POSTER - SYSTEMS BIOLOGY AND MODELING

ENZYME CHARACTERIZATION FOR SYSTEMS BIOLOGY: DEALING WITH UNCERTAINTIES AND COMPLEX EXPERIMENTS WITH A SOFTWARE-AIDED APPROACH

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The characterization of enzyme kinetics is a non-trivial and labor-intensive process, where the experimentalist has to deal with several sources of noise and errors before reaching a conclusion on the values of the kinetic parameters and their uncertainties. First, the choice of the model itself has implications on the experimental design. The classical single-substrate irreversible Michaelis-Menten model is one of the simplest and most widely used options, but is usually unsuitable for posterior use in more elaborate applications in systems biology, due to the fact that additional substrates are not taken into account, and neither is the equilibrium constant of the reaction. On the other hand, if the model is too complicated, some parameters might be difficult to estimate, or

require many more experiments. Second, usually only one of the metabolites in the reaction can be measured in real time, for example NAD(P)+ (through absorbance or fluorescence), and the other chemical species must be inferred from stoichiometry, if at all possible, resulting in incomplete information. This picture is further complicated in coupled assays, where one or more auxiliary reactions/enzymes are required so that the system contains a measurable metabolite. Usually the auxiliary reactions are considered instantaneous, which may lead to errors in the analysis of the data.

In this work we present the obtainment of kinetic parameters for systems biology ready models, performed with the use of a software developed in our lab. It can infer parameters for a user-supplied kinetic model, as well as calculate their uncertainty without any assumptions on the distribution of the parameters. The simplest experiment involves the enzyme Enoyl-CoA hydratase: a simple 1-substrate 1-product enzyme, for the purpose of validation and exemplification. Then, we apply the software to Pyrroline-5-carboxilate synthase, a bifunctional enzyme, where both domains must be taken into account during parameter estimation. Finally, we analyze the activity of Asparaginase A, that requires three auxiliary reactions for the system to display a measurable metabolite, and creates a cycle in the reaction scheme as a side effect. This very challenging experiment can only yield quantitative results when all components are considered in the characterization. All enzymes are recombinant, from Trypanosoma cruzi, and metabolites NAD(P)H and Crotonoyl-CoA were measured through absorbance.

Palavras-chave: metabolism; machine learning; kinetic models.