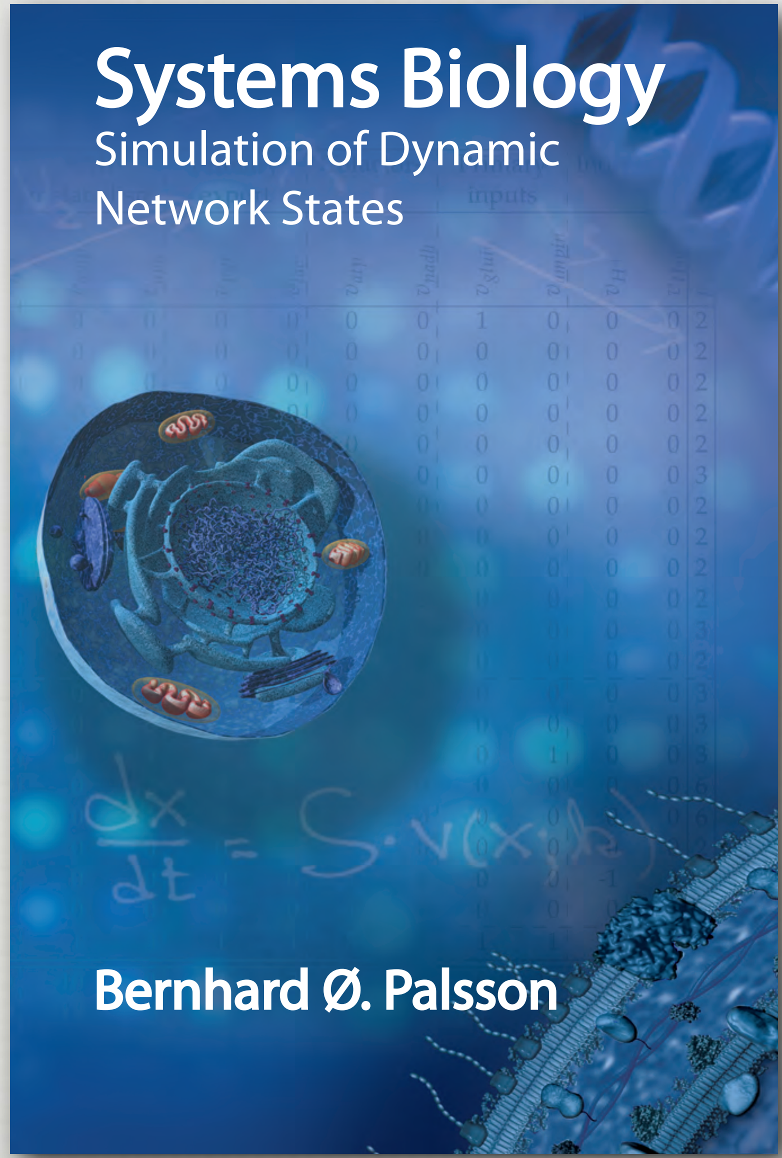


Lecture #11

Coupling Pathways

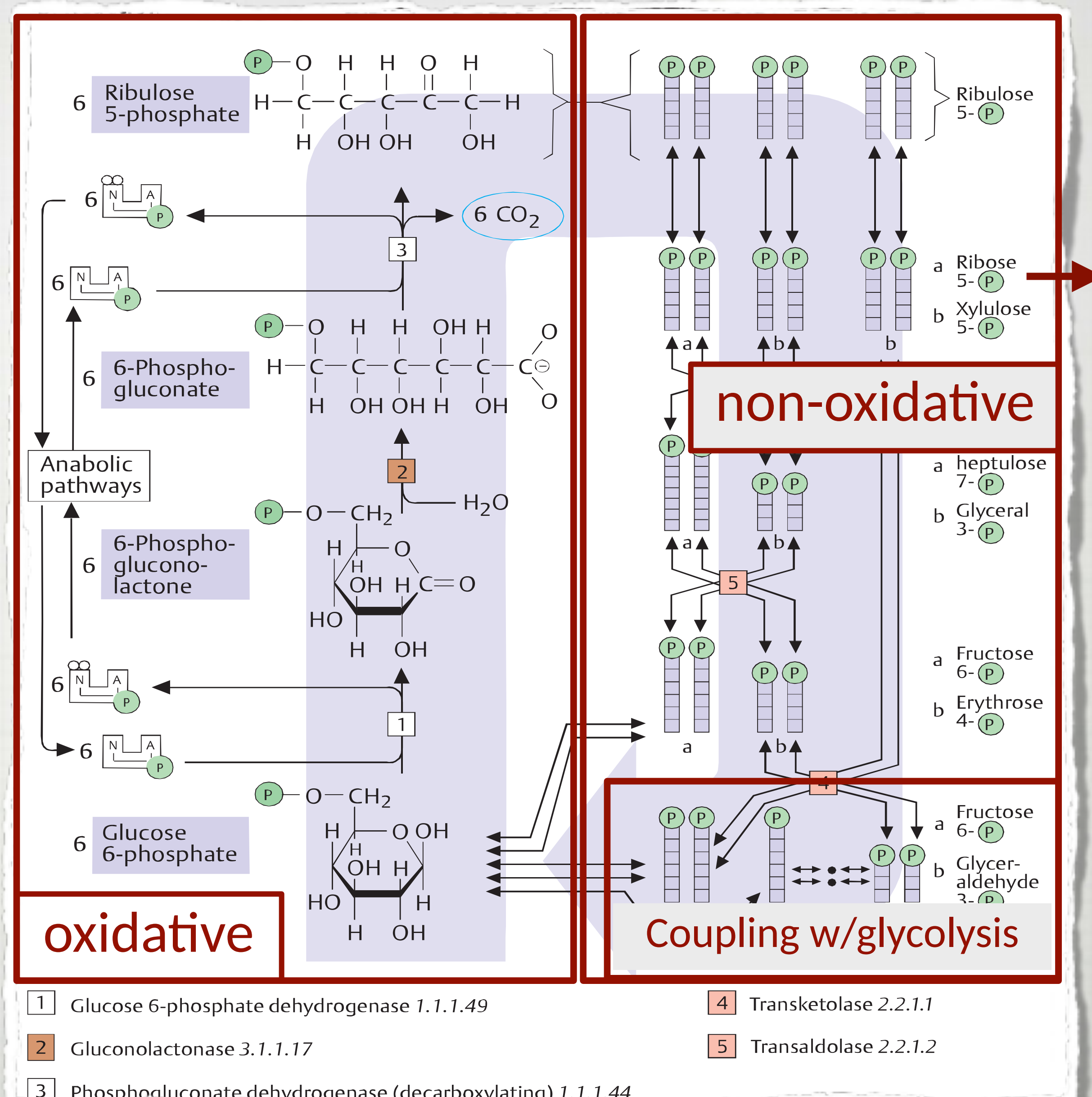
Outline

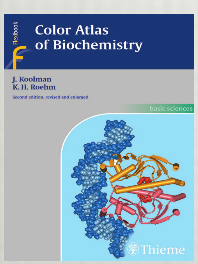
- The pentose pathway;
  - a central metabolic pathway producing pentoses and NADPH
- Co-factor coupling & inorganics
  - NADPH, GSH, CO_2
- The stoichiometric matrix for PPP
  - Its null spaces
- Setting up a simulation model
  - Steady state
- Coupling to glycolysis
  - Dynamic simulation
- Interpreting the results from simulation
  - Concentrations, fluxes, pools, ratios

Basics of pentose metabolism

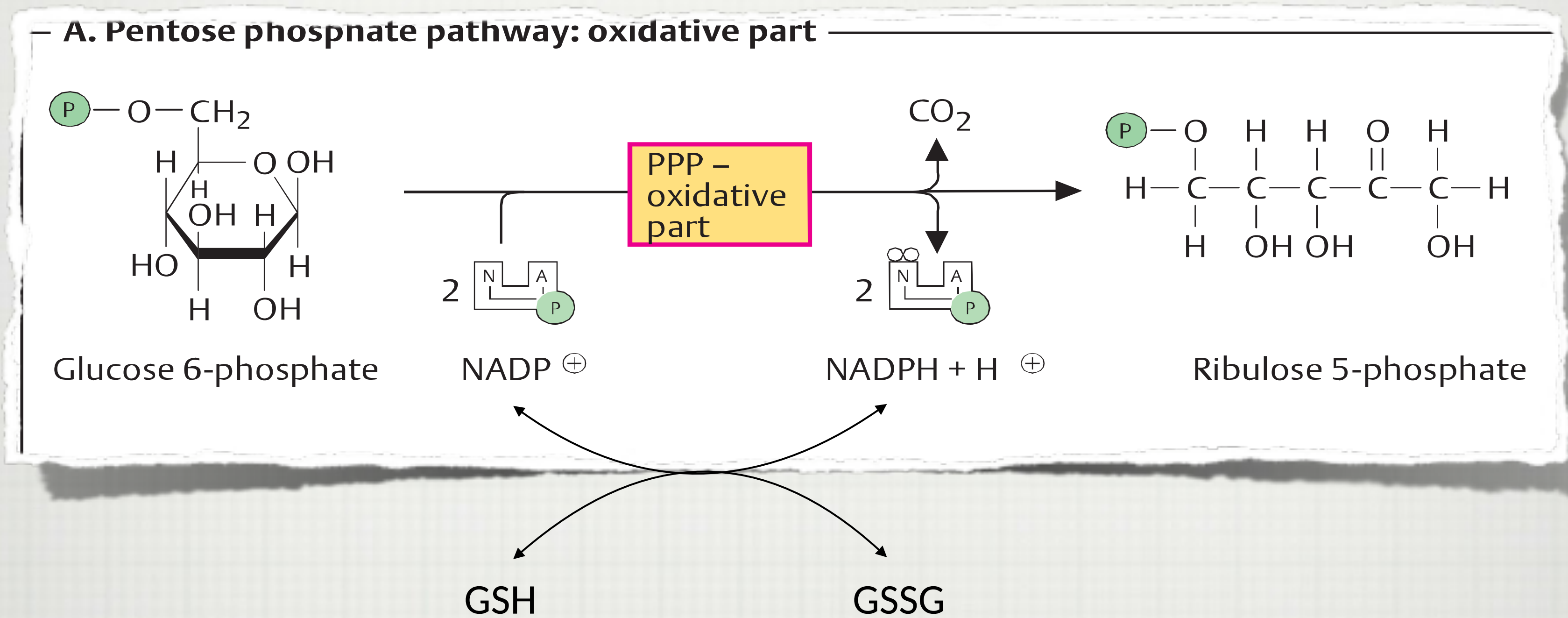
BIOCHEMISTRY PRIMER

PPP: the oxidative and non-oxidative branches



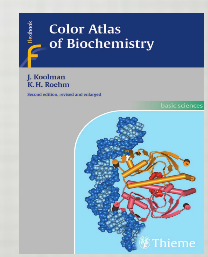


PPP: oxidative branch



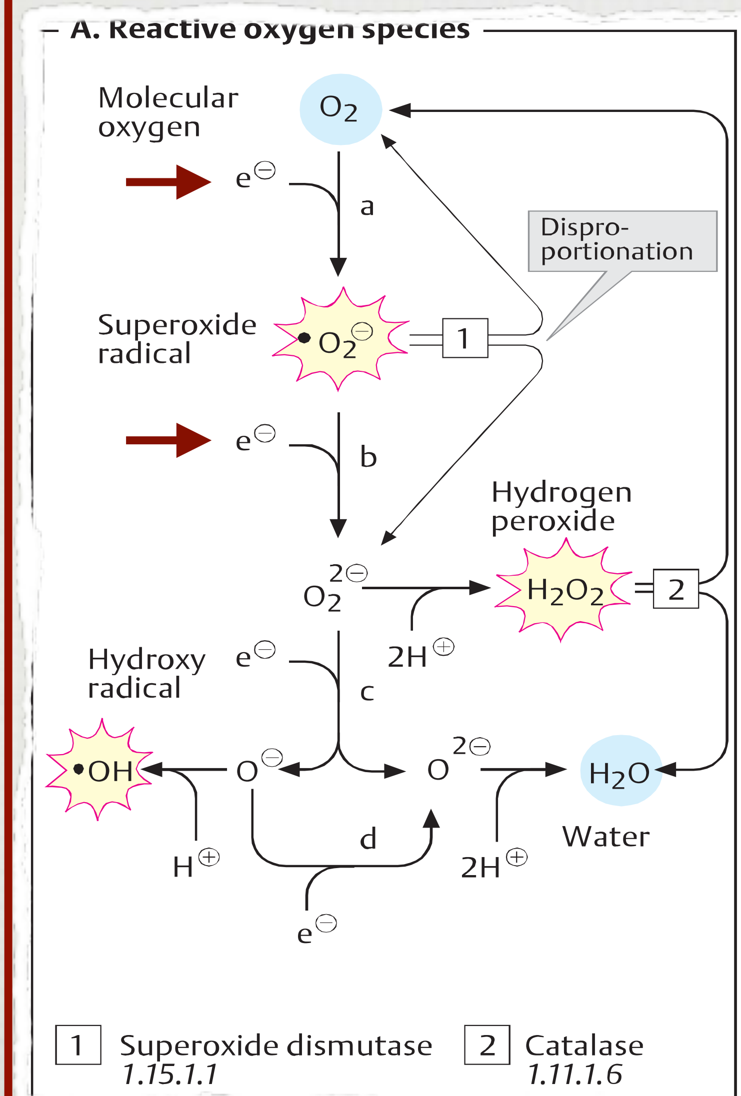
Glutathione buffers NADPH:

GSH + NADP ⇔ GSSG + NADPH



Ox-redox chemistry

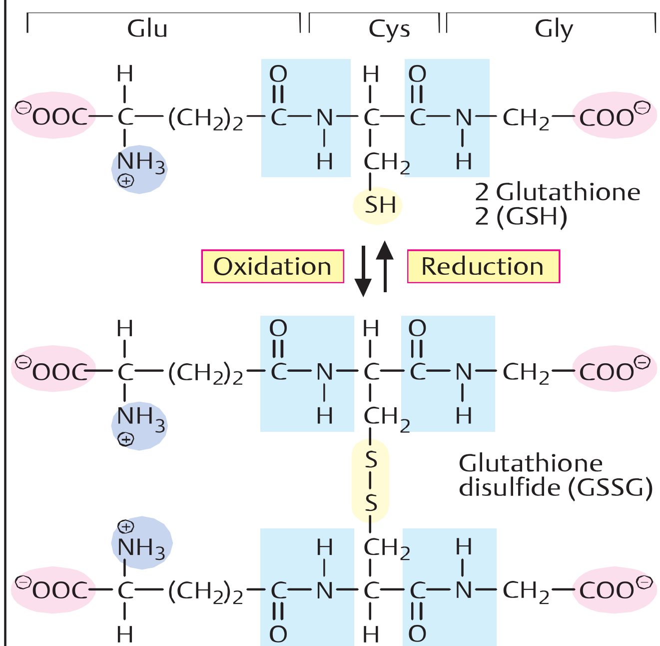
$$-S{:}H + -S{:}H \longrightarrow -S{:}S- + 2e^- + 2H^+$$



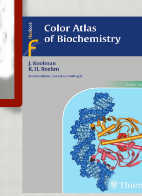
B. Biological antioxidants

Quinols and enols	α-Tocopherol (vitamin E) Ubiquinol (coenzyme Q) Ascorbic acid (vitamin C)
Carotenoids	β-Carotin Lycopin
Others	Glutathione Bilirubin

1. Examples

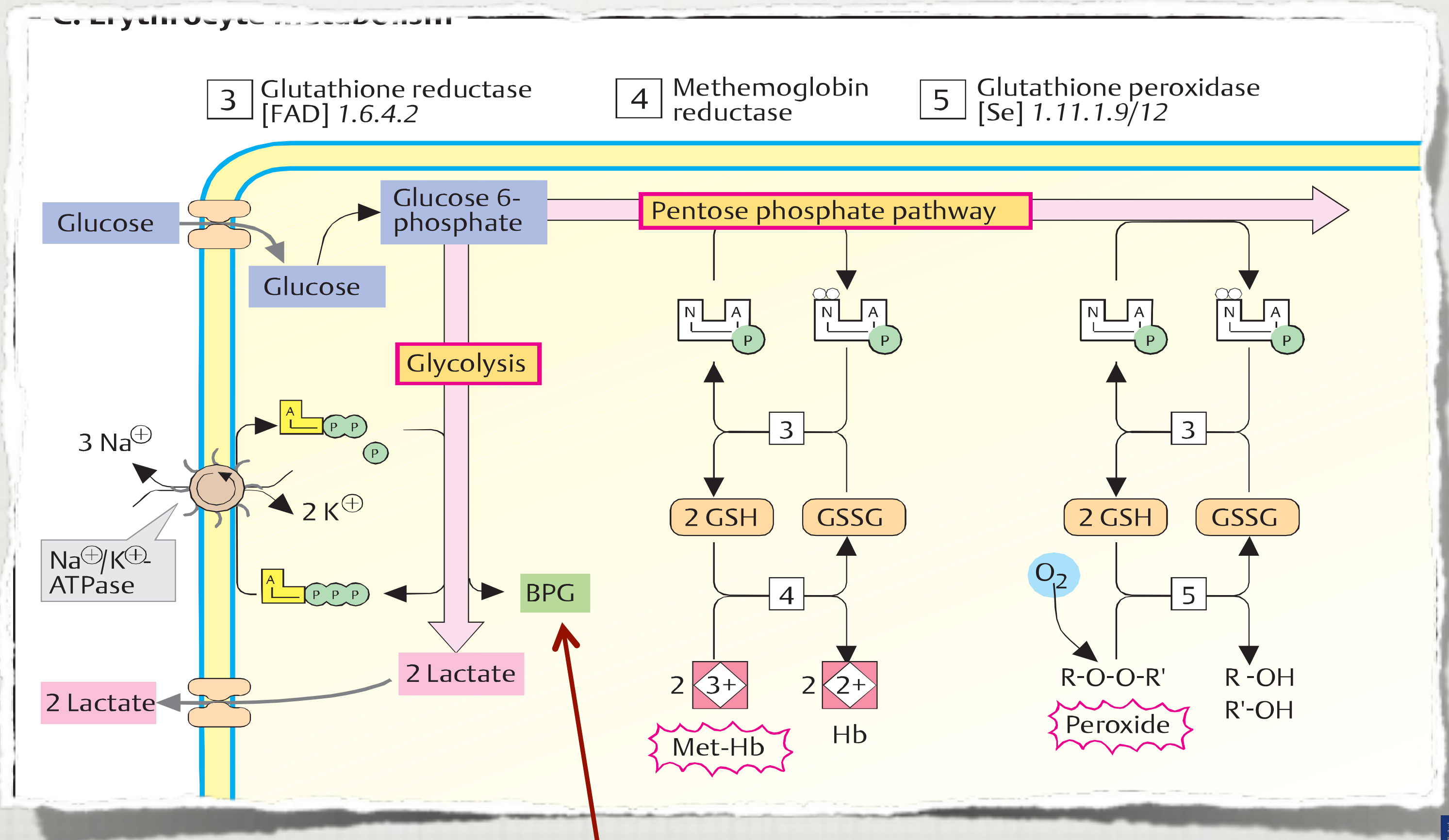


2. Glutathione

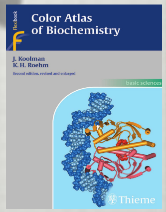


The RBC has a unique metabolite:

More later (CH 13)



Same as 23DPG
Binds to Hb

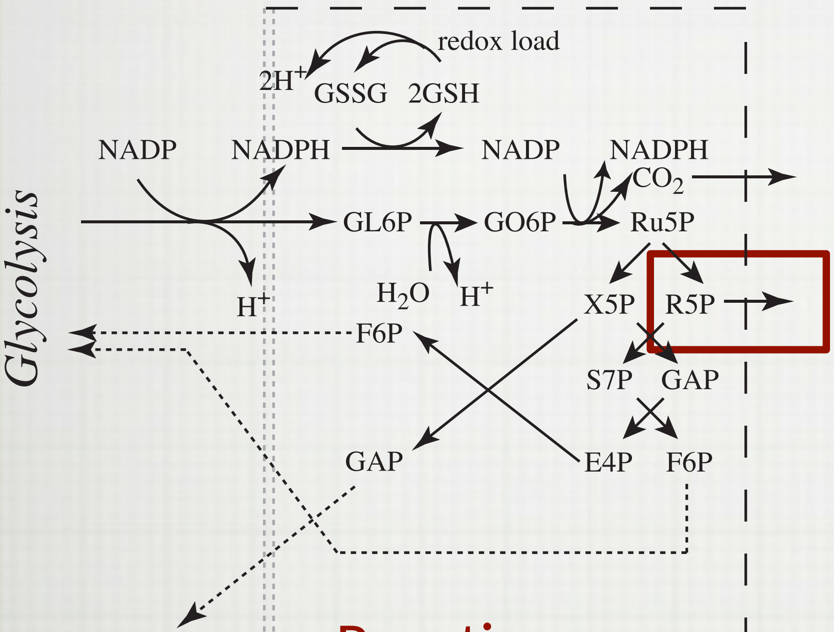


From biochemistry to a simulation model

THE PENTOSE PHOSPHATE PATHWAY

Pentose Phosphate Pathway

Reaction map



Compounds

#	Abbreviation	Intermediates/cofactors	Concentration (mM)
21	GL6P	6-Phosphogluconolactone	0.001 75
22	GO6P	6-Phosphogluconate	0.037 5
23	Ru5P	Ribulose 5-phosphate	0.004 94
24	X5P	Xylose 5-phosphate	0.014 8
25	R5P	Ribose 5-phosphate	0.012 7
26	S7P	Sedoheptulose 7-phosphate	0.024 0
27	E4P	Erythrose 4-phosphate	0.005 08
28	NADP	Nicotinamide adenine dinucleotide phosphate (oxidized)	0.000 2
29	NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)	0.065 8
30	GSH	Glutathione (reduced)	3.2
31	GSSG	Glutathione (oxidized)	0.12
32	CO_2	Carbon dioxide	1.0 (arbitrary)

Reactions

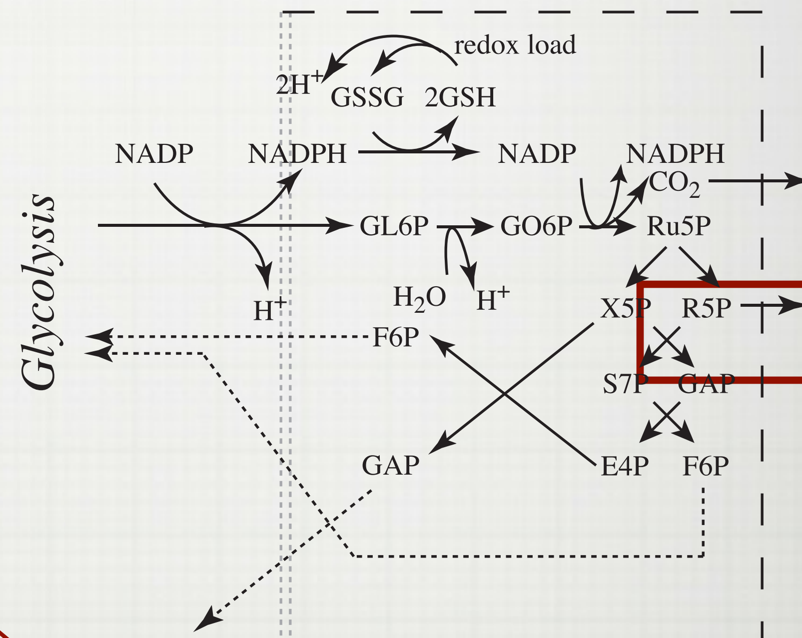
	Abbreviation	Enzymes/transporter/load	Elementally balanced reaction
22	G6PDH	G6P dehydrogenase	G6P + NADP → GL6P + NADPH + H
23	PGLase	6-Phosphogluconolactonase	GL6P + H_2O → GO6P + H
24	GL6PDH	GO6P dehydrogenase	GO6P + NADP → RU5P + NADPH + CO_2
25	R5PE	X5P epimerase	RU5P ⇌ X5P
26	R5PI	R5P isomerase	RU5P ⇌ R5P
27	TKI	Transketolase I	X5P + R5P ⇌ S7P + GAP
28	TKII	Transketolase II	X5P + E4P ⇌ F6P + GAP
29	TALA	Transaldolase	S7P + GAP ⇌ E4P + F6P
30	GSSGR	Glutathione reductase	GSSG + NADPH + H ⇌ 2GSH + NADP
31	GSHR	Glutathione oxidase	2GSH ⇌ GSSG + 2H
32	CO_2 exch	Freely exchanging CO_2	$CO_{2,in} \rightarrow CO_{2,out}$

Elemental composition

	G6P	F6P	GAP	GL6P	GO6P	RU5P	X5P	R5P	S7P	E4P	NADP	NADPH	GSH	GSSG	CO_2	H	H_2O
C	6	6	3	6	6	5	5	5	7	4	0	0	10	20	1	0	0
H	11	11	5	9	10	9	9	9	13	7	0	1	17	32	0	1	2
O	9	9	6	9	10	8	8	8	10	7	0	0	6	12	2	0	1
P	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
N	0	0	0	0	0	0	0	0	0	0	0	0	3	6	0	0	0
S	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0
NADP	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
G_{tot}	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0
NP_{tot}	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0

PPP: S Matrix and Simulation

	I/O				Pentose pathway reactions								Cofactors		Inorganics		
	v_{G6Pin}	v_{F6P}	v_{GAP}	v_{R5P}	v_{G6PDH}	v_{PGLASE}	v_{GL6PDH}	v_{R5PE}	v_{R5PI}	v_{TKI}	v_{TKII}	v_{TALA}	v_{GSSGR}	v_{GSHR}	v_{CO_2}	v_H	v_{H_2O}
G6P	1	0	0	0	−1	0	0	0	0	0	0	0	0	0	0	0	0
F6P	0	−1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
GAP	0	0	−1	0	0	0	0	0	0	1	1	−1	0	0	0	0	0
GL6P	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0
GO6P	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0
RU5P	0	0	0	0	0	0	1	−1	−1	0	0	0	0	0	0	0	0
X5P	0	0	0	0	0	0	0	1	0	−1	−1	0	0	0	0	0	0
R5P	0	0	0	−1	0	0	0	0	1	−1	0	0	0	0	0	0	0
S7P	0	0	0	0	0	0	0	0	0	1	0	−1	0	0	0	0	0
E4P	0	0	0	0	0	0	0	0	0	0	−1	1	0	0	0	0	0
NADP	0	0	0	0	−1	0	−1	0	0	0	0	0	1	0	0	0	0
NADPH	0	0	0	0	1	0	1	0	0	0	0	0	−1	0	0	0	0
GSH	0	0	0	0	0	0	0	0	0	0	0	0	2	−2	0	0	0
GSSG	0	0	0	0	0	0	0	0	0	0	0	0	−1	1	0	0	0
CO_2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	−1	0	0
H	0	0	0	0	1	1	0	0	0	0	0	0	−1	2	0	−1	0
H_2O	0	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	0	−1
C	6	−6	−3	−5	0	0	0	0	0	0	0	0	0	0	−1	0	0
H	11	−11	−5	−9	0	0	0	0	0	0	0	0	0	0	0	−1	−2
O	9	−9	−6	−8	0	0	0	0	0	0	0	0	0	0	−2	0	−1
P	1	−1	−1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0
N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NADP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$\mathbf{p}_1$	1	$\frac{2}{3}$	$\frac{1}{3}$	0	1	1	1	$\frac{2}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	2	2	1	4	−1
$\mathbf{p}_2$	1	0	0	1	1	1	1	0	1	0	0	0	2	2	1	4	−1
$\mathbf{v}_{stst}$	0.21	0.13	0.067	0.01	0.21	0.21	0.21	0.13	0.077	0.067	0.067	0.067	0.42	0.42	0.21	0.84	−0.21

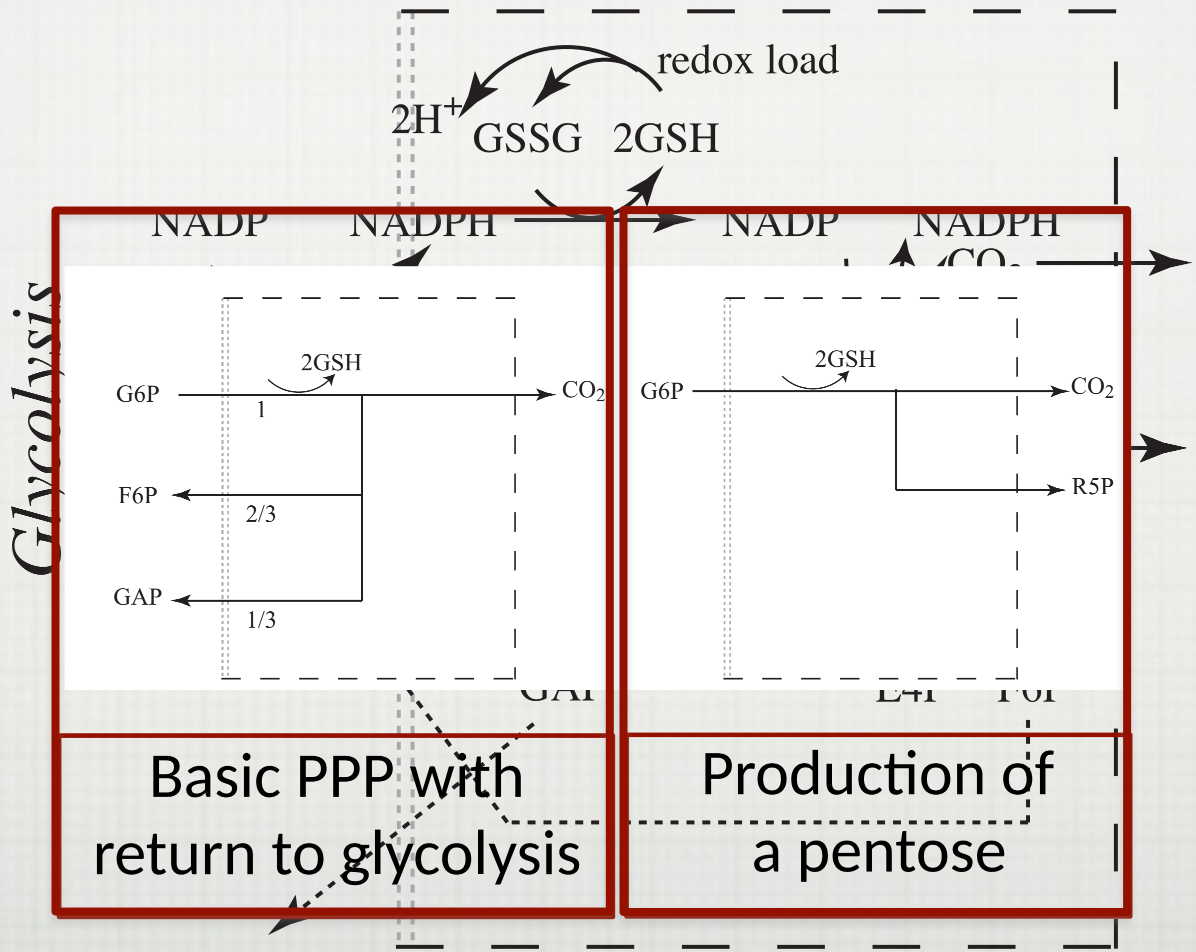


The null space vectors

Stoichiometric matrix

Reaction map

Null Space Basis Vectors: Pathways



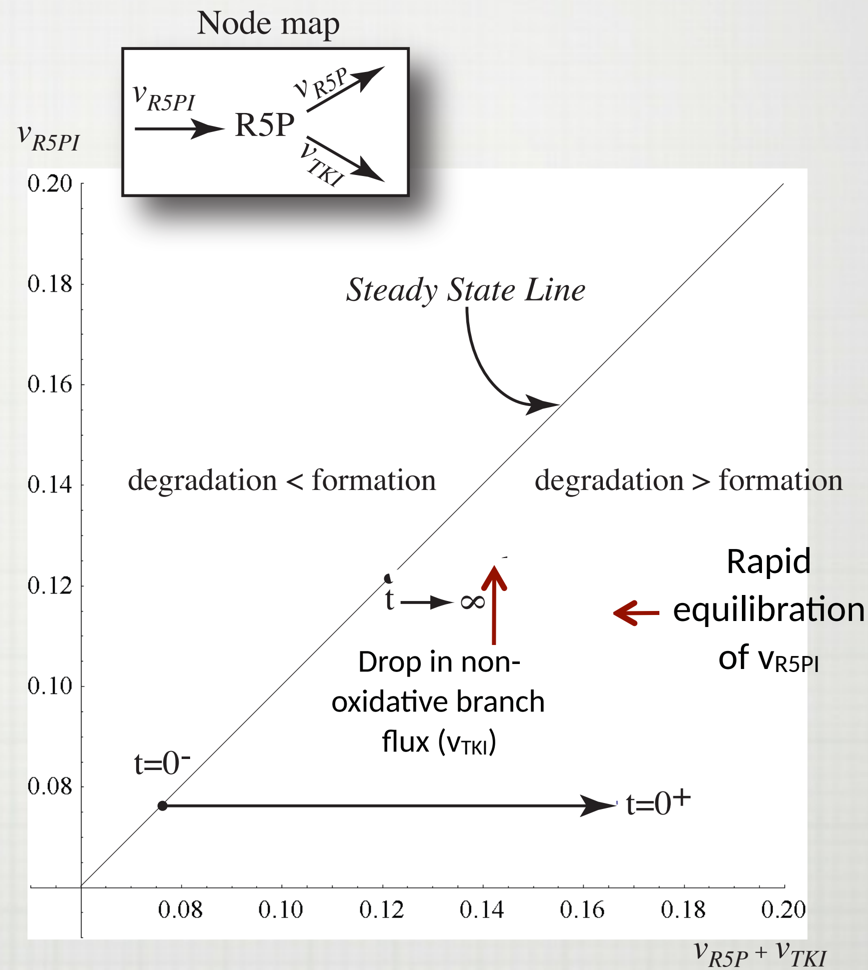
Basic PPP with return to glycolysis

Production of a pentose

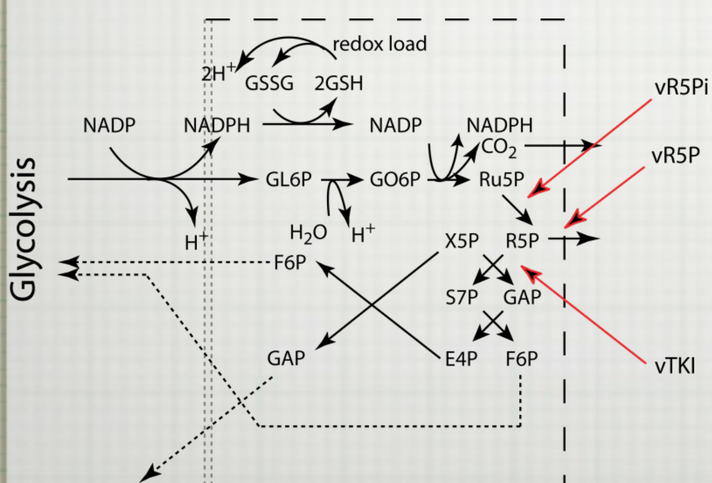
Phase Portraits:

Increasing the rate of R5P production

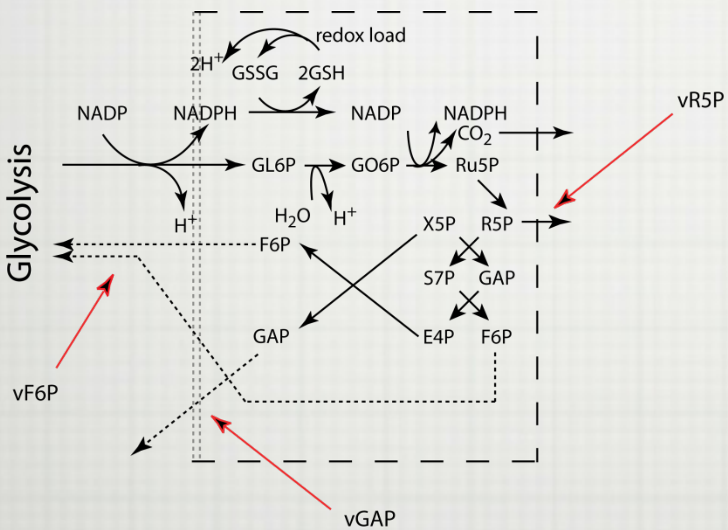
Flux in to R5P node

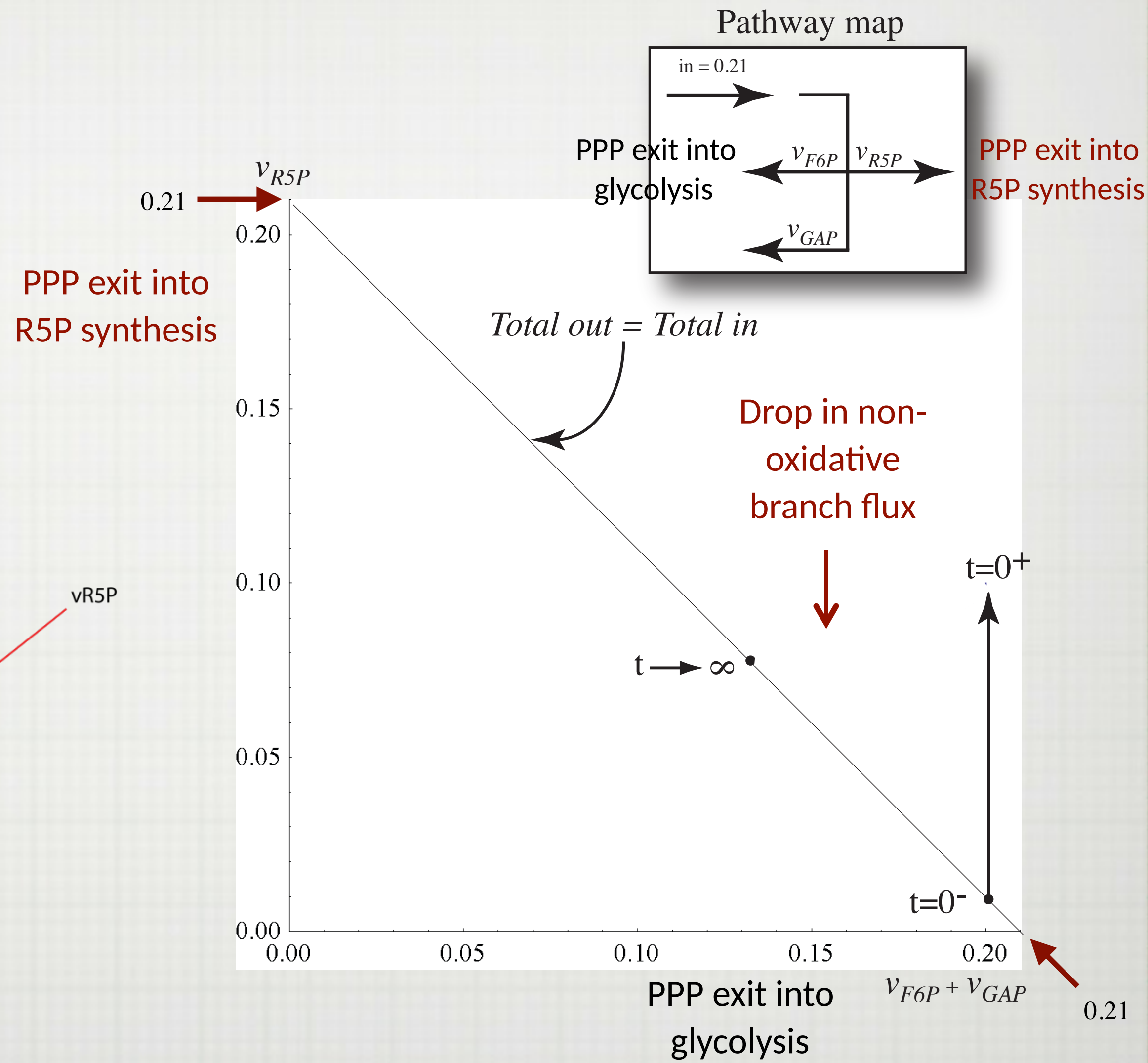


Flux out of R5P node



PPP: Phase Portraits



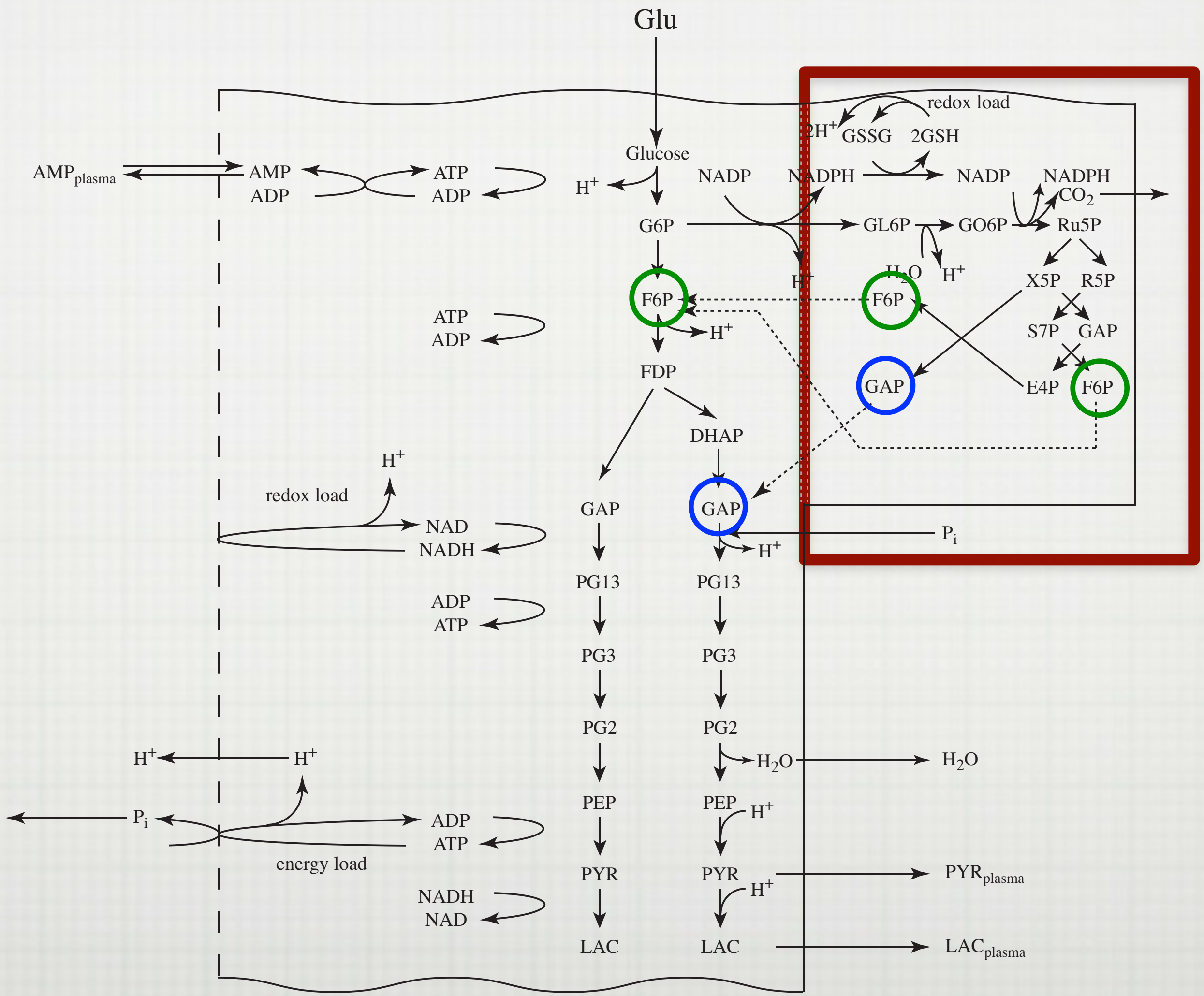


Coupling Pathways

GLYCOLYSIS & THE PENTOSE PHOSPHATE PATHWAY

Coupled pathways:

Glycolysis and the pentose pathway



The integrated S matrix

-S:H

+

-S:H

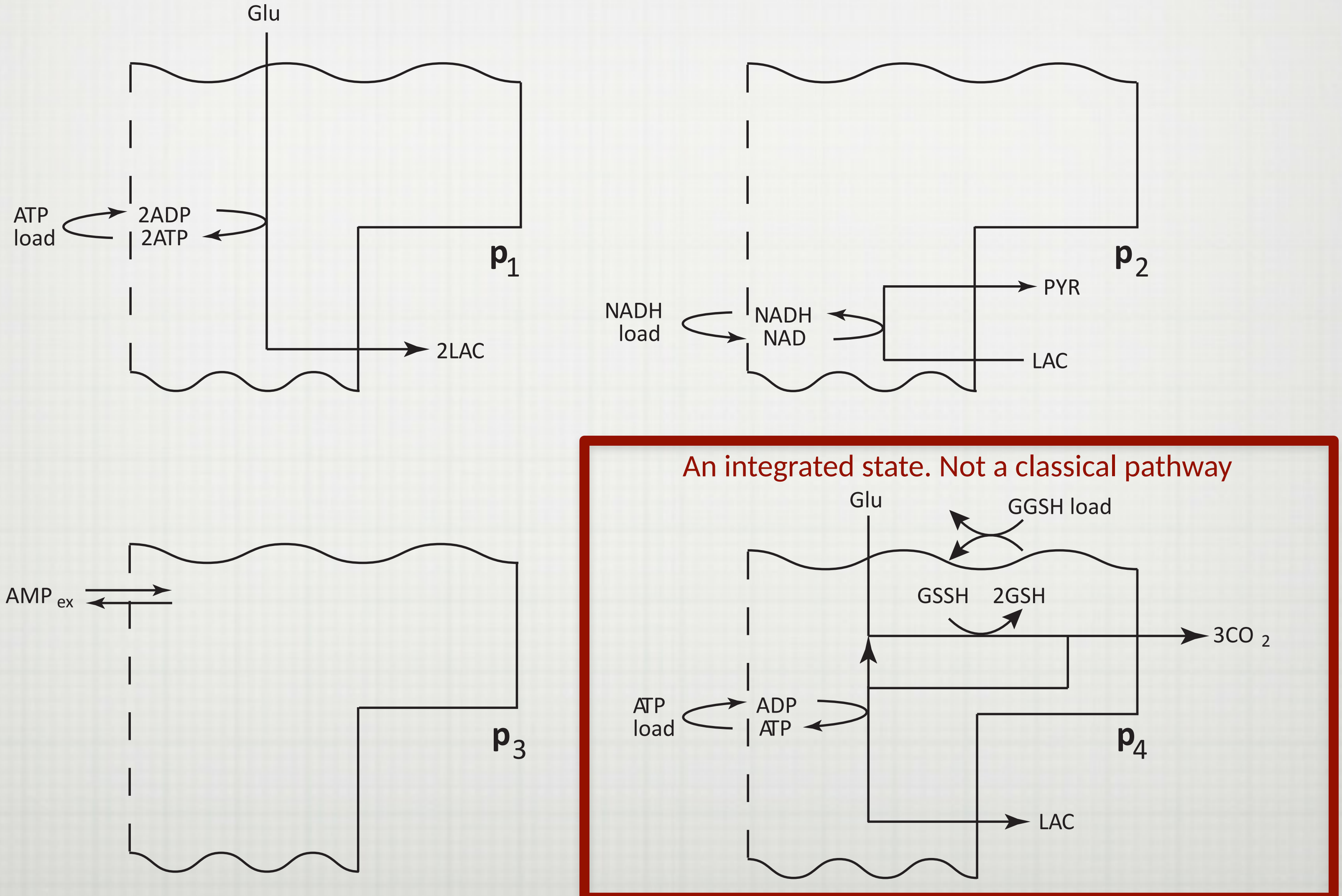
-S:S-

	Glycolysis																					Pentose pathway											
	v_{HK}	v_{PGI}	v_{PFK}	v_{TPI}	v_{ALD}	v_{GADPH}	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_{H_2O}	v_{G6PDH}	v_{PGLASE}	v_{GL6PDH}	v_{R5PE}	v_{R5PI}	v_{TKI}	v_{TKII}	v_{TALA}	v_{GSSGR}	v_{GSHR}	v_{CO_2}	ρ_i
GLU	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
G6P	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	0	3
F6P	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	4
FBP	0	0	1	0	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
DHAP	0	0	0	−1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
GAP	0	0	0	1	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	−1	0	0	0	6
PG13	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
PG3	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								0	0	2
PG2	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0								0	0	2
PEP	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0			Coupling					0	0	2
PYR	0	0	0	0	0	0	0	0	0	1	−1	0	0	−1	0	0	0	0	0	0	0	0	0								0	0	3
LAC	0	0	0	0	0	0	0	0	0	0	1	0	0	0	−1	0	0	0	0	0	0	0	0								0	0	2
NAD	0	0	0	0	0	−1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0								0	0	3
NADH	0	0	0	0	0	1	0	0	0	0	−1	0	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
AMP	0	0	0	0	0	0	0	0	0	0	0	−1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3
ADP	1	0	1	0	0	0	−1	0	0	−1	0	0	−2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
ATP	−1	0	−1	0	0	0	1	0	0	1	0	0	1	0	0	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
PHOS	0	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
H	1	0	1	0	0	1	0	0	0	−1	−1	0	0	0	0	1	1	0	0	−1	0	1	1	0	0	0	0	0	0	−1	2	0	12
H_2O	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	−1	0	0	0	0	−1	0	−1	0	0	0	0	0	0	0	0	0	4
GL6P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	2
GO6P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	2
RU5P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	−1	−1	0	0	0	0	0	0	3
X5P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	−1	−1	0	0	0	0	3
R5P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	2
S7P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	−1	0	0	0	2
E4P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	−1	1	0	0	0	2
NADP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	−1	0	−1	0	0	0	0	0	1	0	0	3
NADPH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	−1	0	0	3
GSH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	−2	0	2
GSSG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	−1	1	0	2
CO_2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	−1	2
π_j	5	2	5	2	3	5	4	2	3	5	5	1	3	1	1	4	3	1	1	1	1	5	4	5	2	2	3	2	2	5	3	1	
$\mathbf{p}_1$	1	1	1	1	1	2	2	2	2	2	2	0	0	0	2	2	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	
$\mathbf{p}_2$	0	0	0	0	0	0	0	0	0	0	−1	0	0	1	−1	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	
$\mathbf{p}_3$	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
$\mathbf{p}_4$	1	−2	0	0	0	1	1	1	1	1	1	0	0	0	1	1	0	1	0	13	−3	3	3	3	2	1	1	1	1	6	6	3	
$\mathbf{v}_{stst}$	1.12	0.91	1.05	1.05	1.05	2.17	2.17	2.17	2.17	2.17	1.95	0.014	0	0.224	1.95	2.17	0.224	1.12	0.014	3.46	−0.21	0.21	0.21	0.21	0.14	0.07	0.07	0.07	0.07	0.42	0.42	0.21	

new measurement

Gly & PPP:

Selected basis based on biochemical intuition

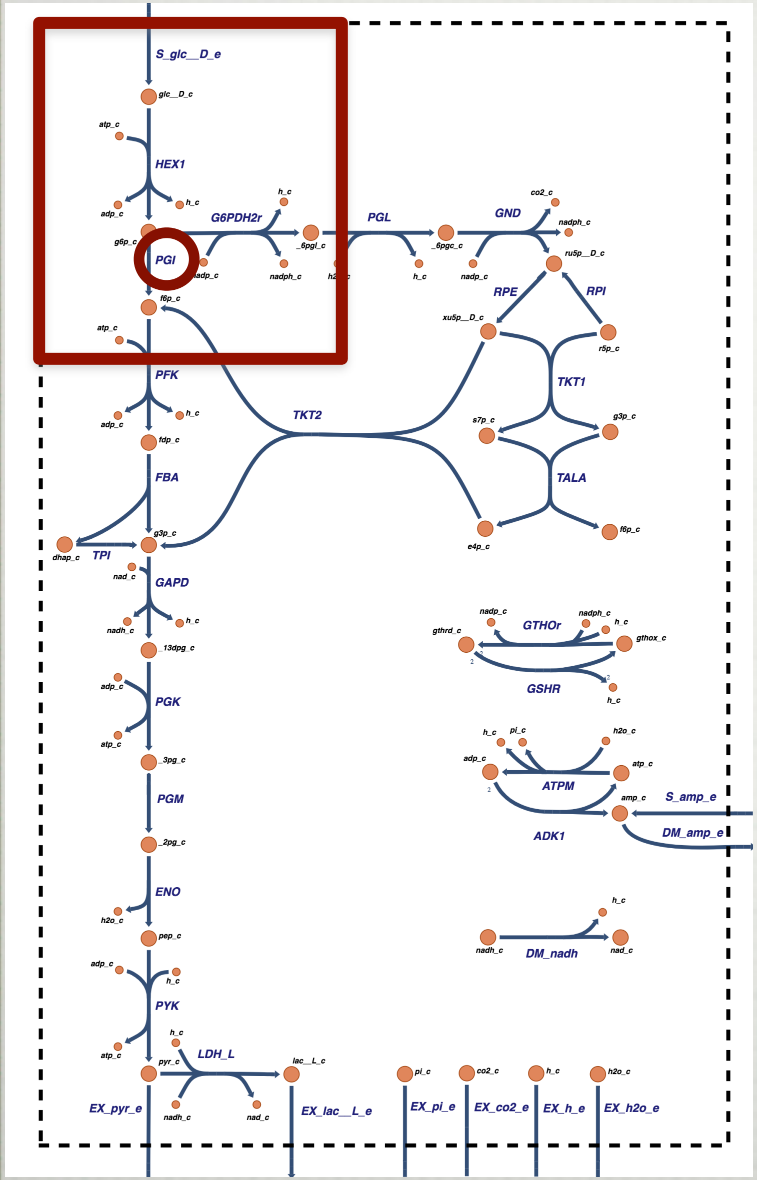


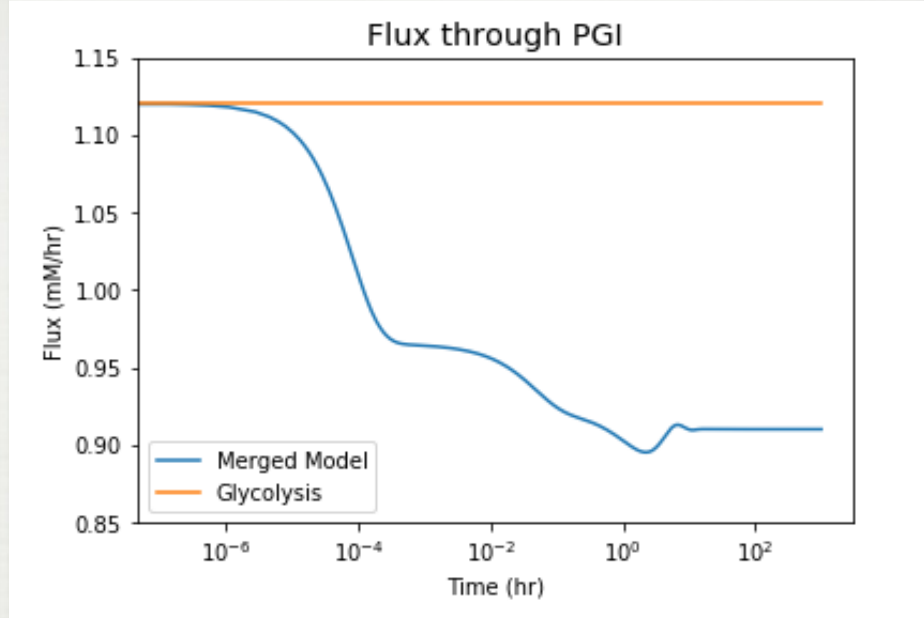
Kinetics Constants: PERCs

The PERCs change a little bit compared to glycolysis alone as the flux map changes due to $\mathbf{p}_4$

#	Reaction	G	K	Γ	k
1	v_{HK}	0.009	850	0	0.700 007
2	v_{PGI}	0.407	0.41	0.994	2961.11
3	v_{PFK}	0.134	310	0	33.1582
4	v_{TPI}	0.046	0.057	0.796	32.2086
5	v_{ALD}	0.08	0.082	0.973	2657.41
6	v_{GAPDH}	0.007	0.018	0.381	3271.23
7	v_{PGK}	1755	1800	0.975	$1.233\,73 \times 10^6$
8	v_{PGLM}	0.146	0.147	0.994	4717.39
9	v_{ENO}	1.504	1.695	0.888	1708.66
10	v_{PK}	19.57	363 000	0	440.186
11	v_{LDH}	44.133	26 300	0.002	1073.94
12	v_{AMP}	0.001	10^6	0	0.161 424
13	v_{ApK}	1.65	1.65	1	1 000 000
14	v_{PYR}	0.995	1	0.995	744.186
15	v_{LAC}	0.735	1	0.735	5.405 56
16	v_{ATP}	0.453	10^6	0	1.356 25
17	v_{NADH}	1.957	10^6	0	7.441 88
18	v_{GLUin}	0	10^6	0	0
19	v_{AMPin}	0	10^6	0	0
20	v_{H}	1	1	1	100 000
21	v_{H_2O}	1	1	1	100 000
22	v_{G6PDH}	11.875	1000	0.012	21 864.6
23	v_{PGLASE}	21.363	1000	0.021	122.323
24	v_{GL6PDH}	43.341	1000	0.043	29 287.8
25	v_{R5PE}	2.995	3	0.998	16 045.9
26	v_{R5PI}	2.566	2.57	0.999	9664.21
27	v_{TKI}	0.932	1.2	0.777	1675.73
28	v_{TKII}	1.921	10.3	0.187	1146.86
29	v_{TALA}	0.575	1.05	0.548	886.848
30	v_{GSSGR}	0.259	100	0.003	53.3298
31	v_{GSHR}	0.012	2	0.006	0.041 26
32	v_{CO_2}	1	1	1	100 000

One PERC Changes





	K_{eq} Symbol	K_{eq} Value	PERC Symbol	PERC Value	Γ	Γ/K_{eq}
HEX1	K_{HEX1}	850.000	k_{HEX1}	0.700	0.008809	0.000010
PGI	K_{PGI}	0.410	k_{PGI}	3644.444	0.407407	0.993677

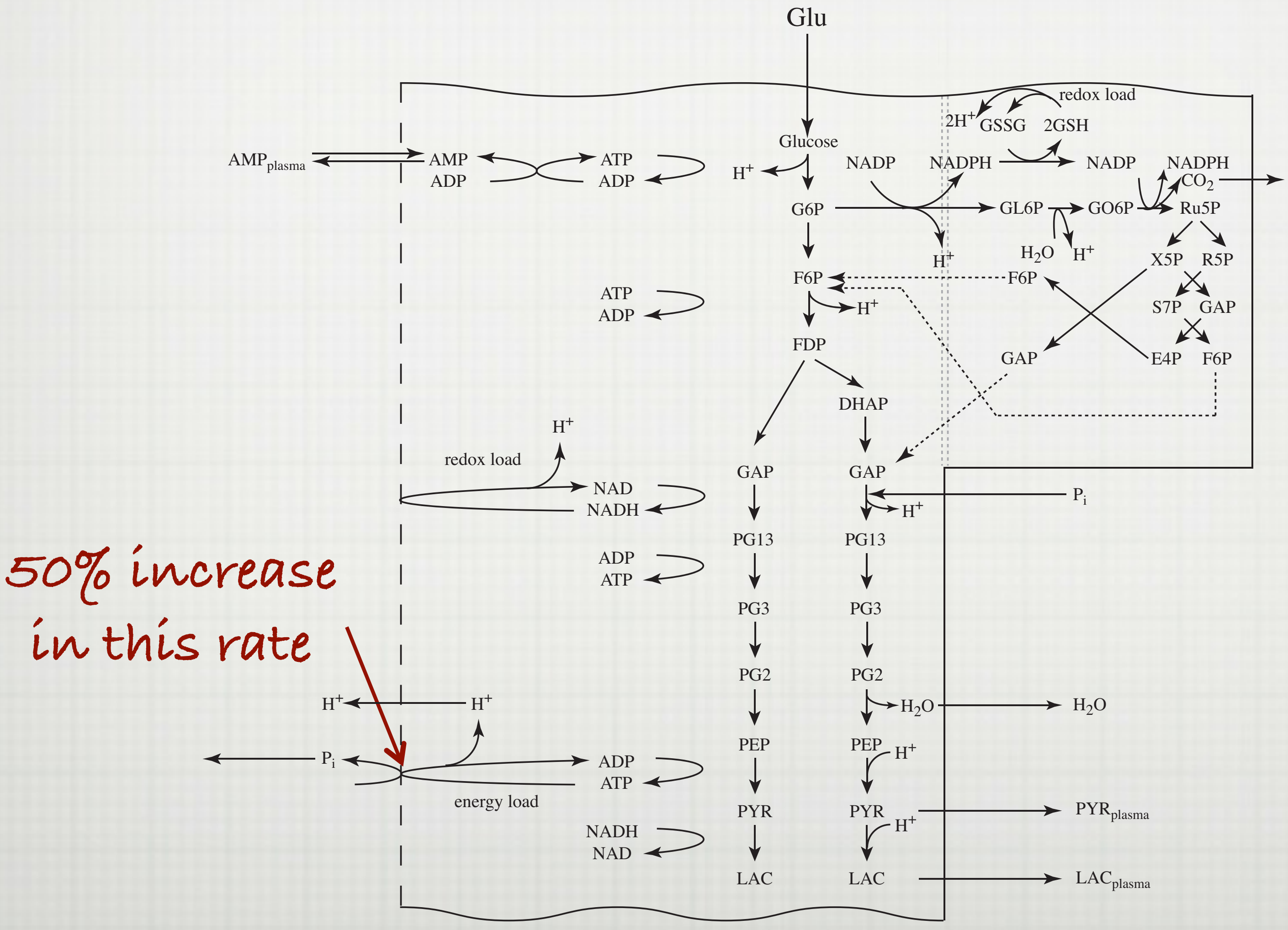
PGI	K_{PGI}	0.410	k_{PGI}	2961.111	0.407407	0.993677

Biochemistry

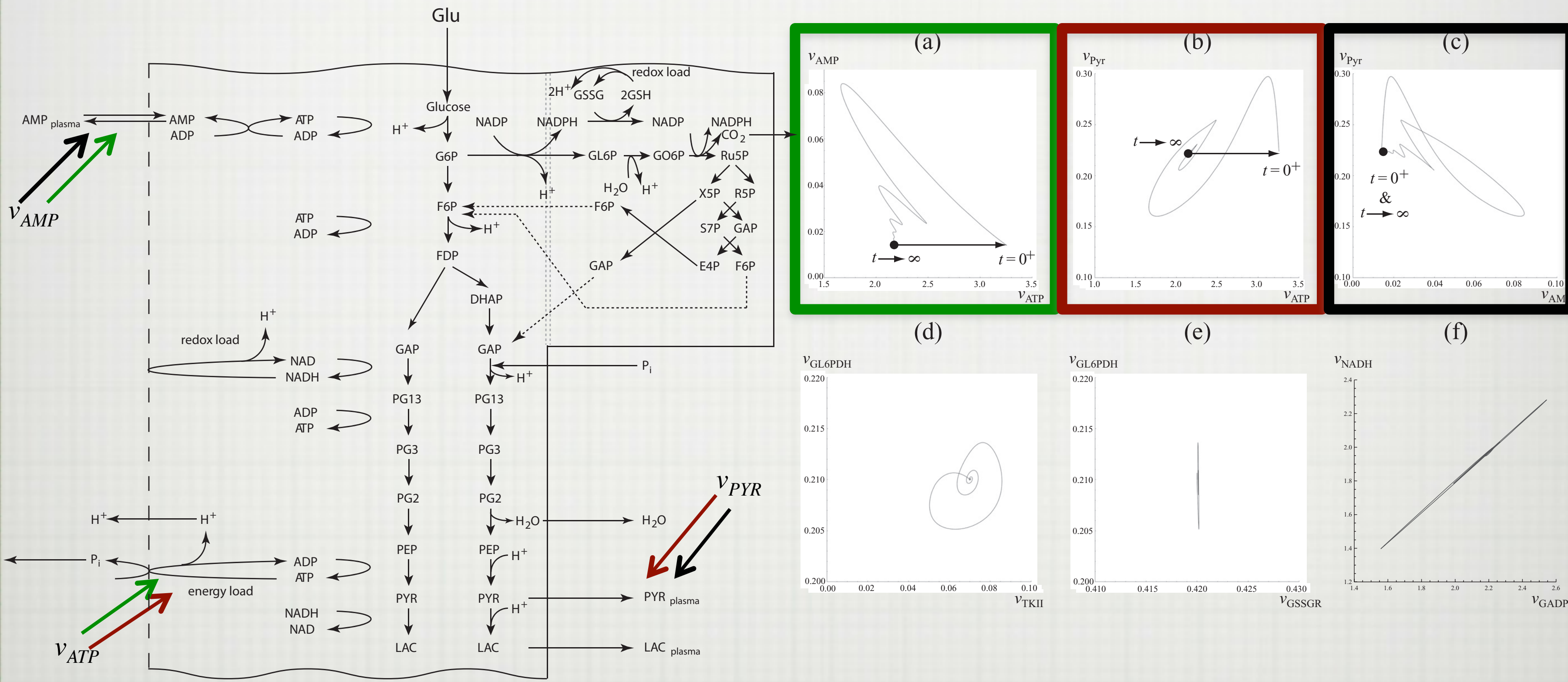
SIMULATING FLUXES AND CONCENTRATIONS

Glycolysis & PPP:

Energy load



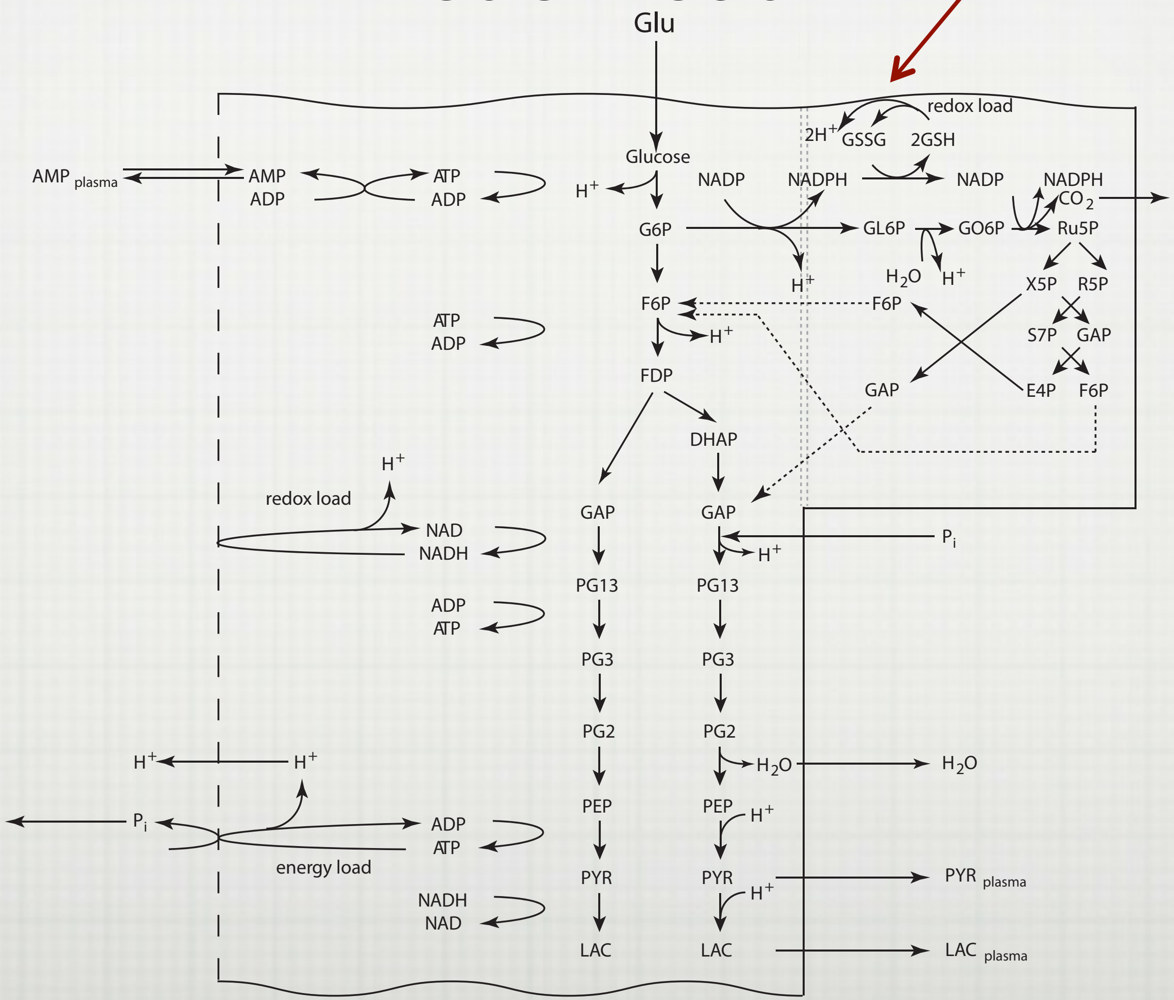
Glycolysis & PPP: Phase Portraits



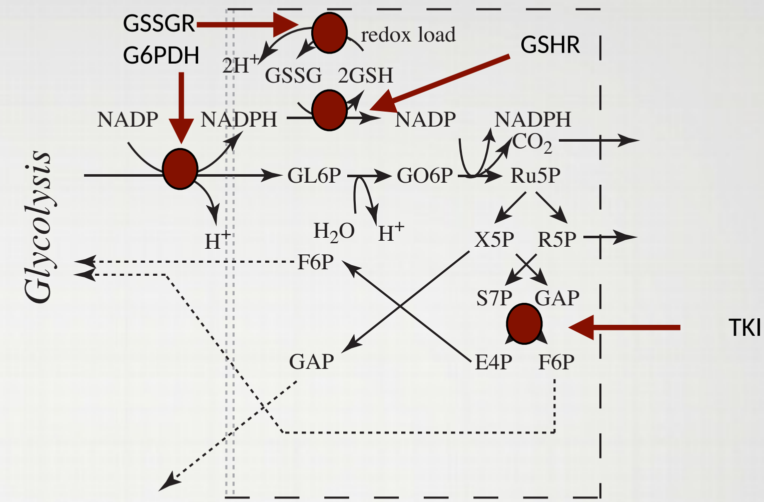
Glycolysis & PPP:

Redox load

Double this rate



Dynamic Simulation



G6PDH

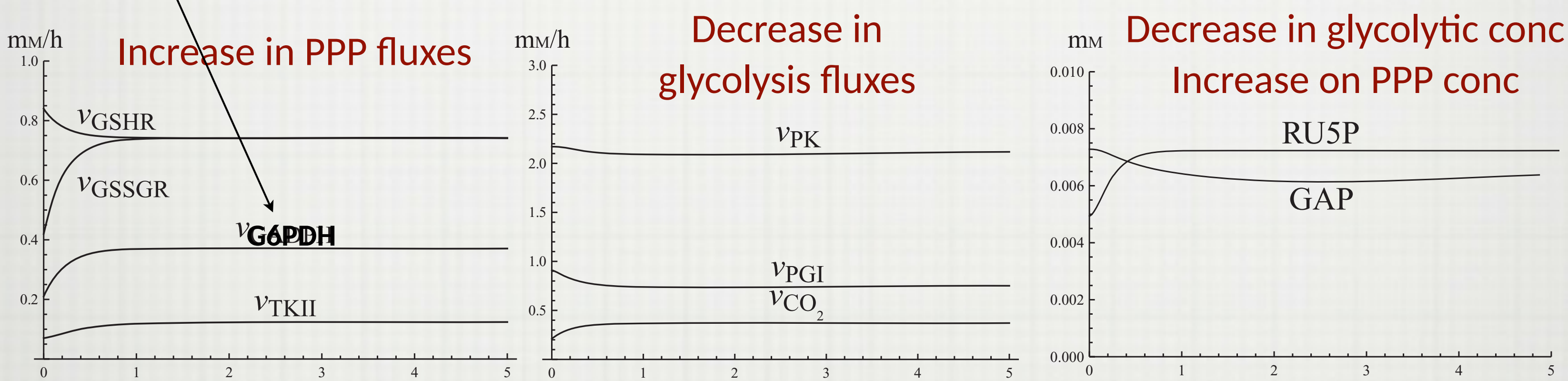


Figure 11.7 Dynamic response of the integrated system of glycolysis and the pentose path way, doubling the rate of GSH utilization. (a) The dynamic response of the fluxes in th oxidative (v_{G6PDH}) and the nonoxidative (v_{TKII}) branches of the pentose pathway as well a GSH reductase and oxidase (the load). (b) The dynamic response of the upper (v_{PGI}) and lowe (v_{PK}) glycolytic fluxes, as well as CO_2 production by the pentose pathway. (c) The dynami response of sample intermediates in glycolysis, GAP, and the pentose pathway, Ru5P.

Summary

- The pentose pathway can be described in a similar way as glycolysis
- A simulation model can be formulated
- The two stoichiometric matrices describing the two pathways can be merged
- The coupling metabolites are G6P, F6P, GAP
- The structure of the null space for glycolysis alone and the coupled pathways is similar
- Simulation to 50% increase in rate of utilization of ATP is similar to that of glycolysis alone
- Simulation to 100% increase in rate of utilization of GSH is fast and does not perturb the network much
- Systems biology shows up
  - Definition of pathways, definition of pools

The End