Supplementary material III: metabolic pathway analysis

Elementary mode analysis

We used elementary mode analysis (1)to systematically identify all circulations and futile cycle in the model. For this, the reactions and metabolites from the Simpheny^{TM} output files where parsed into a MetaTool (2) input file. MetaTool requires definitions of the reaction stoichiometries, reversibility of reactions, and the definition of external metabolites (sources and sinks) and internal metabolites (metabolites that need to be balanced insight each flux mode). By setting only ATP, ADP, phosphate, water and protons as external metabolites, and since all reactions in the network are elementary and charge balanced, only two type of modes were possible:
(i) Futile cycles with net stoichiometry of ATP + H_2O -> ADP + phosphate + H^+.
(ii) Circulations with no net transformation of compounds
In this way, we found 28 futile cycles and 9 circulations (**Table SIII-1**). In **Figure SIII-1** 22 of the futile cycles are schematically depicted, the other 6 are only slight variations of the other EFM's.

EFM#	#rxn	n_{ATP}[a]	Reactions involved in EFM
28 elementary flux modes correspond to futile cycles:			
1	3	0.67	ATPS3r - 3 CHLt6 + 3 CHLabc
2	3	0.67	ATPS3r - 3 GLYBt6 + 3 GLYBabc
3	3	0.67	ATPS3r - 3 ILEt6 + 3 ILEabc
4	3	0.67	ATPS3r - 3 LEUt6 + 3 LEUabc
6	3	0.67	ATPS3r - 3 VALt6 + 3 VALabc
5	3	0.67	ATPS3r - 3 PIt6 + 3 PIabc
7	3	1	G6PDA + GF6PTA + GLNS
8	4	1	GLCP + GLCS1 + GLGC + PPA
9	5	1	G6PI - MALP + PGMT_B + HEX1 + MALT
10	2	1	HYPOE + PYDAMK
11	2	1	PYDXK + PYDXPP
12	2	1	ACKr + ACTPASE
13	3	1	ADK1 + PPS + PYK
14	4	1	HCO3E + PC + PPCK + PYK
15	4	1	HCO3E - MDH + ME1x + PC
16	5	1	HCO3E + LDH_L - MDH + MALLAC + PC
17	4	1	- ACALD + ACKr - PTAr + ALDD2x
18	4	2	ADK1 + ASNN + ASNS2 + PPA
19	5	2	ADK1 + ASNN + ASNS1 + GLNS + PPA
20	4	2	NDPK6 + DUTPDP + PPA + URIDK2
21	9	5	2 ADK1 + PRPPS + ADPT + AHCYSNS + HCYSMT + METAT + 2 PPA + RBK + RHC
22	9	6	3 ADK1 + NNAMr + PRPPS + ADPRDP + NADN + NADS1 + NAPRT + NNAT + 3 PPA

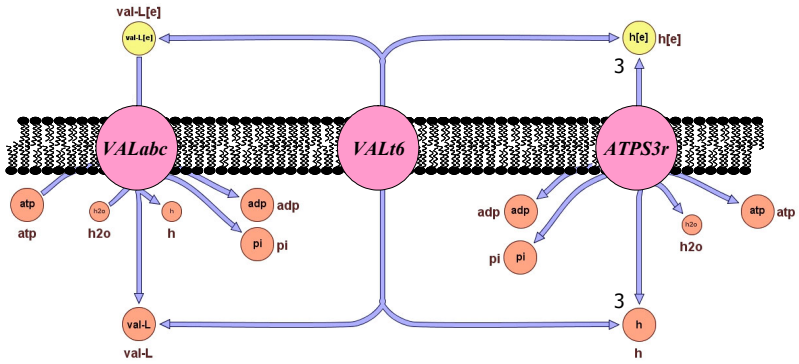
23-28 6 additional EFMs by replacing ADK1 by ADK2 + PPIK, and LDH_L by LDH_D + LAR

9 elementary flux modes correspond to circulations:

29	3	LAR + LDH_D - LDH_L
30	3	GK1 - GK2 - NDPK8
31	2	ASPTA6 - PHETA1
32	2	ASPTA5 - TYRTA
33	2	PSUDS - YUMPS
34	3	PPIK - ADK1 + ADK2
35	3	MALLACap + L-LACt2 - MALt6
36	3	CITLACap - CITt6 L- LACt2
37	3	NADH4 - FRDx - SUCD3BC

Table SIII-1: elementary flux mode analysis recovers all futile cycles and circulations in the genome-scale model. Only ATP, ADP, P_i, H^+ and H2O were set as external metabolites (sources and sinks), and elementary flux modes were calculates using MetaTool (2). EFM's corresponding to futile cycles had an overall stoichiometry of ATP + H2O -> ADP + P_i + H^+.
[a] n_{ATP} corresponds to the number of ATP molecules that is consumed per turnover of the cycle.

#1-6



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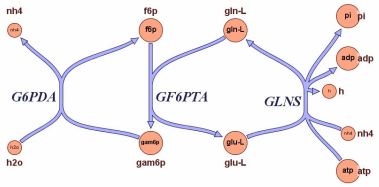

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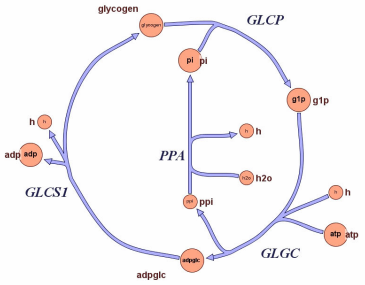

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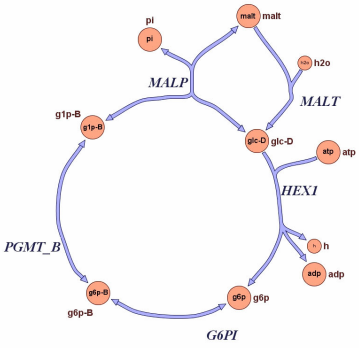

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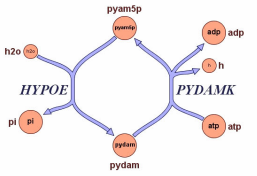

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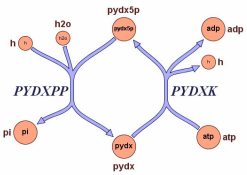

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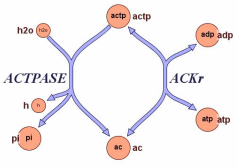

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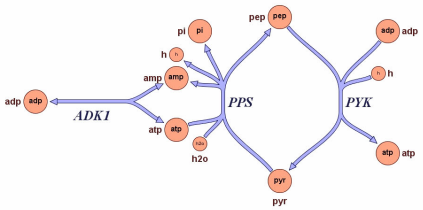

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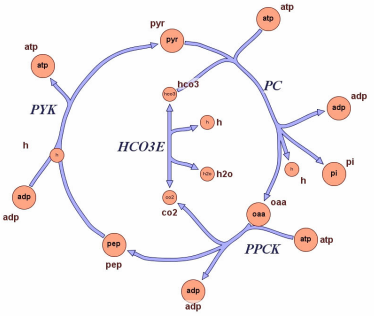

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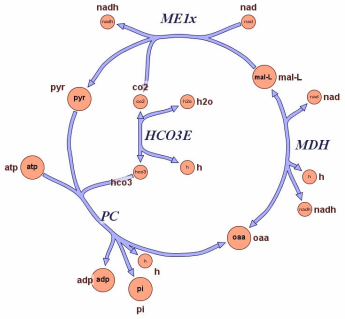

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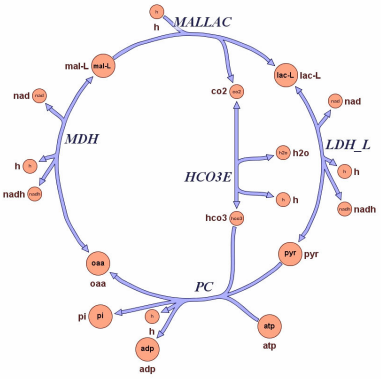

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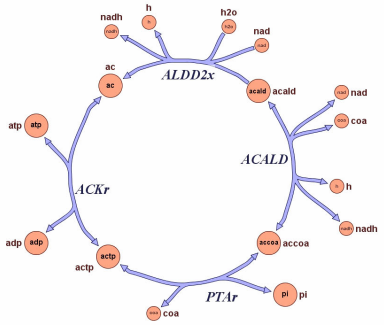

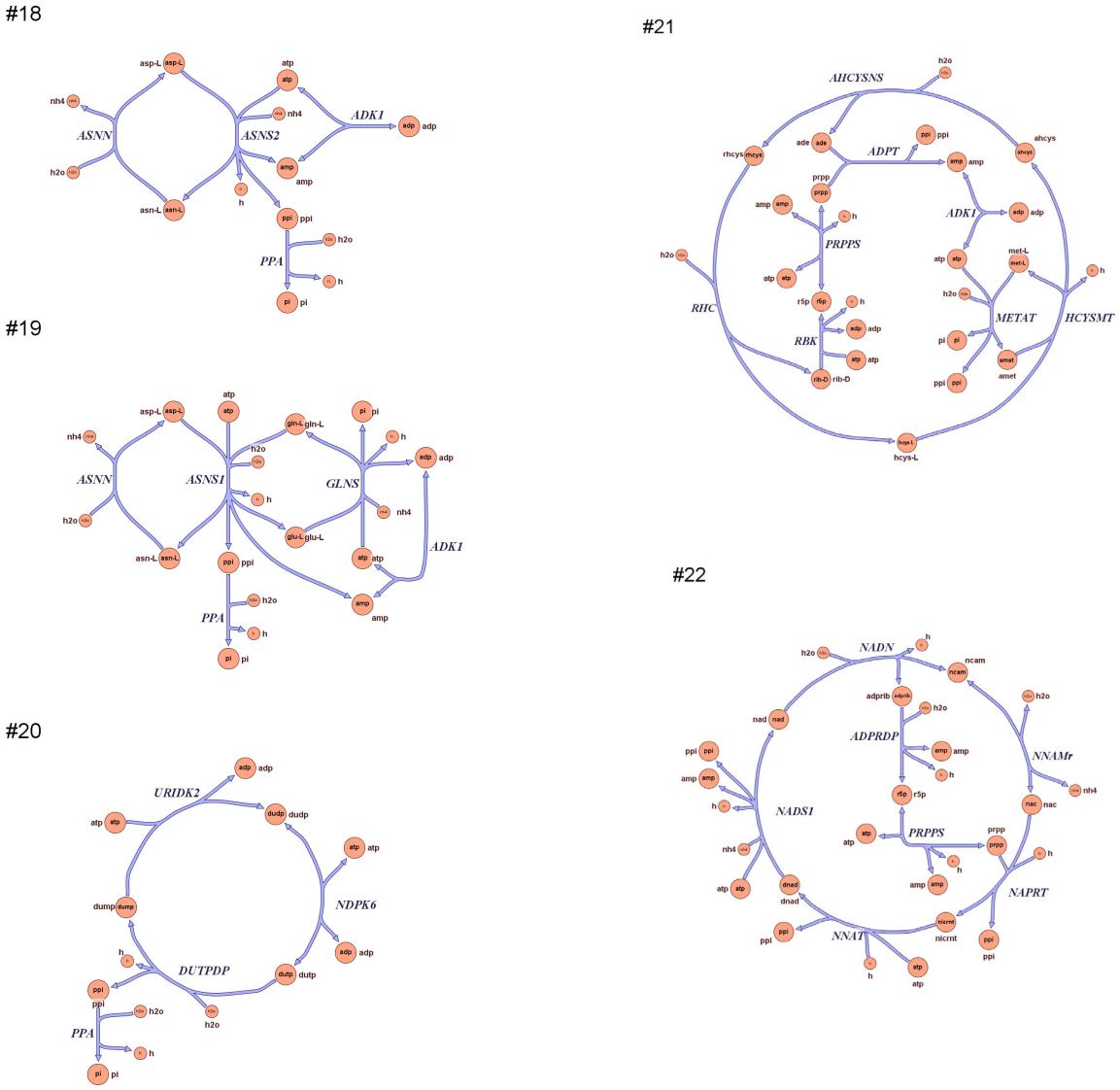

Figure SIII-1. schematic picture of all 22 futile cycles. Pictures were drawn with Simpheny^{TM} software.

Comparison of futile cycle membership and flux span

We also compared the presence of a reaction in a futile cycle or circulation with the span of that reaction (**Table SIII-2**). In all cases where a reaction is involved in a circulation, we found an infinite span, as expected. We also found that many of the reactions involved in a futile cycle had a ATP-dependent span with a normalized flux span that corresponds with the number of ATP molecules hydrolyzed per turn over of the cycle (see column n_{ATP} in **Table SIII-1**).

reaction abbreviation	**# of circulations**	**# of futile cycles #**	**normalized flux span** *max ATP dissipation*	**normalized flux span** *variable ATP dissipation*
MDH	0	3	0.039	2.713
PYK	0	3	0.143	2.203

PTAr	0	1	0.171	1.925
ACALD	0	1	0.100	1.811
VALt6	0	1	0	1.533
LEUt6	0	1	0	1.527
ILEt6	0	1	0	1.515
GLYBt6	0	1	0	1.5
CHLt6	0	1	0	1.5
PIabc	0	1	0	1.5
PIt6	0	1	0	1.5
VALabc	0	1	0	1.5
LEUabc	0	1	0	1.5
ILEabc	0	1	0	1.5
CHLabc	0	1	0	1.5
GLYBabc	0	1	0	1.5
ACKr	0	2	0	1.098
MALLAC	0	2	0.039	1.071
PPCK	0	1	0.039	1.071
ME1x	0	1	0.039	1.071
PYDXPP	0	1	0	1
PPS	0	2	0	1
PC	0	4	0	1
PYDXK	0	1	0	1
HCO3E	0	4	0	1
GLNS	0	3	0	1
GLCP	0	1	0	1
ACTPASE	0	1	0	1
ATPM	0	1	0	1
HYPOE	0	1	0	1
GLGC	0	1	0	1
MALT	0	1	0	1
GLCS1	0	1	0	1
G6PI	0	1	0	1
GF6PTA	0	1	0	1
PYDAMK	0	1	0	1
PPA	0	10	0	1
MALP	0	1	0	1
ALDD2x	0	1	0	1
PGMT_B	0	1	0	1
G6PDA	0	1	0	1
HEX1	0	1	0	1
ATPS3r	0	6	0.036	0.807
ASNS2	0	2	0	0.517
NDPK6	0	1	0	0.5
DUTPDP	0	1	0	0.5
ASNN	0	4	0	0.5
URIDK2	0	1	0	0.5
ASNS1	0	2	0	0.345
ADPT	0	2	0	0.215
RBK	0	2	0	0.202

RHC	0	2	0	0.2
AHCYSNS	0	2	0	0.2
HCYSMT	0	2	0	0.2
METAT	0	2	0	0.2
PRPPS	0	4	0	0.2
NAPRT	0	2	0	0.167
NNAMr	0	2	0	0.167
NADS1	0	2	0	0.167
NADN	0	2	0	0.167
NNAT	0	2	0	0.167
ADPRDP	0	2	0	0.167
L-LACt2	2	0	Infinity	Infinity
ADK1	1	3	Infinity	Infinity
ADK2	1	3	Infinity	Infinity
ASPTA5	1	0	Infinity	Infinity
ASPTA6	1	0	Infinity	Infinity
CITLACap	1	0	Infinity	Infinity
CITt6	1	0	Infinity	Infinity
FRDx	1	0	Infinity	Infinity
GK1	1	0	Infinity	Infinity
GK2	1	0	Infinity	Infinity
LAR	1	1	Infinity	Infinity
LDH_D	1	1	Infinity	Infinity
LDH_L	1	1	Infinity	Infinity
MALLACap	1	0	Infinity	Infinity
MALt6	1	0	Infinity	Infinity
NADH4	1	0	Infinity	Infinity
NDPK8	1	0	Infinity	Infinity
PHETA1	1	0	Infinity	Infinity
PPIK	1	3	Infinity	Infinity
PSUDS	1	0	Infinity	Infinity
SUCD3BC	1	0	Infinity	Infinity
TYRTA	1	0	Infinity	Infinity
YUMPS	1	0	Infinity	Infinity

Table SIII-2. Number of times reactions that are associated with futile cycles and circulations. Only reactions are shown that occur at least 1 time in either a futile cycle or circulation. The span is calculated for the case where the ATP dissipation reaction is constraint to its maximal value obtained by FBA, and for the case where it was free to vary. The span was normalized to the ATP dissipation reaction, i.e. a normalized span of 1 corresponds to a span exactly equal to that of the ATP dissipation reaction. Data are based on a simulation of growth on CDM with 25 mM glucose at $D = 0.11\ h^{-1}$.

We also found reactions with an ATP-dependent variability that did not take part in a futile cycle (**Table SIII-3**). These reactions indicate parallel pathways with different energetic consequences, such as a catabolic route of glucose via the pentose phosphate pathway rather than glycolysis. Other interesting parallel pathways were simultaneous serine biosynthesis and degradation of serine into pyruvate and ammonia (**Fig. 3 in main text**). Serine degradation itself can also occur via two different routes (**Fig. SIII-**

2). Parts of these pathways are easily recognized as they show exactly the same spans (e.g. METACH, SERAT and CYSS, see **Fig SIII-3**).

reaction abbreviation	**normalized flux span** *max ATP dissipation*	**normalized flux span** *variable ATP dissipation*
PKL	0.170750758	2.01944096
GLUDy	0.077990676	1.996700511
PTAr	0.170750758	1.924837454
NH3t	0.155981353	1.465853325
SERD_L	0.077990676	1.404213188
FBA	0.113833839	1.371784813
ENO	0.113833839	1.371784813
PGK	0.113833839	1.371784813
GAPD	0.113833839	1.371784813
PFK	0.113833839	1.371784813
PGM	0.113833839	1.371784813
TPI	0.113833839	1.371784813
ACALDt	0	1.156591309
RPE	0.056916919	1.156277593
PDH	0.075525625	1.019093816
PGI	0	0.964180628
G6PDHy	0	0.964180628
PGL	0	0.964180628
PGDH	0	0.964180628
CYSTL	-6.19637E-14	0.839195646
SHSL5	-1.42146E-15	0.839195646
METACH	0	0.839194488
SERAT	0	0.839194488
CYSS	0	0.839194488
TKT2	0.056916919	0.710375319
P5CD	0	0.708068072
PFL	0.077990676	0.694929526
MTHFC	0.077990676	0.69369078
MTHFD	0.077990676	0.69369078
GHMT	0.077990676	0.693689622
RPI	0.056916919	0.685558511
TAL	0.056916919	0.684917033
TKT1	0.056916919	0.684917033
G5SD	0	0.676582738
GLU5K	0	0.676582738
G5SADs	0	0.676582738
PSP_L	0	0.675332297
PGCD	0	0.675332297
PSERT	0	0.675332297
FTHFL	0.077990676	0.618498695
ACLDC	0	0.525723322

BTDt1-RR	0	0.525723322
ACLS	0	0.525723322
BTDD-RR	0	0.525723322

Table SIII-3. Reactions that are not part of a futile cycle but for which the span was ATP dependent. Only reactions with a span > 0.5 at variable ATP dissipation are shown. Data are based on a simulation of growth on CDM with 25 mM glucose at D = 0.11 h^{-1}.

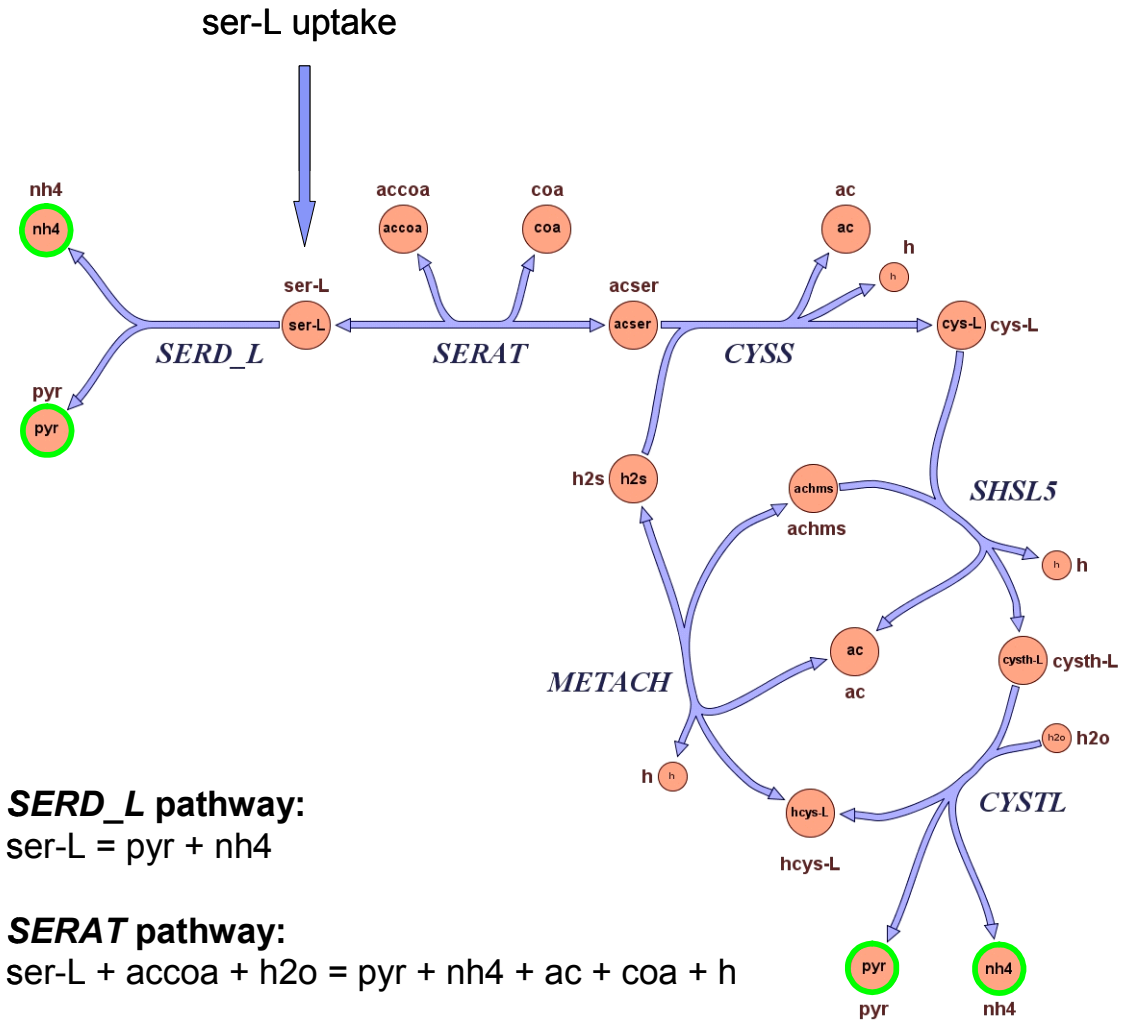

Figure SIII-2. Two alternative pathways for serine catabolism to pyruvate and ammonium. End products are indicated in green.

References

1. Schuster, S., Fell, D. A., and Dandekar, T. (2000) *Nat Biotechnol* 18(3), 326-332.
2. Pfeiffer, T., Sanchez-Valdenebro, I., Nuno, J. C., Montero, F., and Schuster, S. (1999) *Bioinformatics* 15(3), 251-257.