Abstract

Motivation: Elementary flux mode (EFM) analysis allows an unbiased description of metabolic networks in terms of minimal pathways (involving a minimal set of reactions). To date, the enumeration of EFMs is impracticable in genome-scale metabolic models. In a complementary approach, we introduce the concept of a flux tope (FT), involving a maximal set of reactions (with fixed directions), which allows one to study the coordination of reaction directions in metabolic networks and opens a new way for EFM enumeration.

Results: A FT is a (nontrivial) subset of the flux cone specified by fixing the directions of all reversible reactions. In a consistent metabolic network (without unused reactions), every FT contains a ‘maximal pathway’, carrying flux in all reactions. This decomposition of the flux cone into FTs allows the enumeration of EFMs (of individual FTs) without increasing the problem dimension by reaction splitting. To develop a mathematical framework for FT analysis, we build on the concepts of sign vectors and hyperplane arrangements. Thereby, we observe that FT analysis can be applied also to flux optimization problems involving additional (inhomogeneous) linear constraints. For the enumeration of FTs, we adapt the reverse search algorithm and provide an efficient implementation. We demonstrate that (biomass-optimal) FTs can be enumerated in genome-scale metabolic models of \textit{B. cuenoti} and \textit{E. coli}, and we use FTs to enumerate EFMs in models of \textit{M. genitalium} and \textit{B. cuenoti}.

Availability and implementation: The source code is freely available at https://github.com/mpgerstl/FTA.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

The development of constraint-based modeling (CBM) approaches contributed tremendously to our understanding of metabolic processes, in particular, to the analysis of genome-scale metabolic models (GSMMs). Combined with CBM approaches, GSMMs provide a mechanistic basis for our understanding of the genotype-phenotype relationship.

For the analysis of GSMMs, two branches within the CBM spectrum turned out to be most successful: flux-balance analysis and elementary flux mode (EFM) analysis. Both method families use stoichiometric information and consider the linear equalities and inequalities for the reaction rates (fluxes) that arise from the steady-state assumption and irreversibility constraints. Whereas flux-balance analysis identifies optimal solutions (under additional linear...
constraints) and remains computationally practicable even at genome scale, EFM analysis describes all feasible solutions (the flux cone) in terms of minimal metabolic pathways. Due to the combinatorial nature of EFM enumeration, such an analysis faces severe computational challenges already for medium-scale metabolic models (Jungreuthmayer et al., 2013). Despite major advances in algorithm design (Gagneur and Klamt, 2004; Hunt et al., 2014; Terzer and Stelling, 2008; van Klinken and Willems van Dijk, 2016), EFM enumeration for GSMMs is not practicable to date. Hence other approaches focused on the enumeration of subsets of EFMs characterized by particular qualities (De Figueiredo et al., 2009; Kaleta et al., 2009).

In metabolic networks with reversible reactions, (thermodynamically feasible) EFMs can be grouped into largest (thermodynamically) consistent sets (LTCSs) (Gerstl et al., 2016). For all EFMs within one LTCS, the directions of all reactions are fixed (as determined by the Gibbs free energy). Importantly, every flux mode can be written as a sum of EFMs from one LTCS. In fact, a fundamental result of EFM analysis states that every flux mode can be written as a conformal sum of EFMs, that is, if a component of the flux mode has a certain sign, then this component has the same sign (or is zero) in all EFMs involved (Müller and Regensburger, 2016; Urbanczik and Wagner, 2005). In our previous work, it remained open whether LTCSs can be defined without referring to EFMs and computed without enumerating all EFMs beforehand. In the present paper, we show that this is indeed possible.

We introduce the novel concept of a flux tope (FT) as a (nontrivial) subset of the flux cone specified by fixing the directions of all reversible reactions. Obviously, every flux mode is contained in a FT, that is, the flux cone is decomposed into FTs. A feasible combination of reaction directions naturally corresponds to a sign vector (having $-\,\,0$, or $+$ entries) of the flux cone, and every FT corresponds to a support-maximal sign vector of the flux cone. In fact, the term ‘tope’ comes from the theory of oriented matroids, where it refers to a maximal sign vector of a linear subspace (Bachem and Kern, 1992; Bokowski, 2006). Whereas an EFM represents a minimal pathway (involving a minimal set of reactions), a FT contains a maximal ‘pathway’ (involving a maximal set of reactions). As EFMs, FTs need not be thermodynamically feasible, and we discuss the definition and computation of thermodynamically feasible FTs (corresponding to LTCSs) in the outlook. Ultimately, FT analysis can be used to study the coordination of reaction directions in GSMMs, that is, the thermodynamic repertoire of cellular metabolism.

Most importantly, the enumeration of FTs (as opposed to EFMs) is computationally practicable even at larger scale. Our implementation is based on the reverse search algorithm for cell enumeration in hyperplane arrangements (Avis and Fukuda, 1996; Fukuda, 2016). Moreover, FTs can be used to enumerate EFMs in GSMMs with reversible reactions. Indeed, FTs can be computed first, and EFMs (of individual FTs) can be enumerated efficiently (without increasing the problem dimension by reaction splitting) in a second step.

2 Materials and methods

2.1 Sign vectors

For a vector $\mathbf{x} \in \mathbb{R}^n$, we define the sign vector $\text{sign}(\mathbf{x}) \in \{-1, 0, +\}^n$ by applying the sign function component-wise, that is,

$$\text{sign}(\mathbf{x})_i = \text{sign}(x_i) \quad \text{for} \quad i = 1, \ldots, n.$$  

and we write

$$\text{sign}(S) = \{\text{sign}(\mathbf{x}) \mid \mathbf{x} \in S\}.$$  

for a subset $S \subseteq \mathbb{R}^n$.

The relations $0 < -$ and $0 < +$ induce a partial order on $\{-1, 0, +\}$: for sign vectors $\mathbf{z}, \mathbf{w} \in \{-1, 0, +\}^n$, we write $\mathbf{z} \leq \mathbf{w}$ if the inequality holds component-wise and say that $\mathbf{z}$ conforms to $\mathbf{w}$. Analogously, for $\mathbf{x} \in \mathbb{R}^n$ and $\mathbf{z} \in \{-1, 0, +\}^n$, we say that $\mathbf{x}$ conforms to $\mathbf{z}$ if $\text{sign}(\mathbf{x}) \leq \mathbf{z}$, e.g.

$$\text{sign} \left( \begin{array}{c} -1 \\ 0 \\ 2 \end{array} \right) = \left( \begin{array}{c} - \\ 0 \\ + \end{array} \right) \leq \left( \begin{array}{c} - \\ - \\ + \end{array} \right) = \text{sign} \left( \begin{array}{c} -1 \\ -1 \\ 1 \end{array} \right),$$  

that is, $(-1, 0, +)^T$ conforms to $(-1, -1, +)^T$, and $(-1, 0, 2)^T$ conforms to $(-1, 0, +)^T$ (trivially) and $(-1, -1, +)^T$.

Given a subset $S \subseteq \mathbb{R}^n$ and a sign vector $\mathbf{z} \in \{-1, 0, +\}^n$, we define

$$S_{\leq \mathbf{z}} = \{\mathbf{x} \in S \mid \text{sign}(\mathbf{x}) \leq \mathbf{z}\}.$$  

the subset of $S$ conforming to $\mathbf{z}$. (In the application to metabolic networks below, the set $S$ is the flux cone, and the sign vector $\mathbf{z}$ is a maximal sign vector of the flux cone, fixing the directions of all reactions.)

Finally, we call the vectors $\mathbf{x}, \mathbf{y} \in \mathbb{R}^n$ conformal if there exists a sign vector $\mathbf{z} \in \{-1, 0, +\}^n$ such that $\text{sign}(\mathbf{x}), \text{sign}(\mathbf{y}) \leq \mathbf{z}$ or, equivalently, if $x_i, y_i \geq 0$ for $i = 1, \ldots, n$.

2.2 Metabolic networks

A metabolic network is given by $m$ internal metabolites, $r$ reactions and the corresponding stoichiometric matrix $\mathbf{N} \in \mathbb{R}^{m \times r}$, which contains the net stoichiometric coefficients of each metabolite in each reaction. The sets of irreversible and reversible reactions are given by $I_{ir} \subseteq \{1, \ldots, r\}$ and $I_{re} = \{1, \ldots, r\} \setminus I_{ir}$, respectively. A vector of reaction rates that satisfies the steady-state and irreversibility constraints is called a flux mode. In geometric terms, a flux mode is an element of the flux cone

$$C = \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{Nv} = 0 \quad \text{and} \quad v_i \geq 0 \quad \text{for} \quad i \in I_{ir}\}.$$  

a polyhedral cone defined by the nullspace of the stoichiometric matrix and nonnegativity conditions.

2.3 Flux topes

An EFM $e \in C$ is a support-minimal nonzero flux mode, and every element of the ray $\{\lambda e \mid \lambda > 0\}$ is an EFM, too. With respect to the partial order on $\{-1, 0, +\}$ defined above, the sign vector $\text{sign}(e)$ is a minimal nonzero element of

$$\text{sign}(C) = \{\text{sign}(\mathbf{v}) \mid \mathbf{v} \in C\},$$  

the set of all sign vectors of the flux cone. Conversely, a minimal nonzero sign vector $\mathbf{a} \in \text{sign}(C)$ determines the ray

$$C_{\leq \mathbf{a}} = \{\mathbf{v} \in C \mid \text{sign}(\mathbf{v}) \leq \mathbf{a}\} = \{\mathbf{v} \in C \mid \text{sign}(\mathbf{v}) = \mathbf{a}\} = \{\lambda e \mid \lambda > 0\},$$  

where $e \in C$ is some EFM with $\text{sign}(e) = \mathbf{a}$. Analogously, a maximal sign vector $\mathbf{r} \in \text{sign}(C)$ determines the pointed subcone

$$C_{\leq \mathbf{r}} = \{\mathbf{v} \in C \mid \text{sign}(\mathbf{v}) \leq \mathbf{r}\},$$  

which we call a flux tope (FT).
A FT $C_\tau$ consists of all flux modes that conform to the defining sign vector $\tau \in \text{sign}(C)$, in particular, it contains all conforming EFM$s$. Indeed, EFM$s$ are extreme rays of FT$s$, and this property may serve as a definition of EFM$s$ (Klamt et al., 2017; Müller and Regensburger, 2016).

### 2.4 Consistency

A flux cone is called consistent (Acuña et al., 2009) if every reaction (in every possible direction) is supported by a flux mode, that is, if for every $i \in \{1, \ldots, r\}$ there exists $u \in C$ such that $u_i > 0$ and, additionally, for every $i \in I_{irr}$, there exists $v' \in C$ such that $v'_i < 0$. We say that a flux mode has full support, if all its components are non-zero.

**Proposition 1.** If a flux cone is consistent, then every reaction (in every possible direction) is supported by a flux mode with full support.

Proof. Let $C$ be a consistent flux cone and $i \in \{1, \ldots, r\}$. Then there exists $u \in C$ such that $u_i > 0$. Suppose $u$ does not have full support, that is, $u_i = 0$ for some $j \neq i$. By consistency, there exists $w \in C$ such that $w_i > 0$. Now, consider the convex combination $u = (1 - \lambda) v + \lambda w \in C$. For sufficiently small $0 < \lambda < 1$, sign$(u) > \text{sign}(v)$, in particular, $u_j, w_j > 0$. Repetition of the argument eventually yields a flux mode with full support.

Finally, let $i \in I_{irr}$. Then there exists $u \in C$ such that $u_i < 0$, and a flux mode with full support can be constructed as above. □

We say that a FT $C_{\leq s}$, has full support, if the defining maximal sign vector $\tau \in \text{sign}(C)$ has full support, that is, if $\tau \in \{-, +\}^r$.

**Proposition 2.** If a flux cone is consistent, then all FT$s$ have full support.

Proof. Let $C$ be a consistent flux cone. Suppose there exists a FT $C_{\leq s}$ with a maximal sign vector $\tau \in \text{sign}(C)$ that does not have full support, and let $u \in C_{\leq s}$ denote the $i$th row of $K$ and $b^i = \{x \in \mathbb{R}^d | Kx = 0\}$ for $i = 1, \ldots, r$ be the corresponding (central) hyperplane. Then, every flux mode $v \in C$ can be written as

$$v = Kx,$$

where $x \in \mathbb{R}^d$ is unique and $v_i = Kx > 0$ for $i \in I_{irr}$. Most importantly, sign$(v) \in \{-, +\}$ describes the positions of $x$ with respect to the hyperplanes $b^1, \ldots, b^r$. In particular, a sign vector of the flux cone with full support (defining a FT) corresponds to a cell of the hyperplane arrangement that satisfies the irreversibility constraints.

For a general central hyperplane arrangement of $r$ hyperplanes in $\mathbb{R}^d$, there is a well-known upper bound for the number of cells: Out of $2^r$ full sign vectors, $2 \sum_{i=0}^{d-1} \left( \begin{array}{c} r - 1 \\ i \end{array} \right)$ correspond to cells (Buck, 1943; Fukuda, 2016). This upper bound simplifies to $2^r$ if $d \geq r$. In case of irreversibility constraints, where $r = |I_{irr}| + |I_{rev}|$, we have the obvious upper bound $2^{r_{irr}}$ for the number of FT$s$. In case $|I_{rev}| = 0$, there is only one FT.

### 2.6 A toy model

We consider the small network displayed in Figure 1a. It consists of three internal metabolites and six reactions. The resulting stoichiometric matrix amounts to

$$N = \begin{pmatrix} 1 & 0 & 0 & -1 & 0 & -1 \\ 0 & -1 & 0 & 1 & 1 & 0 \\ 0 & 0 & 2 & 0 & -1 & 1 \end{pmatrix}.$$  \hspace{1cm} (9)

A basis of the nullspace of $N$ is given by the columns of the matrix

$$K = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ -1 & 1 \\ -1/2 & 1/2 \\ 0 & 0 \\ 0 & 1 \end{pmatrix}.$$  \hspace{1cm} (10)

Every flux mode can be written as $v = Kx$ with a unique $x \in \mathbb{R}^3$.

Since the submatrix of $K$ consisting of the rows 1, 2 and 4 (corresponding to the reactions R1, R2 and R4) is the identity matrix, we get

$$v = K \begin{pmatrix} v_1 \\ v_2 \\ v_4 \end{pmatrix}.$$  \hspace{1cm} (11)

Now, the irreversible reactions R1, R2 and R6 define the nonnegativity conditions $v_1 \geq 0$, $v_2 \geq 0$, and $v_1 - v_4 \geq 0$ and shape the flux cone, whereas the reversible reactions R3, R4 and R5 determine the hyperplanes $-1/2 v_1 + 1/2 v_2 = 0$, $v_4 = 0$, and $v_1 - v_4 = 0$ and divide the flux cone into FT$s$. The resulting five FT$s$ are listed in Figure 1b. The projection of the FT$s$ on the flux components $v_1$, $v_2$ and $v_4$ is depicted in Figure 1c.

The six (generating) EFM$s$ $e_i$ of the toy network are listed in Figure 1b, and their projections $v_i$ are depicted in Figure 1c. According to Equation (11), we can write them as

$$K(e_1, \ldots, e_6) = K(e_1, \ldots, e_6) = \begin{pmatrix} 2 & 0 & 0 & 2 & 2 & 2 \\ 0 & 0 & 2 & 0 & 2 & 2 \\ 0 & 0 & -2 & 0 & 2 & 2 \end{pmatrix}.$$  \hspace{1cm} (12)

Each FT is generated by three EFM$s$. (This is the smallest possible number since the dimension of the nullspace is three.) The EFM$s$ $e_1, e_2$ and $e_3$ are contained in the largest number of FT$s$ (four and three, respectively), see Figure 1b and c. They generate the most ‘central’ FT $\tau_j$ (depicted in pink), having the largest number of neighbours (three). The EFM$s$ $e_2$ and $e_3$ are contained in two FT$s$ each. Together with the above EFM$s$, they generate four (out of five) FT$s$. The remaining EFM $e_4$ is contained only in the most ‘peripheral’ FT $\tau_j$, having only one adjacent FT. As opposed to the other FT$s$, flux vectors in $\tau_j$ use reaction R5 in reverse direction.
2.7 Reverse search

If (i) the flux cone is consistent, then all maximal sign vectors have full support, by Proposition 2. If (ii) the nullspace matrix does not contain rows which are multiples of each other, then hyperplanes are distinct, and cells can be enumerated using reverse search (Avis and Fukuda, 1996). The algorithm starts from a cell in the hyperplane arrangement (represented by a full sign vector) and recursively checks all adjacent full sign vectors (differing in exactly one component) whether they represent cells.

In our implementation, we use the idea that only adjacent full sign vectors need to be checked, however, for efficiency reasons, we adapt the algorithm. In particular, we do not operate on the hyperplane arrangement, but directly on full sign vectors, see Section 3.2.

In the following, we assume (i) and (ii) which can be ensured using appropriate pre-processing, see Section 3.1.

2.8 Flux optimization

In flux-balance analysis, one often optimizes linear combinations of reaction rates under box constraints, i.e. one solves linear programs (LPs)

\[ \max \mathbf{c}^T \mathbf{v} \quad \text{s.t.} \quad \mathbf{v} \in P, \]

defined on the flux polyhedron

\[ P = \{ \mathbf{v} \in \mathbb{R}^n | \mathbf{Nv} = 0 \ \text{and} \ \ell_i \leq v_i \leq u_i \ \text{for} \ i = 1, \ldots, r \}, \]

where \( \ell_i, u_i \in [-\infty, +\infty] \). The lower and upper bounds define a corresponding flux cone \( C \), in particular, \( i \in I_{irr} \) if and only if \( \ell_i \geq 0 \). If \( \ell_i = -\infty \) or \( u_i = +\infty \) for all \( i \in \{1, \ldots, r\} \), then \( P = C \), otherwise \( P \subset C \).

Let \( \nu^* \) be an optimal flux and \( d = \mathbf{c}^T \nu^* \) the corresponding optimal value. Then \( P^* = \{ \mathbf{v} \in P | \mathbf{c}^T \mathbf{v} = d \} \) is the polyhedron of optimal fluxes. As for the flux cone \( C \), FTs and consistency can be defined for the optimal flux polyhedron \( P^* \) (Klamt et al., 2017). After ensuring consistency using flux variability analysis, all FTs of the flux polyhedron have full support and correspond to cells in a (non-central) hyperplane arrangement that satisfy the box constraints. Finally, after ensuring that hyperplanes are distinct (see Section 3.1), FTs can be enumerated using reverse search.

In our toy model (Fig. 1), assume upper bounds for the uptake reactions R1 and R3 in Figure 1a, in particular, \( v_1 \leq 10 \) and \( v_3 \leq 10 \). Then the projected flux cone in Figure 1c becomes a polyhedron with \( v_2 \leq 30 \), \( v_3 \geq -5 \), \( v_4 \leq 10 \) and \( v_5 \geq -10 \). Still, since EFM \( e_3 \) (the internal cycle) is not constrained by the uptake reactions, there is no lower bound for \( v_4 \) (and no upper bounds for \( v_3 \) and \( v_5 \)). As a consequence, FTs \( t_1 \) and \( t_3 \) become bounded, whereas \( t_2 \) and \( t_4 \) remain unbounded (for negative \( v_4 \)). When the flux through the product reaction R2 is optimized, then the maximum \( v_2 = 30 \) is attained at flux distributions in FTs \( t_1 \) and \( t_3 \), see again Figures 1c and 2. Note that optimal solutions are contained in adjacent FTs, in particular, \( t_1 \) and \( t_3 \) are separated by the hyperplane \( v_4 = 0 \), and the direction of reaction R4 is not determined by the optimum.

2.9 Genome-scale metabolic models

We study GSMMs of *Mycoplasma genitalium, iPS189+* (Suthers et al., 2009 including recent modifications by Hartleb et al., 2016), *Blattabacterium cuenoti* Bge, iCG238 (González-Domenech et al.,...
3.1 Efficient enumeration of flux topes

To check if a full sign vector \( \tau \in \{-, +\}^r \) (with \( \tau_i = + \) for \( i \in I_{irr} \)) determines a FT, we check the feasibility of the LP

\[
Nv = 0, \quad \ell \leq \tau v \leq u \quad \text{for} \quad i = 1, \ldots, r.
\] (15)

For numerical reasons, we set lower and upper bounds, \( \ell = 10^{-6} \) and \( u = 10^4 \), respectively, and a tolerance of the LP solver of at most \( 10^{-10} \).

The algorithm starts with the sign vector having only plus entries. In the first step, it visits all full sign vectors having one minus entry in an independent reversible reaction (and all reactions depending on it) and checks their feasibility, using the above LP (see Fig. 2). In the second step, the algorithm visits all feasible, full sign vectors having two minus entries in an independent reversible reaction, and so on.

More specifically, in step \( n \), the algorithm starts with the set of all feasible full sign vectors having \( n - 1 \) minus entries (the ‘parent’ sign vectors), and visits all full sign vectors with \( n \) minus entries (the ‘child’ sign vectors). Note that ‘child’ sign vectors can be reached from several ‘parent’ sign vectors. If a sign vector is visited for the first time, its feasibility is checked using the above LP and stored in \( \tau \). If \( \tau \) reaches the number of independent reversible reactions, in order to avoid the repetition of the feasibility check, the algorithm terminates if there are no feasible full sign vectors having \( n \) minus entries or if \( n \) reaches the number of independent reversible reactions. For an illustration of our implementation, see Figure 2 and Supplementary Table S1.

Our enumeration algorithm can be threaded efficiently. In particular, checking the feasibility of ‘child’ sign vectors for a given ‘parent’ sign vector forms an independent task.

We implemented the algorithm in C. LPs are solved with CPLEX. The source code is available at https://github.com/mpgerstl/FTA. Unless otherwise stated, computations were carried out using six threads on a Xeon® E5-1650v3 CPU with DDR4 RAM modules running on Debian 8.

4 Results

4.1 FTs correspond to maximal sets of conformal EFM

We analyzed a GSMM of \( M. genitalium \), \( iPS189+ \) (Hartleb et al., 2016; Suthers et al., 2009) and enumerated all FTs and all EFM. (The enumeration of all EFM was possible since the model is sufficiently small.) More than 3 million EFM were found, which are contained in only 672 FTs, see Table 1. The FTs were enumerated within 1 s, whereas EFM computation took 10 h.

We verified that the 672 FTs correspond to maximal sets of conformal EFM (haying matching signs). Thereby, we first computed the set of all EFM and formed the maximal sets of conformal EFM using a mixed integer LP described in Gerstl et al. (2016), which was previously used for the computation of LTCS from the set of EFM.

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Fig. 2. Enumeration of FTs for the toy network in Figure 1 (with colors as in Figure 1). Out of \( 2^{4} = 8 \) full sign vectors, only five define FTs. Two FTs maximize the flux through reaction \( R2 \) (dark blue frames), three are sub-optimal (light blue frames). Three full sign vectors do not represent a FT (red frames), since either \( C \) is only produced or \( B \) is only consumed. Sign vectors are depicted as nodes of a directed acyclic graph (arranged in levels \( n = 0 \) through \( n = 3 \)) with directed edges pointing from ‘parent’ to ‘child’ sign vectors (Color version of this figure is available at Bioinformatics online.)
We also computed the sets of EFMs for all individual FTs and found that their union equals the set of all EFMs.

We conclude that in network containing reversible reactions (i) FTs can be enumerated efficiently, (ii) few FTs condense the information contained in many EFMs and (iii) EFMs can be computed using FTs.

### 4.2 FT analysis may be feasible when EFM analysis is not

We studied a GSMM of *B. cuenoti*, a mutualistic, bacterial endosymbiont living in fat cells of cockroaches. The model iCG238 (González-Domenech et al., 2012) is significantly larger than iPS189+, and a full EFM analysis is infeasible with current methods. However, we were able to enumerate all FTs within 30 h and found 60.2 × 10⁶ FTs, see Table 1.

We note that the number of FTs is much smaller than the obvious upper bound 2^{31} = 2.15 × 10⁹, where 31 is the number of independent reversible reactions. To attain this upper bound, each FT would need to have 31 adjacent FTs. However, most frequently, a FT has only 22 adjacent FTs, see Figure 3.

### 4.3 Optimal FTs can be enumerated in GSMMs

For the model iCG238 (González-Domenech et al., 2012), we were further interested in fluxes that maximize biomass production. As described in Section 2.8, we enumerated the FTs of the optimal flux polyhedron. We found that, out of the 60 million FTs of the flux cone, only 270 are FTs of the optimal flux polyhedron, see Table 1. In fact, the optimal FTs could be identified within 1 s, without first enumerating all FTs (taking 30 h) and then selecting the optimal ones. We verified that both approaches result in the same set of biomass-optimal FTs.

The increase in the number of FTs from 60 million to 270 is a consequence of additional irreversibility constraints arising from the optimality condition. While the model iCG238 contains 31 independent reversible reactions, biomass-optimality enforces 21 additional irreversibility constraints leaving only ten reactions reversible, see Table 1. Interestingly, out of all amino acid transport reactions, only the exchange of Alanine remained reversible. All other amino acids cannot be produced when *B. cuenoti* is growing optimally.

To complete the study of the model iCG238, we randomly selected 10% of the biomass-optimal FTs and performed an EFM analysis. All FTs contained around 10⁹ EFMs, see Figure 4; however, the run times for EFM enumeration varied strongly, ranging from 1 h to more than 60 h in one extreme case.

Finally, we analyzed a GSMM of *E. coli*, iJR904 (Reed et al., 2003). We enumerated all biomass-optimal FTs and found around twelve million FTs within less than 35 h runtime. Interestingly, the number of FTs computed in each step of our algorithm is distributed normally, see left panels in Figure 6 and Supplementary Figure S2.

### Table 1. Algebraic characteristics of consistent GSMMs: dimensions $m \times r$ of the stoichiometric matrix $N$, dimension of the nullspace with basis $K$, $d = \text{rank}(K)$, and number of independent reactions, $r_{\text{ind}}$. (Numbers in brackets refer to the numbers of reversible reactions.)

| Organism                        | model ID | $m \times r$ | $d$ | EFMs | run time | $r_{\text{ind}}$ | FTs | run time | $r_{\text{ind}}$ | FTs (max.BM) | run time |
|---------------------------------|----------|--------------|-----|------|----------|-------------------|-----|----------|-------------------|---------------|----------|
| *M. genitalium*                 | iPS189+  | 271 × 271(21)| 28  | 3 252 686 | 10.3 h   | 83 (13)          | 672 | 1.0 s   | 83 (7)            | 48            | <1.0 s   |
| *B. cuenoti*                    | iCG238   | 306 × 350(45)| 51  | c.i.  | 137 (31) | 60 226 956       | 29.8 h | 137 (10) | 270               | <1.0 s        |          |
| *E. coli*                       | iJR904   | 450 × 667(53)| 233 | c.i.  | —        | 423 (49)         | c.i. | —       | 432 (27)          | 11 796 480    | 34.8 h   |

$\text{c.i.}$, computationally infeasible.

Note: Computational results: number of EFMs [computed by FluxModeCalculator (van Klinken and Willems van Dijk, 2016)], number of FTs (computed by our implementation), and number of FTs that maximize biomass production (max.BM).
For a consistent metabolic network, a FT is a full-dimensional pointed subcone of the flux cone, specified by fixing the directions of all (reversible) reactions. In particular, every FT contains a full ‘pathway’, carrying flux in all reactions. Whereas flux variability analysis allows to study the feasible directions of individual reactions, FT analysis allows to study all feasible (or all optimal) combinations of reaction directions. We developed a mathematical framework for FT analysis, building on the concepts of sign vectors and hyperplane arrangements, we provided an efficient algorithm for the enumeration of FTs, we demonstrated that FTs can be enumerated in large metabolic networks, and we used FTs to enumerate EFMs in metabolic networks with reversible reactions. Ultimately, we are interested in FTs that are both stoichiometrically and thermodynamically feasible and hence characterize the thermodynamic repertoire of cellular metabolism.

To efficiently enumerate FTs, we build on the correspondence between FTs and cells in a (central) hyperplane arrangement. In particular, we adapt the reverse search algorithm for cell enumeration in hyperplane arrangements. Reverse search is both compact and output-polynomial. (Recall that an algorithm is compact if its space requirement is polynomial in the input size only and output-polynomial if its runtime is polynomial in both input and output size.) Moreover, it constantly produces output (not just upon completion). As it turns out, enumerating cells in the hyperplane arrangement (7) is problematic. In particular, solving LPs involving the (dense) null-space matrix \( K \) is slow. Hence, we directly solve the LPs (15) involving the (sparse) stoichiometric matrix \( N \). Further, we trade some space requirements for smaller runtime and store the solutions of LPs to avoid repeated computations. Finally, we change the algorithm from depth-first to breadth-first search. This allows to investigate neighborhoods of a given FT, if the enumeration of all FTs is computationally infeasible or if the reversion of reaction directions increases an objective function (e.g. biomass). In fact, it was suggested that reversing reaction directions can improve strain performance (Nishikawa et al., 2008). Moreover, coordination of reaction directions is key to the study of emergent properties in cross-feeding communities. Currently, it is unclear if members of a community adjust their metabolism in an optimal manner, and unbiased methods like FT analysis are required to identify essential interactions between species (Gottstein et al., 2016).

For EFM enumeration, a metabolic network is often ‘reconfigured’ by splitting reversible reactions, and one considers the resulting higher-dimensional network involving irreversible forward and backward reactions. This approach is not practicable for FT enumeration. For the reconfigured system, there is exactly one (trivial) FT. To identify the FTs of the original system, additional constraints have to be added: For every reversible reaction, either the forward or the backward flux has to be zero. Due to the enforced zero fluxes, the FT enumeration problem is not an LP (but a mixed integer LP), and (efficient) reverse search cannot be used.

All models under study have significantly fewer FTs than EFMs. In fact, in the GSMM of \( \text{B. cuenotii} \), every single FT has more EFMs than the whole network has FTs. This is in contrast to general hyperplane arrangements, in which there are least as many topes (sign vectors with maximal support) as vertices (sign vectors with minimal support) (Fukuda et al., 1991). We conjecture that the lower number of FTs compared to EFMs is a typical feature of GSMMs; a detailed comparison will be the scope of further work. Currently, metabolic pathway analysis is restricted to medium-scale models since the number of EFMs explodes with the size of a model. FTs helps to accommodate this problem in two ways: (i) there are fewer FTs than EFMs, and (ii) they can be enumerated more efficiently. (Recall that the complexity of the double description method for EFM enumeration is not even known).
Finally, the enumeration of FTs opens up a new way for enumerating EFMs in GSMMs. The flux cone is the union of all FTs, which can be subject to EFM analysis, individually. For a given FT, the directions of all (reversible) reactions are fixed, and the double description method can be used without increasing the problem dimension by reaction splitting. On our machines, a conventional EFM analysis of $iCG238$ (B. cenuotii) was infeasible due to memory restrictions. Still, we were able to enumerate all EFMs of individual FTs, cf. Figure 4, which suggests the parallel enumeration of EFMs for all FTs. Clearly, a naive parallelization is inefficient, since EFMs are typically contained in several FTs. Especially EFMs contained in FTs with many adjacent cells are shared frequently. Tests with $iPS189+$ indicate that, on average, an EFM is enumerated more than 100 times. Yet, despite the frequent repetitions, the total CPU run time (compared to a standard EFM analysis) increased only by a factor of ten. Further work is needed to make a FT-based EFM enumeration competitive in terms of run time.

6 Outlook: thermodynamically feasible FTs

Recently, it has been shown that many EFMs are thermodynamically infeasible and hence irrelevant for the characterization of metabolic phenotypes (Gerstl et al., 2016, 2015a,b; Jungreuthmayer et al., 2015; Peres et al., 2017). The same constraints apply to FTs. In our toy model, the FTs $t_1$ and $t_2$ contain the thermodynamically infeasible EFM $e_1$ (the internal cycle), cf. Figures 1b and 2, and hence they are irrelevant biologically. A single thermodynamically infeasible EFM leads to the elimination of two FTs, that is, thermodynamic constraints reduce the number of FTs even more than the number of EFMs.

A thermodynamically feasible FT represents one possible combination of reaction directions and contains all corresponding pathways. Thereby, the thermodynamic feasibility of a FT is determined by the metabolite concentrations via the Gibbs free energy. By cellular control of the metabolite concentrations, a FT can be reached and the corresponding pathways can be activated.

A first generalization of our enumeration algorithm involves the elimination of FTs that do not contain any thermodynamically feasible flux mode: either by straightforward post-processing or by further adaptation of reverse search. In the end, we are not just interested in FTs (defined by full sign vectors) that contain thermodynamically feasible flux modes (possibly with smaller sign vectors), but rather in thermodynamically feasible FTs (defined by maximal sign vectors). The latter definition leads to combinatorial problems which require further theoretical analysis and algorithmic developments.

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References

Acuña, V. et al. (2009) Modes and cuts in metabolic networks: complexity and algorithms. BioSystems, 95, 51–60.

Avis, D. and Fukuda, K. (1996) Reverse search for enumeration. Discrete Appl. Math., 65, 21–46.

Bachem, A. and Kern, W. (1992) Linear Programming Duality. An Introduction to Oriented Matroids. Springer-Verlag, Berlin.

Bokowski, J.G. (2006) Computational Oriented Matroids. Equivalence classes of matrices within a natural framework. Cambridge University Press, Cambridge.

Buck, R.C. (1943) Partition of space. Am. Math. Monthly, 50, 541–544.

De Figueiredo, L.F. et al. (2009) Computing the shortest elementary flux modes in genome-scale metabolic networks. Bioinformatics, 25, 3158–3165.

Fukuda, K. (2016) Lecture: Polyhedral Computation. Lecture notes. ETH Zurich, Switzerland, https://www.inf.ethz.ch/personal/fukudak/lect/pclpect/note2016/PolyComp2016.pdf

Fukuda, K. et al. (1991) Bounding the number of $k$-faces in arrangements of hyperplanes. Discrete Appl. Math., 31, 151–165.

Gagneur, J. and Klamt, S. (2004) Computation of elementary modes: a unifying framework and the new binary approach. BMC Bioinformatics, 5, 175.

Gerstl, M.P. et al. (2015a) Metabolomics integrated elementary flux mode analysis in large metabolic networks. Sci. Rep., 5, 8930.

Gerstl, M.P. et al. (2015b) tEFMA: computing thermodynamically feasible elementary flux modes in metabolic networks. Bioinformatics, 31, 2232–2234.

Gerstl, M.P. et al. (2016) Which sets of elementary flux modes form thermodynamically feasible flux distributions? FEBS J., 283, 1782–1794.

González-Domeñec, C.M. et al. (2012) Metabolic stasis in an ancient symbiosis: genome-scale metabolic networks from two Blattabacterium cenuotii strains, primary endosymbionts of cockroaches. BMC Microbiology, 12, 55.

Gotte, W. et al. (2016) Constraint-based stoichiometric modelling from single organisms to microbial communities. J. R. Soc. Interface, 13, 20160627.

Hartleh, D. et al. (2016) Improved metabolic models for E. coli and Mycoplasma genitalium from GlobalFit, an algorithm that simultaneously matches growth and non-growth data sets. PLoS Comput. Biol., 12, e1005036.

Hunt, K.A. et al. (2014) Complete enumeration of elementary flux modes through scalable demand-based subnetwork definition. Bioinformatics, 30, 1569–1578.

Jungreuthmayer, C. et al. (2013) Comparison and improvement of algorithms for computing minimal cut sets. BMC Bioinformatics, 14, (318).

Jungreuthmayer, C. et al. (2015) Avoiding the enumeration of infeasible elementary flux modes by including transcriptional regulatory rules in the enumeration process saves computational costs. PLoS One, 10, e0129840.

Kaleta, C., De Figueiredo, L. F., et al. (2009). Efmevolver: computing elementary flux modes in genome-scale metabolic networks. In: Lecture Notes in Informatics (LNI) P-157 - Proceedings of the German Conference on Bioinformatics, Bonn. Gesellschaft für Informatik, pp. 179–190.

Klamt, S. et al. (2017) From elementary flux modes to elementary flux vectors: metabolic pathway analysis with arbitrary linear flux constraints. PLoS Comput. Biol., 13, e1005409.

Mahadevan, R. and Schilling, C. (2003) The effects of alternate optimal solutions in constraint-based genome-scale metabolic models. Metab. Eng., 5, 264–276.

Müller, S. and Regensburger, G. (2016) Elementary vectors and conformal sums in polyhedral geometry and their relevance for metabolic pathway analysis. Front. Genet., 7, 90.

Nishikawa, T. et al. (2008) Spontaneous reaction silenced in metabolic optimization. PLoS Comput. Biol., 4, e1000236.

Peres, S. et al. (2017) How important is thermodynamics for identifying elementary flux modes? PLoS One, 12, e0171440.

Reed, J. L., et al. (2003) An expanded genome-scale model of Escherichia coli K-12 (iJR904 GSM/GPR). Genome Biol., 4, R34.

Suthers, F. F., et al. (2009) A genome-scale metabolic reconstruction of Mycoplasma genitalium, ipi189. PLoS Comput. Biol., 5, e1000285.

Terzer, M. and Stelling, J. (2008) Large-scale computation of elementary flux modes with bit pattern trees. Bioinformatics, 24, 2229–2235.

Urbanczik, R. and Wagner, C. (2005) Functional stoichiometric analysis of metabolic networks. Bioinformatics, 21, 4176–4180.

van Klinken, J.B. and Willems van Dijk, K. (2016) FluxModeCalculator: an efficient tool for large-scale flux mode computation. Bioinformatics, 32, 1265–1266.