Effective Dynamic Models of Metabolic Networks

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To Whom It May Concern:

We are thankful to IEEE LSL, and the reviewers for providing feedback on our manuscript: Effective Dynamic Models of Metabolic Networks. We address the reviewer comments below, our response is in red.

**Reviewer 1:**

1) Please clarify if removal of the minor modes is done all at once or one by one.

All the minor modes were removed simultaneously. We have clarified this in the Results Section (II), paragraph 4 sentence 4.

2) Cell mass variable c is not explicitly stated in Section IV.

Thank you for pointing out this important oversight. We have defined the cell mass variable c in the Materials and Methods Section (IV) paragraph 1 sentence 7.

3) Would eliminating the unnecessary modes (which is done for a particular data set) diminish the predictive capability of the model?

We thank the reviewer for this excellent question. Generally, model reduction will diminish the predictive power of most models. However, the minor modes removed here, determined by global sensitivity analysis, represented maintenance states of the cell. Thus, removal of these modes would likely not diminish the predictive capability of the model if the culture was actively growing and maintenance rates were small. However, if the culture growth slowed, these states could become important. In addition, (although not true in this example) minor modes could also represent growth on alternative substrates that were not present when the sensitivity analysis was conducted (thus their influence was negligible). In these cases, the predictive power of the model would definitely be diminished. We have addressed this point in the Discussion Section (III) paragraph 1 sentence 12.

**Reviewer 2:**

1.) Seems odd that the main focus of the paper is a comparison to HCM-EM. This would make sense, if HCM-EM were considered to be the leading method in the field but that case has not been argued. Can you clarify the motivation behind this?

We appreciate the reviewers comment and agree the motivation behind our comparison was not transparent. Previously, HCM-EM has been compared to dynamic flux balance analysis (dFBA), one of the leading methods in the field (reference 12 in the manuscript). In this previous study, HCM-EM was better at predicting extracellular measurements than dFBA. Thus, we only compared our method to HCM-EM because HCM-FBA is a modification of HCM-EM, in particular a reduction of HCM-EM. Thus, we tried to establish that HCM-FBA had at least the same performance as HCM-EM, despite having fewer modes and parameters. We have addressed the reviewers question in the Introduction section (I) paragraph 2 sentence 5, and paragraph 3 sentence 3.

2) In the first example, I found it a bit odd that the authors decided to fit HCM-FBA model to data generated by HCM-EM. I’m not sure what this tells the reader.

We appreciate the reviewers concern, we believe this goes along with the first comment. Since HCM-EM was shown to have superior performance to dFBA, we only compared HCM-FBA to HCM-EM. We wanted to show our method had similar model performance to HCM-EM with fewer parameters and modes. Therefore, we fit HCM-FBA to data generated by HCM-EM to show that we could describe this data with a different formulation. We addressed this issue in the Introduction section (I) paragraph 3 sentence 3.

3) There is an emphasis on the difference in calculated modes when comparing HCM-FBA to HCM-EM but I’m not sure if this is a comment on the objective superiority of HCM-FBA or the overkill of HCM-EM.

We thank the reviewer for this insightful comment (which nicely highlights the underlying problem of HCM-EM). Elementary mode calculations are often intractable for large networks since the computational complexity of these calculations increases exponentially with network size. This is where HCM-FBA has an advantage, because it “naturally” lumps modes into minimal solutions. We addressed this reviewer concern in the Discussion Section (III) paragraph 1 sentence 15.

4) The authors revealed an important limitation of HCM-EM so I am curious about the performance of HCM-FBA when compared to other methods that are feasible?

We thank the reviewer for raising this important question. In general, cybernetic models have superior performance when compared to dFBA (or simple unstructured lumped kinetic models) for estimating extracellular measurements. However, HCM-EM is not feasible for large networks. Thus, HCM-FBA addresses a critical shortcoming of current cybernetic approaches.

In this study, we have not compared the performance of HCM-FBA to other feasible techniques e.g., dFBA or kinetic modeling. We know from previous studies that HCM-EM performs well compared to dFBA, but we have not directly compared HCM-FBA versus dFBA. While we agree this is an important question, we feel this could be better addressed in a follow-on manuscript and not in the current manuscript. In this study, we took a critical first step by showing that HCM-FBA performed at least as well as the most logical cybernetic analog, HCM-EM. Next, we’ll need to compare HCM-FBA directly with other approaches. We expect HCM-FBA will outperform dFBA, but will likely have similar performance to detailed kinetic formulations. However, the advantage of HCM-FBA in this context is its size; it will likely have far fewer parameters than a corresponding kinetic model. These studies are currently underway in our lab.

5) The authors should clearly spell out the distinction between their method and the method presented in [12]. I had to read through the referenced paper [12], which I assume used the method referred to as HCM-EM (I did not see this acronym anywhere so it was a bit confusing). It seems that the EM method uses a dynamic analog of FBA. I would assume that there is a mapping from HCM-EM to HCM-FBA. I’m curious if through the comparisons in the paper the authors can point to which assumptions are valid in FBA. For instance, which states can be assumed to be in steady state and might this shed light on the real system.

We appreciate the reviewers comment and understand that the HCM-EM acronym has not been used before. Thus, we have changed the acronym to HCM throughout the manuscript to be consistent with the original hybrid cybernetic approach used.

The mapping from EM to FBA is explained in the Introduction, paragraph 1 sentence 4. (EMs (or EPs) catalog all possible metabolic behaviors such that any flux distribution predicted by FBA is a convex combination of the EMs (or EPs) [5].)

We also addressed the assumptions of FBA solutions: model intracellular metabolism using the biochemical stoichiometry and other constraints such as thermodynamically feasibility under pseudo-steady state conditions (Introduction, paragraph 1 sentence 3).

Lastly, we addressed the comment on the operating state of the system in the Discussion section paragraph 1 sentence 12 and 13. Insignificant modes were associated with maintenance. Thus, they would not impact the model's predictive capability for a growing culture. A mode consuming a substrate was determined to be active which can give insight to the operating state of the cell.

6) Finally, the authors brought up a good point about comparing to methods with carbon labeling. However I am more interested in how one might be able to incorporate data from 13C into this cybernetic approach. This might be outside the scope of the paper.

We thank the reviewer for this insightful comment. At the core of this future direction is the desire to validate/constrain the flux distribution predicted by HCM-FBA. Carbon labeling is a standard technique that has been utilized with both MFA and FBA to constrain the metabolic solution space. In the context of HCM-FBA, we could incorporate labeling data into the calculation in a similar way as is done with FBA; 13C labeling will establish bounds on the carbon distribution at key branch points in central metabolism. We could then use this information in the parameter estimation problem along with extracellular metabolite information, or measured uptake/secretion rates, e.g., see Varner 2000. However, we agree that this is outside the scope of the current paper and thus we have left it for future work.

Varner J. (2000) Toward the Large-Scale Prediction of Phenotype: Concept. Biotechnol. Bioeng. 69:664-78