

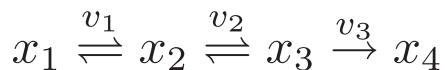
Lecture #10

Metabolic Pathways:

Glycolysis

On to Part III

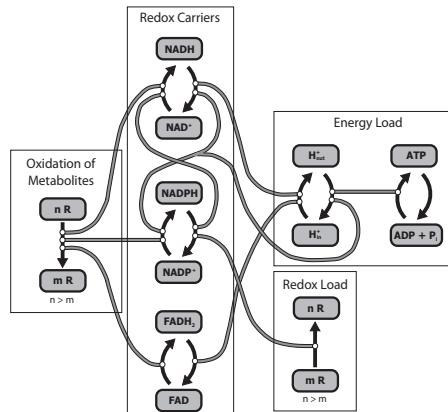
Part I



$$S = \begin{pmatrix} -1 & 0 & 0 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \\ 0 & 0 & 1 \end{pmatrix} \quad \frac{dx}{dt} = S v(x)$$

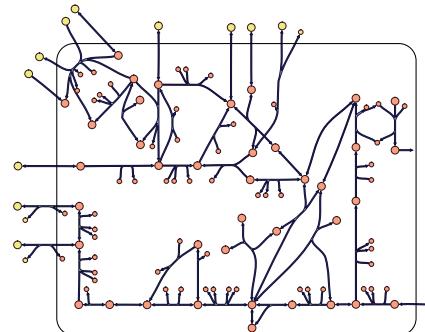
--Basics--

Part II



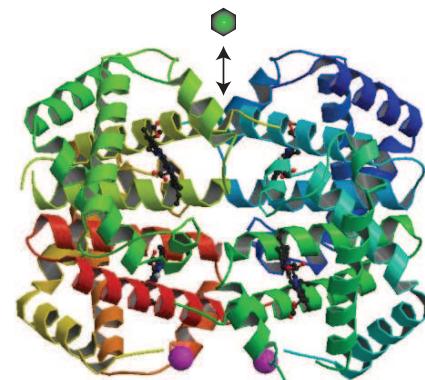
--Biological features--

Part III



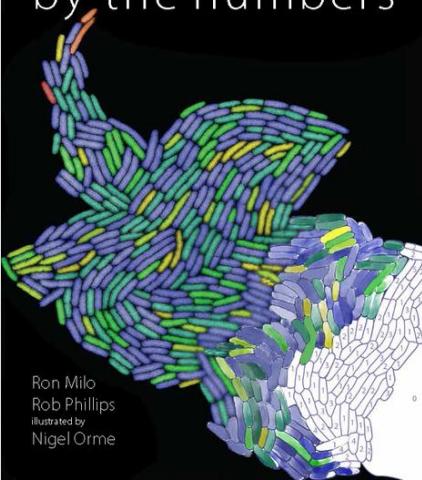
--Metabolic networks--

Part IV



--Macromolecular systems--

CELL BIOLOGY by the numbers



BY NUMBER

BY MASS

BY VOLUME

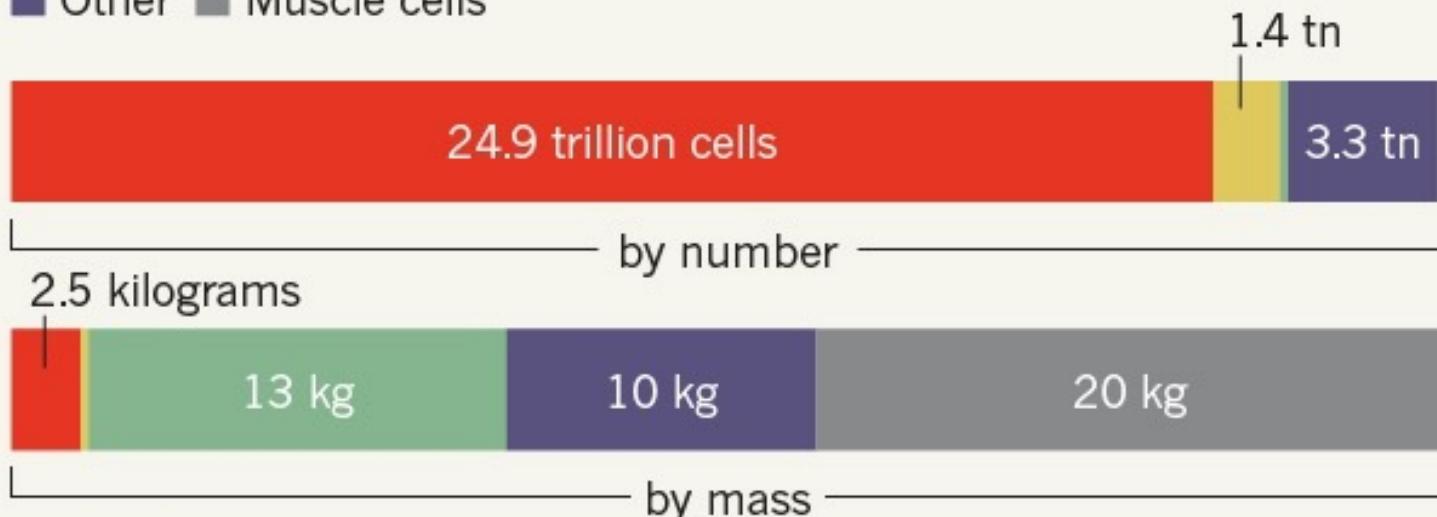
BY SURFACE AREA

BY HUMAN CELLS



body's cells are small red blood cells, although fat cells make up the majority by mass.

- Red blood cells (erythrocytes)
- Platelets
- Fat cells (adipocytes)
- Other
- Muscle cells



©nature

Outline

- Glycolysis: a central metabolic pathway
- Fundamental structure ($m \times n = 20 \times 21$)
- Co-factor coupling (NADH, ATP, P_i)
- The stoichiometric matrix
 - Its null spaces
- Setting up a simulation model
 - Steady state
- Interpreting the results from simulation
 - Concentrations, fluxes, pools, ratios

Building our first biologically meaningful model

GLYCOLYSIS: AN OPEN SYSTEM

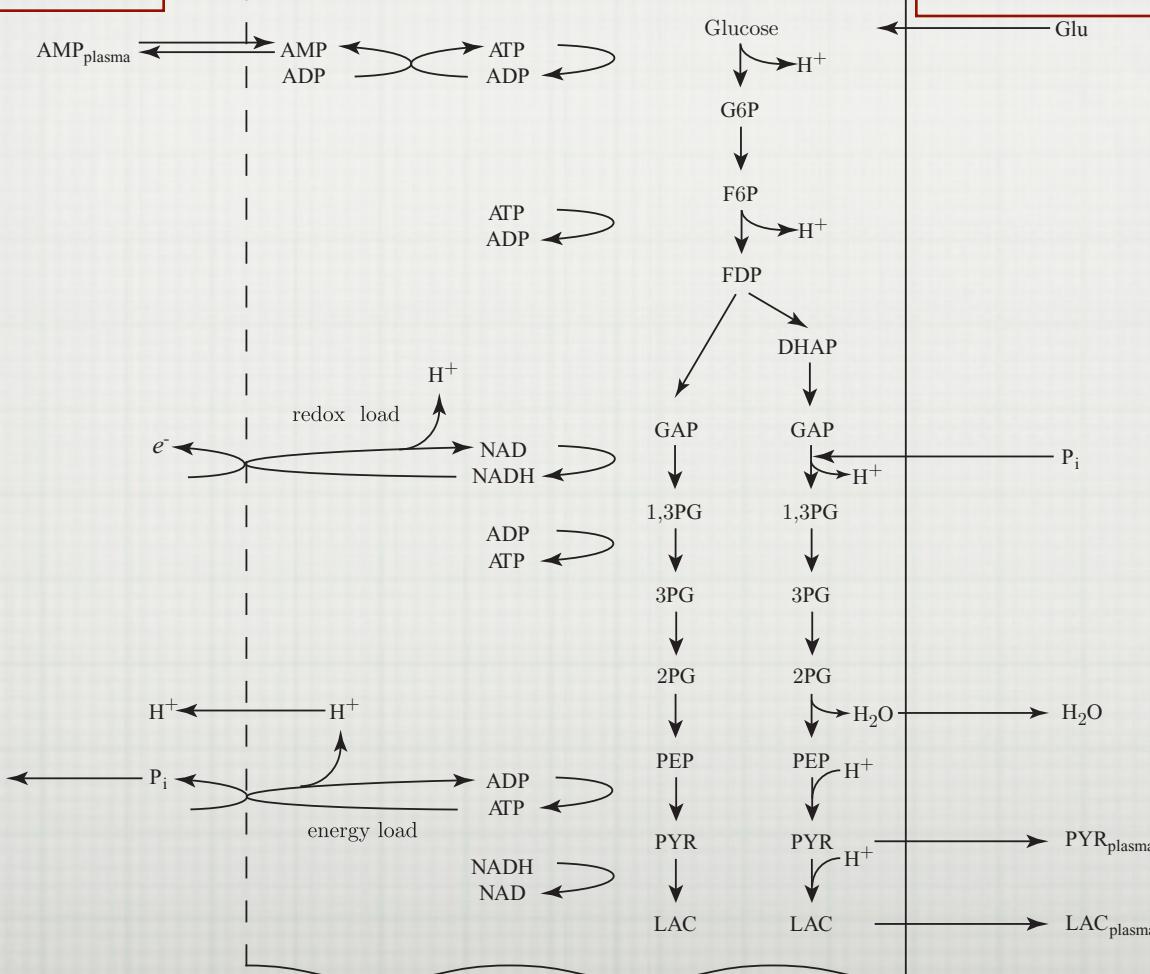
Glycolysis as an Open System

coupling with cellular processes

co-factor coupling

Glycolysis
(reaction schema)

coupling with the environment



Compounds: The nodes

#	Abbreviation	Intermediates and cofactors	Concentration (mM)
1	Gluc	Glucose	1.0
2	G6P	Glucose 6-phosphate	0.048 6
3	F6P	Fructose 6-phosphate	0.019 8
4	FDP	Fructose 1,6-diphosphate	0.014 6
5	DHAP	Dihydroxyacetone phosphate	0.16
6	GAP	Glyceraldehyde 3-phosphate	0.007 28
7	DPG13	1,3-Diphosphoglycerate	0.000 24
8	PG3	3-Phosphoglycerate	0.077 3
9	PG2	2-Phosphoglycerate	0.011 3
10	PEP	Phosphoenolpyruvate	0.017
11	PYR	Pyruvate	0.060 3
12	LAC	Lactate	1.36
13	NAD	Nicotinamide adenine dinucleotide (oxidized)	0.058 9
14	NADH	Nicotinamide adenine dinucleotide (reduced)	0.030 1
15	AMP	Adenosine mono-phosphate	0.086 7
16	ADP	Adenosine di-phosphate	0.29
17	ATP	Adenosine tri-phosphate	1.6
18	P _i	Inorganic phosphate	2.5
19	H ⁺	Proton	10 ⁻⁴
20	H ₂ O	Water	arbitrary

pathway
intermediates

cofactors
carriers

inorganic

Reactions: The links

#	Abbrev.	Enzymes/transporter/load	Elementally balanced reaction
1	HK	Hexokinase	$\text{Gluc} + \text{ATP} \rightarrow \text{G6P} + \text{ADP} + \text{H}^+$
2	PGI	Glucose 6-phosphate isomerase	$\text{G6P} \leftrightarrow \text{F6P}$
3	PFK	Phosphofructokinase	$\text{F6P} + \text{ATP} \rightarrow \text{FDP} + \text{ADP} + \text{H}^+$
4	TPI	Triose-phosphate isomerase	$\text{DHAP} \leftrightarrow \text{GAP}$
5	ALD	Fructose 1,6-diphosphate aldolase	$\text{FDP} \leftrightarrow \text{DHAP} + \text{GAP}$
6	GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	$\text{GAP} + \text{NAD} + \text{P}_i \leftrightarrow \text{DPG13} + \text{NADH} + \text{H}^+$
7	PGK	Phosphoglycerate kinase	$\text{DPG13} + \text{ADP} \leftrightarrow \text{PG3} + \text{ATP}$
8	PGLM	Phosphoglycerate mutase	$\text{PG3} \leftrightarrow \text{PG2}$
9	ENO	Enolase	$\text{PG2} \leftrightarrow \text{PEP} + \text{H}_2\text{O}$
10	PK	Pyruvate kinase	$\text{PEP} + \text{ADP} + \text{H}^+ \rightarrow \text{PYR} + \text{ATP}$
11	LDH	Lactate dehydrogenase	$\text{PYR} + \text{NADH} + \text{H}^+ \leftrightarrow \text{LAC} + \text{NAD}$
12	AMP	AMP export	$\text{AMP} \rightarrow$
13	APK	Adenylate kinase	$2\text{ADP} \leftrightarrow \text{AMP} + \text{ATP}$
14	PYR	Pyruvate exchange	$\text{PYR} \leftrightarrow$
15	LAC	Lactate exchange	$\text{LAC} \leftrightarrow$
16	ATP	ATP hydrolysis	$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i + \text{H}^+$
17	NADH	NADH oxidation	$\text{NADH} \rightarrow \text{NAD} + \text{H}^+$
18	GLU _{in}	Glucose import	$\rightarrow \text{Gluc}$
19	AMP _{in}	AMP import	$\rightarrow \text{AMP}$
20	H	Proton exchange	$\text{H}^+ \leftrightarrow$
21	H ₂ O	Water exchange	$\text{H}_2\text{O} \leftrightarrow$

glycolytic reactions

AMP

metabolism

Primary
export

Cofactor loads

Primary
inputs

Inorganic

Find the homeostatic reference state

THE STEADY STATE

The Stoichiometric Matrix

	Glycolytic reactions										AMP metabolism		Primary export		Cofactors		Primary inputs		Inorganic			
	v_{hk}	v_{pgi}	v_{pfk}	v_{pi}	v_{ald}	v_{gapdh}	v_{pgk}	v_{pgm}	v_{eno}	v_{pk}	v_{ldh}	v_{amp}	v_{ak}	v_{pyr}	v_{ac}	v_{atp}	v_{nadh}	v_{glutin}	v_{ampin}	v_{h+}	v_{H_2O}	
Glu	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
G6P	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F6P	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FBP	0	0	1	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DHAP	0	0	0	-1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GAP	0	0	0	1	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PG13	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PG3	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PG2	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0
PEP	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0
PYR	0	0	0	0	0	0	0	0	0	1	-1	0	0	-1	0	0	0	0	0	0	0	0
LAC	0	0	0	0	0	0	0	0	0	0	1	0	0	0	-1	0	0	0	0	0	0	0
NAD	0	0	0	0	0	-1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
NADH	0	0	0	0	0	1	0	0	0	0	-1	0	0	0	0	0	-1	0	0	0	0	0
AMP	0	0	0	0	0	0	0	0	0	0	0	-1	1	0	0	0	0	0	1	0	0	0
ATP	-1	0	-1	0	0	0	1	0	0	1	0	0	1	0	0	-1	0	0	0	0	0	0
P _i	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
H ⁺	1	0	1	0	0	1	0	0	0	-1	-1	0	0	0	0	1	1	0	0	-1	0	0
H ₂ O	0	0	0	0	0	0	0	0	1	0	0	0	0	0	-1	0	0	0	0	0	-1	0

$m \times n = 20 \times 21$, Rank(S)=18

$$\dim(\text{Null}) = 21 - 18 = 3$$

$$\dim(\text{Left Null}) = 20 - 18 = 2$$

→ 3 pathways

→ 2 conserved quantities

QC/QA on \mathbf{S}

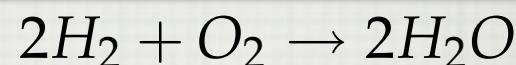
- Every column in \mathbf{S} (s_i ; reaction vector) represents a reaction
 - Chemically accurate, including charge and elemental balancing (see companion book)

Elemental matrix: \mathbf{E}

	compounds	
	H_2O	$C_6H_{12}O_6$
elements		
C	0	6
O	1	6
H	2	12
N	0	0
P	0	0
S	0	0

Elemental balance:

$$\mathbf{E} \bullet \mathbf{s} = 0$$

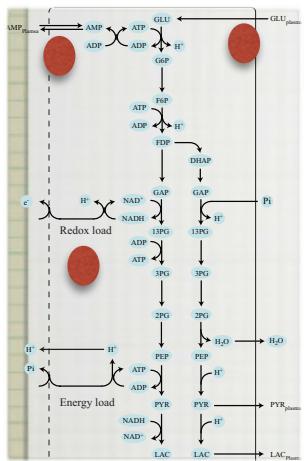


$$O \begin{pmatrix} 0 & 2 & 1 \\ 2 & 0 & 2 \end{pmatrix} \begin{pmatrix} -2 \\ -1 \\ 2 \end{pmatrix} = 0$$

the rows of \mathbf{E} are
in the left null of \mathbf{S}

Glycolysis:

'annotated' S matrix

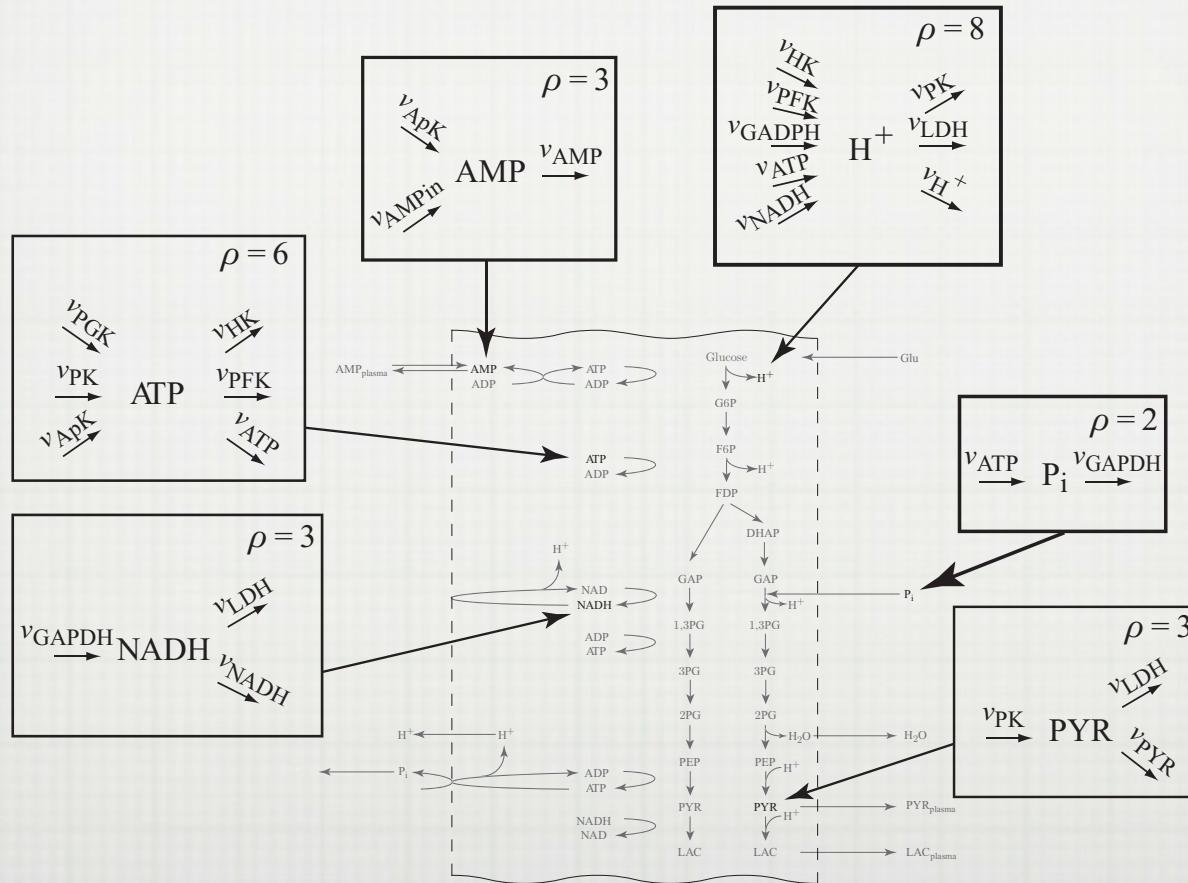


Knowing 3 fluxes
—ie glucose
update, AMP
turnover, Redox
load, on can
compute the flux
vector

	Glycolytic reactions												AMP metabolism		Primary export		Cofactors		Primary inputs		Inorganic	
	V_{hk}	V_{pgi}	V_{pk}	V_{pi}	V_{ald}	V_{gapdh}	V_{pgm}	V_{veno}	V_{pk}	V_{ldh}	V_{amp}	V_{apk}	V_{pyr}	V_{lac}	V_{nadh}	V_{glu}	V_{ampin}	V_{H^+}	V_{H_2O}	ρ_l		
u	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2		
6P	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2		
iP	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2		
P	0	0	1	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2		
HAP	0	0	0	-1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2		
AP	0	0	0	1	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	3		
G13	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	2		
G3	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	2		
G2	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	2		
iP	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	2		
R	0	0	0	0	0	0	0	0	0	1	-1	0	0	-1	0	0	0	0	0	3		
LAC	0	0	0	0	0	0	0	0	0	0	1	0	0	-1	0	0	0	0	0	2		
AD	0	0	0	0	0	-1	0	0	0	0	1	0	0	0	1	0	0	0	0	3		
ADH	0	0	0	0	0	1	0	0	0	-1	0	0	0	0	-1	0	0	0	0	3		
AMP	0	0	0	0	0	0	0	0	0	0	-1	1	0	0	0	0	1	0	0	3		
ADP	1	0	1	0	0	0	-1	0	0	-1	0	0	-2	0	0	1	0	0	0	6		
ATP	-1	0	-1	0	0	0	1	0	0	1	0	0	1	0	-1	0	0	0	0	6		
Pi	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	1	0	0	0	0	2		
H ⁺	1	0	1	0	0	0	1	0	0	-1	-1	0	0	0	1	1	0	0	-1	0		
H ₂ O	0	0	0	0	0	0	0	0	1	0	0	0	0	-1	0	0	0	0	-1	3		
π_j																						
C	0	0	0	0	0	0	0	0	0	0	-10	0	-3	-3	0	0	6	10	0	0		
H	0	0	0	0	0	0	0	0	0	0	-13	0	-3	-5	0	0	12	13	-1	-2		
O	0	0	0	0	0	0	0	0	0	0	-7	0	-3	-3	0	0	6	7	0	-1		
P	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0	0	1	0	0		
N	0	0	0	0	0	0	0	0	0	0	-5	0	0	0	0	0	0	5	0	0		
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
NAD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
p_1																						
p_2	1	1	1	1	1	2	2	2	2	2	0	0	0	2	2	0	1	0	2	0		
p_3	0	0	0	0	0	0	0	0	0	0	-1	0	0	1	-1	0	1	0	0	2		
v_{st}	1.12	1.12	1.12	1.12	2.24	2.24	2.24	2.24	2.24	2.016	0.014	0	0.224	2.016	2.24	0.224	1.12	0.014	2.69	0		

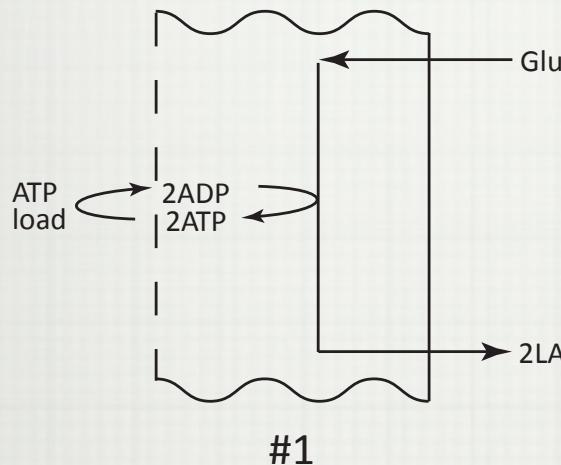
Can you show that a pathway has to be elementally balanced?

Node Maps

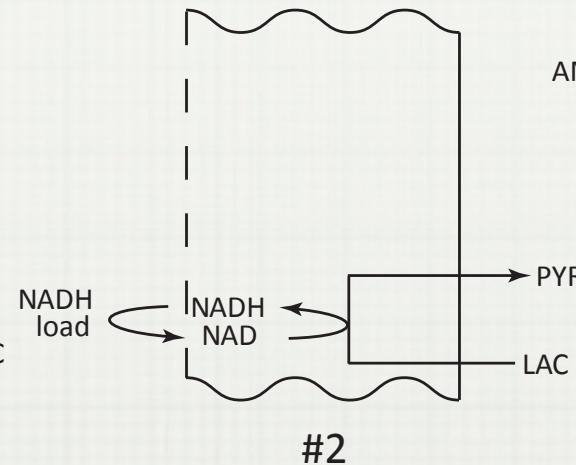


Glycolysis: Pathways in Null(S)

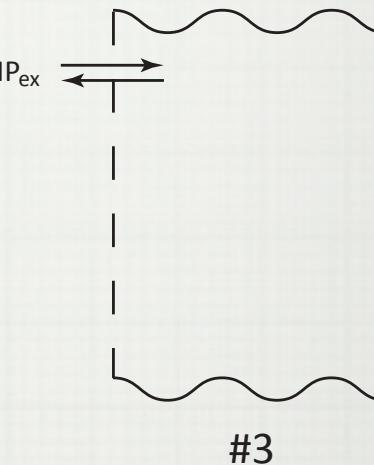
Selected basis based on biochemical intuition



~P synthesis



redox coupling



inventory of AMP

	Glycolytic reactions										AMP metabolism		Primary export		Cofactors		Primary inputs		Inorganic		
	v_{hk}	v_{pgi}	v_{pfk}	v_{tpi}	v_{ald}	v_{gapdh}	v_{pgk}	v_{pglm}	v_{eno}	v_{pk}	v_{ldh}	v_{amp}	v_{apk}	v_{pyr}	v_{lac}	v_{atp}	v_{nadh}	v_{glutin}	v_{ampin}	v_{H^+}	v_{H_2O}
p₁	1	1	1	1	1	2	2	2	2	2	2	0	0	0	2	2	0	1	0	2	0
p₂	0	0	0	0	0	0	0	0	0	0	-1	0	0	1	-1	0	1	0	0	2	0
p₃	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0

The Steady State Fluxes (mM/hr):

fluxes have to balance the network

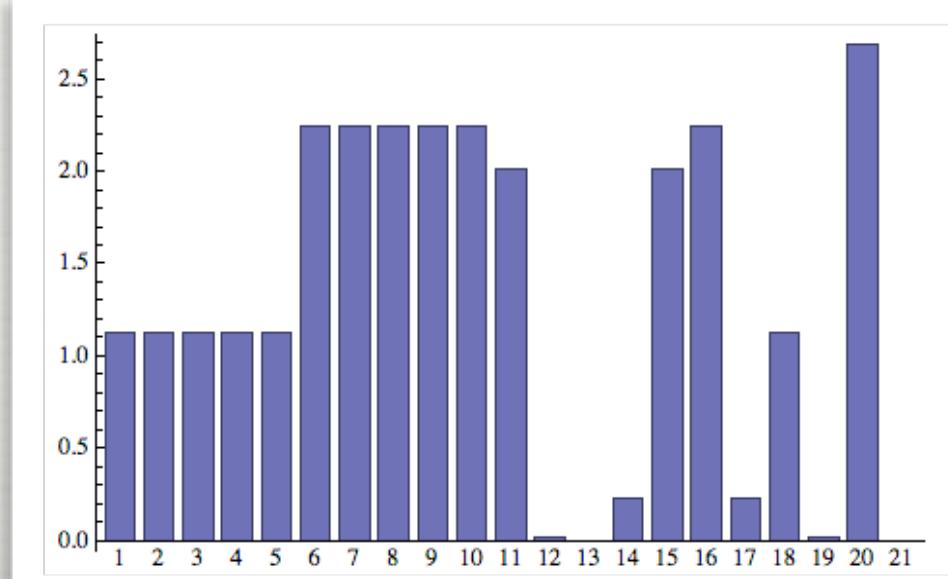
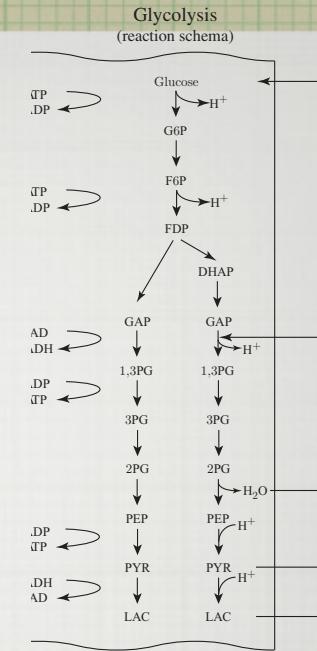
vhk	1.12
vpgi	1.12
vpfk	1.12
vtqi	1.12
vald	1.12
vgapdh	2.24
vpgk	2.24
vpqlm	2.24
veno	2.24
vpk	2.24
vldh	2.016
vamp	0.014
vapk	0
vpyr	0.224
vlac	2.016
vatp	2.24
vnadh	0.224
vgluin	1.12
vampin	0.014
vh	2.688
vh2o	0

upper
glycolysis

lower
glycolysis

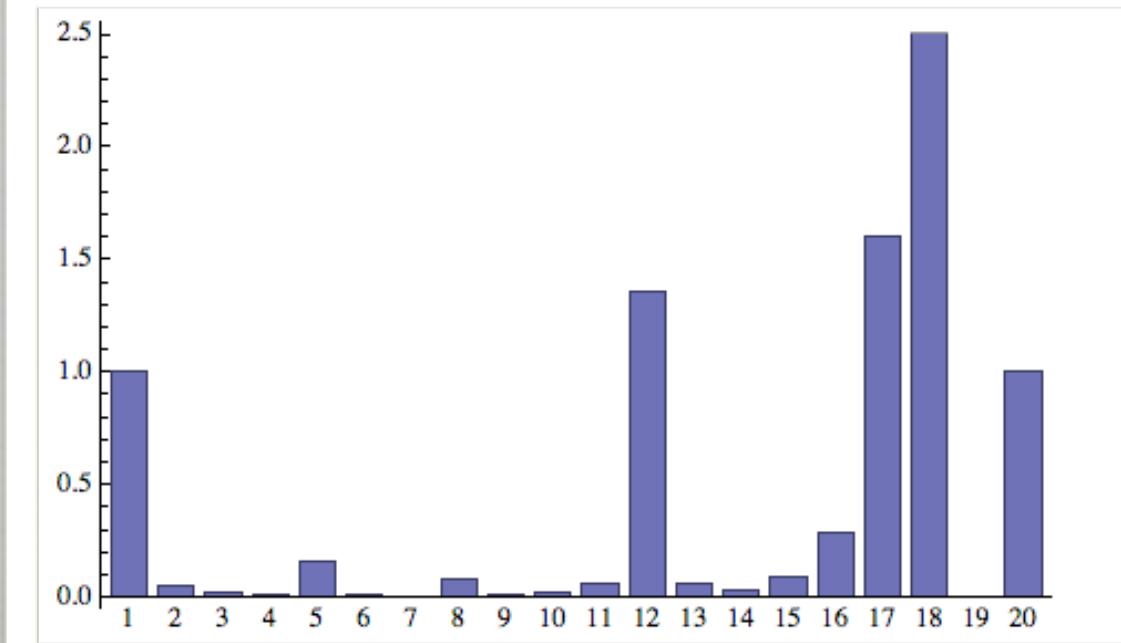
AMP

exchange &
demand
fluxes



The Steady State Concentrations (mM); determined by flux map and kinetic constants

glu	1.
g6p	0.0486
f6p	0.0198
fbp	0.0146
dhap	0.16
gap	0.00728
pg13	0.000243
pg3	0.0773
pg2	0.0113
pep	0.017
pyr	0.060301
lac	1.36
nad	0.0589
nadh	0.0301
amp	0.0867281
adp	0.29
atp	1.6
phos	2.5
h	1.02688×10^{-7}
h2o	1.



Explaining the calculation of pseudo elementary rate constants (PERCs)

- Reaction: A+B → C + D
- Rate: $v_{ss} = k_{\text{PERC}} (A_{ss} \cdot B_{ss} - C_{ss} \cdot D_{ss} / K_{\text{eq}})$
- Only k_{PERC} is unknown--the rest is data from the physiological state of interest

Reactions: the links

pseudo elementary
rate constants (PERC)
distance from
equilibrium

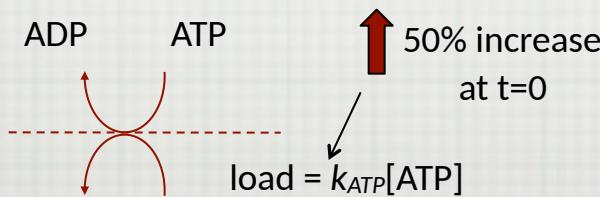
	Abbrev.	Enzymes/transporter/load	Equilibrium constant	Value	PERC	Value	Mass action ratio (Γ)	Γ/K_{eq}
1	HK	Hexokinase	K_{HK}	850	k_{HK}	0.70	0.009	0 ←
2	PGI	Phosphoglucoisomerase	K_{PGI}	0.41	k_{PGI}	3464.4	0.407	0.994 ←
3	PFK	Phosphofructokinase	K_{PFK}	310	k_{PFK}	35.37	0.134	0 ←
4	ALD	Aldolase	K_{ALD}	0.082	k_{ALD}	2834.57	0.08	0.973 ←
5	TPI	Triose phosphate isomerase	K_{TPI}	0.0571	k_{TPI}	34.36	0.046	0.796
6	GAPDH	GAP dehydrogenase	K_{GADPH}	0.018	k_{GADPH}	3376.75	0.007	0.381
7	PGK	Phosphoglycerate kinase	K_{PGK}	1800	k_{PGK}	1.274×10^6	1755.07	0.975
8	PGM	Phosphoglyceromutase	K_{PGM}	0.147	k_{PGM}	4869.57	0.146	0.994 ←
9	ENO	Enolase	K_{ENO}	1.695	k_{ENO}	1763.78	1.504	0.888
10	PK	Pyruvate kinase	K_{PK}	363 000	k_{PK}	454.386	19.57	0 ←
11	LDH	Lactate dehydrogenase	K_{LDH}	26 300	k_{LDH}	1112.57	44.133	0.002
12	AMP	Adenosine monophosphate removal	K_{AMP}	10^6	k_{AMP}	0.161	0.001	0
13	ApK	Adenylate kinase	K_{ApK}	1.65	k_{ApK}	1	1.65	1
14	PYR _{ex}	Pyruvate export	K_{PYR}	1	k_{PYR}	744.186	0.995	0.995
15	LAC _{ex}	Lactate export	K_{LAC}	1	k_{LAC}	5.60	0.735	0.735
16	ATP _{load}	Energy load	K_{ATP}	10^6	k_{ATP}	1.40	0.453	0
17	NADH _{load}	Redox load	K_{NADH}	10^6	k_{NADH}	7.441	1.957	0
18	Glu _{in}	Glucose in (fixed value of 1.12 mm/h)						
19	AMP _{in}	AMP in (fixed value of 0.014 mm/h)						
20	H ⁺ _{exchange}	Freely exchanging proton	K_{H^+}	1				
21	H ₂ O	Freely exchanging water	K_{H_2O}	1				

Model defined and ready for:

DYNAMIC SIMULATION

Simulation: 50% increase in k_{ATP} : dynamic responses of the concentrations

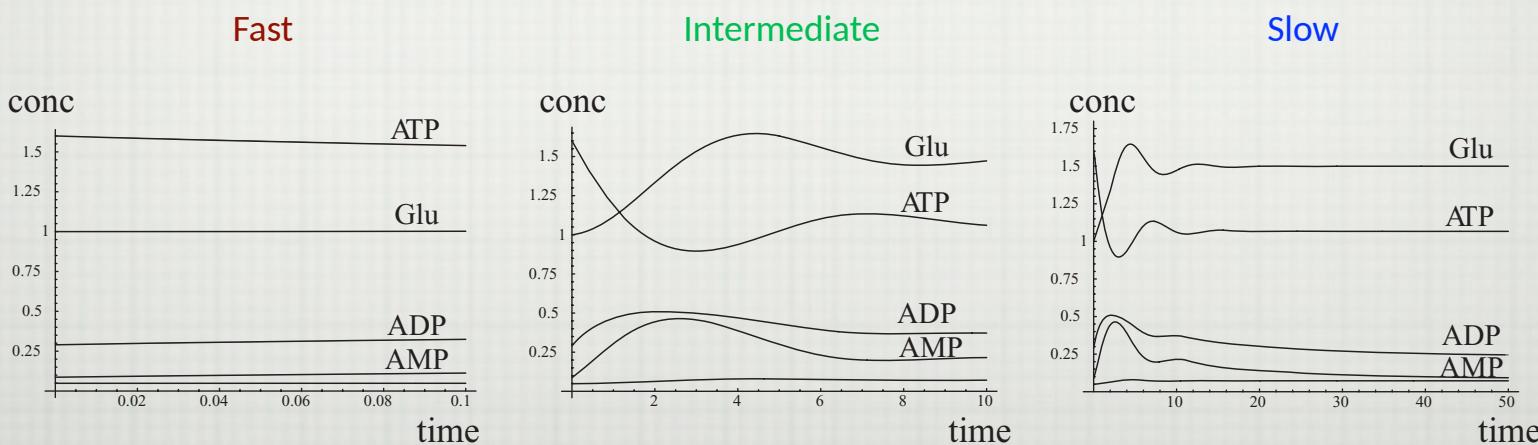
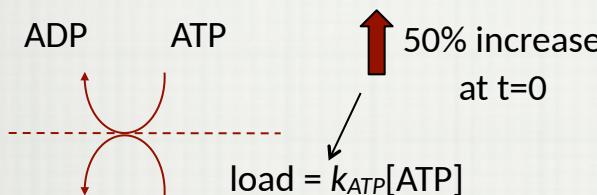
Response to an increased k_{ATP} at a constant glucose input rate Continuing the discussion from Sections 8.3 and 8.4, we focus here on a perturbation in the ATP load, where we increase the k_{ATP} parameter by 50% at $t = 0$ and simulate the dynamic response to a new steady state. This perturbation reflects a change in the rate of usage of ATP. Based on the distribution of rate constants in Table 10.5, we estimate that we have roughly three time scales of interest: <1 hr, ~ 10 hr, and ~ 50 hr.



Key Concepts

1. Time constants
2. Pools
3. Transitions

Simulation: 50% increase in k_{ATP} : dynamic responses of the concentrations

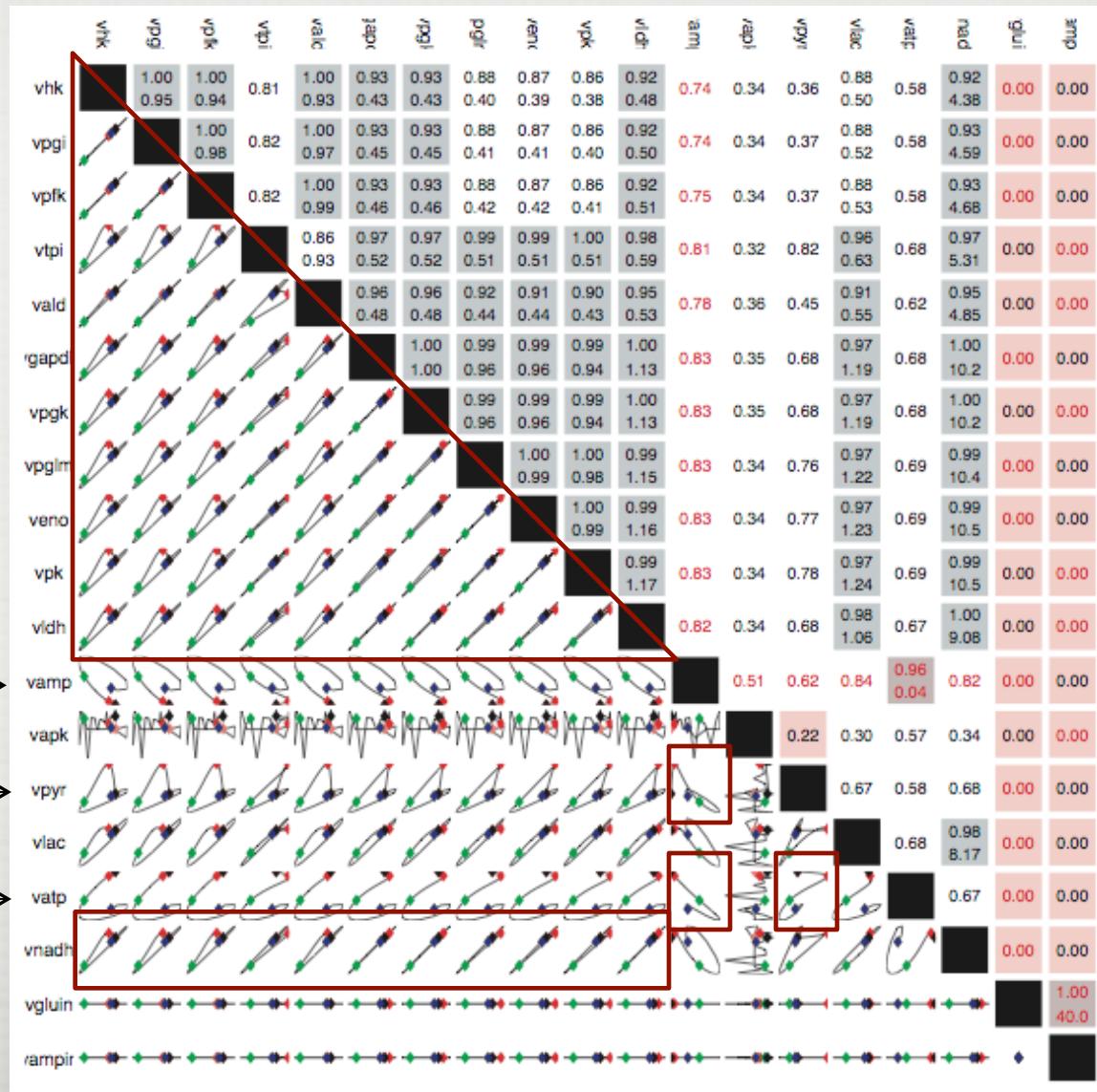


Compound	$t = 0^+$	$t = 50$	Flux	$t = 0^+$	$t = 50$
Glu	1.0	1.50	v_{HK}	1.12	1.12
G6P	0.0486	0.0727	v_{PGI}	1.12	1.12
F6P	0.0198	0.0297	v_{PFK}	1.12	1.12
FBP	0.0146	0.00741	v_{TPI}	1.12	1.12
DHAP	0.16	0.118	v_{ALD}	1.12	1.12
GAP	0.00728	0.00488	v_{GAPDH}	2.24	2.24
PG13	0.000243	0.000224	v_{PGK}	2.24	2.24
PG3	0.0773	0.0898	v_{PGLM}	2.24	2.24
PG2	0.0113	0.0131	v_{ENO}	2.24	2.24
PEP	0.017	0.0201	v_{PK}	2.24	2.24
PYR	0.060301	0.0603	v_{LDH}	2.016	2.016
LAC	1.36	1.36	v_{AMP}	0.014	0.015
NAD	0.0589	0.0589	v_{APK}	0.0	0.0274
NADH	0.0301	0.0301	v_{PYR}	0.224	0.224
AMP	0.0867	0.0930	v_{LAC}	2.016	2.016
ADP	0.29	0.245	v_{ATP}	3.36	2.24
ATP	1.6	1.067	v_{NADH}	0.224	0.224
Phos	2.5	3.619	v_{GLUin}	1.12	1.12
H ⁺	0.000103	0.000103	v_{AMPin}	0.014	0.014
H ₂ O	1.0	1.0	v_{H^+}	2.688	2.688
			v_{H_2O}	0.0	0.0

Tiled Phase Portrait: fluxes

Glycolysis: 0-10 hrs

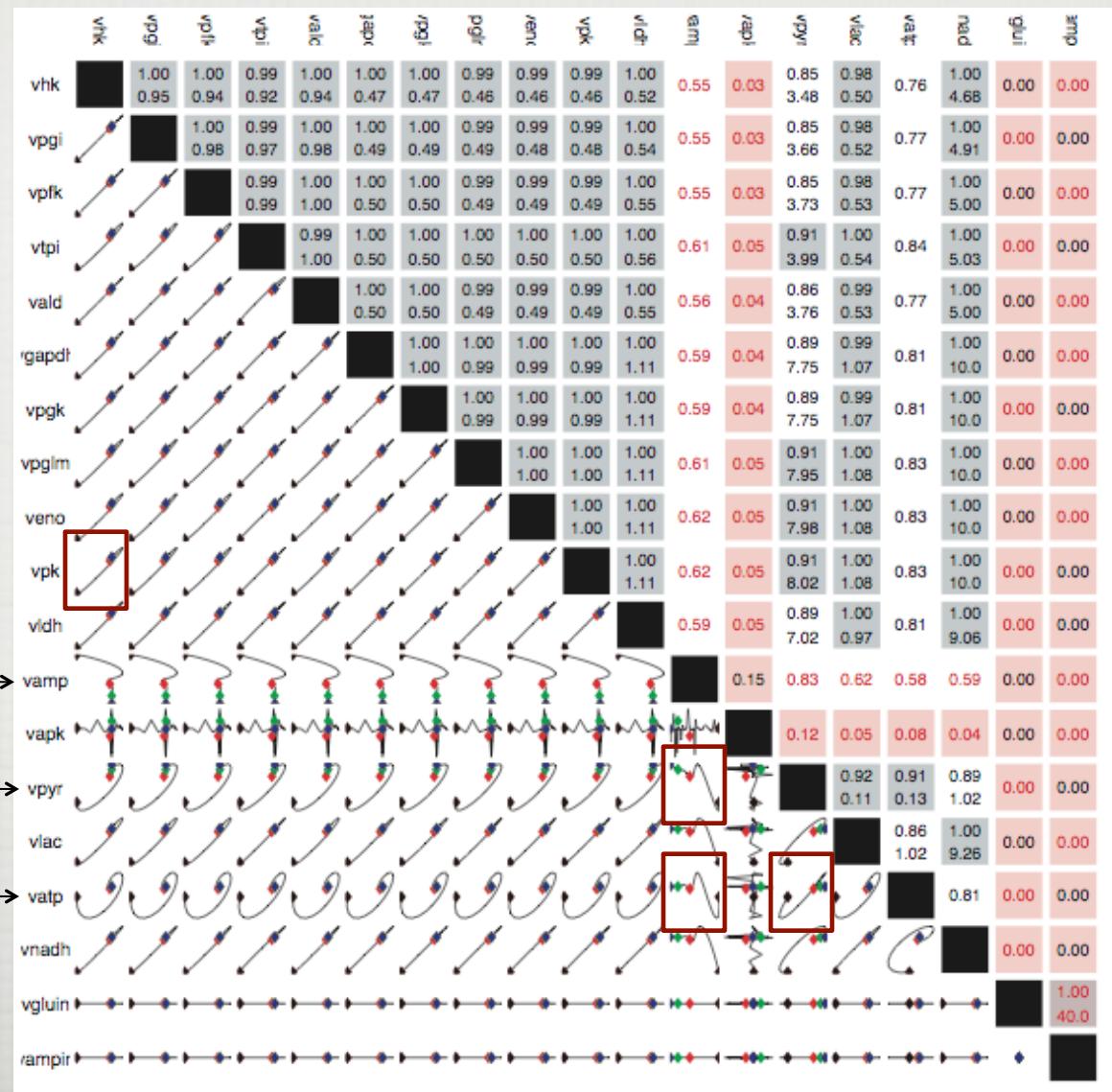
fluxes of interest



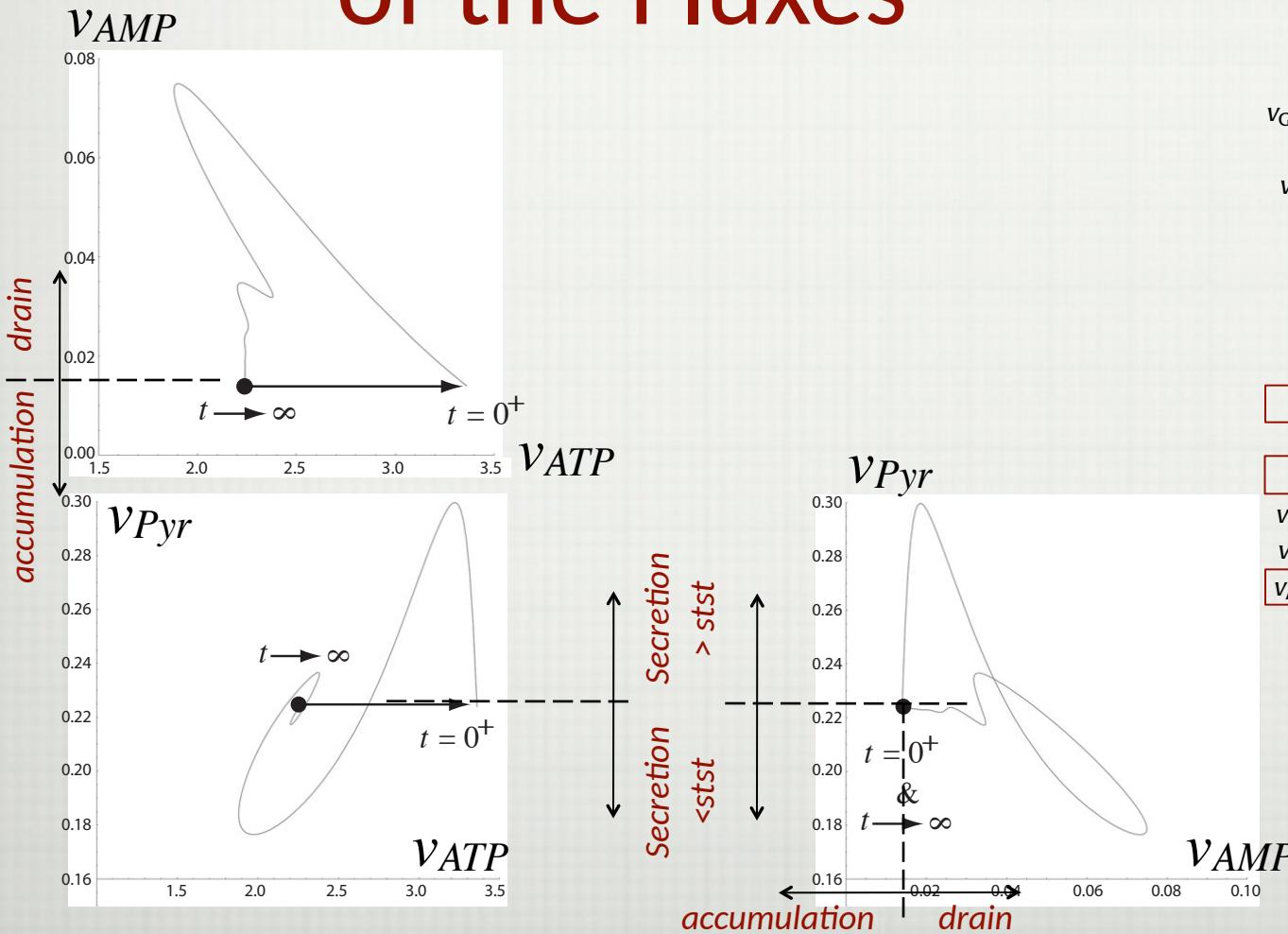
Tiled Phase Portrait: *fluxes*

Glycolysis: > 10 hrs

fluxes of interest

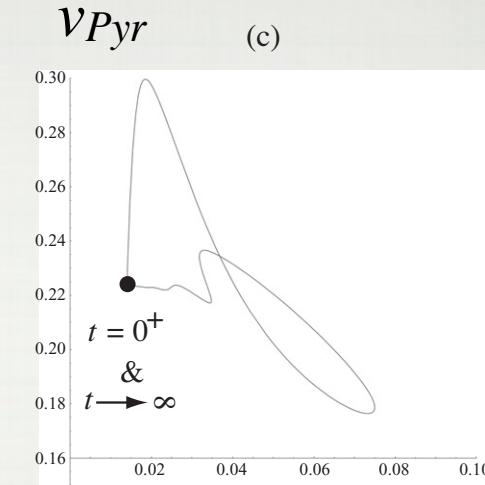
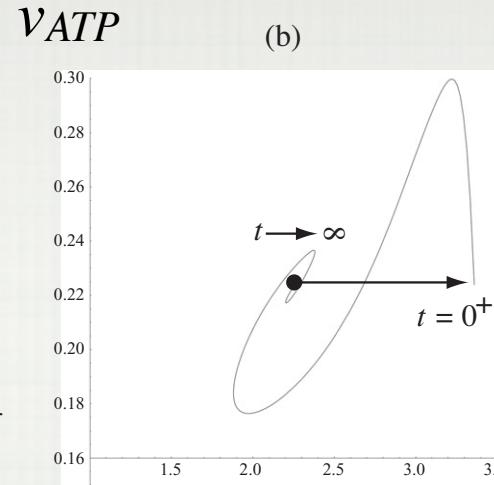
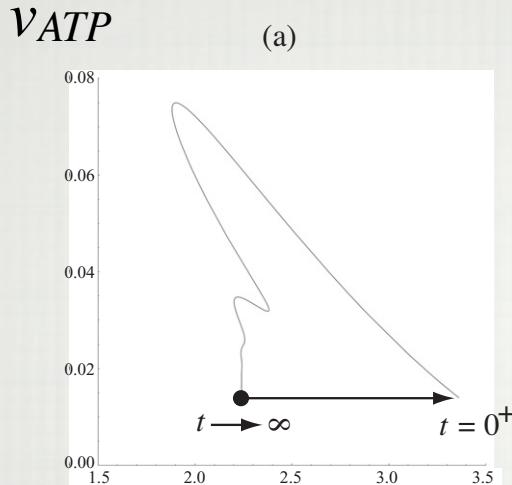


Dynamic Responses of the Fluxes



Flux	$t = 0^+$	$t = 50$
v_{HK}	1.12	1.12
v_{PGI}	1.12	1.12
v_{PFK}	1.12	1.12
v_{TPI}	1.12	1.12
v_{ALD}	1.12	1.12
v_{GAPDH}	2.24	2.24
v_{PGK}	2.24	2.24
v_{PGLM}	2.24	2.24
v_{ENO}	2.24	2.24
v_{PK}	2.24	2.24
v_{LDH}	2.016	2.016
v_{AMP}	0.014	0.015
v_{APK}	0.0	0.0274
v_{PYR}	0.224	0.224
v_{LAC}	2.016	2.016
v_{ATP}	3.36	2.24
v_{NADH}	0.224	0.224
v_{GLUin}	1.12	1.12
v_{AMPin}	0.014	0.014
v_{H^+}	2.688	2.688
v_{H_2O}	0.0	0.0

High dynamic dimension

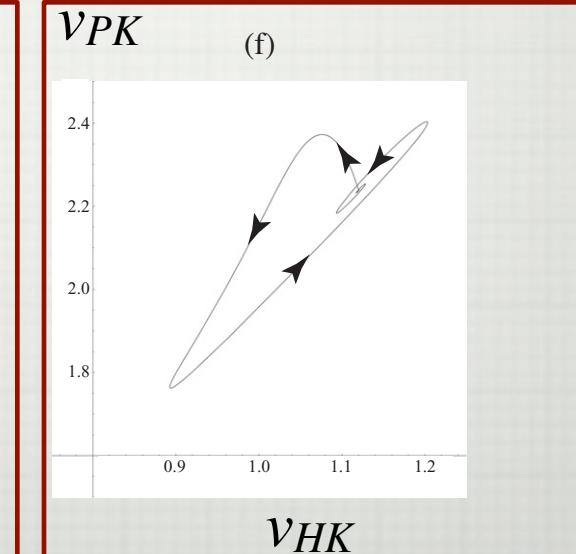
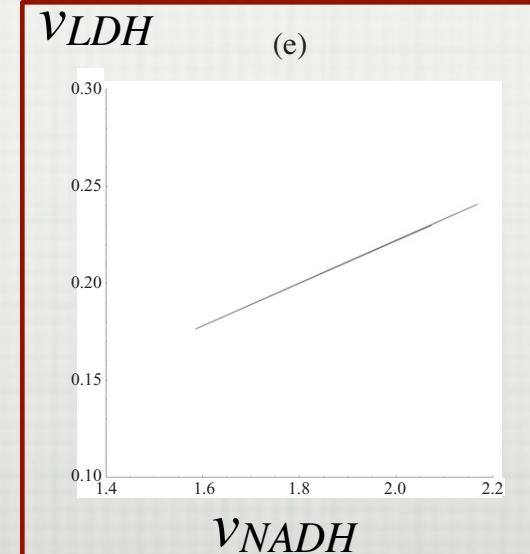
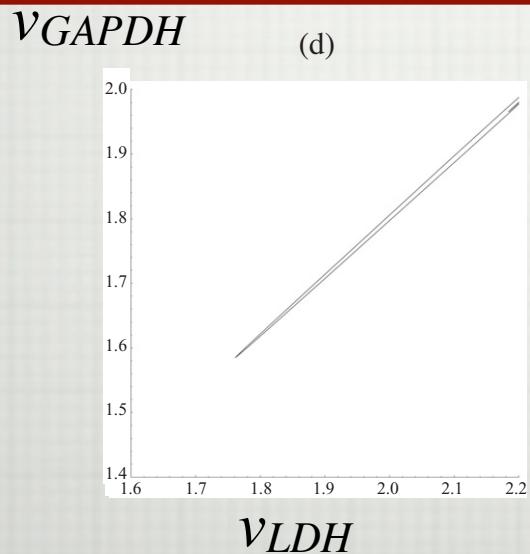


v_{AMP}

v_{Pyr}

v_{AMP}

Low dynamic dimension



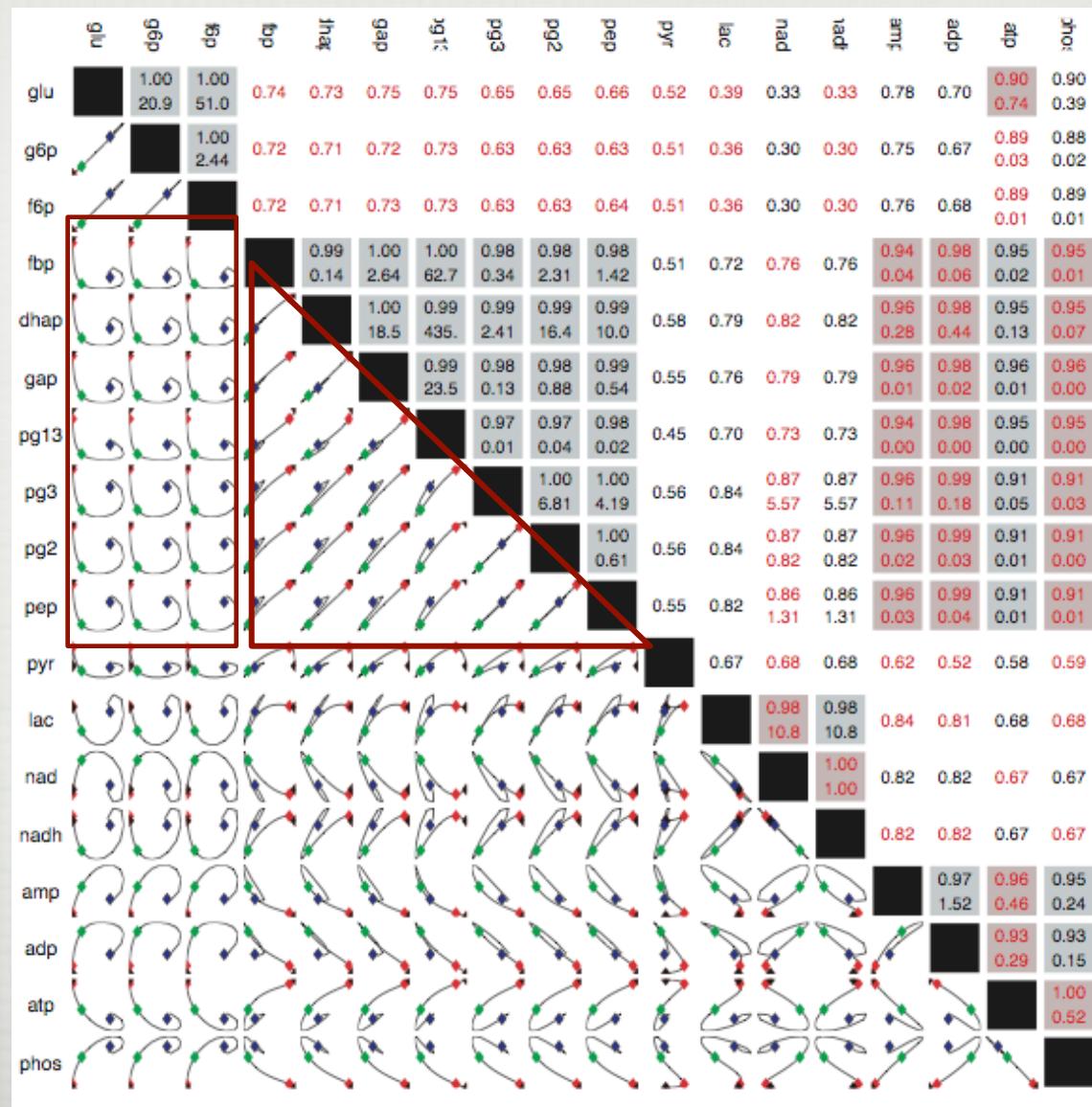
v_{LDH}

v_{NADH}

v_{HK}

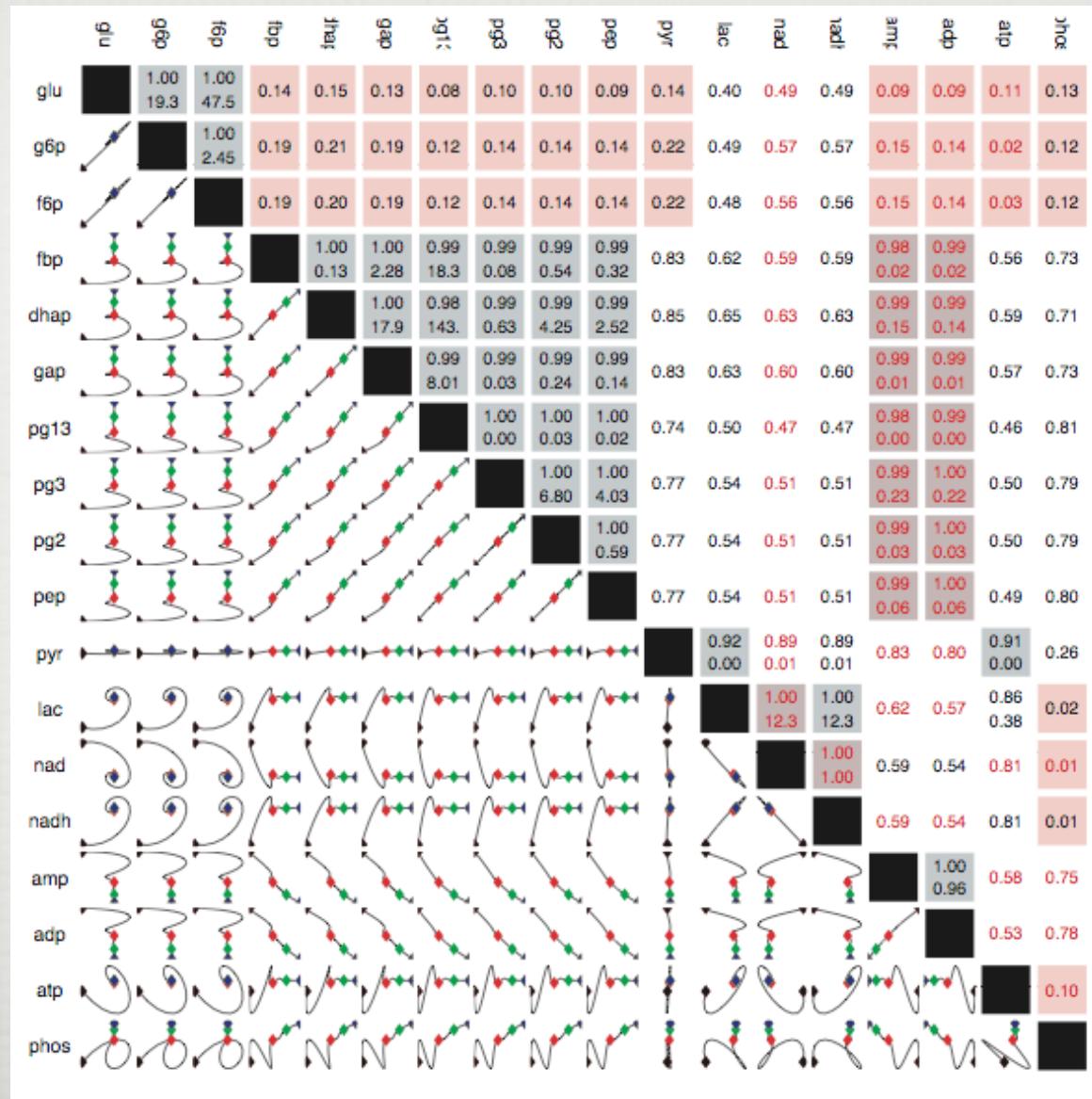
Tiled Phase Portrait:concentrations

Glycolysis: 0-10 hrs



Tiled Phase Portrait: concentrations

Glycolysis:
 > 10 hrs



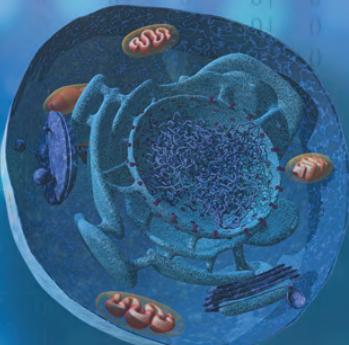
Summary: model building

- First draft dynamic models can be obtained from using measured concentration values, elementary reactions, and associated mass action kinetics.
- Dynamic simulation can be performed for perturbation in environmental parameters and the responses examined in terms of the concentrations and the fluxes.
- This first draft can be used as a scaffold to build more complicated models that include
 - interactions with other pathways (Chapters 11 & 12); and
 - regulatory effects (Chapter 14)
- This leads to a module-by-module approach to building models of complex systems

Break

Systems Biology

Simulation of Dynamic
Network States



$$\frac{dx}{dt} = S \cdot v(x) / k$$

Bernhard Ø. Palsson

Lecture #10B

Glycolysis:
interpreting the dynamic
responses

Outline

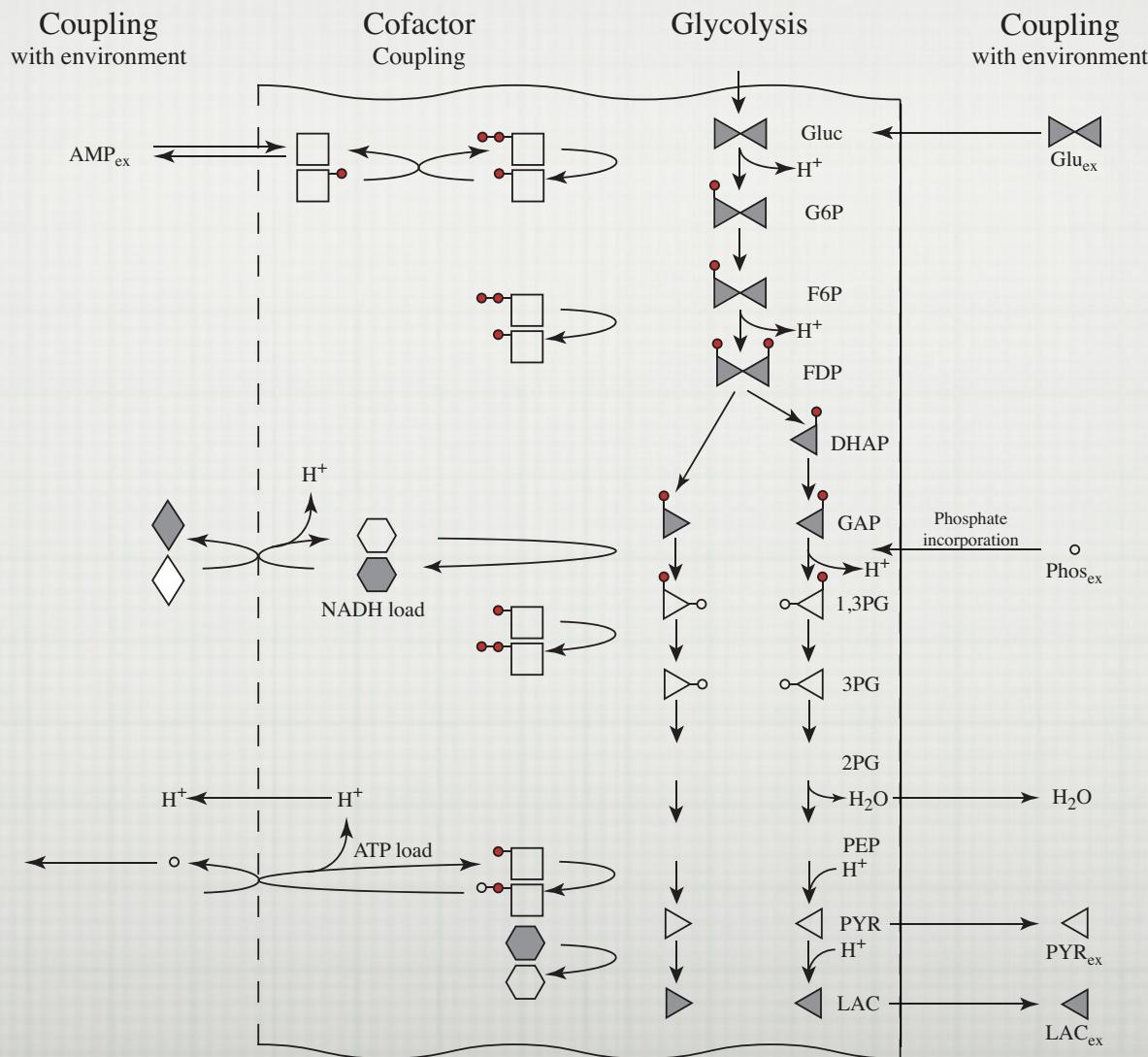
- Structural properties
- Define ‘Pools’
- Transforming the output calculations
- Interpreting the results from simulation
 - Concentrations, fluxes, pools, ratios

Towards systems biology

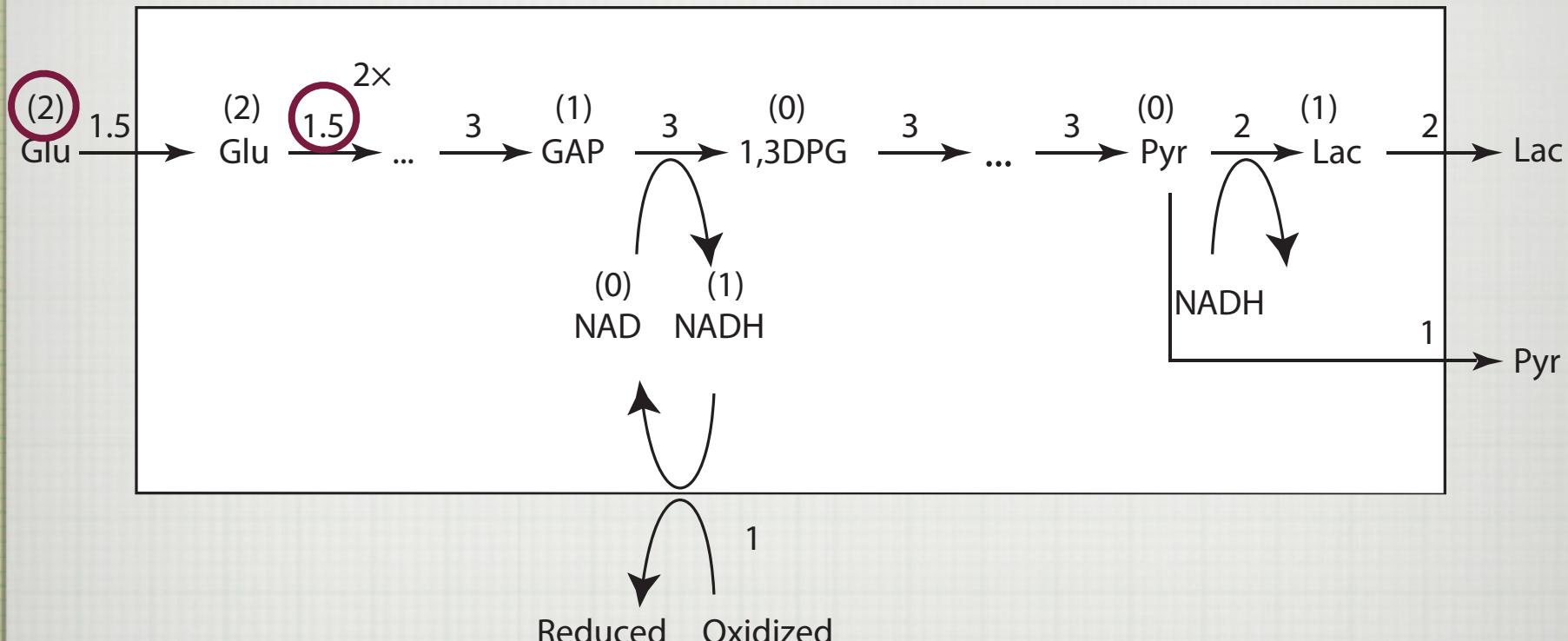
STRUCTURAL NETWORK PROPERTIES

Glycolysis:

the system with symbolic representation



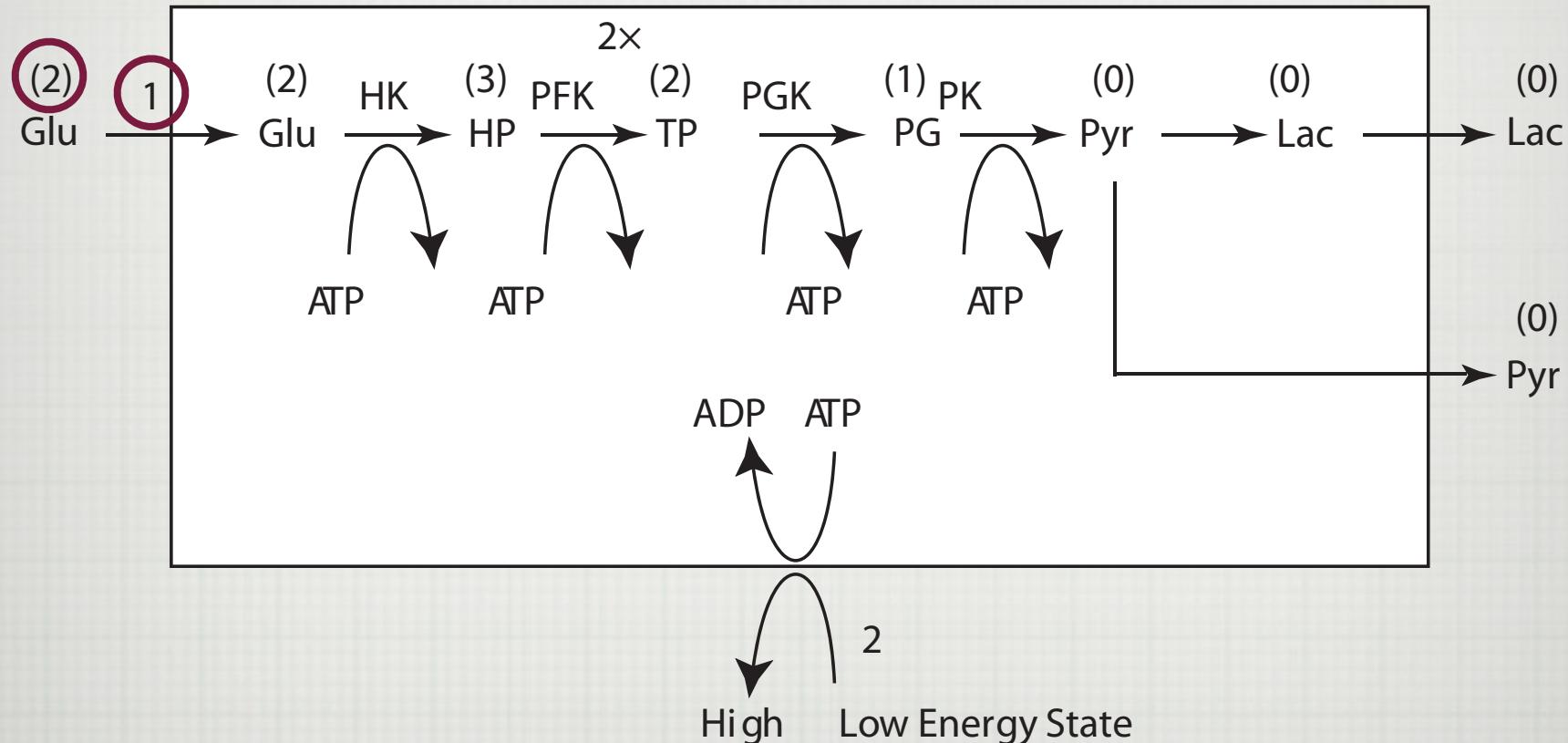
Structural Properties: redox trafficking in glycolysis



(#): Redox value

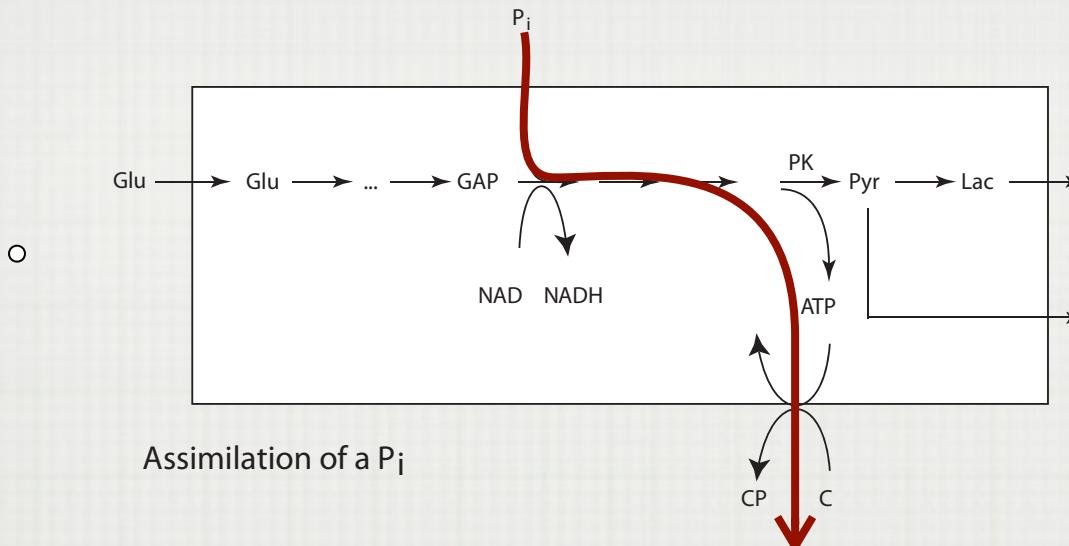
→ #: Flux value

Structural Properties: high-energy bond trafficking in glycolysis

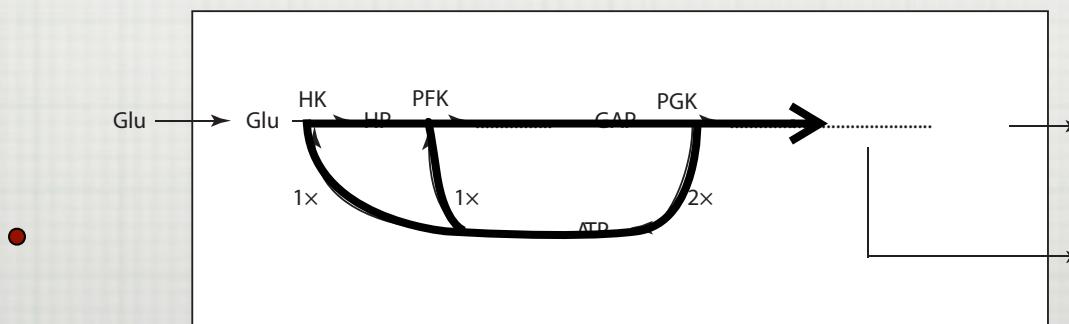


x: Flux value
→

Structural Properties: The Trafficking of Phosphate Groups in Glycolysis



"through"



"cycle"

Internal cycling or
"external use" of $\sim P_i$

Defining pools and ratios

- pool⁺ is the occupied state
- pool⁻ is the vacant state
- pool⁺ + pool⁻ is the total pool size
- thus the ratio(or ‘charge’) is:
 - pool⁺/(pool⁺ + pool⁻)
- Examples;
 1. NADH/(NAD+NADH)
 2. 2ATP+ADP/([2ATP+ADP]+ [ADP+2AMP]) =
2ATP+ADP/(2ATP+2ADP+2AMP) = EC

Pools ($p = Px$): from structural properties

	#	Glu	G6P	F6P	FBP	DHAP	GAP	PG13	PG3	PG2	PEP	PYR	LAC	NAD	NADH	AMP	ADP	ATP	P _i	H ⁺	H ₂ O	ρ_j
GP ⁺	1	2	3	3	4	2	2	2	1	1	1	1	0	0	0	0	0	0	0	0	10	
GP ⁻	2	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	2	
AP ⁺	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	2	
AP ⁻	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	2	
GR ⁺	5	2	2	2	2	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	7	
GR ⁻	6	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	5	
N ⁺	7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	
P ⁺	8	0	1	1	2	1	1	1	0	0	0	0	0	0	0	1	2	0	0	0	8	
P ⁻	9	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	4	
π_j		2	3	3	3	3	3	4	3	3	3	2	2	0	1	1	3	2	0	0	0	
P _{tot}	10	0	1	1	2	1	1	2	1	1	1	0	0	0	0	1	2	1	0	0	12	
N _{tot}	11	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	

	glu	g6p	f6p	fbp	dhap	gap	pg13	pg3	pg2	pep	pyr	lac	nad	nadh	amp	adp	atp	phos
phosphate balance	0	1	1	2	1	1	2	1	1	1	1	0	0	0	0	1	2	1
nadh balance	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0

Redox Value of Intermediates

metabolites

$$p_5 = 2(\text{Gluc} + \text{G6P} + \text{F6P} + \text{FDP}) + (\text{DHAP} + \text{GAP}) + \text{LAC}$$

By the same token, the intermediates in an oxidized state are

$$p_6 = \text{DPG13} + \text{PG3} + \text{PG2} + \text{PEP} + \text{PYR}$$

reduced glycolytic
intermediates



oxidized glycolytic
intermediates

Redox charges We can define the redox charge in glycolysis as

$$r_3 = \frac{p_5}{p_5 + p_6} \quad (10.12)$$

We note that three times the denominator in equation 10.12 is the total carbon inventory in glycolysis. In an analogous fashion, we can define the redox state on the NAD carrier as

$$r_4 = \frac{\text{NADH}}{\text{NADH} + \text{NAD}} = \frac{p_7}{p_{10}} \quad (10.13)$$

carrier

Energy Value of Intermediates

$$p_1 = 2\text{Gluc} + 3(\text{G6P} + \text{F6P}) + 2(2\text{FDP} + \text{DHAP} + \text{GAP} + \text{DPG13}) + (\text{PG3} + \text{PG2} + \text{PEP})$$

energy in glycolytic intermediates

$$p_2 = \text{PYR} + \text{LAC}$$

oxidized glycolytic intermediates

Energy charges We can define an energy charge for glycolysis as the ratio:

$$r_1 = \frac{p_1}{p_1 + p_2} \quad (10.10)$$

that is an analogous quantity to the adenylate energy charge

$$r_2 = \frac{p_3}{p_3 + p_4} = \frac{2\text{ATP} + \text{ADP}}{2(\text{ATP} + \text{ADP} + \text{AMP})} \quad (10.11)$$

Phosphate Bond Trafficking

Incorporated phosphate:

$$p_9 = \text{DPG13} + \text{PG3} + \text{PG2} + \text{PEP}$$

Recycled phosphate:

$$p_8 = \text{G6P} + \text{F6P} + 2\text{FDP} + \text{DHAP} + \text{GAP} + \text{DPG13} + \text{ADP} + 2\text{ATP}$$

*Recycle
ratio:*

$$r_5 = \frac{\text{Recycled}}{\text{Total}} = \frac{p_8}{p_8 + p_9}$$

The Fluxes that Move the Pools:

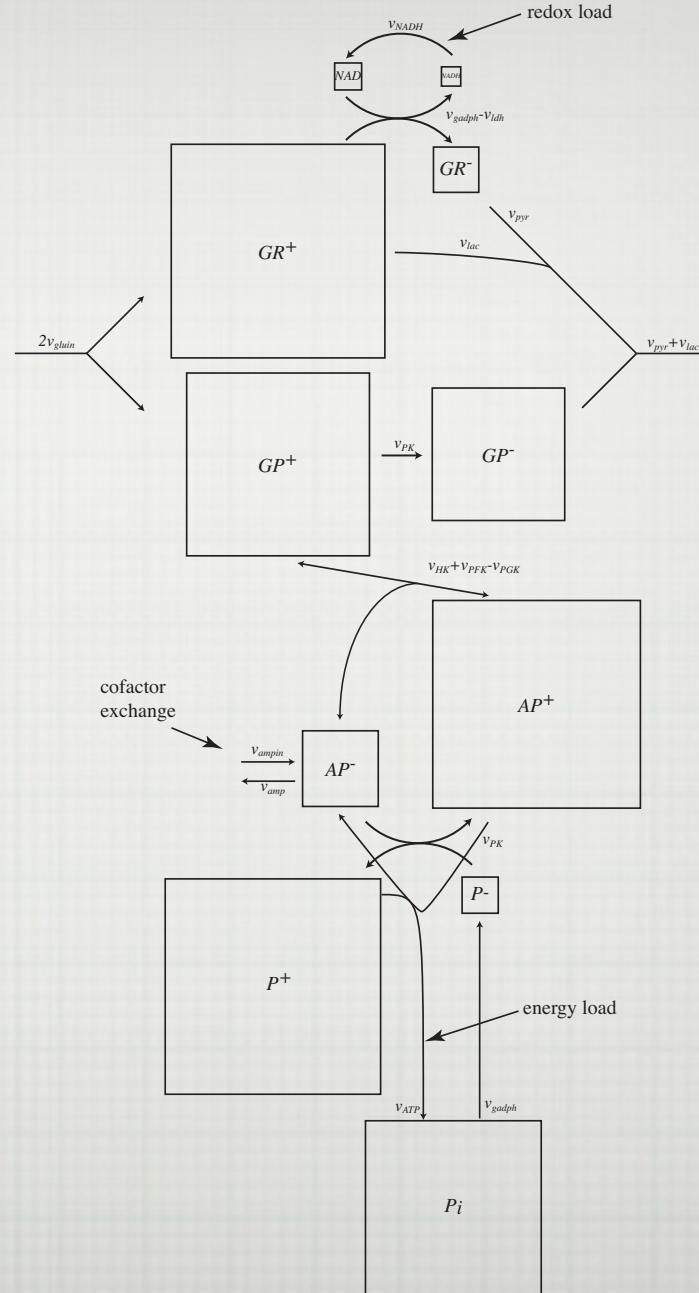
$$d(\mathbf{P}\mathbf{x})/dt = \mathbf{PS} \mathbf{v}(\mathbf{x};\mathbf{k})$$

Table 10.8 The fluxes that move the pools. This table is obtained from the product \mathbf{PS} . The time-invariant pools are in the last two rows. The number of reactions ρ_i that move a pool is shown, as are the pool size, the steady-state flux in and out of the pool, and its turnover time. The number of pools that a reaction moves, π_j , is also given

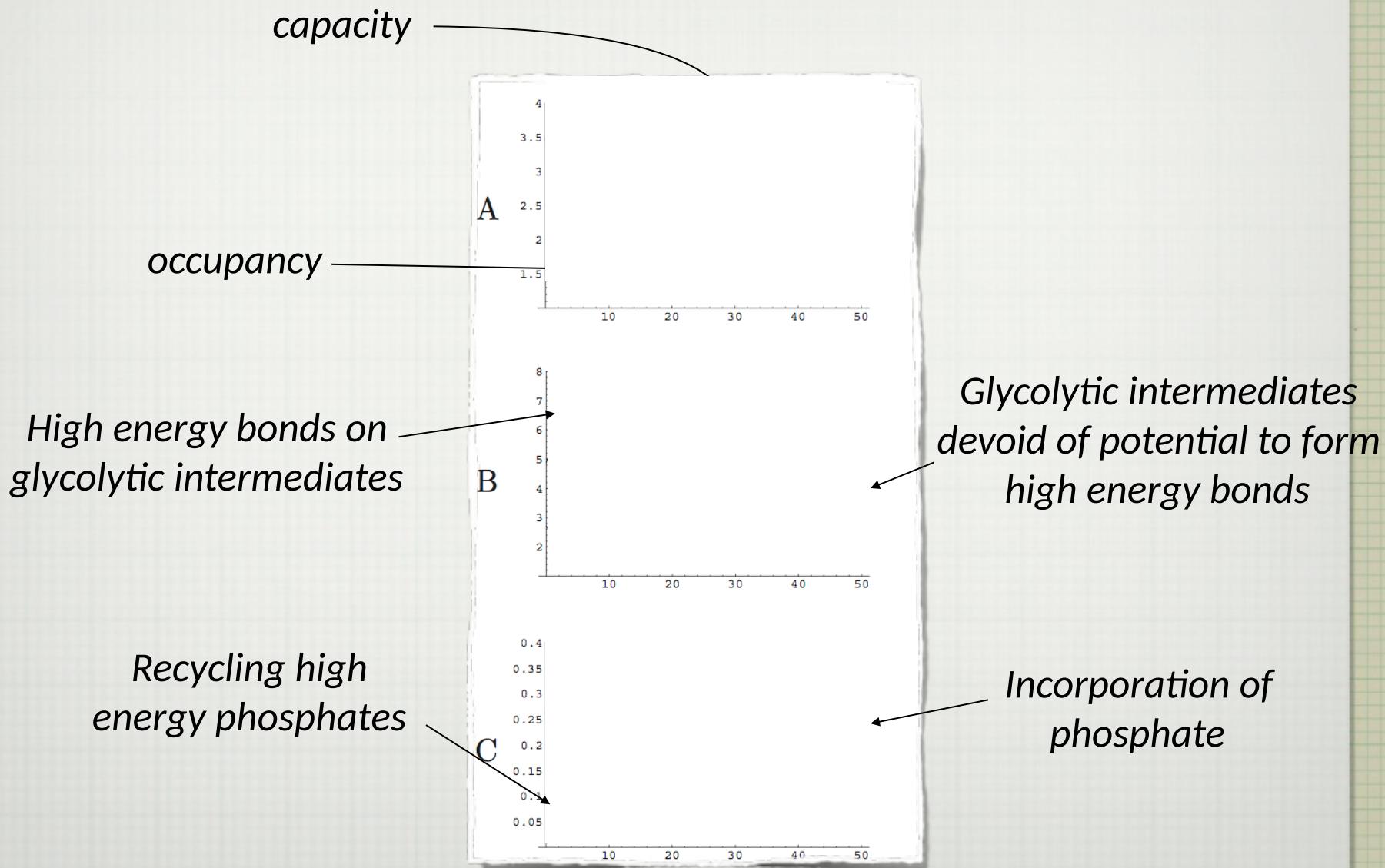
	V_{NADH}	V_{PGI}	V_{PFK}	V_{TPI}	V_{ALD}	V_{GAPDH}	V_{PGK}	V_{PGLM}	V_{ENO}	V_{PK}	V_{LDH}	V_{AMP}	V_{APK}	V_{PYR}	V_{LAC}	V_{ATP}	V_{NADH}	V_{GLUin}	V_{AMPin}	V_{H^+}	$V_{\text{H}_2\text{O}}$	ρ_i	Size (mM)	Net stst flux (mM/h)	τ (h)
GP ⁺	1	0	1	0	0	0	-1	0	0	-1	0	0	0	0	0	0	0	2	0	0	0	5	2.70	4.48	0.60
GP ⁻	0	0	0	0	0	0	0	0	0	1	0	0	0	-1	-1	0	0	0	0	0	0	3	1.42	2.24	0.63
AP ⁺	-1	0	-1	0	0	0	1	0	0	1	0	0	0	0	0	-1	0	0	0	0	0	5	3.49	4.48	0.78
AP ⁻	1	0	1	0	0	0	-1	0	0	-1	0	-1	0	0	0	1	0	0	1	0	0	5	0.46	4.51	0.10
GR ⁺	0	0	0	0	0	-1	0	0	0	0	1	0	0	0	-1	0	0	2	0	0	0	4	3.69	4.26	0.87
GR ⁻	0	0	0	0	0	1	0	0	0	0	-1	0	0	-1	0	0	0	0	0	0	0	3	0.17	2.24	0.07
N ⁺	0	0	0	0	0	1	0	0	0	0	-1	0	0	0	0	-1	0	0	0	0	0	3	0.03	2.24	0.013
P ⁺	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	-1	0	0	0	0	0	2	3.76	2.24	1.68
P ⁻	0	0	0	0	0	1	0	0	0	-1	0	0	0	0	0	0	0	0	0	0	0	2	0.11	2.24	0.05
π_j	3	0	3	0	0	4	3	0	0	6	3	1	0	2	2	3	1	2	1	0	0	0	0.11	2.24	0.05
P _{tot}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.36	8	
N _{tot}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.09	8	

Pool Map:

shows their
interconnections
and steady state
concentrations by
area of square



Dynamic Responses of the Pools

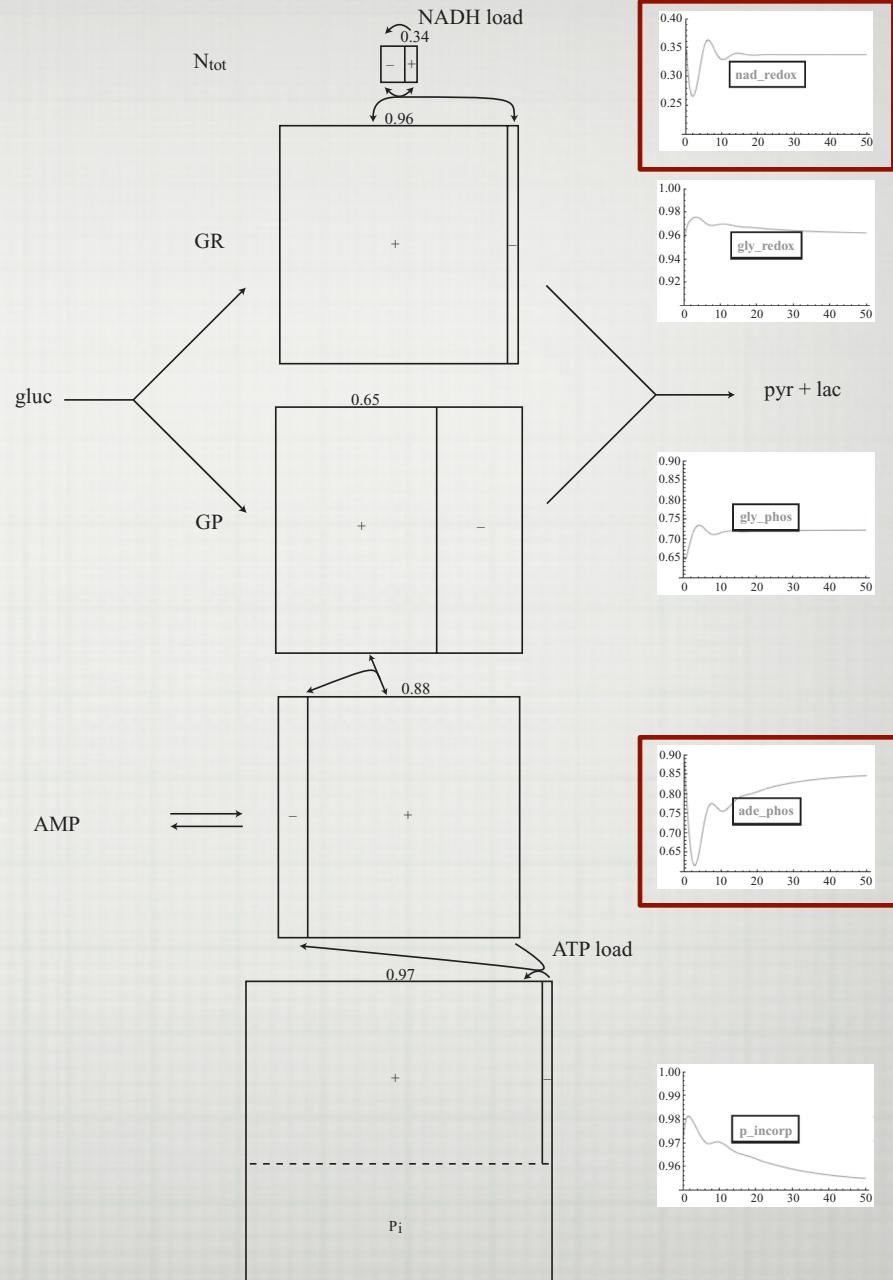


Towards physiology

RATIOS

Property ratios or charges and their dynamic responses

Ratio	#	$t = 0$	$t = 50$
Glycolytic energy charge	1	0.656	0.723
Adenylate energy charge	2	0.883	0.847
Glycolytic redox charge	3	0.957	0.962
NADH redox charge	4	0.338	0.338
Phosphate recycle ratio	5	0.972	0.955



Assumptions

- Time invariant pools
 - NAD+NADH and total phosphate
- Constant input and environment
 - Glucose and AMP_{in} are constants
 - Pyr, Lac, pH in plasma is constant
- Constant volume and electro-neutrality
- No Regulation (see part 4)

Summary: model building

- First draft dynamic models can be obtained from using measured concentration values, elementary reactions, and associated mass action kinetics.
- Dynamic simulation can be performed for perturbation in environmental parameters and the responses examined in terms of the concentrations and the fluxes.
- This first draft can be used as a scaffold to build more complicated models that include
 - interactions with other pathways (Chapters 11 & 12); and
 - regulatory effects (Chapter 14)
- This leads to a module-by-module approach to building models of complex systems

Summary: interpretation

- A metabolic map can be analyzed for its stoichiometric texture to assess co-factor coupling characteristics.
- Such breakdown of the biochemistry helps define pools that are physiologically meaningful from a metabolic perspective, and are context dependent.
- The raw output of the simulation can be post processed with a pooling matrix that allows the pools and their ratios to be graphed to obtain a deeper interpretation of dynamic responses.
- Some of the responses are built into the topological features of a network and require no regulatory action.
- The identification of the reactions that move the key pools is possible by the use of the stoichiometric matrix.

The End