Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Systems Biology

Genome-scale metabolic networks in time and space

Ove Øyås[1,2] and Jörg Stelling[1]

Abstract

Constraint-based models (CBMs) are key tools for elucidating the behavior of genome-scale metabolic networks, but the assumption of steady state hinders their application to spatio-temporally varying and multicellular systems. Models that integrate CBMs with kinetics to allow dynamic simulation through dynamic flux balance analysis (DFBA) can circumvent this problem as well as the limitations of purely kinetic models. With many technical barriers for DFBA overcome in recent years, applications traditionally focused on metabolic engineering have expanded to address problems such as evolution of microbial communities, functions of biomedically relevant biofilms, and diet effects on Parkinson's disease. By addressing substantial computational challenges, we expect that such hybrid metabolic models will pave the way towards whole-cell modeling.

Addresses

[1] Department of Biosystems Science and Engineering, SIB Swiss Institute of Bioinformatics, ETH Zurich, Basel, Switzerland

[2] Systems Biology PhD Program, Life Science Zurich Graduate School, Zurich, Switzerland

Corresponding author: Stelling, Jörg (joerg.stelling@bsse.ethz.ch)

Current Opinion in Systems Biology 2017, ■:1–8

This review comes from a themed issue on **Regulatory and metabolic networks (2018)**

Edited by **Bas Teusink** and **Uwe Sauer**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.coisb.2017.12.003

Keywords

Metabolic networks, Constraint-based models, Genome-scale models, Multi-scale models, Dynamic flux balance analysis.

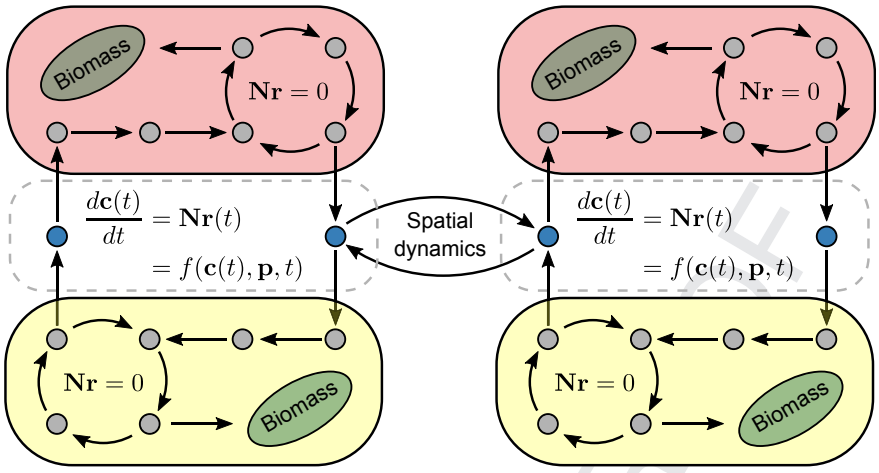
Introduction

Metabolic networks are fundamental in biology, enzymatically converting available substrates into products that include the cellular components needed for survival and growth. Understanding, predicting, and ultimately controlling their behavior is a challenge of crucial importance, not only for basic science but also for manifold applications ranging from metabolic engineering to human health and disease. Experimental technologies, most importantly high-throughput metabolomics, advance rapidly, but system-level mathematical modeling is needed in order to transform data into insight [1].

Constraint-based models (CBMs) are currently the most established and powerful tools for large-scale metabolic network modeling [2]. They are fundamentally based on the quasi-steady-state assumption (QSSA), exploiting that metabolism is fast and therefore approximately invariant on the time-scale of other processes such as gene regulation with which it interacts [3]. This makes CBMs and their analysis linear, parameter-free, and applicable to metabolic networks of virtually any species with a sequenced genome [4,5]. The drawback of relying on the QSSA is that CBMs do not represent metabolite concentrations or their dynamics. Rather, they predict metabolic flux distributions, steady-state reaction rates for all reactions in the network, which have been studied extensively in many organisms, using a plethora of computational methods. The three main computational approaches are (i) flux balance analysis (FBA), which predicts fluxes by assuming cellular objectives such as maximization of growth rate in microorganisms, (ii) pathway analysis, which identifies all possible flux routes through the network, and (iii) random flux sampling, which seeks to determine probability distributions of feasible steady-state fluxes [6].

CBMs have been remarkably successful in areas that range from systematically representing biological knowledge and data [7] to devising metabolic engineering strategies [8]. However, the QSSA limits their applicability beyond the obvious: they do not capture the dynamic behavior of cells in changing environments. Even for a constant environment, CBMs alone cannot predict fluxes from metabolite concentrations (and vice versa). Bridging this gap between fluxes and concentrations is a fundamental challenge of particular importance for multicellular systems such as microbial communities or human tissues because the metabolite concentrations in the environment couple the cells' behaviors to each other (Figure 1). In such cases, methods established for unicellular CBMs are not directly applicable because of many challenges that range from model construction, to assumed cellular objectives, to context-dependent interactions between cells [9]. For example, modified cellular objectives for FBA of microbial communities [10,11] are hard to justify biologically (e.g., why should an individual strain's objective be to maximize the overall growth rate of the community?). For unbiased methods such as pathway analysis and random sampling, the size of multiple interacting metabolic networks exacerbates the computational challenges and makes flux prediction for multicellular systems practically infeasible [12,13].

Figure 1



Metabolic models for multicellular systems. Cells of potentially different types (indicated by colors) contain intracellular metabolic networks composed of chemical species (grey nodes) and reactions (arrows) that are formally represented by a stoichiometric matrix **N**. CBMs for individual cells rely on the QSSA, assuming that the intracellular metabolite concentrations $\mathbf{c}(t)$ do not change over time, such that the mass balances lead to a linear problem in which the flux distribution (set of reaction rates) **r** is the only unknown. For extracellular metabolites (blue nodes) accessible to several cells, kinetics (time-dependent exchange rates $\mathbf{r}(t)$ that are functions of extracellular concentrations $\mathbf{c}(t)$ and kinetic parameters **p**) need to be considered even in a constant environment to capture how resources are distributed between cells. Spatial extensions follow the same logic, where kinetics (for example, describing the diffusion of metabolites) connect different compartments (left and right).

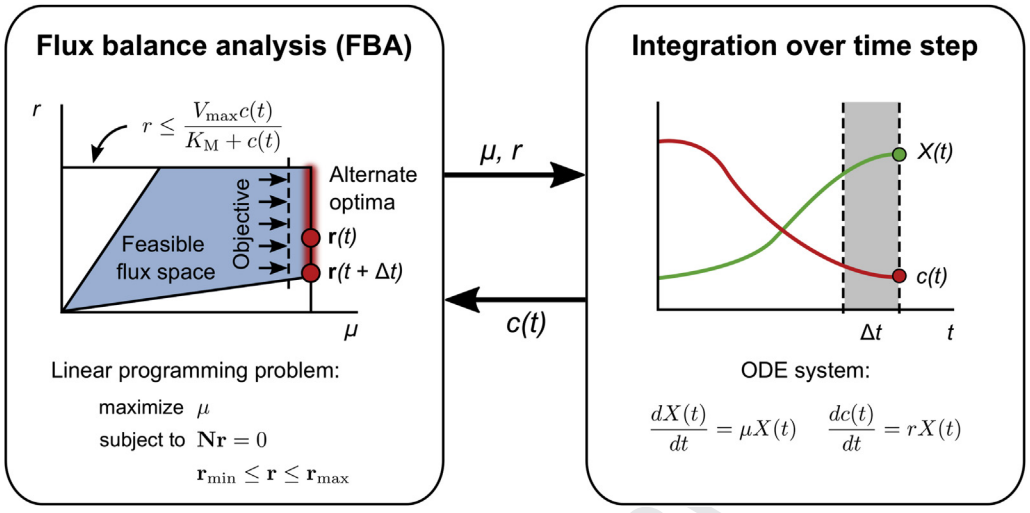
Without fundamental progress in the computational methods, flux predictions for multicellular systems require an integration of CBMs with extracellular concentrations through experimental data, metabolite uptake kinetics, or both. We argue that models that combine CBMs with kinetics are the most promising approach to connect fluxes to concentrations because they rely less on dynamic metabolite data than state-of-the-art methods for integrating metabolomics with CBMs [14,15]. They also need fewer kinetic rate laws and parameters than detailed kinetic models that are often hard to identify and computationally expensive to simulate [16]. Hybrid models can be simulated through dynamic flux balance analysis (DFBA) approaches [17], which connect kinetics to flux predictions from FBA without needing multicellular objectives. Alternative approaches to simplify dynamics, cybernetic models, consider more detailed, optimal pathway-level resource allocation; they are computationally expensive and currently not applicable to genome-scale [18]. Here, we therefore discuss recent advances in DFBA models, and their applications to diverse uni- and multicellular systems with temporal and spatiotemporal resolution.

Metabolic models and dynamic FBA

Mass balances for metabolites that constrain the static or dynamic fluxes are the basis for all metabolic models. DFBA models contain two distinct sets of mass balances, one dynamic and one static (Figure 1). The QSSA virtually always applies to the balances of all intracellular metabolites; the metabolic network is considered static on the extracellular time-scale. Dynamics are modeled by a sequence of instantaneous steady-state responses to environmental changes, mediated by the kinetics of metabolite uptake and secretion. In this case, the dynamic components are biomass and metabolites that can be exchanged between the cell and the environment. Multiple CBMs can share the same pool of dynamic metabolites, which in turn can connect to pools at other points in space.

Because the dynamic and static mass balances are interdependent, one has to solve them together (Figure 2). This amounts to jointly solving ordinary differential equations (ODEs) for dynamic balances and linear algebraic equations for static balances, and integration of the former requires flux predictions from the latter. Any realistic metabolic network contains more reactions than metabolites, such that the static system is generally underdetermined. Together with flux capacity constraints, this defines a CBM whose solution space contains an infinite number of feasible steady-state flux distributions. FBA uses linear programming to identify a specific solution that optimizes an objective function, typically growth rate as represented by the flux of a biomass reaction, and DFBA uses FBA to get the static fluxes needed for integration of the ODE system. The static fluxes, in turn, are constrained by the state of the dynamic system—the environment—through simple binary rules or kinetic equations. In multicellular settings, environmental resources are allocated to individual cells at each time step, and competition and cooperation can emerge over time through dynamic interactions. Importantly, it is not necessary to define

Figure 2



Dynamic flux balance analysis. The general DFBA procedure is illustrated by a hypothetical model consisting only of a growth rate, μ, and a reaction rate, r, which affect the time-dependent extracellular concentrations of biomass, $X(t)$, and of a metabolite, $C(t)$. The feasible flux space is constrained by Michaelis–Menten kinetics with parameters K_M and V_{max}. FBA identifies an optimal flux distribution, $\mathbf{r}(t) = (\mu, r)$, using linear programming. The optimal FBA solution is often non-unique and the algorithm may identify different alternate optima in adjacent time steps, causing numerical instability, even when the feasible space does not change. Fluxes predicted by FBA are used to integrate the ODEs describing extracellular dynamics over the next time step, Δt, which is either fixed or chosen by the ODE solver. Kinetic constraints are updated with the new metabolite concentration before computing the next FBA solution. In principle, one can replace FBA by any procedure that predicts fluxes for all reactions that affect the dynamic concentrations.

(potentially biologically questionable) multicellular objectives.

While conceptually simple, DFBA poses significant computational challenges. Most simulation methods divide the simulation time into sufficiently small intervals, iteratively solving FBA at the start of each interval and integrating the ODEs until the next one begins. The static optimization approach (SOA), which uses pre-defined time intervals [17,19], was developed first, but it is now superseded by the direct approach (DA), in which an ODE solver controls adaptive time-stepping [20]. While both approaches can cope with genome-scale models, the DA is more accurate and less computationally expensive than the SOA because adaptive time steps reduce the number of FBA problems to solve. Still, numerical stiffness, which forces small time steps for all approaches, is frequent in DFBA because of FBA solutions that are non-unique (see Figure 2) or very sensitive to small changes in the external conditions imposed by the dynamic part of the model [21]. To reduce such problems, a recently developed approach uses optimality conditions to reformulate the DFBA problem as a system of differential algebraic equations (DAEs), which allows solutions to be efficiently reused without solving FBA [22,23]. It also reduces instabilities by solving multiple FBA problems sequentially to obtain a solution that is unique, at least for dynamic fluxes of interest [20,22,24]. One can also precompute a large number of FBA solutions and reuse them during simulation [25,26]. Finally, the dynamic optimization approach (DOA) solves the system simultaneously over the entire simulation time in a single nonlinear optimization problem, allowing dynamic objectives such as product or biomass yield, but it is applicable only for very small models [17,27,28]. While appropriate choices of DFBA simulation methods are likely to be model- and context-dependent, how to select a method is currently an open problem.

In addition to the trade-off between simulation accuracy and computational effort (and thus, scalability to large multicellular models), model parametrization is a key challenge unsolved by computational power alone. DFBA requires parametrized kinetics for all relevant exchange fluxes, and their number increases with model complexity and number of CBMs. For example, typical genome-scale CBMs have hundreds of exchange reactions each, and kinetic parameters are mostly unknown [9]. We see three possibilities for dealing with this problem: (i) Using biological knowledge to select relevant exchanges, which may be feasible for well-characterized biological systems, but not for ill-characterized systems such as natural communities. (ii) Brute-force parameter estimation, but current computational methods do not scale sufficiently well [29]. (iii) Systematic model reduction using techniques such as flux variability analysis [30] or structural sensitivity analysis [31] to identify relevant exchanges for individual CBMs or ensembles of CBMs. While the last approach appears most promising, it will require substantial conceptual and technical developments.

Finally, metabolic models that account for spatial heterogeneity usually capture spatial effects by performing DFBA separately in discrete compartments (Figure 1). To connect compartments, the spatial dynamics may include diffusive and convective transport and—if the model considers single cells rather than populations—cell movement and proliferation. This may require solving partial differential equations (PDEs) and executing spatial rules. Consequently, spatiotemporal models inherit at least all the difficulties of DFBA, and spatial discretization essentially multiplies computational cost by the number of compartments. This makes efficient and reliable simulation of large and spatially heterogeneous hybrid models a major challenge that will require new and improved computational approaches, for example, to devise strategies that determine optimal spatial resolution.

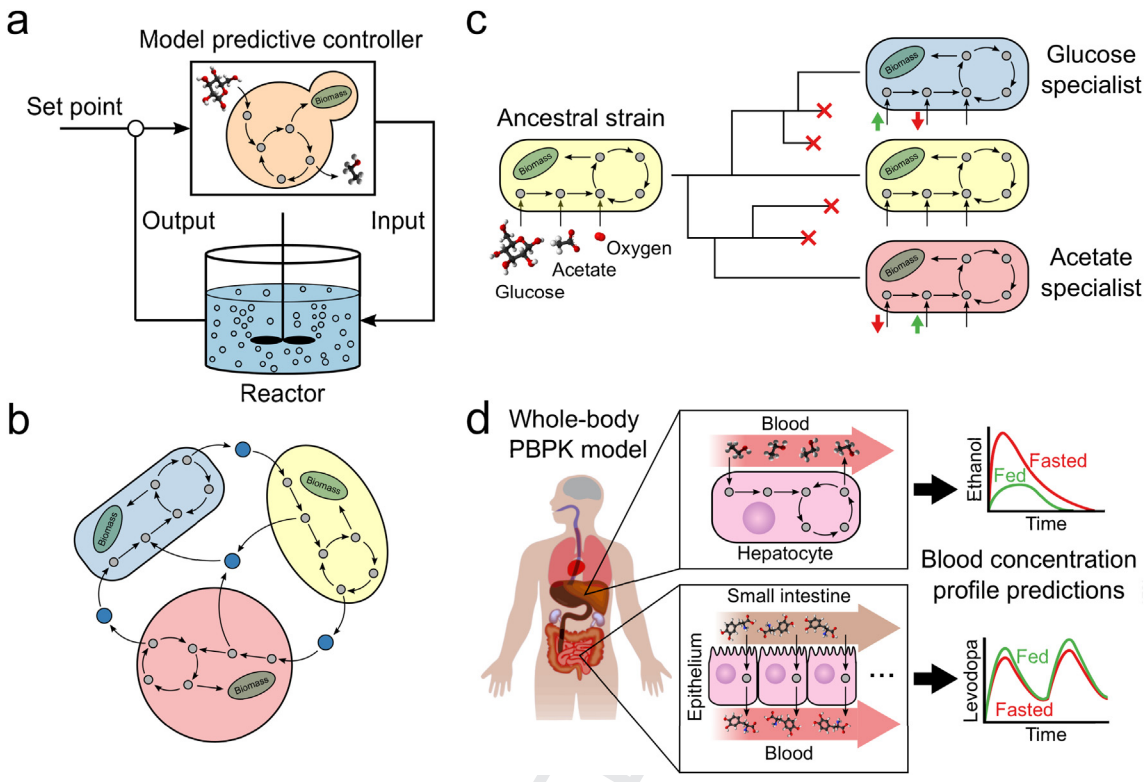
Modeling cells in temporally varying environments

Biochemical and metabolic engineering has been the main application area for DFBA models so far, specifically for the simulation of microbial batch and fed-batch fermentations. Pioneering studies introducing DFBA 20 years ago showed that small-scale DFBA could be used to predict growth and extracellular metabolite dynamics in batch cultures of *Escherichia coli* [17,19] and *Saccharomyces cerevisiae* [32–34], paving the way for genome-scale DFBA analyses [20,35–37]. As DFBA methods and CBMs matured, models were extended to co-cultures, for example, of *E. coli* and *S. cerevisiae* fermenting mixtures of glucose and xylose [38–40]. Only recent studies, however, employed DFBA to predict metabolic engineering targets for improved substrate utilization and production of valuable compounds. For example, Hohenschuh et al. [41] coupled a genome-scale yeast CBM to a kinetic model of hexose uptake, including competitive inhibition and hexose transporter expression. They used DFBA to identify bottlenecks in the xylulose transport and utilization pathway in batch co-fermentation of glucose, xylose, and xylulose. Similarly, Wang et al. [42] predicted gene targets for increased batch production of an immunosuppressive drug by augmenting a genome-scale CBM of *Streptomyces tsukubaensis* with simple substrate uptake and product secretion kinetics. They proposed a novel computational approach that predicts the dynamic effects of engineered perturbations by combining DFBA with minimization of metabolic adjustment (MOMA) [43]. It identified four key targets for gene knockout or overexpression, leading to a superior production strain. Another emerging DFBA application is model-predictive control, as shown by Chang et al. [44] who used a genome-scale yeast CBM with glucose and oxygen uptake kinetics for closed-loop control of fed-batch ethanol production, increasing yield compared to open-loop control (Figure 3a).

Microbial communities are a key current application area for DFBA (Figure 3b). For example, early studies used genome-scale models of *Rhodoferax ferrireducens* and *Geobacter sulfurreducens* with uptake kinetics for acetate and a few inorganic ions to model competition in a uranium-contaminated environment, explaining observed population compositions in terms of nutrient availability and suggesting environmental modulation strategies for bioremediation [45,46]. To investigate (model systems of) microbial ecology, Hanemaajer et al. [47] used a similar approach, combining genome-scale models with uptake kinetics but focusing on medium-dependent metabolic interactions between *Clostridium acetobutylicum* and *Wolinella succinogenes*. DFBA simulations agreed well with measured concentration profiles of eight extracellular metabolites; they identified and quantified metabolite exchanges for four different nitrogen sources. New application areas of DFBA for microbial communities address important biological questions, for example, on the evolutionary emergence of stable populations. Louca et al. [48] and Groβkopf et al. [49] applied DFBA to *E. coli* CBMs with glucose, acetate, and oxygen uptake kinetics to capture and understand the diversification of an ancestral *E. coli* strain into coexisting glucose and acetate specialists in long-term evolution experiments (Figure 3c). The two studies differ substantially in methods and scope, however: one built models of the two strains that reproduced the observed evolutionary dynamics [48], the other also predicted strain emergence from a single model of the ancestral strain through random mutational effects on maximum metabolite uptake rates [49]. Despite these successes, DFBA remains limited to small and well-characterized communities by the availability of high-quality models, kinetic information, and experimental data.

Corresponding models for humans are less frequent, among other reasons, because of the complexity and late availability of human CBMs amenable to simulation. In contrast to the simple metabolite uptake kinetics of microbial models, most human studies employ large physiologically based pharmacokinetic (PBPK) models that quantitatively account for whole-body absorption, distribution, metabolism, and excretion of metabolites and drugs with organ-level resolution [50] (Figure 3d). In the first published example, a genome-scale hepatocyte CBM was coupled to the liver tissue of a PBPK model and DFBA simulated the effects of drugs and metabolic disorders, assuming case-specific liver objectives such as maximum ammonia or uric acid production [51]. Toroghi et al. [24] recently presented a very similar approach, coupling the same hepatocyte model to a PBPK model but using DFBA with sequential optimization and a more general objective—maximizing urea production before minimizing total flux through the liver—to simulate the effects of inborn errors in amino acid metabolism. The number of 237 serum metabolic

Figure 3



Example applications of dynamic flux balance analysis. Most recently, DFBA models have been used to (**a**) optimize and control ethanol production in microbial batch and bubble-column fermentations [44,53,55], (**b**) predict metabolic interactions in microbial communities and their effects on community structure [47,60–62], (**c**) understand the emergence of glucose and acetate specialists from an ancestral *E. coli* strain in long-term evolution experiments [48,49], and (**d**) extend human whole-body physiologically based pharmacokinetic (PBPK) models in order to predict the effect of diet on blood concentration profiles of ethanol and levodopa, a drug used to treat Parkinson's disease [24,52,63].

biomarkers covered by the model illustrates the prediction potential (and complexity of the task). Potential applications are far-ranging, as demonstrated by a study that added oxidative and non-oxidative ethanol metabolism to the model and simulated alcohol clearance from the body, capturing *in vivo* differences in blood alcohol concentration profiles between genders and fed and fasted conditions [52].

Modeling cells in spatiotemporally varying environments

Spatially heterogeneous systems are the most recent application area for DFBA-type models, for example, when the assumption of well-mixed bioreactors for microbial fermentations that only vary in time is not justified. This is the case in bubble column reactors, where liquid and gaseous substrates are fed from the bottom and consumed along the column, creating a spatial gradient that affects cell growth and product formation. For ethanol production from synthesis gas in bubble column reactors with *Clostridium ljungdahlii*, a model with uptake kinetics for the gaseous substrates including inhibitory effects was presented [53,54]. The studies also propose a general framework for spatiotemporal DFBA with one-dimensional convection and diffusion, building on a previously published simulator [22]. Again, the work provided the basis for new process control strategies: model predictive control of the bubble column process was suggested to stabilize ethanol concentration faster than standard (PID) control in response to perturbations [55].

The tightly linked phenotypic and spatial heterogeneity of microbial populations is a central research theme in current microbiology [56], and this is reflected in modeling. Spatial variation in biofilms has received particular attention via population- and individual-based approaches. They consider models as representing a population in which mass grows, or as individuals, leading to model duplication upon cell division. Early examples include a population-based model, in which a genome-scale model of *G. sulfurreducens* provided a physiological underpinning for an existing dynamic model of a microbial fuel cell biofilm [57]. Two different frameworks coupled multiple genome-scale models to a two-dimensional diffusion model to simulate *Pseudomonas*

aeruginosa biofilm development [58] and to predict spatial effects in simple two- and three-species communities in agreement with experiments [59]. As an example for biomedical applications, Phalak et al. [60] combined genome-scale models of *P. aeruginosa* and *Staphylococcus aureus* with uptake kinetics for glucose, oxygen, and three potentially cross-fed metabolites in a spatiotemporal DFBA framework [54]; they predicted spatial partitioning of the two bacteria in a chronic wound biofilm consistent with *in vitro* studies. The same approach could be transferred to a biofilm consisting of three microbes from the human gut, predicting that four key metabolite exchanges are required for community stability [61]. A more complex gut community with seven species represents the current state of the art [62]; its predictions suggest that gradients of complex carbohydrates create niches that determine spatial organization in the human gut.

The greatest challenge for DFBA models is probably the spatiotemporal prediction of metabolism in human tissues. Progress has been made by two recent studies. A population-based study coupled seven genome-scale models of small intestine epithelial cells to a PBPK model to predict the effect of diet on levodopa absorption along the small intestine of Parkinson's disease patients [63]. In an individual-based analysis, generic genome-scale cancer models were coupled to a diffusion model, and used to simulate tumor growth in three dimensions [64]. Towards detailed and reliable whole-body models that may improve diagnosis and treatment, however, one has to overcome many challenges. In addition to the general challenges for spatio-temporal DFBA, this includes the generation of cell type-specific CBMs using functional − omics data, which requires accurate methods for data integration.

Conclusions and outlook

Metabolic network models and DFBA have seen an increased scope of applications over the past few years, ranging from fundamental problems in evolution to practical problems in biomedicine. Several available software packages for DFBA [45,48,65–67] make such studies feasible in practice, and a new modular framework based on Petri nets can integrate DFBA with other modeling formalisms [68] to enable future developments towards whole-cell models [69]. However, we argue that the efficient and accurate simulation via DFBA approaches remains a main bottleneck for their application to multicellular systems. For example, a major open challenge is to use experimental data to calibrate DFBA models, where identifying the parameters of kinetic model parts requires multiple model simulations.

Acknowledgements

Financial support through the SystemsX.ch project GutX, evaluated by the Swiss National Science Foundation, is gratefully acknowledged.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Nielsen J: **Systems biology of metabolism**. *Annu Rev Biochem* 2017. Q2
2. Terzer M, Maynard ND, Covert MW, Stelling J: **Genome-scale metabolic networks**. *Wiley Interdiscip Rev Syst Biol Med* 2009, **1**:285–297.
3. Gunawardena J: **Time-scale separation - Michaelis and Menten's old idea, still bearing fruit**. *FEBS J* 2014, **281**:473–488.
4. Thiele I, Palsson BØ: **A protocol for generating a high-quality genome-scale metabolic reconstruction**. *Nat Protoc* 2010, **5**:93–121.
5. Henry CS, DeJongh M, Best AA, Frybarger PM, Linsay B, Stevens RL: **High-throughput generation, optimization and analysis of genome-scale metabolic models**. *Nat Biotechnol* 2010, **28**:977–982.
6. Lewis NE, Nagarajan H, Palsson BO: **Constraining the metabolic genotype-phenotype relationship using a phylogeny of in silico methods**. *Nat Rev Microbiol* 2012, **10**:291–305.
7. O'Brien EJ, Monk JM, Palsson BO: **Using genome-scale models to predict biological capabilities**. *Cell* 2015, **161**:971–987.
8. Kim WJ, Kim HU, Lee SY: **Current state and applications of microbial genome-scale metabolic models**. *Curr Opin Struct Biol* 2017, **2**:9–17.
9. Gottstein W, Olivier BG, Bruggeman FJ, Teusink B: **Constraint-based stoichiometric modelling from single organisms to microbial communities**. *J R Soc Interface* 2016, **13**.
10. Zomorrodi AR, Islam MM, Maranas CD: **D-optcom: dynamic multi-level and multi-objective metabolic modeling of microbial communities**. *ACS Synthetic Biology* 2014, **3**:247–257.
11. Shoaie S, Ghaffari P, Kovatcheva-Datchary P, Mardinoglu A, Sen P, Pujos-Guillot E, De Wouters T, Juste C, Rizkalla S, Chilloux J, *et al.*: **Quantifying diet-induced metabolic changes of the human gut microbiome**. *Cell Metabol* 2015, **22**:320–331.
12. De Martino D, Mori M, Parisi V: **Uniform sampling of steady states in metabolic networks: Heterogeneous scales and rounding**. *PLoS One* 2015, **10**:1–14.
13. Klamt S, Regensburger G, Gerstl MP, Jungreuthmayer C, Schuster S, Mahadevan R, Zanghellini J, Müller S: **From elementary flux modes to elementary flux vectors: metabolic pathway analysis with arbitrary linear flux constraints**. *PLoS Comput Biol* 2017, **13**:e1005409.
14. Aurich MK, Paglia G, Rolfsson O, Hrafnsdottir S, Magnusdottir M, Stefaniak MM, Palsson B, Fleming RMT, Thiele I: **Prediction of intracellular metabolic states from extracellular metabolomic data**. *Metabolomics* 2015, **11**:603–619.
15. Bordbar A, Yurkovich JT, Paglia G, Rolfsson O, Sigurjónsson ÓE, Palsson BO: **Elucidating dynamic metabolic physiology through network integration of quantitative time-course metabolomics**. *Sci Rep* 2017, **28**:1294–1296, https://doi.org/10.1038/srep46249. Published online: 7 April 2017.
16. Srinivasan S, Cluett WR, Mahadevan R: **Constructing kinetic models of metabolism at genome-scales: a review**. *Biotechnol J* 2015, **10**:1345–1359.
17. Mahadevan R, Edwards JS, Doyle FJ: **Dynamic flux balance analysis of diauxic growth in Escherichia coli**. *Biophys J* 2002, **83**:1331–1340.
18. Ramkrishna D, Song HS: **Dynamic models of metabolism: review of the cybernetic approach**. *AIChE J* 2012, **58**:986–997.
19. Varma A, Palsson BO: **Stoichiometric flux balance models quantitatively predict growth and metabolic by-product secretion in wild-type Escherichia coli W3110**. *Appl Environ Microbiol* 1994, **60**:3724–3731.

20. Hjersted JL, Henson MA, Mahadevan R: **Genome-scale analysis of Saccharomyces cerevisiae metabolism and ethanol production in fed-batch culture**. *Biotechnol Bioeng* 2007, **97**: 1190–1204.

21. Mahadevan R, Schilling CH: **The effects of alternate optimal solutions in constraint-based genome-scale metabolic models**. *Metab Eng* 2003, **5**:264–276.

22. Höffner K, Harwood SM, Barton PI: **A reliable simulator for dynamic flux balance analysis**. *Biotechnol Bioeng* 2013, **110**: 792–802.

23. * Harwood SM, Höffner K, Barton PI: **Efficient solution of ordinary differential equations with a parametric lexicographic linear program embedded**. *Numer Math* 2016, **133**:623–653.
The detailed theory of one the most recent and efficient DFBA simulators (ref. 22).

24. Toroghi MK, Cluett WR, Mahadevan R: **A multi-scale model of the whole human body based on dynamic parsimonious flux balance analysis**. *IFAC-PapersOnLine* 2016, **49**:937–942.

25. Scheibe TD, Mahadevan R, Fang Y, Garg S, Long PE, Lovley DR: **Coupling a genome-scale metabolic model with a reactive transport model to describe in situ uranium bioremediation**. *Microbial Biotechnology* 2009, **2**:274–286.

26. Jeong DH, Yoo SJ, Kim JH, Lee JM: **Computationally efficient dynamic simulation of cellular kinetics via explicit solution of flux balance analysis : xDFBA model**. *Chem Eng Res Des* 2016, **113**:85–95.

27. Luo R-Y, Liao S, Tao G-Y, Li Y-Y, Zeng S, Li Y-X, Luo Q: **Dynamic analysis of optimality in myocardial energy metabolism under normal and ischemic conditions**. *Mol Syst Biol* 2006, **2**:1–6.

28. Luo R, Wei H, Ye L, Wang K, Chen F, Luo L, Liu L, Li Y, Crabbe MJC, Jin L, *et al.*: **Photosynthetic metabolism of C3 plants shows highly cooperative regulation under changing environments: a systems biological analysis**. *Proc Natl Acad Sci Unit States Am* 2009, **106**:847–852.

29. Babtie AC, Stumpf MPH: **How to deal with parameters for whole-cell modelling**. *J R Soc Interface* 2017, **14**:20170237.

30. Mahadevan R, Schilling CH: **The effects of alternate optimal solutions in constraint-based genome-scale metabolic models**. *Metab Eng* 2003, **5**:264–276.

31. Uhr M, Stelling J: **Structural sensitivity analysis of metabolic networks**. *IFAC Proceedings Volumes* 2008, **41**:15879–15884.

32. Sainz J, Pizarro F, Pérez-Correa JR, Agosin E: **Modeling of yeast metabolism and process dynamics in batch fermentation**. *Biotechnol Bioeng* 2003, **81**:818–828.

33. Hjersted JL, Henson MA: **Optimization of fed-batch Saccharomyces cerevisiae fermentation using dynamic flux balance models**. *Biotechnol Prog* 2006, **22**:1239–1248.

34. Pizarro F, Varela C, Martabit C, Bruno C, Pérez-Correa JR, Agosin E: **Coupling kinetic expressions and metabolic networks for predicting wine fermentations**. *Biotechnol Bioeng* 2007, **98**:986–998.

35. Anesiadis N, Cluett WR, Mahadevan R: **Dynamic metabolic engineering for increasing bioprocess productivity**. *Metab Eng* 2008, **10**:255–266.

36. Hjersted JL, Henson MA: **Steady-state and dynamic flux balance analysis of ethanol production by Saccharomyces cerevisiae**. *IET Syst Biol* 2009, **3**:167–179.

37. Meadows AL, Karnik R, Lam H, Forestell S, Snedecor B: **Application of dynamic flux balance analysis to an industrial Escherichia coli fermentation**. *Metab Eng* 2010, **12**:150–160.

38. Hanly TJ, Henson MA: **Dynamic flux balance modeling of microbial co-cultures for efficient batch fermentation of glucose and xylose mixtures**. *Biotechnol Bioeng* 2011, **108**: 376–385.

39. Hanly TJ, Urello M, Henson MA: **Dynamic flux balance modeling of S. cerevisiae and E. coli co-cultures for efficient consumption of glucose/xylose mixtures**. *Appl Microbiol Biotechnol* 2012, **93**:2529–2541.

40. Hanly TJ, Henson MA: **Dynamic metabolic modeling of a microaerobic yeast co-culture: predicting and optimizing ethanol production from glucose/xylose mixtures**. *Biotechnol Biofuels* 2013, **6**:44.

41. Hohenschuh W, Hector R, Murthy GS: **A dynamic flux balance model and bottleneck identification of glucose, xylose, xylulose co-fermentation in Saccharomyces cerevisiae**. *Bioresour Technol* 2015, **188**:153–160.

42. ** Wang C, Liu J, Liu H, Wang J, Wen J: **A genome-scale dynamic flux balance analysis model of Streptomyces tsukubaensis NRRL18488 to predict the targets for increasing FK506 production**. *Biochem Eng J* 2017, **123**:45–56.
A genome-scale model coupled to substrate uptake and product secretion kinetics predicted gene targets for overproduction of an immunosuppressive drug that were experimentally verified and used to construct a superior production strain.

43. Segrè D, Vitkup D, Church GM: **Analysis of optimality in natural and perturbed metabolic networks**. *Proc Natl Acad Sci Unit States Am* 2002, **99**:15112–15117.

44. * Chang L, Liu X, Henson MA: **Nonlinear model predictive control of fed-batch fermentations using dynamic flux balance models**. *J Process Contr* 2016, **42**:137–149.
A genome-scale model with glucose and oxygen uptake kinetics was used for nonlinear model-predictive control of yeast fed-batch fermentation, increasing ethanol yield relative to the open-loop policy.

45. Zhuang K, Izallalen M, Mouser P, Richter H, Risso C, Mahadevan R, Lovley DR: **Genome-scale dynamic modeling of the competition between Rhodoferax and Geobacter in anoxic subsurface environments**. *ISME J* 2011, **5**:305–316.

46. Zhuang K, Ma E, Lovley DR, Mahadevan R: **The design of long-term effective uranium bioremediation strategy using a community metabolic model**. *Biotechnol Bioeng* 2012, **109**: 2475–2483.

47. * Hanemaaijer M, Olivier BG, Röling WFM, Bruggeman FJ, Teusink B: **Model-based quantification of metabolic interactions from dynamic microbial-community data**. *PLoS One* 2017:1–19.
Genome-scale models of *Clostridium acetobutylicum* and *Wolinella succinogenes* with metabolite uptake kinetics were coupled in a co-culture model and used to infer fluxes, including metabolite exchanges, from dynamic experimental data.

48. ** Louca S, Doebeli M: **Calibration and analysis of genome-based models for microbial ecology**. *eLife* 2015, **4**:1–17.
A dynamic framework for microbial community modeling was applied to three interacting genome-scale models of *Escherichia coli* strains, replicating the dynamics of long-term evolution experiments, in which stable strain coexistence emerged.

49. ** Großkopf T, Consuegra J, Gaffé J, Willison JC, Lenski RE, Soyer OS, Schneider D: **Metabolic modelling in a dynamic evolutionary framework predicts adaptive diversification of bacteria in a long-term evolution experiment**. *BMC Evol Biol* 2016:1–15.
An *E. coli* model with metabolite uptake kinetics was evolved *in silico* through random mutations affecting the maximum uptake rates, consistent with long-term evolution *in vitro*.

50. Thiele I, Clancy CM, Heinken A, Fleming RMT: **Quantitative systems pharmacology and the personalized drug-microbiota-diet axis**. *Curr Opin Struct Biol* 2017, **4**:1–10.

51. Krauss M, Schaller S, Borchers S, Findeisen R, Lippert J, Kuepfer L: **Integrating cellular metabolism into a multiscale whole-body model**. *PLoS Comput Biol* 2012, **8**.

52. * Toroghi MK, Cluett WR, Mahadevan R: **Multi-scale metabolic modelling approach for predicting blood alcohol concentration**. *IEEE Life Sciences Letters* 2017, **7685**. 1–1.
The authors coupled a genome-scale human hepatocyte model to a whole-body model and used DFBA to predict blood alcohol concentration profiles in agreement with *in vivo* data.

53. Chen J, Gomez JA, Höffner K, Barton PI, Henson MA: **Metabolic modeling of synthesis gas fermentation in bubble column reactors**. *Biotechnol Biofuels* 2015, **8**:89.

54. * Chen J, Gomez JA, Höffner K, Phalak P, Barton PI, Henson MA: **Spatiotemporal modeling of microbial metabolism**. *BMC Syst Biol* 2016, **10**:21.

An efficient spatiotemporal genome-scale modeling framework that includes metabolite uptake kinetics, diffusion and convection was presented and applied to clostridial syngas production and a *Pseudomonas aeruginosa* chronic wound biofilm.

55. Fu Y, Chang L, Henson MA, Liu XG: **Dynamic matrix control of a bubble-column reactor for microbial synthesis gas fermentation**. *Chem Eng Technol* 2017, **40**:727–736.

56. Ackermann M: **A functional perspective on phenotypic heterogeneity in microorganisms**. *Nat Rev Microbiol* 2015, **13**: 497–508.

57. Jayasinghe N, Mahadevan R: **Metabolic modeling of spatial heterogeneity of biofilms in microbial fuel cells**. *IFAC* 2010.

58. Biggs MB, Papin Ja: **Novel multiscale modeling tool applied to Pseudomonas aeruginosa biofilm formation**. *PLoS ONE* 2013, **8**:1–8.

59. Harcombe WR, Riehl WJ, Dukovski I, Granger BR, Betts A, Lang AH, Bonilla G, Kar A, Leiby N, Mehta P, *et al.*: **Metabolic resource allocation in individual microbes determines ecosystem interactions and spatial dynamics**. *Cell Reports* 2014, **7**:1104–1115.

60. Phalak P, Chen J, Carlson RP, Henson MA: **Metabolic modeling of a chronic wound biofilm consortium predicts spatial partitioning of bacterial species**. *BMC Syst Biol* 2016, **10**:90.

61. Henson M, Phalak P: **Byproduct cross feeding and community stability in an in silico biofilm model of the gut microbiome**. *Processes* 2017, **5**:1–23.

62. ** Bauer E, Zimmermann J, Baldini F, Thiele I, Kaleta C, Noronha A: **BacArena: individual-based metabolic modeling of heterogeneous microbes in complex communities**. *PLoS Comput Biol* 2017, **13**.

An individual-based modeling framework that facilitates coupling of multiple genome-scale models with metabolite uptake kinetics and diffusion, applied to *Pseudomonas aeruginosa* biofilm formation and a seven-member community of bacteria from the human gut.

63. ** Guebila MB, Thiele I: **Model-based dietary optimization for late-stage, levodopa-treated, Parkinson's disease patients**. *npj Systems Biol Appl* 2016, **2**:16013.

Seven genome-scale models of human small intestine epithelial cells were coupled to a whole-body PBPK model and used to predict the effect of diet on levodopa absorption along the gut in Parkinson's disease patients.

64. Ghadiri M, Heidari M, Marashi S-A, Mousavi SH: **A multiscale agent-based framework integrated with a constraint-based metabolic network model of cancer for simulating avascular tumor growth**. *Mol BioSyst* 2017, **68**:35–49.

65. Schellenberger J, Que R, Fleming RMT, Thiele I, Orth JD, Feist AM, Zielinski DC, Bordbar A, Lewis NE, Rahmanian S, *et al.*: **Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox v2.0**. *Nat Protoc* 2011, **6**:1290–1307.

66. Mao L, Verwoerd WS: **ORCA: a COBRA toolbox extension for model-driven discovery and analysis**. *Bioinformatics* 2014, **30**: 584–585.

67. Gomez Ja, Höffner K, Barton PI: **DFBAlab: a fast and reliable MATLAB code for dynamic flux balance analysis**. *BMC Bioinf* 2014, **15**:409.

68. * Wu H, von Kamp A, Leoncikas V, Mori W, Sahin N, Gevorgyan A, Linley C, Grabowski M, Mannan AA, Stoy N, *et al.*: **MUFINS: multi-formalism interaction network simulator**. *npj Systems Biol Appl* 2016, **2**:16032.

A framework for the integration of multiple modeling formalisms based on Petri nets.

69. Karr JR, Sanghvi JC, MacKlin DN, Gutschow MV, Jacobs JM, Bolival B, Assad-Garcia N, Glass JI, Covert MW: **A whole-cell computational model predicts phenotype from genotype**. *Cell* 2012, **150**:389–401.