Biotechnological Applications of Elementary Modes Analysis

Delhi Workshop 1; Structural Modelling of Metabolism; Day 2

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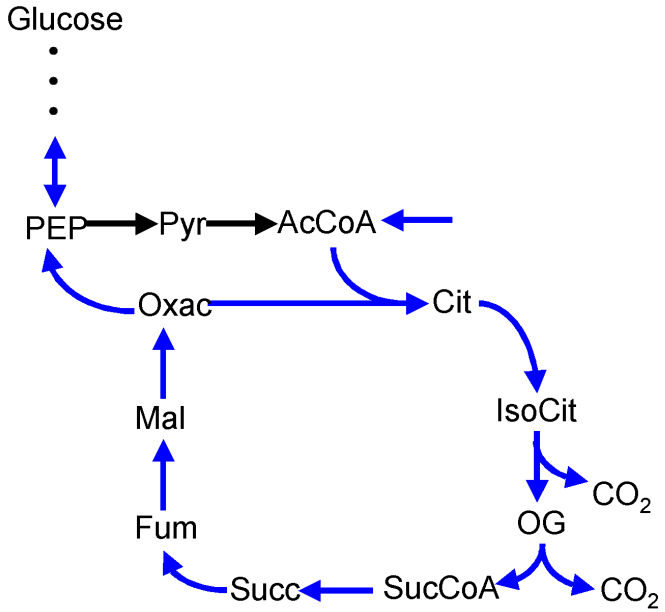
Outline

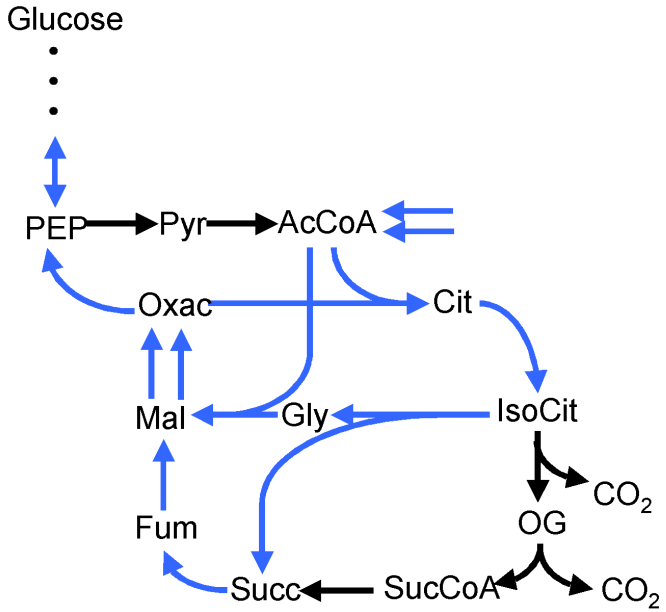
- Predicting new pathways
- Designing metabolic engineering strategies
- Functional analysis of changing metabolic flux patterns

Predicting new pathways

Analysis of Central Carbon Metabolism

In Schuster, Dandekar & Fell, *TIBS* (1999), we were analysing gluconeogenesis and the glyoxylate cycle:





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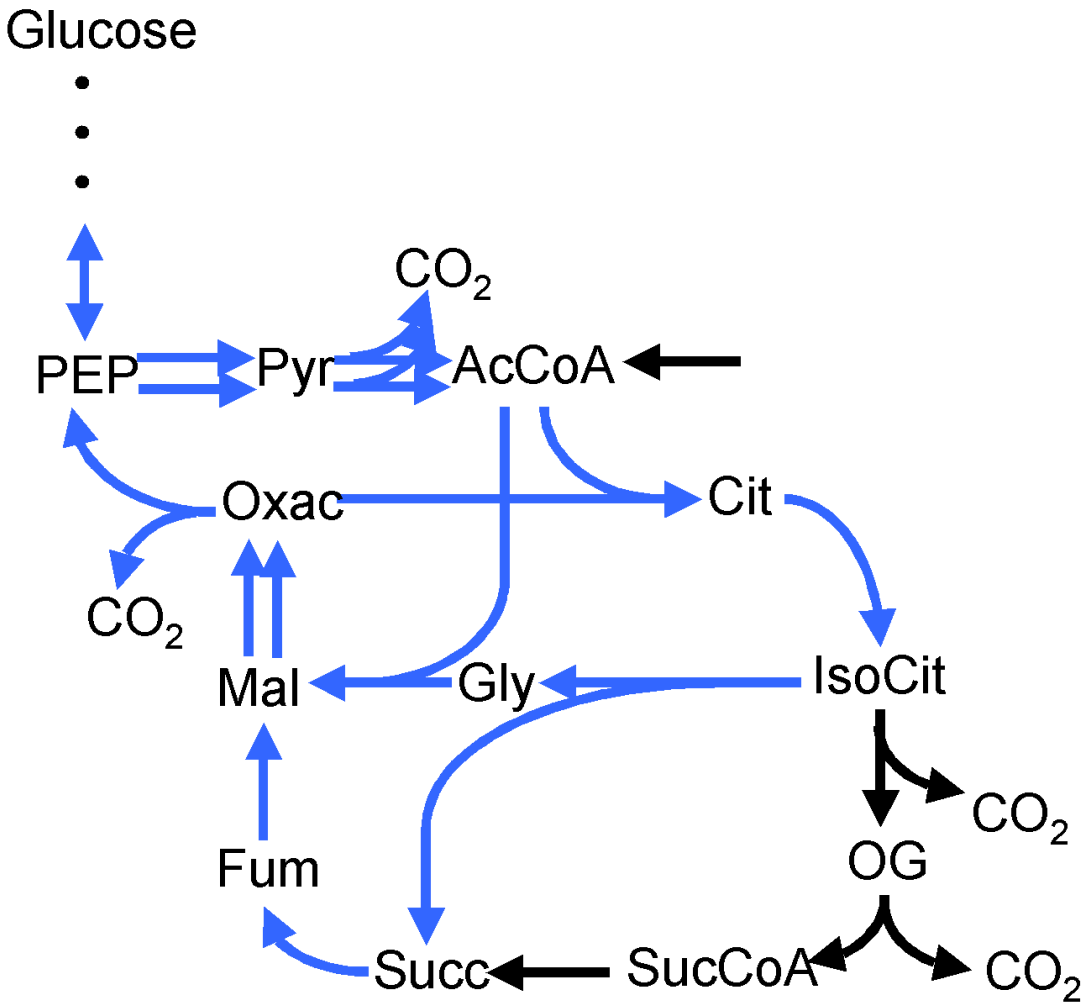
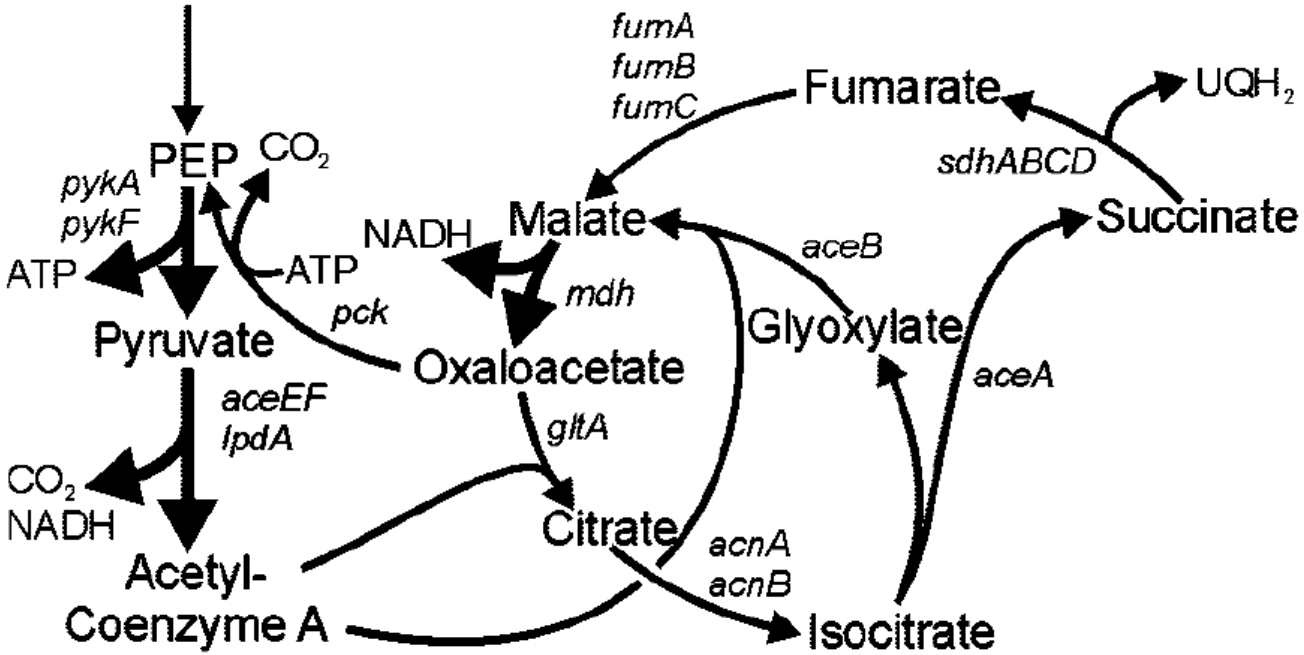
Finding a New Pathway

In Schuster, Dandekar & Fell, *TIBS* (1999), we described the following:



The Experimental Confirmation

In *J Biol Chem* in 2003, Fischer and Sauer observed this pathway in *E coli* cells growing at low glucose levels:



$$2\ PEP \rightarrow 3\ CO_2 + 4\ NADH + UQH_2 + ATP + PEP$$

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Gene ontologies and annotation - 2

How relevant are they? e.g. the Gene Ontology Consortium:

```
glyoxylate cycle
  * Accession: GO:0006097
  * Aspect: process
  * Synonyms: glyoxylate bypass
  * Definition:
    o A modification of the TCA cycle occurring in
      some plants and microorganisms, in which
      isocitrate is cleaved to glyoxylate and
      succinate. Glyoxylate can then react with
      acetyl-CoA to form malate.
```

Designing metabolic engineering strategies

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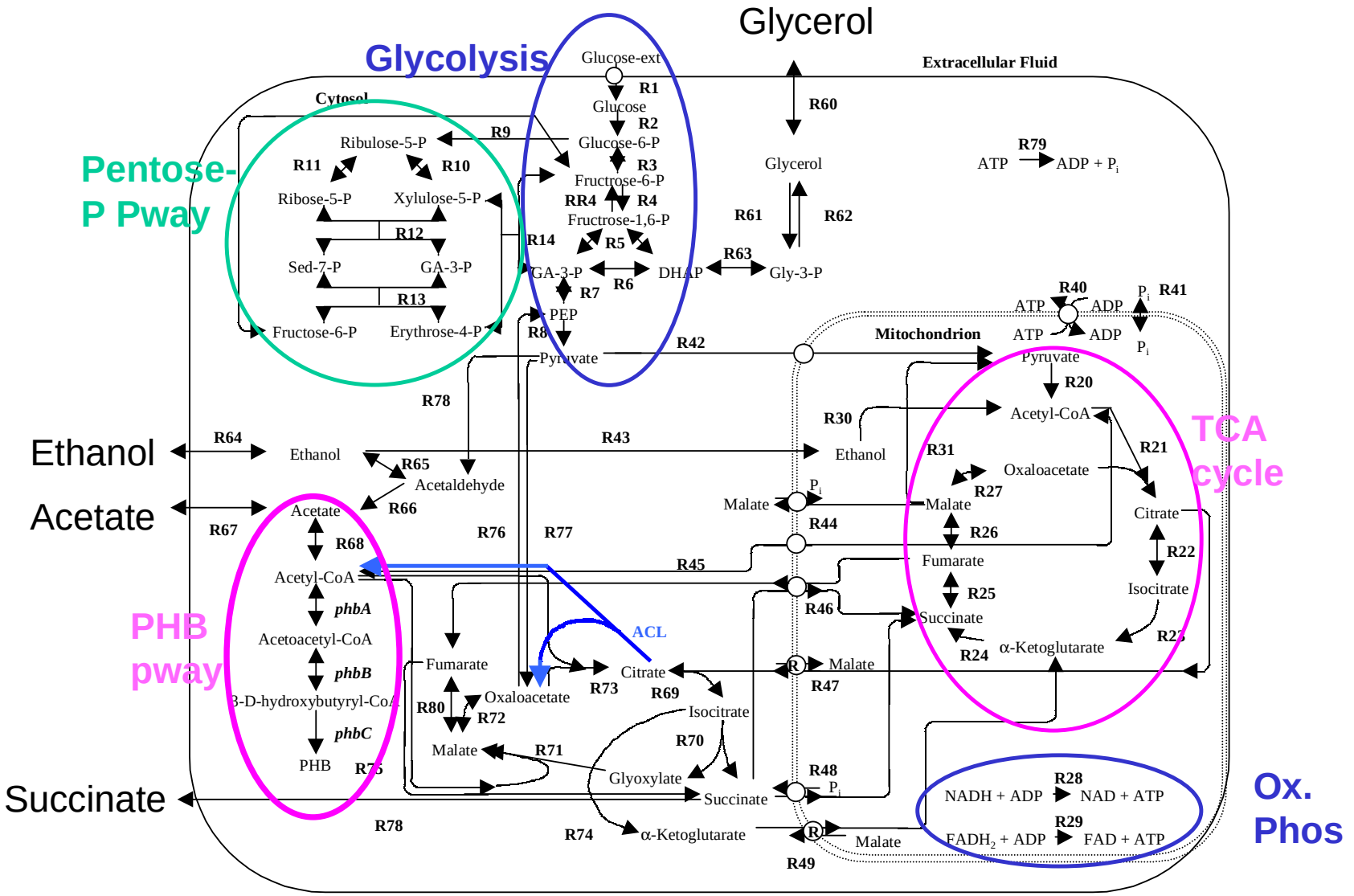
Polyhydroxybutyrate synthesis in yeast



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Optimal yields of PHB synthesis

Wild-type yeast + PHB pathway

1. 2 Acetate + EtOH $\rightarrow$ PHB + 2 CO_2 0.67
2. 65 Ac. + 31 EtOH $\rightarrow$ 30 PHB + 72 CO_2 0.63

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Wild-type yeast + ATP–citrate lyase + PHB pathway

3. 12 EtOH $\rightarrow$ 5 PHB + 4 CO_2 0.83
4. 77 EtOH + 31 Glycerol $\rightarrow$
   48 PHB + 4 Ac. + 47 CO_2 0.78

(Number following each mode is the fractional carbon conversion.)

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Ethanol from Plant Waste

Some of the issues:

- Plant wastes (e.g. straw) contain cellulose and hemicellulose which can be hydrolysed to glucose and pentose sugars.
- Yeasts convert glucose to ethanol, but don't readily use the pentoses.
- *Escherischia coli* can use pentoses as well as glucose, but ethanol is not its preferred product.
- E. coli is easy to engineer, but can it be modified to make ethanol from pentoses in such a way that it cannot mutate back to its original state?

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A Demonstration Solution

- Friedrich Srienc's group (Trinh et al, Appl. Env. Microbiol, 74, 3634-3643, 2008)built a medium-sized structural model of E coli central carbon metabolism.
- They computed the elementary modes leading from glucose and pentoses to products including ethanol and biomass.
- They searched for reactions that were *needed* for modes leading to other products but which were *not needed* for *some* of the routes to biomass and ethanol.
- They found a set of *eight* reactions that between them disabled all the modes except those leading to either ethanol alone or biomass and ethanol.
- They made a the deletion mutants and obtained close to the theoretically-predicted yields of ethanol.

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The Model

- Outline

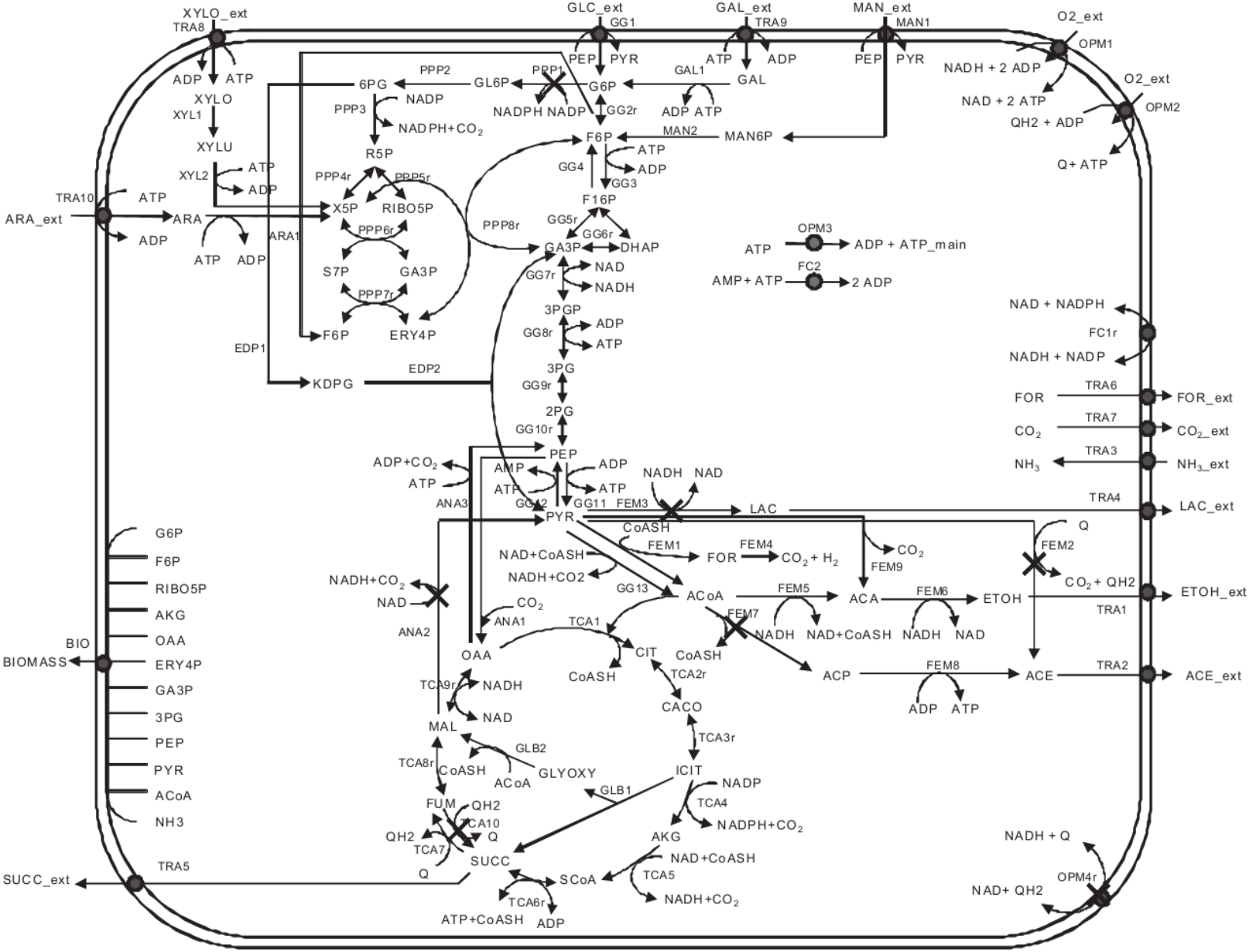
Predicting new pathways

Designing metabolic engineering strategies

- Polyhydroxybutyrate synthesis in yeast
- Optimal yields of PHB synthesis
- Ethanol from Plant Waste
- A Demonstration Solution
- The Model
- The Analysis

Functional analysis of changing metabolic flux patterns

Summary



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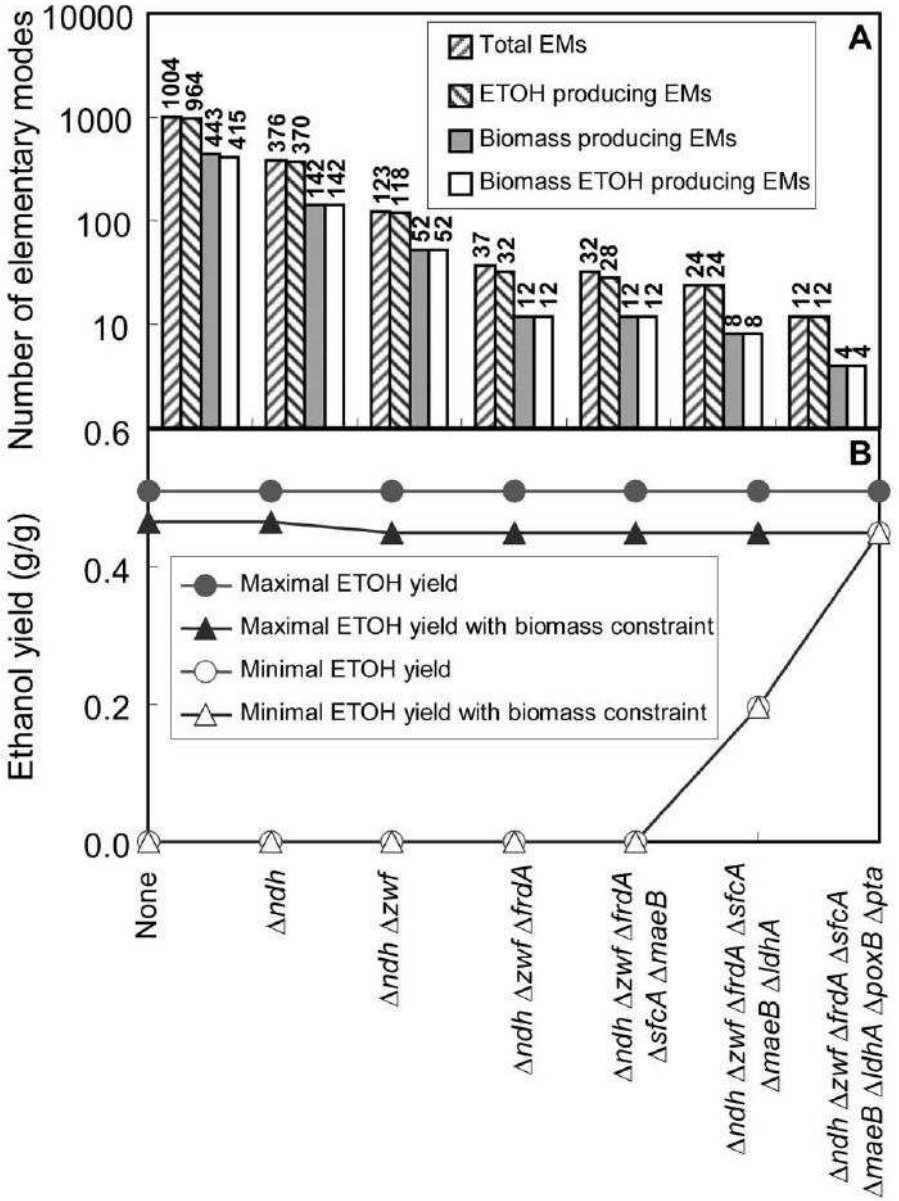
The Analysis



Functional analysis of changing metabolic flux patterns

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Relative flux in elementary modes

The steady state requirement is:

$$\mathbf{N}.\mathbf{v} = \mathbf{0}$$

But each elementary mode vector $\mathbf{e_i}$ in a matrix of elementary mode vectors $\mathbf{E}$ is also a steady state solution, i.e.:

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So the actual velocities, $\mathbf{v}$ are a weighted combination of the elementary modes:

$$\mathbf{v} = \mathbf{E}.\mathbf{w}$$

where $\mathbf{w}$ is a vector of weighting factors.

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Solutions for the weighting vector $\mathbf{w}$

Although there is not a unique solution for $\mathbf{w}$ because $\mathbf{E}$ is generally non–invertible and the system under–determined:

$$\hat{\mathbf{w}} = \mathbf{E}^{\#}\mathbf{v}$$

where $\mathbf{E}^{\#}$ is the generalised Penrose inverse of $\mathbf{E}$. Even if we have not measured all the fluxes in $\mathbf{v}$, we can partition observed $\mathbf{v}_o$ and non–observed $\mathbf{v}_x$ fluxes:

$$\mathbf{v} = [\mathbf{v}_o, \mathbf{v}_x]^T, \text{ and } \mathbf{E} = \begin{bmatrix} \mathbf{E}_o \\ \mathbf{E}_x \end{bmatrix}.$$

Then:

$$\hat{\mathbf{w}} = \mathbf{E}_o^{\#}\mathbf{v}_o$$

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Properties of the solution for $\hat{\mathbf{w}}$

Why select $\hat{\mathbf{w}}$ from the many feasible values of $\mathbf{w}$?

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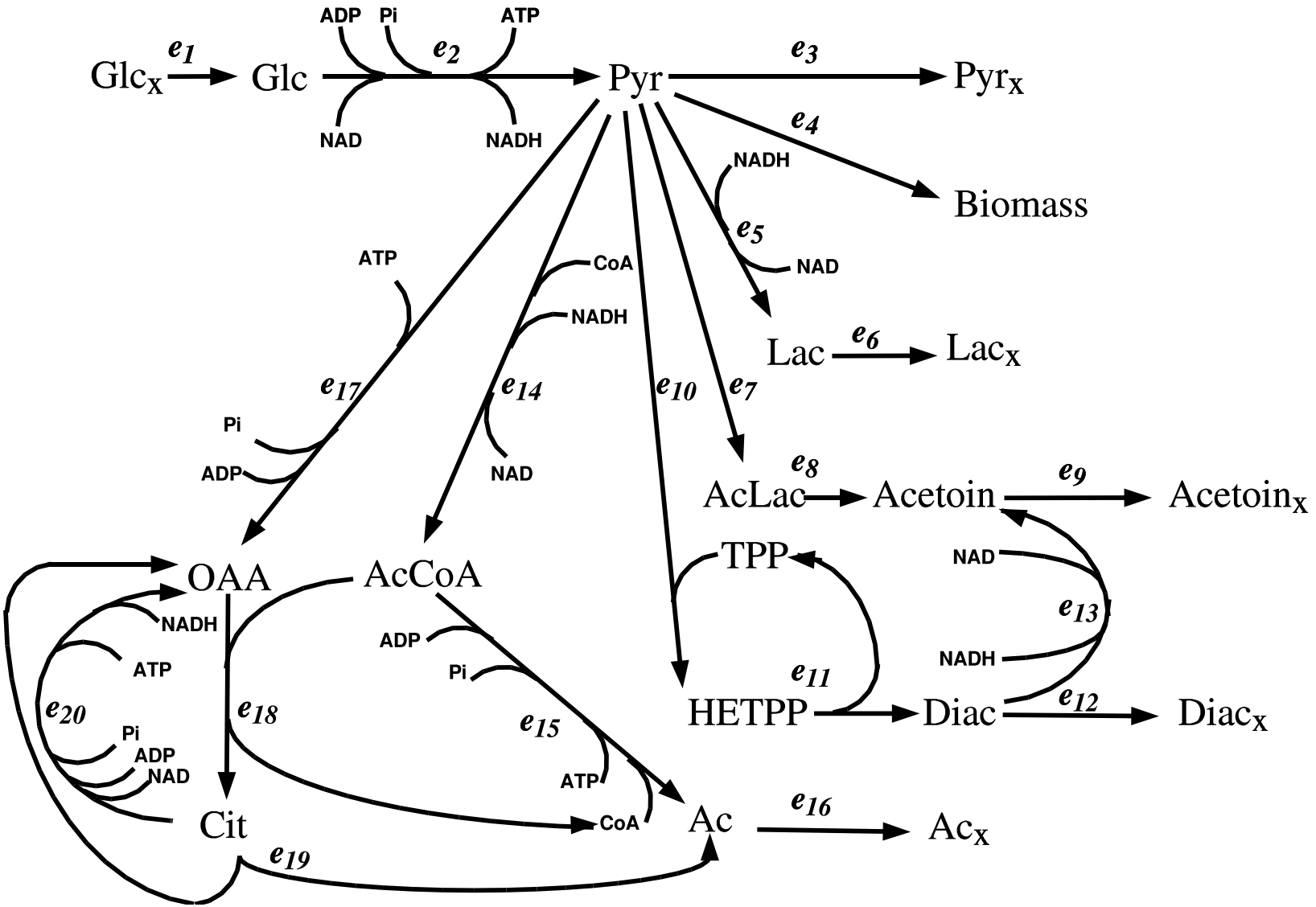
Flux analysis of lactic acid metabolism



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Elementary modes analysis

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- Modes with equivalent overall stoichiometries were grouped and their assigned flux summed to a single figure, giving 23 assigned fluxes.
- The time–dependence of these fluxes defined 4 groups.

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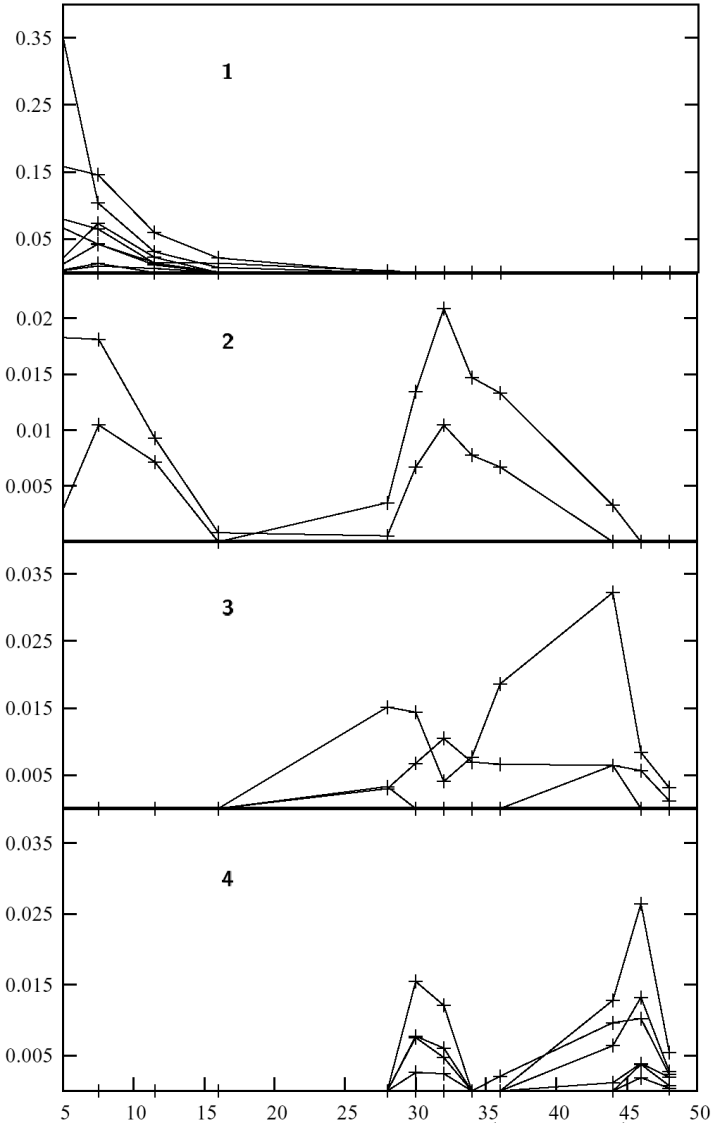
Mode assignment



Stoichiometry			Peak flux $\text{mol.hr}^{-1}.(\text{Mol. Org.})^{-1}$
Group 1			
Glc	$\longrightarrow$	2 Lac	0.45
1/2 + Glc	$\longrightarrow$	2 Biomass	0.16
1/2 + Glc	$\longrightarrow$	2 + Acetoin	0.086
+ Glc	$\longrightarrow$	2 Ac + 2	0.077
1/2 + Glc	$\longrightarrow$	2 Pyr	0.073
Pyr	$\longrightarrow$	5/6 Lac + 1/2	0.042
3/4 + Glc	$\longrightarrow$	Diac + 2	0.014
3/4 + Glc	$\longrightarrow$	Cit	0.01
3 + Glc	$\longrightarrow$	6	0.01
Glc + 6 Pyr	$\longrightarrow$	4 Lac + 2 Cit	0.0003

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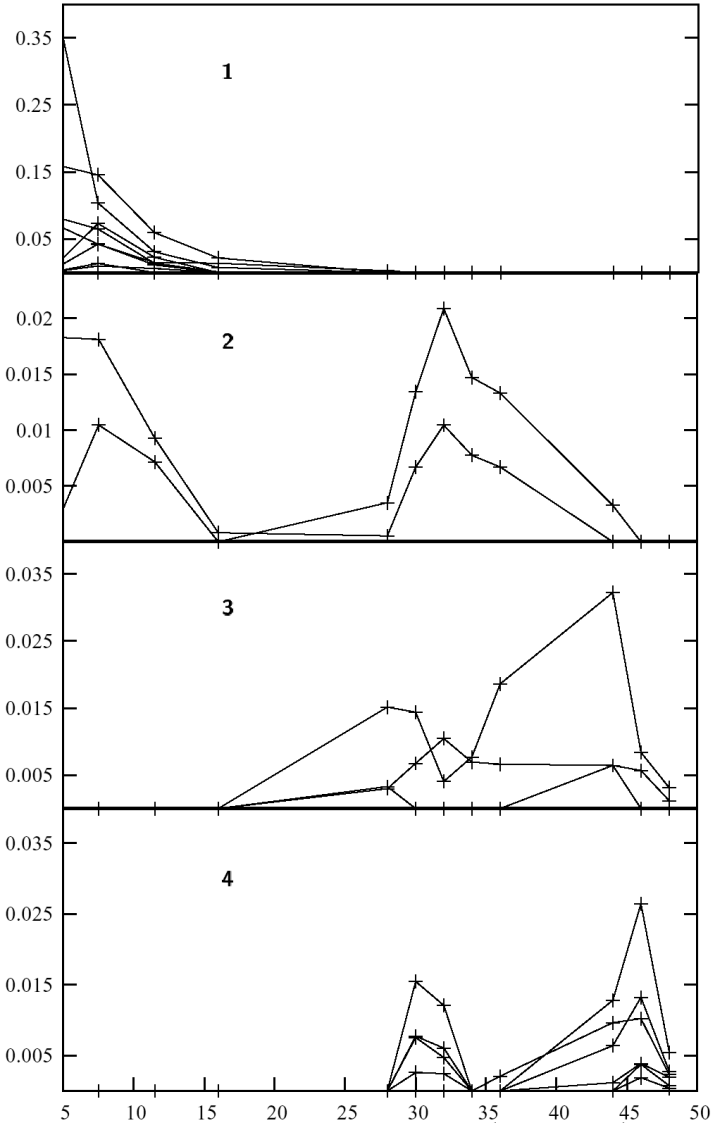
Mode assignment



Stoichiometry	Peak flux mol.hr^{-1}.(Mol. Org.)$^{-1}$
Group 2	
Lac 1/4 + $\longrightarrow$ Biomass	0.021
Pyr $\longrightarrow$ Biomass	0.018

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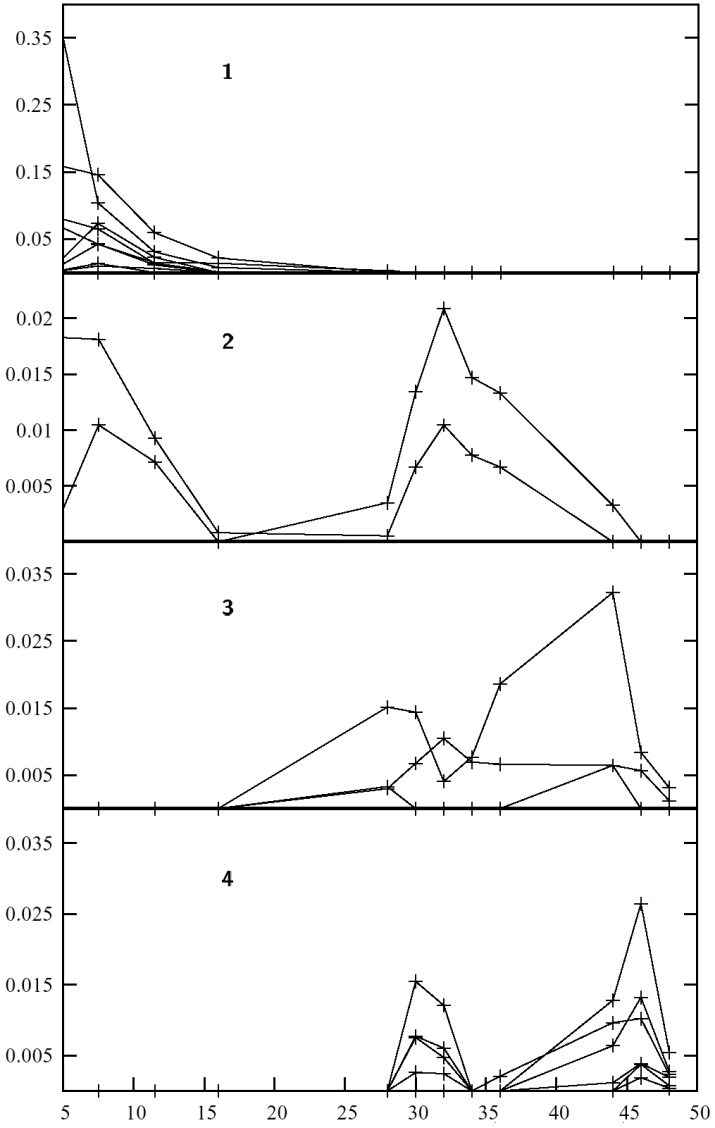
Mode assignment



Stoichiometry			Peak flux $\text{mol.hr}^{-1}.(\text{Mol. Org.})^{-1}$
Group 3			
Lac 1/2 +	$\longrightarrow$	Ac +	0.032
Lac 1/4	$\longrightarrow$	Pyr	0.01
1/4 + Pyr	$\longrightarrow$	Ac +	0.006

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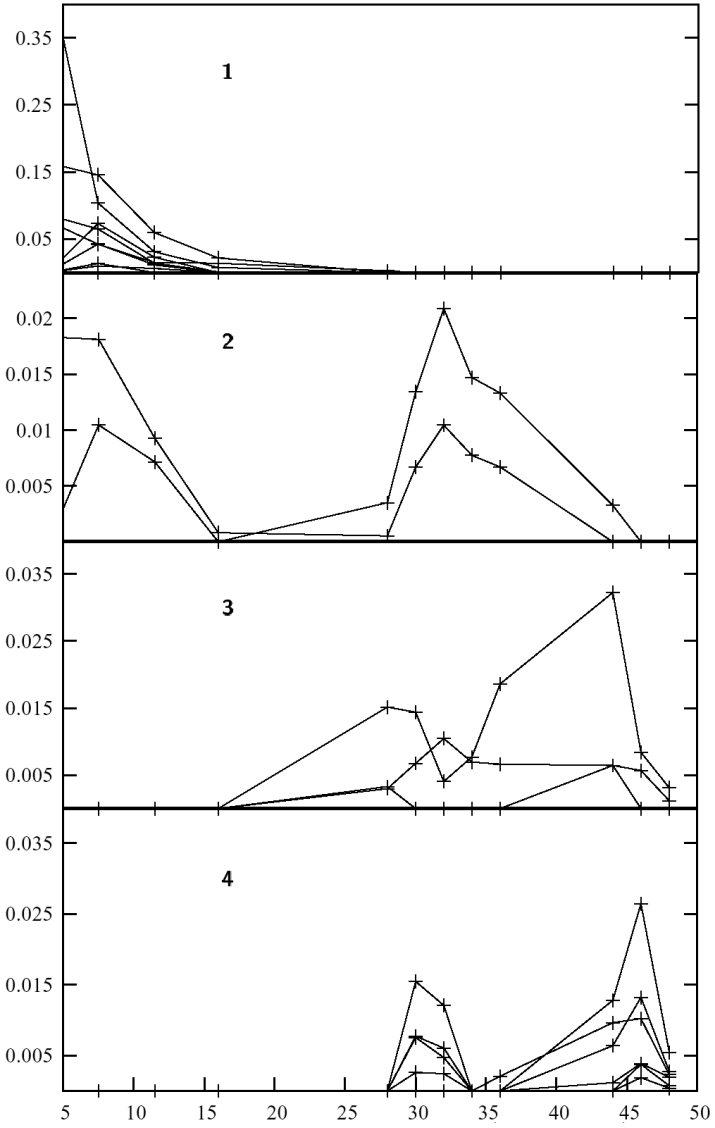
Mode assignment



Stoichiometry			Peak flux $\text{mol.hr}^{-1}.(\text{Mol. Org.})^{-1}$
Group 4			
Lac + 3/2	$\longrightarrow$	3	0.026
Lac + 3/4	$\longrightarrow$	1/3 Cit +	0.013
Lac + 5/12	$\longrightarrow$	1/3 Cit + 1/3 Ac + 1/3	0.010
Lac + 1/4	$\longrightarrow$	1/2 Acetoin	0.0039
5/4 + Pyr	$\longrightarrow$	3	0.0038
1/2 + Pyr	$\longrightarrow$	1/3 Cit +	0.0019

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Summary

- Elementary modes analysis helps to highlight modes of operation of small to medium scale metabolic networks
- Unlike FBA, it provides all routes in one computation.
- It has correctly predicted previously unrecognised pathways.
- It can be used to analyse and design biotechnological processes.

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Acknowledgements — My Group

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