Effective Dynamic Models of Metabolic Networks
IEEE Life Sciences Letters
Manuscript Number: 16-0005

To Whom It May Concern:

We are thankful to IEEE LSL and the reviewers for reading and providing feedback to our manuscript: Effective Dynamic Models of Metabolic Networks. We have read the comments and have addressed them.

For 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.
We appreciate the comment and 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?
The minor modes determined by the global sensitivity analysis represent maintenance states of the cell that would not diminish the predictive capability of the model if the culture is growing. If the culture has stopped growing these states may become more important, however this is typically not a point of interest for fermentation processes. This is a very good question and we address it in the Discussion Section (III) paragraph 1 sentence 12

For 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 comment and we agree the motivation behind our comparison is lacking. HCM-EM has been compared to dynamic flux balance analysis in referenced paper [12] which is one of the leading methods in the field. In [12] HCM-EM shown better performance in predicting extracellular measurements than DFBA, therefore we only compared our method to HCM-EM. We address this issue 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 comment and we believe this goes along with the first comment. Since HCM-EM was shown to have superior performance to DFBA, we found it necessary to only compare it to HCM-EM. We wanted to show our method has similar

model performance to HCM-EM with fewer parameters and modes and therefore we decided to fit HCM-FBA to data generated by HCM-EM. We address 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.

This is a very insightful comment and shows the underlying problem of HCM-EM. Elementary modes show the complexity of a cell and the many possible routes it can take. From a modeling perspective, EM calculations are an overkill for large networks since its decomposition increases exponentially, this is where FBA has an objective superiority to lump these modes into minimal solutions. We address this comment 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?

This is a very important aspect of evaluating the strength of our model. Hybrid cybernetic models have been shown to have superior performance to DFBA in estimating extracellular measurements for batch cultures. Thus we only compared our method to HCM-EM. Cybernetic models have predicted mutant behavior [7, 8], steady-state multiplicity [9], strain specific metabolism [10], and have been used in bioprocess control applications [11] (I Introduction, paragraph 2 sentence 2). Kinetic Models of such scales have large uncertainties in parameter estimations (III Discussion, paragraph 2 sentence 1)

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 comment and understand that HCM-EM has not been used before. Thus, we have changed the acronym to HCM to represent the original hybrid cybernetic approach used. The mapping from EM to FBA is explained in the Introduction section 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 address the assumptions of FBA solutions: model intracellular metabolism using the biochemical stoichiometry and other constraints such as thermodynamical feasibility under pseudo- steady state conditions (Introduction, paragraph 1 sentence 3). We continued to address the comment on the operating state of the system in the Discussion section paragraph 1 sentence 12 and 13 (These insiginificant modes are

associated with maintenance states, thus they would not impact the model's predictive capability for a growing culture. A mode consuming a substrate is 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 ^{13}C into this cybernetic approach. This might be outside the scope of the paper.

 This is a very interesting point of expanding the current HCM-FBA model to incorporate carbon labeling data. Carbon labeling has been utilized with MFA and FBA to constrain the solution space for more realist operating states of the cell. We would incorporate this method the same way, once we are generating FBA modes it would be constrained to ^{13}C data thus this would reduce the generated modes of a network. Thus the model would become reduced but potentially have a stronger predictive capability. We agree this is outside the scope of the paper and thus left it for future work.