Transient catalytic voltammetry of sulfite oxidase reveals rate limiting conformational changes

Supplementary information

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S1 Kinetic schemes and differential equations

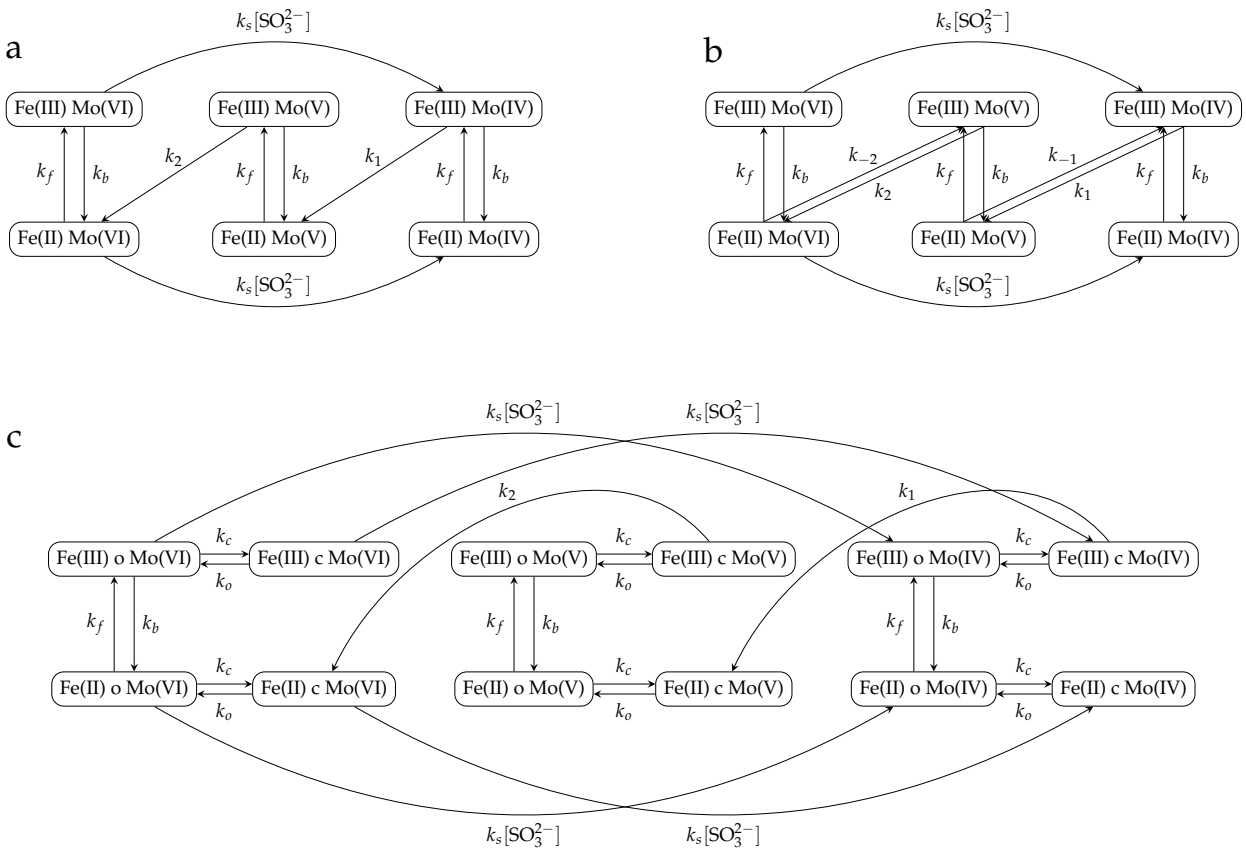

Figure S1: Rigorous presentation of the schemes of models a, b and c. Each of the rectangles represent a microscopic state; they differ in the redox states of the heme (Fe(II) or Fe(III) and of the molybdenum center (Mo(IV), Mo(V) or Mo(VI)). In the case of model c, each state can also be in the open (o) or closed (c) conformation.

Models a and b were fitted using the following differential equations:

$$\frac{\mathrm{d}[\mathrm{Fe(III)Mo(VI)}]}{\mathrm{d}t} = -\left(k_b + k_s\, s\right)[\mathrm{Fe(III)Mo(VI)}] + k_f[\mathrm{Fe(II)Mo(VI)}] \tag{1a}$$

$$\frac{\mathrm{d}[\mathrm{Fe(III)Mo(V)}]}{\mathrm{d}t} = -\left(k_b + k_2\right)[\mathrm{Fe(III)Mo(V)}] + k_f[\mathrm{Fe(II)Mo(V)}] + k_{-2}[\mathrm{Fe(II)Mo(VI)}] \tag{1b}$$

$$\frac{\mathrm{d}[\mathrm{Fe(III)Mo(IV)}]}{\mathrm{d}t} = -\left(k_b + k_1\right)[\mathrm{Fe(III)Mo(IV)}] + k_f[\mathrm{Fe(II)Mo(IV)}] + k_s\, s[\mathrm{Fe(III)Mo(VI)}] + k_{-1}[\mathrm{Fe(II)Mo(V)}] \tag{1c}$$

$$\frac{\mathrm{d}[\mathrm{Fe(II)Mo(VI)}]}{\mathrm{d}t} = -\left(k_f + k_s\, s + k_{-2}\right)[\mathrm{Fe(II)Mo(VI)}] + k_b[\mathrm{Fe(III)Mo(VI)}] + k_2[\mathrm{Fe(III)Mo(V)}] \tag{1d}$$

$$\frac{\mathrm{d}[\mathrm{Fe(II)Mo(V)}]}{\mathrm{d}t} = -\left(k_f + k_{-1}\right)[\mathrm{Fe(II)Mo(V)}] + k_b[\mathrm{Fe(III)Mo(V)}] + k_1[\mathrm{Fe(III)Mo(IV)}] \tag{1e}$$

$$\frac{\mathrm{d}[\mathrm{Fe(II)Mo(IV)}]}{\mathrm{d}t} = -k_f[\mathrm{Fe(II)Mo(IV)}] + k_b[\mathrm{Fe(III)Mo(IV)}] + k_s\, s[\mathrm{Fe(II)Mo(VI)}] \tag{1f}$$

For model a, $k_{-2} = k_{-1} = 0$. s is the concentration of sulfite. The current is computed using the equation:

$$i = nFA\Gamma\Big\{k_f\left([\mathrm{Fe(II)Mo(IV)}] + [\mathrm{Fe(II)Mo(V)}] + [\mathrm{Fe(II)Mo(VI)}]\right) - k_b\left([\mathrm{Fe(III)Mo(IV)}] + [\mathrm{Fe(III)Mo(V)}] + [\mathrm{Fe(III)Mo(VI)}]\right)\Big\} \tag{2}$$

The k_f and k_b rate constants are given by:

$$k_f = k_0\, \exp\frac{F\,(E - E_0)}{2\,R\,T} \qquad\qquad k_b = k_0\, \exp\frac{F\,(E_0 - E)}{2\,R\,T} \tag{3}$$

For model c, we used the following equations in which we used a three-letter coding: in OoO, the first O means Fe(III) (R for Fe(II)); the second o means open (c for closed) and the third O means Mo(VI) (I for Mo(V) and R for Mo(IV)):

$$\frac{\mathrm{d}[\mathrm{OoO}]}{\mathrm{d}t} = -\left(k_b + k_c + k_s\, s\right)[\mathrm{OoO}] + k_f[\mathrm{RoO}] + k_o[\mathrm{OcO}] \tag{4a}$$

$$\frac{\mathrm{d}[\mathrm{OoI}]}{\mathrm{d}t} = -\left(k_b + k_c\right)[\mathrm{OoI}] + k_f[\mathrm{RoI}] + k_o[\mathrm{OcI}] \tag{4b}$$

$$\frac{\mathrm{d}[\mathrm{OoR}]}{\mathrm{d}t} = -(k_b + k_c)[\mathrm{OoR}] + k_f[\mathrm{RoR}] + k_o[\mathrm{OcR}] + k_s\, s[\mathrm{OoO}] \tag{4c}$$

$$\frac{\mathrm{d}[\mathrm{OcO}]}{\mathrm{d}t} = -(k_o + k_s\, s)[\mathrm{OcO}] + k_c[\mathrm{OoO}] \tag{4d}$$

$$\frac{\mathrm{d}[\mathrm{OcI}]}{\mathrm{d}t} = -(k_o + k_2)[\mathrm{OcI}] + k_c[\mathrm{OoI}] \tag{4e}$$

$$\frac{\mathrm{d}[\mathrm{OcR}]}{\mathrm{d}t} = -(k_o + k_1)[\mathrm{OcR}] + k_c[\mathrm{OoR}] + k_s\, s[\mathrm{OcO}] \tag{4f}$$

$$\frac{\mathrm{d}[\mathrm{RoO}]}{\mathrm{d}t} = -(k_f + k_c + k_s\, s)[\mathrm{RoO}] + k_b[\mathrm{OoO}] + k_o[\mathrm{RcO}] \tag{4g}$$

$$\frac{\mathrm{d}[\mathrm{RoI}]}{\mathrm{d}t} = -(k_f + k_c)[\mathrm{RoI}] + k_b[\mathrm{OoI}] + k_o[\mathrm{RcI}] \tag{4h}$$

$$\frac{\mathrm{d}[\mathrm{RoR}]}{\mathrm{d}t} = -(k_f + k_c)[\mathrm{RoR}] + k_b[\mathrm{OoR}] + k_o[\mathrm{RcR}] + k_s\, s[\mathrm{RoO}] \tag{4i}$$

$$\frac{\mathrm{d}[\mathrm{RcO}]}{\mathrm{d}t} = -(k_o + k_s\, s)[\mathrm{RcO}] + k_c[\mathrm{RoO}] + k_2[\mathrm{OcI}] \tag{4j}$$

$$\frac{\mathrm{d}[\mathrm{RcI}]}{\mathrm{d}t} = -k_o[\mathrm{RcI}] + k_c[\mathrm{RoI}] + k_1[\mathrm{OcR}] \tag{4k}$$

$$\frac{\mathrm{d}[\mathrm{RcR}]}{\mathrm{d}t} = -k_o[\mathrm{RcR}] + k_c[\mathrm{RoR}] + k_s\, s[\mathrm{RcO}] \tag{4l}$$

The current is given by:

$$i = nFA\Gamma\Big\{k_f\left([\mathrm{RoO}] + [\mathrm{RoI}] + [\mathrm{RoR}]\right) - k_b\left([\mathrm{OoO}] + [\mathrm{OoI}] + [\mathrm{OoR}]\right)\Big\} \tag{5}$$

Steady-state rates at high potentials for all models

The turnover rate at high potential for both models a and b obey Michaelis-Menten kinetics:

$$k_{\text{cat}} = \frac{k_{\text{cat}}^{\max}}{1 + K_m/s} \tag{6}$$

with the following parameters:

$$k_{\text{cat}}^{\max} = \frac{k_1\, k_2}{k_1 + k_2} \qquad\qquad K_m = k_{\text{cat}}^{\max}/k_s \tag{7}$$

Model c gives a more complicated steady-state rate:

$$\begin{aligned} k_{\text{cat}} = {} & \Big[s k_s\, k_1\, k_o\, k_2\, k_c\, \left(2\, k_s\, s k_o + k_c\, k_s\, s + {k_s}^2 s^2 + {k_o}^2 + k_o\, k_c\right)\Big] \,/ \\ & \Big[3\, k_1\, k_c\, {k_o}^2 {k_s}^2 s^2 + 2\, k_2\, k_1\, {k_s}^3 s^3 k_o + 4\, {k_o}^2 k_2\, k_1\, {k_s}^2 s^2 + 2\, k_2\, {k_o}^3 {k_s}^2 s^2 + k_c\, {k_o}^3 k_1\, k_2 + 2\, k_1\, k_o\, k_2\, {k_c}^2 k_s\, s + k_2\, {k_o}^4 k_s\, s + \\ & 4\, k_2\, {k_o}^2 k_1\, k_c\, k_s\, s + k_1\, {k_o}^2 {k_c}^2 k_s\, s + k_2\, k_o\, k_c\, {k_s}^3 s^3 + k_2\, {k_o}^2 {k_c}^2 k_s\, s + 3\, k_2\, {k_o}^2 k_c\, {k_s}^2 s^2 + 2\, k_1\, {k_o}^3 k_c\, k_s\, s + k_2\, k_o\, {k_c}^2 {k_s}^2 s^2 + \\ & 2\, k_2\, {k_c}^2 k_1\, {k_s}^2 s^2 + 2\, k_2\, k_c\, k_1\, {k_s}^3 s^3 + 2\, k_2\, {k_o}^3 k_1\, k_s\, s + 2\, k_2\, {k_o}^3 k_c\, k_s\, s + 2\, k_1\, {k_o}^3 {k_s}^2 s^2 + \\ & {k_c}^2 {k_o}^2 k_1\, k_2 + k_2\, {k_o}^2 {k_s}^3 s^3 + k_1\, {k_o}^2 {k_s}^3 s^3 + k_1\, {k_o}^4 k_s\, s + 6\, k_2\, k_c\, k_1\, {k_s}^2 s^2 k_o + k_1\, k_c\, {k_s}^3 s^3 k_o + k_1\, {k_c}^2 {k_s}^2 s^2 k_o \Big] \end{aligned} \tag{8}$$

This simplifies at saturating concentrations of substrate to:

$$k_{\text{cat}}^{\max} = \frac{k_1\, k_o\, k_2\, k_c}{k_o\, k_c\, k_1 + k_1\, {k_o}^2 + k_2\, k_o\, k_c + 2\, k_2\, k_1\, k_o + k_2\, {k_o}^2 + 2\, k_2\, k_c\, k_1} \tag{9}$$

Taking the limit for high intramolecular rates constants yields equation (7) in text.

S2 All fits

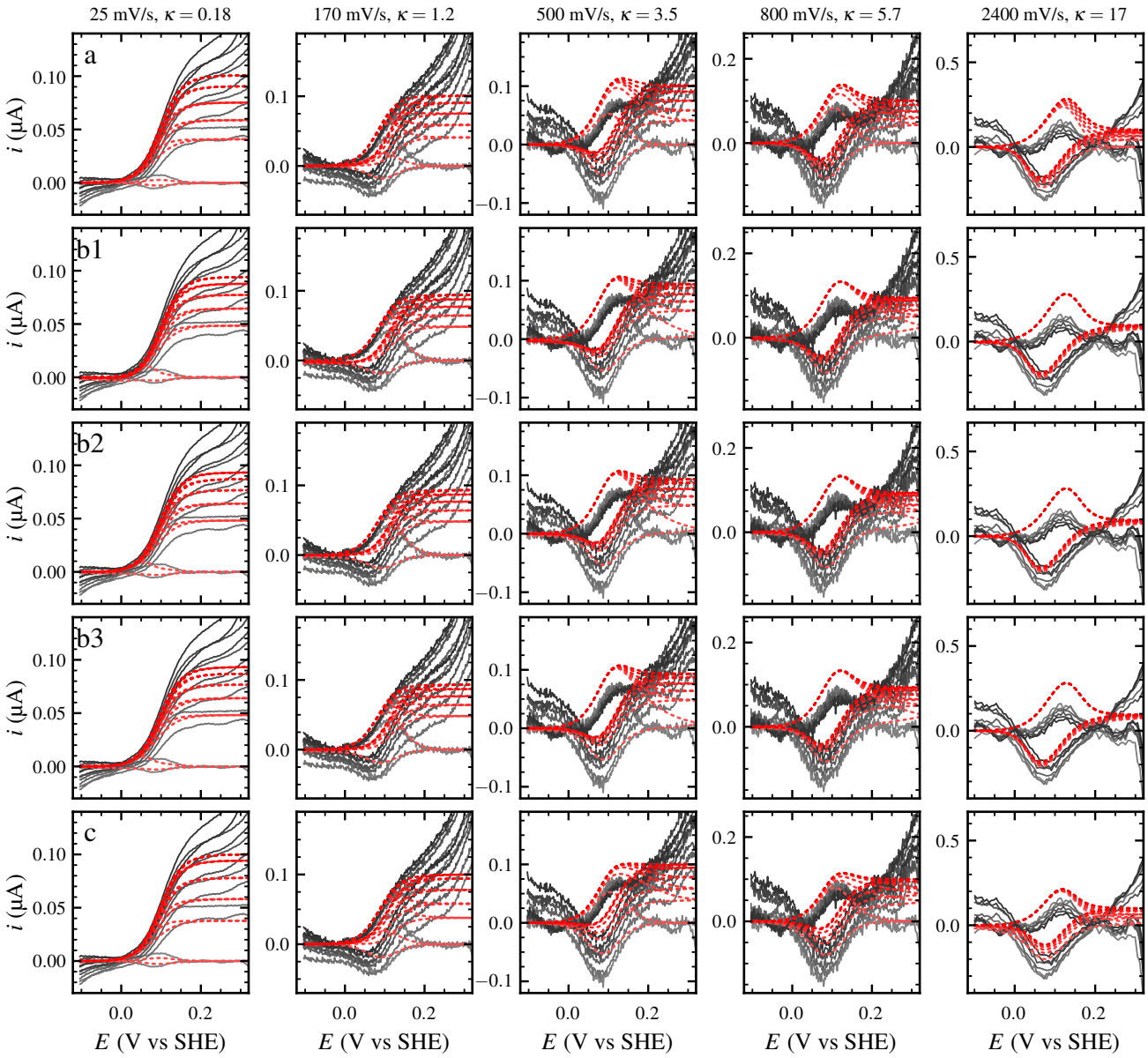

Figure S2: Baseline subtracted voltammograms (solid lines, at sulfite concentrations ranging from 0, light gray to 200 μM, black), along with the fits of models a, b & c (dashed lines), for $k_1/k_2 = 1$. The tree possibilities of the equilibrium constants for model b are named b1, b2 and b3. Conditions: *h*SO immobilized on Au/DTSP/AuNP electrodes, pH 8.4, T = 25°C, scan rates as indicated on the top of each column. The value of κ indicated corresponds to the rate at the maximum concentration of sulfite.

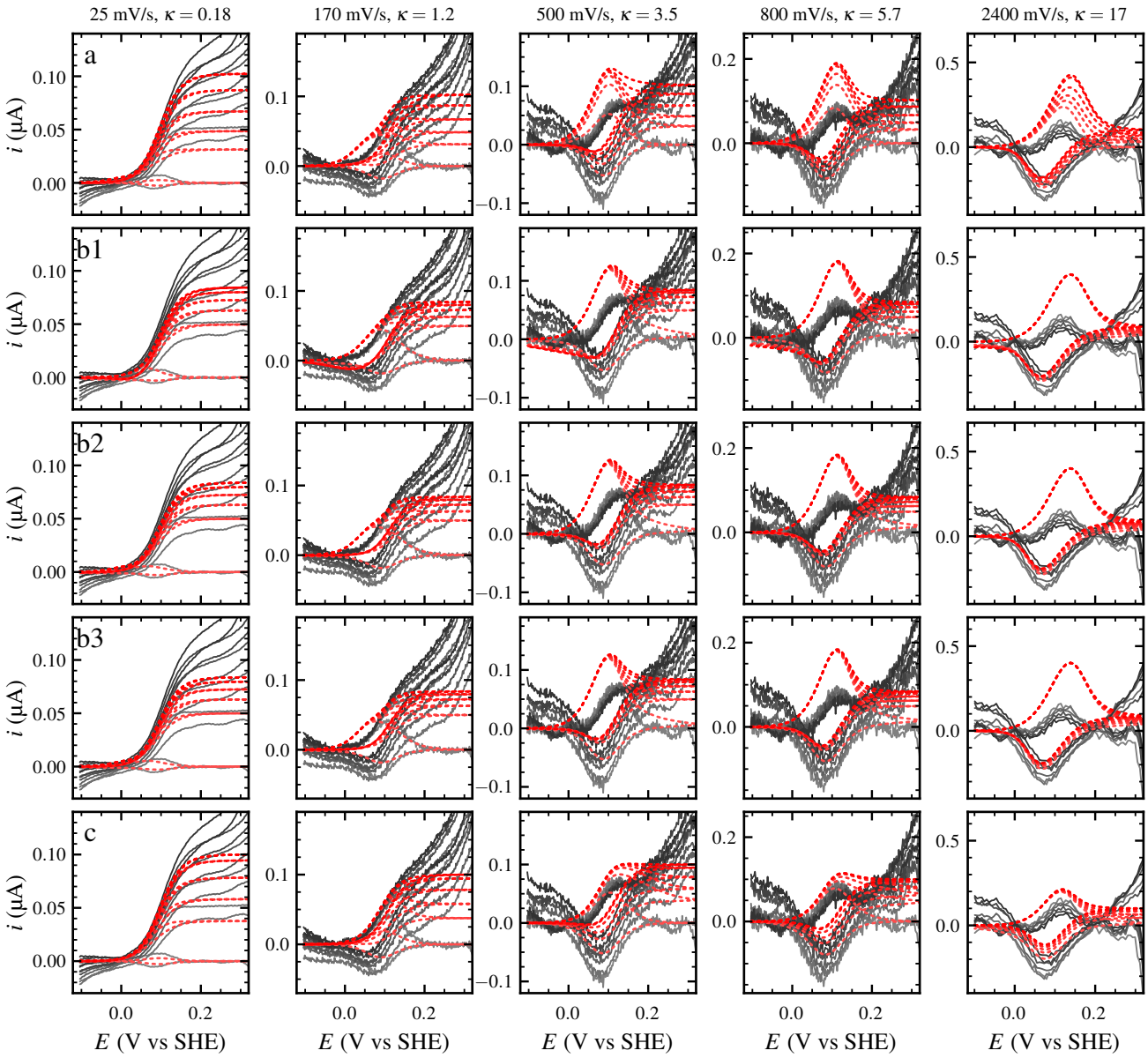

Figure S3: Same as figure S2, for $k_1/k_2 = 10$

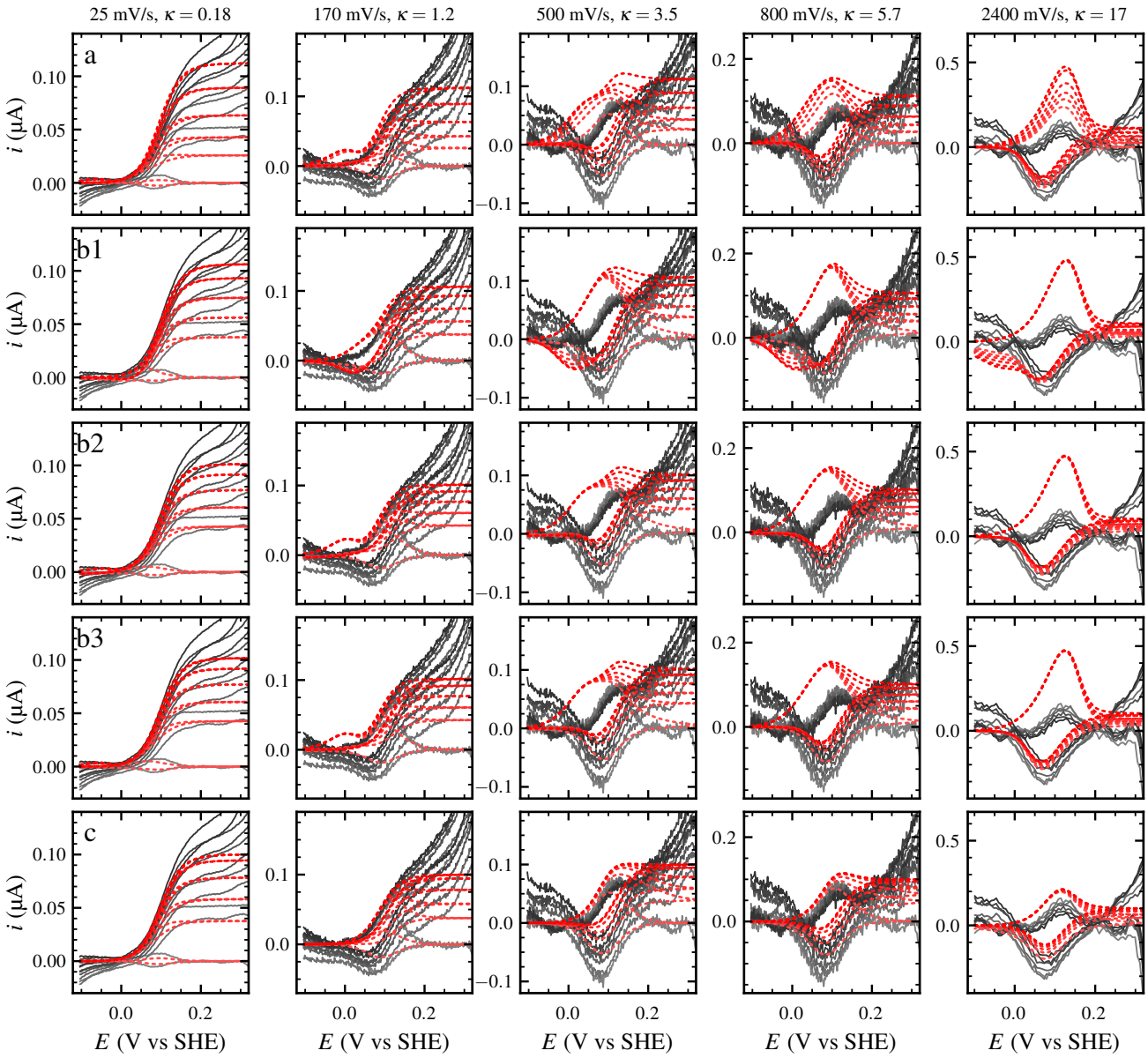

Figure S4: Same as figure S2, for $k_1/k_2 = 100$

S3 Other data

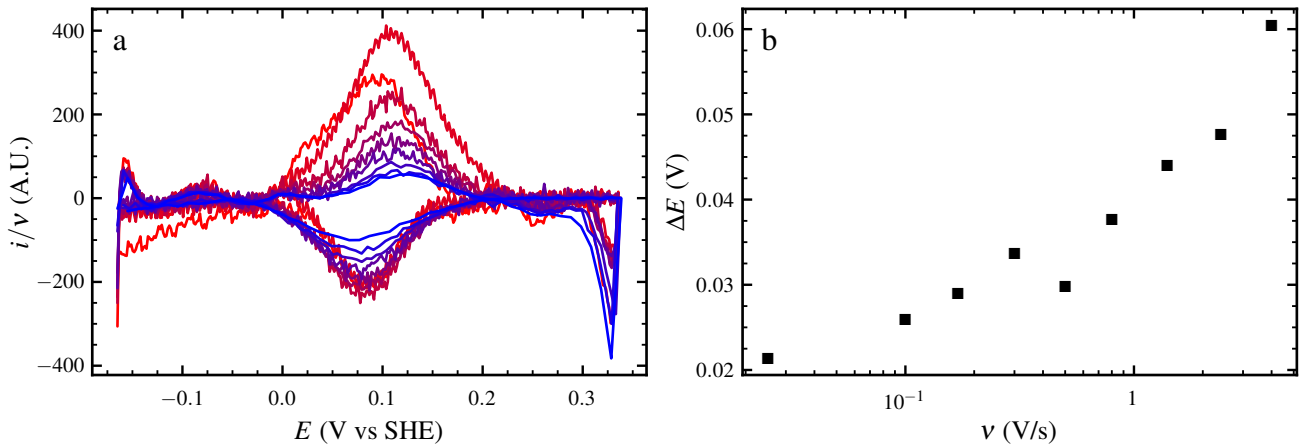

Figure S5: Panel a: baseline-subtracted non-catalytic cyclic voltammograms for scan rates ranging from 25 mV/s (red) to 4 V/s (blue), divided by the scan rate. Panel b: difference of the potentials of the oxidative and the reductive peaks for the voltammograms of panel a. Conditions: *h*SO immobilized on Au/DTSP/AuNP electrodes, pH 8.4, T = 25°C, no sulfite.

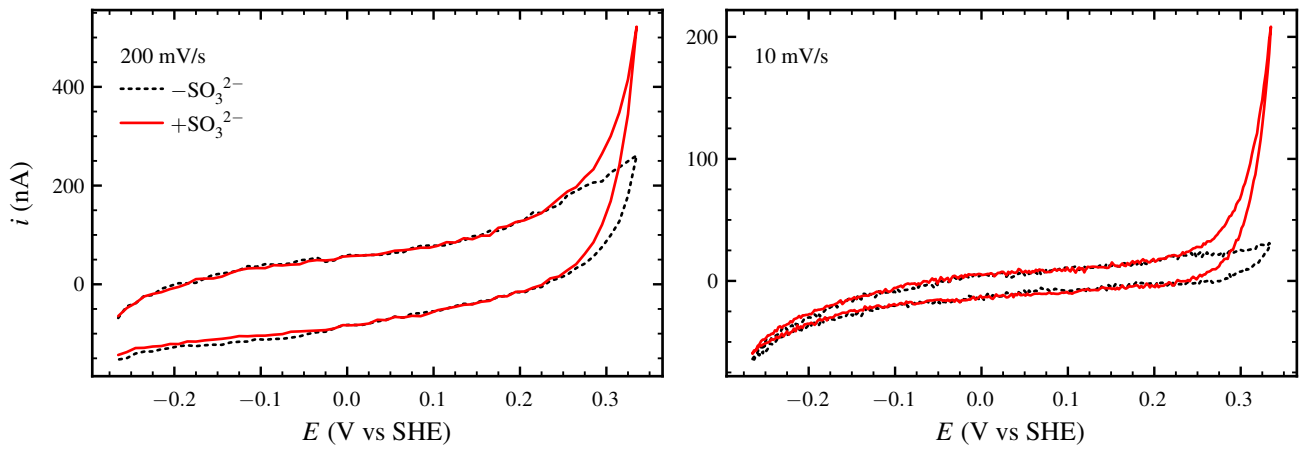

Figure S6: Cyclic voltammograms of a bare Au/DTSP/AuNP electrode, with 0 µM (black dotted line) or 400 µM (red solid line) sulfite. Conditions: pH 8.4, T = 25°C, scan rates as indicated in the graphs.