Currency and commodity metabolites: Their identification and relation to the modularity of metabolic networks

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The large-scale shape and function of metabolic networks are intriguing topics of systems biology. Such networks are on one hand commonly regarded as modular (i.e. built by a number of relatively independent subsystems), but on the other hand they are robust in a way not expected of a purely modular system. To address this question, we carefully discuss the partition of metabolic networks into subnetworks. The practice of preprocessing such networks by removing the most abundant substrates, “currency metabolites,” is formalized into a network-based algorithm. We study partitions for metabolic networks of many organisms and find cores of currency metabolites and modular peripheries of what we call “commodity metabolites.” The networks are found to be more modular than random networks but far from perfectly divisible into modules. We argue that cross-modular edges are the key for the robustness of metabolism.

I. INTRODUCTION

For well over half a century, metabolism has been described as modular, i.e. divisible into relatively autonomous subunits such as the citric acid cycle (10), glycolysis (20), etc. For the pioneers of the 20th century it was a great feat to describe such connected set of reactions. But do these subunits tell us that the organization of metabolic networks is fundamentally modular, or are they a result of the limited knowledge of the time they were discovered?

For systems, such as the metabolism, where a continuous flow is needed throughout a large part of the system, a modular organization of a system is less robust than a more integrated topology (22)—and metabolic networks are robust; metabolite fluxes are restored in minutes after large perturbations (14). A very modular system would be expected to consist of modules with simple (or narrow) inputs and outputs and a more complex interior. Severing the input of a module (in a biochemical context, this could happen through mutation or exposure to atypical conditions, e.g. different types of starvation) would then affect the whole functioning of the module. On the other hand, a homogeneous network without explicit interfaces between modules would be very robust in a way termed “distributed robustness” (22). With the biochemical reaction data available today, answers to questions around modularity and robustness of cellular systems is within reach, and we will argue that biochemical networks are not modular or distributed, but probably best described as having a little bit of both.

To address such general questions one soon gets into technicalities. First of all, one has to choose an appropriate level of description. As many other large-scale studies (24) we simplify the biochemistry to a network and use graph theory to describe its organization. Here we use a substrate graph representation where chemical compounds are nodes and (undirected) edges connect nodes if one of them can produce the other through a reaction; other representations (reaction graphs, enzyme-centric graphs and bipartite compound-reaction graphs) have been used (see Ref. (24) and references therein). Arita (1) introduced yet another representation based on the carbon atoms that are actually transferred during metabolic reactions, and argued that other representations give a skewed estimate of average path lengths. However, he also stated that his structure-based description in a sense gives a compressed view of metabolism, making it difficult to assess the overall robustness of the networks (1), which is one of our aims in this study.

Although biochemical modules are ultimately dynamical entities (6) there is a prevailing supposition that the modules of metabolism can be identified with network clusters (densely connected regions of the networks) (4; 7; 11; 12; 15). But identifying clusters is far from straightforward. One has to choose network representation, cluster-detection algorithm and, last but not least, whether or not to preprocess the data by removing abundant metabolites. The logic behind such preprocessing is that such substrates (like water, carbon dioxide or adenosine triphosphate) are so plentiful in normally functioning cells that their concentrations put no constraints on the activity of a module; in other words, they can be regarded as externally buffered with respect to the system (19). Such ubiquitous substrates are sometimes, by analogy to economy, termed currency metabolites—they have a high turnover and occur in widely different exchange processes. (In some studies they are instead referred to as “current” metabolites, emphasizing their flow through the metabolic networks.) For example, adenosine triphosphate (ATP) can be seen as the energy currency of the cell. Continuing this analogy we will call non-currency metabolites commodity metabolites. So far, the identification of currency metabolites has made based on non-formalized, chemical considerations. Among authors who have chosen to preprocess their metabolic network data some compounds (ATP and NADH, for example) are virtually always considered currency metabolites, others (e.g. small molecules like water and oxygen) are sometimes removed (e.g. Ref. (11)) and sometimes not (e.g. Ref. (23)). Since currency metabolites have high degrees (number of neighbors),
they turn up as “hub metabolites” in studies where abundant substances have not been filtered away (e.g. Ref. 8). Interestingly, versatile currency or hub metabolites like ATP, NADH and H₂O seem to support enzyme variability and stimulate pathway evolution (18). We propose a way to identify a set of currency metabolites from the network structure alone, and, in extension, a scheme to study the modularity of metabolism that does not rely on outer information about the substrates. With this carefully justified network decomposition scheme we look at the large-scale organization of metabolic networks from several different organisms.

In the rest of this paper we will describe how the networks are compiled, state the precise definition of the algorithm, and present results from the analysis of a number of organisms.

II. NETWORK CONSTRUCTION

Interaction networks were computed using data downloaded (between March 13 and 26, 2006) from the anonymous FTP service of KEGG (Kyoto Encyclopedia of Genes and Genomes) at http://www.genome.jp/anonymous/. The following steps were used for each organism (109 in all): 1. A list of the known enzymes encoded by the organism’s genome was downloaded from the “genomes” database of KEGG. 2. The file specifying reactions in the “ligand” database of KEGG was scanned for all reactions catalyzed by enzymes present in the organism in question. 3. Reactants and products were extracted for each of these reactions and all resulting reactant-product pairs were written to an edge-list specifying the connections between the substances. A set of Python scripts automated the process so that all files could be generated in a single batch without manual intervention. In the resulting networks for each organism, substances become nodes and the links between substances from the link file become edges; we will call the number of nodes $N$ and the number of edges $M$ in the following. The adjacency matrix $A$ for a graph is defined as a matrix where the element $A_{ij}$ is set to one if the edge $(i, j)$ exists in the graph and zero if it does not.

III. MODULES AND NETWORK CLUSTERS

The problem to divide a network into subnetworks that are relatively densely connected within, and sparsely interconnected is an old graph theoretical problem that recently has experienced a second blooming. Part of the difficulty to construct such graph clustering algorithms is that the objective is not completely well-defined—the proper definition of a densely-connected cluster is, to some extent, problem dependent. One definition commonly used by many modern clustering algorithms, $\hat{Q}$, is

$$Q = \sum_i \left[ e_{ii} - \left( \sum_j e_{ij} \right)^2 \right],$$

(1)

where the sum is over a partition into clusters and $e_{ij}$ is the fraction of edges that leads between vertices of cluster $i$ and $j$. Given a partition of a network into clusters $Q$ is the fraction of vertices within clusters minus the expected fraction of edges if the edges are wired with no structural bias. The division of networks that maximize $Q$ is then usually taken as the desired partition. Sampling all divisions is clearly infeasible—different algorithms typically differs by their methods to perform this sampling. The specific algorithm we use, presented in Ref. (5) is based one a spectral method that, roughly speaking, iteratively splits clusters until a further split would increase $Q$ (for technical details, see Ref. (13)). More precisely, for a subgraph $H$ define a matrix $B$ with elements

$$B_{ij} = A_{ij} - \frac{k_i k_j}{2M} - \delta_{ij} \left[ h_i - \frac{k_i}{2M} \sum_{k \in H} k_i \right]$$

(2)

where $h_i$ is $i$'s number of neighbors in $H$, $k_i$ is $i$'s number of neighbors in $G$, and $\delta_{ij}$ is Kronecker’s delta. Then if $B$ has a positive leading eigenvalue there is a division of $H$ such that $Q$ increases. The division is given by the signs of the leading eigenvector.

$Q$ is a measure of modularity with respect to different partitions of the same graph. The maximal $Q$ value obtained during the partition process, $\hat{Q}$, is a crude measure the modularity of a whole graph. However, fluctuations make $\hat{Q}$ positive even for random networks in the $N \rightarrow \infty$ limit. Indeed, finite structureless random graphs can have almost any value of $\hat{Q}$. Instead of measuring $\hat{Q}$ we measure the difference between $\hat{Q}$ and $Q$ averaged over an ensemble of null-model networks. Since the degree of a vertex is a rather intrinsic quantity, related to molecular traits (and the set of other present substrates) we choose random graphs conditioned on the set of degrees as our null-model. Such null-model networks can be instantiated by randomly rewiring the original network.

How can one identify currency metabolites from a metabolic network? As previously mentioned, currency metabolites are abundant; not only are they present in relatively high concentrations throughout the cell, they are also

FIG. 1 Subsequent values of the effective modularity $\Delta Q$ during the run of the algorithm for the human metabolic network. The horizontal line marks the $\Delta Q$ value of the original network. The identified currency metabolites are (in the order of deletion, from left to right): water, oxygen, hydrogen ion, nicotinamide adenine dinucleotide phosphate (reduced form, NADPH), adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADP), nicotinamide adenine dinucleotide (NAD’), nicotinamide adenine dinucleotide (reduced form, NADH), phosphate and adenosine diphosphate (ADP). 100 averages of the null-model networks are used for the calculation of $\Delta Q$. Errorbars would be smaller than the symbol size.
FIG. 2. Relative sizes of the detected clusters in the human metabolic networks. Lines mark the connections between clusters. The widths of the lines are proportional to the number of connections. The functional assignments are done by inspection—they should not be viewed as absolute: a given assignment reflects the most common function in a cluster, but other functions are usually also represented to a lesser degree.

IV. NUMERICAL RESULTS

A. The human metabolic network: a case study

Figure 1 shows the effective modularity of the human metabolic network as a function of the number of nodes removed as the algorithm progresses. After ten removed vertices the effective modularity reaches its maximum. After this point $\Delta Q$ decreases roughly monotonically—no larger increase of $\Delta Q$ is observed even if one lets the algorithm run until no vertex remain—so the human currency metabolites seem quite well-defined by this procedure. Their identities correspond to some of the most commonly used currency metabolites from previous studies: ATP, NAD(P)(H), water, oxygen, the hydrogen ion etc. Indeed, our ten currency metabolites are almost identical to the ten most abundant metabolites in enzymatic reactions from all organisms in KEGG [18] (the only difference is that our human network has the hydrogen ion substituted for carbon dioxide).

The partition of nodes into groups for the human metabolic network at its most modular stage of the deletion procedure (after ten currency metabolites have been removed) is shown in Fig. 2. Although the high-degree currency metabolites are deleted (and with them 1988 edges) a large part of the network is still connected. These cross-module edges make the network less than perfectly modular and contribute to a distributed robustness.

Do the groups identified here correspond to biologically meaningful subsets of compounds? It turns out that while

present in many different reactions. This reflects the fact that they are used by many different enzyme superfamilies [18]. That a vertex is present in many reactions means two things: First, it has to have large degree. Second, it will have functionally different substrates as neighbors in the network, i.e. it will have edges to vertices in different modules. The second statement means that the effective modularity will increase when a currency metabolite is deleted. To combine these two precepts, we delete vertices in order of degree and take the network with the highest value of the effective modularity as our set of commodity metabolites. More precisely, let $G_t$ be the network after $t$ vertices are deleted, then the algorithm is as follows:

1. Let $G_1$ be $G_{t-1}$ without its vertex of highest degree and all its incident edges. (If more than one vertex of highest degree exist, select one randomly.)

2. Run the clustering scheme for the current network $G_t$ and $n$ randomizations of $G_t$.

3. Calculate the effective modularity $\Delta Q = \hat{Q} - \langle \hat{Q} \rangle$. If it is higher than the currently highest value, then save the partition.

In practice $\Delta Q$ reaches its maximal value after about ten iterations, seemingly independent of the network size, so the running time of the algorithm will be a constant factor times the running time of the clustering algorithm ($O(N^2 \log N)$ in our case).
some of the groups have a clear biological interpretation, others seem more mixed, and others—due to peculiarities of the algorithm—are isolated islands of substances that would have been expected to end up in one of the larger groups. The largest clusters in Fig. 2 correspond roughly to amino acid metabolism and protein synthesis (ii), sugar metabolism (iii) and citric acid cycle / porphyrin synthesis (iv). However, the groups are to some extent overlapping; for example, although cluster (ii) is the main amino acid metabolism cluster, three nodes representing amino acids occur in the citric acid cycle cluster (iv), and the nitrogen metabolism cluster (i) contains nodes relating to the synthesis of cysteine and methionine. (It also contains many D amino acids, but these never occur in proteins; most L amino acids are, as expected, located in the amino acid metabolism and protein synthesis cluster). Also, the nucleotide metabolism seems to be distributed onto two clusters: one which mainly deals with DNA metabolism and also contains several substances relating to glycolysis, such as phosphoenolpyruvate and D-glucose 1-phosphate (ix), and one more clearly separated nucleoside/nucleotide metabolism cluster (x). Comparing our partitions with previous studies, we note that such cases of overlap and unclean clusters have been found in previous studies. Interestingly, the size difference among the largest detected clusters is so small that the size distribution hardly can be a power-law. If the networks would be truly scale-free one would expect a power-law distribution of cluster sizes. So even if the degree is power-law distributed, the metabolic networks are (using the terminology of Ref. 21) “scale-rich.”

B. General organization of currency metabolites and modules

A comparison of 109 different organisms (Table I) shows the number of currency metabolites is typically rather low and varies little within each group of organisms. However, animals (human, mouse, rat, fruit fly and Caenorhabditis elegans) have significantly more than the other groups which tend to have end up with only two currency metabolites, typically ATP and water. This presumably reflects a general increase in metabolic complexity in higher organisms; as mentioned before, currency metabolites are linked to enzyme variability and pathway evolution 15. All computed networks have markedly higher modularity than null (randomly rewired) networks with the same degree distribution; even before any currency metabolites have been removed. This suggests that a considerable modularity is indeed present in metabolic networks, even if the modules may be only partly mappable to cellular functions as understood by contemporary biochemistry. Furthermore, the presence of inter-modular edges (after deletion of the currency metabolites) and the non-power-law cluster-size distribution of the human metabolic network is observed for the vast majority of other organisms.

### Table I

| taxonomy      | number | \(\langle N \rangle\) | \(\langle M \rangle\) | \(\langle N_{\text{currency}} \rangle\) | \(\langle \Delta Q_{\text{max}} \rangle\) | \(\langle \Delta Q_{0} \rangle\) |
|---------------|--------|----------------------|----------------------|-------------------------------|--------------------------------|--------------------------------|
| animals       | 5      | 1621 ± 124           | 4662 ± 473           | 6.2 ± 1.9                     | 0.157 ± 0.006                  | 0.136 ± 0.002                  |
| plants        | 1      | 1561                 | 4302                 | 1                             | 0.144                          | 0.130                          |
| fungi         | 2      | 1281 ± 97            | 3654 ± 289           | 1.5 ± 0.5                     | 0.150 ± 0.004                  | 0.135 ± 0.007                  |
| bacteria      | 99     | 1059 ± 35            | 2739 ± 108           | 1.7 ± 0.2                     | 0.140 ± 0.001                  | 0.132 ± 0.001                  |

In the present paper we propose a network-based method to partition metabolites into functional groups. In concordance with other works 11 12 19 we propose a fundamental dichotomy between currency and more specific, commodity metabolites. We define the currency metabolites as the substrates that, if omitted, increase the effective modularity of the network. The effective modularity can be calculated by any modern graph clustering algorithm 2 5 13 (we use the one in Ref. 13). The same algorithm can be used (on the fly, while tracing the core of currency metabolites) to partition the specific metabolites into functional subgroups. Our method is thus purely graph theoretical and does not rely on any additional information about the substrates apart from their connections. This is a rather simple network-view of the metabolism, in contrast e.g. Ref. 4 first deletes a set of currency metabolites from the network (based on chemical considerations in Ref. 11), then proposes seven additional functional categories among the remaining commodity vertices (defined by regions in the event space of two vertex-specific network measures). One can indeed proceed from our partitions and group vertices according to other network properties, but more refined levels are not only more prone to errors and incompleteness of the data, they are also closer to the realm where a more complete, dynamical, modeling 17 is called for.

We find that the networks of all 109 organisms show a clearly positive effective modularity. This supports the age-old idea of a modular organization of the cellular biochemistry. But modular architectures of systems like metabolism are also assumed to be fragile 22, and metabolism is known to be robust. We attribute this robustness to the fair amount of cross-modular edges—after the removal of currency metabolites the network is still largely connected. We note that some authors actually associate modularity with robustness—damage may then be confined to a small part of the network 4. While this may be true in some cases (like the
spread of disease in a population or pathogens in an organism, it scarcely seems applicable to metabolism: If (assuming that modules can be mapped onto canonical biochemical pathways) an entire module corresponding to e.g. amino acid metabolism or nucleotide metabolism were knocked out, the organism would hardly be able to survive even though the rest of the modules had remained unscathed. Rather than isolating pathways, it would seem to make sense to intertwine them, so that metabolites whose standard synthesis pathway has been disrupted can be synthesized through alternative pathways.

The view of biochemistry that emerges from our study is that of a core of currency metabolites and a periphery that is strongly, but not completely, modular. We conjecture that of a core of currency metabolites and a periphery that never in the absence of currency.

The degrees of currency metabolites are, by our definition, higher than the average degree of commodity metabolites. This broad degree distribution has been pointed out as a fundamental organizational principle of metabolic networks. That the structure of the network, manifested through a modular organization, is more clearly visible when the currency metabolites are removed, suggests that the functionality and active evolution is dependent on the commodity metabolites. That the presence of high-degree vertices is an inherent property of chemical reaction networks, rather than a result of evolution, is supported by the fact that astrochemical networks also show a broad, power-law-like degree distribution. The analogy with currency and commodities is indeed quite apt: Given that we have a market (metabolism) the presence of currency (metabolites) is inevitable. The development (evolution) of the market occurs at the level of commodities, but never in the absence of currency.

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