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Metrics for regulated biochemical pathway systems

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Abstract

Motivation: The assessment of graphs through crisp numerical metrics has long been a hallmark of biological network analysis. However, typical graph metrics ignore regulatory signals that are crucially important for optimal pathway operation, for instance, in biochemical or metabolic studies. Here we introduce adjusted metrics that are applicable to both static networks and dynamic systems.

Results: The metrics permit quantitative characterizations of the importance of regulation in biochemical pathway systems, including systems designed for applications in synthetic biology or metabolic engineering. They may also become criteria for effective model reduction.

Availability and implementation: The source code is available at <https://gitlab.com/tienbien44/metrics-bsa>

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1 Introduction

Biochemical reaction networks are commonly analyzed within the mathematical framework of graph theory. Specifically, the networks are represented as graphs, with metabolites as nodes and reactions as edges. Two advantages of this representation are its equivalence to an adjacency matrix, which permits a host of algebraic analyses, and the ease with which global metrics intuitively capture the overall structure of otherwise complicated networks. Standard metrics of this type are connectivity, diameter, efficiency, clustering coefficient and the degree distribution. Metrics offer a compact, concise characterization of a network and can be used to evaluate the efficiency of a network design and to obtain a quick impression of how the edges are distributed throughout the network. Metrics also answer questions regarding the control of the network by a few specific nodes and regarding the robustness and resilience of a network. A table of common network metrics is presented in Table 1.

In comparison to randomly connected networks, biological networks tend to have many nodes with very few connections and only a few nodes (*hubs*) with comparably many connections. As a result, every node is connected to every other node through relatively few steps. These facts are reflected in the degree distributions of biological and random networks: the distribution of the former

decreases as a power-law function from highly to lowly connected nodes, for instance, in metabolic networks (Jeong *et al.*, 2000), whereas the latter is binomial. For directed graphs, these distributions are two-dimensional.

While beneficial beyond doubt, metrics-based characterizations are misleading for *regulated biochemical pathway systems*, because the network metrics account only for chemical conversions of metabolites into each other, along with transport steps, but completely disregard regulatory mechanisms that are ubiquitous in metabolism. As an important example, the direct transmission of information from a downstream to an upstream metabolite per feedback inhibition is entirely ignored, even though it is known to be critically important. To remedy this situation, we introduce here effective metrics that adequately address both types of material -or signal-based interactions in a streamlined manner. The account for signals adds ‘functional paths’ between nodes, which permit new means of transmitting information. Furthermore, as signals tend to be faster than enzymatic reactions, it is reasonable to expect that a system using communication signals will reach a new target profile or state faster than a system that relies exclusively on metabolic conversions. An important criterion of effectiveness in this context is a small number of steps that are required for a change in metabolite A

Table 1. Common graph metrics

Metric	Description
Shortest path	Minimal number of edges connecting two nodes
Average path length	Average shortest path among all pairs of nodes
Diameter	Length of the longest shortest path between any two nodes in the graph
Efficiency	Measure of how quickly material or information can be moved within the graph
Connectivity	Average number of edges that connect any node to the rest of the graph
Density	Measure of the completeness of a graph
Centrality	Measure of the dominance of a node in the graph
Clustering coefficient	Measure of the existence of tightly connected groups of nodes within the graph
Degree distribution	Frequency distribution of numbers of edges entering and leaving any of the nodes
Small world property	Measure of how clustered a graph is compared to a random graph

Note: Sourced from Biggs (1993), Boccaletti *et al.* (2006), Diestel (1997), Freeman (1977), Gross and Yellen (2004), Hagberg *et al.* (2006) and Watts and Strogatz (1998).

Fig. 1. Generic metabolic pathway and its corresponding adjacency matrices. The graph of a static network (**A**) is augmented by two regulatory signals (**B**). Expanding this ‘regulated graph’ into a bipartite graph with enzymes and metabolites as different types of nodes (**C**) allows the natural incorporation of the regulatory signals (**D**). The resulting graph is now collapsed back to the same nodes as in the original graph in **A**, but contains new connections that reflect the signals (**E**). Specifically, this step adds functional connections from each signal source to the two nodes that are directly affected by the system; here we add $X_3 \rightarrow X_1$, $X_3 \rightarrow X_2$, $X_4 \rightarrow X_3$ and $X_4 \rightarrow X_5$. Note that what appears to be a reversible reaction between X_2 and X_3 is the combination of a metabolic reaction and a functional connection reflecting a signal. In the resulting graph of the regulated system, both reactions and functional connections are treated the same. Their adjacency matrices (**F** and **G**), correspond to the initial and final graphs, **A** and **E**, respectively.

to effect a change in metabolite B, anywhere in the system, either through mass flux or a signal. Given the natural directionality of biochemical reactions as well as signals, all graphs considered here are directed. Moreover, we exclude by *fiat* graphs that contain no edges.

2 Materials and methods

2.1 Metrics that apply to networks as well as regulated systems

To enable node interactions per information signals, the graph representation of a regulated system model is redesigned to treat every regulatory effect as a *functional connection* that is incorporated into the mathematical representation of the system, in analogy to a typical mass-flow reaction in a static metabolic network. In fact, it may be helpful to think of the signal as ‘short-circuiting’ the flux system,

a design feature that renders the system more efficient in terms of information dissemination.

As an example for the proposed network redesign, consider the generic five-species pathway in Figure 1A. The input species X_1 is converted into X_2 , which is used for two reactions, forming either X_3 and then X_5 , or X_4 . Graph theory prescribes the formulation of an adjacency matrix that accounts for the direction of the mass flow throughout the network (Fig. 1F). Namely, each row and each column represents a node. An entry of 1 in position (i, j) indicates that the network permits direct flow of mass from node X_i to node X_j , while a 0 represents the lack of a direct connection; it is customary to set all diagonal elements (i, i) equal to 0. The number of reactions entering node j , called its *indegree*, corresponds to the sum of all elements in column j , while the number of reactions exiting node i , called its *outdegree*, is the sum of all elements in row i . For a thorough treatment of these concepts see, e.g. Diestel (1997).

Suppose that the pathway in reality contains regulation, as shown in Figure 1B, which could consist of activating or inhibiting

signals. These signals always originate at a node and target an edge; they are customarily represented with different types of arrows than flux arrows, which always connect nodes (Voit, 2018). Here, X_3 could inhibit the enzyme catalyzing the reaction between X_1 and X_2 , and X_4 could be an activator or inhibitor of the reaction between X_3 and X_5 .

Although no mass is transmitted in a signaling process, each signal has an obvious effect on the targeted reaction and its participants. X_3 , for example, affects the reaction from X_1 to X_2 and should therefore be functionally connected to both X_1 and X_2 . To formalize this functional connection, it is initially beneficial to represent the reaction scheme with a different type of node; the result in a *bipartite graph* (Asratian et al., 1998), as it is commonly used in Petri-net representations, where nodes and edges are called *places* and *transitions*, respectively (Koch and Schreiber, 2011). Importantly, metabolite nodes interact directly only with reaction nodes and *vice versa* (Fig. 1C). Note the two-headed arrow between each enzyme and its substrate, which reflects that changes in enzyme amount or activity directly affect the substrate as well as the product.

In a bipartite pathway representation, every signal corresponds to a two-step path between its node of origin and the nodes associated with the targeted reaction. Because our intent here is simply to determine the number of steps necessary to reach one node from another node, whether through mass flow or a functional connection, it is beneficial to collapse the bipartite graph with regulation back into a regular graph, by moving the functional connections from each reaction node to the participants of this reaction (Fig. 1E) and then eliminating all reaction nodes. With this ‘trick’ it is now easy to compare this system graph of the regulated pathway with the standard graph of its unregulated scaffolding network. In particular, we can define a traditional functional adjacency matrix (Fig. 1G) and compare its features to those of the original adjacency matrix in order to quantify the effects of regulation. For these comparisons, we focus primarily on the metrics of reachability and efficiency.

2.1.1 Reachability

For convenience, we introduce the concept of *reachability* and the *reachability coefficient*. This coefficient is the fraction of node pairs that are connected by directed paths. Theoretically, the concept of reachability derives from the field of transitive closure (Aho et al., 1974), which uses binary graphs where each element of the corresponding reachability matrix is 1 if there is a path of finite length from node A to node B, and 0 if no path exists. The concept of transitive closure has become important in theoretical computer science, and much effort has been devoted to developing algorithms for determining optimal ways to compute transitive closure relationships (Nuutila, 1994).

Although computationally not the most efficient solution, matrix multiplication can be used to characterize reachability in an intuitive manner (Oneil and Oneil, 1973). Namely, the original adjacency matrix ($A = A^1$) identifies all paths of length 1 between pairs of nodes, while each higher power A^n identifies the corresponding paths of length n . Thus, A^3 reflects all connections of length 3. By convention, the path length from a node to itself is considered to be 0. Starting with A itself and then increasing the power, the *shortest path* between two nodes is recorded in a new matrix as the numerical value of the minimal path length. This process is repeated up to $N-1$. If no connection between two nodes is found in any A^n up to A^{N-1} , no path exists between these two nodes, as the longest possible

A						B					
	X_1	X_2	X_3	X_4	X_5		X_1	X_2	X_3	X_4	X_5
X_1	0	1	2	2	3	X_1	0	1	1	1	1
X_2	∞	0	1	1	2	X_2	0	0	1	1	1
X_3	∞	∞	0	∞	1	X_3	0	0	0	0	1
X_4	∞	∞	∞	0	∞	X_4	0	0	0	0	0
X_5	∞	∞	∞	∞	0	X_5	0	0	0	0	0

Fig. 2. Distance matrix (A) and reachability matrix (B) of the pathway in Figure 1A

shortest path has a length equaling the total number of nodes minus 1, and the path length is defined as infinite.

The end result of this procedure is the *distance matrix* of the graph (Fig. 2A), and the highest finite value in the distance matrix is its *diameter*. The distance matrix for the pathway in Figure 1A reveals that the diameter (longest shortest path) outside ∞ is 3, but, maybe more importantly, that most paths between node pairs (like $X_5 \rightarrow X_1$) do not even exist.

The reachability matrix is determined by changing the infinite elements of the distance matrix to 0 and changing all remaining non-zero elements to 1 (Fig. 2B). The reachability of node i can directly be computed from the equation:

$$R_i = \frac{\sum_{j=1}^N D_{ij}}{N-1} \quad (1)$$

where D_{ij} are the binary values in the reachability matrix and N is the number of nodes in the graph. The reachability coefficient of a graph is defined as the average of the reachability values of all nodes. The reachability coefficient of a graph is 0 for a completely edgeless graph and 1 if all nodes have paths to all other nodes. For the example in Figure 1A, the reachability of the five nodes is [1, 0.75, 0.25, 0, 0] and the reachability coefficient is 0.4.

The reachability coefficient is a measure of the proportion of nodes that can reach any given node; in other words, it measures *inward reachability*. Depending on the application, it is furthermore useful to compute the number of nodes that can be reached from a given node. This *outward reachability* is readily calculated as the sum over all i instead of all j in Eq. (1) and may be useful for exploratory experiments and selecting drug targets. For instance, a researcher might want to choose a specific metabolite for manipulation based on how much other metabolites in the system would be affected by this choice. In such a situation, a node with high reachability would tend to influence more of the other nodes. Using the reachability matrix, one could also selectively choose or avoid downstream targets through the choice of a particular node subject to perturbation. Note that all outward and inward reachability coefficients have the same average, but different distributions.

Because a systems graph contains as many or more connections than the graph of its scaffolding network, the *system reachability coefficient* is at least as high as the *network reachability coefficient*. To assess the role of signals in the system in comparison to its unregulated scaffold, it is useful to define the *reachability gain* due to regulation as the ratio of the reachability coefficients of the two graphs:

$$G_R = \frac{R_{sys}}{R_{net}} \quad (2)$$

This gain reflects the average reduction in the number of steps needed to transmit information from any one node to another. It is interesting to note that Arceo et al. (2015) also compared regulated systems with corresponding scaffolding networks, using the rigorous

framework of Chemical Reaction Network Theory [CRNT; (Craciun and Feinberg, 2005; Feinberg, 1972; Horn and Jackson, 1972; Wilhelm *et al.*, 1977)]. This theory focuses on the identification of network properties independent of specific parameter values and is therefore of fundamental interest in metabolic systems analysis. With respect to the effects of regulation, the authors used CRNT's central concept of *deficiency*, which they proffered to consist of the sum of two deficiencies, associated with either the stoichiometry or the regulation of the system. The stoichiometric deficiency is a measure of the connectivity of a system, and more specifically, the linear dependence among reactions in the system (Shinar and Feinberg, 2012), while the regulatory deficiency measures the complexity of the system due to regulation. Instead of using a sum of deficiencies, we prefer to define the efficiency gain as the ratio of the system and network efficiencies, as this ratio is independent of scale. Nonetheless, efficiency gain and regulatory deficiency are clearly closely related.

2.1.2 Connectivity

The connectivity of a node is a measure of how many edges must be removed to disconnect this node completely from all other nodes (Gross and Yellen, 2004). The average of all node connectivity values is the connectivity C_G of the graph. C_G is a good measure of the resiliency of a network, expressing how well the system can tolerate the loss of edges. The computation of C_G is straightforward as it consists of counting input and output edges of each node and removing redundant and self-directed edges.

2.1.3 Clustering coefficient (CC)

The clustering coefficient CC_i of node i is a measure of how 'cliquish' the nodes in a network are. It is commonly defined by computing the number of edges among the neighbors of this node, divided by the number of maximally possible edges (e.g. Voit, 2018). It is also possible to calculate CC_i directly from the adjacency matrix. For a directed graph, CC_i can be calculated with the equation:

$$CC_i = \frac{(A + A^T)_{ii}^3}{2[d_i(d_i - 1) - 2A_{ii}^2]} \quad (3)$$

(Fagiolo, 2007) where A and A^T are the adjacency matrix and its transpose, and d is the sum of the number of edges entering or exiting node i (the sum of row i and column i). The clustering coefficient CC_G of a graph G is the average clustering coefficient of all CC_i .

2.1.4 Efficiency

A common metric for static, undirected networks is the average path length. For directed networks and systems, this metric can be very misleading, because non-existing paths between two nodes are customarily assigned the value ∞ . As a stark example, the network in Figure 1A has an average path length of ∞ , as X_1 cannot be reached from any other node. The concept of *efficiency* circumvents the issue of infinite path lengths. It measures how many steps are needed to share information within a system and therefore a measure of how economically a graph is structured (Latora and Marchiori, 2001).

Efficiency is defined based on inverses of distances, with $1/\infty$ defined as 0. Specifically, one takes the average of all inverses of the distances, without including paths from a node to itself, that is, without the diagonal elements of the distance matrix. In the case of the pathway in Figure 1A, its distance matrix is given in Figure 2A and its efficiency is $E \approx 0.292$, while the corresponding value for the regulated system (Fig. 1E) is about 0.46. A perfectly efficient network has an efficiency of 1, whereas an efficiency of 0 signifies no connections between any of the nodes. Efficiency is a very succinct global metric of a network or system.

2.1.5 Summary of general results

As edges are added to a graph, e.g. due to regulation, the values of each of the proposed metrics tend to increase. The extremes are graphs with no edges and fully connected graphs. In most cases, the metrics of the former are 0, while they are 1 for the latter, because the maximum connectivity corresponds to the number of nodes that can connect to each individual node, which in this case is every other node in the graph (see Table 2).

To ascertain the scalability of the metrics, we generated sparse random matrices ranging from four nodes to one hundred thousand nodes. The results of simulations with these graphs and systems show that the values of all metrics in question, namely reachability, clustering coefficient and efficiency, directly scale with the size of the network. For instance, a 1000-node network, in which each node connects on average to 10 other nodes (density = 0.01), has the same metrics as a 100 000-node network that has an average of 1000 connections. However, if the *density* of the network increases (more connections per number of nodes), all metrics increase in value. This fact is true of networks of any size. One direct inference from this information is that an individual signal is relatively more influential in a small network than in a large one, which is probably not surprising.

3 Results

3.1 Five-Node pathway in Figure 1

To demonstrate the effect of regulation on the metrics of a pathway, we start again with the five-node pathway in Figure 1A and E. The results are shown in Table 3. It is evident that the addition of the two signals causes a significant increase in reachability, connectivity and efficiency.

3.2 Branched metabolic pathways

Branched metabolic pathways are often regulated through one of two patterns of feedback inhibition: a sequential pattern (Fig. 3A) or a nested pattern (Fig. 3B) (Savageau, 1976). The two designs have

Table 2. Range of metrics

	Range	Edgeless	Fully connected
Reachability	0—1	0	1
Connectivity	0—(N-1)	0	N-1
Clustering coefficient	0—1	0	1
Efficiency	0—1	0	1

Table 3. Comparison of metrics for the pathway in Figure 1A and E

	Network	System	Gain
Reachability	0.4	0.65	1.65
Connectivity	1.6	2.4	1.5
Clustering coefficient	0	0.1667	N/A
Efficiency	0.2917	0.4583	1.57

Fig. 3. Comparison of sequential and nested branched pathway systems, along with their metrics

slightly different dynamic features, and Savageau determined that the sequential system is usually more reliable than the nested system, but that the nested system is often more robust. In the end, superiority of either design depends on the importance of various features, as well as parameter values and constraints.

In terms of system metrics, the alternative regulatory signals have different effects on the system. Comparison of their metrics with their common scaffold network demonstrates that the nested pattern possesses metrics in terms of connectivity and efficiency that have slightly higher values (Fig. 3). This finding may not really be surprising, as the nested system contains one more signal than the sequential system. As the biological implementation of a signal presumably incurs costs (Bialek and Setayeshgar, 2005), it is difficult to assign superiority to either one of the two designs in an objective manner.

3.3 Amino acid pathway in *Arabidopsis thaliana*

The aspartate-derived amino acid pathway in *Arabidopsis thaliana* includes several activation and inhibition signals (Fig. 4). We use a recent model of the system (Curien et al., 2009) for comparing metrics. The results overall demonstrate that regulation is very effective.

Fig. 4. Aspartate-derived amino acid pathway in *Arabidopsis thaliana* (A) and its metrics (B). The diagram was obtained from Curien et al. (2009)

In particular, all nodes in the system with regulation can be reached from all other nodes, whereas this is only true for about half of all node pairs in the unregulated network. At the same time, the efficiency essentially doubles due to regulation.

As secondary results, these comparisons, for instance, with respect to reachability and efficiency, can be employed to identify the most influential signals, as these can be selectively added and removed to observe the impact on the metrics of the system.

One may also test the effects of individual signals on the system by adding them to the scaffolding network one at a time. Combinatorial explosion precludes a comprehensive exploration but for illustration we may focus on the green potentiation signal from AdoMet (AP), the red inhibition signal from Thr (TI), both inhibition signals from Lys (LI), the inhibition from Ile (II), the activation from AdoMet (AA) and combinations of the signals with respect to reachability and efficiency (see Fig. 4). Note that AP is only biologically important to the system if LI is active. Representative results are displayed in Table 4.

The proposed metrics leave no doubt that the observed regulation ('All' in Table 4) is very effective. Certain signals, such as the green potentiation signal from S-adenosyl-methionine (AdoMet) and the red inhibition signal from Threonine (Thr) drastically raise

Table 4. Increase in information transmission speed for different combinations of signals added to the scaffold network (top left panel) of aspartate derived amino acid production in *Arabidopsis thaliana*

Signal(s)	Reachability	Efficiency
None	0.5364	0.2924
AA	0.5728	0.3197
TI	0.7273	0.3894
LI	0.6273	0.3465
II	0.5455	0.3015
AP LI	0.8273	0.4373
LI TI	0.8182	0.4435
AP TI	0.8182	0.4415
LI AA	0.6637	0.3738
II TI	0.8182	0.4303
AA LI TI	0.9091	0.4950
AP LI TI	0.9091	0.4979
II TI LI	0.9091	0.4844
AP LI TI AA	0.9091	0.5192
ALL	1	0.5602

the efficiency and reachability metrics of the network. This makes intuitively sense, because without signals emanating from these metabolites, changes in these metabolites cannot affect the upstream components of the pathway at all.

4 Discussion

System metrics, proposed here for the first time, permit an effective global quantification of regulated systems and a variety of global comparisons between systems and their scaffold networks. When regulation is taken into account, information sharing is increased, and if a lower average number of steps is considered advantageous, the performance of systems tends to be greatly improved over the performance of the corresponding unregulated network. However, there is no guarantee that increased information sharing always reflects an improved design. For example, a signal incurs an energy cost, which is dependent on the type of binding that occurs at the receptor site (Bialek and Setayeshgar, 2005). Thus, it remains to be explored whether reachability and efficiency correlate with superior designs.

Even though nature's most pertinent optimality criteria for pathways are not necessarily known, the proposed metrics permit new assessment tools of the structures of natural pathway systems as well as of hypothetical systems that one might envision in synthetic biology. For instance, it is possible to add signals to a scaffold network in a random manner, or in a targeted manner based on some hypothesis, and to compare the resulting metrics among these artificial systems with those of natural metabolic systems in terms of their responsiveness and efficiency. This line of investigation could become an additional tool for the search and evaluation of design principles that govern optimally structured pathway systems (Alves and Savageau, 2000; Alves and Sorribas, 2011; Savageau, 1985; Schwacke and Voit, 2004; Voit, 2003). The overall importance of nodes and signals could also be a valuable criterion for different modes of model reduction, where the goal is the elimination of nodes that are not of prime interest without an undue loss of adequate system representation or functionality of the model.

The proposed types of efficiency characterizations are not purely theoretical but could have practical, real-world applications. For instance, when identifying a drug target, it would be helpful to know

which metabolites or enzymes influence the operation of the system most. The same is probably true for model-based metabolic engineering (e.g. Torres and Voit, 2002), where the goal is the enhanced microbial production of expensive organics or the upscaling of microbial bulk products, such as industrial ethanol or citric acid.

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