ARTICLE ADDENDUM
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Large-scale modeling provides insights into *Arabidopsis*'s acclimation to changing light and temperature conditions

Nadine Töpfer and Zoran Nikoloski*
Systems Biology and Mathematical Modeling; Max Planck Institute of Molecular Plant Physiology; Potsdam-Golm, Germany

Classical flux balance analysis predicts steady-state flux distributions that maximize a given objective function. A recent study, Schuetz et al.,[1] demonstrated that competing objectives constrain the metabolic fluxes in *E. coli*. For plants, with multiple cell types, fulfilling different functions, the objectives remain elusive and, therefore, hinder the prediction of actual fluxes, particularly for changing environments. In our study, we presented a novel approach to predict flux capacities for a large collection of metabolic pathways under eight different temperature and light conditions.[2] By integrating time-series transcriptomics data to constrain the flux boundaries of the metabolic model, we captured the time- and condition-specific state of the network. Although based on a single time-series experiment, the comparison of these capacities to a novel null model for transcript distribution allowed us to define a measure for differential behavior that accounts for the underlying network structure and the complex interplay of metabolic pathways.

Keywords: genome-scale modeling, objective functions, acclimation, adjustment, abiotic stress, *Arabidopsis*, transcriptomics data, data integration, metabolism

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*Correspondence to: Zoran Nikoloski; Email: Nikoloski@mpimp-golm.mpg.de

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Genome-scale models of photosynthetic organisms have increased in quality and coverage of processes to include light usage and secondary metabolism.[3,4] These advances pave the way for applications to aid crop breeding and plant metabolic engineering. In a recent study, we presented a constraint-based optimization approach that integrates transcriptomics time-series data to elucidate pathways involved in abiotic stress responses.[2] Here, we present the difference of this approach to the classical analysis of differential gene expression, elaborate on the presented concepts of metabolic sustainer and metabolic modulator, and provide further insights on the proposed optimality indices. Finally, we discuss the implications of the proposed quantities in discerning design principles of metabolic networks.

The classical analysis of differential gene expression tests for difference in transcript abundances with respect to a chosen reference state, usually under ambient conditions.[5] Yet, this reference state does not necessarily reflect the transcriptional state encountered in plants in a natural environment, where biotic and abiotic stress factors shape the plants' cellular state. Therefore, the question arises: Can this selected reference state be regarded as "normal?" By introducing a novel null model for transcript allocation/distribution, we used a theoretical, yet unbiased reference state to elicit differential behavior in the context of a given genome-scale metabolic model.

We determine differential behavior on the pathway level by characterizing flux capacities of selected sets of reactions, so-called metabolic functions. These flux capacities describe the maximum flux thought a pathway in dependence of the transcript-based flux boundaries and were further analyzed with respect to the null model for transcript distribution. Under the null model, we then predicted maximum fluxes that are expected to arise by chance, while accounting for the underlying network structure and overall transcript abundance. The resulting null distribution of flux capacities allowed determining z-scores for every metabolic function. In such a way, the

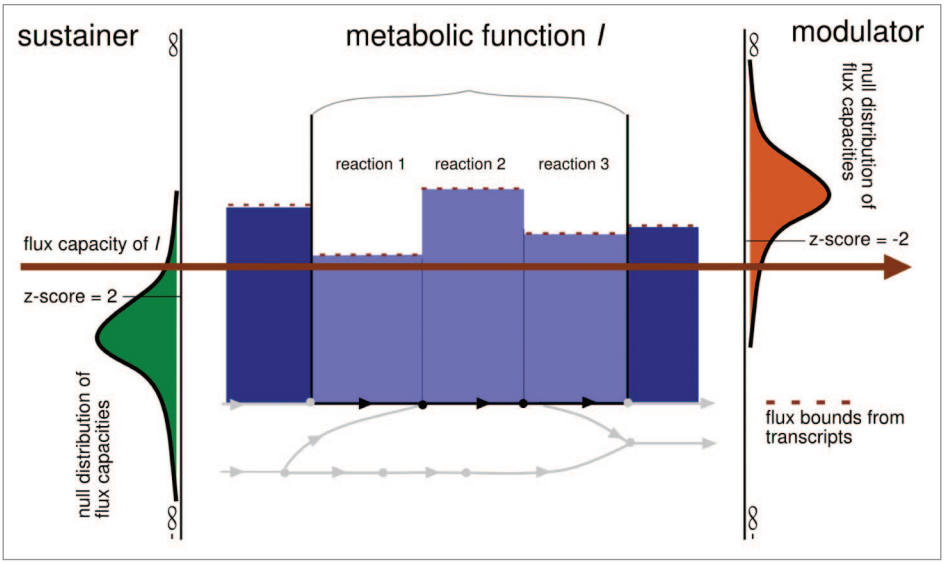

Figure 1. Schematic representation of the concept of metabolic modulators and metabolic sustainers. Shown is a metabolic function in its pathway context. It consists of 3 reactions with upper flux boundaries constrained by the transcript data. The red arrow denotes the respective flux capacity through the metabolic function. (Left) if the flux capacity has a z-score above 2 with respect to the distribution of the null model, the pathway is termed sustainer. (Right) if the flux capacity has an average z-score below –2 with respect to the distribution of the null model, the pathway is termed modulator.

model allowed us to investigate data from single time-series experiments. Given multiple conditions, we focused on the metabolic functions that were found differential in at least one, but not all considered conditions. The later comparison was performed under the assumption that a pathway that shows differential behavior in all or none of the considered conditions does not have the potential of providing condition-specific information.

Based on the null distributions of flux capacities and the resulting z-scores, we defined two classes of pathways, as illustrated in **Figure 1**. Sustainers, i.e., differentially up-regulated metabolic functions, show increased flux capacities in comparison to the null model. Since they do not carry a large flux under all considered conditions they are not part of the "high-flux backbone."[6] In contrast, modulators, i.e., differentially down-regulated metabolic functions, exhibit decreased flux capacity in comparison to the null model. Their down-regulation under certain conditions is likely related to regulatory processes downstream of the pathway. A prominent example for a sustainer is the photorespiration pathway. In combinations of ambient or high-temperature and ambient light or low-light/darkness, the metabolic function shows differential behavior and indicates an increased pathway usage to cope with the adverse condition. Typical modulators are the pathways involved in auxin biosynthesis, which are downregulated in all but 21/32° and darkness. It is well-known that auxin triggers stretching growth to reach possible light sources under light-limited conditions.[7]

It is interesting to see that each considered pathway exclusively falls into one of two classes: A metabolic function that serves as a sustainer of the metabolic state in one condition and is not classified as a modulator in another. It still remains to be elucidated if this behavior it typical only for light and temperature variations or if it persists even over a larger range of environmental conditions.

Further insight into network organization is given by the proposed optimality indices. Metabolic control analysis[8] has already shown that control of fluxes is distributed among the enzymes of the network rather than a distinct feature of a single pathway component. Yet, the approaches applicability is limited to small, well-investigated pathways, since it relies on the knowledge of kinetic parameters and maximal enzyme activities. Here, the introduced dependency index accounts for the large-scale network structure, by determining the extent to which the flux through a pathway under consideration is affected by the state of the remaining network. For instance, if a metabolic function of interest has large dependency on the network, an overexpression of the involved genes is expected to show little molecular phenotypic difference, meaning little increase in the flux through the respective reactions. The priority index can be used for ranking of candidates for metabolic engineering strategies to improve the plants performance under abiotic stresses. Further investigations could aim at determining if overexpression of pathways of highest priorities under a given conditions lead to a more efficient adjustment to the adverse condition. This could be tested by monitoring several factors, such as survival or growth rate. Finally, the efficiency index can be used to point at a relation between the differential regulation of certain genes and its ultimate impact on the flux through the respective pathway.

A striking example for the different aspects of optimality included the methionine degradation functions at 32° and darkness. While we observed a constant increase in priority of those two metabolic functions, they showed a decreasing efficiency, indicating that the reallocation of the transcriptional setup could not be used to its full efficiency. It should be

tested in future experiments if pathways with a high priority and low dependency and/or high efficiency are more susceptible to metabolic engineering strategies that those with a high dependency and/or low efficiency.

To conclude, the presented approach gives novel insights into network organization and temporal pathway usage. Identifying metabolic functions that are involved in acclimation and their characteristics with respect to the optimality indices may serve as a useful tool in metabolic engineering.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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