DEPARTMENT OF SIMULATION

FOMAAS ANALYSIS, SIMULATION & OPTIMIZATION OF COMPLEX SYSTEMS

UNIVERSITY OF SIEGEN

Topological Analysis of Metabolic Isotope Labeling Networks

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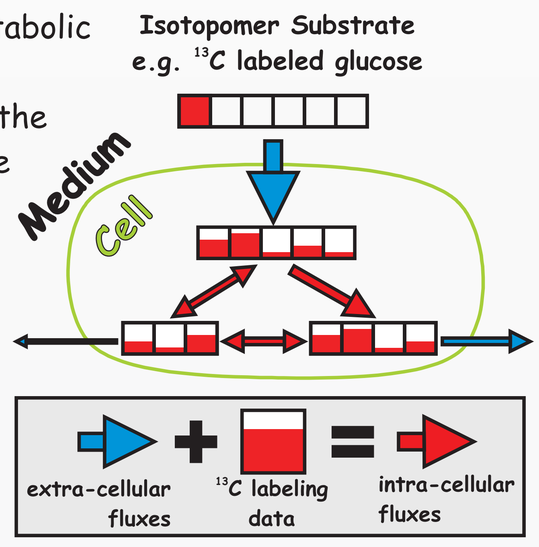
Background: Metabolic Flux Analysis

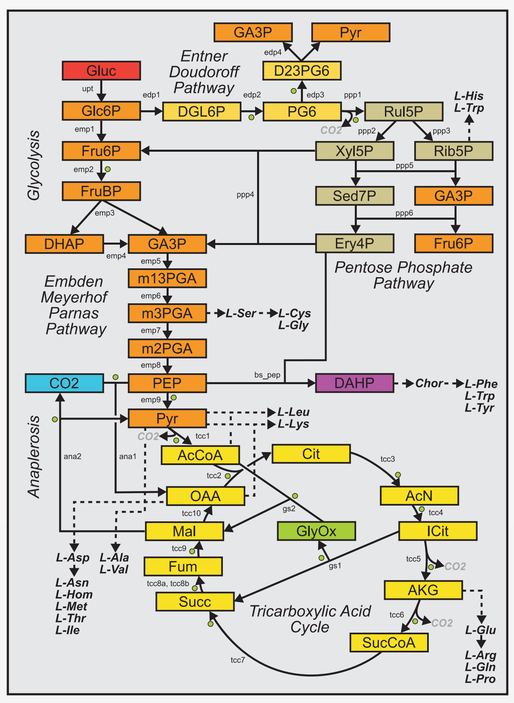
In context of Systems Biology and Metabolic Engineering, Metabolic Flux Analysis (MFA) is a standard tool used for the experimental in-vivo quantification of fluxes through metabolic networks - also called the "Fluxome" of a cell. In contrast to many other biochemical measurement techniques, MFA relies on complex mathematical modeling and costly algorithms.

MFA by the Modeling and Simulation of ILEs

Metabolic Flux Analysis is performed by the Evaluation, and the Modeling and Simulation of Isotope Labeling Experiments (ILEs):

- A specifically labeled isotopomer substrate is fed to the cells as soon as a metabolic stationary state is reached.
- Specific labeling enrichments appear in the different labeling positions as the labeling distributes among the metabolic pools and the cell reaches an isotopic stationary state.
- The positional labeling enrichment is measured using NMR and MS instruments.
- Based on a metabolic model, measured extra cellular fluxes, and the a-priori known substrate labeling the unknown intra cellular fluxes a determined by a parameter fitting which incorporates a (forward-) simulation of the metabolites' positional labeling enrichment.





← ^{13}C-Labeling Network modeling the Central Metabolism and attached Biosynthesis Pathways of E. coli

- 87 metabolite pools (3 sources, 30 sinks)
- 94 reactions (3 input, 30 output)
- 61 intra-cellular reactions (42 bidirectional)
- 11 carbon atoms in largest backbone (amino acid L-tryptophane)
- **10390 cumomer pools**
- **a single forward-simulation run: classical LU-algorithm ≈ 20 sec. new algorithm ≈ $20*10^{-3}$ sec.**

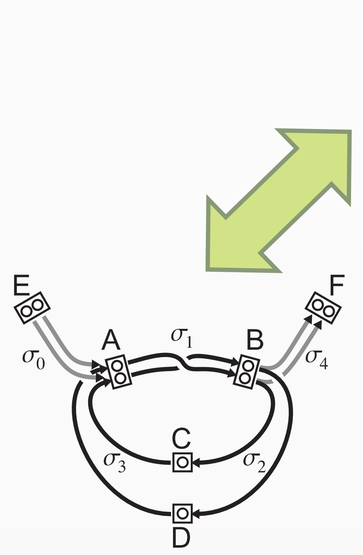
Motivation: The need for faster algorithms

The recent years brought up several new experimental techniques and requirements resulting in increasing performance demands for MFA tools:

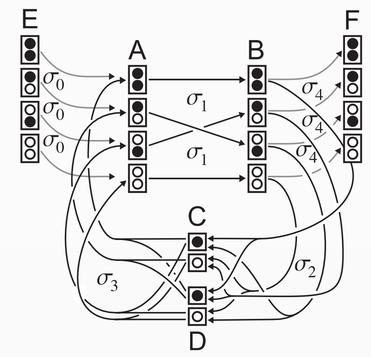
- **High Throughput MFA:** hundreds of carbon labeling experiments running in parallel
- **Model Selection:** detecting the presence or absence of metabolic pathways
- **Larger Metabolic Networks:** more detailed **modeling**, different isotopic labelings
- **Nonlinear Statistics, Optimization by Stochastic Search Techniques:** a large number of simulation runs have to be performed
- **Isotopically Non-Stationary Modeling & Experimental Design:** large differential equation systems, computation of sensitivities

Cumulative Isotopomer (Cumomer) Networks

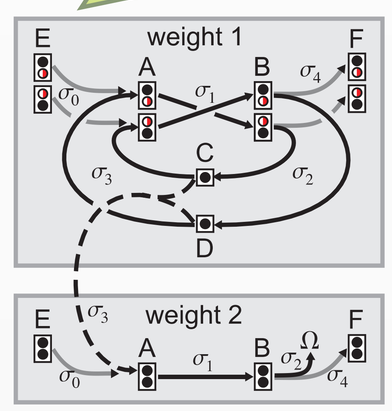
Any simulation of the distribution of an isotopic labeling relies on a metabolic model of the considered organism. For complexity reasons, only the most interesting part of the metabolism can be covered: the central metabolism.



The Atom Network:
- The basic model
- Comprises information about how atoms are transported by metabolic, enzyme-driven reactions



The Isotopomer Net:
- Considers metabolic pools in **all possible labeling** states
- Combinatorial explosion
- **A single huge system** of **nonlinear** balance equations



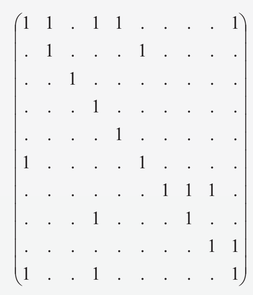
The Cumomer Network:
- Obtained from the Isotopomer Net by a simple **graph transformation**
- Atoms in two distinct states: **"labeled"** and **"don't care"**
- **Natural Partition** in "weight-levels"
- **Linear equations** within weight-levels
- Efficient solution level-by-level

Cumomer and Isotopomer networks are derived from the atom network without additional knowledge. Both represent highly redundant, equivalent representations of the atom network. Solution algorithms suffer from the introduced redundancy.

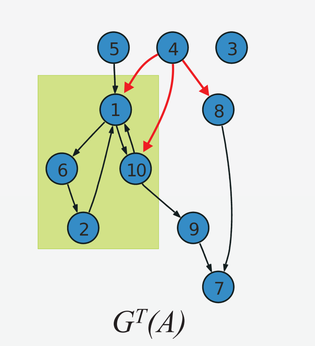
Filtering-Out Redundancy: New Algorithms

The nice properties of Cumomer Networks are the basis for new algorithms:
- Monotonously decreasing connectivity with increasing weight level
- A predictable number of isomorphic subgraphs among the cascade's levels

Consequences:
- Systems of labeling balance equations are highly sparse
- The graph's Connected Components (CCs) represent subsystems within the equations that can be solved in any order
- The graph's Strongly Connected Components (SCCs) correspond to cyclic dependencies between the unknowns
- Isomorphic SCCs are identical subsystems, embedded into different contexts

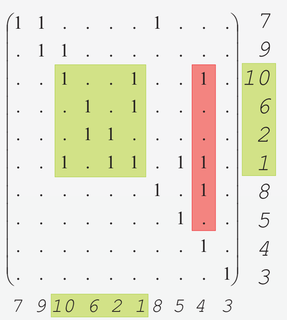


$adj(A)$

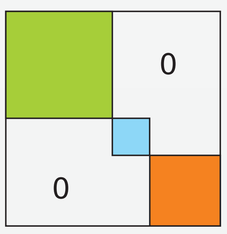


$G^T(A)$

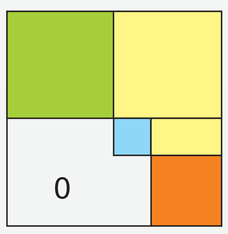
$P=(7,9,10,6,2,1,8,5,4,3)$



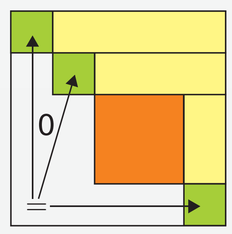
$adj(A(P,P))$



CCs: independent subsystems



SCCs: dependent cyclic subsystems with acyclic dependencies



Isomorphic SCCs: identical subsystems in different contexts