Kinetics of Synthetic Multi-Enzyme Reaction Networks: Dynamic Flux Estimation by use of Piecewise Cubic Hermite Interpolating Polynomials (PCHIP)

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Highlights

- A synthetic reaction network (CETCH), catalyzed by 12 enzymes, is investigated.
- PCHIP is used for estimation of time derivatives from metabolite concentration data.
- Flux estimates are used for parameter estimation.
- Non-hyperbolic dependencies of fluxes on substrate concentrations were found.

1. Introduction

The interest in synthetic *in vitro* metabolic networks is growing over the years. These cell-free systems offer flexibility and precise control over reaction conditions, making them preferable over complex cell-based systems for rapid design and optimization. The CETCH cycle, designed for CO_2 fixation, highlights this through *in vitro* conversion of CO_2 into glycolate through a series of 12 enzymatic core reactions and accessory reactions for cofactor regeneration, byproduct neutralization, and readout [1]. However, kinetic modeling, crucial for computer-aided optimization of these networks, faces challenges due to incomplete, sparse, and noisy data, leading to issues such as overfitting, sloppiness, collinearity, parameter non-identifiability and algorithmic inefficiencies. These problems result in models that perform poorly on new or unseen data. Another issue is the choice of a correct mathematic format that describes the observed dynamics, beyond standard rate laws (e.g. Michaelis-Menten) often used for enzyme kinetic modeling.

To address these challenges, parameter estimation is handled through an initial flux estimation step. This approach, called Dynamic Flux Estimation (DFE), starts with a model-free flux estimation from time-series data, followed by model-based parameter estimation for each flux [2]. This method sidesteps the complexities of simultaneous parameter estimation of an entire model and the rigidity of predefined mathematical formats, presenting a more flexible and potentially accurate modelling strategy.

2. Methods

Time-series concentration measurements of ten chemical species in the CETCH cycle were analyzed, with the dataset comprising three repetitions per experimental conditions, but focusing first only on one specific setting of conditions. Using MATLAB's built-in functions, piecewise cubic Hermite interpolating polynomials (PCHIP) were applied for curve reconstruction from time-series data, thus ensuring a smooth estimation of concentration derivatives w.r.t. time, preserving the monotonicity between data points to accurately reflect the underlying trends without introducing artificial oscillations. For ten observable chemical species, initial temporal gradient estimations were conducted, allowing for the estimation of 10 out of 13 fluxes (12 core reactions and an additional reaction as readout module). A linear system correlating time-series derivatives with the stoichiometric matrix $\tilde{S}$ and the flux vector v was constructed, enabling flux calculation via non-negative least squares (NNLS) from time derivative estimates and a full-rank observable submatrix $\tilde{S}_{obs}$. Some of the flux estimates have then been used to reconstruct the time-series profiles of unobservable chemical species, specifically those of acrylyl-CoA (for Ccr rate equation parametrization) and glyoxylate (for Gor rate equation parametrization), as the fluxes that generate and consume these chemical species can be estimated.

Subsequent parameter estimation for each flux was performed under the assumption that fluxes were predominantly affected by a single limiting substrate, given the excess of cofactors, leading to the application of pseudo-first-order Michaelis-Menten kinetics, except for the crotonyl-CoA

carboxylase/reductase (Ccr) reaction, which is assumed to follow the two-substrate random-order ternary complex mechanism using acrylyl-CoA and crotonyl-CoA as substrates. For parameter estimation, a particle swarm method is used with a swarm size of 200 and up to 1000 stall iterations. It was hybridized with MATLAB's gradient-based *fmincon* using an interior-point algorithm for refining the parameter estimates.

3. Results and discussion

It was expected that many of the estimated flux profiles follow characteristic hyperbolic relationships between the reaction rates (fluxes) and substrate concentrations, typical for Michaelis-Menten kinetics. However, when plotting flux estimates against substrate concentrations, the kinetics indicate hyperbolic behaviour but do not strictly follow Michaelis-Menten kinetics (see Fig.1, r.h.s.). This might due to several factors, in particular competitive inhibition of enzymes by other metabolites involved in the CETCH cycle, and limited specificity of the used enzymes with respect to their substrates. In order to find suitable kinetic descriptions, bigger datasets covering a broader range of operating conditions (cofactor concentrations, pH, temperature etc.) need to be applied. Nevertheless, the here proposed PCHIP-based dynamic flux estimation approach yields highly valuable information about the underlying enzymatic reaction kinetic.

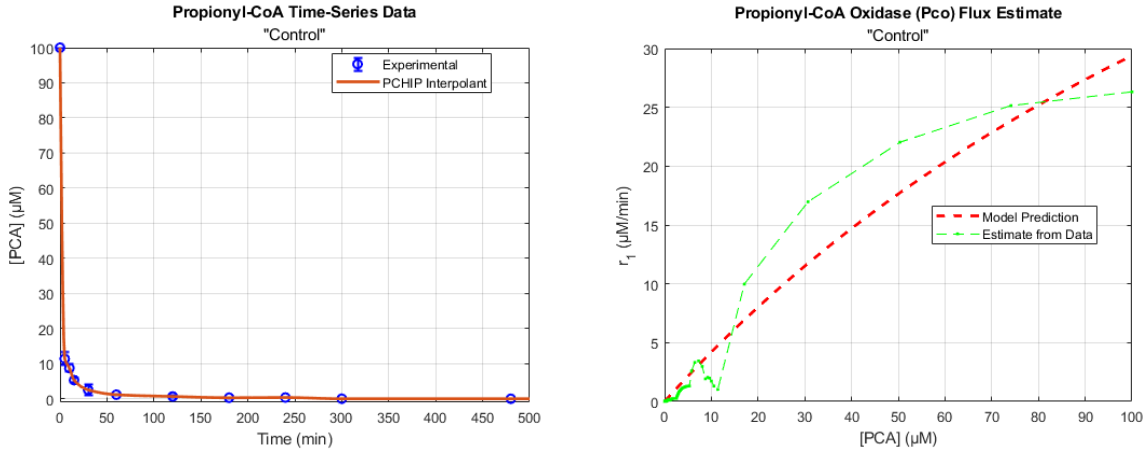

Figure 1. Left: Time-series concentration data for the propionyl-CoA oxidase reaction with the corresponding PCHIP interpolant. Right: Flux estimates (green) and Michaelis-Menten model predictions (red) at "Control" conditions.

4. Conclusions

PCHIP is used for time derivative estimation from time-series data. It turned out to be a very useful tool to obtained reaction flux estimates from dynamic reaction experiments. The flux estimates can then be used for model-based parameter estimation for the CETCH enzyme system. As a first approach, for all reaction steps we used the Michaelis-Menten equation, with the exception of the Ccr step which is modeled to follow a two-substrate random-order mechanism. The overall approach simplifies kinetic analysis, but discrepancies between Michaelis-Menten model predictions and the estimated flux-concentration curves suggest a mismatch between the assumed enzymatic kinetic mechanisms and the system's actual behavior. This indicates the necessity of using more complex kinetic models and including additional datasets at a variety of different operating conditions. We are therefore currently working on kinetic descriptions that account for product inhibition, backward reactions due to the reversibility of several CETCH reaction steps, degradation reaction steps of enzymes, and inhibition caused by the many metabolites involved in the overall CETCH cycle.

References

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Keywords
CETCH Cycle; PCHIP; Dynamic Flux Estimation; Kinetic Modeling