Inferring Dynamic Properties of Biochemical Reaction Networks from Structural Knowledge

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Abstract

Functional properties of biochemical networks depend on both the network structure and the kinetic parameters. Extensive data on metabolic network topologies have been collected in databases, but much less information is available about the kinetic constants or metabolite concentrations. Depending on the values of these parameters, metabolic fluxes and control coefficients may vary within a wide range. Nevertheless, some of the parameters may have little influence on the observables of interest. We address the question whether, despite uncertainty about kinetic parameters, probabilistic statements can be made about dynamic network features. To this end, we perform a variability analysis of the parameters: assuming that the parameters follow statistical distributions, we compute the resulting distributions of the network properties like metabolic fluxes, concentrations, or control coefficients by Monte Carlo simulation. In this manner, we study systematically the possible distributions arising from typical topologies of biochemical networks such as linear chains, branched networks, and signaling and gene expression cascades. This analysis reveals how much information about dynamic behavior can be drawn from structural knowledge.

Keywords: biochemical network, steady state behavior, large-scale simulation, variability analysis

1 Introduction

Systems biology [12] aims at investigating cellular networks by combining experiments, mathematical modeling, and computer simulations. One fundamental prerequisite for the construction of cell models is the analysis of metabolic and signaling networks. The description of such systems comprises both the network structure and the reaction kinetics: only if both are known, stationary states, time courses and responses to parameter changes can be computed. By structure, we understand the stoichiometry and the reversibility of reactions, which both do not depend on the enzymes. The kinetics, i.e. the reaction velocity as a function of substrates, products, and other substances (regulators), is usually described by a fixed mathematical function (for instance, the Michaelis-Menten (MM) kinetics) and parameters (like K_m, V_{max}, and possibly inhibition constants, for MM kinetics). In contrast to the structural features mentioned before, the values of kinetic parameters depend on the enzyme and on the status of the cell. Metabolic control theory studies how the local properties of single reactions affect global properties of the whole metabolic system, such as the qualitative behavior (steady states, oscillations), or global steady-state properties.

High-throughput methods provide large amounts of qualitative data, e.g., on protein interactions networks [17], gene regulatory networks, genome-scale reconstructed metabolic networks [2]. Networks are stored in databases like KEGG [9][24], Genome Knowledgebase [25], MetaCyc [13], EcoCyc [10] which makes them easily available for further analysis. On the other hand, the quantitative knowledge about the interactions and their individual kinetics is rather incomplete. Besides direct measurements, kinetic constants may be fitted to describe experimental data like fluxes estimated from metabolite isotopomer distribution [20,21]. A major problem with this approach is that detailed measurements of concentrations, fluxes, and time courses of individual compounds are rare, at least compared to the number of compounds and the complexity of the networks. Moreover, a part of the system parameters may not be identifiable from the set of experiments. Finally, parameter values remain unreliable due to measurement errors, dependence on experimental conditions, and individual variations in cell composition and state. Anyhow, partial information about their values can be drawn from known equilibrium constants or at least from the reversibility of reactions.

Despite this lack of knowledge, there are several large-scale cell simulation projects (e.g. E-cell, [19,23] also [2], [7]), which have to make kinetic assumptions. In the strict sense, also the choice of small subsystems, as in traditional modeling, is an implicit kinetic assumption. Thus one has to tackle large networks where only partial information is available: but how can we draw functional conclusions from the network structure, combined with uncertain (measurement errors), partial (equilibrium constants), or qualitative (reversibility) information on the network parameters?

The basic idea of this article is that some properties of networks may depend only weakly on the choice of parameters, i.e. be determined with high probability by topology or equilibrium constants. To test this, we assume that the network topology is fixed and known, while the model parameters, that is, kinetic constants of all reactions and the values of external metabolites, follow a statistical distribution. This parameter distribution, together with the fixed network structure, leads to distributions of the observables (concentrations, metabolic fluxes, control coefficients, and mathematical functions thereof, such as signs, order relations etc.). If a distribution is sharp, we conclude that the quantity is strongly determined by the network structure, at least for the ensemble of parameters considered.

The case where parameters are known up to a small uncertainty can be treated by a linear approximation: in first order, a log-normal parameter distribution leads to a corresponding log-normal distribution of the observables. The variance depends on the sensitivity of a logarithmic observable y to a logarithmic parameter x, that is, the respective normalized response coefficient. The case where x is an elasticity and y is a control coefficient has been treated by Small and Fell [16]. If the parameter variance is zero, then also the observables are exactly determined. Here we use Monte Carlo simulations, drawing samples from the parameter space: this allows us to consider large parameter uncertainties and binary network properties like flux directions. This approach has been applied to gene regulatory circuits [11] and a MAP kinase cascade [1].

As examples, we study typical topologies of the biochemical networks: a linear chain of five reactions, a small branching network, a cascade with two/three states on every level, and a glycolysis model based on a list of chemical reactions from KEGG.

2 Methods

Biochemical networks. The dynamics of a biochemical reaction system is described by a set of ordinary differential equations (ODEs)

$$dc_i/dt = \sum_{j=1}^{r} n_{ij} v_j \quad (i = 1,..,m) \tag{1}$$

where m is the number of biochemical species M_i with the concentrations c_i and r the number of reactions with the rates v_j, and the quantities n_{ij} denote the stoichiometric coefficients. The kinetic functions of reactions v_j follow either mass action kinetics

$$v_j = k_j \cdot c_{i1} - k_{-j} \cdot c_{i2} \tag{2}$$

with the rate constants k_j and k_{-j} as parameters, or Michaelis-Menten kinetics (denoted in the following by MM)

$$v_j = \frac{\frac{V^{\rightarrow}_{\max,j}}{K_{m,i1}} \cdot c_{i1} - \frac{V^{\leftarrow}_{\max,j}}{K_{m,i2}} \cdot c_{i2}}{1 + \frac{c_{i1}}{K_{m,i1}} + \frac{c_{i2}}{K_{m,i2}}}. \tag{3}$$

with maximal velocities $V_{\max}$ and K_m-values as kinetic parameters[1]. Given the kinetic parameters and fixed external metabolite concentrations, a stationary state fulfilling $dc_i/dt = 0$ is computed by substituting the kinetic function (equation (2) or (3)) into the right-hand side of equation (1) and solving for the concentrations.

Flux or concentration control coefficients [3,8] are defined as

$$C^{X_i}_{v_j} = \frac{\partial \ln X_i}{\partial \ln v_j} \tag{4}$$

where X_i denotes either the stationary flux through reaction i or the stationary concentration of metabolite i, respectively. The control coefficients provide a quantitative measure for changes of X_i at perturbation of reaction v_j. They can be computed from the stoichiometric matrix (n_{ij}) and the elasticity coefficients, i.e., the linearized kinetics (see, for instance, Heinrich and Schuster [4]).

Parameter distributions. The kinetic constants ($k_{\pm j}, V_{\max}, K_m$) are drawn independently from one of the following distributions: (1) the uniform distribution (UD) with $Min = 10^{-3}, Max = 10^{3}$ or (2) the log-normal distribution (LND) with parameters $\mu = 1, \sigma = 2.5$ (mean and standard deviation of the logarithmic values). The choice of the log-normal distribution is based on a collection of measured and published kinetic constants for various networks from literature [5,14,18] and from the BRENDA database [26]. The distribution of their values in [μM/min] could be approximated by the above log-normal distribution. Moreover, the log-normal distribution is well suited for variables with multiplicative errors, i.e. for the case where it is equally probable that the value is an order of magnitude larger or smaller.
If indicated we also consider constraints by equilibrium constants: for monomolecular reactions with mass action kinetics (equation (1)), the equilibrium constant, i.e. the ratio $q_j = k_j/k_{-j}$ of the rate constants, is determined by the difference of free enthalpies. Similar constraints also hold for the other types of kinetics considered. Choosing independent random values for the rate constants would lead to inconsistencies. To avoid this, we can constrain the rate constants to fulfill energy differences, which are either known, or chosen randomly.

[1] More types of kinetic functions are described, but we restrict the current analysis to mass action and MM kinetics.

Practically, the forward rate constant is drawn from a distribution, while the backwards constant is computed from forward constant and equilibrium constant.
We calculate steady state observables (fluxes, concentrations, control coefficients) for different biochemical networks for the indicated numbers of random choices of parameters values from UD or LND. The distributions of the observables will be characterized by their mean values (Mean) and the coefficients of variation CV (standard deviation divided by mean value), or shown by frequency distributions.
Calculations have been performed with Mathematica®, Wolfram Research and, in the case of the KEGG glycolysis model, with the modeling and simulation environment PyBioS [22].

3 Results

3.1 Unbranched reaction chains

We consider a linear pathway consisting of 5 successive reactions as depicted in Fig. 1. In the basic version all reactions are reversible (i). The kinetic constants are chosen either from UD or LND. Furthermore, we investigate the cases that (ii) all equilibrium constants q_j are known and have a fixed value equal to 5, (iii) negative feedback inhibition from metabolite 4 to reaction 2 occurs, (iv) reaction 2 is irreversible, (v) a combination of (iii) and (iv), (vi) reaction 2 is coupled with another reaction by sharing substrates (like coupling with ATP consumption) with fixed or random kinetics (vi+). In order to get positive fluxes, the concentrations of the external metabolites are chosen as $c_0 = 1$ and $c_5 = 0$, respectively. In each case we compute the steady state for 10^4 to 10^6 parameter choices.

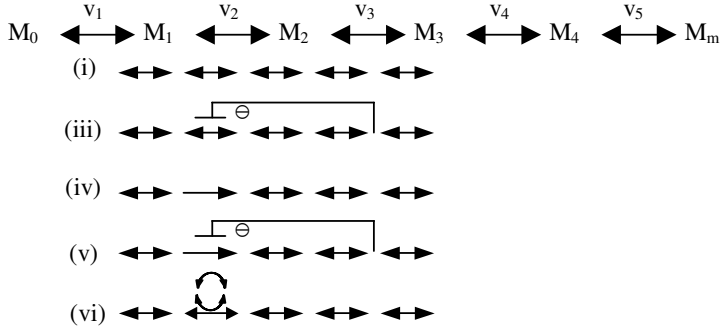

Figure 1: Schematic representation of pathway examples

Fluxes
For all pathway examples, the resulting values for steady state fluxes cover several orders of magnitude: from 4 orders of magnitude for pathway example (vi) with UD to more than 10 orders of magnitude for most of the other examples (e.g. from 10^{-7} to 10^3). With kinetic constants drawn from the UD, the flux values tend to become higher and their coefficients of variation become lower, as compared to the LND. The LND leads to a smeared distribution of fluxes over many orders of magnitude.
This effect is almost independent of features like irreversibility or feedback. For the case of mass action kinetics with parameters from LND the only sharp distribution results from a coupling with another reaction (and fixed total concentration of coupling compounds). The flux values for a reaction chain with MM kinetics are also smeared, but they show sharper peaks for both distributions of kinetic constants. Nevertheless, their coefficients of variation also tend to higher values.

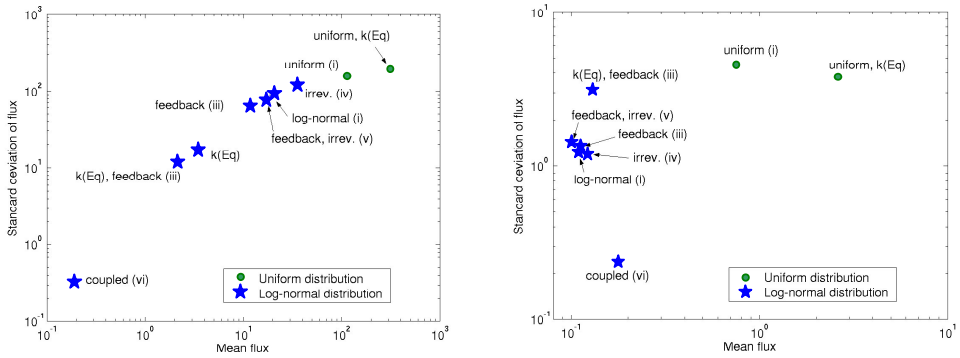

Figure 2: Fluxes in unbranched chains. Standard deviations and mean values of fluxes are plotted against each other for different versions of the unbranched chain (compare Fig. 1) with mass action (left) or Michaelis-Menten kinetics (right).

Fig. 2 shows the distributions of reaction rates (means and standard deviations) for all considered pathway examples, with mass action and MM kinetics (10^4 simulation runs in each case). Fig. 3 shows the frequency distribution of flux values in a logarithmic scale for the different pathways. Note, for comparison, that the steady state fluxes are 0.56 for mass action kinetics and 0.11 for MM kinetics for pathway version (i) in the case that all kinetic constants are equal to 1.

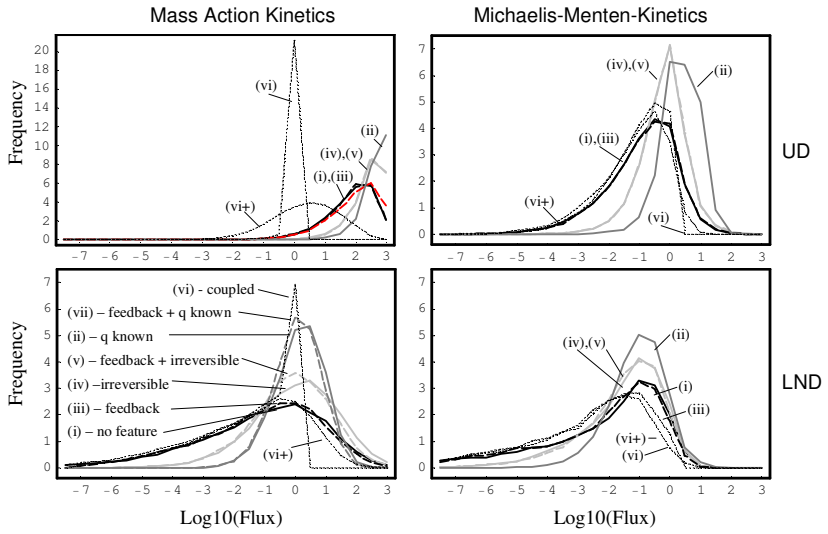

Figure 3: Frequency distribution of flux values for an unbranched reaction chain with mass action or MM kinetics at 10^4 simulation runs with parameters independently taken from LND or UD (see text). Pathway examples as described in Fig. 1 and text.

Flux control coefficients

The flux control coefficients describe the linear effect of parameter perturbations on the flux values. Fig. 4 shows the mean values and the coefficients of variation for flux control coefficients assigned to the reactions in the chain. Note that the flux control coefficients in the unbranched pathway must sum up to unity.

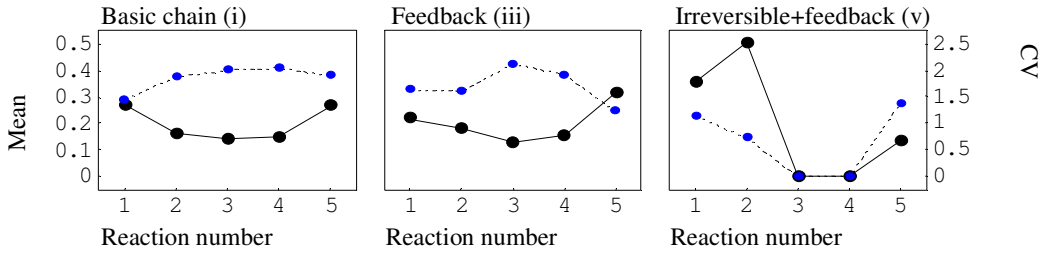

Figure 4: Flux control coefficients for subsequent reactions in the unbranched reaction chain for three different pathway examples: mean values (black dots connected with solid lines) and coefficients of variation (blue dots connected with dashed lines).

As the direction of net flux is fixed (positive) by the choice of external metabolite concentrations ($c_m = 0$), all flux control coefficients are non-negative. For the basic chain (i) we find higher flux control coefficients at the beginning and at the end of the chain compared to the reactions in between. With feedback (iii), reaction 5 shows an enhanced control over the flux, since it degrades metabolite 4, which exerts the feedback inhibition on reaction 2. In case (v) with an irreversible reaction 2, the subsequent reactions 3 and 4 exert no control over the flux. But reaction 5 has flux control, since it degrades the inhibitor. This general pattern remains stable throughout the investigated parameter region. Irreversibility of one reaction lowers the CVs of the flux control coefficients, since some of the control coefficients are completely fixed (here for the flux control of reactions 3 and 4).

Concentration control coefficients

The concentration control coefficients describe whether a metabolite concentration is increased or decreased after the perturbation of the rate of a reaction. Producing reactions tend to have a positive control, while degrading reactions usually exert a negative control over the concentration of a metabolite. This is supported by our simulations, as shown in Fig. 5.

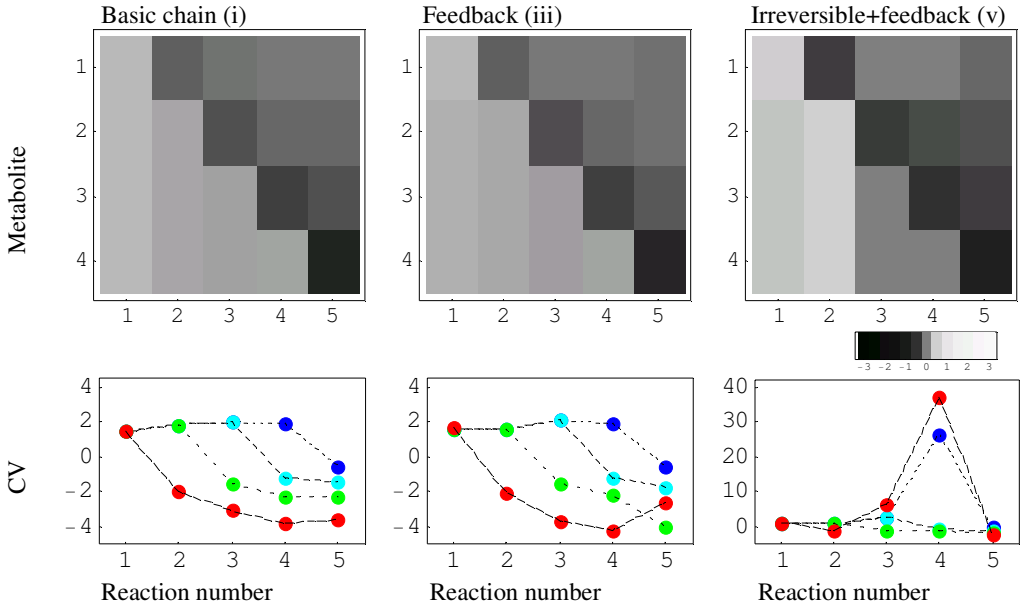

Figure 5: Concentration control coefficients for the unbranched reaction chain. Upper panel: Average values, gray level coded: light or dark squares indicate positive or negative values of control exerted by a reaction on the concentration of the respective metabolite. Lower panel: CV, for each metabolite connected by dotted lines (in online version: metabolite number red – 1, green – 2, light blue – 3, dark blue – 4)

In the basic chain with or without feedback ((i) and (iii)) producing reactions yield positive values (light squares) and degrading reactions yield negative values (dark squares). This pattern is slightly changed in the case (v) of irreversibility and feedback: The negative control of the directly degrading reactions is a bit stronger, and reactions 3 and 4 have on average no or very low positive control over the concentration of metabolite 1. The coefficients of variation vary also systematically: tendentially control coefficients of producing reactions have a positive CV and control coefficients of degrading reactions have negative CVs with lower absolute values for the directly degrading reaction. Only in the case of irreversibility of reaction 2 in conjunction with feedback inhibition the control coefficients of reaction 4 with respect to the concentrations of metabolites 1 and 4 show very large CVs.

3.2 Branched reaction network

We have analyzed a series of non-hierarchical reaction networks of different complexity containing various numbers of features like feedback inhibition or feedback activation, irreversibility of individual reactions or coupling of reactions by common metabolites (not shown). The results are qualitatively the same as for linear reaction chains: the distribution of steady state flux values is smeared over all orders of magnitude considered.

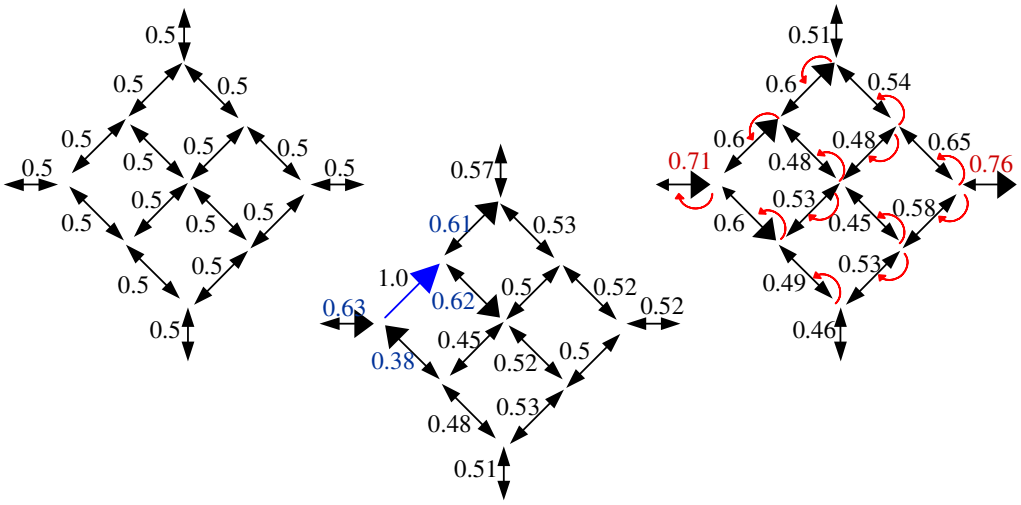

Figure 6: Branched network. Left: basic version, center: with one irreversible reaction, right: with feedbacks from metabolites to forming reactions. All external metabolite concentrations set to 1. Numbers at arrows indicate the relative frequency of forward fluxes (positive flux values) among 10^5 simulation runs.

Also metabolite concentrations are weakly determined. On the contrary, the flux and concentration control coefficients tend to show stable patterns. These patterns are increasingly robust with an increasing number of feedback or feed-forward loops or irreversible reactions. In the example network given in Fig. 6, the fluxes may assume positive and negative values with a probability of 0.5. The absolute values of the CV are in the order of magnitude of 100. Imposing restrictions to the direction of single reactions (making one reaction irreversible) has mainly local effects: the signs of the fluxes through neighboring reactions (involving the same reactant as substrate or product) are affected. If an input or output flux is irreversible, then the signs of the other input/output fluxes are shifted (not shown). Interestingly, this network tends to an accumulation of metabolite. If the values of all kinetic constants are chosen as 1, then the concentration of all metabolites is 1. At random choice of constants, the metabolite concentrations reach an order of magnitude of 10^3 to 10^5 with CV between 10 and 100. Thus, in real systems mechanisms should be implemented that prevent metabolite accumulation. We tested feedback mechanisms as indicated in Fig. 6, right. This imposed a strong restriction on the absolute values of the fluxes (though not on their sign), but no

significant decrease of the metabolite concentrations. Only the CVs of metabolite concentrations decreased to values between 4 and 8.

3.3 Glycolysis network from KEGG

In order to apply the approach to a system relevant for cellular metabolism we downloaded the glycolysis network for Saccharomyces cerevisiae from the KEGG database [27], which is schematically represented in Fig. 7. The reaction network has been populated with linear and bilinear kinetics with kinetic constants chosen from the uniform distribution. We performed 770 simulation runs with the PyBioS simulation environment.

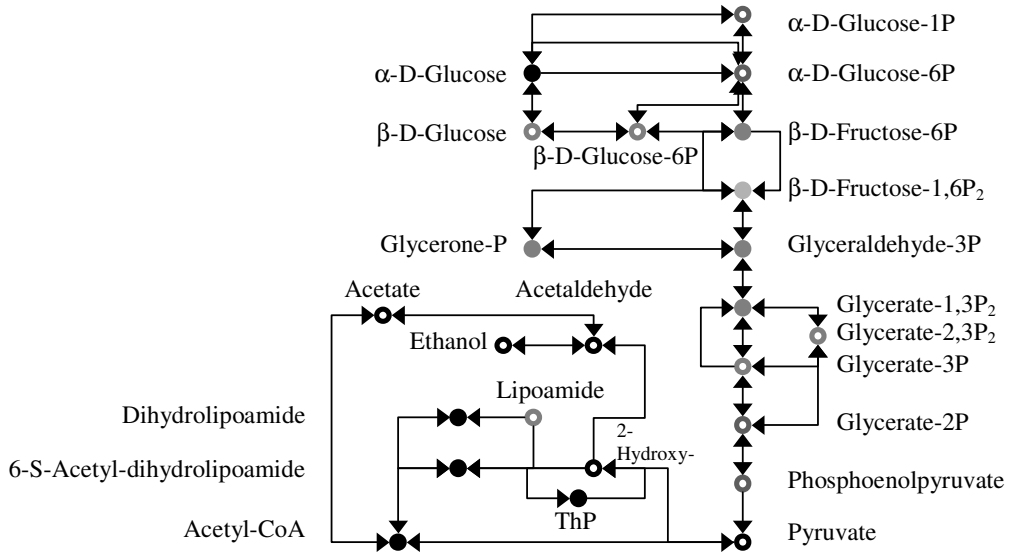

Figure 7: Schematic representation of the glycolysis network from KEGG, circles denote metabolites, arrows denote reactions (for sake of simplicity not all reactions are shown). Circle styles code for CVs of metabolite concentrations: filled black circles – fixed concentrations, black or gray open circles – low or intermediate CVs, resp., gray or light gray filled circles – large or very large CVs.

The results for steady state fluxes and concentration for different choices of kinetic constants vary substantially. Mean concentrations vary between 0.77 (pyruvate) and 496.1 (β-D-fructose-1,6-bisphosphate). The coefficients of variation range between 0.64 (2-(α-Hydroxyethyl) thiamine diphosphate) and 18.68 (β-D-fructose-1,6-bisphosphate). Fig. 8 shows that the coefficients of variation tend to increase with increasing mean concentrations. Less pronounced, this trend can also be observed for the fluxes (not shown).
The prominent result is that the large coefficients of variation are concentrated in the upper part of glycolysis, while lower coefficients are found around pyruvate (see Fig. 7). A possible explanation for this finding is the fact that the respective metabolites are close to external metabolites where the distance is measured by the number of reactions between them. For example, 2-(α-Hydroxyethyl) thiamine diphosphate is directly connected by one reaction to external metabolites and β-D-fructose-1,6-bisphosphate has a distance of 3 reactions to the closest external metabolite.

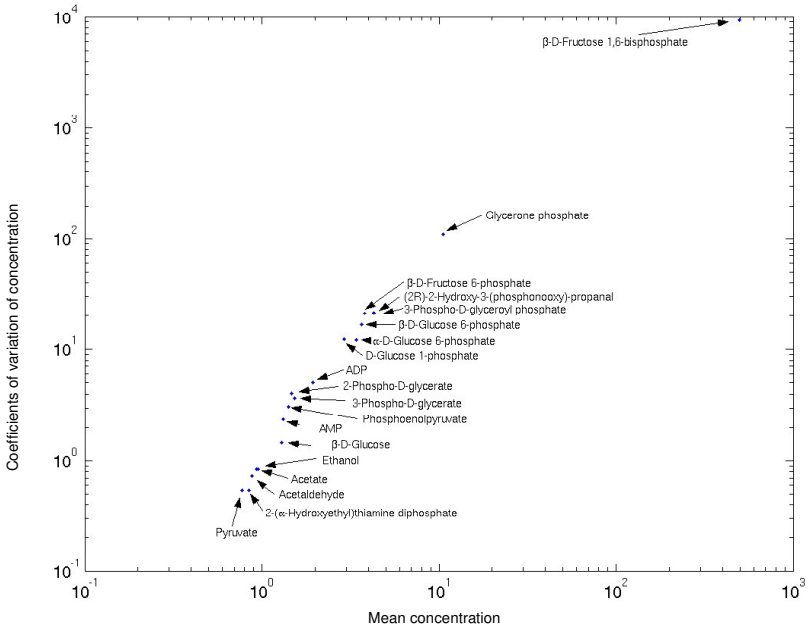

Figure 8: Glycolysis network according to KEGG chart. Coefficients of variation are plotted against mean values for concentrations (logarithmic scale).

Fig. 9 represents linear correlations between metabolite concentrations over the different simulation runs. In the triangle below the diagonal the pairwise correlation coefficients are shown, and the upper triangle illustrates the pairwise distances between the metabolites in the network. Again we find a trend that closer metabolites show higher correlation of their concentrations.

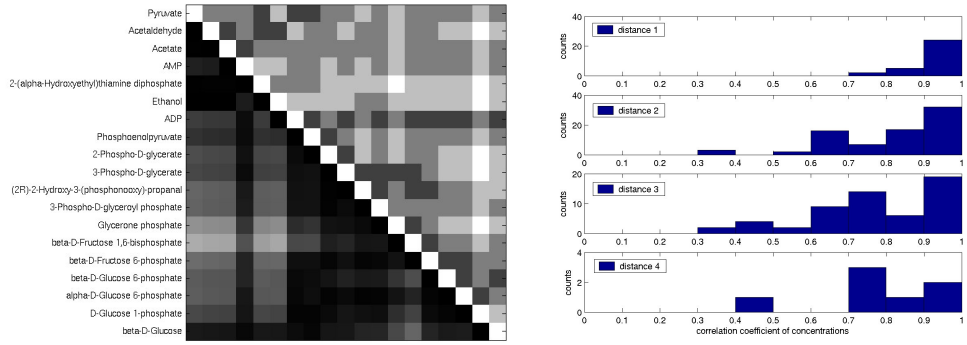

Figure 9: Metabolite correlations and network distances. Left: Color-coded matrix representation, where both rows and columns correspond to the indicated metabolites. The lower triangle shows the correlations (light/dark – low/high correlation), while the upper triangle shows pairwise distances (light…dark – 1...4, with 4 as maximal distance in the considered network). Right: Histograms of correlations for metabolite pairs in given distances.

Based on these simulations we presume that knowledge of certain metabolite concentrations diminishes the uncertainty concerning the concentrations of other metabolites, especially in their vicinity.

3.4 Regulatory Cascades

Regulatory pathways in cells often contain hierarchical structures in which the product of one reaction serves as a catalyst for another reaction. Two examples are shown in Fig. 10.

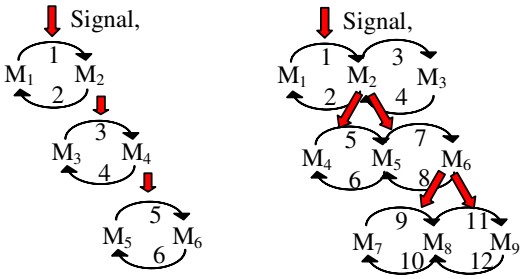

Figure 10: Hierarchical structures with single (left) or double (right) loops on every level.

The first scheme is a simplified form the gene expression cascade comprising DNA activation by transcription factors (upper level), mRNA formation (middle), and protein production (lower level). The second scheme represents the structure of a MAP kinase cascade.
We performed 10^4 simulation runs for low ($S = 0.01$), intermediate ($S = 1$), and high ($S = 100$) input signal strength: mean values and standard deviations are shown in Fig. 11. The metabolite concentrations on each level must fulfill moiety conservation (set arbitrarily to 1).

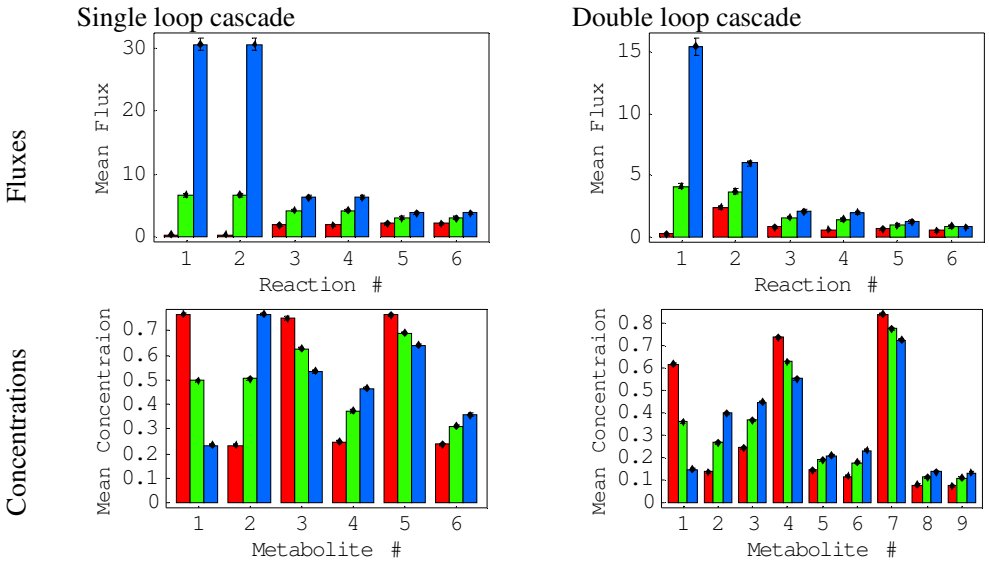

Figure 11: Fluxes and metabolite concentrations at low, intermediate, high activation for the one-loop cascade for the single and double loop cascade. Left (red), middle (green), and right (blue) columns: low, intermediate, and high input signal, respectively.

For both types of cascade (single and double-loop), we find fluxes and concentration patterns with comparably low CVs. Interestingly the coefficients of variation are even lower in the case of double-loop cascade. Furthermore the signal amplification comparing the concentration of the metabolite with the highest number (6 in the single loop case, 9 in the double-loop case) at high or low activation is clearly higher in the double-looped hierarchy.

Concentration control coefficients
The concentration control coefficients for both types of cascade (see Fig. 12 for the double loop cascade) exhibit a remarkably robust pattern with an extremely low CV.

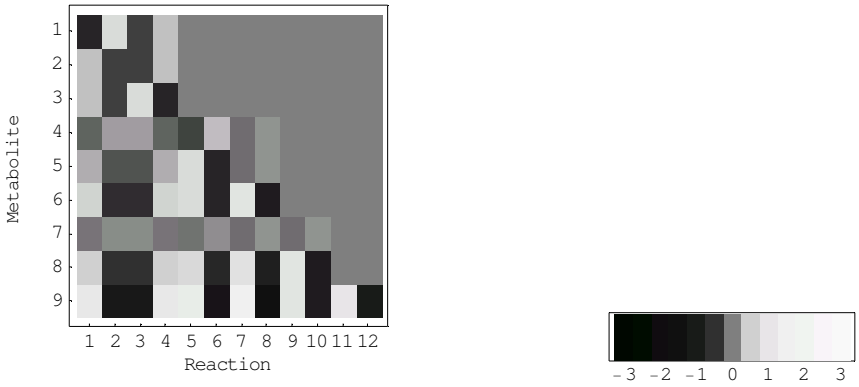

Fig. 12: Concentration control coefficients in the double-loop hierarchy.

Although it is clear from detailed analysis of signaling pathways that effective signal transduction demands appropriate parameters of kinases and phosphatases our results support the idea that the structure of signaling cascades already ensures and stabilizes the transfer of the signal for a wide range of parameters. The individual dynamics is, of course, determined by the precise kinetic values.

4 Discussion

There are two recent developments in systems biology: first, biochemical networks can be easily extracted from databases; second, attempts are made to use this knowledge for large-scale or whole-cell modeling without caring about individual reaction kinetics [28]. To challenge these approaches, we attempted to extract information about biochemical network dynamics from the network structure, with very restricted knowledge about the individual reaction kinetics. From this analysis, we can learn (a) what range of states are possible at all, (b) which structures like feedback or coupling of fluxes limit the range of dynamics.

It is well known that some features of biochemical networks are invariant with respect to the parameters and can be predicted from the structure by algebraic considerations. For instance, some of the flux directions may be constrained by the elementary modes [15]. Moreover, the summation theorems of metabolic control theory [4] imply linear relations between the control coefficients, irrespective of the reaction kinetics.

To go beyond these exact constraints and to deal with uncertain and missing knowledge, we investigated the distributions of steady state fluxes and concentrations and the respective control coefficients while the kinetic constants were chosen from different distributions. The distributions of parameters are supposed to describe a knowledge or belief about their values. We may not know anything, or we may know the order of magnitude, or we may know the value up to an error. Here we chose the kinetic constants from a log-normal distribution, with mean values and standard deviations parameters chosen to describe the distribution of known kinetic constants. To compute the distributions of observables, we used Monte Carlo simulations. When an observable is almost independent of the parameters, we conclude that it is mainly determined by the network structure. We did not consider changes of the qualitative behavior, as it is done in bifurcation theory, but our approach can also be used to study the probabilities for different kinds of qualitative behavior.

We have analyzed the stable steady states of different artificial and real biochemical networks with linear and Michaelis-Menten kinetics. The coefficient of variation was used to measure the robustness of network properties like steady state fluxes, concentrations, and control

coefficients with respect to the kinetic parameters. We found that some of network observables are almost, but not entirely constrained by the network structure. Concerning the structures, we have found two different kinds of behavior:

1. For networks without intrinsic hierarchy, like the metabolic network, we find that flux values are weakly determined by the structure. Even consideration of irreversible reactions or feedback does not contribute much to the robustness of fluxes. This kind of network is designed to adapt fluxes and concentrations to the actual need of the cell. Prediction of dynamics is not possible without detailed knowledge of kinetics (kinetic types of the reactions, respective kinetic parameters, equilibrium constants) as well as concentrations of external metabolites. This is in accordance with many observations. For instance, Ihmels et al. [6] argue that although the structure of metabolic networks is far from linear, reflecting the need for flexibility and diversity of metabolic flow, only the transcriptional regulation leads the metabolic flow towards linearity.

2. In networks with hierarchical structure, i.e. structures where the product of one reaction catalyzes another reaction, flux and concentration values are more strongly determined by the structure: the coefficients of variation are quite low. This corresponds to their function of transferring a signal. The ability of signal amplification is already implemented in this structure, even more in the cascade with two loops. For these structures, the prediction of fluxes and concentrations is more promising – although also here, better predictions can be attained with more prior knowledge. The sign and the value of control coefficients are much more restricted than in networks of the metabolic type.

Altogether, the stochastic approach allowed us to draw information from the network structure that could not be inferred from algebraic constraints. Our results also suggest that the investigation of networks structures must be accompanied or accomplished by studying the kinetics of the individual interactions.

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