Protein-Film Voltammetry of EC′EC″ Processes: A New Theoretical Paradigm for Complex Redox Catalysis

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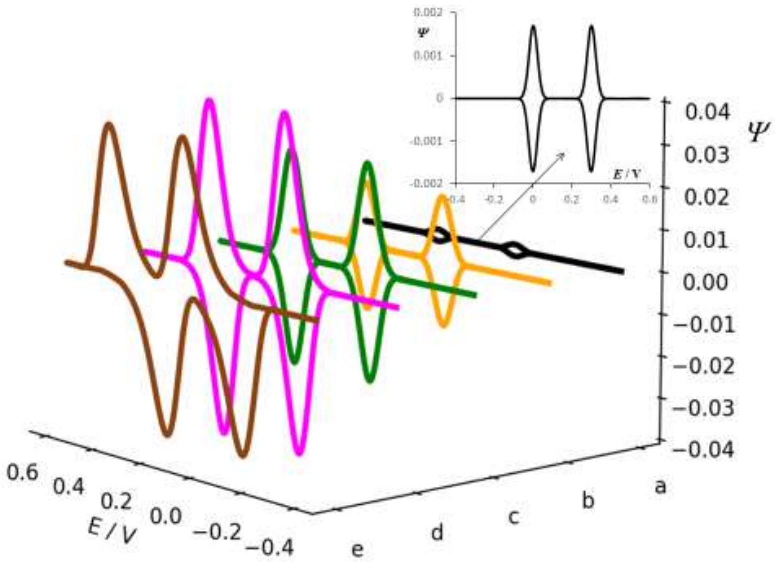
Abstract

Protein-film cyclic voltammetry (PFV) has emerged as a powerful tool for interrogating the redox behavior and catalytic mechanisms of surface-confined biomolecules. While existing theoretical treatments have successfully described single-step electron transfer (E), coupled chemical reactions (EC), and regenerative catalytic schemes (EC′), the extension to more complex tandem catalytic processes remains largely unexplored. In this work, we present, for the first time, resuts of a complete theoretical framework for the EC′EC″ mechanism in protein-film cyclic voltammetry, describing systems in which a redox transformation is followed by two consecutive catalytic (regenerative) chemical steps.

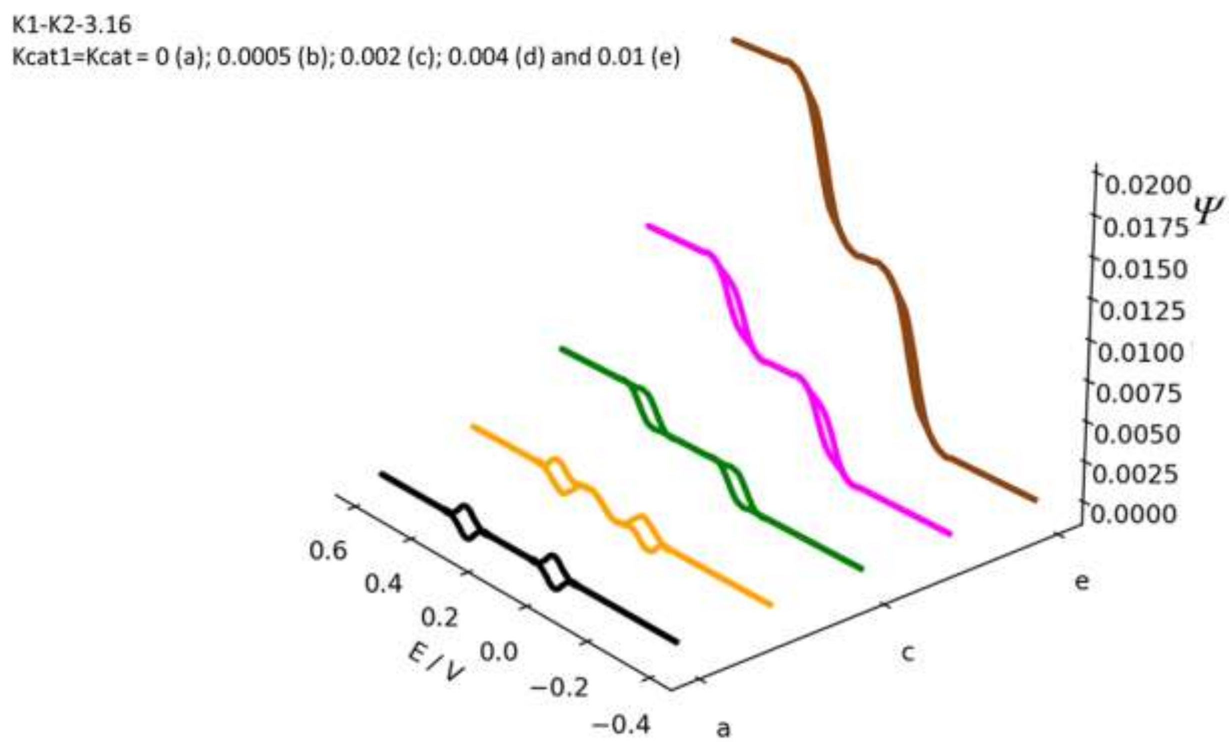
The developed model provides an analytical and numerical description of current–potential responses under conditions of surface confinement, incorporating the interplay between electron transfer kinetics and sequential catalytic regeneration pathways. Importantly, we demonstrate that under well-defined limiting conditions, the general EC′EC″ scheme converges to several simpler and previously established PFV mechanisms, including E, EE, EC’E, EC′, EEC’ and related variants. This establishes the EC′EC″ formalism as a unifying mechanistic framework capable of capturing a broad spectrum of redox processes in bioelectrochemistry.

The relevance of this mechanism is particularly pronounced for lipophilic redox proteins and enzymes embedded in biological membranes, where multi-step catalytic cycles are intrinsic to function. Representative systems include Cytochrome P450, Cytochrome C oxidase, NADPH oxidase, and Ubiquinol-cytochrome C reductase, all of which operate through coupled electron transfer and catalytic turnover steps involving lipophilic substrates or cofactors. In such systems, sequential chemical regeneration steps analogous to EC′EC″ are expected to govern catalytic efficiency, intermediate accumulation, and signal shape in PFV experiments.

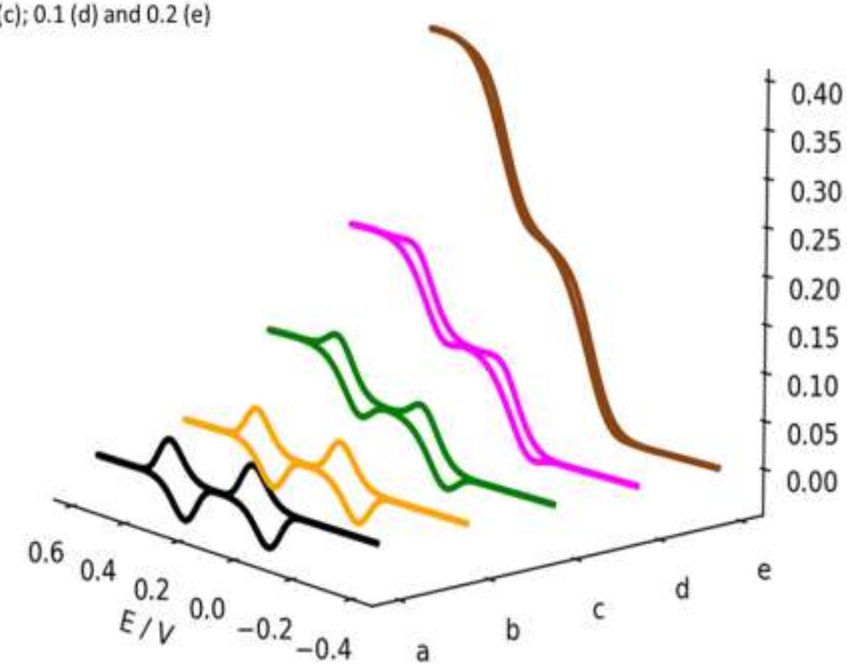
The present theory enables, for the first time, rigorous interpretation of voltammetric signatures arising from tandem catalytic pathways, offering new opportunities for extracting kinetic and mechanistic parameters from experimental PFV data. Beyond its theoretical significance, this work provides a foundation for advancing the electrochemical characterization of complex biological redox systems and designing biomimetic catalytic interfaces. Entire MATHCAD platform is available for free on the Repository of Goce Delcev University, Stip.



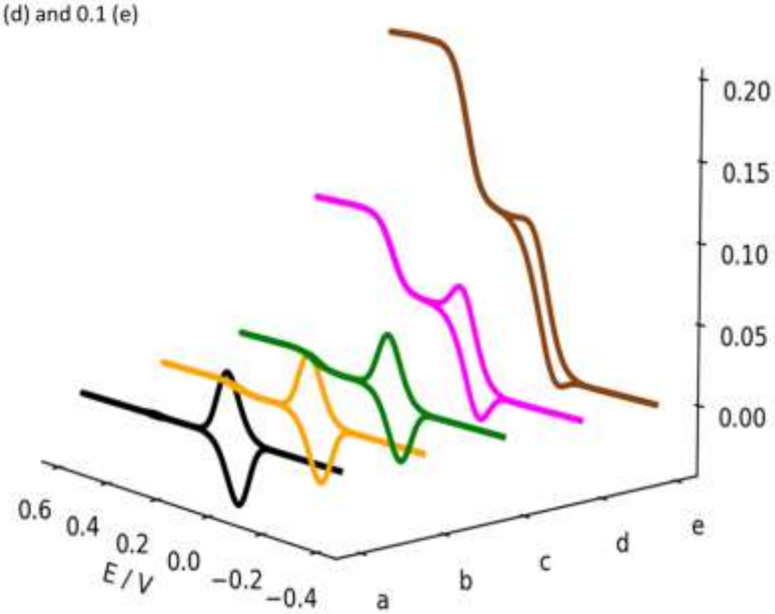
No Catalysis and different kinetics at both electron transfer steps-converge to EE mechanism



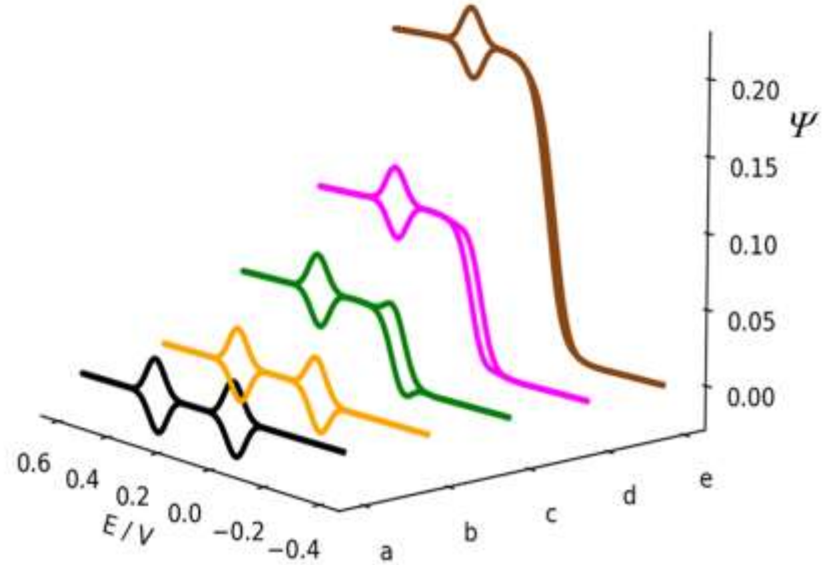
K1-K2=0.1
Kcat1=Kcat = 0 (a); 0.01 (b); 0.05 (c); 0.1 (d) and 0.2 (e)



K1 = 0.141; K2=3.162
Kcat1=Kcat = 0 (a); 0.005 (b); 0.01 (c); 0.05 (d) and 0.1 (e)



K1 = 0.63; K2=0,63
Kcat2 = 0; Kcat1 = 0 (a); 0.01 (b); 0.05 (c); 0.1 (d) and 0.2 (e)



K1 = 1; K2=1
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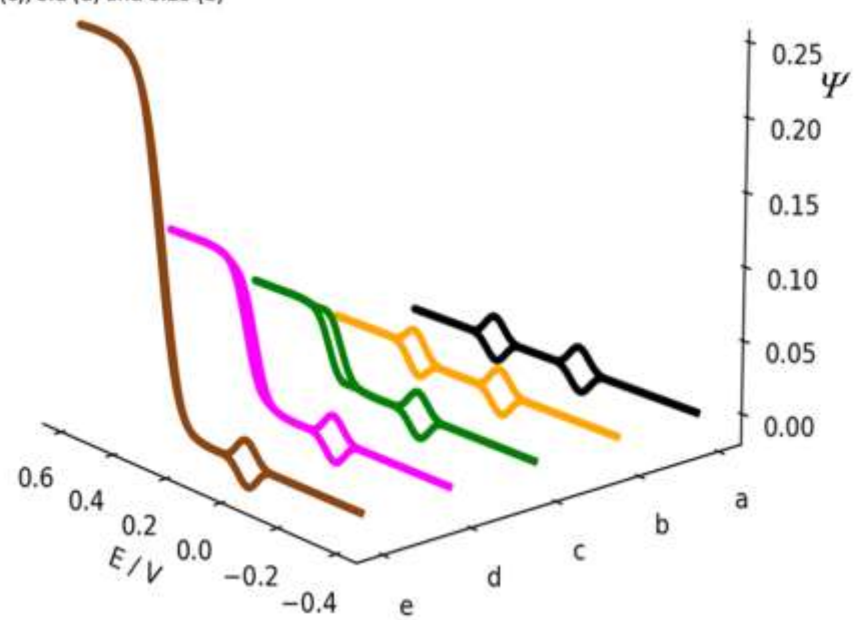

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