equilibration between NiB and I (2) when either I, NiB or A is not accumulated (3) after a rapid equilibration between I and A. We rule out the latter because it would lead to a potential dependent k_I. In the following, we consider the two other cases.

Case 1 A rapid equilibrium between NiB and I occurs when $k_r + k_o \gg k_1 + k_{-1}$. Eq. S15 gives:

$$k_+ \approx k_o + k_r \qquad\qquad k_- \approx \frac{k_1\,k_r}{k_o + k_r} + k_{-1} \tag{S16}$$

The equilibration between NiB and I, which is determined by the high rate k_+, is too fast to be observed experimentally. The relaxation is governed by the slow rate k_-, which matches the value of $(k_A + k_I)$ given by eq. 21.

Case 2. The only possibility is that I is not accumulated. This occurs when $k_o \gg k_r + k_{-1}$ and

$k_1 \gg k_r + k_{-1}$, and leads to:

$$k_+ \approx k_o + k_1 \qquad\qquad k_- \approx \frac{k_r\, k_1 + k_o\, k_{-1}}{k_o + k_1} \tag{S17}$$

The depletion of I, which is determined by the high rate k_+, is not observed. The slow rate k_- determines the relaxation monitored during the experiment, and matches the value of $(k_A + k_I)$ given by eq. 21.

S3   The simplification of equations 22 and 23

Let us now derive the conditions of validity of the simplified equations 24 and 25. Equation 24 only holds when $k_3 \gg k_o$, i.e. for

$$E < E_{max\ A} = E^0 + \frac{1}{f\,(1-\alpha)} \ln \frac{k_3}{k_0} \tag{S18}$$

The fact that the experimental value of k_A does not deviate from that predicted by eq 24, (fig. 7) implies that $E_{max\ A}$ is larger than the potential at which the term k_{Alim} starts to dominate k_A (about +0.2 V for pH 8). Due to the relatively low value of $1 - \alpha = 0.4$, even relatively small values of the ratio k_3/k_0 lead to relatively large values of $E_{max\ A}$ with respect to E^0; for instance, for $k_3/k_0 = 10$, $E_{max\ A}$ is 150 mV greater than E^0.

Similarly, the condition for the validity of equation 25 is:

$$E > E_{lim\ I} \tag{S19}$$

where $E_{lim\ I}$ is the maximum of

$$E^{0\prime} - \frac{1}{(1-\alpha)f} \ln \frac{k_0'}{k_1} \qquad \text{and} \qquad E^{0\prime} - \frac{1}{\alpha f} \ln \frac{k_2}{k_0'} \tag{S20}$$

where the prime parameters characterize the redox couple I_2/I_1, by analogy with eqs 19. $E_{lim\ I}$ only needs to be superior to the lowest potential where the determination of k_I is unambiguous (ie, $k_I > 0.1 \times k_A$).

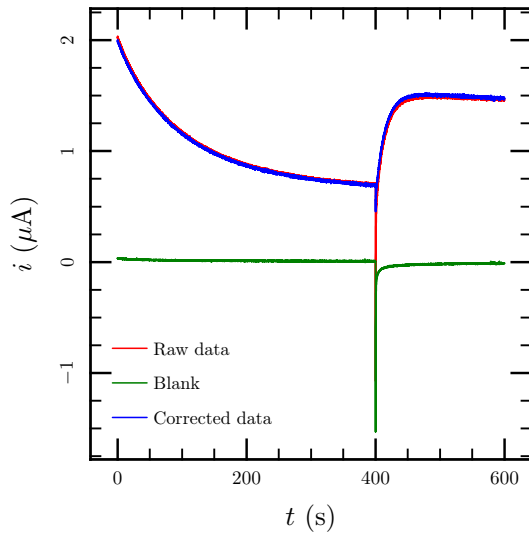## S4   Supplementary figures

Figure S1: Illustration of the effect of subtracting the capacitive current recorded in a control experiment with no adsorbed enzyme.
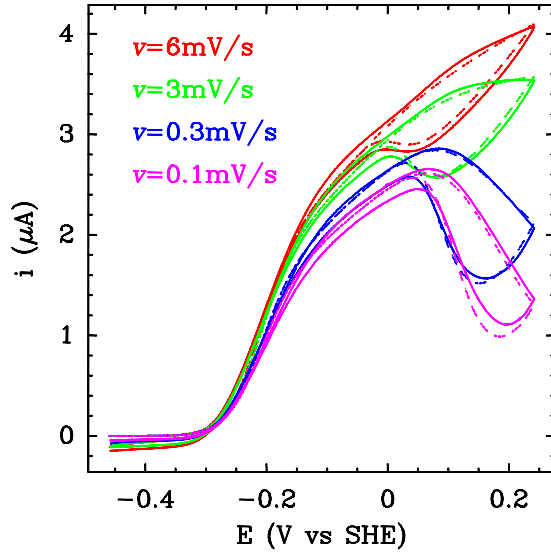
Figure S2: Cyclic voltammograms of adsorbed *A. aeolicus* hydrogenase at different scan rates (solid lines), and the corresponding simulations (dotted lines). Conditions: pH 6.1, 40°C, 1 atm. of H_2, $\omega = 4$ krpm. Parameters: $k_I = 3.5 \times 10^{-3}$ s^{-1}, $\alpha = 0.80$, $k_0 \exp \alpha f E^0 = 0.28$ s^{-1} and $k_{Alim} = 5 \times 10^{-4}$ s^{-1}, and the parameters describing the wave shape: $E_{OH} = -0.212$ V, $E_{HR} = -0.340$ V, $k_2/k_0^{max} = 4 \times 10^{-2}$ and $i_{lim}/\beta d_0 \approx 0.2$ μA (the exact value was adjusted to account for film loss). Film loss during the course of each voltammogram was neglected. The small differences with the parameters used in figure 5 in the main text results from the pH being slightly greater and, possibly, uncertainties in the concentration of dissolved H_2.
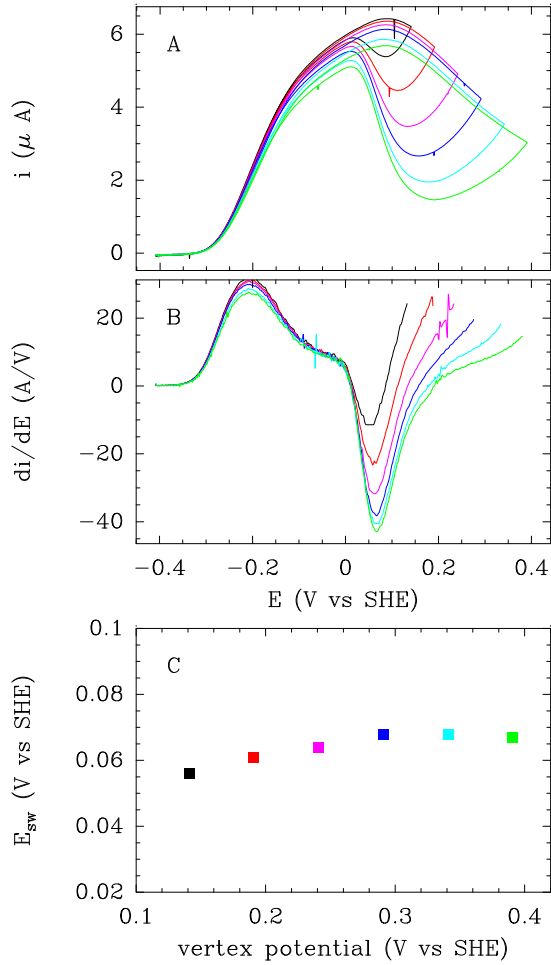

Figure S3: Influence on the value of the vertex (the potential where the direction of the scan is returned) on the value of E_{switch}. Panel A shows the voltammograms recorded with *A. aeolicus* NiFe hydrogenase adsorbed at a rotating electrode, at pH 6, 40°C, under 1 atm. H_2. Electrode rotation rate 3 krpm, scan rate 1.5 mV.s^{-1}. Panel B shows the 1$^{\text{st}}$ derivative of the sweeps towards negative potentials, from which the values of E_{switch} are determined. Panel C shows the values of E_{switch} against vertex potential. The color code is the same in all panels.
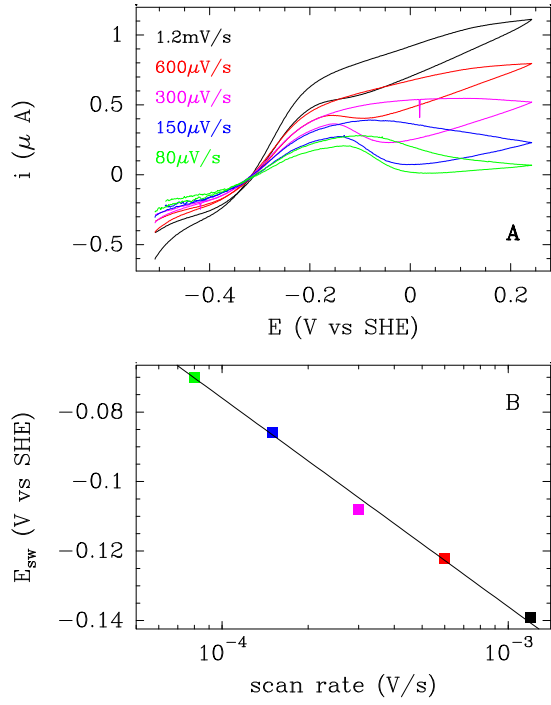
Figure S4: Influence of scan rate on the value of E_{switch} in experiments carried out with *D. fructosovorans* NiFe hydrogenase. Panel A shows the voltammograms recorded at pH 5.5, 40°C, under 0.1 atm. H_2. Electrode rotation rate 2.2 krpm, scan rate as indicated. Panel B shows the values of E_{switch} against scan rate. The color code is the same in panel A and B.
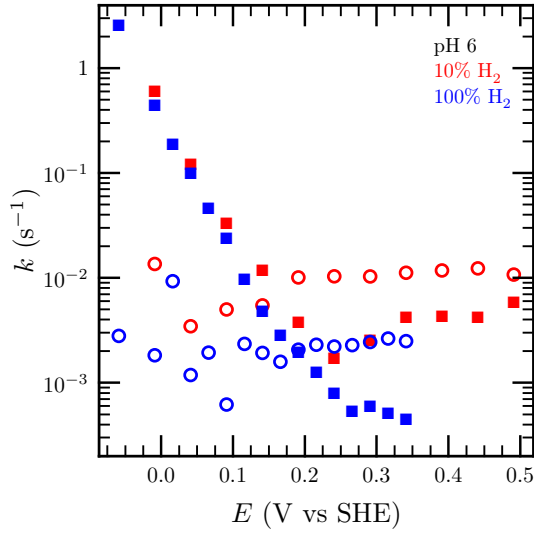

Figure S5: Effect of the variation of H_2 pressure on activation and inactivation rate constants of *A. aeolicus* NiFe hydrogenase. Open circles show the values of k_I, filled squares are the corresponding values of k_A. The red and blue symbol correspond to H_2 pressure of 0.1 and 1 atm, respectively. Other conditions: 40°C, pH 6, $\omega = 4$ krpm.

S5   Supplementary tables

	A. vinosum pH 8.8	*A. aeolicus* pH 8
E_0 (mV)	-284	0
k_1 (s^{-1})	1	4.10^{-2}
k_{Alim} (s^{-1})	$7.10^{-3\,\text{a}}$	3.10^{-4}
k_{-1} (s^{-1})		7.10^{-3}

[a]For *A. vinosum*, as only $k_I + k_A$ is known k_{Alim} and k_{-1} cannot be independently determined, only their sum is known.

Table S1: Values used for the fits of the data for model 1.

	A. vinosum pH 8.8	A. aeolicus pH 8
α	0.8	0.64
$k_0 \exp(-f\alpha E^0)$ (s^{-1})	9.10^{-5}	1.10^{-2}
k_{Alim} (s^{-1})		3.10^{-4}
k_{-1} (s^{-1})	$7.10^{-3\mathrm{a}}$	7.10^{-3}

[a]As for other fits, only the sum of k_{-1} and k_{Alim} can be determined.

Table S3: Values used for the fits of the data for model 3.

	A. vinosum pH 8.8	A. aeolicus pH 8
E_0 (mV)	-290	-160
k_1 (s^{-1})	1.5	8
k_{Alim} (s^{-1})		5.10^{-4}
k_{-1} (s^{-1})	$7.10^{-3\mathrm{a}}$	8.10^{-3}
α	0.55	0.60
k_0 (s^{-1})	0.9	2.3

[a]As for other fits, only the sum of k_{-1} and k_{Alim} can be determined.

Table S2: Values used for the fits of the data for model 2.