Examples of metabolic models

C1net Workshop 2; Day 3

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C1net gas fermentation

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Constructing a threonine pathway model

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Kinetic models

Needed:
a single equation for each enzyme containing all effects from

- substrates
- product inhibition
- reverse reaction
- effectors

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Needed:
a single equation for each enzyme containing all effects from

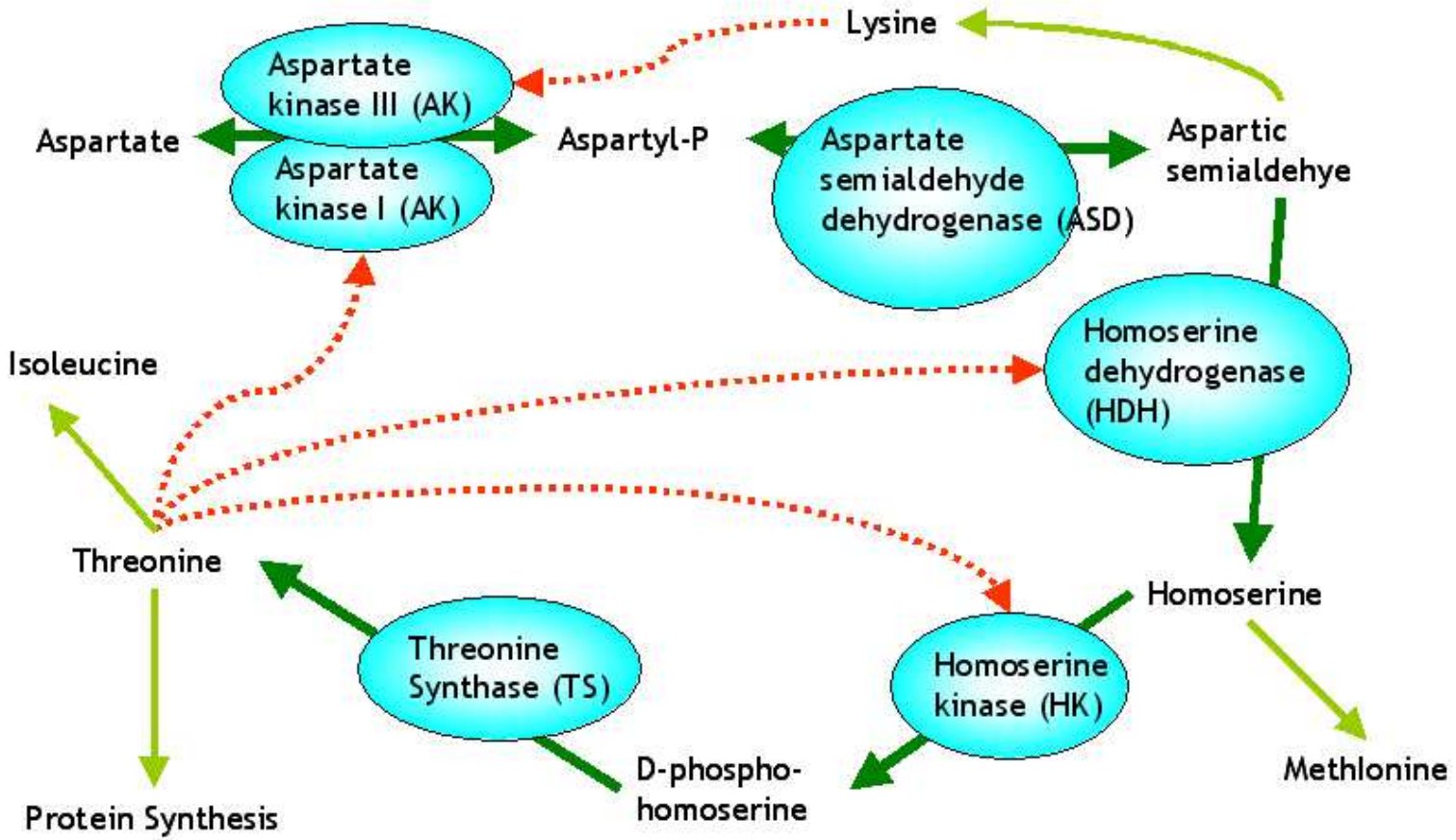
- substrates
- product inhibition
- reverse reaction
- effectors

using parameters determined at

- *in vivo* pH
- temperature
- ion concentrations

in the organism, cell type and compartment under consideration.

The threonine pathway



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Components of the modelling project

1. Kinetics of the pathway enzymes
2. Dynamics of threonine synthesis in cell-free extracts
3. Building a computer model of the pathway based on kinetics
4. Validation of model with cell-free experiments
5. Extrapolating model to intracellular conditions

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1. Kinetic data

Why not use published data?

- pH values.

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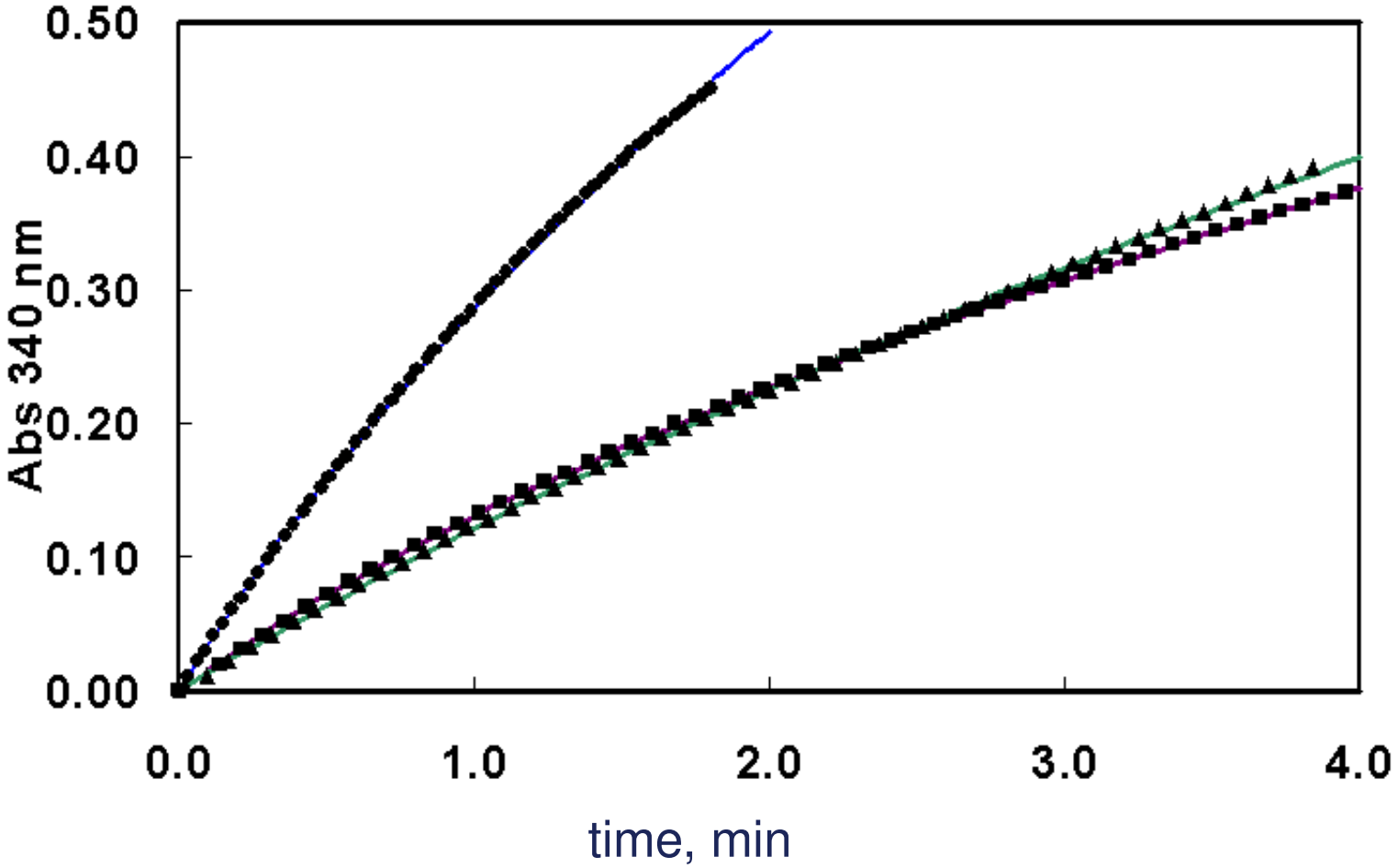
Why not use published data?

- pH values.
- Reaction direction.
- Lack of information on product inhibition.
- Analysis didn't produce a single overall equation in terms of all substrates, products and effectors.

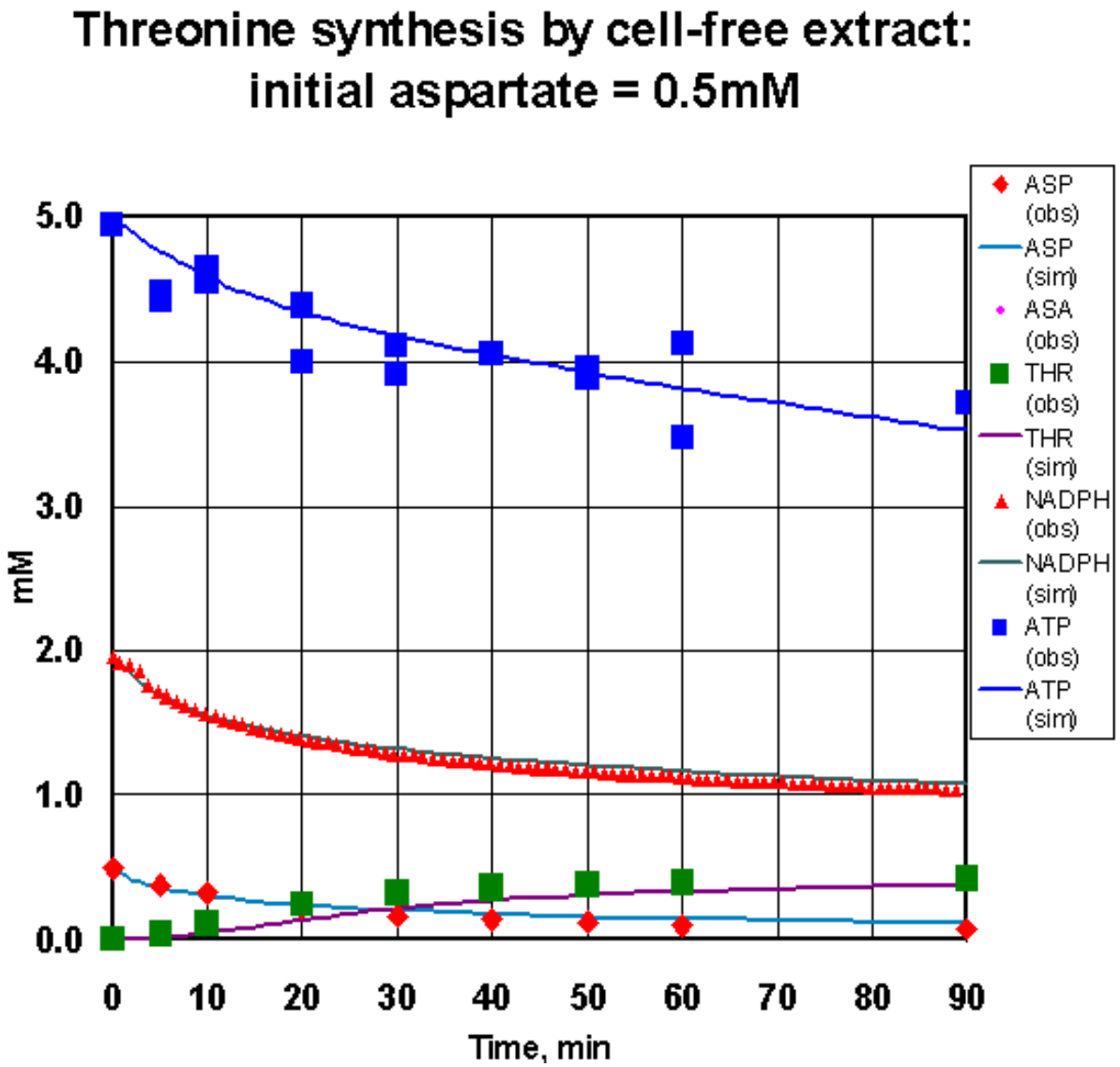
Example: aspartate kinase I

$$
\begin{aligned}
Num &= V_{\mathrm{f}}\left(asp.ATP - \frac{aspp.ADP}{K_{\mathrm{eq}}}\right) \\
D1 &= \left(K_{\mathrm{asp}}\frac{1+\left(\frac{thr}{K_{\mathrm{ithr}}}\right)^{n_h}}{1+\left(\frac{thr}{\alpha K_{\mathrm{ithr}}}\right)^{n_h}} + aspp\frac{K_{\mathrm{asp}}}{K_{\mathrm{aspp}}} + asp\right) \\
D2 &= \left(K_{\mathrm{ATP}}\left(1+\frac{ADP}{K_{\mathrm{ADP}}}\right) + ATP\right) \\
v &= \frac{Num}{D1\times D2}
\end{aligned}
$$

Product inhibition of AK I



2. Generating Pathway Data



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3. Simulator (ScrumPy) Input


```
ak: asp + atp -> aspp + adp

F1*(
vm11*(asp*atp - aspp*adp/keqak)/((k11*(1+(thr/k1thr)
/(1+(thr/(alpha*k1thr))**nak1)+(k11*aspp/k1aspp) +
asp)*(k1atp*(1+adp/k1adp)+atp))
 + vm13*(asp*atp - aspp*adp/keqak)/((1 + (lys/k1lys)
aspp/k13aspp)
 + asp)*(k13atp*(1+adp/k13adp)+atp))
)
# F1 is a factor to allow modulation of enzyme group

asd:  aspp + nadph -> asa + nadp + Pi
(vm2f*(aspp*nadph - asa*nadp*Pi/k2eq))/
((k2aspp*(1 + asa/k2asa)*(1 + Pi/k2p) + aspp)*
(k2nadph*(1 + nadp/k2nadp) + nadph))
```

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ScrumPy Input — 2


```
#Protein content, mg/ml
prot=0.194

#Aspartate kinase 1
vm11 = 0.402*prot*1.49 #1.49 is assay correction fac
k11 = 0.97
k1thr=0.167 alpha=2.47 nak1=4.09
k1aspp = 0.017
k1atp=0.98 k1adp=0.25
keqak=6.39e-04

#aspartate kinase 3
vm13 = 0.283*prot*1.12 #1.12 is assay correction fac
k13 = 0.323 k1lys = 0.391
nak3 = 2.78 k13atp=0.225
k13aspp = 0.017k13adp = 0.25
```

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The Differential Equations

Automatically derived by ScrumPy:

Differential equations:

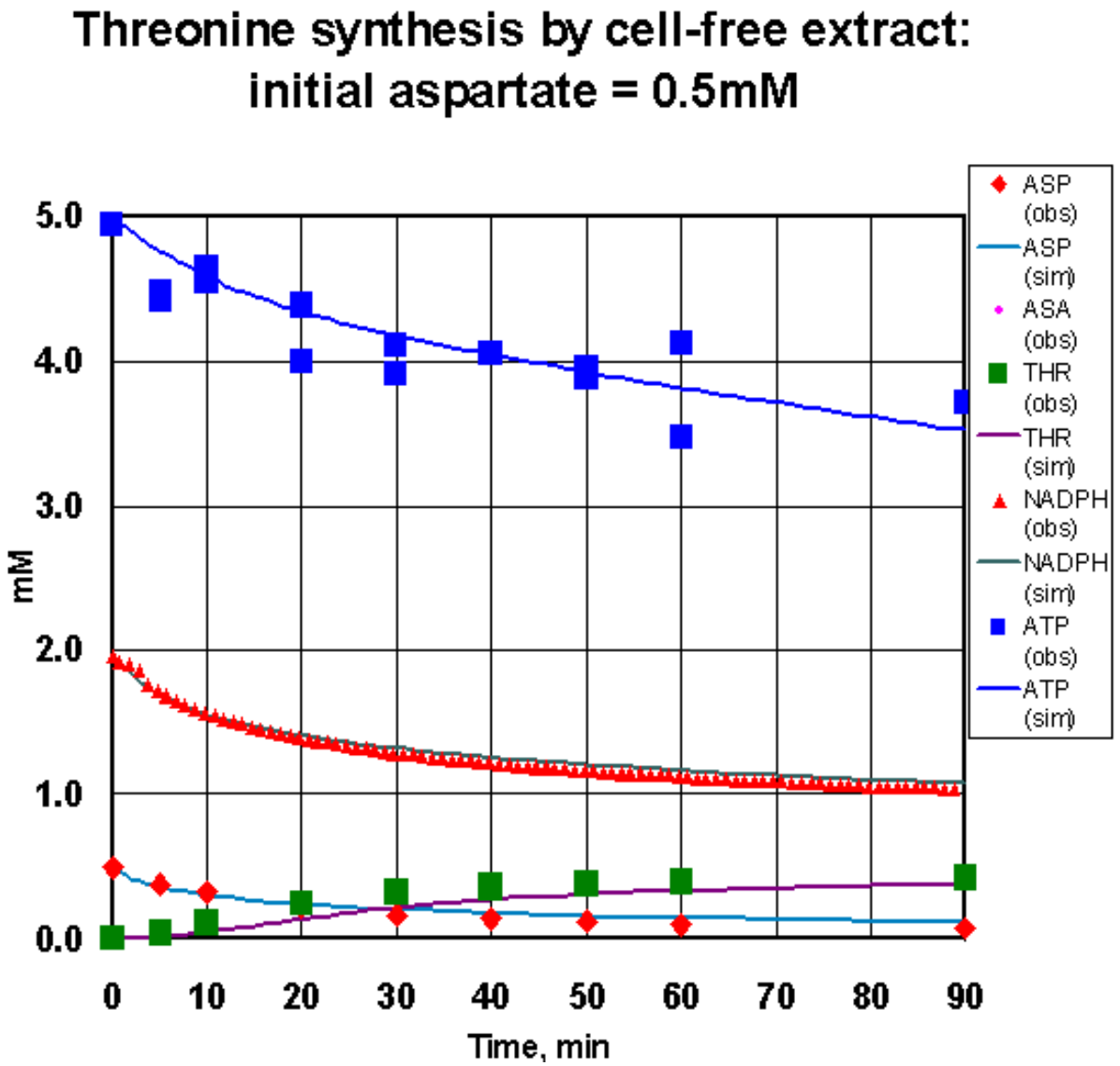
1:aspp' = V[ak] - V[asd]

2:asa' = V[asd] - V[hdh]

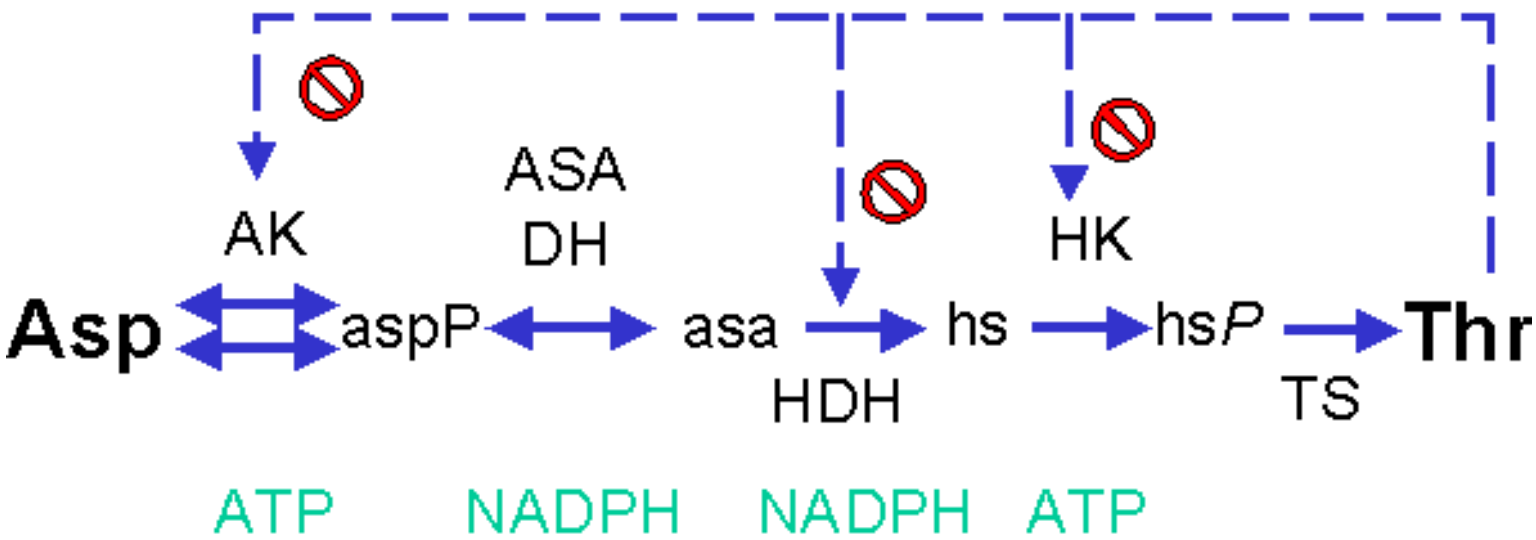
3:hs' = V[hdh] - V[hk]

4:hsp' = - V[ts] + V[hk]

4. Simulating Dynamics



5. Extrapolating to *in Vivo* Behaviour

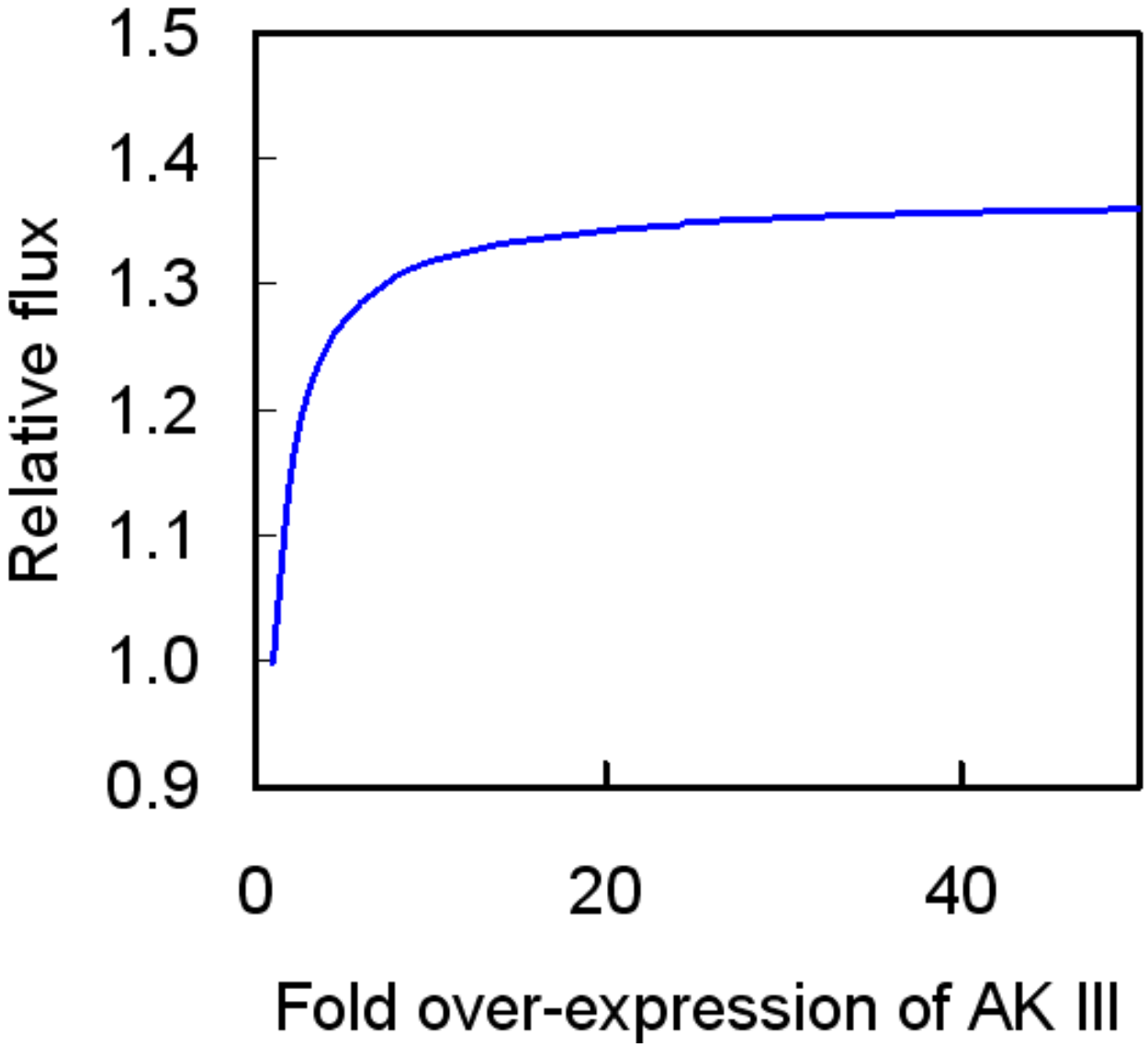


"External" Concentrations

Measured on cells:

Metabolite	Content, nmol.(g dry wt)$^{-1}$	Concentration, mM
asp	2854	1.34
thr	7444	3.49
lys	984	0.46
ATP	2792	1.31
ADP	352	0.17
NADP	1341	0.63
NADPH	1197	0.56
Pi	ND	5

Simulating Enzyme Over–Expression



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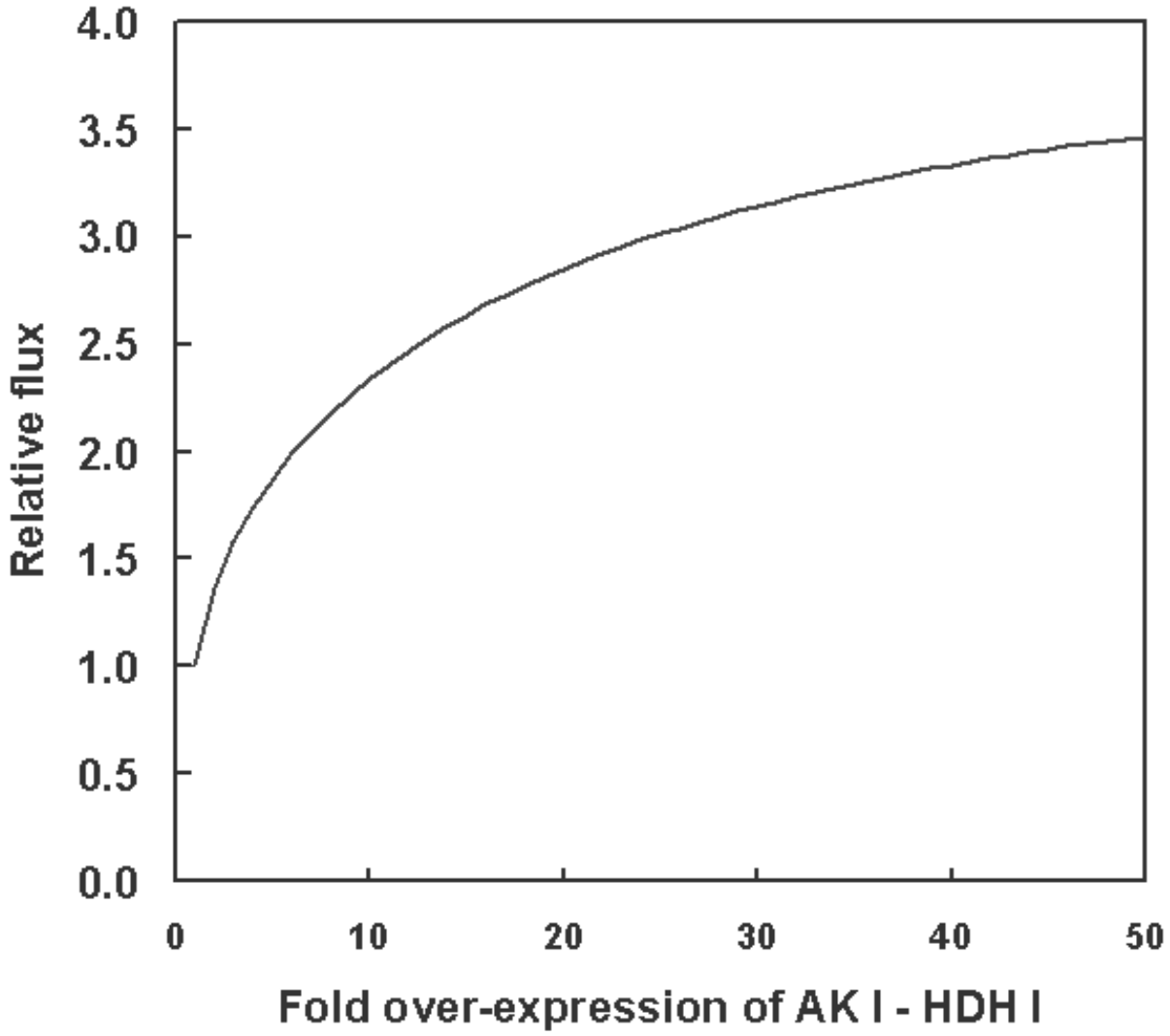
Simulating Enzyme Over–Expression — 2



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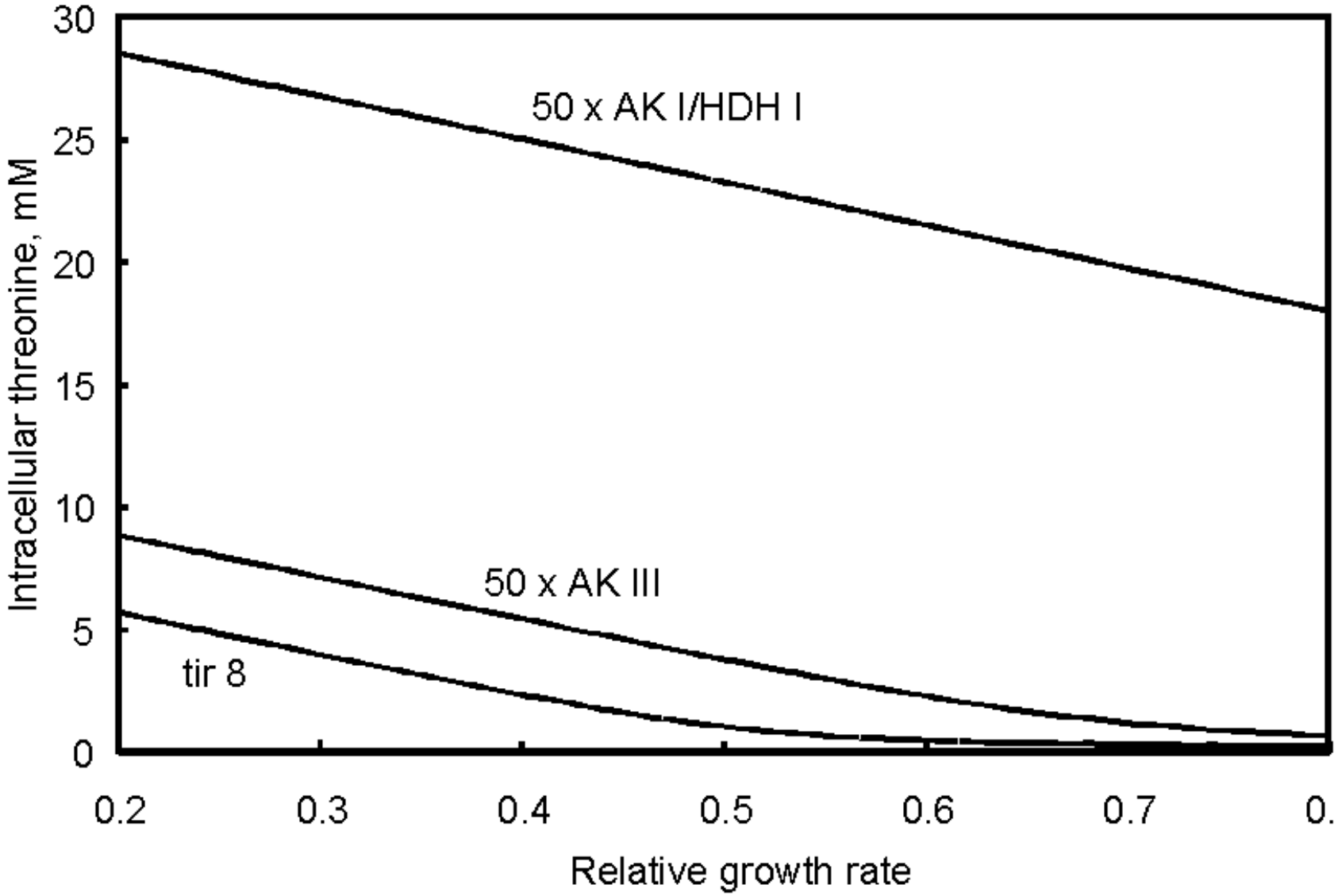
Simulated threonine accumulation



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Further details

References:

- C Chassagnole et al, Biochem J. 356, 415–423, 425–432, 433–434, (2001)

We'll return to modelling the control of threonine synthesis later.

The Entner-Duodoroff Pathway

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Acknowledgements

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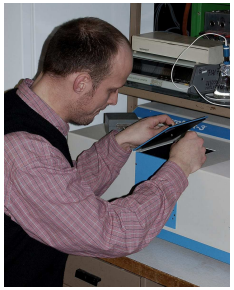

Reinis Rutkis

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http://www.sysbio.lv/

Zymomonas mobilis

- Has very high rates of glucose fermentation to ethanol and high tolerance to both.
- Uses Entner–Doudoroff (ED) pathway of glucose catabolism.
- Uncoupled growth phenomenon — whereby rates of catabolism exceed the requirements of anabolism. (98% glucose is converted to catabolic products.)
- Small genome size and reduced central metabolic network make it attractive for metabolic engineering.
- Electron transport chain poorly coupled to ATP synthesis could allow flexibility over redox state of engineered products.
- Good experimental data on kinetic properties of its ED enzymes, as well as metabolite measurements *in vivo* and in cell-free systems.

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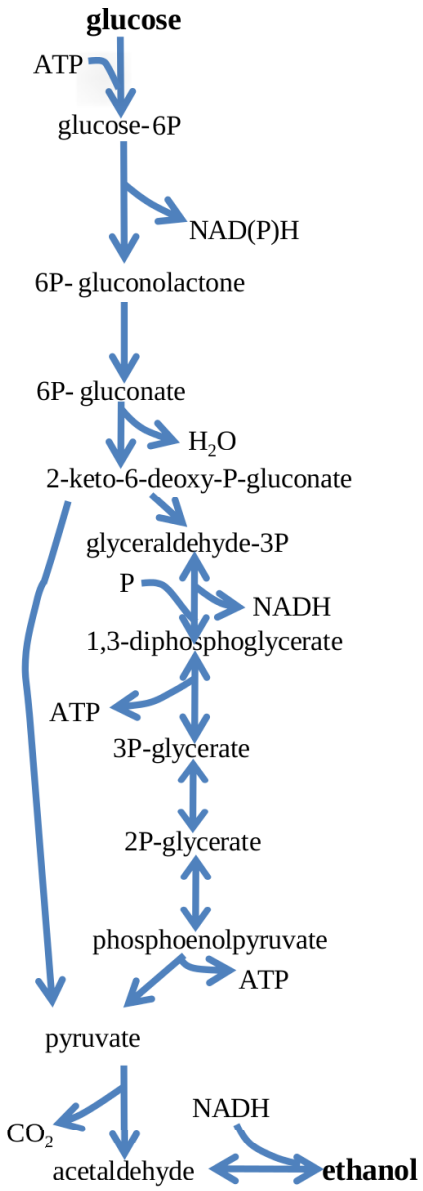
Entner–Doudoroff Pathway



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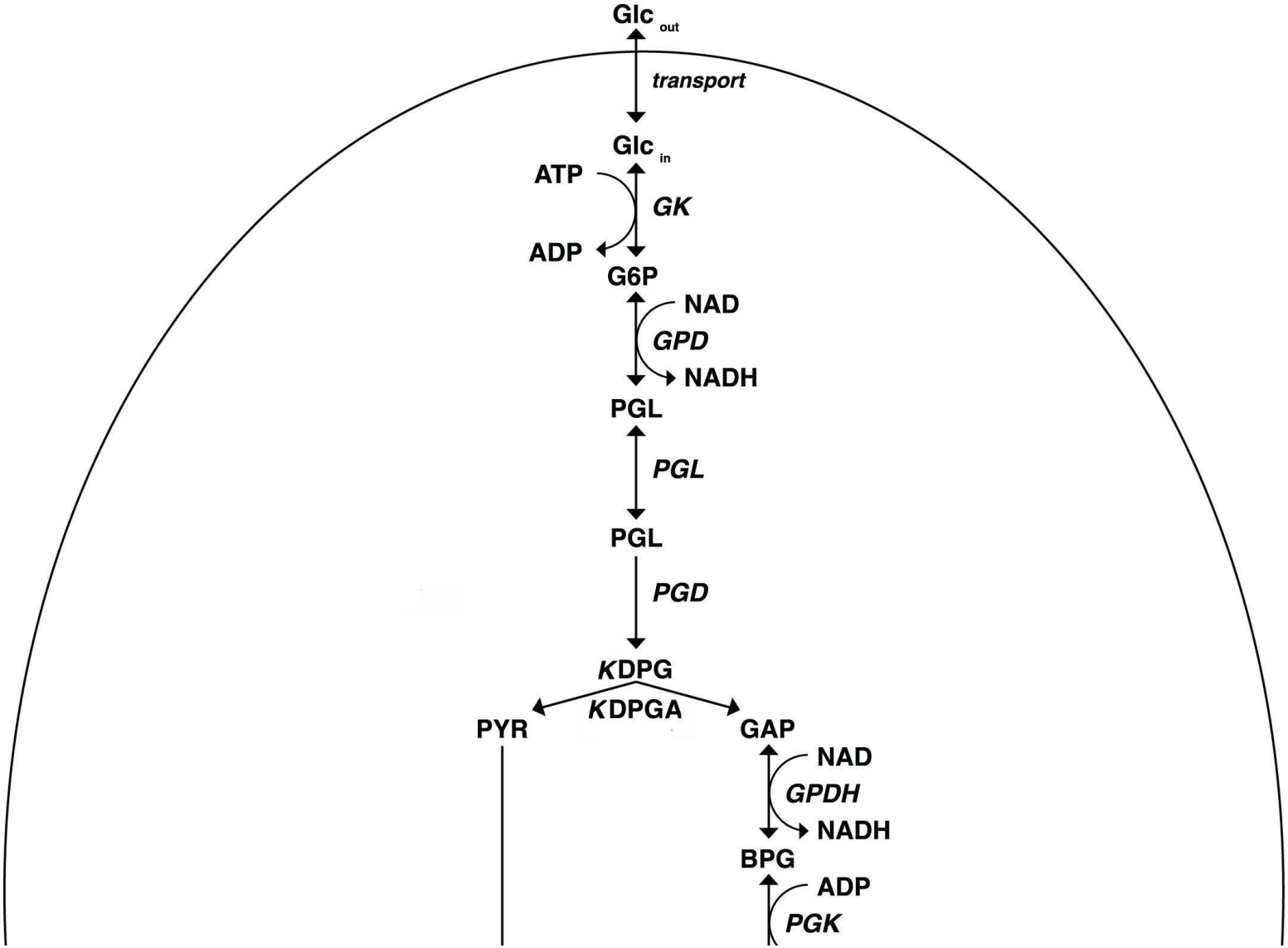
ED Model — Upper Part



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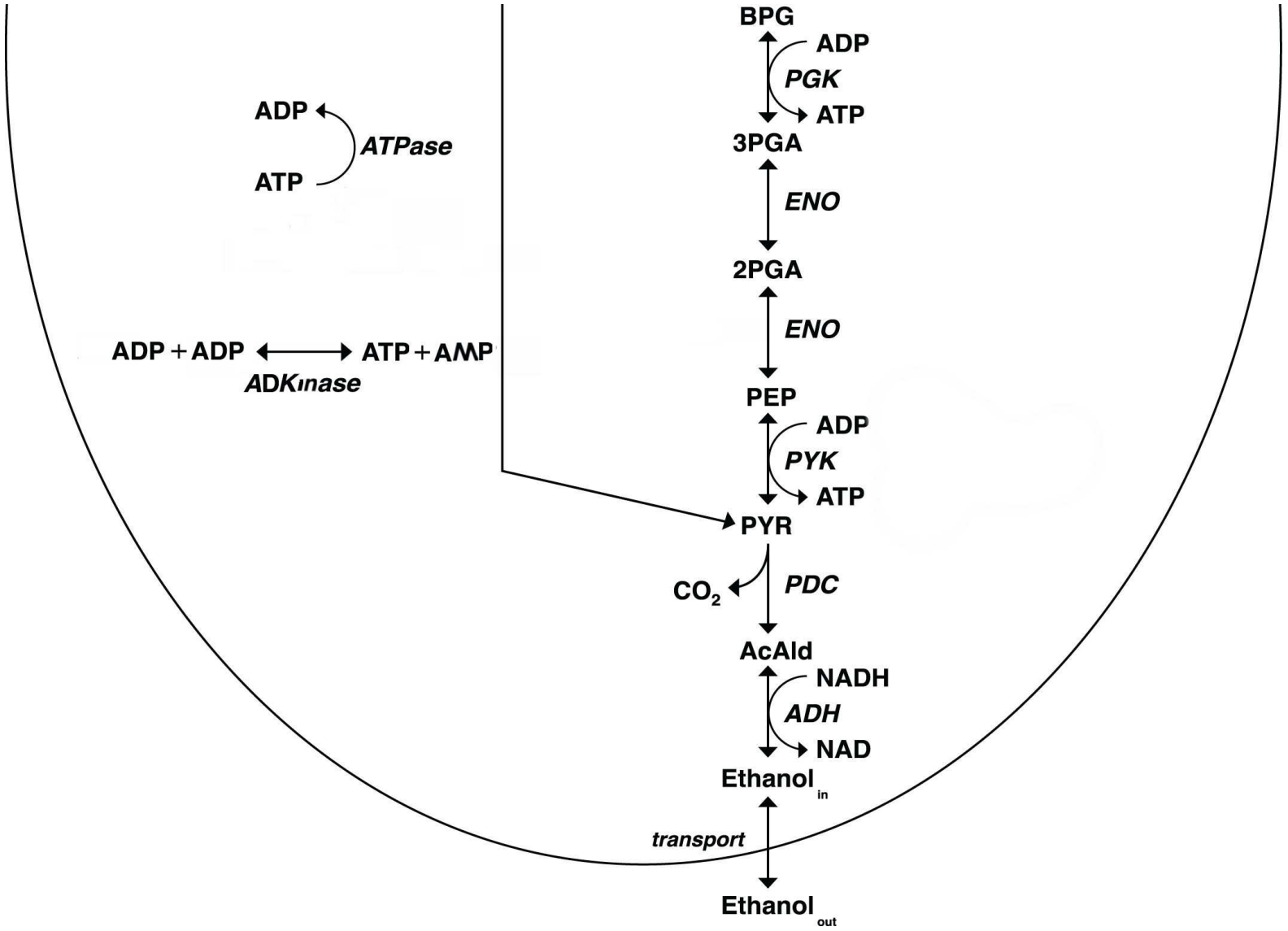
ED Model — Lower Part



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Glucokinase Rate Equation

$$v_{GK} = \frac{\frac{V_{GK}}{GLUC_{in} * K_{ATP} * (1 + \frac{GLUC6P}{K_{iGLUC6P}})} * (GLUC_{in} * ATP - \frac{GLUC6P * ADP}{K_{eq}})}{1 + \frac{GLUC_{in}}{K_{GLUCin}} + \frac{GLUC6P}{K_{GLUC6P}} * (1 + \frac{ATP}{K_{ATP} * (1 + \frac{GLUC6P}{K_{iGLUC6P}})} + \frac{ADP}{K_{ADP}})}$$

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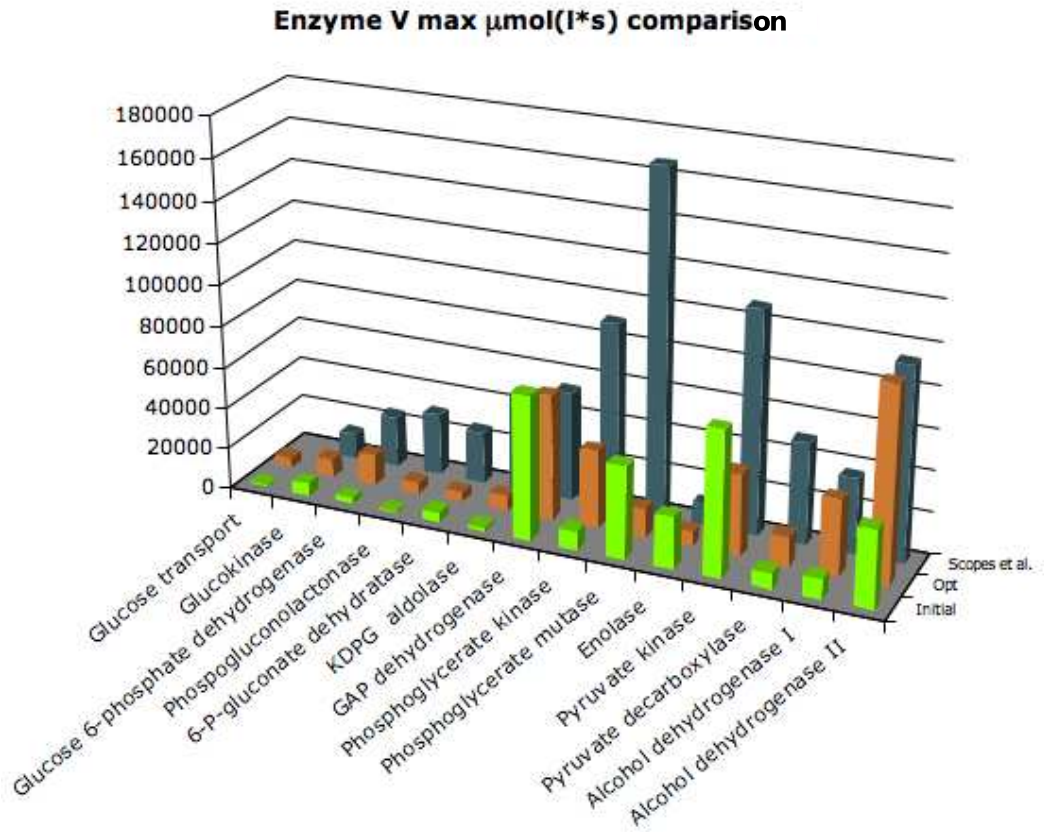
Model Optimization



- With these adjustments, the glucose consumption flux was comparable with that observed in culture.
- The model could also match glucose consumption reported for cell–free extracts with added ATPase.

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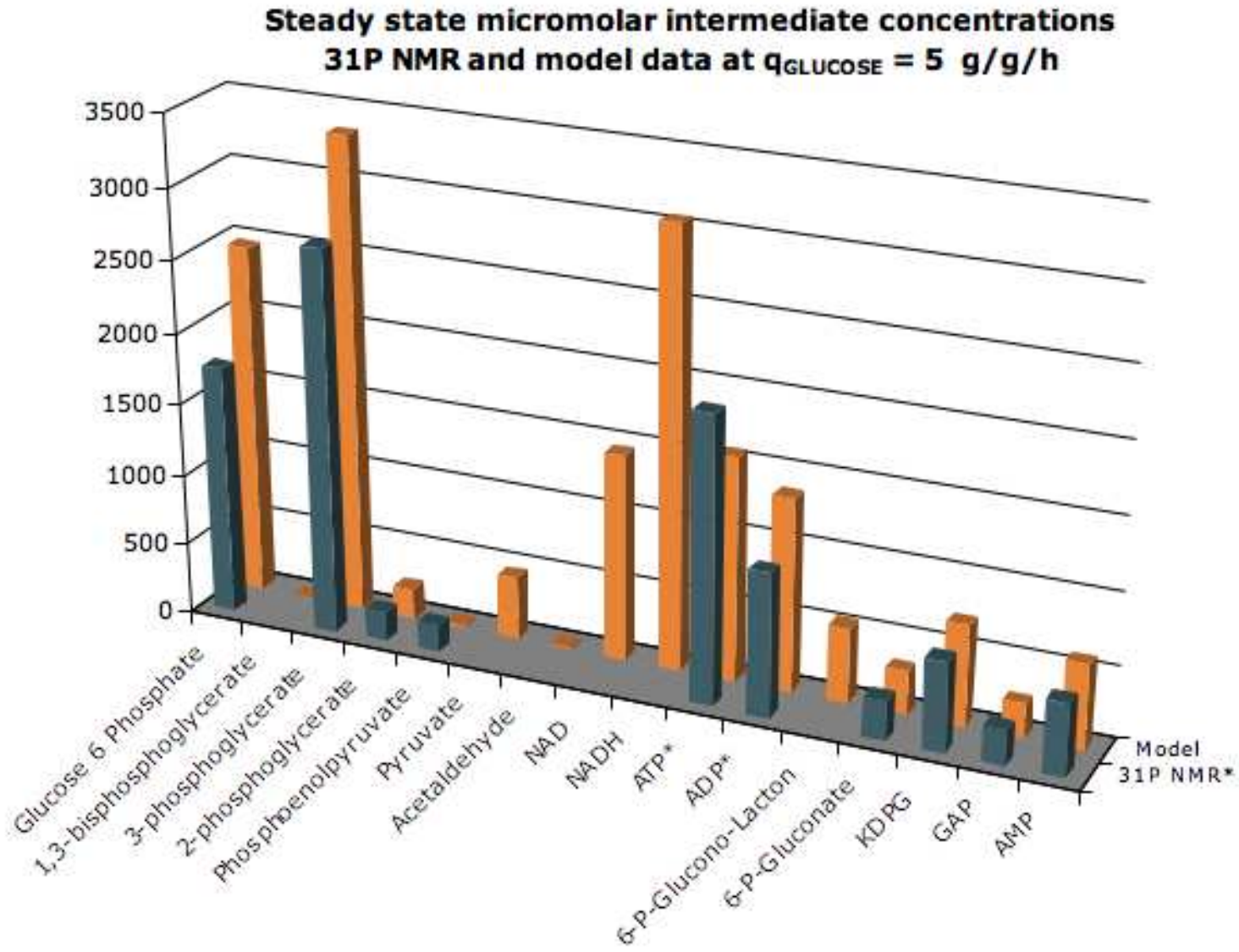
Steady State Metabolite Levels



What controls the high glucose flux?

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Control of Glucose Flux

- Which enzyme activities control the flux?
- We can answer this with the model by calculating the sensitivity of the flux to each of the enzyme activities.
- This sensitivity analysis of metabolic networks is known as **Metabolic Control Analysis**

Control Coefficients on Glucose Flux

	Glucose uptake rate		
Enzyme	4.9	4.5	0.2
Glucokinase	-0.08	-0.08	-0.06
Enolase	0.23	0.11	0.02
Pyruvate decarboxylase	0.27	0.11	0.08
ATPase	0.36	0.70	0.71
Sum of all other enzymes	0.22	0.16	0.25
Total	1.00	1.00	1.00

- The glucose uptake values are for the model, the model adjusted to match an experiment with ATPase inhibition, and to match an experiment with cell-free extract.
- Experiments on over-expression of glycolytic enzymes have not shown increases in glucose flux.

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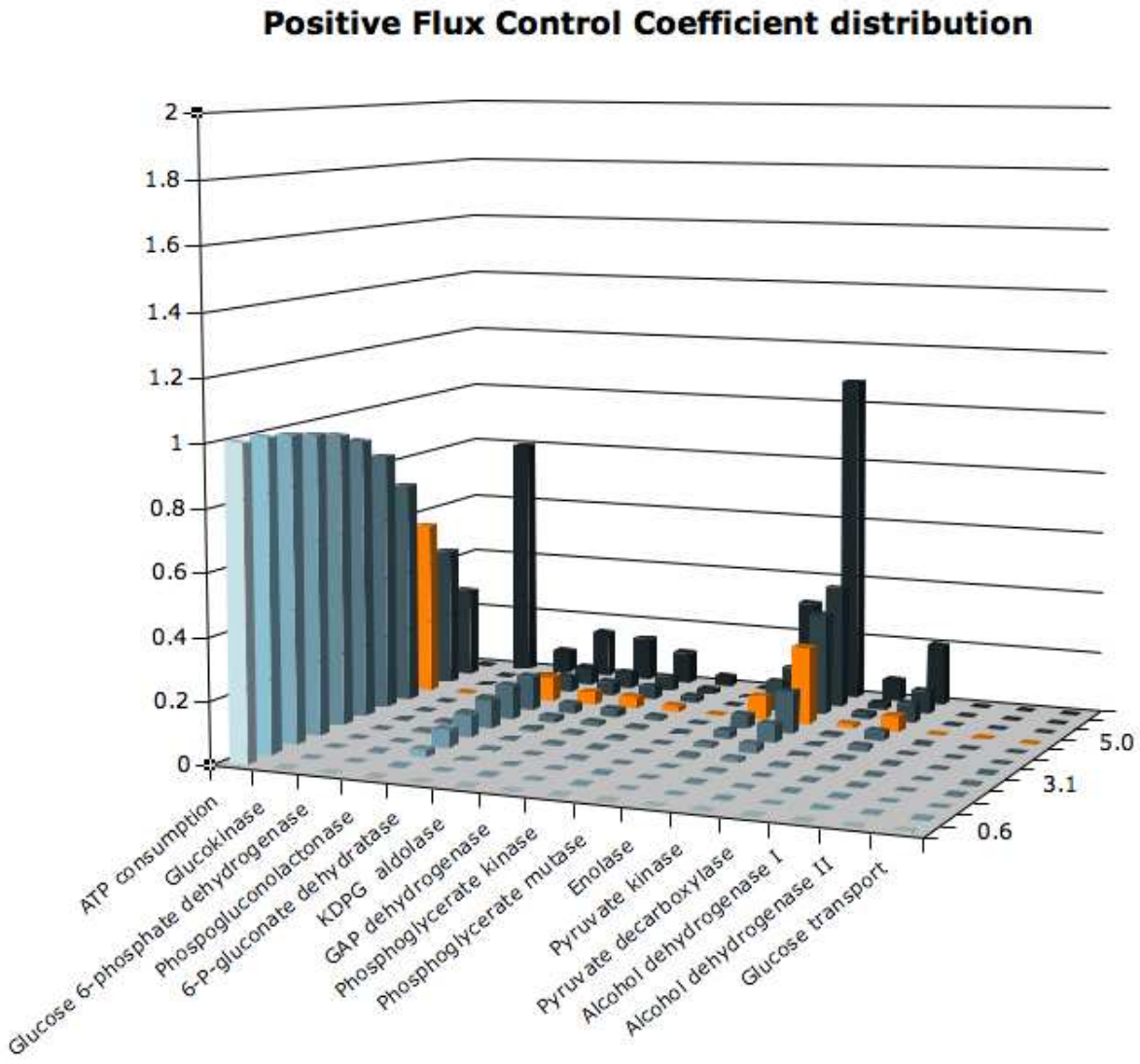
Control Coefficients as a function of ATPase



Model validation via response analysis

Co–Response Coefficients

. . . are ratios of the flux and concentration control coefficients to the same perturbation of enzyme activity.

For a small change in enzyme E affecting flux J and metabolite S, we don't need to know the exact size of the change in E:

$$\Omega_E^{S:J} = \frac{C_E^S}{C_E^J} = \frac{\partial \ln S}{\partial \ln J}$$

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From experimental literature, we may have a finite change response to a perturbation X, affecting one or more enzymes:

$$R_X^{S_i,J} = \frac{\Delta \ln S_i}{\Delta \ln J}$$

Flux and Concentration Responses

- The enzyme's flux control coefficient gives the % change in flux, J, for a 1% change in enzyme activity.
- An enzyme's concentration control coefficient gives the % change in metabolite S for a 1% change in enzyme.
- The enzyme's co–response coefficient gives the % change in metabolite S by an enzyme activity change causing a 1% change in flux J.

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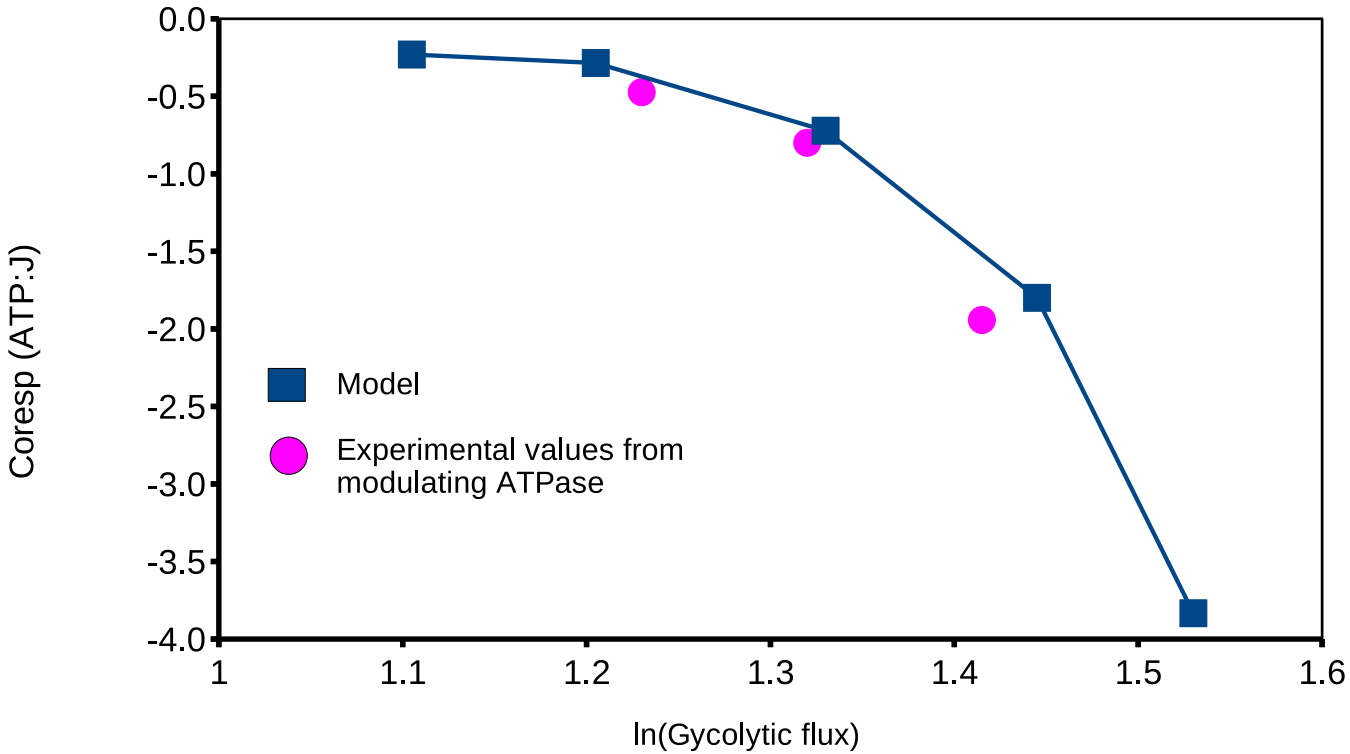
Validation with ATP:Flux Co–Response



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Summary

- A kinetic model of the native ED pathway has been built and shows that the high rates of glucose metabolism are linked to a high consumption of ATP.
- The model is consistent with failure to increase glycolytic flux by over–expression of glycolytic enzymes, and the functional coupling between glycolytic rate and ATP concentrations.
- It remains to apply this model to exploration of metabolic engineering towards the novel products.

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Further Details

- Agris Pentjuss, Ilona Odzina, Andrejs Kostromins, David A Fell, Egils Stalidzans, and Uldis Kalnenieks. *Biotechnological potential of respiring* Zymomonas mobilis*: A stoichiometric analysis of its central metabolism*. J. Biotechnology, **165**:1-10, 2013
- Reinis Rutkis, Uldis Kalnenieks,Egils Stalidzans and David A. Fell. *Kinetic modeling of* Zymomonas mobilis *Entner-Doudoroff pathway: insights into control and functionality.* Microbiology **159**: 2674-2689 (2013)
- Uldis Kalnenieks, Agris Pentjuss, Reinis Rutkis, Egils Stalidzans and David A. Fell.*Modeling of* Zymomonas mobilis *central metabolism for novel metabolic engineering strategies.* Frontiers Microbiol. **5**:42.