Electrochemical Analysis of Coupled Double-Regenerative Enzymatic Pathways in Protein Redox Films

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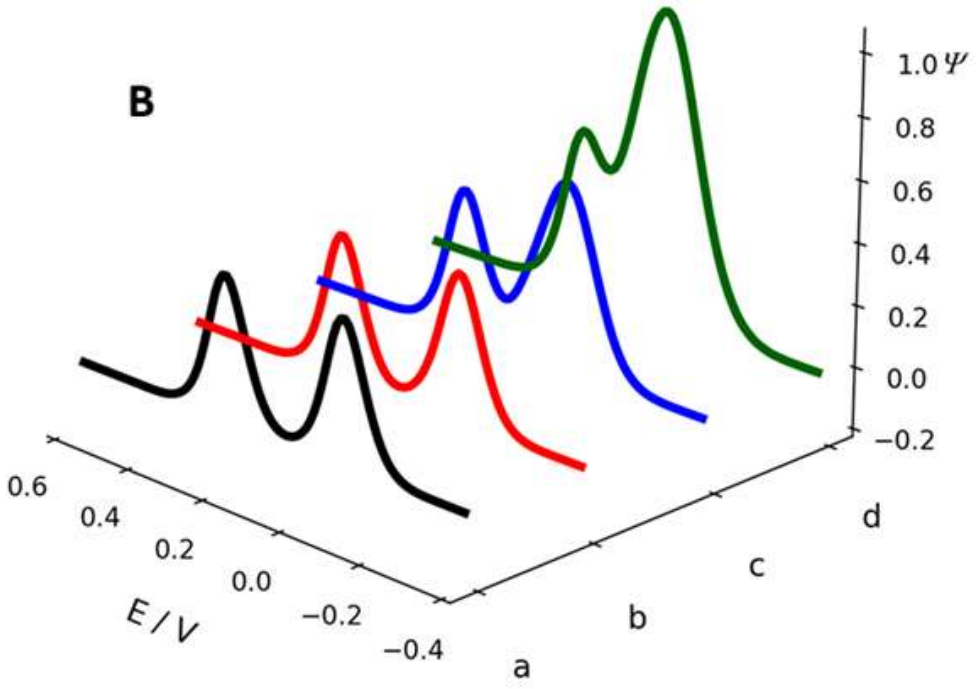
Abstract

Redox-active lipophilic proteins and membrane-associated enzymes such as quinone-interacting oxidoreductases, flavoproteins (FAD/FMN-dependent enzymes), cytochromes, and coenzyme Q–linked systems frequently undergo sequential two-step electron-transfer processes at electrode interfaces. In many of these biologically relevant systems, sustained catalytic turnover is maintained through regenerative chemical steps coupled to each electron-transfer event, leading to complex interfacial electrochemical–catalytic behavior under protein-film voltammetric conditions.

In this study, we develop a theoretical framework tailored for experimental protein-film electrochemistry, describing a surface-confined two-step double-regenerative mechanism (surface EC′EC″) under square-wave voltammetry (SWV) conditions. The model systematically evaluates the combined influence of heterogeneous electron-transfer kinetics, rates of chemical regeneration, surface coverage, and instrumental SWV parameters on the forward, backward, and net current components.

Special emphasis is placed on the diagnostic value of the net SWV responses, whose deconvoluted current components provide direct access to mechanistic signatures of sequential electron transfer and catalytic regeneration. The simulations demonstrate how variations in kinetic rate constants, equilibrium constants, and thermodynamic driving forces modulate peak position, symmetry, amplitude, and splitting in experimentally measurable SWV signals. Both moderate and very fast electron-transfer regimes are analyzed, revealing distinct patterns that enable discrimination between kinetic control and catalytic amplification.

The proposed framework offers a quantitative platform for extracting mechanistic, kinetic, and thermodynamic parameters from experimental SWV data of protein redox films. It thereby provides practical guidance for interpreting complex voltammetric responses of redox proteins and enzymes involved in biological electron-transport chains and catalytic cycles, and establishes clear diagnostic criteria for assessing enzymatic activity and catalytic efficiency directly at electrode surfaces.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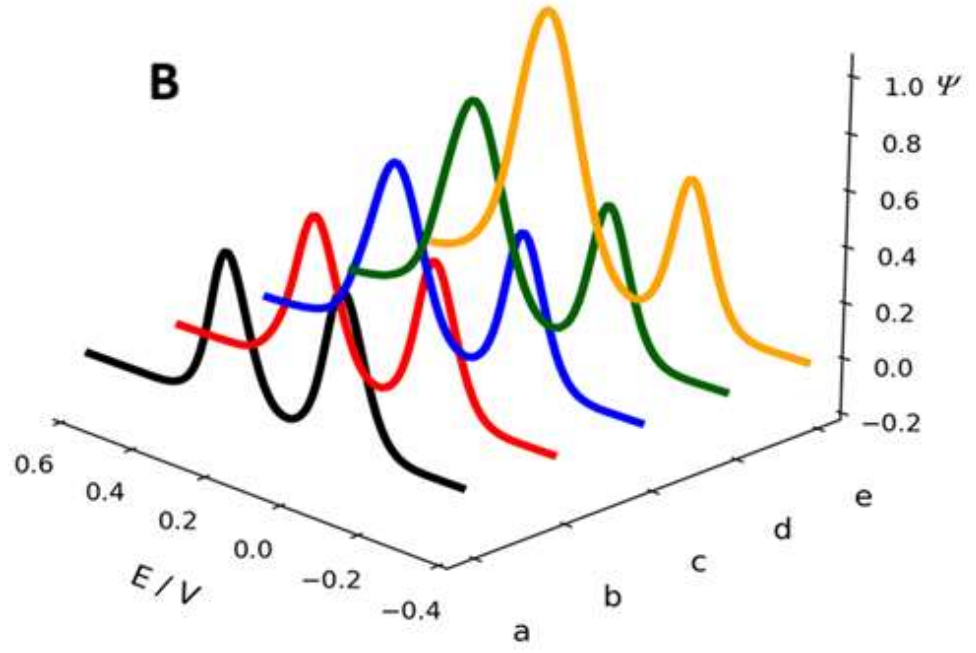
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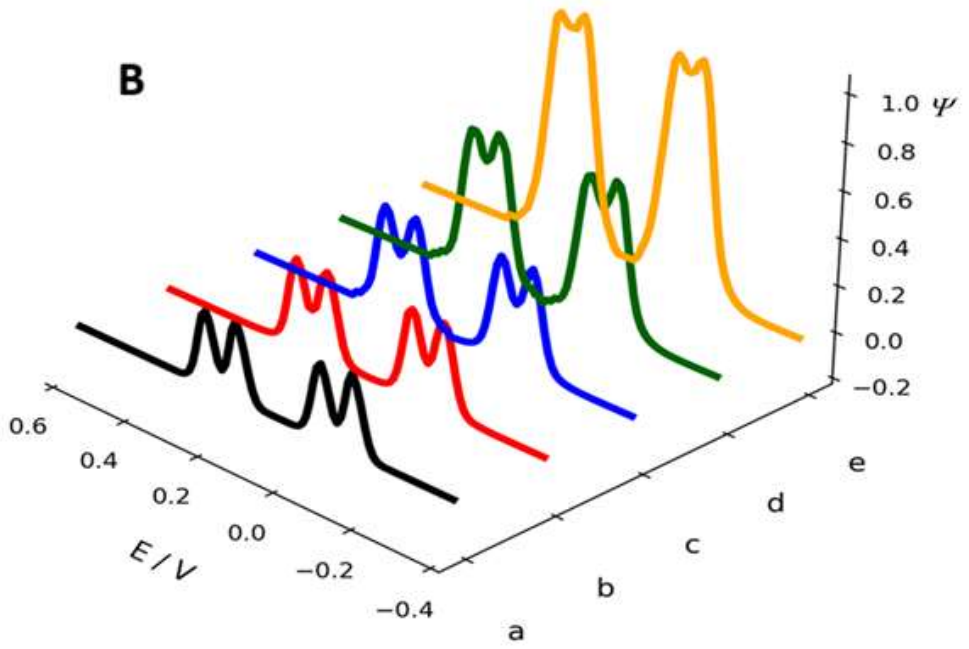
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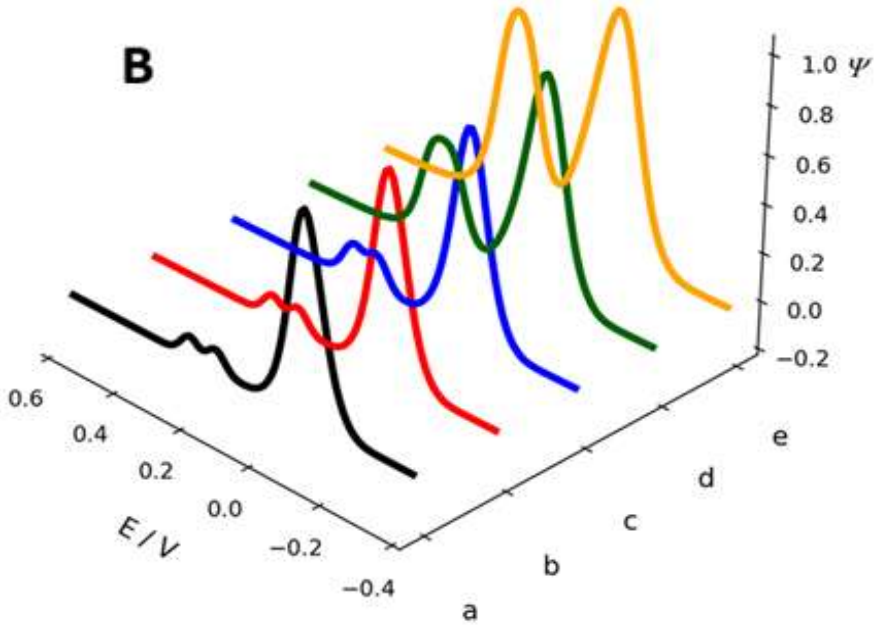
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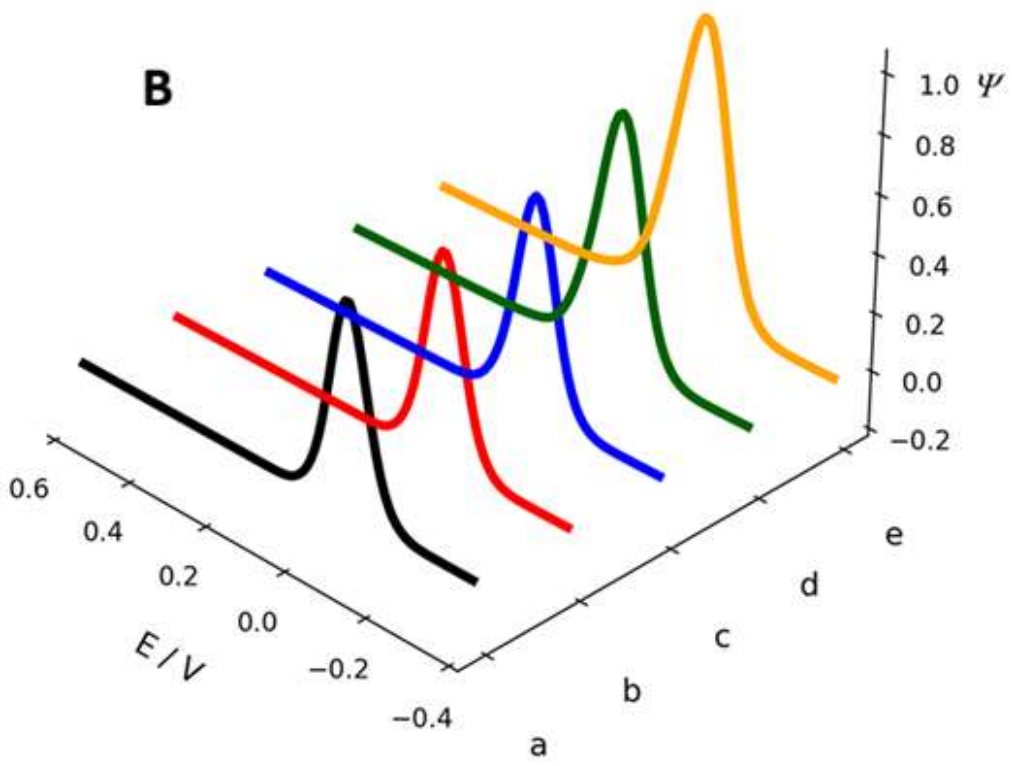

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