From the evolution of metabolic pathways to the emergence of complex properties.

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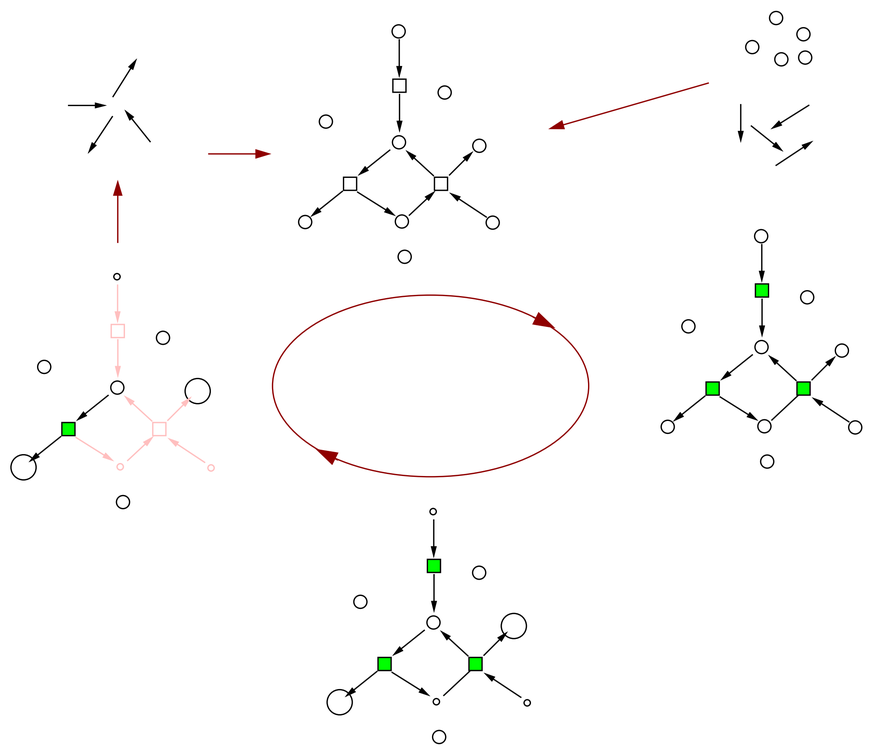
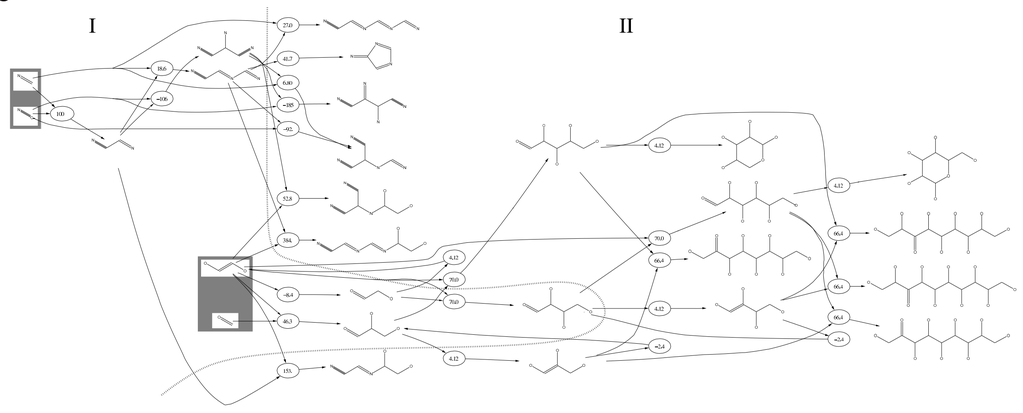
Abstract

The formation of complex systems and their properties is an intriguing field of research in biology with many unresolved questions. The knowledge that can be gained from it is not only beneficial for the understanding and optimization of existing systems but also for constructing many kinds of novel artificial systems.
We are investigating the emergence of complex properties in biological systems by studying the evolution of metabolic pathways and observing the structure and dynamic behavior of metabolic networks. Therefor, we have implemented a multi-level computational model for simulating the evolution of catalyzed reaction-networks, complemented by a versatile metabolic pathway analysis tool that allows us to extract static and dynamic properties of the networks. With the use of meaningful measures for system-level properties we hope to turn our observations of network properties into hypotheses about system-level properties such as robustness and modularity.

Evolution of metabolic Pathways

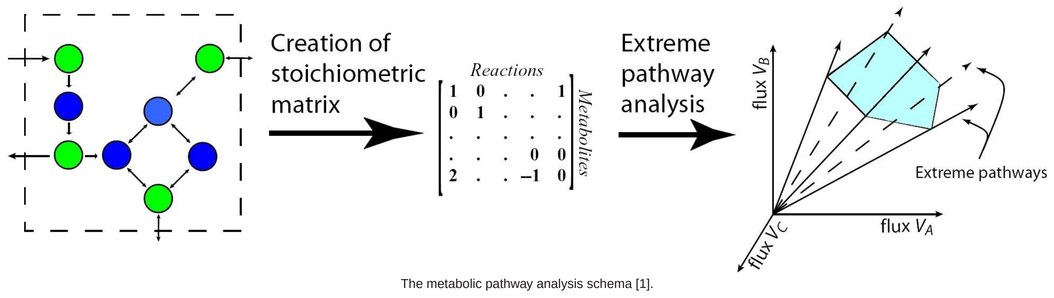


Our model of ribozyme-catalyzed metabolism consists of protocellular entities, containing a genome, a set of catalytic reactions, an algebraic chemistry and molecules which can be involved in chemical reactions and exchanged with the environment. The molecules are abstracted as vertex- and edge-labeled graphs and chemical reactions are realized as Graph-rewrite rules. The graph-based model is supported by an artificial chemistry, to allow for realistic kinetic behavior of the system. The genome codes for a set of catalytic reaction which are applied on the molecules in the protocell. Using the reaction rates, we determine the molecule concentrations. All molecules above a certain concentration threshold are combined further in the next network generation step. New chemical reactions may be introduced through alterations (mutations) in the protocell's genome.



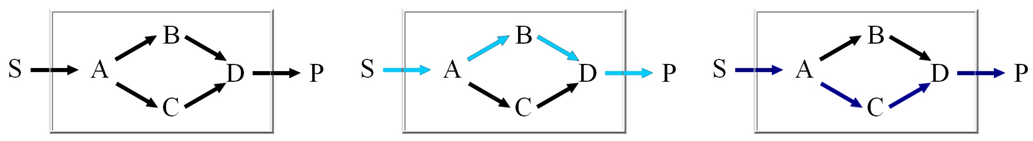
Metabolic Pathway Analysis

Metabolic pathway analysis is the calculation and analysis of the pathway distribution of a steady-state metabolic network to gain insights about its structure, functionality and properties [1]. The calculation starts with the formation of the stoichiometric matrix presentation of the network and delivers the extreme pathways, spanning the entire steady-state flux space, as the final result.



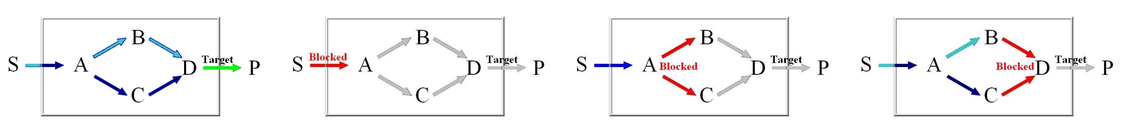
The metabolic pathway analysis schema [1].

Extreme Pathways are the set of essential pathways through which all other possible pathways of the metabolic network can be generated, they are also minimal in the sense that they do not consist of smaller pathways. We calculate the set of extreme pathways with an improved binary Null-space approach. The computation time of the existing approach is dependent on the row-ordering. We integrated a new way to order the rows to reduce the number of candidate pathways.



A simple example network and its two only extreme pathways (light blue and dark blue arrows, respectively) .

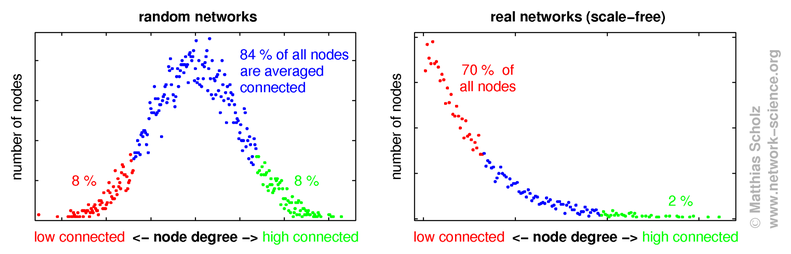
From the set of extreme pathways we can determine further measures, such as the minimal knockout sets, describing the dynamics of the underlying network. Minimal knockout sets are sets of reactions that need to be removed in order to disable the function of a certain target reaction, this means that there may not be any extreme pathway containing this target reaction. For the computation of the minimum knockout sets we implemented an improved depth-first version of Berge's algorithm [2] which avoids the costly superset removal.



Some (three of six) example minimal knockout sets (red arrows) for one target reaction (green arrow).

Network Properties

By examining the network structure with conventional network measures as well as measures suited for metabolic reaction networks, we intend to gain insights about the properties of the investigated networks. Since we also have different stages in the evolution of this networks available, we can even make hypotheses on the emergence of these properties. Here we focus on measures for the robustness of the networks.



Robustness of a network can be defined as a property of the underlying graph structure or as a dynamical property of the system that is represented by the network. Simple graph-based measures for robustness are for example, degree or connectivity distributions, as well as the entropy. Measures for the robustness of the system dynamics, especially in metabolic networks, are related to fluxes through the system and their behavior to elimination of edges (knockout of genes/enzymes) and nodes (depletion of metabolites). Here we use several measures based on the set of extreme pathways (R_{1-3}) [3] and the size distribution of the minimal knockout sets (see Results).

$$R_1 = \frac{\sum_{i=1}^{r} z^i}{r \ * \ z}$$

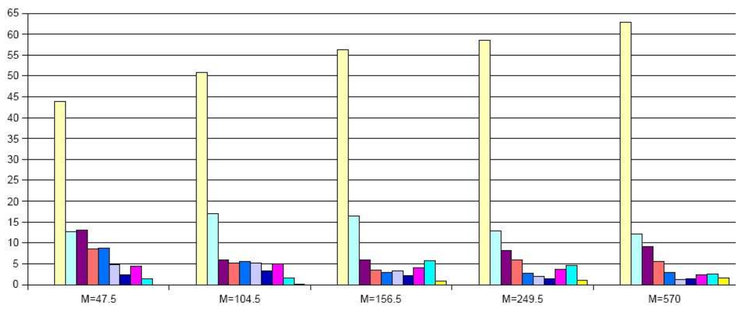
$$R_2 = \frac{\sum_{i=1}^{n} R_1^i}{n}$$

$$R_3 = min\{R_1^1, R_1^2, ..., R_1^n\}$$

r = number of reactions
n = number of metabolites
z^i = number of extreme pathways without r^i
R^i = R if metabolite i is not abundant

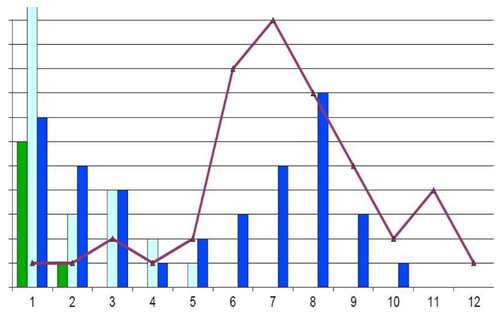
Results

On the one hand, with our model we can make statements about the evolution of metabolic pathways and its elements. On the other hand, we observe the development of certain properties of the networks.
We find that enzymes that were introduced in the system at later stages usually are much more specific than their early counterparts. Another observation is the formation of so called hub metabolites in the course of the simulations. This leads to scale free and robust networks as described in the discussion of the node degree distribution.
The robustness measures (see Network Properties, R_{1-3}) all increase steadily throughout evolution, converging to values close to 1. These results indicate that the overall function of the network is maintained almost perfectly for knockouts (enzymes) or depletions (metabolites) of single components. Furthermore, the discussion of the minimal knockout set size distribution shows that also for specific target functions the network becomes more and more robust against single and multiple knockouts.



Node degree distribution for networks of different size. Average network sizes are: 50, 100, 150, 250, 500 metabolites. Node degrees (bars): 1,2,3,4,5,6,7,8-12,>12 connections.

The degree distribution of the nodes (metabolites) in the network approximates a power law distribution. In further evolved and larger networks we find a much higher percentage of low connected nodes and a few very highly connected nodes. These highly connected nodes resemble so called hub-metabolites which are observed in real-world metabolic networks. Networks with a power law degree distribution, or scale free networks, are known to be highly robust, because most knockouts have only little effect.



Minimal knockout set size distribution. Green = small networks; Light Blue = medium networks; Dark Blue = large networks; Purple Line = real-world complex metabolic networks [2] .

The smaller networks consist almost exclusively of very small minimal knockout sets, this means that target functions can be turned off by the knockout of only few or even single edges (enzymes). However, the networks from later stages in evolution contain larger minimal knockout sets, such that most of their functions are robust against knockouts of several edges at the same time.

Outlook

In future work, we will test the existing measures and hypotheses with more extensive simulation runs and experiment with some realistic scenarios to make statements about real-world metabolic pathways. We also intend to extend the study of the robustness of our networks through barrier trees of the set of extreme pathways. Further we want to introduce measures for modularity of networks, inspired by the notion of biological organizations and autocatalytic sets.
With the use of visualization tools specifically adapted to metabolic networks, we hope to be able to explore the structure and dynamics of our networks on a different levels to gain new insights.
Finally, an extensive comparison of our results on the network property measures with those for real-world networks of different type is desirable for validating our conclusions and possibly further insights.

References

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