

FluxAnalyzer: Studying Structure and Fluxes in Metabolic Networks

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ABSTRACT

In the era of genome sequencing the complete structure of biochemical networks of many organisms can be determined more and more accurately. Mathematical approaches for quantitative and qualitative analysis of such complex networks are rapidly gaining in importance. A well-established approach often used for studying metabolic networks is *Metabolite Balancing*. Its fundamental relation can be expressed by the $m \times q$ stoichiometric matrix \mathbf{N} and the $q \times 1$ vector \mathbf{r} of net reaction rates (m : number of metabolites, q : number of reactions):

$$\mathbf{0} = \mathbf{N}\mathbf{r} \quad (1)$$

As eq. (1) indicates, such metabolic flux distributions are of interest where all metabolites may be balanced to maintain a pseudo-steady state. All vectors \mathbf{r} satisfying eq. (1) lie in the null space of the stoichiometric matrix \mathbf{N} . It is noteworthy, that eq. (1) can be formulated only relying on the network stoichiometry - no kinetic parameters are needed. Thus, stoichiometric analysis supplies fast and valuable information on the capabilities of the complete metabolism of a cell and can also be useful as a complementary tool during dynamic modeling of cellular systems.

The aim of *Metabolic Flux Analysis* (MFA) is the determination of preferably all components of a certain vector \mathbf{r} characterizing the metabolic fluxes during a certain growth experiment [1]. Because the system of linear equations (1) is typically underdetermined, some measured or known rates must be additionally provided to calculate unknown rates.

The aim of *Structural Network Analysis* (SNA) is more general: Hereby, the overall capabilities of a metabolic network are studied by a more detailed analysis of the null space of \mathbf{N} [2, 3]. For this purpose techniques of convex analysis have been introduced successfully also allowing the consideration of reversibility and capacity restrictions of biochemical reactions. For example, the unique sets of extreme pathways (convex basis) or elementary flux modes (minimal non-decomposable flux distributions, [2]) can be calculated which contain valuable information about capabilities and structural features of the network.

However, although MFA is a widely used quantitative technique and SNA has become a promising concept for studying the structure and capabilities of metabolic networks there seems to be a lack of a software tool which integrates methods belonging to both these approaches in an easy-to-use graphical user interface.

Therefore, the *FluxAnalyzer* (FA) has been developed as package for MATLAB. It allows convenient performance of MFA and

SNA and the visualization of calculated results in flux maps. The FA consists of two parts: First, there are several so-called network projects (each containing a symbolic and a graphical network representation), which must be created by the user as follows:

- Arbitrary metabolic network can be composed symbolically with the help of masks where each network element and its properties can be defined. Each element belongs to one of the types *metabolite*, *reaction* or *biomass constituent*.
- Graphical network representations (flux maps) can be linked to the symbolic network representation by user interfaces (small text boxes) predefined in MATLAB. Each text box refers to one network element. They can be positioned at a proper place in the flux map and allow input of values (e.g. reaction rates or biomass composition) or output of calculated fluxes. The flux maps must be provided by the user: either designed by himself (e.g. graphic programs) or by using external sources (e.g. graphics from the KEGG-database).

The second part of the FA comprises various functions related to MFA and SNA, which can easily be started via menu-items, e.g.:

- MFA: calculation of flux distributions based on measured or given rates; consistency checks; sensitivity analysis; optimization of a user-defined objective function (with imposed constraints)
- SNA: general topological properties, calculation and display of elementary flux modes or of a convex basis; feasibility checks of network scenarios
- Documentation: saving of data sets or flux maps

(Further information on the *FluxAnalyzer*: http://www.mpi-magdeburg.mpg.de/people/klamt/klamt_eng.html. A free version for academic users is available from the corresponding author).

REFERENCES

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