

Patterns of Interactions of Reaction Pairs in Metabolic Networks

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Abstract

A large scale structural analysis of metabolic networks is presented focusing on neighbourhood relationships between individual reactions. We define two reactions to be neighbored if one of them provides the necessary set of substances for the other to proceed. A method is developed which allows determining all possible neighborhood relationships categorized as interaction patterns. These patterns differ in the types of participating reactions and in the way they share their reactants. The method is applied to a set of 4795 metabolic reactions contained in the KEGG database. We show that from the 1547 theoretically possible types of interactions 282 patterns are found in metabolism. More than 55% of all interactions occur between reactions with at most two reactants on one side. In these interactions only 25 different patterns play a role. We propose to use these neighborhood relationships as a concept of adjacency in large scale graph theoretical analyses of metabolism.

Keywords: metabolic networks, structural analysis, KEGG database, network expansion

1 Introduction

Traditional modeling approaches describing metabolic reaction networks in terms of systems of differential equations have proven successful [4, 11]. Typically, they involve a detailed description of the kinetic properties of the individual reactions and aim at predicting the metabolite concentrations and fluxes as a function of time. Due to the high level of included details, this class of models is generally restricted to relatively small subsystems of metabolism. However, simulating dynamic properties of metabolic networks is not the only goal of their mathematical analysis. In contrast to chemical reaction networks of inanimate nature, biochemical reaction networks are the result of evolutionary optimization. It is, therefore, also of interest to analyze those of their properties unique to living systems.

In the present paper, we focus on large-scale structural characteristics of metabolic networks. For that kinetic properties of the enzymes are of minor importance and only knowledge on the stoichiometric properties is required. Related methods include flux balance analysis [8, 9] and elementary mode analysis [10]. Here, information on the modeled system enters mainly in form of the stoichiometric matrix N . More recently, graph theoretical descriptions of cellular networks have received considerable interest and produced remarkable results [2, 5, 7, 12]. The information entering these models is merely the list of each reaction's substrates and products which is contained in the sign of the entries of N while the exact stoichiometry is not included.

Obviously, graph theoretical representation of cellular networks is not straightforward. It is rather simple for signal transductions networks where interactions are mainly activations or inhibitions [1].

However, in the case of metabolic networks the construction of graphs turns out to be less clear since individual reactions represent chemical transformations which are usually not unimolecular but often involve many reactants. It can be argued that the widely used ways of constructing graph representations do not account for the complexity of the system. For example, reactions are represented as nodes and two nodes are connected by an edge if the corresponding reactions share at least one metabolite. However, the biological interpretation of two reactions being connected in this way is not obvious since providing a single metabolite is necessary but in general not sufficient for a reaction to proceed. Similar problems arise if nodes do not represent reactions but metabolites.

In the present paper we perform a more thorough analysis of pairwise interactions of reactions. We start from the physico-chemical viewpoint that a given reaction can only proceed if all of its substrates are present. Accordingly, we define two reactions to be neighbored if one of them provides the necessary set of substances for the other to proceed. This approach also allows distinguishing different kinds of interactions depending on the type of the neighbored reaction (uni-, bi-, or trimolecular etc.) and on the way they share each other's reactants. We describe these neighborhood relations in the form of interaction patterns and investigate all possible patterns in metabolism as represented in the KEGG database [6]. Restricting the analysis to reactions with maximally two substrates or products we identified 25 different patterns whereas consideration of the full repertoire of reaction types results in 282 patterns. This multitude of different kinds of interaction paves the way for a graph theoretical analysis where the richness of the structure of metabolism can be better accounted for.

2 Methods and Results

2.1 Patterns of Interacting Reactions

The present paper concerns itself with special structural properties of biochemical networks with a special focus on pairwise and direct interactions between reactions. Information on structural properties of a given network is contained in its stoichiometric matrix N . In this matrix the rows correspond to the chemical species and the columns to the reactions. The entry n_{ij} is the stoichiometric coefficient of the i th species for the j th reaction. Substrates of a reaction enter the matrix with negative coefficients and products with positive coefficients.

For considering direct interactions we define a reaction R_j to be neighbored to another reaction R_i if the set of all reactants (substrates and products) of R_i includes a set of substances which allows reaction R_j to proceed. Depending on thermodynamic properties reactions may take place favorably in one of the two directions. Since it is often not possible to acquire reliable data on these properties we assume, for simplicity's sake, that all reactions may proceed in both directions. Accordingly, the set of substances which is necessary for reaction R_j may either be the set of its substrates or the set of its products. We always assume that water is available and it is therefore not required that H_2O is among the reactants of R_i .

For identifying neighborhood relationships between two reactions some stoichiometric details can be neglected. It is, for example, not of relevance whether one, two or more molecules of the same compound are consumed or produced in these reactions. Accordingly, we do consider only the signs of the stoichiometric coefficients, $\text{sign}(n_{ij})$, for distinguishing between substrates and products of a given reaction, but will not take into account the absolute values of n_{ij} . Therefore, we represent reactions graphically in a simplified way by "diagrams" instead by their common schemes. Whereas reaction schemes usually account for the stoichiometric properties, we include in the corresponding reaction diagrams no information about how many molecules of a given species are produced or consumed. Reaction diagrams can be visualized in a similar way as reactions schemes as shown by the examples in Figure 1. Note that the reaction scheme and the reaction diagram differ significantly only for example d) since here the compound A appears twice on one and the same side of the reaction.

It is worth mentioning that the neighbourhood relationship as defined above is also the basis of the

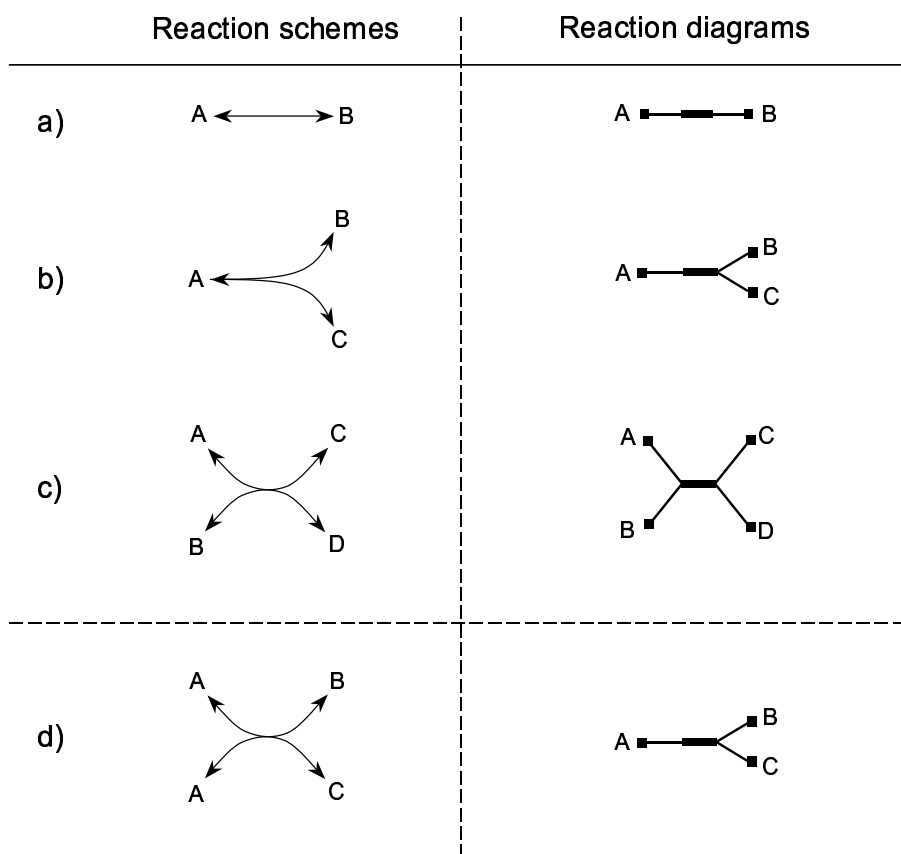


Figure 1: Reaction schemes and reaction diagrams. The left column shows various schemes of single reactions graphically depicted in such way that the stoichiometric properties are fully taken into account. The right column shows the corresponding reaction diagrams. In these diagrams each compound appears only once, even if in the corresponding reaction scheme it appears multiple times (case d). The figure shows all possible types of reaction diagrams for reactions with at most two different chemical species on either side. Similar schemes and diagrams can be depicted for reactions with higher number of substrates or products.

method of network expansion recently developed [3]. There, a reaction R_j turns out to be neighboured to a reaction R_i if and only if R_j is incorporated in the first generation of the expansion when the process is started with all reactants of reaction R_i as seed compounds.

In the following, all specific calculations are based on a set of reactions derived from the KEGG database. Prior to the analysis we have excluded all reactions containing unspecified reactants (such as sugar, electron acceptor or a chain of unspecified length of certain subunits). Reactions with incorrect stoichiometric balance were either corrected by addition of one compound from the database, or if this was not possible, they were excluded from the analysis. From the 4807 remaining reactions 12 contain a reactant appearing both on the substrate and on the product side. These reactions were excluded as well for a closer inspection showed that these cases were artefacts of the database (for example, Fe^{2+} and Fe^{3+} are treated as the same chemical species).

Reaction diagrams can be categorized into “reaction types”. These types contain, in contrast to the diagrams, no information on the identity substrates and products but only on their numbers. We say that a reaction is of type (n, m) if it has n different substrates and m different products. Without loss of generality, we may assume $n \leq m$ since this can always be achieved by an appropriate choice of

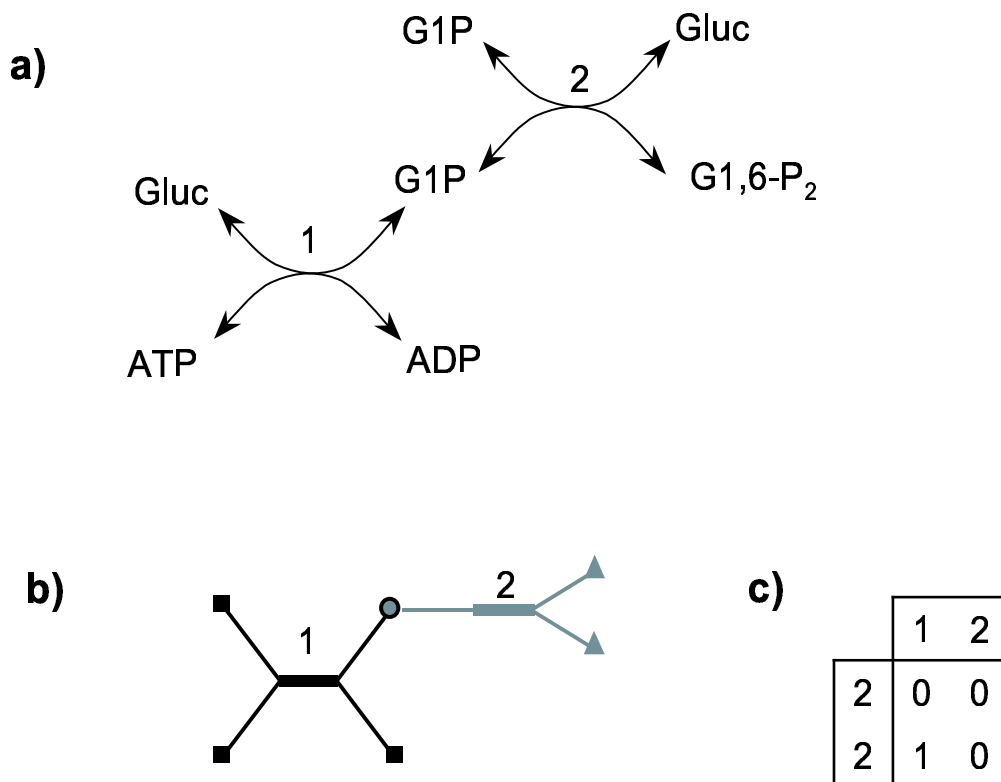


Figure 2: A specific example of an interaction of two neighbored reactions. Reactions 1 and 2 represent the reactions catalyzed by glucokinase and phosphodismutase, respectively. (a) Graphical representation of the two interacting reactions taking into account the details of stoichiometry. (b) Graphical representation of the interaction pattern based on reaction types (see Figure 1). Reaction 1 allows reaction 2 to proceed. The opposite holds not true since reaction 2 does not provide ADP which would be necessary for the course of reaction 1. Accordingly the interaction pattern is unidirectional. (c) An interaction table representing the pattern shown in(b).

the forward direction of the reaction. Since water is assumed to be present we have omitted H₂O from both reaction diagrams and reaction types. An inspection shows that the KEGG database contains 12 reaction types with types as complex as (4, 4), (3, 5) or (2, 6). The frequencies of the occurrence of the various types of reactions are listed in Table 1.

Neighborhood relationships between two different reactions R_i and R_j can be further specified by considering which types of reactions are involved and in which way they share substrates and products. This results in a distinction of various “interaction patterns”. Figure 2a shows a specific example from carbohydrate metabolism where two reactions, 1 and 2, share the metabolite G1P (glucose 1-phosphate) in such a way that the presence of reaction 1 allows reaction 2 to proceed.

For all pairs of reactions containing not more than two substrates or products, that is, of type (n, m) with $n, m \leq 2$, there exist 27 possible patterns which are depicted in Figure 3. There are cases where the products of one reaction are the substrates of the neighbored reaction (examples are cases 1, 2, or 16), and other cases with more intricate interrelations between substrates and products (for example, case 4 where the two products of one reaction are both substrate and product of the other reaction). Interaction patterns can be bidirectional or unidirectional. Given two reactions R_i and R_j where R_i allows R_j to proceed we say that the interaction pattern is bidirectional if R_j also allows R_i to proceed. Otherwise the interaction pattern is called unidirectional. Examples of bidirectional patterns are cases 1, 3, and 4 whereas case 2 is an example of a unidirectional pattern. Note that the

interaction pattern shown in Figure 2b belongs to the class of unidirectional patterns.

Table 1: Occurrences of the different reaction types in the KEGG database. Since we assume the reversibility of the reactions, we can choose to call those side of a reaction as “substrate side” which has a lower number of reactants. The number in the field $[i, j]$ denotes the occurrence of the reaction type (i, j) in the analysed set of 4795 reactions in the database.

		Number of products					
		1	2	3	4	5	6
Number of substrates	1	600	1110	59	7	0	0
	2	0	1814	755	165	0	1
	3	0	0	229	41	5	0
	4	0	0	0	9	0	0

2.2 Determination of Possible Interaction Patterns

For patterns involving reactions with more than two substrates or two products it would be difficult to construct all possible cases by mere intuition. Therefore, a more thorough treatment of interaction patterns is required. To this end, we construct a table of interactions for each pattern in the following way:

$$P = \begin{array}{c|cc} & T_{j,s} & T_{j,p} \\ \hline T_{i,s} & C_{ss} & C_{sp} \\ \hline T_{i,p} & C_{ps} & C_{pp} \end{array}$$

In the first column the symbols $T_{i,s}$ and $T_{i,p}$ denote the numbers of different substrates and different products of reaction R_i , respectively. Analogously, $T_{j,s}$ and $T_{j,p}$ in the first row denote the numbers of substrates and products of reaction R_j , respectively. Accordingly, reactions R_i and R_j are of type $(T_{i,s}, T_{i,p})$ and $(T_{j,s}, T_{j,p})$, respectively. The other entries describe how the two reactions share substrates and products: C_{ss} , number of reactants being both substrates of R_i and substrates of R_j ; C_{sp} , number of reactants being both substrates of R_i and products of R_j ; C_{ps} , number of reactants being both products of R_i and substrates of R_j and C_{pp} , number of reactants being both products of R_i and products of R_j . A specific example for a table of interaction is shown in Figure 2c corresponding to the interaction pattern shown in Figure 2b. All entries of the table P have to be nonnegative integers. Moreover, they have to fulfill the following relations

$$C_{ss} + C_{ps} \leq T_{j,s}, \quad C_{sp} + C_{pp} \leq T_{j,p}, \quad (1a, b)$$

$$C_{ss} + C_{sp} \leq T_{i,s}, \quad C_{ps} + C_{pp} \leq T_{i,p}. \quad (1c, d)$$

Relation (1a), for example, expresses the fact that the number of substrates of reaction R_j serving as substrates or products of reaction R_i cannot be higher than the total number of substrates of

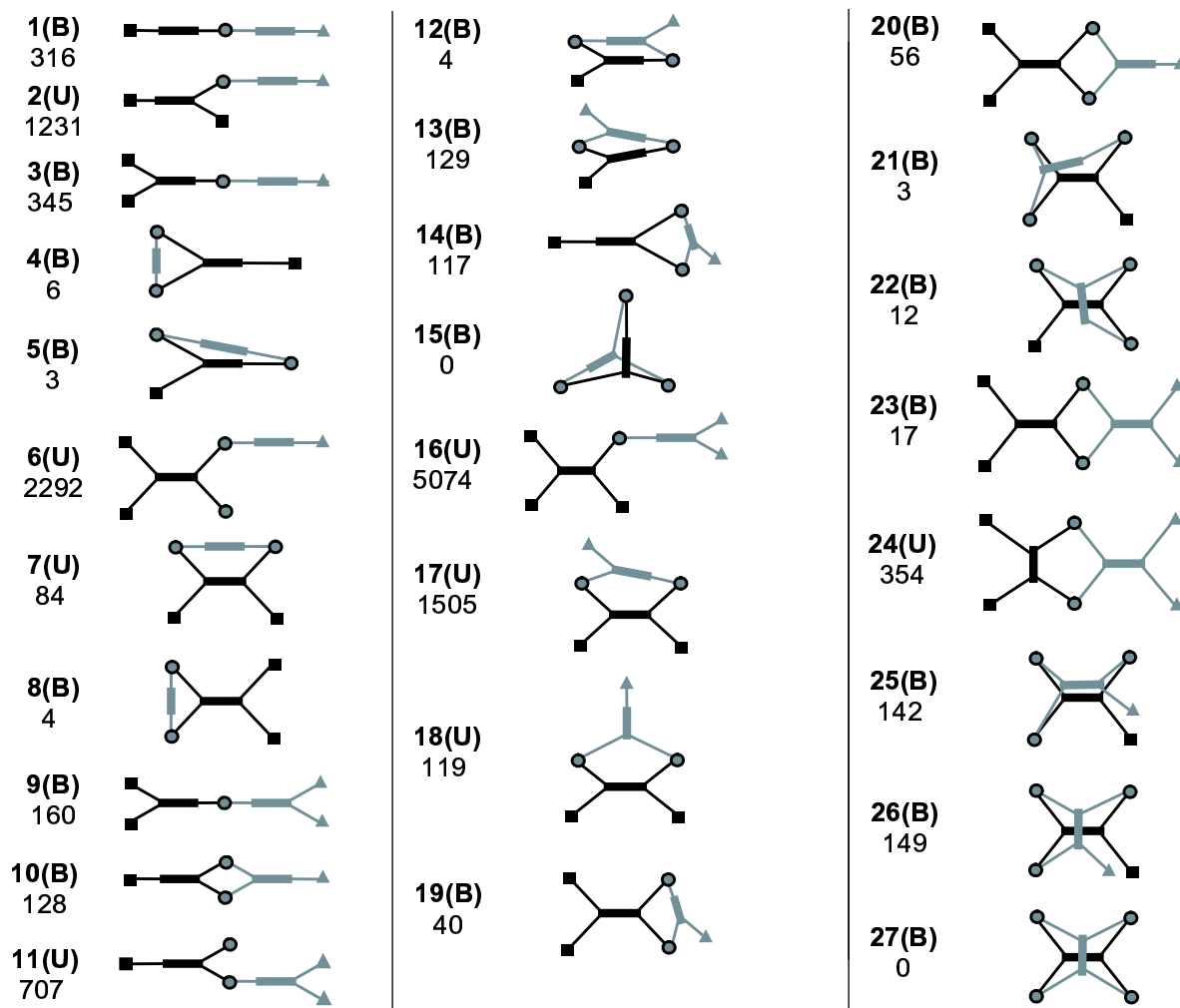


Figure 3: Interaction patterns of neighbored reactions. Shown are all possible 27 interaction patterns for pairs of reactions of types (1, 1), (1, 2) and (2, 2). 19 patterns are bidirectional (B) and 8 patterns are unidirectional (U). Squares (■) stand for the compounds of reaction 1 and triangles (▲) for the compounds of reaction 2. Circles (●) mark compounds shared by both reactions. In each case, reaction 1 allows reaction 2 to proceed. For the unidirectional patterns the opposite does not hold true. The numbers represent the frequencies of the given pattern in the KEGG database.

reaction R_j . Similar arguments yield relations (1b) to (1d). According to the definition given above, reaction R_j is neighbored to reaction R_i if at least one of the relations (1a) and (1b) is fulfilled as an equality relation. This expresses that either all substrates or all products of R_j (or both) are provided by reaction R_i . Analogously, equality signs in relations (1c) and (1d) imply that reaction R_i is neighbored to reaction R_j . Clearly, application of the formalism easily allows to determine whether an interaction patterns is unidirectional or bidirectional.

Based on this formalism all interaction patterns for a given pair of reaction types can be determined in the following way: first the numbers $T_{i,s}$, $T_{i,p}$, $T_{j,s}$, and $T_{j,p}$ have to be fixed according to the considered reaction pair, and second, those nonnegative sets of integers have to be determined which satisfy relations (1a)-(1d) under the condition that at least one of these relations is fulfilled as an equality relation. Importantly, different sets of integers may correspond to one and the same interaction pattern. This follows from the fact that interaction patterns should be invariant against renumbering of the reactions. Accordingly, transposition of an interaction table gives rise to a table representing

Table 2: Numbers of theoretically possible types of reactions, interaction patterns depending on the maximal number of different compounds appearing on the product side of the reactions. See main text for the meaning of bracketed numbers.

	Maximal number of products, m_{\max}					
	1	2	3	4	5	6
Number of possible reaction types	1	3	6	10	15 (11)	21 (12)
Number of possible interaction patterns	1	27	186	832	2806 (1185)	7895 (1547)
Fraction of bidirectional interaction patterns	1	0.704	0.543	0.442	0.371 (0.414)	0.320 (0.409)

the same interaction pattern. Moreover, since all reactions are considered to be reversible, interaction patterns should also be invariant against exchanging the role of substrates and products. Therefore, exchanging the last two rows or exchanging the last two columns also results in tables representing the same interaction pattern. From that it follows that one and same interaction can be represented by up to 8 different tables. For example, the pattern shown in Figure 2 can be represented not only the table given in Figure 2c but also by any other table constructed by a combination of transposition and exchanging the last two rows or last two columns.

We applied the formalism to determine the number of theoretically possible interaction patterns depending on the types of the participating reactions. Specifically, we performed two types of calculations: (1) all conceivable reaction types (n, m) , with $n \leq m \leq m_{\max}$ for some fixed number m_{\max} , have been taken into account, and (2) only the reaction types appearing in metabolism have been taken into account. The information whether or not this is the case for a certain reaction type can be inferred from Table 1. The numbers of possible interaction patterns are shown in the second row of Table 2 up to a value of $m_{\max} = 6$ which is the highest m value in the KEGG database. For $m_{\max} \leq 4$ all possible types of reactions can be found in this database, hence both types of calculations, (1) and (2), yield the same results. For $m_{\max} = 5$ and 6 this is not the case, and the results of calculation (2) based on existing reaction types are given in brackets. The third row of Table 2 contains the information on the fraction of bidirectional interaction patterns.

Note, that the numbers for $m_{\max} = 2$ in the second column of Table 2 correspond to the set of reaction patterns depicted in Figure 3. Inspection of columns with higher m_{\max} values shows that the number of possible interaction patterns increases dramatically when more complex reactions are taken into account. Moreover, the fraction of bidirectional patterns becomes smaller with increasing m_{\max} values since for reactions with multiple substrates or multiple products the conditions for bidirectionality are harder to fulfill.

2.3 Types and Frequencies of Interaction Patterns as Determined from the KEGG Database

Up to this point we were only concerned with all theoretically possible interactions patterns for given types of reactions. The formalism introduced in the previous section also allows identifying all kinds of interaction patterns which occur in metabolism and determine the frequencies of their occurrence. The corresponding results are shown in Table 3 for $m_{\max} = 2, 3,$ and 6 .

Let us discuss first the case $m_{\max} = 2$ where interaction patterns occur for pairs of reactions with up to two different substrates or different products. The KEGG database contains $R = 3524$ reactions of these types resulting in $N_R = R(R - 1)/2 = 6,207,526$ possible pairs. However, only a few of these pairs (0.209 %) represent pairs of neighbored reactions. From the 27 theoretically possible interaction patterns $T = 25$ patterns can be found in the database. The frequencies of their occurrence are given in Figure 3. As seen from this figure the three most abundant patterns contain a reaction of type (2,2) interacting with a reaction of type (1,2), (patterns 16 and 17) or with a reaction of type (1,1), (pattern 6). This result is not very surprising since reactions of types (2,2) and (2,1) occur with highest frequencies and also reactions of type (1,1) are highly abundant (see Table 1). However, inspection of the numbers given in Figure 3 reveals that the frequencies of interactions are not only determined by the abundances of the participating reaction types. This becomes clear by comparing, for example, patterns 2 and 4 which both are formed by reactions of types (1,1) and (1,2) but occur with very different frequencies. One might argue that a reason for this difference could be that the two reactions share a different number of compounds (two compounds in pattern 4 but only one compound in pattern 2) and that higher numbers of shared compounds in a pattern would tend to lower the probability of its occurrence. Such an effect could also be the reason why pattern 6 occurs much more frequently than patterns 7 or 8. However, there are several counterexamples for this purely statistical reasoning. For example, patterns 17–20 occur with very different frequencies although all of them are formed by the same types of reactions and also the number of shared compounds is the same. A more thorough understanding of the distribution of pattern occurrences would be a topic of future research by considering also in detail how a given pattern can be realized chemically.

The last two columns of Table 3 contain numbers regarding statistical properties of interaction patterns taking into account reactions with up to three or up to six products ($m_{\max} = 3$ and 6 , respectively). We discuss the given numbers mainly in comparison to those obtained for $m_{\max} = 2$. As seen the total number of reactions increases only moderately when more complex types of reactions are taken into account. Interestingly, the fraction of interacting pairs remains almost constant while the total number of reaction pairs increases considerably by a factor of 1.85 from about 6.2×10^6 to 11.5×10^6 . There is a strong increase in the number of observed interaction patterns. However, in contrast to the case $m_{\max} = 2$ where 25 out of 27 theoretically possible patterns are found the corresponding fractions are much smaller for $m_{\max} = 3$ and 6 (116 out of 186 and 282 out of 1547, respectively; c.f. 2nd row of Table 2 and 5th row of Table 3). Increasing m_{\max} leads to a significant decrease in the fraction of bidirectional patterns concerning their types.

Due to the high numbers of patterns found for $m_{\max} = 3$ and 6 (116 and 282, respectively) we refrained from a graphical representation as has been done in Figure 3 for the case $m_{\max} = 2$. However, our method allowed determining the frequencies of all individual patterns. The resulting distribution is shown in Figure 4 split in two parts, one for the bidirectional patterns (Figure 4a) and one for the unidirectional patterns (Figure 4b). Note that sorting of the patterns according to frequencies (ordinate) has been performed irrespective of their directionality. The higher number of points in Figure 4b reflects the fact that pairwise interactions in metabolism are dominated by unidirectional interaction patterns (c.f. last row of Table 3). In line with this statement is the observation that the patterns with highest frequencies are unidirectional. The most abundant bidirectional pattern and the most abundant unidirectional pattern occur with frequencies 345 and 5074, respectively.

Table 3: Basic statistical properties regarding the occurrences of patterns of interacting reactions derived from the KEGG database.

	Maximal number of products, m_{\max}		
	2	3	6
Number of reactions, R	3524	4567	4795
Total number of reaction pairs, $N_R = R(R - 1)/2$	6207526	10426461	11493615
Number of interacting pairs of reactions, N_R^{int}	12997	20562	22913
Fraction of interacting pairs of reactions, N_R^{int}/N_R	0.00209	0.00197	0.00199

Number of different interaction patterns, $T = T_{bidirect} + T_{unidirect}$	25	116	282
Number of different bidirectional interaction patterns, $T_{bidirect}$	17	47	73
Fraction of bidirectional interaction patterns, $F_{bidirect} = T_{bidirect}/T$	0.680	0.405	0.259

Total frequency of bidirectional patterns, $N_{R,bidirect}^{int}$	1631	2016	2141
Fraction of pairs interacting via bidirectional patterns, $N_{R,bidirect}^{int}/N_R^{int}$	0.125	0.098	0.093

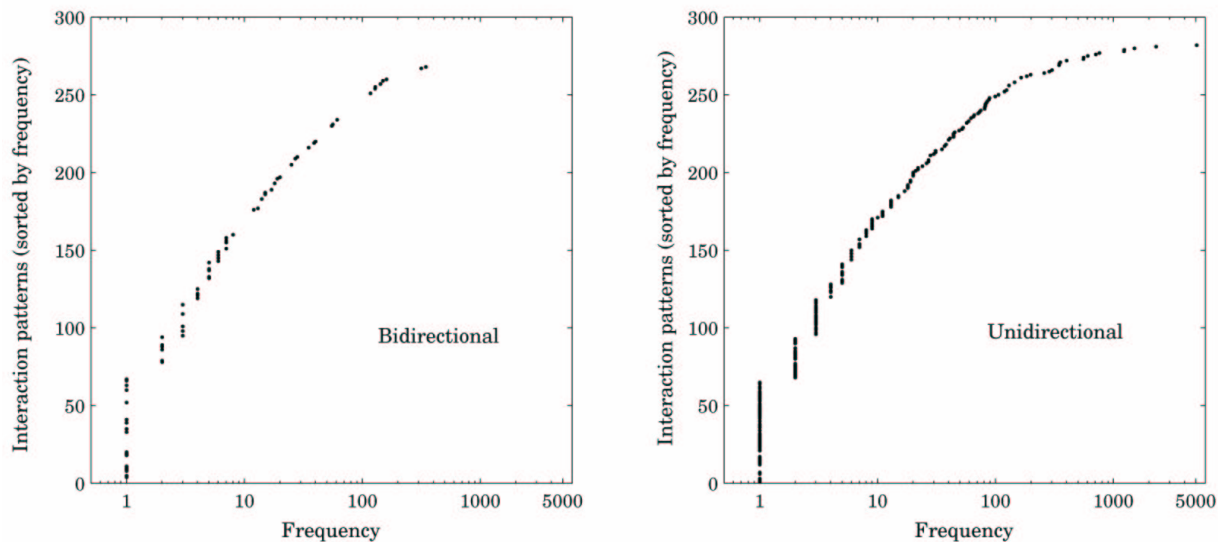


Figure 4: Abundances of interaction patterns. Shown is the distribution of the frequencies of all 282 interaction patterns derived from the KEGG database. Frequencies are shown on the abscissa and the ranks of the corresponding patterns on the ordinate. For the sake of clarity, data for bidirectional interactions and unidirectional patterns have been plotted separately (a and b, respectively). Moreover, patterns occurring with the same frequencies have been ranked consecutively resulting in vertical lines for frequencies occurring more than once.

3 Discussion

In this work we present a new model representation of metabolic networks based on patterns of interacting reactions. Our method is a graph representation of metabolic networks in that it defines a (directed) connection between two reactions. It uses stoichiometric information in the form of a list of each reaction's substrates and products. Compared to previous methods which consider reactions to be adjacent if they share any compound we propose to use a more stringent definition of neighbourhood relationship. Based on a more physico-chemical reasoning we define two reactions to interact with each other if one of them enables the other to proceed by providing it with all necessary substrates. Moreover, in contrast to standard graph theoretical approaches, our concept allows categorizing neighbourhood relationships between reactions as interaction patterns depending on the way how the reactions share their reactants. Calculations based on the KEGG database revealed that possible interaction patterns occur with very different frequencies. Interestingly, this phenomenon cannot be derived purely from differences in the abundances of the constituting reactions, since distinct patterns containing the same types of reactions are found in very different abundances. This raises the question whether patterns which are particularly abundant have been selected during evolution and play a special role in the functioning of metabolism. In this sense, patterns of reactions could play the role of motifs for metabolism, in a similar way as motifs of interconnections have been detected in networks of gene regulation or food webs [7]. A reasonable hypothesis could be that those patterns supporting the formation of branches and therefore chemical diversity of metabolic networks have been favoured by evolution. These questions will be tackled in our future work. Another interesting track will be the analysis of the presented model from the graph theoretical point of view. However, existing graph analytical methods will have to be modified to account for the fact that in our framework many different kinds of adjacency relationships exist representing the whole variety of possible interaction patterns.

Acknowledgments

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