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Analysis and Design of Metabolic Networks - Experiments and Computer Simulation

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Abstract

Understandig of the function of complex metabolic networks and methods for their rational design, e.g. for the overproduction of metabolites or for the abatement of metabolically related diseases, are still in its infancy compared to their real complexity. Using the increasing knowledge about genome sequences and its bioinformatic exploration, whole genome network analysis and design is increasingly possible, presently primarily using stoichiometric information. In a next higher level kinetic information that cannot be derived from genome analysis is used to describe also the dynamics of network. This allows a much more detailed understanding and application for design, as has been shown for the production of citric acid [1]. We will briefly describe the state-of-the-art and present and likely future developments.

Using stoichiometric information, methods like elementary mode analysis permit direct genome scale studies providing, e.g. valuable information about potential maximal yields of overproduction also using mixed substrates. They also provide a certain guidance for the design of potentially useful mutants, e.g. by deletion of genes promoting by-product formation. We have applied this technique to analyze methionine production in *C. glutamicum* and *E. coli* [2]. On essentially the same basis genome wide metabolic flux analysis is possible delivering in vivo activities of a network at specific conditions. Combining flux balancing with, e.g. ^{13}C-labelling techniques, a detailed picture can be routinely achieved for many bacterial systems and applied for designing mutants [3]. This requires, however, the combination of well defined cultivation, sampling, (bio)chemical analysis, modeling and parameter estimation techniques. Steady-

state modelling of networks, particularly of atom transfer throughout a network, is less developed for eukaryotic systems, where the analysis is complicated by extensive compartmentation and related transport. In mammalian cells widespread reversible transport is further complicating the analysis. Relevant physiological information about transport is, however, rather incomplete. Due to the higher complexity significantly more experimental data is required to determine the larger number of unknown parameters of a network. We have started work on CHO-cultures, today most frequently used for the production of biopharmaceuticals. We studied transport of metabolites using special ^{13}C experiments designed on the basis of network simulations. Several amino acids are exclusively consumed whereas others are consumed and secreted depending on the growth phase. ^{13}C enrichment is anlysed in external metabolites as well as in macromolecular components of the cell, i.e. proteins, lipids, carbohydrates and nucleic acids using GC-MS techniques.

For the quantification of metabolic activity of the production of secondary metabolites in potatoe tubers we used a dynamic labeling technique based on the HPLC-MS analysis of intracellular metabolites [4]. A dynamic model using power law kinetics was developed that allowed simulation of experimental data and the estimation of in vivo fluxes. On the basis of this model, flux control coefficients could be determined that provide guidance for the manipulation of genes to allow improved defense of e.g. microbial or viral attack.

References

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