September 26, 2022

Dear Editor,

We wish to submit an original research paper entitled 'Elucidating yeast glycolytic dynamics at steady state growth and glucose pulse through kinetic metabolic modeling' for consideration by *Metabolic Engineering*. In this work, we have developed a physiologically informed kinetic metabolic model of *Saccharomyces cerevisiae* glycolysis. This tool has the potential to help the academic and industrial communities to understand, monitor and manipulate the response of this microbial cell factory to changing environments.

To understand and ultimately manipulate central metabolism, we developed a kinetic model of glycolysis connected to the effect of anabolic precursor sink reactions, mitochondria and the trehalose cycle. To deal with the challenge of model complexity, a parameter estimation pipeline was developed, consisting of a divide and conquer approach, supplemented with regularization and global optimization. The resulting model simultaneously describes for the first time a growing cell at different steady states and under a 110 mM glucose perturbation, is robust to parametric uncertainty, explains the contribution of the different pathways in the network, and indicates locations in the model where regulation is missing. This model is also relevant for the yeast metabolic engineering community because it shows how to bring kinetic metabolic models a step forward to be useful for both academia and industry, physiological information has to be considered, and not enzyme kinetics alone.

We believe that this manuscript could be interesting for the *Metabolic Engineering* readers for several reasons: First, this metabolic reconstruction is a tool for the yeast biotechnology community, which has struggled to develop a model of such a coverage and that can be implemented in the industrial setup. Second, glycolysis is a key pathway in the central metabolism of most microbial cell factories, and its modulation by genetic engineering or environmental perturbation has a critical impact in the bioprocess outcome. Third, a new parameter estimation pipeline is developed, fit for dealing with complex metabolic models and integrating different data types.

If the reviewers want to check the code, we can send the it. We declare no conflicts of interest and confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with its submission.

Thank you for your consideration of this manuscript.

Sincerely,

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