**Improving the accuracy of flux balance analysis through the addition of carbon availability constraints for intracellular reactions**

Maximilian Lularevic1,2, Andy Racher2, Colin Jaques2, Alexandros Kiparissides1

*1 Department of Biochemical Engineering, University College London, WC1E 6BT, UK;*

*2 Lonza Biologics PLC, 228 Bath Road, Slough SL1 4DX, UK*

*\*Corresponding author:* *alex.kiparissides@ucl.ac.uk*

**Highlights**

* A new method to refine the results of Flux Balance Analysis (FBA) is presented.
* Predictions from Carbon-constrained FBA (ccFBA) are in good agreement with 13C data
* ccFBA can improve our ability to predict reaction directionalities compared to FBA.

**1. Introduction**

Constraint based modelling methods, such as Flux Balance Analysis (FBA), have been extensively used to decipher complex, information rich -omics datasets in order to elicit system-wide behavioral patterns of cellular metabolism. FBA has been successfully used to gain insight in a wide range of applications, such as range of substrate utilization, product yields and to design metabolic engineering strategies to improve bioprocess performance [1]. A well-known challenge associated with large genome-scale metabolic networks (GEMs) is that they result in underdetermined problem formulations. Consequently, rather than single-point solutions, FBA and related methods examine ranges of reaction flux values that are consistent with the studied physiological conditions. The wider the reported flux ranges, the higher the uncertainty in the determination of basic reaction properties, limiting interpretability of and confidence in the results.

**2. Methods**

Herein we propose a new, computationally efficient approach that refines flux range predictions by introducing an additional set of constraints based on the elemental balance of carbon. Carbon constrained FBA (ccFBA) attempts to refine the feasible solution space, by constraining the permissible flux through intracellular reactions based on the amount of carbon taken up by the cell under the studied physiological conditions While at its core, ccFBA is a set of constraints based on the elemental balance of carbon, several aspects of cellular metabolism need to be taken into account.

**3. Results and discussion**

We compared carbon constrained FBA (ccFBA) with standard FBA using the latest CHO genome scale metabolic model (iCHO1766) [2] and were able to achieve significantly improved predictions for intracellular reactions. We showed that permissible flux ranges estimated by ccFVA contained the experimentally measured intracellular fluxes in the majority of cases and lead to quantitative predictions in the same order of magnitude as 13C measurements. This can be attributed to ccFVA’s ability to mitigate the impact of internal loops or futile cycles by constraining the amount of flux able to pass through any single reaction based on the amount of carbon entering the cell. Finally, when used in combination with random sampling, ccFVA substantially improved our ability to predict reaction directionalities compared to normal FVA.



**Figure 1.** Comparison between FVA (grey bars), ccFVA (pink bars) and intracellular flux measurements (yellow bars) for central carbon metabolism (Glycolysis, TCA, PPP). Data from Templeton et al., 2017 [3]

**4. Conclusions**

ccFBA is an easy to use and computationally efficient method for reducing flux variability in and improving the accuracy of constrained-based metabolic networks. It can be used as a stand-alone method or as a complimentary tool to most other methods currently available for stoichiometric metabolic network analysis.

**References**

1. J. Schellenberger, R. Que, R.M.T. Fleming, I. Thiele, J.D. Orth, A.M. Feist, …, B.Ø. Palsson, Nature Protocols 6(49) (2011), 1290-1307
2. H. Hefzi, K.S. Ang, M. Hanscho, A. Bordbar, D. Ruckerbauer, M. Lakshmanan, …, N.E. Lewis, Cell Systems 3(5) (2016), 434-443
3. N. Templeton, S. Xu, D.J. Roush & H. Chen, Met. Eng. 44 (2017), 126-133