

Elucidating compartmentalized flux direction patterns in yeast

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Based on thermodynamic principles, we determined possible flux patterns within the pyruvate/acetate metabolism of ethanol-grown *Saccharomyces cerevisiae* from measured quantitative metabolome concentrations. By combining elementary flux mode (EFM) analysis and network-embedded thermodynamic analysis (NET analysis) all possible flux distributions derived from a detailed stoichiometric model of central carbon metabolism could systematically be analyzed for thermodynamic feasibility (Figure 1). Since our model includes cellular compartmentalization, we could distinguish between the reactions' activity in mitochondria and cytosol. Overall, we provide a detailed view on potential flux direction patterns in a region of metabolism where fluxes have so far not fully been resolved experimentally by ¹³C metabolic flux analysis.

First, to exhaustively describe all possible flux distributions we exploited EFM analysis of a metabolic model: Each EFM represents a distinct possibility to distribute the metabolic fluxes in a mass balanced manner, and the true flux distribution is a linear combination of EFMs (2). For growth on ethanol we reconstructed a model that describes yeast's central carbon metabolism, and carefully accounts for the compartmental localization of TCA cycle enzymes and alternative reactions within the pyruvate/acetate metabolism (Figure 2). This stoichiometric network consists of 155 reactions, 42 transporters, and 20 lumped pathways for biomass synthesis. Enumeration of the flux solution space resulted in 313,841 EFMs in total.

Second, to sort out infeasible flux patterns, we exploited thermodynamic principles: According to the second law of thermodynamics, reactions can only proceed in the direction of negative Gibbs energy of reaction, i.e. the products possess a lower Gibbs energy than the substrates. As the Gibbs energies depend on the reactants' amounts, metabolite concentrations can exclude the occurrence of a net flux in one direction. We measured concentrations of central carbon intermediates and free amino acids in a pH-controlled bioreactor by combined utilization of GC-TOF and LC-MS/MS analytics (Figure 2). To test for thermodynamic feasibility in a systematic and automated fashion, we subjected the measured concentrations to NET analysis. This optimization based tool was previously established in our group (1), and we here applied it on all 313,841 possible flux configurations (i.e. all EFMs) for growth on ethanol.

As a result, a large fraction of the analyzed EFMs were shown to be thermodynamically infeasible and the solution space could be reduced drastically. As all feasible EFMs display a flux through certain reactions in the same direction, we testified that these reactions are proceeding. Also, we demonstrated the inactivity of other reactions based on the fact that no feasible EFM exhibits a flux through them. Notably, as our analysis considers the metabolites' network interactions, we also could approve or disprove activity for reactions for which not all of the participating reactants were measured. Overall, detailed information on possible flux distributions for a highly flexible part of metabolism could be obtained.

References

1. Kümmel, A., S. Panke, and M. Heinemann, *Putative regulatory sites unraveled by network-embedded thermodynamic analysis of metabolome data*. *Molecular Systems Biology*, 2006. **2**: p. 2006.0034.
2. Papin, J.A., et al., *Comparison of network-based pathway analysis methods*. *Trends in Biotechnology*, 2004. **22**(8): p. 400-405.

Figures

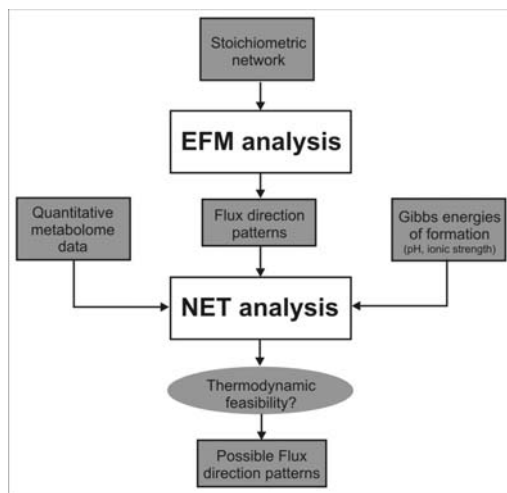


Figure 1. EFM and NET analysis framework By integrating the metabolites' thermodynamic properties and concentrations, NET analysis systematically tests for feasibility of all potential flux directions patterns that are derived from a stoichiometric model by EFM analysis. Thus, the solution space that contains the true flux distribution is reduced according to the remaining possible flux direction patterns.

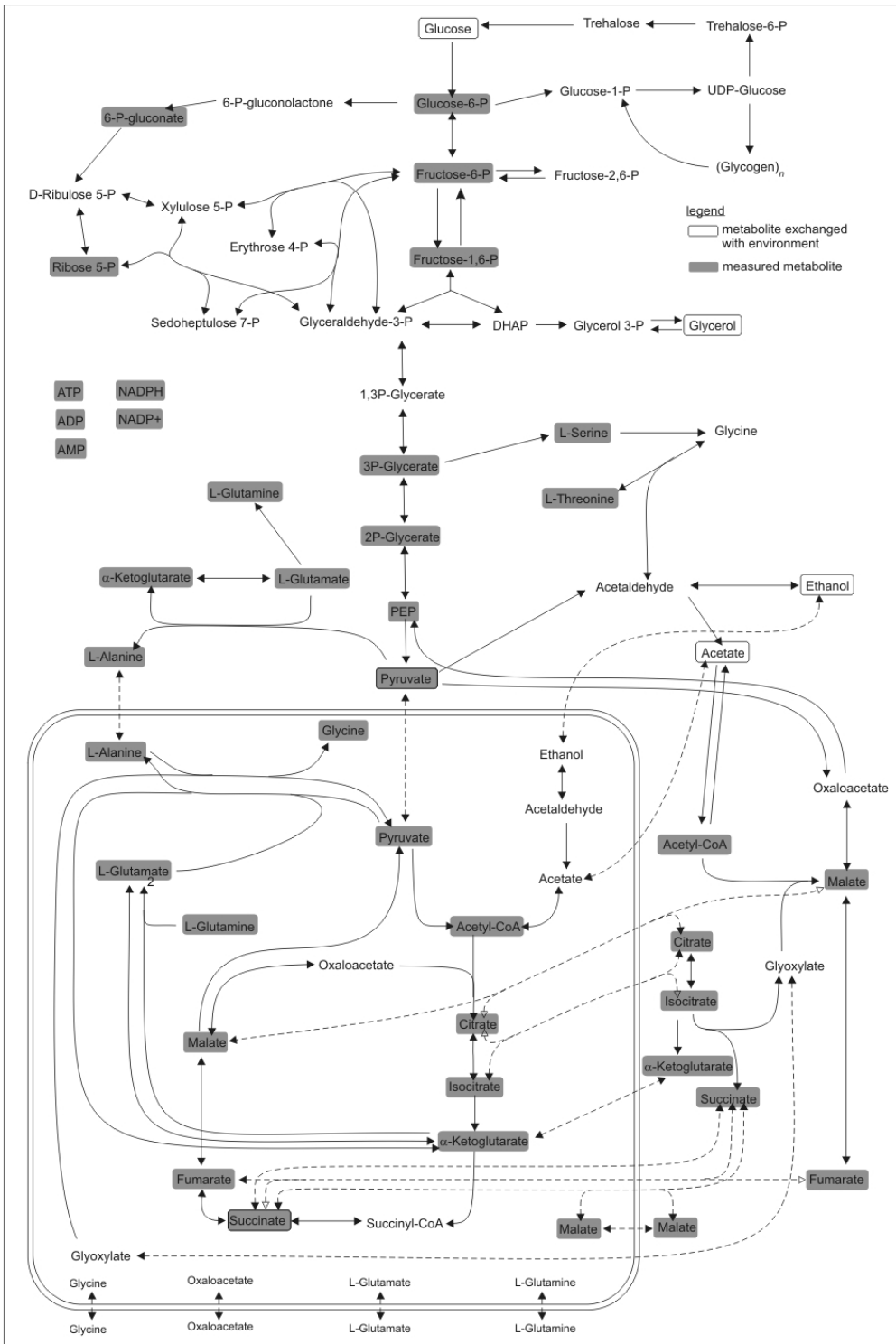


Figure 2. Metabolic map of central carbon metabolism in yeast The employed metabolic model contains amongst others all the displayed reactions and transporters. Metabolites for which concentrations were measured are labeled in gray.