Summer School on
Design and Control of Self-Organization in Physical, Chemical, and Biological Systems

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Functional Dynamics Modelling of Biological Systems

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Modelling Biological Function

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- Reproduction

- Reproduction

- Energy utilization
glycolytic flux

- Reproduction

- Energy utilization glycolytic flux

- Physiological rythms (oscillations)

examples of biological function

- Reproduction

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- Physiological rythms (oscillations)

- Mimicry (patterns)

examples of biological function

- Reproduction

- Energy utilization glycolytic flux

- Physiological rythms (oscillations)

- Mimicry (patterns)

- Biological function is a possible process in an intact biological system at a certain timescale and under biological meaningfull conditions.

- Fast muscle contraction is a function which is obviously of importance for survival.

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- Can the associated changes of flux be understood by current knowledge of the influence of metabolites on flux.

metabolome of muscle power plant
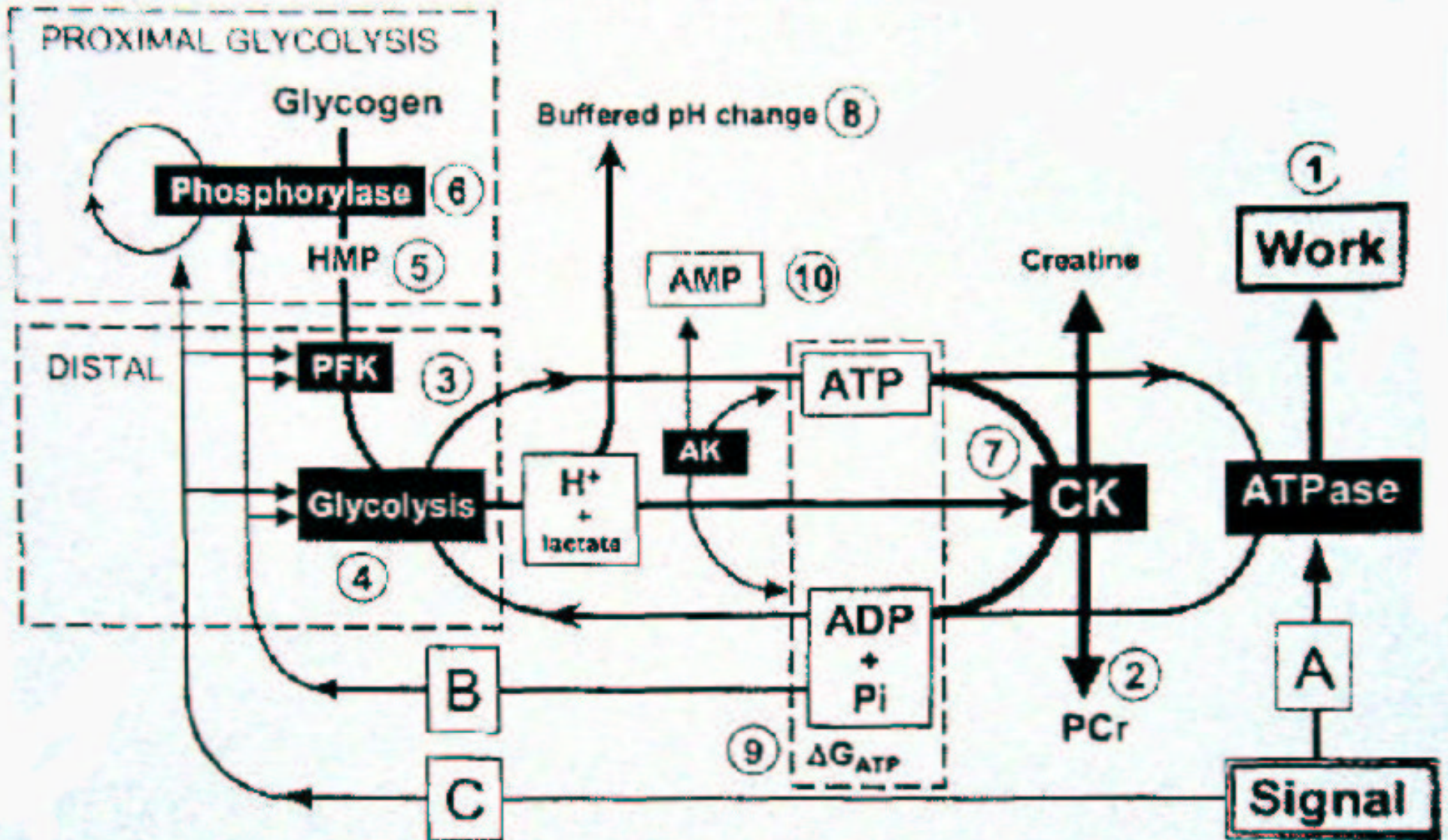
experimental results on muscle work in humans

NMR measurements of
pCr (phosphocreatine)
P (phosphate) and pH at
(a) 70% MVC
(maximal voluntary contraction)
(b) 90% MVC.

$1^{st} \downarrow$ Contraction

$2^{st} \downarrow$ Anaerobic "recovery"

$3^{st} \downarrow$ Aerobic recovery
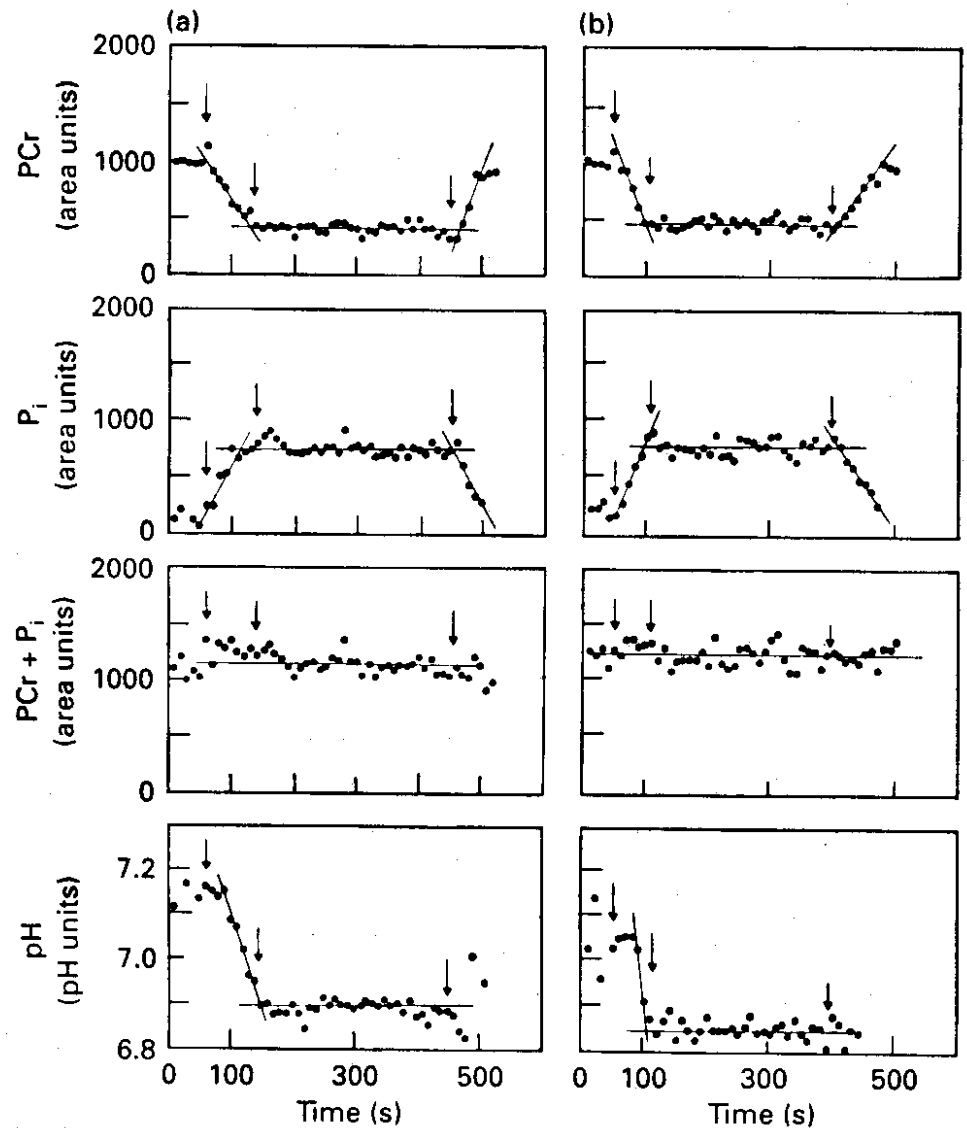

Figure 3 Effect on PCr, P_i and pH of isometric contraction and recovery during ischaemia followed by aerobic recovery

Genome

↓

Proteome

↓

Metabolome

↓

Cellular structure and function

- Gene interactions (secs - hours)

- Gene interactions (secs - hours)

- Protein synthesis and degradation (secs - hours)

timescales for cell processes

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- Protein synthesis and degradation (secs - hours)

- Protein interactions (millisecs - hours)

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- Conformation changes and phosphorylation f.ex by kinases (millisecs).

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- Protein synthesis and degradation (secs - hours)

- Protein interactions (millisecs - hours)

- Conformation changes and phosphorylation f.ex by kinases (millisecs).

- Protein function (enzymatic reactions) (millisecs - hours)

Modelling of metabolic networks

- Systematic methods for quantitative modelling of steady states in metabolic networks.
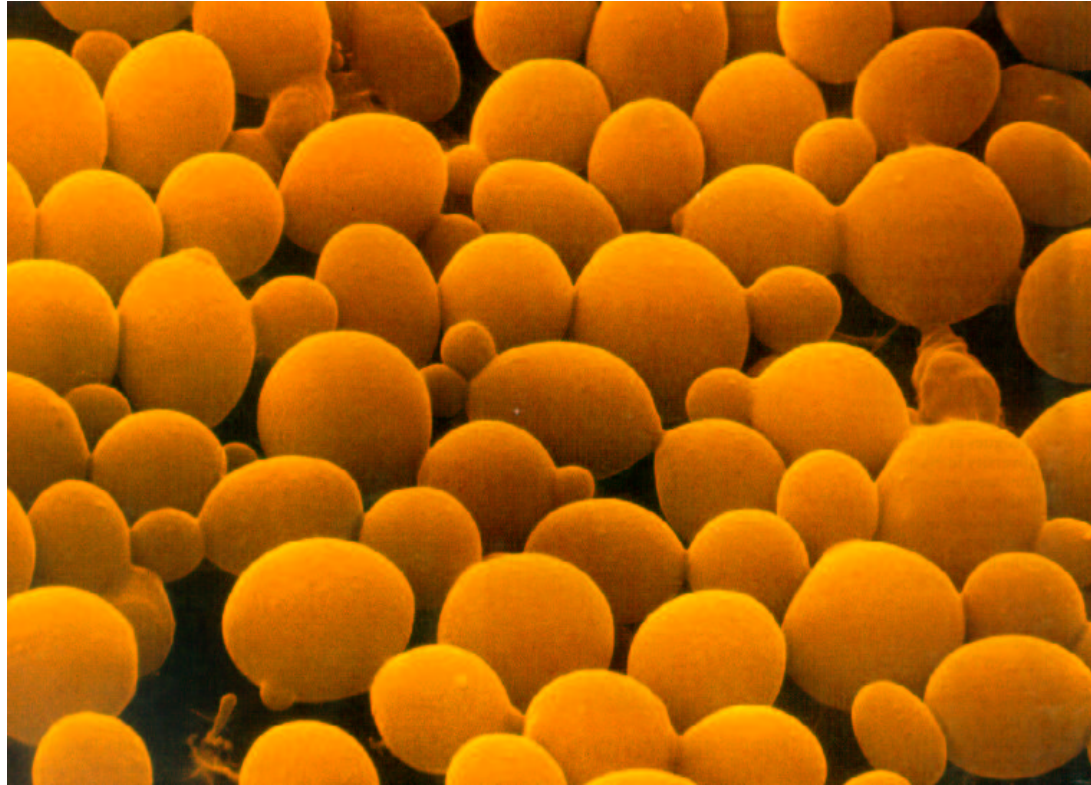
Modelling of metabolic networks

- Systematic methods for quantitative modelling of steady states in metabolic networks.

- Example on modelling glycolysis in yeast cells which in many respects is similar to glycolysis in muscle cells.
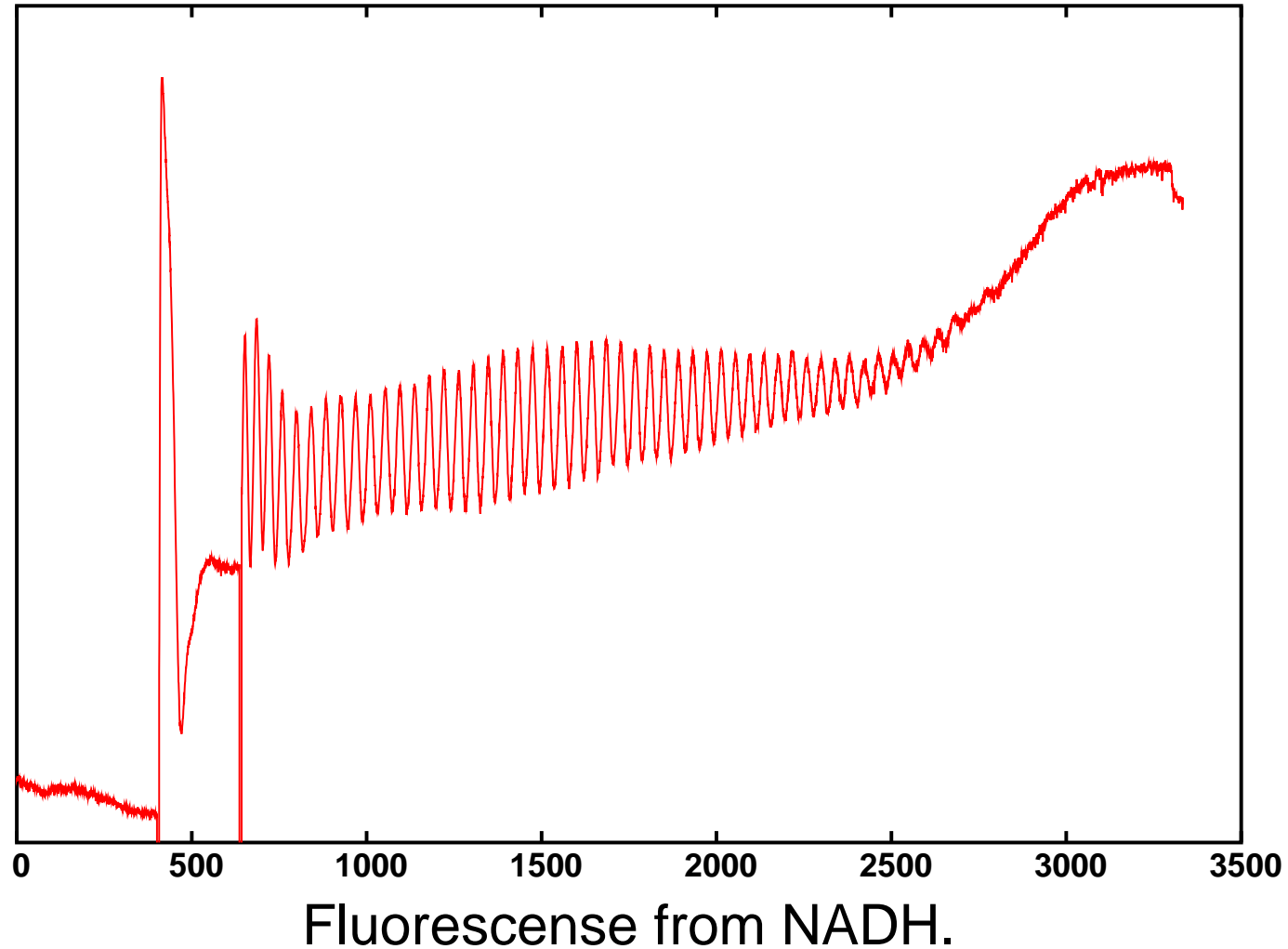
the quest for the oscillophore of yeast cells

Cells of *Saccharomyces cerevisiae* are grown aerobically in batch. They are harvested at glucose depletion (diauxic shift) and then starved for some hours in a buffer. At 5°C the cells can be stored for several days.

Oscillations in a closed system

Fluorescense from NADH.

Addition of glucose at t=400s. Addition of cyanide at t = 650.

Continuous oscillations using a flow reactor

Glucose →

Cyanide →

Cell suspens →

c_3 c_2 c_1 c_4 c_5 c

Outflow →
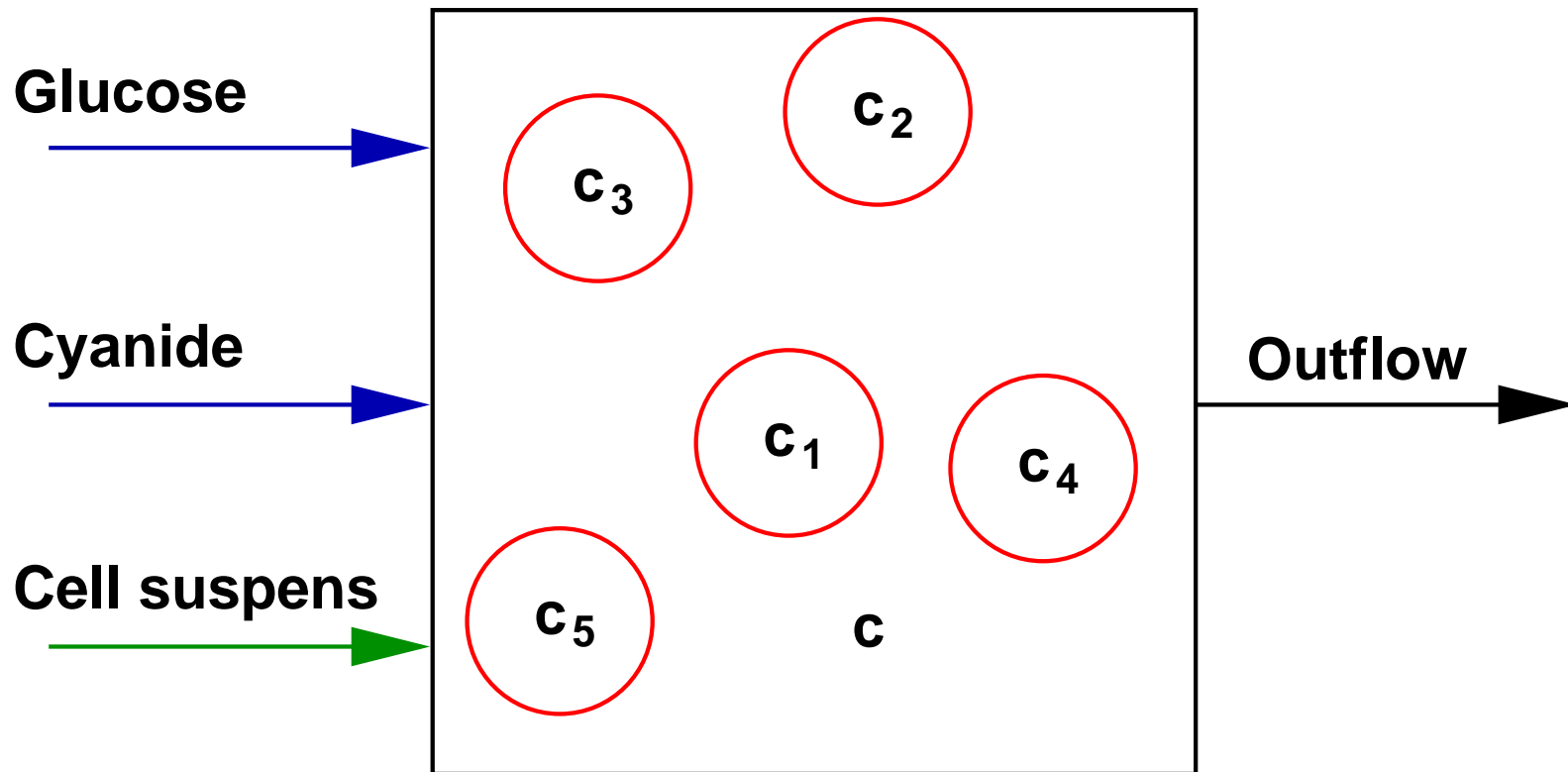
The reactor is fed with constant flow of cell suspension together with glucose and cyanide. Oscillations are observed if the cells oscillate in phase. Amplitude depends on glucose concentration.
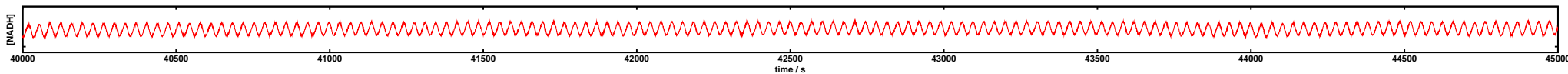
stationary experimental data for the cell suspension

- Measurements of stationary concentrations

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stationary experimental data for the cell suspension

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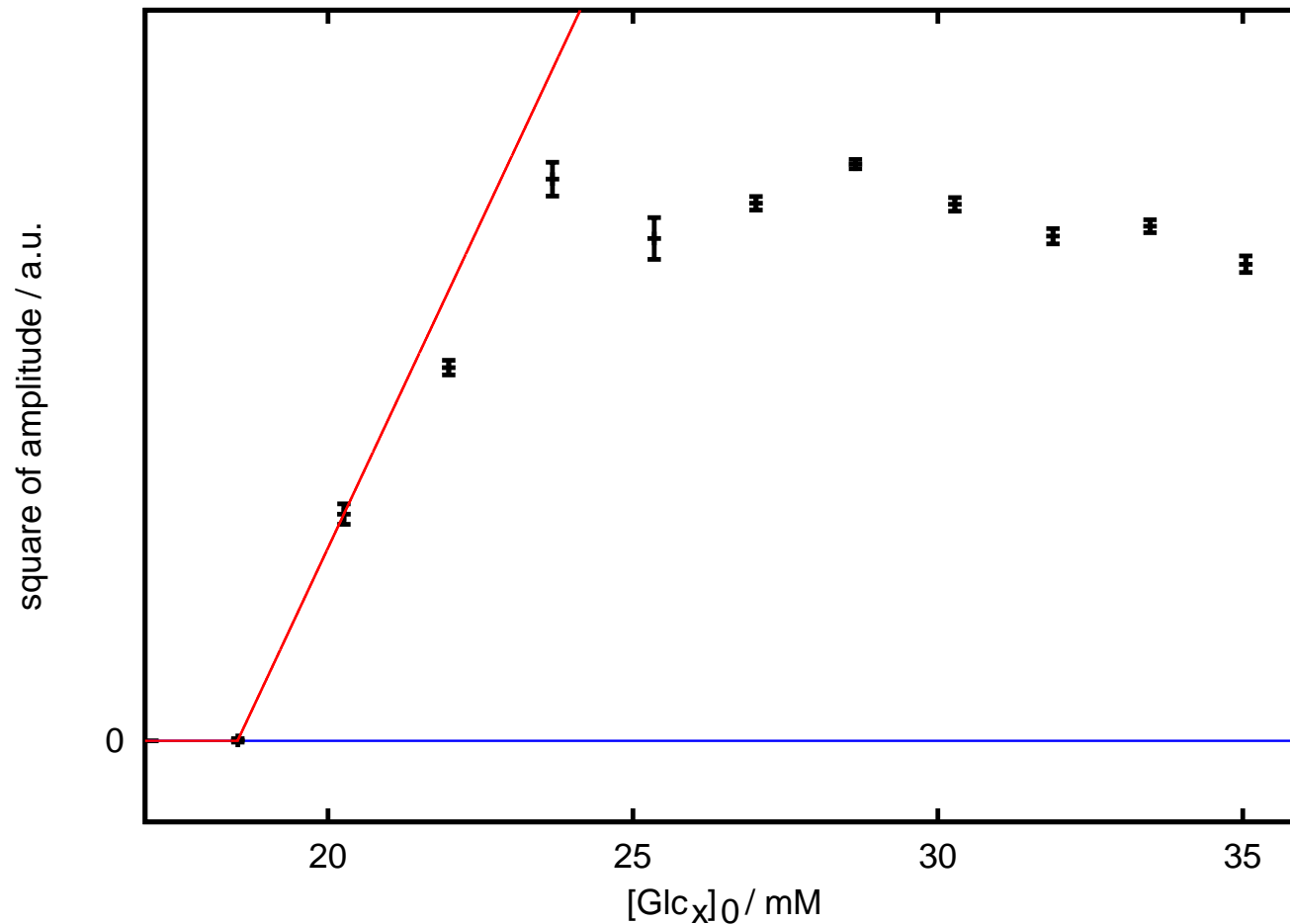- Measurements of amplitude and phases for metabolite oscillations

- Can a complete model using *in vitro* determined parameters explain the experimental data
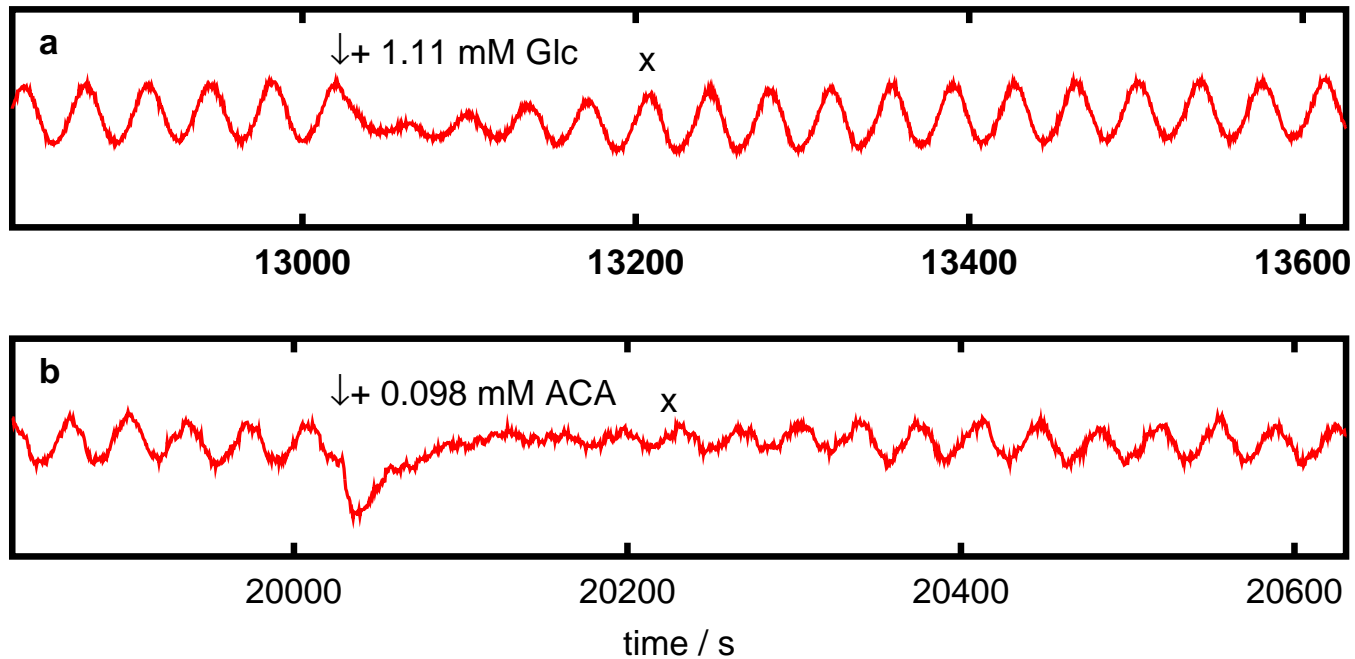
- NO

Dependence of amplitude on glucose concentrations.

Quenchings of the oscillations.

The reactor is perturbed with instantaneous additions of
a: glucose and b: acetaldehyde.
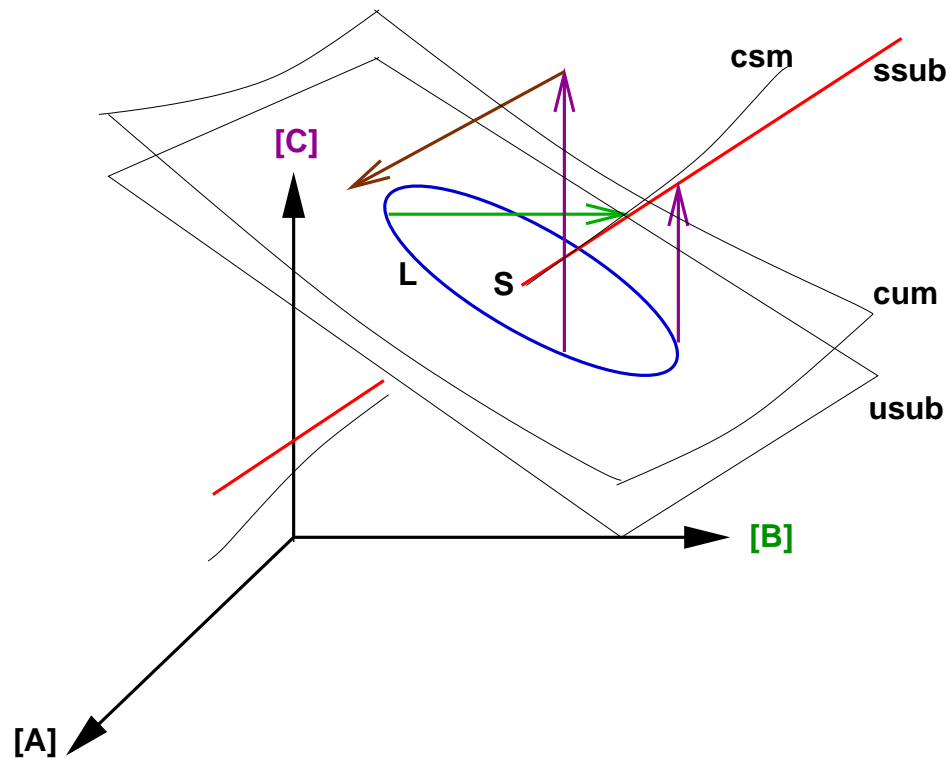
Hopf bifurcation in 3 dimensional concentration space. **ssub** and **usub** are the stable and unstable subspace of the unstable stationary state **S**. **L** is the stable limit cycle embedded in the center-unstable manifold **cum** tangent to **usub** at **S**. **csm** is the center-stable manifold of **S** tangent to **ssub** at **S**.

network for reactions in flow reactor

Intracellular reactions

Extracellular reactions

Transport across cellular membrane

ODE model at metabolome level

20 variables

24 reactions

60 parameters

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

  c_s is concentration of species s
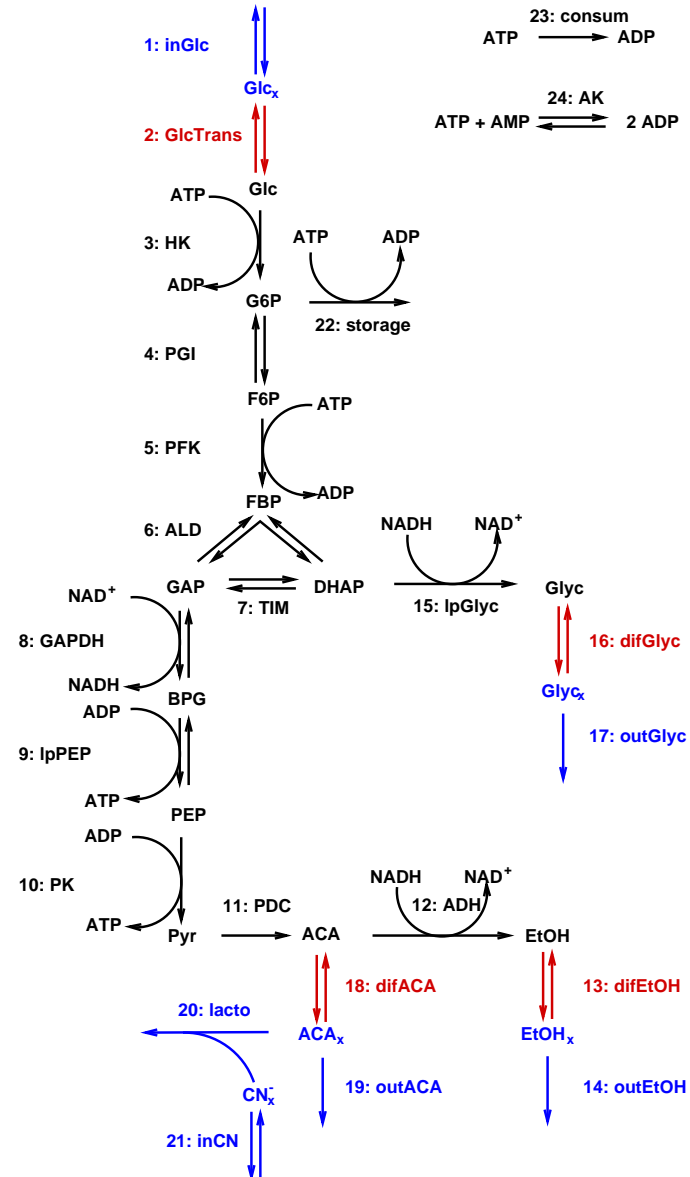
  ν is the stoichiometric matrix

  v_r is the rate of reaction r

2:
$$v_\rightarrow = \frac{V_{2m}\frac{[\text{Glc}_x]}{K_{2\text{Glc}}}}{1+\frac{[\text{Glc}_x]}{K_{2\text{Glc}}}+\frac{P_2\frac{[\text{Glc}_x]}{K_{2\text{Glc}}}+1}{P_2\frac{[\text{Glc}]}{K_{2\text{Glc}}}+1}\left(1+\frac{[\text{Glc}]}{K_{2\text{Glc}}}+\frac{[\text{G6P}]}{K_{2\text{IG6P}}}+\frac{[\text{Glc}][\text{G6P}]}{K_{2\text{Glc}}K_{2\text{IIG6P}}}\right)}$$

$$v_\leftarrow = \frac{V_{2m}\frac{[\text{Glc}]}{K_{2\text{Glc}}}}{1+\frac{[\text{Glc}]}{K_{2\text{Glc}}}+\frac{P_2\frac{[\text{Glc}]}{K_{2\text{Glc}}}+1}{P_2\frac{[\text{Glc}_x]}{K_{2\text{Glc}}}+1}\left(1+\frac{[\text{Glc}_x]}{K_{2\text{Glc}}}\right)+\frac{[\text{G6P}]}{K_{2\text{IG6P}}}+\frac{[\text{Glc}][\text{G6P}]}{K_{2\text{Glc}}K_{2\text{IIG6P}}}}$$

5:
$$v_\rightarrow = \frac{V_{5m}[\text{F6P}]^2}{K_5\left(1+\kappa_5\frac{[\text{ATP}]^2}{[\text{AMP}]^2}\right)+[\text{F6P}]^2}$$

6:
$$v_\rightarrow = \frac{V_{6f}[\text{FBP}]}{K_{6\text{FBP}}+[\text{FBP}]+\frac{[\text{GAP}]K_{6\text{DHAP}}V_{6f}}{K_{6eq}V_{6r}}+\frac{[\text{DHAP}]K_{6\text{GAP}}V_{6f}}{K_{6eq}V_{6r}}+\frac{[\text{FBP}][\text{GAP}]}{K_{6\text{IGAP}}}+\frac{[\text{GAP}][\text{DHAP}]V_{6f}}{K_{6eq}V_{6r}}}$$

$$v_\leftarrow = \frac{V_{6f}\frac{[\text{GAP}][\text{DHAP}]}{K_{6eq}}}{K_{6\text{FBP}}+[\text{FBP}]+\frac{[\text{GAP}]K_{6\text{DHAP}}V_{6f}}{K_{6eq}V_{6r}}+\frac{[\text{DHAP}]K_{6\text{GAP}}V_{6f}}{K_{6eq}V_{6r}}+\frac{[\text{FBP}][\text{GAP}]}{K_{6\text{IGAP}}}+\frac{[\text{GAP}][\text{DHAP}]V_{6f}}{K_{6eq}V_{6r}}}$$

8:
$$v_\rightarrow = \frac{V_{8m}[\text{GAP}][\text{NAD}^+]}{K_{8\text{GAP}}K_{8\text{NAD}}\left(1+\frac{[\text{GAP}]}{K_{8\text{GAP}}}+\frac{[\text{BPG}]}{K_{8\text{BPG}}}\right)\left(1+\frac{[\text{NAD}^+]}{K_{8\text{NAD}}}+\frac{[\text{NADH}]}{K_{8\text{NADH}}}\right)}$$

$$v_\leftarrow = \frac{V_{8m}\frac{[\text{BPG}][\text{NADH}]}{K_{8eq}}}{K_{8\text{GAP}}K_{8\text{NAD}}\left(1+\frac{[\text{GAP}]}{K_{8\text{GAP}}}+\frac{[\text{BPG}]}{K_{8\text{BPG}}}\right)\left(1+\frac{[\text{NAD}^+]}{K_{8\text{NAD}}}+\frac{[\text{NADH}]}{K_{8\text{NADH}}}\right)}$$

15:
$$v_\rightarrow = \frac{V_{15m}[\text{DHAP}]}{K_{15\text{DHAP}}\left(1+\frac{K_{15\text{INADH}}}{[\text{NADH}]}\left(1+\frac{[\text{NAD}^+]}{K_{15\text{INAD}}}\right)\right)+[\text{DHAP}]\left(1+\frac{K_{15\text{NADH}}}{[\text{NADH}]}\left(1+\frac{[\text{NAD}^+]}{K_{15\text{INAD}}}\right)\right)}$$

Other reaction rates are Michaelis-Menten or mass action.

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

direct optimization method

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- Rate expression

rate parameter

$\downarrow$

The rate has the form: $v_r = V_r g_r(c, K_r)$

$\uparrow$

intrinsic parameters

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- Rate expression

  rate parameter

  $\downarrow$

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  $\uparrow$

  intrinsic parameters

- Null space: $\sum_r \nu_{s,r} v_r^{ss} = 0$

Extreme currents in a 3 dimensional reaction space
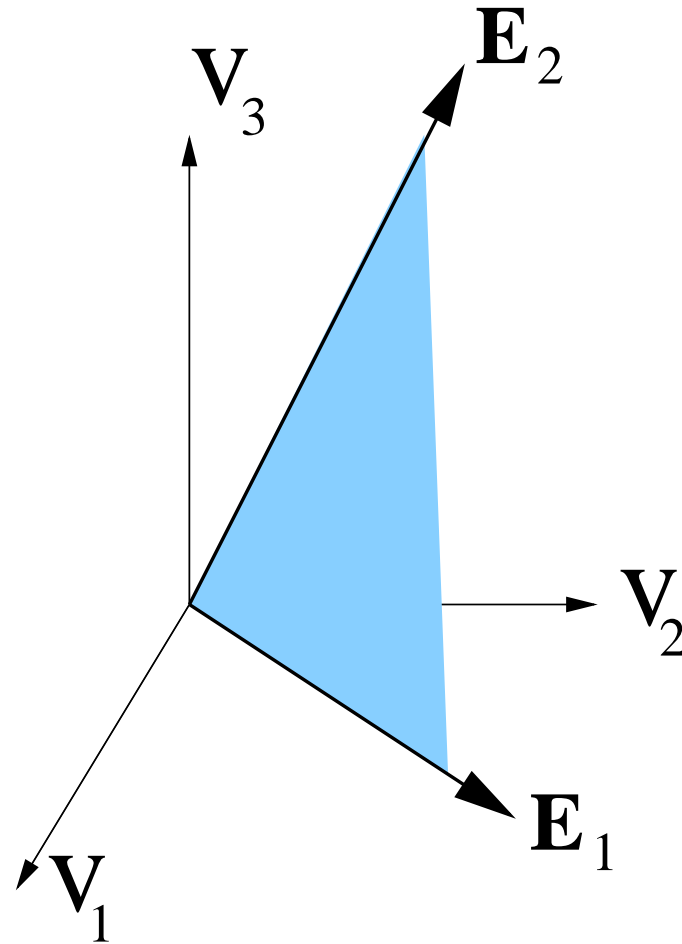
Extreme currents for glycolysis at Hopf point.
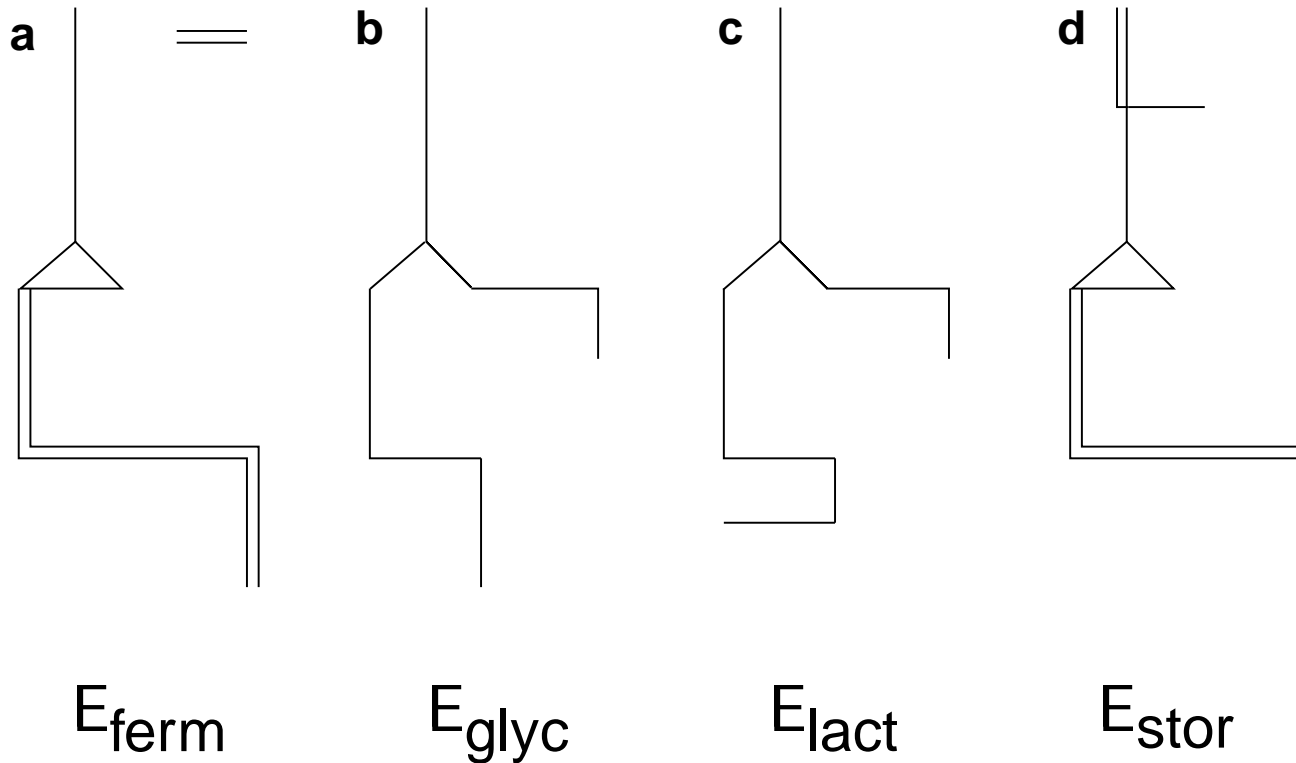
$$E^{ss} = a_1 E_{ferm} + a_2 E_{glyc} + a_3 E_{lact} + a_4 E_{stor}$$

a: Select a_i, c^{ss} and K

direct optimization method

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b: Calculate Jacobian and find eigenvalues.

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e: Calculate right and left eigenvectors of Jacobian and amplitudes and phases of oscillations and quenchings.

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f: If fit to experiments are better than previous best fit then store parameters.

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Parameter optimization

	c_s/mM	a_s/a	$\theta_s/\deg$	$q_s/q_{\mathrm{ACA_x}}$	$\phi_s/\deg$
$\mathrm{Glc_x}$	1.55 (1.6)	0.013	135	5.3 (11)	355 (4)
Glc	0.57	1.83	12	19	81
G6P	4.2 (4.1)	15.8 (21)	190 (260)	1.7	67
F6P	0.49 (0.5)	2.16 (2.7)	178 (250)	1.7	72
FBP	4.64 (5.1)	22.2 (26)	32 (70)	4.4	218
GAP	0.115 (0.12)	0.295 (0.04)	30	7.0	255
DHAP	2.95 (2.5)	6.97 (0.5)	38	7.9	195
BPG	0.0003 (n.d)	0.002	136	0.53	287
PEP	0.04 (0.04)	0.023 (0.07)	18	1.1	286
Pyr	8.7 (8.7)	4.06 (7)	79	125	180
ACA	1.48	0.894	196	2.5	268
$\mathrm{EtOH_x}$	16.5	0.035	114	∞ (n.p)	undef
EtOH	19.2	1.22	26	∞	undef
Glyc	4.2	1.68	98	∞	undef
$\mathrm{Glyc_x}$	1.68	0.005	188	∞ (n.p)	undef
$\mathrm{ACA_x}$	1.29	0.037 (0.3)	284 (200)	1 (1)	181 (172)
$\mathrm{CN_x^-}$	5.2	5×10^{-5}	193	2400 (n.p)	271
ATP	2.1 (2.1)	10.8 (8)	139 (180)	0.50	289
ADP	1.5 (1.5)	6.32 (9.4)	319 (0)	1.0	290
AMP	0.33 (0.33)	4.5 (3.6)	319 (0)		
NADH	0.33 (0.33)	1 (1)	0 (0)	0.68	106
$\mathrm{NAD^+}$	0.65 (0.65)	1 (0.6)	180 (180)		

Comparison of optimized model with experimental results (in paranthesis).
c_s : concentrations of the stationary state. a_s : relative amplitudes of oscillations. θ_s: angles of oscillations. q_s : relative quenching amplitudes. ϕ_s : quenching phases.

Control coefficients for amplitude and frequence

The quest for the oscillophore can be closed by calculating control coefficients for the oscillations from the comprehensive model.

- Control coefficient for square amplitude and frequence

$$\Gamma_E^{a^2} = E\frac{\partial a^2}{\partial E} \qquad\qquad C_E^{\omega_{lc}} = \frac{E}{\omega_{lc}}\frac{\partial \omega_{lc}}{\partial E}$$

Control coefficients for amplitude and frequence

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- The control coefficients satisfy summation rules which can be obtained from the timescale invariance of trajectories by differentiating a^2 with a scale factor h for time
  $$\frac{\partial a^2}{\partial h} = \sum_r \frac{\partial a^2}{\partial(hE_r)}\frac{\partial(hE_r)}{\partial h} \stackrel{h=1}{\equiv} \sum_r \frac{\partial a^2}{\partial E_r}E_r = \sum_r \Gamma^{a^2}_{E_r} = 0$$

Control coefficients for amplitude and frequence

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- The control coefficients satisfy summation rules which can be obtained from the timescale invariance of trajectories by differentiating a^2 with a scale factor h for time
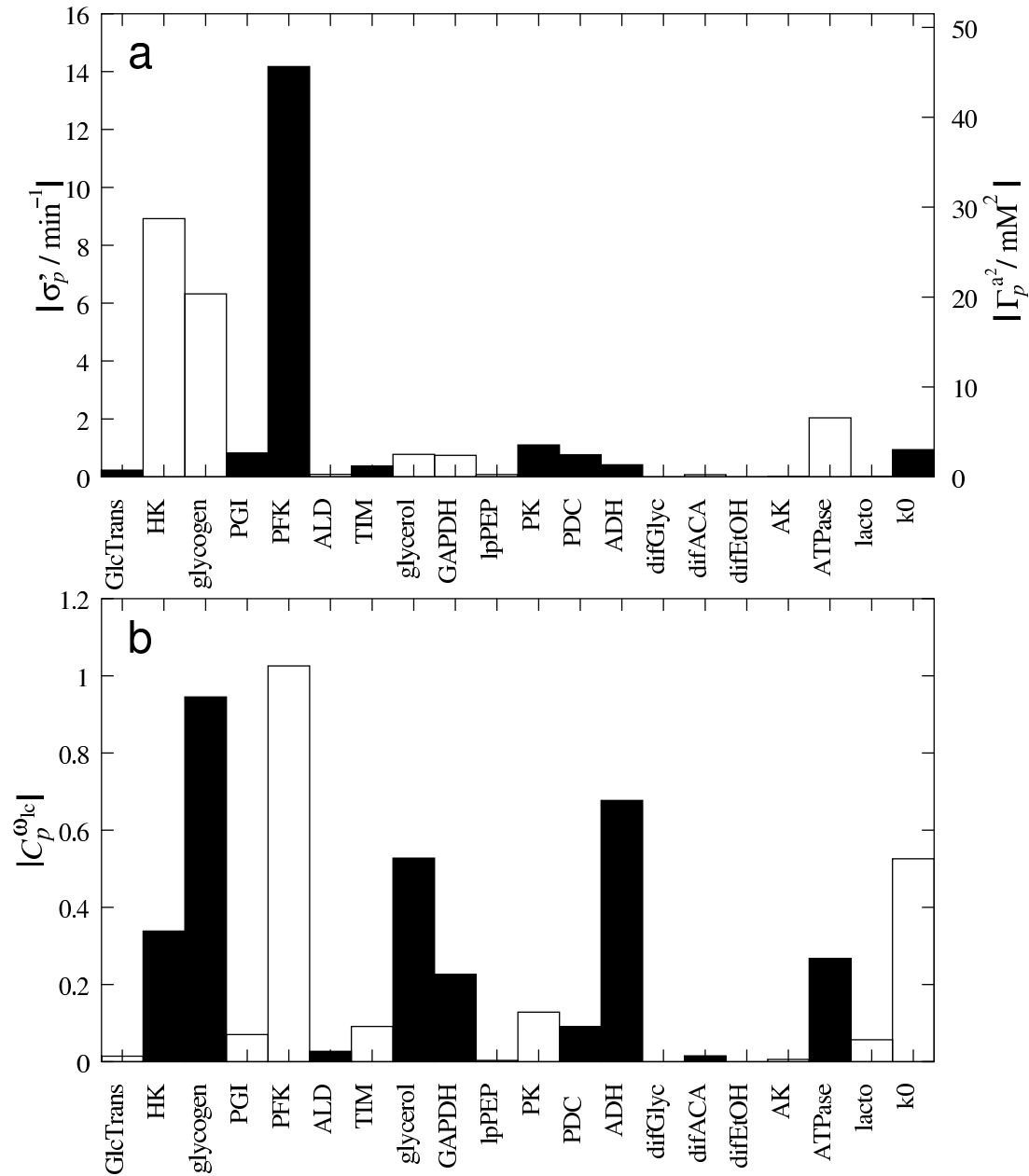$$\frac{\partial a^2}{\partial h} = \sum_r \frac{\partial a^2}{\partial (hE_r)} \frac{\partial (hE_r)}{\partial h} \stackrel{h=1}{=} \sum_r \frac{\partial a^2}{\partial E_r} E_r = \sum_r \Gamma^{a^2}_{E_r} = 0$$

- Similarly
$$\frac{\partial \omega_{lc}}{\partial h} = \sum_r \frac{\partial \omega_{lc}}{\partial (hE_r)} \frac{\partial (hE_r)}{\partial h} \stackrel{h=1}{=} \sum_r \frac{\partial \omega_{lc}}{\partial E_r} E_r = \omega_{lc} \sum_r C^{\omega_{lc}}_{E_r} = \omega_{lc}$$
such that $\sum_r C^{\omega_{lc}}_{E_r} = 1$

Control of amplitude and frequence

Hopf interpretation of controlcoefficients

- Stuart-Landau equation: $\dot{z} = (i\omega_0 + \sigma\mu)z + g^z|z|^2$

- Control coefficients for Hopf:

$$\frac{d\mathrm{Re}(\lambda)}{dp/p_0} = \frac{d\mathrm{Re}(\lambda)}{d\mu} = \sigma'_p$$

$$C_p^{\omega_{\mathrm{lc}}} = \frac{d\ln(\omega)}{d\ln(p)} = \frac{1}{\omega}\frac{d(\omega)}{d(\mu)} = \frac{1}{\omega_0}\left(\sigma''_p - \sigma'_p\frac{g''}{g'}\right)$$

Conclusion on control of function

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- Control coefficients of a metabolic network depends on the stationary state.

Conclusion on modelling

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- Measurements of stationary concentrations are usually not sufficient to discriminate between different sets of model parameters.

- The simples dynamic information is obtained from small instantaneous perturbations of the state of biological systems.

- From a timeseries of responses to random perturbations with all metabolites you can determine the Jacobian.

coworkers

Sune Danø

Finn Hynne

Mads Ipsen

Mads Madsen

Bjørn Quistorff

Henrik Skødt