Review of “A compact model of Escherichia coli core and biosynthetic metabolism”

The medium-scale metabolic model of *E. coli* that is created by the authors will likely be a useful resource for the metabolic modeling community. Although the work is not necessarily creative or novel, the goal of this research also doesn’t require that. The work has been done carefully and is undoubtedly useful. The collection of various types of information about the metabolic model, as summarized in the ‘functional annotation graph’, will be useful as an information resource even if no metabolic modeling is performed. Finally, it is good to see that some commonly-used modeling methods are tried on the model, and that these turn out to be both feasible and that they give realistic results.

Since the work has been done carefully, I only have minor comments and suggestions on how to improve the current version, which I list below. In addition, I attach the filled-in survey as suggested by PCI to the reviewers.

Minor comments and questions

In Section 2.2, the authors compare the behavior of the model by performing FBA with various substrate constraints, and by making “production envelopes”. They show that the new model mostly agrees with the larger iML1515 model, and that where there is a deviation the new model is more reasonable. To me, this is slightly unconvincing since it is not clarified why the old model made the wrong predictions, and how this can be avoided in the future. Of course, it is not the authors’ task to explain the mistakes in the larger model, but explaining these mistakes could give more guarantee that no similar mistakes are present in the iCH360 model.

In Section 2.4 the authors distinguish catalysis connections as primary and secondary. Connections are noted as secondary if the reaction “accounts only for negligible activity for the reaction in the wild-type strain”. My question is if this will not be highly dependent on the conditions in which the wild-type strain is grown in the experiment. Can the authors provide some evidence that the experimental conditions that they take into account are broad enough, such that their primary/secondary annotations are applicable to almost all relevant conditions in which *E. coli* can grow?

“We did not find as significant differences” -> “We did not find any significant differences”

The authors perform enzyme predictions using “Enzyme-constrained FBA”, and state that their predicted enzyme abundances are quite good: “This analysis led to generally good predictions, with root mean squared error (RMSE, computed for log10-transformed enzyme abundances) ranging from 0.53 to 0.62 (Supplementary Figure 9).”
Looking at Supplemental Figure 9, one can see that many enzymes are 10-fold (to even 50-fold) off. Although this statement is subjective, I think it is a bit of a stretch to call these “good predictions”.

Next, the authors fit the turnover numbers that they previously found to fit the enzyme data better, “To increase the predictive ability of the model”. I don’t think it is sufficiently made clear that the predictive ability of the model has been improved. Clearly, the fit gets better if hundreds of parameters can be adjusted. However, it is not clear whether this increases the predictive ability in other conditions. Maybe the authors could at least do a standard splitting of the data into a training and test dataset, and then seeing if fitting the data on the training set improves the predictive ability on the test set. The authors could do this by leaving one of the experimental conditions out.

I don’t understand the phrase “to be attributed to variability in enzyme demand across conditions”, maybe it can be clarified.

The authors mention that EFMs cannot be calculated for the full model due to redundancies. First of all, there are several alternative elementary pathway definitions that should make their enumeration feasible. I would, clearly, suggest calculating Elementary Conversion Modes

(10.1016/j.patter.2020.100177) for this model, but a less biased overview of such alternative elementary pathways can be found in (https://doi.org/10.1371/journal.pcbi.1012472).
Second, when only redundant reaction pathways are deleted to produce the iCH360red model, can't knowledge of these deleted reactions be used to get the EFMs for the full model as well based on the EFMs that were calculated on the reduced model?

When Muller et al. [28] are cited for proving that an optimal solution will only use one EFM, I think it is more correct to also cite Wortel et al. (10.1111/febs.12722) since these papers found this result simultaneously.

"However, the satFBA formalism can also be used with additional flux bounds, thus going beyond EFM-based analysis." This is not true, it has been shown that the number of active EFMs is equal to the number of constraints (https://doi.org/10.1371/journal.pcbi.1006858). So by adding one or two additional constraints, the number of EFMs is still very limited, and one can easily find a small set of EFMs that give rise to the optimal flux distribution.
I think adding a small analysis of this would be better, as the currently shown discrete increase in acetate production does not reflect the measured behavior from Basan et al. at all.

The abbreviation "PTA" is introduced without being described.

General questions

- Is the manuscript well written?

Yes, the research clearly and consisely presented.

- Is the description of the rationale and methods clear and comprehensive?

Yes.

- Are there flaws in the design of the research?

Not as far as I can see.

- Are there flaws in the analysis?

Only minor points. See the comments above.

- Are there flaws in the interpretation of results?

Only minor points. See the comments above.

- Do you have concerns about ethics or scientific misconduct?

No.

- Did you detect a spin on the results, discussion or abstract? (a spin is a way of twisting the reporting of results such that the true nature and range of the findings are not faithfully represented, https://doi.org/10.1073/pnas.1710755115)

No.

- Is something critical missing?

Not as far as I can see.

Evaluation of the various components of the article

Title/abstract/introduction

- Does the title clearly reflect the content of the article?

Yes.

- Does the abstract present the supported findings of the study concerned and no other?

Yes.

- Does the introduction clearly explain the motivation for the study?

Yes.

- Is the research question/hypothesis/prediction clearly presented?

Yes.

- Does the introduction build on relevant recent and past research performed in the field?

Yes.

Materials and Methods

- Are the methods and analysis described in sufficient detail to allow replication by other researchers?

Not completely. The model is largely built by manual curation, and to me the choices made during that process are not entirely clear. I understand that it is hard to fully rationalize such manual curation steps, but some of the rationales could be more clearly presented with some examples. For example, what type of reactions from the iML1515 model were left out, and why?

- Is the experimental plan consistent with the questions?

Not applicable.

- Are the statistical analyses appropriate?

Yes.

- Have you evaluated the statistical scripts and program codes?

No.

Results

- Have you checked the raw data and their associated description?

Not applicable.

- Have you run the data transformations and statistical analyses and checked that you get the same results?

No.

- To the best of your ability, can you detect any obvious manipulation of data (e.g. removal)?

No.

- Do the statistical results strongly support the conclusion ($p < 10\text{-}3$ or $BF > 20$)?

I think this is a bad question, we can't judge statistical results on a p-value only, without taking into account the type of test and the type of data.

- In the case of negative results, was a statistical power analysis (or an appropriate Bayesian analysis) performed?

Not applicable.

- Did the authors conduct many experiments but retain only some of the results?

No.

Discussion

- Do the interpretations of the analysis go too far?

No.

- Are the conclusions adequately supported by the results?

Yes.

- Does the discussion take into account relevant recent and past research performed in the field?

Most of it. See minor comments above.

- Did the authors test many hypotheses but consider only a few in the discussion?

No.

References

- Are all the references appropriate?

Most of them. See minor comments above.

- Are the necessary references present?

Most of them. See minor comments above.

- Do the references seem accurate?

Yes.

Tables and figures

- Are the tables and figures clear and comprehensive?

Yes.

- Are all the tables/figures useful?

Yes.

- Are there too many/too few tables and figures?

No.

- Do the tables and figures have suitable captions such that they can be understood without having to read the main text?

Yes.