# Getting Insight into Enzymes Kinetics and Thermodynamics via Theoretical Models in Protein-film Square-wave Voltammetry

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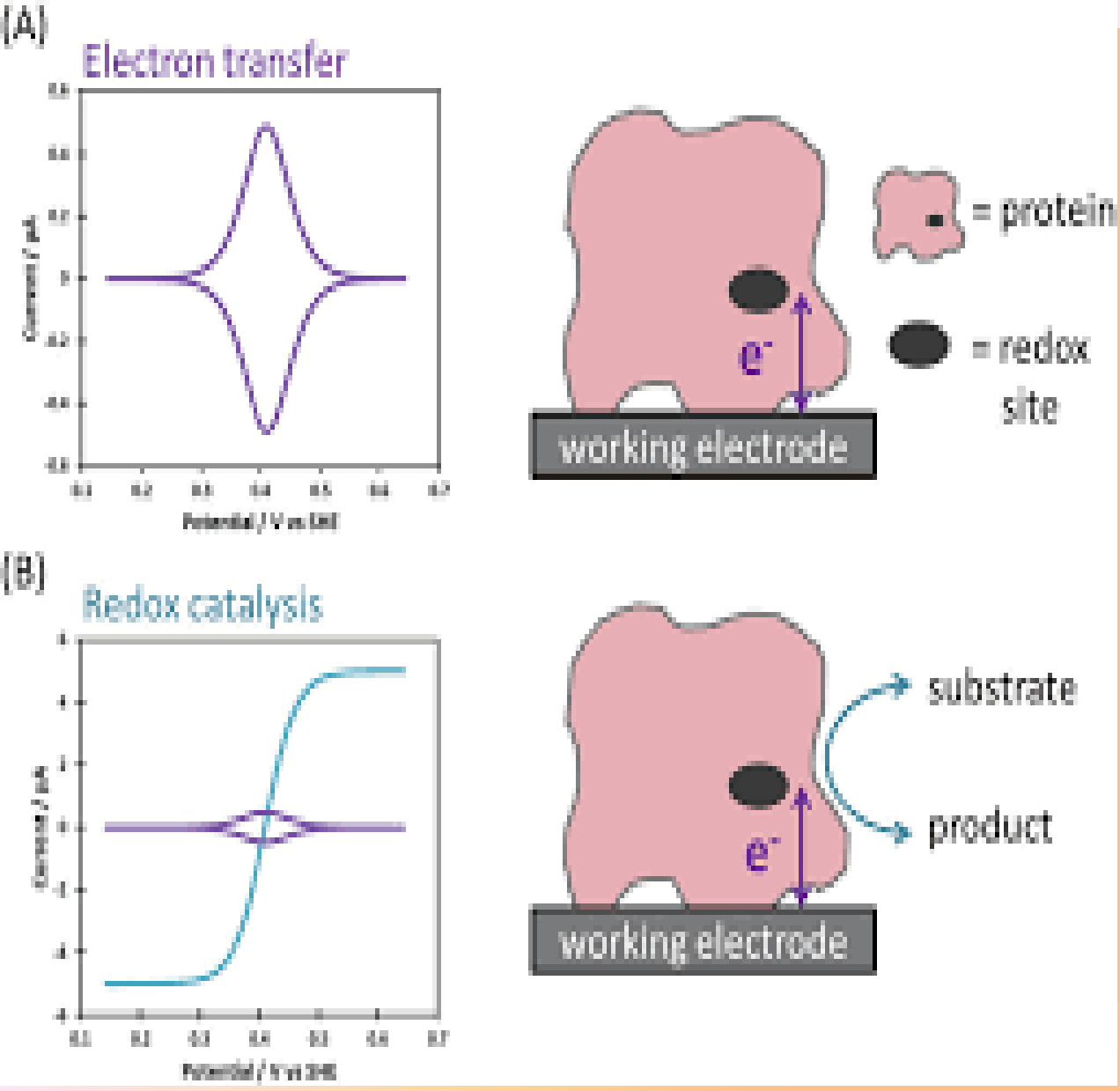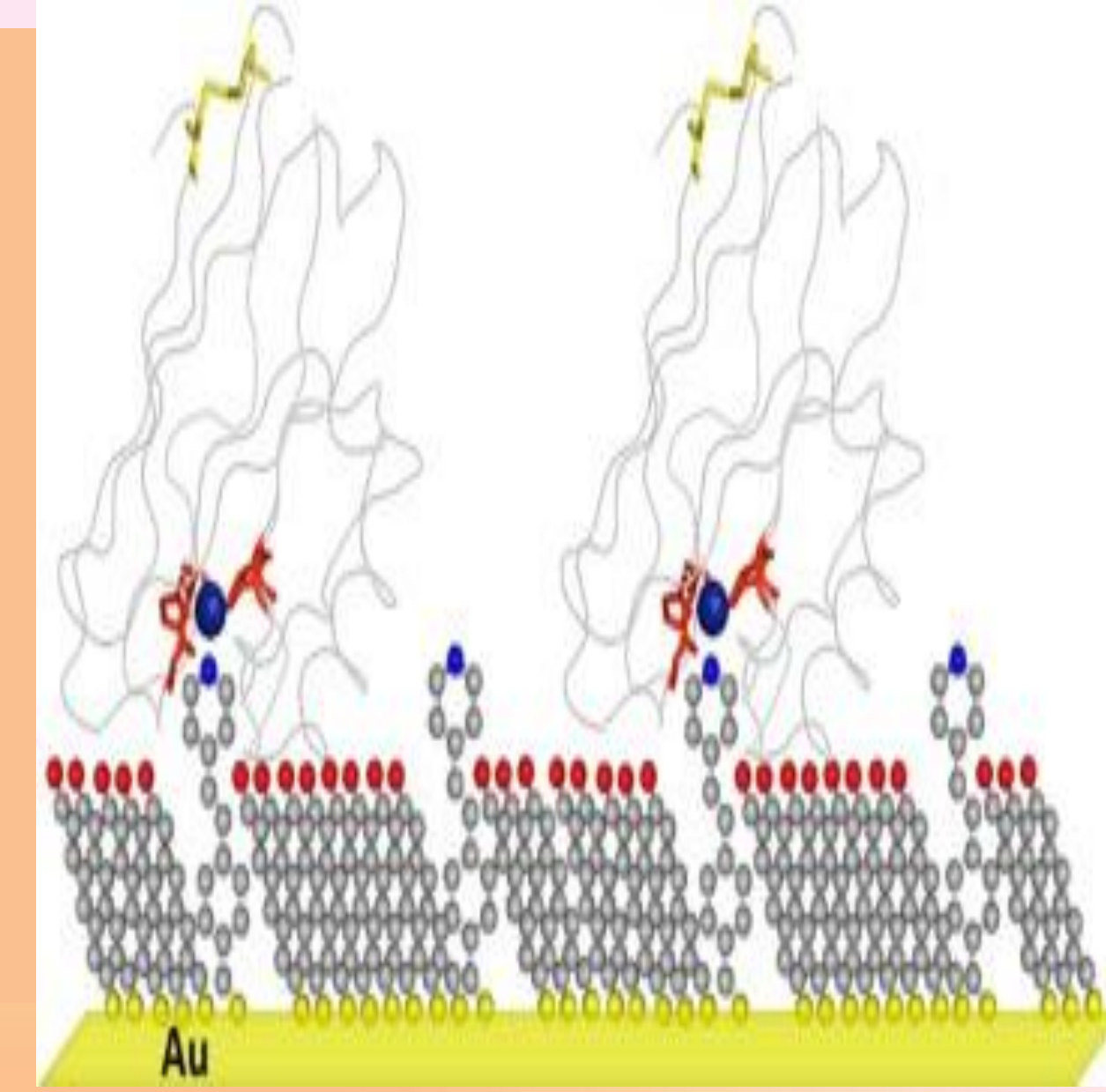
Introduction

In the last few decades, protein film voltammetry is known as a simple and useful tool for studying redox properties of many classes of lipophilic enzymes. The simplicity of this technique refers to the experimental set up and experimental conditions, but not to understanding of the obtained voltammetric results. If a given enzyme is firmly adsorbed on a working electrode surface forming a monolayer of non-interacting redox molecules, then its chemical features can be explored by developing theoretical models of so-called "surface redox reactions". When square-wave voltammetry (shortly SWV) is applied as a working technique, then the redox behaviour of many lipophilic enzyme featuring fast electron-exchange between working electrode and enzyme's redox active site(a) cannot be considered as a simple task. The main intriguing features in such scenario are portrayed via phenomena of "split net-SWV peak" [1] and the "quasireversible maximum" .

Mechanisms

In this work we present theoretical SWV results of several one step or two-step protein-film electrode reactions, in which the electrochemical steps going on at the electrode surface are coupled to preceding, follow-up or regenerative chemical steps. The adequate description of these mechanisms is "surface ECrev", "CrevE", ECatalityc, ECirevE, EECrev., EECirev. and EECatalytic mechanism", where "E" stands for the electrode reaction, while "C" stands for the chemical step. "catalytic" stands for the regenerative chemical reaction, "rev" means reversible and "irr" means ireevrsible chemical step

Ongoing theoretical modeling

We have focused mainly on the effects of kinetics of chemical steps to the features of the electrode enzymatic reactions, while we give time independent methodologies for getting insight into the rate of chemical as well as equilibrium rate constants of reversible chemical steps. Alongside to the applied potential instrumental parameters, fhe voltammetric outputs of considered mechanisms depend on several dimensionless parameters defined as follow: dimensionless electrode kinetic parameter K ($K = k_s/f$), where k_s is the standard rate constant of electron transfer, and f is the SW frequency; dimensionless rate parameter of the reversible chemical step Kchem defined as $Kchem = (k_f+k_b)/f$, where k_f and k_b are the rate constants of the direct and backward chemical steps; Equilibrium constant of chemical stek $Keqiulibrium = k_f/k_b$. We show on the next few figures several aspects of the effect of chemical step to simple one-electron enzymatic electrode reactions coupled with preceding and follow up chemical steps.
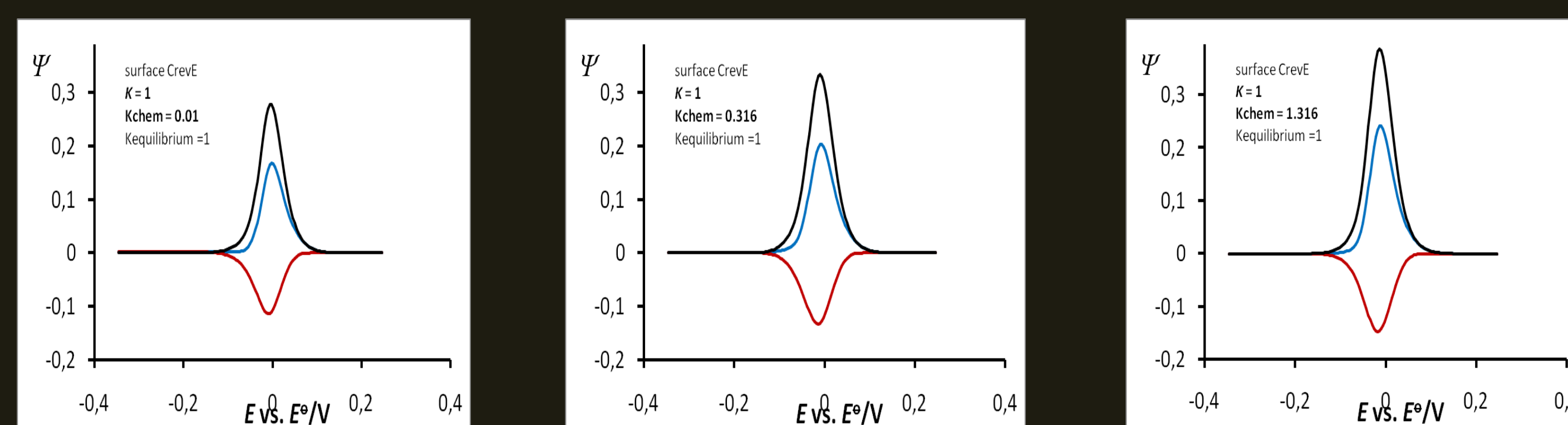

Figure 1. Surface CrevE mchanism: Effect of the dimensionless chemical parameter Kchem to the SW voltammograms featuring slow electron transfer

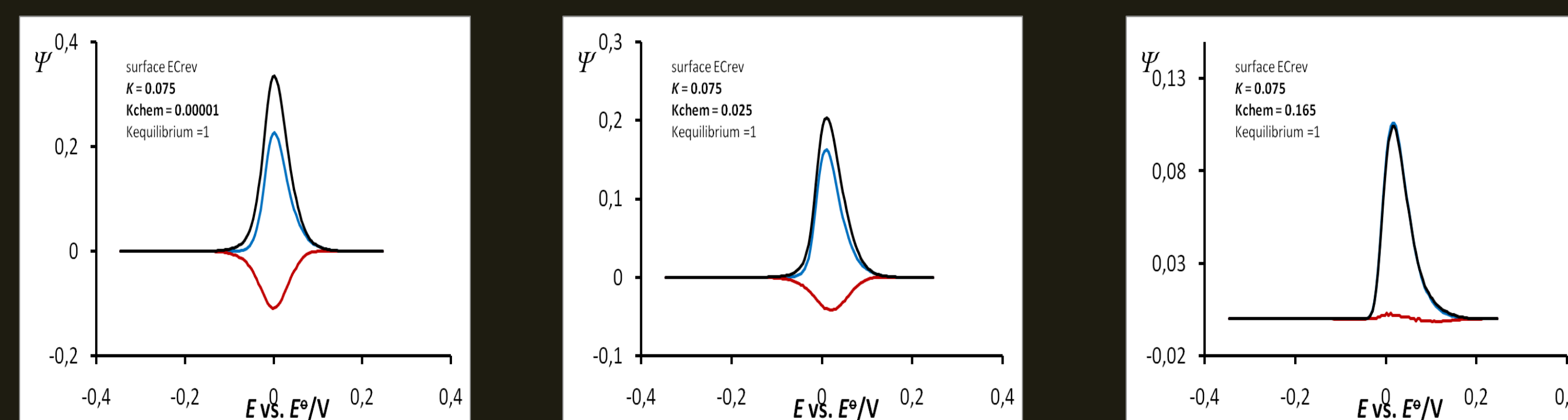Figure 2 Surface ECrev mechanism: Effect of the dimensionless chemical parameter Kchem to the SW voltammograms featuring slow electron transfer

For fast electron transfer enzymatic reactions at the electrode surface, a "splitting" phenomenon of the SWV net-peak occurs (figure 3a). In such reaction mechanisms, when the kinetics of follow-up chemical step is comparable to the rate of electrode reaction, we see effect of increasing of both reduction and oxidation (and also of SWV net) current components as the value of chemical rate parameter Kchem increases (figure 3b, 3c), and vanishing of "splitting" (figure 3d).
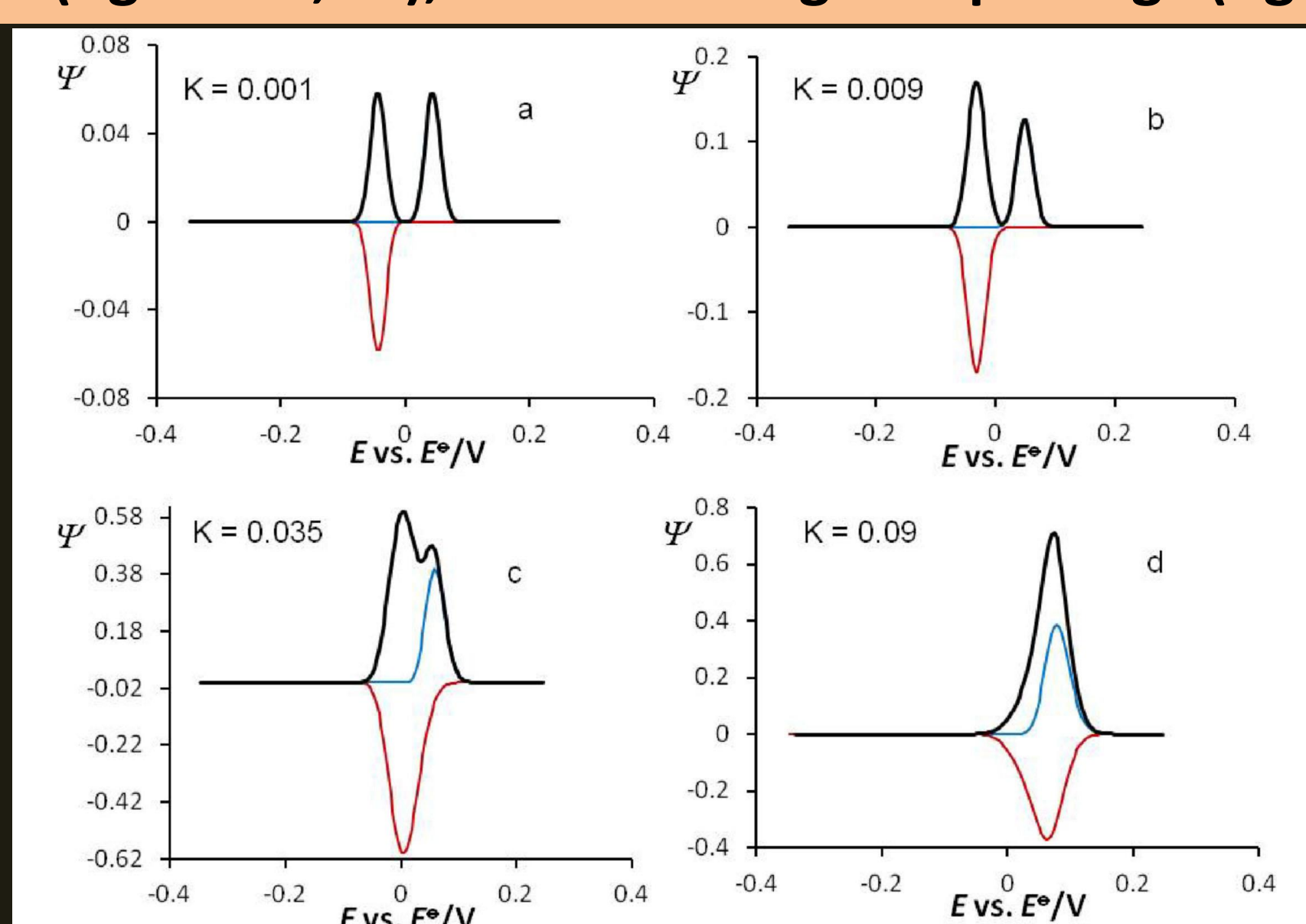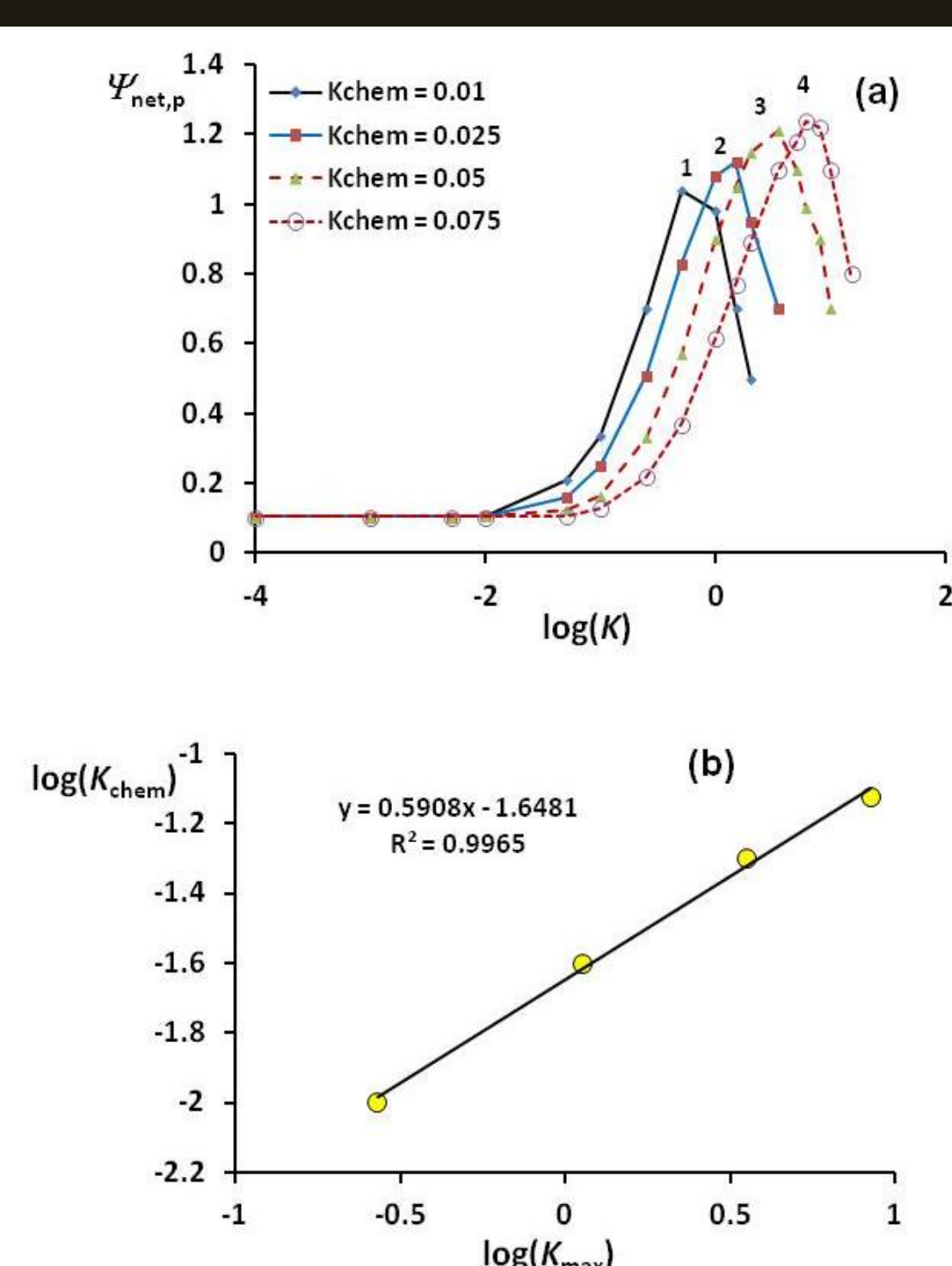

Figure 3 Surface ECrev reaction: Effect of the dimensionless chemical kinetic parameter Kchem to the SW voltammograms featuring fast electron transfer simulated for K = 5 (situation of split SW peaks).

Another remarkable feature of surface enzymatic reactions studied in SWV is phenomenom of "quasireversible maximum" (figure 4a), which can be used for electrode kinetic determination. As this phenomenon by surface reactions coupled to chemical steps is affected by the rate of chemical step (figure 4a and figure 4b), we can use the equation on linear line in figure 4b to determine the chemical rate kinetics of follow up chemical reaction.

Figure 4. Surface ECrev mechanism with slow and moderate electron reaction rates and equilibrium constant of follow-up chemical reaction of Keq = 1. (a) "Quasireversile maxima" simulated for four different values of dimensionless chemical parameter Kchem. Kchem was set to 0.01 (1); 0.025 (2); 0.05 (3) and 0.75 (4). (b) Dependence of log(Kchem) as a function of logarithm of maximal values of dimensionless electrode kinetic parameter log(Kmax).

Conclusions

Many surface enzymatic reactions are considered as "simple" in terms of their redox behavior at working electrodes. However, some of these "simple" protein-film reactions mechanisms, when studied in square-wave voltammetry, can be strongly affected in unusual way by kinetics and thermodynamics of preceding, follow-up, or regenerative chemical steps, as it is shown in figures 3 and 4 in the results of this short paper. While we present plenty of relevant voltammetric situations useful for recognizing relevant and most common protein-film mechanisms in square-wave voltammetry, we also propose several new approaches in square-wave voltammetry to get access to kinetics and thermodynamics of chemical steps at all those mechanisms. Most of the results in this work throw new insight into the features of protein-film systems that are coupled with chemical reactions.

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