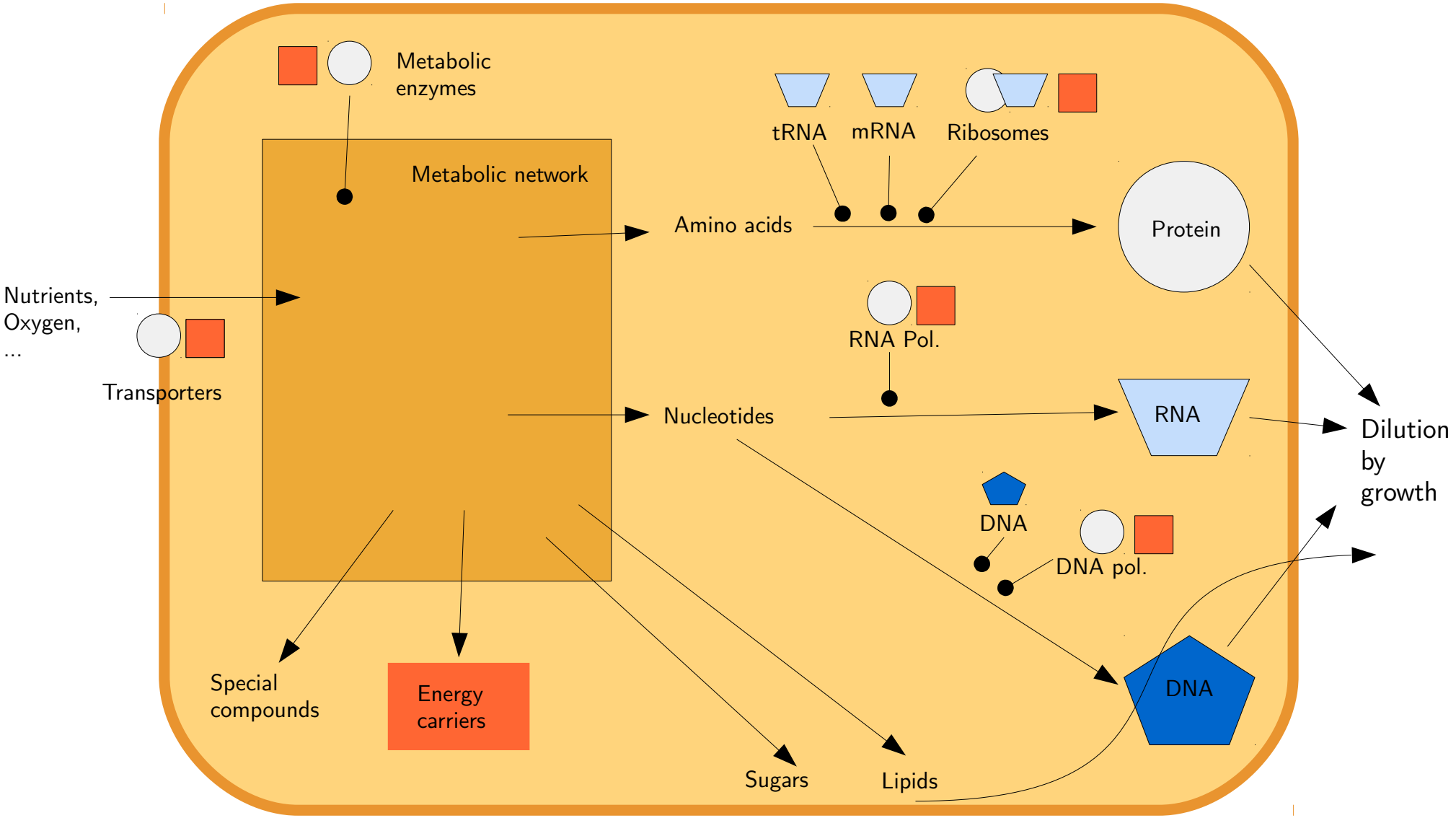
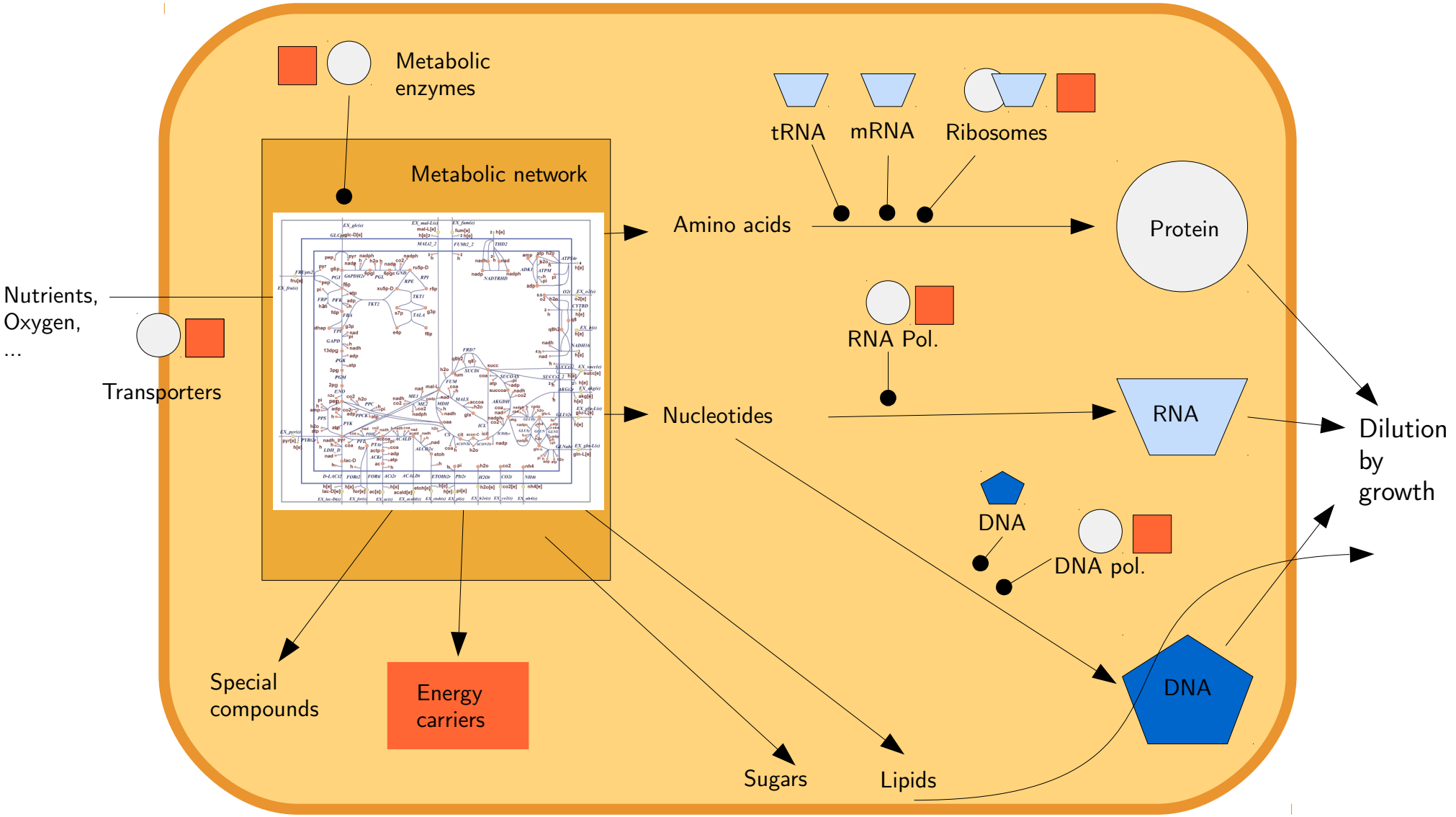
Part 3: Flux prediction by constraint-based models

The cell as a self-replicating factory



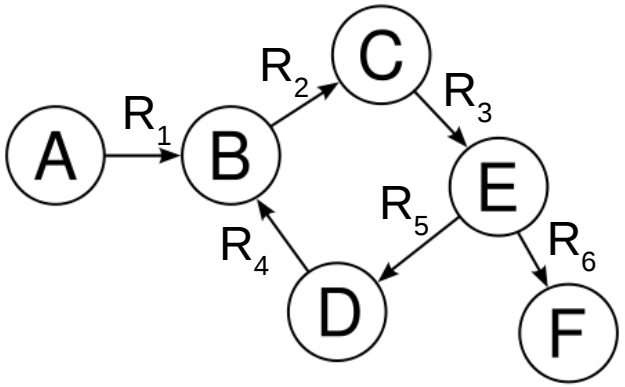
What fluxes exist in the metabolic network?



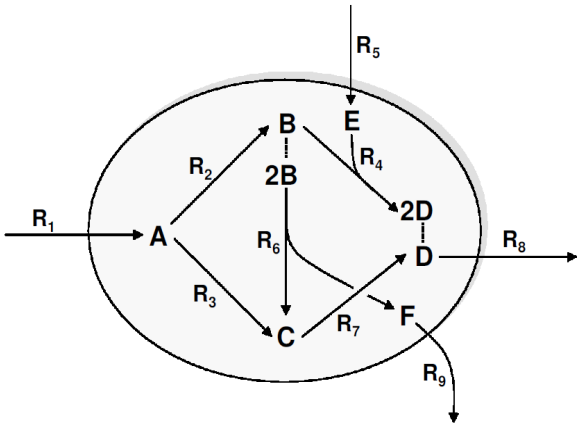
Stoichiometric network reconstruction

Metabolic network are not simple graphs

simple directed graph

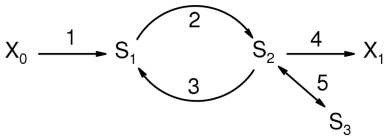


metabolic graph

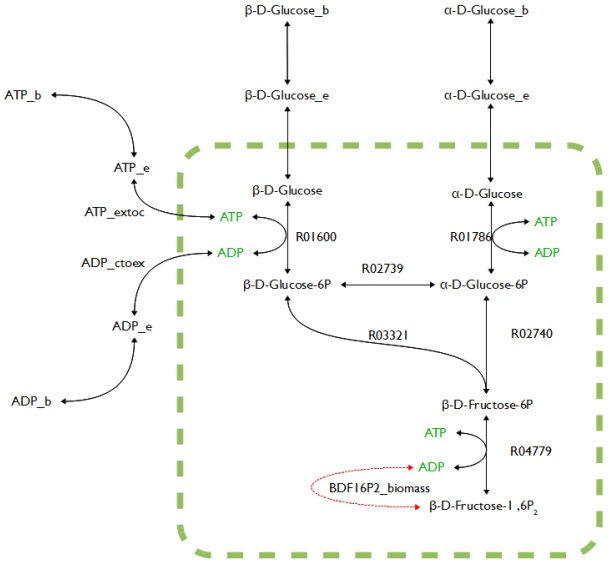


- Two types of entities (metabolites, enzymes)
- Possible representations:
  - hypergraph - enzymes are hyper-edges (not always 1:1)
  - bipartite graph - enzymes are “special” nodes
- Stoichiometry (encoded as weights in a bipartite graph)

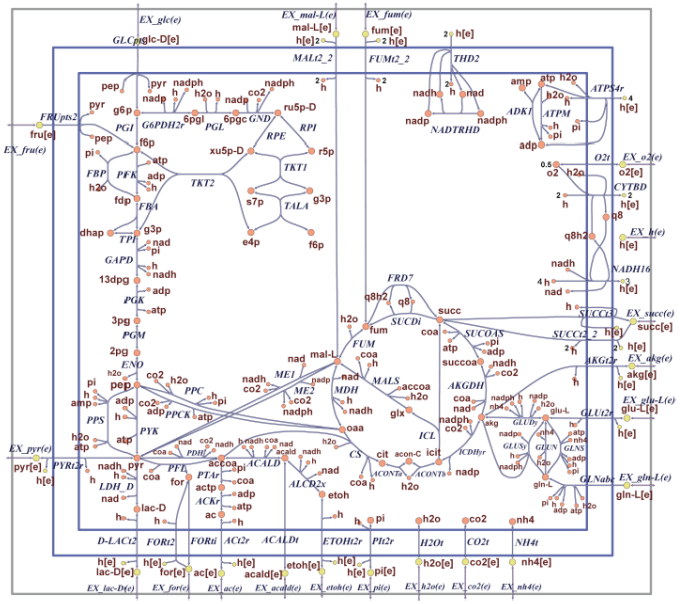
Metabolic networks: from small scale to genome scale



toy model

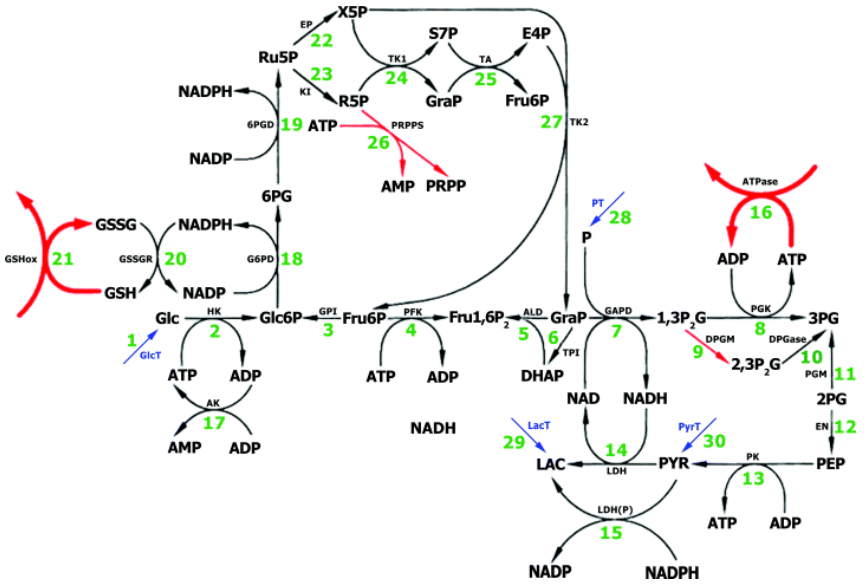


hexose transport model



core metabolism model

A realistic example: erythrocyte metabolism



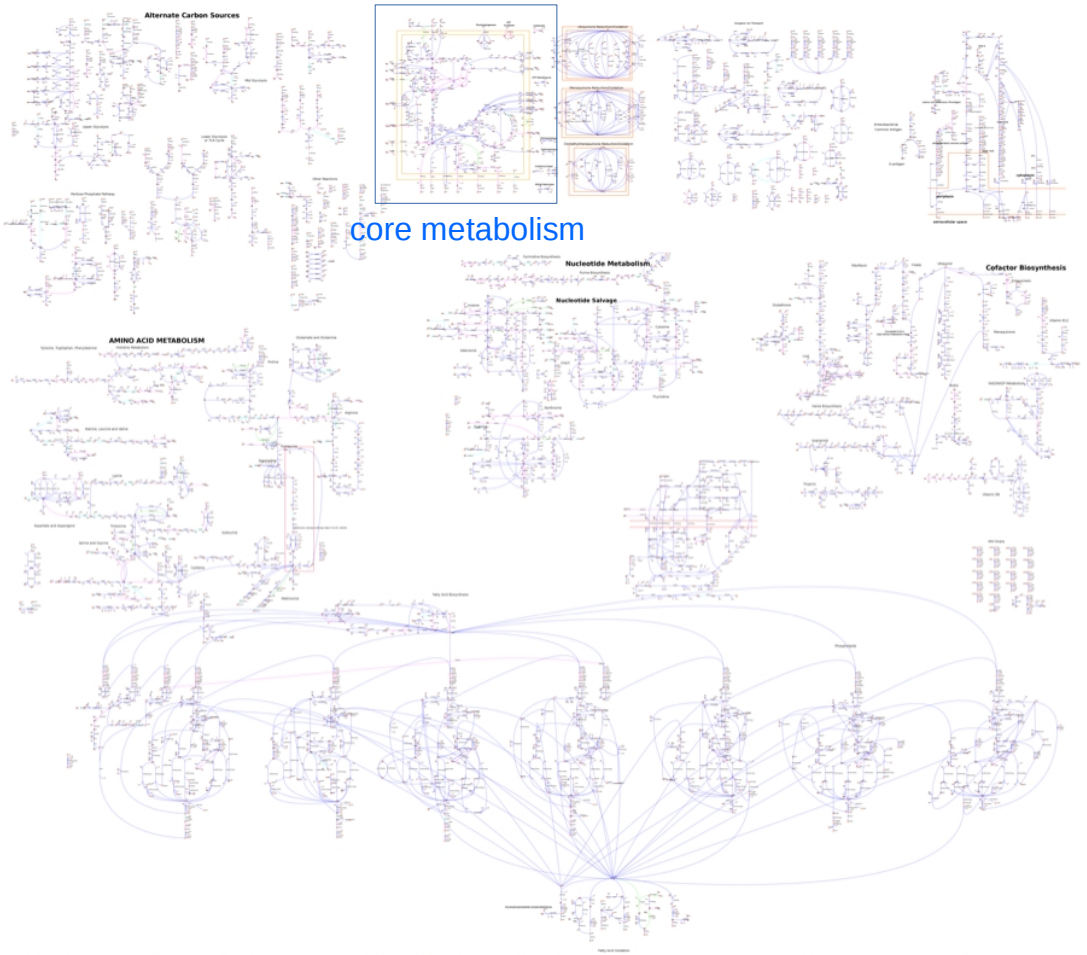
metabolites

reactions

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
		Glct	HK	GPI	PFK	ALD	TPI	GAPDH	PGK	DPGM	DPGase	PGM	EN	PK	LDH	LDH(P)	ATPase	AK	G6PD	6PGD	GSSGR	GSHox	EP	KI	TK1	TA	PRPPS	TK2	Pt	Lact	Pyrt
1	Glcin	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Glc6P	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	0	0	0
3	Fru6P	0	0	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
4	Fru16P2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	GraP	0	0	0	0	-1	-1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	1	0	0
6	DHAP	0	0	0	0	-1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	13P2Gri	0	0	0	0	0	0	-1	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	23P2Gri	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	3PGri	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	2PGri	0	0	0	0	0	0	0	0	0	0	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	PEP	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	ATP	0	-1	0	-1	0	0	0	1	0	0	0	0	1	0	0	-1	1	0	0	0	0	0	0	0	0	-1	0	0	0	0
13	ADP	0	1	0	1	0	0	0	-1	0	0	0	0	-1	0	0	1	-2	0	0	0	0	0	0	0	0	0	0	0	0	0
14	6PGlcA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0
15	NADP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	-1	-1	1	0	0	0	0	0	0	0	0	0	0
16	GSH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	-2	0	0	0	0	0	0	0	0	0
17	Rul5P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	-1	-1	0	0	0	0	0	0	0	0
18	Xul5P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	-1	0	0	-1	0	0	0	0
19	Rib5P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	-1	0	0	0	0	0
20	Sed7P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0
21	E4P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	-1	0	0	0	0
22	NAD	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	Pi	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
24	Lac	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
25	Pyr	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

H.G. Holzhütter (2004) The principle of flux minimization and its application to estimate stationary fluxes in metabolic networks European Journal of Biochemistry / FEBS, 271(14), 2905–22

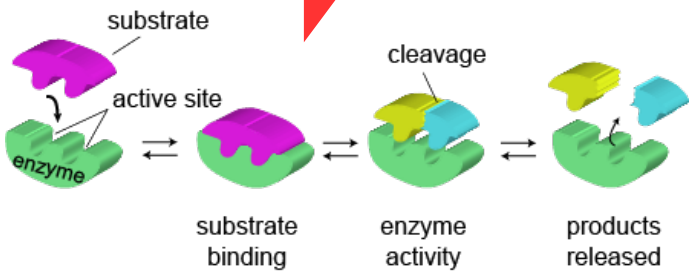
Genome-scale metabolic models of *Escherichia coli*

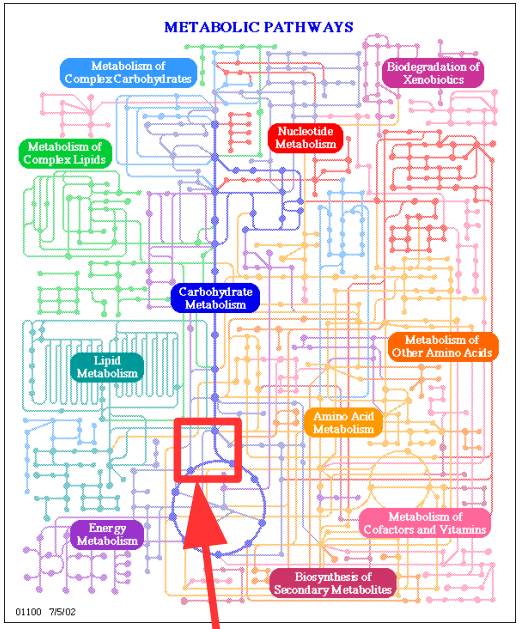


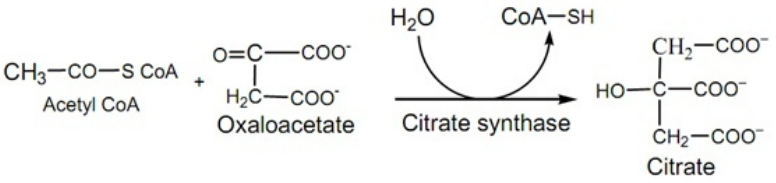
Model	Year	Reactions	Metabolites
iJE660	2000	627	438
iJR904	2003	931	625
iAF1260	2007	1260	1039
iJO1366	2011	2077	1136
core model	2007	95	72

http://systemsbiology.ucsd.edu/InSilicoOrganisms/Ecoli/EcoliSBML

Stoichiometric network reconstruction

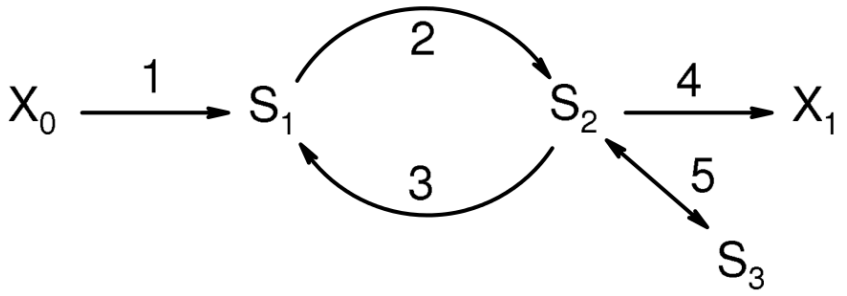






Flux-balance analysis

Metabolic network and stoichiometric matrix



Graph

$$\begin{aligned}\frac{dS_1}{dt} &= v_1 - v_2 + v_3\\ \frac{dS_2}{dt} &= v_2 - v_3 - v_4 - v_5\\ \frac{dS_3}{dt} &= v_5\end{aligned}$$

Kinetic model (ODE)

reactions

metabolites

$$\begin{array}{c} \\ S_1 \\ S_2 \\ S_3 \end{array} \begin{bmatrix} 1 & -1 & 1 & 0 & 0 \\ 0 & 1 & -1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

Stoichiometric matrix

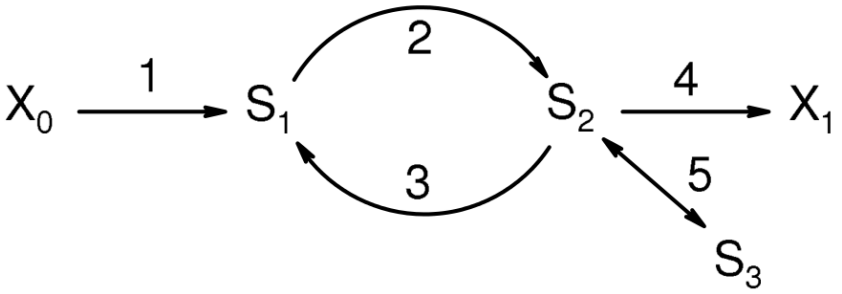
$$\begin{bmatrix} \frac{dS_1}{dt} \\ \frac{dS_2}{dt} \\ \frac{dS_3}{dt} \end{bmatrix} = \begin{bmatrix} 1 & -1 & 1 & 0 & 0 \\ 0 & 1 & -1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix}$$

$$\dot{S} = N \cdot v$$

System of differential equations

Metabolic fluxes in steady state

A kinetic model would have to contain rate laws for each reaction



$$\frac{dS_1}{dt} = v_1 - v_2 + v_3$$
$$\frac{dS_2}{dt} = v_2 - v_3 - v_4 - v_5$$
$$\frac{dS_3}{dt} = v_5$$

$$v_1 = V_{max,1}\frac{X_0}{K_1 + X_0}$$

$$v_2 = V_{max,2}\frac{S_1}{K_2 + S_1}$$

$$v_3 = V_{max,3}\frac{S_2}{K_3 + S_2}$$

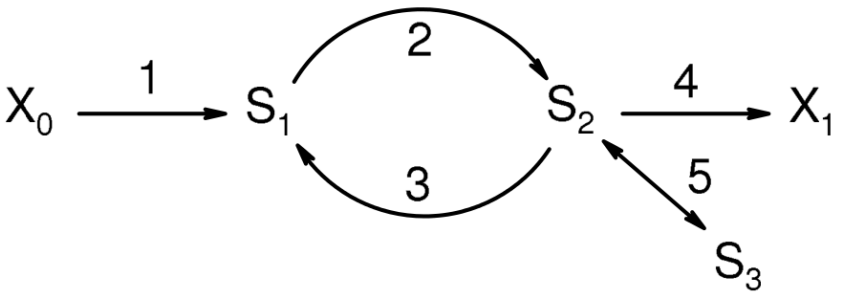
$$v_4 = V_{max,4}\frac{S_2}{K_4 + S_2}$$

$$v_5 = E_5\frac{k_{f,5}S_2/K_{f,5} - k_{b,5}S_3/K_{b,5}}{1 + S_2/K_{f,5} + S_3/K_{b,5}}$$

Metabolic fluxes in steady state

A kinetic model would have to contain rate laws for each reaction

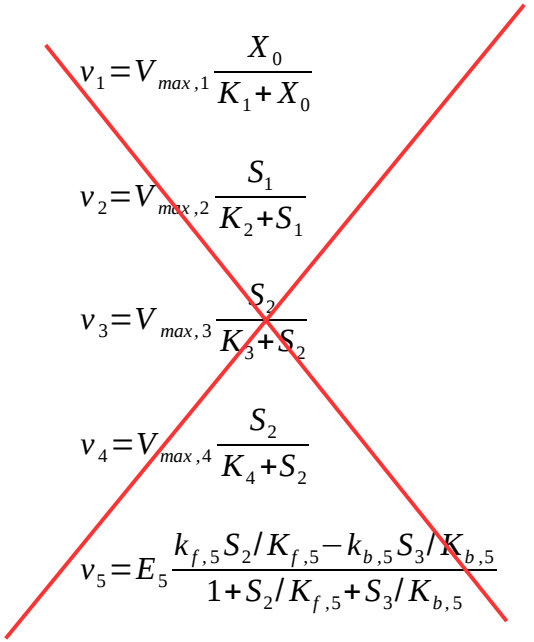
.. however, one can also do quite a lot without it!



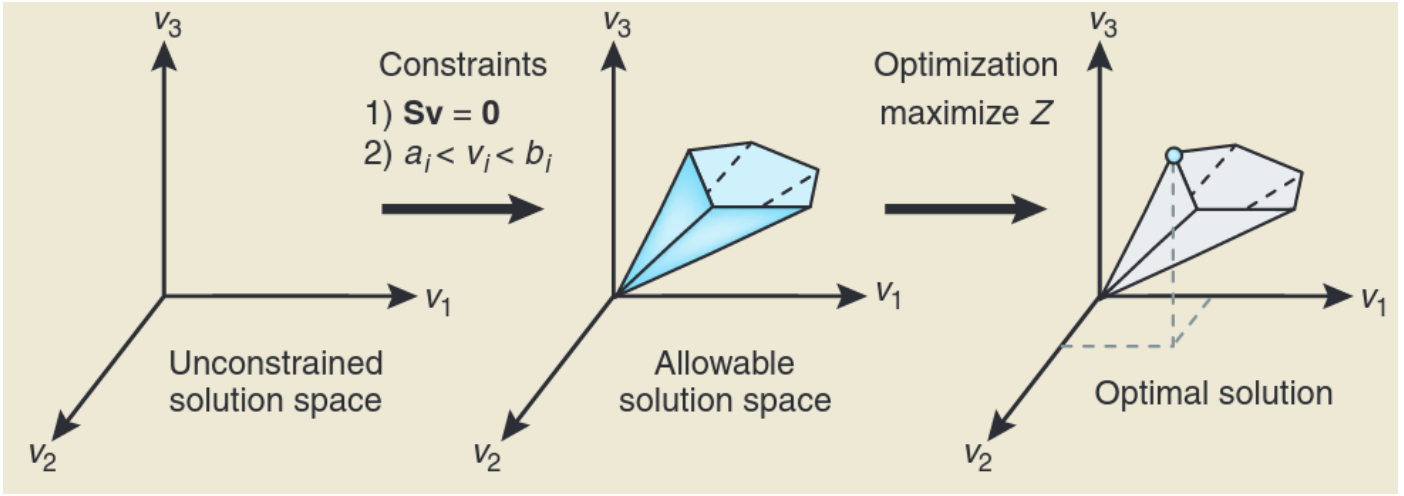
$$\frac{dS_1}{dt} = v_1 - v_2 + v_3 = 0$$

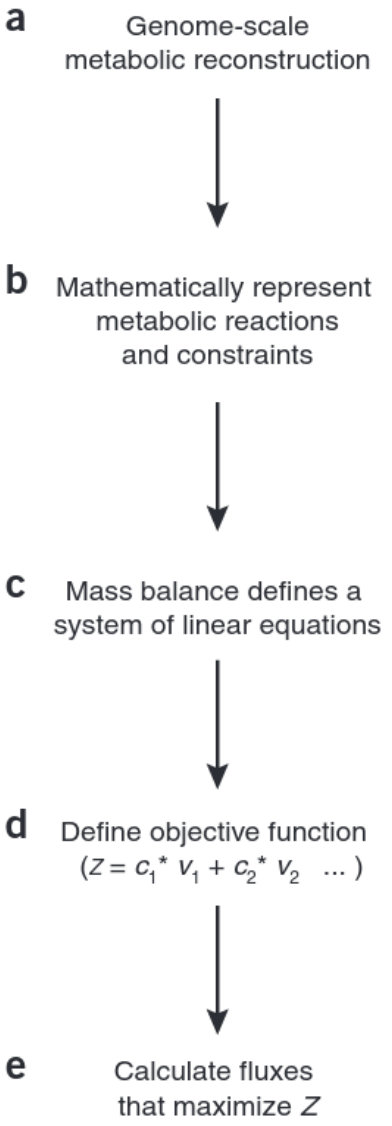
$$\frac{dS_2}{dt} = v_2 - v_3 - v_4 - v_5 = 0$$

$$\frac{dS_3}{dt} = v_5 = 0$$



Predicting metabolic fluxes by Flux balance analysis





What is Flux balance analysis (FBA)?

- First, define the scope: define a stoichiometric network ***N***

$$\begin{matrix} S_1 \\ S_2 \\ S_3 \end{matrix} \begin{bmatrix} 1 & -1 & 1 & 0 & 0 \\ 0 & 1 & -1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

N

What is Flux balance analysis (FBA)?

- First, define the scope: define a stoichiometric network ***N***
- Apply a steady state assumption*, i.e. all internal metabolite concentrations are constant (mass balance)

* In realistic models, ***N*** has more reactions than metabolites, which means that this system of linear equations is under-determined

$$\frac{dS_1}{dt} = v_1 - v_2 + v_3 = 0$$
$$\frac{dS_2}{dt} = v_2 - v_3 - v_4 - v_5 = 0$$
$$\frac{dS_3}{dt} = v_5 = 0$$

$$N \cdot v = 0$$

What is Flux balance analysis (FBA)?

- First, define the scope: define a stoichiometric network ***N***
- Apply a steady state assumption, i.e. all internal metabolite concentrations are constant (mass balance)
- Add individual constraints* for each reaction flux

* there is still usually a large solution space

$$N \cdot v = 0$$
$$a_i \leqslant v_i \leqslant b_i$$

What is Flux balance analysis (FBA)?

- First, define the scope: define a stoichiometric network ***N***
- Apply a steady state assumption, i.e. all internal metabolite concentrations are constant (mass balance)
- Add individual constraints for each reaction flux
- Maximize an objective function, typically biomass production rate

$$\max_{v} \quad c \cdot v$$

$$N \cdot v = 0$$

$$a_i \leqslant v_i \leqslant b_i$$

c·v – a linear combination of biomass precursor synthesis fluxes

What is Flux balance analysis (FBA)?

- First, define the scope: define a stoichiometric network ***N***
- Apply a steady state assumption, i.e. all internal metabolite concentrations are constant (mass balance)
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- Maximize an objective function, typically biomass production rate

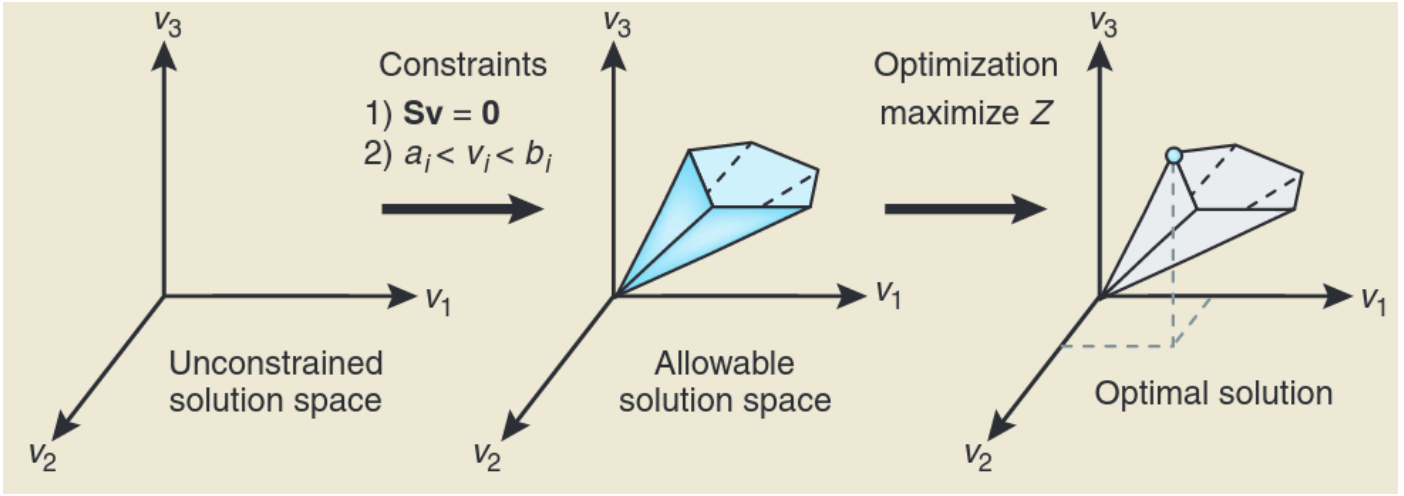
Solve using linear programming

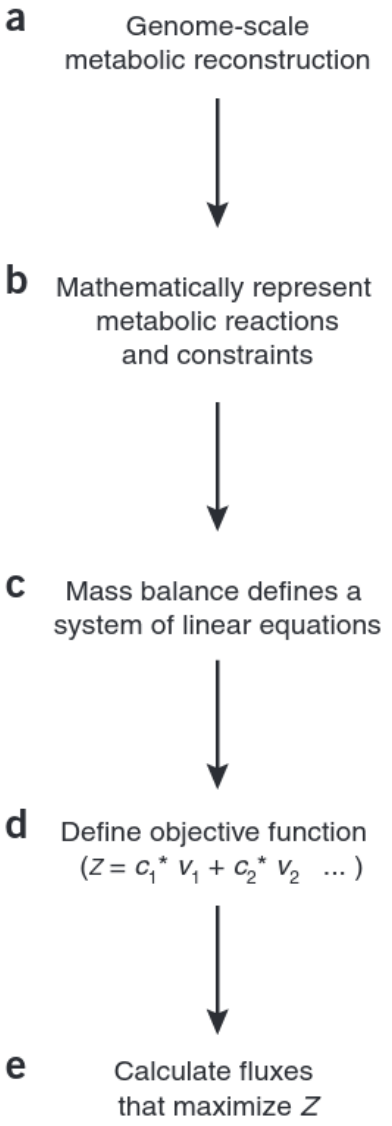
$$\max_{v} \quad c \cdot v$$

$$N \cdot v = 0$$

$$a_i \leqslant v_i \leqslant b_i$$

Predicting metabolic fluxes by Flux balance analysis

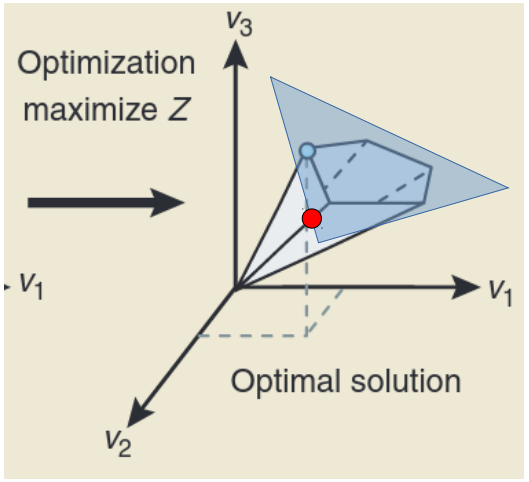




Variants of flux-balance analysis

FBA with Molecular Crowding: Limited space for enzymes!

- Catalyzing a reaction at a certain rate requires some minimal concentration of enzyme
- Physiological constraints on protein concentrations put an upper bound on the sum of all enzyme concentrations:



$$\Sigma\, \alpha_i\, E_i \leq V/M$$

- α_i: enzyme *i* specific volume
- E_i: enzyme *i* concentration
- V: total cell volume
- M: total cell dry weight

Beg, et al. (2007) Intracellular crowding defines the mode and sequence of substrate uptake by Escherichia coli and constrains its metabolic activity PNAS 104(31), 12663–8.

Principle of flux minimization: Minimal usage of enzymes!

- Sometimes called parsimonious FBA (pFBA)
- Rather than maximizing the biomass flux, minimize the sum of all fluxes

$$\underset{v}{minimize} \sum_{j=1}^{r} |v_j|$$

H.G. Holzhütter (2004) The principle of flux minimization and its application to estimate stationary fluxes in metabolic networks European Journal of Biochemistry / FEBS, 271(14), 2905–22

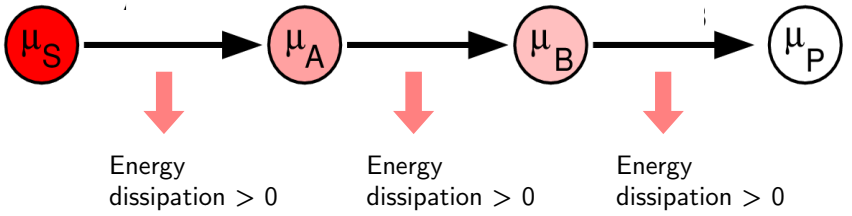
Thermodynamic constraint on flux directions

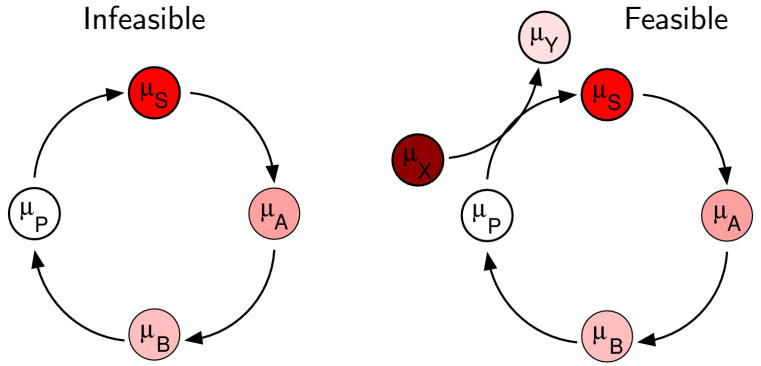
Chemical potential $\mu_i = \dfrac{\partial G}{\partial n_i}$

Gibbs free energy dissipation

$$-\Delta G \cdot v > 0$$

Sign constraint: $\operatorname{sign}(v) = \operatorname{sign}(-\Delta G)$





What parts of the cell are ignored by FBA?

- Metabolism doesn't end in precursor metabolites

- All following processes are “lumped” into one step called the “biomass function”:
  - transcription
  - translation
  - protein modification / assembly / trafficking
  - DNA replication
  - membrane assembly / division
  - macromolecule degradation

- What can we gain by extending the model to encompass the entire cell?