Metabolic pathway analysis in presence of biological constraints

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Abstract

Metabolic pathway analysis is a key method to study metabolism at steady state and the elementary fluxes (`EFs`) is one major concept allowing one to analyze the network in terms of non-decomposable pathways. The supports of the `EFs` contain in particular the supports of the elementary flux modes (`EFMs`), which are the support-minimal pathways, and `EFs` coincide with `EFMs` when the only flux constraints are given by the irreversibility of certain reactions. Practical use of both `EFMs` and `EFs` has been hampered by the combinatorial explosion of their number in large, genome-scale, systems. The `EFs` give the possible pathways at steady state but the real pathways are limited by biological constraints, such as thermodynamic or, more generally, kinetic constraints and regulatory constraints from the genetic network. We provide results about the mathematical structure and geometrical characterization of the solutions space in presence of such biological constraints and revisit the concept of `EFMs` and `EFs` in this framework. We show that most of those results depend only on a very general property of compatibility of the constraints with sign: either sign-based for regulatory constraints or sign-monotone (a stronger property) for thermodynamic and kinetic constraints. We show in particular that `EFs` for sign-monotone constraints are just those original `EFs` that satisfy the constraint and show how to efficiently integrate their computation in the double description method, the most widely used method in the tools dedicated to `EFMs` computation.

1 Introduction

1.1 Metabolic networks

With the objective for this paper to be self-contained and to require no prerequisite to be read, we summarize in this introduction the state of the art related to the subject and fix the notations adopted in the following throughout the paper. The results of this introduction, being known, are thus given without proof and the reader is invited to refer for the proofs or for historical surveys to [41, 45, 40, 25, 47, 29, 22] in addition to the references quoted in the text.

A metabolic network is made up of a set of r biochemical enzymatic reactions. Each reaction consumes some metabolites (the substrates of the reaction) and produces some metabolites (the products of the reaction). Each metabolite is assigned a coefficient in the reaction, its stoichiometric coefficient (counted negatively for substrates and positively for products). One distinguishes internal and external metabolites w.r.t. the system under study (e.g., a bacterium, an eukaryotic cell), the reactions involving both internal and external metabolites being transfer reactions. If m is the number of internal metabolites ($r > m$), the network is thus given by its stoichiometric matrix $\mathbf{S} \in \mathbb{R}^{m\times r}$, where coefficient $\mathbf{S}_{ji}$ is the stoichiometric coefficient of internal metabolite j in reaction i (positive if j is a product of reaction i and negative if it is a substrate). A state of the network at a given time t is given by the net rates (or fluxes) in each of its reactions at t, i.e., by a flux vector (or rate vector, or flux distribution) $\mathbf{v}(t) \in \mathbb{R}^r$. Denoting by $\underline{\mathbf{M}}(t) \in \mathbb{R}_+^{*m}$ the vector of the concentrations of internal metabolites at t, the time evolution of the network is thus given by:

$$\frac{d\underline{\mathbf{M}}(t)}{dt} = \mathbf{S}\mathbf{v}. \tag{1}$$

1.2 Steady state behavior and flux subspace

We are interested in the steady-state behavior of the network. The steady-state assumption means that the concentrations of internal metabolites remain constant along time (no accumulation or reduction of internal metabolites inside the system, an approximation which is valid for short time periods, e.g., a few minutes) and leads thus to the fundamental equation:

$$\mathbf{S}\mathbf{v} = \mathbf{0}. \tag{2}$$

With only this assumption, the solutions space $Sol_{\mathbf{S}}$, i.e., the space of all admissible flux vectors $\mathbf{v}$, is thus the linear subspace FS of $\mathbb{R}^r$ given by the kernel, or nullspace [37], of $\mathbf{S}$:

$$Sol_{\mathbf{S}} \triangleq FS \triangleq \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{S}\mathbf{v} = \mathbf{0}\}. \tag{3}$$

The dimension of the flux subspace is given by $dim(FS) = r - rank(\mathbf{S}) \geq r - m$. Often, possibly after a pre-processing eliminating its linearly dependent rows, $\mathbf{S}$ is assumed to be of full rank and then $dim(FS) = r - m$.

The support of a vector $\mathbf{x} \in \mathbb{R}^r$ is defined by:

$$supp(\mathbf{x}) \triangleq \{i \mid \mathbf{x}_i \neq 0\}. \tag{4}$$

The support of a flux vector $\mathbf{v}$ has thus an important biological signification as it represents the reactions involved in the subnetwork (that we will call pathway) defined by $\mathbf{v}$ (i.e., those through which the flux given by $\mathbf{v}$ is not null).

1.3 Irreversible reactions, flux cones and polyhedral cones

In addition to the homogeneous equality constraints provided by the steady-state assumption, the flux vectors have also in general to satisfy homogeneous inequality constraints corresponding to those reactions known as irreversible, whose set will be noted $\mathbf{Irr}$:

$$\mathbf{v}_i \geq 0 \text{ for } i \in \mathbf{Irr}. \tag{5}$$

This means that fluxes in irreversible reactions are constrained to be nonnegative (in reversible reactions, fluxes may be either positive, or negative or null and the direction fixed as positive is arbitrary, the role between substrates and products being able to switch). If $r_I = |\mathbf{Irr}|$, with $0 \leq r_I \leq r$, is the number of irreversible reactions, the solutions space $Sol_{\mathbf{S},\mathbf{Irr}}$ is the intersection of the linear subspace FS with r_I nonnegative half-spaces, it is thus a particular case of convex polyhedral cone, called s-cone (subspace cone or special cone) or flux cone and noted FC:

$$Sol_{\mathbf{S},\mathbf{Irr}} \triangleq FC \triangleq \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{S}\mathbf{v} = \mathbf{0}, \mathbf{v}_i \geq 0 \text{ for } i \in \mathbf{Irr}\}. \tag{6}$$

A (general) convex polyhedral cone is defined implicitly (or by intension) by finitely many homogeneous linear inequalities:

$$C \triangleq \{\mathbf{x} \in \mathbb{R}^r \mid \mathbf{A}\mathbf{x} \geq \mathbf{0}\} \tag{7}$$

with a suitable matrix $\mathbf{A} \in \mathbb{R}^{n \times r}$ (called a representation matrix of C) and is thus the intersection of n half-spaces passing though the origin. The dimension of C, noted $dim(C)$, is defined as the dimension of its affine span. A flux cone corresponds thus to a particular matrix $\mathbf{A} \in \mathbb{R}^{(2m+r_I)\times r}$ given by:

$$\mathbf{A} = \begin{pmatrix} \mathbf{S} \\ -\mathbf{S} \\ \mathbf{I}_{\mathbf{Irr}} \end{pmatrix} \tag{8}$$

where $\mathbf{I}_{\mathbf{Irr}} \in \mathbb{R}^{r_I \times r}$ is the extension of the $(r_I \times r_I)$ identity matrix by zero columns corresponding to reversible reactions. This means that, for a flux cone, the homogeneous linear inequalities are of a special type: part of them (m) are actually equalities defining a lower-dimensional subspace given by the nullspace of the stoichiometric matrix $\mathbf{S}$, the others (r_I) being nonnegativity constraints about

some single coordinate variables (given by the irreversible reactions) corresponding thus to particular half-spaces defined by such positive coordinate axes.

Conversely, to any convex polyhedral cone C in $\mathbb{R}^r$ defined by a representation matrix $\mathbf{A} \in \mathbb{R}^{n \times r}$ one can associate a flux cone FC_C of same dimension in $\mathbb{R}^{r+n}$ defined by:

$$FC_C \triangleq \{\begin{pmatrix} \mathbf{x} \\ \mathbf{A}\mathbf{x} \end{pmatrix} \in \mathbb{R}^{r+n} \mid \mathbf{x} \in C\} = \{\mathbf{v} \in \mathbb{R}^{r+n} \mid \begin{pmatrix} \mathbf{A} & -\mathbf{I} \end{pmatrix} \mathbf{v} = \mathbf{0}, \mathbf{v}_i \geq 0 \text{ for } r+1 \leq i \leq r+n\}. \quad (9)$$

Using this correspondence (which defines a bijection of C onto FC_C), several properties, proven for flux cones, can actually be lifted up to general convex polyhedral cones.

From the definition of a cone, for every nonzero element $\mathbf{x}$ of C, the whole half-line $\{\alpha\mathbf{x} \mid \alpha \geq 0\}$ is contained in C. It is called a ray of C. Thus a flux vector is defined up to a positive scalar multiplication. The lineality space of C is the union of all lines of C, i.e. $\{\mathbf{x} \in C \mid -\mathbf{x} \in C\}$. If C is defined by a representation matrix $\mathbf{A}$, its lineality space equals thus the nullspace of $\mathbf{A}$, i.e. $\{\mathbf{x} \in \mathbb{R}^r \mid \mathbf{A}\mathbf{x} = \mathbf{0}\}$. For a flux cone FC given by (6), its lineality space is thus constituted by the flux vectors $\mathbf{v}$ such that $\mathbf{v}_i = 0$ for $i \in \mathbf{Irr}$, i.e., those flux vectors involving only reversible reactions (and thus the global flux can go either in one direction or the other). The cone C is called pointed if it does not contain a line, i.e., if its lineality space is reduced to $\{\mathbf{0}\}$. For example, if C is contained in a closed orthant, it is pointed (where the 3^r closed orthants are defined by $\{\mathbf{x} \in \mathbb{R}^r \mid \mathbf{x}_i \ \mathbf{op}_i \ 0 \text{ for } i = 1, \ldots, r\}$ for an operators vector $\mathbf{op} \in \{\leq, =, \geq\}^r$). In particular a flux cone FC with only irreversible reactions ($r_I = r$) is necessarily pointed as it is included in the positive r-orthant (i.e., of dimension r). Actually, reversible flux vectors occur only rarely in metabolic networks, which thus often give rise to pointed flux cones.

1.4 Extreme vectors and generating sets

We are interested in finding an explicit (by extension) representation of C in the form of a (minimal) set of generators. A nonzero vector $\mathbf{x} \in C$ is called extreme (or extreme pathway [36, 17] if $\mathbf{x}$ is a flux vector), if

$$\mathbf{x} = \mathbf{x}^1 + \mathbf{x}^2, \text{ with nonzero } \mathbf{x}^1, \mathbf{x}^2 \in C, \text{ implies } \mathbf{x}^1 = \lambda\mathbf{x}^2 \text{ with } \lambda > 0. \quad (10)$$

If $\mathbf{x} \in C$ is extreme, then $\{\alpha\mathbf{x} \mid \alpha \geq 0\}$ is called an extreme ray of C as all its nonzero elements are extreme (and thus for simplifying notations, we will not distinguish extreme vectors and extreme rays when it does not create confusion). In fact, C has an extreme ray if and only if C is pointed and, in this case, the extreme rays are the edges (faces of dimension one) of C and, from Minkowski's theorem, constitute the unique minimal (finite) set of generators of C for conical (i.e., nonnegative linear) combination:

$$C = \{\sum\nolimits_{k \in K} \beta_k \mathbf{y}^k \mid \beta_k \geq 0\} \triangleq cone(\{\mathbf{y}^k\}) \quad (11)$$

where the $\mathbf{y}^k$'s, $k \in K$ (finite index set), are representatives of the extreme rays (unique up to positive scalar multiplication) and *cone* is the conical hull. More precisely, we get an upper bound of the number of extreme vectors that are sufficient to decompose any given nonzero vector $\mathbf{x} \in C$: $\mathbf{x} = \sum_{k \in K_\mathbf{x}} \beta_k \mathbf{y}^k$ with $|K_\mathbf{x}| \leq min(dim(C), |supp(\mathbf{x})| + |supp(\mathbf{A}\mathbf{x})|)$. This result can be demonstrated first for a flux vector $\mathbf{v}$ of a pointed flux cone FC with an upper bound given by $|K_\mathbf{v}| \leq min(dim(C), |supp(\mathbf{v})|)$ and then for a vector $\mathbf{x}$ of a general pointed polyhedral cone C by using the correspondence (9) between C and FC_C which maps the extreme vectors of C onto the extreme vectors of FC_C. Extreme vectors of a pointed cone C will be noted `ExVs`.

The Double Description (DD) method [28, 8], known as Fourier-Motzkin, is an incremental algorithm (which processes one by one each linear inequality $(\mathbf{A}\mathbf{x})_j \geq 0$) to build an explicit description of a pointed cone C, as a minimal generating matrix (whose columns are in 1-to-1 correspondence with the extreme rays), from an implicit description of C by a representation matrix, i.e., to enumerate its extreme rays.

If C is not pointed, it is still finitely generated:

$$C = \{\sum\nolimits_{k \in K} \beta_k \mathbf{y}^k + \sum\nolimits_{l \in L} \gamma_l \mathbf{z}^l \mid \beta_k \geq 0, \gamma_l \in \mathbb{R}\} = cone(\{\mathbf{y}^k, \mathbf{z}^l, -\mathbf{z}^l\}) \quad (12)$$

with (not unique this time) minimal set of generators consisting of basis vectors $\mathbf{z}^l$ of the lineality space and suitable vectors $\mathbf{y}^k$ not in the lineality space (e.g., the extreme vectors of the pointed cone obtained by intersecting C with the orthogonal complement of its lineality space). Actually, Minkowski-Weyl's theorem for cones states that it is equivalent for a set C to be a polyhedral cone (7) or to be a finitely generated cone, i.e., the conical hull of a finite set of vectors (as the $\mathbf{y}^k$'s, $\mathbf{z}^l$'s and $-\mathbf{z}^l$'s).

1.5 Elementary vectors and conformal generating sets

Now, if the existence and uniqueness of a minimal set of generators for conical decomposition in a pointed polyhedral cone C is satisfactory for an explicit geometric description of C, it is not in general meaningful for a flux cone FC representing the steady-state flux vectors of a metabolic network. Actually, for a metabolic pathway, only a conical decomposition without cancelations is biochemically meaningful, since a reversible reaction cannot have a net rate in opposite directions in the contributing pathways. Indeed, the second law of thermodynamics states that a reaction can only carry flux in the direction of negative Gibbs free energy of reaction, which is imposed by the values of metabolites concentrations. This means that, when decomposing a flux vector, only so-called conformal sums, i.e., sums without cancelations, are biochemically admissible. A sum $\mathbf{v} = \mathbf{v}^1 + \mathbf{v}^2$ of vectors is called conformal if, for all $i \in \{1, \ldots, r\}$:

$$\mathbf{v}_i = 0 \text{ implies } \mathbf{v}_i^1 = \mathbf{v}_i^2 = 0, \mathbf{v}_i > 0 \text{ implies } \mathbf{v}_i^1, \mathbf{v}_i^2 \geq 0, \mathbf{v}_i < 0 \text{ implies } \mathbf{v}_i^1, \mathbf{v}_i^2 \leq 0. \tag{13}$$

An equivalent definition is to define a sum $\mathbf{v} = \mathbf{v}^1 + \mathbf{v}^2$ as conformal if

$$sign(\mathbf{v}^1), sign(\mathbf{v}^2) \leq sign(\mathbf{v}) \tag{14}$$

where the sign vector $sign(\mathbf{v}) \in \{-, 0, +\}^r$ is defined by applying the sign function component-wise, i.e., $sign(\mathbf{v})_i = sign(\mathbf{v}_i)$, and the partial order $\leq$ on $\{-, 0, +\}^r$ is defined by applying component-wise the partial order on $\{-, 0, +\}$ induced by $0 < -$ and $0 < +$. For example, there is a one-to-one mapping between closed orthants O and sign vectors $\boldsymbol{\eta}$, defined by: $O = \{\mathbf{x} \in \mathbb{R}^r \mid sign(\mathbf{x}) \leq \boldsymbol{\eta}\}$ (O will be said defined by $\boldsymbol{\eta}$ and noted $O_{\boldsymbol{\eta}}$), which induces a one-to-one mapping between closed r-orthants and full support (i.e., with only nonzero components) sign vectors. For $\boldsymbol{\xi}, \boldsymbol{\eta}$ sign vectors and $\mathbf{v}$ a vector, we say that $\boldsymbol{\xi}$ conforms to $\boldsymbol{\eta}$ if $\boldsymbol{\xi} \leq \boldsymbol{\eta}$ and that $\mathbf{v}$ conforms to $\boldsymbol{\eta}$ if $sign(\mathbf{v}) \leq \boldsymbol{\eta}$. We call two vectors $\mathbf{v}^1, \mathbf{v}^2$ conformal if $sign(\mathbf{v}^1), sign(\mathbf{v}^2) \leq \boldsymbol{\eta}$ for some sign vector $\boldsymbol{\eta}$ or, equivalently, if $\mathbf{v}_i^1 \mathbf{v}_i^2 \geq 0$ for all i (so, $\mathbf{v}^1 + \mathbf{v}^2$ is a conformal sum iff $\mathbf{v}^1$ and $\mathbf{v}^2$ are conformal). A conformal sum $\mathbf{v} = \mathbf{v}^1 + \mathbf{v}^2$, i.e., verifying (14), will be noted $\mathbf{v} = \mathbf{v}^1 \oplus \mathbf{v}^2$. It is thus natural to look for generators as conformally non-decomposable vectors, where a nonzero vector $\mathbf{x}$ of a convex polyhedral cone C is called conformally non-decomposable, if

$$\mathbf{x} = \mathbf{x}^1 \oplus \mathbf{x}^2, \text{ with nonzero } \mathbf{x}^1, \mathbf{x}^2 \in C, \text{ implies } \mathbf{x}^1 = \lambda \mathbf{x}^2 \text{ with } \lambda > 0. \tag{15}$$

A vector $\mathbf{x}$ (resp., flux vector $\mathbf{v}$) of a convex polyhedral cone C (resp., a flux cone FC) is called elementary [22] if it is conformally non-decomposable. All nonzero elements of the ray defined by an elementary vector are elementary, i.e., elementary vectors are unique up to positive scalar multiplication (and thus we will often not distinguish elementary vectors and elementary rays). Elementary vectors (resp., flux vectors) will be noted `EVs` (resp., `EFVs`).

The elementary rays constitute the unique minimal (finite) set of conformal generators of C, i.e., generators for conformal conical sum:

$$C = \{\bigoplus\nolimits_{g \in VG} \beta_g \mathbf{e}^g \mid \beta_g \geq 0\} \triangleq cone_{\oplus}(\{\mathbf{e}^g\}) \tag{16}$$

where the $\mathbf{e}^g$'s, $g \in VG$ (finite index set), are representatives of the elementary rays (unique up to positive scalar multiplication) and $cone_{\oplus}$ is the conical conformal hull. More precisely, we get an upper bound of the number of elementary vectors that are sufficient to decompose any given nonzero vector $\mathbf{x} \in C$: $\mathbf{x} = \bigoplus_{g \in VG_{\mathbf{x}}} \beta_g \mathbf{e}^g$ with $|VG_{\mathbf{x}}| \leq min(dim(C), |supp(\mathbf{x})| + |supp(\mathbf{A}\mathbf{x})|)$. This result can be demonstrated first for a flux vector $\mathbf{v}$ of a flux cone FC with an upper bound given by $|VG_{\mathbf{v}}| \leq min(dim(C), |supp(\mathbf{v})|)$ and then for a vector $\mathbf{x}$ of a general polyhedral cone C by using the correspondence (9) between C and FC_C which maps the elementary vectors of C onto the elementary flux vectors of FC_C.

It results from (10) and (15) that an extreme vector of C is elementary, i.e., `ExVs` $\subseteq$ `EVs`, but the converse is false in general as a conformally non-decomposable vector may be conically decomposable. Nevertheless, if C is contained in a closed orthant, there is identity between extreme vectors and elementary vectors, i.e., `ExVs` = `EVs`. More precisely, for nonzero $\mathbf{x} \in C$ and O a closed orthant with $\mathbf{x} \in O$, then $\mathbf{x}$ is elementary in C if and only if it is extreme in $C \cap O$. It results that the elementary vectors of C are the extreme vectors of intersections of C with any closed orthant:

$$\texttt{EVs}(C) = \bigcup\nolimits_{O \ orthant} \texttt{ExVs}(C \cap O). \tag{17}$$

Elementary vectors of C can thus be obtained by using algorithms, such as DD, to compute extreme vectors `ExVs` of the pointed polyhedral cones $C \cap O$. Doing this, it is convenient to select in (17) only a minimal subset of closed orthants O, in order to avoid equality or inclusion between the $C \cap O$'s (nonempty intersection cannot obviously be avoided as orthants are closed). It is clearly enough to consider only the 2^r closed r-orthants of maximal dimension r, but this does not avoid equality or inclusion. Let $\{\boldsymbol{\eta}^i\}$ be the maximal (for the partial order defined above on $\{-,0,+\}^r$) sign vectors of $sign(C) = \{sign(\mathbf{x}) \mid \mathbf{x} \in C\}$. Then, it is enough in (17) to consider the closed orthants $O_i = \{\mathbf{x} \in \mathbb{R}^r \mid sign(\mathbf{x}) \leq \boldsymbol{\eta}^i\}$ and there is no equality or inclusion between the $C \cap O_i$'s, where $C \cap O_i = C_{\leq \boldsymbol{\eta}^i} = \{\mathbf{x} \in C \mid sign(\mathbf{x}) \leq \boldsymbol{\eta}^i\}$. The $C_{\leq \boldsymbol{\eta}^i}$'s are called topes, noted `Ts` (flux topes [12] noted `FTs` for a flux cone FC). C is thus decomposed into topes and (17) can be rewritten as:

$$\texttt{EVs}(C) = \bigcup\nolimits_{\boldsymbol{\eta}^i \ maximal \ in \ sign(C)} \texttt{ExVs}(C_{\leq \boldsymbol{\eta}^i}). \tag{18}$$

Note that, for a flux cone FC (6), a `FT` is defined by specifying a maximal subset of reactions with fixed directions (thus fixing the directions of reversible reactions), the other ones having necessarily a zero flux. This simplifies if FC is consistent, i.e., without unused reaction, which means that every reaction, in every possible direction for reversible ones, is supported by a flux vector: $\forall i \in \{1,\ldots,r\}$ $\exists \mathbf{v} \in FC$ $\mathbf{v}_i > 0$ and $\forall i \in \{1,\ldots,r\} \backslash \mathbf{Irr}$ $\exists \mathbf{v} \in FC$ $\mathbf{v}_i < 0$. We can always assume FC consistent after a pre-processing step (practically, this can be achieved by using flux variability analysis [14]) that removes all reactions that cannot carry nonzero steady-state flux and changes all reversible reactions that cannot carry flux in both directions into irreversible ones. In this case, every remaining reaction in every possible direction is supported by a flux vector with full support (i.e., with nonzero flux in any reaction) and all `FTs` $FC_{\leq \boldsymbol{\eta}^i}$ have full support, i.e., the $\boldsymbol{\eta}^i$'s have full support or, equivalently, the O_i's are r-orthants. An obvious upper bound for the number of `FTs` is thus 2^{r-r_I}.

Now, for a flux cone FC, another commonly used method is, at the extreme opposite, to make it included into a single (positive) orthant in higher dimension by splitting up each reversible reaction i into a forward i^+ and a backward i^- irreversible reaction. This means decomposing a flux in i as $\mathbf{v}_i = \mathbf{v}_i^+ - \mathbf{v}_i^-$ with $\mathbf{v}_i^+ = \mathbf{v}_{i^+} \geq 0$ and $\mathbf{v}_i^- = \mathbf{v}_{i^-} \geq 0$. Columns of the stoichiometric matrix $\mathbf{S}$ corresponding to reversible reactions i are negated (which means exchanging the roles of substrates and products in i) and appended to $\mathbf{S}$ as new columns to form the new stoichiometric matrix $\widetilde{\mathbf{S}} \in \mathbb{R}^{m \times \tilde{r}}$, where $\tilde{r} = 2r - r_I$ is the new reactions number and all $\tilde{r}$ reactions are now irreversible, $\widetilde{\mathbf{Irr}} = \{1,\ldots,\tilde{r}\}$. FC is in one-to-one correspondence with those vectors of $\widetilde{FC}$ such that $\mathbf{v}_{i^+}.\mathbf{v}_{i^-} = 0$ for i reversible. In particular, the fluxes of the form $\mathbf{v}_{i^+} = \mathbf{v}_{i^-} > 0$ with all other components being null are obtained as extreme vectors of $\widetilde{FC}$ but represent futile cycles (involving reactions i^+ and i^-) without biological reality and must be eliminated. Finally, $\texttt{EFVs}(FC)$ is in one-to-one correspondence with $\texttt{ExVs}(\widetilde{FC}) \backslash \{\text{futile cycles}\}$ (called at the origin extreme currents in stoichiometric network analysis [6]). $\widetilde{FC}$ is included in the positive $\tilde{r}$-orthant and has thus only one `FT`.

We have thus two opposite ways of dealing with reversible reactions for computing `EFVs` of a flux cone FC: either splitting up each reversible reaction into two irreversible ones, such that FC is reduced to a single `FT` at the price of an increase of the space dimension by $r - r_I$ (which can cause serious efficiency problems to algorithms such as DD) or keeping unchanged the reversible reactions and decomposing FC into `FTs`, in each of which directions of reversible reactions are fixed, at the price of a potentially exponential (in terms of $r - r_I$) number of `FTs` to consider. All intermediate cases, where only a subset of reversible reactions are split into irreversible ones and the others are processed by decomposition into `FTs`, are obviously possible. Independently of the solution adopted, we will work most of the time in a given `FT` for FC, defined by a (full support if FC is consistent) sign vector $\boldsymbol{\eta}$, and the `EFVs` of FC which conform to $\boldsymbol{\eta}$ are thus given by the `ExVs` of this `FT` $FC_{\leq \boldsymbol{\eta}}$.

1.6 Elementary modes

The null value 0 plays a component-wise crucial role in definitions of the support of a vector (4), of a flux cone (6) and of a conformal sum (13),(14). It results a close relationship between support-minimal vectors and elementary vectors in a flux cone. A nonzero vector $\mathbf{x} \in C$ is called support-minimal, if

$$supp(\mathbf{x}') \subseteq supp(\mathbf{x}), \text{ with nonzero } \mathbf{x}' \in C, \text{ implies } supp(\mathbf{x}') = supp(\mathbf{x}). \tag{19}$$

A nonzero vector $\mathbf{x}$ (resp., flux vector $\mathbf{v}$) of a convex polyhedral cone C (resp., a flux cone FC) is called elementary mode (resp., elementary flux mode) if it is support-minimal (the concept of elementary flux mode was first introduced, under the name of elementary vector [34], for a subspace of $\mathbb{R}^r$, i.e., for a flux linear subspace FS (3) and then [38] for a flux cone FC with a definition actually closer to that of support-wise non-decomposable vector). All nonzero elements of the ray defined by an elementary mode are elementary modes having the same support, i.e., elementary modes are unique up to positive scalar multiplication (and thus we will in general identify two positively proportional elementary modes). Elementary modes (resp., flux modes) will be noted `EMs` (resp., `EFMs`). The concept of `EFM` in a flux cone FC is biologically significant as it represents a minimal pathway operating at steady state, i.e., with all reactions involved necessarily active (with a nonzero net rate), which means that no proper sub-pathway can operate at steady state.

Note that, if $\mathbf{x}$ is an `EFM`, then $sign(\mathbf{x})$ is a minimal (for the partial order defined above on $\{-,0,+\}^r$) nonzero element of $sign(FC)$ and, conversely, one shows that a minimal nonzero sign vector $\boldsymbol{\sigma} \in sign(FC)$ determines an `EFM` $\mathbf{x}$ with $sign(\mathbf{x}) = \boldsymbol{\sigma}$ by: $FC_{\leq\boldsymbol{\sigma}} = \{\mathbf{v} \in FC \mid sign(\mathbf{v}) \leq \boldsymbol{\sigma}\} = \{\mathbf{v} \in FC \mid sign(\mathbf{v}) = \boldsymbol{\sigma}\} \cup \{\mathbf{0}\} = \{\lambda\mathbf{x} \mid \lambda \geq 0\}$. There is thus a one-to-one mapping between `EFMs` and minimal nonzero sign vectors of $sign(FC)$. Comparing with the one-to-one mapping between `FTs` and maximal sign vectors of $sign(FC)$, we see that `EFMs` and `FTs` are dual concepts.

Now, for a flux cone FC, support-minimality and conformal non-decomposability are equivalent properties, i.e., there is identity between elementary flux modes and elementary flux vectors: `EFMs` = `EFVs`. For metabolic pathways in a flux cone FC, there is thus identity between minimal (for support inclusion) pathways and non-decomposable (for conformal sum) pathways. From (16), the `EFMs` constitute a conformal generating set (i.e., generating set for conformal sum) for FC, and actually the unique minimal one (in fact one way of proving (16) for flux cones FC is to prove this result about `EFMs` as conformal generating set and to prove that `EFMs` = `EFVs`). From (18), for any maximal sign vector $\boldsymbol{\eta}$ of $sign(FC)$, the `EFMs` of FC which conform to $\boldsymbol{\eta}$ are the `EFMs` of the `FT` $FC_{\leq\boldsymbol{\eta}}$ and coincide with the `ExVs` of this `FT`, this result being at the basis of the methods for computing `EFMs` [9, 7]. `EFMs` are thus decomposed into subsets according to the decomposition of FC into flux topes [12]: the `EFMs` of the `FT` $FC_{\leq\boldsymbol{\eta}}$ correspond to the $FC_{\leq\boldsymbol{\sigma}}$'s, where the $\boldsymbol{\sigma}$'s are the minimal nonzero sign vectors of $sign(FC)$ such that $\boldsymbol{\sigma} \leq \boldsymbol{\eta}$.

For a general polyhedral cone C, there is no direct relationship between elementary modes and elementary vectors but the correspondence (9) between C and the higher dimensional flux cone FC_C maps the elementary vectors of C onto the elementary flux vectors of FC_C, i.e., the elementary flux modes of FC_C:

$$\texttt{EVs}(C) = \{\mathbf{x} \in \mathbb{R}^r \mid \begin{pmatrix}\mathbf{x}\\ \mathbf{y}\end{pmatrix} \in \texttt{EFMs}(FC_C)\} \text{ with } FC_C \text{ given by (9).} \tag{20}$$

And it remains true that any vector which is support-minimal in some $C_{\leq\boldsymbol{\eta}}$ is actually support minimal in C, as it depends only on the convexity of C: if $\mathbf{x}$ and $\mathbf{x}'$ are vectors in a convex set with $supp(\mathbf{x}') \subset supp(\mathbf{x})$, then it exists a vector $\mathbf{x}''$ in this convex set with $sign(\mathbf{x}'') \leq sign(\mathbf{x})$ and $supp(\mathbf{x}'') \subset supp(\mathbf{x})$ (one takes $\mathbf{x}'' = \lambda\mathbf{x}' + (1-\lambda)\mathbf{x}$ with λ minimal in $(0,1]$ such that $\mathbf{x}''_i = 0$ for some i with $\mathbf{x}_i \neq 0$). Thus `EMs` of C can be computed tope by tope:

$$\texttt{EMs}(C) = \bigcup\nolimits_{\boldsymbol{\eta}^i \ maximal\ in\ sign(C)} \texttt{EMs}(C_{\leq\boldsymbol{\eta}^i}). \tag{21}$$

1.7 Inhomogeneous linear constraints and polyhedra

Additionally, in this standard setting, fluxes may be constrained by other constraints, typically lower and upper reaction rates bounds:

$$\mathbf{v}_i^- \leq \mathbf{v}_i \leq \mathbf{v}_i^+ \tag{22}$$

or, more generally, any set **ILC** of inhomogeneous linear constraints that can be written in the general form:

$$\mathbf{G}\mathbf{v} \geq \mathbf{h} \tag{23}$$

where $\mathbf{G} \in \mathbb{R}^{l\times r}$ is a matrix and $\mathbf{h} \in \mathbb{R}^l$ a vector with nonzero components, defining a general inhomogeneous convex polyhedron $P_{\mathbf{ILC}} = \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{G}\mathbf{v} \geq \mathbf{h}\}$. The solutions space $Sol_{\mathbf{S},\mathbf{Irr},\mathbf{ILC}}$ is thus now a s-polyhedron or flux polyhedron noted FP and defined by:

$$Sol_{\mathbf{S},\mathbf{Irr},\mathbf{ILC}} \triangleq FP \triangleq \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{S}\mathbf{v} = \mathbf{0}, \mathbf{v}_i \geq 0 \text{ for } i \in \mathbf{Irr}, \mathbf{G}\mathbf{v} \geq \mathbf{h}\} = FC \cap P_{\mathbf{ILC}}. \tag{24}$$

It is a particular case of (general) convex polyhedron that is defined implicitly by finitely many linear inequalities:

$$P \triangleq \{\mathbf{x} \in \mathbb{R}^r \mid \mathbf{A}\mathbf{x} \geq \mathbf{b}\} \tag{25}$$

with a suitable matrix $\mathbf{A} \in \mathbb{R}^{n \times r}$ and vector $\mathbf{b} \in \mathbb{R}^n$ and is thus the intersection of n (affine) half-spaces. Its dimension is defined as the dimension of its affine span. A flux polyhedron FP corresponds thus to a particular matrix $\mathbf{A} \in \mathbb{R}^{(2m+r_I+l)\times r}$ and vector $\mathbf{b} \in \mathbb{R}^{2m+r_I+l}$ given by:

$$\mathbf{A} = \begin{pmatrix} \mathbf{S} \\ -\mathbf{S} \\ \mathbf{I_{Irr}} \\ \mathbf{G} \end{pmatrix} \qquad \mathbf{b} = \begin{pmatrix} \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{h} \end{pmatrix} \tag{26}$$

meaning that those inequalities that are homogeneous divide actually in m equalities defining a lower-dimensional subspace given by the nullspace of the stoichiometric matrix $\mathbf{S}$ and in r_I nonnegativity constraints about some single coordinate variables (given by the irreversible reactions). A polyhedral cone (resp., flux cone) is a special case of polyhedron (resp., flux polyhedron) where $\mathbf{b} = \mathbf{0}$ (resp., $\mathbf{ILC} = \emptyset$).

To any nonempty polyhedron P given by (25) is associated its so-called recession cone $C_P = \{\mathbf{x} \in \mathbb{R}^r \mid \mathbf{A}\mathbf{x} \geq \mathbf{0}\}$, which is the polyhedral cone containing all unbounded directions (rays) of P (if P is a polyhedral cone, then $P = C_P$). A bounded polyhedron is called a polytope and thus P is a polytope if and only if its recession cone is trivial: $C_P = \{\mathbf{0}\}$. P is called pointed if its recession cone C_P is pointed, i.e., if its lineality space $\{\mathbf{x} \in \mathbb{R}^r \mid \mathbf{A}\mathbf{x} = \mathbf{0}\}$, also called the lineality space of P (as it contains all unbounded lines of P), is trivial. For a flux polyhedron FP, we have: $C_{FP} = FC \cap C_{P_{\mathbf{ILC}}}$ (thus FP can be a polytope without $P_{\mathbf{ILC}}$ being so and be pointed without neither FC nor $P_{\mathbf{ILC}}$ being so). Note that C_{FP} is not in general a flux cone.

1.7.1 Extreme points and vectors and generating sets

A vector $\mathbf{x} \in P$ is called an extreme point, if it cannot be written as a convex combination of distinct vectors of P:

$$\mathbf{x} = \lambda\mathbf{x}^1 + (1-\lambda)\mathbf{x}^2, \text{ with } \mathbf{x}^1, \mathbf{x}^2 \in P \text{ and } 0 < \lambda < 1, \text{ implies } \mathbf{x}^1 = \mathbf{x}^2. \tag{27}$$

Extreme points coincide with vertices of P, where a vertex of P is defined as a face of dimension 0. P is pointed if and only if it has a vertex and in this case, from Minkowski's theorem, the vertices of P and the extreme rays of C_P constitute the unique minimal (finite) set of ("bounded" and "unbounded", resp.) generators of P for (convex and conical, resp.) combination:

$$P = \{\sum\nolimits_{j\in J} \alpha_j \mathbf{p}^j + \sum\nolimits_{k\in K} \beta_k \mathbf{y}^k \mid \alpha_j, \beta_k \geq 0, \sum\nolimits_{j\in J} \alpha_j = 1\} \triangleq conv(\{\mathbf{p}^j\}) + cone(\{\mathbf{y}^k\}) \tag{28}$$

where the $\mathbf{p}^j$'s, $j \in J$ (finite index set), are the extreme points (vertices) of P, noted `ExPs`, and the $\mathbf{y}^k$'s, $k \in K$ (finite index set), are the extreme vectors of C_P (unique up to positive scalar multiplication), noted `ExVs`, and *conv* is the convex hull (if P is a pointed polyhedral cone, then it has for only vertex the zero vector and the formula (28) reduces to (11)). More precisely, we get an upper bound of the number of extreme points and vectors that are sufficient to decompose any given vector $\mathbf{x} \in P$: $\mathbf{x} = \sum_{j\in J_\mathbf{x}} \alpha_j \mathbf{p}^j + \sum_{k\in K_\mathbf{x}} \beta_k \mathbf{y}^k$ with $|J_\mathbf{x}| + |K_\mathbf{x}| \leq min(dim(P)+1, |supp(\mathbf{x})| + |supp(\mathbf{A}\mathbf{x}-\mathbf{b})| + 1)$. This result can be deduced from the result (11) for a pointed flux cone FC by using the following correspondence between P and such a flux cone.

Actually, to any convex polyhedron P in $\mathbb{R}^r$ defined by a matrix $\mathbf{A} \in \mathbb{R}^{n\times r}$ and vector $\mathbf{b} \in \mathbb{R}^n$ (25), one can associate a flux cone FC_P in higher dimension $\mathbb{R}^{r+1+n}$ defined by:

$$FC_P \triangleq \{\begin{pmatrix} \mathbf{x} \\ \xi \\ \mathbf{A}\mathbf{x} - \xi\mathbf{b} \end{pmatrix} \in \mathbb{R}^{r+1+n} \mid \xi \geq 0, \mathbf{A}\mathbf{x} - \xi\mathbf{b} \geq \mathbf{0}\}$$

$$= \{\mathbf{v} \in \mathbb{R}^{r+1+n} \mid \begin{pmatrix} \mathbf{A} & -\mathbf{b} & -\mathbf{I} \end{pmatrix} \mathbf{v} = \mathbf{0}, \mathbf{v}_i \geq 0 \text{ for } r+1 \leq i \leq r+1+n\}. \tag{29}$$

This introduces a correspondence between vectors $\mathbf{x}$ of P and vectors $\begin{pmatrix} \mathbf{x} \\ 1 \\ \mathbf{Ax}-\mathbf{b} \end{pmatrix}$ of FC_P, which maps vertices of P onto extreme vectors of FC_P with component $\xi = 1$, and between vectors $\mathbf{x}$ of C_P and vectors $\begin{pmatrix} \mathbf{x} \\ 0 \\ \mathbf{Ax} \end{pmatrix}$ of FC_P, which maps extreme vectors of C_P onto extreme vectors of FC_P with component $\xi = 0$. Thanks to this correspondence, several properties, proven for flux cones, can actually be lifted up to general convex polyhedra.

For the particular case where P is a flux polyhedron FP given by (24), the correspondence (29) simplifies by associating to FP the flux cone FC_{FP} in dimension $\mathbb{R}^{r+1+l}$ defined by:

$$FC_{FP} \triangleq \{\begin{pmatrix} \mathbf{v} \\ \xi \\ \mathbf{Gv}-\xi\mathbf{h} \end{pmatrix} \in \mathbb{R}^{r+1+l} \mid \mathbf{Sv} = \mathbf{0}, \mathbf{v}_i \geq 0 \text{ for } i \in \mathbf{Irr}, \xi \geq 0, \mathbf{Gv}-\xi\mathbf{h} \geq \mathbf{0}\}$$

$$= \{\mathbf{v}' \in \mathbb{R}^{r+1+l} \mid \begin{pmatrix} \mathbf{S} & \mathbf{0} & \mathbf{0} \\ \mathbf{G} & -\mathbf{h} & -\mathbf{I} \end{pmatrix} \mathbf{v}' = \mathbf{0}, \mathbf{v}'_i \geq 0 \text{ for } i \in \mathbf{Irr} \text{ and for } r+1 \leq i \leq r+1+l\}. \quad (30)$$

The DD method builds an explicit description of a pointed polyhedron P, in the form of two generating matrices whose columns are respectively the $\mathbf{p}^j$'s and the $\mathbf{y}^k$'s, from an implicit description of P as in (25), i.e., enumerates its vertices `ExPs` and extreme vectors `ExVs`.

If P is not pointed, it is still finitely generated:

$$P = \{\sum\nolimits_{j\in J} \alpha_j \mathbf{p}^j + \sum\nolimits_{k\in K} \beta_k \mathbf{y}^k + \sum\nolimits_{l\in L} \gamma_l \mathbf{z}^l \mid \alpha_j, \beta_k \geq 0, \gamma_l \in \mathbb{R}, \sum\nolimits_{j\in J} \alpha_j = 1\}$$

$$= conv(\{\mathbf{p}^j\}) + cone(\{\mathbf{y}^k, \mathbf{z}^l, -\mathbf{z}^l\}) \quad (31)$$

with (not unique this time) minimal set of generators consisting of basis vectors $\mathbf{z}^l$ of the lineality space and suitable vectors $\mathbf{p}^j$ and $\mathbf{y}^k$ (e.g., the vertices and extreme vectors of the pointed polyhedron obtained by intersecting P with the orthogonal complement of its lineality space; if P is a non-pointed polyhedral cone, there is no nonzero $\mathbf{p}^j$ and the formula (31) reduces to (12)). Actually, Minkowski-Weyl's theorem for polyhedra states that it is equivalent for a set P to be a polyhedron (25) or to be finitely generated, i.e., to be the Minkowski sum of the convex hull of a finite set of vectors (as the $\mathbf{p}^j$'s) and of the conical hull of a finite set of vectors (as the $\mathbf{y}^k$'s, $\mathbf{z}^l$'s and $-\mathbf{z}^l$'s).

1.7.2 Elementary points and vectors and conformal generating sets

But, as for flux cones, such decomposition into a finite set of generators is not in general satisfactory for a flux polyhedron as only a decomposition without cancelations is biochemically meaningful. Such, in the same way we replaced as generators for a polyhedral cone extreme vectors by conformally non-decomposable vectors, we will replace as generators for a polyhedron P extreme points (vertices) by convex-conformally non-decomposable vectors (and for its recession cone C_P extreme vectors by conformally non-decomposable vectors). A vector $\mathbf{x}$ of a polyhedron P is called convex-conformally non-decomposable, if

$$\mathbf{x} = \lambda\mathbf{x}^1 \oplus (1-\lambda)\mathbf{x}^2, \text{ with } \mathbf{x}^1, \mathbf{x}^2 \in P \text{ and } 0 < \lambda < 1, \text{ implies } \mathbf{x}^1 = \mathbf{x}^2. \quad (32)$$

Given a polyhedron P (resp., flux polyhedron FP), a vector (resp., flux vector) $\mathbf{x}$ is called an elementary point (resp., elementary flux point) - also called "bounded" elementary vector - of P if $\mathbf{x} \in P$ is convex-conformally non-decomposable and is called an elementary vector (resp., elementary flux vector) - also called "unbounded" elementary vector - of P if $\mathbf{x} \in C_P$ is conformally non-decomposable (it is unique only up to positive scalar multiplication) [22]. Elementary points (resp., flux points) will be noted `EPs` (resp., `EFPs`) and elementary vectors (resp., flux vectors) will be noted `EVs` (resp., `EFVs`), which is consistent with the same notation for polyhedral cones and flux cones. We will note `Es` = `EPs` $\cup$ `EVs` (resp., `EFs` = `EFPs` $\cup$ `EFVs`) the elementary elements (resp., elementary fluxes) of P (resp., FP).

The elementary points and the elementary rays constitute the unique minimal (finite) set of conformal generators of P, i.e., generators for convex-conformal (for elementary points) and conformal (for elementary vectors) sum:

$$P = \{\bigoplus\nolimits_{g\in PG} \alpha_g \mathbf{e}^g \oplus \bigoplus\nolimits_{g\in VG} \beta_g \mathbf{e}^g \mid \alpha_g, \beta_g \geq 0, \sum\nolimits_{g\in PG} \alpha_g = 1\}$$

$$\triangleq \; conv_{\oplus}(\{\mathbf{e}^g \mid g \in PG\}) \oplus cone_{\oplus}(\{\mathbf{e}^g \mid g \in VG\}) \tag{33}$$

where the $\mathbf{e}^g$'s, $g \in PG$ (finite index set), are the elementary points and the $\mathbf{e}^g$'s, $g \in VG$ (finite index set), are the elementary vectors (unique up to positive scalar multiplication), and $conv_{\oplus}$ is the convex conformal hull. More precisely, we get an upper bound of the number of elementary points and vectors that are sufficient to decompose any given vector $\mathbf{x} \in P$: $\mathbf{x} = \bigoplus_{g \in PG_{\mathbf{x}}} \alpha_g \mathbf{e}^g \oplus \bigoplus_{g \in VG_{\mathbf{x}}} \beta_g \mathbf{e}^g$ with $|PG_{\mathbf{x}}| + |VG_{\mathbf{x}}| \leq min(dim(P)+1, |supp(\mathbf{x})| + |supp(\mathbf{A}\mathbf{x}-\mathbf{b})|+1)$. This result can be demonstrated from the result (16) for a flux cone by using the correspondence (29) between P and FC_P which maps the elementary points of P onto the elementary flux vectors of FC_P with component $\xi = 1$ and the elementary vectors of P onto the elementary flux vectors of FC_P with component $\xi = 0$.

We already know that an extreme vector of C_P is elementary, thus is an elementary vector of P, i.e., $\texttt{ExVs} \subseteq \texttt{EVs}$. It results from (27) and (32) that an extreme point (vertex) of P is an elementary point of P, i.e., $\texttt{ExPs} \subseteq \texttt{EPs}$, but the converse is false in general as a convex-conformally non-decomposable vector may be convex decomposable. Nevertheless, if P is contained in a closed orthant (and thus C_P too), any sum of vectors of P (resp., C_P) is conformal and thus there is identity between extreme points (vertices) and elementary points (resp., between extreme vectors and elementary vectors), i.e., $\texttt{ExPs} = \texttt{EPs}$ and $\texttt{ExVs} = \texttt{EVs}$. More precisely, for $\mathbf{x} \in P$ (resp., $\mathbf{x} \in C_P$ and nonzero) and O a closed orthant with $\mathbf{x} \in O$, then $\mathbf{x}$ is an elementary point (resp., elementary vector) of P if and only if it is a vertex in $P \cap O$ (resp., an extreme vector in $C_P \cap O = C_{P \cap O}$). It results that the elementary points (resp., elementary vectors) of P are the vertices (resp., extreme vectors) of intersections of P (resp., C_P) with any closed orthant, which are pointed subpolyhedra (resp., pointed subcones):

$$\texttt{EPs}(P) = \bigcup\nolimits_{O \text{ orthant}} \texttt{ExPs}(P \cap O) \qquad \texttt{EVs}(P) = \bigcup\nolimits_{O \text{ orthant}} \texttt{ExVs}(C_P \cap O). \tag{34}$$

Note in particular that, if $\mathbf{0} \in P$, then $\mathbf{0} \in \texttt{EPs}$. Elementary points and vectors can thus be obtained by using algorithms, such as DD, to compute vertices $\texttt{ExPs}$ and extreme vectors $\texttt{ExVs}$ of the pointed polyhedra $P \cap O$. It is obvious that considering only the 2^r closed r-orthants is enough. Now, as for polyhedral cones, decomposing the polyhedron into topes is better:

$$\texttt{EPs}(P) = \bigcup\nolimits_{\boldsymbol{\eta}^i \text{ maximal in } sign(P)} \texttt{ExPs}(P_{\leq \boldsymbol{\eta}^i}) \qquad \texttt{EVs}(P) = \bigcup\nolimits_{\boldsymbol{\eta}^j \text{ maximal in } sign(C_P)} \texttt{ExVs}(C_{P \leq \boldsymbol{\eta}^j}). \tag{35}$$

If O_i is the closed orthant defined by $\boldsymbol{\eta}^i$, then the corresponding tope for P is $P \cap O_i = P_{\leq \boldsymbol{\eta}^i} = \{\mathbf{x} \in P \mid sign(\mathbf{x}) \leq \boldsymbol{\eta}^i\}$ and, as seen for polyhedral cones, $C_P \cap O_j = C_{P \leq \boldsymbol{\eta}^j}$ is a tope for the recession cone C_P. Examine how the equality $C_{P_{\leq \boldsymbol{\eta}}} = C_{P \leq \boldsymbol{\eta}}$, for $\boldsymbol{\eta}$ an arbitrary sign vector, can be expressed in terms of topes. Note first that any $\boldsymbol{\eta}^j$ is dominated by at least one $\boldsymbol{\eta}^i$ for the partial order $\leq$ on $\{-,0,+\}^r$: $\forall \boldsymbol{\eta}^j \exists \boldsymbol{\eta}^i \boldsymbol{\eta}^j \leq \boldsymbol{\eta}^i$, which means that O_j is a sub-orthant of O_i, and we have $C_{P \leq \boldsymbol{\eta}^j} = C_{P_{\leq \boldsymbol{\eta}^i}}$, expressing the relation between the topes for the recession cone of the polyhedron and the recession cones of some of the polyhedron topes (precisely those topes $P_{\leq \boldsymbol{\eta}^i}$ for which $\boldsymbol{\eta}^i$ dominates an $\boldsymbol{\eta}^j$, necessarily unique). More generally, the recession cone of any tope $P_{\leq \boldsymbol{\eta}^i}$ for P can be expressed as a subcone of a tope for the recession cone of P by: $C_{P_{\leq \boldsymbol{\eta}^i}} = C_{P \leq \mathbf{c}(\boldsymbol{\eta}^i)}$, where $\mathbf{c}(\boldsymbol{\eta}^i) = max\{\boldsymbol{\eta} \in sign(C_P) \mid \boldsymbol{\eta} \leq \boldsymbol{\eta}^i\}$ is the greatest (it is necessarily unique) sign vector in $sign(C_P)$ dominated by $\boldsymbol{\eta}^i$ (thus, if $\boldsymbol{\eta}^i$ does not dominate any $\boldsymbol{\eta}^j$, $C_{P \leq \mathbf{c}(\boldsymbol{\eta}^i)}$ is not a tope for C_P; this is the case for example if P is not a polytope but $P_{\leq \boldsymbol{\eta}^i}$ is, implying $\mathbf{c}(\boldsymbol{\eta}^i) = \mathbf{0}$ and $C_{P \leq \mathbf{c}(\boldsymbol{\eta}^i)} = \{\mathbf{0}\}$ not a tope for $C_P \neq \{\mathbf{0}\}$).

For the particular case of a flux polyhedron FP (24), we have $FP_{\leq \boldsymbol{\eta}} = FC_{\leq \boldsymbol{\eta}} \cap P_{\mathbf{ILC}}$ and $C_{FP_{\leq \boldsymbol{\eta}}} = C_{FP \leq \boldsymbol{\eta}} = FC_{\leq \boldsymbol{\eta}} \cap C_{P_{\mathbf{ILC}}}$, for any sign vector $\boldsymbol{\eta}$. Any flux tope $FP_{\leq \boldsymbol{\eta}^i}$ for FP can thus be expressed as: $FP_{\leq \boldsymbol{\eta}^i} = FC_{\leq \boldsymbol{\eta}^k} \cap P_{\mathbf{ILC}}$, for some flux tope $FC_{\leq \boldsymbol{\eta}^k}$ for FC, i.e., by applying the constraints **ILC** to a flux tope for FC. The same holds for the recession cone: $C_{FP_{\leq \boldsymbol{\eta}^i}} = C_{FP \leq \mathbf{c}(\boldsymbol{\eta}^i)} = FC_{\leq \boldsymbol{\eta}^k} \cap C_{P_{\mathbf{ILC}}}$, i.e., by applying the homogeneous counterparts of constraints **ILC** to a flux tope for FC. If FP is assumed to be consistent (same definition as for a flux cone, i.e., without unused reaction with always a zero flux), all $\texttt{FTs}$ $FP_{\leq \boldsymbol{\eta}^i}$ have then full support, i.e., the $\boldsymbol{\eta}^i$'s have full support or, equivalently, the O_i's are r-orthants. An obvious upper bound for the number of $\texttt{FTs}$ is thus 2^{r-r_I}. Note that C_{FP} consistent is a sufficient (but not necessary) condition for FP to be consistent. And that, if FP is consistent, so is FC (but FP can be inconsistent even if both FC and $P_{\mathbf{ILC}}$ are consistent).

For a flux polyhedron FP, the method used for a flux cone to make it included into a single (positive) orthant in higher dimension by splitting up each reversible reaction i into a forward i^+ and a backward i^- irreversible reaction applies as well. The matrix $\mathbf{G}$ is extended into the matrix $\tilde{\mathbf{G}} \in \mathbb{R}^{l \times \tilde{r}}$ in the same

way that $\mathbf{S}$ is extended into $\widetilde{\mathbf{S}} \in \mathbb{R}^{m\times\tilde{r}}$, where $\tilde{r} = 2r - r_I$ is the new reactions number and all $\tilde{r}$ reactions are now irreversible, $\widetilde{\mathbf{Irr}} = \{1, \ldots, \tilde{r}\}$. FP is in one-to-one correspondence with those vectors of $\widetilde{FP}$ such that $\mathbf{v}_{i^+}.\mathbf{v}_{i^-} = 0$ for i reversible. In particular, the fluxes with $\mathbf{v}_{i^+}, \mathbf{v}_{i^-} > 0$ for some i, which are obtained as vertices or extreme vectors of $\widetilde{FP}$, are futile (involving a net rate in opposite directions in reactions i^+ and i^-) without biological reality and must be eliminated (they are not limited in general to futile cycles as in flux cones). Finally, the set of elementary fluxes $\texttt{EFs}(FP)$ is in one-to-one correspondence with $(\texttt{ExPs}(\widetilde{FP}) \cup \texttt{ExVs}(\widetilde{FP}))\backslash\{\text{futile fluxes}\}$. As $\widetilde{FP}$ is included in the positive $\tilde{r}$-orthant, it has only one FT.

As for flux cones, it exists thus two opposite ways of dealing with reversible reactions for computing $\texttt{EFs} = \texttt{EFPs} \cup \texttt{EFVs}$ of a flux polyhedron FP: either splitting up each reversible reaction into two irreversible ones, reducing FP to a single FT in higher dimension, or keeping unchanged the reversible reactions and decomposing FP into FTs without increasing the dimension. And all intermediate cases are possible. Whatever the solution adopted, EFs are obtained as the union of ExPs and ExVs for each flux tope for FP and thus we will work in general in a given FT for FP, defined by a (full support if FP is consistent) sign vector $\boldsymbol{\eta}$, and the EFs of FP which conform to $\boldsymbol{\eta}$ are thus given by the ExPs and ExVs of this FT $FP_{\leq\boldsymbol{\eta}}$.

1.7.3 Elementary modes

For a general polyhedron P (and even for a flux polyhedron FP), there is no direct relationship between support-minimal (19) vectors and elementary elements as it was the case for flux cones FC. Nevertheless, from (33), it results that, for any support-minimal nonzero vector in P (still called elementary mode EM), it exists an elementary element with same support. Thus, all minimal support patterns of vectors appear in the set of supports of elementary elements and are actually the minimal elements in this set for subset inclusion:

$$supp(\texttt{EMs}) = Min_{\subseteq}\{supp(\texttt{Es})\}. \tag{36}$$

In particular, for a metabolic network, it means that any set of reactions involved in a minimal pathway (EFM) appears as set of reactions involved in some elementary flux (EF).

In addition, for a general polyhedron P, the correspondence (29) between P and the higher dimensional flux cone FC_P maps the elementary points (resp., elementary vectors) of P onto the elementary flux vectors, i.e., the elementary flux modes, of FC_P with component $\xi = 1$ (resp., with component $\xi = 0$).

$$\texttt{EPs}(P) = \{\mathbf{x} \in \mathbb{R}^r \mid \begin{pmatrix}\mathbf{x}\\1\\\mathbf{y}\end{pmatrix} \in \texttt{EFMs}(FC_P)\} \qquad \texttt{EVs}(P) = \{\mathbf{x} \in \mathbb{R}^r \mid \begin{pmatrix}\mathbf{x}\\0\\\mathbf{y}\end{pmatrix} \in \texttt{EFMs}(FC_P)\} \tag{37}$$

with FC_P given by (29). The same formula holds for the particular case of a flux polyhedron FP (24) with FC_{FP} given by (30).

And, as seen in the proof of (21), any vector which is support-minimal in some $P_{\leq\boldsymbol{\eta}}$ is actually support minimal in P, thus EMs of P can be computed tope by tope:

$$\texttt{EMs}(P) = \bigcup_{\boldsymbol{\eta}^i \ maximal\ in\ sign(P)} \texttt{EMs}(P_{\leq\boldsymbol{\eta}^i}). \tag{38}$$

1.8 Complexity results

Enumerating the extreme vectors of a polyhedral cone or the vertices of a polytope has been proven to be a #P-complete problem. A consequence is that enumerating EFs or EFMs of a metabolic network is #P-complete too [1, 2]. The problem of enumerating the extreme vectors of a polyhedral cone or the vertices of a polytope with polynomial delay (i.e., the time between one output item and the next one is bounded by a polynomial function of the input size) is an open problem. So the possibility of enumerating EFs or EFMs of a metabolic network with polynomial delay is an open problem (except for a flux cone with all reactions being reversible, i.e., a flux linear subspace (3), as the EFMs are then the circuits of a linear matroid [34]). Given a metabolic network, deciding if it exists an EF or EFM with given support of size at least two is an NP-complete problem. The same result holds for deciding if it exists an EF or EFM whose support size is bounded above by a given positive integer [1, 2].

The McMullen's upper bound theorem [26] states that, for any fixed positive integers d and n, the maximum number of j-faces of a d-polytope with n facets (i.e., faces of dimension $d-1$) is attained by the dual cyclic polytope $c^\star(d,n)$ for all $j = 0, 1, \ldots, d-2$. A consequence is that the maximum number of vertices of a d-polytope with n facets is given by $\binom{n-\lceil d/2\rceil}{n-d} + \binom{n-\lfloor d/2\rfloor-1}{n-d} \sim 2\binom{n-\lfloor d/2\rfloor}{n-d}$. One obtains thus that the number of `EFs` or `EFMs` in a metabolic network (after having split each reversible reaction into two irreversible ones) is bounded above by a quantity approximately equal to $2\binom{\lfloor(\tilde{r}+m)/2\rfloor}{m}$ with $\tilde{r} = 2r - r_I$ (so $\tilde{r}$ varies between r and $2r$). If the number m of internal metabolites is small compared to the total number $\tilde{r}$ of reactions (after having split up reversible reactions), the number of `EFs` or `EFMs` is thus bounded above by a quantity of order $\Theta((\tilde{r}/2)^m)$, if m is close to $\tilde{r}$, this number is bounded above by a quantity of order $\Theta(\tilde{r}^{(\tilde{r}-m)/2})$. The worst case occurs when m is close to $\tilde{r}/3$ with an upper bound approximately equal to $2\binom{2m}{m}$. One obtains the same results with r instead of $\tilde{r}$ if we do not split reversible reactions but fix their signs arbitrarily, i.e., if we consider `EFs` or `EFMs` in an arbitrary closed r-orthant O, i.e., in an arbitrary flux tope for FC (or FP).

2 Metabolic pathways in presence of biological constraints

Although the current improved implementations of the DD method [39, 42] allow the computation of millions, even billions, of `EFMs` or `EFs`, it is insufficient to tackle genome-scale metabolic models (`GSMMs`). Moreover, most of those computed `EFMs` or `EFs` are not biologically valid, because only stoichiometry and some fluxes constraints as irreversibility of reactions or reactions rates bounds are taken into account. For both scaling up to large systems and limiting the number of biologically invalid solutions, it is necessary to consider additional biological constraints, such as thermodynamic, kinetic or regulatory constraints.

2.1 The biological constraints

2.1.1 Thermodynamic constraints

Assuming constant pressure and a closed system, according to the second law of thermodynamics, a reaction i proceeds spontaneously only in the direction of its negative Gibbs free energy $\Delta_r G_i$ [3], given by:

$$\Delta_r G_i = \Delta_r G_i'^0 + RT\ln(\prod\nolimits_j \mathbf{M}_j^{\overline{\mathbf{S}}_{ji}}) \tag{39}$$

where: $\Delta_r G_i'^0$ is the standard Gibbs free energy of reaction i, R the molar gas constant, T the absolute temperature, $\mathbf{M}_j$ the (positive) concentration of metabolite j and $\overline{\mathbf{S}}_{ji}$ is the stoichiometric coefficient of metabolite j in reaction i (i.e., $\overline{\mathbf{S}} \in \mathbb{R}^{(m+\overline{m})\times r}$ is the extension of the stoichiometric matrix $\mathbf{S}$ to the $\overline{m}$ external metabolites). This means that $\Delta_r G_i < 0$, (resp., $\Delta_r G_i > 0$) is a necessary condition to get $\mathbf{v}_i > 0$ (resp., $\mathbf{v}_i < 0$), which can be expressed by the constraint: $sign(\mathbf{v}_i) \leq -sign(\Delta_r G_i)$. And that a flux vector $\mathbf{v}$ is thermodynamically feasible [16] if and only if all its components $\mathbf{v}_i$ satisfy such a constraint (it is enough to consider those $i \in supp(\mathbf{v})$ as the constraint is trivially satisfied when $\mathbf{v}_i = 0$). The thermodynamic constraint for $\mathbf{v}$, that depends on the vector $\mathbf{M}$ of metabolites concentrations, can thus be defined as:

$$\mathbf{TC}_{\mathbf{M}}(\mathbf{v}) \triangleq \forall i \in supp(\mathbf{v}), sign(\mathbf{v}_i) = -sign(\Delta_r G_i'^0 + RT\ln(\prod\nolimits_j \mathbf{M}_j^{\overline{\mathbf{S}}_{ji}})). \tag{40}$$

As at equilibrium $\Delta_r G_i$ is null, we obtain: $\Delta_r G_i'^0 = -RT\ln(K_{eq}^i)$, where $K_{eq}^i = \prod_j M_{j_{eq}}^{\overline{\mathbf{S}}_{ji}}$ is the equilibrium constant of reaction i. Thus, $\Delta_r G_i$ can be rewritten as $\Delta_r G_i = RT\ln(\prod_j \mathbf{M}_j^{\overline{\mathbf{S}}_{ji}}/K_{eq}^i)$ and the thermodynamic constraint $\mathbf{TC}_{\mathbf{M}}(\mathbf{v})$ as:

$$\mathbf{TC}_{\mathbf{M}}(\mathbf{v}) \triangleq \forall i \in supp(\mathbf{v}), sign(\mathbf{v}_i) = -sign(\prod\nolimits_j \mathbf{M}_j^{\overline{\mathbf{S}}_{ji}} - K_{eq}^i). \tag{41}$$

Often, the concentrations of external metabolites can be measured and included in the constraint as known parameters, keeping only a dependency of the constraint on the concentrations of internal metabo-

lites. The formula $\Delta_r G_i = RT\ln(\prod_j \mathbf{M}_j^{\overline{\mathbf{S}}_{ji}}/K_{eq}^i)$ can be rewritten, by dividing the numerator and denominator of the fraction by the terms dealing with external metabolites, as $\Delta_r G_i = RT\ln(\prod_j \underline{\mathbf{M}}_j^{\mathbf{S}_{ji}}/\hat{K}_{eq}^i)$, where $\hat{K}_{eq}^i = K_{eq}^i/\prod_j \overline{\mathbf{M}}_j^{\overline{\mathbf{S}}_{ji}}$ is the apparent equilibrium constant of the reaction i and $\underline{\mathbf{M}}$ (resp., $\overline{\mathbf{M}}$) the vector of internal (resp., external) metabolites concentrations. The thermodynamic constraint (41) can thus be rewritten as:

$$\underline{\mathbf{TC}}_{\underline{\mathbf{M}}}(\mathbf{v}) \triangleq \forall i \in supp(\mathbf{v}), sign(\mathbf{v}_i) = -sign(\prod_j \underline{\mathbf{M}}_j^{\mathbf{S}_{ji}} - \hat{K}_{eq}^i). \tag{42}$$

For given metabolites concentrations vector $\mathbf{M}$ (resp., internal metabolites concentrations vector $\underline{\mathbf{M}}$), let $\mathbf{ts}_{\mathbf{M}} \in \{-,0,+\}^r$ (resp., $\mathbf{ts}_{\underline{\mathbf{M}}}$) the fixed thermodynamic sign vector defined by:

$$(\mathbf{ts}_{\mathbf{M}})_i = -sign(\Delta_r G_i'^0 + RT\ln(\prod_j \mathbf{M}_j^{\overline{\mathbf{S}}_{ji}})) = -sign(\prod_j \mathbf{M}_j^{\overline{\mathbf{S}}_{ji}} - K_{eq}^i)$$

$$(\mathbf{ts}_{\underline{\mathbf{M}}})_i = -sign(\prod_j \underline{\mathbf{M}}_j^{\mathbf{S}_{ji}} - \hat{K}_{eq}^i) \qquad \text{for } 1 \leq i \leq r. \tag{43}$$

Then the thermodynamic constraint $\mathbf{TC}_{\mathbf{M}}$ (resp., $\underline{\mathbf{TC}}_{\underline{\mathbf{M}}}$) can be rewritten as:

$$\mathbf{TC}_{\mathbf{M}}(\mathbf{v}) \triangleq sign(\mathbf{v}) \leq \mathbf{ts}_{\mathbf{M}} \qquad \underline{\mathbf{TC}}_{\underline{\mathbf{M}}}(\mathbf{v}) \triangleq sign(\mathbf{v}) \leq \mathbf{ts}_{\underline{\mathbf{M}}}. \tag{44}$$

Thus the set $Sol_{\mathbf{TC}_{\mathbf{M}}}$ (resp., $Sol_{\underline{\mathbf{TC}}_{\underline{\mathbf{M}}}}$) of vectors $\mathbf{v}$ satisfying the constraint $\mathbf{TC}_{\mathbf{M}}(\mathbf{v})$ (resp., $\underline{\mathbf{TC}}_{\underline{\mathbf{M}}}(\mathbf{v})$), given by $\{\mathbf{v} \in \mathbb{R}^r \mid sign(\mathbf{v}) \leq \mathbf{ts}_{\mathbf{M}}\}$ (resp., $\{\mathbf{v} \in \mathbb{R}^r \mid sign(\mathbf{v}) \leq \mathbf{ts}_{\underline{\mathbf{M}}}\}$), is the closed orthant $O_{\mathbf{ts}_{\mathbf{M}}}$ (resp., $O_{\mathbf{ts}_{\underline{\mathbf{M}}}}$) defined by $\mathbf{ts}_{\mathbf{M}}$ (resp., $\mathbf{ts}_{\underline{\mathbf{M}}}$), of dimension r if $\mathbf{ts}_{\mathbf{M}}$ (resp., $\mathbf{ts}_{\underline{\mathbf{M}}}$) has full support, i.e., $(\mathbf{ts}_{\mathbf{M}})_i \neq 0$ (resp., $(\mathbf{ts}_{\underline{\mathbf{M}}})_i \neq 0$) for all i, of lesser dimension otherwise.

Lemma 2.1. *Given metabolites concentrations $\boldsymbol{M}$ (resp., internal metabolites concentrations $\underline{\boldsymbol{M}}$), the set $Sol_{\boldsymbol{TC_M}}$ (resp., $Sol_{\underline{\boldsymbol{TC}}_{\underline{\boldsymbol{M}}}}$) of vectors in $\mathbb{R}^r$ satisfying the thermodynamic constraint $\boldsymbol{TC_M}$ (resp., $\underline{\boldsymbol{TC}}_{\underline{\boldsymbol{M}}}$) is the set of vectors that conform to the thermodynamic sign vector $\boldsymbol{ts_M}$ (resp., $\boldsymbol{ts}_{\underline{\boldsymbol{M}}}$) (43), i.e., the closed orthant $O_{\boldsymbol{ts_M}}$ (resp., $O_{\boldsymbol{ts}_{\underline{\boldsymbol{M}}}}$) defined by $\boldsymbol{ts_M}$ (resp., $\boldsymbol{ts}_{\underline{\boldsymbol{M}}}$).*

2.1.2 Kinetic constraints

Metabolic reactions are catalyzed by enzymes. The catalytic mechanisms of key enzymes have been investigated in great detail and described by mathematical formulas. But many kinetic equations are still unknown and have to be substituted by standard rate laws such as mass-action kinetics, power laws, reversible Hill kinetics, lin-log kinetics, convenience kinetics, generic rate equations or TKM rate laws [24]. What is important for our study is that those modular rate laws share the general form:

$$\mathbf{v}_i = \mathbf{E}_i \kappa_i(\mathbf{M}) \quad \text{with} \quad \kappa_i = f_i^{reg} \frac{T_i}{D_i + D_i^{reg}} \tag{45}$$

where $\mathbf{E}_i$ is the (nonnegative) level of the enzyme catalyzing the reaction i (given either as an amount or as a concentration, in what case the rate law is pre-multiplied by the compartment volume) and κ_i depends on the concentrations of the metabolites occurring in i (reactants of i) and on reaction i stoichiometry, rate law considered, allosteric regulation and parameters. In the general form of κ_i, T_i is the thermodynamic numerator (which can be written in the compact form $k_i^+\theta_i^+ - k_i^-\theta_i^-$ with turnover rates parameters $k_i^\pm$) that gives its sign to κ_i (and thus to $\mathbf{v}_i$) and reflects the relation between chemical potentials and reaction directions and ensures that the rate vanishes at chemical equilibrium, f_i^{reg} and D_i^{reg}, both positive, implement enzyme regulation (partial or complete for the first one, specific for the second one) and D_i is the (positive) kinetic denominator, a polynomial of scaled reactants concentrations whose terms correspond to different binding states of the enzyme (reducing the enzyme amount available for catalysis), which depends on the rate law considered.

The kinetic constraint for $\mathbf{v}$ depends both on the vector $\mathbf{E}$ of enzymes concentrations and on the vector $\mathbf{M}$ of metabolites concentrations and can thus be defined as:

$$\mathbf{KC}_{\mathbf{E},\mathbf{M}}(\mathbf{v}) \triangleq \mathbf{v} = \mathbf{E} \circ \boldsymbol{\kappa}(\mathbf{M}) \tag{46}$$

where $\circ$ is the component-wise vectors product: $(\mathbf{E} \circ \boldsymbol{\kappa})_i = \mathbf{E}_i \kappa_i$. This means that the flux vector is a component-wise linear function of the enzymes concentrations vector (and nonlinear function of the metabolites concentrations vector). For nonzero $\mathbf{E}_i$, the sign of T_i gives the direction in which reaction i proceeds, so in this sense the kinetic constraint includes the thermodynamic constraint.

This can be highlighted on a widely used rate law, the reversible Michaelis-Menten kinetics [31]. In the simple case of a reaction i where the enzyme can only exist in one of three distinct states: free, all substrates bound, or all products bound, it can be written as:

$$\kappa_i = \frac{k_i^+ \prod_{j|\overline{\mathbf{S}}_{ji}<0} (\mathbf{M}_j / K_{ij}^M)^{-\overline{\mathbf{S}}_{ji}} - k_i^- \prod_{j|\overline{\mathbf{S}}_{ji}>0} (\mathbf{M}_j / K_{ij}^M)^{\overline{\mathbf{S}}_{ji}}}{1 + \prod_{j|\overline{\mathbf{S}}_{ji}<0} (\mathbf{M}_j / K_{ij}^M)^{-\overline{\mathbf{S}}_{ji}} + \prod_{j|\overline{\mathbf{S}}_{ji}>0} (\mathbf{M}_j / K_{ij}^M)^{\overline{\mathbf{S}}_{ji}}} \tag{47}$$

where k_i^+ and k_i^- are the maximal forward and backward rates in reaction i per unit of enzyme and the K_{ij}^M's are the Michaelis constants. Equating the numerator to zero at equilibrium, one obtains: $\frac{k_i^+}{k_i^-} \prod_j (K_{ij}^M)^{\overline{\mathbf{S}}_{ji}} = K_{eq}^i$. This gives:

$$\kappa_i = k_i^+ \left(\frac{\prod_{j|\overline{\mathbf{S}}_{ji}<0} (\mathbf{M}_j / K_{ij}^M)^{-\overline{\mathbf{S}}_{ji}}}{1 + \prod_{j|\overline{\mathbf{S}}_{ji}<0} (\mathbf{M}_j / K_{ij}^M)^{-\overline{\mathbf{S}}_{ji}} + \prod_{j|\overline{\mathbf{S}}_{ji}>0} (\mathbf{M}_j / K_{ij}^M)^{\overline{\mathbf{S}}_{ji}}} \right) \left(1 - \frac{\prod_j \mathbf{M}_j^{\overline{\mathbf{S}}_{ji}}}{K_{eq}^i} \right) \tag{48}$$

that is, the product of three terms: the positive capacity term per unit of enzyme, the positive (smaller than one) fractional saturation term depending on $\mathbf{M}$ and the thermodynamic term, which can be rewritten as $1 - e^{\Delta_r G_i / RT}$ and gives the sign of κ_i (and thus the sign of $\mathbf{v}_i$): $\kappa_i > 0 \Leftrightarrow \Delta_r G_i < 0$, i.e., the thermodynamic constraint. Thus $sign(\boldsymbol{\kappa}(\mathbf{M})) = \mathbf{ts}_{\mathbf{M}}$ and we will assume that this equality holds for all kinetic laws we consider.

Note that, for given metabolites and enzymes concentrations $\mathbf{M}$ and $\mathbf{E}$, the kinetic constraint $\mathbf{KC}_{\mathbf{E},\mathbf{M}}$ defines completely and uniquely the only vector that satisfies it: $Sol_{\mathbf{KC}_{\mathbf{E},\mathbf{M}}} = \{\mathbf{E} \circ \boldsymbol{\kappa}(\mathbf{M})\}$.

2.1.3 Regulatory constraints

Coupling metabolic networks with Boolean transcriptional regulatory networks allows one to express the additional constraints imposed by gene regulatory information on a metabolic network and to take them into account when computing `EFMs` [20, 21, 19]. In whole generality, such a constraint may be given by an arbitrary Boolean formula in terms of the reactions i, viewed as propositional symbols, i.e., the positive literal i meaning that reaction i is active (nonzero flux) and the negative literal $\neg i$ meaning it is inactive (zero flux). Thus, when applying this Boolean constraint, noted Bc, to a flux vector $\mathbf{v}$, the positive literal i is interpreted as $\mathbf{v}_i \neq 0$, i.e., $i \in supp(\mathbf{v})$ (4), and the negative literal $\neg i$ as $\mathbf{v}_i = 0$, i.e., $i \notin supp(\mathbf{v})$.

The regulatory constraint $\mathbf{RC}_{Bc}$ for $\mathbf{v}$ induced by Bc can thus be defined as:

$$\mathbf{RC}_{Bc}(\mathbf{v}) \triangleq Bc(\mathbf{v}_i). \tag{49}$$

Note that coupled reactions as used by Flux Coupling Analysis (FCA) [5, 23, 35] can be easily represented by such constraints. For example, i directionally coupled to j, meaning that zero flux through i implies zero flux through j, is expressed by $Bc = i \vee \neg j$, and i partially coupled to j, meaning that zero flux through i is equivalent to zero flux through j, is expressed by $Bc = (i \wedge j) \vee (\neg i \wedge \neg j)$.

By rewriting the Boolean constraint Bc in DNF (Disjunctive Normal Form), $Bc = \bigvee_k D_k$, the set $Sol_{\mathbf{RC}_{Bc}} = \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{RC}_{Bc}(\mathbf{v})\}$ of vectors $\mathbf{v}$ satisfying the constraint $\mathbf{RC}_{Bc}$ is a union of the solutions spaces for each disjunct D_k (and this union can be assumed to be disjoint by taking the disjuncts D_k two by two inconsistent). Now a disjunct is a conjunction of literals, where a negative literal $\neg i$ corresponds to the constraint $\mathbf{v}_i = 0$ and a positive literal i to the constraint $\mathbf{v}_i \neq 0$, which can be rewritten as the disjunctive constraint $(\mathbf{v}_i < 0) \vee (\mathbf{v}_i > 0)$. Finally, a propositional symbol i that does not appear in the disjunct corresponds to an absence of constraint on $\mathbf{v}_i$, which can be rewritten as the disjunction $(\mathbf{v}_i < 0) \vee (\mathbf{v}_i = 0) \vee (\mathbf{v}_i > 0)$. Thus, the solutions space for a disjunct is itself the disjoint union of subspaces defined each one by constraints of the type $\mathbf{v}_i \; op_i \; 0$ for all i, with $op_i \in \{<, =, >\}$, that is, defined by $\{\mathbf{v} \in \mathbb{R}^r \mid sign(\mathbf{v}) = \mathbf{rs}\}$ for a given sign vector $\mathbf{rs} \in \{-, 0, +\}^r$, i.e., an open orthant $\mathring{O}_{\mathbf{rs}}$ (which, for $\mathbf{rs} \neq \mathbf{0}$, is topologically open in the vector subspace it spans and is the interior in this

subspace of the closed orthant $O_{\mathbf{rs}} = \{\mathbf{v} \in \mathbb{R}^r \mid sign(\mathbf{v}) \leq \mathbf{rs}\}$, which is the closure of $\mathring{O}_{\mathbf{rs}}$ in $\mathbb{R}^r$). In total, $Sol_{\mathbf{RC}_{Bc}}$ is thus the disjoint union of such open orthants. Now, instead of keeping this partition of $Sol_{\mathbf{RC}_{Bc}}$ into open orthants, it can be more practical to generalize this concept and deal with what we will call semi-open orthants O°, i.e., orthants O without some of their faces of lesser dimension (open orthant is thus a particular case of semi-open orthant, without any facet thus without any face of lesser dimension). To do this, we can group together with any given open orthant $\mathring{O}_{\mathbf{rs}_j}$ in $Sol_{\mathbf{RC}_{Bc}}$, in a same cluster $\mathcal{C}$, all other open orthants $\mathring{O}_{\mathbf{rs}_k}$ in $Sol_{\mathbf{RC}_{Bc}}$ such that $O_{\mathbf{rs}_k} \subset O_{\mathbf{rs}_j}$, i.e. $O_{\mathbf{rs}_k}$ is a face of $O_{\mathbf{rs}_j}$, and, for any arbitrary orthant $O_{\mathbf{rs}_l}$ with $O_{\mathbf{rs}_k} \subset O_{\mathbf{rs}_l} \subset O_{\mathbf{rs}_j}$ (which is equivalent to $\mathbf{rs}_k < \mathbf{rs}_l < \mathbf{rs}_j$), then $\mathring{O}_{\mathbf{rs}_l} \in \mathcal{C}$. In this case $\bigcup_{\mathring{O} \in \mathcal{C}} \mathring{O}$ is a semi-open orthant O°. We can iteratively process like this by considering each time the open orthants in $Sol_{\mathbf{RC}_{Bc}}$ that have not been selected yet in any cluster, which guarantees that the semi-open orthants built in this way are disjoint. In addition, by choosing as open orthant $\mathring{O}_{\mathbf{rs}_j}$ to start with at each iteration a maximal one among those who remain, we are sure that no two of the semi-open orthants thus built can be grouped together to constitute a bigger semi-open orthant, i.e., the collection obtained is minimal in this sense. We obtain finally that $Sol_{\mathbf{RC}_{Bc}}$ can be written as a disjoint union of semi-open orthants, with no merging possible between two of them. But notice that such a decomposition is not unique and that it can still exist an inclusion between the closures of two such semi-open orthants. If we consider the particular case of a Boolean constraint which is an arbitrary disjunct D, then for any closed r-orthant $O_{\boldsymbol{\eta}}$, i.e., for any full support sign vector $\boldsymbol{\eta}$, $Sol_{\mathbf{RC}_D \leq \boldsymbol{\eta}}$ is a semi-open orthant, which is the face of $O_{\boldsymbol{\eta}}$ defined by $\mathbf{v}_i = 0$ for all i such that $\neg i$ is a literal of D, without its facets of equation $\mathbf{v}_i = 0$ for all i such that i is a literal of D.

Lemma 2.2. *The set $Sol_{\boldsymbol{RC}_{Bc}}$ of vectors in $\mathbb{R}^r$ satisfying the regulatory constraint $\boldsymbol{RC}_{Bc}$ is a disjoint union of open orthants $\mathring{O}$. They can be grouped together such that $Sol_{\boldsymbol{RC}_{Bc}}$ is a disjoint union of semi-open orthants O°, i.e., orthants O without some of their faces, such that no two of them can be grouped together to constitute a bigger semi-open orthant. If Bc is a conjunction of literals and $\boldsymbol{\eta}$ an arbitrary full support sign vector, then $Sol_{\boldsymbol{RC}_{Bc} \leq \boldsymbol{\eta}}$ is itself a semi-open orthant.*

2.2 Characterizing the solutions space

One starts from a flux cone (6) (or more generally a flux polyhedron (24)) representing the flux vectors of a metabolic network at steady state, satisfying the stoichiometric equations, some reactions irreversibility inequalities (and possibly some inhomogeneous linear constraints). And one considers additional biological constraints, as those described in section (2.1). In whole generality, these constraints will be noted $\mathbf{C}_{\mathbf{x}}(\mathbf{v})$, where $\mathbf{v}$ represents a flux vector in $\mathbb{R}^r$ and $\mathbf{x}$ a vector of biochemical quantities involved in the constraint (typically, metabolites concentrations and enzymes concentrations). Some parameters (such as stoichiometric coefficients) are also present that are not made explicit in the notation for sake of simplicity. In the following, the solutions space in $\mathbb{R}^r$ of an arbitrary constraint $\mathbf{C}$ will be noted $Sol_{\mathbf{C}}$, defined as: $Sol_{\mathbf{C}} = \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{C}(\mathbf{v})\}$. For given values of quantities $\mathbf{x}$, the solutions space is thus the constrained flux cone subset (not necessarily a cone, depending on $\mathbf{C}_{\mathbf{x}}$):

$$\begin{aligned} Sol_{\mathbf{S},\mathbf{Irr},\mathbf{C}}(\mathbf{x}) &\triangleq CFC_{\mathbf{C}}(\mathbf{x}) \triangleq \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{Sv} = \mathbf{0}, \mathbf{v}_i \geq 0 \text{ for } i \in \mathbf{Irr}, \mathbf{C}_{\mathbf{x}}(\mathbf{v})\} \\ &= Sol_{\mathbf{S},\mathbf{Irr}} \cap Sol_{\mathbf{C}_{\mathbf{x}}} = FC \cap Sol_{\mathbf{C}_{\mathbf{x}}} \end{aligned} \tag{50}$$

or, more generally, the constrained flux polyhedron subset (not necessarily a polyhedron, depending on $\mathbf{C}_{\mathbf{x}}$):

$$\begin{aligned} Sol_{\mathbf{S},\mathbf{Irr},\mathbf{ILC},\mathbf{C}}(\mathbf{x}) &\triangleq CFP_{\mathbf{C}}(\mathbf{x}) \triangleq \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{Sv} = \mathbf{0}, \mathbf{v}_i \geq 0 \text{ for } i \in \mathbf{Irr}, \mathbf{Gv} \geq \mathbf{h}, \mathbf{C}_{\mathbf{x}}(\mathbf{v})\} \\ &= Sol_{\mathbf{S},\mathbf{Irr},\mathbf{ILC}} \cap Sol_{\mathbf{C}_{\mathbf{x}}} = FP \cap Sol_{\mathbf{C}_{\mathbf{x}}} = CFC_{\mathbf{C}}(\mathbf{x}) \cap P_{\mathbf{ILC}}. \end{aligned} \tag{51}$$

Now, most of the time, the values of the quantities $\mathbf{x}$, if not all at least most of them, are not known. Then we require only consistency of the set of constraints $\mathbf{C}_{\mathbf{x}}$, i.e., the existence of values for variables $\mathbf{x}$ such that the constraints are satisfied. This means that we replace the constraint $\mathbf{C}_{\mathbf{x}}$ by the constraint $\exists \mathbf{x} \mathbf{C}_{\mathbf{x}}$ whose solutions space is $Sol_{\exists \mathbf{x} \mathbf{C}_{\mathbf{x}}} = \{\mathbf{v} \in \mathbb{R}^r \mid \{\mathbf{x} \mid \mathbf{C}_{\mathbf{x}}(\mathbf{v})\} \neq \emptyset\} = \bigcup_{\mathbf{x}} Sol_{\mathbf{C}_{\mathbf{x}}}$. The solutions space becomes:

$$Sol_{\mathbf{S},\mathbf{Irr},\mathbf{C}} \triangleq CFC_{\mathbf{C}} \triangleq \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{Sv} = \mathbf{0}, \mathbf{v}_i \geq 0 \text{ for } i \in \mathbf{Irr}, \exists \mathbf{x} \mathbf{C}_{\mathbf{x}}(\mathbf{v})\}$$

$$= Sol_{\mathbf{S},\mathbf{Irr}} \cap Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}} = \bigcup_\mathbf{x} Sol_{\mathbf{S},\mathbf{Irr},\mathbf{C}}(\mathbf{x}) = FC \cap \bigcup_\mathbf{x} Sol_{\mathbf{C}_\mathbf{x}} \tag{52}$$

or:

$$Sol_{\mathbf{S},\mathbf{Irr},\mathbf{ILC},\mathbf{C}} \triangleq CFP_\mathbf{C} \triangleq \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{S}\mathbf{v} = \mathbf{0}, \mathbf{v}_i \geq 0 \text{ for } i \in \mathbf{Irr}, \mathbf{G}\mathbf{v} \geq \mathbf{h}, \exists \mathbf{x}\mathbf{C}_\mathbf{x}(\mathbf{v})\}$$

$$= Sol_{\mathbf{S},\mathbf{Irr},\mathbf{ILC}} \cap Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}} = \bigcup_\mathbf{x} Sol_{\mathbf{S},\mathbf{Irr},\mathbf{ILC},\mathbf{C}}(\mathbf{x}) = FP \cap \bigcup_\mathbf{x} Sol_{\mathbf{C}_\mathbf{x}} = CFC_\mathbf{C} \cap P_{\mathbf{ILC}}. \tag{53}$$

If some, but not all, of the values of quantities $\mathbf{x}$ are known, $\exists \mathbf{x}$ concerns only those $\mathbf{x}$ whose value is unknown, the known values being integrated in $\mathbf{C}$ as parameters to simplify the notations.
We will have examples of such constraints $\exists \mathbf{x}\mathbf{C}_\mathbf{x}(\mathbf{v})$ by considering the cases of thermodynamic constraint **TC** and of kinetic constraint **KC**.

Depending on the constraint considered, the solutions space is in general no longer a cone or polyhedron and no longer convex. Nevertheless, the definitions of extreme vectors and extreme points, of elementary flux vectors and elementary flux points, of elementary flux modes are still valid, as, respectively, non-decomposable and convex non-decomposable vectors, conformally non-decomposable and convex-conformally non-decomposable vectors, support-minimal vectors, where decomposition and support minimality has to be understood w.r.t. vectors of the solutions space. It is then clear that if a vector of FC (resp., FP) satisfies one of those properties in FC (resp., FP) and satisfies the given constraint, then it satisfies the same property in the solutions space, i.e.:

$$\begin{aligned}
\texttt{ExVs}(FC) \cap Sol_{\mathbf{C}_\mathbf{x}} &\subseteq \texttt{ExVs}(CFC_\mathbf{C}(\mathbf{x})) & \texttt{ExVs}(FC) \cap Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}} &\subseteq \texttt{ExVs}(CFC_\mathbf{C}) \\
\texttt{ExVs}(C_{FP}) \cap Sol_{\mathbf{C}_\mathbf{x}} &\subseteq \texttt{ExVs}(CFP_\mathbf{C}(\mathbf{x})) & \texttt{ExVs}(C_{FP}) \cap Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}} &\subseteq \texttt{ExVs}(CFP_\mathbf{C}) \\
\texttt{ExPs}(FP) \cap Sol_{\mathbf{C}_\mathbf{x}} &\subseteq \texttt{ExPs}(CFP_\mathbf{C}(\mathbf{x})) & \texttt{ExPs}(FP) \cap Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}} &\subseteq \texttt{ExPs}(CFP_\mathbf{C}) \\
\texttt{EFVs}(FC) \cap Sol_{\mathbf{C}_\mathbf{x}} &\subseteq \texttt{EFVs}(CFC_\mathbf{C}(\mathbf{x})) & \texttt{EFVs}(FC) \cap Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}} &\subseteq \texttt{EFVs}(CFC_\mathbf{C}) \\
\texttt{EFVs}(C_{FP}) \cap Sol_{\mathbf{C}_\mathbf{x}} &\subseteq \texttt{EFVs}(CFP_\mathbf{C}(\mathbf{x})) & \texttt{EFVs}(C_{FP}) \cap Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}} &\subseteq \texttt{EFVs}(CFP_\mathbf{C}) \\
\texttt{EFPs}(FP) \cap Sol_{\mathbf{C}_\mathbf{x}} &\subseteq \texttt{EFPs}(CFP_\mathbf{C}(\mathbf{x})) & \texttt{EFPs}(FP) \cap Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}} &\subseteq \texttt{EFPs}(CFP_\mathbf{C}) \\
\texttt{EFMs}(FC) \cap Sol_{\mathbf{C}_\mathbf{x}} &\subseteq \texttt{EFMs}(CFC_\mathbf{C}(\mathbf{x})) & \texttt{EFMs}(FC) \cap Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}} &\subseteq \texttt{EFMs}(CFC_\mathbf{C}) \\
\texttt{EFMs}(FP) \cap Sol_{\mathbf{C}_\mathbf{x}} &\subseteq \texttt{EFMs}(CFP_\mathbf{C}(\mathbf{x})) & \texttt{EFMs}(FP) \cap Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}} &\subseteq \texttt{EFMs}(CFP_\mathbf{C})
\end{aligned} \tag{54}$$

For `EFMs`, those formulas are valid whatever the structure of $Sol_\mathbf{C}$ is. It is also the case for `ExPs` and `EFPs` but in practice really meaningful only if $CFP_\mathbf{C}(\mathbf{x})$ (resp., $CFP_\mathbf{C}$) is a convex polyhedron. Finally, for `ExVs` and `EFVs`, this is meaningful only if $CFC_\mathbf{C}(\mathbf{x})$ (resp., $CFC_\mathbf{C}$) is a convex polyhedral cone and $CFP_\mathbf{C}(\mathbf{x})$ (resp., $CFP_\mathbf{C}$) is a convex polyhedron. This is ensured when $Sol_{\mathbf{C}_\mathbf{x}}$ (resp., $Sol_{\exists \mathbf{x}\mathbf{C}_\mathbf{x}}$) is itself a convex polyhedral cone and we will see that, for almost all common biological constraints, this solutions space is actually a union of such cones or of semi-open cones and thus the formulas will apply for each conical component. We have in this case $C_{CFP_\mathbf{C}} = CFC_\mathbf{C} \cap C_{P_{\mathbf{ILC}}} = C_{FP} \cap Sol_\mathbf{C}$. This can be generalized when $Sol_\mathbf{C}$ is a convex polyhedron, and thus $CFC_\mathbf{C}$ and $CFP_\mathbf{C}$ too, with $C_{CFC_\mathbf{C}} = FC \cap C_{Sol_\mathbf{C}}$ and $C_{CFP_\mathbf{C}} = C_{CFC_\mathbf{C}} \cap C_{P_{\mathbf{ILC}}} = C_{FP} \cap C_{Sol_\mathbf{C}}$, by replacing $Sol_\mathbf{C}$ by $C_{Sol_\mathbf{C}}$ in the formulas above about `ExVs` and `EFVs`.

But in general, in all cases, we have not the reciprocal subset inclusion. It is what we will study for particular constraints.

2.2.1 Application to thermodynamics

Assume first that the concentrations of the metabolites (resp., of the internal metabolites) are known and given. From (50), (51) and Lemma 2.1, we obtain:

$$CFC_{\mathbf{TC}}(\mathbf{M}) = FC_{\leq \mathbf{ts}_\mathbf{M}} \qquad CFC_{\underline{\mathbf{TC}}}(\underline{\mathbf{M}}) = FC_{\leq \mathbf{ts}_{\underline{\mathbf{M}}}} \tag{55}$$

$$CFP_{\mathbf{TC}}(\mathbf{M}) = FP_{\leq \mathbf{ts}_\mathbf{M}} \qquad CFP_{\underline{\mathbf{TC}}}(\underline{\mathbf{M}}) = FP_{\leq \mathbf{ts}_{\underline{\mathbf{M}}}}. \tag{56}$$

$CFC_{\mathbf{TC}}(\mathbf{M})$, $CFC_{\underline{\mathbf{TC}}}(\underline{\mathbf{M}})$ are thus flux cones and $CFP_{\mathbf{TC}}(\mathbf{M})$, $CFP_{\underline{\mathbf{TC}}}(\underline{\mathbf{M}})$ flux polyhedra (possibly equal to $\{\mathbf{0}\}$ or empty (for polyhedra) if the fluxes directions imposed by the concentrations of the

metabolites and the second law of thermodynamics are incompatible with the steady-state assumption). When nonempty, they consist of a single flux tope. From (18), (35) and (38), it results that:

$$\texttt{EFVs}(CFC_{\mathbf{TC}}(\mathbf{M})) = \texttt{EFVs}(FC)_{\leq \mathbf{ts_M}} = \texttt{EFMs}(CFC_{\mathbf{TC}}(\mathbf{M})) = \texttt{EFMs}(FC)_{\leq \mathbf{ts_M}} = \texttt{ExVs}(FC_{\leq \mathbf{ts_M}}) \quad (57)$$

$$\texttt{EFPs}(CFP_{\mathbf{TC}}(\mathbf{M})) = \texttt{EFPs}(FP)_{\leq \mathbf{ts_M}} = \texttt{ExPs}(FP_{\leq \mathbf{ts_M}})$$

$$\texttt{EFVs}(CFP_{\mathbf{TC}}(\mathbf{M})) = \texttt{EFVs}(C_{FP})_{\leq \mathbf{ts_M}} = \texttt{ExVs}(C_{FP_{\leq \mathbf{ts_M}}})$$

$$\texttt{EFMs}(CFP_{\mathbf{TC}}(\mathbf{M})) = \texttt{EFMs}(FP)_{\leq \mathbf{ts_M}} \quad (58)$$

and the same for $CFC_{\underline{\mathbf{TC}}}(\underline{\mathbf{M}})$ and $CFP_{\underline{\mathbf{TC}}}(\underline{\mathbf{M}})$. Now, considering the decomposition of FC (resp., FP) into flux topes, we can proceed flux tope by flux tope for the computation of the sets above. Starting from a FT $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$), we have $(FC_{\leq \boldsymbol{\eta}})_{\leq \mathbf{ts_M}} = FC_{\leq \boldsymbol{\eta}'}$ (resp., $(FP_{\leq \boldsymbol{\eta}})_{\leq \mathbf{ts_M}} = FP_{\leq \boldsymbol{\eta}'}$) with $\boldsymbol{\eta}' = inf(\boldsymbol{\eta}, \mathbf{ts_M})$ (where inf is defined component-wise with $inf(-,+) = 0$) and the four sets above are equal to $\texttt{ExVs}(FC_{\leq \boldsymbol{\eta}'})$, $\texttt{ExPs}(FP_{\leq \boldsymbol{\eta}'})$, $\texttt{ExVs}(C_{FP_{\leq \boldsymbol{\eta}'}})$ and $\texttt{EFMs}(FP_{\leq \boldsymbol{\eta}'})$, respectively. This is in particular the case if we split up each reversible reaction into two irreversible ones as, in higher dimension, $\widetilde{FC}$ (resp., $\widetilde{FP}$) is reduced to a single flux tope defined by $\boldsymbol{\eta} = +$, the all-positive sign vector, and thus $\boldsymbol{\eta}'$ is obtained from $\mathbf{ts_M}$ by changing each $-$ into 0. Note also that FC (resp., FP) consistent does not imply that $CFC_{\mathbf{TC}}(\mathbf{M})$ (resp., $CFP_{\mathbf{TC}}(\mathbf{M})$) is consistent ($\boldsymbol{\eta}$ being full support does not imply that $\boldsymbol{\eta}'$ is full support).

Proposition 2.3. *Given metabolites concentrations $\boldsymbol{M}$, the space of flux vectors in FC (resp., FP) satisfying the thermodynamic constraint $\boldsymbol{TC_M}$ is a flux cone (resp., a flux polyhedron), made up of vectors of FC (resp., FP) which conform to the thermodynamic sign vector $\boldsymbol{ts_M}$ given in (43). Its elementary flux vectors, identical to elementary flux modes, (resp., elementary flux points, elementary flux vectors and elementary flux modes) are exactly those of FC (resp., FP) that satisfy the constraint, i.e., that conform to $\boldsymbol{ts_M}$. The same result holds for internal metabolites concentrations $\underline{\boldsymbol{M}}$ and thermodynamic constraint $\underline{\boldsymbol{TC}}_{\underline{\boldsymbol{M}}}$ with $\boldsymbol{ts}_{\underline{\boldsymbol{M}}}$.*

Consider now the more usual case where the concentrations of the metabolites (at least those of internal metabolites) are unknown. From (44), we obtain one or the other form for the thermodynamic constraint (existentially quantified on metabolites concentrations):

$$\mathbf{TC}(\mathbf{v}) \triangleq \exists \mathbf{M}\ \mathbf{TC_M}(\mathbf{v}) \triangleq \exists \mathbf{M}\ sign(\mathbf{v}) \leq \mathbf{ts_M}$$

$$\underline{\mathbf{TC}}(\mathbf{v}) \triangleq \exists \underline{\mathbf{M}}\ \underline{\mathbf{TC}}_{\underline{\mathbf{M}}}(\mathbf{v}) \triangleq \exists \underline{\mathbf{M}}\ sign(\mathbf{v}) \leq \mathbf{ts}_{\underline{\mathbf{M}}}. \quad (59)$$

Though the metabolites concentrations $\mathbf{M}_j$ are unknown, often some lower bounds $\mathbf{M}_j^-$ and upper bounds $\mathbf{M}_j^+$ on those concentrations are known. They are thus added as additional constraints:

$$\mathbf{TC}^b(\mathbf{v}) \triangleq \exists \mathbf{M}\ (sign(\mathbf{v}) \leq \mathbf{ts_M} \wedge \mathbf{M}^- \leq \mathbf{M} \leq \mathbf{M}^+)$$

$$\underline{\mathbf{TC}}^b(\mathbf{v}) \triangleq \exists \underline{\mathbf{M}}\ (sign(\mathbf{v}) \leq \mathbf{ts}_{\underline{\mathbf{M}}} \wedge \underline{\mathbf{M}}^- \leq \underline{\mathbf{M}} \leq \underline{\mathbf{M}}^+). \quad (60)$$

The solutions space in $\mathbb{R}^r$ of those constraints is thus $Sol_{\mathbf{TC}} = \bigcup_{\mathbf{M}} Sol_{\mathbf{TC_M}}$ (idem for $\underline{\mathbf{TC}}$) and $Sol_{\mathbf{TC}^b} = \bigcup_{\mathbf{M}|\mathbf{M}^- \leq \mathbf{M} \leq \mathbf{M}^+} Sol_{\mathbf{TC_M}}$ (idem for $\underline{\mathbf{TC}}^b$). From the result of lemma 2.1, it thus follows: $Sol_{\mathbf{TC}} = \bigcup_{\mathbf{M}} O_{\mathbf{ts_M}}$ and $Sol_{\mathbf{TC}^b} = \bigcup_{\mathbf{M}|\mathbf{M}^- \leq \mathbf{M} \leq \mathbf{M}^+} O_{\mathbf{ts_M}}$ (idem for $\underline{\mathbf{TC}}$ and $\underline{\mathbf{TC}}^b$ with $O_{\mathbf{ts}_{\underline{\mathbf{M}}}}$). It is obviously enough to take the union on the maximal $\mathbf{ts_M}$'s (resp., $\mathbf{ts}_{\underline{\mathbf{M}}}$'s) when $\mathbf{M}$ varies. As these are at most 2^r, the union is finite.

Lemma 2.4. *The set $Sol_{\boldsymbol{TC}}$ (resp., $Sol_{\boldsymbol{TC}^b}$) of vectors in $\mathbb{R}^r$ satisfying the thermodynamic constraint $\boldsymbol{TC}$ (resp., $\boldsymbol{TC}^b$) is a union of closed orthants. More precisely, it is the (finite) union for all $\boldsymbol{M}$ (resp., all bounded $\boldsymbol{M}$) of the sets of vectors that conform to $\boldsymbol{ts_M}$, i.e., $O_{\boldsymbol{ts_M}}$'s. The same result holds for $\underline{\boldsymbol{TC}}$ and $\underline{\boldsymbol{TC}}^b$ with $\underline{\boldsymbol{M}}$ and $O_{\boldsymbol{ts}_{\underline{\boldsymbol{M}}}}$.*

It follows from (52), (53), (55), (56) that:

$$CFC_{\mathbf{TC}} = \bigcup_{\mathbf{M}} FC_{\leq \mathbf{ts_M}} \qquad CFC_{\mathbf{TC}^b} = \bigcup_{\mathbf{M}|\mathbf{M}^- \leq \mathbf{M} \leq \mathbf{M}^+} FC_{\leq \mathbf{ts_M}} \tag{61}$$

$$CFP_{\mathbf{TC}} = \bigcup_{\mathbf{M}} FP_{\leq \mathbf{ts_M}} \qquad CFP_{\mathbf{TC}^b} = \bigcup_{\mathbf{M}|\mathbf{M}^- \leq \mathbf{M} \leq \mathbf{M}^+} FP_{\leq \mathbf{ts_M}} \tag{62}$$

and the same for $\underline{\mathbf{TC}}$ and $\underline{\mathbf{TC}}^b$ with $\underline{\mathbf{M}}$ and $\mathbf{ts}_{\underline{\mathbf{M}}}$, where all unions are finite.
From this and (18), (35) and (38), it results that:

$$\begin{aligned}
&\mathtt{EFVs}(CFC_{\mathbf{TC}}) = \bigcup_{\mathbf{M}} \mathtt{EFVs}(FC_{\leq \mathbf{ts_M}}) = \bigcup_{\mathbf{M}} \mathtt{EFVs}(FC)_{\leq \mathbf{ts_M}} = \mathtt{EFMs}(CFC_{\mathbf{TC}}) = \\
&\bigcup_{\mathbf{M}} \mathtt{EFMs}(FC_{\leq \mathbf{ts_M}}) = \bigcup_{\mathbf{M}} \mathtt{EFMs}(FC)_{\leq \mathbf{ts_M}} = \bigcup_{\mathbf{M}} \mathtt{ExVs}(FC_{\leq \mathbf{ts_M}})
\end{aligned} \tag{63}$$

$$\begin{aligned}
&\mathtt{EFPs}(CFP_{\mathbf{TC}}) = \bigcup_{\mathbf{M}} \mathtt{EFPs}(FP_{\leq \mathbf{ts_M}}) = \bigcup_{\mathbf{M}} \mathtt{EFPs}(FP)_{\leq \mathbf{ts_M}} = \bigcup_{\mathbf{M}} \mathtt{ExPs}(FP_{\leq \mathbf{ts_M}}) \\
&\mathtt{EFVs}(CFP_{\mathbf{TC}}) = \bigcup_{\mathbf{M}} \mathtt{EFVs}(C_{FP_{\leq \mathbf{ts_M}}}) = \bigcup_{\mathbf{M}} \mathtt{EFVs}(C_{FP})_{\leq \mathbf{ts_M}} = \bigcup_{\mathbf{M}} \mathtt{ExVs}(C_{FP_{\leq \mathbf{ts_M}}}) \\
&\mathtt{EFMs}(CFP_{\mathbf{TC}}) = \bigcup_{\mathbf{M}} \mathtt{EFMs}(FP_{\leq \mathbf{ts_M}}) = \bigcup_{\mathbf{M}} \mathtt{EFMs}(FP)_{\leq \mathbf{ts_M}}
\end{aligned} \tag{64}$$

and the analog for $\mathbf{TC}^b$, $\underline{\mathbf{TC}}$ and $\underline{\mathbf{TC}}^b$. Now, considering the decomposition of FC (resp., FP) into flux topes, we can proceed flux tope by flux tope for the computation of the sets above. Starting from a FT $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$), we have $\bigcup_{\mathbf{M}}(FC_{\leq \boldsymbol{\eta}})_{\leq \mathbf{ts_M}} = \bigcup_i FC_{\leq \boldsymbol{\eta}^i}$ (resp., $\bigcup_{\mathbf{M}}(FP_{\leq \boldsymbol{\eta}})_{\leq \mathbf{ts_M}} = \bigcup_i FP_{\leq \boldsymbol{\eta}^i}$) where $\{\boldsymbol{\eta}^i\}$ are the maximal sign vectors in $\{inf(\boldsymbol{\eta}, \mathbf{ts_M}) \mid \mathbf{M} \in \mathbb{R}_+^{*(m+\overline{m})}\}$ and the four sets above are equal to $\bigcup_i \mathtt{ExVs}(FC_{\leq \boldsymbol{\eta}^i})$, $\bigcup_i \mathtt{ExPs}(FP_{\leq \boldsymbol{\eta}^i})$, $\bigcup_i \mathtt{ExVs}(C_{FP_{\leq \boldsymbol{\eta}^i}})$ and $\bigcup_i \mathtt{EFMs}(FP_{\leq \boldsymbol{\eta}^i})$, respectively. This is in particular the case if we split up each reversible reaction into two irreversible ones as, in higher dimension, $\widetilde{FC}$ (resp., $\widetilde{FP}$) is reduced to a single flux tope defined by $\boldsymbol{\eta} = +$. The analog holds for $\mathbf{TC}^b$, $\underline{\mathbf{TC}}$ and $\underline{\mathbf{TC}}^b$.

Proposition 2.5. *The space of flux vectors in FC (resp., FP) satisfying the thermodynamic constraint $\boldsymbol{TC}$, or $\boldsymbol{TC}^b$, is a finite union of flux cones (resp., flux polyhedra), obtained as those vectors of FC (resp., FP) which conform to some $\boldsymbol{ts_M}$, for all $\boldsymbol{M}$, or bounded $\boldsymbol{M}$. It is thus no longer convex but made up of particular faces $FC_{\leq \boldsymbol{\eta}^i}$ (resp., $FP_{\leq \boldsymbol{\eta}^i}$) of each flux tope $FC_{\leq \boldsymbol{\eta}}$ for FC (resp., $FP_{\leq \boldsymbol{\eta}}$ for FP), with $\boldsymbol{\eta}^i \leq \boldsymbol{\eta}$. Its elementary flux vectors, identical to elementary flux modes, (resp., elementary flux points, elementary flux vectors and elementary flux modes) are exactly those of FC (resp., FP) that satisfy the constraint, i.e., that conform to some $\boldsymbol{ts_M}$, and coincide thus with the extreme vectors (resp., the extreme points, extreme vectors and elementary modes) of the $FC_{\leq \boldsymbol{\eta}^i}$'s (resp., $FP_{\leq \boldsymbol{\eta}^i}$'s). The same result holds for $\underline{\boldsymbol{TC}}$, or $\underline{\boldsymbol{TC}}^b$, with $\underline{\boldsymbol{M}}$ and $\boldsymbol{ts}_{\underline{\boldsymbol{M}}}$.*

We will now refine this result in order to characterize the faces involved. Consider the case of a flux cone where the concentrations of external metabolites are given and assume first there are no bounds on the concentrations of the internal metabolites [32]. As $CFC_{\underline{\mathbf{TC}}} = FC \cap \bigcup_{\underline{\mathbf{M}}} O_{\mathbf{ts}_{\underline{\mathbf{M}}}}$ and $CFP_{\underline{\mathbf{TC}}} = FP \cap \bigcup_{\underline{\mathbf{M}}} O_{\mathbf{ts}_{\underline{\mathbf{M}}}}$, let begin by studying $\bigcup_{\underline{\mathbf{M}}} O_{\mathbf{ts}_{\underline{\mathbf{M}}}}$. We have: $\mathbf{x} \in \bigcup_{\underline{\mathbf{M}}} O_{\mathbf{ts}_{\underline{\mathbf{M}}}} \Leftrightarrow \exists \underline{\mathbf{M}} \in \mathbb{R}_+^{*m}$ $(sign(\mathbf{x}) \leq \mathbf{ts}_{\underline{\mathbf{M}}}) \Leftrightarrow \exists \underline{\mathbf{M}} \in \mathbb{R}_+^{*m}$ $\forall i, 1 \leq i \leq r, (sign(\mathbf{x}_i) \leq -sign(\prod_j \underline{\mathbf{M}}_j^{\mathbf{S}_{ji}} - \hat{K}_{eq}^i))$ from (43). By applying the monotonic logarithm function and noting $\mathbf{L}\underline{\mathbf{M}} \in \mathbb{R}^m$ the vector whose components are given by $\mathbf{L}\underline{\mathbf{M}}_j = ln(\underline{\mathbf{M}}_j)$, one obtains: $\mathbf{x} \in \bigcup_{\underline{\mathbf{M}}} O_{\mathbf{ts}_{\underline{\mathbf{M}}}} \Leftrightarrow \exists \mathbf{L}\underline{\mathbf{M}} \in \mathbb{R}^m$ $\forall i, 1 \leq i \leq r, (sign(\mathbf{x}_i) \leq -sign(\sum_j \mathbf{S}_{ji} \mathbf{L}\underline{\mathbf{M}}_j - ln(\hat{K}_{eq}^i))) \Leftrightarrow \exists \mathbf{L}\underline{\mathbf{M}} \in \mathbb{R}^m$ $\forall i \in supp(\mathbf{x})$ $(sign(\mathbf{x}_i) \sum_j \mathbf{S}_{ji} \mathbf{L}\underline{\mathbf{M}}_j < sign(\mathbf{x}_i) ln(\hat{K}_{eq}^i))$. We will now make use of the Gale's theorem (or Kuhn-Fourier theorem), which is one of the forms of the Farkas duality lemma (for two vectors $\mathbf{x}$, $\mathbf{y}$, $\mathbf{x} < \mathbf{y}$ means $\mathbf{x}_i < \mathbf{y}_i$ for all i):

Gale's theorem. *For any $\boldsymbol{A} \in \mathbb{R}^{p \times q}$ and $\boldsymbol{b} \in \mathbb{R}^p$, exactly one of the following statements holds:*
(a) there exists $\boldsymbol{y} \in \mathbb{R}^q$ such that $\boldsymbol{A}\boldsymbol{y} < \boldsymbol{b}$;
(b) there exists $\boldsymbol{z} \in \mathbb{R}^p \backslash \{\boldsymbol{0}\}$ such that $\boldsymbol{z} \geq \boldsymbol{0}$, $\boldsymbol{z}^T \boldsymbol{A} = \boldsymbol{0}$ and $\boldsymbol{z}^T \boldsymbol{b} \leq 0$.

Applying this theorem in the formula above to $\mathbf{A} \in \mathbb{R}^{supp(\mathbf{x}) \times m}$ (where we note $\mathbb{R}^{supp(\mathbf{x})} \triangleq \mathbb{R}^r \cap \bigcap_{i|\mathbf{x}_i=0} \{\mathbf{z}_i = 0\}$ the subspace of vectors having null component outside the support of $\mathbf{x}$), given by $\mathbf{A}_{ij} = sign(\mathbf{x}_i)\mathbf{S}_{ji}$, and $\mathbf{b} \in \mathbb{R}^{supp(\mathbf{x})}$, given by $\mathbf{b}_i = sign(\mathbf{x}_i) ln(\hat{K}_{eq}^i)$, provides: $\mathbf{x} \in \bigcup_{\underline{\mathbf{M}}} O_{\mathbf{ts}_{\underline{\mathbf{M}}}} \Leftrightarrow$

$\forall \mathbf{z} \in \mathbb{R}^{supp(\mathbf{x})}\backslash\{\mathbf{0}\}(\mathbf{z} \geq \mathbf{0}, \forall j, 1 \leq j \leq m, \sum_i \mathbf{S}_{ji} sign(\mathbf{x}_i)\mathbf{z}_i = 0 \Rightarrow \sum_i sign(\mathbf{x}_i)\mathbf{z}_i ln(\hat{K}^i_{eq}) > 0) \Leftrightarrow \forall \mathbf{z} \in \mathbb{R}^r\backslash\{\mathbf{0}\}(sign(\mathbf{z}) \leq sign(\mathbf{x}), \mathbf{Sz} = \mathbf{0} \Rightarrow \mathbf{z}^T \mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}} > 0)$ where $\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}} \in \mathbb{R}^r$ is defined by: $(\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}})_i = ln(\hat{K}^i_{eq})$.
We obtain thus: $CFC_{\underline{\mathbf{TC}}} = \{\mathbf{v} \in FC \mid \forall \mathbf{z} \in FC\backslash\{\mathbf{0}\}(sign(\mathbf{z}) \leq sign(\mathbf{v}) \Rightarrow \mathbf{z}^T \mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}} > 0)\}$. This can be rewritten: $CFC_{\underline{\mathbf{TC}}} = \{\mathbf{v} \in FC \mid FC_{\leq sign(\mathbf{v})}\backslash\{\mathbf{0}\} \subseteq H^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}\}$, where $H^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}} \triangleq \{\mathbf{z} \in \mathbb{R}^r \mid \mathbf{z}^T \mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}} > 0\}$ is an open half-space with frontier the hyperplane $H_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}} \triangleq \{\mathbf{z} \in \mathbb{R}^r \mid \mathbf{z}^T \mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}} = 0\}$. Considering the decomposition of FC into FTs $FC_{\leq \boldsymbol{\eta}}$, this is equivalent to: $CFC_{\underline{\mathbf{TC}}\leq \boldsymbol{\eta}} = \{\mathbf{v} \in FC_{\leq \boldsymbol{\eta}} \mid FC_{\leq sign(\mathbf{v})}\backslash\{\mathbf{0}\} \subseteq H^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}\}$ for all $\boldsymbol{\eta}$'s maximal sign vectors in $sign(FC)$. Now, notice that, in this formula, $FC_{\leq sign(\mathbf{v}}$ is the minimal (for set inclusion) face of $FC_{\leq \boldsymbol{\eta}}$ containing $\mathbf{v}$. We obtain thus finally:

$$CFC_{\underline{\mathbf{TC}}\leq \boldsymbol{\eta}} = \bigcup_i FC_{\leq \boldsymbol{\eta}^i} \text{ for all maximal } \boldsymbol{\eta}^i \leq \boldsymbol{\eta} \text{ s.t. } FC_{\leq \boldsymbol{\eta}^i}\backslash\{\mathbf{0}\} \subseteq H^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}. \tag{65}$$

That is to say that $CFC_{\underline{\mathbf{TC}}}$ is the union of all the maximal faces $FC_{\leq \boldsymbol{\eta}^i}$ of the FTs for FC such that $FC_{\leq \boldsymbol{\eta}^i}\backslash\{\mathbf{0}\} \subseteq H^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}$ or, equivalently, the union of all maximal faces F_i of the $FC \cap O$'s for all r-orthants O such that $F_i\backslash\{\mathbf{0}\} \subseteq H^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}$. A topological consequence of this characterization is that $CFC_{\underline{\mathbf{TC}}}\backslash\{\mathbf{0}\}$ is a connected set (actually arc-connected). Another consequence, about EFMs (or, equivalently, EFVs), is:

$$\mathtt{EFMs}(CFC_{\underline{\mathbf{TC}}}) = \mathtt{EFMs}(FC) \cap Sol_{\underline{\mathbf{TC}}} = \mathtt{EFMs}(FC) \cap H^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}} \tag{66}$$

i.e., the elementary flux modes in the (non-convex) cone of those flux vectors in FC satisfying the thermodynamic constraint $\underline{\mathbf{TC}}$ are exactly those elementary flux modes in FC that satisfy $\underline{\mathbf{TC}}$, i.e., that belong to the open half-space $H^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}$. We can equivalently write:

$$\mathtt{EFMs}(CFC_{\underline{\mathbf{TC}}}) = \mathtt{EFMs}(FC^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}})\backslash H_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}$$

$$\text{with } FC^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}} \triangleq \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{Sv} = \mathbf{0}, \mathbf{v}_i \geq 0 \text{ for } i \in \mathbf{Irr}, \mathbf{v}^T \mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}} \geq 0\} \tag{67}$$

where $FC^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}$ is the polyhedral cone obtained from FC by just adding to it the homogeneous linear inequality $\mathbf{v}^T \mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}} \geq 0$ [33]. Each flux vector of $CFC_{\underline{\mathbf{TC}}}$ is a conformal conical sum of those EFMs, but the converse is false as $CFC_{\underline{\mathbf{TC}}}$ is not convex. Thus the set of EFMs, considered globally, does not characterize the solutions space $CFC_{\underline{\mathbf{TC}}}$. More precisely, we have:

$$CFC_{\underline{\mathbf{TC}}\leq \boldsymbol{\eta}} = \bigcup_i cone_{\oplus}(E_i) \text{ with } E_i = \mathtt{EFMs}(FC_{\leq \boldsymbol{\eta}^i}) \text{ and } FC_{\leq \boldsymbol{\eta}^i} \text{ as in (65)} \tag{68}$$

i.e., $CFC_{\underline{\mathbf{TC}}}$ is characterized by the decomposition of the set of EFMs into the (non-disjoint) subsets E_i. Now, the E_i's are exactly the maximal subsets of EFMs included in a given flux tope for FC (i.e., in a given r-orthant O) and whose conical hull is included in $CFC_{\underline{\mathbf{TC}}}$, i.e., all vectors in this hull must satisfy the constraint $\underline{\mathbf{TC}}$: E_i maximal s.t. $E_i \subseteq \mathtt{EFMs}(CFC_{\underline{\mathbf{TC}}})$ and $E_i \subseteq O$ with O r-orthant and $cone_{\oplus}(E_i) \subseteq CFC_{\underline{\mathbf{TC}}}$. Such an E_i is called a largest thermodynamically consistent set (LTCS) of EFMs [10] in O (or, equivalently, in the flux tope $FC_{\leq \boldsymbol{\eta}} = FC \cap O$ defined by O).

We can wish to estimate the ratio of thermodynamically feasible EFMs on all EFMs, i.e., the ratio of $\mathtt{EFMs}(CFC_{\underline{\mathbf{TC}}})$ on $\mathtt{EFMs}(FC)$. If all reactions are reversible ($r_I = 0$), then the function $\mathbf{v} \mapsto -\mathbf{v}$ maps the EFMs on one side of the hyperplane $H_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}$ onto the EFMs on the other side. Thus, if we neglect the EFMs that would possibly belong to this hyperplane, it means that 50% of the EFMs are thermodynamically feasible (and thus only 50% eliminated). Now, intuitively, given irreversible reactions in **Irr** come from an expert knowledge that can be seen as a form of compiled thermodynamic knowledge, as we saw that it is thermodynamics which imposes the direction in which a reaction may proceed. So, one can assume, provided the adequacy of the model for some given environment (such as the concentrations of external metabolites, supposed here to be known), that any thermodynamically feasible EFM satisfies those irreversibility constraints. Under this assumption, the irreversibility constraints rule out only thermodynamically unfeasible EFMs so that applying then the thermodynamic constraint $\underline{\mathbf{TC}}$ can only eliminate less than 50% of the remaining EFMs (from 50% when all reactions are reversible to 0% when all are irreversible, without splitting up any reversible reaction into two irreversible ones).

If inhomogeneous linear constraints are added, we have $CFP_{\underline{\mathbf{TC}}} = CFC_{\underline{\mathbf{TC}}} \cap P_{\mathbf{ILC}}$, with $P_{\mathbf{ILC}} = \{\mathbf{v} \in \mathbb{R}^r \mid \mathbf{Gv} \geq \mathbf{h}\}$ (24) and we obtain in the same way, for all $\boldsymbol{\eta}$'s maximal sign vectors in $sign(FC)$:

$$CFP_{\underline{\mathbf{TC}}\,\leq\boldsymbol{\eta}} = \bigcup_i FP_{\leq\boldsymbol{\eta}^i} \text{ for all maximal } \boldsymbol{\eta}^i \leq \boldsymbol{\eta} \text{ s.t. } FC_{\leq\boldsymbol{\eta}^i}\backslash\{\mathbf{0}\} \subseteq H^+_{\mathbf{ln\hat{K}_{eq}}}. \tag{69}$$

That is to say that $CFP_{\underline{\mathbf{TC}}}$ is the union of all the $FP_{\leq\boldsymbol{\eta}^i} = FC_{\leq\boldsymbol{\eta}^i} \cap P_{\mathbf{ILC}}$ such that $FC_{\leq\boldsymbol{\eta}^i}$ is a maximal face of a FT for FC verifying $FC_{\leq\boldsymbol{\eta}^i}\backslash\{\mathbf{0}\} \subseteq H^+_{\mathbf{ln\hat{K}_{eq}}}$ or, equivalently, the union of the $F_i \cap P_{\mathbf{ILC}}$ for all maximal faces F_i of the $FC \cap O$'s for all r-orthants O such that $F_i\backslash\{\mathbf{0}\} \subseteq H^+_{\mathbf{ln\hat{K}_{eq}}}$. Take care that the $FP_{\leq\boldsymbol{\eta}^i}$'s involved are faces of $FP_{\leq\boldsymbol{\eta}}$ included in $H^+_{\mathbf{ln\hat{K}_{eq}}} \cup \{\mathbf{0}\}$, but that not any face of $FP_{\leq\boldsymbol{\eta}}$ included in $H^+_{\mathbf{ln\hat{K}_{eq}}} \cup \{\mathbf{0}\}$ is included in $CFP_{\underline{\mathbf{TC}}}$ (as such a face may be defined by inhomogeneous equality constraints coming from **ILC** and not only by nullity constraints of the form $\mathbf{v}_i = 0$). $CFP_{\underline{\mathbf{TC}}}$ is no longer a connected set in general.

We can sum up these results as follows.

Proposition 2.6. *The space of flux vectors in FC satisfying the thermodynamic constraint $\underline{\boldsymbol{TC}}$ is a finite union of flux cones, obtained as all the maximal faces of all the flux topes $FC_{\leq\boldsymbol{\eta}}$ that are entirely contained (except the null vector) in the open half-space $H^+_{\boldsymbol{ln\hat{K}_{eq}}} = \{\boldsymbol{z} \mid \boldsymbol{z}^T\boldsymbol{ln\hat{K}_{eq}} > 0\}$. The thermodynamically feasible* EFMs *are thus those* EFMs *which belong to this half-space and can be simply computed as the* EFMs *of the flux cone obtained from FC by adding to it the homogeneous linear inequality $\boldsymbol{v}^T\boldsymbol{ln\hat{K}_{eq}} \geq 0$ (and removing those* EFMs *that would belong to the frontier hyperplane of $H^+_{\boldsymbol{ln\hat{K}_{eq}}}$). They can be decomposed into (non-disjoint) maximal subsets of* EFMs *belonging to a same flux tope (i.e., a same r-orthant) and whose conical hull is made up of thermodynamically feasible vectors, each of these subsets representing thus the set of* EFMs *of one of the maximal faces above. At most 50% of the* EFMs *can thus be ruled out as thermodynamically infeasible, if we assume that no thermodynamically feasible flux vector violates given irreversibility constraints. In presence of additional inhomogeneous linear constraints on flux vectors given by $\boldsymbol{Gv} \geq \boldsymbol{h}$, the space of flux vectors in FP satisfying $\underline{\boldsymbol{TC}}$ is a finite union of flux polyhedra, obtained as intersections of the flux cones above with the polyhedron defined by $\boldsymbol{Gv} \geq \boldsymbol{h}$. All these results hold for the constraint $\boldsymbol{TC}$ by just replacing the $\hat{K}^i_{eq}$'s by the K^i_{eq}'s.*

This applies in particular to $\widetilde{FC}$ and $\widetilde{FP}$ (after splitting up each reversible reaction into two irreversible ones) with the simplification, as $\widetilde{FC}$ (resp., $\widetilde{FP}$) is reduced to a single flux tope defined by $\boldsymbol{\eta} = +$, that one has just to consider the maximal faces of $\widetilde{FC}$ that are entirely contained (except the null vector) in the open half-space $H^+_{\mathbf{ln\hat{K}_{eq}}}$.

Consider now the case where some bounds on the concentrations of internal metabolites are known, i.e., the case of the thermodynamic constraint $\underline{\mathbf{TC}}^b$. We have $CFC_{\underline{\mathbf{TC}}^b} = FC \cap \bigcup_{\underline{\mathbf{M}}|\underline{\mathbf{M}}^- \leq \underline{\mathbf{M}} \leq \underline{\mathbf{M}}^+} O_{\mathbf{ts}_{\underline{\mathbf{M}}}}$. From what precedes, we obtain: $\mathbf{x} \in \bigcup_{\underline{\mathbf{M}}|\underline{\mathbf{M}}^- \leq \underline{\mathbf{M}} \leq \underline{\mathbf{M}}^+} O_{\mathbf{ts}_{\underline{\mathbf{M}}}} \Leftrightarrow \exists \mathbf{L}\underline{\mathbf{M}} \in \mathbb{R}^m (\forall i \in supp(\mathbf{x})\ (sign(\mathbf{x}_i) \sum_j \mathbf{S}_{ji}\mathbf{L}\underline{\mathbf{M}}_j < sign(\mathbf{x}_i) ln(\hat{K}^i_{eq})) \wedge \mathbf{L}\underline{\mathbf{M}} \leq ln(\underline{\mathbf{M}}^+) \wedge -\mathbf{L}\underline{\mathbf{M}} \leq -ln(\underline{\mathbf{M}}^-))$. Applying Gale's theorem in this formula to $\begin{pmatrix} \mathbf{A} \\ \mathbf{I}_m \\ -\mathbf{I}_m \end{pmatrix} \in \mathbb{R}^{(supp(\mathbf{x})+2m)\times m}$, with $\mathbf{A}_{ij} = sign(\mathbf{x}_i)\mathbf{S}_{ji}$, and $\begin{pmatrix} \mathbf{b} \\ ln\underline{\mathbf{M}}^+ \\ -ln\underline{\mathbf{M}}^- \end{pmatrix} \in \mathbb{R}^{supp(\mathbf{x})+2m}$, with $\mathbf{b}_i = sign(\mathbf{x}_i)\ ln(\hat{K}^i_{eq})$, provides: $\mathbf{x} \in \bigcup_{\underline{\mathbf{M}}|\underline{\mathbf{M}}^- \leq \underline{\mathbf{M}} \leq \underline{\mathbf{M}}^+} O_{\mathbf{ts}_{\underline{\mathbf{M}}}} \Leftrightarrow \forall \begin{pmatrix} \mathbf{z} \\ \underline{\mathbf{z}} \\ \overline{\mathbf{z}} \end{pmatrix} \in \mathbb{R}^{r+2m} (\mathbf{z} \neq \mathbf{0}, \underline{\mathbf{z}} \geq \mathbf{0}, \overline{\mathbf{z}} \geq \mathbf{0}, sign(\mathbf{z}) \leq sign(\mathbf{x}),\ \mathbf{Sz} + \underline{\mathbf{z}} - \overline{\mathbf{z}} = \mathbf{0} \Rightarrow (\mathbf{z}^T\underline{\mathbf{z}}^T\overline{\mathbf{z}}^T)\mathbf{ln\hat{K}^b_{eq}} > 0)$ where $\mathbf{ln\hat{K}^b_{eq}} \in \mathbb{R}^{r+2m}$ is defined by: $\mathbf{ln\hat{K}^b_{eq}} = \begin{pmatrix} \mathbf{ln\hat{K}_{eq}} \\ ln\underline{\mathbf{M}}^+ \\ ln(1/\underline{\mathbf{M}}^-) \end{pmatrix} = ln \begin{pmatrix} \mathbf{\hat{K}_{eq}} \\ \underline{\mathbf{M}}^+ \\ 1/\underline{\mathbf{M}}^- \end{pmatrix}$. Let $FC^b \triangleq \{\begin{pmatrix} \mathbf{z} \\ \underline{\mathbf{z}} \\ \overline{\mathbf{z}} \end{pmatrix} \in \mathbb{R}^{r+2m} \mid (\mathbf{S}\ \mathbf{I}_m\ -\mathbf{I}_m) \begin{pmatrix} \mathbf{z} \\ \underline{\mathbf{z}} \\ \overline{\mathbf{z}} \end{pmatrix} = \mathbf{0}$, $\mathbf{z}_i \geq 0$ for $i \in \mathbf{Irr}, \underline{\mathbf{z}} \geq \mathbf{0}, \overline{\mathbf{z}} \geq \mathbf{0}\} = \{\begin{pmatrix} \mathbf{z} \\ \mathbf{w} + (\mathbf{Sz})^- \\ \mathbf{w} + (\mathbf{Sz})^+ \end{pmatrix} \in \mathbb{R}^{r+2m} \mid \mathbf{z}_i \geq 0$ for $i \in \mathbf{Irr}, \mathbf{w} \geq \mathbf{0}\}$, where $(\mathbf{Sz})^+_j = max((\mathbf{Sz})_j, 0)$ and $(\mathbf{Sz})^-_j = max(-(\mathbf{Sz})_j, 0)$. FC^b is a flux cone in $\mathbb{R}^{r+2m}$ of dimension $r+m$ and $FC^b \cap (\mathbb{R}^r \times \{\mathbf{0}\}) = FC$. We obtain thus: $CFC_{\underline{\mathbf{TC}}^b} = \{\mathbf{v} \in FC \mid \forall (\mathbf{z}^T\underline{\mathbf{z}}^T\overline{\mathbf{z}}^T)^T \in FC^b\ (\mathbf{z} \neq \mathbf{0}, sign(\mathbf{z}) \leq$

$sign(\mathbf{v}) \Rightarrow (\mathbf{z}^T\underline{\mathbf{z}}^T\overline{\mathbf{z}}^T)\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}} > 0)\}$. This is equivalent to: $CFC_{\underline{\mathbf{TC}}^b \leq \boldsymbol{\eta}} = \{\mathbf{v} \in FC_{\leq \boldsymbol{\eta}} \mid \forall (\mathbf{z}^T\underline{\mathbf{z}}^T\overline{\mathbf{z}}^T)^T \in FC^b\ (\mathbf{z} \in O_{sign(\mathbf{v})}\backslash\{\mathbf{0}\} \Rightarrow (\mathbf{z}^T\underline{\mathbf{z}}^T\overline{\mathbf{z}}^T)\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}} > 0)\} = \{\mathbf{v} \in FC_{\leq \boldsymbol{\eta}} \mid \forall \mathbf{z} \in \mathbb{R}^r(\mathbf{z} \in O_{sign(\mathbf{v})}\backslash\{\mathbf{0}\} \Rightarrow (\mathbf{z}^T(\mathbf{Sz})^{-T}(\mathbf{Sz})^{+T})\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}} > 0)\}$ for all flux topes $FC_{\leq \boldsymbol{\eta}}$ for FC. This can be rewritten: $CFC_{\underline{\mathbf{TC}}^b \leq \boldsymbol{\eta}} = \{\mathbf{v} \in FC_{\leq \boldsymbol{\eta}} \mid FC^b \cap (O_{sign(\mathbf{v})}\backslash\{\mathbf{0}\} \times \mathbb{R}^{2m}_+) \subseteq H^+_{\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}}}\}$, where we note $H^+_{\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}}} \triangleq \{\mathbf{Z} \in \mathbb{R}^{r+2m} \mid \mathbf{Z}^T\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}} > 0\}$ the open half-space with frontier the hyperplane $H_{\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}}} \triangleq \{\mathbf{Z} \in \mathbb{R}^{r+2m} \mid \mathbf{Z}^T\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}} = 0\}$. We have $H^+_{\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}}} \cap \mathbb{R}^r = H^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}$ and $H_{\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}}} \cap \mathbb{R}^r = H_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}}$. Now, notice that, in the formula above, $FC^b \cap (O_{sign(\mathbf{v})} \times \mathbb{R}^{2m}_+)$ is a face of the cone $FC^b_{\leq \boldsymbol{\eta}+}$ (we note $\boldsymbol{\eta}+$ the sign vector of dimension $r+2m$ obtained by concatenation of $\boldsymbol{\eta}$ of dimension r and $+$ of dimension $2m$, thus $FC^b_{\leq \boldsymbol{\eta}+} = FC^b \cap (O_{\boldsymbol{\eta}} \times \mathbb{R}^{2m}_+)$) whose intersection with $\mathbb{R}^r \times \{\mathbf{0}\}$ is equal to $FC_{\leq sign(\mathbf{v})}$, the minimal face of the tope $FC_{\leq \boldsymbol{\eta}}$ containing $\mathbf{v}$. Actually, among all the faces of $FC^b_{\leq \boldsymbol{\eta}+}$ whose intersection with $\mathbb{R}^r \times \{\mathbf{0}\}$ is equal to $FC_{\leq sign(\mathbf{v})}$, it is the maximal one (without any constraint on the $\mathbb{R}^{2m}_+$ factor). We obtain thus finally:

$$CFC_{\underline{\mathbf{TC}}^b \leq \boldsymbol{\eta}} = \bigcup_j FC_{\leq \boldsymbol{\eta}^j} \text{ for all maximal } \boldsymbol{\eta}^j \leq \boldsymbol{\eta} \text{ s.t. } F^b_j\backslash(\{\mathbf{0}\} \times \mathbb{R}^{2m}) \subseteq H^+_{\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}}},$$

$$\text{where } F^b_j \text{ is the maximal face of } FC^b_{\leq \boldsymbol{\eta}+} \text{ whose intersection with } \mathbb{R}^r \times \{\mathbf{0}\} \text{ is equal to } FC_{\leq \boldsymbol{\eta}^j}. \quad (70)$$

Notice that any $FC_{\leq \boldsymbol{\eta}^j}$ given by (70) is a face of some $FC_{\leq \boldsymbol{\eta}^i}$ given by (65) (i.e., $\forall \boldsymbol{\eta}^j \exists \boldsymbol{\eta}^i\ \boldsymbol{\eta}^j \leq \boldsymbol{\eta}^i$). $CFC_{\underline{\mathbf{TC}}^b}\backslash\{\mathbf{0}\}$ is no longer a connected set in general. A consequence, about EFMs (or, equivalently, EFVs), is:

$$\mathtt{EFMs}(CFC_{\underline{\mathbf{TC}}^b \leq \boldsymbol{\eta}}) = \mathtt{EFMs}(FC_{\leq \boldsymbol{\eta}}) \cap Sol_{\underline{\mathbf{TC}}^b} = \{\mathbf{v} \in \mathtt{EFMs}(FC_{\leq \boldsymbol{\eta}}) \mid F^b_{\mathbf{v}}\backslash(\{\mathbf{0}\} \times \mathbb{R}^{2m}) \subseteq H^+_{\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}}}\},$$

$$\text{where } F^b_{\mathbf{v}} \text{ is the maximal face of } FC^b_{\leq \boldsymbol{\eta}+} \text{ s.t. } F^b_{\mathbf{v}} \cap (\mathbb{R}^r \times \{\mathbf{0}\}) = \mathbb{R}_+\mathbf{v} \quad (71)$$

i.e., the elementary flux modes in the (non-convex) cone of those flux vectors in $FC_{\leq \boldsymbol{\eta}}$ satisfying the thermodynamic constraint $\underline{\mathbf{TC}}^b$ are exactly those elementary flux modes $\mathbf{v}$ in $FC_{\leq \boldsymbol{\eta}}$ (or in $CFC_{\underline{\mathbf{TC}} \leq \boldsymbol{\eta}}$) that satisfy $\underline{\mathbf{TC}}^b$, i.e., such that the maximal face of $FC^b_{\leq \boldsymbol{\eta}+}$ whose intersection with $\mathbb{R}^r \times \{\mathbf{0}\}$ is equal to the ray $\mathbb{R}_+\mathbf{v}$ is included in $H^+_{\mathbf{ln}\hat{\mathbf{K}}_{\mathbf{eq}}} \cup (\{\mathbf{0}\} \times \mathbb{R}^{2m})$. Note that $\mathtt{EFMs}(CFC_{\underline{\mathbf{TC}}^b})$ is thus given by:

$$\{\mathbf{v} \in \mathtt{EFMs}(FC) \mid \forall \mathbf{z} \in \mathbb{R}^r(sign(\mathbf{z}) \leq sign(\mathbf{v}), (\mathbf{z}^T(\mathbf{Sz})^{-T}(\mathbf{Sz})^{+T})\mathbf{ln}\hat{\mathbf{K}}^b_{\mathbf{eq}} \leq 0 \Rightarrow \mathbf{z} = \mathbf{0})\}. \quad (72)$$

Each flux vector of $CFC_{\underline{\mathbf{TC}}^b}$ is a conformal conical sum of those EFMs, but the converse is false as $CFC_{\underline{\mathbf{TC}}^b}$ is not convex. More precisely, we have:

$$CFC_{\underline{\mathbf{TC}}^b \leq \boldsymbol{\eta}} = \bigcup_j cone_{\oplus}(E_j) \text{ with } E_j = \mathtt{EFMs}(FC_{\leq \boldsymbol{\eta}^j}) \text{ and } FC_{\leq \boldsymbol{\eta}^j} \text{ as in (70)} \quad (73)$$

i.e., $CFC_{\underline{\mathbf{TC}}^b}$ is characterized by the decomposition of the set of EFMs into the (non-disjoint) subsets E_j. Now, the E_j's are exactly the maximal subsets of EFMs included in a given flux tope for FC (i.e., in a given r-orthant O) and whose conical hull is included in $CFC_{\underline{\mathbf{TC}}^b}$, i.e., all vectors in this hull must satisfy the constraint $\underline{\mathbf{TC}}^b$: E_j maximal s.t. $E_j \subseteq \mathtt{EFMs}(CFC_{\underline{\mathbf{TC}}^b})$ and $E_j \subseteq O$ with O r-orthant and $cone_{\oplus}(E_j) \subseteq CFC_{\underline{\mathbf{TC}}^b}$. Such an E_j is called a largest thermodynamically consistent (for bounded concentrations of internal metabolites) set (LTCbS) of EFMs [10] in O (or, equivalently, in the flux tope $FC_{\leq \boldsymbol{\eta}} = FC \cap O$ defined by O) and is included in some LTCS E_i as in (68).

If inhomogeneous linear constraints **ILC** are added, we have, for all $\boldsymbol{\eta}$'s maximal sign vectors in $sign(FC)$:

$$CFP_{\underline{\mathbf{TC}}^b \leq \boldsymbol{\eta}} = \bigcup_j FP_{\leq \boldsymbol{\eta}^j} \text{ with } \boldsymbol{\eta}^j \text{ as in (70).} \quad (74)$$

That is to say that $CFP_{\underline{\mathbf{TC}}^b}$ is the union of all the $FP_{\leq \boldsymbol{\eta}^j} = FC_{\leq \boldsymbol{\eta}^j} \cap P_{\mathbf{ILC}}$ such that $FC_{\leq \boldsymbol{\eta}^j}$ is given as in (70).

We can sum up these results as follows.

Proposition 2.7. *For FC a flux cone in $\mathbb{R}^r$, define its lift to $\mathbb{R}^{r+2m}$ as the flux cone $FC^b = \{\boldsymbol{Z} \in \mathbb{R}^r \times \mathbb{R}^{2m}_+) \mid (\boldsymbol{S}\ \boldsymbol{I}_m\ -\boldsymbol{I}_m)\boldsymbol{Z} = \boldsymbol{0}, \boldsymbol{Z}_i \geq 0$ for $i \in \boldsymbol{Irr}, 1 \leq i \leq r\}$. Thus, $FC^b \cap (\mathbb{R}^r \times \{\boldsymbol{0}\}) = FC$. For any flux tope $FC_{\leq \boldsymbol{\eta}}$ for FC and any face FC' of $FC_{\leq \boldsymbol{\eta}}$, its lift to $\mathbb{R}^{r+2m}$ is defined as the maximal face of $FC^b_{\leq \boldsymbol{\eta}+}$ whose intersection with $\mathbb{R}^r \times \{\boldsymbol{0}\}$ is equal to FC'. The space of flux vectors in FC satisfying the thermodynamic constraint $\underline{\boldsymbol{TC}}^b$ is a finite union of flux cones, obtained as all the maximal faces of all the flux topes $FC_{\leq \boldsymbol{\eta}}$ whose lifts to $\mathbb{R}^{r+2m}$ are entirely contained (except vectors from $\{\boldsymbol{0}\} \times \mathbb{R}^{2m}$) in the open half-space $H^+_{\boldsymbol{ln}\hat{\boldsymbol{K}}^b_{\boldsymbol{eq}}} = \{\boldsymbol{Z} \in \mathbb{R}^{r+2m} \mid \boldsymbol{Z}^T \boldsymbol{ln}\hat{\boldsymbol{K}}^b_{\boldsymbol{eq}} > 0\}$, where $\boldsymbol{ln}\hat{\boldsymbol{K}}^b_{\boldsymbol{eq}} = ln(\hat{\boldsymbol{K}}_{\boldsymbol{eq}}\ \underline{\boldsymbol{M}}^+\ 1/\underline{\boldsymbol{M}}^-)^T$. The thermodynamically feasible* `EFMs` *in $FC_{\leq \boldsymbol{\eta}}$ are those* `EFMs` *in $FC_{\leq \boldsymbol{\eta}}$ whose lifts (as rays) are contained (except vectors from $\{\boldsymbol{0}\} \times \mathbb{R}^{2m}$) in this half-space. They can be decomposed into (non-disjoint) maximal subsets of* `EFMs` *belonging to a same flux tope (i.e., a same r-orthant) and whose conical hull is made up of thermodynamically feasible vectors, each of these subsets representing thus the set of* `EFMs` *of one of the maximal faces above. In presence of additional inhomogeneous linear constraints on flux vectors given by $\boldsymbol{Gv} \geq \boldsymbol{h}$, the space of flux vectors in FP satisfying $\underline{\boldsymbol{TC}}^b$ is a finite union of flux polyhedra, obtained as intersections of the flux cones above with the polyhedron defined by $\boldsymbol{Gv} \geq \boldsymbol{h}$.*

2.2.2 Application to kinetics

In the same way, we obtain for the kinetic constraint in absence of knowledge about values of enzymes and metabolites concentrations:

$$\mathbf{KC}(\mathbf{v}) \triangleq \exists \mathbf{E}, \mathbf{M}\ \mathbf{KC}_{\mathbf{E},\mathbf{M}}(\mathbf{v}) \triangleq \exists \mathbf{E}, \mathbf{M}\ \mathbf{v} = \mathbf{E} \circ \boldsymbol{\kappa}(\mathbf{M}). \tag{75}$$

And again we can add optionally lower and upper bounds $\mathbf{M}^-_j$ and $\mathbf{M}^+_j$ on metabolites concentrations and/or lower and upper bounds $\mathbf{E}^-_i$ and $\mathbf{E}^+_i$ on enzymes concentrations if they are known, and also a global enzymatic resource constraint, which is often considered, as $\mathbf{c}^T\mathbf{E} \leq W$, where $\mathbf{c}$ is a constant positive vector of size r and W a positive constant:

$$\mathbf{KC}^b(\mathbf{v}) \triangleq \exists \mathbf{E}, \mathbf{M}\ (\mathbf{v} = \mathbf{E} \circ \boldsymbol{\kappa}(\mathbf{M}) \wedge \mathbf{c}^T\mathbf{E} \leq W \wedge \mathbf{E}^- \leq \mathbf{E} \leq \mathbf{E}^+ \wedge \mathbf{M}^- \leq \mathbf{M} \leq \mathbf{M}^+). \tag{76}$$

We can also consider intermediate constraints, existentially quantified only on metabolites concentrations if enzymes concentrations are known:

$$\mathbf{KC}_{\mathbf{E}}(\mathbf{v}) \triangleq \exists \mathbf{M}\ \mathbf{KC}_{\mathbf{E},\mathbf{M}}(\mathbf{v}) \tag{77}$$

possibly with bounds on metabolites concentrations in the quantification:

$$\mathbf{KC}^b_{\mathbf{E}}(\mathbf{v}) \triangleq \exists \mathbf{M}\ (\mathbf{KC}_{\mathbf{E},\mathbf{M}}(\mathbf{v}) \wedge \mathbf{M}^- \leq \mathbf{M} \leq \mathbf{M}^+) \tag{78}$$

or only on enzymes concentrations if metabolites concentrations are known:

$$\mathbf{KC}_{\mathbf{M}}(\mathbf{v}) \triangleq \exists \mathbf{E}\ \mathbf{KC}_{\mathbf{E},\mathbf{M}}(\mathbf{v}) \tag{79}$$

possibly with enzymatic resource constraint and bounds on enzymes concentrations in the quantification:

$$\mathbf{KC}^b_{\mathbf{M}}(\mathbf{v}) \triangleq \exists \mathbf{E}\ (\mathbf{KC}_{\mathbf{E},\mathbf{M}}(\mathbf{v}) \wedge \mathbf{c}^T\mathbf{E} \leq W \wedge \mathbf{E}^- \leq \mathbf{E} \leq \mathbf{E}^+). \tag{80}$$

The solutions space in $\mathbb{R}^r$ of the constraint $\mathbf{KC}_{\mathbf{M}}$ is $Sol_{\mathbf{KC}_{\mathbf{M}}} = \bigcup_{\mathbf{E}} Sol_{\mathbf{KC}_{\mathbf{E},\mathbf{M}}} = \bigcup_{\mathbf{E} \in \mathbb{R}^r_+} \{\mathbf{E} \circ \boldsymbol{\kappa}(\mathbf{M})\} = \{\mathbf{v} \in \mathbb{R}^r \mid sign(\mathbf{v}) \leq sign(\boldsymbol{\kappa}(\mathbf{M}))\} = \{\mathbf{v} \in \mathbb{R}^r \mid sign(\mathbf{v}) \leq \mathbf{ts}_{\mathbf{M}}\} = Sol_{\mathbf{TC}_{\mathbf{M}}}$, as the sign of $\boldsymbol{\kappa}(\mathbf{M})$ is the thermodynamic sign vector $\mathbf{ts}_{\mathbf{M}}$. It is thus the same as the solutions space of the thermodynamic constraint $\mathbf{TC}_{\mathbf{M}}$ and is the closed orthant $O_{\mathbf{ts}_{\mathbf{M}}}$. It results that the solutions space of the constraint $\mathbf{KC}$, given by $Sol_{\mathbf{KC}} = \bigcup_{\mathbf{M}} Sol_{\mathbf{KC}_{\mathbf{M}}} = \bigcup_{\mathbf{M}} Sol_{\mathbf{TC}_{\mathbf{M}}} = Sol_{\mathbf{TC}}$, is the same as the solutions space of the thermodynamic constraint $\mathbf{TC}$ and is the finite union of the closed orthants $O_{\mathbf{ts}_{\mathbf{M}}}$. Analogously, $Sol_{\mathbf{KC}^b} = Sol_{\mathbf{TC}^b}$ if the bounds are only on the metabolites concentrations, i.e., there are no bounds on enzymes concentrations. From $Sol_{\mathbf{KC}^b_{\mathbf{M}}} = \bigcup_{\mathbf{E}^- \leq \mathbf{E} \leq \mathbf{E}^+ \wedge \mathbf{c}^T\mathbf{E} \leq W} \{\mathbf{E} \circ \boldsymbol{\kappa}(\mathbf{M})\}$, it results that the flux vectors in $Sol_{\mathbf{KC}^b_{\mathbf{M}}}$ are defined by the linear inequalities: $\kappa_i(\mathbf{M})\mathbf{E}^-_i \leq \mathbf{v}_i \leq \kappa_i(\mathbf{M})\mathbf{E}^+_i$ for those i's such that $\kappa_i(\mathbf{M}) > 0$, $\kappa_i(\mathbf{M})\mathbf{E}^+_i \leq \mathbf{v}_i \leq \kappa_i(\mathbf{M})\mathbf{E}^-_i$ for those i's such that $\kappa_i(\mathbf{M}) < 0$, $\mathbf{v}_i = 0$ for those i's such that

$\kappa_i(\mathbf{M}) = 0$ and $\sum_{i|\kappa_i(\mathbf{M})\neq 0} \mathbf{c}_i\kappa_i(\mathbf{M})^{-1}\mathbf{v}_i \leq W - \sum_{i|\kappa_i(\mathbf{M})=0} \mathbf{c}_i\mathbf{E}_i^-$. Thus $Sol_{\mathbf{KC}^b_\mathbf{M}}$ is a convex polyhedron (a parallelepiped contained in the closed orthant $O_{\mathbf{ts}_\mathbf{M}}$, truncated by a hyperplane). Now, if $\mathbf{E}^- = \mathbf{0}$, i.e., in absence of positive lower bounds for enzymes concentrations, this polyhedron has $\mathbf{0}$ as vertex and is nothing else that $Sol_{\mathbf{KC}_\mathbf{M}}$, i.e., the closed orthant $O_{\mathbf{ts}_\mathbf{M}}$, truncated as a parallelepiped by the faces given by $\mathbf{v}_i = \kappa_i(\mathbf{M})\mathbf{E}_i^+$ for those i's such that $\kappa_i(\mathbf{M}) \neq 0$ and by the hyperplane $\sum_{i|\kappa_i(\mathbf{M})\neq 0} \mathbf{c}_i\kappa_i(\mathbf{M})^{-1}\mathbf{v}_i = W$. It results that $Sol_{\mathbf{KC}^b} = \bigcup_\mathbf{M} Sol_{\mathbf{KC}^b_\mathbf{M}}$ is equal to some truncation of $Sol_\mathbf{KC} = Sol_\mathbf{TC}$, defined as the finite union of truncations of the closed orthants $O_{\mathbf{ts}_\mathbf{M}}$ (truncation of each $O_\mathbf{ts}$ being, as a parallelepiped, by hyperplanes of equations $\mathbf{v}_i = sup(\kappa_i(\mathbf{M}))\mathbf{E}_i^+$ if $\mathbf{ts}_i = +$ (resp., $\mathbf{v}_i = inf(\kappa_i(\mathbf{M}))\mathbf{E}_i^+$ if $\mathbf{ts}_i = -$), where sup (resp., inf) is taken on those $\mathbf{M}$ such that $sign(\boldsymbol{\kappa}(\mathbf{M})) = \mathbf{ts}$, which gives a polyhedron, but also, in presence of an enzymatic resource constraint, by an algebraic, non-linear, surface, which gives in this case a local solutions space in $O_\mathbf{ts}$ that is no longer a polyhedron and is not necessarily convex).

Proposition 2.8. *Given metabolites concentrations $\boldsymbol{M}$, the kinetic constraint $\boldsymbol{KC_M}$ is identical to the thermodynamic constraint $\boldsymbol{TC_M}$ and thus the set $Sol_{\boldsymbol{KC_M}}$ of vectors in $\mathbb{R}^r$ satisfying $\boldsymbol{KC_M}$ is the closed orthant defined by the thermodynamic sign vector $\boldsymbol{ts_M}$. The kinetic constraint $\boldsymbol{KC}$ is identical to the thermodynamic constraint $\boldsymbol{TC}$ and thus the set $Sol_{\boldsymbol{KC}}$ of vectors in $\mathbb{R}^r$ satisfying $\boldsymbol{KC}$ is a union of closed orthants. In presence of bounds on metabolites concentrations only (and not on enzymes concentrations), the kinetic constraint $\boldsymbol{KC}^b$ is identical to the thermodynamic constraint $\boldsymbol{TC}^b$ and thus the set $Sol_{\boldsymbol{KC}^b}$ of vectors in $\mathbb{R}^r$ satisfying $\boldsymbol{KC}^b$ is a union of closed orthants. So, those kinetic constraints boil down to thermodynamic constraints and the results about the geometrical structure of the corresponding spaces of flux vectors and the characterization of elementary flux vectors (or elementary flux modes) given by propositions 2.3, 2.5, 2.6 and 2.7 apply: in particular, $CFC_{\boldsymbol{KC}}(\boldsymbol{M}) = CFC_{\boldsymbol{TC}}(\boldsymbol{M})$ is a flux cone and $CFC_{\boldsymbol{KC}} = CFC_{\boldsymbol{TC}}$ and $CFC_{\boldsymbol{KC}^b} = CFC_{\boldsymbol{TC}^b}$ are finite unions of flux cones. For given $\boldsymbol{M}$ and in presence of bounds for enzymes concentrations, the set of vectors in $\mathbb{R}^r$ satisfying $\boldsymbol{KC}^b_{\boldsymbol{M}}$ is a convex polyhedron and $CFC_{\boldsymbol{KC}^b}(\boldsymbol{M})$ is thus a flux polyhedron. In the particular case of absence of positive lower bounds for enzymes concentrations, $CFC_{\boldsymbol{KC}^b}(\boldsymbol{M})$ is just the flux cone $CFC_{\boldsymbol{KC}}(\boldsymbol{M}) = CFC_{\boldsymbol{TC}}(\boldsymbol{M})$ truncated, as a parallelepiped, by hyperplanes originating from the upper bounds on enzymes concentrations and by a hyperplane originating from the enzymatic resource constraint and coincides thus with this flux cone in some adequate neighborhood of $\boldsymbol{0}$, i.e., for sufficiently small values of the fluxes. In this case, $CFC_{\boldsymbol{KC}^b}$ is thus a truncation (by an algebraic surface) of $CFC_{\boldsymbol{TC}}$ and coincides with this union of flux cones in some adequate neighborhood of $\boldsymbol{0}$ and the characterization of elementary flux vectors remains valid in this neighborhood. These results extend in presence of additional inhomogeneous linear constraints on flux vectors given by $\boldsymbol{Gv} \geq \boldsymbol{h}$ by intersecting the solutions spaces above with the polyhedron defined by $\boldsymbol{Gv} \geq \boldsymbol{h}$, giving rise to flux polyhedra (results in a neighborhood of $\boldsymbol{0}$ holding only if $\boldsymbol{h} < \boldsymbol{0}$).*

But the geometric structure of $Sol_{\mathbf{KC}_E}$, of $Sol_{\mathbf{KC}^b_E}$ and of $Sol_{\mathbf{KC}^b}$ in presence of positive lower bounds for enzymes concentrations, depends on the kinetic function $\boldsymbol{\kappa}(\mathbf{M})$ and $CFC_\mathbf{KC}(\mathbf{E})$, $CFC_{\mathbf{KC}^b}(\mathbf{E})$ and $CFC_{\mathbf{KC}^b}$ are in general neither polyhedra nor convex.

Proposition 2.8 has important consequences on constrained enzyme allocation problems in kinetic metabolic networks. Considering a kinetic metabolic network, with possibly bounds on metabolites concentrations, but not on enzymes concentrations, i.e., with solutions space $CFC_\mathbf{KC} = CFC_\mathbf{TC}$, or $CFC_{\mathbf{KC}^b} = CFC_{\mathbf{TC}^b}$, which is a finite union, for $\mathbf{M}$ varying, of the flux cones $FC_{\leq \mathbf{ts}_\mathbf{M}}$, the generic enzyme allocation problem consists in maximizing the specific flux (or specific rate, i.e., rate expressed per unit biomass amount) of a given reaction, say k, defined as $\mathbf{v}_k/E_T$, where $\mathbf{v}$ is a flux vector in $CFC_\mathbf{KC}$ or $CFC_{\mathbf{KC}^b}$ and E_T denotes the total protein content in the system. E_T is expressed in whole generality as a fixed weighted sum of the enzymes concentrations, $E_T = \sum_{i=1}^r w_i\mathbf{E}_i$ (the w_i's being given positive weights that denote the fraction of the resource used per unit enzyme), able to encode different enzymatic constraints (such as limited cellular or membrane surface space) as well as other constraints for the abundance of certain enzymes. Likewise, the steady-state flux component $\mathbf{v}_k > 0$ may stand for diverse metabolic processes, ranging from the synthesis rate of a particular product within a specific pathway to the rate of overall cellular growth. The formation rate of a metabolic product expressed per gram biomass and the specific growth rate of a cell are both examples of such specific rates. The maximization is looked for on the solutions space by varying the metabolites concentrations (inside their bounds, if any) and the enzymes concentrations (without bounds), giving rise to a complex non-convex optimization problem. Now, maximizing $\mathbf{v}_k/E_T$ is equivalent to fix the rate $\mathbf{v}_k$ to a positive value, e.g., to 1, and minimize the E_T needed to attain this level of $\mathbf{v}_k$. This means minimizing the

function $\sum_{i=1}^{r}(w_i/\kappa_i(\mathbf{M}))\mathbf{v}_i$ by varying $\mathbf{M}$ (with possible bounds) and, for each given $\mathbf{M}$, the flux vector $\mathbf{v}$ in $FP_{\mathbf{M}} = FC_{\leq \mathbf{ts_M}} \cap \{\mathbf{v} \mid \mathbf{v}_k = 1\}$ (without bounds on $\mathbf{v}$ as we assume no bounds on enzymes concentrations). If all $FP_{\mathbf{M}}$'s are empty, the problem is unfeasible, i.e., $\mathbf{v}_k > 0$ is incompatible with the kinetics. Otherwise, i.e., when the problem is feasible, consider successively each nonempty $FP_{\mathbf{M}}$. Such $FP_{\mathbf{M}}$ is a flux polyhedron whose elementary flux points (which are equal to the extreme points or vertices) correspond to the intersections of the hyperplane $\{\mathbf{v} \mid \mathbf{v}_k = 1\}$ with extreme rays (edges) of $FC_{\leq \mathbf{ts_M}}$, i.e., EFMs of $CFC_{\mathbf{KC}}$ or $CFC_{\mathbf{KC}^b}$, and elementary vectors (equal to extreme vectors), if any, correspond to the extreme rays of $FC_{\leq \mathbf{ts_M}} \cap \{\mathbf{v} \mid \mathbf{v}_k = 0\}$. As, for $\mathbf{M}$ fixed, the function to minimize is linear in $\mathbf{v}$, its minimum on $FP_{\mathbf{M}}$ is reached on a lower-dimensional face of $FP_{\mathbf{M}}$ (as $\mathbf{v}_i/\kappa_i(\mathbf{M}) \geq 0$, $w_i > 0$ and $FP_{\mathbf{M}}$ is included in a closed orthant, this face is necessarily a convex hull of some of the extreme points of $FP_{\mathbf{M}}$ even if it is not a polytope, i.e., no extreme vector may occur as one of the generators of this face), and thus reached in particular at at least one extreme point, i.e., at an EFM. Now, as the total number of EFMs is finite, so is the number of those for which the minimum of the function occurs for any given $\mathbf{M}$, considering all nonempty $FP_{\mathbf{M}}$'s. So, when $\mathbf{M}$ varies in its domain, we obtain the result that the maximum of the specific flux $\mathbf{v}_k/E_T$ occurs (at least) at an EFM of $CFC_{\mathbf{KC}}$ or $CFC_{\mathbf{KC}^b}$. In the case of $CFC_{\mathbf{KC}^b}$ and assuming the $\kappa_i(\mathbf{M})$'s continuous, this maximum is attained at an EFM at finite metabolite concentrations as $\mathbf{M}$ varies then in the compact set $\prod_j[\mathbf{M}_j^-, \mathbf{M}_j^+]$. In the case of $CFC_{\mathbf{KC}}$, it may happen that the maximum is not attained at finite metabolite concentrations. It is the result already obtained in [30, 46] and we followed an analog proof, but relying this time on a precise characterization of the solutions space $CFC_{\mathbf{KC}}$ or $CFC_{\mathbf{KC}^b}$ and of the EFMs given by the Proposition 2.8, which was not the case in the works quoted. Finally, the enzyme allocation problem can be theoretically solved by computing all the thermodynamically feasible EFMs having k in their support (i.e., satisfying the Boolean constraint $Bc = k$, see next subsection), say $\{\mathbf{e}^l\}$ (whose all components are fixed by $\mathbf{e}^l_k = 1$), and, for each one, compute the minimum (if it exists) of $\sum_{i \in supp(\mathbf{e}^l)}(w_i\mathbf{e}^l_i)/\kappa_i(\mathbf{M})$ for $\mathbf{M}$ varying in its domain, such that $sign(\kappa_i(\mathbf{M})) = sign(\mathbf{e}^l_i)$ for all $i \in supp(\mathbf{e}^l)$, which is a much more simpler optimization problem than the initial one. The global minimum, if it exists, is then the smallest of these local minima, for all $\mathbf{e}^l$'s. We obtain then the maximum value of the specific flux $\mathbf{v}_k/E_T$, an EFM where this maximum occurs and the values of the concentrations metabolites for which it occurs.

Proposition 2.9. *Given a kinetic metabolic network with possible bounds on metabolites concentrations, but not on enzymes concentrations, i.e., with solutions space $CFC_{\boldsymbol{KC}}$ or $CFC_{\boldsymbol{KC}^b}$, if the enzyme allocation problem, which consists in maximizing the specific flux (rate per unit biomass amount) in a given reaction k, i.e., $\boldsymbol{v}_k/E_T$, where $\boldsymbol{v}$ is a flux vector in $CFC_{\boldsymbol{KC}}$ or $CFC_{\boldsymbol{KC}^b}$ and $E_T = \sum_{i=1}^{r} w_i\boldsymbol{E}_i$ is the total protein content in the system (the w_i's being fixed positive weights), has an optimal solution (which is always the case for $CFC_{\boldsymbol{KC}^b}$ if the problem is feasible), then this solution is reached in particular at an* EFM *of $CFC_{\boldsymbol{KC}}$ or $CFC_{\boldsymbol{KC}^b}$.*

2.2.3 Application to regulatory constraints

From Lemma 2.2, we obtain that $CFC_{\mathbf{RC}_{Bc}}$ (resp., $CFP_{\mathbf{RC}_{Bc}}$) is the disjoint union of $FC \cap \mathring{O}$'s (resp., $FP \cap \mathring{O}$'s), for some open orthants $\mathring{O}$ and, after merging, the disjoint union of $FC \cap O^\circ$'s (resp., $FP \cap O^\circ$'s), for some semi-open orthants O° (orthants without some of their faces), without any further merging possible.

We will call open polyhedral cone (resp., open polyhedron) in dimension $r > 0$ an r-polyhedral cone C (resp., r-polyhedron P) without its facets, and we will note it $\mathring{C}$ (resp., $\mathring{P}$) as it coincides with the topological interior of C (resp., P) in the affine span of C (resp., P). Reciprocally, C (resp., P) is the topological closure of $\mathring{C}$ (resp., $\mathring{P}$) in $\mathbb{R}^r$. $\mathring{C}$ (resp., $\mathring{P}$) is defined as in (7) (resp., in (25)) but with strict inequalities (and equalities for defining its affine space). We will in particular consider open faces of a cone C or polyhedron P as $\mathring{F}$ for F a face of C or P. C (resp., P) is the disjoint union of its open faces (by convention, a face of dimension 0, i.e., a vertex, is equal to its open face).

It results from these definitions that, for any closed r-orthant O and any open orthant $\mathring{O}' \subset O$, $FC \cap \mathring{O}'$ (resp., $FP \cap \mathring{O}'$) is an open face of $FC \cap O$ (resp., a disjoint union of open faces of $FP \cap O$, as we have to keep faces corresponding to $\mathbf{Gv} = \mathbf{h}$). In total, we obtain that $CFC_{\mathbf{RC}_{Bc}} \cap O$ (resp., $CFP_{\mathbf{RC}_{Bc}} \cap O$) is a disjoint union of open faces of the flux cone $FC \cap O$ (resp., the flux polyhedron $FP \cap O$). Or, equivalently, that, for any flux tope $FC_{\leq \boldsymbol{\eta}}$ for FC (resp., $FP_{\leq \boldsymbol{\eta}}$ for FP), $CFC_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}$ (resp., $CFP_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}$) is a disjoint union of open faces of $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$). As we grouped together

open orthants into semi-open orthants in Lemma 2.2, one can also group together with such an open face $\mathring{F}$ all those other open faces $\mathring{F}'$ in question where F' is a face of F to obtain thus a (minimal) disjoint union of semi-open polyhedral cones (resp., semi-open polyhedra). Here, we call semi-open polyhedral cone C° (resp., semi-open polyhedron P°) a polyhedral cone C (resp., polyhedron P) without some (between zero and all) of its faces of lesser dimension, that can be thus expressed as a disjoint union of some (between all and only $\mathring{C}$, resp., $\mathring{P}$) of the open faces of C (resp., P). We have: $\mathring{C} \subseteq C^\circ \subseteq C$ (resp., $\mathring{P} \subseteq P^\circ \subseteq P$).

Proposition 2.10. *Given an arbitrary Boolean constraint Bc, the solutions space $CFC_{\mathbf{RC}_{Bc}}$ (resp., $CFP_{\mathbf{RC}_{Bc}}$) for the regulatory constraint $\mathbf{RC}_{Bc}$ is a finite disjoint union of open polyhedral cones (resp., open polyhedra), which are some open faces of all the flux topes $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$). Grouping together some of those open faces according to Lemma 2.2, one obtains a disjoint union of semi-open (i.e., without some of their faces of lesser dimension) faces of the $FC_{\leq \boldsymbol{\eta}}$'s (resp., $FP_{\leq \boldsymbol{\eta}}$'s), without any possible further merging of two of them to make up a bigger semi-open face.*

Note that the rules presented in the proof of Lemma 2.2 to group together open faces into semi-open faces are applied globally to all flux topes. If choice is done to apply them separately for each flux tope, then the union of semi-open faces obtained is no more disjoint in general as two such semi-open faces for two different flux topes may have a nonempty intersection (as $CFC_{\mathbf{RC}_{Bc}} \cap O$ (resp., $CFP_{\mathbf{RC}_{Bc}} \cap O$) and $CFC_{\mathbf{RC}_{Bc}} \cap O'$ (resp., $CFP_{\mathbf{RC}_{Bc}} \cap O'$), for two different closed r-orthants O and O' may have open faces in common). Note also that, even after this merging, there may exist a strict inclusion relationship between the closures of two semi-open faces.

From this geometrical structure of the solutions space, we will now deduce what are its `EFMs` and its `EFVs` (or `EFPs`). Begin by a preliminary remark. We know that, by definition of `EFVs`, we have, for a flux cone FC: $\texttt{EFVs}(FC) = \bigcup_{\boldsymbol{\eta} \text{ maximal in } sign(FC)} \texttt{EFVs}(FC_{\leq \boldsymbol{\eta}})$ (and idem for a flux polyhedron FP with `EFPs` and `EFVs`), which remains true for any constrained flux cone subset $CFC_{\mathbf{C}}$ (or constrained flux polyhedron subset $CFP_{\mathbf{C}}$) whatever the biological constraint $\mathbf{C}$ is. We saw that this equality was also satisfied by `EFMs` for FC: $\texttt{EFMs}(FC) = \bigcup_{\boldsymbol{\eta} \text{ maximal in } sign(FC)} \texttt{EFMs}(FC_{\leq \boldsymbol{\eta}})$ (and idem for FP) and remained true for $CFC_{\mathbf{C}}$ for $\mathbf{C}$ any thermodynamic constraint or any kinetic constraint of the form $\mathbf{KC_M}$, $\mathbf{KC}$ or $\mathbf{KC}^b$ (in absence of bounds on enzymes concentrations), allowing thus in those cases to decompose the identification of `EFMs` flux tope by flux tope, as for `EFVs`. But this is no longer true for regulatory constraints. Actually, counter-examples can be found in presence of reversible reactions, used in a given direction in an `EFM` local to some flux tope and in the other direction in an `EFM` local to another flux tope, choosing the Boolean constraint such that it imposes a strict set inclusion between the supports of those two local `EFMs`.

Example 2.1. Consider the simple network made of one reaction $R : A \to B$, where A and B are the two internal metabolites, and four transfer reactions $T1 : \to A$, $T2 : \to B$, $T3 : A \to$, $T4 : B \to$, and assume that R and $T1$ are reversible (we will note par $rev()$ the reversed reaction), the three other reactions being irreversible. It is obvious that $\mathbf{e}^1 = (1,1,0,0,1)^T$, made up of $T1, R, T4$, $\mathbf{e}^4 = (-1,-1,1,0,0)^T$, made up of $T2, rev(R), rev(T1)$, and $\mathbf{e}^3 = (0,0,1,0,1)^T$, made up of $T2, T4$, are `EFMs` of FC. Consider now the Boolean constraint $Bc = T1 \wedge T4$. Then, $\mathbf{e}^1$ is an `EFM` of $CFC_{\mathbf{RC}_{Bc}}$, belonging to $CFC_{\mathbf{RC}_{Bc} \leq (+,+,+,+,+)^T}$. And any $\mathbf{e} = \lambda \mathbf{e}^3 + \mathbf{e}^4$ with $\lambda > 0$ is an `EFM` of $CFC_{\mathbf{RC}_{Bc} \leq (-,-,+,+,+)^T}$ (obviously, the sub-pathways $\mathbf{e}^3$ and $\mathbf{e}^4$ of $\mathbf{e}$ do not belong to $CFC_{\mathbf{RC}_{Bc}}$). As $supp(\mathbf{e}^1) = \{R, T1, T4\}$ and $supp(\mathbf{e}) = \{R, T1, T2, T4\}$, we have $supp(\mathbf{e}^1) \subset supp(\mathbf{e})$ and thus $\mathbf{e}$ is not an `EFM` of $CFC_{\mathbf{RC}_{Bc}}$.

Now, from a biological point of view, it is not relevant to compare supports of two pathways with some reaction in a given direction in the first support and in the other direction in the second support (case of R and $T1$ in the example). This means that the useful concept concerning minimality is not support-minimality, but sign-minimality (exactly in the same way as, concerning decomposition, we saw that the useful concept is not non-decomposability but conform non-decomposability), which is equivalent to compare supports separately for each closed r-orthant, i.e., for each flux tope. We will thus identify the `EFMs` flux tope by flux tope (note that it is analog to distinguish a positive flux from a negative flux in a regulatory constraint, e.g., distinguish the constraints $T1^+ \wedge T4$ and $T1^- \wedge T4$ in the example above, which could be done by adopting a tri-valued logic instead of Boolean logic to represent those constraints; this is obviously automatically done when splitting each reversible reaction into two irreversible ones, where only the positive r-orthant has to be considered).

So, we will consider in the following an arbitrary closed r-orthant O given by a full support sign vector $\boldsymbol{\eta}$ (with $\boldsymbol{\eta}_i = +$ for $i \in \mathbf{Irr}$) and consider the part of the solutions space limited to this orthant, i.e., $CFC_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}$ (resp., $CFP_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}$), thus we can limit to sign vectors $\boldsymbol{\eta}$ that are maximal in $sign(CFC_{\mathbf{RC}_{Bc}})$ (resp., $sign(CFP_{\mathbf{RC}_{Bc}})$). We saw that we can rewrite the Boolean constraint as a disjunction of two by two exclusive disjuncts, $Bc = \bigvee_k D_k$, decomposing thus the solutions space $CFC_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}$ (resp., $CFP_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}$) into the disjoint solutions spaces for each disjunct, $CFC_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}$ (resp., $CFP_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}$). The elementary flux vectors (i.e., faces of dimension one) of $FC_{\leq \boldsymbol{\eta}}$ that satisfy the constraint $\mathbf{RC}_{D_k}$ are obviously elementary flux vectors of $CFC_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}$ and the reciprocal is also true: if a flux vector of the semi-open polyhedral cone $CFC_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}$ is not an elementary flux vector of $FC_{\leq \boldsymbol{\eta}}$, i.e., is not a face of dimension one, then it belongs to the interior of a face of dimension at least two of $CFC_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}$ and is thus conformally decomposable in this face, i.e., is not elementary in $CFC_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}$. It results that the elementary flux vectors of $CFC_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}$ are made up of all the elementary flux vectors of the $CFC_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}$'s. We can sum up the results about EFVs as:

$$\text{For } Bc = \bigvee_k D_k, \quad \texttt{EFVs}(CFC_{\mathbf{RC}_{Bc}}) = \bigcup_{\boldsymbol{\eta} \text{ maximal in } sign(CFC_{\mathbf{RC}_{Bc}})} \texttt{EFVs}(CFC_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}})$$

$$= \bigcup_{\boldsymbol{\eta}} \bigcup_k \texttt{EFVs}(CFC_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}) = \bigcup_{\boldsymbol{\eta}} \bigcup_k \texttt{EFVs}(FC_{\leq \boldsymbol{\eta}}) \cap Sol_{\mathbf{RC}_{D_k}}. \tag{81}$$

This means that EFVs can be computed flux tope by flux tope and constraint-disjunct by constraint-disjunct. And the result holds also for EFPs (by considering vertices of $FP_{\leq \boldsymbol{\eta}}$) and EFVs of $CFP_{\mathbf{RC}_{Bc}}$.

But, for EFMs, we have to take care that a phenomenon analog to that described in the example above still arises and that, even in a given flux tope, an EFM of $CFC_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}$ is not necessarily an EFM of $CFC_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}$.

Example 2.2. Consider the network made of three irreversible reactions and one internal metabolite A: $R1 : \rightarrow A$, $R2 : \rightarrow A$, $R3 : A \rightarrow$, and the Boolean constraint $Bc = \neg R1 \vee R2$, decomposed as $Bc = D_1 \vee D_2$, with $D_1 = \neg R1$ and $D_2 = R1 \wedge R2$. Take $\boldsymbol{\eta} = +$. Then $\mathbf{e}^1 = (0, 1, 1)^T$, made up of $R2, R3$, which is the only EFM of $CFC_{\mathbf{RC}_{D_1} \leq \boldsymbol{\eta}}$, is also the only EFM of $CFC_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}$. But any $\mathbf{e}^2 = (\lambda, 1, 1 + \lambda)^T$ with $\lambda > 0$, made up of $R1, R2, R3$, is an EFM of $CFC_{\mathbf{RC}_{D_2} \leq \boldsymbol{\eta}}$ and is not an EFM of $CFC_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}$. Note that the way the constraint is decomposed matters. With the decomposition $Bc = D'_1 \vee D'_2$ with $D'_1 = R2$ and $D'_2 = \neg R1 \vee \neg R2$, the result for D'_1 is identical to that for D_1, but $CFC_{\mathbf{RC}_{D'_2} \leq \boldsymbol{\eta}} = \{\mathbf{0}\}$.

This means that, if it is natural to study each $CFC_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}$ (resp., $CFP_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}}$) separately in order to characterize the solutions space and if EFVs are obtained like this by gathering all the local EFVs, it is not the case for EFMs and, after gathering all local EFMs, one has to keep only those with minimal support:

$$\texttt{EFMs}(CFC_{\mathbf{RC}_{Bc} \leq \boldsymbol{\eta}}) = Min_{supp\text{-}\subseteq}\{\texttt{EFMs}(CFC_{\mathbf{RC}_{D_k} \leq \boldsymbol{\eta}})\}_k. \tag{82}$$

Proposition 2.11. *Given an arbitrary regulatory constraint $\boldsymbol{RC}_{Bc}$ with $Bc = \bigvee_k D_k$, where the disjuncts D_k are taken two by two inconsistent, and the associated constrained flux cone subset $CFC_{\boldsymbol{RC}_{Bc}}$ (resp., constrained flux polyhedron subset $CFP_{\boldsymbol{RC}_{Bc}}$), its* EFVs *(resp.,* EFPs *and* EFVs*) are obtained by collecting those for each flux tope (i.e., in each r-orthant $O_{\leq \boldsymbol{\eta}}$) and for each disjunct, i.e., for each $CFC_{\boldsymbol{RC}_{D_k} \leq \boldsymbol{\eta}}$ (resp., $CFP_{\boldsymbol{RC}_{D_k} \leq \boldsymbol{\eta}}$), and are nothing else that the* EFVs *(resp.,* EFPs *and* EFVs*) of FC (resp., FP) satisfying the constraint $\boldsymbol{RC}_{Bc}$. This is not the case for* EFMs*. First, an* EFM *of $CFC_{\boldsymbol{RC}_{Bc} \leq \boldsymbol{\eta}}$ is not necessarily an* EFM *of $CFC_{\boldsymbol{RC}_{Bc}}$, but actually the biologically relevant minimality concept being sign-minimality and not support-minimality, we will consider* EFMs *for each flux tope $CFC_{\boldsymbol{RC}_{Bc} \leq \boldsymbol{\eta}}$. Second, an* EFM *of $CFC_{\boldsymbol{RC}_{D_k} \leq \boldsymbol{\eta}}$ is not necessarily an* EFM *of $CFC_{\boldsymbol{RC}_{Bc} \leq \boldsymbol{\eta}}$.* EFMs *of $CFC_{\boldsymbol{RC}_{Bc} \leq \boldsymbol{\eta}}$ are actually obtained by collecting* EFMs *of $CFC_{\boldsymbol{RC}_{D_k} \leq \boldsymbol{\eta}}$ for all k and keeping only those with minimal support.*

This being said, we will thus focus now on an arbitrary disjunct D of the form $\bigwedge_{i \in I} \mathbf{v}_i \wedge \bigwedge_{j \in J} \neg \mathbf{v}_j$ with $I, J \subseteq \{1, \ldots, r\}, I \cap J = \emptyset$. Thus $CFC_{\mathbf{RC}_D \leq \boldsymbol{\eta}}$ (resp., $CFP_{\mathbf{RC}_D \leq \boldsymbol{\eta}}$) is the semi-open face F° of $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$), obtained from the face F defined by $\{\mathbf{v}_j = 0, j \in J\}$ (i.e., $F = FC_{\leq \boldsymbol{\eta}} \cap \bigcap_{j \in J}\{\mathbf{v}_j = 0\}$, idem with FP) by removing its facets $\{\mathbf{v}_i = 0\}$ for all $i \in I$. Let's note $\texttt{EFMs}^{\mathbf{RC}_D} \triangleq \texttt{EFMs}(FC_{\leq \boldsymbol{\eta}}) \cap Sol_{\mathbf{RC}_D}$ those EFMs of $FC_{\leq \boldsymbol{\eta}}$ that satisfy the constraint $\mathbf{RC}_D$ and $\texttt{EFMs}_{\mathbf{RC}_D} \triangleq \texttt{EFMs}(CFC_{\mathbf{RC}_D \leq \boldsymbol{\eta}})$ the EFMs of the part of the solutions space in $O_{\boldsymbol{\eta}}$. Obviously, $\texttt{EFMs}^{\mathbf{RC}_D} \subseteq \texttt{EFMs}_{\mathbf{RC}_D}$. If $I = \emptyset$, $F^\circ = F$ and

$\texttt{EFMs}^{\mathbf{RC}_D} = \texttt{EFMs}_{\mathbf{RC}_D}$, so we will consider the case $I \neq \emptyset$. In this case, and contrary to what happen for thermodynamic and kinetic (as described in Proposition 2.8) constraints, there is in general no longer identity between $\texttt{EFMs}^{\mathbf{RC}_D}$ and $\texttt{EFMs}_{\mathbf{RC}_D}$ [27].

Example 2.3. Consider the network of Example 2.1 (thus $D = T1 \wedge T4$) and let $\boldsymbol{\eta} = +$ and $\mathbf{e}^2 = (0,1,0,1,0)^T$ be the EFM of $FC_{\leq \boldsymbol{\eta}}$ made up of $T1$ and $T3$. Then, for any $\lambda > 0$, $\mathbf{e}^2 + \lambda \mathbf{e}^3$, positive conformal combination of the two EFMs $\mathbf{e}^2$ and $\mathbf{e}^3$ of $FC_{\leq \boldsymbol{\eta}}$, has support $\{T1, T2, T3, T4\}$ and belongs to $\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D}$.

$\texttt{EFMs}^{\mathbf{RC}_D}$ correspond to the faces of dimension one (edges or extreme rays), if any, of the semi-open face F°, i.e., the edges of the face F that have not been removed, which means that they are not included in any hyperplane of equation $\mathbf{v}_i = 0$, for some $i \in I$, or equivalently that their supports contain I. Consider now a face F' of F, of dimension at least two, such that $\mathring{F'} \subseteq F^\circ$ but not any facet of F' has its interior included in F° (i.e., any facet of F' is included in an hyperplane of equation $\mathbf{v}_i = 0$, for some $i \in I$), if any. Note that several such F' may exist, but none can be included in another one, i.e., they are not comparable for inclusion in the lattice of the faces of F. Then all vectors of $\mathring{F'}$ have the same minimal support, i.e., $\mathring{F'} \subseteq \texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D}$. If $\{\mathbf{e}^k\}_{k \in K}$ (with $|K| \geq 2$) are representatives of the extreme vectors of F', we have $F' = cone_{\oplus}(\{\mathbf{e}^k\})$ (which is actually the same as $cone(\{\mathbf{e}^k\})$ as we are in the orthant $O_{\leq \boldsymbol{\eta}}$) and thus $\mathring{F'} = \{\bigoplus_{k \in K} \beta_k \mathbf{e}^k \mid \beta_k > 0\} \triangleq cone^+_{\oplus}(\{\mathbf{e}^k\})$ and the common minimal support of all vectors of $\mathring{F'}$ is $supp(\mathring{F'}) = \bigcup_{k \in K} supp(\mathbf{e}^k)$. Conversely, if $\mathbf{v} \in \texttt{EFMs}_{\mathbf{RC}_D}$, let F' be the minimal face of F containing $\mathbf{v}$. If F' has dimension one (extreme ray), then $F' \backslash \{\mathbf{0}\} \subseteq F^\circ$ and $\mathbf{v} \in \texttt{EFMs}^{\mathbf{RC}_D}$. If F' has dimension at least two, then not any facet of F' has its interior included in F°, because if it was the case for one facet, then any vector of its interior would have its support strictly included in the support of $\mathbf{v}$, which would contradict the minimality of the latter. Finally, $\mathbf{v} \in \mathring{F'} \subseteq F^\circ$ and all vectors of $\mathring{F'}$ have same support than $\mathbf{v}$ and belong to $\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D}$. We have thus characterized both $\texttt{EFMs}^{\mathbf{RC}_D}$ and $\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D}$. Precise now, for the latter one, the decomposition of its vectors into $\mathbf{e}^k \in \texttt{EFMs}(F) \backslash \texttt{EFMs}^{\mathbf{RC}_D}$, in order to compute their supports, which is generally the only useful information (the precise decomposition into fluxes being often not so relevant). So, we consider a face F' of F, of dimension at least two, such that $\mathring{F'} \subseteq F^\circ$ but not any facet of F' has its interior included in F° and $\{\mathbf{e}^k\}_{1 \leq k \leq N}$ a minimal set of vectors in $\texttt{EFMs}(F')$ such that $supp(\mathring{F'}) = \bigcup_{1 \leq k \leq N} supp(\mathbf{e}^k)$. Note that, for any k, $supp(\mathbf{e}^k) \cap I \neq \emptyset$ (and, as we have seen, $I \nsubseteq supp(\mathbf{e}^k)$), because if for some k_0 we had $supp(\mathbf{e}^{k_0}) \cap I = \emptyset$, then $\mathbf{e}^{k_0}$ would belong to all hyperplanes of equation $\mathbf{v}_i = 0$ for $i \in I$, thus to all facets of F', which is impossible for a non-null vector. Let note $S_1 = supp(\mathbf{e}^1) \cap I$ and, for any k, $2 \leq k \leq N$, $S_k = (supp(\mathbf{e}^k) \cap I) \backslash \bigcup_{1 \leq j \leq k-1} S_j$. Then, for any k, $S_k \neq \emptyset$, because if for some k_0 we had $S_{k_0} = \emptyset$, then the vectors of $cone^+_{\oplus}(\{\mathbf{e}^k\}_{k \neq k_0})$ would verify the constraint $\mathbf{RC}_D$ and have their supports included in $supp(\mathring{F'})$, thus equal to it as it is minimal for vectors in $CFC_{\mathbf{RC}_D \leq \boldsymbol{\eta}}$, which would contradict the minimality of $\{\mathbf{e}^k\}$. Finally, as by construction $I = \bigcup_{1 \leq k \leq N} S_k$ and $S_k \cap S_j = \emptyset$ for $k \neq j$, we obtain that $\{S_k\}_{1 \leq k \leq N}$ constitutes a partition of I and $\{\mathbf{e}^k\}_{1 \leq k \leq N}$ is a set of vectors in $\texttt{EFMs}(F') \subseteq \texttt{EFMs}(F) \backslash \texttt{EFMs}^{\mathbf{RC}_D}$ such that $supp(\mathbf{e}^k) \supseteq S_k$ by construction and $supp(\mathbf{e}^k) \nsupseteq S_j$ for any $j \neq k$, otherwise, by the same reasoning that above, $\mathbf{e}^j$ could be suppressed from the set $\{\mathbf{e}^k\}$, contradicting its minimality. Finally, we obtain that the support of any vector in $\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D}$ can be written as $\bigcup_{1 \leq k \leq N} supp(\mathbf{e}^k)$, where $\{\mathbf{e}^k\}_{1 \leq k \leq N}$, $N \geq 2$, are vectors in $\texttt{EFMs}(F)$ verifying $supp(\mathbf{e}^k) \supseteq S_k$ and $supp(\mathbf{e}^k) \nsupseteq S_j$ for all k and $j \neq k$, where $\{S_k\}_{1 \leq k \leq N}$ is a partition of I (note that we have the analog result for $\texttt{EFMs}^{\mathbf{RC}_D}$ by taking $N = 1$). Now, a given $\bigcup_{1 \leq k \leq N} supp(\mathbf{e}^k)$ is not necessarily minimal among the whole collection when $N, \{\mathbf{e}^k\}$ and $\{S_k\}$ are varying. It is also possible that it strictly contains the support of a vector in $\texttt{EFMs}^{\mathbf{RC}_D}$. So, to obtain exactly the supports of vectors in $\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D}$, one has to keep only the minimal elements for inclusion w.r.t. the whole collection extended by $supp(\texttt{EFMs}^{\mathbf{RC}_D})$.

Example 2.4. Continue with the network of Examples 2.1 and 2.3, so $D = T1 \wedge T4$ and $\boldsymbol{\eta} = +$. We have $\texttt{EFMs}(F) = \{\mathbf{e}^1, \mathbf{e}^2, \mathbf{e}^3\}$ and $\texttt{EFMs}^{\mathbf{RC}_D} = \{\mathbf{e}^1\}$. The only partition of $\{T1, T4\}$ with size ≥ 2 is given by: $S_1 = \{T1\}$ and $S_2 = \{T4\}$. The only vector in $\texttt{EFMs}(F)$ whose support contains S_1 and not S_2 is $\mathbf{e}^2$ and the only one whose support contains S_2 and not S_1 is $\mathbf{e}^3$. Thus, $supp(\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D}) = \{supp(\mathbf{e}^2) \cup supp(\mathbf{e}^3)\} = \{\{T1, T3\} \cup \{T2, T4\}\} = \{\{T1, T2, T3, T4\}\}$. Actually, we have from Example 2.3: $\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D} = \{\mathbf{e}^2 + \lambda \mathbf{e}^3 \mid \lambda > 0\}$.

Consider now the following network made up of two internal metabolites and seven irreversible reactions, whose six are transfer reactions, $R : A \to B$, $T1 :\to A$, $T2 : B \to$, $T3 :\to A$, $T4 : B \to$, $T5 : A \to$, $T6 :\to B$. Let $D = T1 \wedge T2$ and $\boldsymbol{\eta} = +$. Let $\mathbf{e}^1 = (1,1,1,0,0,0,0)^T$ made up of $T1, R, T2$, $\mathbf{e}^2 = (1,0,0,1,1,0,0)^T$ made up of $T3, R, T4$, $\mathbf{e}^3 = (1,1,0,0,1,0,0)^T$ made up of $T1, R, T4$, $\mathbf{e}^4 = (1,0,1,1,0,0,0)^T$ made up of $T3, R, T2$, $\mathbf{e}^5 = (0,1,0,0,0,1,0)^T$ made up of $T1, T5$, $\mathbf{e}^6 = (0,0,0,1,0,1,0)^T$ made up of $T3, T5$, $\mathbf{e}^7 = (0,0,1,0,0,0,1)^T$ made up of $T6, T2$ and $\mathbf{e}^8 = (0,0,0,0,1,0,1)^T$ made up of $T6, T4$. We have $\texttt{EFMs}(F) = \{\mathbf{e}^1, \mathbf{e}^2, \mathbf{e}^3, \mathbf{e}^4, \mathbf{e}^5, \mathbf{e}^6, \mathbf{e}^7, \mathbf{e}^8\}$ and $\texttt{EFMs}^{\mathbf{RC}_D} = \{\mathbf{e}^1\}$. The only partition of $\{T1, T2\}$ with size ≥ 2 is given by: $S_1 = \{T1\}$ and $S_2 = \{T2\}$. The vectors in $\texttt{EFMs}(F)$ whose support contains S_1 and not S_2 are $\mathbf{e}^3$ and $\mathbf{e}^5$ and those whose support contains S_2 and not S_1 are $\mathbf{e}^4$ and $\mathbf{e}^7$. We have $supp(\mathbf{e}^3) \cup supp(\mathbf{e}^4) = \{R, T1, T2, T3, T4\}$, $supp(\mathbf{e}^3) \cup supp(\mathbf{e}^7) = \{R, T1, T2, T4, T6\}$, $supp(\mathbf{e}^5) \cup supp(\mathbf{e}^4) = \{R, T1, T2, T3, T5\}$ and $supp(\mathbf{e}^5) \cup supp(\mathbf{e}^7) = \{T1, T2, T5, T6\}$. Each one of those four supports obtained is minimal in this collection, but the three first contain $supp(\mathbf{e}^1) = \{R, T1, T2\}$. Thus, $supp(\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D}) = \{\{T1, T2, T5, T6\}\}$. Actually, $\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D} = \{\mathbf{e}^5 + \lambda \mathbf{e}^7 \mid \lambda > 0\}$.
Consider finally another network made up of three internal metabolites and seven irreversible reactions, whose five are transfer reactions, $R1 : B \to A$, $R2 : C \to A$, $T1 :\to B + C$, $T2 : A \to$, $T3 : A \to$, $T4 :\to B$, $T5 :\to C$. Let $D = R1 \wedge R2 \wedge T2$ and $\boldsymbol{\eta} = +$. Let $\mathbf{e}^1 = (1,1,1,2,0,0,0)^T$ made up of $T1, R1, R2, T2$, $\mathbf{e}^2 = (1,1,1,0,2,0,0)^T$ made up of $T1, R1, R2, T3$, $\mathbf{e}^3 = (1,0,0,1,0,1,0)^T$ made up of $T4, R1, T2$, $\mathbf{e}^4 = (1,0,0,0,1,1,0)^T$ made up of $T4, R1, T3$, $\mathbf{e}^5 = (0,1,0,1,0,0,1)^T$ made up of $T5, R2, T2$ and $\mathbf{e}^6 = (0,1,0,0,1,0,1)^T$ made up of $T5, R2, T3$. We have $\texttt{EFMs}(F) = \{\mathbf{e}^1, \mathbf{e}^2, \mathbf{e}^3, \mathbf{e}^4, \mathbf{e}^5, \mathbf{e}^6\}$ and $\texttt{EFMs}^{\mathbf{RC}_D} = \{\mathbf{e}^1\}$. There are four partitions of $\{R1, R2, T2\}$ with size ≥ 2. The partition $\{\{R1\}, \{R2\}, \{T2\}\}$ gives nothing because because there is no vector in $\texttt{EFMs}(F)$ whose support contains $\{T2\}$ but neither $\{R1\}$ nor $\{R2\}$. For the partition $S_1 = \{R1, T2\}$ and $S_2 = \{R2\}$, the vector in $\texttt{EFMs}(F)$ whose support contains S_1 and not S_2 is $\mathbf{e}^3$ and those whose support contains S_2 and not S_1 are $\mathbf{e}^2$, $\mathbf{e}^5$ and $\mathbf{e}^6$, providing $supp(\mathbf{e}^3) \cup supp(\mathbf{e}^2) = \{R1, R2, T1, T2, T3, T4\}$, $supp(\mathbf{e}^3) \cup supp(\mathbf{e}^5) = \{R1, R2, T2, T4, T5\}$ and $supp(\mathbf{e}^3) \cup supp(\mathbf{e}^6) = \{R1, R2, T2, T3, T4, T5\}$. For the partition $S_1 = \{R2, T2\}$ and $S_2 = \{R1\}$, the vector in $\texttt{EFMs}(F)$ whose support contains S_1 and not S_2 is $\mathbf{e}^5$ and those whose support contains S_2 and not S_1 are $\mathbf{e}^2$, $\mathbf{e}^3$ and $\mathbf{e}^4$, providing $supp(\mathbf{e}^5) \cup supp(\mathbf{e}^2) = \{R1, R2, T1, T2, T3, T5\}$, $supp(\mathbf{e}^5) \cup supp(\mathbf{e}^3) = \{R1, R2, T2, T4, T5\}$ and $supp(\mathbf{e}^5) \cup supp(\mathbf{e}^4) = \{R1, R2, T2, T3, T4, T5\}$. For the partition $S_1 = \{R1, R2\}$ and $S_2 = \{T2\}$, the vector in $\texttt{EFMs}(F)$ whose support contains S_1 and not S_2 is $\mathbf{e}^2$ and those whose support contains S_2 and not S_1 are $\mathbf{e}^3$ and $\mathbf{e}^5$, providing $supp(\mathbf{e}^2) \cup supp(\mathbf{e}^3) = \{R1, R2, T1, T2, T3, T4\}$ and $supp(\mathbf{e}^2) \cup supp(\mathbf{e}^5) = \{R1, R2, T1, T2, T3, T5\}$. The minimal elements of this collection of supports are $\{R1, R2, T1, T2, T3, T4\}$, $\{R1, R2, T1, T2, T3, T5\}$ and $\{R1, R2, T2, T4, T5\}$. Minimizing also w.r.t. $supp(\mathbf{e}^1) = \{R1, R2, T1, T2\}$ gives thus $supp(\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D}) = \{\{R1, R2, T2, T4, T5\}\}$. Actually, $\texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D} = \{\mathbf{e}^3 + \lambda \mathbf{e}^5 \mid \lambda > 0\}$.

We have thus proved the following result.

Proposition 2.12. *Given a Boolean constraint D as a conjunction of literals $\bigwedge_{i \in I} \boldsymbol{v}_i \wedge \bigwedge_{j \in J} \neg \boldsymbol{v}_j$ and an arbitrary closed r-orthant $O_{\leq \boldsymbol{\eta}}$, let $CFC_{\boldsymbol{RC}_D \leq \boldsymbol{\eta}}$ be the constrained flux cone subset for the regulatory constraint $\boldsymbol{RC}_D$ in $O_{\leq \boldsymbol{\eta}}$. Let F be the face of the flux cone $FC_{\leq \boldsymbol{\eta}}$ defined as $FC_{\leq \boldsymbol{\eta}} \cap \bigcap_{j \in J} \{\boldsymbol{v}_j = 0\}$, then $CFC_{\boldsymbol{RC}_D \leq \boldsymbol{\eta}}$ is the semi-open flux cone F° obtained from F by removing its facets $\{\boldsymbol{v}_i = 0\}$ for all $i \in I$. Let $\texttt{EFMs}^{\boldsymbol{RC}_D} \triangleq \texttt{EFMs}(FC_{\leq \boldsymbol{\eta}}) \cap Sol_{\boldsymbol{RC}_D}$ be the $\texttt{EFMs}$ of $FC_{\leq \boldsymbol{\eta}}$ that satisfy $\boldsymbol{RC}_D$ and $\texttt{EFMs}_{\boldsymbol{RC}_D} \triangleq \texttt{EFMs}(CFC_{\boldsymbol{RC}_D \leq \boldsymbol{\eta}})$ be the $\texttt{EFMs}$ of $CFC_{\boldsymbol{RC}_D \leq \boldsymbol{\eta}}$. We have $\texttt{EFMs}^{\boldsymbol{RC}_D} \subseteq \texttt{EFMs}_{\boldsymbol{RC}_D}$ but, unlike the case of thermodynamic and kinetic (as in Proposition 2.8) constraints, there is in general no longer identity between $\texttt{EFMs}^{\boldsymbol{RC}_D}$ and $\texttt{EFMs}_{\boldsymbol{RC}_D}$. $\texttt{EFMs}^{\boldsymbol{RC}_D}$ correspond to the extreme rays (faces of dimension one) of F°, i.e., the edges of F whose support contains I. $\texttt{EFMs}_{\boldsymbol{RC}_D} \backslash \texttt{EFMs}^{\boldsymbol{RC}_D}$ are the vectors of the $\mathring{F'}$'s for all F' faces of F of dimension at least two, such that $\mathring{F'} \subseteq F^\circ$ (i.e., F' is not included in any hyperplane $\{\boldsymbol{v}_i = 0\}$ with $i \in I$) but not any facet of F' has its interior included in F° (i.e., any facet of F' is included in some hyperplane $\{\boldsymbol{v}_i = 0\}$ with $i \in I$). The supports of those vectors in $\texttt{EFMs}_{\boldsymbol{RC}_D} \backslash \texttt{EFMs}^{\boldsymbol{RC}_D}$ are obtained as $\bigcup_{1 \leq k \leq N} supp(\boldsymbol{e}^k)$, where $\{\boldsymbol{e}^k\}_{1 \leq k \leq N}$, $N \geq 2$, are vectors in $\texttt{EFMs}(F)$ verifying $supp(\boldsymbol{e}^k) \supseteq S_k$ and $supp(\boldsymbol{e}^k) \not\supseteq S_j$ for all k and $j \neq k$, where $\{S_k\}_{1 \leq k \leq N}$ is a partition of I (they are actually the minimal elements, for subset inclusion and including the supports of $\texttt{EFMs}^{\boldsymbol{RC}_D}$ when checking minimality, obtained like this, for any partition $\{S_k\}$ of I, of size at least two, and any choice of $\{\boldsymbol{e}^k\}$ as above).*

This characterization of $\texttt{EFMs}$ for flux cones in presence of regulatory constraints does not hold so

simply for flux polyhedra, as the added inhomogeneous linear constraints **ILC**, given by $\mathbf{Gv} \geq \mathbf{h}$, may not respect at all the structure of $CFC_{\mathbf{RC}_{Bc}}$ and cut the interior of flux cones: we already mentioned that there is no direct relationship between EFMs and extreme or elementary fluxes for a flux polyhedron. Nevertheless, the ideas developed above for flux cones can be applied to flux polyhedra $FP_{\leq \boldsymbol{\eta}}$, when some of their faces have to be removed due to the constraint D because they are included in some hyperplane $\{\mathbf{v}_i = 0\}$, in order to determine $\mathtt{EFMs}_{\mathbf{RC}_D} \backslash \mathtt{EFMs}^{\mathbf{RC}_D}$. And in practice, **ILC** is generally only used to bound (below and/or above) fluxes, in what case each extremal ray $\mathbb{R}_+\mathbf{e}$ of $FC_{\leq \boldsymbol{\eta}}$ is still partly present in $FP_{\leq \boldsymbol{\eta}}$ as for example an edge $[\alpha^-, \alpha^+]\mathbf{e}$, defined by the two vertices $\alpha^-\mathbf{e}$ and $\alpha^+\mathbf{e}$. The results above can then be transposed by using convex-conformal decomposition into vertices and conformal decomposition into elementary vectors to characterize $\mathtt{EFMs}_{\mathbf{RC}_D} \backslash \mathtt{EFMs}^{\mathbf{RC}_D}$.

We are now interested in looking for vectors in the solutions space that are (in some sense) non-decomposable while being not support-minimal and in characterizing them, if any. We could think to EFVs (or EFPs) but, from the study above about EFMs, we note that, for a flux cone constrained by the regulatory constraint D, the vectors in $\mathtt{EFMs}_{\mathbf{RC}_D} \backslash \mathtt{EFMs}^{\mathbf{RC}_D}$ are necessarily conformally decomposable, as they can be described as the interiors of polyhedral cones in $O_{\leq \boldsymbol{\eta}}$ of dimension at least two (for example, $\mathbf{e}^2 + \mathbf{e}^3$ in Example 2.3 can be decomposed as $(0.7\mathbf{e}^2 + 0.3\mathbf{e}^3) + (0.3\mathbf{e}^2 + 0.7\mathbf{e}^3)$). More straightforwardly, it can be noticed that $\mathtt{EFMs}^{\mathbf{RC}_D}$ is equal to $\mathtt{EFVs}(CFC_{\mathbf{RC}_D \leq \boldsymbol{\eta}})$, i.e., precisely the conformally non-decomposable vectors. As already pointed, more than the precise decomposition into fluxes, what matters is the decomposition of the supports (in the decomposition above of $\mathbf{e}^2 + \mathbf{e}^3$, the supports of the components are unchanged, i.e., equal to $supp(\mathbf{e}^2) \cup supp(\mathbf{e}^3) = \{T1, T2, T3, T4\}$). It follows that a relevant concept for a solution vector is not to be conformally non-decomposable but, less strictly, to be (conformally) support-wise non-decomposable, in the sense that the vector cannot be conformally decomposed into two vectors of different (necessarily not greater) supports. Now, for all faces F' of F of dimension at least two such that $\mathring{F}' \subseteq F^\circ$, not covered by Proposition 2.12, i.e., owning at least one facet F'' with interior included in F°, it happens that all vectors of $\mathring{F}'$ are actually support-wise decomposable, as such a vector can always be decomposed in a vector of $\mathring{F}'$ of same support and a vector of $\mathring{F}''$ of smaller support. This leads to not authorize in the decomposition to get the same support for one of the component vectors. Thus the really relevant (less strict) concept is when the vector cannot be conformally decomposed into two vectors of smaller supports and we call a nonzero vector $\mathbf{x}$ of a convex polyhedral cone C as (conformally) support-wise non-strictly-decomposable, if

$$\mathbf{x} = \mathbf{x}^1 \oplus \mathbf{x}^2, \text{ with nonzero } \mathbf{x}^1, \mathbf{x}^2 \in C, \text{ implies } supp(\mathbf{x}^1) = supp(\mathbf{x}) \text{ or } supp(\mathbf{x}^2) = supp(\mathbf{x}). \tag{83}$$

The (conformally) support-wise non-strictly-decomposable vectors in C (resp., flux vectors in a flux cone FC) will be noted swNSDVs (resp., swNSDFVs). Note that we could have defined support-wise non-strictly-decomposability more generally without imposing a conformal decomposition, but, as already pointed out, this is not relevant for biological fluxes and we will use this concept only in some tope. It is obvious that, in such a tope (resp., flux tope), $\mathtt{EMs}, \mathtt{ExVs} = \mathtt{EVs} \subseteq \mathtt{swNSDVs}$ (resp., $\mathtt{ExVs} = \mathtt{EFVs} = \mathtt{EFMs} = \mathtt{swNSDFVs}$, so that all four definitions coincide). We will introduce the analog definition with convex combination for a polyhedron P (the previous definition and the associated relationships above being valid for its recession cone C_P). A vector $\mathbf{x}$ of a polyhedron P is called support-wise convex(-conformally) non-strictly-decomposable, if

$$\mathbf{x} = \lambda\mathbf{x}^1 \oplus (1-\lambda)\mathbf{x}^2, \text{ with } \mathbf{x}^1, \mathbf{x}^2 \in P,\ 0 < \lambda < 1, \text{ implies } supp(\mathbf{x}^1) = supp(\mathbf{x}) \text{ or } supp(\mathbf{x}^2) = supp(\mathbf{x}). \tag{84}$$

The support-wise convex(-conformally) non-strictly-decomposable vectors in P (resp., flux vectors in a flux polyhedron FP) will be called support-wise non-strictly-decomposable points (resp., support-wise non-strictly-decomposable flux points) and noted swNSDPs (resp., swNSDFPs). It is obvious that, in any given tope (resp., flux tope), $\mathtt{EMs}, \mathtt{ExPs} = \mathtt{EPs} \subseteq \mathtt{swNSDPs}$ (resp., $\mathtt{EFMs}, \mathtt{ExPs} = \mathtt{EFPs} \subseteq \mathtt{swNSDFPs}$).

With the notations of Proposition 2.12, let $\mathtt{swNSDFVs}^{\mathbf{RC}_D} \triangleq \mathtt{swNSDFVs}(FC_{\leq \boldsymbol{\eta}}) \cap Sol_{\mathbf{RC}_D}$ and $\mathtt{swNSDFVs}_{\mathbf{RC}_D} \triangleq \mathtt{swNSDFVs}(CFC_{\mathbf{RC}_D \leq \boldsymbol{\eta}})$. We have thus $\mathtt{EFMs}^{\mathbf{RC}_D} = \mathtt{swNSDFVs}^{\mathbf{RC}_D} \subseteq \mathtt{EFMs}_{\mathbf{RC}_D} \subseteq \mathtt{swNSDFVs}_{\mathbf{RC}_D}$ but, unlike the case of thermodynamic and kinetic (as in Proposition 2.8) constraints for flux cones, not only there is no longer identity between $\mathtt{swNSDFVs}^{\mathbf{RC}_D}$ and $\mathtt{swNSDFVs}_{\mathbf{RC}_D}$ (consequence of the non-identity between $\mathtt{EFMs}^{\mathbf{RC}_D}$ and $\mathtt{EFMs}_{\mathbf{RC}_D}$), but we will see now that there is in general no longer identity between $\mathtt{EFMs}_{\mathbf{RC}_D}$ and $\mathtt{swNSDFVs}_{\mathbf{RC}_D}$.

Example 2.5. Continuing with the network of Example 2.3, $\mathbf{e}^1 + \mathbf{e}^3 \in \texttt{swNSDFVs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}_{\mathbf{RC}_D}$. More precisely, we have (by considering only a representative for each ray) $\texttt{EFMs}^{\mathbf{RC}_D} = \texttt{swNSDFVs}^{\mathbf{RC}_D} = \{\mathbf{e}^1\} \subset \texttt{EFMs}_{\mathbf{RC}_D} = \texttt{EFMs}^{\mathbf{RC}_D} \cup \{\mathbf{e}^2 + \lambda \mathbf{e}^3 \mid \lambda > 0\} \subset \texttt{swNSDFVs}_{\mathbf{RC}_D} = \texttt{EFMs}_{\mathbf{RC}_D} \cup \{\mathbf{e}^1 + \lambda \mathbf{e}^2 \mid \lambda > 0\} \cup \{\mathbf{e}^1 + \lambda \mathbf{e}^3 \mid \lambda > 0\}$.

So, we extend the results of Proposition 2.12 by precising the structure in F of $\texttt{swNSDFVs}_{\mathbf{RC}_D}$. Let $\{\mathbf{e}^m\}_{m \in M}$ be representatives of the extreme vectors of F, thus $F = cone_{\oplus}(\{\mathbf{e}^m\}_{m \in M})$. We have the following structure for F° regarding $\texttt{EFMs}$ and $\texttt{swNSDFVs}$, given in the form of an algorithm:

- Let $R = \{m \in M \mid \mathbf{e}^m \in F^\circ\} = \{m \in M \mid \forall i \in I \ \mathbf{e}^m_i \neq 0\}$. Then $\texttt{EFMs}^{\mathbf{RC}_D} = \{\mathbf{e}^m\}_{m \in R}$. Note that R can vary from $\emptyset$ to M, thus $\texttt{EFMs}^{\mathbf{RC}_D}$ from $\emptyset$ to $\texttt{EFMs}(F)$. If $R = M$, then $\texttt{EFMs}^{\mathbf{RC}_D} = \texttt{EFMs}_{\mathbf{RC}_D} = \texttt{swNSDFVs}_{\mathbf{RC}_D} = \texttt{EFMs}(F)$ and the analysis is done (if $\boldsymbol{\eta}$ has been chosen maximal in $sign(CFC_{\mathbf{RC}_D})$, this case corresponds to $I = \emptyset$). We consider in the following the case $R \subset M$.

- Let consider successively all faces F' of F of dimension at least two, such that $\mathring{F}' \subseteq F^\circ$, i.e., F' is not included in any hyperplane $\{\mathbf{v}_i = 0\}$ with $i \in I$ (the lattice of faces of F can be explored for example in a way such that a sub-face is visited before a super-face; once such a F' has been found, all faces of F containing it suit also). Let $\{\mathbf{e}^k\}_{k \in K}$, with $K \subseteq M$, be representatives of the extreme vectors of F'. Thus $\mathring{F}' = cone^+_{\oplus}(\{\mathbf{e}^k\}_{k \in K})$ and $I \subseteq \bigcup_{k \in K} supp(\mathbf{e}^k)$. For each of those F', three exclusive cases are now distinguished.

- If not any facet of F' has its interior included in F°, i.e., any facet of F' is included in some hyperplane $\{\mathbf{v}_i = 0\}$ with $i \in I$ (a necessary but not sufficient condition is: $K \subseteq M \backslash R$), then $\mathring{F}' \subseteq \texttt{EFMs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}^{\mathbf{RC}_D}$ and $\bigcup_{k \in K} supp(\mathbf{e}^k)$ is a minimal support for vectors in F°.

- If exactly one facet of F' has its interior included in F°, i.e., it is the only facet not included in any hyperplane $\{\mathbf{v}_i = 0\}$ with $i \in I$, then $\mathring{F}' \subseteq \texttt{swNSDFVs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}_{\mathbf{RC}_D}$ and $\bigcup_{k \in K} supp(\mathbf{e}^k)$ is a non-strictly-decomposable non-minimal support for vectors in F° (non-strictly-decomposable support means that any vector which is a conical sum of the $\mathbf{e}^k$'s having this support is support-wise non-strictly-decomposable, independently of the choice of the nonnegative coefficients fixing the contribution of each $\mathbf{e}^k$ in the distribution of the fluxes). This result follows immediately from the facts that one facet is not enough to decompose some vector in $\mathring{F}'$ strictly in terms of supports and that the support of the vectors in the interior of the facet in question is strictly included in the support of the vectors in $\mathring{F}'$.

- If at least two facets of F' have their interior included in F°, i.e., those facets are not included in any hyperplane $\{\mathbf{v}_i = 0\}$ with $i \in I$, then let $\{\mathbf{e}^l\}_{l \in L}$, with $L \subseteq K$, be representatives of the extreme vectors of all those facets (note that we have then necessarily $K \cap R \subseteq L$, i.e., $K \backslash L \subseteq K \backslash R$). Thus, the strict conical sum of the interiors of those facets, which is equal to $cone^+_{\oplus}(\{\mathbf{e}^l\}_{l \in L})$, is not empty in $\mathring{F}'$ (as there are at least two such facets) and is made up of the support-wise strictly-decomposable vectors of $\mathring{F}'$ (by construction): $cone^+_{\oplus}(\{\mathbf{e}^l\}_{l \in L}) = \mathring{F}' \backslash \texttt{swNSDFVs}_{\mathbf{RC}_D}$. Two subcases have thus to be distinguished.

- If $L = K$, i.e., the strict conical sum of the interiors of those facets is equal to $\mathring{F}'$, then $\mathring{F}' \subseteq F^\circ \backslash \texttt{swNSDFVs}_{\mathbf{RC}_D}$ and $\bigcup_{k \in K} supp(\mathbf{e}^k)$ is a strictly-decomposable support for vectors in F° (which means that any vector which is a conical sum of the $\mathbf{e}^k$'s having this support is support-wise strictly-decomposable, independently of the choice of the nonnegative coefficients fixing the contribution of each $\mathbf{e}^k$ in the distribution of the fluxes).

- If $L \subset K$, then $\mathring{F}'$ is split into two nonempty subsets: $cone^+_{\oplus}(\{\mathbf{e}^l\}_{l \in L}) \subseteq F^\circ \backslash \texttt{swNSDFVs}_{\mathbf{RC}_D}$ and $\mathring{F}' \backslash cone^+_{\oplus}(\{\mathbf{e}^l\}_{l \in L}) \subseteq \texttt{swNSDFVs}_{\mathbf{RC}_D} \backslash \texttt{EFMs}_{\mathbf{RC}_D}$ (note that $\mathring{F}' \backslash cone^+_{\oplus}(\{\mathbf{e}^l\}_{l \in L})$ is made up of those vectors of $cone^+_{\oplus}(\{\mathbf{e}^k\}_{k \in K})$ the conical decomposition of which on the $\mathbf{e}^k$'s requires at least one $\mathbf{e}^k$ with $k \in K \backslash L$). This means that part of the vectors of $\mathring{F}'$ are support-wise non-strictly-decomposable and part are support-wise strictly-decomposable, while having the same non-minimal support $\bigcup_{k \in K} supp(\mathbf{e}^k)$ (still equal to $\bigcup_{l \in L} supp(\mathbf{e}^l)$). This proves that support-wise strict decomposability does not depend in general only on the support but on the positive values of the fluxes and that, unlike the particular cases above, one cannot speak of a strictly-decomposable

or non-strictly-decomposable support. Note that, in this subcase, the support-wise non-strictly-decomposable vectors of $\mathring{F}'$ constitute the complementary in the open cone $cone^+_\oplus(\{\mathbf{e}^k\}_{k\in K})$ of the open sub-cone $cone^+_\oplus(\{\mathbf{e}^l\}_{l\in L})$, which is thus a finite disjoint union of semi-open cones, each of which is conically generated (with positive or nonnegative coefficients according to faces that are present or not) by extreme vectors $\mathbf{e}^k$'s with either $k \in K\backslash L \subseteq K\backslash R$ or $k \in L\backslash R$, thus in any case with $k \in K\backslash R$, i.e., by extreme vectors $\mathbf{e}^k \in \mathtt{EFMs}(F)\backslash\mathtt{EFMs}^{\mathbf{RC}_D}$.

We have finally proved the following result (keeping the notations of Proposition 2.12).

Proposition 2.13. *Let* $\mathtt{swNSDFVs}_{\boldsymbol{RC}_D} \triangleq \mathtt{swNSDFVs}(CFC_{\boldsymbol{RC}_D\,\leq\boldsymbol{\eta}})$ *be the support-wise non-strictly-decomposable vectors of* $CFC_{\boldsymbol{RC}_D\,\leq\boldsymbol{\eta}}$. *We have* $\mathtt{EFMs}_{\boldsymbol{RC}_D} \subseteq \mathtt{swNSDFVs}_{\boldsymbol{RC}_D}$ *but, unlike the case of thermodynamic and kinetic (as in Proposition 2.8) constraints, there is in general no longer identity between* $\mathtt{EFMs}_{\boldsymbol{RC}_D}$ *and* $\mathtt{swNSDFVs}_{\boldsymbol{RC}_D}$. *Consider all faces* F' *of* F *of dimension at least two, such that* $\mathring{F}' \subseteq F^\circ$ *(i.e.,* F' *is not included in any hyperplane* $\{\boldsymbol{v}_i = 0\}$ *with* $i \in I$*), and let* $D(F') \triangleq cone^+_\oplus(\{\mathring{F}'' \mid F''$ *facet of* F' *with* $\mathring{F}'' \subseteq F^\circ\})$. *Result of Proposition 2.12 can be stated as:* $\mathtt{EFMs}_{\boldsymbol{RC}_D}\backslash\mathtt{EFMs}^{\boldsymbol{RC}_D} = \bigcup_{\{F'|D(F')=\emptyset\}} \mathring{F}'$. *Now we have:* $\mathtt{swNSDFVs}_{\boldsymbol{RC}_D}\backslash\mathtt{EFMs}_{\boldsymbol{RC}_D} = \bigcup_{\{F'|\emptyset\neq D(F')\not\subseteq\mathring{F}'\}} \mathring{F}'\backslash D(F')$. *Note that the* F'*'s considered here own necessarily at least one facet* F'' *that is not included in any hyperplane* $\{\boldsymbol{v}_i = 0\}$ *with* $i \in I$. *There are actually two cases. For those* F'*'s which own exactly one such facet* F'' *(thus* $D(F') = \mathring{F}'' \subseteq F^\circ\backslash\mathring{F}'$*), one obtains* $\mathring{F}' \subseteq \mathtt{swNSDFVs}_{\boldsymbol{RC}_D}\backslash\mathtt{EFMs}_{\boldsymbol{RC}_D}$ *and the common support of vectors in* $\mathring{F}'$ *is thus a non-strictly-decomposable (independently of the choice of the distribution of the fluxes) non-minimal support for vectors in* F°. *For those* F'*'s which own at least two such facets* F''*, one obtains* $\mathring{F}'\backslash D(F') \subseteq \mathtt{swNSDFVs}_{\boldsymbol{RC}_D}\backslash\mathtt{EFMs}_{\boldsymbol{RC}_D}$ *and* $D(F') \subseteq F^\circ\backslash\mathtt{swNSDFVs}_{\boldsymbol{RC}_D}$*, thus part of the vectors of* $\mathring{F}'$ *(consisting of a finite disjoint union of semi-open cones) are support-wise non-strictly-decomposable and part (consisting of an open cone) are support-wise strictly-decomposable (depending on the choice of the distribution of the fluxes), while having the same non-minimal support.*

Example 2.6. Consider the simple network made of one reaction $R : A \to B$, where A and B are the two internal metabolites, and four transfer reactions $T1 : \to A$, $T2 : \to A$, $T3 : B \to$, $T4 : B \to$, and assume the five reactions irreversible. FC is a pointed cone of dimension 3 included in the positive orthant of $\mathbb{R}^5$ with axes $\{T1, T2, T3, R, T4\}$ in this order. We have: $FC = \{(x\ y\ z\ x+y\ x+y-z)^T \mid x, y, z \geq 0, x+y \geq z\}$. FC has 4 facets and 4 extremal rays. Representatives of those extremal rays ($\mathtt{EFMs}$) are: $\mathbf{e}^1 = (1\ 0\ 1\ 1\ 0)^T$, $\mathbf{e}^2 = (1\ 0\ 0\ 1\ 1)^T$, $\mathbf{e}^3 = (0\ 1\ 1\ 1\ 0)^T$ and $\mathbf{e}^4 = (0\ 1\ 0\ 1\ 1)^T$, defined by their supports: $\{T1, R, T3\}$, $\{T1, R, T4\}$, $\{T2, R, T3\}$ and $\{T2, R, T4\}$, respectively.
Consider the Boolean constraint $D = T1 \wedge T3$. Then $F = FC$ and the solutions space is the semi-open cone $CFC_{\mathbf{RC}_D} = F^\circ = \{(x\ y\ z\ x+y\ x+y-z)^T \mid x, z > 0, y \geq 0, x+y \geq z\}$, i.e., F without its two facets $\{x = 0\}$ and $\{z = 0\}$.
The only $\mathtt{EFM}$ still present in F° is $\mathbf{e}^1$, thus $\mathtt{EFMs}^{\mathbf{RC}_D} = \{\mathbf{e}^1\}$ with support $\{T1, R, T3\}$.
There are two faces of F of dimension two with their interiors included in F°: $F'_1 = cone_\oplus(\{\mathbf{e}^1, \mathbf{e}^2\})$ and $F'_2 = cone_\oplus(\{\mathbf{e}^1, \mathbf{e}^3\})$, both with $\mathbb{R}_+\mathbf{e}^1$ as their only facet with interior included in F°. Thus $D(F'_1) = D(F'_2) = \mathbb{R}^*_+\mathbf{e}^1$, $\mathtt{EFMs}_{\mathbf{RC}_D}\backslash\mathtt{EFMs}^{\mathbf{RC}_D} = \emptyset$, $\mathring{F}'_1 = cone^+_\oplus(\{\mathbf{e}^1, \mathbf{e}^2\}) \subseteq \mathtt{swNSDFVs}_{\mathbf{RC}_D}\backslash\mathtt{EFMs}_{\mathbf{RC}_D}$ and $\mathring{F}'_2 = cone^+_\oplus(\{\mathbf{e}^1, \mathbf{e}^3\}) \subseteq \mathtt{swNSDFVs}_{\mathbf{RC}_D}\backslash\mathtt{EFMs}_{\mathbf{RC}_D}$. $\{T1, R, T3, T4\}$ and $\{T1, T2, R, T3\}$ are thus non-strictly-decomposable (independently of the respective values of the fluxes in $T3$ and $T4$ or in $T1$ and $T2$, respectively) non-minimal supports.
The last face of F with its interior included in F° is F itself, of dimension three, with $\mathring{F} = cone^+_\oplus(\{\mathbf{e}^1, \mathbf{e}^2, \mathbf{e}^3, \mathbf{e}^4\})$ defined in F° by $\{y > 0, x+y > z\}$. F has exactly two facets with their interiors included in F°, namely F'_1 and F'_2 and thus $D(F) = cone^+_\oplus(\{\mathbf{e}^1, \mathbf{e}^2, \mathbf{e}^3\}) = F^\circ\backslash\mathtt{swNSDFVs}_{\mathbf{RC}_D}$. Those support-wise strictly-decomposable vectors constitute the open sub-cone of $\mathring{F}$ defined by $\{y < z\}$: $F^\circ\backslash\mathtt{swNSDFVs}_{\mathbf{RC}_D} = \{(x\ y\ z\ x+y\ x+y-z)^T \mid x, y, z > 0, y < z < x+y\}$. They are the pathways of support $\{T1, T2, R, T3, T4\}$ such that the flux in $T2$ is smaller than the flux in $T3$. And actually any vector $(x\ y\ z\ x+y\ x+y-z)^T$ with $x, y, z > 0, y < z < x+y$ can be decomposed as: $(k(z-y)\mathbf{e}^1 + (x+y-z)\mathbf{e}^2) \oplus ((1-k)(z-y)\mathbf{e}^1 + y\mathbf{e}^3)$, with arbitrary k, $0 < k < 1$, i.e., into two support-wise non-strictly-decomposable vectors respectively in $\mathring{F}'_1$ with support $\{T1, R, T3, T4\}$ and in $\mathring{F}'_2$ with support $\{T1, T2, R, T3\}$.
We have thus $\mathring{F}\backslash D(F) = cone^+_\oplus(\{\mathbf{e}^2, \mathbf{e}^3, \mathbf{e}^4\}) \cup cone^+_\oplus(\{\mathbf{e}^2, \mathbf{e}^3\}) \subseteq \mathtt{swNSDFVs}_{\mathbf{RC}_D}\backslash\mathtt{EFMs}_{\mathbf{RC}_D}$. Those support-wise non-strictly-decomposable vectors constitute the semi-open sub-cone of $\mathring{F}$ defined by $\{z \leq$

$y\}$: $\{(x\ y\ z\ x+y\ x+y-z)^T \mid x,y,z>0, z\leq y\}$. They are the pathways of support $\{T1,T2,R,T3,T4\}$ such that the flux in $T2$ is not smaller than the flux in $T3$. Any vector $(x\ y\ z\ x+y\ x+y-z)^T$ with $x,y,z>0, z\leq y$ is equal to $x\mathbf{e}^2+z\mathbf{e}^3+(y-z)\mathbf{e}^4$ and belongs to $cone^+_\oplus(\{\mathbf{e}^2,\mathbf{e}^3,\mathbf{e}^4\})$ if $z<y$ and to $cone^+_\oplus(\{\mathbf{e}^2,\mathbf{e}^3\})$ if $z=y$.

But take care that, in case of a general constraint $Bc=\bigvee_k D_k$, if the support-wise strictly-decomposable vectors of each $CFC_{\mathbf{RC}_{D_k}\leq\boldsymbol{\eta}}$ are support-wise strictly-decomposable in $CFC_{\mathbf{RC}_{Bc}\leq\boldsymbol{\eta}}$, it is not true for support-wise non-scrictly-decomposable vectors and some of them for $CFC_{\mathbf{RC}_{D_k}\leq\boldsymbol{\eta}}$ may be decomposable in $CFC_{\mathbf{RC}_{Bc}\leq\boldsymbol{\eta}}$.

This characterization of support-wise non-strictly-decomposable vectors in presence of regulatory constraints does not extend directly from flux cones to flux polyhedra. The reason is that, due to inhomogeneous linear constraints **ILC**, some faces of $FP_{\leq\boldsymbol{\eta}}$ are not defined by equalities of the form $\mathbf{v}_k=0$ and thus play no role in the definition of the support of the vectors they contain. In consequence, the basis of the reasoning above, namely that the support of vectors of the interior of any face is larger than the supports of vectors of the interior of any facet of this face, does not hold. Nevertheless, the ideas developed for flux cones for dealing with faces included in some hyperplane $\{\mathbf{v}_i=0\}$ with $i\in I$, and thus removed from the solutions space due to the Boolean constraint considered, can be applied. But it is necessary at each step, when considering an arbitrary face, to distinguish its facets resulting from **ILC**, not involved in the definition of the support, its facets included in some hyperplane $\{\mathbf{v}_k=0\}$ with $k\notin I$ that are still present and contribute to the definition of the support, and its facets included in some hyperplane $\{\mathbf{v}_i=0\}$ with $i\in I$ that are removed.

2.2.4 Case of several types of constraints

In general, when analyzing a metabolic pathway, all known biological constraints will have to be taken into account together, typically kinetic constraints and regulatory constraints. For two such constraints $\mathbf{C}_1$ (say, a kinetic constraint **KC**, equivalent to **TC**, or $\mathbf{KC}^b$ in absence of bounds on enzymes concentrations, equivalent to $\mathbf{TC}^b$) and $\mathbf{C}_2$ (say a regulatory constraint $\mathbf{RC}_{Bc}$), the solutions space, in the case of a flux cone FC (the reasoning would be analog for a flux polyhedron FP) is given by: $CFC_{\mathbf{C}_1\wedge\mathbf{C}_2}=FC\cap Sol_{\mathbf{C}_1\wedge\mathbf{C}_2}=FC\cap Sol_{\mathbf{C}_1}\cap Sol_{\mathbf{C}_2}=CFC_{\mathbf{C}_1}\cap Sol_{\mathbf{C}_2}$. Now, from propositions 2.5 and 2.8, $CFC_{\mathbf{C}_1}$ is a finite union of flux cones FC_i, which are some particular faces of each flux tope of FC and the constraint $\mathbf{C}_2$ can be applied to each one as to an original flux cone: $CFC_{\mathbf{C}_1\wedge\mathbf{C}_2}=\bigcup_i FC_i\cap Sol_{\mathbf{C}_2}=\bigcup_i CFC_{i\ \mathbf{C}_2}$. From proposition 2.10, each $CFC_{i\ \mathbf{C}_2}$ is a disjoint union of particular open faces of FC_i. In total, the solutions space is a disjoint union of particular open faces of the flux topes of FC. From propositions above and proposition 2.11, one can also conclude that the `EFVs` of $CFC_{\mathbf{C}_1\wedge\mathbf{C}_2}$ are exactly the `EFVs` of FC that satisfy both constraints $\mathbf{C}_1$ and $\mathbf{C}_2$.

Proposition 2.14. *The space of flux vectors in FC (resp., FP) satisfying both the kinetic constraint **KC** (or $\mathbf{KC}^b$ in absence of bounds on enzymes concentrations) and the regulatory constraint $\mathbf{RC}_{Bc}$ is a finite disjoint union of open polyhedral cones (resp., open polyhedra) which are some open faces of the flux topes of FC (resp., FP). The elementary flux vectors (resp., elementary flux points and vectors) are those of FC (resp., FP) that satisfy both constraints.*

Note that proposition 2.13 applies also, by starting from each FC_i instead of each flux tope of FC, to determine elementary flux modes and support-wise non-strictly-decomposable vectors.

3 General case of sign-compatible constraints

We are interested in determining, for general biological constraints $\mathbf{C}$, what can be said about the structure of the solutions spaces $CFC_{\mathbf{C}}$ or $CFP_{\mathbf{C}}$. More precisely, in drawing some general features about constraints $\mathbf{C}$ (some of which present in particular in those biological constraints (41),(46),(49) we have considered so far), allowing one to clarify the mathematical and geometrical structure of the solutions space, to determine the `EFs` (conformal non-decomposable fluxes) or `EFMs` (support-minimal fluxes) for this space and if they characterize it and how to compute them efficiently, in particular by checking the integration of this computation into the DD method. We will focus on deducing interesting geometrical characteristics of the solutions space only from general properties of compatibility of the constraints with vectors signs (i.e., with vector supports in each closed r-orthant or flux tope) and we

will see that part of the results obtained above for thermodynamic, kinetic and regulatory constraints depends actually only on these general global properties.

3.1 Sign-based constraints

A constraint $\mathbf{C_x}(\mathbf{v})$ (resp., $\exists\mathbf{x}\mathbf{C_x}(\mathbf{v})$), is said sign-based if it depends only on $sign(\mathbf{v})$, i.e., on the signs of the $\mathbf{v}_i$'s but not on their values (in the formulas below, free variables are assumed universally quantified):

$$\mathbf{C_x}(\mathbf{v}), sign(\mathbf{v}') = sign(\mathbf{v}) \Rightarrow \mathbf{C_x}(\mathbf{v}') \tag{85}$$

$$\exists\mathbf{x}\mathbf{C_x}(\mathbf{v}), sign(\mathbf{v}') = sign(\mathbf{v}) \Rightarrow \exists\mathbf{x}\mathbf{C_x}(\mathbf{v}'). \tag{86}$$

It follows from these definitions that, if the $\mathbf{C_x}(\mathbf{v})$'s are sign-based for all $\mathbf{x}$, then $\exists\mathbf{x}\mathbf{C_x}(\mathbf{v})$ is sign-based. Note that the property for a constraint to be support-based (i.e., to depend only on $supp(\mathbf{v})$) is stronger than the property to be sign-based. Actually, in any flux tope (or in any given closed orthant), having the same sign for two vectors is equivalent to have the same support and thus being sign-based for a constraint means being support-based in each flux tope independently.

Example 3.1. It results then from definitions (40), (41) or (42) that the thermodynamic constraint $\mathbf{TC_M}(\mathbf{v})$ or $\underline{\mathbf{TC_M}}(\mathbf{v})$ is sign-based. This is thus also the case for $\mathbf{TC}(\mathbf{v})$ and $\underline{\mathbf{TC}}(\mathbf{v})$ (59) and for $\mathbf{TC}^b(\mathbf{v})$ and $\underline{\mathbf{TC}}^b(\mathbf{v})$ (60).
On the other hand, the kinetic constraint $\mathbf{KC_{E,M}}(\mathbf{v})$ (46) is not sign-based, and this is also the case of $\mathbf{KC_E}(\mathbf{v})$ (77), because $\kappa_i(\mathbf{M})$ is bounded below and above (e.g., for Michaelis-Menten kinetics, $-k_i^- < \kappa_i < k_i^+$) and thus, for a given nonzero enzyme concentration $\mathbf{E}_i$, there does not exist metabolites concentrations vector $\mathbf{M}$ allowing an arbitrary flux value $\mathbf{v}_i$ in reaction i (actually as soon as the value of $\mathbf{v}_i/\mathbf{E}_i$ is outside the κ_i bounds).
But the kinetic constraint $\mathbf{KC_M}(\mathbf{v})$ (79) is sign-based. This results from the linear dependency of $\mathbf{v}_i$ on $\mathbf{E}_i$. Actually, if $\mathbf{KC_M}(\mathbf{v})$ is satisfied (for some $\mathbf{E}$) and $sign(\mathbf{v}') = sign(\mathbf{v})$, then, for all $i \in supp(\mathbf{v}')$, $\mathbf{v}_i$ and $\kappa_i(\mathbf{M})$ have same sign, thus it is also the case for $\mathbf{v}'_i$ and $\kappa_i(\mathbf{M})$. Thus by taking $\mathbf{E}'_i = \mathbf{v}'_i/\kappa_i(\mathbf{M}) = \mathbf{E}_i\mathbf{v}'_i/\mathbf{v}_i$ when $\mathbf{v}'_i \neq 0$, and $\mathbf{E}'_i = 0$ when $\mathbf{v}'_i = 0$, $\mathbf{KC_M}(\mathbf{v}')$ is satisfied (for $\mathbf{E}'$). This sign-based property holds thus also for $\mathbf{KC}(\mathbf{v})$ (75) and for $\mathbf{KC}^b(\mathbf{v})$ (76) if the only bounds are on metabolites concentrations. But it does not hold for $\mathbf{KC}^b_\mathbf{M}(\mathbf{v})$ (80) and for $\mathbf{KC}^b(\mathbf{v})$ (76) in presence of bounds on enzymes concentrations.
Finally, by definition, the regulatory constraint $\mathbf{RC}_{Bc}(\mathbf{v})$ (49) depends only on $supp(\mathbf{v})$, so is support-based and thus sign-based.

Lemma 3.1. *All thermodynamic constraints are sign-based. Only those kinetic constraints $\boldsymbol{KC_M}$ and $\boldsymbol{KC}$ are sign-based, as well as $\boldsymbol{KC}^b$ with only bounds on metabolites concentrations (but not on enzymes concentrations, thus in particular without enzymatic resource constraint). The regulatory constraints are support-based, thus sign-based.*

The structure of the solutions space of a sign-based constraint follows directly from its definition. Actually, if $\mathbf{v}$ satisfies a given sign-based constraint $\mathbf{C} = \mathbf{C_x}(\mathbf{v})$ (resp., $\mathbf{C} = \exists\mathbf{x}\mathbf{C_x}(\mathbf{v})$), then $\mathring{O}_{sign(\mathbf{v})} = \{\mathbf{x} \in \mathbb{R}^r \mid sign(\mathbf{x}) = sign(\mathbf{v})\}$ is included in $Sol_\mathbf{C}$ and thus $Sol_\mathbf{C} = \bigcup_{\{\mathbf{v}|\mathbf{C}(\mathbf{v})\}} \mathring{O}_{sign(\mathbf{v})}$ is a disjoint union of open orthants. This result can be compared to Lemma 2.2. More precisely, if we consider a support-based constraint $\mathbf{C}$, the same reasoning applies and gives $Sol_\mathbf{C} = \bigcup_{\{\mathbf{v}|\mathbf{C}(\mathbf{v})\}} S_{supp(\mathbf{v})}$, where $S_\boldsymbol{\rho} = \{\mathbf{x} \in \mathbb{R}^r \mid supp(\mathbf{x}) = \boldsymbol{\rho}\}$ denotes the set of vectors of support $\boldsymbol{\rho}$, where we code a support as a binary vector $\boldsymbol{\rho}$ (1 coding support membership and 0 non-membership). A support-based constraint $\mathbf{C}$ is thus equivalent (in extension) to a family $\{S_{\boldsymbol{\rho}^i}\}_i$ of such support sets and, as there are 2^r possible binary vectors, there are thus 2^{2^r} different support-based constraints. Now a given $S_\boldsymbol{\rho}$ is equivalent to the Boolean constraint $D_\boldsymbol{\rho} = \bigwedge_{\{i|\boldsymbol{\rho}_i=1\}} \mathbf{v}_i \wedge \bigwedge_{\{j|\boldsymbol{\rho}_j=0\}} \neg\mathbf{v}_j$ and thus an arbitrary support-based constraint $\mathbf{C}$ is equivalent to the Boolean constraint $Bc = \bigvee_i D_{\boldsymbol{\rho}^i}$, thus to the regulatory constraint $\mathbf{RC}_{Bc}$. Thus support-based constraints identify to regulatory constraints and all properties we have demonstrated for the latter apply to the first. For example, associating to any sign vector $\boldsymbol{\eta}$ its support $\boldsymbol{\rho}$ (i.e., the support of any vector having the given sign), given by $\boldsymbol{\rho}_i = 1$ if $\boldsymbol{\eta}_i = +$ or $-$ and $\boldsymbol{\rho}_i = 0$ if $\boldsymbol{\eta}_i = 0$, we deduce that, for any binary vector $\boldsymbol{\rho}$, there are $2^{|supp(\boldsymbol{\rho})|}$ sign vectors $\boldsymbol{\eta}$ with support $\boldsymbol{\rho}$, given by $\boldsymbol{\eta}_i = +$ or $-$ if $\boldsymbol{\rho}_i = 1$ and 0 else, and we obtain $S_\boldsymbol{\rho} = \bigcup_{\{\boldsymbol{\eta}^i|supp(\boldsymbol{\eta}^i)=\boldsymbol{\rho}\}} \mathring{O}_{\boldsymbol{\eta}^i}$ and $Sol_\mathbf{C} = \bigcup_{\{\boldsymbol{\eta}^i|supp(\boldsymbol{\eta}^i)=supp(\mathbf{v})\wedge\mathbf{C}(\mathbf{v})\}} \mathring{O}_{\boldsymbol{\eta}^i}$, i.e.,

that $Sol_{\mathbf{C}}$ is a disjoint union of open orthants, which is precisely the statement of Lemma 2.2. Notice that a support based constraint $\mathbf{C}$ is entirely defined by its restriction to an arbitrary closed r-orthant $O_{\boldsymbol{\eta}}$, i.e., for $\boldsymbol{\eta}$ an arbitrary full support sign vector, as $Sol_{\mathbf{C}}$ can be reconstituted from $Sol_{\mathbf{C}} \cap O_{\boldsymbol{\eta}}$. And, as to be sign-based and to be support-based coincide in any closed r-orthant, a sign-based constraint is nothing else that a constraint whose restriction to any closed r-orthant is support-based, i.e., which coincides in any closed r-orthant with a well defined regulatory constraint (but those regulatory constraints differ in general from one r-orthant to another, while obviously coinciding on their intersection). A sign-based constraint $\mathbf{C}$ is equivalent to a family $\{\mathring{O}_{\boldsymbol{\eta}^i}\}_i$ of open orthants and, as there are 3^r possible sign vectors, there are thus 2^{3^r} different sign-based constraints. Applying the merging method described in the proof of Lemma 2.2, those open orthants can be grouped together in order to obtain a family of semi-open orthants, without any possible further merging between two of them.

Proposition 3.2. *Support-based constraints coincide with Boolean constraints, i.e., regulatory constraints. They are completely characterized by their restriction to any closed r-orthant and number 2^{2^r}. Sign-based constraints coincide with constraints whose restriction to any closed r-orthant is given by a regulatory constraint (with identity of those regulatory constraints on the intersections of any two of those orthants). They number 2^{3^r}. The set $Sol_{\boldsymbol{C}}$ of vectors in $\mathbb{R}^r$ satisfying the sign-based constraint $\boldsymbol{C} = \boldsymbol{C_x}(\boldsymbol{v})$ (resp., $\boldsymbol{C} = \exists \boldsymbol{x} \boldsymbol{C_x}(\boldsymbol{v})$) is a disjoint union of open orthants, which can be grouped together according to Lemma 2.2 to provide a disjoint union of semi-open orthants without any possible further merging between two of them.*

An important consequence is that, if we reason for each closed r-orthant separately, i.e., flux tope by flux tope in the solutions space, then all results demonstrated in subsection 2.2.3 for regulatory constraints apply to sign-based constraints. With the previous definitions of open or semi-open polyhedral cones and open or semi-open polyhedra and, more generally, of open or semi-open faces of polyhedral cones or polyhedra, we get the following result.

Theorem 3.3. *Let $\boldsymbol{C} = \boldsymbol{C_x}(\boldsymbol{v})$ (resp., $\boldsymbol{C} = \exists \boldsymbol{x} \boldsymbol{C_x}(\boldsymbol{v})$) be a sign-based constraint and $CFC_{\boldsymbol{C}}$ (resp., $CFP_{\boldsymbol{C}}$) be the associated constrained flux cone subset (resp., the associated constrained flux polyhedron subset). Then $CFC_{\boldsymbol{C}}$ (resp., $CFP_{\boldsymbol{C}}$) is a finite disjoint union of open polyhedral cones (resp., open polyhedra), which are some open faces of the flux topes $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$) for all $\boldsymbol{\eta}$ maximal sign vectors in $sign(FC)$ (resp., $sign(FP)$). They can be grouped together according to Lemma 2.2 to provide a disjoint union of semi-open faces of the $FC_{\leq \boldsymbol{\eta}}$'s (resp., $FP_{\leq \boldsymbol{\eta}}$'s) without any possible further merging between two of them. Elementary fluxes are obtained by collecting those for each flux tope (which is not the case for elementary flux modes, where after collecting them, only those with minimal support have to be kept) and are nothing else that the elementary fluxes of FC (resp., FP) that satisfy the constraint $\boldsymbol{C}$ (which again is not the case for elementary flux modes):*

$$\texttt{EFVs}(CFC_{\boldsymbol{C}}) = \texttt{EFVs}(FC) \cap Sol_{\boldsymbol{C}} = \texttt{EFMs}(FC) \cap Sol_{\boldsymbol{C}} \subseteq \texttt{EFMs}(CFC_{\boldsymbol{C}}) \tag{87}$$

$$\texttt{EFPs}(CFP_{\boldsymbol{C}}) = \texttt{EFPs}(FP) \cap Sol_{\boldsymbol{C}} \qquad \texttt{EFVs}(CFP_{\boldsymbol{C}}) = \texttt{EFVs}(C_{FP}) \cap Sol_{\boldsymbol{C}}. \tag{88}$$

Proof. The disjoint decomposition of the solutions space into open faces of the flux topes $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$) results from Proposition 3.2 or directly from the fact that all vectors of the strict conical hull $cone^+(\mathbf{v}^1, \ldots, \mathbf{v}^n) = \{\beta_1 \mathbf{v}^1 + \ldots + \beta_n \mathbf{v}^n \mid \beta_1, \ldots, \beta_n > 0\}$ and of the strict convex hull $conv^+(\mathbf{v}^1, \ldots, \mathbf{v}^n) = \{\alpha_1 \mathbf{v}^1 + \ldots + \alpha_n \mathbf{v}^n \mid \alpha_1, \ldots, \alpha_n > 0, \alpha_1 + \ldots + \alpha_n = 1\}$ of given vectors $\mathbf{v}^1, \ldots, \mathbf{v}^n$ in a flux tope, thus in a closed orthant (so the sum is conformal), have the same support $supp(\mathbf{v}^1) \cup \ldots \cup supp(\mathbf{v}^n)$, thus the same sign, and from the fact that an open face of a polyhedron is the Minkowski sum of the strict convex hull of its vertices and the strict conical hull of its extreme vectors. Thus an open face of a flux tope $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$) either does not intersect the solutions space or is completely included in it. We have seen (54) that any elementary flux or any elementary flux mode of FC (resp., FP) that satisfies the constraint is an elementary flux or an elementary flux mode of the solutions space (the conditions of validity of (54) for elementary fluxes are satisfied from the structure of the solutions space). Those elementary fluxes or elementary flux modes identify actually with the extreme vectors of the $FC_{\leq \boldsymbol{\eta}}$'s (resp., extreme points of the $FP_{\leq \boldsymbol{\eta}}$'s and extreme vectors of the $C_{FP\ \leq \boldsymbol{\eta}}$'s) that satisfy the constraint, i.e., whose sign satisfies the constraint. Reciprocally, any vector of the solutions space that is not in this case belongs necessarily to an open face of some $FC_{\leq \boldsymbol{\eta}}$ (or $C_{FP\ \leq \boldsymbol{\eta}}$) of dimension at least two (resp., of some $FP_{\leq \boldsymbol{\eta}}$ of dimension at least one) and is thus conformally (resp., convex-conformally) decomposable

in this open face and in consequence cannot be an elementary vector (resp., elementary point) of the solutions space (obviously, the decomposition involves two vectors of same support, thus nothing can be deduced about elementary flux modes). This gives the result about elementary fluxes. □

Theorem 3.3 applies in particular to all thermodynamic constraints, to regulatory constraints and to those kinetic constraints described in Lemma 3.1. Especially, we obtain directly the results of Propositions 2.10 and 2.11 for regulatory constraints.

It is important to notice that the only knowledge of the EFVs for $CFC_{\mathbf{C}}$ (resp., the EFPs and EFVs for $CFP_{\mathbf{C}}$) is really far to characterize the solutions space $CFC_{\mathbf{C}}$ (resp., $CFP_{\mathbf{C}}$): actually they are just the ExVs of each tope $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$ (resp., the ExPs and ExVs of each $CFP_{\mathbf{C}\ \leq\boldsymbol{\eta}}$), i.e., the only one-dimension open polyhedral cones, i.e., edges (resp., zero-dimension polyhedra, i.e., vertices, and edges for the recession cones) among all the open cones (resp., open polyhedra) of any dimension that constitute $CFC_{\mathbf{C}}$ (resp., $CFP_{\mathbf{C}}$).

As in each closed r-orthant, i.e., in each flux tope, a sign-based constraint identifies to a regulatory constraint, Propositions 2.12 and 2.13 demonstrated for regulatory constraints apply thus to sign-based constraints. Nevertheless, they are stated for constraints that are conjunctions of literals and, even if a decomposition into such disjuncts is always possible from the identity above, it is not necessarily natural for an arbitrary sign-based constraint and a global characterization for each flux tope is preferable, in particular for what concerns support-wise non-strictly-decomposable vectors. The difference is that one has to deal for $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$ with an arbitrary family of open faces of $FC_{\leq\boldsymbol{\eta}}$ instead of a single semi-open face of $FC_{\leq\boldsymbol{\eta}}$, obtained specifically as a face of $FC_{\leq\boldsymbol{\eta}}$ without some of its facets, for $CFC_{\mathbf{RC}_D\ \leq\boldsymbol{\eta}}$.

Adopt notations analog to those used in Propositions 2.12 and 2.13, i.e., let $\texttt{EFMs}^{\mathbf{C}} \triangleq \texttt{EFMs}(FC_{\leq\boldsymbol{\eta}}) \cap Sol_{\mathbf{C}}$ be the elementary flux modes of $FC_{\leq\boldsymbol{\eta}}$ that satisfy $\mathbf{C}$ (i.e., the elementary flux vectors of $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$), $\texttt{EFMs}_{\mathbf{C}} \triangleq \texttt{EFMs}(CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}})$ be the elementary flux modes of $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$ and $\texttt{swNSDFVs}_{\mathbf{C}} \triangleq \texttt{swNSDFVs}(CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}})$ be the support-wise non-strictly-decomposable vectors of $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$. We have thus $\texttt{EFMs}^{\mathbf{C}} \subseteq \texttt{EFMs}_{\mathbf{C}} \subseteq \texttt{swNSDFVs}_{\mathbf{C}}$. The proof of Proposition 2.12 adapts straightforwardly: $\texttt{EFMs}^{\mathbf{C}}$ correspond to the open faces of dimension one (extreme rays) of $FC_{\leq\boldsymbol{\eta}}$ in $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$ and $\texttt{EFMs}_{\mathbf{C}} \backslash \texttt{EFMs}^{\mathbf{C}}$ are made up of all the open faces $\mathring{F}$ in $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$ where F is a face of dimension at least two of $FC_{\leq\boldsymbol{\eta}}$ but not any proper (i.e., with positive dimension, less than the dimension of F) face F' of F is such that $\mathring{F}'$ is in $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$. The result is based on the properties that all vectors of an open face $\mathring{F}$ in $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$ have same support and the common support of vectors of $\mathring{F}'$, where F' is a face of F, is strictly included in that of vectors of $\mathring{F}$. In the same way, the proof of Proposition 2.13 is easily adapted for what concerns $\texttt{swNSDFVs}_{\mathbf{C}} \backslash \texttt{EFMs}_{\mathbf{C}}$. For this we consider successively all the open faces $\mathring{F}$ in $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$ where F is a face of dimension at least two of $FC_{\leq\boldsymbol{\eta}}$ which owns at least one proper face F' such that $\mathring{F}'$ is in $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$. For each such given $\mathring{F}$, let $\{F'_j\}_{j\in J}$ be the family of all such F'. Let $\{\mathbf{e}^k\}_{k\in K}$ be representatives of extreme vectors of F and $\{\mathbf{e}^l\}_{l\in L}$, with $L \subseteq K$, be representatives of the extreme vectors of all those F'_j. Thus $cone^+_{\oplus}(\{\mathbf{e}^k\}_{k\in K}) = \mathring{F}$ and $cone^+_{\oplus}(\{\mathbf{e}^l\}_{l\in L}) = cone^+_{\oplus}(\{\mathring{F}'_j\}_{j\in J})$ and one obtains $\mathring{F} \cap cone^+_{\oplus}(\{\mathbf{e}^l\}_{l\in L}) = \mathring{F} \backslash \texttt{swNSDFVs}_{\mathbf{C}}$, as by construction those vectors of $\mathring{F}$ which belong to $cone^+_{\oplus}(\{\mathring{F}'_j\}_{j\in J})$ are precisely those in $\mathring{F}$ which are support-wise strictly-decomposable in $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$, the decomposition being achieved along vectors in the $\mathring{F}'_j$'s, whose support is strictly included into the common support of vectors in $\mathring{F}$. So three different exclusive cases can be distinguished for $\mathring{F}$:

- $cone^+_{\oplus}(\{\mathbf{e}^l\}_{l\in L}) \cap \mathring{F} = \emptyset$, i.e., the F'_j's are all included in a same facet of F, that is in a same hyperplane $\{\mathbf{v}_i = 0\}$ for some dimension i of the affine span of F, which is still equivalent to $\bigcup_{l\in L} supp(\mathbf{e}^l) \subset \bigcup_{k\in K} supp(\mathbf{e}^k)$ (this is for example the case if $|J| = 1$). Then $\mathring{F} \subseteq \texttt{swNSDFVs}_{\mathbf{C}} \backslash \texttt{EFMs}_{\mathbf{C}}$ is entirely made up of support-wise non-strictly-decomposable vectors and $\bigcup_{k\in K} supp(\mathbf{e}^k)$ is a non-strictly-decomposable non-minimal support for vectors in $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$.

- $cone^+_{\oplus}(\{\mathbf{e}^l\}_{l\in L}) = \mathring{F}$, i.e., $L = K$. Then $\mathring{F} \subseteq CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}} \backslash \texttt{swNSDFVs}_{\mathbf{C}}$ is entirely made up of support-wise strictly-decomposable vectors and $\bigcup_{k\in K} supp(\mathbf{e}^k)$ is a strictly-decomposable support for vectors in $CFC_{\mathbf{C}\ \leq\boldsymbol{\eta}}$.

- $cone^+_{\oplus}(\{\mathbf{e}^l\}_{l\in L}) \subset \mathring{F}$, i.e., $L \subset K$ and not all F'_j's are included in a same facet of F, which means that, for all i dimension of the affine space of F, it exists $j \in J$ such that F'_j is not included in the hyperplane $\{\mathbf{v}_i = 0\}$, or equivalently, it exists $l \in L$ such that $\mathbf{e}^l_i \neq 0$, which

is still equivalent to $\bigcup_{l\in L} supp(\mathbf{e}^l) = \bigcup_{k\in K} supp(\mathbf{e}^k)$. Then $\mathring{F}$ is split into two nonempty subsets: $cone^+_\oplus(\{\mathbf{e}^l\}_{l\in L}) = \mathring{F}\backslash \mathtt{swNSDFVs}_\mathbf{C}$, made up of support-wise strictly-decomposable vectors, and $\mathring{F}\backslash cone^+_\oplus(\{\mathbf{e}^l\}_{l\in L}) \subseteq \mathtt{swNSDFVs}_\mathbf{C}\backslash \mathtt{EFMs}_\mathbf{C}$, made up of support-wise non-strictly-decomposable vectors (note that $\mathring{F}\backslash cone^+_\oplus(\{\mathbf{e}^l\}_{l\in L})$ is made up of those vectors of $\mathring{F}$ the conical decomposition of which on the $\mathbf{e}^k$'s requires at least one $\mathbf{e}^k$ with $k \in K\backslash L$). All vectors of $\mathring{F}$ having the same non-minimal support $\bigcup_{k\in K} supp(\mathbf{e}^k)$, this proves that support-wise strict decomposability does not depend in general only on the support but on the positive values of the fluxes and that, unlike the two particular cases above, one cannot speak of a strictly-decomposable or non-strictly-decomposable support. Note that the support-wise non-strictly-decomposable vectors of $\mathring{F}$ are obtained as the complementary in this open cone of the open sub-cone $cone^+_\oplus(\{\mathbf{e}^l\}_{l\in L})$, which is thus a disjoint union of semi-open cones (its connected components), each of which is conically generated both by some extreme vectors $\mathbf{e}^k$ with $k \in K\backslash L$ (that necessarily do not belong to $\mathtt{EFMs}^\mathbf{C}$), with nonnegative coefficients, and by some extreme vectors $\mathbf{e}^k$ with $k \in L$ (that may belong or not to $\mathtt{EFMs}^\mathbf{C}$), with positive coefficients (in order to keep the concerned facets common with $cone_\oplus(\{\mathbf{e}^l\}_{l\in L})$).

We have finally proved the following result (keeping the notations of Theorem 3.3).

Theorem 3.4. *For $\boldsymbol{C}$ a sign-based constraint, $FC_{\leq\boldsymbol{\eta}}$ a flux tope and $CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}}$ the associated subset of the solutions space (disjoint union of open faces of $FC_{\leq\boldsymbol{\eta}}$ from Theorem 3.3), let $\mathtt{EFMs}^{\boldsymbol{C}} \triangleq \mathtt{EFMs}(FC_{\leq\boldsymbol{\eta}}) \cap Sol_{\boldsymbol{C}}$ be the elementary flux modes of $FC_{\leq\boldsymbol{\eta}}$ that satisfy $\boldsymbol{C}$ (equal to $\mathtt{EFVs}(CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}})$), $\mathtt{EFMs}_{\boldsymbol{C}} \triangleq \mathtt{EFMs}(CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}})$ be the elementary flux modes of $CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}}$ and $\mathtt{swNSDFVs}_{\boldsymbol{C}} \triangleq \mathtt{swNSDFVs}(CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}})$ be the support-wise non-strictly-decomposable vectors of $CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}}$, with $\mathtt{EFMs}^{\boldsymbol{C}} \subseteq \mathtt{EFMs}_{\boldsymbol{C}} \subseteq \mathtt{swNSDFVs}_{\boldsymbol{C}}$. $\mathtt{EFMs}^{\boldsymbol{C}}$ correspond to the open faces of dimension one (extreme rays) of $FC_{\leq\boldsymbol{\eta}}$ belonging to $CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}}$. Consider now successively all the open faces $\mathring{F}$ in $CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}}$ where F is a face of dimension at least two of $FC_{\leq\boldsymbol{\eta}}$, and for each such given F, let $\{\boldsymbol{e}^k\}_{k\in K}$ be representatives of the extreme vectors of F and $\{\boldsymbol{e}^l\}_{l\in L}$, with $L \subseteq K$, be representatives of the extreme vectors of all proper (i.e., with positive dimension, less than the dimension of F) faces F' of F such that $\mathring{F}'$ belongs to $CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}}$. Thus $cone^+_\oplus(\{\boldsymbol{e}^k\}_{k\in K}) = \mathring{F}$ and let $D(F) \triangleq cone^+_\oplus(\{\boldsymbol{e}^l\}_{l\in L})$. Then, if $L = \emptyset$, $\mathring{F} \subseteq \mathtt{EFMs}_{\boldsymbol{C}}\backslash\mathtt{EFMs}^{\boldsymbol{C}}$; if $L \neq \emptyset$ and $\bigcup_{l\in L} supp(\boldsymbol{e}^l) \subset \bigcup_{k\in K} supp(\boldsymbol{e}^k)$, $\mathring{F} \subseteq \mathtt{swNSDFVs}_{\boldsymbol{C}}\backslash\ \mathtt{EFMs}_{\boldsymbol{C}}$ and $\bigcup_{k\in K} supp(\boldsymbol{e}^k)$ is a non-strictly-decomposable non-minimal support for vectors in $CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}}$; if $L = K$, $\mathring{F} \subseteq CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}}\backslash\mathtt{swNSDFVs}_{\boldsymbol{C}}$ and $\bigcup_{k\in K} supp(\boldsymbol{e}^k)$ is a strictly-decomposable support for vectors in $CFC_{\boldsymbol{C}\leq\boldsymbol{\eta}}$; if $L \subset K$ and $\bigcup_{l\in L} supp(\boldsymbol{e}^l) = \bigcup_{k\in K} supp(\boldsymbol{e}^k)$, $D(F) = \mathring{F}\backslash\mathtt{swNSDFVs}_{\boldsymbol{C}}$, a non-empty open cone, constitutes the support-wise strictly-decomposable vectors in $\mathring{F}$ and $\mathring{F}\backslash D(F) \subseteq \mathtt{swNSDFVs}_{\boldsymbol{C}}\backslash\mathtt{EFMs}_{\boldsymbol{C}}$, a non-empty finite disjoint union of semi-open cones, constitutes the support-wise non-strictly-decomposable vectors in $\mathring{F}$, all having the same non-minimal support (decomposability depending in this case not only on the support but on the distribution of the fluxes). One obtains finally $\mathtt{EFMs}_{\boldsymbol{C}}\backslash\mathtt{EFMs}^{\boldsymbol{C}}$ and $\mathtt{swNSDFVs}_{\boldsymbol{C}}\backslash\mathtt{EFMs}_{\boldsymbol{C}}$ by gathering those vectors for each $\mathring{F}$.*

Notice that even the knowledge of $\mathtt{swNSDFVs}_\mathbf{C}$ is not enough to completely reconstruct the tope $CFC_{\mathbf{C}\leq\boldsymbol{\eta}}$ of the solutions space.

In order to deal with the usual enzymatic resource constraint (more generally capacity constraints) in the kinetic constraints, we generalize the sign-based criterion. A constraint $\mathbf{C_x}(\mathbf{v})$ (resp., $\exists\mathbf{x}\mathbf{C_x}(\mathbf{v})$), is said contracting-sign-based if, when satisfied by one vector, it is satisfied by any vector having the same sign which is not greater (on each component), i.e., that belongs to the open rectangle parallelepiped defined by the null vector and the given vector:

$$\mathbf{C_x}(\mathbf{v}), sign(\mathbf{v}') = sign(\mathbf{v}), \forall i|\mathbf{v}'_i| \leq |\mathbf{v}_i| \Rightarrow \mathbf{C_x}(\mathbf{v}') \tag{89}$$

$$\exists\mathbf{x}\mathbf{C_x}(\mathbf{v}), sign(\mathbf{v}') = sign(\mathbf{v}), \forall i|\mathbf{v}'_i| \leq |\mathbf{v}_i| \Rightarrow \exists\mathbf{x}\mathbf{C_x}(\mathbf{v}'). \tag{90}$$

Obviously a sign-based constraint is contracting-sign-based and, if the $\mathbf{C_x}(\mathbf{v})$'s are contracting-sign-based for all $\mathbf{x}$, then $\exists\mathbf{x}\mathbf{C_x}(\mathbf{v})$ is contracting-sign-based.

Example 3.2. The kinetic constraints $\mathbf{KC}^b_\mathbf{M}(\mathbf{v})$ (80) and $\mathbf{KC}^b(\mathbf{v})$ (76) are contracting-sign-based in absence of positive lower bounds for enzymes concentrations (i.e., when $\mathbf{E}^- = \mathbf{0}$). Actually, if $\mathbf{KC}^b_\mathbf{M}(\mathbf{v})$

is satisfied (for some $\mathbf{E}$ verifying $\mathbf{c}^T\mathbf{E} \leq W$ and $\mathbf{E} \leq \mathbf{E}^+$) and $sign(\mathbf{v}') = sign(\mathbf{v})$ with $|\mathbf{v}'_i| \leq |\mathbf{v}_i|$ for all i, we saw that, by taking $\mathbf{E}'_i = \mathbf{v}'_i/\kappa_i(\mathbf{M}) = \mathbf{E}_i\mathbf{v}'_i/\mathbf{v}_i$ when $\mathbf{v}'_i \neq 0$, and $\mathbf{E}'_i = 0$ when $\mathbf{v}'_i = 0$, then $\mathbf{KC_M}(\mathbf{v}')$ is satisfied (for $\mathbf{E}'$). Now, as $|\mathbf{v}'_i| \leq |\mathbf{v}_i|$, we have $\mathbf{E}' \leq \mathbf{E}$ and thus $\mathbf{c}^T\mathbf{E}' \leq \mathbf{c}^T\mathbf{E} \leq W$ and $\mathbf{E}' \leq \mathbf{E}^+$, so $\mathbf{KC}^b_\mathbf{M}(\mathbf{v}')$ is satisfied.

Lemma 3.5. *The kinetic constraints $\boldsymbol{KC^b_M}$ and $\boldsymbol{KC^b}$ are contracting-sign-based in absence of positive lower bounds for enzymes concentrations (i.e., when $\boldsymbol{E^-} = \boldsymbol{0}$).*

We will call $\mathbf{0}$-star domain a subset SD of $\mathbb{R}^r$ which has the property that the whole open segment joining $\mathbf{0}$ to any element of SD is included in SD: $\mathbf{v} \in SD, 0 < \lambda < 1 \Rightarrow \lambda\mathbf{v} \in SD$. Any cone is a $\mathbf{0}$-star domain.

Theorem 3.6. *If $\boldsymbol{C} = \boldsymbol{C_x}(\boldsymbol{v})$ (or $\boldsymbol{C} = \exists \boldsymbol{x}\boldsymbol{C_x}(\boldsymbol{v})$) is contracting-sign-based, then $CFC_{\boldsymbol{C}}$ is a $\boldsymbol{0}$-star domain and it exists a neighborhood $N =]-\delta, +\delta[^r$ of $\boldsymbol{0}$ for some $\delta > 0$ such that $CFC_{\boldsymbol{C}} \cap N$ is a finite disjoint union of N-truncated open polyhedral cones, which are the intersection with N of open faces of the flux topes $FC_{\leq \boldsymbol{\eta}}$. In addition, results (87) about* `EFVs` *and* `EFMs` *hold in N, i.e., for sufficiently small fluxes. The same holds for $CFP_{\boldsymbol{C}}$ with the flux topes $FP_{\leq \boldsymbol{\eta}}$ if $\boldsymbol{0}$ is an interior point of FP, and in this case results (88) about* `EFVs` *and* `EFPs` *hold in N.*

Proof. The property of being a $\mathbf{0}$-star domain is a direct consequence of the definition: if a contracting-sign-based constraint is satisfied by a vector $\mathbf{v}$, it is satisfied by $\lambda\mathbf{v}$ for all $0 < \lambda < 1$. From the proof of Theorem 3.3, it results that if a nonzero vector $\mathbf{v} \in FC_{\leq \boldsymbol{\eta}}$ verifies a given contracting-sign-based constraint, then any vector $\mathbf{v}'$ of the minimal open face of $FC_{\leq \boldsymbol{\eta}}$ containing $\mathbf{v}$ (thus having same sign as $\mathbf{v}$) and belonging to $]-\delta_\mathbf{v}, +\delta_\mathbf{v}[^r$ with $\delta_\mathbf{v} = min_{i \in supp(\mathbf{v})}|\mathbf{v}_i|$ (contracting condition) verifies also the constraint. The result is obtained by taking for δ the minimum of the $\delta_\mathbf{v}$'s on all open faces of the $FC_{\leq \boldsymbol{\eta}}$'s (whose number is smaller than the number of possible signs, i.e., 3^r). The proof is still valid for FP but the result is meaningful only if N is included in FP, i.e., if $\mathbf{0}$ is an interior point of FP, which means that the inhomogeneous linear constraints defining FP, given by $\mathbf{Gv} \geq \mathbf{h}$, verify $\mathbf{h} < \mathbf{0}$. □

Theorem 3.6 tells that the result of Theorem 3.3 for sign-based constraints about the geometrical structure of the solutions space applies identically for contracting-sign-based constraints locally in a neighborhood of $\mathbf{0}$, i.e., when considering only pathways with sufficiently small amounts of fluxes. It applies in particular to those kinetic constraints described in Lemma 3.5.

3.2 Sign-monotone constraints

We consider now a property of compatibility of a constraint with signs that is stronger than to be sign-based. A constraint $\mathbf{C_x}(\mathbf{v})$ (resp., $\exists\mathbf{x}\mathbf{C_x}(\mathbf{v})$), is said sign-monotone if, when satisfied by a vector, is satisfied by any other vector with a smaller or equal sign (for the partial order on signs).

$$\mathbf{C_x}(\mathbf{v}), sign(\mathbf{v}') \leq sign(\mathbf{v}) \Rightarrow \mathbf{C_x}(\mathbf{v}') \tag{91}$$

$$\exists\mathbf{x}\mathbf{C_x}(\mathbf{v}), sign(\mathbf{v}') \leq sign(\mathbf{v}) \Rightarrow \exists\mathbf{x}\mathbf{C_x}(\mathbf{v}'). \tag{92}$$

It follows from these definitions that, if the $\mathbf{C_x}(\mathbf{v})$'s are sign-monotone for all $\mathbf{x}$, then $\exists\mathbf{x}\mathbf{C_x}(\mathbf{v})$ is sign-monotone and that any sign-monotone constraint is sign-based. Note that, in any given closed orthant, thus in any flux tope, $sign(\mathbf{v}') \leq sign(\mathbf{v})$ is equivalent to $supp(\mathbf{v}') \subseteq supp(\mathbf{v})$ and thus being sign-monotone for a constraint means being support-monotone in each flux tope. Note also that if a sign-monotone constraint has a solution, then the null vector $\mathbf{0}$ is solution.

Example 3.3. It results then from definitions (40), (41) or (42) that the thermodynamic constraint $\mathbf{TC_M}(\mathbf{v})$ or $\underline{\mathbf{TC}}_\mathbf{M}(\mathbf{v})$ is sign-monotone. This is thus also the case for $\mathbf{TC}(\mathbf{v})$ and $\underline{\mathbf{TC}}(\mathbf{v})$ (59) and for $\mathbf{TC}^b(\mathbf{v})$ and $\underline{\mathbf{TC}}^b(\mathbf{v})$ (60).
The argument given previously to establish that the kinetic constraint $\mathbf{KC_M}(\mathbf{v})$ (79) is sign-based proves in fact that it is sign-monotone. This sign-monotone property holds thus also for $\mathbf{KC}(\mathbf{v})$ (75) and for $\mathbf{KC}^b(\mathbf{v})$ (76) in absence of bounds on enzymes concentrations.
The regulatory constraint $\mathbf{RC}_{Bc}(\mathbf{v})$ (49) is not sign-monotone in general: consider for example for Bc a positive literal. Actually, $\mathbf{RC}_{Bc}(\mathbf{v})$ is sign-monotone if and only if Bc in DNF contains no positive literal, which is a very special case, requiring only some fluxes to be zero but unable to express that a given flux is nonzero (in this particular case, it is support-monotone, which is stronger than sign-monotone).

Lemma 3.7. *All thermodynamic constraints are sign-monotone. Only those kinetic constraints* $\boldsymbol{KC_M}$ *and* $\boldsymbol{KC}$ *are sign-monotone, as well as* $\boldsymbol{KC}^b$ *in absence of bounds on enzymes concentrations. The regulatory constraint* $\boldsymbol{RC}_{Bc}$ *is not sign-monotone, except if Bc in DNF contains only negative literals (i.e., assigns only zero fluxes, but no nonzero flux), in what case it is support-monotone.*

The structure of the solutions space of a sign-monotone constraint follows directly from its definition.

Lemma 3.8. *The set* $Sol_{\boldsymbol{C}}$ *of vectors in* $\mathbb{R}^r$ *satisfying the sign-monotone constraint* $\boldsymbol{C} = \boldsymbol{C_x}(\boldsymbol{v})$ *(resp.,* $\boldsymbol{C} = \exists \boldsymbol{x} \boldsymbol{C_x}(\boldsymbol{v})$*) is a union of closed orthants.*

Actually, if $\mathbf{v}$ satisfies $\mathbf{C}$, then $O_{sign(\mathbf{v})} = \{\mathbf{x} \in \mathbb{R}^r \mid sign(\mathbf{x}) \leq sign(\mathbf{v})\}$ is included in $Sol_{\mathbf{C}}$ and thus $Sol_{\mathbf{C}} = \bigcup_{\{\mathbf{v}|\mathbf{C}(\mathbf{v})\}} O_{sign(\mathbf{v})}$. Obviously we can keep only those $\mathbf{v}$'s for which $sign(\mathbf{v})$ is maximal, in order to get no inclusion between the closed orthants. This result can be compared to Lemmas 2.1, 2.4 and first part of Proposition 2.8, which are obviously more precise but it shows that the mathematical structure of the solutions space of those thermodynamic and kinetic constraints is for the most part the only consequence of their sign-monotonicity.

Theorem 3.9. *Consider indifferently a sign-monotone constraint* $\boldsymbol{C_x}(\boldsymbol{v})$ *or* $\exists \boldsymbol{x} \boldsymbol{C_x}(\boldsymbol{v})$ *(e.g., if* $\boldsymbol{C_x}(\boldsymbol{v})$ *is sign-monotone for any* $\boldsymbol{x}$*) and let us name it* $\boldsymbol{C}$ *and the associated constrained flux cone subset* $CFC_{\boldsymbol{C}}$ *(resp., the associated constrained flux polyhedron subset* $CFP_{\boldsymbol{C}}$*). Then* $CFC_{\boldsymbol{C}}$ *(resp.,* $CFP_{\boldsymbol{C}}$*) is a finite union of polyhedral cones (resp., polyhedra), which are faces of the flux topes* $FC_{\leq \boldsymbol{\eta}}$ *(resp.,* $FP_{\leq \boldsymbol{\eta}}$*) for all* $\boldsymbol{\eta}$ *maximal sign vectors in* $sign(FC)$ *(resp.,* $sign(FP)$*) and we have:*

$$\texttt{EFVs}(CFC_{\boldsymbol{C}}) = \texttt{EFMs}(CFC_{\boldsymbol{C}}) = \texttt{EFVs}(FC) \cap Sol_{\boldsymbol{C}} = \texttt{EFMs}(FC) \cap Sol_{\boldsymbol{C}} \tag{93}$$

$$\texttt{EFPs}(CFP_{\boldsymbol{C}}) = \texttt{EFPs}(FP) \cap Sol_{\boldsymbol{C}} \qquad \texttt{EFVs}(CFP_{\boldsymbol{C}}) = \texttt{EFVs}(C_{FP}) \cap Sol_{\boldsymbol{C}}. \tag{94}$$

Proof. The (non-disjoint) decomposition of the solutions space into faces of the flux topes $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$) results from Lemma 3.8 or directly from the fact that all vectors of the conical hull $cone(\mathbf{v}^1, \ldots, \mathbf{v}^n) = \{\beta_1 \mathbf{v}^1 + \ldots + \beta_n \mathbf{v}^n \mid \beta_1, \ldots, \beta_n \geq 0\}$ and of the convex hull $conv(\mathbf{v}^1, \ldots, \mathbf{v}^n) = \{\alpha_1 \mathbf{v}^1 + \ldots + \alpha_n \mathbf{v}^n \mid \alpha_1, \ldots, \alpha_n \geq 0, \alpha_1 + \ldots + \alpha_n = 1\}$ of given vectors $\mathbf{v}^1, \ldots, \mathbf{v}^n$ in a flux tope, thus in a closed orthant (so the sum is conformal), have their supports included in $supp(\mathbf{v}^1) \cup \ldots \cup supp(\mathbf{v}^n)$, which is the support of any vector $\mathbf{v}$ in $cone^+(\mathbf{v}^1, \ldots, \mathbf{v}^n)$ or $conv^+(\mathbf{v}^1, \ldots, \mathbf{v}^n)$, thus their signs less than or equal to the sign of $\mathbf{v}$, and from the fact that a face of a polyhedron is the Minkowski sum of the convex hull of its vertices and the conical hull of its extreme vectors. Thus, if a vector $\mathbf{v}$ of a flux tope $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$) belongs to the solutions space, the minimal face of $FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$) containing $\mathbf{v}$ is completely included in it. As a sign-monotone constraint is sign-based, results of Theorem 3.3 apply and provide formulas for `EFVs` and `EFPs`. Remains thus only the case of `EFMs`. Now, if an elementary flux mode $\mathbf{v}$ of the solutions space was not support-minimal in FC, it would exist a nonzero vector $\mathbf{v}'$ in FC with $supp(\mathbf{v}') \subset supp(\mathbf{v})$. But we could choose $\lambda \in \mathbb{R}$ such that $\mathbf{v}'' = \mathbf{v} - \lambda \mathbf{v}'$ is nonzero, belongs to FC and verifies $supp(\mathbf{v}'') \subset supp(\mathbf{v})$ and $sign(\mathbf{v}'') \leq sign(\mathbf{v})$. By the sign-monotonicity property of the constraint, $\mathbf{v}''$ would also satisfy the constraint, thus would belong to the solutions space, which would contradict the fact that $\mathbf{v}$ is an elementary flux mode in this space. So, $\mathbf{v}$ is an elementary flux mode in FC and we get the result for `EFMs`. □

Obviously, in the decomposition of $CFC_{\mathbf{C}}(\mathbf{x})$ or $CFC_{\mathbf{C}}$ (resp., $CFP_{\mathbf{C}}(\mathbf{x})$ or $CFP_{\mathbf{C}}$) as union of some faces of the $FC_{\leq \boldsymbol{\eta}}$'s (resp., $FP_{\leq \boldsymbol{\eta}}$'s), one can keep only those faces which are maximal. Theorem 3.9 applies in particular to all thermodynamic constraints, to those kinetic constraints and to those very few regulatory constraints described in Lemma 3.7. In particular, we obtain directly (except obviously the reference to $\mathbf{ts_M}$) Proposition 2.5 for thermodynamic constraints $\mathbf{TC}$ and $\mathbf{TC}^b$, as well as the structure of the solutions space as union of flux cones (resp., flux polyhedra) for kinetic constraints $\mathbf{KC}$ and $\mathbf{KC}^b$ (in absence of bounds on enzymes concentrations) and the characterization of elementary fluxes and elementary flux modes given by Proposition 2.8, which proves that these results depend only on the fact that those constraints are sign-monotone.

Notice that the knowledge of `EFVs`, equal to `EFMs`, of $CFC_{\mathbf{C}}$ (resp., and of `EFPs` of $CFP_{\mathbf{C}}$) is not enough to reconstruct the structure of the solutions space $CFC_{\mathbf{C}}$ (resp., $CFP_{\mathbf{C}}$) as union of polyhedral cones (resp., polyhedra). For this, it is necessary to know the (non-disjoint) decomposition $\{E_i\}$ of those `EFVs` (resp., and `EFPs`) as extreme vectors (resp., and extreme points) of the faces F_i of the flux topes

$FC_{\leq \boldsymbol{\eta}}$ (resp., $FP_{\leq \boldsymbol{\eta}}$) that constitute the solutions space. The E_i's are exactly the maximal subsets of `EFVs` (resp., and `EFPs`) whose conformal conical hull (resp., conformal convex hull) is entirely contained in the solutions space: $cone_{\oplus}(E_i) \subseteq CFC_{\mathbf{C}}$ (resp., and $conv_{\oplus}(E_i) \subseteq CFP_{\mathbf{C}}$). By analogy with LTCS, we will call each such E_i a largest $\mathbf{C}$-consistent set of `EFVs` or `EFMs` (resp., and of `EFPs`), noted $\mathrm{LCS}_{\mathbf{C}}$.

In order to deal with enzymatic capacity constraints, we generalize the sign-monotone criterion exactly in the same way we generalized the sign-based criterion. A constraint $\mathbf{C}_{\mathbf{x}}(\mathbf{v})$ (resp., $\exists \mathbf{x} \mathbf{C}_{\mathbf{x}}(\mathbf{v})$), is said contracting-sign-monotone if, when satisfied by one vector, is satisfied by any vector having a less or equal sign which is not greater (on each component), i.e., that belongs to the rectangle parallelepiped defined by the null vector and the given vector:

$$\mathbf{C}_{\mathbf{x}}(\mathbf{v}), sign(\mathbf{v}') \leq sign(\mathbf{v}), \forall i |\mathbf{v}'_i| \leq |\mathbf{v}_i| \Rightarrow \mathbf{C}_{\mathbf{x}}(\mathbf{v}') \tag{95}$$

$$\exists \mathbf{x} \mathbf{C}_{\mathbf{x}}(\mathbf{v}), sign(\mathbf{v}') \leq sign(\mathbf{v}), \forall i |\mathbf{v}'_i| \leq |\mathbf{v}_i| \Rightarrow \exists \mathbf{x} \mathbf{C}_{\mathbf{x}}(\mathbf{v}'). \tag{96}$$

Obviously a sign-monotone constraint is contracting-sign-monotone and, if the $\mathbf{C}_{\mathbf{x}}(\mathbf{v})$'s are contracting-sign-monotone for all $\mathbf{x}$, then $\exists \mathbf{x} \mathbf{C}_{\mathbf{x}}(\mathbf{v})$ is contracting-sign-monotone. Also, any contracting-sign-monotone constraint is contracting-sign-based.

Example 3.4. The argument given in Example 3.2 to establish that the kinetic constraints $\mathbf{KC}^b_{\mathbf{M}}(\mathbf{v})$ (80) and $\mathbf{KC}^b(\mathbf{v})$ (76) are contracting-sign-based in absence of positive lower bounds for enzymes concentrations (i.e., when $\mathbf{E}^- = \mathbf{0}$), proves in fact that they are contracting-sign-monotone.

Lemma 3.10. *The kinetic constraints $\boldsymbol{KC}^b_{\boldsymbol{M}}$ and $\boldsymbol{KC}^b$ are contracting-sign-monotone in absence of positive lower bounds for enzymes concentrations (i.e., when $\boldsymbol{E}^- = \boldsymbol{0}$).*

Theorem 3.11. *If $\boldsymbol{C} = \boldsymbol{C}_{\boldsymbol{x}}(\boldsymbol{v})$ (or $\boldsymbol{C} = \exists \boldsymbol{x} \boldsymbol{C}_{\boldsymbol{x}}(\boldsymbol{v})$) is contracting-sign-monotone, then $CFC_{\boldsymbol{C}}$ is a $\boldsymbol{0}$-star domain and it exists a neighborhood $N =]-\delta, +\delta[^r$ of $\boldsymbol{0}$ for some $\delta > 0$ such that $CFC_{\boldsymbol{C}} \cap N$ is a finite union of N-truncated polyhedral cones, which are the intersection with N of faces of the flux topes $FC_{\leq \boldsymbol{\eta}}$. In addition, results (93) about `EFVs` and `EFMs` hold in N, i.e., for sufficiently small fluxes. The same holds for $CFP_{\boldsymbol{C}}$ with the flux topes $FP_{\leq \boldsymbol{\eta}}$ if $\boldsymbol{0}$ is an interior point of FP, and in this case results (94) about `EFVs` and `EFPs` hold in N.*

Proof. The proof is identical to the proof of Theorem 3.6, by using the proof of Theorem 3.9. □

Theorem 3.11 tells that the result of Theorem 3.9 for sign-monotone constraints about the geometrical structure of the solutions space and the determination of elementary fluxes and elementary flux modes applies identically for contracting-sign-monotone constraints locally in a neighborhood of $\mathbf{0}$, i.e., when considering only pathways with sufficiently small amounts of fluxes. It applies in particular to those kinetic constraints described in Lemma 3.10, giving the result about the structure of $CFC_{\mathbf{KC}^b_{\mathbf{M}}}$ and $CFC_{\mathbf{KC}^b}$ quoted in the last part of Proposition 2.8.

Finally, proposition 2.14 extends straightforwardly to the case where one has to deal both with one sign-monotone constraint and one sign-based constraint. As a sign-monotone constraint is sign-based the conjunction of the two constraints is sign-based and theorem 3.3 applies.

Theorem 3.12. *The space of flux vectors in FC (resp., FP) satisfying both a sign-monotone constraint and a sign-based constraint is a finite disjoint union of open polyhedral cones (resp., open polyhedra) which are some open faces of the flux topes of FC (resp., FP). The elementary flux vectors (resp., elementary flux points and vectors) are those of FC (resp., FP) that satisfy both constraints.*

Note that, in the case of FC, theorem 3.4 applies also to determine elementary flux modes and support-wise non-strictly-decomposable vectors.

3.3 Consequences on elementary fluxes computation

From the results above, we will see that the computation of elementary fluxes in presence of a sign-monotone constraint can be efficiently performed with the Double Description (DD) method.

Let first remind briefly the principle of the DD method [28]. It is an algorithm that takes as input the implicit description of a pointed convex polyhedral cone C as its representation matrix, i.e., a finite set of homogeneous linear inequalities defining C as the intersection of the corresponding vector half-spaces,

and produces as output the explicit description of C as a (minimal) generating matrix, i.e., the set of extreme rays of C. And, more generally, it can deal in the same way with a pointed convex polyhedron P producing, from a finite set of linear inequalities defining P as the intersection of the corresponding affine half-spaces, the explicit description of P as two generating matrices providing respectively the vertices of P and the extreme rays of C_P. As the first are obtained as the extreme rays of the pointed cone obtained from P by adding one dimension to the space and considering the conical hull of P from an origin of this extended vector space, it is enough to explain how the DD method works on pointed convex polyhedral cones. The DD method is an incremental algorithm that processes one by one each of the n homogeneous linear inequalities defining C. At each step $i, 1 \leq i \leq n$, it builds the intermediate extreme rays of the intermediate current cone C_i defined by the i first linear inequalities from the knowledge of the extreme rays of C_{i-1} built at previous steps and of the ith linear inequality. At the end, for $i = n$, $C_n = C$ and the extreme rays are thus obtained. The ith linear inequality defines a half-space H_i^+ with frontier a vector hyperplane H_i. All the extreme rays of C_{i-1} that are on the right side of H_i, i.e., belong to H_i^+, are extreme rays of C_i and thus kept; all those that are on the wrong side do not belong to C_i and will be discarded; the new extreme rays at step i appear from the intersection of C_{i-1} with H_i and are obtained as the intersection with H_i of the 2-faces of C_{i-1} defined by two adjacent extreme rays, one of each side of H_i. Thus it is just needed to keep and update at each step this list of pairs of adjacent extreme rays and, when the ith linear inequality is processed, each such pair with elements on both sides of H_i determines with H_i an extreme ray for C_i. The key fact is that each new extreme ray built at step i is the conical sum of two extreme rays existing at step $i-1$ and, as we will proceed by flux tope, i.e., in a given closed r-orthant, this sum is conformal and the support of this new extreme ray is the union of the supports of two previously existing extreme rays. And, analogously, this new extreme ray, if it is involved in the next steps, as a member of a relevant pair of adjacent rays, to build a new extreme ray, will have its support included in the support of this latter one. Finally, it remains just to initialize C_1. For a flux cone FC defined by (6), what has been shown as the most efficient is to start from a basis of the nullspace of $\mathbf{S}$ ([44] proposed a method to compute `EFMs` directly as linear combinations of the vectors of a basis of this nullspace that turned out to be less efficient than DD), and this basis (of size $r - m$ by assuming $\mathbf{S}$ of full rank) can be chosen so that $r - m$ of the r linear inequalities are satisfied (we consider here the worst case corresponding to the highest number of such inequalities, i.e., $r_I = r$, which happens in particular if each reversible reaction is split into two irreversible ones). It remains thus m linear inequalities to satisfy, i.e., $n = m$ steps in the DD algorithm.

Consider now a sign-monotone constraint $\mathbf{C}$. From Theorem 3.9, the `EFVs` (identical to `EFMs`) of the constrained flux cone subset $CFC_{\mathbf{C}}$ are just those of FC, i.e., of all its flux topes $FC_{\leq \boldsymbol{\eta}}$ successively, that satisfy the constraint $\mathbf{C}$ (and the same is true for `EFPs` of $CFP_{\mathbf{C}}$). So, one has just to build the extreme rays of each $FC_{\leq \boldsymbol{\eta}}$ by the DD algorithm as presented above and filter those that satisfy $\mathbf{C}$. Obviously a filtering at the end, once all extreme rays built, will not improve at all the efficiency. But exploiting the key fact that any newly built extreme ray at some step, if used in some next steps to build other new extreme rays will have necessarily its support included in each support of those latter ones and the sign-monotonicity of $\mathbf{C}$, one can conclude that, each time a newly built extreme ray does not satisfy the constraint $\mathbf{C}$, it can be immediately discarded because not only it has no use at the step of its discovery but no use also at the future steps as all extreme rays, building of which it could be involved in, would have a larger support and thus would not satisfy $\mathbf{C}$ too. Thus the computation of elementary fluxes or elementary flux modes in presence of a sign-monotone constraint can be fully integrated to the incremental DD algorithm, filtering of the extreme rays satisfying the constraint being achieved at each step when they are newly created. The extra-cost is the checking of all intermediate extreme rays built for satisfiability by $\mathbf{C}$ (for most constraints, this can be practically instantaneous) and the gain is that all intermediate extreme rays for which this checking gives a negative answer are suppressed.

Proposition 3.13. *Given a sign-monotone constraint $\boldsymbol{C}$, the* `EFVs` *(or* `EFMs`*) of the constrained flux cone subset $CFC_{\boldsymbol{C}}$ are obtained by the DD algorithm as the extreme rays of each flux tope $FC_{\leq \boldsymbol{\eta}}$ by filtering out at each step all those newly built extreme rays that do not satisfy $\boldsymbol{C}$.*

This result applies in particular to thermodynamic constraints $\mathbf{TC}$ and $\mathbf{TC}^b$ [11, 13] and to kinetic constraints $\mathbf{KC}$ and $\mathbf{KC}^b$ (in absence of bounds on enzymes concentrations). Actually, for $\mathbf{TC}$ (or $\mathbf{KC}$ which is identical from Proposition 2.8), Proposition 2.6 demonstrated that the criterion for an extreme ray to be thermodynamically satisfiable is to belong to a given fixed open half-space delimited by a vector hyperplane. Checking this satisfiability boils down thus to compute the scalar product of the extreme

ray with a fixed vector (normal to the hyperplane and oriented towards the half-space) and verify it is positive. In this case, as it has been shown, it is yet much simpler to integrate this thermodynamic constraint as one supplementary $(n+1)$th homogeneous linear inequality and one can choose for example that it is processed in first by the DD algorithm. But, as it has been already pointed out, providing the list of the extreme flux vectors of $CFC_{\mathbf{C}}$ does not give its structure in terms of union of polyhedral cones. For this, it is necessary to identify all the $\mathrm{LCS}_{\mathbf{C}}$'s, i.e., the maximal subsets of those extreme flux vectors whose conformal conical hull is entirely contained in $CFC_{\mathbf{C}}$. For each flux tope $FC_{\leq \boldsymbol{\eta}}$, it amounts to compute all maximal upper bounds of signs of some of the extreme rays built from this flux tope such that any vector of this tope having for sign such an upper bound satisfies the sign constraint $\mathbf{C}$, as the vectors of the strict conical hull of a subset of extreme rays have for common sign the upper bound of the signs of those extreme rays. To each such upper bound identified is associated the $\mathrm{LCS}_{\mathbf{C}}$ made up of all the extreme rays whose signs are smaller than this upper bound.

For sign-based constraints, thus for regulatory constraints, no such incremental filtering is possible as a newly built intermediate extreme ray may have a sign that does not satisfy the constraint but may participate later at some next steps to the construction of other new extreme rays (with necessarily larger support) whose sign will satisfy the constraint. Thus such an extreme ray cannot be discarded. In addition we saw that anyway there is no longer identity between those `EFMs` of FC that satisfy the constraint, the `EFMs` of $CFC_{\mathbf{C}}$ and the support-wise non-strictly-decomposable vectors in $CFC_{\mathbf{C}}$ and, as only the two last ones are biologically relevant, computing the first ones would have no interest.

4 Conclusion

The analysis of metabolic networks at steady state takes classically into account only stoichiometric and reactions irreversibility constraints. In this context, well known pathways have been introduced, such as elementary flux modes or elementary flux vectors, which are biologically relevant in their own and from which all pathways, whose structure is a convex polyhedral flux cone FC, can be reconstructed. But first the computation of those `EFMs` or `EFVs` is hampered by the combinatorial explosion of their number in genome-scale metabolic models, second most of them are biologically irrelevant, because other important biological constraints are not taken into account. With the objective of both enumerating only biologically feasible `EFMs` or `EFVs` and, as they are much less, of improving the scalability of this computation, we took in consideration in this paper from one side thermodynamic and, more generally, kinetic constraints and from the other side regulatory constraints, and we tackled the problem of revisiting in this new extended framework the concept of `EFMs` and `EFVs` and, more largely, of characterizing the geometry of the solutions space. Actually, we considered a more general conceptual framework for constraints, namely the property for them to be compatible with vectors sign (i.e., with vectors support separately in each closed r-orthant), as it happens that most properties of the geometrical structure of the solutions space depend only on this very general sign-compatibility of the constraints. That is how we demonstrated that, for constraints which are sign-monotone (i.e., once satisfied by some vector are satisfied by any vector with smaller or equal sign), which is the case of thermodynamic constraints and of kinetic constraints in absence of bounds on enzymes concentrations, the solutions space is a union of convex polyhedral cones (which are faces of the flux topes of FC) and the `EFMs`, that coincide with the `EFVs`, are just those of FC that satisfy the constraint. In addition, we showed that their computation can be efficiently integrated to the classical double description algorithm, as each newly built intermediate extreme ray that does not satisfy the constraint can be filtered out at each incremental step. For the specific case of thermodynamic constraints or of kinetic constraints in absence of bounds on enzymes concentrations, we demonstrated that their solutions spaces are identical and, when there are also no bounds on metabolites concentrations, made up of those maximal faces of the flux topes of FC which are entirely contained into a fixed open vector half-space and thus the `EFMs` are just those of FC which belong to this half-space and are computed simply by adding one homogeneous linear inequality to the initial (stoichiometric and reactions irreversibility) ones. The situation is more complex for constraints which are only sign-based (i.e., once satisfied by some vector are satisfied by any vector with same sign). We demonstrated that in fact support-based constraints coincide with regulatory (Boolean) constraints, so, in this sense, it is not a generalization. For sign-based constraints (i.e., support-based in each closed r-orthant separately), we demonstrated that the solutions space is a finite disjoint union of open (i.e., without their facets) convex polyhedral cones and that the `EFVs` are just those of FC (equal to the `EFMs` of FC) that satisfy the

constraint. But they cannot be efficiently computed by the double description algorithm because they cannot be filtered out during the incremental process. In addition it is no longer true that EFVs identify to EFMs and that EFMs identify to support-wise non-strictly-decomposable vectors: there are in general strict inclusions between those three sets and we provided again a complete characterization of the two latter ones. Finally, we extended all these results to the case where inhomogeneous linear constraints (expressing for example capacity constraints or bounds on fluxes) exist, dealing thus with a convex flux polyhedron FP instead of a flux cone FC. Basically, most of results about the geometrical structure of the solutions space in presence of the above biological constraints remain the same with cones replaced by polyhedra.

Future work will be carried out along two directions. Firstly the present theoretical work will be extended to minimal cut sets (MCSs). Such MCSs are defined as minimal (for set inclusion) sets of reactions whose deletion will block the operation of some given objectives or target reactions (as, e.g., those producing some toxic or undesirable product), i.e., removal of an MCS (that is, knock-out or cut of its reactions) implies a zero flux for the target reactions in steady state. MCSs are important for computing intervention strategies, e.g., for metabolic engineering. It is obvious from this definition that, for a metabolic network modeled at steady state by a flux cone FC, the MCSs are the minimal hitting sets of the set of target EFMs (identical to EFVs) of the given metabolic network (i.e., the set of EFMs that comprise at least one of the target reactions), where a hitting set of the target EFMs is a set (of reactions) that has a nonempty intersection with each one of those EFMs. And this generalizes to a metabolic network modeled by a flux polyhedron FP by using EFPs. This gives an indirect method for computing MCSs [15, 18] from preliminary computation of EFVs (or EFPs) that can be applied in presence of biological constraints by using the results obtained in this paper. But it is known that it exists also a method for computing MCSs directly as the EFVs of a dual network [4, 43], obtained basically by transposing the stoichiometric matrix of the original matrix (thus in some sense, exchanging reactions and metabolites). Thus we will study how to properly define this dual operation for metabolic networks in presence of biological constraints in order to preserve this result (or most of it). Secondly, algorithms described in this paper will be implemented and testing conducted on metabolic networks described in the literature for which biological (thermodynamic, kinetic or regulatory) data are known. As it has been shown, the computation of elementary flux vectors (or elementary flux points) boils down to filter those of extreme rays (or vertices) in each flux tope which satisfy the biological constraints. And for sign-monotone constraints as thermodynamic constraints or kinetic constraints in absence of bounds on enzymes concentrations, this filtering can be easily integrated at each step of the incremental double description method, so it is natural to rely first on this method which benefits from very efficient implementations. In absence of bounds on metabolites concentrations, the advantage is obvious as it is just needed to add one step (i.e., one homogeneous linear constraint to deal with) in the DD algorithm. But it has been shown that at most 50% of the EFVs can be ruled out that way, which is clearly insufficient to scale up to GSMMs. Adding bounds on metabolites concentrations has already proved to rule out an higher percentage of EFVs. This is achieved by checking the extreme rays at each step of the DD algorithm thanks to a call to a linear programming solver and it will be interesting to compare its efficiency with the method given by proposition 2.7 which does not need using an LP solver but has the inconvenient to require reasoning in higher dimension. As the structure of the solutions space cannot be deduced from the only knowledge of the EFVs but requires the identification of the largest consistent (w.r.t. the constraints) sets of EFVs, efficient computation of those $\mathrm{LCS}_{\mathbf{C}}$'s will be studied. One can reasonably think that scaling up will be obtained only by dealing with all biological constraints together. But, as it has been shown, handling regulatory constraints poses serious problems as those constraints are not sign-monotone and thus filtering of the EFVs that satisfy them cannot be integrated incrementally in the DD algorithm. In addition the structure of the solutions space is more complex as union of open polyhedral cones (or open polyhedra) and other pathways of interest for biologists, such as elementary flux modes or support-wise non-strictly-decomposable vectors, do not coincide any more with EFVs and require more complex computations to be identified. Algorithms will be carefully studied for maximal efficiency and novel ways of using DD method or use of other methods recently proposed as local reverse search or satisfiability based methods will be investigated.

Acknowledgments

I would like to express my sincere thanks to my colleagues Sabine Peres, who introduced me a few years ago to bioinformatics and more particularly to metabolic pathway analysis, that was a field completely unknown to me, and Antoine Deza, who explained me all I had to know about convex geometry and the double description algorithm. Without their help and the huge number of hours we spent working and discussing together, this work could never have been accomplished.

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