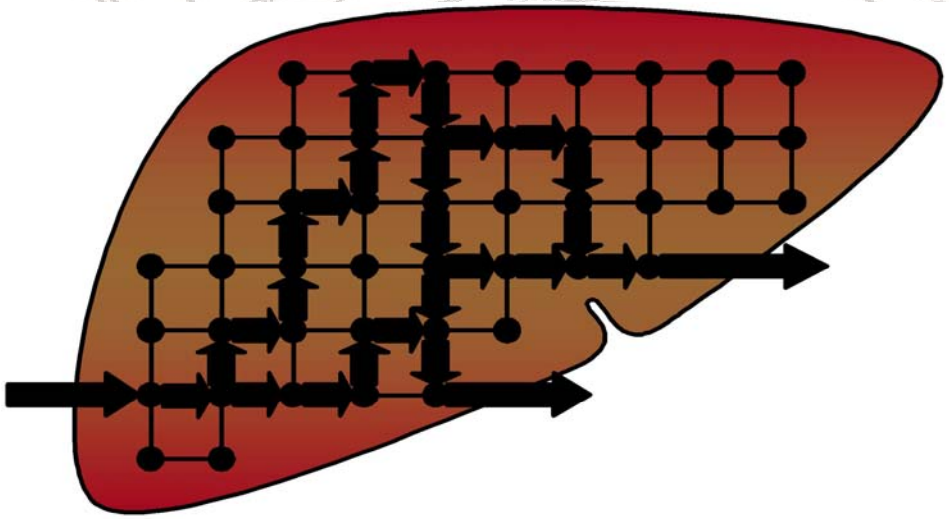
Thermodynamics Constraints Flux-balance Models



Andreas Hoppe
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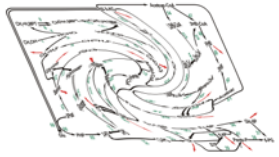

1. E. coli growth (Introductionary example)
2. Reversibility
3. Thermodynamic Realizability (TR)
4. Estimating Gibb's energies
5. TR for the Hepatocyte

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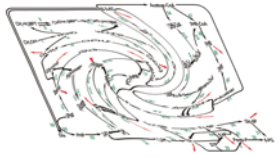

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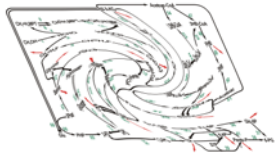
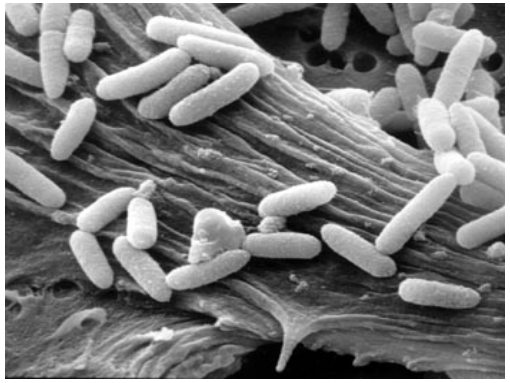
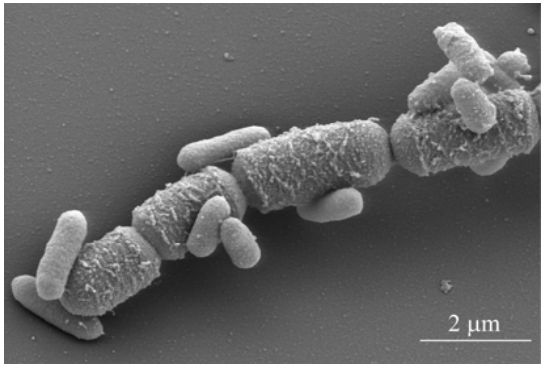
Introduction: E. coli

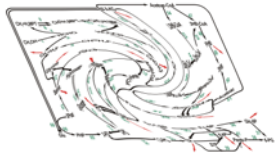


- Reed&Palsson 2004 jR904 network:
  - 904 metabolites
  - 932 reactions, transporters
  - Growth function: energy equivalents, amino acids, lipid pools, macromolecules



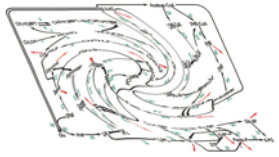


Reversibility

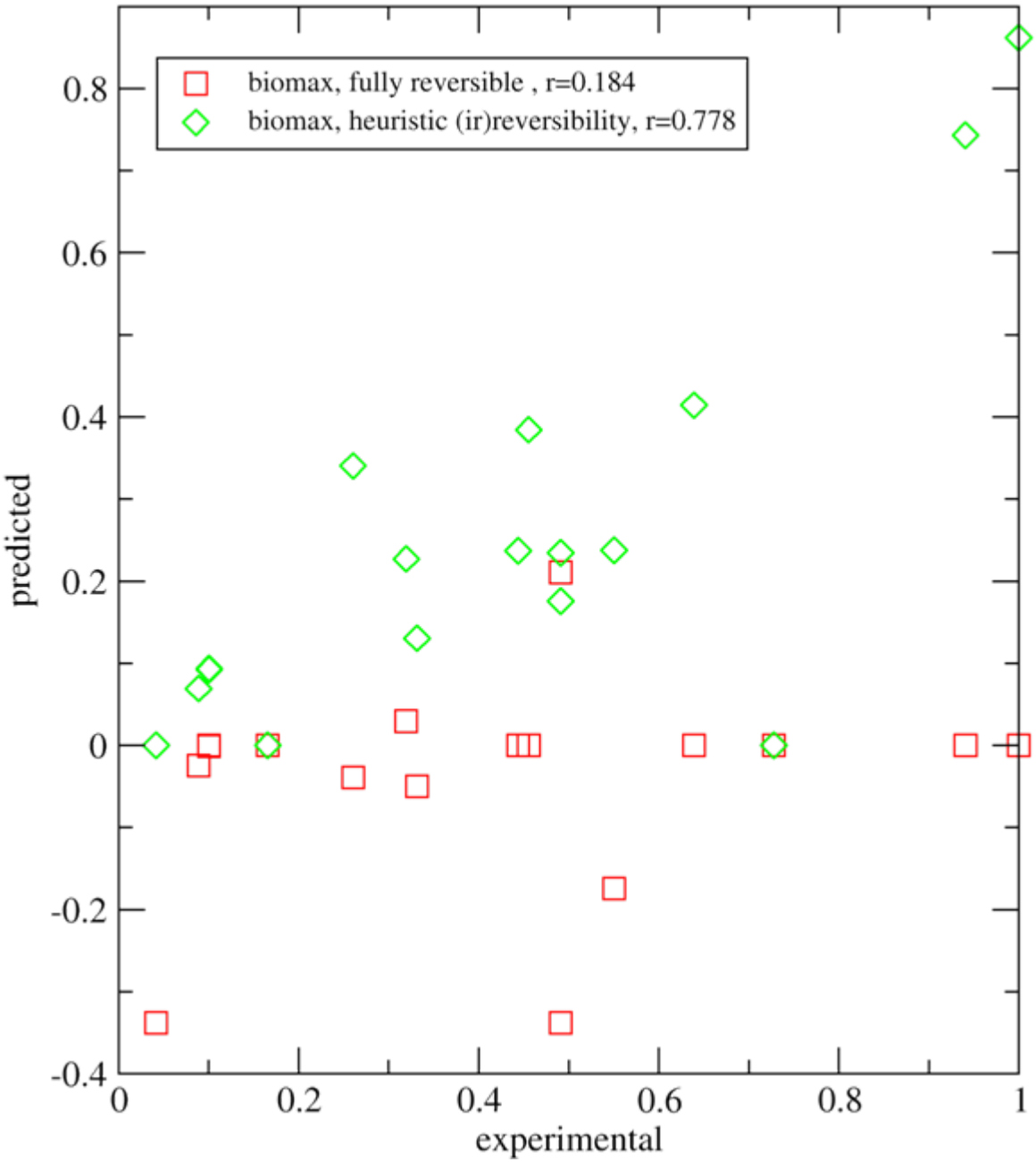


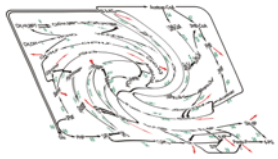
- Heuristic setting:
  - 245 reversible reactions
  - 687 reactions fixed to one direction
- Based on
  - Biochemical knowledge
  - Thermodynamical considerations
  - Purpose

Flux measurements

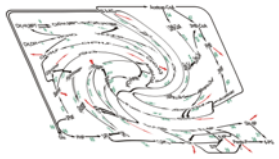


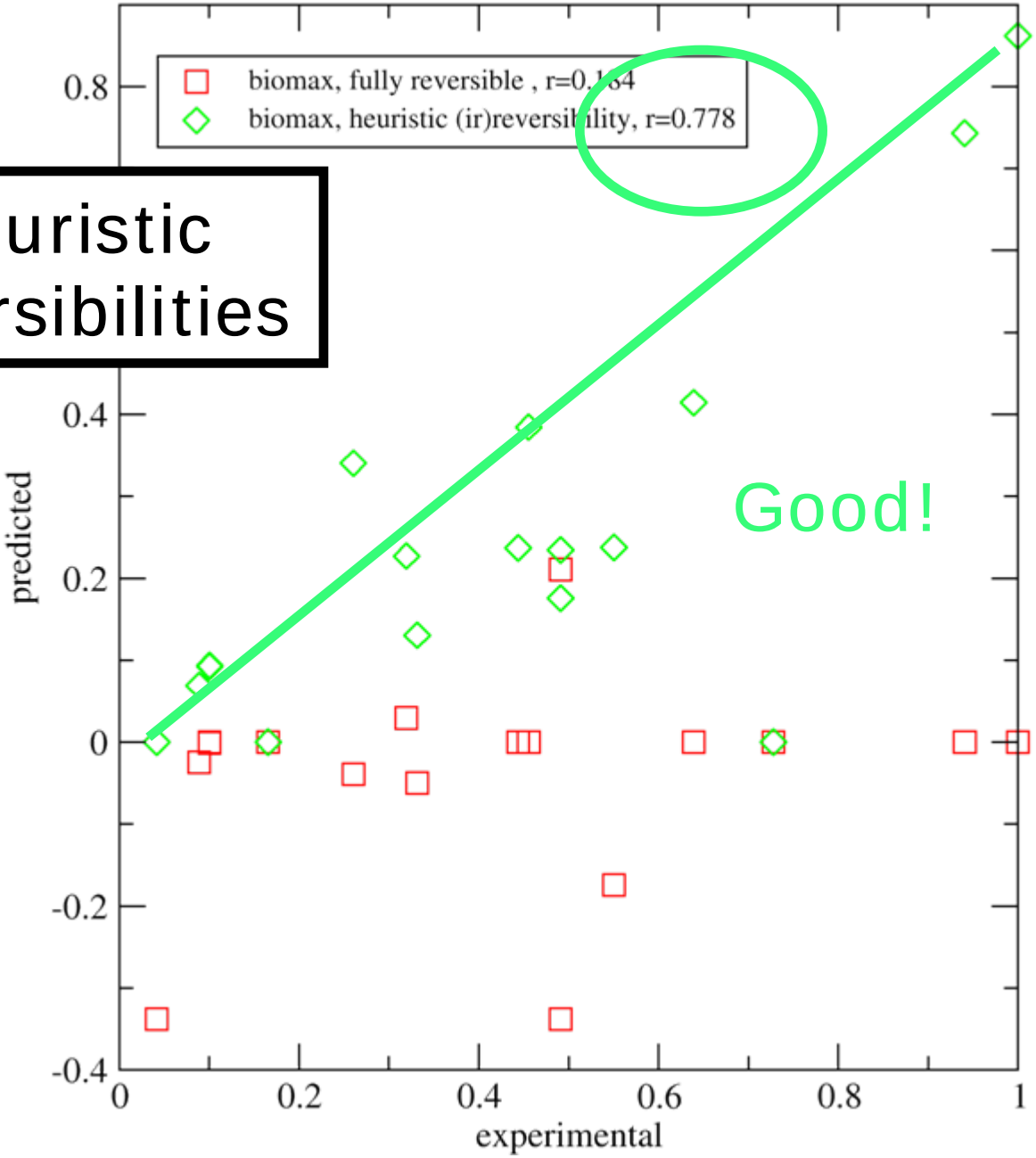
- Emmerling et al. J. Bacteriol. 2002
- Comparison with values predicted by FBA (biomass maximization)
  - Heuristic reversibilities (Reed)
  - Fully reversible model


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

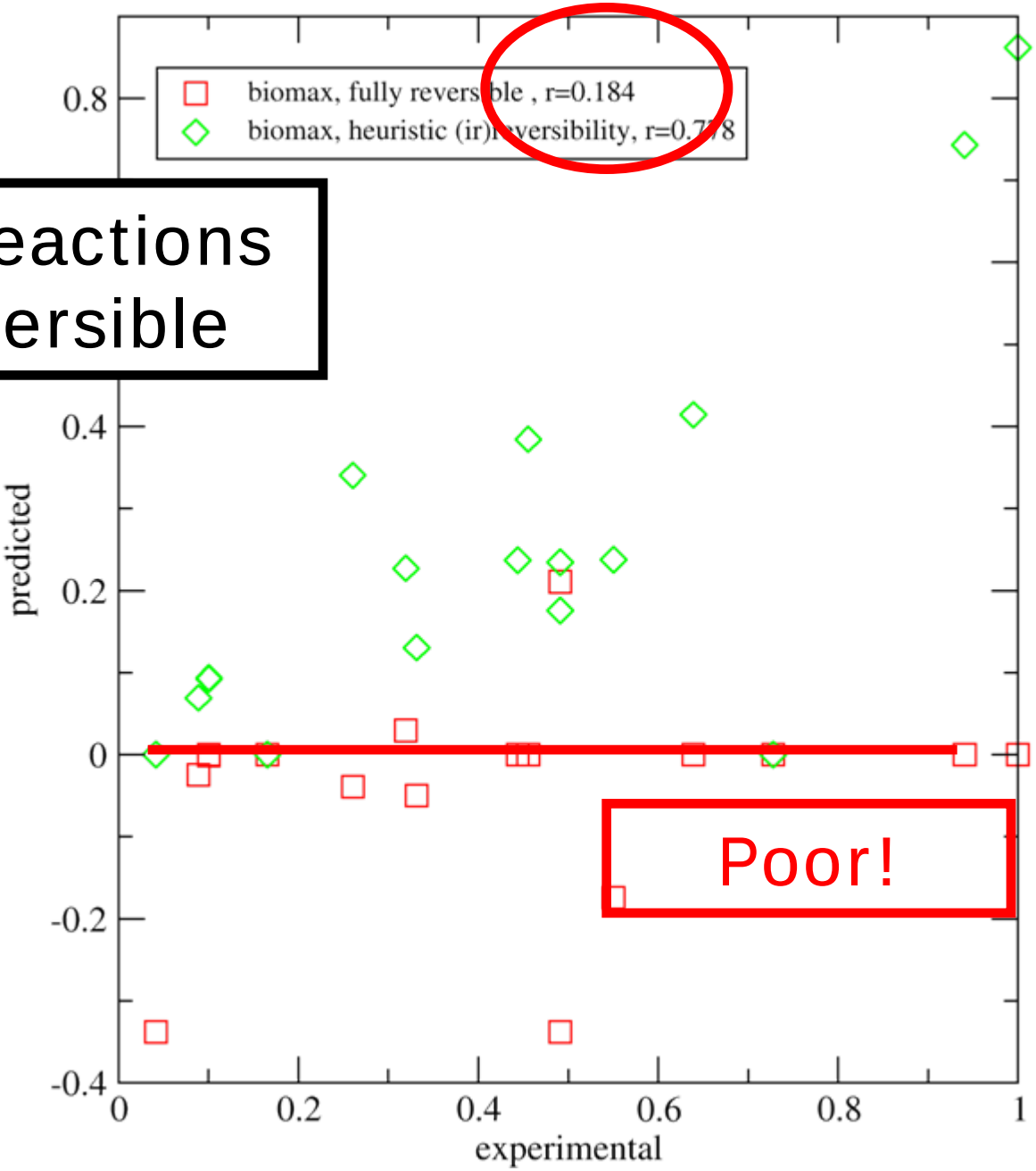


Heuristic reversibilities

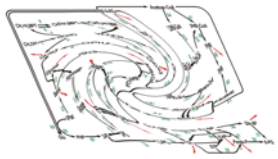




All reactions reversible



Poor!



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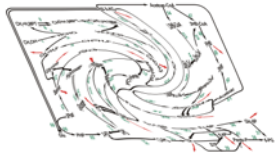

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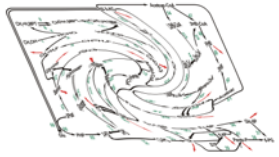
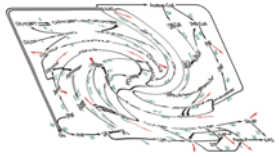
Reversibility



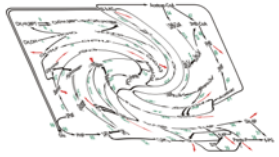
- Every reaction is reversible in principle
- Concentration gradient may sometimes not be sufficient
- Only a few reactions are strictly irreversible for cellular concentrations (Henry 06)

Aspects of heuristic settings



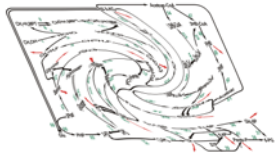
- E. coli network is designed for normal growth
- Predictions may be poor for extreme cellular states
- Requires ad hoc assignments

Aim:



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

Contents



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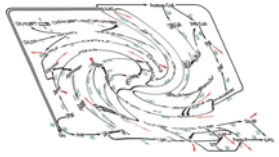
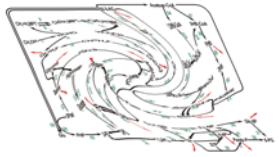
Thermodynamic Feasibilty



Implication from basic thermodynamic laws:

Chemical reaction proceeds (voluntarily) in the direction of negative Gibb‘s free energy ΔG_r

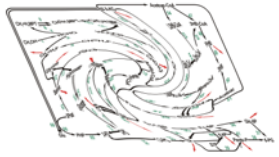
Dependence on concentrations



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

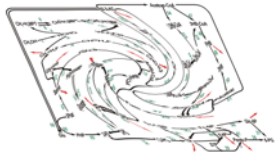
- R ... gas constant
- T ... temperature
- $[M]$ ... active concentration
- ΔG_r^0 ... standard Gibb‘s free energy

History



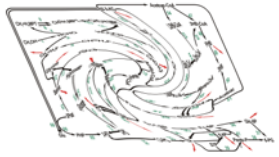
- First application to metabolic networks (paths): Mavrovouniotis 1993
- Beard/Qian 2004 (Milwaukee/Wisconsin)
- Kümmel/Heinemann 2006 (Zürich)
- Henry/Hatzimanikatis 2006 (Evanston/Illinois)

Why now?



- Networks increased in size and validity
- Prospect to high-throughput Metabolomics, Thermodynamics
- Limitations of pure topological analysis
- Large-scale kinetics – still a long way to go

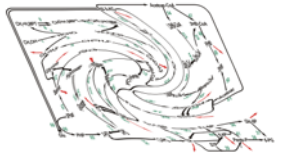
Thermodynamic Realizability (TR)



- Flux distribution is called TR if there exist concentrations (within physiological boundaries) such that the system is thermodynamically feasible, i.e. every flux proceeds in the direction of negative ΔG_r

Hoppe et. al. 2007, BMC Systems Biology

Thermodynamic Realizability (TR)



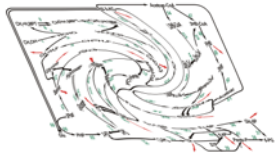
- Crucial problem moved:

  Heuristic setting of direction

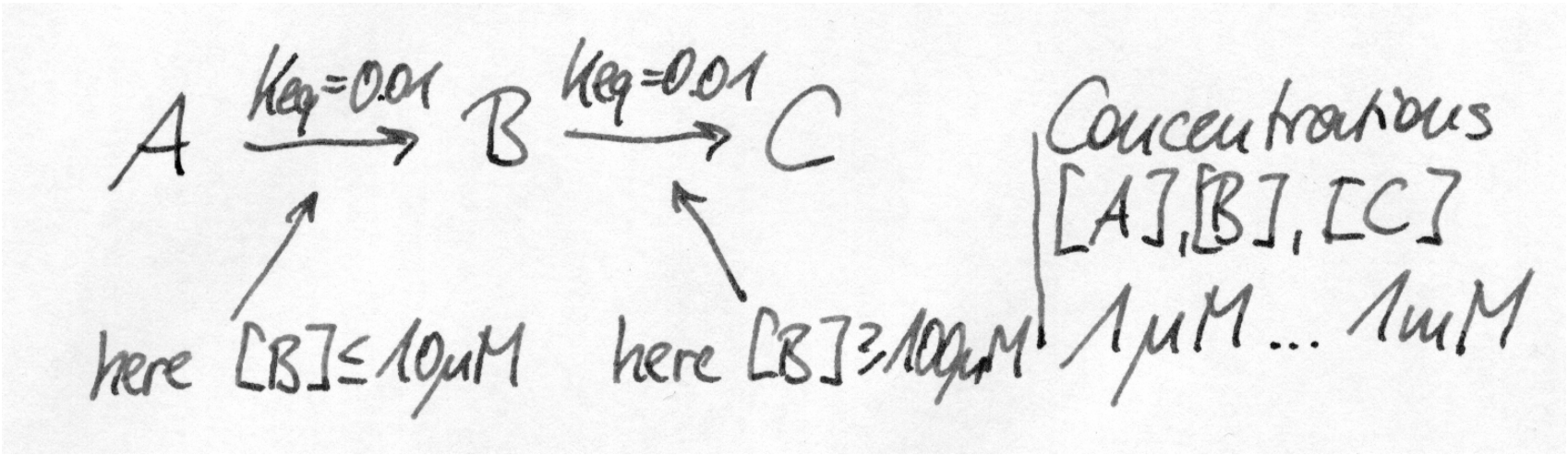
  → Metabolite concentration ranges

  & Accurate Gibb's free energy values

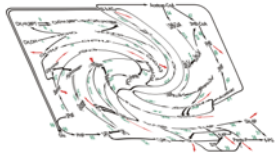
TR is a systemic property



- 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:

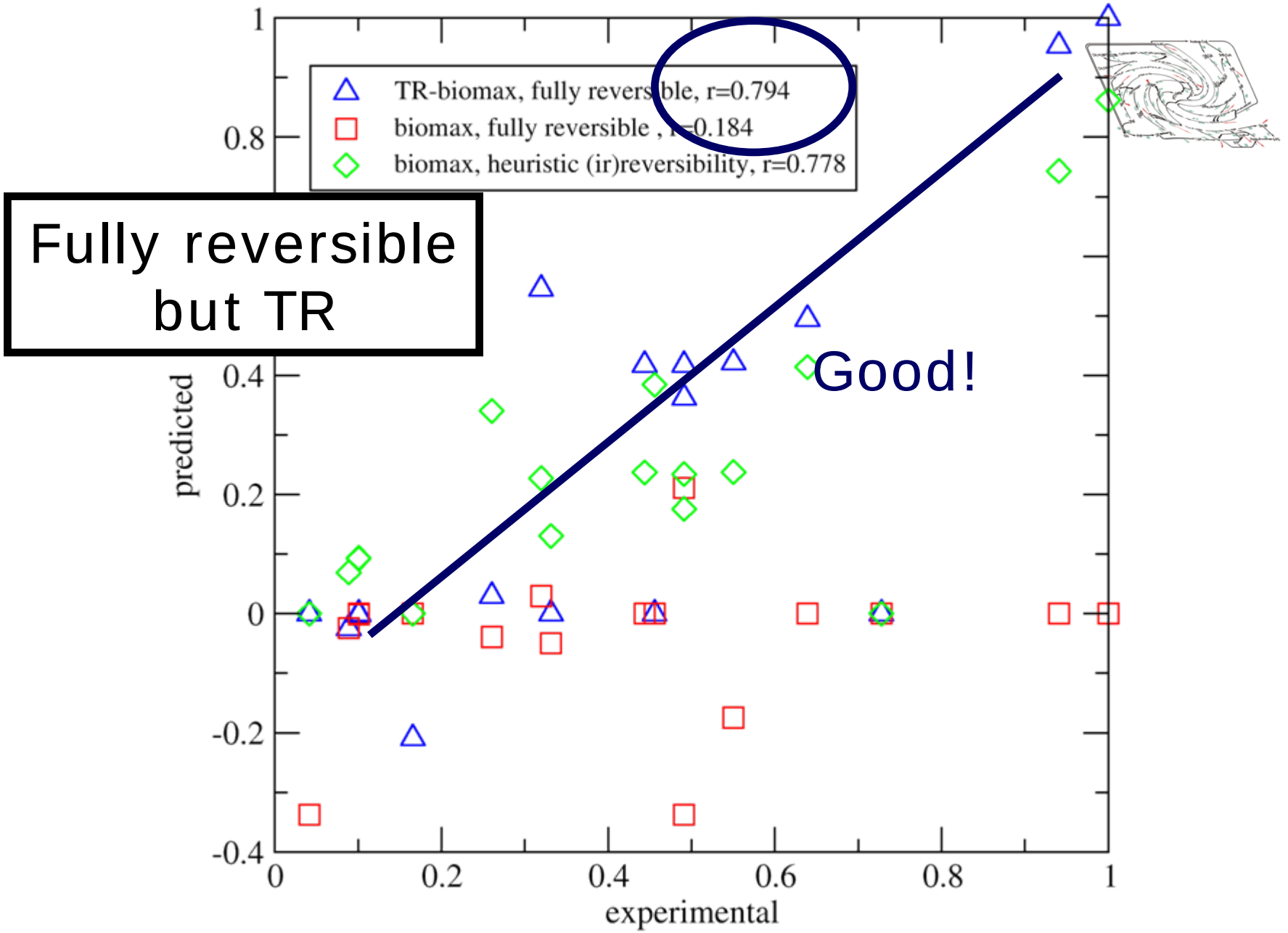
$\mathbf{S}$ ... stoichiometric matrix (given)

$\Delta_{\mathrm{r}}\mathrm{G}_{0}$ ... standard Gibb's free energies

Variables:

V ... (column) vector flux distribution

C ... (column) vector of log-concentrations multiplied with $\mathrm{R}T$


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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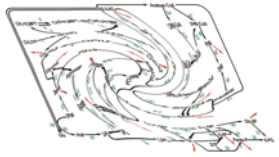

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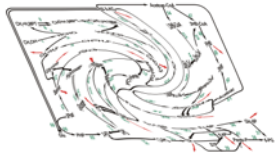
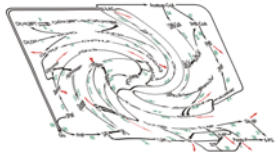
Measuring Gibb's energies



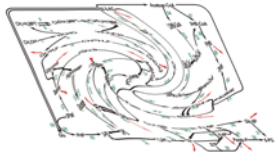
- Caloric measures, equilibrium points
- NIST 74 database, collection of literature data
- Low coverage of genome-size models (Kümmel 2006)
- Different experimental essays: not fully comparable values

Computing Gibb's energies



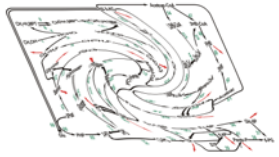
- Group Contribution method (Mavrovouniotis 1990)
  - Recent implementations (Jankowski 2008)
- Reaction-classification method (Rother)

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

Contents



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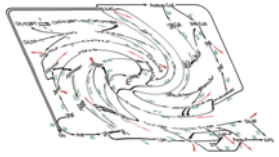
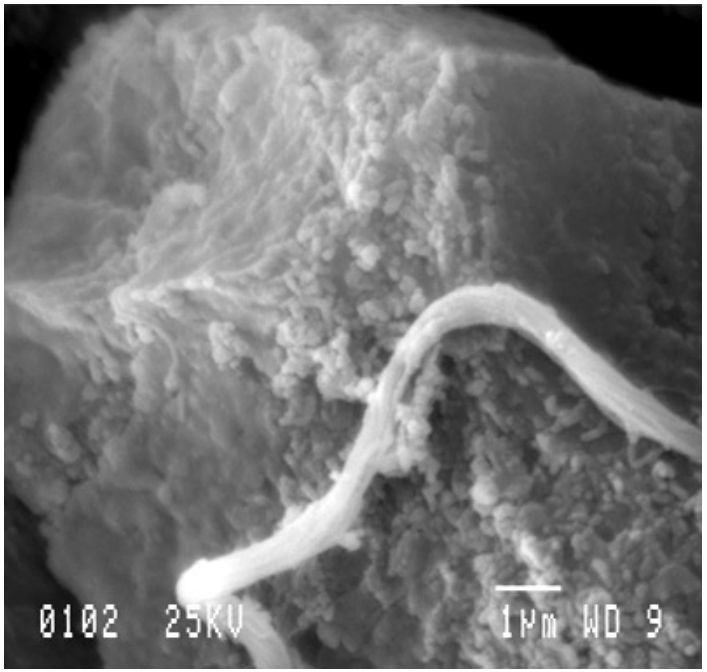
TR for the hepatocyte

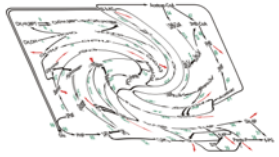


- Stoichiometric model almost complete
- Gibb's energy estimation (Jankowski)
- Concentration ranges (MetabolomeDB)



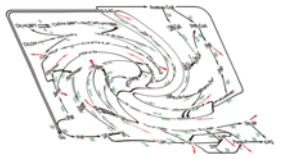
A single hepatocyte and threadlike contaminant. (Gregory Gavelis '08)

TR for the hepatocyte



- Many functions are “extreme”
  - Glycolysis/Gluconeogenesis
  - Substrate shortage/abundance
  - Homeostasis
- Presumably many reactions are used in both directions (depending on the function)

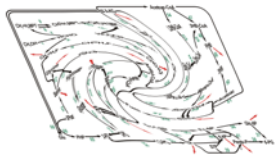
Acknowledgements



Hermann-Georg Holzhütter
Sabrina Hoffmann
Sascha Bulik

Thank You very much for your attention!

Take-Home Message



- Thermodynamic realizability: systemic and universal approach to reversibility in FBA
- Similar yield as knowledgeable setting of (ir)reversibility (for E. coli)
- Particularly suited to model extreme cellular states (in the hepatocyte)