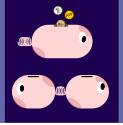
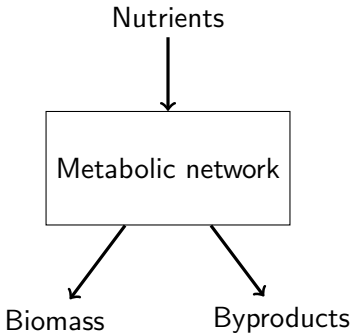


Optimization of metabolic fluxes

Steffen Waldherr Felipe Scott

A whole-cell perspective on metabolism



Properties that a model can try to describe

- Exchange fluxes / biomass production under given environmental conditions
- What is the internal network state to achieve certain exchange fluxes?
- How do the exchange fluxes / the internal network state react to external / internal perturbations?

Dimensions of metabolism

General overview

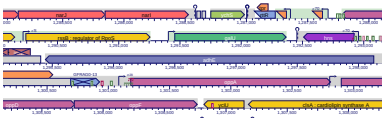
- **19 090** known biochemical compounds (KEGG COMPOUND database)
- **11 911** known biochemical reactions (KEGG REACTION database)
- **8 423** known enzymes (BRENDA database)

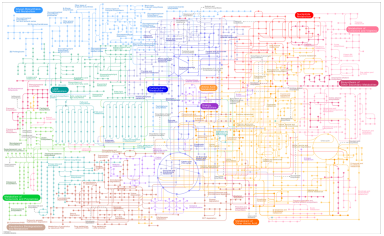
Organism specific view (`biocyc.org`)

Organism	# of reactions	# of metabolites
Escherichia coli	2 201	2 967
Saccharomyces cerevisiae	1 650	1 160
Homo sapiens	2 900	2 121
Arabidopsis thaliana	3 193	2 777

Reconstruction of metabolic networks from genome data

Genome ⟶ Metabolic network



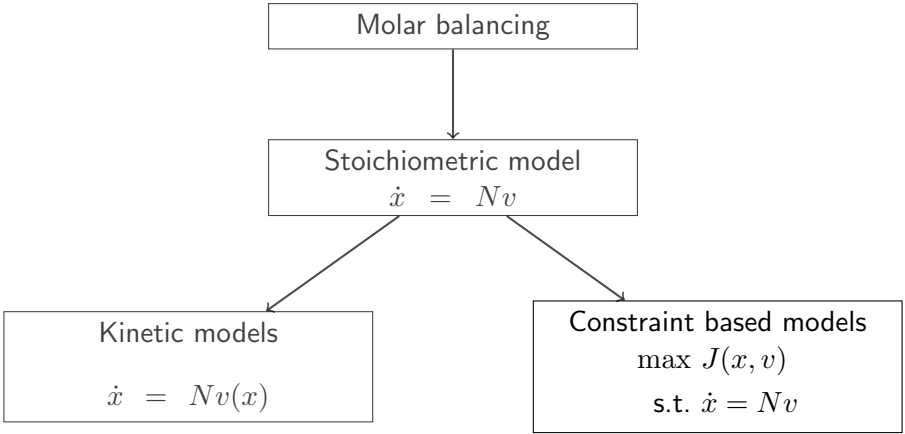


(kegg.jp)

1. Identify genes with enzymatic function (annotation / sequence homology)
2. Find matching reactions in reaction database
3. Add a biomass reaction (metabolic building blocks + energy (ATP) turnover)

Genome-scale metabolic network model

From metabolic networks to models



Flux balance analysis

Constraints applied to the network

- Intermediate / intracellular metabolites are assumed to be in a quasi-steady state:

  $$\text{flux of producing reactions} = \text{flux of consuming reactions}$$

- "Irreversible" reactions can only have flux in one direction
- Maintenance / housekeeping reactions can be constrained to have a minimum flux value (empirical)
- Nutrient uptake (exchange) reactions are constrained according to availability of nutrients in the considered environment

Optimization principle

- **Hypothesis**: Cells regulate fluxes within constraints to achieve an "optimal" configuration from an evolutionary perspective.
- In many applications, network solutions that **maximize flux through the biomass reaction** are taken

Constraints on fluxes

1. Steady state constraint

$$Nv = 0$$

   - Fluxes constrained to **subspace**

2. Irreversibility constraints on some fluxes (from thermodynamics/heuristics/empirical evidence)

$$v_i \geq 0, \qquad i \text{ irreversible}$$

   - Fluxes constraint to **flux cone**

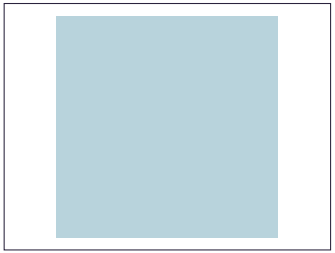
3. Flux bounds from capacity constraints, maintenance, ...

$$v_{i,min} \leq v \leq v_{i,max}$$

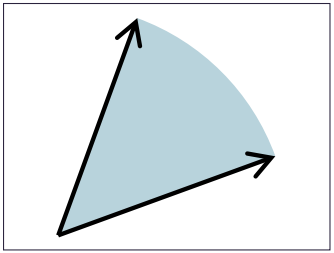
   - Fluxes constraint to **convex polytope**

Geometric illustration

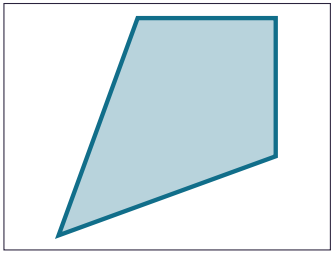
Flux space



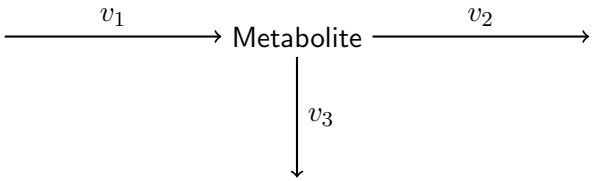
Flux cone



Flux polytope



Flux space $\rightarrow$ cone $\rightarrow$ polytope example



Construct the ...

- flux space;
- flux cone assuming $v_2,\ v_3 \geq 0$;
- flux polytope assuming $v_1 \leq 0.5$.

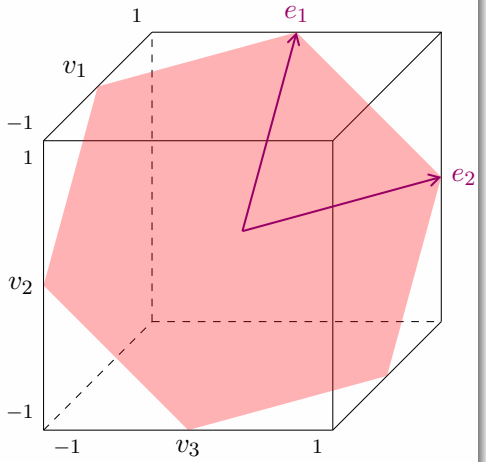
Molar balancing:

$$\dot{x} = \begin{pmatrix} 1 & -1 & -1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \end{pmatrix}$$

Flux space from $Sv = 0$

- Plane defined by

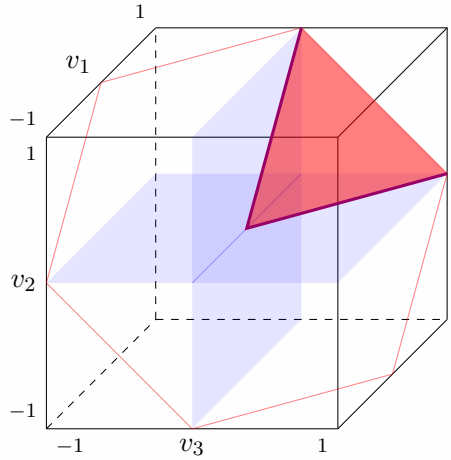
$$v_1 - v_2 - v_3 = 0$$



Flux cone

- Add irreversibility

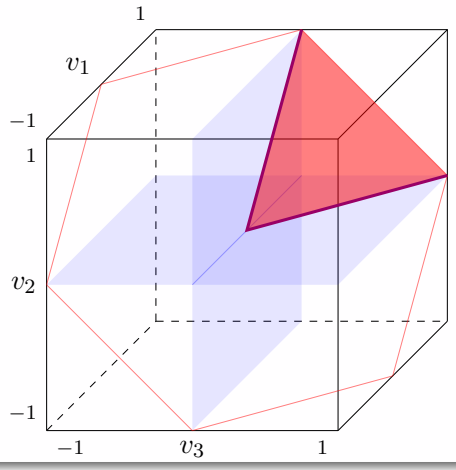
$$v_2,\ v_3 \geq 0$$



Flux cone

- Add irreversibility

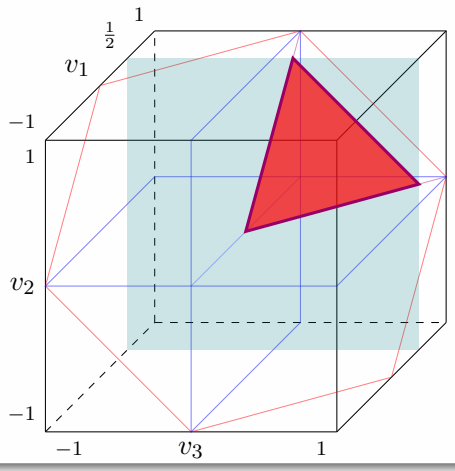
$$v_2,\ v_3 \geq 0$$



Flux polytope

- Add upper bound(s)

$$v_1 \leq 0.5$$



Setting up the constraint based model (CBM)

Constraint based model useful if non-trivial steady state fluxes exist

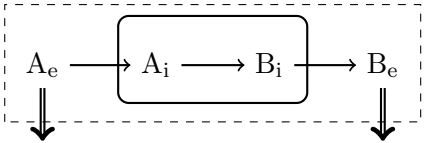
- The steady state equation
$$Nv = 0$$
should have a non-zero solution $v \Rightarrow$ non-trivial steady state flux space
- We need $\operatorname{rank} N < m$; most models have more reactions than metabolites anyway.

Metabolite / flux units

- In CBMs, metabolites are usually considered in **molar amounts per dry biomass**: $\mathrm{mmol/g}$
- Fluxes are then in $\mathrm{mmol/(gh)}$

Exchange reactions

- Exchange reactions are added for all metabolites that are either **consumed** or **produced** in a metabolic steady state.
- They normally involve only **extracellular** metabolites.
- By convention, the reaction **direction** is towards the outside of the system



$\longrightarrow$ normal reaction

$\Longrightarrow$ exchange reaction

Positive vs. negative flux on exchange reaction

- Negative flux = actually goes into the system = **supply** (consumption) of a metabolite
- Positive flux = goes outside of system = **removal** (production) of a metabolite

Elementary Flux Modes (EFMs)

The flux cone

$$\mathcal{C} = \{v \mid Nv = 0,\, v^{\rightarrow} \geq 0\}$$

Elements of the flux cone are called *flux modes*.

with $\mathcal{R}^{\rightarrow} \subseteq \{1, \ldots, n\}$ be the index set of the irreversible reactions, then $v^{\rightarrow} := v_{\mathcal{R}^{\rightarrow}} \geq 0$, that is, $v_i \geq 0$ if $i \in \mathcal{R}^{\rightarrow}$

Remarks

- Irreversibility arises from thermodynamic constraints or biological knowledge.
- Writing all reversible reaction as two irreversible rates, the flux cone can be defined in the semipositive orthant of the flux-space.
- If the the original flux-space is used, we get EFMs
- If all internal reversible reactions are decomposed as two irreversible ones, but the reversible exchange reactions unchanged, the edges of this cone are termed extreme pathways

Lets find EFMs!

Find as many *unique* pathways allowing flux trough the network.

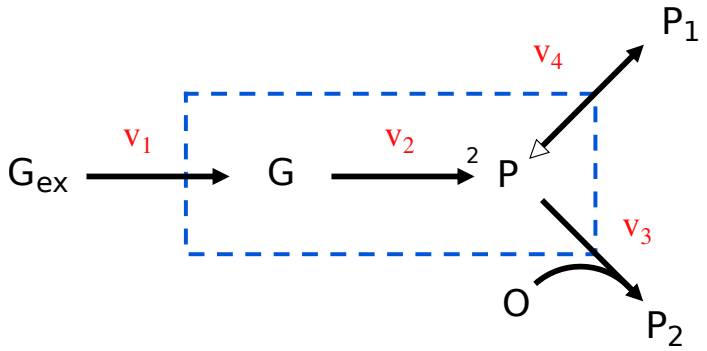


Figure: Central carbon metabolism as a metabolic network. Extracellular glucose, G_{ex}, pyruvate, P, fermentation product, P_1, oxidative phosphorylation product P_2

Lets find EFMs II!

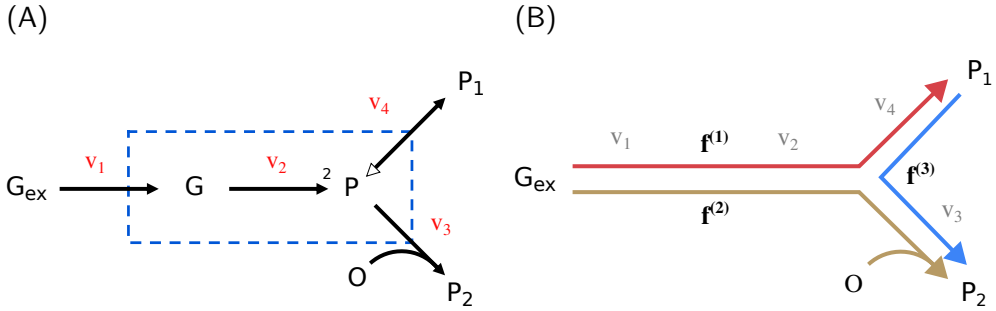


Figure: Central carbon metabolism as a metabolic network. Extracellular glucose, G_{ex}, pyruvate, P, fermentation product, P_1, oxidative phosphorylation product P_2. EFMs $^{(1)}$,$^{(2)}$,$^{(3)}$. From our understanding of central carbon metabolism, $^{(1)}$ represents glycolytic fermentation, $^{(2)}$ the oxidative metabolism of glucose, and $^{(3)}$ the oxidative metabolism of the fermentation product.

A formal definition of EFMs

Define the *support* of a vector v as t $supp(v) = \{i \mid v_i \neq 0\}$, that is, the support of a flux vector is the index set of reactions that have a nonzero rate.

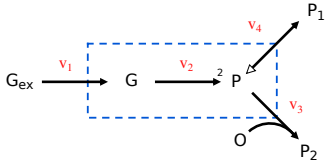
Elementary flux modes properties

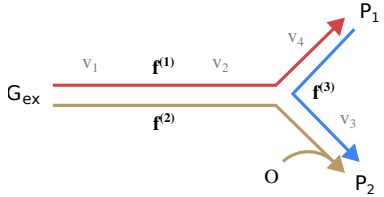
- v is an admissible (flux) mode if $v \neq 0$, vR^n, solves $Nv = 0$,
- and obeys irreversibility: $v^{\rightarrow} \geq 0$.
- A mode is called an EFM, e, if $supp(v) \subseteq supp(e) \Longrightarrow supp(v) = supp(e)$

Remarks

- an EFM is a *minimal*, unique set of flux-carrying reactions operating in steady-state
- if any flux-carrying reactions in an EFM is deleted, the EMF can no longer operate in steady-state and the EFM is killed.

Conformal sums of EFMs





$$N = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 2 & -1 & -1 \end{pmatrix} \quad (1)$$

with $v = (v_1, v_2, v_3, v_4)^T$, where $v_1, v_2, v_3 \geq 0$ and $v_1 = 1$, and $Nv = 0$.
The set of EFMs is given by

$$f^{(1)} = \begin{pmatrix} 1 \\ 1 \\ 0 \\ 2 \end{pmatrix}, \quad f^{(2)} = \begin{pmatrix} 1 \\ 1 \\ 2 \\ 0 \end{pmatrix}, \quad f^{(3)} = \begin{pmatrix} 0 \\ 0 \\ 1 \\ -1 \end{pmatrix}. \quad (2)$$

How can $v = (1, 1, 1, 1)^T$ be represented as a sum of EFMs?

Optimization principle

Constraint based model

$$Nv = 0$$

$$v_{i,min} \leq v_i \leq v_{i,max}$$

- **Underdetermined** system of equalities / inequalities: flux polytope
- How do we determine fluxes v that we expect to occur in nature?

Add an optimization objective

- **Hypothesis:** Cells regulate fluxes within constraints to achieve an "optimal" configuration from an evolutionary perspective.

$$\begin{aligned} &\max\ J(v) \\ &\text{s.t.}\quad Nv = 0 \\ &\qquad v_{i,min} \leq v_i \leq v_{i,max} \end{aligned}$$

Useful objective functions

Type	Objective $J(v)$	Principle
Biomass yield	$\max v_{bio}$	Biomass flux at fixed max. substrate uptake
ATP yield	$\max v_{ATP}$	ATP flux at fixed max. substrate uptake
Minimal flux	$\min \|v\|^2$	Minimization of overall flux ($\sim$ enzyme usage)
Biomass flux yield	$\max v_{bio}/\|v\|^2$	Biomass yield per overall flux unit

Empirical evaluation of objective functions: Schuetz, R., Kuepfer, L., & Sauer, U. (2007). Systematic evaluation of objective functions for predicting intracellular fluxes in *Escherichia coli*. Molecular Systems Biology, 3, 119.

Biomass composition

E. coli biomass composition

Compound	Proportion [% g/g DW]
Protein	72
DNA	4
RNA	10
Lipids	9
Polysaccharides	2.5
Mureine	2.5

Chassagnole *et al.* 2002, via bionumbers.hms.harvard.edu, ID 108705
Varies depending on environmental conditions (nutrients, aerobic/anaerobic, growth rate, ...)

Break down to metabolites

- 20 proteinogenic amino acids
- 8 D/R nucleotides
- phospholipids
- cofactors / vitamins
- ATP hydrolysis required for biomass assembly ("**growth associated maintenance**" GAM)

Biomass reaction

- Biomass reaction formalizes consumption of metabolites to generate biomass

$$v_{bio}: \qquad \sum_{i=1}^{n} c_i X_i \rightarrow 1 \text{ g dry biomass}$$

- Based on pre-determined constant biomass composition
- Coefficients c_i commonly in mmol / g dry biomass
- Unit of v_{bio} becomes $1/\mathrm{h}$: interpretable as dry biomass growth rate μ!

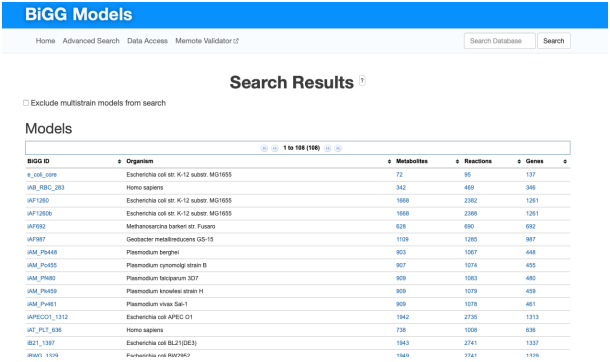
Maintenance

- "**Non-growth associated maintenance**" (NGAM):
  - membrane voltage gradients and osmolarity (ion pumps)
  - movement (flagella)
  - macromolecule (RNA/protein/carbohydrates) turnover
- Energy demand is commonly represented by a single ATP hydrolysis reaction

$$v_{maint}: \qquad \mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{ADP} + \mathrm{Pi} + \mathrm{H^+}$$

- Put as constraint into constraint based model
  - $v_{maint} \geq \alpha$ [mmol / (h · g biomass)]
  - NGAM rate estimates: *E. coli* 8.4 mmol/g/h; *S. cerevisiae* 1.0 mmol/g/h

Collections of constraint based models



- BiGG models database: `http://bigg.ucsd.edu/models`
- ModelSEED (plant models): `https://modelseed.org/genomes/`
- BioModels database: `https://biomodels.net` (filter for "constraint-based model")

Linear programs

A linear program in standard form:

$$\begin{aligned} \max\ & c^{\mathrm{T}} v \\ \text{s.t. } & Av = b \\ & v \geq 0 \end{aligned}$$

Objective

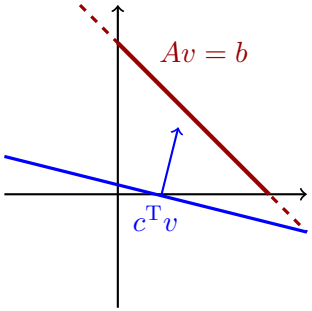
$c^{\mathrm{T}} v$

Equality constraint

$Av = b$

Inequality constraint

(Cone constraint) $v \geq 0$

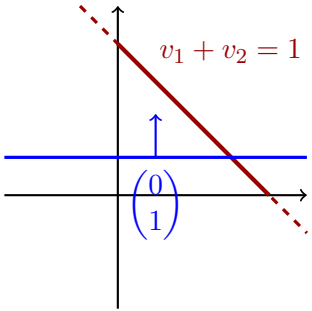


Example

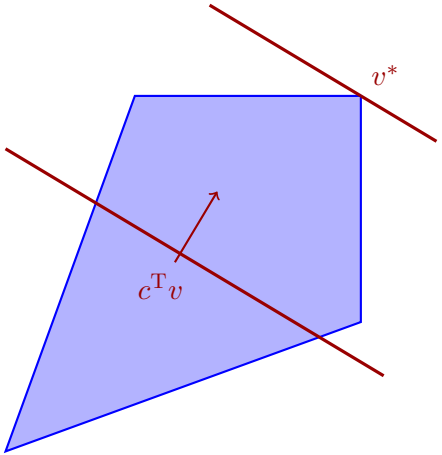
$$\max_{v_1, v_2} \; v_2$$
$$\text{s.t. } v_1 + v_2 = 1$$
$$v \geq 0$$

Thus:

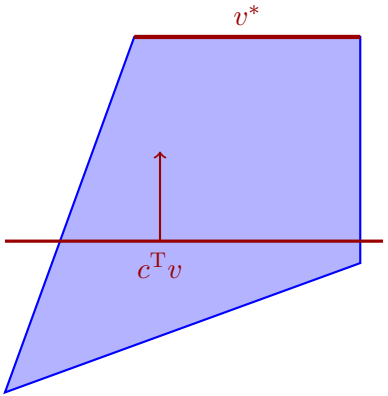
$$c^{\mathrm{T}} = \begin{pmatrix} 0 & 1 \end{pmatrix}$$
$$A = \begin{pmatrix} 1 & 1 \end{pmatrix}$$
$$b = 1$$



Generalized geometrical interpretation

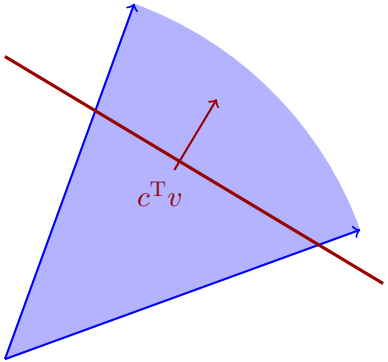


Non-uniqueness of optimal solutions



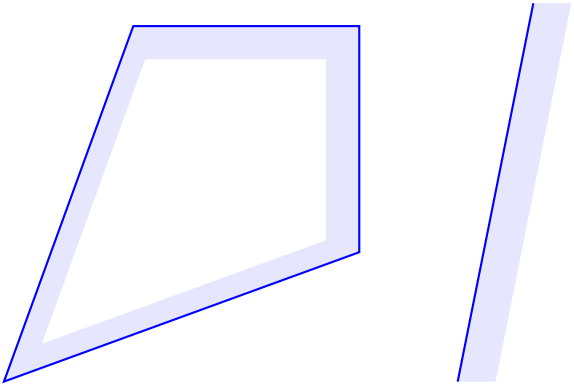
Set of optimal solutions is a **face of the polytope**

Unboundedness



Unboundedness: $\max c^{\mathrm{T}}v = \infty$

Infeasibility: Constraint set is empty



Example

$$v_1 + v_2 \leq -1$$

$$v_1, v_2 \geq 0$$

Flux balance analysis (FBA)

FBA to maximize biomass yield as LP

$$\begin{aligned} J^* = \max\ & v_{bio} \\ \text{s.t. } & Nv = 0 \\ & v_{i,min} \leq v_i \leq v_{i,max} \end{aligned}$$

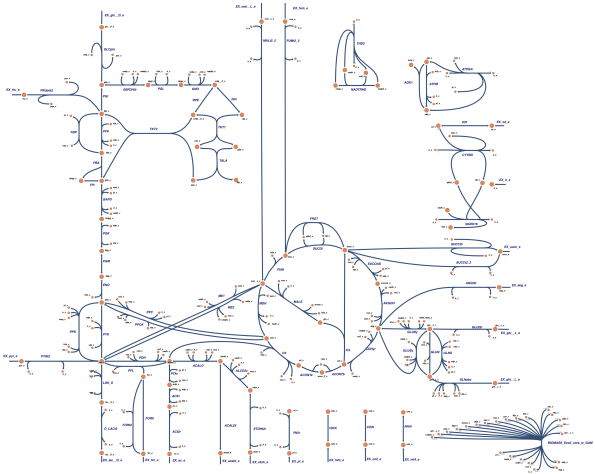
- Typical relevant constraint is glucose / oxygen uptake rate

$$-v_{e,gluc,max} \leq v_{e,gluc} \leq 0$$

- For practical reasons $v_{i,max} = M$ (10^6 mole/h/g) even if no capacity constraint
- Typically no unique optimal flux distribution v^*

FBA example: *E. coli* core

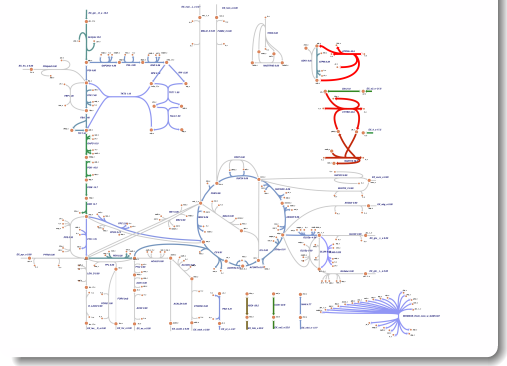
- Core carbon network from BiGG database: 72 metabolites, 95 reactions
- Network visualization from `https://escher.github.io/`



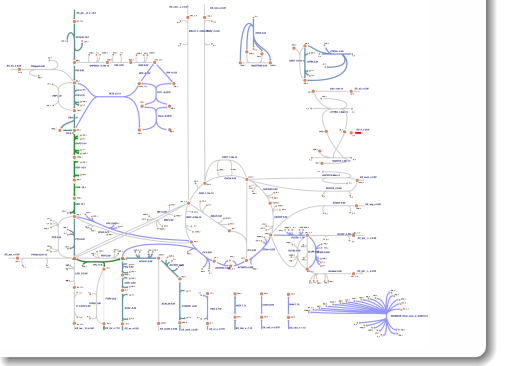
FBA results: comparing intracellular flux states

- With a graphical layout of the metabolic network is available: **graphical illustration of intracellular metabolic state**

Aerobic growth in *E. coli* core



Anaerobic growth in *E. coli* core



Made with `escher.github.io`

Outlook: further extensions of FBA

- Dynamic FBA
- Thermodynamic FBA
- Resource allocation models:
  - ME models
  - Resource balance analysis
  - Dynamic enzyme-cost FBA

Exercise on `https://principlescellphysiology.org/book-economic-principles/index.html`

Run FBA on the carbon core model (Jupyter notebook → Google Colaboratory)