Supplementary Material for: MetDFBA: incorporating time-resolved metabolomics measurements into dynamic flux balance analysis

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1 Adaptation of *Penicillium chrysogenum* during feast-famine cycles

In order to study the effects of substrate gradients that occur during large scale-up of fermentation processes and the resulting product formation reduction, intermittent feeding cycles of 360 s consisting of 36 s of feeding with glucose and 324 s of no feed were applied to *Penicillium chrysogenum* cultivation. For details see [2]. After 100 hours block wise regime intracellular and extracellular metabolite concentrations were measured using GC-MS and and LC-MS, during one cycle at 24 time points with smaller intervals in the beginning. The studied metabolic network consists of the upper glycolysis, the pentose phosphate pathway (PPP) and storage metabolism, including trehalose, glycogen and mannitol. Time-series of 17 metabolites, participating in 22 reactions were used to apply our method. See Supplementary Tables 1 and 2 for an overview of the metabolic network and used abbreviations.

Since xylitol 5-phosphate was not measured, we lumped reactions *r2_4* and *r2_5* into *r2_4-r2_5* and reactions *r2_4* and *2_7* into *r2_4-r2_7*. We constrained the optimization problem further by making the reactions irreversible by setting the lower flux bounds to zero with an exception of the reactions *r1_2, r2_4-r2_5, r2_6* and *r2_4-r2_7* [2]. Because the feed was known we fixed this flux by setting both upper and lower bound of this flux to 7.3140 mmol/gDW/h for the first four time points, and to zero for the remaining time points. In this case study we focussed on testing how well our method performs in estimating dynamic fluxes. Therefore we minimized only the sum of squared fluxes to obtain a solution for the optimization.

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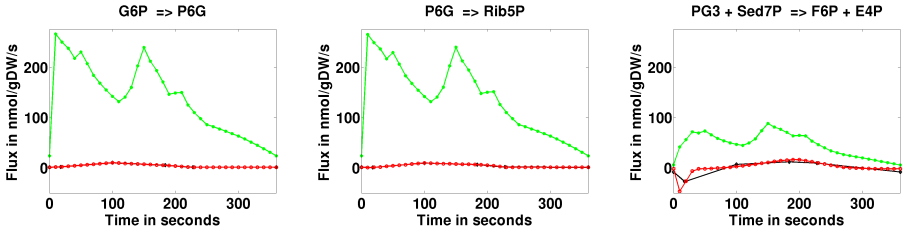
Supplementary Table 1: Abbreviations of measured metabolites

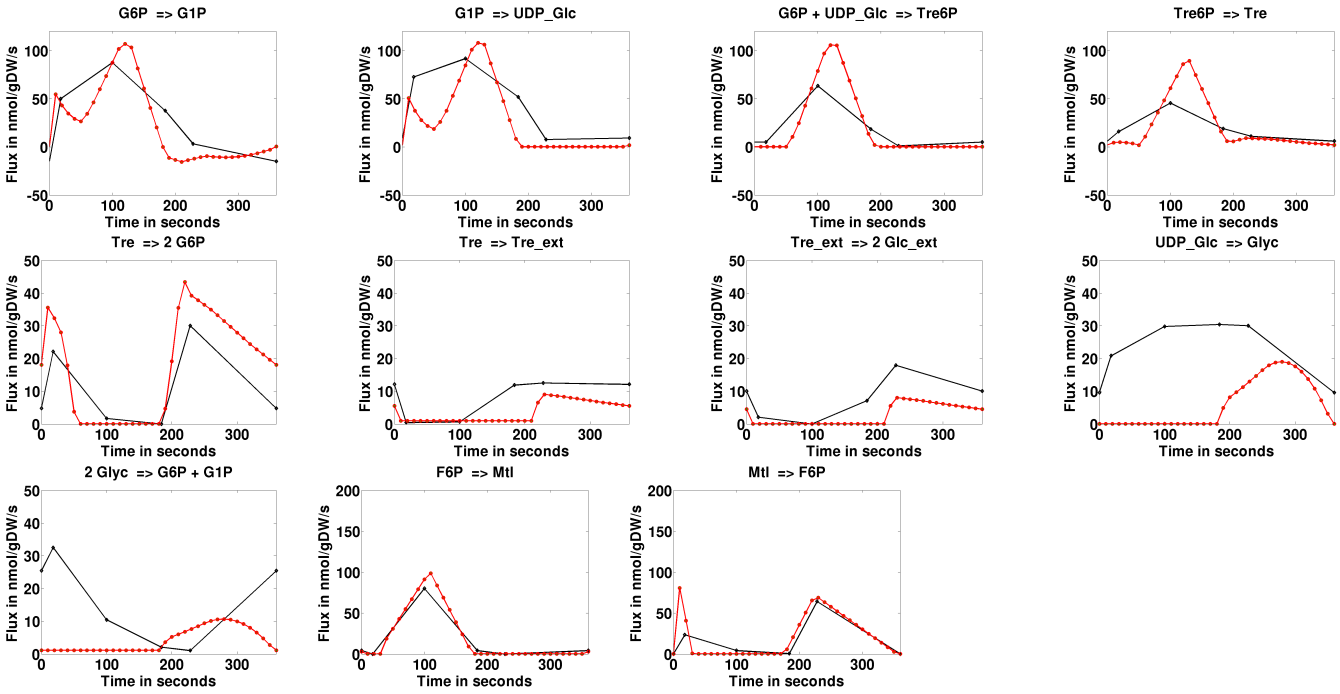
abbreviation	**metabolite**
3PG	3-Phospho-glycerate
6PG	6-Phospho-gluconate
E4P	Erythrose 4-phosphate
F6P	Fructose-6-phosphate
FBP	Fructose-1,6-bisphosphate
G1P	Glucose-1-phosphate
G6P	Glucose-6-phosphate
GAP	Glyceraldehyde-3-phosphate
Glc_ext	Extracellular Glucose
Glyc	Glycogen
Mtl	Mannitol
Rib5P	Ribose-5-phosphate
Sed7P	Sedoheptulose-7-phosphate
Tre	Trehalose
Tre6P	Trehalose-6-phosphate
UDP_Glc	Uridine diphosphate glucose
Tre_ext	Extracellular Trehalose



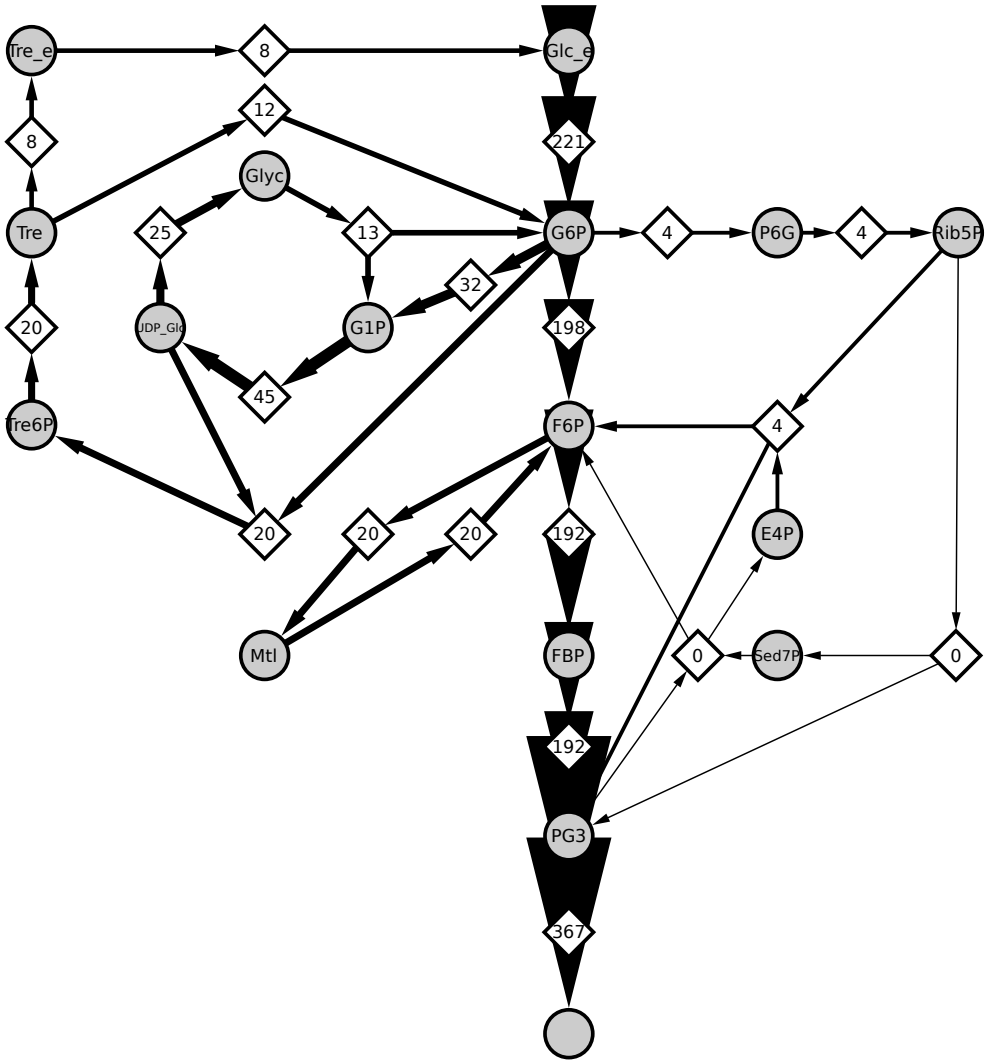
Supplementary Figure 1: Estimated dynamic fluxes in nmol/gDW/s through the pentose phosphate pathway. Black indicates the experimental reference (^{13}C MFA), green indicates MetDFA estimations, red indicates the MetDFBA fixing the conversion of G6P into 6PG (reaction *r2_1*).

Supplementary Table 2: Metabolic network

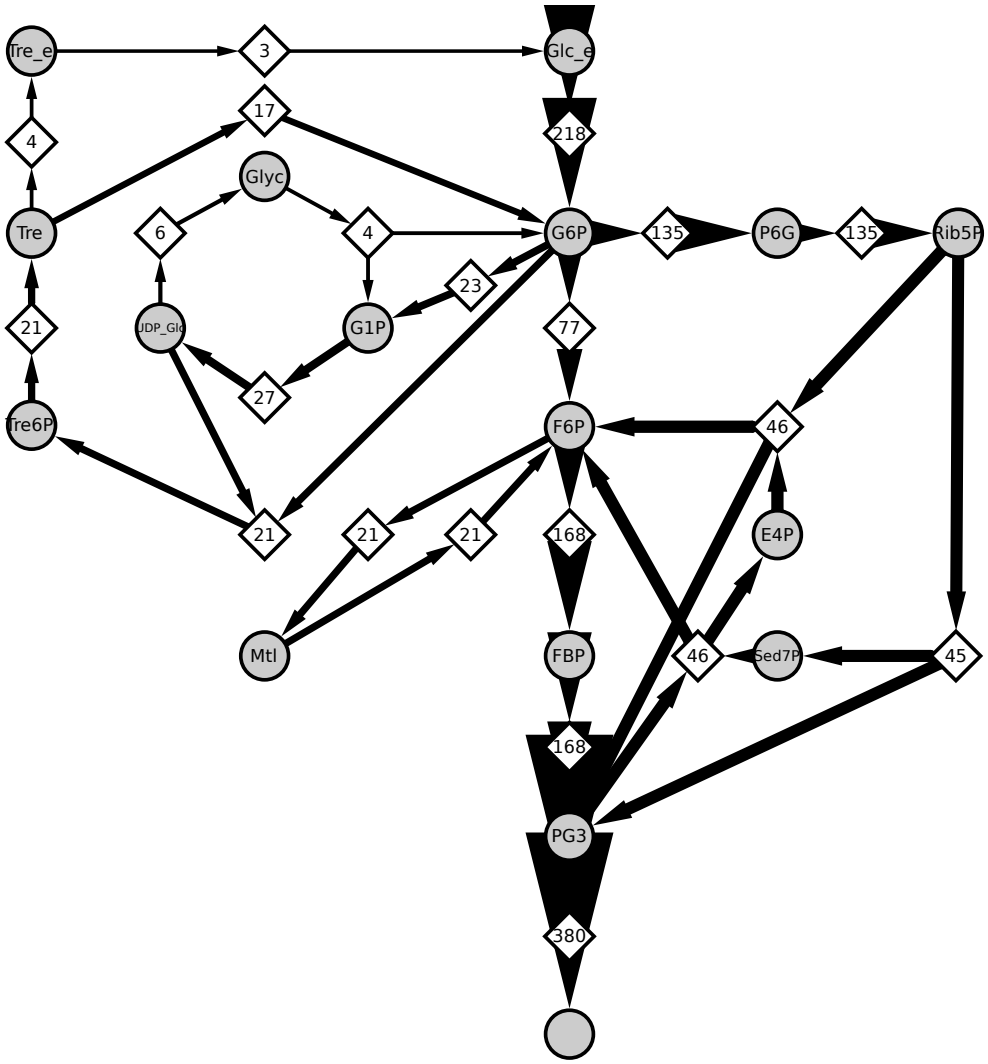
reaction	**name**	**lower bound**	**upper bound**
=> Glc_ext	vfeedA	fixed	fixed
1 Glc_ext => 1 G6P	r1_1	0	1000
1 G6P => 1 F6P	r1_2	-1000	1000
1 F6P => 1 FBP	r1_3	0	1000
1 FBP => 2 3PG	r1_4	0	1000
1 3PG =>	r1_5	0	1000
1 G6P => 1 P6G	r2_1	0	1000
1 P6G => 1 Rib5P	r2_2	0	1000
2 Rib5P => 1 3PG + 1 Sed7P	r2_4-r2_5	-1000	1000
1 3PG + 1 Sed7P => 1 F6P + 1 E4P	r2_6	-1000	1000
1 Rib5P + 1 E4P => 1 F6P + 1 3PG	r2_4-r2_7	-1000	1000
1 G6P => 1 G1P	tre_1	-1000	1000
1 G1P => 1 UDP_Glc	tre_2	0	1000
1 G6P + 1 UDP_Glc => 1 Tre6P	tre_3	0	1000
1 Tre6P => 1 Tre	tre_4	0	1000
1 Tre => 2 G6P	tre_deg	0	1000
1 Tre => 1 Tre_ext	tre_exp	0	1000
1 Tre_ext => 2 Glc_ext	tre_ext_deg	0	1000
1 UDP_Glc => 1 Glyc	glyc_1	0	1000
2 Glyc => 1 G6P + 1 G1P	glyc_deg	0	1000
1 F6P => 1 Mtl	mtl_syn	0	1000
1 Mtl => 1 F6P	mtl_deg	0	1000



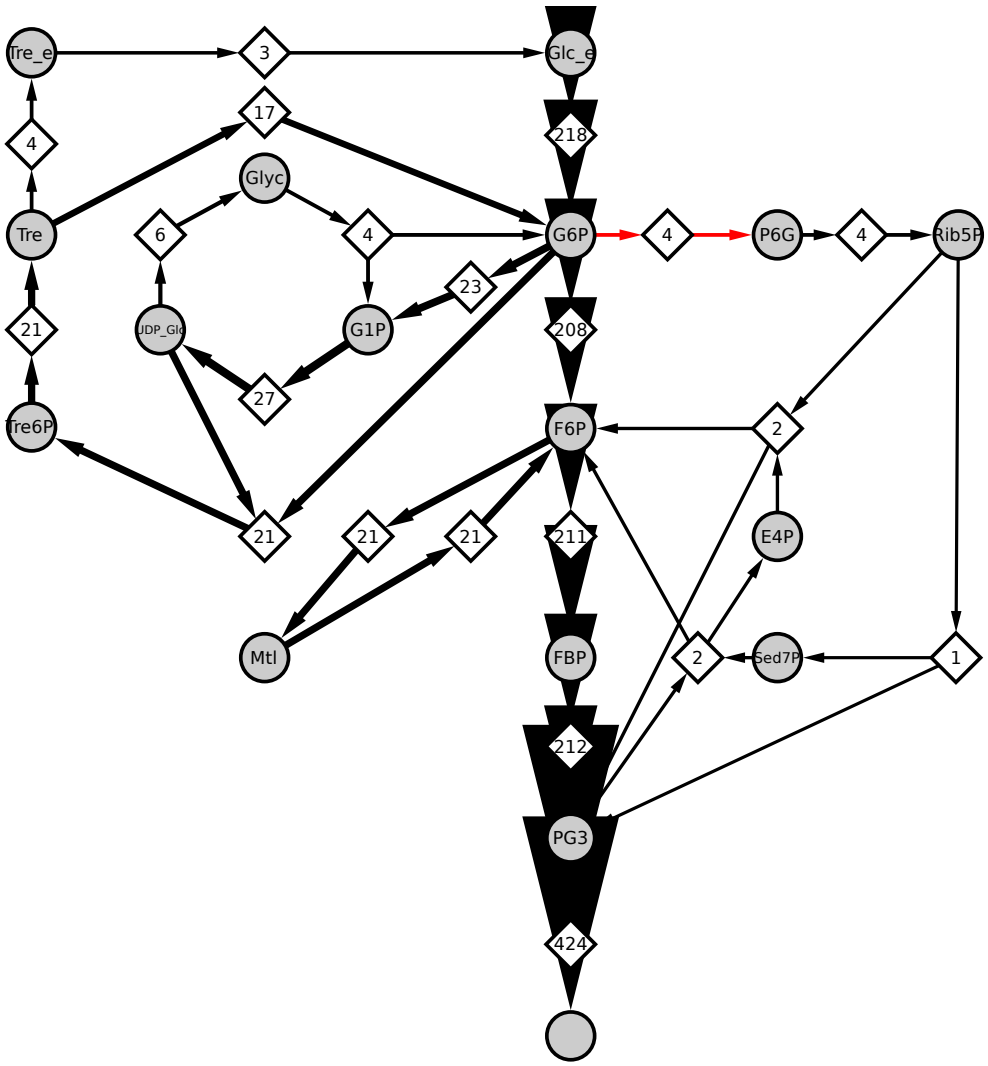
Supplementary Figure 2: Estimated dynamic fluxes in nmol/gDW/s through storage metabolism. Black indicates the experimental reference (^{13}C MFA), green indicates MetDFA estimations, red indicates the MetDFBA fixing the conversion of G6P into 6PG (reaction *r2_1*).



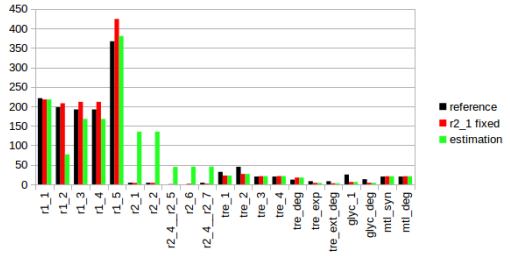
Supplementary Figure 3: Estimations of the average fluxes based on ^{13}C-labeling through the considered metabolic network in nmol/gDW/s including upper glycolysis, pentose phosphate pathway (PPP) and storage metabolism (mannitol, glycogen and trehalose). The width of the arrow is proportional to the hight of the flux.



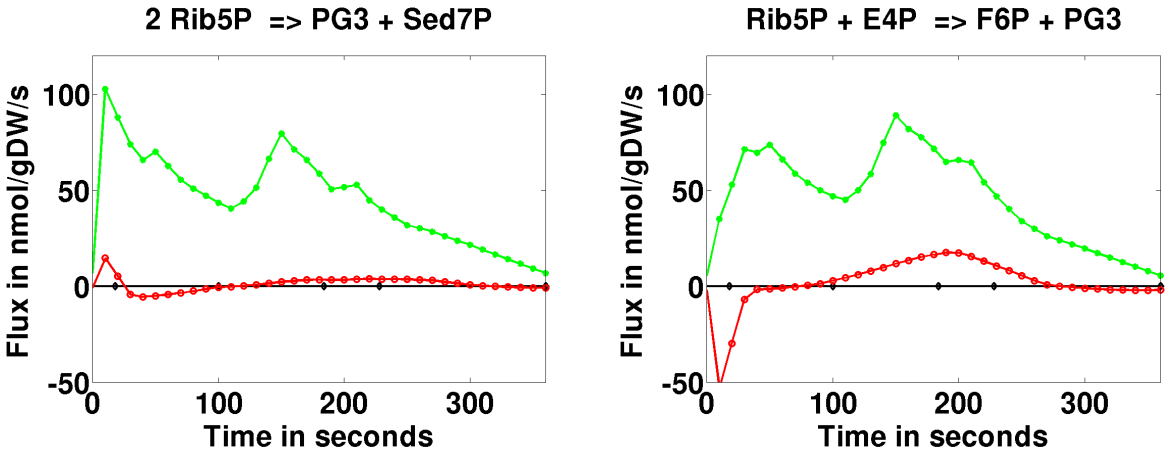
Supplementary Figure 4: MetDFBA estimations of the average fluxes through the considered metabolic network in nmol/gDW/s including upper glycolysis, pentose phosphate pathway (PPP) and storage metabolism (mannitol, glycogen and trehalose). The width of the arrow is proportional to the hight of the flux.



Supplementary Figure 5: MetDFBA estimation with the entrance reaction to the PPP (r2_1, red arrow) fixed through the considered metabolic network in nmol/gDW/s including upper glycolysis, pentose phosphate pathway (PPP) and storage metabolism (mannitol, glycogen and trehalose). The width of the arrow is proportional to the hight of the flux.



Supplementary Figure 6: Bar plot showing the same fluxes as in Supplementary Figures 3-5. Black indicates the experimental reference (^{13}C MFA), green indicates MetDFA estimations, red indicates the MetDFBA fixing the conversion of G6P into 6PG (reaction *r2_1*).



Supplementary Figure 7: Estimated dynamic fluxes in nmol/gDW/s going through the lumped reactions (part of the PPP). Black indicates the experimental reference (^{13}C MFA), green indicates MetDFA estimations, red indicates the MetDFBA fixing the conversion of G6P into 6PG (reaction *r2_1*).

2 Response of *Saccharomyces cerevisiae* to a glucose pulse

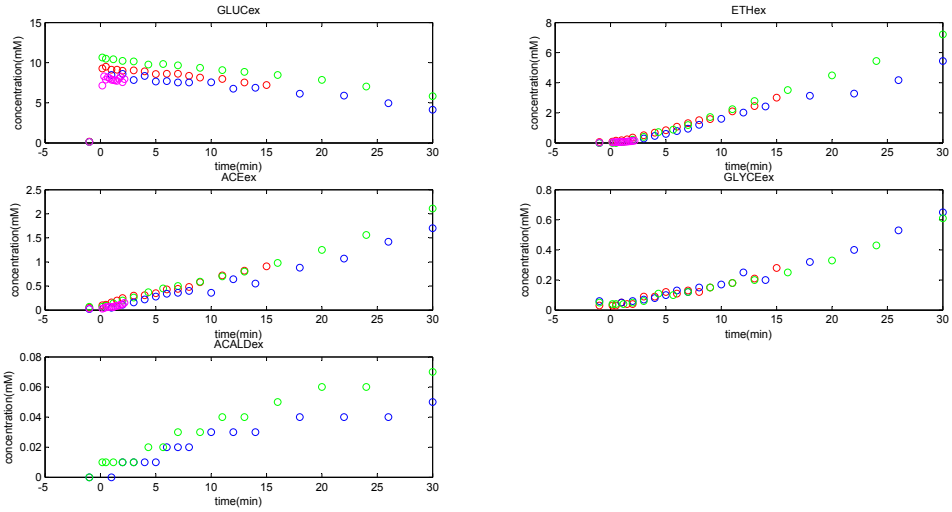
Cells were cultivated in aerobic chemostats (dilution rate $D = 0.1\ h^{-1}$), as described elsewhere. [1, 8, 11] Short-term perturbation-response experiments were carried out by introducing a sudden increase of 10 mM in the extracellular glucose concentration (also called a 10 mM glucose pulse). The data set consists of time-series of quantitative data on extracellular and intracellular metabolite concentration levels (in mM). Extracellular metabolite levels were obtained enzymatically as described elsewhere.[1] Each time-series consists of 14-16 time points. Supplementary Table 3 gives an overview of the extracellular metabolites in the data set. Intracellular metabolite concentrations were determined by LC-MS/MS.[1] The time-series consist of 11-15 time points. The metabolites measured are from glycolysis and its branches, the tricarboxylic acid (TCA) cycle and the pentose phosphate pathway (PPP). Supplementary Table 4 gives an overview. Data were measured on the second scale. The shortest window of observation was from 0 to 130 seconds. The concentrations of the external metabolites are plotted in Supplementary Figure 8. Plots of the concentrations of the internal metabolites from glycolysis (except GAP and DHAP) and the TCA cycle are available in the Supplementary Material of [9]. Concentrations for the remaining internal metabolites (PPP, branches of glycolysis, GAP, DHAP, cofactors) are plotted in Supplementary Figures 9 and 10.

Supplementary Table 3: Extracellular metabolites in the data set.

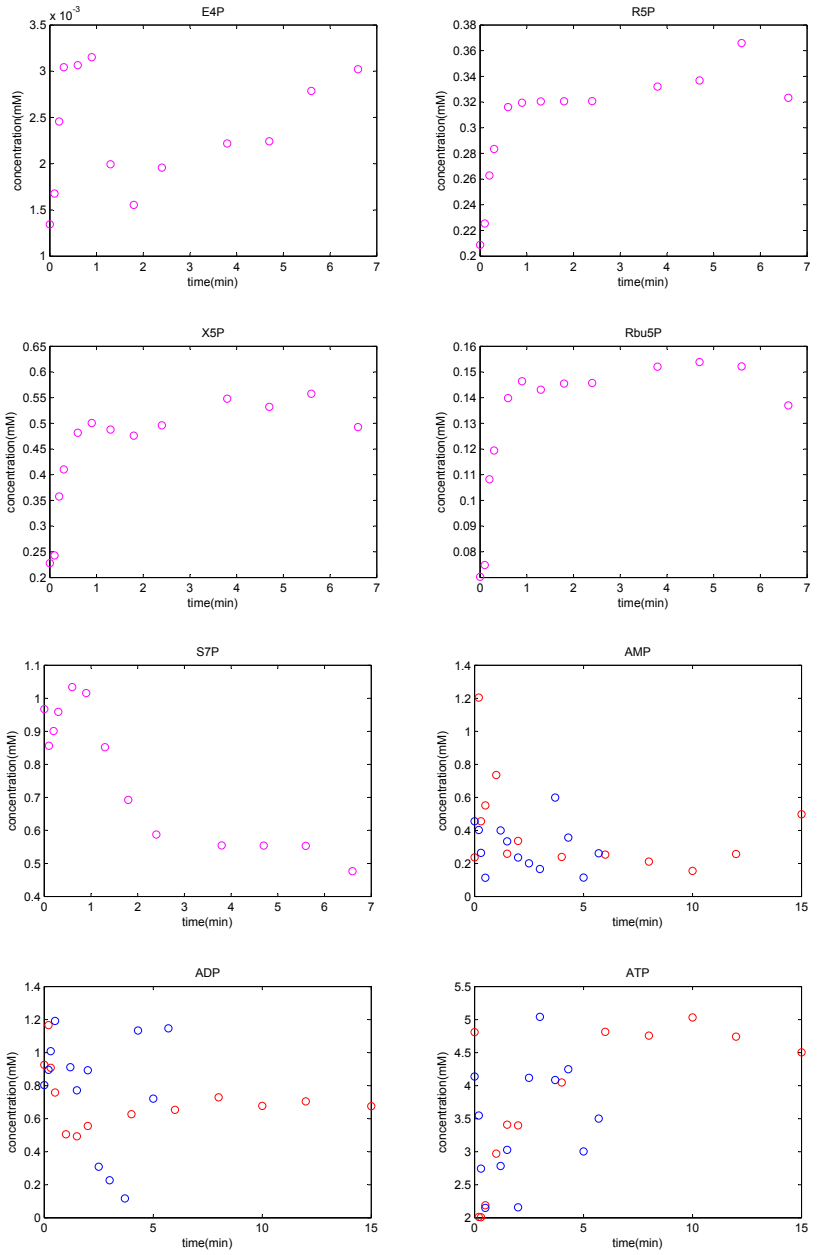
metabolite	**number of biological replicates**
glucose (GLUC)	4
ethanol (ETH)	4
acetate (ACE)	4
glycerol (GLYCE)	3
acetaldehyde (ACALD)	2

Supplementary Table 4: Intracellular metabolites in the data set.

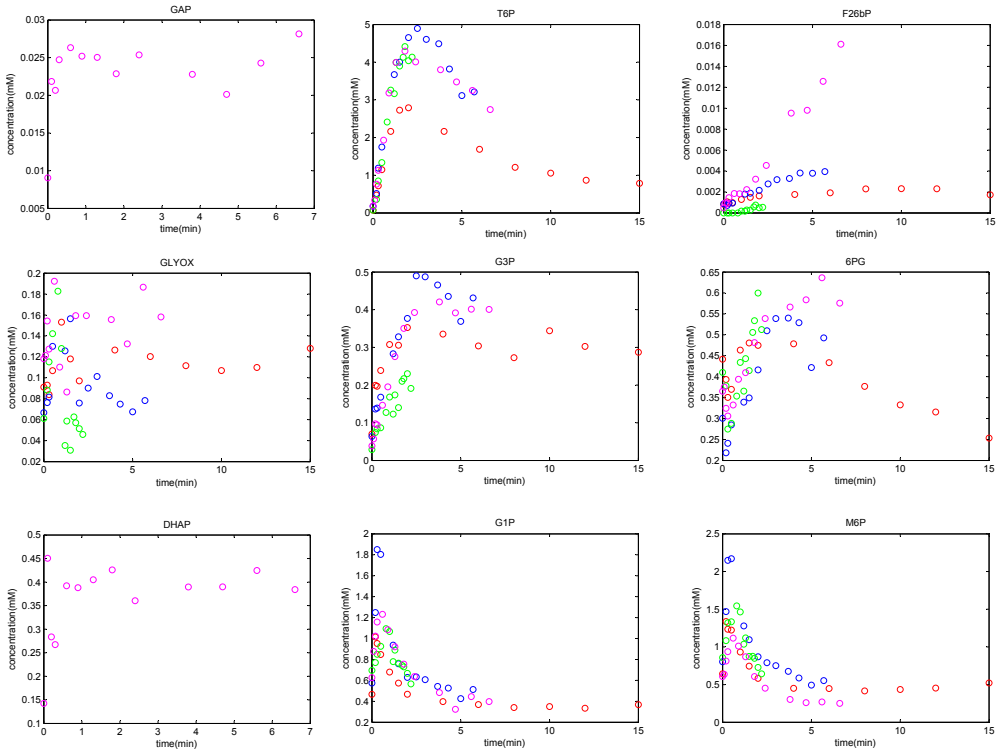
metabolite	**pathway/branch**	**number of biological replicates**
glucose-6-phosphate (G6P)	glycolysis	4
fructose-6-phosphate (F6P)	glycolysis	4
fructose-1,6-bisphosphate (FBP)	glycolysis	4
3-phosphoglycerate (3PG)	glycolysis	4
phosphoenolpyruvate (PEP)	glycolysis	4
pyruvate (PYR)	glycolysis	4
glyceraldehyde-3-phosphate (GAP)	glycolysis	1
dihydroxyacetonephosphate (DHAP)	glycolysis	1
fructose-2,6-bisphosphate (F26bP)	regulator of glycolysis	4
trehalose-6-phosphate (T6P)	trehalose branch	4
glycerol-3-phosphate (G3P)	glycerol branch	4
glucose-1-phosphate (G1P)	glycogen branch	4
mannose-6-phosphate (M6P)	mannose branch	4
citrate (CIT)	TCA cycle	4
oxoglutarate (OGL)	TCA cycle	4
succinate (SUC)	TCA cycle	4
fumarate (FUM)	TCA cycle	4
malate (MAL)	TCA cycle	4
glyoxylate (GLYOX)	glyoxylate shunt	4
6-phosphogluconate (6PG)	pentose phosphate pathway	4
erythrose-4-phosphate (E4P)	pentose phosphate pathway	1
ribose-5-phosphate (R5P)	pentose phosphate pathway	1
xylulose-5-phosphate (X5P)	pentose phosphate pathway	1
ribulose-5-phosphate (Rbu5P)	pentose phosphate pathway	1
sedoheptulose-7-phosphate (S7P)	pentose phosphate pathway	1
adenosine monophosphate (AMP)	cofactors	2
adenosine diphosphate (ADP)	cofactors	2
adenosine triphosphate (ATP)	cofactors	2



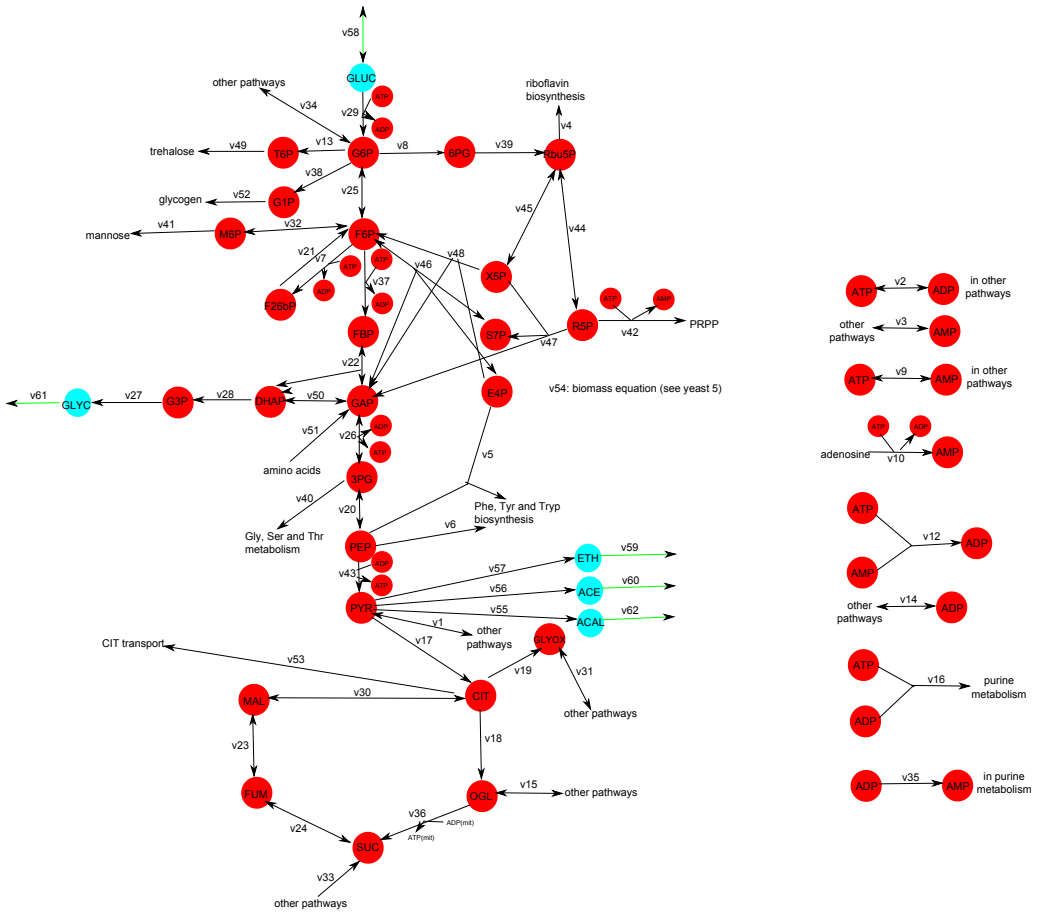
Supplementary Figure 8: Experimental data *S.cerevisiae* - external metabolites. Each color represents a different biological experiment.



Supplementary Figure 9: Experimental data *S.cerevisae* - internal metabolites (E4P, R5P, X5P, Rbu5P, S7P, AMP, ADP, ATP). Each color represents a different biological experiment.



Supplementary Figure 10: Experimental data *S.cerevisae* - internal metabolites (GAP, T6P, F26bP, GLYOX, G3P, 6PG, DHAP, G1P, M6P). Each color represents a different biological experiment.



Supplementary Figure 11: Case study *S.cerevisiae* - reaction scheme after lumping of reactions. See Supplementary Table 5 for a reaction list and abbreviations. The biomass reaction (v54) is taken from the genome scale model (called yeast 5) [7] and is linked to the rest of the lumped model through AMP, ADP and ATP. The reaction coefficients of AMP, ADP and ATP are the same as in the genome scale model (see Supplementary Table 5). The measured concentrations of AMP, ADP and ATP are influenced by several reactions forming or consuming AMP, ADP or ATP outside the central carbon metabolism. Those reactions are lumped to v2, v3, v9, v10, v12, v14, v16 and v35: v2 lumps all interconversions between ATP and ADP; v3 lumps all reactions forming or consuming AMP; v9 lumps all interconversions between ATP and AMP; v10 lumps all reactions consuming ATP and forming ADP + AMP; v12 is the reaction ATP + AMP $\rightarrow$ 2 ADP; v14 lumps all reactions forming or consuming ADP; v16 is the consumption of ATP + ADP in the purine metabolism; v35 is the reaction that converts ADP to AMP in the purine metabolism. Detailed information on the original reactions can be found in the genome scale model. [7]

Supplementary Table 5: Detailed list of the reactions in the lumped model. For more information, see caption Supplementary Figure 11. Abbreviations: see Supplementary Table 6.

v1	pyruvate ↔ other pathways	v32	M6P ↔ F6P (PMI)
v2	ATP ↔ ADP in non-central carbon pathways, transport	v33	non-central carbon pathways, transport → SUC
v3	non-central carbon pathways, transport ↔ AMP	v34	G6P ↔ non-central carbon pathways, transport
v4	Rbu5P → riboflavin biosynthesis (DHBPS)	v35	ADP → AMP in purine metabolism (NDP)
v5	E4P + PEP → Phe, Tyr and Tryp biosynthesis (DAHPS)	v36	OGL + ADP(mit) → SUC + ATP(mit) (OGDH)
v6	PEP → Phe, Tyr and Tryp biosynthesis (EPSPS)	v37	F6P + ATP → FBP + ADP (PFK)
v7	F6P + ATP → F26bP + ADP (6PF2K)	v38	G6P → G1P (PGM)
v8	G6P → 6PG (G6PDH + PGLS)	v39	6PG → Rbu5P (6PGDH)
v9	ATP ↔ AMP in non-central carbon pathways, transport	v40	3PG → Gly, Ser and Thr metabolism (PGDH)
v10	adenosine + ATP ↔ AMP + ADP in purine metabolism (AdK)	v41	fructose and mannose metabolism ← M6P (PMM)
v11	ATP ↔ non-central pathways, transport	v42	R5P + ATP → AMP + PRPP in purine metabolism (PRPS)
v12	ATP + AMP → 2ADP (ADK)	v43	PEP + ADP → PYR + ATP (PYK)
v13	G6P → T6P (TPS)	v44	Rbu5P ↔ R5P (R5PI)
v14	non-central carbon pathways, transport ↔ ADP	v45	Rbu5P ↔ X5P (Ru5PE)
v15	OGL ↔ non-central carbon pathways, transport	v46	GAP + S7P ↔ F6P + E4P (TAL)
v16	ADP + ATP → purine metabolism (APA)	v47	R5P + X5P → S7P + GAP (TKL1)
v17	PYR + ATP → CIT + ADP (PYC + CIT)	v48	E4P + X5P → GAP + F6P (TKL2)
v18	CIT → OGL (ACO + IDH / OGL transport)	v49	T6P → trehalose (TPP)
v19	CIT → GLYOX (ACO + ICL)	v50	DHAP ↔ GAP (TPI)
v20	3PG ↔ PEP (GPM + ENO)	v51	amino acids → GAP (TRP)
v21	F26bP → F6P (FBP26)	v52	G1P → glycogen
v22	FBP ↔ GAP + DHAP (FBA)	v53	citrate transport ← CIT
v23	FUM ↔ MAL (FMH)	v54	biomass pseudoreaction: non measured + 0.051 AMP + 59.3 ATP - 59.3 ADP → 1 biomass
v24	FUM ↔ SUC (FMR/SDH/SUC-FUM transport)	v55	PYR → ACALDex (PDC + ACALD transport)
v25	G6P ↔ F6P (PGI)	v56	PYR → ACEex (PDC + ALDH + ACE transport)
v26	GAP + ADP ↔ 3PG + ATP (GAPDH + PGK)	v57	PYR → ETHex (PDC + ADH + ETH transport)
v27	G3P → glycerol (G3PP + GT)	v58	GLUCex ↔ (glucose exchange)
v28	DHAP → G3P (G3PDH)	v59	ETHex → (ethanol exchange)
v29	glucose + ATP → G6P + ADP (glucose transport + HXK (glucose))	v60	ACEex → (acetate exchange)
v30	MAL → CIT (MDH + CIT / citrate transport)	v61	GLYCex → (glycerol exchange)
v31	GLYOX ↔ non-central carbon pathways, transport	v62	ACALDex → (acetaldehyde exchange)

Supplementary Table 6: List of abbreviations

6PF2K	6-phosphofructo-2-kinase	6PGDH	phosphogluconate dehydrogenase
ACO	aconitate hydratase (aconitase)	ADH	alcohol dehydrogenase
AdK	adenosine kinase	ADK	adenylate kinase
ALDH	aldehyde dehydrogenase	APA	ATP adenyltranferase
CIT	citrate synthase	DAHPS	3-deoxy-D-arabino-heptulosonate 7 phosphate synthetase
DHBPS	3,4-dihydroxy-2-butanone-4-phosphate synthase	ENO	enolase
EPSPS	3-phosphoskikimate 1 carboxyvinyltransferase (5-enolpyruvylskikimate-3-phosphate synthase)	FBA	fructose-bisphosphate aldolase
FBP26	fructose-2,6-bisphosphate 2-phosphatase	FMH	fumarase
FMR	fumarate reductase	G3PDH	glycerol-3-phosphate dehydrogenase
G3PP	glycerol-3-phosphatase	G6PDH	glucose 6-phosphate dehydrogenase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	GPM	phosphoglycerate mutase
GT	glycerol transport	HXK	hexokinase
ICL	isocitrate lyase	IDH	isocitrate dehydrogenase
MDH	malate dehydrogenase	NDP	nucleoside diphosphatase
OGDH	oxoglutarate dehydrogenase	PDC	pyruvate decarboxylase
PFK	phosphofructokinase	PGDH	phosphoglycerate dehydrogenase
PGI	glucose-6-phosphate isomerase	PGK	phosphoglycerate kinase
PGLS	6-phosphoglucolactonase	PGM	phosphoglucomutase
PMI	mannose-6-phosphate isomerase	PMM	phosphomannomutase
PRPS	phosphoribosylpyrophosphate synthetase	PYC	pyruvate carboxylase
PYK	pyruvate kinase	R5PI	ribose-5-phosphate isomerase
Ru5PE	ribulose 5-phosphate 3-epimerase	SDH	succinate dehydrogenase
TAL	transaldolase	TKL1	transketolase 1
TKL2	transketolase 2	TPI	triose-phosphate isomerase
TPP	trehalose-phosphatase	TPS	alpha,alpha-trehalose-phosphate synthase
TRP	tryptophan synthase	ACALDex	external acetaldehyde
ACEex	external acetate	ETHex	external ethanol
GLUCex	external glucose	Gly	glycine
GLYCex	external glycerol	Phe	phenylalanine
PRPP	phosphoribosylpyrophosphate	Ser	serine
Thr	threonine	Tryp	tryptophan
Tyr	tyrosine		

2.1 MetDFBA: Objective functions used in the *S.cerevisiae* study.

Maximize biomass yield. In most (D)FBA studies of microorganisms it is assumed that survival is equivalent with growth [3] and, consequently, the objective is biomass (max $v_{biomass}$) maximization . However, there are conditions where the cell does not grow optimally [17].

Maximize ATP yield. The objective of maximizing ATP yield (max v_{ATP}) is based on the assumption that cells maximize energy production when oxygen is available.[16]

Maximize ATP yield in the cytosol. Studies of perturbation-response experiments showed that after a glucose pulse there is a switch from respiratory to respiro-fermentative metabolism and as a consequence low TCA activity.[5] This means that there is low ATP production in the mitochondria. Therefore maximization of cytosolic ATP (max v_{ATPcyt}) was incorporated.

Maximize glucose uptake. Maximizing glucose uptake is motivated by the fact that after a glucose pulse *S.cerevisiae* rapidly takes up the excess of glucose.[6]

Maximize ethanol yield. *S.cerevisiae* is known to ferment an excess of glucose to ethanol.[19] Maximization of ethanol (max $v_{ethanol}$) is therefore also included.

Minimization of the overall flux. Cells are assumed to minimize their enzyme usage, which corresponds to minimizing their overall flux.[17, 10] This can be mathematically formulated as minimizing the sum of the absolute values of all fluxes.[19] The following dynamic optimization problem has to be solved:

$$\begin{aligned}
&\text{minimize} \int_{t_0}^{t_f} \left(\sum_{j=1}^{r} |v_j(t)| \right) dt \\
&\text{subject to} \\
&A \cdot v_t = b \\
&v_{min} \leq v_t \leq v_{max}
\end{aligned}$$

This problem can be converted to a linear optimization problem [14] by introducing r more (slack) variables $v_{j+r} = |v_j| \ \ (j = 1, \cdots, r)$. The optimization problem above is equivalent with: [4]

$$\begin{aligned}
&\text{minimize } F = \int_{t_0}^{t_f} \left(\sum_{j=1}^{r} v_{j+r} \right) dt \\
&\text{subject to} \\
&A \cdot v_t = b \\
&v_{min} \leq v_t \leq v_{max} \\
&v_{j+r} \geq -v_j \ \ (j = 1, \cdots, r) \\
&v_{j+r} \geq v_j \ \ (j = 1, \cdots, r)
\end{aligned}$$

Approximating the integral in the objective function by using the trapezoidal rule results in a linear objective function.

Minimization of Metabolic Adjustment (MOMA). Organisms are assumed to adjust their metabolism with minimal effort after a perturbation. This is called Minimization of Metabolic Adjustment (MOMA).[18] The following quadratic optimization problem is solved:

$$\begin{aligned}
&\text{minimize } \sum_{i=1}^{n}\sum_{j=1}^{r}\left(v_j\left(t_0\right)-v_j\left(t_i\right)\right)^2\\
&\text{subject to}\\
&A\cdot v_t = b\\
&v_{min}\leq v_t \leq v_{max}
\end{aligned}$$

This results in an optimum F_{opt} for the objective function and optimal dynamic rate profiles.

Supplementary Table 7: Overview of the objective functions used in the *S.cerevisiae* study.

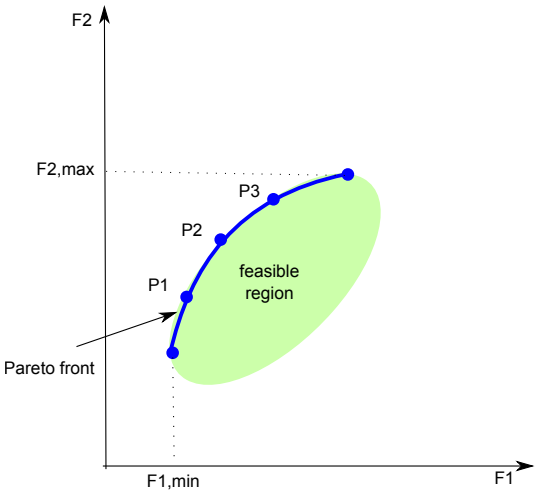
Objective function	**Explanation**
max $v_{biomass}$ subject to $A \cdot v_t = b$ irreversibility constraints (see FBA)	• maximal biomass yield (v_{54}) • most common objective function in FBA studies[13, 15]
max v_{ATP} subject to $A \cdot v_t = b$ irreversibility constraints rate to TCA cycle $\leq$ steady state flux to TCA cycle growth rate = 0.1 h^{-1}	• maximal energetic efficiency[17, 13] • reactions contributing to the objective function: v_2,v_7,v_9,v_{10},v_{11},v_{12},v_{16},v_{17},v_{26},v_{29}, v_{36},v_{37},v_{42},v_{43},v_{54} • switch to respiro-fermentative metabolism (excess glucose (from the pulse) fermented to ethanol)[12] • growth rate does not change significantly during a small time interval
max v_{ATPcyt} subject to $A \cdot v_t = b$ irreversibility constraints rate to TCA cycle $\leq$ steady state flux to TCA cycle growth rate = 0.1 h^{-1}	• maximize energetic efficiency in the cytosol • like previous objective, but without v_{36} • respiro-fermentative metabolism[5] $\rightarrow$ low TCA cycle activity $\rightarrow$ low ATP production in mitochondria • excess glucose fermented to ethanol • growth rate does not change significantly during a small time interval
max glucose uptake rate subject to $A \cdot v_t = b$ irreversibility constraints rate to TCA cycle $\leq$ steady state flux to TCA cycle growth rate = 0.1 h^{-1}	• organism consumes the excess of glucose as fast as possible • glucose uptake rate = v_{29} • excess glucose fermented to ethanol • growth rate does not change significantly during a small time interval
max $v_{ethanol}$ subject to $A \cdot v_t = b$ irreversibility constraints growth rate = 0.1 h^{-1}	• maximize ethanol yield (v_{57}) • excess glucose fermented to ethanol • growth rate does not change significantly during a small time interval
min sum of absolute fluxes subject to $A \cdot v_t = b$ irreversibility constraints $0.1 \leq$ growth rate ≤ 1000	• minimize enzyme usage • same constraints as FBA
min $\sum_{i=1}^{n} \sum_{j=1}^{r} \left(v_j\left(t_0\right) - v_j\left(t_i\right)\right)^2$ subject to $A \cdot v_t = b$ irreversibility constraints	• minimization of metabolic adjustment (MOMA)

Supplementary Table 8: *S.cerevisiae* study. Results of the evaluation of the five criteria described in section 3.2.1. in the main text for MetDFBA with the objective functions in Supplementary Table 7.

objective function	**order of the optimum**	**contradiction with**
max $v_{biomass}$	10^4	1
max v_{ATP}	10^5	1
max v_{ATPcyt}	10^{-1}	1
max $v_{glucose\ uptake\ rate}$	10^5	1
max $v_{ethanol}$	10^5	1
min sum of absolute fluxes	10^3	2 and 5
MOMA	10^2	5

Supplementary Table 9: Overview of the multi-objective optimization problems solved in the *S.cerevisiae* study.

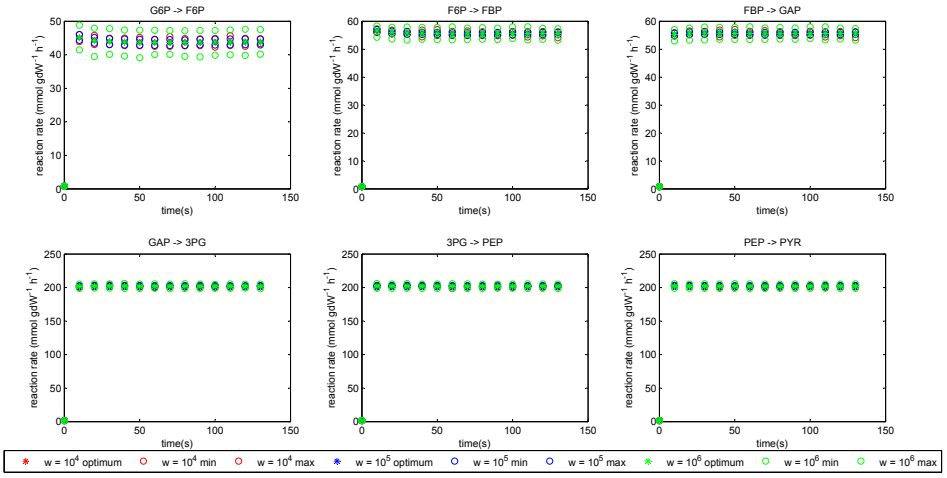
objective function
$\min \left\{ \left(\sum_{i=1}^{n} \sum_{j=1}^{r} \left(v_j\left(t_0\right) - v_j\left(t_i\right) \right)^2 \right) - w \cdot v_{ATP} \right\}$
$\min \left\{ \left(\sum_{i=1}^{n} \sum_{j=1}^{r} \left(v_j\left(t_0\right) - v_j\left(t_i\right) \right)^2 \right) - w \cdot v_{gluc\ uptake} \right\}$
$\min \left\{ \left(\sum_{i=1}^{n} \sum_{j=1}^{r} \left(v_j\left(t_0\right) - v_j\left(t_i\right) \right)^2 \right) - w \cdot v_{ETH} \right\}$
$\min \left\{ \left(\sum_{i=1}^{n} \sum_{j=1}^{r} \left(v_j\left(t_0\right) - v_j\left(t_i\right) \right)^2 \right) - w \cdot v_{biomass} \right\}$
$\min \left\{ \left(\sum_{i=1}^{n} \sum_{j=1}^{r} \left(v_j\left(t_0\right) - v_j\left(t_i\right) \right)^2 \right) - w \cdot v_{ATPcyt} \right\}$
$\min \left\{ sum\ of\ absolute\ fluxes\ - w \cdot v_{ATP} \right\}$
$\min \left\{ sum\ of\ absolute\ fluxes\ - w \cdot v_{gluc\ uptake} \right\}$
$\min \left\{ sum\ of\ absolute\ fluxes\ - w \cdot v_{ETH} \right\}$
$\min \left\{ sum\ of\ absolute\ fluxes\ - w \cdot v_{biomass} \right\}$
$\min \left\{ sum\ of\ absolute\ fluxes\ - w \cdot v_{ATPcyt} \right\}$



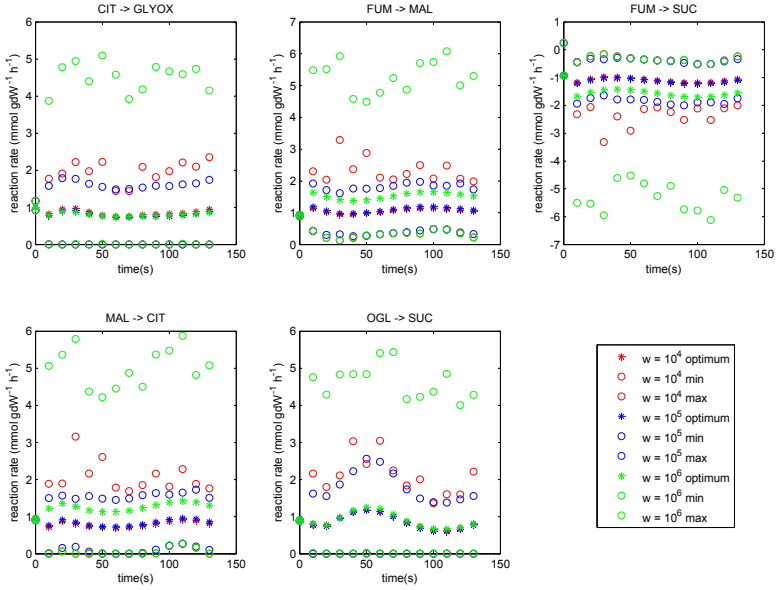
Supplementary Figure 12: Different Pareto optimal solutions $P_1, P_2, P_3, \cdots$ can be calculated by varying the weight w in the multi-objective optimization.

Supplementary Table 10: *S.cerevisiae* study. Results of the evaluation of the five criteria described in section 3.2.1. when solving the multi-objective optimization problems of Supplementary Table 9.

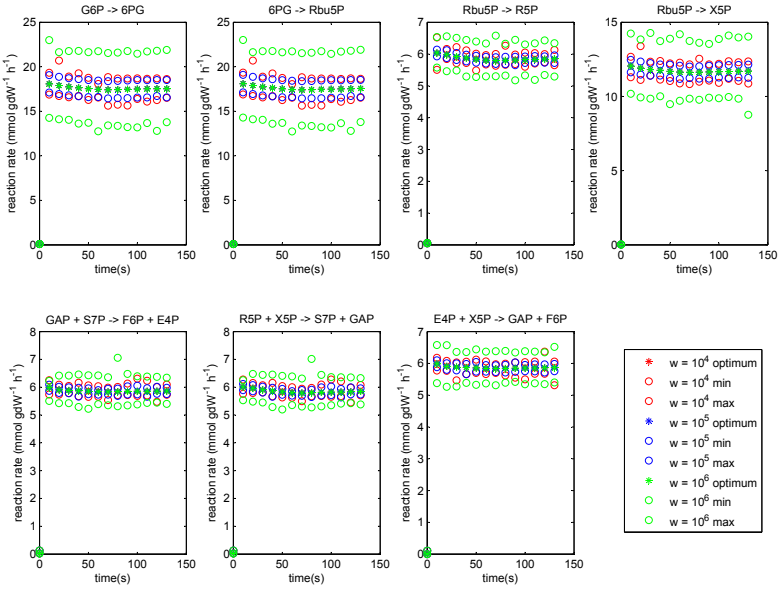
objective $F1 - w \cdot F2$	w	**order of** $F1$	**order of** $F2$	**Contradiction with literature?**
$\min\left\{\left(\sum_{i=1}^{n}\sum_{j=1}^{r}\left(v_j\left(t_0\right)-v_j\left(t_i\right)\right)^2\right)-w\cdot v_{ATP}\right\}$	$0.001-10$	10^2	10^2	contradiction with 5
	$100-10^5$	10^4-10^7	10^3-10^5	contradiction with 3 and 5
	10^6	10^7	10^5	contradiction with 3
$\min\left\{\left(\sum_{i=1}^{n}\sum_{j=1}^{r}\left(v_j\left(t_0\right)-v_j\left(t_i\right)\right)^2\right)-w\cdot v_{gluc\ uptake}\right\}$	$0.001-10$	10^2-10^3	10^2	contradiction with 5
	$100-10^5$	10^4-10^7	10^3-10^5	contradiction with 4 and 5
	10^6	10^7	10^5	contradiction with 4
$\min\left\{\left(\sum_{i=1}^{n}\sum_{j=1}^{r}\left(v_j\left(t_0\right)-v_j\left(t_i\right)\right)^2\right)-w\cdot v_{ETH}\right\}$	$0.001-10^3$	10^2-10^6	10^2-10^4	contradiction with 5
	10^4-10^6	10^7	10^5	**no contradiction**
$\min\left\{\left(\sum_{i=1}^{n}\sum_{j=1}^{r}\left(v_j\left(t_0\right)-v_j\left(t_i\right)\right)^2\right)-w\cdot v_{biomass}\right\}$	$0.001-10^3$	10^2-10^3	10^1	contradiction with 5
	10^4-10^6	10^5-10^8	10^2-10^4	contradiction with 2
$\min\left\{\left(\sum_{i=1}^{n}\sum_{j=1}^{r}\left(v_j\left(t_0\right)-v_j\left(t_i\right)\right)^2\right)-w\cdot v_{ATPcyt}\right\}$	$0.001-10^6$	10^2	10^{-1}	contradiction with 5
$\min\{sum\ of\ absolute\ fluxes\ -w\cdot v_{ATP}\}$	$0.001-1$	10^3	10^0	contradiction with 4 and 5
	$10-10^6$	10^5	10^5	contradiction with 3, 4 and 5
$\min\{sum\ of\ absolute\ fluxes\ -w\cdot v_{gluc\ uptake}\}$	$0.001-1$	10^3	10^1	contradiction with 4 and 5
	$10-10^6$	10^5	10^5	contradiction with 5
$\min\{sum\ of\ absolute\ fluxes\ -w\cdot v_{ETH}\}$	$0.001-10^6$	10^3-10^5	10^2-10^5	contradiction with 4 and 5
$\min\{sum\ of\ absolute\ fluxes\ -w\cdot v_{biomass}\}$	$0.001-1$	10^3	10^1	contradiction with 2, 4 and 5
	10	10^3	10^1	contradiction with 4 and 5
	100	10^5	10^3	contradiction with 2 and 5
	1000	10^6	10^4	contradiction with 5
	10^4-10^6	10^6	10^4	contradiction with 2 and 5
$\min\{sum\ of\ absolute\ fluxes\ -w\cdot v_{ATPcyt}\}$	$0.001-10^6$	10^3	10^{-1}	contradiction with 4 and 5



Supplementary Figure 13: *S.cerevisiae* study. Plots glycolysis for MOMA + max $w \cdot v_{ETH}$, $w = 10^4 - 10^6$.



Supplementary Figure 14: *S.cerevisiae* study. Plots TCA cycle for MOMA + max $w \cdot v_{ETH}$, $w = 10^4 - 10^6$.



Supplementary Figure 15: *S.cerevisiae* study. Plots pentose phosphate pathway for MOMA + max $w \cdot v_{ETH}$, $w = 10^4 - 10^6$.

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