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Linear programming applied to genome-scale metabolic network models

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Introduction

General idea: optimise a linear function under inequality constraints

Variables: $x_i\,, \quad i = 1 \ldots N$

Constraints: $l_i \leq x_i \leq u_i$

Objective: $\Omega = \sum_i^N c_i \cdot x_i$

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EXAMPLE

Application to metabolic networks

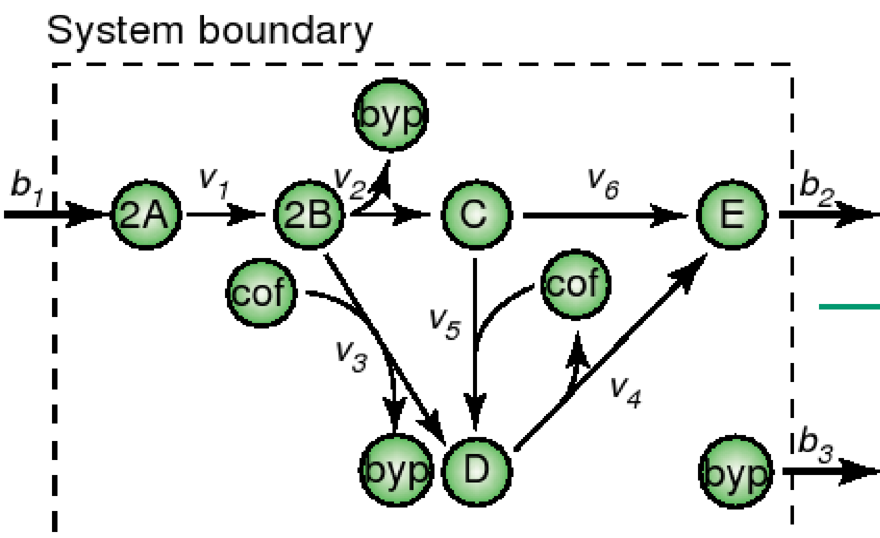
Variables: *FLUXES* $v_i, \quad i = 1 \ldots R$

Constraints: *?*

Objective: *?*

Application to metabolic networks

A metabolic system is defined by
internal reactions and ***exchange fluxes***



$N =$

	v_1	v_2	v_3	v_4	v_5	v_6	b_1	b_2	b_3
A	−1	0	0	0	0	0	+1	0	0
B	+1	−2	+2	0	0	0	0	0	0
C	0	+1	0	0	−1	−1	0	0	0
D	0	0	+1	−1	+1	0	0	0	0
E	0	0	0	+1	0	+1	0	−1	0
byp	0	+1	+1	0	0	0	0	0	−1
cof	0	0	−1	+1	−1	0	0	0	0

The temporal change of the concentrations is given by $$\frac{dX}{dt} = N \cdot v$$

Steady state is characterised by $$N \cdot v = 0$$

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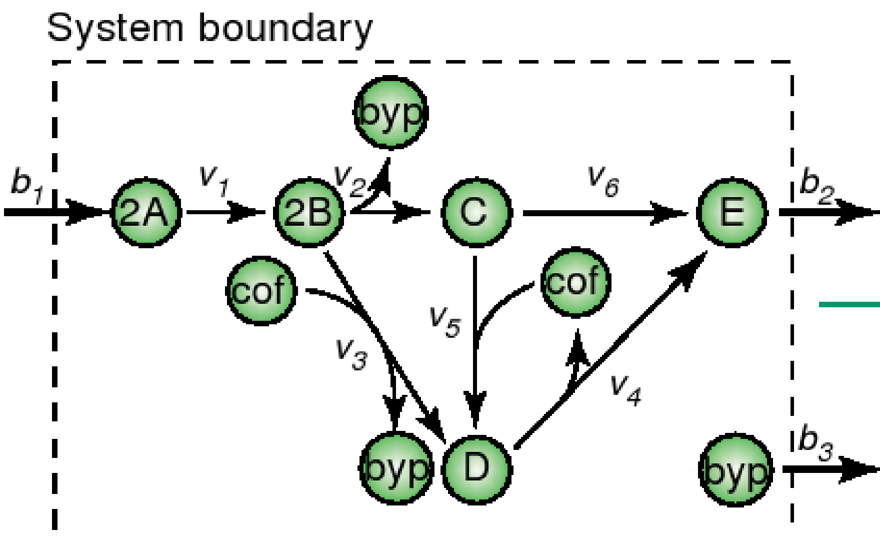

$$N = \left\{ \begin{array}{cccccc|ccc} v_1 & v_2 & v_3 & v_4 & v_5 & v_6 & b_1 & b_2 & b_3 \\ -1 & 0 & 0 & 0 & 0 & 0 & +1 & 0 & 0 \\ +1 & -2 & +2 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & +1 & 0 & 0 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & +1 & -1 & +1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & +1 & 0 & +1 & 0 & -1 & 0 \\ 0 & +1 & +1 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & -1 & +1 & -1 & 0 & 0 & 0 & 0 \end{array} \right\} \begin{array}{l} \\ A \\ B \\ C \\ D \\ E \\ byp \\ cof \end{array}$$

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Constraint #1

Other constraints

Because of thermodynamic reasons, some reactions can only proceed in one direction

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E.g. $\mathrm{Glc} + \mathrm{ATP} \rightarrow \mathrm{G6P} + \mathrm{ADP}$ $\qquad \Delta G^0 = -24.9\,\mathrm{kJ/mole}$

$$K_{\mathrm{eq}} = \frac{[\mathrm{ADP}]_{\mathrm{eq}} \cdot [\mathrm{G6P}]_{\mathrm{eq}}}{[\mathrm{ATP}]_{\mathrm{eq}} \cdot [\mathrm{Glc}]_{\mathrm{eq}}} = e^{-\Delta G^0 / RT} = e^{10.05} = 23000$$

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With $[\text{ATP}]/[\text{ADP}] = 3$ and $[\text{Glc}] = 1\,\text{mM}$ the reaction runs in reverse if

$$[\text{G6P}] > K_{\text{eq}} \cdot [\text{Glc}] \cdot \frac{[\text{ATP}]}{[\text{ADP}]} = 69000\,\text{mM} = 69\,\text{M} \quad !!!$$

(pure water has 55.5 M)

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⇨ directionality implies $v_j \geq 0$ Constraints #2

Other constraints

Some process have upper bounds

- maximal uptake rates
- known maximal enzyme activities

⇨ directionality implies $v_j \leq v_j^{\max}$ Constraints #3

What is constraint based modelling?

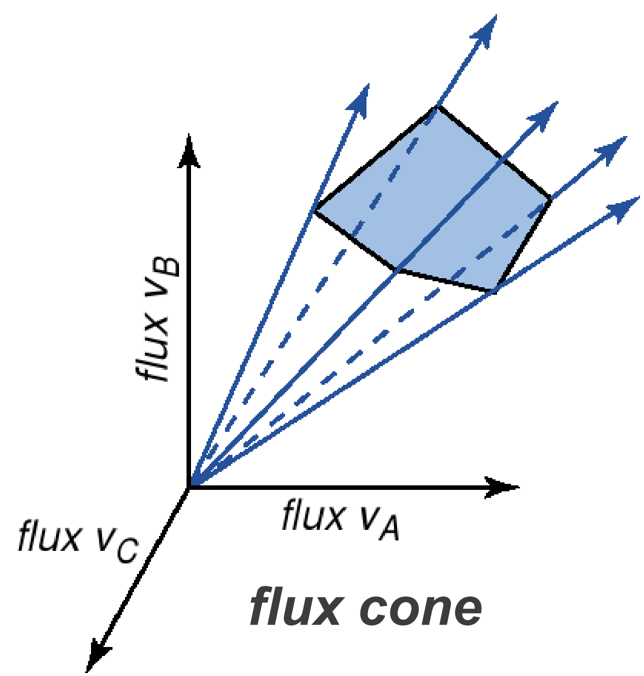
Fluxes in metabolic networks are subject to *constraints*

- Thermodynamic (directionality) $v_i \geq 0$
- Enzyme concentrations $v_i \leq v_{i,max}$

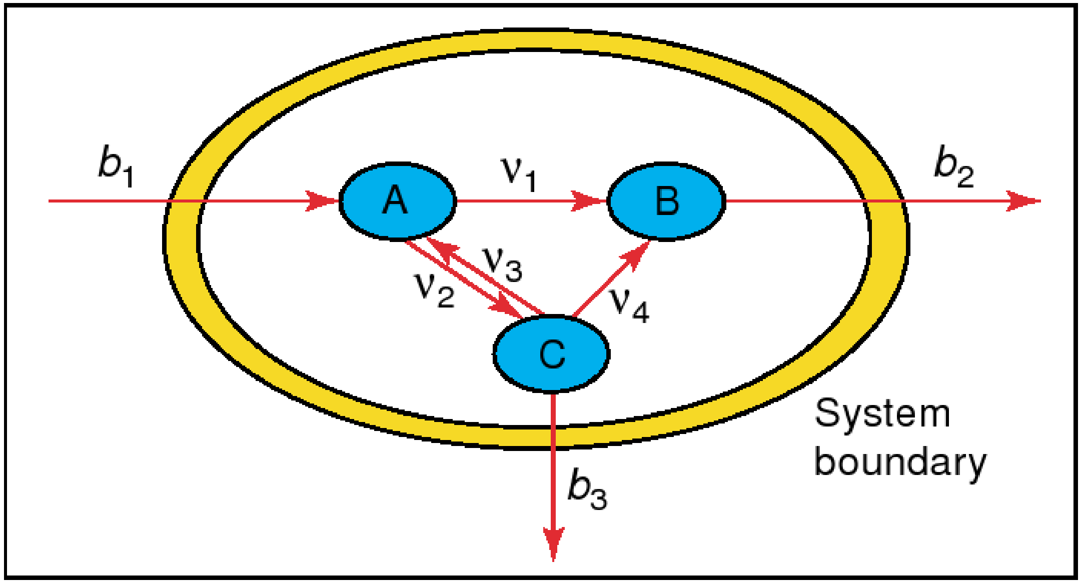
Constraint based models analyse steady state solutions which fulfill the given constraints.

Find a solution vector $v = (v_1, \ldots, v_r)^T$ such that

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



Example: constraint based model



(Kauffman et. al, 2003)

constraints

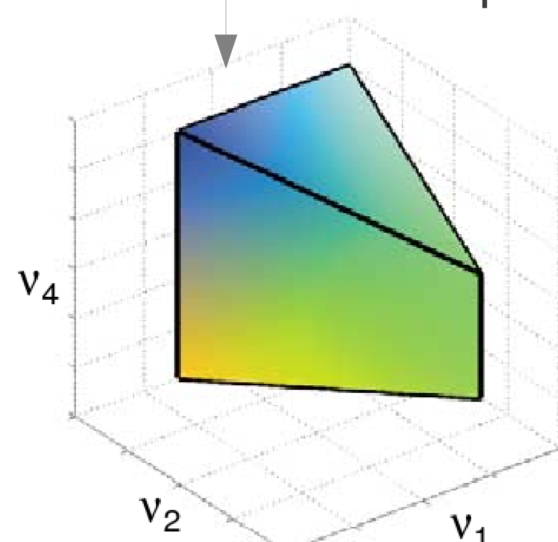
$v_3 = 0$ (thermodynamic)

$0 \leq b_1 \leq 1 \Rightarrow 0 \leq v_1 + v_2 \leq 1$

$0 \leq b_2 \leq 2 \Rightarrow 0 \leq v_1 + v_4 \leq 2$

$0 \leq b_3 \Rightarrow 0 \leq v_2 - v_4$

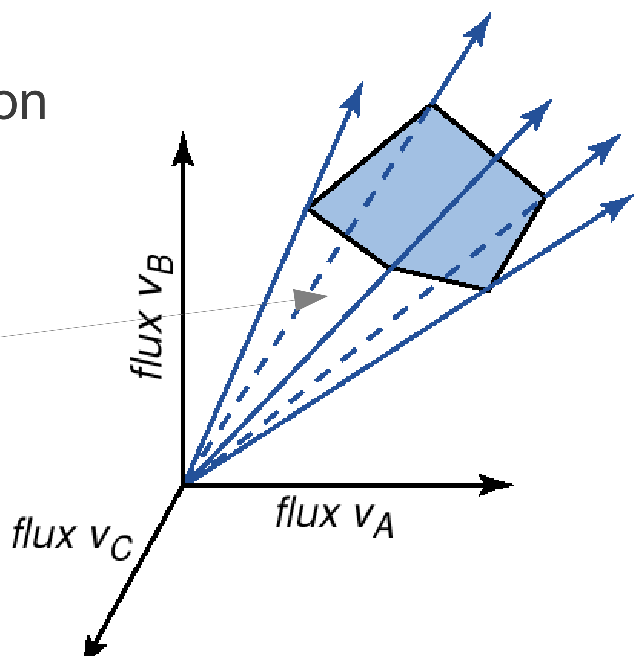
Solution space



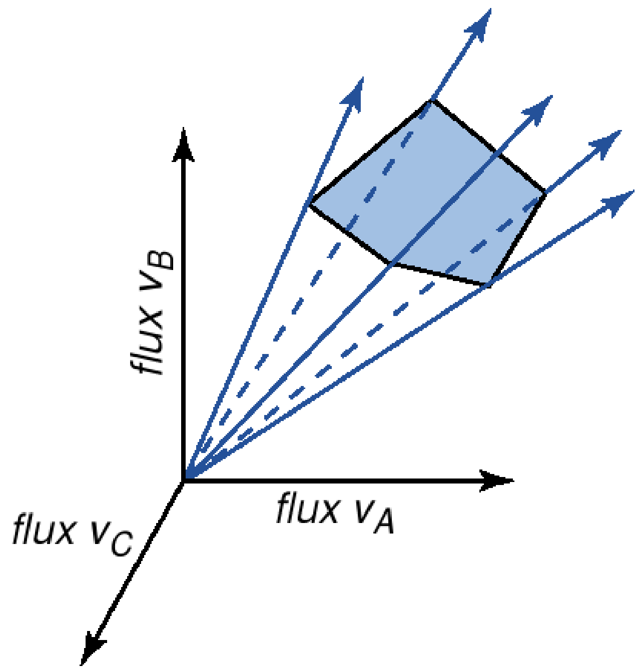
$\mathbf{S} \cdot \mathbf{v} = 0$

In general, the solution is a convex cone:

flux cone



Which solution?



Application to metabolic networks

Variables: *FLUXES* $v_i, \quad i = 1 \ldots R$

Constraints: *Stationarity, maximal rates* $N \cdot v = 0, \quad 0 \leq v_i \leq v_i^{\max}$

Objective: *?*

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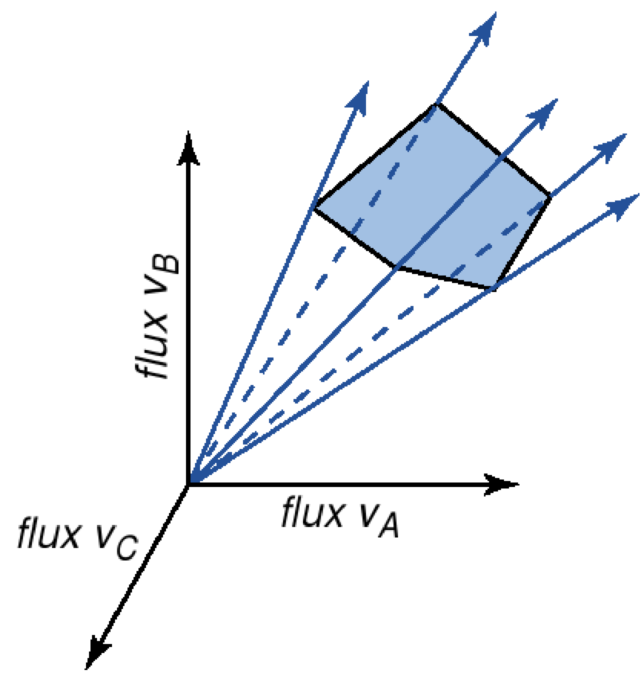
Objective: *?*

The whole purpose of linear programming is to find <u>one flux distribution</u> from the solution cone which is "optimal"



What is optimal?

No general answer!

Plausible assumptions:

- maximal growth / biomass production
- most 'economic' solution (minimal enzyme usage)

Even if the objective is not 'correct', the computation is useful:

We can investigate the question “what if...?”

A typical LP problem maximising biomass

- assemble *r* x *n* stoichiometry matrix *N* (*r* reactions, *n* metabolites)
- identify irreversible reactions $R \subset \{1 \ldots r\}$
- define boundary fluxes $B \subset \{1 \ldots r\}$
- define “biomass reaction” $v_{\text{biomass}}: \sum_i \alpha_i \cdot S_i \rightarrow \text{biomass}$

Example from *E.coli* model (Feist et al, 2007)

(54.613) cpd00001 + (59.98) cpd00002 + (0.001787) cpd00003 + (0.000045) cpd00004 + (0.000335) cpd00005 + (0.000112) cpd00006 + (0.000168) cpd00010 + (0.01128) cpd00013 + (0.000223) cpd00015 + (0.000223) cpd00016 + (0.000223) cpd00017 + (0.000279) cpd00022 + (0.2557) cpd00023 + (0.000223) cpd00028 + (0.003008) cpd00030 + (0.5953) cpd00033 + (0.003008) cpd00034 + (0.4991) cpd00035 + (0.2091) cpd00038 + (0.3334) cpd00039 + (0.2342) cpd00041 + (0.000223) cpd00042 + (0.00376) cpd00048 + (0.2874) cpd00051 + (0.1298) cpd00052 + (0.2557) cpd00053 + (0.2097) cpd00054 + (0.000223) cpd00056 + (0.003008) cpd00058 + (0.1493) cpd00060 + (0.1401) cpd00062 + (0.004512) cpd00063 + (0.05523) cpd00065 + (0.18) cpd00066 + (0.134) cpd00069 + (0.000031) cpd00070 + (0.000098) cpd00078 + (0.08899) cpd00084 + (0.000223) cpd00087 + (0.004512) cpd00099 + (0.4378) cpd00107 + (0.02481) cpd00115 + (0.03327) cpd00118 + (0.0921) cpd00119 + (0.000223) cpd00125 + (0.2148) cpd00129 + (0.2342) cpd00132 + (0.003008) cpd00149 + (0.1542) cpd00155 + (0.4119) cpd00156 + (0.2465) cpd00161 + (0.000223) cpd00166 + (0.000223) cpd00201 + (0.1692) cpd00205 + (0.000223) cpd00216 + (0.000223) cpd00220 + (0.02561) cpd00241 + (0.007519) cpd00254 + (0.006744) cpd00264 + (0.2823) cpd00322 + (0.000223) cpd00345 + (0.02561) cpd00356 + (0.02481) cpd00357 + (0.000223) cpd00557 + (0.000055) cpd02229 + (0.000223) cpd03453 + (0.006767) cpd10515 + (0.006767) cpd10516 + (0.000223) cpd11313 + (0.003008) cpd11574 + (0.000223) cpd15353 + (0.002944) cpd15428[p] + (0.00229) cpd15429[p] + (0.00118) cpd15431[p] + (0.008151) cpd15432[e] + (0.000223) cpd15499 + (0.001345) cpd15501[p] + (0.000605) cpd15503[p] + (0.005381) cpd15505[p] + (0.005448) cpd15506[p] + (0.000673) cpd15508[p] + (0.0318) cpd15531[p] + (0.02473) cpd15532[p] + (0.01275) cpd15534[p] + (0.004897) cpd15538[p] + (0.003809) cpd15539[p] + (0.001963) cpd15541[p] + (0.000223) cpd15561 => (59.81) cpd00008 + (58.8062) cpd00009 + (0.7498) cpd00012 + (59.81) cpd00067 + cpd11416

- define upper bounds for uptake rates (boundary fluxes): $v_i \leq v_i^{\max}$ for $i \in B$

The LP-problem:

maximise v_{biomass}

under the constraints

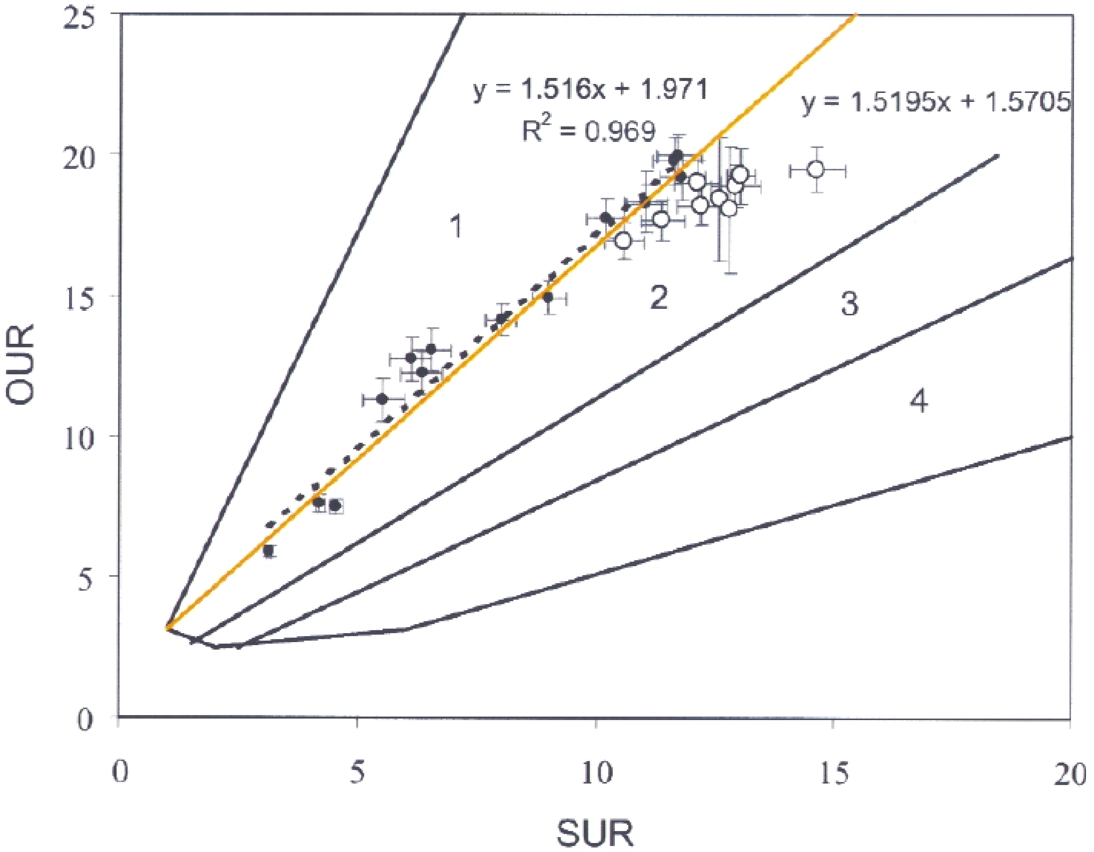
$$N \cdot v = 0$$
$$v_i \leq v_i^{\max} \quad \text{for} \quad i \in B$$
$$v_j \geq 0 \quad \text{for} \quad i \in R$$

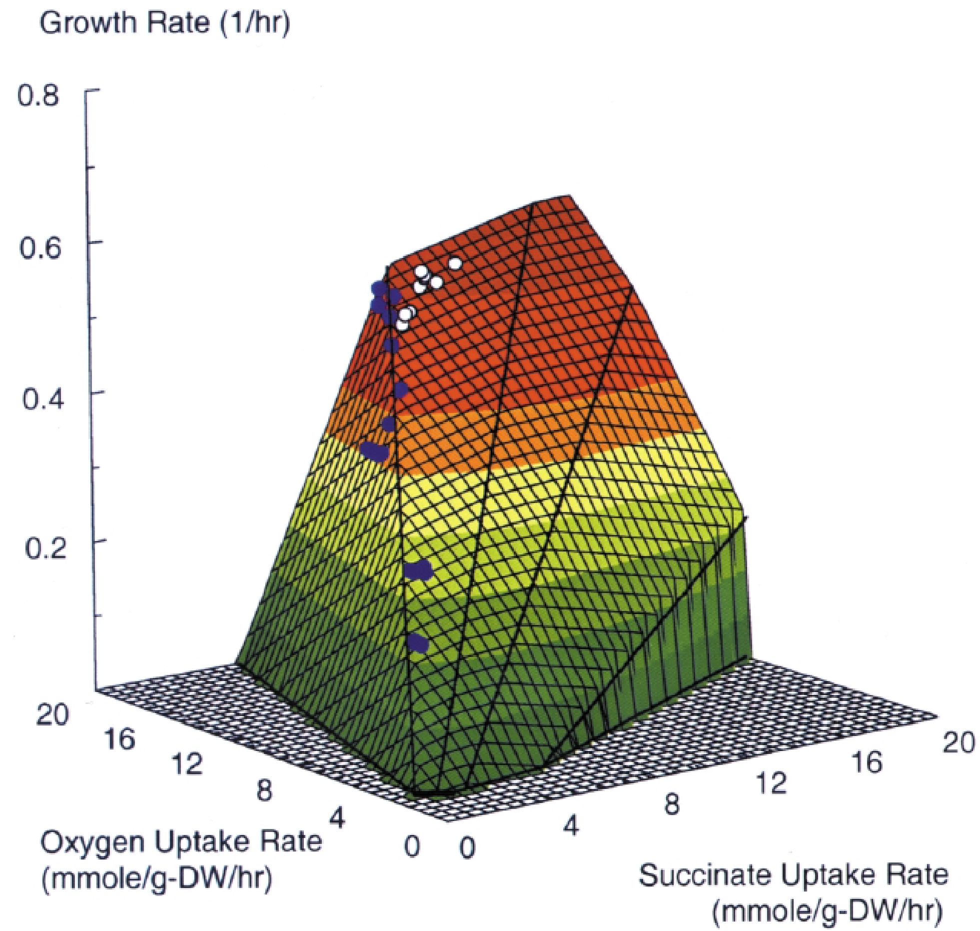
Result:

Flux distribution *v*

Optimality studies in *E. coli*

- *E. coli* was grown on succinate
- Optimal growth rates were predicted as extreme fluxes
- Oxygen and succinate uptake rates were measured





(Edwards and Palsson, 2000)

A typical LP problem minimising costs

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- define "biomass reaction" $v_{\text{biomass}}: \sum_i \alpha_i \cdot S_i \rightarrow \text{biomass}$
- Fix biomass (e.g. from experiments) $v_{\text{biomass}} = v_{\text{biomass}}^{\text{exp}}$

<u>The LP-problem:</u>

minimise $\sum_i^r w_i \cdot v_i$

under the constraints $N \cdot v = 0$

$$v_{\text{biomass}} = v_{\text{biomass}}^{\text{exp}}$$

$$v_j \geq 0 \quad \text{for} \quad i \in R$$

Variation of constraints to query the model

Objective: study how optimal fluxes change upon perturbation of external conditions

Example: impose additional ATP demand (reflecting e.g. external stress conditions)

⇨ Additional constraint $v_{\mathrm{ATPdemand}} = \gamma$ ← tunable parameter

(Additional ATP consuming process: $\mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{ADP} + \mathrm{P_i}$)

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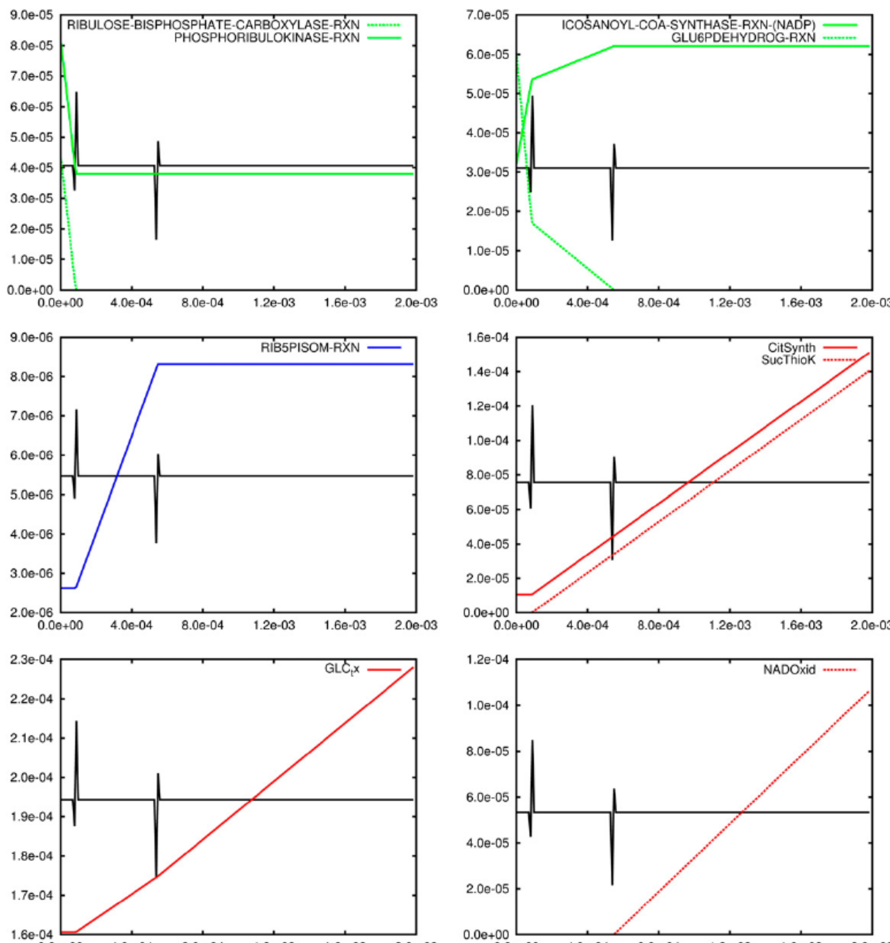

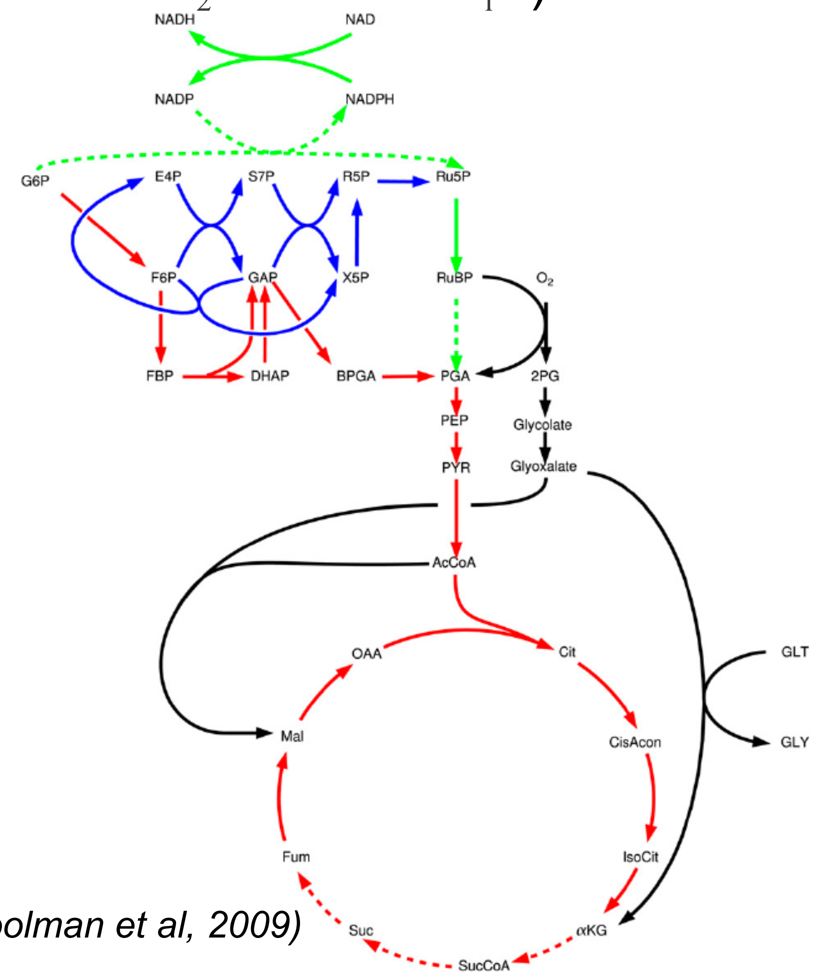

(Poolman et al, 2009)