The Mathematics Behind Surface CirrE1CrevE2C' Mechanism in Protein-Film Square-Wave Voltammetry

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1. Reaction sequence of the surface CirrE₁CrevE₂C′ mechanism in protein-film square-wave voltammetry

The model considers a fully surface-confined protein film. All species participating in the redox sequence are strongly adsorbed ("ads") at the working-electrode surface. The mechanism contains five elementary steps, labeled (a)–(e) in Reaction scheme 1.

$$A_{ads} \overset{k_{c,irr}}{\rightarrow} Red_{ads} \qquad (a) \quad (Cirr)$$

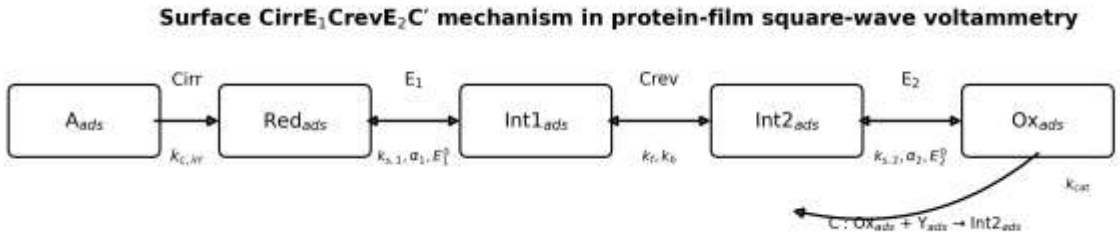
$$Red_{ads} \overset{k_{s,1},\,\alpha_1}{\rightleftharpoons} Int1_{ads} + e^- \qquad (b) \quad (E_1)$$

$$Int1_{ads} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} Int2_{ads} \qquad (c) \quad (Crev)$$

$$Int2_{ads} \overset{k_{s,2},\,\alpha_2}{\rightleftharpoons} Ox_{ads} + e^- \qquad (d) \quad (E_2)$$

$$Ox_{ads} + Y_{ads} \overset{k_{cat}}{\rightarrow} Int2_{ads} \qquad (e) \quad (C')$$

Reaction scheme 1. Elementary steps forming the surface CirrE₁CrevE₂C′ mechanism.



Scheme 1. Reaction scheme of the surface CirrE₁CrevE₂C′ mechanism considered in protein-film square-wave voltammetry.

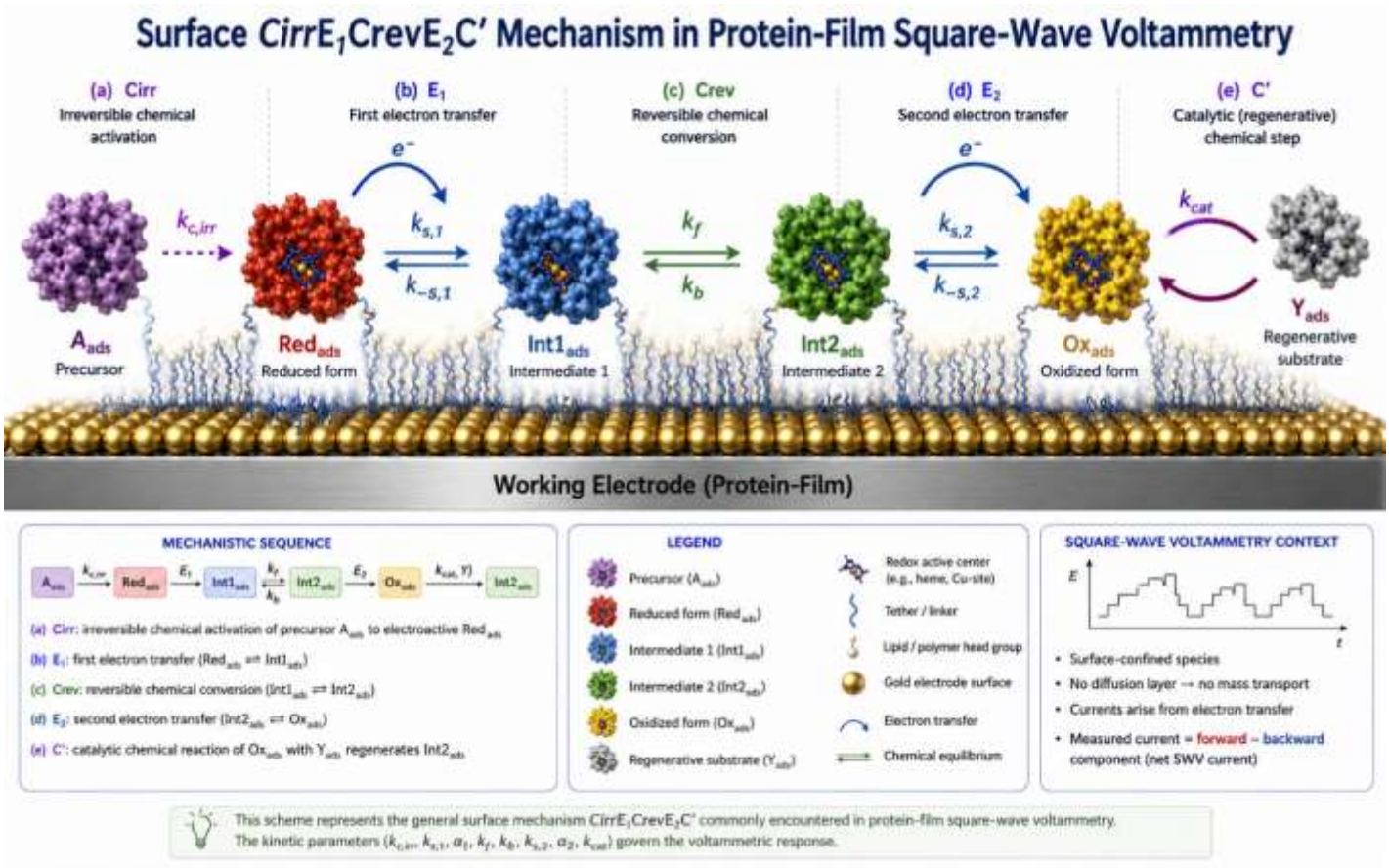


Figure 1. *Reaction sequences of the surface CirrE₁CrevE₂C' mechanism considered in protein-film square-wave voltammetry*

The initial irreversible chemical reaction, Cirr, is governed by the first-order rate constant kc,irr. This step converts the adsorbed precursor A into the electroactive reduced form Red.

The first electron-transfer step, E1, converts adsorbed Red into the first intermediate, Int1. Its kinetics are characterized by the standard rate constant ks,1 (s^{-1}) and the transfer coefficient α1.

The reversible chemical step, Crev, interconverts Int1 and Int2. The forward and backward rate constants are kf and kb. Two useful derived quantities are Keq, the equilibrium constant, and kchem,r, the cumulative chemical rate parameter, defined by equations (1) and (2):

$$K_{\mathrm{eq}} = \frac{k_f}{k_b} \qquad (1)$$

$$k_{\mathrm{chem,r}} = k_f + k_b \qquad (2)$$

The second electron-transfer step, E2, transforms Int2 into the final oxidized form Ox. This process is described by the standard rate constant ks,2 and the transfer coefficient α2.

The final C′ step is an irreversible regenerative surface reaction between Ox and the adsorbed substrate Y. It regenerates Int2 and establishes the catalytic pathway. The rate is controlled by the catalytic

constant kcat. When Y is present in large excess and its surface coverage is effectively unchanged, the catalytic reaction can be treated in pseudo-first-order form using kcat′:

$$k'_{\mathrm{cat}} = k_{\mathrm{cat}}\Gamma_{\mathrm{Y}} \qquad (3),$$

Here, ΓY denotes the surface concentration of the regenerative substrate Y.

3. Mathematical model for protein-film square-wave voltammetry

The CirrE₁CrevE₂C′ mechanism is treated as a fully surface-confined system under protein-film square-wave voltammetric conditions. All redox species remain immobilized at the working electrode. Concentration gradients in solution are therefore absent, so diffusion equations are not required. Migration and convection do not contribute to the immobilized reaction sequence. Electron transfer occurs directly between the electrode and the adsorbed redox centers and is described by Butler–Volmer kinetics. The following formulation defines the model and the physical meaning of its individual terms.

3.1. Initial conditions and mass conservation for the adsorbed species

Each species is represented by its surface concentration, Γ. The time-dependent coverages of A, Red, Int1, Int2, Ox, and Y are ΓA(t), ΓRed(t), ΓInt1(t), ΓInt2(t), ΓOx(t), and ΓY(t), respectively, in mol cm⁻². In the absence of desorption or exchange with the bulk solution, the total surface concentration ΓT of the redox-active sequence remains constant:

$$\Gamma_T = \Gamma_A + \Gamma_{Red} + \Gamma_{Int1} + \Gamma_{Int2} + \Gamma_{Ox} = \text{constant} \qquad (4)$$

If Y is also immobilized and is not replenished from the solution, its coverage may be described by a separate conservation relation. Alternatively, it can be treated independently according to its role in the catalytic step.

3.2. Rates of the surface chemical reactions

The chemical rate expressions are defined first. They are then used in the differential equations describing the complete CirrE₁CrevE₂C′ sequence.

a) a) Rate of the preceding irreversible chemical step (Cirr)

The rate of the irreversible A → Red surface conversion, v_{irr}, is defined by:

$$v_{\mathrm{irr}} = k_{c,\mathrm{irr}}\Gamma_A \qquad (5)$$

Equation (5) represents first-order depletion of precursor A together with formation of Red. A reverse contribution is absent because Cirr is defined as irreversible.

b) b) Rate of the reversible chemical conversion (Crev)

The net rate of the reversible Int1 ⇌ Int2 transformation, v_{rev}, is given by equation (6):

$$v_{\mathrm{rev}} = k_f\Gamma_{Int1} - k_b\Gamma_{Int2} \qquad (6)$$

In equation (6), the first contribution describes Int1 → Int2, whereas the second describes Int2 → Int1. Accordingly, both the surface coverages and the values of kf and kb determine the magnitude and direction of vrev.

c) Rate of the catalytic regenerative chemical step (C′)

For surface-confined Ox and Y, the rate of the regenerative C′ step, v_{cat}, is expressed by equation (7):

$$v_{\mathrm{cat}} = k_{\mathrm{cat}}\Gamma_{\mathrm{Ox}}\Gamma_{\mathrm{Y}} \qquad (7)$$

When Y is in sufficiently large excess, its surface coverage changes negligibly during a square-wave voltammetric experiment. Under this condition, equation (7) simplifies to:

$$v_{\mathrm{cat}} = k'_{\mathrm{cat}}\Gamma_{\mathrm{Ox}} \qquad (8), \text{ where}$$

$$k'_{\mathrm{cat}} = k_{\mathrm{cat}}\Gamma_{\mathrm{Y}} \qquad (9).$$

3.3. Butler–Volmer kinetics of the two surface electron-transfer steps

The potential dependence of E1 and E2 is expressed through the overpotential η. The corresponding definitions are given by equations (10) and (11):

$$\eta_1(t) = E(t) - E_1^0 \qquad (10)$$

$$\eta_2(t) = E(t) - E_2^0 \qquad (11).$$

Here, E(t) denotes the potential imposed by the square-wave waveform. E_1^0 and E_2^0 are the formal potentials of the E1 and E2 surface redox couples, respectively.

3.4. Differential equations for the surface CirrE₁CrevE₂C′ mechanism

Because every redox species is surface confined, the model is written as ordinary differential equations for the time-dependent surface concentrations Γ. No diffusion terms are included.

a) a) Differential equation for the initial species A

Precursor A participates only in the preceding irreversible chemical reaction. Its surface concentration therefore decreases according to first-order kinetics, as given by equation (13):

$$\frac{d\Gamma_A}{dt} = -k_{c,\mathrm{irr}}\Gamma_A \qquad (13)$$

The negative term in equation (13) describes irreversible consumption of A. Since Cirr has no reverse branch, A is not regenerated through this step.

b) b) Differential equation for the electroactive species Red

Red is produced from A by Cirr and participates in the first electron-transfer step. Its temporal evolution is given by equation (14):

$$\frac{d\Gamma_{Red}}{dt} = k_{c,\mathrm{irr}}\Gamma_A - v_1 \qquad (14)$$

In equation (14), the first term accounts for formation of Red from A. The contribution −v1 represents its net electrochemical conversion to Int1.

c) c) Differential equation for the intermediate Int1

Int1 is generated in E1 and subsequently enters the reversible Int1 ⇌ Int2 chemical step. Its surface concentration is therefore described by:

$$\frac{d\Gamma_{Int1}}{dt} = v_1 - v_{\mathrm{rev}} = v_1 - k_f\Gamma_{Int1} + k_b\Gamma_{Int2} \qquad (15)$$

The term v1 represents electrochemical production of Int1. The term −vrev accounts for its net consumption through Crev.

d) d) Differential equation for the intermediate Int2

Int2 is linked to Int1 through Crev and to Ox through E2. It is also regenerated from Ox by the C′ reaction. Its balance therefore contains contributions from all three processes and is written as:

$$\frac{d\Gamma_{Int2}}{dt} = v_{\text{rev}} - v_2 + v_{\text{cat}} = k_f\Gamma_{Int1} - k_b\Gamma_{Int2} - v_2 + v_{\text{cat}} \qquad (16)$$

In equation (16), vrev is the net chemical contribution from Crev. The term −v2 describes conversion of Int2 to Ox by E2, while +vcat represents catalytic regeneration of Int2.

e) e) Differential equation for the final oxidized species Ox

Ox is produced by the second electron-transfer step and consumed by the regenerative C′ reaction with Y. Its time dependence is given by equation (17):

$$\frac{d\Gamma_{(\text{Ox})}}{dt} = v_2 - v_{\text{cat}} \qquad (17)$$

In equation (17), v2 accounts for electrochemical formation of Ox. The term −vcat describes its removal through the irreversible catalytic reaction.

f) f) Differential equation for the electrochemically inactive regenerative substrate Y

When Y is immobilized and consumed by the catalytic reaction, its surface coverage varies with time according to:

$$\frac{d\Gamma_Y}{dt} = -v_{\text{cat}} \qquad (18)$$

If Y is supplied in large excess, its availability can be regarded as constant during the square-wave experiment. The corresponding condition is:

$$\Gamma_{Y,(t)} \approx \Gamma_{Y,0} = \text{constant} \qquad (19),$$

Here,$\Gamma_{Y,0}$is the initial surface concentration of Y. Under this condition, an explicit differential equation for Y is unnecessary, and the C′ step becomes pseudo-first-order with respect to Ox.

3.6. Kinetic boundary conditions for the surface-confined mechanism

For a fully immobilized system, conventional diffusion boundary conditions are unnecessary. Instead, the Butler–Volmer rates v1 and v2 enter directly into the ordinary differential equations for the corresponding surface coverages.

a) a) Net rate of the first electron-transfer step (E_1)

Equation (27) gives the Butler–Volmer rate for the surface Red/Int1 couple. Under the adopted IUPAC sign convention, v1 > 0 denotes oxidation of Red to Int1.

$$v_1 = k_{s,1}\left[\Gamma_{Red}\exp\left(\frac{\alpha_1 F\eta_1}{RT}\right) - \Gamma_{Int1}\exp\left(-\frac{(1-\alpha_1)F\eta_1}{RT}\right)\right] \qquad (27).$$

In equation (27), ks,1 is the standard surface electron-transfer rate constant (s^{-1}), α1 is the transfer coefficient, F is the Faraday constant, R is the universal gas constant, and T is the thermodynamic temperature.

b) b) Net rate of the second electron-transfer step (E_2)

Equation (28) defines the Butler–Volmer rate for the Int2/Ox surface couple. A positive v2 corresponds to oxidation of Int2 to Ox.

$$v_2 = k_{s,2}\left[\Gamma_{Int2}\exp\left(\frac{\alpha_2 F\eta_2}{RT}\right) - \Gamma_{Ox}\exp\left(-\frac{(1-\alpha_2)F\eta_2}{RT}\right)\right] \qquad (28).$$

The overpotentials η1 and η2 used in equations (27) and (28) are defined by equations (10) and (11), respectively.

c) c) Total Faradaic current in square-wave voltammetry

The instantaneous Faradaic current contains contributions from both one-electron-transfer steps. With positive v1 and v2 assigned to oxidation, the total current is:

$$I(t) = FS(v_1 + v_2) \qquad (29)$$

In equation (29), I(t) is the instantaneous Faradaic current, F is the Faraday constant, and S is the geometric active area of the working electrode. For a protein film, the current scales with the amount of electroactive material immobilized at the surface. During square-wave voltammetry, equation (29) is evaluated over each potential pulse to obtain the forward and backward current components. Their difference gives the net square-wave response. The coupled differential equations, initial conditions, and

Butler–Volmer relations are solved numerically for the applied square-wave potential program. The complete Mathcad simulation protocol supplied with this work allows systematic variation of kinetic and thermodynamic parameters and direct simulation of the corresponding protein-film square-wave voltammograms.

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