Challenges and opportunities at the interface of network science and metabolic modelling

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Metabolism plays a central role in cell physiology and provides the cellular machinery for building biomolecules essential for growth. At the genome-scale, metabolism is made up of thousands of reactions interacting with one another. Untangling this complexity is key to understand how cells respond to genetic, environmental or therapeutic perturbations. Here we discuss the roles of two complementary strategies for the analysis of genome-scale metabolic models: constraint-based methods, such as flux balance analysis, and network science. Whereas constraint-based methods estimate metabolic flux on the basis of an optimization principle, network-theoretic approaches reveal emergent properties of the global metabolic connectivity. We highlight how the integration of both approaches promises to deliver insights on the structure and function of metabolic systems with wide-ranging implications in basic discovery science, precision medicine and industrial biotechnology.

I. INTRODUCTION

Metabolism comprises the biochemical reactions that convert nutrients into biomolecules and energy to sustain cellular functions. Advances in high-throughput screening technologies have enabled the quantitative characterization of metabolites, proteins and nucleic acids at the genome-scale, revealing previously unknown links between metabolism and many other cellular processes. For example, gene regulation, signal transduction, immunity, and epigenetic modifications have been shown to interact closely with metabolic pathways. The increasing availability of data and the fundamental roles of metabolism in various cellular phenotypes have triggered a surge in metabolic research, together with a revived need for computational tools to untangle its complexity.

At the genome scale, metabolism is made of multiple interconnected reactions devoted to the synthesis of specific biomolecules (e.g. proteins, lipids or nucleic acids) and to the production of energy. The notion of a metabolic pathway is typically employed to organize sets of related reactions into functionally cohesive subsystems. Thus, lipid pathways, for example, are traditionally studied as distinct subsystems from amino acid or aerobic respiration pathways. Although conveniently descriptive, such a priori partitioning can obscure the links between relevant layers of metabolic organization. Furthermore, metabolic connectivity is not static but actively responds and adapts to extracellular cues. Indeed, through various layers of transcriptional, translational and post-translational regulation, metabolic pathways can be activated or shutdown depending on external perturbations. These metabolic adaptations underpin fundamental biological processes, such as microbrial adaptations to growth conditions or the ability of pathogens to rewire their metabolism and evade the action of antimicrobial drugs. Metabolic adaptations are also thought to modulate the onset of complex diseases such as cancer, diabetes, Alzheimer’s, and others. As a result, there is a growing need for computational methods that go beyond classical pathway definitions and uncover hidden groupings and interactions between metabolic components.

The complexity of metabolism has prompted the development of a myriad of methods to analyse its connectivity. For specific pathways, kinetic models based on differential equations are widely employed to describe the temporal dynamics of metabolic intermediates and products. At the genome scale, however, such kinetic models face substantial challenges in their construction and analysis. The most widespread method for genome scale modelling is Flux Balance Analysis (FBA), a powerful framework to predict how metabolic fluxes are distributed on the reaction network under an optimization principle, such as maximizing growth. Therefore, the concept of a metabolic network in FBA refers to the stoichiometric connectivity relating the enzymes and the metabolites they catalyze. Such a definition, however, is at odds with the discipline of network science, in which complex systems are mathematically described through graphs that are amenable to computational and mathematical analyses.

In this paper we discuss the relationship between FBA and graph-based analyses of metabolism, and we highlight the different perspectives they bring to the problem. On the one hand, FBA has been shown to usefully predict metabolic activity in various environmental and genetic contexts; on the other, network science can shed light on the emergent properties of global metabolic con-
nectivity. As illustrated in Figure 1, both approaches share a common basis in that they represent genome-scale metabolism in terms of a stoichiometric matrix, yet they offer different toolkits for analysis. In the following, we discuss their advantages and caveats, and highlight the need for integrated methods that combine flux optimisation with the topological and graph-theoretical methods of network science.

II. GENOME-SCALE METABOLIC MODELLING

A widely adopted strategy for genome-scale modelling is constraint-based analysis (CBA), an umbrella term for various algorithms that predict metabolic fluxes using optimisation principles. Most popular among these is Flux Balance Analysis (FBA), which predicts metabolic fluxes at steady state by solving the following optimization problem:

$$\text{max}_v \ J(v)$$

subject to: $S_v = 0$

$$v_{i}^{\text{min}} \leq v_{i} \leq v_{i}^{\text{max}}, \ i = 1, \ldots, m,$$

where $S$ is the $n \times m$ stoichiometry matrix for a system with $n$ metabolites and $m$ reactions; $v$ is a vector containing the $m$ reaction fluxes; and $(v_{i}^{\text{min}}, v_{i}^{\text{max}})$ are bounds on each flux. $J(v)$ is the objective function and is suitably chosen to describe the optimization principle assumed to underpin the physiology of the particular organism under study. In microbes, the most common choice for the objective function is biomass production, in which case $J(v) = c^T \cdot v$, i.e., $J(v)$ is assumed to be a linear combination of specific biosynthetic fluxes describing biomass output as given by the vector $c$. There exists a broad range of dedicated FBA software packages and the popularity of FBA has led to a myriad of extensions that account for other complexities of cell physiology, such as gene regulation and dynamic adaptation, among many others.

Flux Balance Analysis has found applications in diverse domains, including cell biology, metabolic engineering, microbiome studies, and personalized medicine. A salient feature of FBA is its ability to incorporate various types of ‘omics datasets into its predictions. Various approaches have been developed for this purpose, most of which incorporate experimental data into the metabolic model through adjustments of the stoichiometric matrix $S$ or the flux bounds $v_i^{\text{min}}$ and $v_i^{\text{max}}$ in $J(v)$.

A popular use case of FBA is the identification of essential genes, i.e., genes that severely impact cellular growth when knocked out. Through simulation of gene deletions, FBA can serve as a systematic tool for in silico screening of lethal mutations, and identification of biomarkers and drug targets in disease. A related application of FBA is the study of metabolic robustness. Since only a fraction of all metabolic reactions are essential in a given environment, knocking out non-essential reactions often has little effect on the phenotype. This is because many reactions have functional backups through other pathways, so as to preserve cellular function in face of perturbations. By providing insights into the reorganization of fluxes under different conditions, FBA can also help improve our understanding of robustness to gene knockouts, gene mutations and different growth conditions.

One limitation of FBA is the crucial importance of the objective function to be optimized, which needs to be designed to represent cellular physiology. In microbes, a common choice is maximization of growth rate, but it is questionable whether this is a realistic cellular objective across organisms or in different growth conditions. Although the vast majority of FBA studies rely on the maximization of cellular growth, other objective functions have been proposed, including maximization of ATP production and minimization of substrate uptake minimization.

III. APPLICATIONS OF NETWORK SCIENCE IN METABOLIC MODELLING

Network science represents complex systems as graphs where the nodes describe the components of the system and the edges describe interactions between components. This general description provides a backbone for the modelling of large, interconnected systems across many disciplines, including biology, sociology, economics and others. There have been numerous attempts to formalize the analysis of metabolism under the lens of network science. Graph-theoretic concepts such as degree distributions and centrality measures can reveal structural features of the connectivity of the overall system, while clustering algorithms can uncover substructures hidden in the network topology. Such tools can be combined with the analysis of perturbations, such as deletions of network nodes or edges, which can represent changes in the environment, gene knockouts, or therapeutic drugs that target specific metabolic enzymes. Unlike FBA, in which the analysis depends on the choice of a specific objective function, network-theoretical methods rely on the metabolic stoichiometry alone.

Metabolic modularity is an area where network science has shown promising results. Intuitively, a network module is a subset of the network containing nodes that are more connected among themselves than to the rest of the network. Numerous works have studied the modularity of metabolic networks, and how the network modules can be used to coarse-grain the metabolic network into subunits. The modules identified using network analysis have been found to mirror the organization of textbook biochemical pathways while uncovering novel links and relationships between them. A recurring theme in these analyses is the bow-tie topology, whereby a metabolic network can be divided into an input component, an output component and a strongly connected
internal component. This architecture aligns well with an intuitive understanding of metabolism, which comprises nutrient uptake, waste production and secretion, and a large number of internal cycles which produce biomass and energy.

Despite promising results in the analysis of modularity, network science has achieved mixed success in metabolic research. For example, from a network perspective it is natural to assume that essential genes should be associated with high centrality scores. This idea draws parallels from other domains, such as the internet and social networks, where highly central nodes are deemed critical for network connectivity. However, the correlation between gene essentiality and node centrality are weak, with various essential metabolites and reactions exhibiting low centrality scores, possibly as a result of poorly connected nodes in pathways that supply resources essential for growth. Other studies have attempted to resolve this problem with new network metrics specifically tailored to describe important features of metabolism.

A key challenge for the use of network science in metabolic modelling is the lack of consensus on how to build a graph from a metabolic model. For a network with $q$ nodes, the graph is encoded through the $q \times q$ adjacency matrix $A$, which has an entry $A_{ij} \neq 0$ if nodes $i$ and $j$ are connected, and $A_{ij} = 0$ otherwise. Depending on how nodes and edges are defined, one can build different graphs for the same metabolic model described by the stoichiometry matrix $S$ in (1). For example, one can build a graph where the nodes are metabolites and the edges are reactions between them. In this case the adjacency matrix is

$$A_{n \times n} = \hat{S}S^T,$$

where $\hat{S}$ is the binary version of the stoichiometry matrix $S$ (i.e. $\hat{S}_{ij} = 1$ when $S_{ij} \neq 0$, and $\hat{S}_{ij} = 0$ otherwise). Conversely, a graph where the nodes are reactions and the edges describe the sharing of metabolites as reactants or products has an adjacency matrix

$$A_{m \times m} = S^T \hat{S}. \quad (3)$$

One can also build bipartite graphs, where both metabolites and reactions are nodes of different types, or even hypergraphs where an edge connects a set of reactants to a set of products. In addition, all of these graphs can be directed/undirected (when the matrix $A$ is symmetric/asymmetric), or weighted/unweighted (where the elements $A_{ij}$ can have weights encoding different properties). Such modelling choices have a dramatic influence on the results and conclusions drawn from network analyses. For example, the existence of power law degree distribution and the small-world property, two widespread concepts in network science, have been disputed and attributed to specific ways of constructing the metabolic network graph.

A further limitation of graph-based analyses is their ad hoc treatment of pool metabolites, e.g., $H_2O$, ATP, NADH and other enzymatic co-factors. Because pool metabolites participate in a large number of reactions, they distort and dominate the topological properties of the network. A common approach to minimize this problem is to prune pool metabolites from the graph; yet there is no accepted standard on how to do this or how to mitigate the potential loss of information in so doing. Another challenge arises from the representation of the reversibility of metabolic reactions in the graph. Although all biochemical reactions are reversible, they take one direction depending on the physiological conditions. Graph-based studies either pre-define a direction for the flux of the reaction, or they split them into forward and backward components. Neither of these approaches is ideal: assigning the direction of a reaction based on one condition may not generalize across other
conditions, whereas incorporating bi-directional edges increases the complexity of the analysis.

IV. INTEGRATING FLUX INFORMATION AND NETWORK SCIENCE

As discussed, network-theoretical models of metabolism can be built in multiple ways. Each particular choice represents particular modelling assumptions and caveats that shape the conclusions that can be drawn from them. Recent work, however, strongly suggests that integration of flux information into network analyses offers a promising avenue to address these challenges.

A well-established approach relies on the notion of Elementary Flux Modes (EFM)\cite{89}. Roughly speaking, EFMs are steady state flux vectors (i.e., satisfying $Sv = 0$) with a minimal, unique set of flux-carrying reactions. EFMs are non-decomposable steady-state pathways, in the sense that if any of its contributing reactions is deleted, the EFM will not be able to carry a steady-state flux\cite{89}. A key result is that every steady state flux distribution can be represented as a non-negative linear combination of EFMs. A number of algorithms have been proposed for the efficient enumeration of EFMs\cite{90}. Some of these algorithms exploit topological connectivity and flux information simultaneously, e.g., by using graph-based approaches on cellular function. Here we have discussed the complementary roles of Flux Balance analysis and network science in the analysis of metabolism at the genome scale. Although both approaches start from the metabolic stoichiometry (Figure 1), they differ in their mathematical foundations and the type of predictions they produce. FBA predictions can be accurate but their effectiveness requires high quality ‘omics datasets. Network theory, in contrast, requires nothing more than the metabolic stoichiometry, yet can lead to misleading predictions depending on how the network graph is built. As a result, so far FBA has led to more successful connections with experimental results than network science.

When used in isolation, both FBA and network-theoretical methods can be insufficient to understand changes in metabolic connectivity triggered by physiological or environmental perturbations. The integration of FBA and network-theoretical methods can close this gap in many application domains. For example, with the rise of big data in the life sciences, there is a growing interest in using metabolic signatures of patients to tailor treatments...
suited to their individual needs. Computational methods can play a key role in detecting drug targets involved in metabolic activity, and how their targeting can disrupt metabolic connectivity. A particularly promising area is cancer treatment, where there is considerable interest on drugs that target specific metabolic enzymes.

Another exciting application domain is industrial biotechnology, where so called “microbial cell factories” are engineered for production of commodity chemicals and fine product. In this field, FBA is widely employed for strain design, with the goal of finding combinations of genetic interventions that maximize production of a desired metabolite. A recent trend is to increase production with synthetic biology tools and dynamic control of gene expression. This approach needs computational methods that capture the dynamic reallocation of metabolic flux. Integrating FBA solutions with network models can provide a versatile tool to identify suitable genetic modifications for microbial strains with increased production.

Further developments at the interface of FBA and network science offer a novel way to explore the impact of perturbations on metabolic connectivity. The flexibility of FBA allows for the modeling of metabolic perturbations of various kinds, including changes in growth conditions, deletion of metabolic genes or the action of enzyme inhibitors, whereas the application of graph-theoretical tools from network theory can bring a broadened understanding of emergent properties of the overall system. This flexibility is a key advantage and offers promising potential to deploy network science tools across a range of questions in basic science, biomedicine and industrial biotechnology.

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