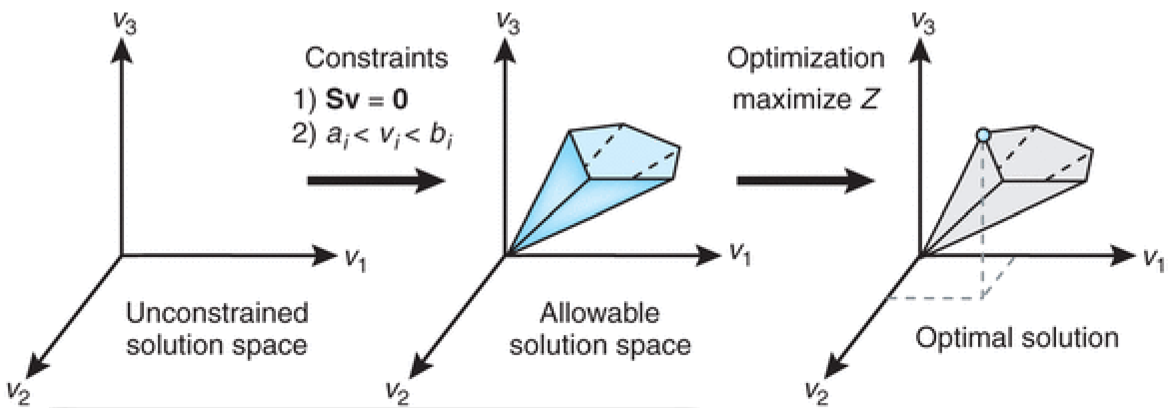
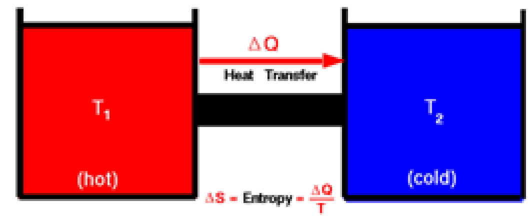
CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

virtual liver network

Flux-balance Optimization Thermodynamics Constraints





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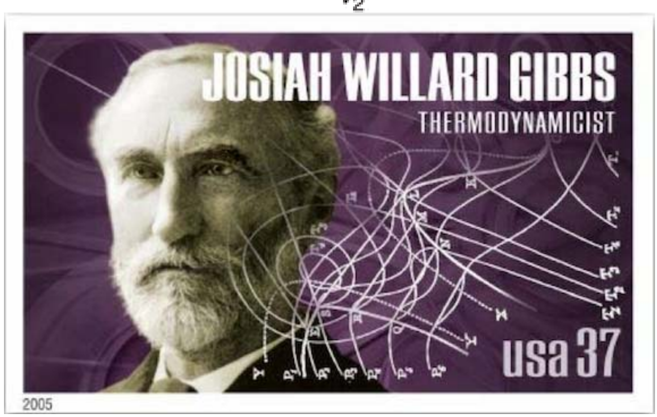

COMPUTATIONAL SYSTEMS BIOCHEMISTRY

Overview

1. What is flux-balance optimization?
2. Problem of reversibility (E. coli growth 1)
3. Thermodynamic Realizability (TR)
4. What defies thermodynamics?
5. Testing with known concentrations (E. coli growth 2)
6. Estimating standard Gibbs' energies

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What is Flux-balance optimization?

- **Mathematical framework** for the modeling of a biochemical reaction system

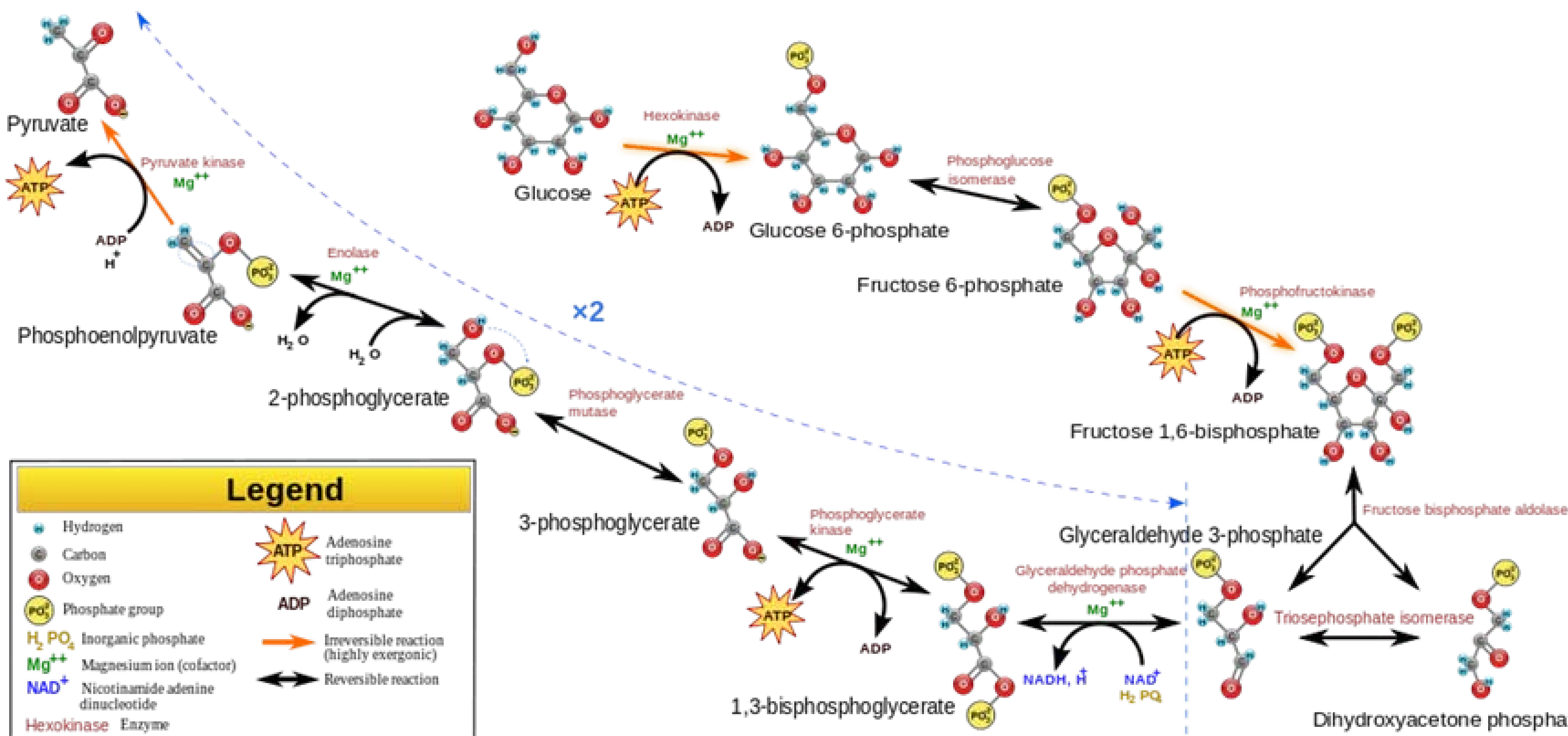
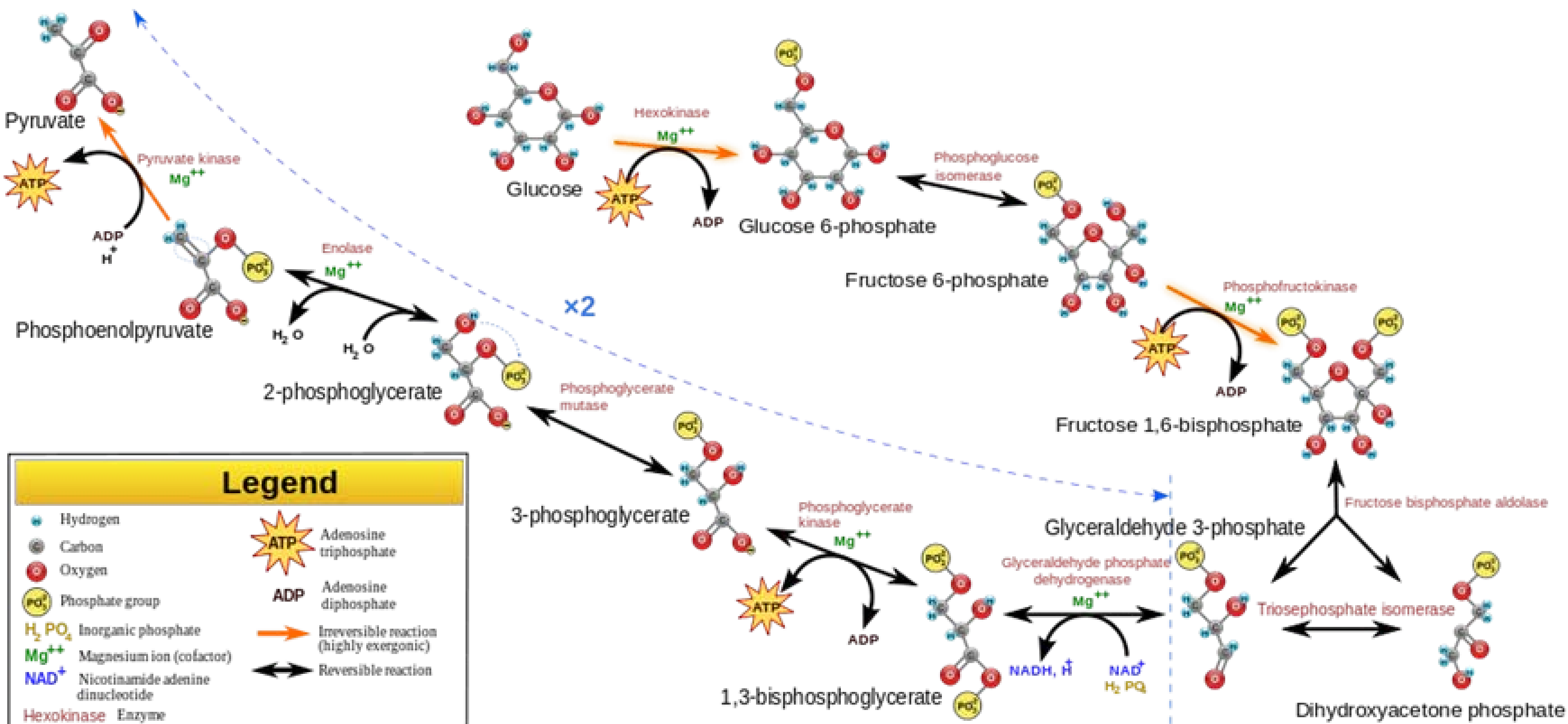


image source: Wikipedia/JohnyAbb

What is Flux-balance optimization?

- Mathematical framework for the modeling of a biochemical reaction system
- Flux-balance: no internal metabolite accumulated/drained



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What is Flux-balance optimization?

- **Mathematical framework** for the modeling of a biochemical reaction system
- **Flux-balance**: no internal metabolite accumulated/drained
- Optimization: way to compute reaction fluxes

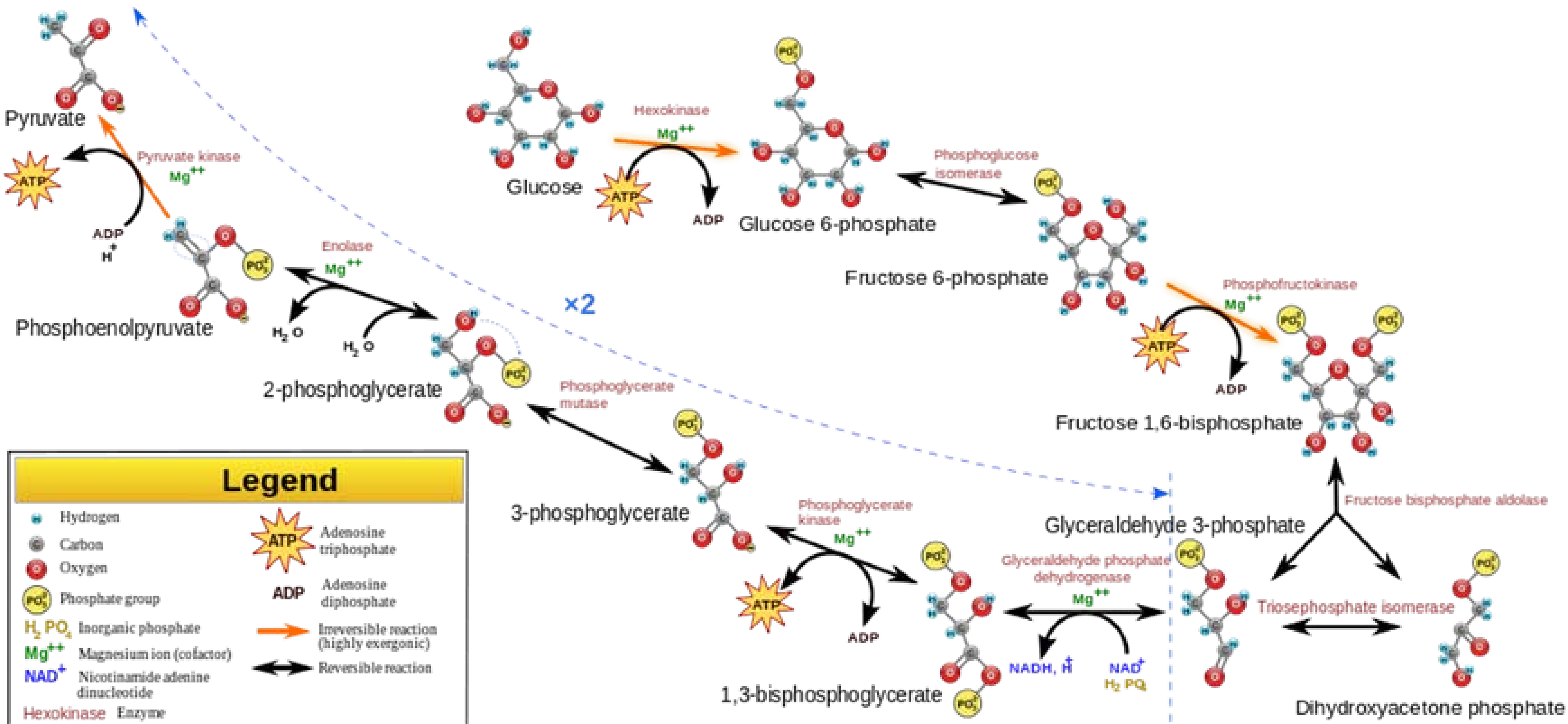
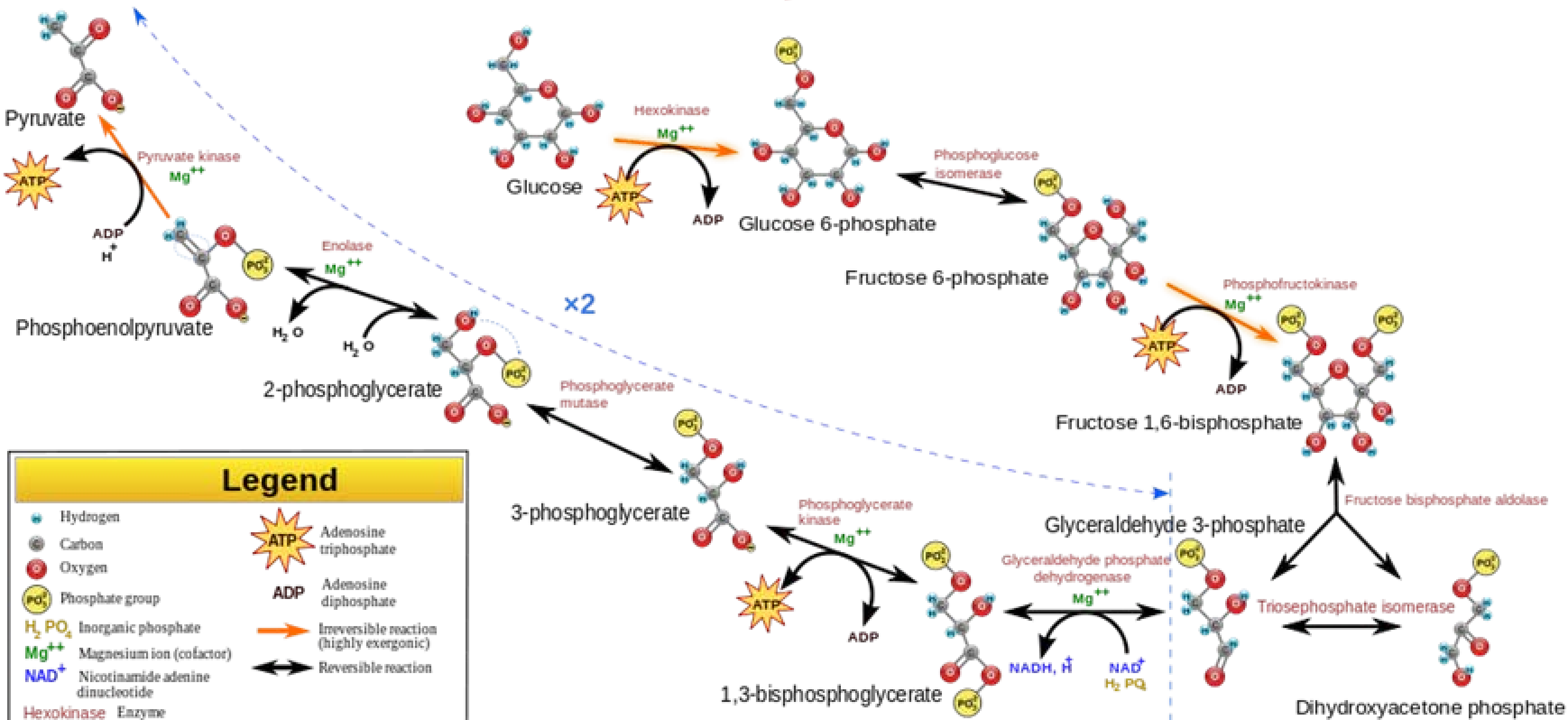


image source: Wikipedia/JohnyAbb

HK: Glc + ATP -> G6P + ADP
PGI: G6P -> F6P
PFK: F6P + ATP -> F1,6BP + ADP
FBA: F1,6BP -> DHAP + GAP
TPI: GAP -> DHAP
GAPD: DHAP + NAD + PI -> NADH + 13DPG
PGK: 13DPG + ADP -> 3PG + ATP
PGM: 3PG -> 2PG
ENO: 2PG -> PEP + H2O
PYK: PEP + ADP -> PYR + ATP



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PGK: 13DPG + ADP -> 3PG + ATP
PGM: 3PG -> 2PG
ENO: 2PG -> PEP + H2O
PYK: PEP + ADP -> PYR + ATP

S	HK	PGI	PFK	FBA	TPI	GAPD	PGK	PGM	ENO	PYK
Glc	-1	0	0	0	0	0	0	0	0	0
G6P	1	-1	0	0	0	0	0	0	0	0
F6P	0	1	-1	0	0	0	0	0	0	0
FBP	0	0	1	-1	0	0	0	0	0	0
GAP	0	0	0	1	-1	0	0	0	0	0
DHAP	0	0	0	1	1	-1	0	0	0	0
13DPG	0	0	0	0	0	1	-1	0	0	0
3PG	0	0	0	0	0	0	1	-1	0	0
2PG	0	0	0	0	0	0	0	1	-1	0
PEP	0	0	0	0	0	0	0	0	1	-1
PYR	0	0	0	0	0	0	0	0	0	1
ATP	-1	0	-1	0	0	0	1	0	0	1
ADP	1	0	1	0	0	0	-1	0	0	-1
NADH	0	0	0	0	0	1	0	0	0	0
NAD	0	0	0	0	0	-1	0	0	0	0
PI	0	0	0	0	0	-1	0	0	0	0
H2O	0	0	0	0	0	0	0	0	1	0

$$\mathbf{S} \cdot \mathbf{v} = \frac{dC}{dt}$$

S

	HK	PGI	PFK	FBA	TPI	GAPD	PGK	PGM	ENO	PYK
Glc	-1	0	0	0	0	0	0	0	0	0
G6P	1	-1	0	0	0	0	0	0	0	0
F6P	0	1	-1	0	0	0	0	0	0	0
FBP	0	0	1	-1	0	0	0	0	0	0
GAP	0	0	0	1	-1	0	0	0	0	0
DHAP	0	0	0	1	1	-1	0	0	0	0
13DPG	0	0	0	0	0	1	-1	0	0	0
3PG	0	0	0	0	0	0	1	-1	0	0
2PG	0	0	0	0	0	0	0	1	-1	0
PEP	0	0	0	0	0	0	0	0	1	-1
PYR	0	0	0	0	0	0	0	0	0	1
ATP	-1	0	-1	0	0	0	1	0	0	1
ADP	1	0	1	0	0	0	-1	0	0	-1
NADH	0	0	0	0	0	1	0	0	0	0
NAD	0	0	0	0	0	-1	0	0	0	0
PI	0	0	0	0	0	-1	0	0	0	0
H2O	0	0	0	0	0	0	0	0	1	0

v

$$\mathbf{v} = \begin{pmatrix} V_{HK} \\ V_{PGI} \\ V_{PFK} \\ V_{FBA} \\ V_{TPI} \\ V_{GAPD} \\ V_{PGK} \\ V_{PGM} \\ V_{ENO} \\ V_{PYK} \end{pmatrix}$$

=

$$\frac{dC}{dt} = \begin{pmatrix} \text{Glc} \\ \text{G6P} \\ \text{F6P} \\ \text{FBP} \\ \text{GAP} \\ \text{DHAP} \\ \text{13DPG} \\ \text{3PG} \\ \text{2PG} \\ \text{PEP} \\ \text{PYR} \\ \text{ATP} \\ \text{ADP} \\ \text{NADH} \\ \text{NAD} \\ \text{PI} \\ \text{H2O} \end{pmatrix}$$

= vector of change of metabolite amount

FB optimization – Reversibility – Thermodynamic Realizability – Defied TD – Concentrations – Gibbs' energies

internal metabolites

S

	HK	PGI	PFK	FBA	TPI	GAPD	PGK	PGM	ENO	PYK
Glc	-1	0	0	0	0	0	0	0	0	0
G6P	1	-1	0	0	0	0	0	0	0	0
F6P	0	1	-1	0	0	0	0	0	0	0
FBP	0	0	1	-1	0	0	0	0	0	0
GAP	0	0	0	1	-1	0	0	0	0	0
DHAP	0	0	0	1	1	-1	0	0	0	0
13DPG	0	0	0	0	0	1	-1	0	0	0
3PG	0	0	0	0	0	0	1	-1	0	0
2PG	0	0	0	0	0	0	0	1	-1	0
PEP	0	0	0	0	0	0	0	0	1	-1
PYR	0	0	0	0	0	0	0	0	0	1
ATP	-1	0	-1	0	0	0	1	0	0	1
ADP	1	0	1	0	0	0	-1	0	0	-1
NADH	0	0	0	0	0	1	0	0	0	0
NAD	0	0	0	0	0	-1	0	0	0	0
PI	0	0	0	0	0	-1	0	0	0	0
H2O	0	0	0	0	0	0	0	0	1	0

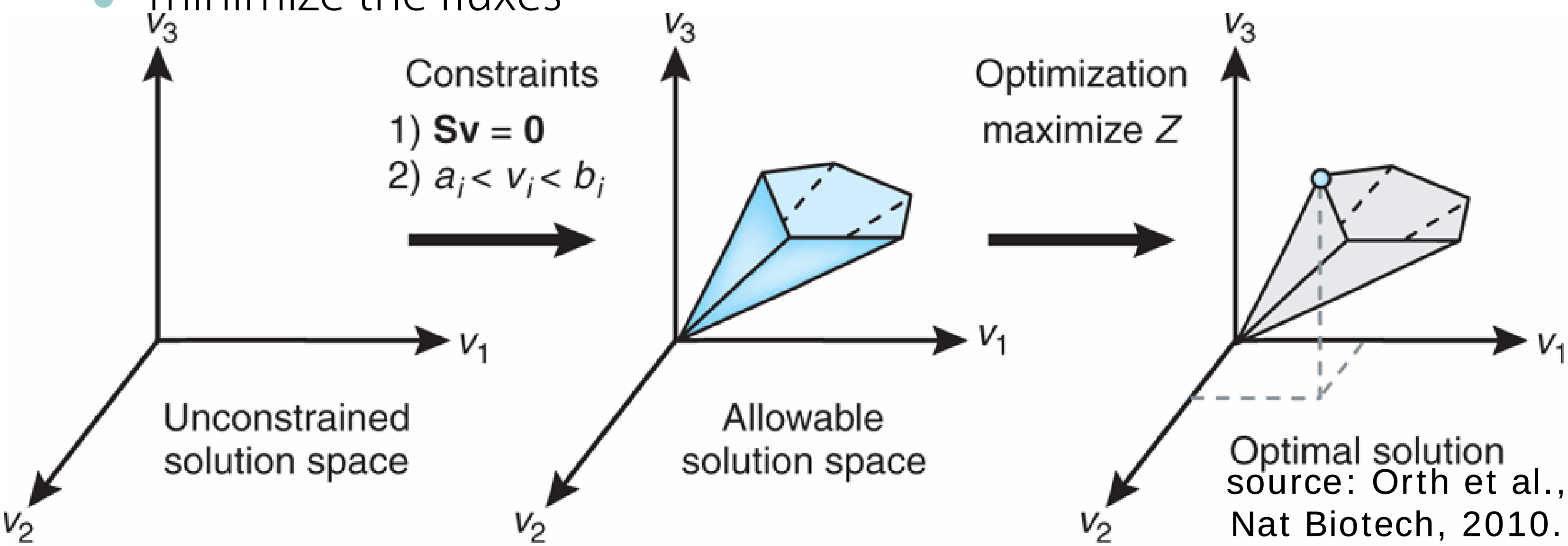
$$\mathbf{v} = \begin{pmatrix} V_{HK} \\ V_{PGI} \\ V_{PFK} \\ V_{FBA} \\ V_{TPI} \\ V_{GAPD} \\ V_{PGK} \\ V_{PGM} \\ V_{ENO} \\ V_{PYK} \end{pmatrix} = \frac{dC}{dt} = \begin{pmatrix} \text{Glc} \\ \text{G6P} \\ \text{F6P} \\ \text{FBP} \\ \text{GAP} \\ \text{DHAP} \\ \text{13DPG} \\ \text{3PG} \\ \text{2PG} \\ \text{PEP} \\ \text{PYR} \\ \text{ATP} \\ \text{ADP} \\ \text{NADH} \\ \text{NAD} \\ \text{PI} \\ \text{H2O} \end{pmatrix}$$

= vector of change of metabolite amount

FB optimization – Reversibility – Thermodynamic Realizability – Defied TD – Concentrations – Gibbs' energies

What is Flux-balance optimization?

- $\mathbf{Sv}|_{\mathbf{intern}} = \mathbf{0}$
- Solution space: all flux vectors satisfying FBC
- Constraints: e.g. $0 \leq v_{HK} \leq 2$
- Flux-balance optimization: select one solution as maximum of a scoring function φ( ), e.g.
  - maximize the biomass production
  - maximize ATP yield
  - minimize the fluxes



source: Orth et al., Nat Biotech, 2010.

How to solve?

- : linear equations
- $\alpha_i \leq v_i \leq \beta_i$: linear inequalities
- $\varphi(\;)$: linear optimization function
- Called "**linear program**"
  - efficient solvers available
  - millions of variables
  -

lp_solve, glpk ... GPL CPLEX ... commercial, free academic licenses MATLAB, LINDO ... commercial

Flux-balance analysis

- Knockout strain prediction
  - Edwards & Palsson. BMC Bioinformatics, 2000.
- Prediction of unknown reactions: gluconate utilization
  - Rolfsson et al., BMC Syst Biol, 2011.
- Unknown reaction paths: sugar from fat
  - Kaleta et al., PLoS Comput Biol, 2011.

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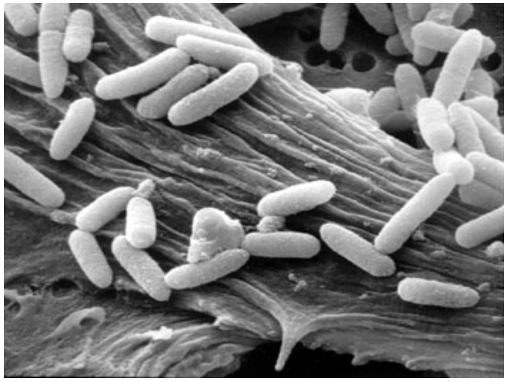
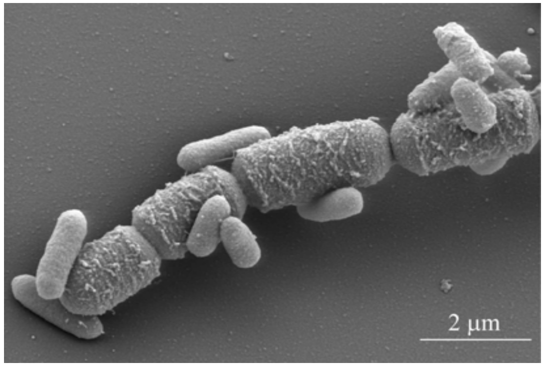
E. coli model

- Reed&Palsson 2004 jR904 network:
  - 904 metabolites
  - 932 reactions, transporters
  - Growth function: energy equivalents, amino acids, lipid pools, macromolecules



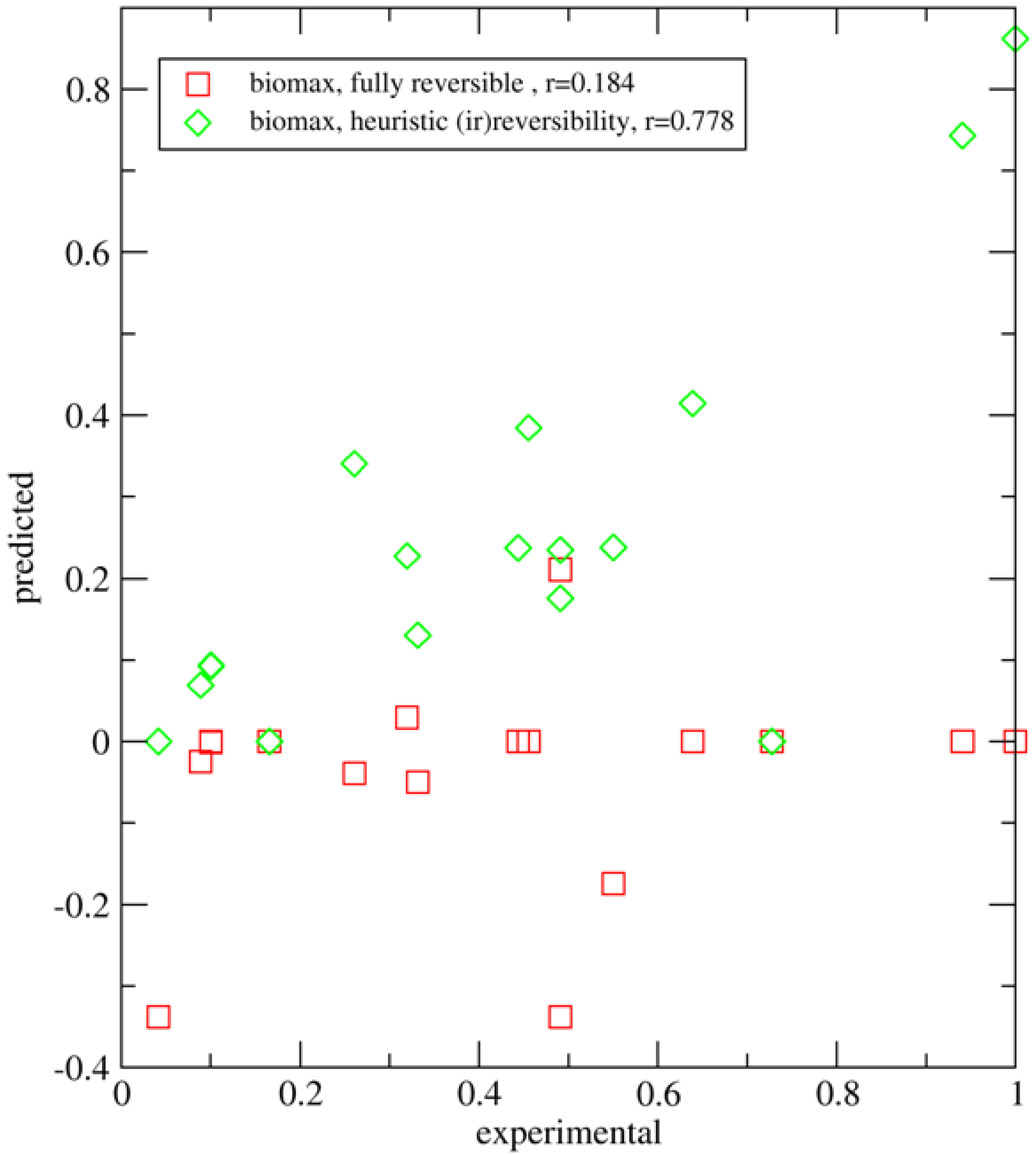


Reversibility

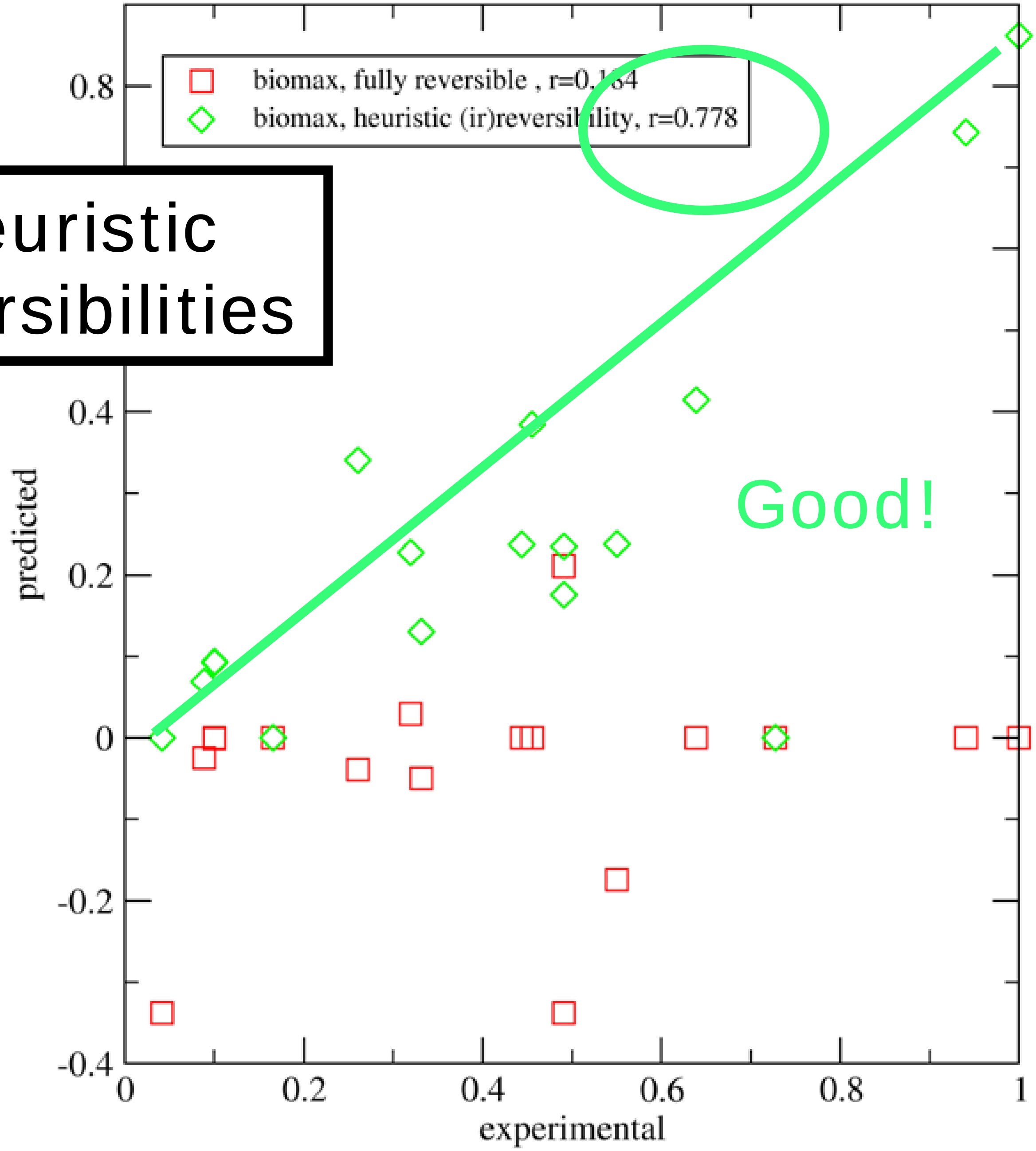
- Heuristic setting:
  - 245 reversible reactions
  - 687 reactions fixed to one direction
- Based on
  - Biochemical knowledge
  - Thermodynamical considerations
  - Purpose

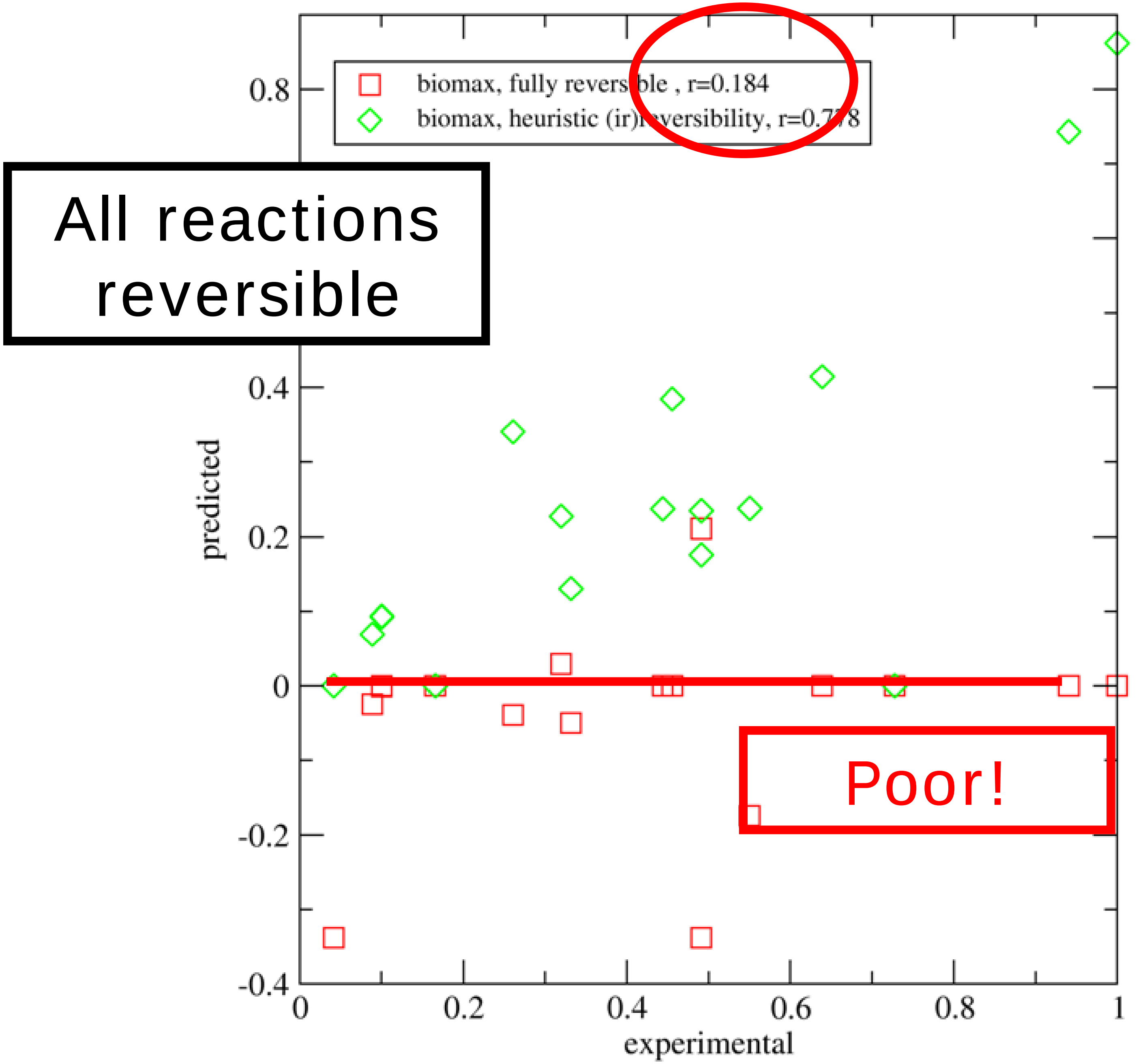
Flux measurements

- Emmerling et al. J. Bacteriol. 2002
- Comparison with values predicted by FBA (biomass maximization)
  - Heuristic reversibilities (Reed et al., Genome Biol, 2003.)
  - Fully reversible model


biomax, fully reversible , r=0.184
biomax, heuristic (ir)reversibility, r=0.778
0.8
0.6
0.4
0.2
0
-0.2
-0.4
predicted
0
0.2
0.4
0.6
0.8
1
experimental

Heuristic reversibilities





biomax, fully reversible , r=0.184
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All reactions reversible
Poor!
predicted
experimental
0.8
0.4
0.2
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0
0.2
0.4
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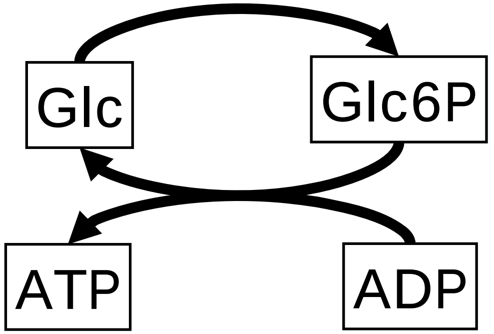
0.8
1

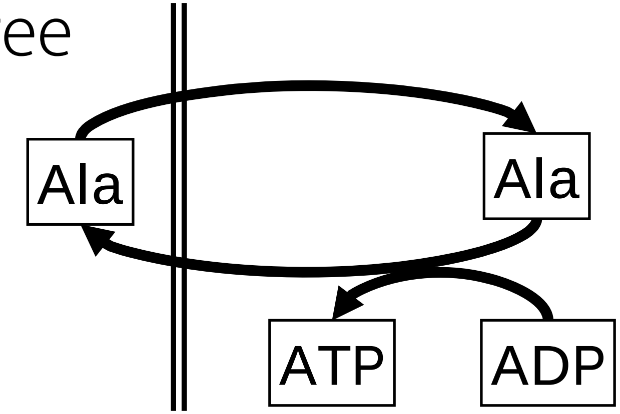
Importance of Reversibility

- Rephosphorylation of ATP for free



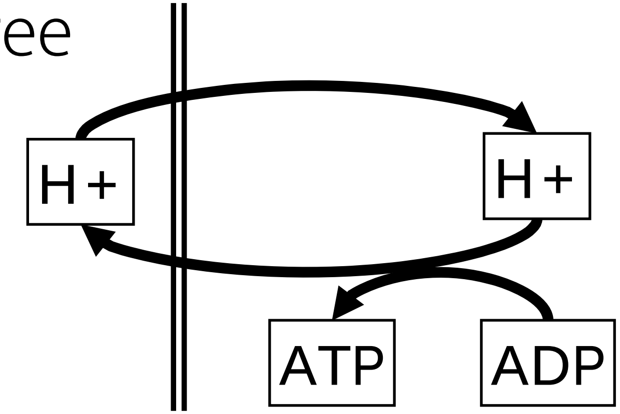
Importance of Reversibility

- Rephosphorylation of ATP for free
- Active transports



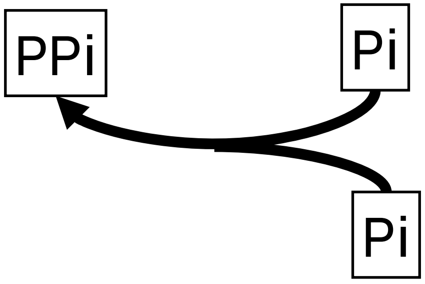
Importance of Reversibility

- Rephosphorylation of ATP for free
- Active transports
- Pumped protons



Importance of Reversibility

- Rephosphorylation of ATP for free
- Active transports
- Pumped protons
- Highly exergone reactions



Reversibility of reactions

- Chemical reactions are governed by thermodynamics

Net reaction flux proceeds in the direction of negative Gibbs' free energy ΔG_r

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> **Net reaction flux proceeds in the direction of negative Gibbs' free energy** ΔG_r

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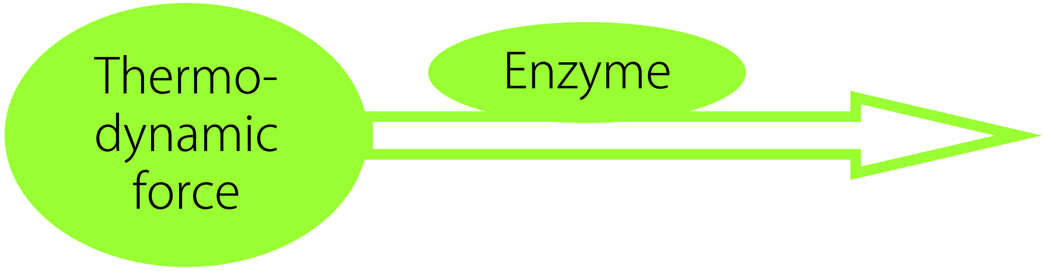
- Catalysts such as enzymes do not change this



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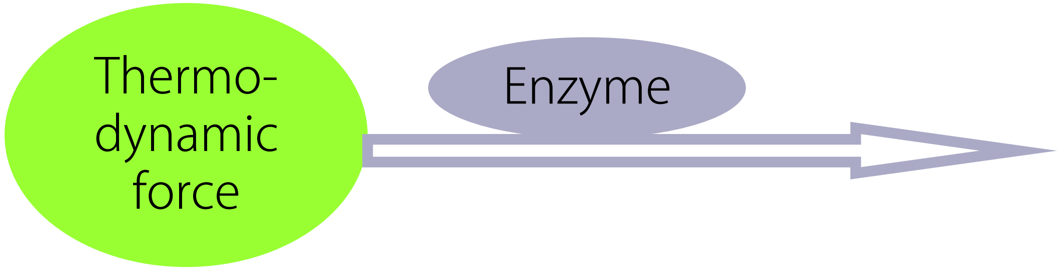

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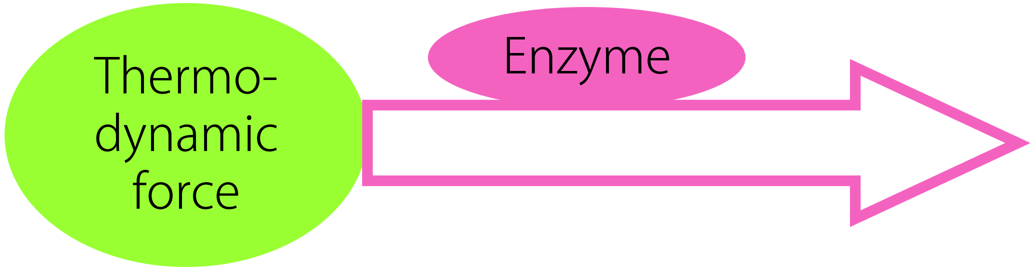


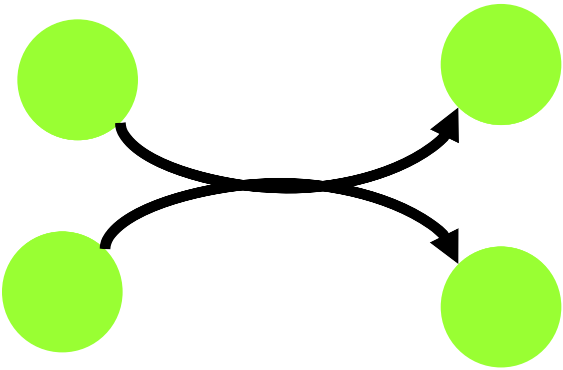
Dependence on concentrations

$$\Delta G_r = \Delta G_r^0 + RT \sum_{\text{products}} \ln[M] - RT \sum_{\text{substrates}} \ln[M]$$

- R ··· gas constant
- T ··· temperature
- $[M]$ ··· active concentration
- ΔG_r^0 ··· standard Gibbs' free energy

Reversibility

- Almost every reaction is reversible in principle
- Concentration gradient may sometimes not be sufficient
- Only few reactions are strictly irreversible for cellular concentrations (Henry et al., Biophys J, 2006.)



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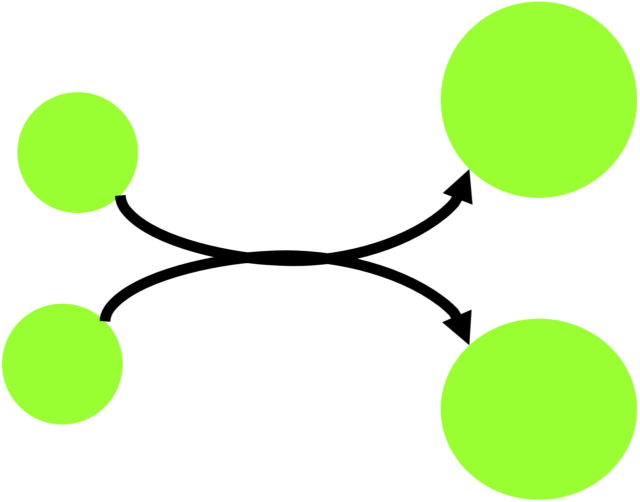

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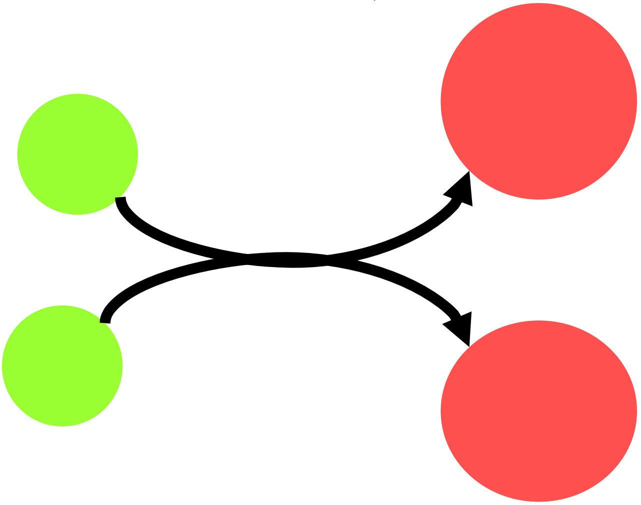

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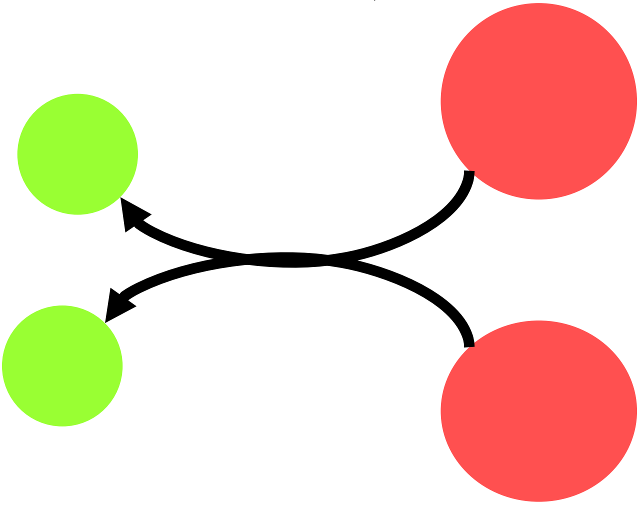

Heuristic reversibility settings

- E. coli network is designed for normal growth
- Predictions may be poor for extreme cellular states
- Requires ad hoc assignments

Aim:

- Flexible as the fully reversible setting
- Effective as the heuristic setting
- Based on objective criterion
- Not be based on “purpose”

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Thermodynamics applied to metabolic networks

- First application to metabolic networks (paths):
  - Mavrovouniotis Proc Int Conf Intell Syst Mol Biol. 1993
- Exclude flux distributions infeasible for arbitrary concentrations
  - Beard/Qian, J Theor Biol, 2004, PLoS One. 2007.
- Thermodynamic assessment: is a given flux distribution compatible with concentrations?
  - Kümmel et al., BMC Bioinf, 2006.
  - Henry et al., Biophys J, 2007.

Thermodynamic Feasibility

- Flux distribution is consistent with given concentrations, if every flux proceeds in the direction of negative ΔG_r

  Henry et. al., Biophys J, 2004.

Thermodynamic Realizability (TR)

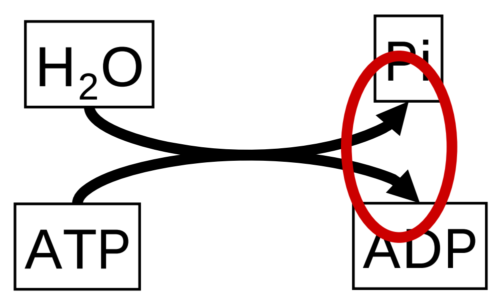
- Flux distribution is called TR if there exist concentrations (within physiological boundaries) such that the system is thermodynamically feasible.

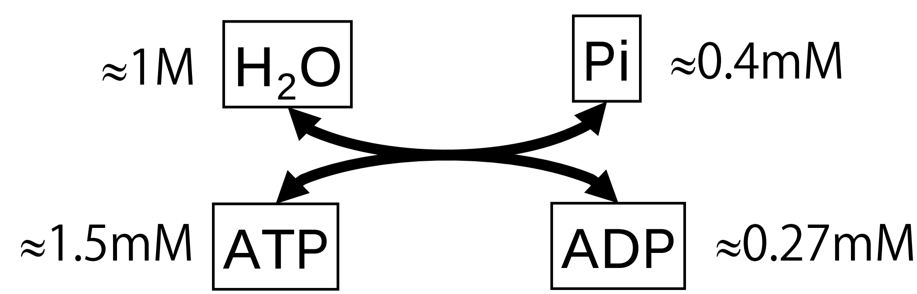
Hoppe et. al., BMC Systems Biology, 2007.

Thermodynamic Realizability (TR)

Problem moved:

Heuristic setting of direction → Metabolite concentration ranges & Accurate Gibbs' free energy values

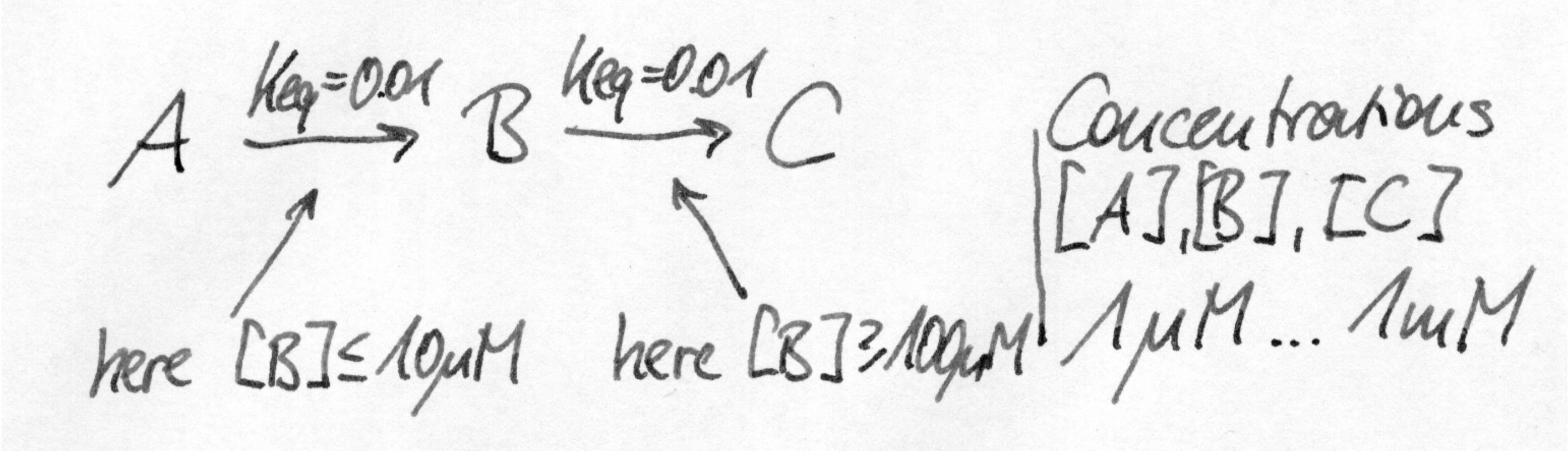




$$\overline{\Delta G_r^0} = -30.5\,\frac{kJ}{mol}$$

TR is a systemic property

- Thermodynamics has been frequently used to fix (single) directions ⋯ but



TR as a constraint for FBA

$$\mathrm{sgn}(\mathrm{V}) = -\mathrm{sgn}(\overline{\Delta \mathrm{G}_{\mathrm{r}}^{0}} + \mathbf{S}\mathrm{C})$$

Constants:

$\mathbf{S}$ ··· stoichiometric matrix (given)

$\Delta_{r} G_{0}$··· standard Gibbs‘ free energies

Variables:

$\mathbf{V}$ ··· (column) vector flux distribution

$\mathbf{C}$ ··· (column) vector of log-concentrations *R*T*

Constraints:

Ranges for $\mathbf{C}$

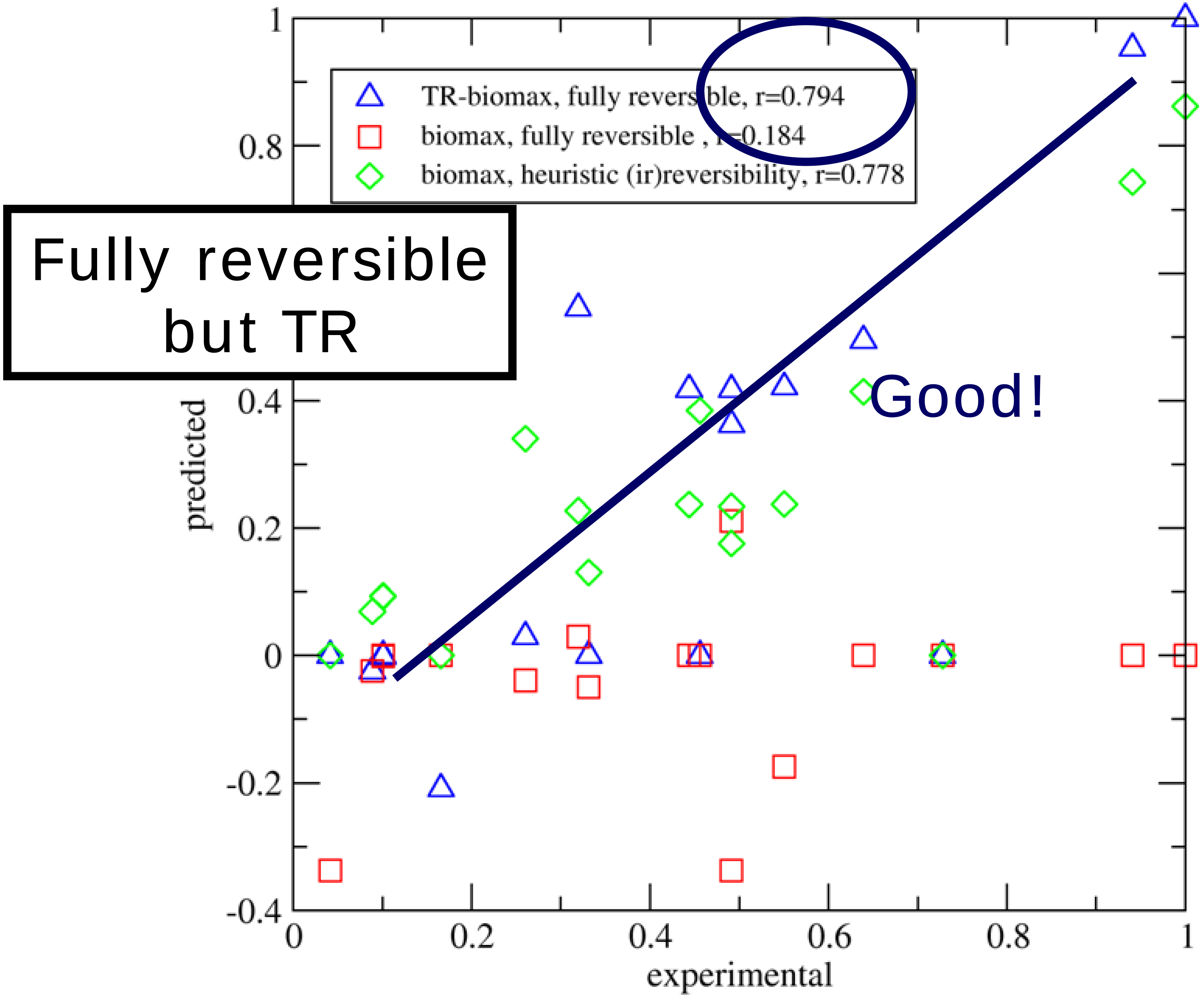
Computability

$$\mathrm{sgn}(V) = -\mathrm{sgn}(\overline{\Delta G_r^0} + \mathbf{S}C)$$

- Calculation with log concentrations
- Constants: $\overline{\Delta G_r^0}$
- Linear equation set: $\overline{\Delta G_r^0} + \mathbf{S}C$
- sgn(), boolean variables
- FBA becomes a mixed-boolean linear program
- efficient implementation in CPLEX: clauses
- vs. logarithmic optimization
  - anNET: Zamboni et al,. BMC Bioinf, 2008.

Concentrations – not so unknown

- Some metabolites measured
- From other cell types/organisms
- General assumptions for unknown concentrations
  - <1M – general chemical properties
  - >1pM – substrates must find enzymes
  - Greater size – lower concentration
  - High conversion fluxes – higher concentrations
  - Normally, confined to 6 orders


TR-biomax, fully reversible, r=0.794
biomax, fully reversible , r=0.184
biomax, heuristic (ir)reversibility, r=0.778
Fully reversible but TR
Good!
predicted
experimental
1
0.8
0.4
0.2
0
-0.2
-0.4
0
0.2
0.4
0.6
0.8
1

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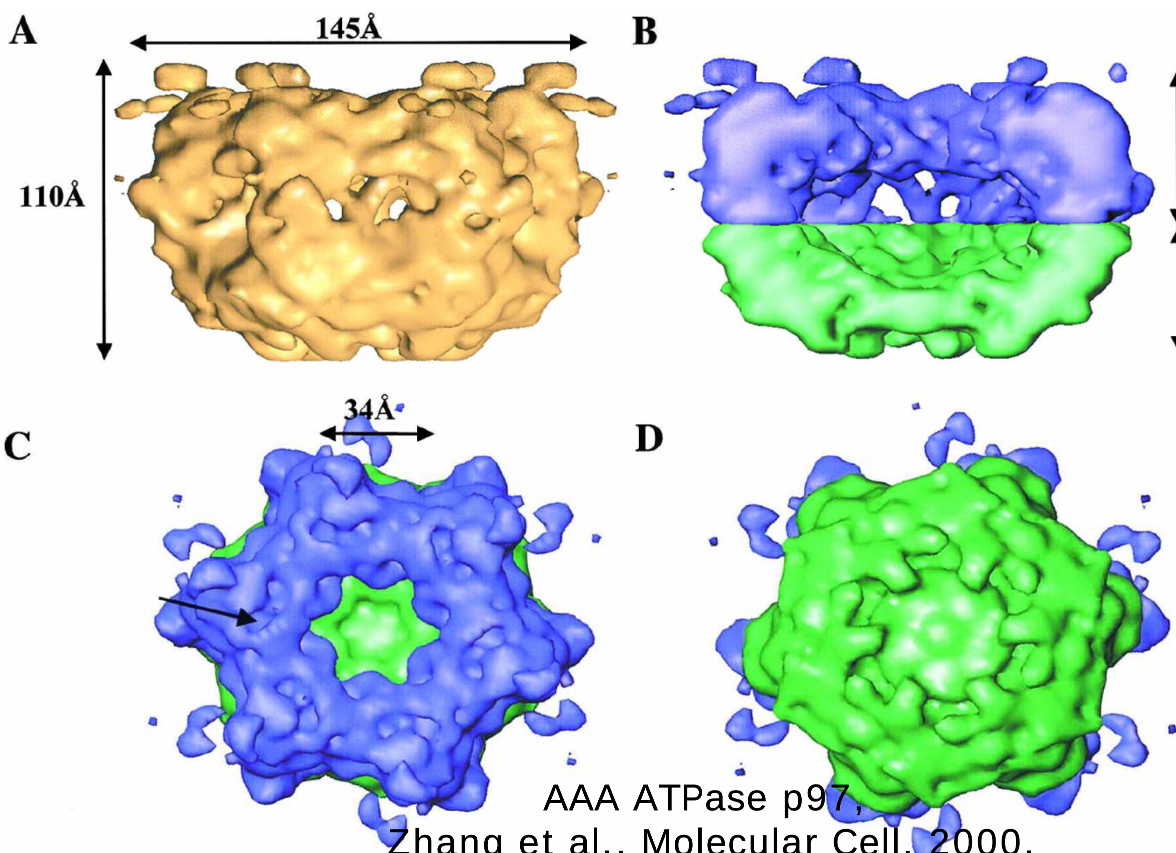
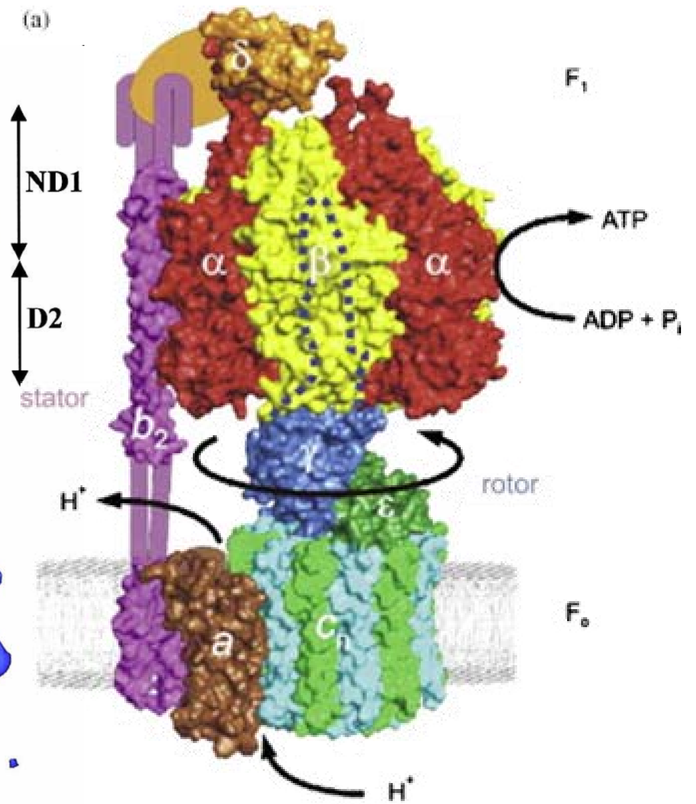
What defies thermodynamics?

Photo: Shepherd's Bush Blog

What defies thermodynamics?

- Ratchet enzyme (still hypothetical in metabolism)



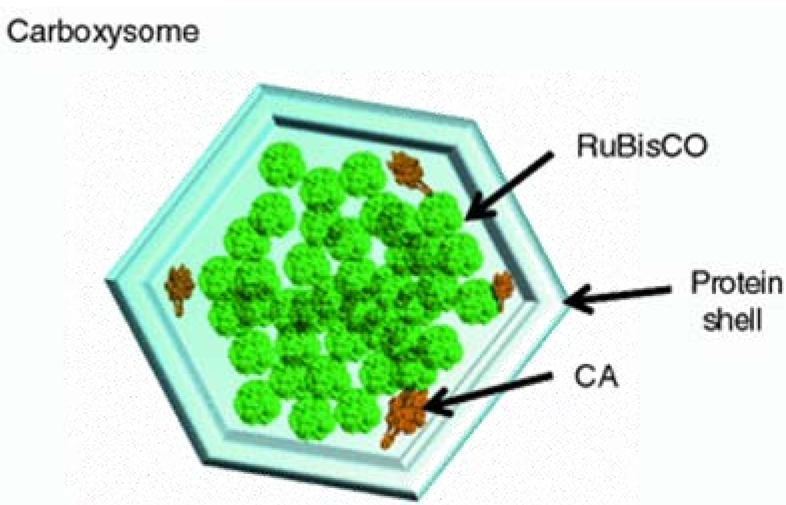


AAA ATPase p97,
Zhang et al., Molecular Cell, 2000.

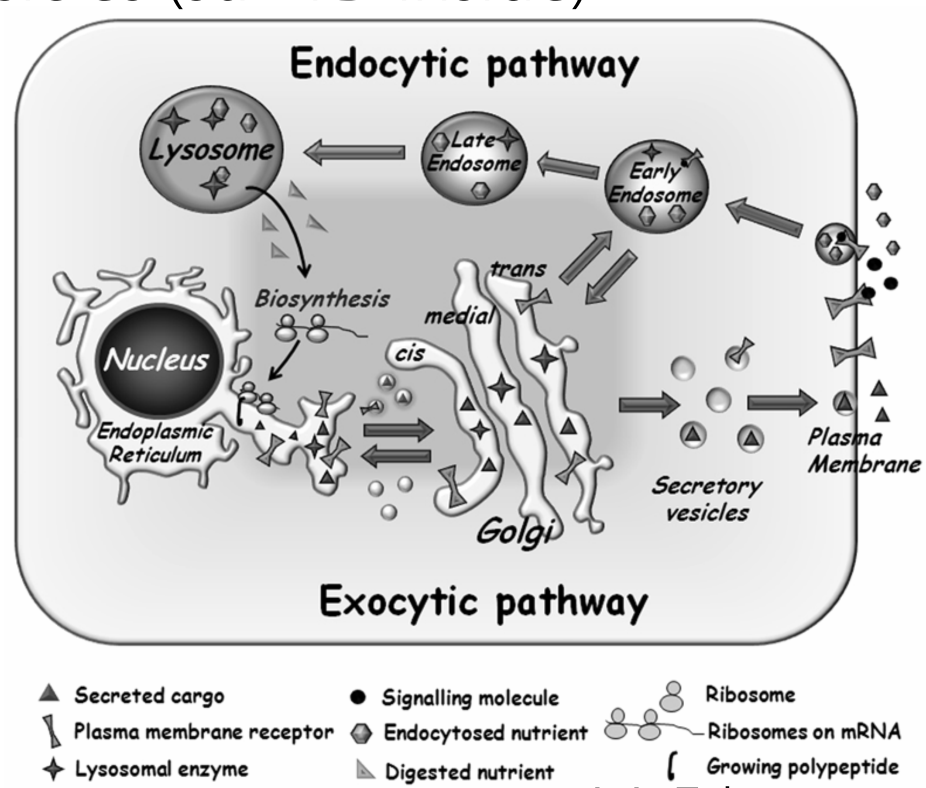
ATPase, Demirel,
J Non-Newt Fluid Dyn, 2008.

What defies thermodynamics?

- Ratchet enzyme (still hypothetical in metabolism)
- Compartments and vesicles (still TD inside)



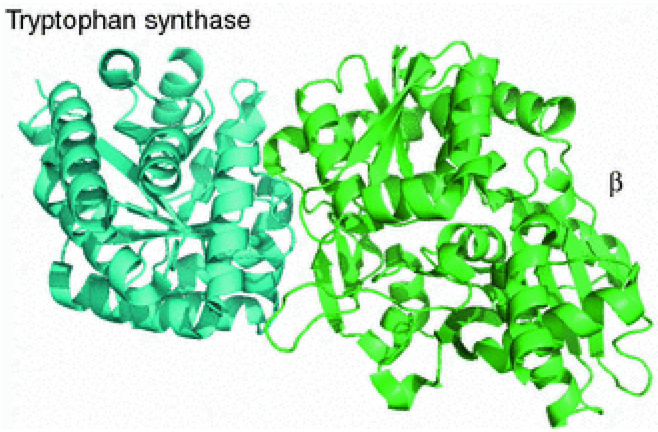
source: U. Jandt

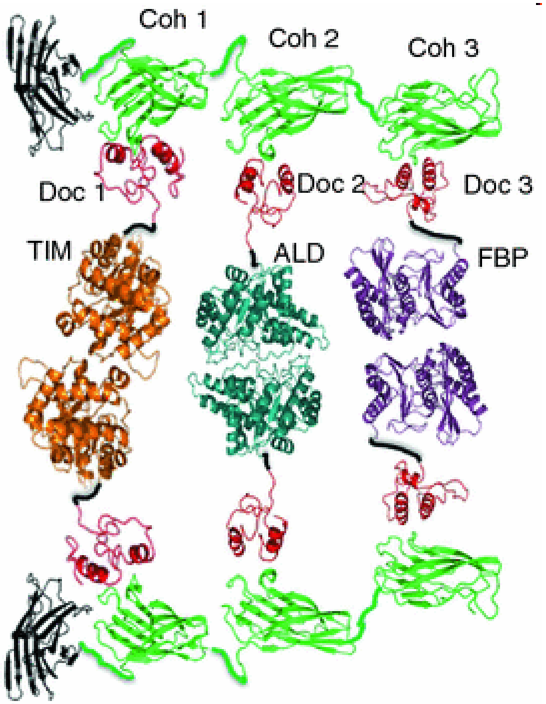


source: A.A. Tokarev

What defies thermodynamics?

- Ratchet enzyme (still hypothetical in metabolism)
- Compartments and vesicles (still valid inside)
- Molecular channeling

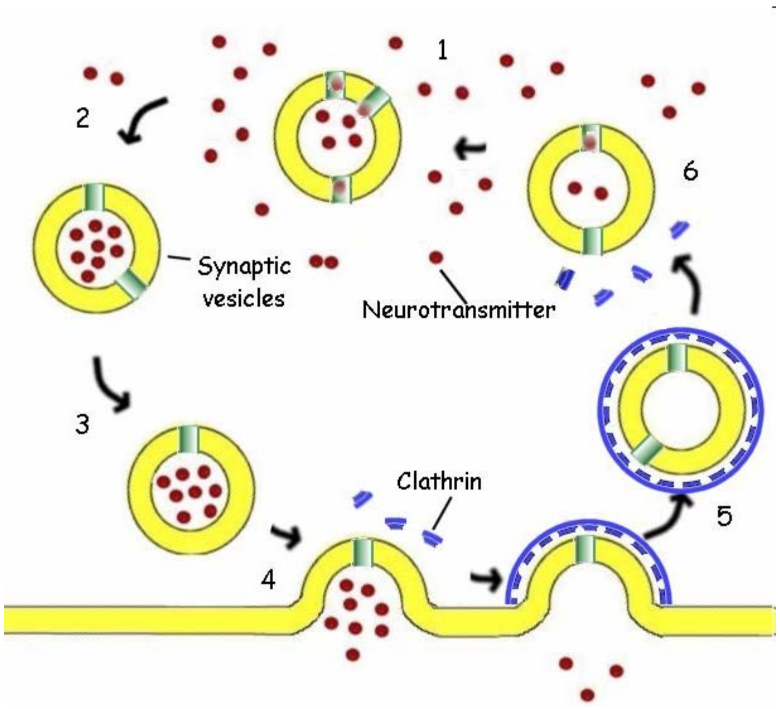




source: U. Jandt

What defies thermodynamics?

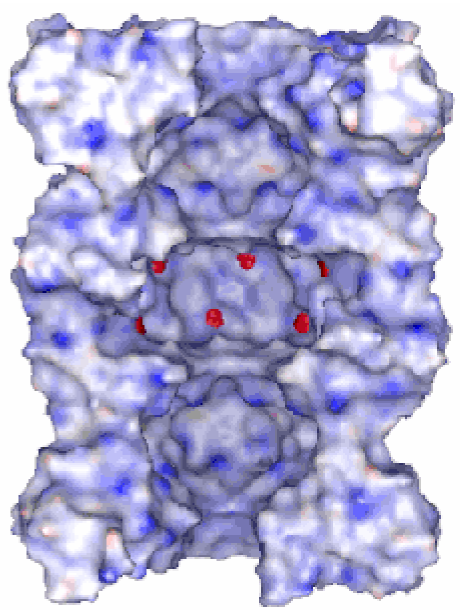
- Ratchet enzyme (still hypothetical in metabolism)
- Compartments and vesicles (still valid inside)
- Molecular channeling
- Directed vesicle transport



source: VidaLuz

What defies thermodynamics?

- Ratchet enzyme (still hypothetical in metabolism)
- Compartments and vesicles (still valid inside)
- Molecular channeling
- Directed vesicle transport
- Proteasome peptide transport



source: Maupin-Furlow

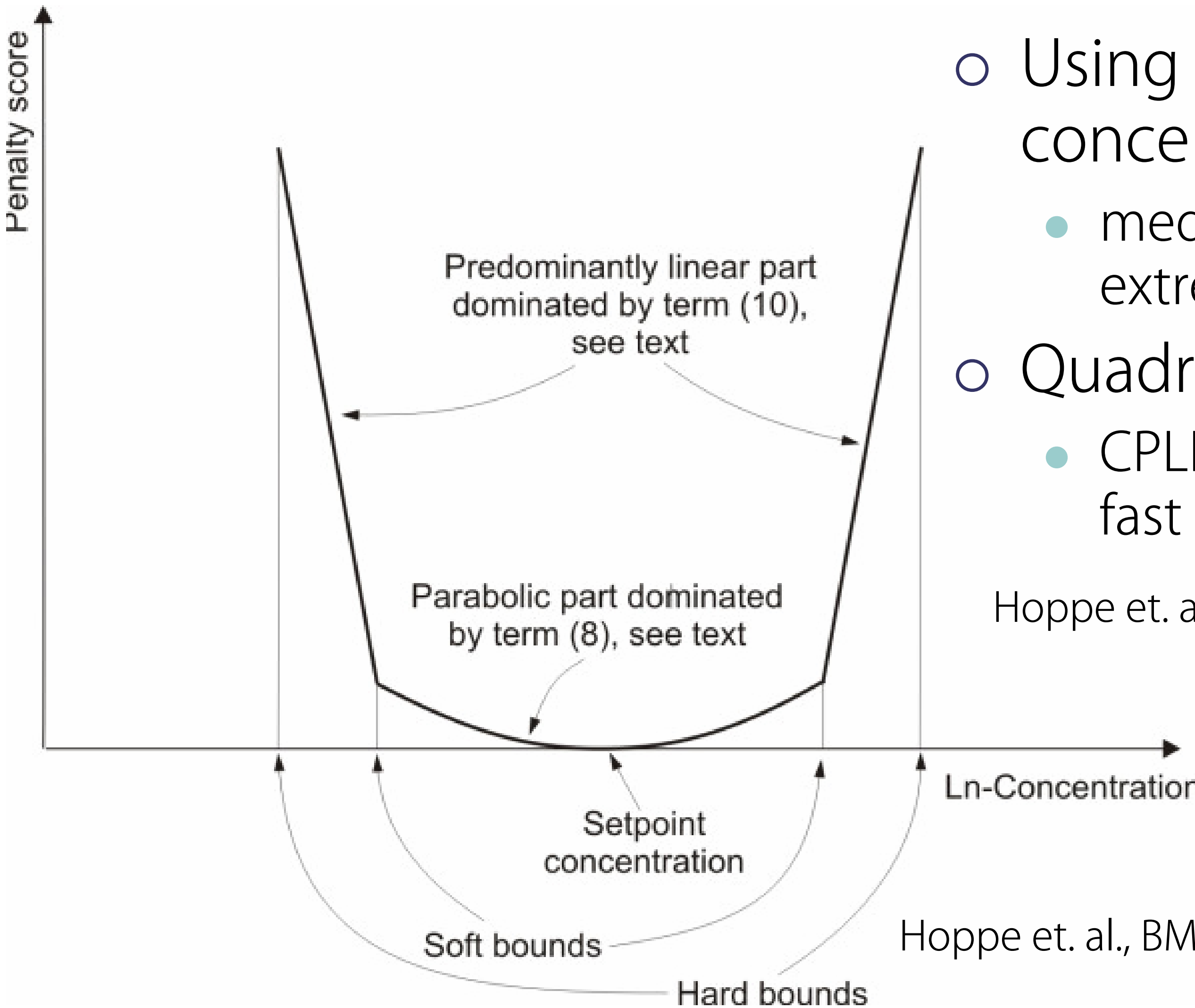
Overview

1. What is flux-balance optimization?
2. Problem of reversibility (E. coli growth 1)
3. Thermodynamic Realizability (TR)
4. What defies thermodynamics?
5. Testing with known concentrations (E. coli growth 2)
6. Estimating standard Gibbs' energies

Testing flux predictions in many conditions

- Chemostat with various flow velocities, 24 mutants
  - Ishii et al., Science, 2007.
- Metabolite concentrations, fluxes measured
- FBA tested with different algorithms
  - Hoffmann, PhD thesis, 2012.

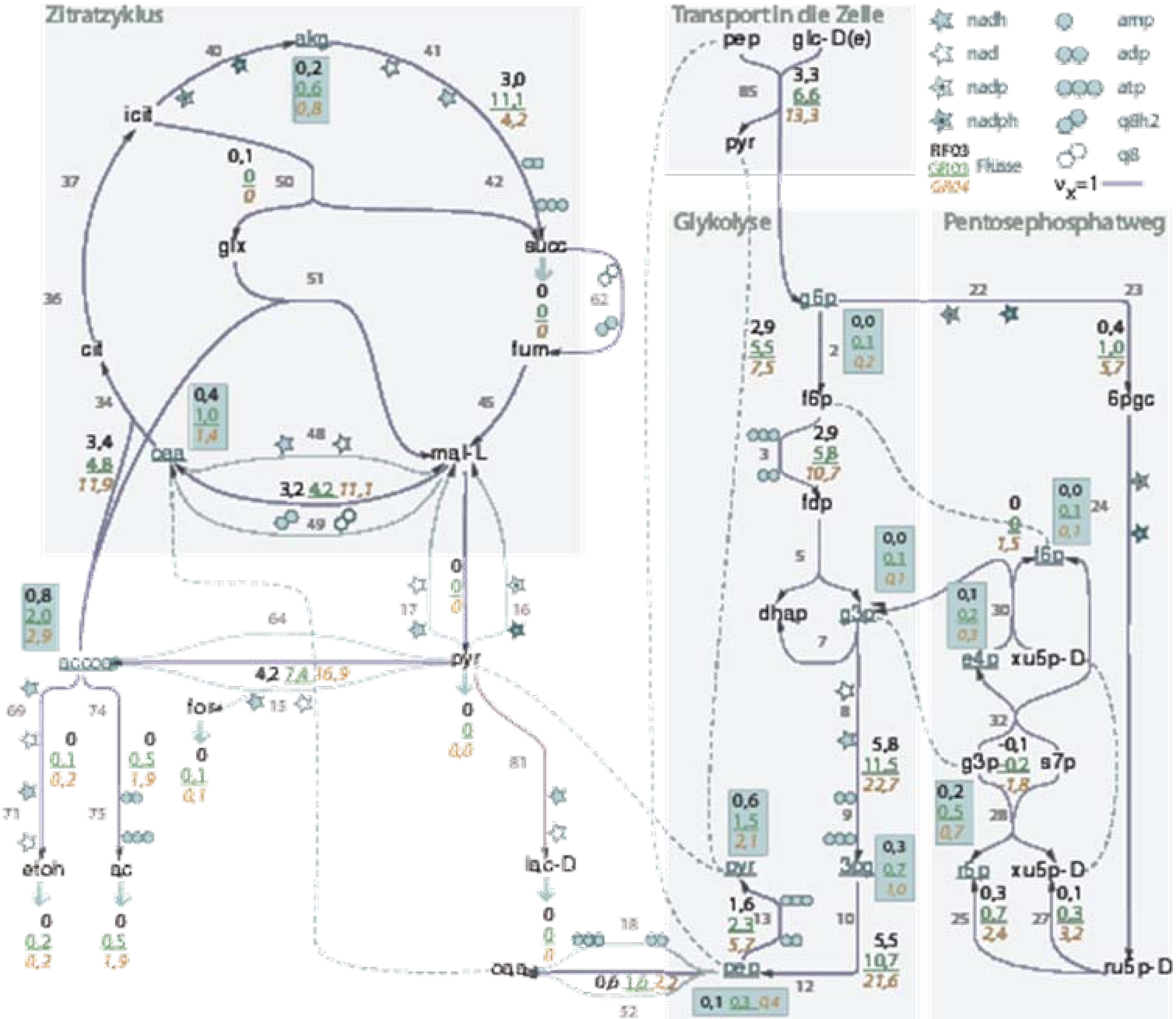
Finer use of concentration ranges



- Using measured concentrations
  - median, typical, extreme
- Quadratic objective
  - CPLEX sufficiently fast

Hoppe et. al., BMC Bioinf, 2011.

Hoppe et. al., BMC Systems Biology, 2007.



FB optimization – Reversibility – Thermodynamic Realizability – Defied TD – Concentrations – Gibbs' energies

K ··· individually determined

Kümmel et al., BMC Bioinf, 2006.

S ··· TR computed

F ··· heuristic

Feist et al., MSB, 2007

P/FP ··· deduced by system's function

Hoffmann, Genome Inf, 2007.

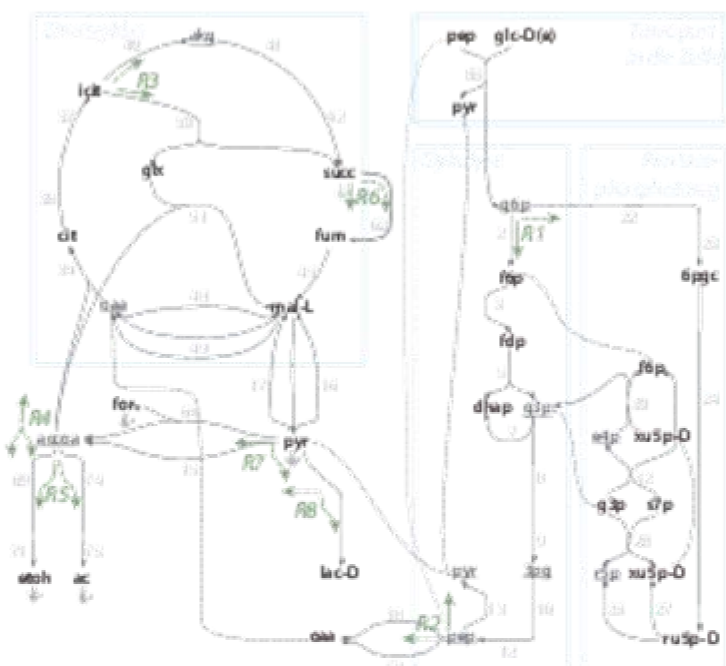
I ··· flux measured

Ishii et l., Science, 2010.

RID	Name	$\Delta_R G_{min}$	$\Delta_R G^0$	$\Delta_R G_{max}$	K	S	P	F	FP	I
2	PGI	-17.18	-2.93	5.69						
3	PFK	-40.4	-15.92	6.29			→	→	→	→
5	FBA*	-16.92	-17.6	39.06			←		←	←
7	TPI*	-22.53	-5.87	14.07			←		←	←
8	GAPD	-17.6	-0.42	34.79		→	→		→	→
9	PGK*	-22.56	-11.73	10.05		→	→		→	→
10	PGM*	-10.35	0	6.05		→	→		→	
12	ENO	-13.51	-3.77	3.98		→	→		→	
13	PYK	-33.73	-22.21	-0.28	→	→	→	→	→	→
15	PDH	-74.47	-34.78	-24	→	→	→	→	→	→
16	ME2*	-9.84	-6.7	40.84				←	←	←
17	ME1*	11.68	-5.45	55.89	←	←	←	←	←	←
18	PPCK*	4.75	-0.84	45.74	←	←	←	←	←	n.e.
22	G6PDH2r	-21.73	-6.7	25.99		→	→		→	→
23	PGL	-37.65	-21.37	-14.04	→	→	→	→	→	→
24	GND*	-19.63	-3.49	47.94			←	←	←	←
25	RPI*	-19.35	-2.09	9.87			→		→	→
27	RPE	-21.74	0	6.49						→
28	TKT1*	-40.16	-7.96	13.93						
30	TKT2	-15.66	-7.12	25.97						
32	TALA	-36.67	-7.12	7.18						
34	CS	-59.24	-36.03	-2.02	→	→	→	→	→	→
36	ACONTa*	-17.88	-6.29	20.28		←	←		←	←
37	ACONTb	-9.63	-0.84	19.26		→	→		→	→
40	ICDHyr*	-22.62	-14.25	32.28		←	←		←	←
41	AKGDH	-81.2	-34.78	-30.45	→	→	→	→	→	→
42	SUCOAS	-32.08	-4.19	20.01		←	←		←	←
45	FUM	-18.09	-2.51	17.59						→
48	MDH*	-34.34	-26.82	17.41						n.e.
49	MDH2	-83.34	-45.67	-26.8	→	→	→	→	→	n.e.
52	PPC	-35.55	-28.49	-3.95	→	→	→	→	→	n.e.
53	THD2pp*	-42.35	-22.14	17.35				→	→	n.e.
54	NADTRHD	-46.43	-1.26	9.83				→	→	n.e.
55	ATPS4rpp*	-15.9	-7.45	13.48				→	→	n.e.
56	NADH16pp	-47.2	-37.39	12.54				→	→	n.e.
57	NADH5	-90.83	-72.49	-36.24	→	→	→	→	→	n.e.
58	CYTBO3_4pp	-46.4	-62.27	38.13				→	→	n.e.
59	CYTBDpp	-104.57	-109.07	-26.91	→	→	→	→	→	n.e.
62	SUCDi	-39.98	-8.8	20.65				→	→	→
64	PFL	-41.37	-21.37	-0.95	→	→	→			n.e.
66	FDH4pp&FORtppi	-99.33	-74.2	-53.08	→	→	→	→	→	n.e.
69	ACALD	-33.15	-18.44	28.29		←	←		←	←
71	ALCD2x*	-43.48	-25.14	11.11		→	→		→	→
74	PTAr*	-27.17	-15.92	6.83		←	←	←	←	←
75	ACKr*	-38.16	-18.02	-1.62	→	→	→		→	→
81	LDH_D*	-29.86	-26.82	0.17		→	→		→	→

Tested: independent flux ratios

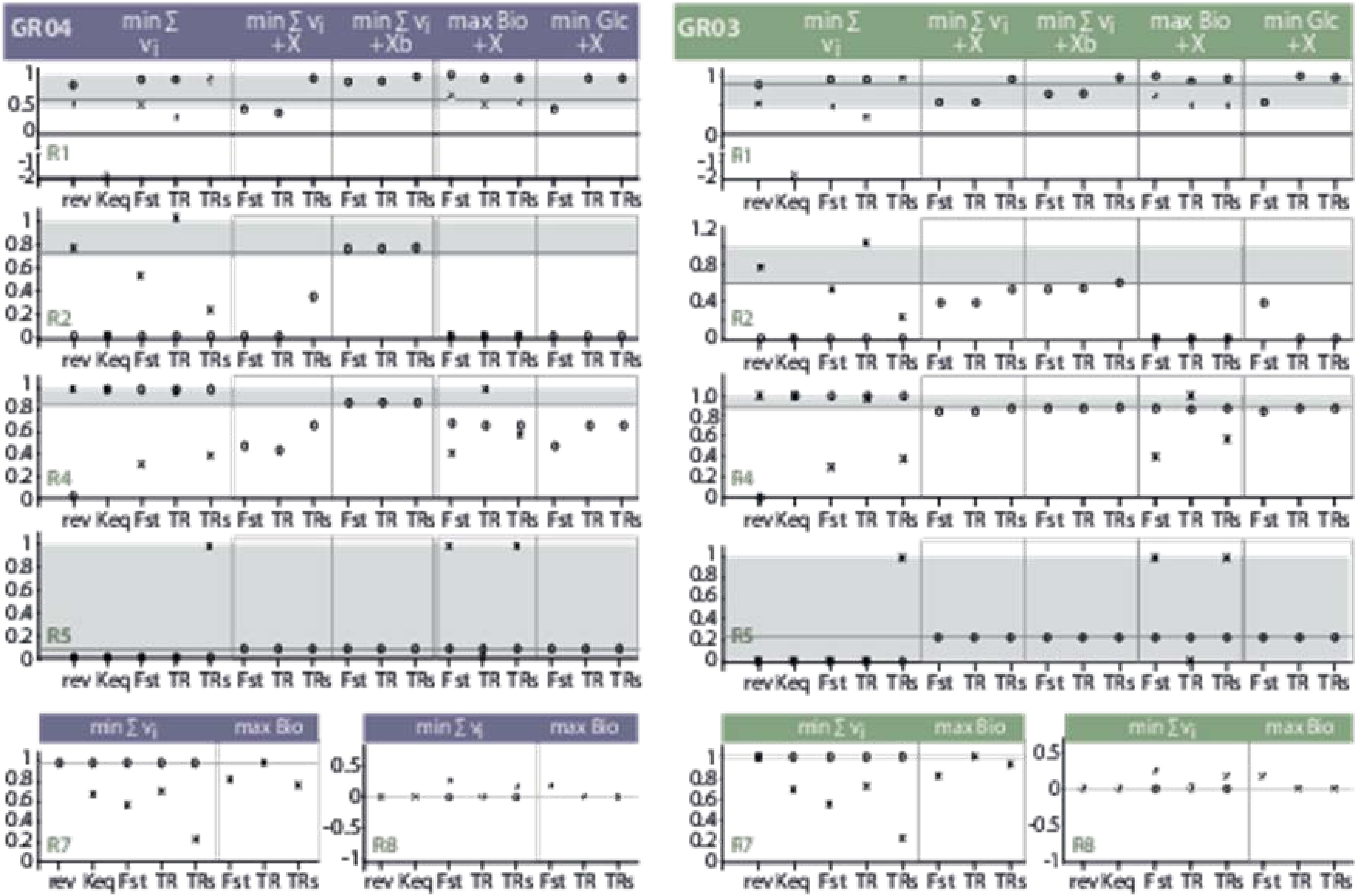
$R_1 = \frac{v_2}{v_{22}+v_2}$	Fluss in die Glykolyse (und nicht in den Pentose-Phosphat-Weg)
$R_2 = \frac{v_{13}}{v_{13}+v_{18}+v_{52}}$	(Netto)Fluss von Pep zu Pyruvat via PYK (und nicht zum Oxalacetat)
$R_3 = \frac{v_{51}}{v_{37}}$	Fluss in den Glyoxalatshunt (und nicht weiter im Zitratzyklus)
$R_4 = \frac{v_{34}}{v_{34}+v_{71}+v_{75}}$	Fluss von Acetyl-CoA in den Zitratzyklus (und nicht in Ethanol oder Acetat)
$R_5 = \frac{v_{71}}{v_{71}+v_{75}}$	Ethanol-Export (und nicht Acetatexport)
$R_6 = \frac{v_{92}}{-v_{42}}$	Succinat-Export (und nicht Umsetzung zu Fumarat)
$R_7 = \frac{v_{84}+v_{15}}{v_{84}+v_{15}+v_{81}+v_{94}}$	Fluss von Pyruvat zu Acetyl-CoA (und nicht ins Lactat)
$R_8 = \frac{v_{81}}{v_{84}+v_{15}+v_{81}+v_{94}}$	Fluss von Pyruvat nach Lactat (und nicht Umsetzung zu Acetyl-CoA oder Pyruvatexport)



Flussverhältnisse experimenteller Werte der Studie von Ishii et al.:

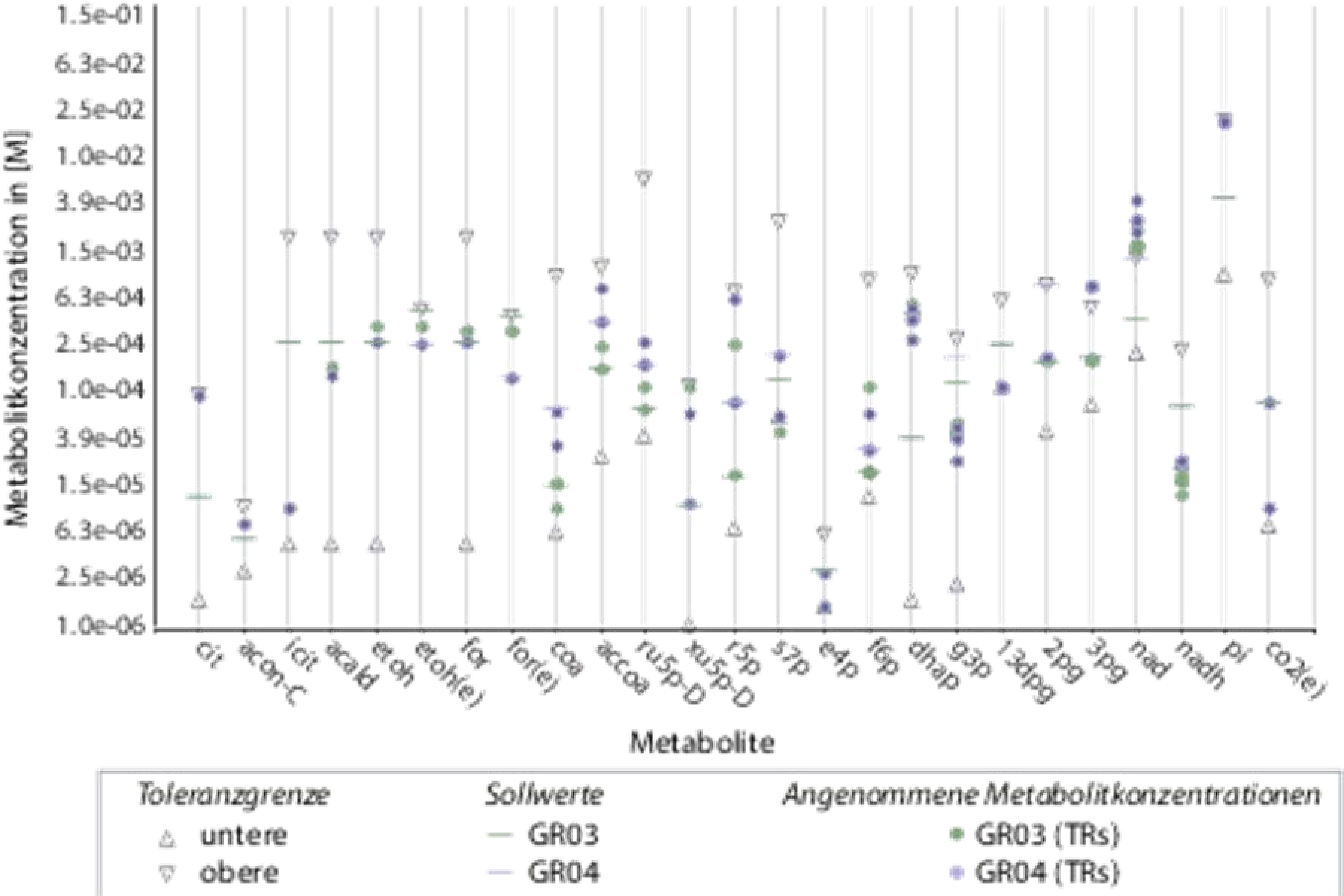
	GR03	GR04	RF03	min	max
R_1	0,85	0,57	0,88	0,47	1
R_2	0,59	0,72	0,74	0,5	1
R_3	-	-	0,02	0	0,41
R_4	0,88	0,85	1	0,85	1
R_5	0,23	0,07	0	0	1
R_6	0	0	0	0	0
R_7	1	1	1	1	1
R_8	0	0	0	0	0

TR with setpoint most robust criterium (TRs)



FB optimization – Reversibility – Thermodynamic Realizability – Defied TD – Concentrations – Gibbs' energies

TR with setpoint predicts metabolite conentrations



FB optimization – Reversibility – Thermodynamic Realizability – Defied TD – Concentrations – Gibbs' energies

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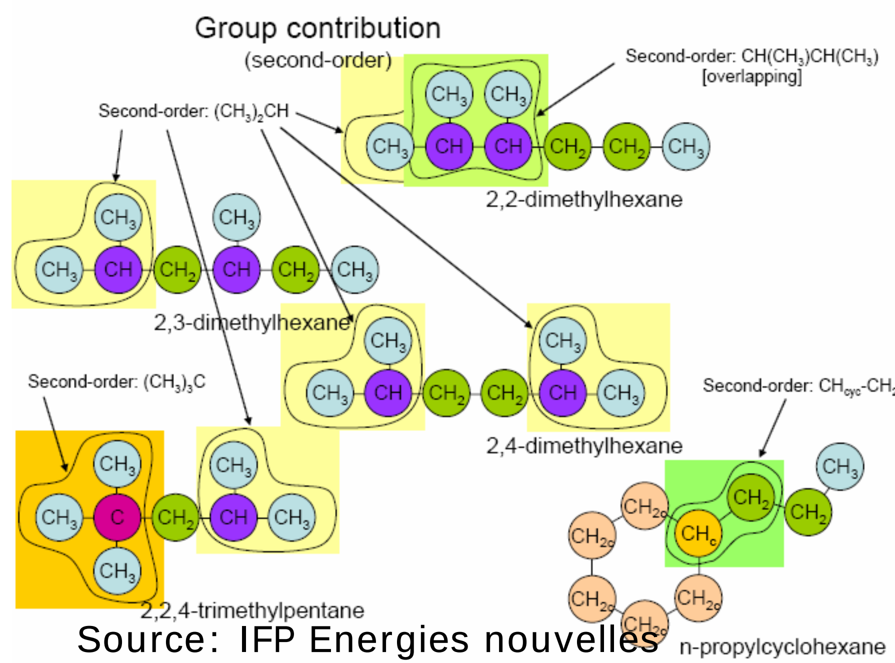
Measuring Gibb's energies

- Caloric measures, equilibrium points
- NIST 74 database, collection of literature data
- Low coverage of genome-size models
  - Kümmel et al., BMC Syst Biol,2006.
- Different experimental essays: not fully comparable values

Source: AdvoCare Types of calorimeters

Computing Gibb's energies

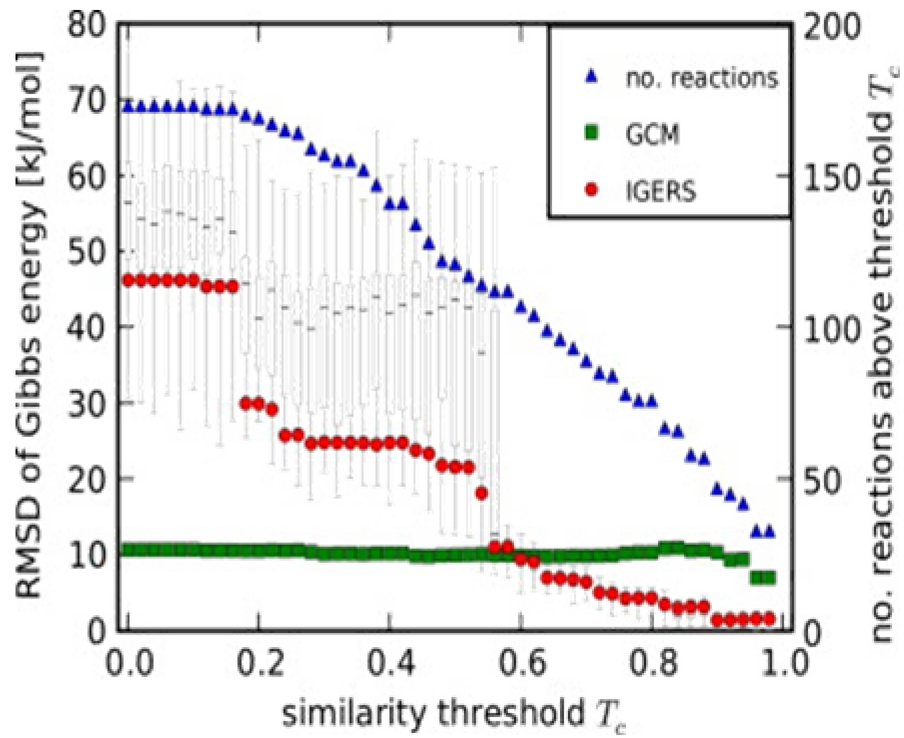
- Group Contribution method (Mavrovouniotis 1990)
  - Recent implementation: Jankowski et al., Biophys J, 2008.
- Calculating effect of pH, temperature etc.
  - Thermodynamics of Biochemical Reactions, Alberty, 2003 (book).



Source: IFP Energies nouvelles

IGERS: Reaction-classification method

- Molecule decomposition algorithm
  - 59 alpha position groups
  - 126 chemical groups
- Atom transition matrices (BIOPATH, KEGG)
- Reaction classification
  - 2210 reaction types (in KEGG)
- Inference on reaction type similarity
- Rother et al., Biophys J, 2010.



Take-Home Message

- Reversibility critical for flux-balance optimization
- Thermodynamic realizability: systemic and universal approach to reversibility in FBA
- Similar yield as knowledgeable setting of (ir)reversibility (for E. coli)
- Concentrations known: TR with setpoint

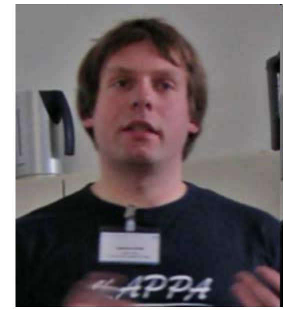
Have a look at:

- www.charite.de/sysbio/hoppe
- www.bioinformatics.org/fasimu

Acknowledgements

Hermann-Georg Holzhütter

Sabrina Hoffmann



Sascha Bulik

Kristian Rother

Thank You very much for your attention!