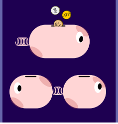


Constraint-based modeling of microbial communities: from polyhedral geometry to ecology

S. Müller, M. Predl, D. Széliová, J. Zanghellini

Joint work: math/biochem

Stefan Müller

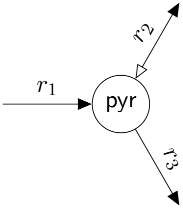
Faculty of Mathematics
University of Vienna

Michael Predl, Diana Széliová, Jürgen Zanghellini

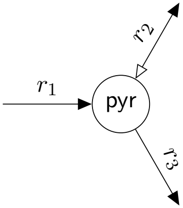
Biochemical Network Analysis
Department of Analytical Chemistry
University of Vienna

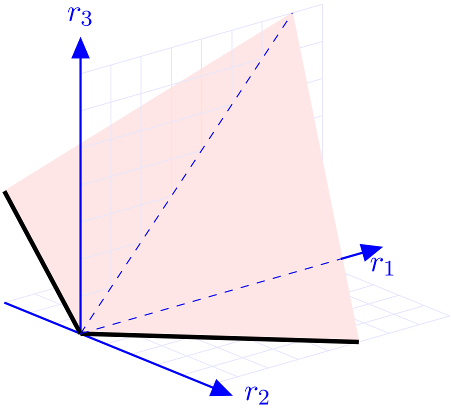
Preprint on bioRxiv by end of July . . .

Reminder: What are elementary flux modes?

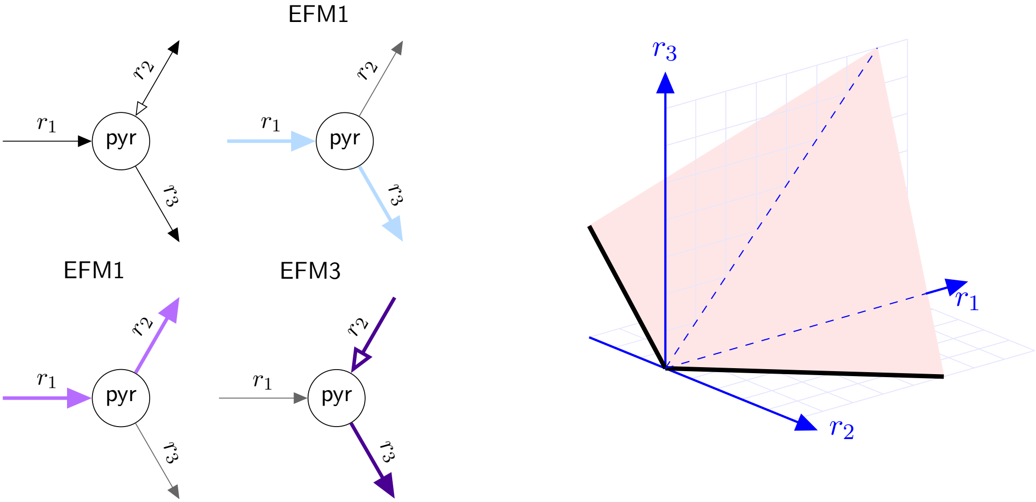


Reminder: What are elementary flux modes?

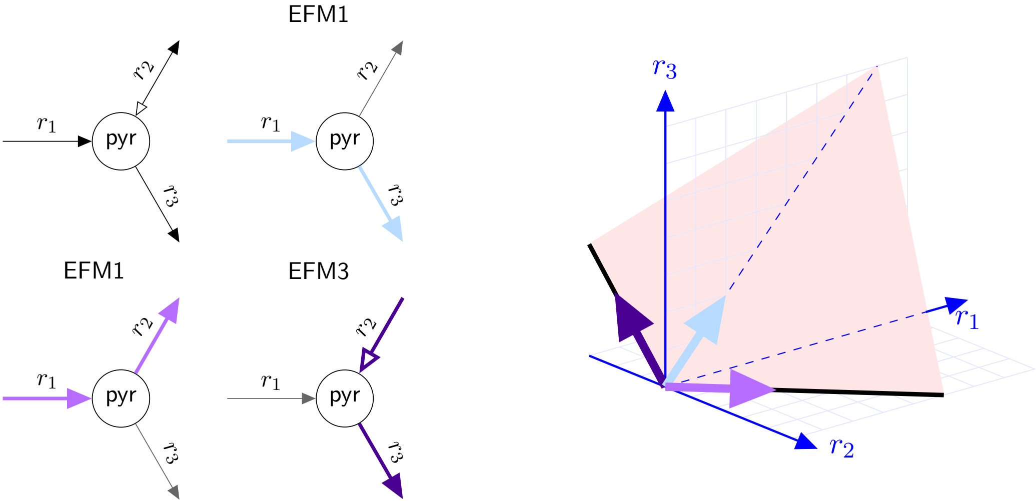




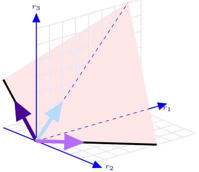
Reminder: What are elementary flux modes?



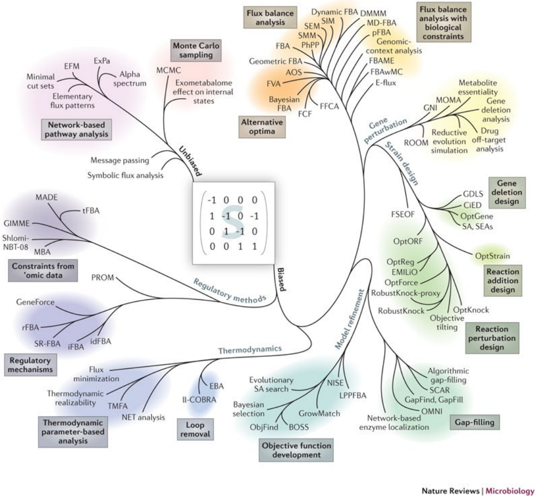
Reminder: What are elementary flux modes?



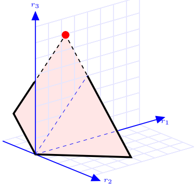
The forest of methods



+ principle importance
+ metabolic lego blocks
+ characterize full space
– computationally difficult
– constraints $\geq 0, = 0$
– yields only

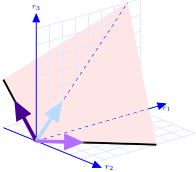


doi:10.1038/nrmicro2737

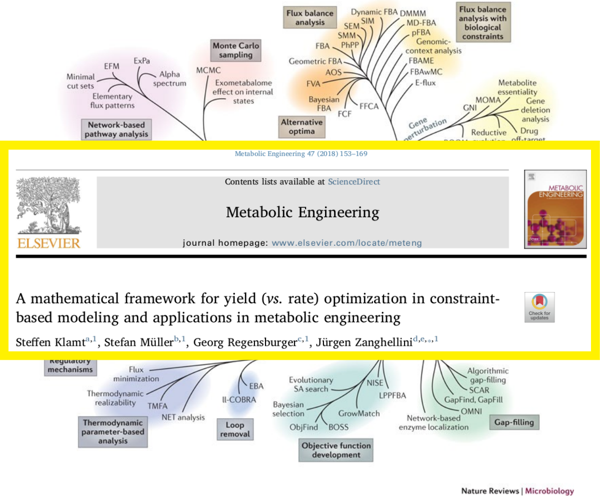


+ any linear constraints
+ computationally easy
+ flux rates & yields
– only one point
– optimally

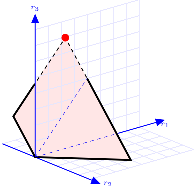
The forest of methods



+ principle importance
+ metabolic lego blocks
+ characterize full space
– computationally difficult
– constraints $\geq 0, = 0$
– yields only

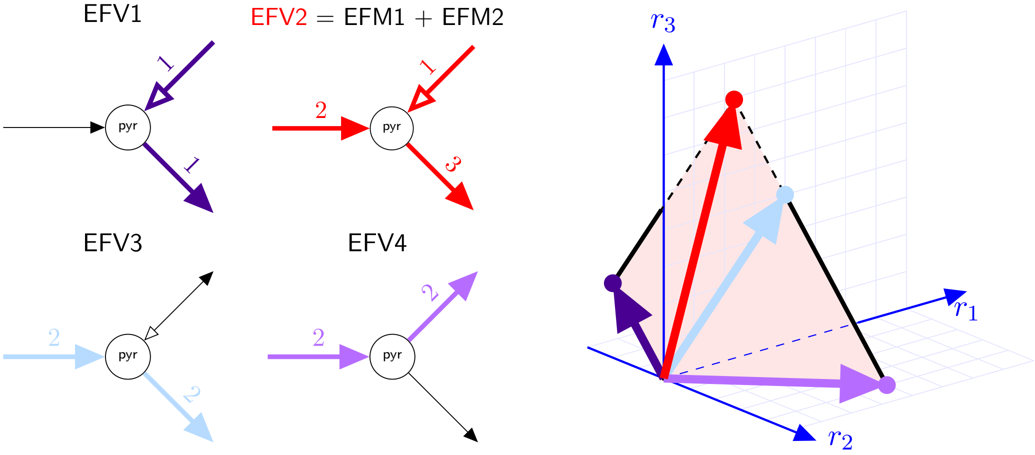


doi:10.1038/nrmicro2737



+ any linear constraints
+ computationally easy
+ flux rates & yields
– only one point
– optimally

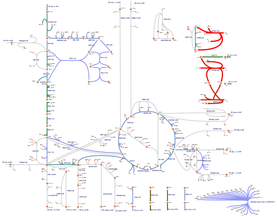
Zoo of minimal pathways: Elementary flux vectors (EFVs)

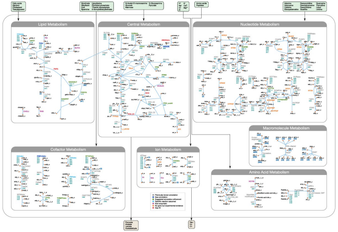


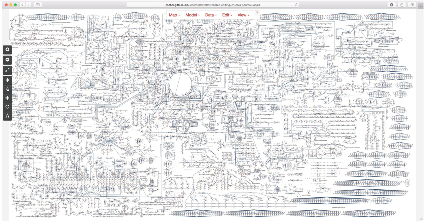
- unique, but not support-minimal
- defined rates and yields
- respect capacity constraints
- any flux convex sums of pathways

How many EFMs does ... have?

- *E. coli*'s central carbon metabolism, doi:10.1186/s12918-018-0607-5
- a Minimal cell, JCVI-syn3A, doi:10.7554/eLife.36842
- a Human cell doi:10.1038/nbt.4072

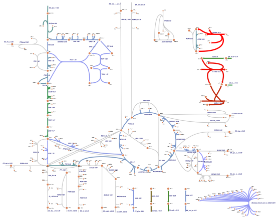


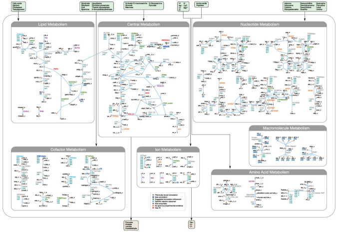


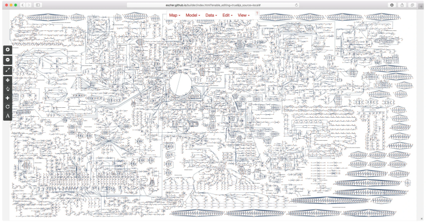


How many EFMs does ... have?

- *E. coli*'s central carbon metabolism, doi:10.1186/s12918-018-0607-5
- a Minimal cell, JCVI-syn3A, doi:10.7554/eLife.36842
- a Human cell doi:10.1038/nbt.4072

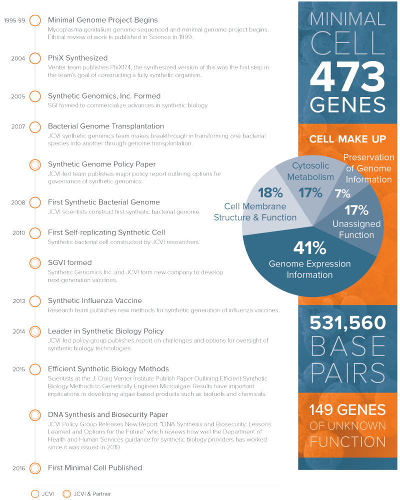




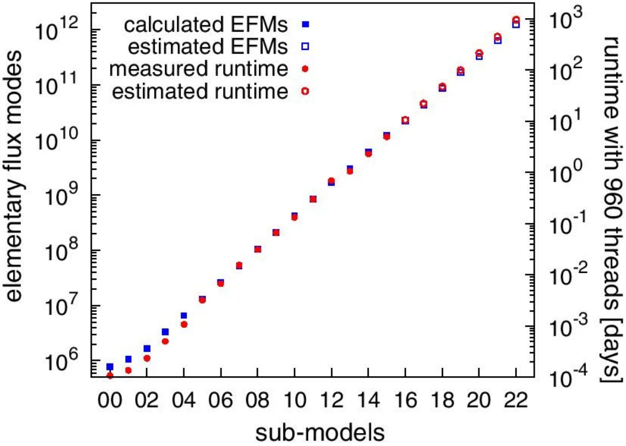


124,341,216 $\approx 10^{12}$ (one trillion) $> 10^{29}$

Functional space of a minimal cell



- >1 trillion EFMs, $\approx 1\% = 12,051,382,513$ computed
- 2.5 years w/ 1000 CPUs and 33×10^6GB $=$ 33PB storage for full set



Why do we do this? (Personal motivation)

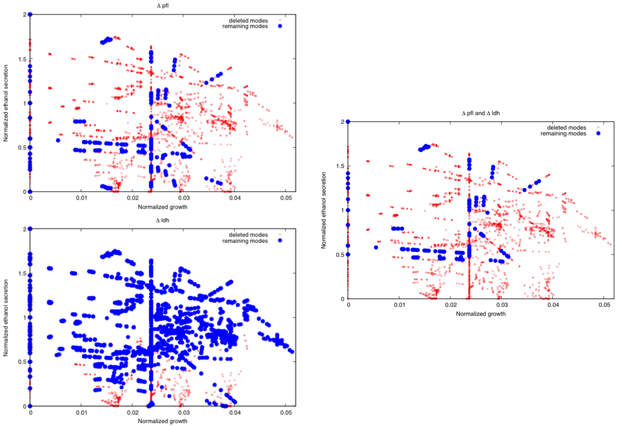
- fundamental understanding of biological processes

Theorem 1. *The flux distribution that maximizes an objective flux over the total enzyme cost in a metabolic network without additional constraints is an Elementary Flux Mode.*

Why do we do this? (Personal motivation)

- fundamental understanding of biological processes

Theorem 1. *The flux distribution that maximizes an objective flux over the total enzyme cost in a metabolic network without additional constraints is an Elementary Flux Mode.*



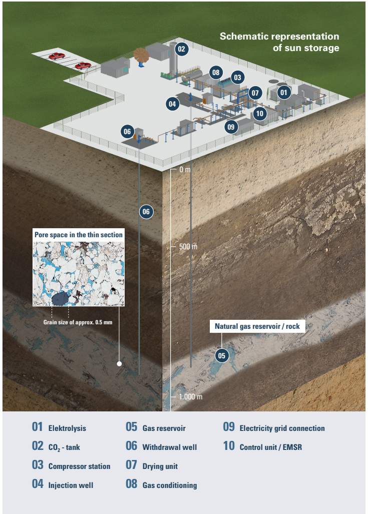
- metabolic engineering
- synthetic biology

The Problem: Pilsbach, Upper Austria

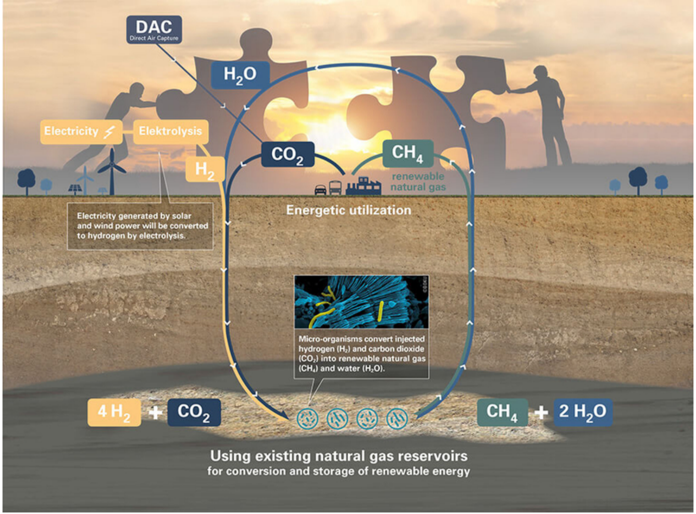
© RAG (photography: RAG-Archiv)

The Problem: Pilsbach, Upper Austria

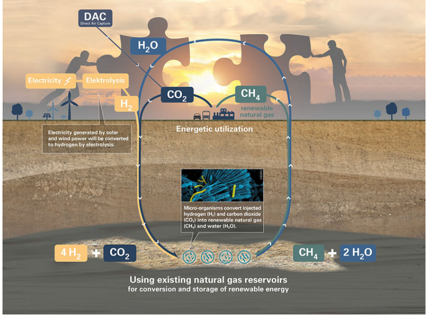
© RAG (photography: RAG-Archiv)



Underground Sun Conversion

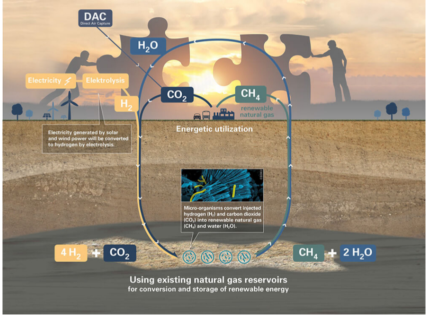


How can we maximize “biogas” production?

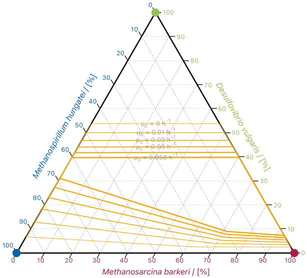


- What community compositions are feasible?
- What community composition is optimal?

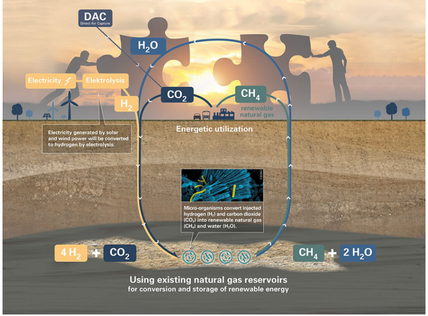
How can we maximize “biogas” production?



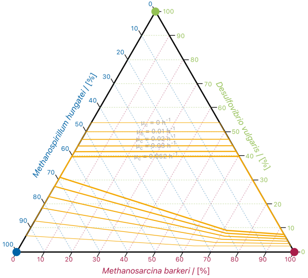
- What community compositions are feasible?
- What community composition is optimal?

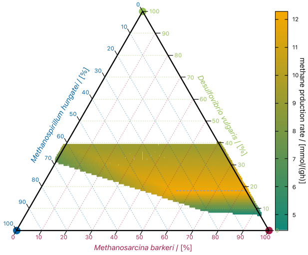


How can we maximize “biogas” production?

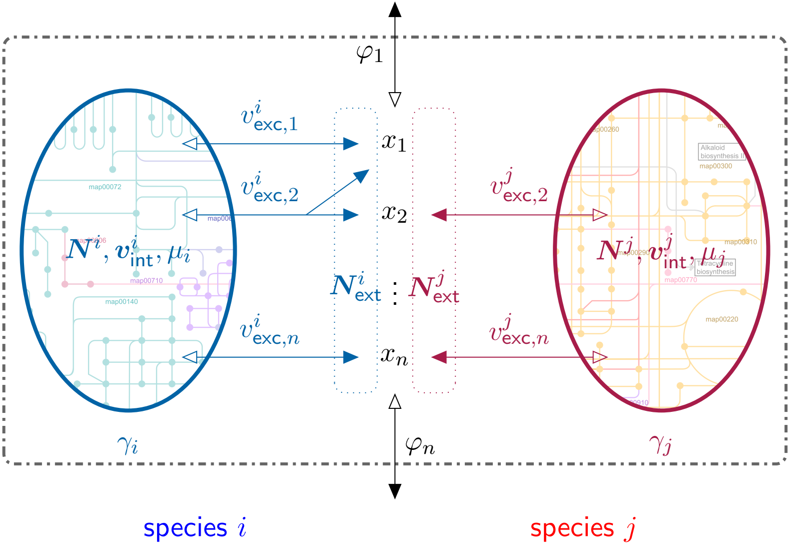


- What community compositions are feasible?
- What community composition is optimal?





Microbial community



Q&A

Given

- a set of microbial species (their metabolic models),
- a medium, and
- a growth rate:

What are all feasible community compositions and metabolic interactions?
What are the minimal communities?

Q&A

Given
– a set of microbial species (their metabolic models),
– a medium, and
– a growth rate:

What are all feasible community compositions and metabolic interactions?
What are the minimal communities?

We define:

community metabolic space (a polytope)
elementary compositions & exchange fluxes (ECXs)

Elementary vectors in metabolic pathway analysis

- Elementary flux modes (EFMs) — flux cone
  Elementary flux vectors (EFVs) — flux polyhedron (FBA)
- Elementary conversion modes (ECMs) — exchange fluxes
- Elementary growth modes (EGMs) — next-generation models, resource balance analysis (RBA)
- ECXs — microbial communities
  ECs, EXs

Elementary vectors

Linear subspace S:

elementary vector (EV): $e \in S$ with minimal support

Theorem (Rockafellar 1969)

Every $f \in S$ is a finite, conformal sum of EVs:

$$f = \sum_e e \quad \textit{with} \quad \mathrm{sign}(e) \leq \mathrm{sign}(f)$$

Elementary vectors

Linear subspace S:

elementary vector (EV): $e \in S$ with minimal support

Theorem (Rockafellar 1969)

Every $f \in S$ is a finite, conformal sum of EVs:

$$f = \sum_e e \quad \textit{with} \quad \mathrm{sign}(e) \leq \mathrm{sign}(f)$$

Reaction directions, thermodynamics

Elementary vectors

Linear subspace S:

elementary vector (EV): $e \in S$ with minimal support

Theorem (Rockafellar 1969)

Every $f \in S$ is a finite, conformal sum of EVs:

$$f = \sum_e e \quad \textit{with} \quad \operatorname{sign}(e) \leq \operatorname{sign}(f)$$

Reaction directions, thermodynamics

Generalization (Müller, Regensburger 2016)

linear subspace $S \rightarrow$ general polyhedral cone (e.g. flux cone),
polyhedron (e.g. flux polyhedron)

Elementary vectors

Linear subspace S:
elementary vector (EV): $e \in S$ with minimal support

Theorem (Rockafellar 1969)

Every $f \in S$ is a finite, conformal sum of EVs:

$$f = \sum_e e \quad \textit{with} \quad \mathrm{sign}(e) \leq \mathrm{sign}(f)$$

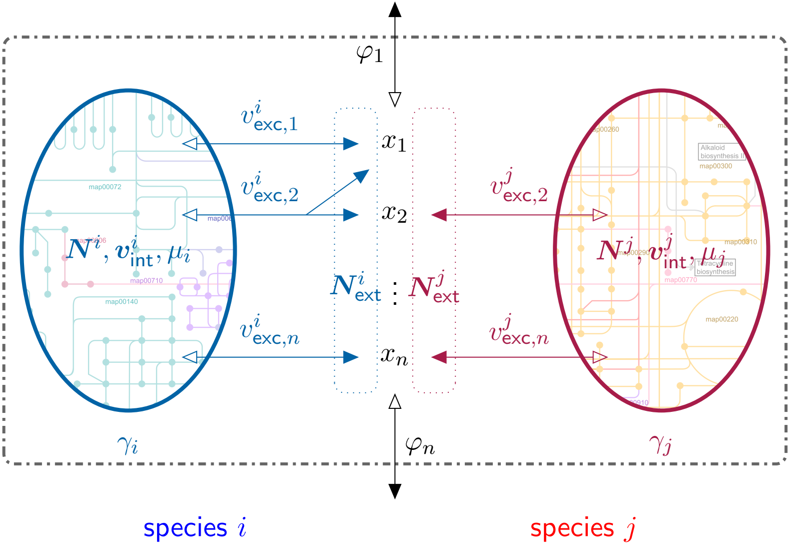
Reaction directions, thermodynamics

Generalization (Müller, Regensburger 2016)

linear subspace $S \to$ general polyhedral cone (e.g. flux cone),
polyhedron (e.g. flux polyhedron)

elementary vector: (convex-)conformally non-decomposable

Microbial community



(Constraint-based) metabolic modeling of microbial communities

- Single species growth
- Exchange with medium
- From dynamics to steady state

Single species growth

Πάντα αὐξάνεται (Panta auxanetai) ... ‘Everything grows’

Single species growth

Πάντα αὐξάνεται (Panta auxanetai) ... 'Everything grows'

Self-fabricating cell:

$$\frac{\mathsf{d}}{\mathsf{d}t}\begin{pmatrix} x \\ y \end{pmatrix} = \underbrace{\begin{pmatrix} N & S \\ 0 & \mathrm{I} \end{pmatrix}}_{N_{\text{nxt-gen}}} \begin{pmatrix} v(x,y) \\ w(x,y) \end{pmatrix} - \mu \begin{pmatrix} x \\ y \end{pmatrix}$$

x ... metabolites, y ... macromolecules

Single species growth

Πάντα αὐξάνεται (Panta auxanetai) … 'Everything grows'

Self-fabricating cell:

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} x \\ y \end{pmatrix} = \underbrace{\begin{pmatrix} N & S \\ 0 & \mathrm{I} \end{pmatrix}}_{N_{\text{nxt-gen}}} \begin{pmatrix} v(x,y) \\ w(x,y) \end{pmatrix} - \mu \begin{pmatrix} x \\ y \end{pmatrix}$$

x … metabolites, y … macromolecules

CBM: RBA (resource balance analysis)

Single species growth

Dynamics of growth:

$$\frac{\mathsf{d}}{\mathsf{d}t}\begin{pmatrix} x \\ y \end{pmatrix} = \underbrace{\begin{pmatrix} N & -S \\ 0 & \mathrm{I} \end{pmatrix}}_{N_{\text{nxt-gen}}} \begin{pmatrix} v(x,y) \\ w(x,y) \end{pmatrix} - \mu \begin{pmatrix} x \\ y \end{pmatrix}$$

CBM: FBA (flux balance analysis) and EFM analysis

Single species growth

Dynamics of growth:

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} x \\ y \end{pmatrix} = \underbrace{\begin{pmatrix} N & -S \\ 0 & \mathrm{I} \end{pmatrix}}_{N_{\text{nxt-gen}}}\begin{pmatrix} v(x,y) \\ w(x,y) \end{pmatrix} - \mu \begin{pmatrix} x \\ y \end{pmatrix}$$

CBM: FBA (flux balance analysis) and EFM analysis

$$\frac{\mathrm{d}y}{\mathrm{d}t} = 0 \quad \rightarrow \quad w = \mu\, y \quad \rightarrow \quad Sw = \mu \underbrace{Sy}_{x_{\text{bound}}} \quad \rightarrow \quad \frac{\mathrm{d}x}{\mathrm{d}t} = Nv(x) - \mu \underbrace{(x_{\text{bound}} + x)}_{x_{\text{total}}}$$

Single species growth

Dynamics of growth:

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} x \\ y \end{pmatrix} = \underbrace{\begin{pmatrix} N & -S \\ 0 & \mathrm{I} \end{pmatrix}}_{N_{\text{nxt-gen}}} \begin{pmatrix} v(x,y) \\ w(x,y) \end{pmatrix} - \mu \begin{pmatrix} x \\ y \end{pmatrix}$$

CBM: FBA (flux balance analysis) and EFM analysis

$$\frac{\mathrm{d}y}{\mathrm{d}t} = 0 \quad \rightarrow \quad w = \mu\, y \quad \rightarrow \quad Sw = \mu \underbrace{Sy}_{x_{\text{bound}}} \quad \rightarrow \quad \frac{\mathrm{d}x}{\mathrm{d}t} = Nv(x) - \mu \underbrace{(x_{\text{bound}} + x)}_{x_{\text{total}}}$$

$$\frac{\mathrm{d}x}{\mathrm{d}t} = 0 \quad \rightarrow \quad 0 = Nv - \mu\, x_{\text{total}} \quad \rightarrow \quad 0 = \underbrace{\begin{pmatrix} N & -x_{\text{total}} \frac{\text{g}}{\text{mol}} \end{pmatrix}}_{N_{\text{bm}}} \begin{pmatrix} v \\ \mu \frac{\text{mol}}{\text{g}} \end{pmatrix}$$

Single species growth

$$0 = N_{\mathsf{bm}} \begin{pmatrix} v \\ \mu \, \frac{\mathsf{mol}}{\mathsf{g}} \end{pmatrix},$$

$$l \leq v \leq u$$

Single species growth

$$0 = N_{\mathsf{bm}} \begin{pmatrix} v \\ \mu \, \frac{\mathsf{mol}}{\mathsf{g}} \end{pmatrix},$$

$$l \leq v \leq u$$

Multiple species: $i = 1, \ldots, \#\text{species}$

$$\gamma_i = \frac{m_i}{m} \quad \text{with} \quad m = \sum_i m_i$$

Single species growth

$$0 = N_{\text{bm}} \begin{pmatrix} v \\ \mu \, \frac{\text{mol}}{\text{g}} \end{pmatrix},$$

$$l \leq v \leq u$$

Multiple species: $i = 1, \ldots, \#\text{species}$

$$\gamma_i = \frac{m_i}{m} \quad \text{with} \quad m = \sum_i m_i \quad \implies \quad 0 \leq \gamma_i \leq 1 \quad \text{and} \quad \sum_i \gamma_i = 1$$

Single species growth

$$0 = N_{\mathsf{bm}} \begin{pmatrix} v \\ \mu \, \frac{\mathsf{mol}}{\mathsf{g}} \end{pmatrix},$$

$$l \leq v \leq u$$

Multiple species: $i = 1, \ldots, \#\text{species}$

$$\gamma_i = \frac{m_i}{m} \quad \text{with} \quad m = \sum_i m_i \quad \Longrightarrow \quad 0 \leq \gamma_i \leq 1 \quad \text{and} \quad \sum_i \gamma_i = 1$$

Individual species i:

$$0 = N^i_{\mathsf{bm}} \begin{pmatrix} v^i \\ \mu_i \, \frac{\mathsf{mol}}{\mathsf{g}} \end{pmatrix},$$

$$l^i \leq v^i \leq u^i,$$

$$\gamma_i \geq 0$$

Exchange with medium

Internal metabolites:

$$N = \text{Met} \begin{pmatrix} \overset{\text{Exc}}{N_{\text{exc}}} & \overset{\text{Int}}{N_{\text{int}}} \end{pmatrix}$$

Exchange with medium

Internal metabolites:

$$N = \text{Met} \; \overset{\text{Exc} \qquad \text{Int}}{\begin{pmatrix} N_{\text{exc}} & N_{\text{int}} \end{pmatrix}}$$

Metabolites in medium:

$$\begin{pmatrix} * \\ N \end{pmatrix} = \begin{matrix} \text{Med} \\ \text{Met} \end{matrix} \overset{\text{Exc} \qquad \text{Int}}{\begin{pmatrix} N_{\text{ext}} & 0 \\ N_{\text{exc}} & N_{\text{int}} \end{pmatrix}}$$

Exchange with medium

Internal metabolites:

$$N = \begin{array}{c} \\ \text{Met} \end{array} \begin{array}{c} \begin{array}{cc} \text{Exc} & \text{Int} \end{array} \\ \begin{pmatrix} N_{\text{exc}} & N_{\text{int}} \end{pmatrix} \end{array}$$

Metabolites in medium:

$$\begin{pmatrix} * \\ N \end{pmatrix} = \begin{array}{c} \\ \text{Med} \\ \text{Met} \end{array} \begin{array}{c} \begin{array}{cc} \text{Exc} & \text{Int} \end{array} \\ \begin{pmatrix} N_{\text{ext}} & 0 \\ N_{\text{exc}} & N_{\text{int}} \end{pmatrix} \end{array}$$

Dynamics in medium:

$$\frac{\mathrm{d}X_{\text{Med}}}{\mathrm{d}t} = m \sum_i \gamma_i \, N^i_{\text{ext}} v^i_{\text{exc}} - \Phi$$

Exchange with medium

Internal metabolites:

$$N = \text{Met} \begin{pmatrix} \overset{\text{Exc}}{N_{\text{exc}}} & \overset{\text{Int}}{N_{\text{int}}} \end{pmatrix}$$

Metabolites in medium:

$$\begin{pmatrix} * \\ N \end{pmatrix} = \begin{matrix} \text{Med} \\ \text{Met} \end{matrix} \begin{pmatrix} \overset{\text{Exc}}{N_{\text{ext}}} & \overset{\text{Int}}{0} \\ N_{\text{exc}} & N_{\text{int}} \end{pmatrix}$$

Dynamics in medium:

$$\frac{\mathrm{d}X_{\text{Med}}}{\mathrm{d}t} = m \sum_i \gamma_i \, N^i_{\text{ext}} v^i_{\text{exc}} - \Phi$$

$$\frac{\mathrm{d}X_{\text{Med}}}{\mathrm{d}t} = 0 \quad \rightarrow \quad 0 = \sum_i \gamma_i \, N^i_{\text{ext}} v^i_{\text{exc}} - \Phi/m \quad \rightarrow \quad \Phi_j \geq 0\text{: } \sum_i \gamma_i \, N^i_{\text{ext}} v^i_{\text{exc}} \geq 0, \quad \ldots$$

Community model in μ, γ and v^i

$$0 = N^i_{\text{bm}} \begin{pmatrix} v^i \\ \mu \, \frac{\text{mol}}{\text{g}} \end{pmatrix},$$

$$l^i \leq v^i \leq u^i,$$

$$\gamma_i \geq 0,$$

for $i = 1, \ldots, \#$species, and

$$\sum_i \gamma_i \, (N^i_{\text{ext}} v^i_{\text{exc}})_j \begin{cases} = 0, & \text{if } j \in \text{Med}_0, \\ \leq 0, & \text{if } j \in \text{Med}_{\text{in}}, \\ \geq 0, & \text{if } j \in \text{Med}_{\text{out}}, \end{cases}$$

$$\sum_i \gamma_i = 1.$$

Community model in μ, γ and v^i

$$0 = N^i_{\text{bm}} \begin{pmatrix} v^i \\ \mu \, \frac{\text{mol}}{\text{g}} \end{pmatrix},$$

$$l^i \leq v^i \leq u^i,$$

$$\gamma_i \geq 0,$$

for $i = 1, \ldots, \#\text{species}$, and

$$\sum_i \gamma_i \, (N^i_{\text{ext}} v^i_{\text{exc}})_j \begin{cases} = 0, & \text{if } j \in \text{Med}_0, \\ \leq 0, & \text{if } j \in \text{Med}_{\text{in}}, \\ \geq 0, & \text{if } j \in \text{Med}_{\text{out}}, \end{cases}$$

$$\sum_i \gamma_i = 1.$$

Introduce $\bar{v}^i = \gamma_i \, v^i$ (scaled fluxes)

Koch, ..., Klamt (2019), Plos Comp. Biol.

Community model in μ, γ and $\bar{v}^i$

$$0 = N^i_{\mathsf{bm}} \begin{pmatrix} \bar{v}^i \\ \gamma_i \, \mu \, \frac{\mathsf{mol}}{\mathsf{g}} \end{pmatrix},$$

$$\gamma_i \, l^i \leq \bar{v}^i \leq \gamma_i \, u^i,$$

$$\gamma_i \geq 0,$$

for $i = 1, \ldots, \#\text{species}$, and

$$\sum_i (N^i_{\mathsf{ext}} \bar{v}^i_{\mathsf{exc}})_j \begin{cases} = 0, & \text{if } j \in \mathsf{Med}_0, \\ \leq 0, & \text{if } j \in \mathsf{Med}_{\mathsf{in}}, \\ \geq 0, & \text{if } j \in \mathsf{Med}_{\mathsf{out}}, \end{cases}$$

$$\sum_i \gamma_i = 1.$$

Community model in μ, γ and $\bar{v}^i$

$$
\begin{gathered}
0 = N^i_{\text{bm}} \begin{pmatrix} \bar{v}^i \\ \gamma_i \, \mu \, \frac{\text{mol}}{\text{g}} \end{pmatrix}, \\
\gamma_i \, l^i \le \bar{v}^i \le \gamma_i \, u^i, \\
\gamma_i \ge 0,
\end{gathered}
$$

for $i = 1, \ldots, \#\text{species}$, and

$$
\begin{gathered}
\sum_i (N^i_{\text{ext}} \bar{v}^i_{\text{exc}})_j \begin{cases} = 0, & \text{if } j \in \text{Med}_0, \\ \le 0, & \text{if } j \in \text{Med}_{\text{in}}, \\ \ge 0, & \text{if } j \in \text{Med}_{\text{out}}, \end{cases} \\
\sum_i \gamma_i = 1.
\end{gathered}
$$

Project fluxes to exchange fluxes ($\bar{v}^i \to \bar{v}^i_{\text{exc}}$)

Community model in μ, γ and $\bar{v}^i_{\text{exc}}$

$$A^i\,\bar{v}^i_{\text{exc}} + b^i(\mu)\,\gamma_i \geq 0,$$

$$\gamma_i\, l^i_{\text{exc}} \leq \bar{v}^i_{\text{exc}} \leq \gamma_i\, u^i_{\text{exc}},$$
$$\gamma_i \geq 0,$$

for $i = 1, \ldots, \#\text{species}$, and

$$\sum_i (N^i_{\text{ext}} \bar{v}^i_{\text{exc}})_j \begin{cases} = 0, & \text{if } j \in \text{Med}_0, \\ \leq 0, & \text{if } j \in \text{Med}_{\text{in}}, \\ \geq 0, & \text{if } j \in \text{Med}_{\text{out}}, \end{cases}$$
$$\sum_i \gamma_i = 1.$$

Community model in μ, γ and $\bar{v}^i_{\text{exc}}$

$$A^i \, \bar{v}^i_{\text{exc}} + b^i(\mu)\,\gamma_i \geq 0,$$

$$\gamma_i \, l^i_{\text{exc}} \leq \bar{v}^i_{\text{exc}} \leq \gamma_i \, u^i_{\text{exc}},$$
$$\gamma_i \geq 0,$$

for $i = 1, \ldots, \#\text{species}$, and

$$\sum_i (N^i_{\text{ext}} \bar{v}^i_{\text{exc}})_j \begin{cases} = 0, & \text{if } j \in \text{Med}_0, \\ \leq 0, & \text{if } j \in \text{Med}_{\text{in}}, \\ \geq 0, & \text{if } j \in \text{Med}_{\text{out}}, \end{cases}$$
$$\sum_i \gamma_i = 1.$$

Fix μ (define polytope)

Community model $\rightarrow$ community modes

Fix μ

$\downarrow$

composition & exchange flux polytope in $\gamma, \bar{v}_{\text{exc}}$

$\swarrow \quad \searrow$

composition polytope in γ exchange flux polytope in $\bar{v}_{\text{exc}}$

Community model → community modes

Fix μ

$\downarrow$

composition & exchange flux polytope in $\gamma, \bar{v}_{\text{exc}}$

$\swarrow \quad \searrow$

composition polytope in γ exchange flux polytope in $\bar{v}_{\text{exc}}$

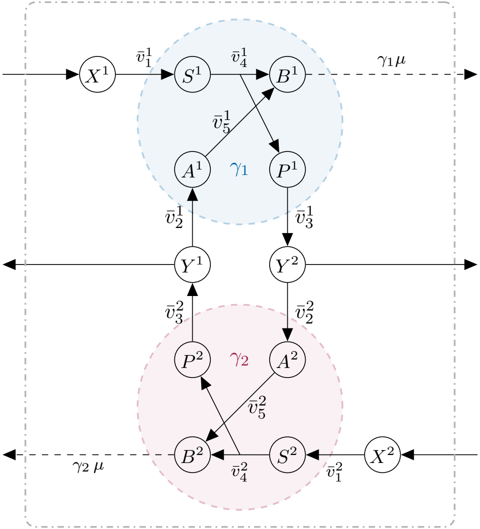
Convex-conformally non-decomposable vectors:

ECXs

$\swarrow \quad \searrow$

ECs   EXs

Example



X^1
$\bar{v}_1^1$
S^1
$\bar{v}_4^1$
B^1
$\gamma_1 \mu$
$\bar{v}_5^1$
A^1
γ_1
P^1
$\bar{v}_2^1$
$\bar{v}_3^1$
Y^1
Y^2
$\bar{v}_3^2$
$\bar{v}_2^2$
P^2
γ_2
A^2
$\bar{v}_5^2$
B^2
S^2
X^2
$\gamma_2 \, \mu$
$\bar{v}_4^2$
$\bar{v}_1^2$

Example: elementary compositions & exchange fluxes

$\hat{\mu} \in [0, 1]$:

$$
\begin{array}{lcccc}
 & \gamma_1, \gamma_2; & \hat{v}_1^1, \hat{v}_2^1, \hat{v}_3^1; & \hat{v}_1^2, \hat{v}_2^2, \hat{v}_3^2 & \\
\mathsf{ECX}_1 = (& 1, 0; & \hat{\mu}, 0, \hat{\mu}; & 0, 0, 0 &)^\top, \\
\mathsf{ECX}_2 = (& 0, 1; & 0, 0, 0; & \hat{\mu}, 0, \hat{\mu} &)^\top, \\
\mathsf{ECX}_3 = (& \frac{1}{2}, \frac{1}{2}; & \frac{\hat{\mu}}{2}, 0, \frac{\hat{\mu}}{2}; & 0, \frac{\hat{\mu}}{2}, 0 &)^\top, \\
\mathsf{ECX}_4 = (& \frac{1}{2}, \frac{1}{2}; & 0, \frac{\hat{\mu}}{2}, 0; & \frac{\hat{\mu}}{2}, 0, \frac{\hat{\mu}}{2} &)^\top.
\end{array}
$$

Example: elementary compositions & exchange fluxes

$\hat{\mu} \in [0,1]$:

$$\begin{array}{lcccc} & \gamma_1, \gamma_2; & \hat{v}_1^1, \hat{v}_2^1, \hat{v}_3^1; & \hat{v}_1^2, \hat{v}_2^2, \hat{v}_3^2 & \\ \mathsf{ECX}_1 = (& 1, 0; & \hat{\mu}, 0, \hat{\mu}; & 0, 0, 0 &)^\top, \\ \mathsf{ECX}_2 = (& 0, 1; & 0, 0, 0; & \hat{\mu}, 0, \hat{\mu} &)^\top, \\ \mathsf{ECX}_3 = (& \frac{1}{2}, \frac{1}{2}; & \frac{\hat{\mu}}{2}, 0, \frac{\hat{\mu}}{2}; & 0, \frac{\hat{\mu}}{2}, 0 &)^\top, \\ \mathsf{ECX}_4 = (& \frac{1}{2}, \frac{1}{2}; & 0, \frac{\hat{\mu}}{2}, 0; & \frac{\hat{\mu}}{2}, 0, \frac{\hat{\mu}}{2} &)^\top. \end{array}$$

$\hat{\mu} \in [1,2]$:

$$\begin{array}{lcccc} \mathsf{ECX}_5 = (& \frac{1}{\hat{\mu}}, \frac{\hat{\mu}-1}{\hat{\mu}}; & \frac{1}{\hat{\mu}}, \frac{\hat{\mu}-1}{\hat{\mu}}, \frac{1}{\hat{\mu}}; & \frac{\hat{\mu}-1}{\hat{\mu}}, \frac{(\hat{\mu}-1)^2}{\hat{\mu}}, \frac{\hat{\mu}-1}{\hat{\mu}} &)^\top, \\ \mathsf{ECX}_6 = (& \frac{\hat{\mu}-1}{\hat{\mu}}, \frac{1}{\hat{\mu}}; & \frac{\hat{\mu}-1}{\hat{\mu}}, \frac{(\hat{\mu}-1)^2}{\hat{\mu}}, \frac{\hat{\mu}-1}{\hat{\mu}}; & \frac{1}{\hat{\mu}}, \frac{\hat{\mu}-1}{\hat{\mu}}, \frac{1}{\hat{\mu}} &)^\top, \\ \mathsf{ECX}_7 = (& \frac{1}{2}, \frac{1}{2}; & \frac{1}{2}, \frac{\hat{\mu}-1}{2}, \frac{1}{2}; & \frac{\hat{\mu}-1}{2}, \frac{1}{2}, \frac{\hat{\mu}-1}{2} &)^\top, \\ \mathsf{ECX}_8 = (& \frac{1}{2}, \frac{1}{2}; & \frac{\hat{\mu}-1}{2}, \frac{1}{2}, \frac{\hat{\mu}-1}{2}; & \frac{1}{2}, \frac{\hat{\mu}-1}{2}, \frac{1}{2} &)^\top. \end{array}$$

Example: elementary compositions

$\hat{\mu} \in [0, 1]$:

$$\gamma_1, \gamma_2$$

$$\mathsf{EC}_1 = (1, 0)^\top,$$
$$\mathsf{EC}_2 = (0, 1)^\top.$$

$\hat{\mu} \in [1, 2]$:

$$\mathsf{EC}_5 = (\tfrac{1}{\hat{\mu}}, \tfrac{\hat{\mu}-1}{\hat{\mu}})^\top,$$
$$\mathsf{EC}_6 = (\tfrac{\hat{\mu}-1}{\hat{\mu}}, \tfrac{1}{\hat{\mu}})^\top.$$

Example: elementary compositions

$\hat{\mu} \in [0, 1]$:

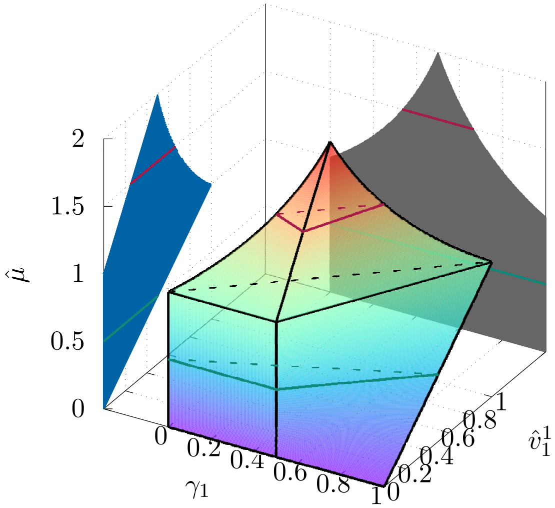
$$\begin{aligned} & \quad\quad \gamma_1, \gamma_2 \\ \mathsf{EC}_1 &= (1, 0)^\top, \\ \mathsf{EC}_2 &= (0, 1)^\top. \end{aligned}$$

$\hat{\mu} \in [1, 2]$:

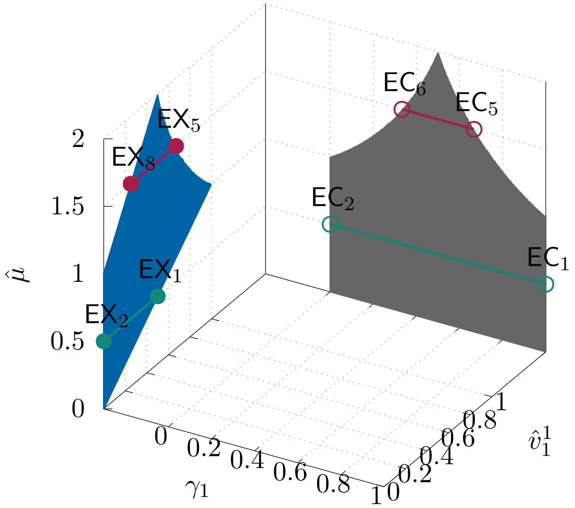
$$\begin{aligned} \mathsf{EC}_5 &= (\tfrac{1}{\hat{\mu}}, \tfrac{\hat{\mu}-1}{\hat{\mu}})^\top, \\ \mathsf{EC}_6 &= (\tfrac{\hat{\mu}-1}{\hat{\mu}}, \tfrac{1}{\hat{\mu}})^\top. \end{aligned}$$

analogously for elementary exchange fluxes (EXs)

Example: projection to $\mu, \gamma_1, \bar{v}_1^1$



Example: ECs and EXs

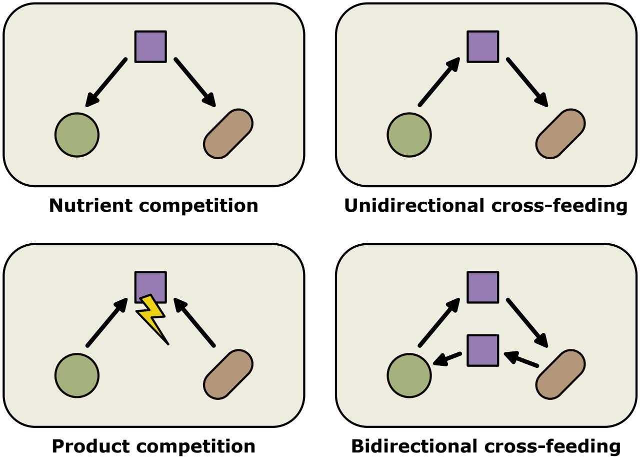


Ecological significance

	$\hat{\mu} \in [0, 1]$				$\hat{\mu} \in (1, 2]$			
ECX	1	2	3	4	5	6	7	8
specialization	✓	✓						
commensalism			✓	✓				
mutualism					✓	✓	✓	✓
maximum uptake					✓	✓		
maximum yield			✓	✓			✓	✓
nonlinear in $\hat{\mu}$					✓	✓		

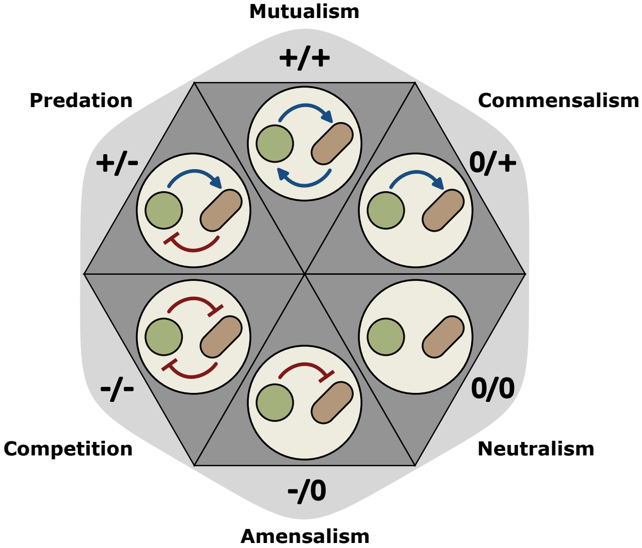
Interpretation of flux patterns: Topology

- Based on network structure
- Independent of growth or objective
- Applicable to any flux pattern
- Descriptive
- Biological implication not straight forward

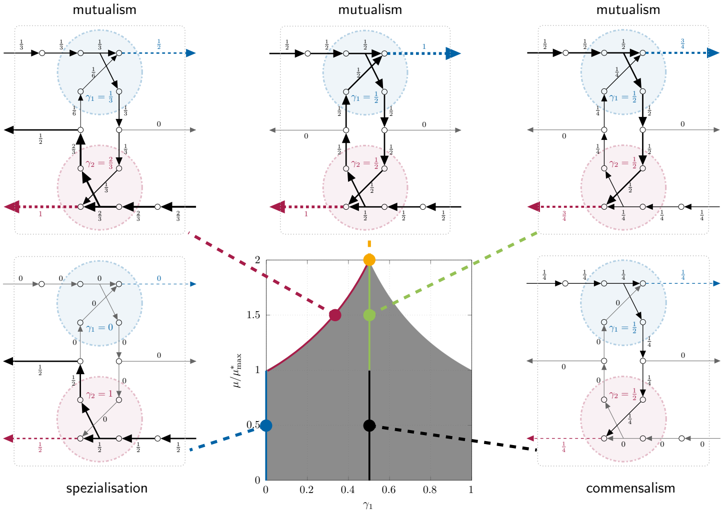


Interpretation of flux patterns: Ecology

- Classical ecology framework
- Sign-based classification (+/-/0)
- Requires *ecological outcome*
- Requires comparison of states
- Direct translation to biological significance



Interpretation of flux patterns: Example



Isotypic vs. anisotypic mutualism

Isotypic Mutualism

When deconstructed into ECFMs, *at least one* ECFM is mutualistic.

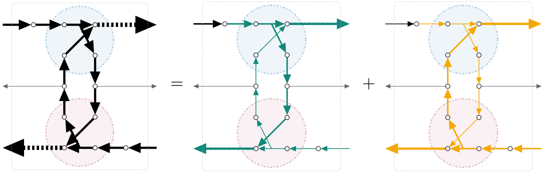
In example (a) all ECFM are mutualistic.

Anisotypic Mutualism

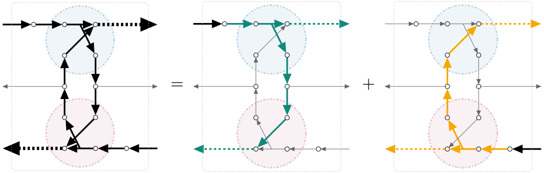
When deconstructed into ECFMs, *no* ECFM is mutualistic.

In example (b) all ECFM are commensalistic, and non is mutualistic.

(a) $\mu/\mu^*_{\max} = 1.5$



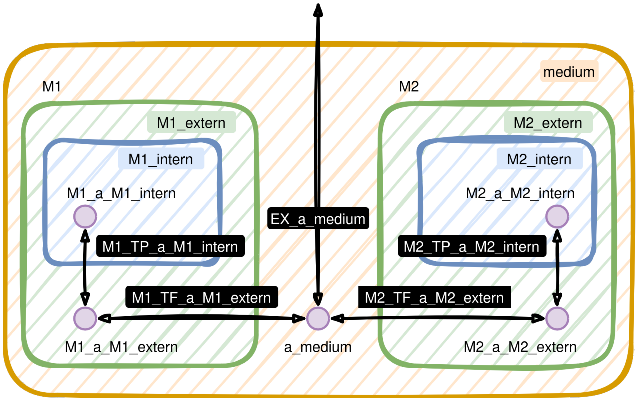
(b) $\mu/\mu^*_{\max} = 0.5$



Community model generation

Additional structure

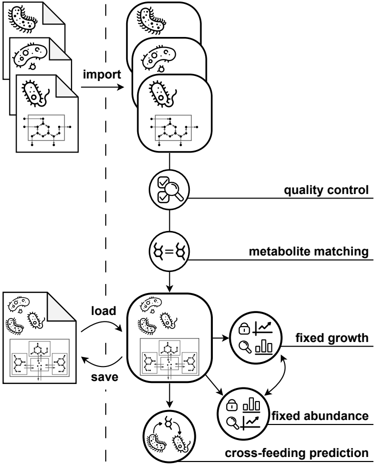
- Shared medium compartment
- Transfer reactions
- Community exchange reactions
- Community biomass reaction



Python package for community modeling (PyCoMo)

Model generation steps

- Match external metabolites
- Merge models
- Add shared medium compartment
- Manage exchange reactions
- Add community biomass function
- Scale member fluxes by mass fraction
- Check for mass and charge balance



Genome-scale example

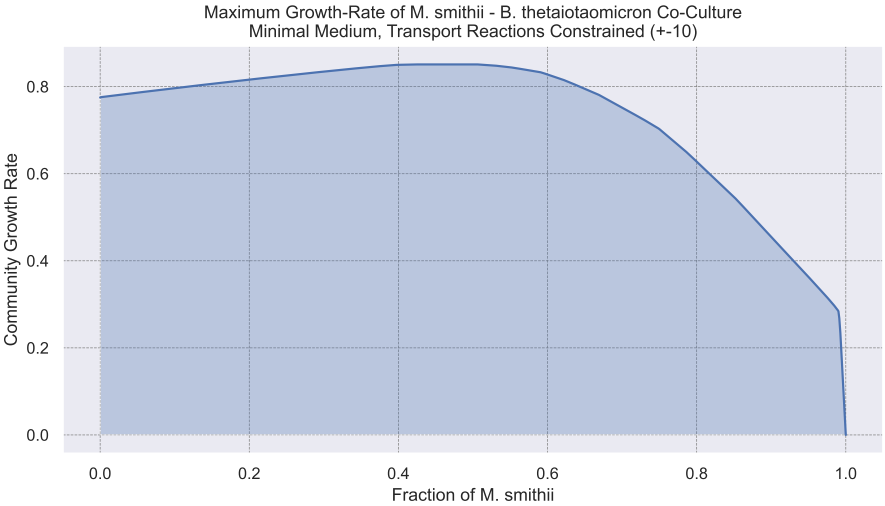
A co-culture of two human gut microbes

M. smithii: A methanogenic archaeon
B. thetaiotaomicron: A polysaccharide degrading bacterium

The community metabolic model is big!

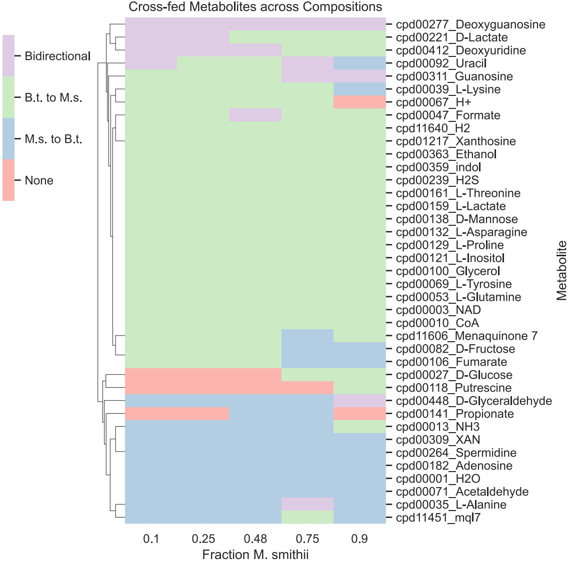
- # Reactions: 3860
- # Metabolites: 3355
- # Genes: 1141

Analysis options: growth rate



Analysis options: interactions

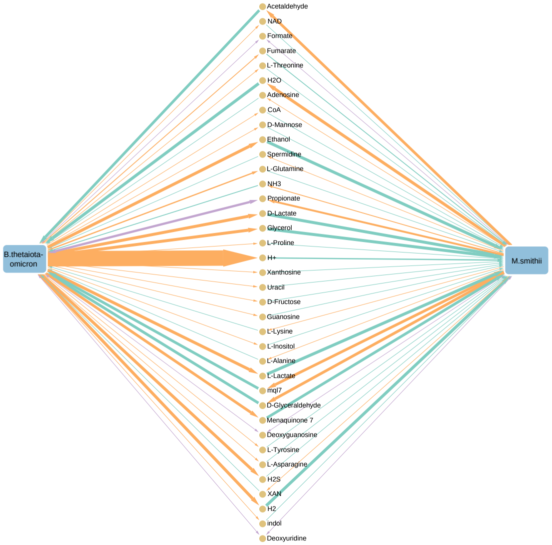
- FBA and FVA allow the detection of feasible cross-feeding
- Metabolic plasticity of the model: some cross-feeding interactions can occur in either direction
- Feasible cross-feeding patterns change across growth rate and composition



Analysis options: visualization

Visualization with
ScyNet and Cytoscape

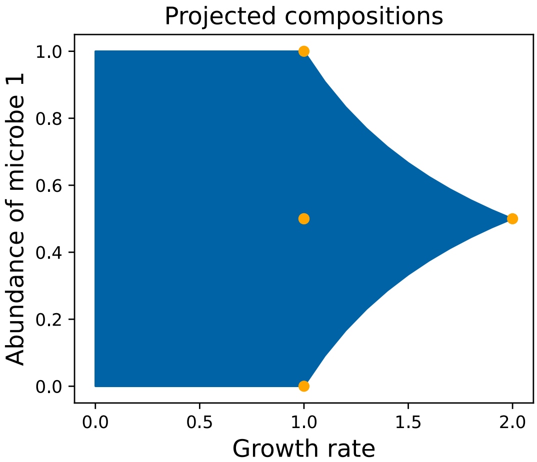
- Allows exploration of the model
- Reduced complexity by hiding internal reactions
- Reactions arrows can be contextualized with flux data



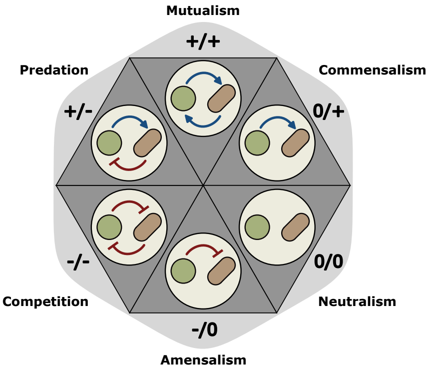
Analysis options: ECFMs

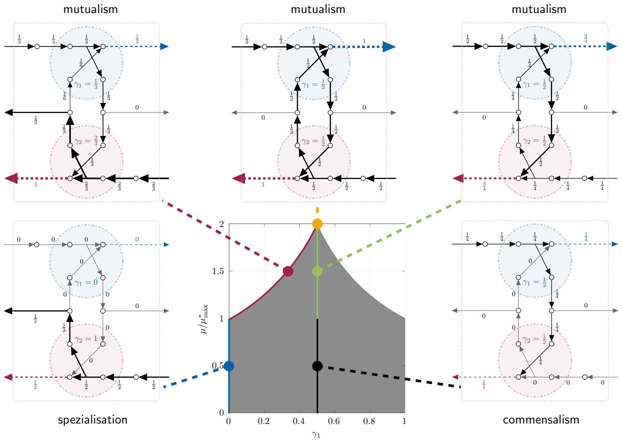
Computation of ECFMs with PyCoMo and efmtool

- PyCoMo community models can be used as input
- efmtool calculates ECFMs for fixed μ
- As of now, computation only feasible for smaller models (e.g. two E. coli core models)



Summary


Mutualism
+/+
Predation
Commensalism
+/-
0/+
-/-
0/0
Competition
Neutralism
-/0
Amensalism


mutualism
mutualism
mutualism
spezialisation
commensalism
$\mu/\mu^*_{\max}$
γ_1