Mathematical descriptions of the steady-state flux cone of a metabolic network

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The set of all possible flux distributions over a metabolic network at steady state defines a polyhedral cone, the steady-state flux cone. As any polyhedral cone, this cone may be described mathematically in two different ways: either by an inner description using sets of generating vectors, or an outer description based on systems of linear equations and inequalities. Some of the most popular concepts in metabolic pathway analysis such as elementary flux modes or extreme pathways correspond to special inner descriptions of the cone. An alternative approach is the use of an outer description based on sets of non-negativity constraints (or irreversible reactions), resulting in so-called minimal metabolic behaviors and the reversible metabolic space. The goal of this talk is to overview these different approaches, and to discuss their relationship. In particular, we will propose a general framework to show how different inner descriptions may be obtained from the outer one, and discuss the possible impact on the description size.

Relating structural and functional properties of large-scale metabolic networks

Oliver Ebenhoeh

In this talk, I will review the method of network expansion. This simple and extremely efficient method can be used to study various aspects of metabolic networks. In its simplest form, it mimics an evolutionary process by subsequently building networks of increasing size, starting from a predefined set of initial seed compounds. The resulting network contains all those metabolites which a metabolic network is in principle capable of producing, provided with the seed metabolites as resources. We term this set of metabolites the scope of the seed. Scopes link in a natural way the structure of a network with functional properties, namely biosynthetic capacities on predefined sets of resources.

When investigating the global metabolic network, comprising all known biochemical reactions, an intricate hierarchical organisation of the scopes was identified and allowed to distinguish between the chemical and the biological complexity of substances. We revealed remarkable functional properties that are not expected to have evolved by chance and we argue that present day metabolism is organised in such a way that RNA and DNA metabolism is separated even on a very fundamental, that is structural, level. Systematic comparison of the biosynthetic capacities of single organisms provided with different carbon sources results in the notion of carbon utilisation spectra. We outline how this concept may be used to infer typical habitat conditions from the network structure alone.

Because the network expansion algorithm is extremely fast, it can be applied a large number of times and, applying clever heuristics, computationally challenging problems may be solved. Inverting the scope problem and asking for minimal sets of nutrients that are required to produce a given set of target metabolites, allows in principle to predict minimal nutritional requirements from the network structure. With a similar algorithm, it is possible to identify minimal sets of reactions which have to be added to incomplete draft networks in order to make them compliant with experimental observations. I will present results for the recently sequence green alga Chlamydomonas reinhardtii.

On the complexity of some enumeration problems related to polyhedra

Khaled Elbassioni

For a given graph G and a weight function on the edges w, we consider the polyhedron P(G,w) of negative-weight flows on G, and get a complete characterization of the vertices of P(G,w). For any CNF formula f, we give a construction mapping f into a weighted graph (G(f),w) such that the existence of a negative-weight cycle of length greater than 2 in G is equivalent to the satisfiability of f.

We illustrate that this characterization and construction can be used to show that a number of problems are NP-hard, including generating all vertices of a 0/1-polyhedron, computing Minkowski sums of given two polytopes, checking if a given integral polyhedron is 0/1, or if a given polyhedron is half-integral, and approximating the vertex centroid (which the average of the vertices) of a given polyhedron to within any non-trivial distance.

The Duality between Elementary Modes and Minimal Cut Sets: A paradigm for functional analysis of biological networks?

Steffen Klamt

Minimal functional units and *minimal failure modes* play a fundamental role in the functional analysis of biological networks. In metabolic (stoichiometric) networks, these objects are conceptually described by *elementary modes* and *minimal cut sets*. Elementary modes (EMs) are useful for a number of applications, e.g. to identify biologically meaningful metabolic pathways and cycles or to assess network flexibility and the functional importance of single network elements. Minimal cut sets (MCSs) provide a dual perspective on network function; they are support-minimal sets of reactions whose removal will repress certain network functions. MCSs are useful for target identification or for fragility (robustness) analysis in metabolic networks. As I will point out, MCSs and EMs are in fact dual to each other in a logical sense [1]: the MCSs represent the *minimal hitting sets* of the EMs and vice versa and algorithms for computing MCSs from the EMs (and possibly EMs from MCSs) exploit this dual relationship [2]. The duality between EMs and MCSs is well understood in metabolic networks and may represent a paradigm for functional network analysis Systems Biology. In fact, it turns out that minimal functional units and minimal failure modes play a similar fundamental role in structural models (Boolean networks, interaction graphs) of signaling and regulatory network networks, though, conceptually, they have to be defined in a different way [3,4].

[1] Klamt S. 2006. Generalized concept of minimal cut sets in biochemical networks. Biosystems, 83: 233-247.
[2] Haus U-U, Klamt S and Stephen T. 2008. Computing knock-out strategies in metabolic networks. Journal of Computational Biology, 15:259-268
[3] Klamt S et al. 2006. A methodology for the structural amd functional analysis of signaling and regulatory networks. BMC Bioinformatics, 7:56.
[4] Samaga R, von Kamp A, Klamt S. 2009. Computing combinatorial intervention strategies and failure modes in signaling networks. Submitted.

Construction and analysis of a genome scale metabolic model of Arabidopsis thaliana

Mark Poolman

In this talk I will describe the construction and analysis of a genome scale metabolic model of Arabidopsis thaliana and it's subsequent use to investigate the metabolism of a cell suspension culture, growing under heterotrophic conditions, from minimal media.

The complete model contains ~1500 reactions and metabolites, but it has been possible to show (via linear programming) that all biomass components can be synthesised by ~230 reactions.

Analysis of the response of this minimal model to varying ATP demand, revealed a set of 40 reactions whose flux responded to this variation in demand, and further analysis allowed a decomposition of this set of reactions into to smaller groups with biochemically distinct functionality.

The work is more fully described in Poolman et. al. (2009), Plant Physiology, in press.

Expanding frontiers of genome-scale in silico models for biotechnology and medicine

Nathan D. Price

High-throughput experimental technologies have made possible the reconstruction of biochemical networks at the genome-scale. In silico models based on these networks have now been successfully used in a wide variety of biotechnology applications. Within this context, I will discuss ongoing efforts to expand the frontiers of metabolic pathway analysis, including: 1) the use of metabolic models in human systems, 2) the incorporation of dynamics in a constraint-based manner, 3) the introduction of probabilistic approaches to flux analysis, and 4) the integration of metabolic and regulatory networks.

An overview of the history of Metabolic Pathway Analysis

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Stoichiometric analysis in chemistry has a very long tradition, while its use in biochemistry started in the middle of the 20th century. This talk reviews stoichiometric and pathway analyses back to that era. For example, Horiuti and Nakamura introduced the concept of nullspace of the stoichiometry matrix (under a different name) in 1957, which was later successfully applied by Christine Reder in Metabolic Control Analysis and by David Fell in Metabolic Pathway Analysis. The overview will then cover the work by Horn, Jackson and Feinberg in the 1970's and by Mavrovouniotis and others in the 1980's. A breakthrough happened by the work of Bruce Clarke in the 1980's, who was the first to use concepts from convex analysis and coined the term "extreme currents". Building on that work, the "fundamental modes" proposed by Leiser and Blum in 1987 and the nullspace approach, Claus Hilgetag, Ronny Schuster and I came up with the elementary flux modes and worked both on algorithmic issues and applications to biochemistry. This was then continued in a very fruitful cooperation with David Fell, Thomas Dandekar and others.
Elementary-modes analysis has become a well-established theoretical tool in metabolic pathway analysis. It allows one to decompose complex metabolic networks into the smallest functional entities, which can be interpreted as biochemical pathways. This has manifold applications in bioengineering, such as predicting maximum yields and determining the robustness to knockouts.
The talk will also cover several other concepts such as that of extreme pathways established by Bernard Palsson's lab. Moreover, flux coupling analysis as developed by Costas Maranas and coworkers (based on the concept of "enzyme subsets" suggested in 1999 by Thomas Pfeiffer and myself) will be discussed. Recent developments in tackling the problem of combinatorial explosion in genome-scale networks will be reviewed.

Metabolic networks and how they evolve

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Recently developed computational tools enable the rigorous identification of all possible elementary modes in a reaction network. Based on this knowledge one can identify the most efficient pathways that support the synthesis of desired metabolites. Such pathways can be experimentally realized to engineer minimal cells with reduced metabolic functionality by genetically eliminating undesired reactions. This rational metabolic engineering approach has immense potential for biotechnology applications. In addition to their practical importance, elementary modes have significant fundamental implications. They represent discrete, permissible states of the metabolism that can be interpreted with the tools of statistical thermodynamics which offers a theory of how they evolve.

Computing elementary modes: new capabilities through an efficient implementation

Marco Terzer

The number of elementary modes or extreme fluxes of a metabolic network depends exponentially on the network size. The computation is therefore challenging already for medium scale networks. Focusing on the double description method - the algorithm usually chosen due to the degeneracy of problems arising in biology - we review some of the most important algorithmic aspects indispensable for any useful implementation. The efficiency of the proposed techniques has been demonstrated by our open source tool implemented in Java. It is one of the most powerful tools available today for the computation of elementary modes or - in mathematical terms - for generating the extreme rays of (highly degenerate) polyhedral cones. We also highlight some of the most interesting biological discoveries made possible through the enhanced capability. Finally, we conclude with potential future applications that have now come into range.

Using metabolic pathway analysis for biotechnological applications

Bas Teusink

In this presentation I will illustrate the use of combinations of constraint-based modeling techniques, elementary flux mode analysis and bioinformatics to address specific biological questions related to biotechnological application, of lactic acid bacteria in particular. For a number of such lactic acid bacteria, pioneered in Lactobacillus plantarum, we have made genome-scale metabolic models, which are of great help in understanding data related to complex fermentations. I will illustrate this with examples, and then address the use and limitations of Flux Balance Analysis and EFM analysis to predict the outcome of adaptive evolution experiments. Finally I will share some ideas and first results on extensions of metabolic pathway analysis into the multicellular domain, i.e. the interaction of two lactic acid bacteria in yoghurt production.

A computational framework for estimating metabolic fluxes from 13C tracer experiments

Esko Ukkonen

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We present a novel computational framework for estimating metabolic flux ratios in the cell from 13C isotopomer measurement data. In the framework, equation systems constraining the fluxes are derived automatically from the model of the metabolism of an organism. The framework is designed to be applicable with all metabolic network topologies, 13C isotopomer measurement techniques, substrates and substrate labelling patterns. The core of our approach constitutes of flow and independence analysis of metabolic fragments and techniques for manipulating isotopomer measurements with vector space techniques. These methods facilitate efficient computation of the ratios between the fluxes of pathways that converge to a common junction metabolite. By analyzing NMR and MS measurement data obtained from the micro-organisms Bacillus subtilis and Saccharomyces cerevisiae, our framework automatically produced the flux ratios discovered so far by the domain experts with tedious manual analysis.