INVITED PAPER

Metabolic Networks, Microbial Consortia, and Analogies to Smart Grids

This tutorial article introduces approaches to predict fluxes in chemical reaction networks inside living cells, with an emphasis on similarities to (smart) electrical grids.

By Axel Theorell and Jörg Stelling

ABSTRACT | Metabolic network analysis is an accessible and versatile modeling approach for biology that has taken much inspiration from electric circuit analysis. After introducing its main concepts, we focus on numerical tools, such as optimization and sampling, to predict cellular features and behaviors at a large scale. Optimization approaches exploit that metabolic networks are shaped by evolution and are, thus, assumed to embed a fitness condition reflecting the environment that they evolved in. In the past ten years, there is a trend to generalize metabolic network analysis to consortia of interacting species. This raises technical questions on, for example, optimality in consortia but also more general ones on metabolic coevolution, information exchange, and adaptation. This suggests and allows us to explore interesting analogies to technological systems, specifically to smart grids.

KEYWORDS | Computational systems biology; mathematical programming; molecular communication (telecommunication); Monte Carlo methods; smart grids.

I. INTRODUCTION

Metabolism refers to the set of (bio)chemical reactions operating on chemical compounds (metabolites) that sustain life in biological cells and organisms. It converts food to energy and chemical building blocks, which are used to compose cellular building blocks, such as proteins, and ultimately enable self-replication and the generation of new biomass. Metabolism and its conceptualizations, therefore, have a central role in the life sciences [1]–[3].

Metabolism is commonly conceptualized via metabolic networks, that is, complex collections of thousands of connected chemical reactions acting as devices. Like other scientific abstractions, such as coordinate systems or electric circuit diagrams, we hypothesize that the concept of metabolic networks significantly influences how scientists and engineers think about their objects of study. Metabolic networks are not merely used for the visualization and qualitative analysis of metabolism, but the study of metabolism has benefited from an ecosystem of formal and quantitative analysis methods for a long time.

More than being common scientific abstractions, metabolic networks and electric circuit diagrams, which conceptualize flows (of matter and current, respectively) on graphs, share some important attributes, In particular, domain-specific versions of Kirchhoff's first and second laws are important in both abstractions [4], [5]; in this review, we will use the laws as starting points for conveying the concepts of metabolic network analysis to readers beyond its core enthusiasts. Yet, analogies between metabolic networks and electric circuits continue beyond modeling the metabolism of a single organism. Conceptually, smart grids are circuits of circuits, and network representations of communities of biological organisms that interact via their metabolisms are networks of networks. In both cases, the generalization from one to many introduces intriguing new challenges in terms of the decision-making of distributed agents and internal or environmental influences on collective behaviors.

In this article, we review the field of metabolic network analysis and its recent developments in characterizing communities of organisms. Distinct from the many existing reviews [3], [6]–[8], we focus on the mathematical

Manuscript received July 21, 2021; revised November 11, 2021; accepted February 27, 2022. Date of publication March 23, 2022; date of current version May 19, 2022. This work was supported by the Swiss National Science Foundation Sinergia Project under Grant 177164. *(Corresponding author: Jörg Stelling.)*

The authors are with the Department of Biosystems Science and Engineering and the SIB Swiss Institute of Bioinformatics, ETH Zürich, 4058 Basel, Switzerland (e-mail: axel.theorell@bsse.ethz.ch; joerg.stelling@bsse.ethz.ch).

Digital Object Identifier 10.1109/JPROC.2022.3158396

methodology employed in metabolic network analysis and its parallels with, as well as differences to methodologies in electrical and information engineering. Thereby, we aim to make the text accessible to readers outside the life sciences who, through their theoretical and computational expertise, may make valuable contributions to the field. For example, we detail optimization and Monte Carlo sampling problems frequently generated by applications, as well as decision-making problems that arise in going from metabolic networks of single organisms to communities of metabolically interacting organisms. In the other direction, we argue that important distinctions between living and engineered systems exist. This has led to problem definitions, theoretical concepts, and computational approaches that could be interesting for "traditional" engineering disciplines.

More specifically, we focus on quantitative methods for predicting and determining the so-called metabolic fluxes, the rates of (bio)chemical reactions, in a metabolic network. Flux prediction is a ubiquitous problem in applications. For example, in metabolic engineering, which aims to design organisms for efficient biotechnological processes, fluxes are the central entity ultimately determining the value-generating yields [9], [10]. Fluxes are also targets for interventions in medical research, such as the development of antibiotics [2] and cancer research [1].

To limit the scope, we assume that metabolic network models, mostly constructed from genome sequences, are given. Briefly, in the reconstruction process, sections of the genome are compared to a list of sequences of enzymes that catalyze known chemical reactions. If a match is found, the chemical reaction is added to the network. To ensure high quality, the resulting draft reconstruction undergoes a sequence of curation steps [11]. For details on metabolic network reconstruction, we refer to recent reviews [3], [8], [12], [13].

Even with an established model, as detailed in the following, the metabolic flux analysis poses challenging problems because of the peculiarities of biological systems. For example, observability is often limited: fluxes occurring inside a cell (intracellular fluxes) are, in general, not measurable directly such that modeling is required if their magnitude is to be estimated or predicted. Another important, complicating factor is uncertainty in biological systems [14]. Ultimately, specific metabolic fluxes emerge as a consequence of the extracellular environment and the abundance of metabolites and active enzymes. The process of how a cell controls the fluxes to keep functioning despite changes in the environment is called metabolic regulation. However, in contrast to engineered systems, the structure and parameters of the corresponding control circuits are often unknown. This spurred the development of a large variety of methods to determine metabolic fluxes without direct references to control.

Section II introduces key elements of metabolic networks, such as metabolic fluxes, kinetics, and thermodynamics more formally. The computational methods

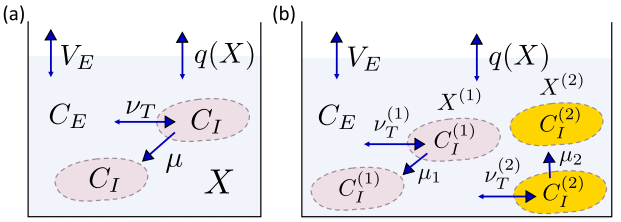

Fig. 1. *Overview of mass flows in hypothetical cell cultures. (a) Culture of cells of a single biological species (pink ellipses) with mass exchanges (arrows). V_E is a vector of metabolite influxes and effluxes of the culture, q(X) is the biomass efflux ("washout"), ν_T is a vector of transport fluxes crossing the cell boundary, C_E and C_I are vectors of concentrations of metabolites outside and inside the cells, respectively, μ is the rate at which cells (biomass) produce biomass, and X is the concentration of biomass. Large arrow heads indicate the primary reaction directions. (b) Generalization to a coculture of two species (pink and yellow ellipses), where the symbols ν_T, C_I, and X become species-specific (numerical superscripts). The coculture opens the possibility of cross-feeding of compounds via the culture medium and transport reactions.*

described in Section III for predicting metabolic fluxes were mainly developed for single biological species. Section IV proposes conceptual extensions to interacting species, which suggests interesting parallels to smart grids and multilevel control problems more generally. Overall, we focus on concepts and theory; to complement our highlighted applications, we refer to recent reviews [8], [15].

II. METABOLIC NETWORKS

Metabolic networks describe metabolism, which is a process taking place in cells residing in cell culture. Thus, to introduce metabolic networks and their associated methods, we start by describing a generic cell culture hosting a metabolic process.

A. Cells in Culture

Fig. 1(a) shows a schematic culture of one type of cells, e.g., of a single species of microbes. Metabolites enter and exit the culture via $N_{M,E}$ exchange reactions; their fluxes are given by the vector $V_E \in \mathcal{R}^{N_{M,E}}$ in concentration units (e.g., mol/l) per time. Exchanges influence the concentrations of metabolites in the culture medium, $C_E \in \mathcal{R}_+^{N_{M,E}}$. Cells may take up metabolites from the medium via $N_{\nu,T}$ transport reactions with fluxes $\nu_T \in \mathcal{R}^{N_{\nu,T}}$ in concentration units per time and biomass concentration. Observe that V_E and ν_T have different units because the amount of transport depends on the concentration of biomass, $X \in \mathcal{R}_+$. The transport matrix $T \in \mathcal{R}^{N_{M,E} \times N_{\nu,T}}$ maps the compounds transported by ν_T to their corresponding extracellular compounds C_E. The biomass concentration is enriched by self-replication at the rate $\mu \in \mathcal{R}$ but also changes over time by processes such as cell death and cells being washed out of the culture, given by the function $q(X) \in \mathcal{R}$. Assuming that mass is conserved, we get a system of ordinary differential equations (ODEs) describing the time development of the extracellular metabolite and

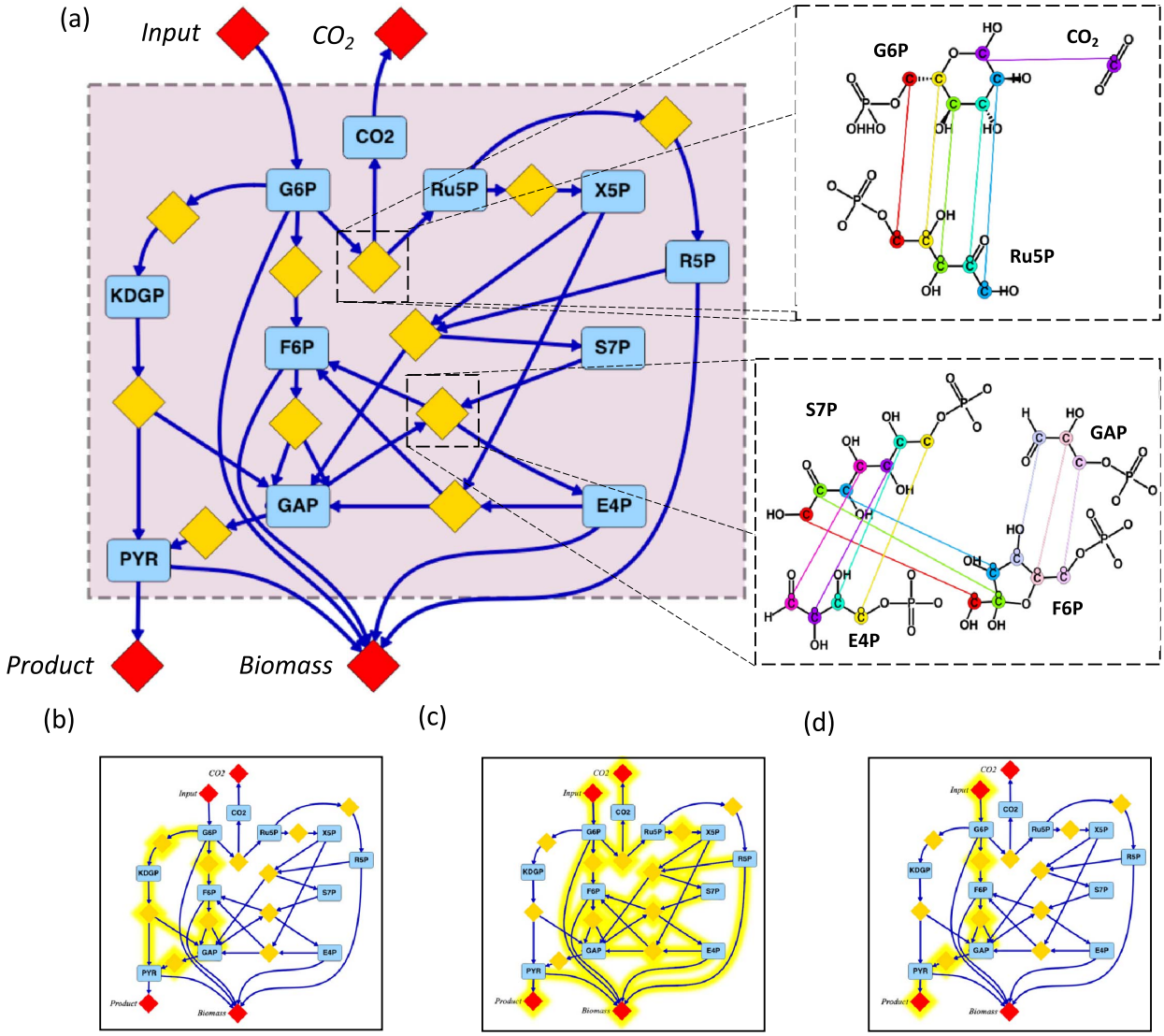

Fig. 2. *Example metabolic network with atom-mappings and EFMs. (a) Stoichiometry describing how 15 reactions (diamonds) convert 11 metabolites (rectangles) in a graph structure. Yellow diamonds represent reactions that conserve the number of carbon atoms (intracellular reactions), and red diamonds represent reactions that exchange carbon atoms across the system boundary (exchange reactions). All intracellular reactions are a priori bidirectional; they may proceed backward (against the indicated arrows). The exchange reactions are unidirectional. The two zooms on the right show how individual carbon atoms are passed between specific positions of the carbon backbone of the molecules in the reactions. (b)–(d) Examples of EFMs (minimal subnetworks that may carry a nonzero flux) of the network. The graphic was created using Omix [16].*

biomass concentrations

$$\frac{dC_E}{dt} = -V_E - X \cdot T\nu_T \tag{1}$$

$$\frac{dX}{dt} = X\mu - q(X). \tag{2}$$

The minus signs in (1) are due to the primary directions chosen for the reactions [see Fig. 1(a)].

B. Fluxes in Metabolic Networks

Similar mass balances are also upheld in the interior of the cell. How the reactions in the cell interrelate is what is described by a metabolic network. Formally, the metabolic network is a hypergraph, which shows how metabolites (nodes) can be converted into each other by reactions (hyperedges).

Fig. 2 shows a simplified metabolic network inspired by central metabolic pathways (Entner–Doudoroff, Embden–Meyerhof–Parnas, and pentose phosphate pathways) in the bacterium *Escherichia coli* [17]. In the figure, the hyperedges have been enhanced with diamond symbols that clarify which arrows belong together. The four red diamonds represent reactions that exchange material with the outside of the cell and correspond to ν_T fluxes. The *Input* reaction is an uptake of nutrients that the cell catabolizes

to gain energy and building blocks for synthesizing new compounds. The CO_2 reaction is an example of a biproduct secretion and the *Product* reaction indicates that the cell can produce a compound that, for example, could be of biotechnological interest. In the example, the product reaction emanates from the metabolite PYR (pyruvate). Rather than just producing pyruvate, we chose the product reaction to represent a large range of compounds that are derived from pyruvate. Fundamentally, cells are self-replicating entities. The *Biomass* reaction, corresponding to μ in Fig. 1, takes the precursors of the molecules corresponding to one unit of biomass to represent the cell growth process. How much of each precursor metabolite is needed is based on measurements of the molecular composition of the cell [18].

We now present the mass balance equation for the $N_{M,I}$ intracellular metabolites with concentrations $C_I \in \mathcal{R}_+^{N_{M,I}}$. For this, we need the vector of metabolic fluxes $\nu \in \mathcal{R}^{N_\nu}$ (with units concentration per time and biomass concentration), which includes the transport fluxes ν_T and the biomass flux μ (despite its deviating unit). The so-called stoichiometric matrix, $S \in \mathcal{R}^{N_{M,I} \times N_\nu}$, governs how the material is transported through the network. An entry $s_{h,l}$ of S determines how much of metabolite h reaction l produces ($s_{h,l} > 0$) or consumes ($s_{h,l} < 0$) per unit of flux. Regular reactions consume or produce whole molecules, implying that entries in S corresponding to regular reactions are integer-valued. The biomass production reaction is not a regular reaction because it lumps together several regular reactions and produces a composite (biomass) rather than defined molecules; it has noninteger entries in S. By combining the elements to form mass balances and assuming constant density (due to time-scale separation), the time development of the intracellular metabolite concentrations is described by the system

$$\frac{dC_I}{dt} \cdot \frac{1}{X} = S\nu. \tag{3}$$

Equation (3) describes how $N_{M,I}$ potentially measurable time courses of metabolite concentrations relate to N_ν time courses of nonmeasurable fluxes. Apart from time-varying fluxes, a particular challenge for metabolic network analysis is that $N_\nu \gg N_{M,I}$. For the stationary analysis, this implies dealing with underdetermined systems (see the following).

C. Steady States and Constraints

To reduce the complexity of this dynamic system (with respect to fluxes), (3) is combined with further assumptions. Based on arguments of time-scale separations, one common fundamental assumption is the metabolically stationary state (or steady state), meaning that fluxes ν and intracellular metabolite concentrations C_I are time constant. Investigating the steady state is interesting because it presumably describes the long-term (cell and time) average behavior of cell culture. Under the steady state assumption, (3) reduces to the linear homogeneous, underdetermined system that is referred to as the stoichiometric constraints

$$0 = S\nu. \tag{4}$$

This is analogous to Kirchhoff's first circuit law: The sum of currents (fluxes) entering a node equals the sum of currents (fluxes) exiting that node.

For metabolic networks, the solution space of (4) is called the feasible flux space; geometrically, it is a polytope (referred to as flux polytope). Network couplings induced by stoichiometry directly constrain this space because they reduce the number of degrees of freedom of fluxes by the rank of the stoichiometric matrix. In our example in Fig. 2, $\text{rank}(S) = 11$, implying that (4) reduces the dimensionality of the feasible flux space from 15 to 4.

Apart from the stoichiometry and regardless of whether metabolism is assumed to be in steady state or not, the feasible ranges of fluxes are restricted by N_C so-called capacity constraints. For a matrix $B \in \mathcal{R}^{N_C \times N_\nu}$ and vector $b \in \mathcal{R}^{N_C}$, we impose

$$B\nu \leq b. \tag{5}$$

On the one hand, to ease computations by restraining the fluxes from attaining infinite values, upper and lower bounds for all fluxes are often introduced. They are intentionally chosen to be orders of magnitude wider than any physiologically realistic flux. On the other hand, capacity constraints can ensure that fluxes or combinations of fluxes remain in physiologically relevant ranges. The difficulty here is that we do not know what an accurate physiological range for most intracellular fluxes is. However, some extracellular fluxes and the biomass flux are directly measurable and may be provided with (condition-specific) bounds reflecting the uncertainty in these measurements. In addition, thermodynamics knowledge of a reaction may be used to constrain the direction of a reaction (see Section II-D).

Apart from these single flux capacity constraints, mechanistic hypotheses restraining weighted sums of fluxes have been proposed and tested [19]. To increase the value of flux, an increase in the concentration of a corresponding enzyme with a specific size, efficiency, and *cost* in terms of synthesis from precursors is needed. Since the cell has a finite (and roughly known) volume and a known density, there is a limit on how crowded the cell can be in terms of the total number of enzymes, weighted by size. Via the efficiencies of the enzymes, this limits the total sum of fluxes [20]. Instead of focusing on cell density, we can consider that cells are self-replicating entities, and the metabolic machinery facilitating the self-replication must be synthesized too. Thus, large fluxes through reactions catalyzed by expensive enzymes operating at low efficiency

will lead to a large metabolic burden for the cell. This motivates the idea that the sum of fluxes may be restricted in terms of the total metabolic cost of synthesizing the enzymes that facilitate these fluxes [21], [22].

D. Thermodynamics

To derive capacity constraints from first principles, we can exploit that, as all processes, the metabolic machinery is driven by thermodynamic potential energy. Each reaction ν_l is associated with a change in Gibbs free energy, $\Delta_r G'_l$. Although many reactions proceed both in the forward and backward directions at the same time (bidirectional flux), the net flux always proceeds in the direction that yields a lower potential energy ($\Delta_r G'_l$ is negative). $\Delta_r G'_l$ depends on temperature, the relative concentration of the reactants, and (condition-dependent) molecular properties of the reactants. The part relating to molecular properties is referred to as the standard Gibbs reaction energy, $\Delta_r G'^{\circ}_l$. In most cases, values for $\Delta_r G'^{\circ}_l$ are available either in databases [23] or via estimation [24]. We restrict attention to reactions operating fully in the interior of the cell with their corresponding stoichiometric matrix $S_I \in \mathcal{R}^{N_{M,I} \times N_{\nu,I}}$, which is a submatrix of S. Denoting the gas constant by R, temperature by T, and the transpose of the stoichiometric matrix by S_I^T, the vector of reaction energies $\Delta_r G'$ depends on the vector $\Delta_r G'^{\circ}$ via

$$\Delta_r G' = \Delta_r G'^{\circ} + R \cdot T \cdot S_I^T \cdot \ln(C_I). \tag{6}$$

Since $\Delta_r G'$ depends on the logarithm of the metabolite concentrations, $\ln(C_I)$, and these concentrations are condition-dependent, it is *a priori* unknown in which direction a reaction proceeds. Also, applying the deceptively simple equation to biochemistry is not straightforward because correct concentration units and potentially chemical activities need to be considered [25]. However, in cases where a standard reaction energy $\Delta_r G'^{\circ}_l$ is so far from equilibrium (far from zero) that, from (6), it can be concluded that the sign of $\Delta_r G'$ will be constant for all physiologically relevant metabolite concentrations, we can assume that the reaction always proceeds in one direction. Such reactions are referred to as unidirectional reactions, as opposed to bidirectional reactions, and are often equipped with directionality constraints (≥ 0) in computations.

With the introduction of thermodynamics comes Kirchhoff's second circuit law (the loop law), which states that the sum of voltages around any closed loop is zero. Analogously, the reaction energies $\Delta_r G'$ around any closed loop in a metabolic network have zero sum. A corollary to the loop law is that the fluxes in a metabolic network can, in general, not proceed in cycles [5]. This corollary can be applied, even if quantitative thermodynamics is not considered explicitly. Fig. 2(b) highlights a loop in the example network. From the stoichiometric constraints alone [see (4)], flux around the indicated cycle is feasible. To enforce the loop law explicitly, additional integer constraints are necessary (detailed in Section III-B) [26].

E. Kinetics

Reaction kinetics functionally relate fluxes (ν) to states (C). For the analysis of engineered systems, such relations are known, which is in stark contrast to biological systems where, often, even the functional form is unknown. Even with the increasing availability of experimental data for inference, kinetic modeling of metabolism remains challenging. We refer to recent reviews, such as [27], for details and focus on kinetics of extracellular transport because they are critical for metabolic exchanges in communities of cells.

For single-species cultivations, one can estimate transport fluxes from changes in extracellular metabolite concentrations and constrain transport fluxes ν_T in (5). For transport fluxes not constrained this way, one needs to specify fluxes as a function of the extracellular concentrations C_E because active (via dedicated transport proteins) and passive (via diffusion) transports are generally considered to depend on them. For active transport, assuming that the index l refers to the same compound for ν_T and C_E, most commonly irreversible Michaelis–Menten kinetics are assumed [28]

$$\nu_{T,l} = \frac{\nu_{\max,l} \cdot C_{E,l}}{K_l + C_{E,l}} \tag{7}$$

with uptake rate of compound l, ν_l, concentration $C_{E,l}$, and two parameters for maximal uptake rate, $\nu_{\max,l}$, and transporter affinity, K_l. Apart from being a vital link that limits intracellular metabolism to the reality of the nutrient supply, (7) is also a source of challenges and uncertainty for computational methods because the values of the two parameters are often unknown (and flux specific). A computationally favorable feature of (7) is that it is monotone in $C_{E,l}$. However, that does not always have to be the case. For example, approximately 20% of enzymes possess nonmonotone substrate inhibition kinetics, where fluxes increase for low substrate concentrations, but decline again for high concentrations [29]. A key aim for metabolic network analysis is, therefore, to predict metabolic behaviors without having to specify (all) reaction kinetics.

III. COMPUTATIONAL METHODS

In biology, model-based computer experiments are a lot cheaper and faster to perform than exhaustive testing on living organisms in laboratory experiments. Therefore, having once reconstructed a metabolic network of an organism from its genome, it is often conducive to retrieve as much relevant information as possible using computational methods.

Here, we distinguish between: 1) structural methods to characterize all feasible fluxes in a network (leading to convex analysis problems); 2) optimization methods for

Table 1 Seminal and Summarizing Papers of Computational Techniques

Computational Technique	Short Description	Reference
Elementary Flux Modes	Minimal functional pathways of metabolic network	[30], [31]
Minimal Cut Sets	Minimal interventions to disable a target functionality of a network	[32]
Flux Balance Analysis	Metabolic fluxes of metabolic network predicted using (steady state) constrained optimization	[33], [34]
Dynamic Flux Balance Analysis	Time resolved metabolic fluxes of metabolic network predicted using (quasi steady state) constrained optimization	[35]
Multi species Flux Balance Analysis	Flux Balance Analysis extended to conserve metabolite mass balances across species	[36]
Flux Variability Analysis	Explore (flux wise) ranges in which metabolic objective may be achieved	[37], [38]
Flux Sampling	Characterize feasible (steady state) flux space by (uniform) sampling	[39], [40]
^{13}C Metabolic Flux Analysis	Model based inference of metabolic fluxes from measured ^{13}C enrichment in metabolites	[41], [42]

predicting fluxes in a specific condition (leading to complex optimization problems); 3) probabilistic methods with the same aim (leading to sampling problems); and 4) flux inference methods to estimate nonmeasurable intracellular fluxes from condition-specific measurements and models (leading to identification problems). To facilitate navigating the literature for the computational methods discussed, we compiled Table 1.

A. Structural Methods

The structural analysis facilitates the exploration of all capabilities of a metabolic network (at metabolically stationary state). Its use ranges from investigating fundamental scientific questions in biology [43] to biotechnological applications, for example, to increase product yields [44].

The associated methods directly interrogate the network structure as represented by the stoichiometric matrix S [45], [46]. However, metabolic networks are hypergraphs (e.g., because a reaction may use more than one reactant, see examples in Fig. 2), which hinders the analysis using regular graph theory [47]. It is possible to increase the resolution of a metabolic network and trace single atoms (see zoom in Fig. 2) or conserved chemical moieties rather than whole molecules. This turns the network into a regular graph (with some exceptions) but significantly increases network size [48] and requires additional constraints on fluxes, for example, to avoid unwanted molecule cleavage. In general, engineered analogs to metabolic networks are electronic circuits with active components, requiring approaches beyond regular graph theory.

A typical problem is to decompose the network into subunits, for example, to answer the long-standing question if biology is modular akin to engineered systems [49]. There, metabolic networks provide an additional challenge: S is usually sparse but not (block-)diagonalizable because common chemical energy, and other "currencies" introduce dense rows to S [48]. In terms of general analysis methods, for example, transferring hypergraph partitioning approaches in integrated circuit chip design, especially for very large scale integration (VLSI) [50], to metabolism seems promising.

To decompose the network into minimal (overlapping) *functional* subunits, the structural analysis of metabolic networks uses application-specific methods. Chronologically, the first important structural approach involved so-called elementary flux modes (EFMs) [30], [31]. By separating all bidirectional fluxes into forward and backward fluxes, all flux variables are made nonnegative. This irreversible space, in combination with *homogeneous* stoichiometry and capacity constraints [see (4) and (5) with $b = 0$], is a pointed convex polyhedral cone (the flux cone). Its extreme rays (see Fig. 3(a) illustrating the geometry) are feasible flux distributions with minimal support, in the sense that setting any nonzero flux to zero forces an all-zero solution. Fig. 2(b)–(d) shows examples of EFMs. The EFM in Fig. 2(d) is of hypothetical biotechnological interest—it uses all input for product generation. In contrast, the EFM in Fig. 2(c) produces both biomass and the byproduct CO_2. In the long run, some production of biomass is necessary to keep a cell culture healthy. Hence, a goal for metabolic engineering could be to balance the two EFMs for a healthy culture that generates a product at a high yield.

Importantly, EFMs are dual to (minimal) cut sets, here defined as (minimal) reaction sets that need to be jointly blocked to deactivate a target metabolic function [32]. Such "failure modes" are familiar to engineers in reliability analysis, and cut sets specifically have gained renewed interest in the analysis of decentralized power grids [51], indicating potential synergies of the domains.

However, the original formulation of EFMs does not admit inhomogeneous constraints. The recent generalization of EFMs to elementary flux vectors (EFVs) addresses this limitation [46]. Furthermore, although high-performance algorithms for enumerating EFMs exist [52], capable of enumerating billions of EFMs, a fundamental challenge of EFMs is that their number increases combinatorially with the network size [53]. Therefore, recent efforts in structural analysis focused on developing pathway concepts that reduce the number of modes. For example, when investigating which chemicals a network can produce from which substrates, instead of enumerating the EFMs or EFVs, it suffices to enumerate the elementary conversion modes (ECMs). ECMs are a minimal set of metabolite conversions that span the space of possible metabolite conversions using positive

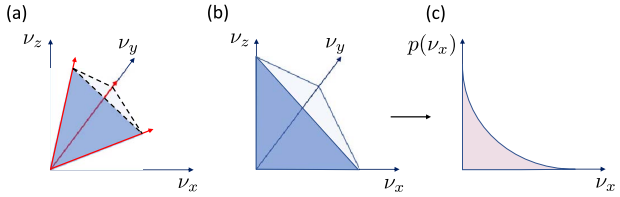

Fig. 3. *Geometry of flux spaces. (a) Hypothetical flux cone in three dimensions. The red arrows are the extreme rays of the cone. The dashed line indicates that the cone may be limited from above (bounded cone). (b) and (c) Given a uniform probability distribution on a simplex in three dimensions (b), the emerging marginal distribution in ν_x declines quadratically with increasing flux values.*

coefficients [54], [55]. The number of ECMs is much smaller than the set of EFMs since, in general, one metabolite conversion can be mediated by many intracellular pathways. In another concept, minimal pathways (MPs), the network is first divided into (user-defined) enumerated fluxes and nonenumerated fluxes. Under constraints such as requiring a certain growth rate, MPs are support minimal combinations of the enumerated fluxes that allow for feasible solutions in the complete network (including nonenumerated fluxes) [56]. Analogously to ECMs, by not elaborating which path the flux takes over the nonenumerated fluxes, pathway numbers may be reduced greatly. Contrary to ECMs, MPs are not designed to span a specific space, and they allow to incorporate capacity constraints, resulting in a bounded cone [see Fig. 3(a)].

Given the link between minimality and cut sets, EFMs and other pathway definitions are interesting for engineering applications. For example, a typical problem of bioprocess design is the tradeoff between biomass and product yield. Minimal cuts sets can be used to achieve strict coupling of the two. A recent study achieved a commercially viable strain design for the production of the pigment indigoidine by 14 simultaneous gene knockouts predicted through MCSs [57].

B. Optimization Methods

Predicting fluxes for specific, given conditions faces the challenge of underdetermined systems. Given a network with capacity constraints [see (5)] and, if the metabolic stationary state is assumed, stoichiometric constraints [see (4)], there are, in general, more degrees of freedom in the fluxes than there are constraints. Additional assumptions have to be made to lock the remaining degrees of freedom. The prominent approach in the field relies on optimization methods, assuming that the fluxes fulfill biologically motivated optimality criteria. Similar to structural methods, optimization-based methods are useful for metabolic engineering with applications in industrial biotechnology [58], [59]. However, they also have numerous applications in medicine, such as predicting the effects of drug treatments [60] or predicting metabolic changes in SARS-CoV2 infected [61] or cancer cells [1].

When predicting condition-specific fluxes using metabolic networks, the workhorse method, easily showcasing the majority of applications, is called flux balance analysis (FBA) [34]. It is founded upon the idea of evolutionary optimality. Since natural cells are products of evolution, their phenotype (and, in particular, their metabolism) should, in some sense, be "optimal" in their natural environment. For single-celled organisms, such as bacteria, one commonly assumes that the cells grow at maximal rate. However, many alternative objective functions, including multiobjective functions, which are appropriate in different scenarios, have been proposed and tested [62], [63]. In the metabolically stationary state, given an objective function $f(\nu)$, the FBA problem is denoted as

$$\begin{aligned} &\max f(\nu) \\ &S\nu = 0 \\ &B\nu \leq b. \end{aligned} \tag{8}$$

In particular, if $f(\nu)$ is a linear function, (8) defines a linear program, which is easy to solve for thousands of flux variables.

To highlight one application of FBA, it was recently used to engineer bacterial strains that are highly efficient in using methanol as a (sole) carbon source. This substrate can be produced sustainably from carbon dioxide or methane [64].

However, solutions to the imposed optimization problems are often not unique (in particular, for the linear objectives), leaving fluxes nondetermined. To characterize the space of alternative optima, the so-called flux variability analysis (FVA) has become common practice [37], [38]. In FVA, after solving the initial optimization problem [see (8)], an additional linear constraint is introduced, which restricts the flux space to optimal solutions. Then, the smallest and largest feasible values of each flux under the new constraint are computed by minimizing and maximizing each flux using linear programming.

In addition, (8) does not necessarily respect the loop law; it may generate thermodynamically infeasible solutions. To enforce the loop law in FBA without including quantitative reaction energies, one can add a vector of hypothetical reaction energies $G \in \mathcal{R}^{N_{\nu,I}}$, a binary vector a of the same length, and a large number Ω to the FBA problem [see (8)]. The extra constraints of loop less FBA are [26]

$$\begin{aligned} -\Omega(1-a) \leq \nu_I \leq \Omega a \\ -\Omega a + 1(1-a) \leq G \leq -a + \Omega(1-a) \\ S_I \cdot G = 0. \end{aligned} \tag{9}$$

In particular, the second line enforces all G elements to be nonzero, thereby avoiding the solution $G = 0$, which does

allow for loops. Similarly, FVA has been extended to respect the thermodynamic loop law [65].

If the metabolic stationary state is not assumed, so-called dynamic FBA (dFBA) may be applied. Conceptually, dFBA introduces additional complexity since it allows for both instantaneous (optimized for all time points) and terminal (time-integrated) objective functions. In a general formulation, given initial conditions for C_E, C_I, and X, dFBA solves for the optimal fluxes with respect to instantaneous or terminal objective functions, subject to mass balance and capacity constraints [see (1)–(3) and (5)]. One can also include further constraints, for example, to limit the rate of change in fluxes. By discretizing (and/or parameterizing) metabolite concentrations and fluxes, the resulting system can be solved as one nonlinear optimization problem [35]. With only instantaneous (and linear) objectives, by discretizing the time domain, the optimal (constant) fluxes in each time interval can be calculated via a sequence of (linear) optimization problems. Metabolite and biomass concentrations are then updated via their differential equations in each time interval using the computed fluxes. A further, often applied, simplification is that intracellular fluxes are stationary, implying that the stoichiometric constraint [see (4)] is fulfilled at each time point [66]. Recalling that ν_T and μ are elements of ν, this gives an optimization problem for the time dependent fluxes $\nu(t)$

$$\begin{aligned} &\max_{\nu(t)} f(\nu(t)) \\ &\text{s.t. } \frac{dC_E(t)}{dt} = -V_E(t) - X(t)\cdot T\nu_T(t) \\ &\quad \frac{dX(t)}{dt} = X(t)\cdot\mu(t) - q(X(t)) \\ &\quad S\nu(t) = 0 \\ &\quad B\nu(t) \le b(C_E(t)) \end{aligned} \tag{10}$$

where $C_E(t), X(t)$, and $\nu(t)$ are unknown functions to be solved for. The second line of (10), enforcing mass balance of the extracellular metabolites, couples the metabolite and biomass concentrations with the optimization problem. The maximal magnitude of the transport fluxes ν_T often depends on the extracellular concentrations C_E, as in (7), implying a dependence of b on C_E in the last line. Although (10) may be addressed directly by discretization in the time domain (the inner optimization problem is solved in every step), this formulation may become computationally prohibitive, particularly for nonlinear objective functions. As an alternative, one can convert the optimization problem to an algebraic system using the Karush–Kuhn–Tucker (KKT) theorem and solve the emerging differential algebraic equation (DAE) system with dedicated DAE solvers [67].

In comparison, metabolically stationary FBA and dFBA handle transport reactions differently. FBA can circumvent the often unfulfilled need for a detailed and accurate description of the transport capacity constraints [7] by assuming optimal behavior. Any cellular objective that minimizes the flux required to sustain a certain growth rate, such as yield optimization or efficient enzyme usage [68], will impose effective bounds on transport reactions. In contrast, dFBA models the extracellular metabolite concentrations explicitly. It requires some kind of kinetics, for example, to capture how a declining nutrient abundance leads to declining nutrient uptake. This distinction will become important for community models in Section IV.

C. Probabilistic Methods

Optimization-based methods overcome the indeterminacy of the high-dimensional flux spaces by imposing biologically motivated objectives. Unfortunately, which objective function to choose is subject to debate [63]; in some scenarios, there is no obvious objective (e.g., for multicellular organisms). Probabilistic methods for flux predictions endow fluxes with probability distributions and, thereby, do not rely on (biological) objectives. In addition to (derived) point estimates, they produce useful uncertainty estimates [40].

Probabilistic methods are commonly applied to the metabolically stationary state, under the stoichiometry and capacity constraints [see (4)–(5)]. In the (common) absence of a function that grades the probability of fluxes, it is often assumed that all fluxes within the feasible space are equally likely, thus imposing a uniform probability distribution on the fluxes. If we denote the metabolically stationary flux polytope P as $P = \{\nu : S\nu = 0, B\nu \le b\}$, then the probability density of fluxes is

$$p(\nu) = \begin{cases} \dfrac{1}{\int_{\nu\in P} d\nu}, & \text{if } \nu \in P \\ 0, & \text{otherwise.} \end{cases} \tag{11}$$

From this joint flux distribution, marginal distributions and statistics, such as moments, may be computed. Denote by $\nu_{\setminus l}$ all fluxes except ν_l and by $P|_{\nu_l}$ the flux polytope with flux ν_l fixed to a specific value. The marginal distribution of ν_l is then

$$p(\nu_l) = \int_{\nu_{\setminus l}\in P|_{\nu_l}} d\nu_{\setminus l}. \tag{12}$$

Notably, despite the uniform joint distribution, due to the nontrivial shape of the flux polytope, these marginal flux distributions are often nonuniform. This is visualized in Fig. 3(b) and (c); the marginal densities of a 3-D simplex decline quadratically. Computationally, marginal flux distributions are mostly obtained via Markov chain Monte Carlo (MCMC) sampling [69], but an analytic approximation approach has also been developed [70].

Such computed marginal flux distributions can be predictive in their own right [71], but they tend to underestimate the (often measured) growth rate μ. When

some measurements, such as moments of extracellular fluxes or the growth rate, are available, it is desirable to modulate the joint density. Instead of being *a priori* uniform, it should be as *uninformative* as possible while still matching the measured moments. According to information science, the least *informative* distribution is the distribution that maximizes entropy, $\mathbb{E}[p(\nu)]$ [72]. The maximum entropy distribution constrained to match first moments is the exponential distribution. Imposing an exponential distribution on the growth rate and adapting the distribution's parameter until the expected value of the growth rate matched measurements were shown to improve the agreement between measured (inferred) and predicted fluxes substantially compared to when assuming a uniform distribution [71]. Despite this encouraging result, the maximum entropy approach has been little explored.

Nonuniform flux densities that may be challenging to sample arise also in other contexts. For example, consideration of quantitative thermodynamics creates a joint space of fluxes and reaction energies $(\nu, \Delta_r G')$. In this joint distribution, the fluxes ν are *a priori* uniform, but the reaction directions are constrained by the values of the reaction energies $\Delta_r G'$. Hence, any outcome of sampling $\Delta_r G'$ forces the fluxes to take values in some specific (hyper)quadrant. Having approximate $\Delta_r G'^{\circ}$ values [24] and accounting for the uncertainty in intracellular metabolite concentrations, it is natural that the reaction energies $\Delta_r G'$ are distributed according to some distribution that concentrates around the measured values (for example, a normal distribution). The marginal distribution of the fluxes, $p(\nu)$, after integrating out the reaction energies of the joint distribution, $p(\nu, \Delta_r G')$, is then constant in each (hyper)quadrant of the flux space. However, it will have different densities in different quadrants. Computationally, assessing the joint distribution $p(\nu, \Delta_r G')$ by MCMC is a much more difficult task than assessing $p(\nu)$ in (11) because the definition space of the fluxes is no longer convex. This problem of sampling the nonconvex space has currently only partial solutions [73], [74].

D. Flux Inference

Flux inference aims to identify metabolic fluxes from large sets of experimental measurements for a given condition using metabolic networks [10], [42]. For this identification, measurements of growth rate and extracellular fluxes, which predictive methods employ as well, are augmented by measurements of so-called isotope label enrichment [42]. The concept of flux inference has been explored for nonmetabolically stationary states, where the fluxes are functions [75], [76]. Here, however, we will focus on the bulk of the literature and assume a metabolically stationary state.

Using (tandem) mass and nuclear magnetic resonance spectroscopy, we can differentiate between compounds with different masses. These highly precise technologies

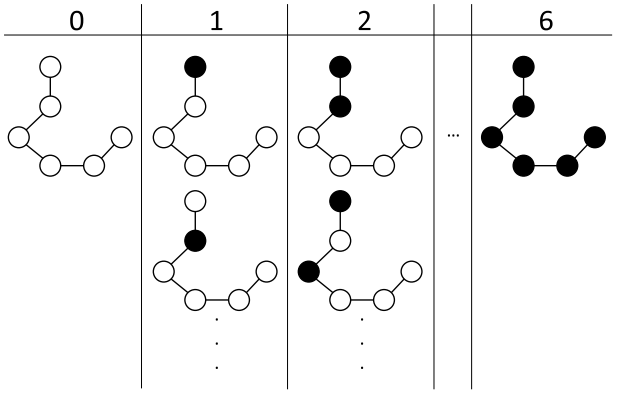

Fig. 4. *Isotopologues of a six atom (glucose-like) molecule ordered in columns by number of labeled atoms (weight). The filled circles represent positions with isotopic label, such as ^{13}C atoms.*

can also differentiate between different isotopologues: versions of the same molecule composed of different isotopes of the same atoms. For example, carbon has two stable isotopes, ^{12}C and ^{13}C. A typical nutrient such as glucose with six carbon atoms may, therefore, appear in 2^6 different isotopic configurations referred to as isotopologues (see Fig. 4). When measuring isotopologue concentrations, isotopologues with different numbers of labeled atoms may be differentiated in that they have different masses. However, to some extent, also isotopologues with identical mass may be differentiated [77]. For standard reactions, we not only know how the molecules map to each other but we also know how individual atoms map, which is visualized for two reactions in Fig. 2(a). Thus, if we know the fluxes, the concentrations of labeled intracellular metabolites, and the label distribution of the nutrient supply, we can calculate the time-resolved label distribution for all molecules in the network, where the (absolute) abundance of each label of each metabolite at each time point is a potential measurement. The label distribution is sensitive to the flux values. In Fig. 2(a), the reaction of G6P to Ru5P and CO_2, the carbon atom going to CO_2, leaves the system. If only this atom in G6P is ^{13}C labeled and the reaction G6P to Ru5P and CO_2 dominates the other reactions from G6P, most labeling will exit the network with CO_2 and, thus, not enrich in downstream metabolites. The lower zoom in Fig. 2(a) also gives rise to flux-dependent patterns in the measured labeling.

For brevity, we refer to the dedicated literature for the full formalism of ^{13}C metabolic flux analysis [78]. Importantly, by performing an experiment in which the labeling of the substrate is known, hundreds or thousands of labeling measurements are possible [79], [80], and from these, an inference problem can be posed. Denoting the measurements by $\eta \in \mathcal{R}^{N_\eta}$ (including flux and growth rate measurements) with associated covariance matrix $\Sigma \in \mathcal{R}^{N_\eta \times N_\eta}$, the simulation function $w(\nu) \in \mathcal{R}^{N_\eta}$, and some distance function $d_\Sigma \in \mathcal{R}_+$ that depends on the

measurement covariance, we can denote the flux inference problem as

$$\begin{aligned} \hat{\nu} &= \arg\min_{\nu} d_{\Sigma}(\eta, w(\nu)) \\ S\nu &= 0 \\ B\nu &= b. \end{aligned} \tag{13}$$

Depending on the measurements at hand, the simulation function $w(\nu)$ comes in two flavors. If a time course of successive label enrichment is available, simulation amounts to solving a system of ODEs by numerical integration [79], [81]. If only the asymptotically stable labeling pattern is measured, a system of algebraic equations is solved [78], potentially by deriving an explicit formula for $w(\nu)$ [82].

With rare exceptions [83], measurement errors are assumed to be additive and Gaussian. Then, solving the inference problem (13) with the Mahalanobis distance as distance function d_{Σ} yields a maximum likelihood (point) estimate; to be informative, it should be equipped with uncertainty estimates [84]–[86]. Alternatively, one can directly approximate the (Bayesian) distribution emerging from the assumptions of the measurement errors using MCMC to obtain (Bayesian) point and uncertainty estimators of the fluxes from the sampled distribution [87]. Again (see Section III-C), the emerging MCMC problems are constrained to nonsymmetric polytopes, imposed by the stoichiometry and capacity constraints [see (4) and (5)]. They also imply nonuniform flux densities, here stemming from how well the fluxes fit the measurements. These problems evade straightforward application of modern high performance MCMC algorithms [88], [89]; they are solved using basic rejection-based strategies [83], [87]. Potentially, great computational speed-ups could be realized with more sophisticated methods.

Considering labeling data also introduces a number of nuisance parameters that need fitting. Most importantly, for a chemical reaction between two metabolites A and B, not only the net flux but also the bidirectional flux impacts the labeling [84]. Bidirectional fluxes relate to the thermodynamics of metabolic reactions (see Section II-D). The closer a reaction operates to thermodynamic equilibrium, the smaller the net flux is relative to the bidirectional flux and will thus strongly influence the measured labeling [90], [91]. Despite the apparent bridge between flux inference and quantitative thermodynamics, the two concepts have been combined explicitly only recently [92]. Instead, most applications incorporate thermodynamics implicitly, by inferring bidirectional flux parameters solely for reactions assumed to be close to equilibrium. The dependence on bidirectionality complicates the inference problem: it may increase the uncertainty in (net) flux estimates and introduce biases. Note that the maximal number of degrees of freedom in bidirectional fluxes is higher than the number of net fluxes (the target of inference), which are constrained by stoichiometry [see (4)]. Again, this leads to challenging inference problems, for example, to jointly infer the values of bidirectional fluxes and whether reactions should be modeled as bidirectional [93]. Such advanced concepts could help exploit that labeling experiments carry information about reaction reversibility *in vivo*, information that cannot be measured directly [84].

IV. MICROBIAL CONSORTIA

Cellular consortia, and especially microbial communities (microbiomes), perform indispensable functions ranging from (bio)geochemical cycles to human health via the gut microbiome. Corresponding to increased experimental capabilities for characterizing communities, the past decade has brought multiple advances in developing and analyzing metabolic network community models (MNCMs) [7].

Applications of MNCMs fall into three major categories. The first set of studies investigates the function of specific consortia. Examples include the analysis of interactions between photosynthetic and nonphotosynthetic microbes [36] or of microbial communities relevant for ecological restoration [94] and the human gut [95], [96]. A second category focuses on deriving general properties of cellular consortia, for example, to correlate environment richness with cooperation levels in consortia [97] or to explore mechanisms leading to community cooperation [98], [99]. The final set primarily develops methods for MNCM simulations, targeting hurdles such as tradeoffs between community and individual fitness in FBA [100]–[102], efficient dFBA solutions [103], engineering of biotechnologically relevant consortia [104], and spatial or single-cell resolution [105]. As one specific application example, NMCM simulations were instrumental in establishing a functioning community of a strain that uses photosynthesis to provide sugars to sustain the life of another strain that could serve as a producer of valuable biocompounds [106].

Importantly, most of these studies use FBA, which was originally developed for single species. This, as well as extensions of structural and probabilistic methods, pose substantial conceptual challenges. We argue here that analogies between microbial consortia and smart grids could help addressing them.

A. Analogies to Smart Grids

A smart grid in electricity supply is a system of communicating agents that strive to control supply and demand with the objective of reliably performing tasks while minimizing the cost of electricity production. A central challenge is to account for the fluctuations in supply and demand, which are strongly influenced by external factors, such as weather. Because of the connectivity in a grid, such fluctuations imply challenges even when one has good characterizations of individual power consumers and generators. Behaviors of a smart grid have to be predicted with models that include the smart grid itself [107].

A cellular consortium is also a system of communicating agents (cells). Each cell consumes (externally supplied)

nutrients, but it may also produce nutrients that are consumed by other cells (so-called cross-feeding). Cross-feeding is an example of division of labor, where, by performing chemical reactions for each other, the number of reactions individual cells have to perform is reduced. Compared to modeling single organisms, a central challenge for systems analysis of consortia is that, due to unknown production of nutrients from other community members, the available nutrient supply of individual cells is generally unknown; it has to be predicted using models that include the whole consortium.

In a smart grid, power consumers and generators may belong to many different beneficiaries. Therefore, whose cost to minimize is not given. One route to resolve this conflict of interest is by employing game theory [108]–[110]. Similarly, even if the division of labor is proven to improve some fitness of a cellular consortium, certain community members may choose not to engage in division of labor if it does not benefit them specifically. Thus, for division of labor to be a realistic scenario, the incentives of the consortium members must align. As for smart grids, game theory can provide a suitable analysis framework [111].

A fundamental difference is that smart grids are engineered and human-controlled systems, whereas natural microbial communities have evolved. This implies that we can tune and interpret components of a smart grid much better than species in microbial communities. The same holds for interactions in both types of networks. This also affects our ability to impose top-down rules, such as pricing systems, which may be possible for smart grids [109]; for microbial consortia, reverse-engineering such rules remains an open problem. Though not completely evolved, from a modeling perspective, engineered microbial communities retain most challenges of their natural counterparts, such as largely unknown metabolic regulation and interactions.

B. Consortia in Chemostats

As for single species [see Fig. 1(a)], we first need to consider culture conditions when formalizing interactions in cell consortia; see Fig. 1(b) for a schematic cell culture with two species. The leap from one to more than one species has three important implications for modeling. First, in a single species culture, one can measure the (time and cell average) transport fluxes ν_T by monitoring the extracellular concentrations C_E and the species concentration X. For cocultures, which divide ν_T into species-specific transport fluxes, such measurements are no longer possible. Second, a single species in a controlled environment can only take up compounds added to the medium (or in rare cases produced by itself). For a coculture, a wide and partly unknown range of compounds may be crossfed between species. Finally, a metabolically stationary state implies constant fluxes ν. In a N_S species coculture with species fluxes $\nu^{(i)},\ i \in 1,\ldots,N_S$, recalling that the fluxes are in units per biomass $X^{(i)}$, a minimal requirement for metabolic stationarity is that the relative species concentrations

$$\frac{X^{(i)}}{\sum_j X^{(j)}}, \quad i \in 1,\ldots,N_S \tag{14}$$

are constant over time. Unless an external mechanisms equilibrates the relative species concentrations by adding and removing cells, (14) requires that $\mu^{(i)} = \mu^{(j)},\ \forall i,j \in 1,\ldots,N_S$, referred to as balanced growth [112]. Realistically, (14) will never be upheld exactly in actual cultures. Instead, it represents a time average of a pseudostationary state that is subject to some fluctuations.

For a so-called chemostat culturing environment with time-constant (nutrient) influxes and effluxes of rate D (volume per time), potential metabolically stationary states maintain constant absolute species concentrations $X^{(i)},\ i \in 1,\ldots,N_S$ and growth rates $\mu^{(i)} = D,\ i \in 1,\ldots,N_S$. Such stationary states emerge from a self-stabilizing (negative feedback) process: Growth rates above D increase biomass concentration and concomitantly nutrient consumption. This leads to lower external nutrient concentrations, which (often) decreases the growth rate [113]. Natural stable ecosystems are inherently chemostat-like, in which they maintain a long-term species balance that is enforced by (cyclic) nutrient limitations. Therefore, the study of the chemostat and other long-term stable systems that maintain a species equilibrium by nutrient limitation even under cyclic fluctuations is of high interest. It also raises interesting control problems, for example, to maintain relative species concentrations in chemostats [114].

Modeling self-equilibrating stationary states requires an explicit representation of the extracellular metabolite concentrations C_E. They are not present in the stationary state FBA formulation [see (8)]. However, dFBA (with or without the assumption of pseudostationary metabolism) explicitly represents all relevant concentrations and fluxes (see Fig. 1). Thus, dFBA is directly applicable for MNCM simulations, theoretically under any cultivation condition. Despite posing significant computational challenges, dFBA simulations with large consortia are possible [99], [103], [105]. The main challenge for reliable dFBA community simulations is that the kinetics relating extracellular concentrations to (maximal) substrate uptake rates are largely unknown (see Section III-B). Current solutions to address this limitation include combining assumed values with database approximations [103] and sensitivity analysis of transport capacities by random sampling [115].

Because dFBA represents the extracellular environment explicitly, it also provides a viable route for studying (possibly chemostat-like) long-term emerging community structures. However, there is no guarantee that such structures exist or that they are unique. Contrarily, in repeated batch experiments with natural consortia, seemingly identical experimental sequences lead to functionally similar

stable microbial communities but with different species' compositions [116]. In view of MNCM models, deviating compositions may arise deterministically, as a result of varying initial conditions, or stochastically, for example, as a result of low cell numbers or environmental heterogeneity. At present, stochastic dynamic simulations of MNCM models are unexplored, and they are probably computationally prohibitive. Thus, deterministic MNCM simulation can help to explore the emergence of deviating cellular communities under similar conditions. The number of long-term community structures arising will depend on specific assumptions made, in particular, regarding uptake kinetics [see (7)]. In addition, nonmonotone relationships between metabolite concentrations and growth are likely in the presence of growth-inhibiting (toxic) compounds [117]; they may influence the number of long-term solutions. To investigate alternative long-term solutions of MNCMs, instead of performing repeated dFBA simulations, we have recently proposed a direct analysis of the stationary states [118]. In contrast to normal FBA, the extracellular environment is modeled explicitly; we argue that there exist ample opportunities for method development in similar directions.

C. Community Decision-Making

Naturally evolved multispecies communities often show complex interactions and many examples of mutualistic interactions have been established. In this context, a fascinating hypothesis is that mitochondria, today organelles in cells of higher organisms (eukaryotes) that provide most of the chemical energy, were once bacteria living symbiotically with ancestors of today's eukaryotic cells. Through evolution, this cellular consortium merged into a single organism [119]. Other, less dedicated, examples are mutualistic relationships between a host organism and its gut microbiome [120], and between the organisms within the microbiome [121].

For the formal analysis, the prevalence of mutualistic communities adds a new level of complexity to FBA-type simulations. On the one hand, cells evolved to optimize their own fitness; on the other hand, through the evolution of communities, cells evolved to optimize community fitness. What community fitness means is context-specific. For example, in the gut, successful communities have high resistance to invasion by pathogenic species [122]. Such objectives are not necessarily correlated with classic single-species objectives such as fast growth, thus opening up potential conflicts of interest between the (fitness of) the cell and the community.

Probably due to the lower mathematical and computational complexity, most of the advanced decision-making models in metabolic network analysis were developed for stationary state cellular communities. Many dFBA applications have no representation of a community decision-maker [94], [99], [103]. An early decision-making mechanism, applicable for predicting metabolic behaviors of cellular communities at metabolically stationary state, is the so-called OptCom algorithm [100]. In OptCom, the community is modeled as a bilevel optimization problem with a community objective $f(\nu, X)$, constrained by the individual objectives of the participating species $g^{(i)}(\nu^{(i)\prime})$. Denoting species-specific symbols with superscript (i) as before ($\nu = [\nu^{(1)}, \ldots, \nu^{(N_S)}]$) and community symbols without superscript, the OptCom formulation is

$$\begin{aligned} &\max_{\nu, X, V_E} f(\nu, X) \\ &\quad V_E + \sum_i X^{(i)} T^{(i)} \nu_T^{(i)} = 0 \\ &\text{s.t. } V_{E,\min} \le V_E \le V_{E,\max} \\ &\quad \begin{bmatrix} \nu^{(i)} = \arg\max_{\nu^{(i)\prime}} g^{(i)}(\nu^{(i)\prime}) \\ S^{(i)} \nu^{(i)\prime} = 0 \\ B^{(i)} \nu^{(i)\prime} \le b^{(i)} \\ \nu_T^{(i)\prime} = \nu_T^{(i)} \end{bmatrix} \quad \forall i. \end{aligned} \tag{15}$$

The two first lines of (15) constitute the outer problem pertaining to the whole community with the second line enforcing steady state in the extracellular compartment and the third line keeping the system fluxes within a range defined by lower and upper bound vectors $V_{E,\min}$ and $V_{E,\max}$. The equations in brackets constitute the inner problems (one per organism i, where $B^{(i)}$ and $b^{(i)}$ are the strain's capacity matrix and capacity bounds, respectively). Note that the definition space of the outer problem only includes (ν, X) for which the inner problems have a feasible solution. This formulation differs slightly from the original one in [100]; we introduced species concentrations X explicitly to use consistent units throughout. Note also that, in its original metabolically stationary formulation, OptCom does not enforce balanced growth [see (14)], but it has been extended to a dFBA formulation [123]. With the last line, $\nu_T^{(i)\prime} = \nu_T^{(i)}$, the outer problem in (15) dictates the transport fluxes of the inner problem. Thus, one can argue that OptCom gives the community objective precedence over the individual objective. Alternative formulations that assign more importance to the individual cellular objectives include considering the space of the Pareto optimal solutions of the individual objectives [101] and the Nash equilibrium solutions by using game theory explicitly [102]. Again, we see abundant space for future conceptual developments, in particular those making explicit use of the game theory [111].

D. Coculture Flux Inference

We introduced how the fluxes of a single species can be inferred from measured enrichment of isotopes (typically ^{13}C; see Section III-D). In microbial communities, this approach faces additional challenges. In cultivations where the species are not spatially separated and without additional separation steps [124], standard measurement techniques cannot differentiate in which species a label enrichment of a standard compound occurs; only the

average label enrichment across all species will be measurable. However, because of nonlinear mappings between fluxes and label enrichment, one can, sometimes, infer species-specific fluxes from community average measurements [125]. Another route to coculture flux inference is to measure (nonstandard) species-specific metabolites, such as peptides, short chains of amino acids [126].

A generally important topic when modeling cocultures is to identify crossfed compounds. In theory, this can be addressed with flux inference. However, the crossfed compounds are often not known *a priori*. Then, all reasonable cross-feeding reactions should be added to the coculture model prior to inference. This greatly increases the number of degrees of freedom and, thus, decreases the statistical power of the inference.

Despite these limitations, combining optimization-based results [127] with evidence from coculture flux inference [128] shows promising results, For example, in certain environments, *E. coli* cells can bifurcate into two distinct subpopulations with different metabolic behaviors. This exemplifies phenotypic heterogeneity, a widely studied phenomenon in biology [129]. For the bifurcated culture, modeling required two coupled metabolic networks, one for each subpopulation, to achieve an acceptable fit to the labeling data [128]. Methodologically, this study highlights that, when inferring fluxes of a coculture (or a single-species culture), each species may need to be modeled by multiple metabolic networks to fit the measurements. However, such subpopulations are generally problematic for flux inference because they inflate (multiply) the number of inferred parameters. In addition, commonly, the number of subpopulations is unknown; estimating this number from data imposes additional model selection problems. For the general case, a continuum of highly variable phenotypes, no approaches to flux inference have been proposed yet.

E. Challenges and Open Problems

Given that cellular communities constitute a system of distributed decision-makers, it is natural that game theory enters into the picture [111], sometimes explicitly [98], [102]. OptCom [see (15)] and related formulations [112] do not refer to game theory explicitly, but they impose a structure of objectives to mimic the behavior of distributed agents performing a task, which is a game-like scenario. The proposed decision-making formalisms assume different levels of cooperativity. Thus, when modeling a specific community, the choice of formalism should take into account *how much* cooperation is expected. This is often uncertain; it depends on whether organisms have coevolved in the modeled environment and on properties of the environment itself [97]. However, such choices may fundamentally change the predicted community behavior [102]. Considering this and the general complexity of simulating cellular communities, performing quantitatively accurate simulations with MNCMs appears very challenging. Instead, simulations with multiple sets of assumptions (that is, model structures or objectives) may provide a route to explore the space of phenotypic possibilities of a community. Then, outcomes that are not predicted under any assumptions can be rejected conclusively.

Characterizing the space of long-term (asymptotic) solutions of community dFBA simulations is another open problem (see Section IV-B). For small, purely ODE-based models, the analysis of asymptotic community behavior has a long tradition in the chemostat literature [130]. For an asymptotic solution to be of interest, it should also be (locally) stable, meaning that the system will return to a stable state after a small perturbation. For MNCMs in dFBA, we have systems of ODEs with optimality and algebraic constraints, which complicates stability analysis. As mentioned in Section III-B, the optimality constraints can be converted to algebraic constraints [67]. However, even then, the stability analysis of differential algebraic systems is challenging [131] and not nearly as explored as regular stability analysis. In general, we expect cellular decision-making, represented by optimization in FBA, to have the capacity to stabilize steady states. This would be analogous to a Segway standing up upheld by a controller although standing up is an unstable solution of the inverse pendulum equation. The alternative to an objective function–incorporating corresponding cellular control circuits *explicitly* into metabolic network analysis–represents a major challenge, even if these circuits were known.

For cellular community modeling, extending single-species FBA to multispecies FBA is a straightforward formal task. However, as we discussed, this extension has strong conceptual implications with regard to (assumed) cell culture and decision-making. In general, this makes the results of community simulations less reliable (quantitatively). However, for some scientific inquiries, such as when investigating the capacity of organisms to crossfeed metabolites, quantitative simulations may not be necessary. Instead, the complementarity of organisms can be assessed qualitatively by comparing their metabolic pathways [132]. Novel extensions of structural methods to communities could further develop such analyses.

If you have a hammer, everything is a nail—the computational ease of FBA-type analyses for MNCMs has probably influenced the scientific questions addressed. Since metabolic capabilities are encoded in the metabolic network, most MNCM studies investigate cellular interactions in terms of cross-feeding. Resource competition, on the other hand, a very common and fundamental interaction type, has not been systematically investigated with MNCNs. In the biological scenario where two organisms compete for a single resource, the best adapted organism will outgrow the other. Best adaptation may mean a better capacity to turn the resource into biomass, which includes not wasting resources on maintaining unnecessary capabilities in this particular condition. Such metabolic and regulatory overhead is not considered in standard FBA. However, corresponding extensions that also account for organism-induced changes in the environment may offer a

good starting point for modeling competition between specific organisms and cross-feeding.

V. CONCLUSION

The quantitative analysis of metabolic networks is a mature field with a wide array of techniques and applications. It has been covered in many reviews focusing on different subfields [3], [6]–[8], [12], [31], [34], [52]. Here, we gave an introduction and overview to the computational methods of the field, aimed at readers with advanced knowledge of engineering and computational sciences. We raised a number of current research questions that are important in our view. They often relate to the developing field of metabolic network simulations of cellular communities. Specific topics include the emergence of differing stable cellular consortia under similar conditions, decision-making of cellular communities, stability of solutions to ODE systems in the presence of decision-makers, and mechanisms for community interactions in cooperation and competition. By relating the entities and challenges in metabolic networks to the study of electric circuits and grids with their strong similarities, we demonstrate interesting biology-inspired challenges for engineers and computer scientists, in the hope that it will invite cross-disciplinary contributions. ■

Acknowledgment

The authors would like to thank Mattia Gollub for inspiring discussions.

REFERENCES

[1] A. Nilsson and J. Nielsen, "Genome scale metabolic modeling of cancer," *Metabolic Eng.*, vol. 43, pp. 103–112, Sep. 2017.
[2] W. Y. Chung, Y. Zhu, M. H. M. Maifiah, N. K. H. Shivashekaregowda, E. H. Wong, and N. A. Rahim, "Novel antimicrobial development using genome-scale metabolic model of Gram-negative pathogens: A review," *J. Antibiotics*, vol. 74, pp. 95–104, Sep. 2020.
[3] Á. Altamirano, P. A. Saa, and D. Garrido, "Inferring composition and function of the human gut microbiome in time and space: A review of genome-scale metabolic modelling tools," *Comput. Struct. Biotechnol. J.*, vol. 18, pp. 3897–3904, 2020.
[4] D. Segrè, D. Vitkup, and G. M. Church, "Analysis of optimality in natural and perturbed metabolic networks," *Proc. Nat. Acad. Sci. USA*, vol. 99, no. 23, pp. 15112–15117, Nov. 2002.
[5] N. D. Price, I. Famili, D. A. Beard, and B. Ø. Palsson, "Extreme pathways and Kirchhoff's second law," *Biophys. J.*, vol. 83, no. 5, pp. 2879–2882, Nov. 2002.
[6] A. Bordbar, J. M. Monk, Z. A. King, and B. O. Palsson, "Constraint-based models predict metabolic and associated cellular functions," *Nature Rev. Genet.*, vol. 15, no. 2, pp. 107–120, Feb. 2014.
[7] M. B. Biggs, G. L. Medlock, G. L. Kolling, and J. A. Papin, "Metabolic network modeling of microbial communities," *Wiley Interdiscipl. Rev., Syst. Biol. Med.*, vol. 7, no. 5, pp. 317–334, Sep. 2015.
[8] C. Gu, G. B. Kim, W. J. Kim, H. U. Kim, and S. Y. Lee, "Current status and applications of genome-scale metabolic models," *Genome Biol.*, vol. 20, no. 1, pp. 1–18, Dec. 2019.
[9] G. Stephanopoulos, "Metabolic fluxes and metabolic engineering," *Metabolic Eng.*, vol. 1, no. 1, pp. 1–11, Jan. 1999.
[10] U. Sauer, "Metabolic networks in motion: ^{13}C-based flux analysis," *Mol. Syst. Biol.*, vol. 2, no. 1, p. 62, 2006.
[11] I. Thiele and B. Ø. Palsson, "A protocol for generating a high-quality genome-scale metabolic reconstruction," *Nature Protocols*, vol. 5, no. 1, pp. 93–121, Jan. 2010.
[12] L. Wang, S. Dash, C. Y. Ng, and C. D. Maranas, "A review of computational tools for design and reconstruction of metabolic pathways," *Synth. Syst. Biotechnol.*, vol. 2, no. 4, pp. 243–252, Dec. 2017.
[13] S. N. Mendoza, B. G. Olivier, D. Molenaar, and B. Teusink, "A systematic assessment of current genome-scale metabolic reconstruction tools," *Genome Biol.*, vol. 20, no. 1, pp. 1–20, Dec. 2019.
[14] P. Kirk, A. C. Babtie, and M. P. Stumpf, "Systems biology (un)certainties," *Science*, vol. 350, no. 6259, pp. 386–388, 2015.
[15] A. Heinken, A. Basile, J. Hertel, C. Thinnes, and I. Thiele, "Genome-scale metabolic modeling of the human microbiome in the era of personalized medicine," *Annu. Rev. Microbiol.*, vol. 75, no. 1, pp. 199–222, Oct. 2021.
[16] P. Droste, S. Miebach, S. Niedenführ, W. Wiechert, and K. Nöh, "Visualizing multi-omics data in metabolic networks with the software omix—A case study," *Biosystems*, vol. 105, no. 2, pp. 154–161, 2011.
[17] J. D. Orth *et al.*, "A comprehensive genome-scale reconstruction of *Escherichia coli* metabolism–2011," *Mol. Syst. Biol.*, vol. 7, no. 1, p. 535, 2011.
[18] F. C. Neidhardt, J. L. Ingraham, and M. Schaechter, *Physiology of the Bacterial Cell; A Molecular Approach*. Sunderland, MA, USA: Sinauer Associates, 1990.
[19] D. H. de Groot, J. Lischke, R. Muolo, R. Planqué, F. J. Bruggeman, and B. Teusink, "The common message of constraint-based optimization approaches: Overflow metabolism is caused by two growth-limiting constraints," *Cellular Mol. Life Sci.*, vol. 77, no. 3, pp. 441–453, Feb. 2020.
[20] Q. K. Beg *et al.*, "Intracellular crowding defines the mode and sequence of substrate uptake by *Escherichia coli* and constrains its metabolic activity," *Proc. Nat. Acad. Sci. USA*, vol. 104, no. 31, pp. 12663–12668, Jul. 2007.
[21] B. J. Sánchez, C. Zhang, A. Nilsson, P.-J. Lahtvee, E. J. Kerkhoven, and J. Nielsen, "Improving the phenotype predictions of a yeast genome-scale metabolic model by incorporating enzymatic constraints," *Mol. Syst. Biol.*, vol. 13, no. 8, p. 935, 2017.
[22] P. S. Bekiaris and S. Klamt, "Automatic construction of metabolic models with enzyme constraints," *BMC Bioinf.*, vol. 21, no. 1, pp. 1–13, Dec. 2020.
[23] R. N. Goldberg, Y. B. Tewari, and T. N. Bhat, "Thermodynamics of enzyme-catalyzed reactions—A database for quantitative biochemistry," *Bioinformatics*, vol. 20, no. 16, pp. 2874–2877, Nov. 2004.
[24] E. Noor, H. S. Haraldsdóttir, R. Milo, and R. M. T. Fleming, "Consistent estimation of Gibbs energy using component contributions," *PLoS Comput. Biol.*, vol. 9, no. 7, Jul. 2013, Art. no. e1003098.
[25] C. Held and G. Sadowski, "Thermodynamics of bioreactions," *Annu. Rev. Chem. Biomol. Eng.*, vol. 7, no. 1, pp. 395–414, Jun. 2016, doi: 10.1146/annurev-chembioeng-080615-034704.
[26] J. Schellenberger, N. E. Lewis, and B. Ø. Palsson, "Elimination of thermodynamically infeasible loops in steady-state metabolic models," *Biophys. J.*, vol. 100, no. 3, pp. 544–553, Feb. 2011.
[27] M. M. Islam, W. L. Schroeder, and R. Saha, "Kinetic modeling of metabolism: Present and future," *Current Opinion Syst. Biol.*, vol. 26, pp. 72–78, Jun. 2021. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S2452310021000147
[28] A. Cornish-Bowden, "One hundred years of Michaelis–Menten kinetics," *Perspect. Sci.*, vol. 4, pp. 3–9, Mar. 2015.
[29] M. C. Reed, A. Lieb, and H. F. Nijhout, "The biological significance of substrate inhibition: A mechanism with diverse functions," *BioEssays*, vol. 32, no. 5, pp. 422–429, Apr. 2010.
[30] S. Schuster and C. Hilgetag, "On elementary flux modes in biochemical reaction systems at steady state," *J. Biol. Syst.*, vol. 2, no. 2, pp. 165–182, Jun. 1994.
[31] J. Zanghellini, D. E. Ruckerbauer, M. Hanscho, and C. Jungreuthmayer, "Elementary flux modes in a nutshell: Properties, calculation and applications," *Biotechnol. J.*, vol. 8, no. 9, pp. 1009–1016, Sep. 2013.
[32] S. Klamt and E. D. Gilles, "Minimal cut sets in biochemical reaction networks," *Bioinformatics*, vol. 20, no. 2, pp. 226–234, Jan. 2004.
[33] D. A. Fell and J. R. Small, "Fat synthesis in adipose tissue. An examination of stoichiometric constraints," *Biochem. J.*, vol. 238, no. 3, pp. 781–786, Sep. 1986.
[34] K. Raman and N. Chandra, "Flux balance analysis of biological systems: Applications and challenges," *Briefings Bioinf.*, vol. 10, no. 4, pp. 435–449, Jul. 2009.
[35] R. Mahadevan, J. S. Edwards, and F. J. Doyle, "Dynamic flux balance analysis of diauxic growth in *Escherichia coli*," *Biophys. J.*, vol. 83, no. 3, pp. 1331–1340, Sep. 2002.
[36] S. Stolyar *et al.*, "Metabolic modeling of a mutualistic microbial community," *Mol. Syst. Biol.*, vol. 3, no. 1, p. 92, Jan. 2007.
[37] R. Mahadevan and C. H. Schilling, "The effects of alternate optimal solutions in constraint-based genome-scale metabolic models," *Metabolic Eng.*, vol. 5, no. 4, pp. 264–276, Oct. 2003.
[38] S. Gudmundsson and I. Thiele, "Computationally efficient flux variability analysis," *BMC Bioinf.*, vol. 11, no. 1, pp. 1–3, Dec. 2010.
[39] J. Schellenberger and B. Ø. Palsson, "Use of randomized sampling for analysis of metabolic networks," *J. Biol. Chem.*, vol. 284, no. 9, pp. 5457–5461, Feb. 2009.
[40] H. A. Herrmann, B. C. Dyson, L. Vass, G. N. Johnson, and J.-M. Schwartz, "Flux sampling is a powerful tool to study metabolism under changing environmental conditions," *NPJ Syst. Biol. Appl.*, vol. 5, no. 1, pp. 1–8, Dec. 2019.
[41] J. J. Vallino and G. Stephanopoulos, "Metabolic flux distributions in *Corynebacterium glutamicum* during growth and lysine overproduction," *Biotechnol. Bioeng.*, vol. 41, no. 6, pp. 633–646, 1993.
[42] W. Wiechert, "^{13}C metabolic flux analysis," *Metabolic Eng.*, vol. 3, no. 3, pp. 195–206, Jul. 2001.

[43] M. T. Wortel, E. Noor, M. Ferris, F. J. Bruggeman, and W. Liebermeister, "Metabolic enzyme cost explains variable trade-offs between microbial growth rate and yield," *PLOS Comput. Biol.*, vol. 14, no. 2, Feb. 2018, Art. no. e1006010.

[44] A. Neuner and E. Heinzle, "Mixed glucose and lactate uptake by corynebacterium glutamicum through metabolic engineering," *Biotechnol. J.*, vol. 6, no. 3, pp. 318–329, Mar. 2011.

[45] M. Terzer, N. D. Maynard, M. W. Covert, and J. Stelling, "Genome-scale metabolic networks," *Wiley Interdiscipl. Rev., Syst. Biol. Med.*, vol. 1, no. 3, pp. 285–297, 2009.

[46] S. Klamt *et al.*, "From elementary flux modes to elementary flux vectors: Metabolic pathway analysis with arbitrary linear flux constraints," *PLOS Comput. Biol.*, vol. 13, no. 4, Apr. 2017, Art. no. e1005409.

[47] S. Klamt, U.-U. Haus, and F. Theis, "Hypergraphs and cellular networks," *PLoS Comput. Biol.*, vol. 5, no. 5, May 2009, Art. no. e1000385.

[48] H. S. Haraldsdóttir and R. M. T. Fleming, "Identification of conserved moieties in metabolic networks by graph theoretical analysis of atom transition networks," *PLOS Comput. Biol.*, vol. 12, no. 11, Nov. 2016, Art. no. e1004999.

[49] L. H. Hartwell, J. J. Hopfield, S. Leibler, and A. W. Murray, "From molecular to modular cell biology," *Nature*, vol. 402, no. 6761, pp. C47–C52, Dec. 1999.

[50] T. Heuer and S. Schlag, "Improving coarsening schemes for hypergraph partitioning by exploiting community structure," in *Proc. 16th Int. Symp. Exp. Algorithms (SEA)*, 2017.

[51] C. Caro-Ruiz, J. Ma, D. J. Hill, A. Pavas, and E. Mojica-Nava, "A minimum cut-set vulnerability analysis of power networks," *Sustain. Energy, Grids Netw.*, vol. 21, Mar. 2020, Art. no. 100302.

[52] M. Terzer and J. Stelling, "Large-scale computation of elementary flux modes with bit pattern trees," *Bioinformatics*, vol. 24, no. 19, pp. 2229–2235, Oct. 2008.

[53] S. Klamt and J. Stelling, "Combinatorial complexity of pathway analysis in metabolic networks," *Mol. Biol. Rep.*, vol. 29, no. 1, pp. 233–236, 2002.

[54] R. Urbanczik and C. Wagner, "Functional stoichiometric analysis of metabolic networks," *Bioinformatics*, vol. 21, no. 22, pp. 4176–4180, Nov. 2005.

[55] T. J. Clement, E. B. Baalhuis, B. Teusink, F. J. Bruggeman, R. Planqué, and D. H. de Groot, "Unlocking elementary conversion modes: Ecmtool unveils all capabilities of metabolic networks," *Patterns*, vol. 2, no. 1, Jan. 2021, Art. no. 100177.

[56] O. Øyås and J. Stelling, "Scalable metabolic pathway analysis," *bioRxiv*, to be published, doi: 10.1101/2020.07.31.230177.

[57] D. Banerjee *et al.*, "Genome-scale metabolic rewiring improves titers rates and yields of the non-native product indigoidine at scale," *Nature Commun.*, vol. 11, no. 1, pp. 1–11, Dec. 2020.

[58] K. A. Curran, N. C. Crook, and H. S. Alper, "Using flux balance analysis to guide microbial metabolic engineering," in *Microbial Metabolic Engineering*. New York, NY, USA: Springer, 2012, pp. 197–216.

[59] A. P. Burgard, P. Pharkya, and C. D. Maranas, "Optknock: A bilevel programming framework for identifying gene knockout strategies for microbial strain optimization," *Biotechnol. Bioeng.*, vol. 84, no. 6, pp. 647–657, Dec. 2003.

[60] O. Øyås *et al.*, "Model-based integration of genomics and metabolomics reveals SNP functionality in mycobacterium tuberculosis," *Proc. Nat. Acad. Sci. USA*, vol. 117, no. 15, pp. 8494–8502, Apr. 2020.

[61] P. Nanda and A. Ghosh, "Genome scale-differential flux analysis reveals deregulation of lung cell metabolism on SARS-CoV-2 infection," *PLOS Comput. Biol.*, vol. 17, no. 4, Apr. 2021, Art. no. e1008860.

[62] R. Schuetz, L. Kuepfer, and U. Sauer, "Systematic evaluation of objective functions for predicting intracellular fluxes in *Escherichia coli*," *Mol. Syst. Biol.*, vol. 3, no. 1, p. 119, Jan. 2007.

[63] C. E. García Sánchez and R. G. Torres Sáez, "Comparison and analysis of objective functions in flux balance analysis," *Biotechnol. Prog.*, vol. 30, no. 5, pp. 985–991, Sep. 2014.

[64] P. Keller *et al.*, "Methanol-dependent *Escherichia coli* strains with a complete ribulose monophosphate cycle," *Nature Commun.*, vol. 11, no. 1, pp. 1–10, Dec. 2020.

[65] A. C. Müller and A. Bockmayr, "Fast thermodynamically constrained flux variability analysis," *Bioinformatics*, vol. 29, no. 7, pp. 903–909, Apr. 2013.

[66] K. Höffner, S. M. Harwood, and P. I. Barton, "A reliable simulator for dynamic flux balance analysis," *Biotechnol. Bioeng.*, vol. 110, no. 3, pp. 792–802, Mar. 2013.

[67] X. Zhao, S. Noack, W. Wiechert, and E. V. Lieres, "Dynamic flux balance analysis with nonlinear objective function," *J. Math. Biol.*, vol. 75, nos. 6–7, pp. 1487–1515, Dec. 2017.

[68] N. E. Lewis *et al.*, "Omic data from evolved *E. coli* are consistent with computed optimal growth from genome-scale models," *Mol. Syst. Biol.*, vol. 6, no. 1, p. 390, 2010.

[69] H. S. Haraldsdóttir, B. Cousins, I. Thiele, R. M. T. Fleming, and S. Vempala, "CHRR: Coordinate hit-and-run with rounding for uniform sampling of constraint-based models," *Bioinformatics*, vol. 33, no. 11, pp. 1741–1743, Jun. 2017.

[70] A. Braunstein, A. P. Muntoni, and A. Pagnani, "An analytic approximation of the feasible space of metabolic networks," *Nature Commun.*, vol. 8, no. 1, pp. 1–9, Apr. 2017.

[71] D. De Martino, A. M. C. Andersson, T. Bergmiller, C. C. Guet, and G. Tkačik, "Statistical mechanics for metabolic networks during steady state growth," *Nature Commun.*, vol. 9, no. 1, pp. 1–9, Dec. 2018.

[72] E. T. Jaynes, "Information theory and statistical mechanics," *Phys. Rev.*, vol. 106, no. 4, p. 620, 1957.

[73] M. G. Gollub, H. M. Kaltenbach, and J. Stelling, "Probabilistic thermodynamic analysis of metabolic networks," *Bioinformatics*, vol. 37, no. 18, pp. 2938–2945, Mar. 2021.

[74] P. A. Saa and L. K. Nielsen, "Ll-ACHRB: A scalable algorithm for sampling the feasible solution space of metabolic networks," *Bioinformatics*, vol. 32, no. 15, pp. 2330–2337, Aug. 2016.

[75] H. Link, K. Kochanowski, and U. Sauer, "Systematic identification of allosteric protein-metabolite interactions that control enzyme activity *in vivo*," *Nature Biotechnol.*, vol. 31, no. 4, pp. 357–361, Apr. 2013.

[76] R. Schumacher and S. Wahl, "Effective estimation of dynamic metabolic fluxes using ^{13}C labeling and piecewise affine approximation: From theory to practical applicability," *Metabolites*, vol. 5, no. 4, pp. 697–719, Dec. 2015.

[77] J. Choi, M. T. Grossbach, and M. R. Antoniewicz, "Measuring complete isotopomer distribution of aspartate using gas chromatography/tandem mass spectrometry," *Anal. Chem.*, vol. 84, no. 10, pp. 4628–4632, May 2012.

[78] W. Wiechert and K. Nöh, *Quantitative Metabolic Flux Analysis Based on Isotope Labeling*, 13th ed. Hoboken, NJ, USA: Wiley, 2021.

[79] K. Nöh, K. Grönke, B. Luo, R. Takors, M. Oldiges, and W. Wiechert, "Metabolic flux analysis at ultra short time scale: Isotopically non-stationary ^{13}C labeling experiments," *J. Biotechnol.*, vol. 129, no. 2, pp. 249–267, Apr. 2007.

[80] S. B. Crown, C. P. Long, and M. R. Antoniewicz, "Integrated ^{13}C-metabolic flux analysis of 14 parallel labeling experiments in *Escherichia coli*," *Metabolic Eng.*, vol. 28, pp. 151–158, Mar. 2015.

[81] J. D. Young, "INCA: A computational platform for isotopically non-stationary metabolic flux analysis," *Bioinformatics*, vol. 30, no. 9, pp. 1333–1335, May 2014.

[82] W. Wiechert, K. Nöh, and M. Weitzel, "Metabolic isotopomer labeling systems. Part III: Path tracing," *Math. Biosci.*, vol. 244, no. 1, pp. 1–12, Jul. 2013.

[83] V. Kadirkamanathan, J. Yang, S. A. Billings, and P. C. Wright, "Markov chain Monte Carlo algorithm based metabolic flux distribution analysis on corynebacterium glutamicum," *Bioinformatics*, vol. 22, no. 21, pp. 2681–2687, Nov. 2006.

[84] W. Wiechert and A. A. de Graaf, "Bidirectional reaction steps in metabolic networks: I. Modeling and simulation of carbon isotope labeling experiments," *Biotechnol. Bioeng.*, vol. 55, no. 1, pp. 101–117, Jul. 1997.

[85] M. R. Antoniewicz, J. K. Kelleher, and G. Stephanopoulos, "Determination of confidence intervals of metabolic fluxes estimated from stable isotope measurements," *Metabolic Eng.*, vol. 8, no. 4, pp. 324–337, Jul. 2006.

[86] C. Wittmann and E. Heinzle, "Genealogy profiling through strain improvement by using metabolic network analysis: Metabolic flux genealogy of several generations of lysine-producing corynebacteria," *Appl. Environ. Microbiol.*, vol. 68, no. 12, pp. 5843–5859, Dec. 2002.

[87] A. Theorell, S. Leweke, W. Wiechert, and K. Nöh, "To be certain about the uncertainty: Bayesian statistics for ^{13}C metabolic flux analysis," *Biotechnol. Bioeng.*, vol. 114, no. 11, pp. 2668–2684, 2017.

[88] M. D. Hoffman and A. Gelman, "The no-u-turn sampler: Adaptively setting path lengths in Hamiltonian Monte Carlo," *J. Mach. Learn. Res.*, vol. 15, no. 1, pp. 1593–1623, Apr. 2014.

[89] M. Girolami and B. Calderhead, "Riemann manifold Langevin and Hamiltonian Monte Carlo methods," *J. Roy. Stat. Soc., B (Stat. Methodol.)*, vol. 73, no. 2, pp. 123–214, Mar. 2011.

[90] W. Wiechert, "The thermodynamic meaning of metabolic exchange fluxes," *Biophys. J.*, vol. 93, no. 6, pp. 2255–2264, Sep. 2007.

[91] E. Noor, A. Flamholz, W. Liebermeister, A. Bar-Even, and R. Milo, "A note on the kinetics of enzyme action: A decomposition that highlights thermodynamic effects," *FEBS Lett.*, vol. 587, no. 17, pp. 2772–2777, Sep. 2013.

[92] J. Saldida *et al.*, "Unbiased metabolic flux inference through combined thermodynamic and ^{13}C flux analysis," *bioRxiv*, to be published, doi: 10.1101/2020.06.29.177063.

[93] A. Theorell and K. Nöh, "Reversible jump MCMC for multi-model inference in metabolic flux analysis," *Bioinformatics*, vol. 36, no. 1, pp. 232–240, Jan. 2020.

[94] K. Zhuang *et al.*, "Genome-scale dynamic modeling of the competition between rhodoferax and geobacter in anoxic subsurface environments," *ISME J.*, vol. 5, no. 2, pp. 305–316, Feb. 2011.

[95] S. Shoaie *et al.*, "Quantifying diet-induced metabolic changes of the human gut microbiome," *Cell Metabolism*, vol. 22, no. 2, pp. 320–331, Aug. 2015.

[96] S. H. J. Chan, E. S. Friedman, G. D. Wu, and C. D. Maranas, "Predicting the longitudinally and radially varying gut microbiota composition using multi-scale microbial metabolic modeling," *Processes*, vol. 7, no. 7, p. 394, Jun. 2019.

[97] D. Machado *et al.*, "Polarization of microbial communities between competitive and cooperative metabolism," *Nature Ecol. Evol.*, vol. 5, pp. 195–203, Jan. 2020.

[98] A. R. Zomorrodi and D. Segrè, "Genome-driven evolutionary game theory helps understand the rise of metabolic interdependencies in microbial communities," *Nature Commun.*, vol. 8, no. 1, pp. 1–12, Dec. 2017.

[99] M. J. A. V. Hoek and R. M. H. Merks, "Emergence of microbial diversity due to cross-feeding interactions in a spatial model of gut microbial metabolism," *BMC Syst. Biol.*, vol. 11, no. 1, pp. 1–18, Dec. 2017.

[100] A. R. Zomorrodi and C. D. Maranas, "OptCom: A multi-level optimization framework for the metabolic modeling and analysis of microbial communities," *PLoS Comput. Biol.*, vol. 8, no. 2, Feb. 2012, Art. no. e1002363.

[101] M. Budinich, J. Bourdon, A. Larhlimi, and

D. Eveillard, "A multi-objective constraint-based approach for modeling genome-scale microbial ecosystems," *PLoS ONE*, vol. 12, no. 2, Feb. 2017, Art. no. e0171744.
[102] J. Cai, T. Tan, and S. H. J. Chan, "Predicting Nash equilibria for microbial metabolic interactions," *Bioinformatics*, vol. 36, no. 24, pp. 5649–5655, Apr. 2021.
[103] D. Popp and F. Centler, "μbialSim: Constraint-based dynamic simulation of complex microbiomes," *Frontiers in Bioeng. Biotechnol.*, vol. 8, p. 574, Jun. 2020.
[104] S. Koch *et al.*, "RedCom: A strategy for reduced metabolic modeling of complex microbial communities and its application for analyzing experimental datasets from anaerobic digestion," *PLOS Comput. Biol.*, vol. 15, no. 2, Feb. 2019, Art. no. e1006759.
[105] E. Bauer, J. Zimmermann, F. Baldini, I. Thiele, and C. Kaleta, "BacArena: Individual-based metabolic modeling of heterogeneous microbes in complex communities," *PLOS Comput. Biol.*, vol. 13, no. 5, May 2017, Art. no. e1005544.
[106] C. Zuñiga *et al.*, "Synthetic microbial communities of heterotrophs and phototrophs facilitate sustainable growth," *Nature Commun.*, vol. 11, no. 1, pp. 1–13, Dec. 2020.
[107] I. Alotaibi, M. A. Abido, M. Khalid, and A. V. Savkin, "A comprehensive review of recent advances in smart grids: A sustainable future with renewable energy resources," *Energies*, vol. 13, no. 23, p. 6269, Nov. 2020.
[108] J. H. Wang, Z. Zhou, and A. Botterud, "An evolutionary game approach to analyzing bidding strategies in electricity markets with elastic demand," *Energy*, vol. 36, no. 5, pp. 3459–3467, May 2011.
[109] K. Wang, Z. Ouyang, R. Krishnan, L. Shu, and L. He, "A game theory-based energy management system using price elasticity for smart grids," *IEEE Trans. Ind. Informat.*, vol. 11, no. 6, pp. 1607–1616, Dec. 2015.
[110] S. Abapour, M. Nazari-Heris, B. Mohammadi-Ivatloo, and M. Tarafdar Hagh, "Game theory approaches for the solution of power system problems: A comprehensive review," *Arch. Comput. Methods Eng.*, vol. 27, no. 1, pp. 81–103, Jan. 2020.
[111] T. Pusa, M. Wannagat, and M.-F. Sagot, "Metabolic games," *Frontiers Appl. Math. Statist.*, vol. 5, p. 18, Apr. 2019.
[112] R. A. Khandelwal, B. G. Olivier, W. F. M. Röling, B. Teusink, and F. J. Bruggeman, "Community flux balance analysis for microbial consortia at balanced growth," *PLoS ONE*, vol. 8, no. 5, May 2013, Art. no. e64567.
[113] Z. Li, B. Liu, S. H.-J. Li, C. G. King, Z. Gitai, and N. S. Wingreen, "Modeling microbial metabolic trade-offs in a chemostat," *PLOS Comput. Biol.*, vol. 16, no. 8, Aug. 2020, Art. no. e1008156.
[114] D. Fiore, F. D. Rossa, A. Guarino, and M. D. Bernardo, "Feedback ratiometric control of two microbial populations in a single chemostat," *IEEE Control Syst. Lett.*, vol. 6, pp. 800–805, 2022.
[115] S. H. J. Chan, M. N. Simons, and C. D. Maranas, "SteadyCom: Predicting microbial abundances while ensuring community stability," *PLOS Comput. Biol.*, vol. 13, no. 5, May 2017, Art. no. e1005539.
[116] J. E. Goldford *et al.*, "Emergent simplicity in microbial community assembly," *Science*, vol. 361, no. 6401, pp. 469–474, Aug. 2018.
[117] R. Taffs *et al.*, "*In silico* approaches to study mass and energy flows in microbial consortia: A syntrophic case study," *BMC Syst. Biol.*, vol. 3, no. 1, pp. 1–16, Dec. 2009.
[118] A. Theorell and J. Stelling, "Microbial community decision making models in batch and chemostat cultures," in *Proc. Int. Conf. Comput. Methods Syst. Biol.* Cham, Switzerland: Springer, 2021.
[119] M. W. Gray, G. Burger, and B. F. Lang, "Mitochondrial evolution," *Science*, vol. 283, no. 5407, pp. 1476–1481, 1999.
[120] J. E. van Hylckama Vlieg, P. Veiga, C. Zhang, M. Derrien, and L. Zhao, "Impact of microbial transformation of food on health—From fermented foods to fermentation in the gastro-intestinal tract," *Current Opinion Biotechnol.*, vol. 22, no. 2, pp. 211–219, 2011.
[121] S. Rakoff-Nahoum, K. R. Foster, and L. E. Comstock, "The evolution of cooperation within the gut microbiota," *Nature*, vol. 533, no. 7602, pp. 255–259, May 2016.
[122] S. Brugiroux *et al.*, "Genome-guided design of a defined mouse microbiota that confers colonization resistance against Salmonella enterica serovar typhimurium," *Nature Microbiol.*, vol. 2, no. 2, pp. 1–12, Feb. 2017.
[123] A. R. Zomorrodi, M. M. Islam, and C. D. Maranas, "D-OptCom: Dynamic multi-level and multi-objective metabolic modeling of microbial communities," *ACS Synth. Biol.*, vol. 3, no. 4, pp. 247–257, Apr. 2014.
[124] D. J. V. Beste *et al.*, "^{13}C-flux spectral analysis of host-pathogen metabolism reveals a mixed diet for intracellular *Mycobacterium tuberculosis*," *Chem. Biol.*, vol. 20, no. 8, pp. 1012–1021, Aug. 2013.
[125] N. A. Gebreselassie and M. R. Antoniewicz, "^{13}C-metabolic flux analysis of co-cultures: A novel approach," *Metabolic Eng.*, vol. 31, pp. 132–139, Sep. 2015.
[126] A. Ghosh *et al.*, "A peptide-based method for ^{13}C metabolic flux analysis in microbial communities," *PLoS Comput. Biol.*, vol. 10, no. 9, Sep. 2014, Art. no. e1003827.
[127] J. A. Cole, L. Kohler, J. Hedhli, and Z. Luthey-Schulten, "Spatially-resolved metabolic cooperativity within dense bacterial colonies," *BMC Syst. Biol.*, vol. 9, no. 1, pp. 1–17, Dec. 2015.
[128] E. Wolfsberg, C. P. Long, and M. R. Antoniewicz, "Metabolism in dense microbial colonies: ^{13}C metabolic flux analysis of *E. coli* grown on agar identifies two distinct cell populations with acetate cross-feeding," *Metabolic Eng.*, vol. 49, pp. 242–247, Sep. 2018.
[129] M. Ackermann, "A functional perspective on phenotypic heterogeneity in microorganisms," *Nature Rev. Microbiol.*, vol. 13, no. 8, pp. 497–508, Aug. 2015.
[130] H. L. Smith and P. Waltman, *The Theory of the Chemostat: Dynamics of Microbial Competition*, vol. 13. Cambridge, U.K.: Cambridge Univ. Press, 1995.
[131] T. Stykel, "On criteria for asymptotic stability of differential-algebraic equations," *ZAMM-J. Appl. Math. Mech./Zeitschrift für Angew. Mathematik und Mech.*, vol. 82, no. 3, pp. 147–158, Mar. 2002.
[132] A. Zelezniak, S. Andrejev, O. Ponomarova, D. R. Mende, P. Bork, and K. R. Patil, "Metabolic dependencies drive species co-occurrence in diverse microbial communities," *Proc. Nat. Acad. Sci. USA*, vol. 112, no. 20, pp. 6449–6454, May 2015.

ABOUT THE AUTHORS

Axel Theorell received the M.S. degree in applied and computational mathematics from the KTH Royal Institute of Technology, Stockholm, Sweden, in 2015, and the Ph.D. degree from the Faculty of Mechanical Engineering, RWTH Aachen University, Aachen, Germany, in 2020.

Since 2020, he has been a Post-Doctoral Researcher with the Department of Biosystems Science and Engineering, ETH Zürich, Basel, Switzerland. He has a strong interest in mathematical modeling of biological systems, in particular, stoichiometric models and ^{13}C metabolic flux analysis, and computational statistics, with the main emphasis on Markov chain Monte Carlo methods.

Jörg Stelling received the M.S. degree in biotechnology from the Technical University of Braunschweig, Braunschweig, Germany, in 1996, and the Ph.D. degree in control and dynamic systems from the University of Stuttgart, Stuttgart, Germany, in 2004.

From 2005 to 2008, he was an Assistant Professor with the Computer Science Department, ETH Zürich, Basel, Switzerland, where he has been a tenured Professor with the Department of Biosystems Science and Engineering. His research interests include the analysis and synthesis of biological networks using—and further developing—methods from systems theory, computer science, and experimental biology.