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Metabolic Control Analysis (MCA)

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Preben Graae Sørensen

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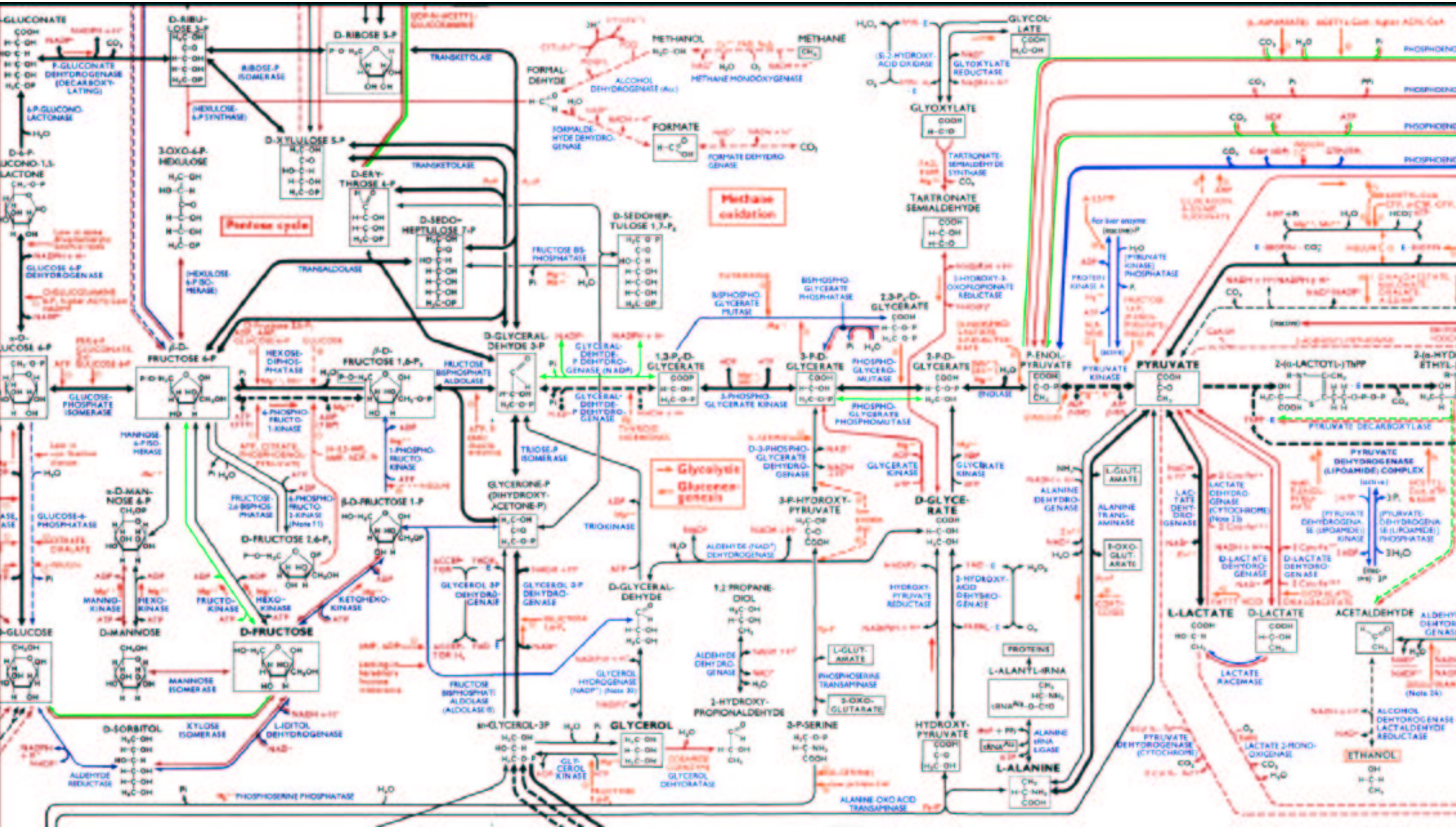
- “Thermodynamics” of a metabolic stationary state

biochemical pathways adjacent to glycolysis



rate determining step

What determines the efficiency of metabolism?

- Metabolic chain: $\overset{E_1}{\rightarrow} X_1 \overset{E_2}{\rightarrow} X_2 \overset{E_3}{\rightarrow} X_3 \overset{E_4}{\rightleftharpoons} X_4 \overset{E_5}{\rightarrow} X_5 \rightarrow$

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- Higher flux by adding metabolites after the rate limiting step.

rate determining step

Non-equilibrium step.

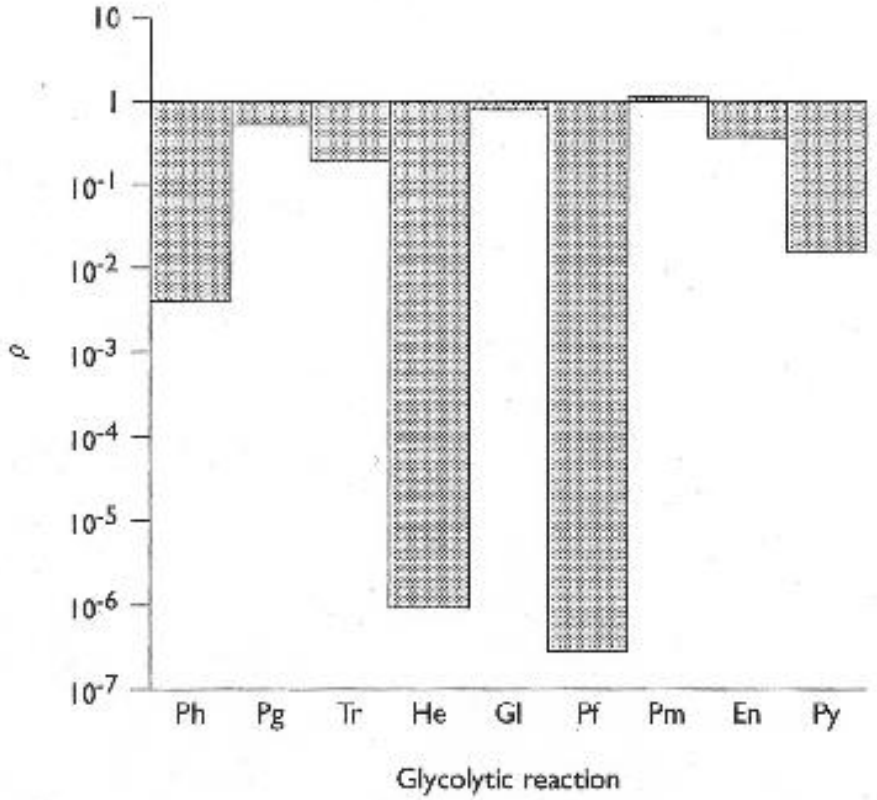

Figure 4.1 Displacement of reactions from equilibrium in glycolysis in working rat heart The measurements were of metabolites in working perfused heart supplied with glucose but no insulin. Both external glucose and endogenous glycogen were being used as fuels. Equilibrium constants were corrected to measured intracellular conditions. Key: Ph, phosphorylase; Pg, phosphoglucomutase; Tr, glucose transport; He, hexokinase; Gl, glucose–6–phosphate isomerase; Pf, phosphofructokinase; Pm, phosphoglycerate mutase; En, enolase; Py, pyruvate kinase. Data from Kashiwaya et al.[125]

Figure from Fell.

rate determining step

Enzyme capacity.

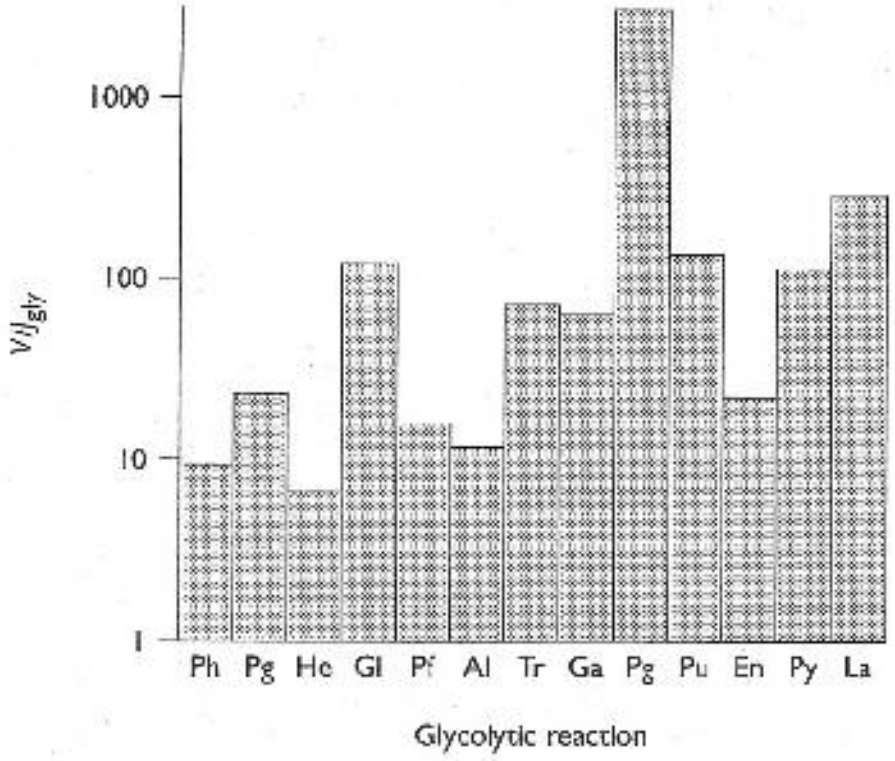

Figure 4.4 Relative enzyme activities in glycolysis in working rat heart
The limiting rates (V) were all measured at pH 7.2 in the presence of 150 mM K^+ and 5 mM Mg^{2+}. They are given relative to the glycolytic flux, J_{gly}, measured for working rat hearts using glucose as a fuel. Key: Ph, phosphorylase; Pg, phosphoglucomutase; He, hexokinase; Gl, glucose–6–P isomerase; Pf, phosphofructokinase; Al, aldolase; Tr, triose phosphate isomerase; Ga, glyceraldehyde–3–phosphate dehydrogenase; Pg, phosphoglycerate kinase; Pu, phosphoglycerate mutase; En, enolase; Py, pyruvate kinase; La, lactate dehydrogenase. Data from Kashiwaya et al.[125]

Figure from Fell.

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- What determines the flux at reaction step ydh for a pathway at a stationary state $[S]_{ss}$?

metabolic control analysis

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  E_{xase} is the activity (concentration) of xase

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- Summation theorem for particular flux $\sum_{i=1}^{m} C_i^J = 1$ summation is over all reactions in the cell.

metabolic control analysis MCA

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  $v_{\mathsf{xase}} = \frac{V[S]}{K_m + [S]}$ giving $\epsilon_S^{xase} = \frac{K_m}{K_m + [S]}$

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- Connectivity theorem for a particular flux J and a particular substrate S $\sum_{\mathsf{i}=1}^{\mathsf{m}} \mathsf{C}_{\mathsf{i}}^{\mathsf{J}} \epsilon_{\mathsf{S}}^{\mathsf{i}} = 0$
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- if $|\epsilon_j^j|$ small (irreversible reaction) then C_j^J large and E_j controls flux.

rate determining step

Irreversible metabolic chain:

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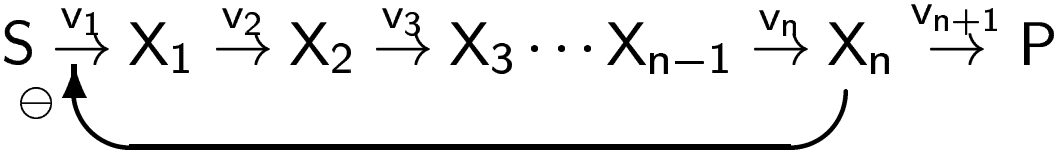
- The summation theorem gives $C_1^J = 1$ such that all control is by E_1.

rate determining step

Irreversible metabolic chain with negative feedback:

- 

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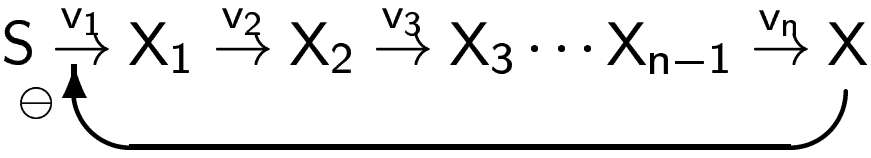
- 

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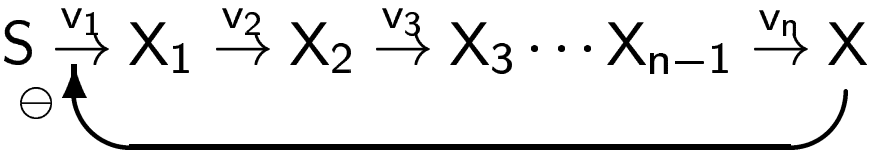
- 

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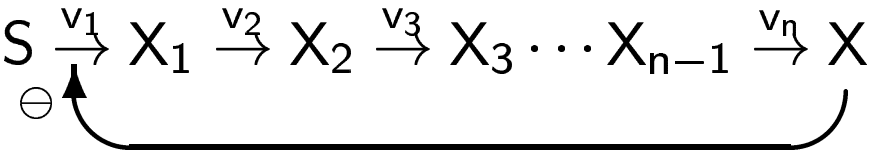
- 

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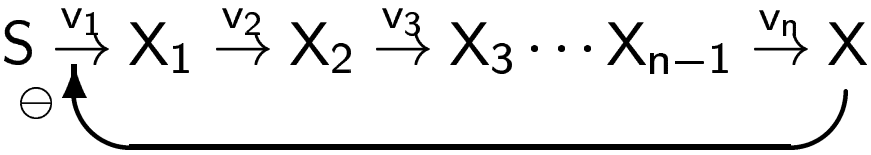
- 

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- We get $C_1^J = \frac{\epsilon_n^{n+1}}{\epsilon_n^{n+1} - \epsilon_n^1}$ and $C_{n+1}^J = -\frac{\epsilon_n^1}{\epsilon_n^{n+1} - \epsilon_n^1}$

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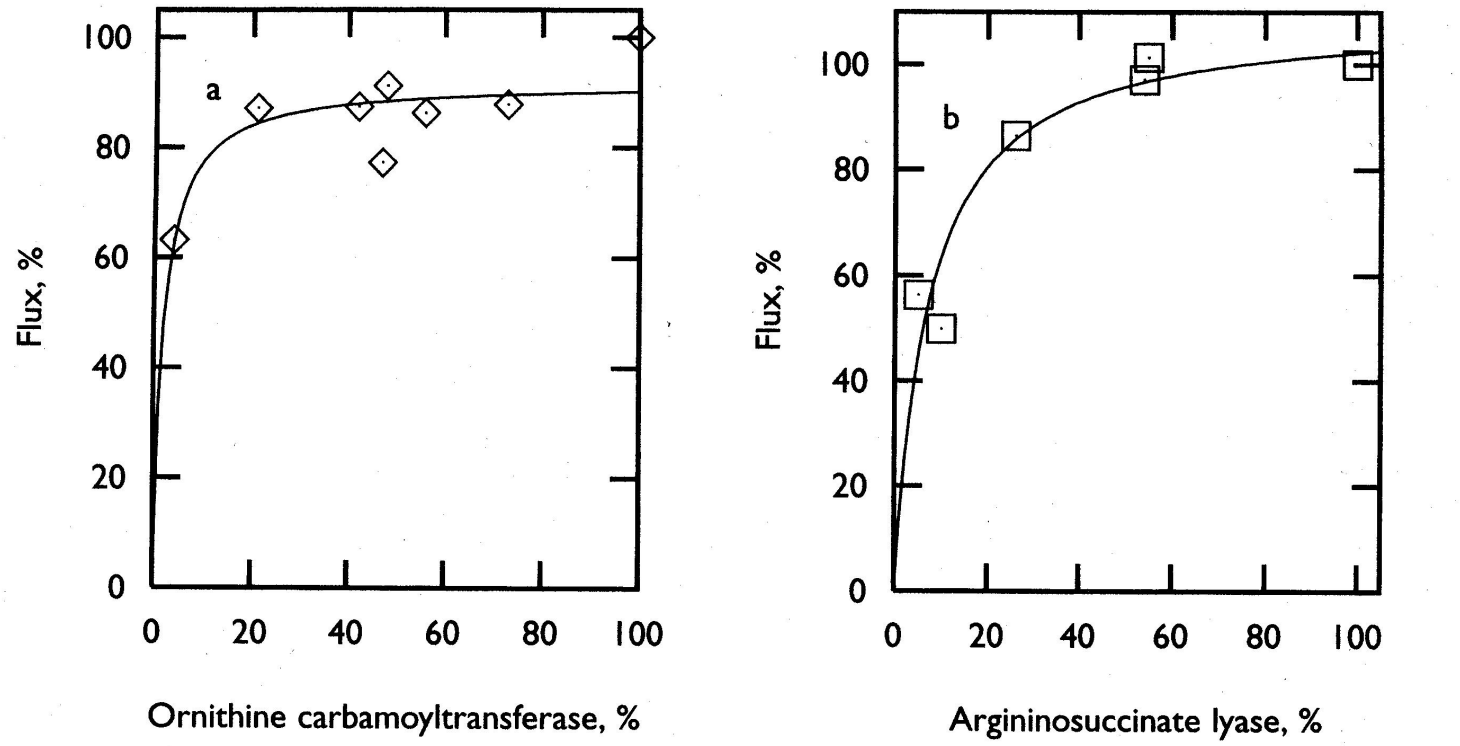

Figure 6.2 Dependence of arginine synthesis flux in *Neurospora* on enzyme levels
The results are those of Flint et al.[74] with my best–fit hyperbolic curves. Most of the points were obtained by forming heterokaryons with different ratios of wild–type and mutant nuclei. In each graph, however, the point at the lowest enzyme activity is not a heterokaryon, but a partial revertant from the mutant. (a) The dependence of the flux to arginine through argininosuccinate lyase on the activity of ornithine carbamoyltransferase, both expressed as a % of wild–type levels. (b) The dependence of the same flux on the activity of argininosuccinate lyase itself.

Figure from Fell.

alteration by titration with enzymes

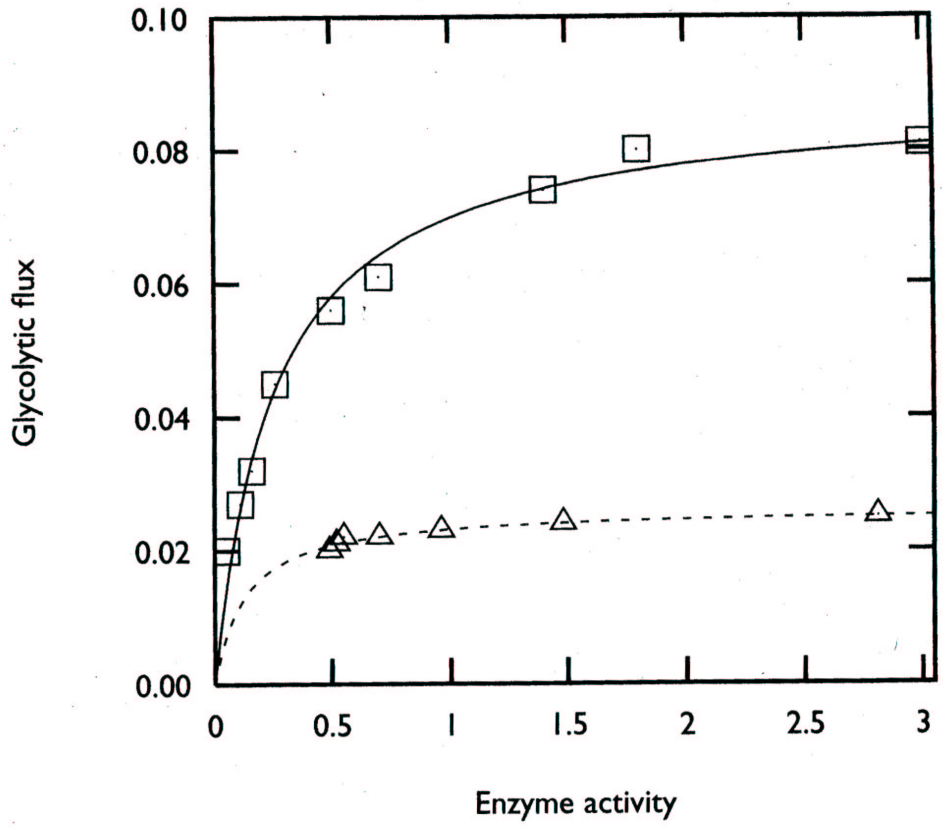

Figure 6.8 Dependence of glycolytic flux in a rat liver homogenate on added enzymes
The enzymes added were hexokinase (□) and phosphofructokinase (△). The results are those of Torres et al.[248] with computed best–fit rectangular hyperbolas. The leftmost point on each curve represents the original activity in the homogenate. The phosphofructokinase activity has been multiplied by a factor of 10 for display purposes. Titration with glucose–6–phosphate isomerase gave no change in flux.

Figure from Fell.

measurements of elasticities

In vivo modulation (Kacser& Burns)

$\cdots \rightarrow$ 2PG $\overset{\text{enolase}}{\rightarrow}$ PEP $\rightarrow$ ... $\qquad v_e = f(2PG, PEP) = J$

- Measure from three different experiments

$$\Delta J_1 = J_1 - J_c, \quad \Delta 2PG_1 = 2PG_1 - 2PG_c, \quad \Delta PEP_1 = PEP_1 - PEP_c$$
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- By differentiation of J_1, J_2 and J_c from three different experiments we have
  $\Delta J_1 \approx \frac{\partial v_e}{\partial 2PG}\Delta 2PG_1 + \frac{\partial v_e}{\partial PEP}\Delta PEP_1, \Delta J_2 \approx \frac{\partial v_e}{\partial 2PG}\Delta 2PG_2 + \frac{\partial v_e}{\partial PEP}\Delta PEP_2$

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- Scaling these equation by division with J_c gives
  $\frac{\Delta J_1}{J_c} \approx \epsilon^e_{2PG}\frac{\Delta 2PG_1}{2PG_c} + \epsilon^e_{PEP}\frac{\Delta PEP_1}{PEP_c}$ and $\frac{\Delta J_2}{J_c} \approx \epsilon^e_{2PG}\frac{\Delta 2PG_2}{2PG_c} + \epsilon^e_{PEP}\frac{\Delta PEP_2}{PEP_c}$

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- These equation can now be solved for the two elasticities ϵ^e_{2PG} and ϵ^e_{PEP}

kinetic equations for a metabolic network

- Kinetic equations: $\frac{dc_s}{dt} = \sum_r \nu_{s,r} v_r$

  c_s is concentration of species s

  ν is the stoichiometric matrix

  v_r is the rate of reaction r

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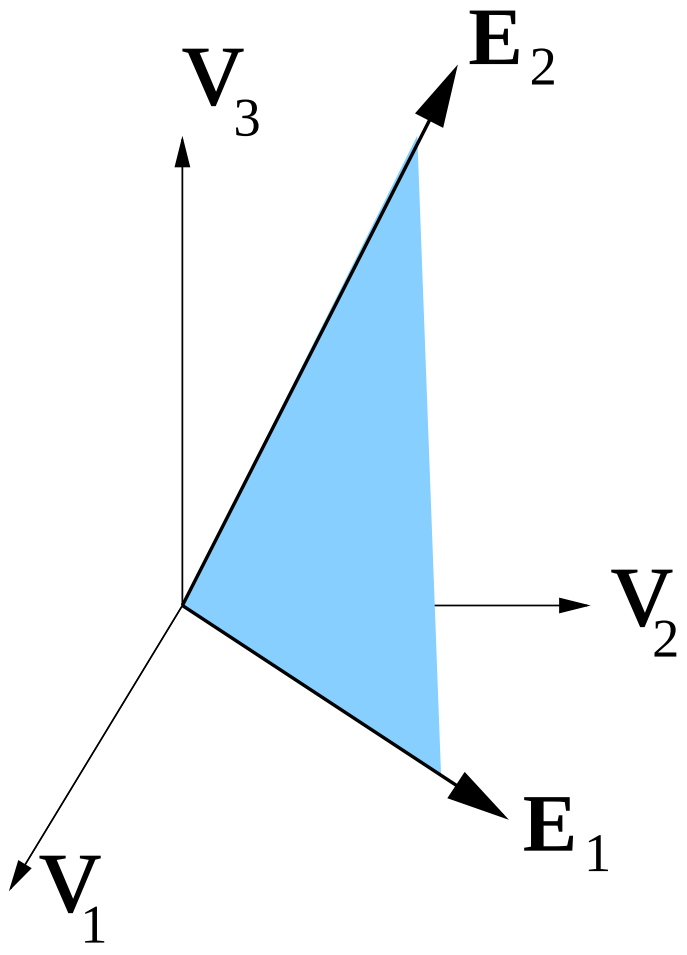
- The null space is a convex cone in R bounded by extreme currents.

example of stationary states in rate space

Two extreme currents in a 3 dimensional rate space



network for "glycolysis" in flow reactor

Intracellular reactions

Extracellular reactions

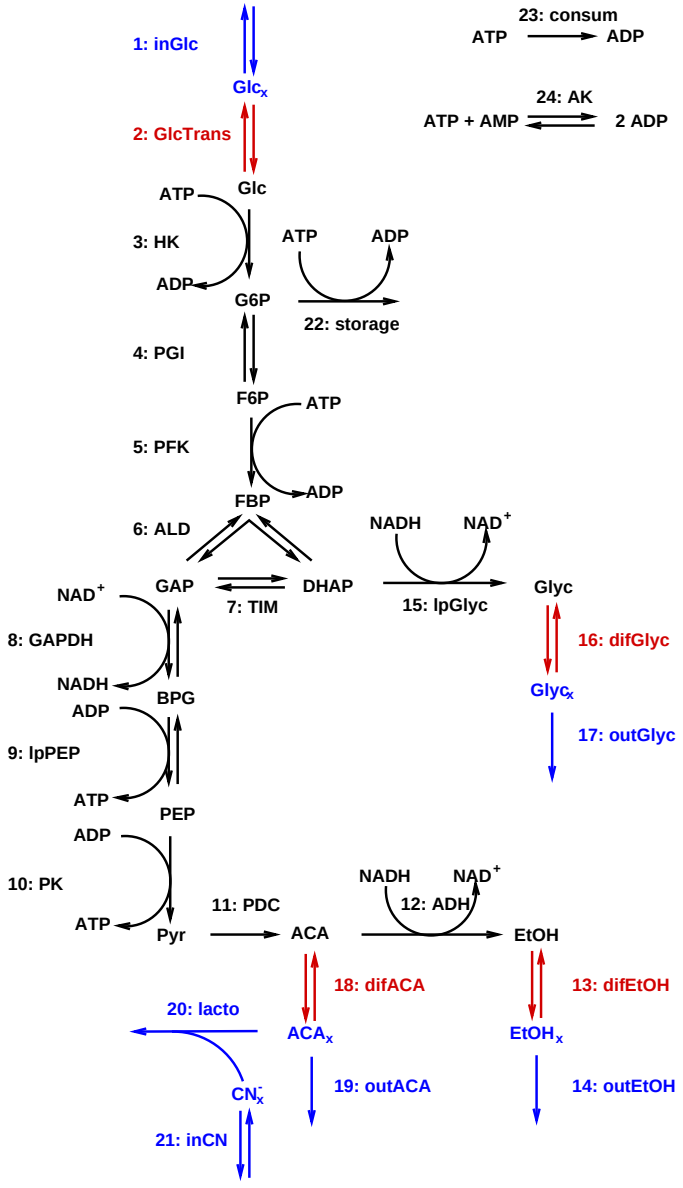
Transport across cellular membrane

ODE model at metabolome level

20 variables

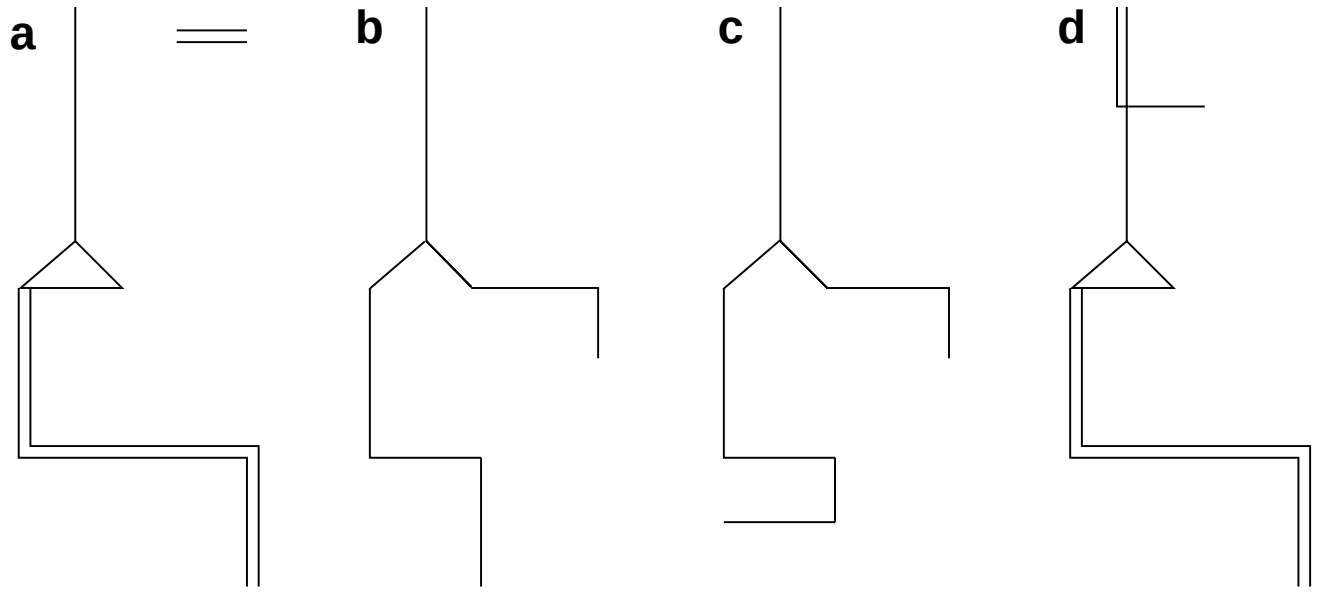
24 reactions

60 parameters



extreme currents of stationary states

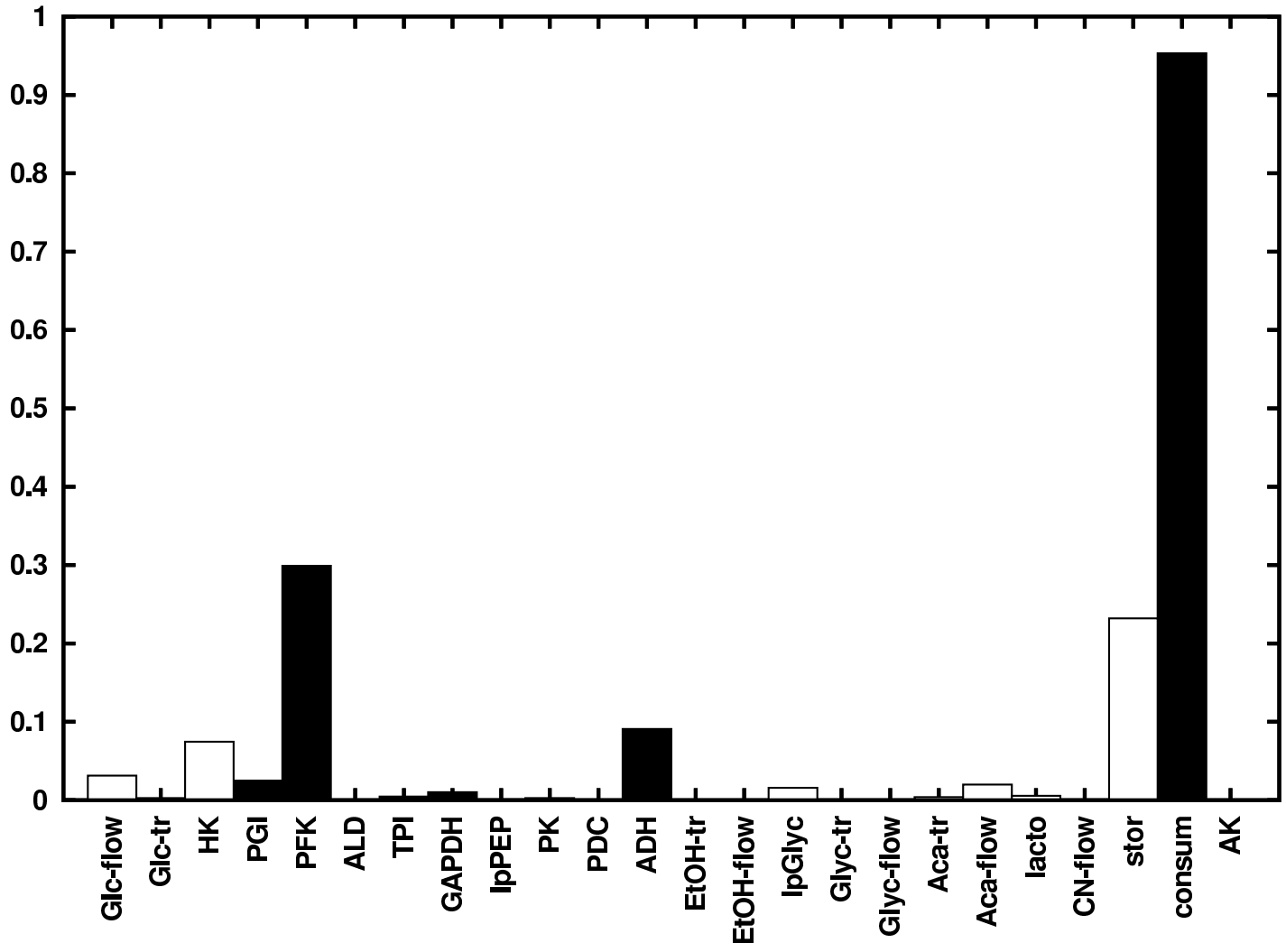
Extreme currents for glycolysis at Hopf point.



$\mathbf{E}_{\text{ferm}}$ $\mathbf{E}_{\text{glyc}}$ $\mathbf{E}_{\text{lact}}$ $\mathbf{E}_{\text{stor}}$

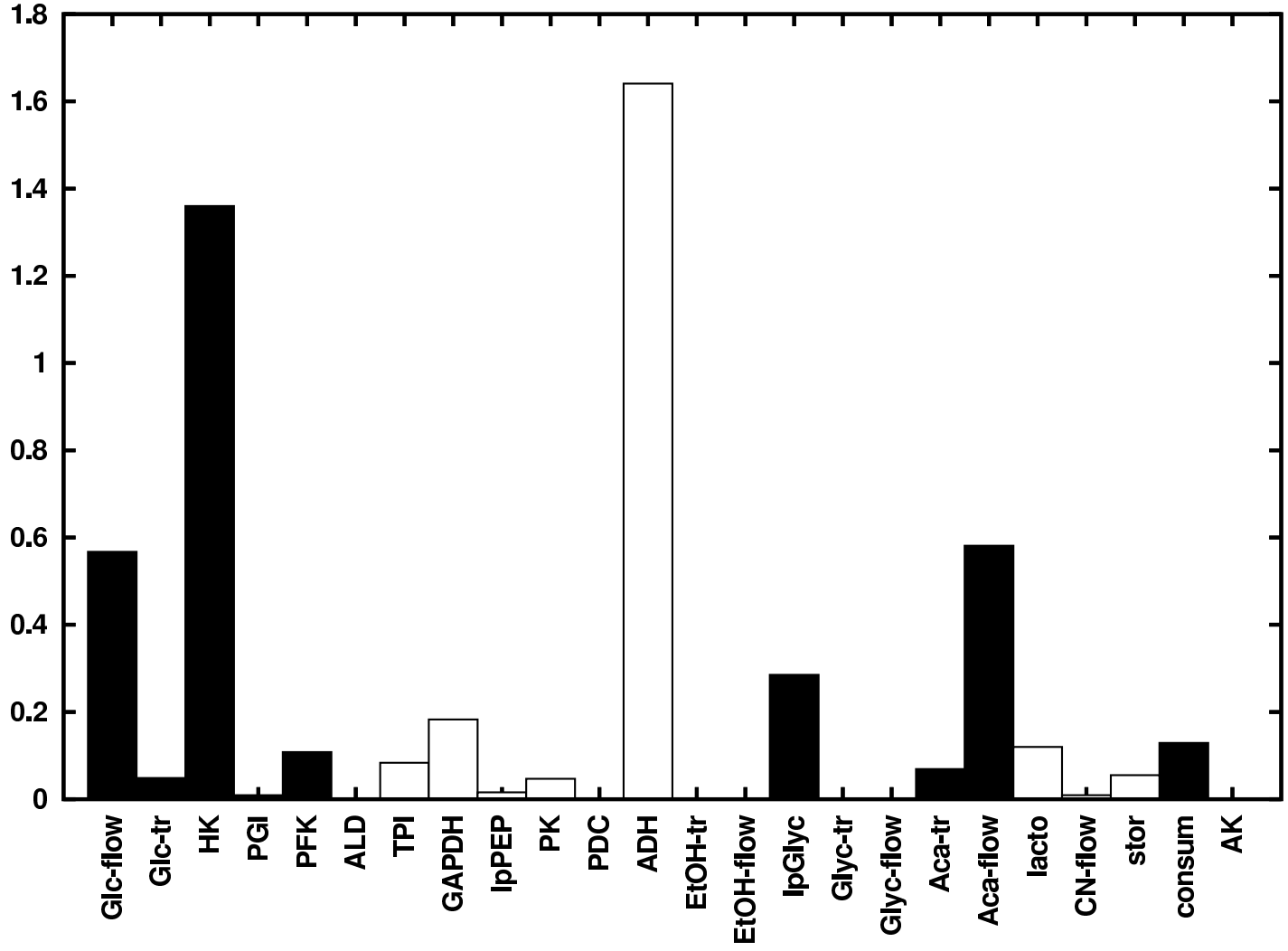
control of extreme currents

$C_{E_r}^{J_{consum}}$ E_{ferm}



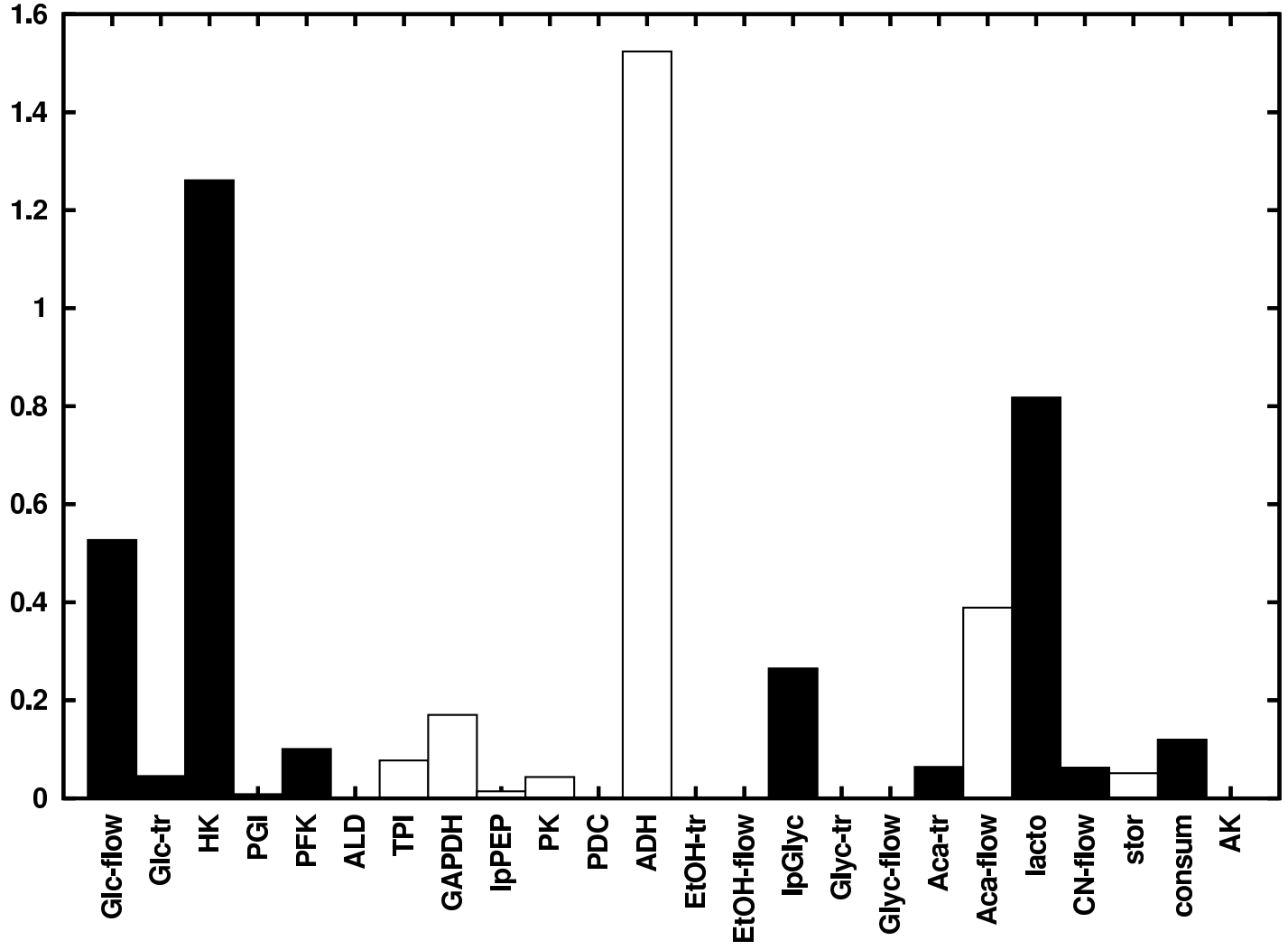
control of extreme currents

$C_{E_r}^{J_{outACA}}$ $\mathbf{E}_{glyc}$



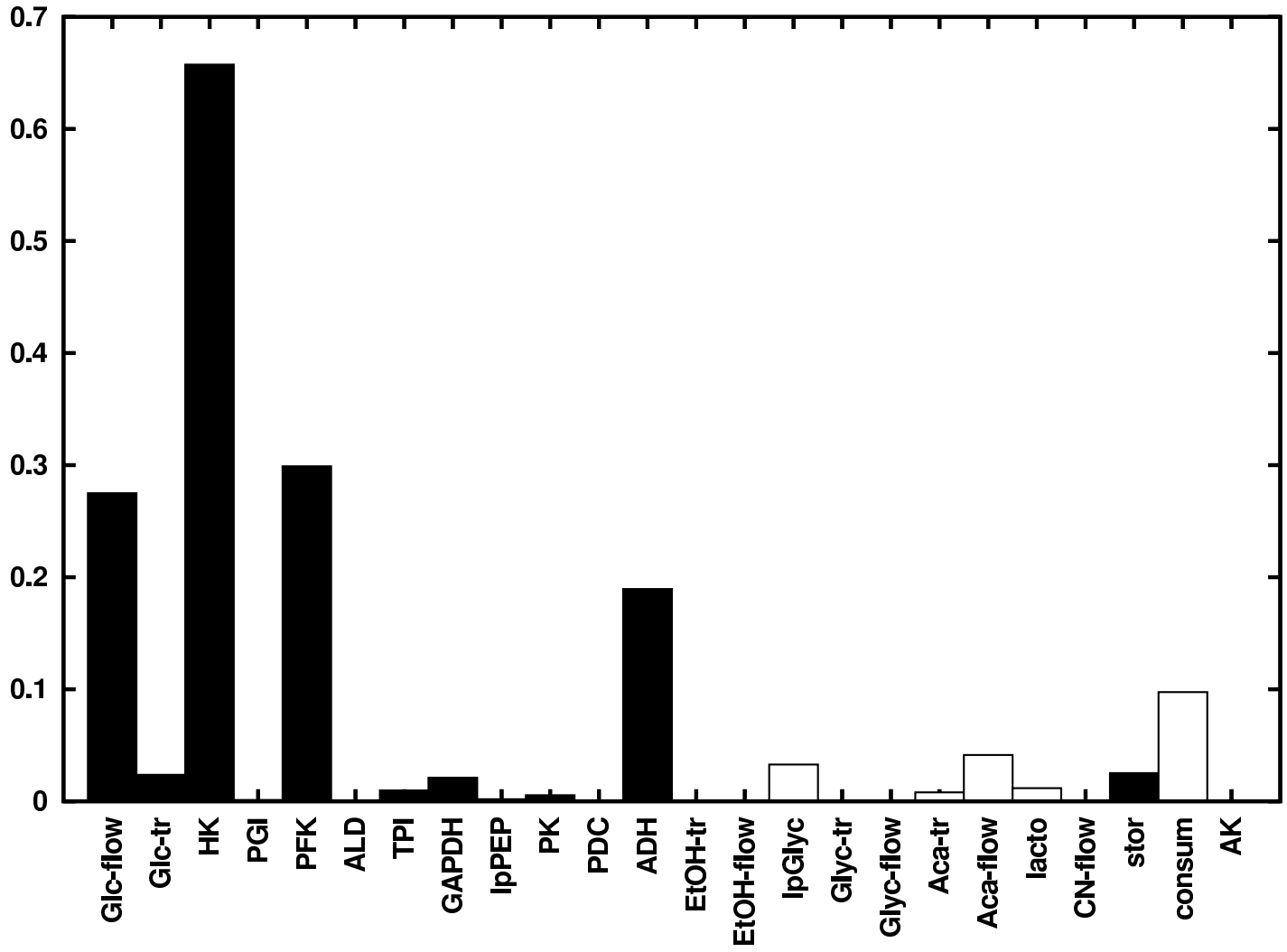
control of extreme currents

$C_{E_r}^{J_{lacto}}$ $\mathbf{E}_{lact}$



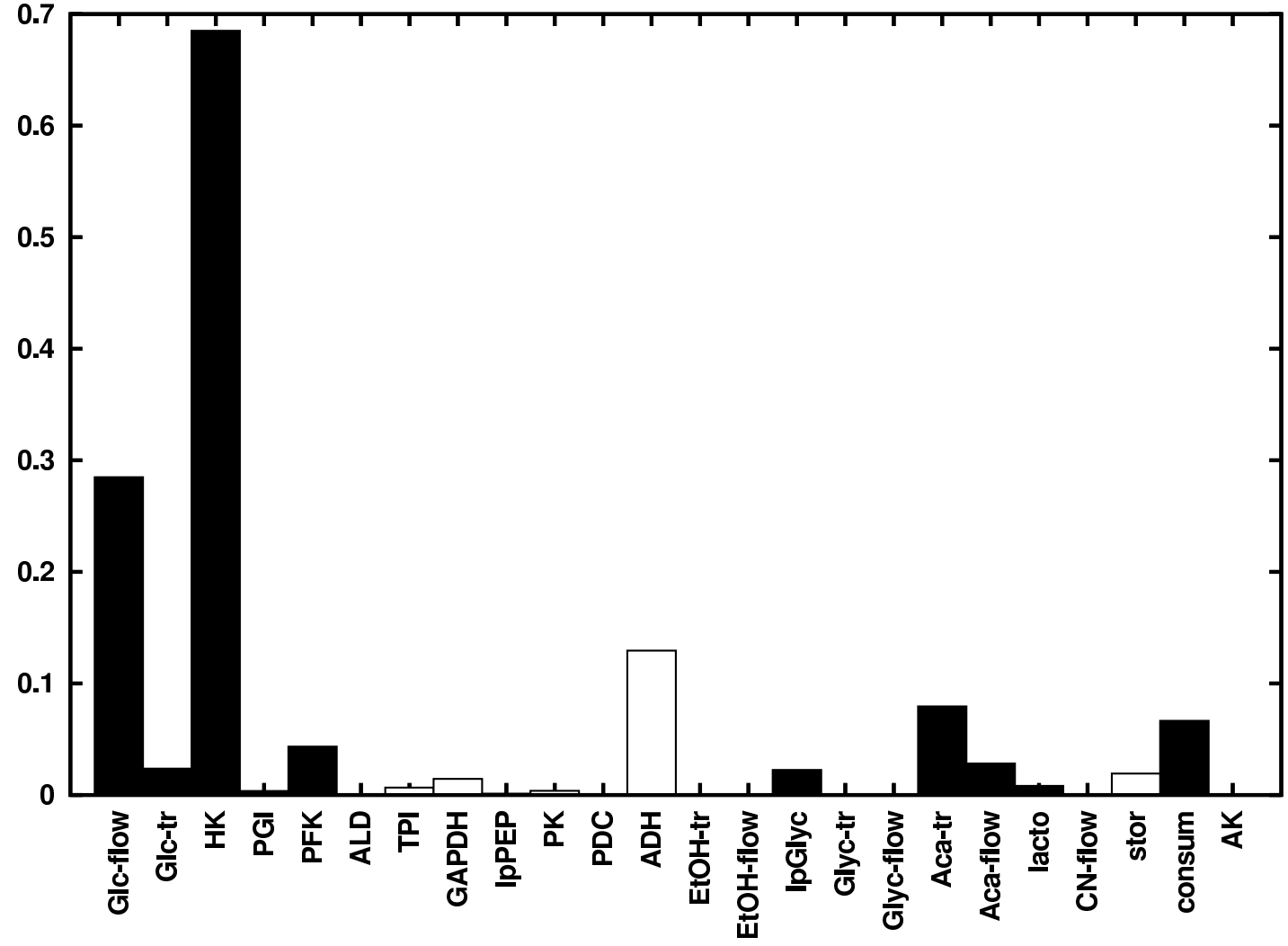
control of extreme currents

$C_{E_r}^{J_{storage}}$ $\mathbf{E}_{stor}$



control of flux at Hopf point

$C^{J_{Ald}}_{E_r}$



perspectives

- Flux control coefficients describe the influence of genetic manipulation on metabolic flux at a stationary state

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- Redirection of flux requires simultaneous changes in many enzyme activities

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- Is a stationary state a valid description of a biological system.
- On long timescales changes in metabolite concentrations feed back on gene expression.

References

Fell David Fell, Understanding the Control of Metabolism Portland Press, London 2003.