

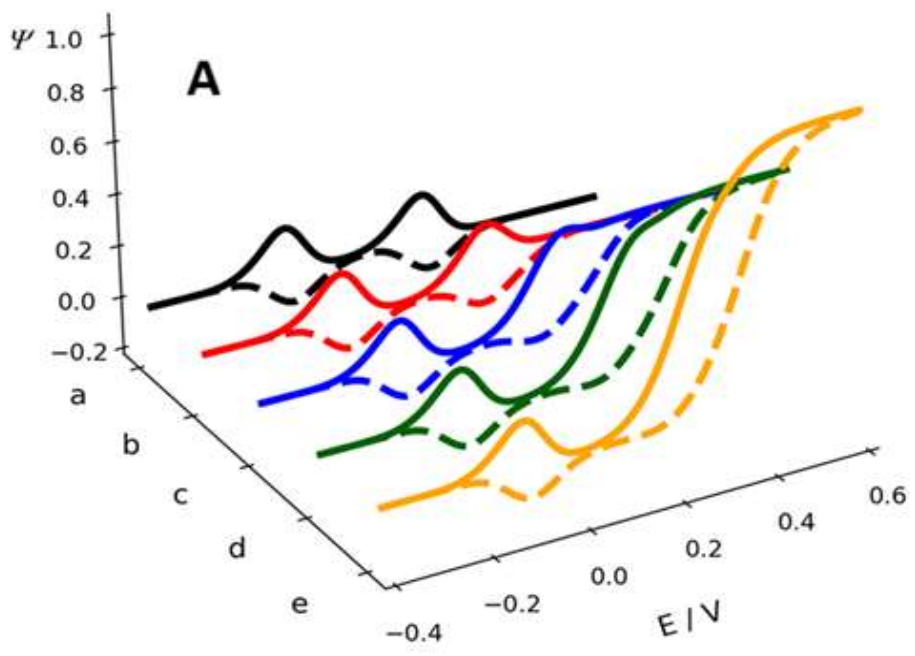
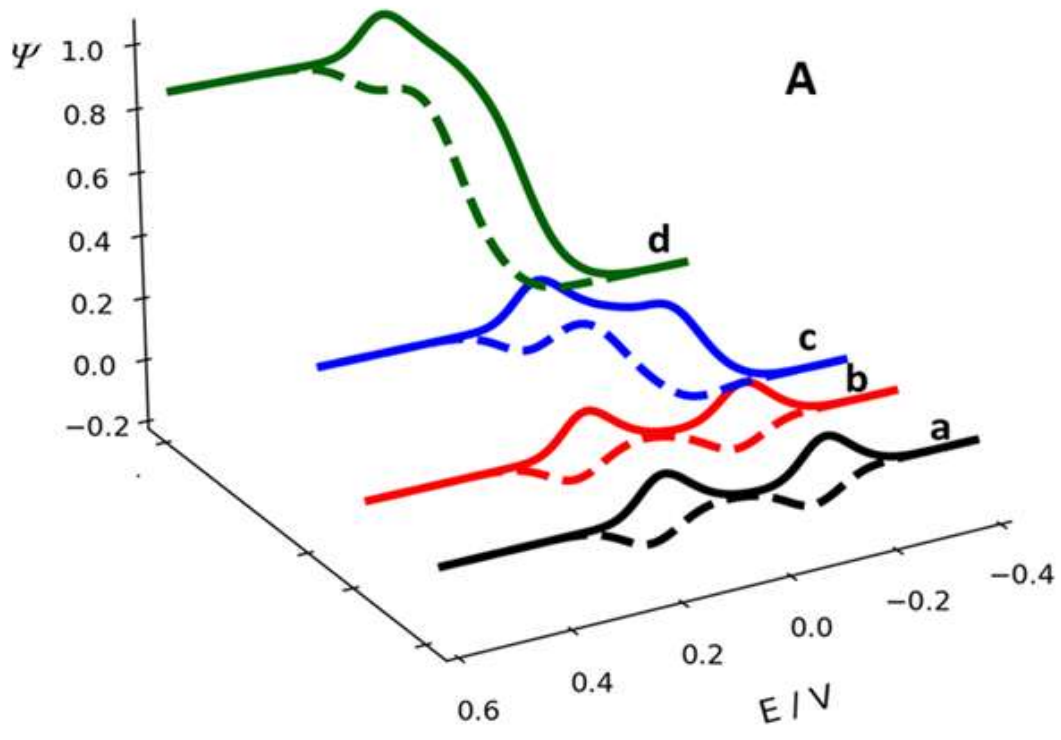
# Probing the Two-Step Double-Regenerative Enzymatic Mechanism by Protein-Film Voltammetry

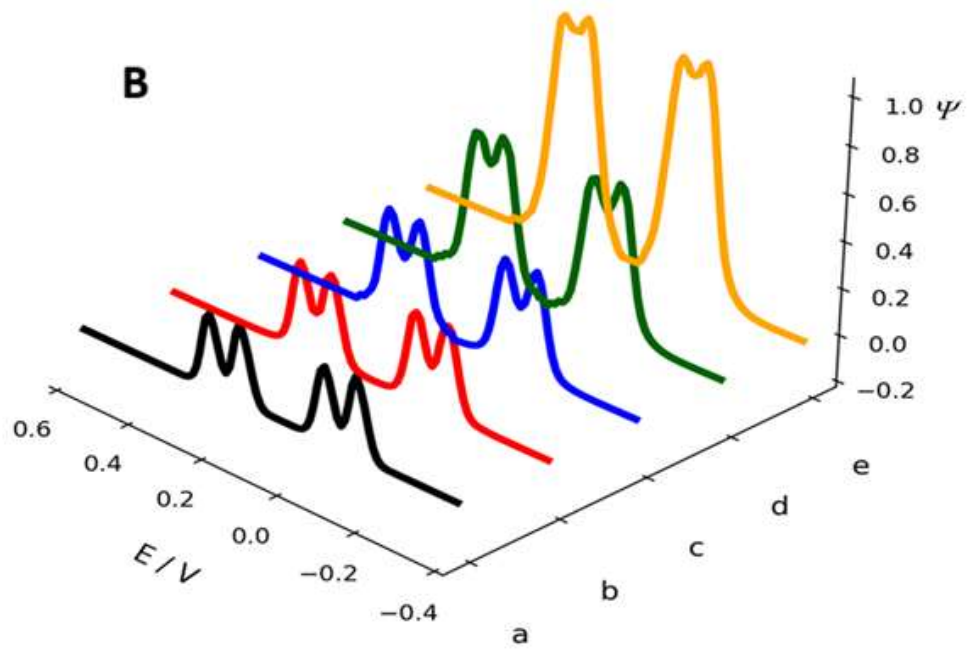
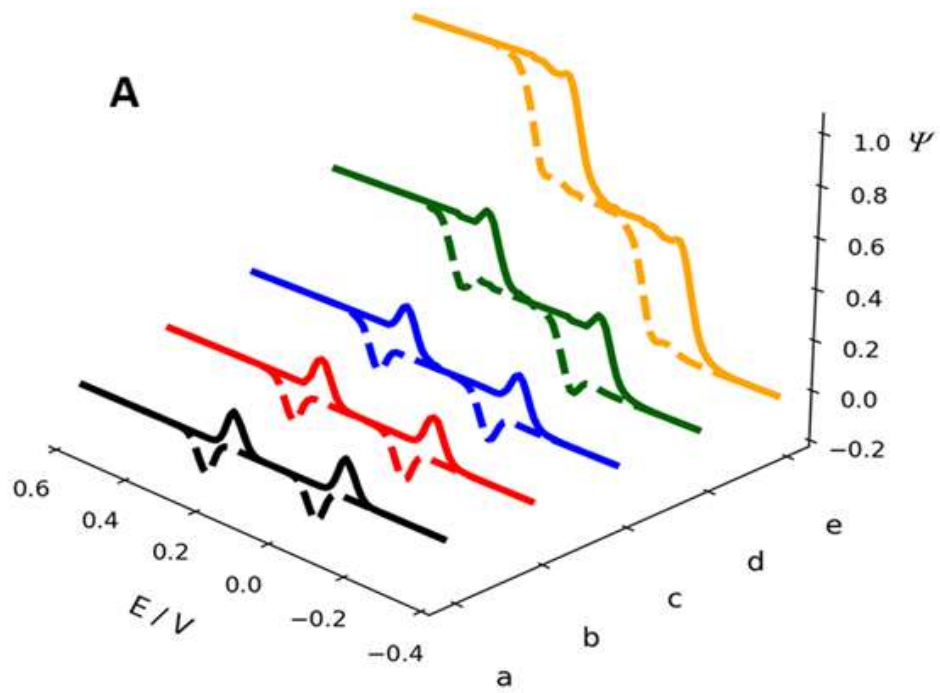
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## Abstract

Redox transformations of many lipophilic proteins and related biochemical systems proceed via sequential two-step electron-transfer processes. In a large number of such systems, sustained catalytic turnover is enabled by regenerative chemical reactions that are coupled to the electron-transfer steps, resulting in complex multistep electrochemical–catalytic behavior. In this work, we present the first theoretical model of a surface-confined two-step double-regenerative mechanism, schematically denoted as a surface  $EC'EC''$  mechanism, formulated under square-wave voltammetry (SWV) conditions. A systematic analysis is carried out to elucidate the combined effects of electron-transfer kinetics, chemical regeneration rates, and instrumental parameters on the resulting square-wave voltammetric response. The simulated voltammograms reveal distinct mechanistic signatures arising from the interplay between sequential electron-transfer steps and catalytic regeneration at the electrode surface. The findings of this study are particularly relevant for researchers employing experimental protein-film voltammetry, as the model provides a robust theoretical framework for interpreting complex voltammetric responses of lipophilic redox proteins and enzymes. Furthermore, the proposed approach offers a practical platform for establishing diagnostic criteria and for developing quantitative methodologies aimed at extracting kinetic parameters directly related to enzymatic activity and catalytic efficiency in biologically relevant redox systems. Forward and backward voltammetric curves of this mechanism are given, simulated for moderate and very fast rates at both electron transfer steps. These voltammograms present playground for deeper insights in the mechanisms in which many redox enzyme undergo redox transformation in living systems.





## REFERENCES

1. R. Gulaboski, *Journal of Solid State Electrochemistry* 24 (2020) 2081-2081
2. R. Gulaboski, E. S. Ferreira, C. M. Pereira, M. N. D. S. Cordeiro, A. Garau, V. Lippolis, A. F. Silva, *Journal of Physical Chemistry C* 112 (2008) 153-161
3. R. Gulaboski, V. Mirceski, M. Lovric, I. Bogeski, *Electrochemistry Communications* 7 (2005) 515-522.
4. R Gulaboski, V Mirceski, *Macedonian Journal of Chemistry and Chemical Engineering* 39 (2020) 153-166
5. V. Mirceski, R. Gulaboski, *Macedonian Journal of Chemistry and Chemical Engineering* 33 (2014), 1-12
6. V. Mirceski, R. Gulaboski, *Journal of Solid State Electrochemistry* 7 (2003) 157-165
7. M. Janeva, P. Kokoskarova, V. Maksimova, R. Gulaboski, *Electroanalysis* 31 (2019) 2488-2506
8. R. Gulaboski, V. Mirceski, S. Komorsky-Lovric, M. Lovric, *Electroanalysis* 16 (2004) 832-842
9. R. Gulaboski, C.M. Pereira, M.N.D.S Cordeiro, I. Bogeski, F. Silva, *Journal of Solid State Electrochemistry* 9 (2005) 469-474
10. B. Sefer, R. Gulaboski, V. Mirceski, *Journal of Solid State Electrochemistry* 16 (2012) 2373-2381.
11. P. Kokoskarova, Rubin Gulaboski, *Electroanalysis* 32 (2020) 333-344.  
<https://doi.org/10.1002/elan.201900491>
12. R. Gulaboski, C. M. Pereira, *Electroanalytical Techniques and Instrumentation in Food Analysis; in Handbook of Food Analysis Instruments* (2008) 379-402.
13. M. Jorge, R. Gulaboski, C. M. Pereira, M. N. D. S. Cordeiro, *Journal of Physical Chemistry B* 110 (2006) 12530-12538.

14. V. Mirceski, D. Guziejewski, L. Stojanov, R. Gulaboski, *Analytical Chemistry* 91 (2019) 14904-14910.
15. V. Mirceski, R. Gulaboski, F. Scholz, *Journal of Electroanalytical Chemistry* 566 (2004) 351-360.
16. R. Gulaboski, M. Chirea, C. M. Pereira, M. N. D. S. Cordeiro, R. B. Costa, A. F. Silva, J. *Phys. Chem. C* 112 (2008) 2428-2435
17. R. Gulaboski, V. Mirceski, S. Komorsky-Lovric, M. Lovric, *Electroanalysis* 16 (2004) 832-842
18. R. Gulaboski, C. M. Pereira, M. N. D. S. Cordeiro, A. F. Silva, M. Hoth, I. Bogeski, *Cell Calcium* 43 (2008) 615-621
19. R. Gulaboski, V. Mirceski, F. Scholz, *Amino Acids* 24 (2003) 149-154
20. V. Mirceski, R. Gulaboski, *Croatica Chemica Acta* 76 (2003) 37-48.
21. F. Scholz, R. Gulaboski, *Faraday Discussions* 129 (2005) 169-177.
22. R. Gulaboski, K. Caban. Z. Stojek, F. Scholz, *Electrochemistry Communications* 6 (2004) 215-218.
23. V. Mirceski, R. Gulaboski, *Journal of Physical Chemistry B*, 110 (2006) 2812-2820.
24. V. Mirceski, R. Gulaboski, B. Jordanoski, S. Komorsky-Lovric, *Journal of Electroanalytical Chemistry*, 490 (2000) 37-47.
25. R. Gulaboski, *Macedonian Journal of Chemistry and Chemical Engineering* 41 (2022) 151-162
26. R. Gulaboski, P. Kokoskarova, S. Petkovska, *Analytical&Bioanalytical Electrochemistry*, 12 (2020) 345-364.
27. V. Mirčeski, R. Gulaboski, F. Scholz, *Electrochemistry Communications* 4 (2002) 814-819
28. M. Jorge, R. Gulaboski, C. M. Pereira, M. N. D. S Cordeiro, *Molecular Physics* 104 (2006) 3627-3634.

29. R. Gulaboski, V. Mirceski, M. Lovric, *Macedonian Journal of Chemistry and Chemical Engineering* 40 (2021) 1-9.
30. R. Gulaboski, P. Kokoskarova, S. Risafova, J. *Electroanal. Chem.* 868 (2020) 114189.
31. R. Gulaboski, V. Mirceski, *Journal of Solid State Electrochemistry* 28 (2024) 1121-1130.
32. V. Mirceski, B. Mitrova, V. Ivanovski, N. Mitreska, A. Aleksovska, R. Gulaboski, *Journal of Solid State Electrochemistry* 19 (2015) 2331-2342.
33. I. Spirevska, L. Soptrajanova, R. Gulaboski, *Analytical Letters* 33 (2000) 919-928.
34. R. Gulaboski, B. Jordanoski, *Bulletin of Chemists and Technologist of Macedonia* 19 (2000) 177-181
35. R. Gulaboski, M. Lovrić, V. Mirčeski, I. Bogeski, M. Hoth, *Biophysical Chemistry* 137 (2008) 49-55.
36. R. Gulaboski, V. Mirčeski, S. Mitrev, *Food Chemistry*, 138 (2013) 116-121.
37. R. Gulaboski, V. Mirčeski, M. Lovrić, *Journal of Solid State Electrochemistry* 23 (2019) 2493-2506
38. V. Mirceski, R. Gulaboski, F. Scholz, *Electrochemistry Communications* 4 (2019) 814-819.
39. Rubin Gulaboski, V. Mirceski, *Journal of Solid State Electrochemistry* 28 (2024) 1121-1130.
40. R Gulaboski, F Borges, CM Pereira, M Cordeiro, J Garrido, AF Silva, *Combinatorial chemistry & high throughput screening* 10 (2007), 514-526