Abstract

Cells adapt their metabolism to survive changes in their environment. We present a framework for the construction and analysis of metabolic reaction networks that can be tailored to reflect different environmental conditions. Using context-dependent flux distributions from Flux Balance Analysis (FBA), we produce directed networks with weighted links representing the amount of metabolite flowing from a source reaction to a target reaction per unit time. Such networks are analyzed with tools from network theory to reveal salient features of metabolite flows in each biological context. We illustrate our approach with the directed network of the central carbon metabolism of *Escherichia coli*, and study its properties in four relevant biological scenarios. Our results show that both flow and network structure depend drastically on the environment: networks produced from the same metabolic model in different contexts have different edges, components, and flow communities, capturing the biological re-routing of metabolic flows inside the cell. By integrating FBA-based analysis with tools from network science, our results provide a framework to interrogate cellular metabolism beyond standard pathway descriptions that are blind to the environmental context.

1 Introduction

The metabolism of a cell is the set of chemical reactions that ensure life is sustained. The classic way to categorise metabolic reactions is into *metabolic pathways*, which consist of reactions (often consecutive) that serve a specific purpose; many such pathways have been identified in cells [1]. A metabolic reaction network can be constructed from the reactions in these pathways. The nodes of the network are the reactions, and their connections are created by their interactions via the production and consumption of compounds.

A substantial amount of research has been devoted to the development of methods and tools to describe and classify biological and metabolic networks. Many of these studies have focused on properties of the networks such as the degree distribution, the small-world property, roles, or the existence of communities [2–6]. Although intuitive and useful on many levels, the insights that can be obtained from these networks is severely limited by two main factors:

1. *Interpretation of edges*. The connections in many networks are undirected and only describe whether two reactions share a metabolite [7]. These networks make no distinctions between reactions that consume/produce the same metabolite (competitors/co-producers) or reactions that have a supplier-consumer relationship. In addition, the edge weights denote the number of different metabolites that the reactions have in common; this neglects the stoichiometry (i.e., the number of molecules) of the metabolites in the reactions. These two characteristics of edges mean that it is not possible to investigate the structure of metabolism from a dynamics or flow perspective; undirected connections would make every reaction reversible, and the rates of flow among the reactions cannot be ascertained.
2. Absence of context. Many networks are constructed from metabolic models (or lists of reactions) that are known to exist in the cell. These models do not specify the biological context of the cell, which has a large effect on the flux and relationships among the reactions the reactions. Incorporating context into metabolic networks is necessary in order to understand how cells adapt their metabolism to survive in a variety of scenarios.

As mentioned above, cells can adapt their metabolism in response to a wide range of situations or stimuli; different reactions are present only under particular scenarios, and with varying fluxes. Some reactions may only be relevant under specific internal and external conditions (e.g., importing and processing a type of molecule). This fact is often overlooked in the study of metabolic networks; however, some recent studies have begun to recognise the importance of biological context to understand metabolism [8–11]. These studies use extracellular, expression or proteomic data to infer the connectivity of the network, or to test whether the metabolic models are able to predict cellular behaviour.

We present a methodology to address the need to study metabolism in a flexible manner in which connections have a clear physical interpretation and that is able to incorporate different biological contexts. We showcase our method on the core *Escherichia coli* metabolic model [12]. This model is encapsulated by a matrix that contains the stoichiometric coefficients of all the reactions [13,14]. Such a representation of metabolism assumes stationarity, so the total amount created and consumed of each metabolite is constant. In this framework, a biological context can be encoded as constraints on the reaction rates. Using Flux Balance Analysis (FBA) [15,16] it is possible to endow a metabolic reaction network with information about specific contexts of interest. Given a set of constraints on the reaction fluxes, FBA maximises the flux through a objective function, usually the biomass reaction (e.g., using linear programming, see Methods) [15]. FBA allows us to obtain optimal fluxes, which we incorporate to the construction of metabolic reaction networks to obtain a more informed description of the cell’s metabolic state in a given context.

These context-dependent metabolic networks can be studied using tools from network science and can be compared to see how their structure changes as we change the biological context. For example, network-based metabolic pathways or communities have been studied previously [17,18], albeit in context-independent settings. We use diffusion processes on our context-dependent networks to identify groups of reactions that retain flow at different scales. These groups can tailor the notion of metabolic pathway to specific contexts of interest. Our results show that when biological context is taken into account, the structure of the metabolic reaction networks can vary drastically. Furthermore, the structure of our networks is consistent with the main biological features that define each specific context.

2 Networks of metabolic reactions

We consider metabolic networks composed of *n* metabolites and *m* reactions:

\[
R_j : \sum_{i=1}^{n} \alpha_{ij} x_i \overset{j}{\rightarrow} \sum_{i=1}^{n} \beta_{ij} x_i, \quad j = 1, 2, \ldots, m. \tag{1}
\]

The numbers \(\alpha_{ij}\) and \(\beta_{ij}\) are the depletion and production coefficients of the species in each reaction. The evolution of the metabolite concentrations follows the differential equation

\[
\dot{x} = S \mathbf{v}, \tag{2}
\]

where \(x(t)\) is an *n*-dimensional vector of metabolite concentrations \(x_i(t)\) and \(\mathbf{v}\) is an *m*-dimensional vector of reaction rates. The \(n \times m\) matrix \(S\) is the stoichiometric matrix with entries \(s_{ij} = \beta_{ij} - \alpha_{ij}\), that is, the net number of \(x_i\) molecules produced \((s_{ij} > 0)\) or consumed \((s_{ij} < 0)\) by the \(j\)-th reaction. Due to chemical or thermodynamic constraints, some reactions in \(\mathbf{v}\) are known to be irreversible (i.e., \(v_j \geq 0\)
for certain reactions $R_j$). This information can be summarised in an $m$-dimensional reversibility vector $r$ with entries

$$r_i = \begin{cases} 1 & \text{if } R_i \text{ is reversible}, \\ 0 & \text{otherwise}. \end{cases}$$

(3)

We seek useful graph descriptions of metabolic models that we can interrogate with a wide range of tools from network science. To illustrate the construction of the different networks described in this paper, we use a toy example (Figure 1A) of a metabolic network that describes nutrient uptake, biosynthesis of metabolic intermediates, secretion of waste products, and biomass production [19], all of which are key components of genome-scale metabolic models.

A unipartite network with $m$ nodes can be described by its $m \times m$ adjacency matrix with entries that represent the connection between nodes $i$ and $j$. In contrast, a bipartite network has two types of nodes and connections only occur between nodes of different type, resulting in an $n \times m$ adjacency matrix. One of the simplest ways [7] to construct a network from a metabolic model is to create a bipartite network with adjacency matrix $\tilde{S}$, a boolean version of $S$ such that

$$\tilde{s}_{ij} = \begin{cases} 1 & \text{if } s_{ij} \neq 0, \\ 0 & \text{otherwise}. \end{cases}$$

This network connects metabolites to reactions based on whether metabolites participate on a given reaction, either as reactants or products. Figure 1B shows the bipartite network associated to our toy model. The most common approach to construct a unipartite network of reactions from $\tilde{S}$ is via the $m \times m$ adjacency matrix

$$A = \tilde{S}^T \tilde{S}. \quad (4)$$

In the network $A$, two reaction nodes are connected if they share metabolites as reactants or products (Figure 1B), while self loops represent the total number of metabolites that participate in a reaction. Though widely studied [2,7], the network $A$ suffers from three key limitations: (i) It does not distinguish between forward and backward flow of metabolites between reactions (its adjacency matrix is symmetric), and hence it cannot incorporate information on the reversibility of reactions. (ii) It does not distinguish between connections caused by important metabolites, such as carbon sources or metabolic intermediates, and connections caused by pool metabolites that participate in numerous reactions, such as water, ions or enzymatic cofactors. As a consequence, $A$ has a large number of connections that obscure its structure and the connectivity between reaction nodes. (iii) It is a rigid description that does not account for varying cellular contexts such as changes in carbon sources, growth conditions or environmental shocks.

### 2.1 Constructing networks that incorporate directionality and context

To address the limitations of the network $A$, we propose the construction of two types of networks that can be applied to any stoichiometric model:

- A directed network in the absence of context, yet capturing flows, that penalises the connections caused by pool metabolites.

- A family of context-dependent directed networks that re-balance the edge weights according to flux distributions predicted by Flux Balance Analysis (FBA).

In both networks, we include the directionality of reactions by redefining the connections between reaction nodes, namely: two reactions are connected if one produces a metabolite that is consumed by the other. This definition leads to networks that naturally account for the reversibility of reactions and allow for a seamless integration of different biological contexts modelled through FBA.
Figure 1. (Colour online) Different network representations for metabolic models. (A) Toy metabolic model describing nutrient uptake, biosynthesis of metabolic intermediates, secretion of waste products, and biomass production [19]. Reaction $R_4$ is reversible and reaction $R_8$ represents biomass production defined as $R_8 : x_3 + 2x_4 + x_5$. (B) Construction of networks from the stoichiometric matrix of the toy model ($S$). The bipartite network is defined by the boolean matrix $\hat{S}$, from which the traditional undirected reaction network can be constructed [7]. By splitting reactions into their forward and backward rates, we build the production and consumption bipartite networks, and construct the directed network ($D$). The proposed normalised network ($D_{\text{norm}}$) and context-dependent networks ($M_v$) are edge re-weighted versions of $D$. The Supplementary Information includes all the matrices to create these networks.
Inspired by techniques for the analysis of kinetic models for biochemical reaction networks \cite{20}, we first decompose the reaction vector into its forward and backward rates as \( \mathbf{v} = \mathbf{v}^+ - \mathbf{v}^- \), where \( \mathbf{v}^+ \) and \( \mathbf{v}^- \) are both non-negative. For irreversible reactions, the backward rates are \( \mathbf{v}^- = 0 \), and thus the following relation holds \( \mathbf{v} = \mathbf{v}^+ = \mathbf{v}^+ - \text{diag}(\mathbf{r}) \mathbf{v}^- \), where \( \mathbf{r} \) is the reversibility vector defined in \cite{3}. We then rewrite the metabolic model as

\[
\dot{\mathbf{x}} = \mathbf{S} \mathbf{v} = \left[ \mathbf{S} \mathbf{S}^{-1} \right] \begin{bmatrix} \mathbf{I}_m & 0 \\ 0 & \text{diag}(\mathbf{r}) \end{bmatrix} \begin{bmatrix} \mathbf{v}^+ \\ \mathbf{v}^- \end{bmatrix},
\]

where \( \mathbf{I}_m \) is the \( m \times m \) identity matrix. The matrix \( \mathbf{S}_2 \mathbf{S}^{-2} \mathbf{m} \) and vector \( \mathbf{v}_2 \mathbf{m} \) are augmented versions of original stoichiometric matrix \( \mathbf{S} \) and reaction vector \( \mathbf{v} \). As a result, the augmented model in \cite{5} has \( n \) metabolites and \( 2m \) reactions. In the following sections we detail how to construct the proposed networks over the augmented set of reaction nodes.

### 2.1.1 Directed networks of metabolism in the absence of context

To construct directed networks we first extract the auxiliary production and consumption stoichiometric matrices from \( \mathbf{S}_2 \mathbf{m} \):

\[
\begin{align*}
\mathbf{S}^+_2 &= \frac{1}{2} (\text{abs} (\mathbf{S}_2 \mathbf{m}) + \mathbf{S}_2 \mathbf{m}), \\
\mathbf{S}^-_2 &= \frac{1}{2} (\text{abs} (\mathbf{S}_2 \mathbf{m}) - \mathbf{S}_2 \mathbf{m}),
\end{align*}
\]

where \( \text{abs}(\mathbf{S}_2 \mathbf{m}) \) is a matrix whose entries are the absolute values of \( \mathbf{S}_2 \mathbf{m} \). Note that the matrices satisfy \( \mathbf{S}_2 \mathbf{m} = \mathbf{S}^+_2 \mathbf{m} - \mathbf{S}^-_2 \mathbf{m} \). The entries \( s^+_{ij} \) of the matrix \( \mathbf{S}^+_2 \mathbf{m} \) are the number of molecules of metabolite \( x_i \) produced by reaction \( R_j \), whereas the entries \( s^-_{ij} \) of \( \mathbf{S}^-_2 \mathbf{m} \) are the number of molecules of metabolite \( x_i \) consumed by reaction \( R_j \).

Similarly, as in the bipartite network \( \mathbf{S} \), the boolean versions of the matrices \( \mathbf{S}^+_2 \mathbf{m} \) and \( \mathbf{S}^-_2 \mathbf{m} \) define two bipartite networks, a production network and a consumption network, shown in Figure 1B for our toy metabolic model. From these two matrices we construct the adjacency matrix of a directed network:

\[
\mathbf{D} = \mathbf{S}^+_2 \mathbf{S}^-_2 \mathbf{m},
\]

where the tilde denotes the boolean versions of the respective matrices. The entries \( d_{ij} \) of \( \mathbf{D} \) represent the total number of metabolites produced by reaction \( R_i \) that are consumed by reaction \( R_j \). The adjacency matrix of \( \mathbf{D} \) has size \( 2m \times 2m \) and defines a directed network on the space of reactions split into their forward and backward components. In Figure 1B we summarise the steps to construct the network \( \mathbf{D} \). In contrast to the undirected \( \mathbf{A} \) network, the adjacency matrix of \( \mathbf{D} \) is not symmetric and thus captures the existence of irreversible reactions in the original metabolic model. We note that \( \mathbf{D} \) and \( \mathbf{A} \) are not directly comparable as they are defined on different sets of nodes. We can obtain a directed network on the same set of nodes as \( \mathbf{A} \) with the \( m \times m \) adjacency matrix

\[
\mathbf{A}_{\text{dir}} = \mathbf{C}^T \mathbf{D} \mathbf{C},
\]

where \( \mathbf{C} = [\mathbf{I}_m \mathbf{I}_m]^T \). The directed network defined by \( \mathbf{A}_{\text{dir}} \) contains the connections in \( \mathbf{A} \), but excludes those edges caused by the non-existent backward rates in irreversible reactions. If the metabolic model contains only reversible reactions, i.e., when the reversibility vector \( \mathbf{r} \) contains only ones, from equations \( \cite{3} \) and \( \cite{8} \) we recover \( \mathbf{A}_{\text{dir}} = \mathbf{A} \).

To avoid the effect of pool metabolites on the structure of a metabolic network, a common approach is to remove those highly-connected metabolites from the network description \cite{21,23}. However, this
can change the network structure drastically and without a clear definition of which pool metabolites should be pruned, it is typically done with heuristics that depend on the particular network and scientific question at hand. As an alternative, we propose not to remove any pool metabolites, but instead to normalise the network edges and include their relative importance in the network connectivity \[24\].

We construct the normalised network, called \( \bm{D}_{\text{norm}} \), by scaling the rows of the boolean consumption \( (\hat{\bm{S}}_{2m}^+) \) and production adjacency matrices \( (\hat{\bm{S}}_{2m}^-) \) by the total number of reactions in which each metabolite participates. If \( \hat{s}_{ij}^+ \) and \( \hat{s}_{ij}^- \) are the entries of the respective adjacency matrices, we have that metabolite \( x_i \) is produced by a total of \( d_i^+] = \sum_{i=j}^{2m} \hat{s}_{ij}^+ \) reactions, and consumed by a total of \( d_i^- = \sum_{i=j}^{2m} \hat{s}_{ij}^- \) reactions. We collect this information in the vectors

\[
\begin{align*}
\bm{d}^+ &= \hat{\bm{S}}_{2m}^+ \mathbf{1}_{2m}, \\
\bm{d}^- &= \hat{\bm{S}}_{2m}^- \mathbf{1}_{2m},
\end{align*}
\]

where \( \mathbf{1}_{2m} \) is a \( 2m \)-dimensional vector of ones. The adjacency matrix of our proposed normalised network is then

\[
\bm{D}_{\text{norm}} = \left( \bm{D}_+ \hat{\bm{S}}_{2m}^+ \right)^T \left( \bm{D}_- \hat{\bm{S}}_{2m}^- \right),
\]

where \( \bm{D}_+ \) and \( \bm{D}_- \) are the pseudo-inverses of \( \text{diag} (\bm{d}^+) \) and \( \text{diag} (\bm{d}^-) \), respectively. The construction of \( \bm{D}_{\text{norm}} \) is analogous to \( \bm{D} \) in \[7\], but instead uses normalised versions of the production and consumption stoichiometry. As a result, \( \bm{D}_{\text{norm}} \) is a directed network that penalises those connections caused by highly-connected pool metabolites and gives a higher importance to interactions caused by relevant metabolites.

### 2.1.2 Context-dependent networks

To incorporate the effect of different cellular contexts on the graph of a metabolic network, we propose to re-weight the edges of the directed network using the metabolic flux distributions predicted by Flux Balance Analysis (FBA). FBA computes a vector of fluxes \( \mathbf{v}^* \) that optimize a cellular objective, usually maximisation of biomass or growth, assuming that cellular metabolism is in quasi steady state with respect to the remaining cellular processes, i.e., \( \dot{\mathbf{x}} = \mathbf{S} \mathbf{v} = 0 \). A key advantage of FBA is that specific environmental conditions can be included as upper and lower bounds on the reaction fluxes, which in turn act as constraints for the optimisation problem. These constraints describe, for example, the availability of nutrients, oxygen, and toxins. We have summarised the key elements of FBA in Sec. A.1.

We define the weight of an edge between reactions nodes \( R_i \) and \( R_j \) as the total flow of metabolites produced by \( R_i \) that are consumed by \( R_j \). Under such definition, the entries of the adjacency matrix of the context-dependent network, which we call \( \bm{M}_v \), are

\[
m_{ij} = \sum_{k=1}^{n} \left( \text{flow of } x_k \text{ produced by } v_i \right) \times \left( \frac{\text{flow of } x_k \text{ consumed by } v_j}{\text{total flow of } x_k} \right),
\]

The main assumption behind our definition is that the amount of metabolite produced by one reaction gets distributed among the reactions that consume it in proportion to their flux. For example, if in the FBA solution the total flux of metabolite \( x_k \) is 10 mmol gDW\(^{-1}\)h\(^{-1}\) with reaction \( R_i \) producing \( x_k \) at a rate 1.5 mmol gDW\(^{-1}\)h\(^{-1}\), and reaction \( R_j \) consuming \( x_k \) at a rate 3.0 mmol gDW\(^{-1}\)h\(^{-1}\), then the mass flow of \( x_k \) from \( R_i \) to \( R_j \) is 1.5 mmol gDW\(^{-1}\)h\(^{-1}\) \times (3.0/10) = 0.45 mmol gDW\(^{-1}\)h\(^{-1}\). The definition in equation \[10\] adds together the mass flows of all metabolites produced by \( R_i \) and consumed by \( R_j \), and thus \( m_{ij} \) represents the total flow between the two reactions. In this network, self loops describe the metabolic flux of autocatalytic reactions, i.e., those in which some products are also reactants.
Figure 2. (Colour online) Context-dependence in metabolic reaction networks. Three examples of context-dependent networks ($M_v$) for the toy example in Figure 1. The networks were constructed with equation (12) applied to the stoichiometric matrix in Figure 1 (see also SI) and with three solutions from FBA under different constraints. In contexts 2 and 3, the lower flux bounds for reactions 6 and 7 were perturbed, causing changes in the flux distribution that translate into different weighting of the graph edges. The dashed boxes represent the flux bounds employed in each case, and solid lines describe the optimal fluxes predicted by FBA in units of $\text{mmol gDW}^{-1} \cdot \text{h}^{-1}$. In all cases, the optimal flux for reaction $R_4$ is positive and thus the corresponding backward reaction node $R_4$ is disconnected.

We can compute the edge weights $m_{ij}$ directly from the stoichiometric matrices $S_{2m}^+$ and $S_{2m}^-$ defined in equation [6], and the FBA solution $v^*$. We build the augmented flux vector

$$v_{2m}^* = \begin{bmatrix} v^+ \\ v^- \end{bmatrix} = \frac{1}{2} \begin{bmatrix} \text{abs}(v^*) + v^* \\ \text{abs}(v^*) - v^* \end{bmatrix},$$

which splits the forward and backward fluxes from the FBA solution in a similar way as $v_{2m}$ in equation [6]. If we denote the $i$-th and $j$-th entries of $v_{2m}^*$ as $v_{2m}^i$ and $v_{2m}^j$, respectively, we have that reaction $R_i$ produces the metabolite $x_k$ at a rate $s_{ki}^+ v_{2m}^i$, while reaction $R_j$ and consumes $x_k$ at a rate $s_{kj}^- v_{2m}^j$. Substituting in equation (10) we get

$$m_{ij} = \sum_{k=1}^{n} \frac{s_{ki}^+ v_{2m}^i}{s_{kj}^- v_{2m}^j} \times \left( \frac{s_{kj}^- v_{2m}^j}{\sum_{j=1}^{2m} s_{kj}^- v_{2m}^j} \right).$$

(11)

The adjacency matrix of the network with entries $m_{ij}$ is then

$$M_v = (S_{2m}^+ V^*)^T J_v^+ (S_{2m}^- V^*),$$

(12)

where $V^* = \text{diag}(v_{2m}^*)$, $J_v^+$ is the pseudo-inverse of $J_v$, and $J_v$ is the vector of production and consumption fluxes

$$J_v = S_{2m}^+ v_{2m}^* = S_{2m}^- v_{2m}^*.$$

The equality is a consequence of the steady state condition $\dot{x} = S_{2m} v_{2m}^* = (S_{2m}^+ - S_{2m}^-) v_{2m}^* = 0$. 

---

[6] For context, please refer to the cited literature.
Figure 3. (Colour online) The E. coli core metabolic model. (A) Map of the E. coli core metabolic model [12] created with the online tool Escher [25]. (B) Directed reaction network $D_{\text{norm}}$ of the metabolic model constructed from equation (9). The nodes (reactions) in the network are coloured according to the pathway to which they belong. Reversible reactions are represented by two overlapping nodes (one node for the forward reaction and another for the backward). There are 154 nodes in this network (95 forward and 59 backward reactions) and 1,604 directed connections. The relative location of the reactions in the network and the map is the same.

A fundamental feature of the networks generated by $M_v$ is that the connections have a precise physical interpretation. The weights of the connections correspond to the total flow of metabolites between reactions in units of $\text{mmol} \cdot \text{s}^{-1} \cdot \text{gDW}^{-1}$. This feature allows to directly link the connectivity of the network to the mass flow of metabolites through the network. Since there is a network $M_v$ specific to each FBA solution $v^*$, our approach is a versatile framework to produce metabolic reaction networks for different environmental conditions.

Figure 2 shows three context-dependent networks for the example in Figure 1A. In each case we computed FBA solutions under a fixed uptake flux and constrained the remaining fluxes to account for different cellular contexts. In Context 1 we constrained the fluxes to be strictly positive and no larger than the nutrient uptake flux. In contexts 2 and 3, we imposed additional lower bounds for the fluxes through reactions $R_6$ and $R_7$, respectively. The results illustrate how changes in the optimal flux distributions translate into different network connectivities and edge weights. Context 2 leads to a network with a similar connectivity to Context 1, but with a noticeable redistribution of edge weights in the graph, while context 3 displays an extra connection between reactions $R_4$ and $R_7$, which is absent in the other two cases.

3 Analysis of network representations of the core E. coli metabolic model

We now construct and analyse the networks described in the previous section for the core E. coli metabolic model [12]. This model is relatively small, with 72 metabolites (20 extracellular and 52 intracellular) and 95 reactions (20 exchange reactions, 25 transport reactions, 49 metabolic reactions and one biomass reac-
tion). The canonical way to describe metabolic reactions is in terms of pathways that consist of reactions that serve a specific function \[18, 26, 27\]. For example, the reactions that form glycolysis convert D-glucose into pyruvate and produce adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH). In this model, the reactions are grouped into 11 metabolic pathways that represent the main biochemical routes in the central carbon metabolism (Figure 3A). The directed network representation of the metabolic model in the absence of context \(D_{\text{norm}}\) (Fig. 3B) reveals the underlying complexity of the connectivity of the reactions which is typically absent from pathway representations.

The notion of directed flow is at the core of the construction of our networks. We are therefore interested in finding groups of reactions that are tightly linked by flows, and observing how these groups change in different contexts. These groups of reactions can be understood as a generalisation of the notion of pathway, which are tailored to specific contexts. The analysis of these groups will help us understand which reactions form groups of interaction, under what circumstances, and what are their defining features. The \(M_v\)-networks we propose can help to transition from analyses that do not incorporate networks nor context, to a framework of flexible, context-dependent, network-based analyses of the cell's metabolism.

We use the Markov Stability community detection framework \[28, 29\] to extract groups of reactions in the different networks. This framework employs diffusion processes (flows) of different duration on networks, which is ideally suited to analyse our reaction networks. Markov stability defines a community in precisely the way that interests us: a group of nodes in which flows are retained at specific scales. The duration of the diffusion process acts as a resolution parameter on the size of the communities \[30\]. A consequence of defining communities in terms of flow-retention is that Markov Stability can naturally incorporate the directionality of the connections \[31, 32\] (see Sec. A.2), which is crucial to analyse metabolism in a realistic way. Therefore, we study communities in reaction networks: groups of reactions that are tightly linked by the flow of metabolites they produce and consume. Each community is formed by reactions that retain metabolites as much as possible.

We analyse the community structure of the networks we obtain from the core \(E. coli\) metabolic model (\(A, D_{\text{norm}}, \text{and} M_v\) for a selection of \(v\)). This analysis will enable us to answer questions such as: How does context affect the community structure of the network?, is it useful to describe the networks in terms of the same pathways in very different scenarios?, or what is the multiscale organisation of metabolism and how does it relate to the standard pathways? Below we provide answers to these questions.

### 3.1 Analysis of the \(E. coli\) core network in the absence of context

As previously mentioned, the network \(A\), obtained from equation (4), contains connections between reactions that share metabolites in any capacity and does not distinguish between reversible and irreversible reactions (Fig. 4A). This network has 95 reactions and 1158 connections. In contrast the network \(D_{\text{norm}}\) contains 154 reactions (i.e., all forward reactions and all legitimate reverse reactions) and 1,604 connections. By its construction in equation (9), the connections created by pool metabolites are penalised, so that more weight is placed on connections that describe the flow of less-abundant, yet more informative, metabolites (Fig. 3D).

We use Markov Stability to extract the community structure of the networks \(A\) and \(D_{\text{norm}}\). The undirected network \(A\) has a robust partition into six communities (see Fig. 4A and SI for a detailed description of each community). These communities are largely determined by the connections created by pool metabolites. For example, community \(C_2A\) is formed by reactions that consume/produce ATP. The biomass reaction (the largest consumer of ATP when we include fluxes) is not a member of this community. This reaction uses the majority of the ATP produced in the cell for cellular growth; however, the construction of the network considers any connection that involves ATP equally. Other communities in this network are also determined by pool metabolites such as NAD\(^+\) and NADP\(^+\) (\(C_1A\)), or water (\(C_3A\)). The community structure in the network \(A\) highlights its limitations due to the absence of biological context and the overwhelming amount of uninformative connections.
Figure 4. (Colour online) **Communities in networks in the absence of context** (A) The network $A$ has a robust partition into six communities. Note that this network does not include information about reverse reactions, which is why they are not shown here. (B) The network $D_{\text{norm}}$ has a robust partition into four communities. Figure 4B shows the network $D_{\text{norm}}$ with the re-balanced edge weights and reverse reaction nodes. This network has a robust partition into four communities (see SI for a detailed description of each community). The communities in this network emphasise flows of metabolites that are important for specific functions, and can be described in terms of pathways. For example community $C_2$ contains the pentose phosphate pathway and the first steps of glycolysis. Communities tend to contain reactions that belong to the same pathways. Although an improvement on $A$, the network $D_{\text{norm}}$ is still context-independent and thus not suitable to study metabolism in specific scenarios.

### 3.2 Context-dependent networks of the *E. coli* core metabolic model

We now examine four different context-dependent networks from the core *E. coli* metabolic model. These networks illustrate how different circumstances force important changes in the pattern of metabolite flows. The scenarios we analyse are:

- $M_{\text{glc}}$: Aerobic growth with D-glucose as a carbon source.
- $M_{\text{etoh}}$: Aerobic growth with ethanol.
- $M_{\text{anaero}}$: Anaerobic growth on D-glucose.
- $M_{\text{lim}}$: Aerobic growth with D-glucose, and limited phosphate and ammonium.

In each case we solve an FBA problem in which the constraints encode each scenario. The optimal reaction fluxes represent the state of the cell’s metabolism in each context. We construct the networks using each optimal flux $v^*$ and equation (12). In all cases, the connected components of the networks...
Figure 5. (Colour online) Metabolic reaction networks of E. coli in different contexts. Each of these networks is obtained from equation (12) and the solution of a FBA problem in four different contexts: (A) Aerobic growth with glucose as a source of carbon; (B) aerobic growth with ethanol; (C) anaerobic with glucose; (D) aerobic growth with limited ammonium and phosphate. The reactions are coloured according to their community (see text); the inactive reactions in each context (i.e., with zero flux) are shown in grey. The thickness of the connections is proportional to the edge weights within each network.
have fewer nodes and connections than $D_{\text{norm}}$ because the solution of the FBA contains numerous nil fluxes (i.e., reactions with zero net flux).

Figure 5 shows that the four networks are remarkably different from each other: the nodes in the giant component and the weights of the connections are different, and each network has a community structure with a different biological interpretation. We explain the main features of the communities of each network below (see also the SI for a detailed description of all communities).

The network $M_{\text{glc}}$ has a robust partition into three communities (see Fig. 5A and SI) which divide the network in three parts with a concrete interpretation: community $C_0^{\text{glc}}$ contains reactions in charge of processing carbon from D-glucose to pyruvate. Community $C_1^{\text{glc}}$ contains the reactions in charge producing NADH and NADPH (cell’s reductive power). Community $C_2^{\text{glc}}$ harbours the bulk of the cell’s ATP production. The network from growth in ethanol ($M_{\text{etoh}}$ Fig. 5B) also has a partition into three communities resembling those in $M_{\text{glc}}$ with subtle, yet important differences. For example, in $C_0^{\text{etoh}}$ the change of source of carbon from D-glucose to ethanol has transformed glycolysis into gluconeogenesis by a reversal of the flux in the reactions in $C_0^{\text{glc}}$. Moreover, $C_0^{\text{etoh}}$ contains new reactions (e.g., ME2, PPCK, GLUDy, and ICDHyr) in charge of the production of growth precursors. The biomass reaction is now contained in $C_0^{\text{etoh}}$ due the increased flow of precursors relative to ATP production.

The absence of oxygen has, predictably, a profound impact in the cell. The network $M_{\text{anaero}}$ (Fig. 5C) reflects this new metabolic regime. The connectivity and the communities in this network are different from the other aerobic scenarios. For example the reactions CYTBD and NADH16 which are the first two steps of the electron transport chain are absent; the absence of these reactions (due to their high connectivity in normal circumstances) has a profound effect in the flow of metabolites and, consequently, on the community structure of the network.

The network $M_{\text{lim}}$ (Fig. 5D) depicts the network under severe conditions. In this scenario, the community structure reflects a phenomenon known as overflow metabolism [33], which occurs when the cell takes in more carbon than it can process. In this case overflow metabolism is due to limited phosphate and NH$_4$: the overabundance of carbon is secreted from the cell, there is a strong decrease in the growth rate, and a partial shut-down of the TCA cycle. Community $C_1^{\text{lim}}$ is similar to $C_1^{\text{glc}}$ and $C_1^{\text{etoh}}$, with the addition of the secretion routes of acetate and formate.

### 3.2.1 Structure of context-dependent networks at multiple resolutions

We have analysed one partition in each of the four context-dependent networks. However, complex networks such as these often have important partitions into communities at different levels of granularity [28,34]. In the Markov Stability framework, we can explore these scales by scanning through different values of Markov time (see Sec. A.2).

Figure 6A shows the number of communities found in the $M_{\text{glc}}$ network as we scan through a range of Markov times. Short Markov times result in fine-grained partitions (e.g., near $t = 0.3$ there are over 20 communities); as we increase Markov times, we will find coarser partitions until (as Markov time tends to infinity) we find a single community containing all the nodes. We have selected four Markov times at which we find robust partitions into 10,7, 3, and 2 communities. To analyse the partitions (and the partition into pathways) simultaneously, we construct an alluvial diagram [35] (Fig. 6B). These diagrams allow us to visualise the composition of the different partitions and how they are related in terms of their members.

In the example from Fig. 6B, we start with the partition of the reactions of $M_{\text{glc}}$ into their metabolic pathways. As we move to the right, we can see how the reactions in each of the pathways assemble into the partitions we have obtained with Markov Stability. This figure highlights different features and properties of the $E.\ coli$ metabolic network in aerobic conditions and with glucose as the sole source of carbon. In some cases, the reactions of pathways such as the oxidative phosphorylation or glycolysis are grouped mostly together in the same community. Interestingly, the TCA cycle, although it appears as a cohesive unit for most Markov times is split in two at $t = 22.5$. The pathways with reactions
Figure 6. (Colour online) Multiscale community structure of M_{gc}. (A) Number of communities (dashed-blue line) and Variation of Information (solid-red line) in the M_{gc} network as we scan Markov times (see text and Methods section) for communities. We have selected four Markov times in which the network can be split into 10, 7, 3, and 2 communities and the VI is low or has a pronounced dip. (B) Alluvial diagram [35] showing how the reactions that form each of the pathways (left) assemble in communities of different size as we scan Markov time. Note that in this network there are no reactions (with positive flux) in the pyruvate metabolism pathway.
in charge of exchanging substances with the exterior of the cell (exchange and transport) are spread among the partitions in all Markov times; these are pathways in which the reactions do not interact amongst themselves. These pathways act more like ‘roles’ (i.e., importing and moving substrates) than like cohesive metabolic sub-units. Other pathways such as the pentose phosphate pathway is divided into different communities except at \( t = 7.17 \) when, as the TCA cycle, comes together before splitting up again. This phenomenon illustrates that some biological features may only become relevant at specific resolutions.

4 Discussion

We have proposed a method to construct reaction networks from metabolic models that go beyond standard pathway representations. By accounting for the directionality of metabolic flows and penalising uninformative connections, we obtain the network \( D_{\text{norm}} \), that reflects the flow relationships among reactions, rather than mere adjacencies collected under previous network descriptions of metabolism (i.e., network \( A \)). The network \( D_{\text{norm}} \) provides an overall description of a metabolic subsystem, yet does not capture the specific influence of the context in which the cell may be found. To address this need, we have incorporated information obtained from constraint-based modelling (e.g., FBA) to create context-dependent networks \( M_v \) that are tailored to specific biological scenarios. These networks include the reaction fluxes thus reflecting the availability of nutrients and other essential compounds, as well as a constrained optimization for cellular growth. Our network-theoretic analysis shows that under different conditions the relationships among the reactions and the structure of the network can be fundamentally different. For example, in the core \( E. \ coli \) metabolic model, our method is able to correctly reflect important biological features such as the transition from glycolysis to gluconeogenesis, overflow metabolism, and the effects of anoxia.

Such context-dependent networks can be used to analyse the properties of metabolism in a wide variety of scenarios; for example, to find metabolic conditions in which a drug or a combination of drugs may be maximally effective \([36–39]\). In addition, these networks can be easily adapted to a multi-layer network setting \([40]\) (e.g., modifying equation (12) to obtain an \( m \times m \times n \) adjacency tensor) to study the contribution by each metabolite to the network. Finally, our approach can also be extended to obtain context-dependent networks beyond the stationarity paradigm of FBA and incorporate dynamical features, for example by using Dynamic FBA \([41,42]\), or by the incorporation of enzyme levels \([43]\).

A Methods

A.1 Flux balance analysis

Flux Balance Analysis (FBA) \([15,19]\) is a widely-adopted approach to analyse metabolism and cellular growth. FBA calculates the reaction fluxes that optimise growth in specific biological contexts. The main hypothesis behind FBA is that cells adapt their metabolism to maximise growth in different biological conditions. The conditions are encoded as constraints on the fluxes of certain reactions; for example, constraints reactions that import nutrients and other necessary compounds from the exterior.

The mathematical formulation of the FBA is described in the following constrained optimisation problem:

\[
\text{maximise: } c^T v \\
\text{subject to } \begin{cases} 
Sv = 0 \\
v_{lb} \leq v \leq v_{ub},
\end{cases}
\]

where \( S \) is the stoichiometry matrix of the model, \( v \) the vector of fluxes, \( c \) is an indicator vector (i.e., \( c(i) = 1 \) when \( i \) is the biomass reaction and zero everywhere else) so that \( c^T v \) is the flux of the biomass reaction.
The constraint $Sv = 0$ enforces mass-conservation at stationarity, and $v_{lb}$ and $v_{ub}$ are the lower and upper bounds of each reaction’s flux. Through these vectors, one can encode a variety of different scenarios [12]. The biomass reaction represents the most widely-used flux that is optimised, although there are others can be used as well [44, 45].

In our simulations, we set the individual carbon intake rate to $18.5 \text{ mmol gDW}^{-1} \text{h}^{-1}$ for every source available in each scenario. We allowed oxygen intake to reach the maximum needed in to consume all the carbon except in the anaerobic condition scenario, in which the upper bound for oxygen intake was $0 \text{ mmol gDW}^{-1}$. In the scenario with limited phosphate and ammonium intake, the levels of NH$_4$ and phosphate intake were fixed at $4.5 \text{ mmol gDW}^{-1}$ and $3.04 \text{ mmol gDW}^{-1}$ respectively (a reduction of 50% compared to a glucose-fed aerobic scenario with no restrictions).

A.2 Markov Stability community detection framework

We extract the communities in each network using the Markov Stability (MS) community detection framework [28, 29]. This framework uses diffusion processes on the network to find groups of nodes (i.e., communities) that retain flows for longer than one would expect on a comparable random network; in addition, MS incorporates directed flows seamlessly into the analysis [31, 32].

The diffusion process we use is a continuous-time Markov process on the network. From the adjacency matrix $A$ we construct a rate matrix for the process: $M = K^{-1} - A$, where $K$ is the diagonal matrix of out-strengths, $k_{out,i} = \sum_j a_{i,j}$. When a node has no outgoing edges then we simply let $k_{out,i} = 1$.

In general, a directed network will not be strongly-connected and thus a Markov process on $M$ will not have a unique steady state. To ensure the uniqueness of the steady state we must add a teleportation component to the dynamics by which a random walker visiting a node can follow an outgoing edge with probability $\lambda$ or jump (teleport) uniformly to any other node in the network with probability $1 - \lambda$ [46]. The rate matrix of a Markov process with teleportation is:

$$B = \lambda M + \frac{1}{N} [(1 - \lambda) I_N + \lambda \text{diag}(a)] 11^T,$$

where the $N \times 1$ vector $a$ is an indicator for dangling nodes: if node $i$ has no outgoing edges then $a_i = 1$, and $a_i = 0$ otherwise. Here we use $\lambda = 0.85$. The Markov process is described by the ODE:

$$\dot{x} = -L^T x,$$

where $L = I_N - B$. The solution of (15) is $x(t) = e^{-tL} x(0)$ and its stationary state (i.e., $\dot{x} = 0$) is $x = \pi$, where $\pi$ is the leading left eigenvector of $B$.

A hard partition of the network into $C$ communities can be encoded into the $N \times C$ matrix $H$, where $h_{ic} = 1$ if node $i$ belongs to community $c$ and zero otherwise. The $C \times C$ clustered autocovariance of (15) is

$$R(t, H) = H^T \left( \Pi e^{-tL} - \pi \pi^T \right) H.$$

The entry $(c,s)$ of $R(t, H)$ is how likely it is that a random walker that started the process in community $c$ at finds itself in community $s$ at time $t$. Crucially, the diagonal elements of $R(t, H)$ show how good are the communities in $H$ at retaining flows. The stability of the partition is then

$$r(t, H) = \text{trace} R(t, H).$$

We find the communities in the network by optimising (17) over the space of partitions for a given time $t$ using the Louvain greedy optimisation heuristic [47]. The Louvain algorithm does not guarantee a globally optimal partition of the network into communities; for this reason we optimise (17) 100 times for each value of $t$. We assess the consistency of the resulting partition using the Variation of Information (VI)
metric [48], as described in [30, 32]. A low value of the VI implies that the 100 partitions obtained from Louvain are similar; a VI of exactly zero means that all the partitions in each of the 100 optimisations are identical.

The value of the Markov time $t$, i.e. the duration of the Markov process, is also a resolution parameter for the partition of the network into communities [28, 30]. In the limit $t \to 0$, Markov stability will assign each node to its own community; as $t$ grows, we will obtain larger communities because the random walkers have more time to explore the network. Finally, when $t \to \infty$ all nodes merge into a single community comprising the entire network [29]. We scan through a range of values of $t$ to explore the multiscale community structure of the network.

B Acknowledgments

M.B.D. acknowledges support from the James S. McDonnell Foundation Postdoctoral Program in Complexity Science/Complex Systems Fellowship Award (#220020349-CS/PD Fellow), and the Oxford-Emirates Data Science Lab. G.B. acknowledges the support from the Spanish Ministry of Economy FPI Program (BES-2012-053772). D.O. acknowledges support from the Human Frontier Science Program through a Young Investigator Grant (RGY0076-2015), and from Imperial College London through a Junior Research Fellowship. J.P. acknowledges the support from the Spanish Ministry of Economy through the Multiscales project (DPI2011-28112-C04-01). M.B. acknowledges funding from the EPSRC through grants EP/I017267/1 and EP/N014529/1.
## Supplementary Information

### A Toy model example

Matrices for the examples in Figs. 1 and 2 from Ref. [19]. Stoichiometric matrix and unipartite projection using equation (4):

$$
S = \begin{bmatrix}
R_1 & R_2 & R_3 & R_4 & R_5 & R_6 & R_7 & R_8 \\
A & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\
B & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\
C & 0 & 0 & 1 & 0 & -1 & 0 & 0 & -1 \\
D & 0 & 0 & 0 & 1 & 0 & -1 & -1 & -2 \\
E & 0 & 0 & 0 & 0 & 1 & 1 & 0 & -1 \\
\end{bmatrix}, \quad \mathbf{A} = \tilde{\mathbf{S}}^T \tilde{\mathbf{S}} = \begin{bmatrix}
1 & 1 & 0 & 0 & 0 & 0 & 0 \\
1 & 2 & 1 & 1 & 0 & 0 & 0 \\
0 & 1 & 2 & 1 & 0 & 0 & 1 \\
0 & 1 & 1 & 2 & 0 & 1 & 1 \\
0 & 1 & 0 & 2 & 1 & 0 & 2 \\
0 & 0 & 0 & 1 & 1 & 2 & 1 \\
0 & 0 & 0 & 1 & 0 & 1 & 1 \\
0 & 0 & 0 & 1 & 1 & 2 & 1 & 3 \\
\end{bmatrix}.
$$

FBA scenario 1 setting and context-dependent network:

$$
\begin{align*}
R_1 &: v_{lb1} & v_{ub1} & v_1^* \\
R_2 &: 10 & 10 & 10 \\
R_3 &: 0 & 10 & 10 \\
R_4 &: 0 & 10 & 4.992 \\
R_5 &: -10 & 10 & 5.008 \\
R_6 &: 0 & 10 & 2.492 \\
R_7 &: 0 & 10 & 0.008 \\
R_8 &: 0 & 10 & 2.5 \\
R_{4r} &: -10 & 10 & 0 \\
\end{align*}
$$

$$
\mathbf{M}_{c1} = \begin{bmatrix}
R_1 & R_2 & R_3 & R_4 & R_5 & R_6 & R_7 & R_8 \\
R_1 & 0 & 10 & 0 & 0 & 0 & 0 & 0 \\
R_2 & 0 & 0 & 4.992 & 5.008 & 0 & 0 & 0 \\
R_3 & 0 & 0 & 0 & 0 & 2.492 & 0 & 0 & 2.5 \\
R_4 & 0 & 0 & 0 & 0 & 0 & 0.008 & 5 \\
R_5 & 0 & 0 & 0 & 0 & 0 & 2.492 & 0 & 0 \\
R_6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
R_7 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
R_8 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{bmatrix}
$$

FBA scenario 2 setting and context-dependent network:

$$
\begin{align*}
R_1 &: v_{lb2} & v_{ub2} & v_2^* \\
R_2 &: 10 & 10 & 10 \\
R_3 &: 0 & 10 & 10 \\
R_4 &: 0 & 10 & 3.877 \\
R_5 &: -10 & 10 & 6.123 \\
R_6 &: 0 & 10 & 1.877 \\
R_7 &: 2 & 10 & 2 \\
R_8 &: 0 & 10 & 2 \\
R_{4r} &: -10 & 10 & 0 \\
\end{align*}
$$

$$
\mathbf{M}_{c2} = \begin{bmatrix}
R_1 & R_2 & R_3 & R_4 & R_5 & R_6 & R_7 & R_8 \\
R_1 & 0 & 10 & 0 & 0 & 0 & 0 & 0 \\
R_2 & 0 & 0 & 3.877 & 6.123 & 0 & 0 & 0 \\
R_3 & 0 & 0 & 0 & 0 & 1.877 & 0 & 0 & 2 \\
R_4 & 0 & 0 & 0 & 0 & 0 & 0.123 & 2 & 4 \\
R_5 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.123 \\
R_6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
R_7 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
R_8 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{bmatrix}
$$

FBA scenario 3 setting and context-dependent network:

$$
\begin{align*}
R_1 &: v_{lb3} & v_{ub3} & v_3^* \\
R_2 &: 10 & 10 & 10 \\
R_3 &: 0 & 10 & 10 \\
R_4 &: 0 & 10 & 3 \\
R_5 &: -10 & 10 & 7.001 \\
R_6 &: 0 & 10 & 0.5 \\
R_7 &: 2 & 10 & 2.001 \\
R_8 &: 0 & 10 & 2.5 \\
R_{4r} &: -10 & 10 & 0 \\
\end{align*}
$$

$$
\mathbf{M}_{c3} = \begin{bmatrix}
R_1 & R_2 & R_3 & R_4 & R_5 & R_6 & R_7 & R_8 \\
R_1 & 0 & 10 & 0 & 0 & 0 & 0 & 0 \\
R_2 & 0 & 0 & 2.999 & 7.001 & 0 & 0 & 0 \\
R_3 & 0 & 0 & 0 & 0 & 0.499 & 0 & 2.5 \\
R_4 & 0 & 0 & 0 & 0 & 0 & 2.001 & 5 \\
R_5 & 0 & 0 & 0 & 0 & 0 & 0.499 & 0 \\
R_6 & 0 & 0 & 0 & 0 & 0 & 2.001 & 0 \\
R_7 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
R_8 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{bmatrix}
$$
Figure 7. (Colour online) Number of communities (dashed blue line) and Variation of Information (solid red line) in Markov time for networks A and $D_{\text{norm}}$.

B Communities from Markov stability

B.1 Network A

The communities in this network found at Markov time $t = 22$ (Fig. 7). The communities at this resolution (Fig. 4A) are:

- Community C0$_{A}$ (purple) includes the reactions NADH dehydrogenase (NADH16) and cytochrome oxidase (CYTBD). These two reactions involve pool metabolites (such as H$_2$O, H$^+$, NADH) which create a large number of connection. Other members include fumarate reductase (FR7) and succinate dehydrogenase (SUCCI) which couple the TCA cycle with the electron transport chain (through ubiquinone-8 reduction and ubiquinol-8 oxidation). Reactions that include export and transport of most secondary carbon sources (such as pyruvate, ethanol, lactate, acetate, malate, fumarate, succinate or glutamate) are included in the community as well. These reactions are included in the community because of their influence in the proton balance of the cell. Most of these reactions do not occur under normal circumstances. This community highlights the fact that in the absence of biological context, many reactions that do not normally interact can be grouped together.

- Community C1$_{A}$ (light blue) contains reactions that produce or consume nicotinamide adenine dinucleotide (NAD$^+$), nicotinamide adenine dinucleotide phosphate (NADP$^+$), or their reduced variants NADH and NADPH. The main two reactions of the community are NAD(P) transhydrogenase (THD2) and NAD$^+$ transhydrogenase (NADTRHD). There are also reactions related to the production of NADH or NADPH in the TCA cycle such as isocitrate dehydrogenase (ICDHyr), 2-oxoglutarate dehydrogenase (AKGDH) and malate dehydrogenase (MDH). The community also includes reactions that are not frequently active such as malic enzme NAD (ME1) and malic enzime NADH (ME2) or acetate dehydrogenase (ACALD) and ethanol dehydrogenase (ALCD2x).

- Community C2$_{A}$ (red) contains all the reactions that consume or produce ATP, another pool metabolite. Production of ATP comes mostly from oxidative phosphorylation (ATPS4r) and substrate level phosphorylation reactions such as phosphofructokinase (PFK), phosphoglycerate kinase (PGK) and succinil-CoA synthase (SUCOAS). Reactions that consume ATP include glutamine synthetase (GLNS) and ATP maintenance equivalent reaction (ATPM). The reactions L-glutamine transport via ABC system (GLNabc), acetate transport in the form of phosphotransacetilase (PTAr), and acetate kinase (ACKr) are also part of this community.

- Community C3$_{A}$ (green) contains reactions that involve H$_2$O. Under normal conditions water is assumed to be abundant in the cell, thus the biological link that groups these reactions together is tenuous.
Figure 8. (Colour online) Number of communities (dashed blue line) and Variation of Information (solid red line) in Markov time for the context-dependent networks $M_{\text{glc}}$, $M_{\text{etoh}}$, $M_{\text{anaero}}$, and $M_{\text{lim}}$.

- Community C4 (orange) contains the main carbon intake of the cell (glucose), the initial steps of glycolysis, and most of the pentose phosphate shunt. These reactions are found in this community because the metabolites involved in these reactions (e.g., alpha-D-ribose-5-phosphate (r5p) or D-erythrose-4-phosphate (e4p)) are only found in these reactions. This community includes the biomass reaction due to the number of connections created by growth precursors.

- Community C5 (magenta) is a small community that contains oxygen intake reactions.

B.2 Network Dnorm

The communities in this network found at Markov time $t = 13$ (Fig. 7). The communities at this resolution (Fig. 4B) are:

- Community C0 (purple) contains the main reaction that produces ATP oxidative phosphorylation (ATPS4r). The flow of metabolites among the reactions in this community includes some pool metabolites such as ATP, ADP, H$_2$O, and phosphate; however, there are connections created by metabolites that only appear in a handful of reactions such as adenosine monophosphate (AMP) whose sole producer is phosphoenolpyruvate synthase (PPS) and its sole consumer is ATPS4r (this connection is clearly visible in Fig. 4B).

- Community C1 (green) includes the core of the citric acid (TCA) cycle such as citrate synthase (CS), aconitase A/B (ACONTa/b), and anaplerotic reactions such as malate synthase (MALS), malic enzyme NAD (ME1), and malic enzyme NADP (ME2). This community also includes the intake of cofactors such as CO$_2$ and H$_2$O and most of the nitrogen-related metabolism.

- Community C2 (yellow) includes the first half of the glycolysis and the complete pentose phosphate pathway. The metabolites that create the connections among these reactions such as D-fructose, D-glucose, or D-ribulose. This community also contains the biomass reaction.

- Community C3 (red) contains reactions that are secondary sources of carbon such as malate and succinate, as well as oxidative phosphorylation reactions.
B.3 Network \( M_{\text{glc}} \)

This network has 48 reactions with nonzero flux and 227 edges. At Markov time \( t = 7 \) (Fig. 8) this network has a partition into three communities (Fig. 5A):

- **Community \( C_0_{\text{glc}} \) (green)** comprises the intake of glucose and most of the glycolysis and pentose phosphate pathway. The function of the reactions in this community consists of carbon intake and processing glucose into phosphoenolpyruvate (PEP). This community produces essential biocomponents for the cell such as alpha-D-Ribose 5-phosphate (rp5), D-Erythrose 4-phosphate (e4p), D-fructose-6-phosphate (f6p), glyceraldehyde-3-phosphate (g3p) or 3-phospho-D-glycerate (3pg). Other reactions produce energy ATP and have reductive capabilities for catabolism.

- **Community \( C_1_{\text{glc}} \) (red)** contains the TCA cycle at its core. The reactions in this community convert PEP into ATP, NADH and NADPH. In contrast with \( C_0_{\text{glc}} \), there is no precursor formation here. Beyond the TCA cycle, pyruvate kinase (PYK), phosphoenolpyruvate carboxylase (PPC) and pyruvate dehydrogenase (PDH) appear in this community. These reactions highlight the two main carbon intake routes in the cycle: oxalacetate from PEP through phosphoenol pyruvate carboxylase (PPC), and citrate from acetyl coenzyme A (acetyl-CoA) via citrate synthase (CS). Furthermore, both routes begin with PEP, so it is natural for them to belong to the same community along with the rest of the TCA cycle. Likewise, the production of L-glutamate from 2-oxoglutarate (AKG) by glutamate dehydrogenase (GLUDy) is strongly coupled to the TCA cycle.

- **Community \( C_2_{\text{glc}} \) (purple)** contains the electron transport chain which produces the majority of the energy of the cell. In the core \( E. coli \) metabolic model the chain is represented by the reactions NADH dehydrogenase (NADH16), cytochrome oxidase BD (CYTBD) and ATP synthase (ATPS4r). This community also contains associated reactions to the electron transport such as phosphate intake (EXpi(e), PIt2), oxygen intake (EXo2(e), O2t) and proton balance (EXh(e)). This community also includes the two reactions that represent energy maintenance costs (ATPM), and growth (biomass); this is consistent with the biological scenario because ATP is the main substrate for both ATPM, and the biomass reaction.

B.4 Network \( M_{\text{etoh}} \)

This network contains 49 reactions and 226 edges. At Markov time \( t = 6 \) (Fig. 8) this network has a partition into three communities (Fig. 5B):

- **Community \( C_0_{\text{etoh}} \) (green)** in this network is similar to its counterpart in \( M_{\text{glc}} \), but with important differences. For example, the reactions in charge of the glucose intake (EXglc(e) and GLCpts) are no longer part of the network (i.e., they have zero flux), and reactions such as malic enzyme NAPD (ME2) and phosphoenolpyruvate carboxykinase (PPCK), which now appear in the network, belong to this community. This change in the network reflect the cell’s response to a new biological situation. The carbon intake through ethanol has changed the direction of glycolysis into gluconeogenesis \( [1] \) (the reactions in \( C_0_{\text{glc}} \) in Fig. 5A are now operating in the reverse direction in Fig. 5B). The main role of the reactions in this community is the production of bioprecursors such as PEP, pyruvate, 3-phospho-D-glycerate (3PG) glyceraldehyde-3-phosphate (G3P), D-fructose-6-phosphate (F6P), and D-glucose-6-phosphate, all of which are substrates for growth. Reactions ME2 and PPCK also belong to this community due to their production of PYR and PEP. Reactions that were in a different community in \( M_{\text{glc}} \), such as GLUDy and ICDHyr which produce precursors L-glutamate and NADPH respectively, are now part of \( C_0_{\text{etoh}} \). This community also includes the reactions that produce inorganic substrates of growth such as NH\(_4\), CO\(_2\) and H\(_2\)O.

- **Community \( C_1_{\text{etoh}} \) (red)** contains most of the TCA cycle. The main difference between this community and \( C_1_{\text{glc}} \) is that here acetyl-CoA is extracted from acetaldehyde (which comes from ethanol)
by the reaction acetaldehyde dehydrogenase reaction (ACALD), instead of the classical pyruvate from glycolysis. The glyoxylate cycle reactions isocitrate lyase (ICL) and malate synthase (MALS) which now appear in the network, also belong to this community. These reactions are tightly linked to the TCA cycle and appear when the carbon intake is acetate or ethanol to prevent the loss of carbon as CO$_2$.

- Community C$_{2\text{etoh}}$ (purple) contains the electron transport chain and the bulk of ATP production, which is similar to C$_{2\text{glc}}$. However, there are subtle differences that reflect changes in this new scenario. Ethanol intake and transport reactions (EX$_{\text{etoh}(e)}$ and ETOHt2r) appear in this community due to their influence in the proton balance of the cell. In addition, C$_{2\text{etoh}}$ contains NADP transhydrogenase (THD2) which is in charge of NADH/NADPH balance. This reaction is present here due to the NAD consumption involved in the reactions ACALD and ethanol dehydrogenase (ALCD2x), which belong to this community as well.

### B.5 Network M$_{\text{anaero}}$

This network contains 47 reactions and 212 edges. At Markov time $t = 4.4$ (Fig. 8) this network has a partition into three communities (Fig. 5C):

- Community C$_{0\text{anaero}}$ (green) contains the reactions responsible D-glucose intake (EX$_{\text{glc}}$) and most of the glycolysis. The reaction that represents the cellular maintenance energy cost, ATP maintenance requirement (ATPM), is included in this community because of the increased strength of its connection to the substrate-level phosphorilation reaction phosphoglycerate kinase (PGK). Also note that reactions in the pentose phosphate pathway do not belong to the same community as the glycolysis reactions (unlike in M$_{\text{glc}}$ and M$_{\text{etoh}}$).

- Community C$_{1\text{anaero}}$ (red) contains all the reactions in the TCA cycle that are present in the network. These reactions produce two growth precursors are 2-oxalacetate and NADPH. In addition, this community contains reactions CO$_2$t and EX$_{\text{co2}(e)}$ in charge of CO$_2$ transport. The reaction phosphoenolpyruvate carboxylase (PPC) is the only other reaction in this network that consumes CO$_2$.

- Community C$_{2\text{anaero}}$ (purple) includes the biomass reaction and the reactions in charge of supplying it with substrates. These reactions include the pentose phosphate pathway (now detached from C$_{0\text{glc}}$), which produce essential growth precursors such as alpha-D-ribose-5-phosphate (r5p) or D-erythrose-4-phosphate (e4p). The reactions in charge of acetate production (ACKr, ACt2r and EX$_{\text{ac}(e)}$) are also members of this community through the ability of ACKr to produce ATP. Glutamate metabolism reactions GLUDy and GLNS are also included in this community. It is worth mentioning that the reverse of ATP synthase (ATPS4r) is present in this community because here, unlike in M$_{\text{glc}}$, ATPS4r consumes ATP instead of producing it. When this flux is reversed, then ATPS4r is in part responsible for pH homeostasis.

- Community C$_{3\text{anaero}}$ (yellow) includes the main reactions involved in NADH production and consumption, which occurs via glyceraldehyde-3-phosphate dehydrogenase (GAPD). NADH consumption occurs in two consecutive steps in ethanol production: in ACALD and ALCD2x. The phosphate intake and transport reactions EX$_{\text{pi}(e)}$ and Pi2r belong to this community because most of the phosphate consumption takes place at GAPD.

- Community C$_{4\text{anaero}}$ (light blue) contains the conversion of PEP into formate through the sequence of reactions PYK, PFL, FORt1 and EX$_{\text{for}(e)}$. More than half of the carbon secreted by the cell becomes formate.
B.6 Network $M_{\text{lim}}$

This network has 52 nodes and 228 edges. At Markov time $t = 13$ this network (Fig. 8) has a partition into three communities (Fig. 5D):

- Community $C_0_{\text{lim}}$ (green) contains the glycolysis pathway (detached from the pentose phosphate pathway). This community is involved in precursor formation, ATP production, substrate-level phosphorylation and processing of D-glucose into PEP.

- Community $C_1_{\text{lim}}$ (red) is the community that differs the most from those in the other aerobic growth networks ($M_{\text{glc}}$ and $M_{\text{etoh}}$). This community gathers reactions that under normal circumstances would not be so strongly related but that the limited availability of ammonium and phosphate have forced together; its members include reactions from the TCA cycle, the pentose phosphate pathway, nitrogen metabolism and by-product secretion. The core feature of the community is carbon secretion as formate and acetate. Reactions PPC, malate dehydrogenase (MDH) reverse and ME2 channel most of the carbon to the secretion routes in the form of formate and acetate. The production of L-glutamine seems to be attached to this subsystem through the production of NADPH in ME2 and its consumption in the glutamate dehydrogenase NAPD (GLUDy).

- Community $C_2_{\text{lim}}$ (blue) contains the bioenergetic machinery of the cell; the main difference to the previous scenarios is that the electron transport chain has a smaller role in ATP production (ATPS4r), and substrate-level phosphorylation (PGK, PYK, SUCOAS, ACKr) becomes more important. In $M_{\text{lim}}$ the electron transport chain is responsible for the 21.8% of the total ATP produced in the cell while in $M_{\text{glc}}$ it produces 66.5%. The reactions in charge of intake and transport of inorganic ions such as phosphate ($\text{EXpi}(e)$ and $\text{Plt2}(r)$), $O_2$ ($\text{EXO}_2(e)$ and $O_2t$) and $H_2O$ ($\text{EXH}_2O$ and $H_2Ot$) belong to this community as well. This community includes the reactions in the pentose phosphate pathway that produce precursors for growth: transketolase (TKT2) produces e4p, and ribose-5-phosphate isomerase (RPI) produces r5p.

References

1. Berg J, Tymoczko J, Stryer L (2002) Biochemistry, Fifth Edition. W. H. Freeman.
2. Wagner A, Fell DA (2001) The small world inside large metabolic networks. Proc R Soc Lond B 268: 1803–1810.
3. Barabási AL, Oltvai ZN (2004) Network biology: understanding the cell’s functional organization. Nature Reviews Genetics 5: 101-113.
4. Girvan M, Newman MEJ (2002) Community structure in social and biological networks. Proceedings of the National Academy of Sciences 99: 7821-7826.
5. Guimerá R, Nunes Amaral LA (2005) Functional cartography of complex metabolic networks. Nature 433: 895-900.
6. Winterbach W, Mieghem PV, Reinders M, Wang H, de Ridder D (2013) Topology of molecular interaction networks. BMC Systems Biology 7: 90.
7. Palsson BO (2006) Systems Biology: Properties of Reconstructed Networks. New York, NY, USA: Cambridge University Press.
8. Almaas E, Kovács B, Vicsek T, Oltvai ZN, Barabási AL (2004) Global organization of metabolic fluxes in the bacterium escherichia coli. Nature 427: 839–843.
9. Colijn C, Brandes A, Zucker J, Lun DS, Weiner B, et al. (2009) Interpreting expression data with metabolic flux models: Predicting mycobacterium tuberculosis mycolic acid production. PLoS Comput Biol 5: e1000489.
23

10. Becker SA, Palsson BO (2008) Context-specific metabolic networks are consistent with experiments. PLoS Comput Biol 4: e1000082.
11. Tobalina L, Bargiela R, Pey J, Herbst FA, Lores I, et al. (2015) Context-specific metabolic network reconstruction of a naphthalene-degrading bacterial community guided by metaproteomic data. Bioinformatics 31: 1771-1779.
12. Orth J, Fleming R, Palsson B (2010) Reconstruction and use of microbial metabolic networks: the core escherichia coli metabolic model as an educational guide. EcoSal Plus.
13. Feist AM, Henry CS, Reed JL, Krummenacker M, Joyce AR, et al. (2007) A genome-scale metabolic reconstruction for escherichia coli k-12 mg1655 that accounts for 1260 orfs and thermodynamic information. Molecular systems biology 3: 121.
14. Flahaut NAL, Wiersma A, van de Bunt B, Martens DE, Schaap PJ, et al. (2013) Genome-scale metabolic model for lactococcus lactis MG1363 and its application to the analysis of flavor formation. Applied Microbiology and Biotechnology 97: 8729-8739.
15. Orth JD, Thiele I, Palsson B (2010) What is flux balance analysis? Nature Biotechnology 28: 245-248.
16. McCloskey D, Palsson B, Feist AM (2013) Basic and applied uses of genome-scale metabolic network reconstructions of escherichia coli. Molecular Systems Biology 9: 661.
17. Papin JA, Price ND, Wiback SJ, Fell DA, Palsson BO (2003) Metabolic pathways in the post–genome era. Trends in Biochemical Sciences 28: 250–258.
18. Folch-Fortuny A, Tortajada M, Prats-Montalbán JM, Llaneras F, Picó J, et al. (2015) MCR–ALS on metabolic networks: Obtaining more meaningful pathways. Chemometrics and Intelligent Laboratory Systems 142: 293–303.
19. Rabinowitz JD, Vastag L (2012) Teaching the design principles of metabolism. Nat Chem Biol 8: 497–501.
20. Chellaboina V, Bhat SP, Haddad WM, Bernstein DS (2009) Modeling and analysis of mass-action kinetics. IEEE Control Systems 29: 60-78.
21. Ma H, Zeng AP (2003) Reconstruction of metabolic networks from genome data and analysis of their global structure for various organisms. Bioinformatics 19: 270-277.
22. Silva MRd, Sun J, Ma H, He F, Zeng AP (2008) Metabolic networks. In: Junker BH, Schreiber F, editors, Analysis of Biological Networks, John Wiley & Sons, Inc. pp. 233–253.
23. Samal A, Martin OC (2011) Randomizing genome-scale metabolic networks. PLoS ONE 6: e22295.
24. Croes D, Couche F, Wodak SJ, van Helden J (2006) Inferring meaningful pathways in weighted metabolic networks. Journal of Molecular Biology 356: 222-236.
25. King ZA, Drğer A, Ebrahim A, Sonnenschein N, Lewis NE, et al. (2015) Escher: A web application for building, sharing, and embedding data-rich visualizations of biological pathways. PLoS Comput Biol 11: 1-13.
26. Schuster S, Fell DA, Dandekar T (2000) A general definition of metabolic pathways useful for systematic organization and analysis of complex metabolic networks. Nat Biotechnol 18: 326–332.
27. Schilling CH, Letscher D, Palsson BO (2000) Theory for the systemic definition of metabolic pathways and their use in interpreting metabolic function from a pathway-oriented perspective. J Theor Biol 203: 229–248.
28. Delvenne JC, Yaliraki S, Barahona M (2010) Stability of graph communities across time scales. Proc Nat Acad Sci USA 107: 12755-12760.
29. Delvenne JC, Schaub MT, Yaliraki SN, Barahona M (2013) The stability of a graph partition: A dynamics-based framework for community detection. In: Mukherjee A, Choudhury M, Peruani F, Ganguly N, Mitra B, editors, Dynamics On and Of Complex Networks, Volume 2, Springer New York, Modeling and Simulation in Science, Engineering and Technology. pp. 221-242.
30. Schaub MT, Delvenne JC, Yaliraki SN, Barahona M (2012) Markov dynamics as a zooming lens for multi-scale community detection: non clique-like communities and the field-of-view limit. PLoS ONE 7: e32210.
31. Beguerisse-Díaz M, Garduño Hernández G, Vangelov B, Yaliraki SN, Barahona M (2014) Interest communities and flow roles in directed networks: the Twitter network of the UK riots. J R Soc Interface 11.

32. Lambiotte R, Delvenne J, Barahona M (2014) Random walks, markov processes and the multiscale modular organization of complex networks. Network Science and Engineering, IEEE Transactions on 1: 76-90.

33. Vemuri GN, Eiteman MA, McEwen JE, Olsson L, Nielsen J (2007) Increasing nadh oxidation reduces overflow metabolism in saccharomyces cerevisiae. Proc Natl Acad Sci U S A 104: 2402–2407.

34. Bacik KA, Schaub MT, Beguerisse-Díaz M, Billeh YN, Barahona M (2015) Flow-based network analysis of the caenorhabditis elegans connectome. arXiv:151100673 .

35. Rosvall M, Bergstrom CT (2010) Mapping change in large networks. PLoS ONE 5: e8694.

36. Csermely P, Ágoston V, Pongor S (2005) The efficiency of multi-target drugs: the network approach might help drug design. Trends in Pharmacological Sciences 26: 178 - 182.

37. Cantor JR, Sabatini DM (2012) Cancer cell metabolism: one hallmark, many faces. Cancer Discov 2: 881–898.

38. Galluzzi L, Kepp O, Vander Heiden MG, Kroemer G (2013) Metabolic targets for cancer therapy. Nat Rev Drug Discov 12: 829–846.

39. Chang RL, Xie L, Xie L, Bourne PE, Palsson BO (2010) Drug off-target effects predicted using structural analysis in the context of a metabolic network model. PLoS Comput Biol 6: e1000938.

40. Kivelä M, Arenas A, Barthelemy M, Gleeson JP, Moreno Y, et al. (2014) Multilayer networks. Journal of Complex Networks 2: 203-271.

41. Covert MW, Xiao N, Chen TJ, Karr JR (2008) Integrating metabolic, transcriptional regulatory and signal transduction models in escherichia coli. Bioinformatics 24: 2044–2050.

42. Mahadevan R, Edwards JS, Doyle FJ 3rd (2002) Dynamic flux balance analysis of diauxic growth in escherichia coli. Biophys J 83: 1331–1340.

43. Fell DA (2005) Enzymes, metabolites and fluxes. Journal of Experimental Botany 56: 267-272.

44. Feist AM, Palsson BO (2010) The biomass objective function. Current Opinion in Microbiology 13: 344-349.

45. Schuetz R, Kuepfer L, Sauer U (2007) Systematic evaluation of objective functions for predicting intracellular fluxes in escherichia coli. Molecular Systems Biology 3.

46. Page L, Brin S, Motwani R, Winograd T (1999) The PageRank citation ranking: Bringing order to the web. Technical Report 1999-66, Stanford InfoLab. URL http://ilpubs.stanford.edu:8090/422/ Previous number = SIDL-WP-1999-0120.

47. Blondel VD, Guillaume JL, Lambiotte R, Lefebvre E (2008) Fast unfolding of communities in large networks. Journal of Statistical Mechanics: Theory and Experiment 2008: P10008.

48. Meila M (2007) Comparing clusterings: an information based distance. Journal of Multivariate Analysis 98: 873 - 895.