Swiss Federal Institute of Technology Zürich

In Vivo Operation of Metabolic Networks

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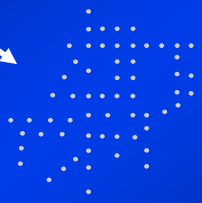
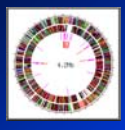
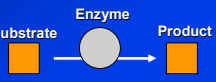
Genome annotation



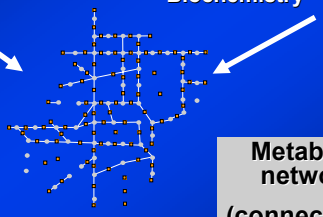
Parts list
(enzymes)



Genome annotation



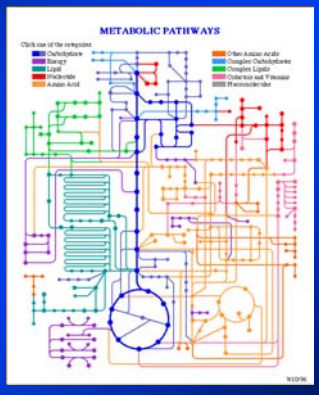
Biochemistry



Metabolic network
(connections)

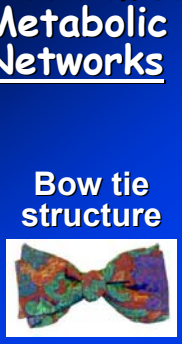
Metabolic Networks

Complex but ....
..... structured!

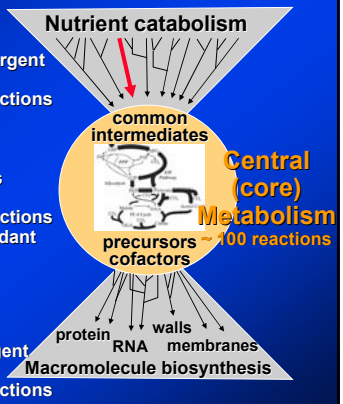


800-1100 reactions
not to mention regulation

Biochemistry of Metabolic Networks

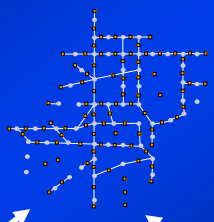


Bow tie structure



Csete & Doyle 2004. Trends Biotechnol. 22: 446

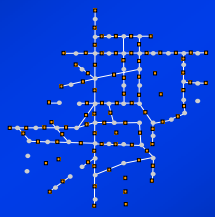
How to Analyze Metabolic Networks as a System ?



Computationally

Experimentally

Computational Network Analysis



Interactions

Topology

Stoichiometric

$A + B \longleftrightarrow C$

Influence of constraints on network function

Mechanisms

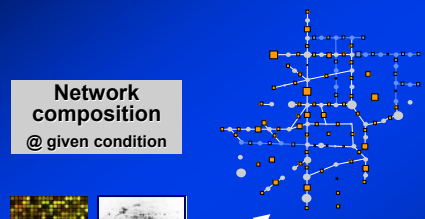
$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C$

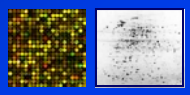
Quantitative, dynamic behavior

Stelling 2004. Curr. Opin. Microbiol. 7:513

Experimental Network Analysis

Network composition
@ given condition

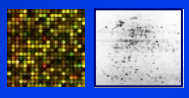




Transcriptome / Proteome
Metabolome

Experimental Network Analysis

Network composition
@ given condition



Transcriptome / Proteome
Metabolome

Network operation
(molecular traffic)
@ given condition

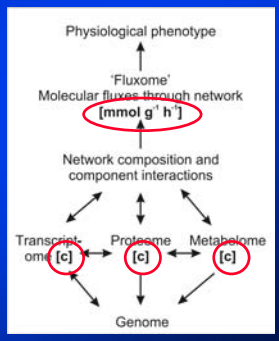
'Fluxome'
Systems-level in vivo activity

Fluxome vs Other 'Omes

Lots of biochemical & regulatory interactions

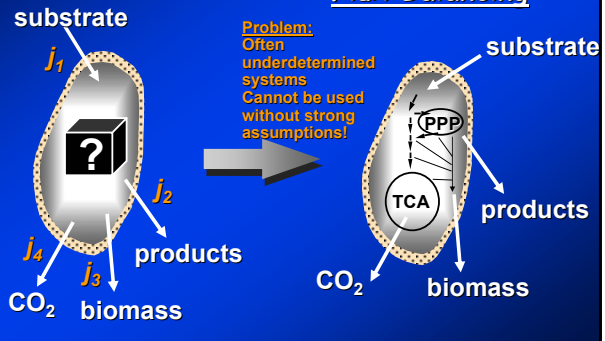
Network Operation

Network Composition

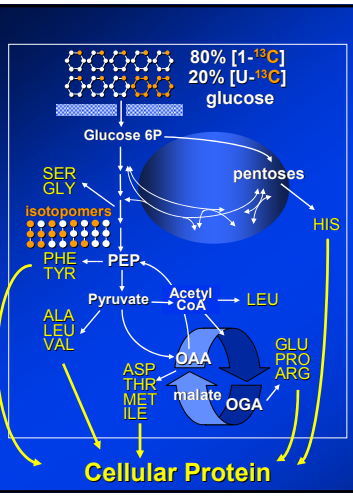


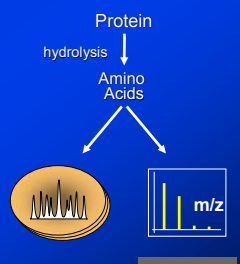
Unlike other 'omes, flux data quantify the integrated response of the network!

How to Quantify Fluxes?



The Principle of ^{13}C-Labeling Experiments





Metabolic Flux Analysis

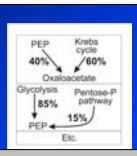
^{13}C tracer

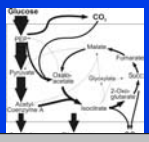
Cell

^{13}C pattern (protein-bound amino acids)

MS (NMR)

Mass isotope distribution reflects pathway usage





- 19 15 indep. ratios
- analytical solution
- probabilistic equations

In

- one solution
- iterative fitting
- within an isotopomer model

Quantitative Physiology

Sauer 2004 Curr. Opin. Biotech. 15: 58

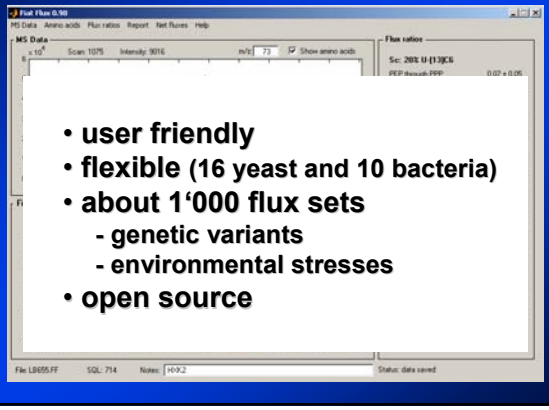
Current Status of Flux Methods

- Ready-to-use methodology
  ~ 5 - 8 key groups!
- But tedious & expensive
- ~ 200 published data sets!
- 3 key issues:
  accuracy - observability - throughput

parallel microtiter cultivation
sensitive MS-methods

Fischer, Zamboni & Sauer 2004. Anal Biochem 325:308
Sauer 2004 Curr. Opin. Biotech. 15: 58

Software for ^{13}C-Constrained Flux Analysis: FiatFlux (N. Zamboni & E. Fischer)



- user friendly
- flexible (16 yeast and 10 bacteria)
- about 1'000 flux sets
  - genetic variants
  - environmental stresses
- open source

Large-Scale Flux Analysis

Instead of investigating intuitively chosen strains/conditions systematic analyses are feasible.

Are there general principles of metabolic network responses and what are they?

Systematic 'Larger-Scale' Analysis: Baseline Study

Scope:
Quantitative network responses to genetic perturbations across all functional groups!

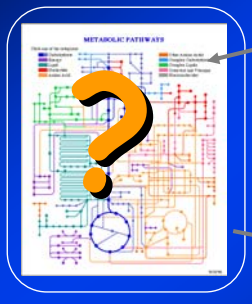
> 500 altered expression levels

B. subtilis
a spore forming soil bacterium
175 mutants

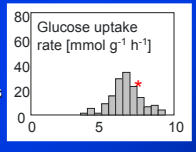
- transport
- metabolic
- regulators
- stress
- replication
- structure
- .....
- unknown

Mutants from K. Kobayashi (Nara, Japan) & S. Aymerich (INRA France)

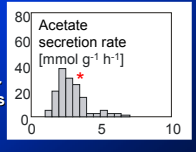
Fluxes In and Out of the Cell Vary Significantly!



number mutants

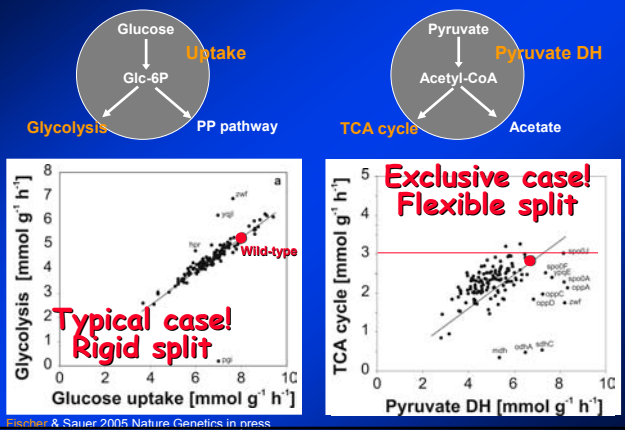


number mutants



Fischer & Sauer 2005 Nature Genetics in press

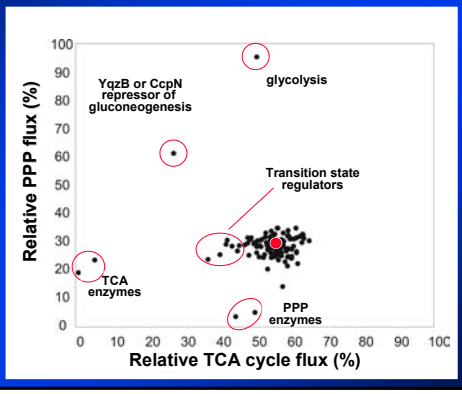
Absolute Fluxes at Key Branch Points!



Fischer & Sauer 2005 Nature Genetics in press

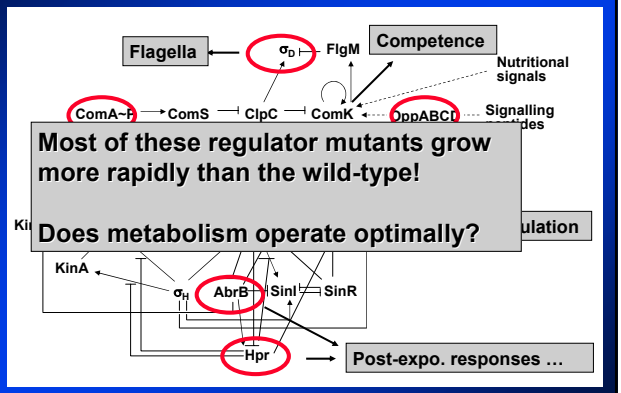
Main Routes of Carbon Flow: PP Pathway vs TCA Cycle in *B. subtilis*

(from analytically determined flux ratios!)

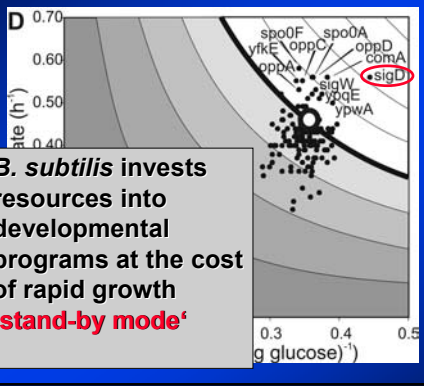


Fischer & Sauer 2005 Nature Genetics in press

Developmental Regulation Network



Trade-off Between Growth Rate and Yield: Non-Optimal Growth on Glucose



B. subtilis **invests resources into developmental programs at the cost of rapid growth ‚stand-by mode‘**

Only mutants in developmental regulators but not in general regulators (e. g. AbrB, Hpr, CcpA) grow more optimally!

Fischer & Sauer 2005 Nature Genetics in press

Conclusions:

Large-Scale Flux Analysis in *B. subtilis*

- metabolism not necessarily optimized
- absolute flux level flexible!
- unlike other ‘omes- the relative flux distribution is robust to genetic changes!
  - Flexibility is restricted to few key nodes in the network
- @ a given condition, cultures are in a stable metabolic state
  - robust to random genetic perturbation (knockout & overexpression)
  - *sensitive to environmental changes - mediated through complex regulation networks*

What Needs to be Investigated at Genome-Scale to Characterize the Metabolic State ?

Regulatory gene knockouts - ALL !
many alter the metabolic state- most of them have no known metabolic target

- experimental data to identify the connections

Metabolic gene knockouts - ALL .....??

- computational analysis to focus on key experiments *(an important aspect of SB)*

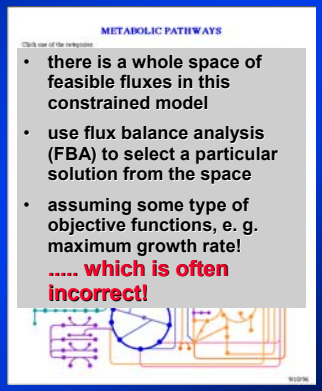
Which flux responses are obvious?
Where do we have to do experiments?

Stoichiometric model of *S. cerevisiae*

A + B ⟷ C
A + B ⟶ C
A + B ⟶ C
ATP

Constraints on reactions

1038 reactions



- there is a whole space of feasible fluxes in this constrained model
- use flux balance analysis (FBA) to select a particular solution from the space
- assuming some type of objective functions, e. g. maximum growth rate!

..... which is often incorrect!

672 genes

Genome-Scale FBA Yeast Model

(Förster et al 2003) - see also Blank, Küpfer & Sauer Poster U-P03

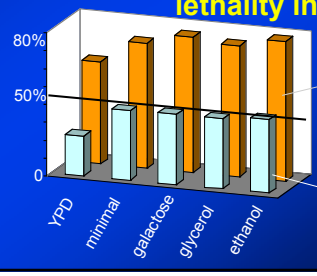
If one reaction is knocked out, can biomass be made - Predict Lethality ?

Correct prediction of lethality in yeast

Model verified against experimental data

- 4600 knockout mutants
- 5 env. Conditions
- Growth +/-

With Matthias Peter, ETH Biochemistry



Modified network
636 metabolites
1038 reactions

Original network

Förster et al., Genome Res. 2003

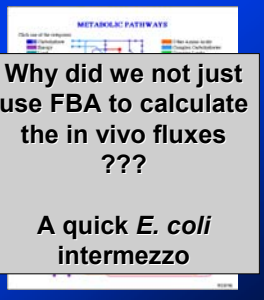
Genome-Scale Flux Analysis

To identify the active reactions under the investigated conditions

Explicit mapping from the normal 50 100 fluxes from ^{13}C experiments to genome scale:

Total 1038 reaction
339 active on glucose
Similar on
- ethanol
- glycerol
- galactose

similar to Papp et al 2004 Nature

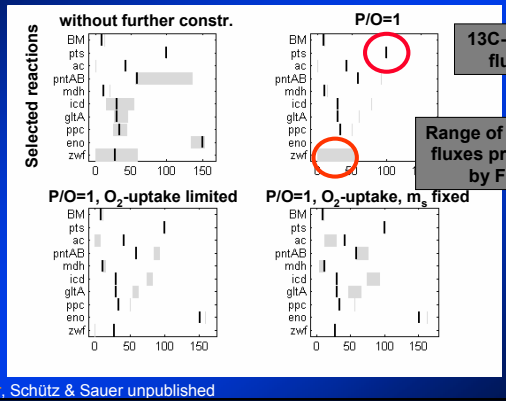


Why did we not just use FBA to calculate the in vivo fluxes ???

A quick *E. coli* intermezzo

Blank, Küpfer & Sauer unpublished

FBA Limitations to Predict In Vivo Fluxes: Alternate Optima

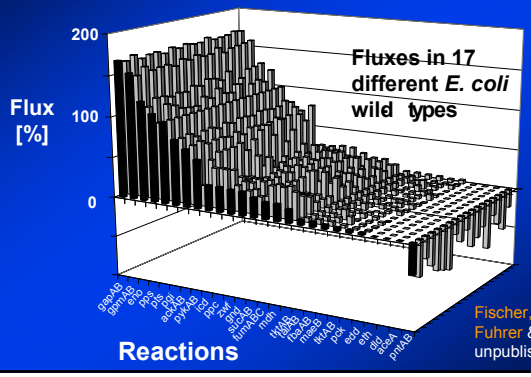


13C-exp. flux

Range of feasible fluxes predicted by FBA

Küpfer, Schütz & Sauer unpublished

Biological Variance ... Is E coli = E. coli ?



Fischer, Perrenoud, Fuhrer & Sauer unpublished

Back to Yeast: Where is Metabolism Flexible?

Flux balance analysis with the verified genome-scale model of *S. cerevisiae*

1038 reactions (total)

339 active on glucose (from ^{13}C)
most knockouts are lethal!

106 flexible reactions:
alternative pathways exist
‚unpredictable' critical cases!

80% trivial to predict flux

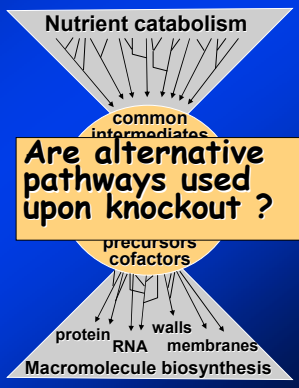
Only 51 have a flux higher than 1%
Encoded by 46 genes!

Blank, Küpfer & Sauer unpublished

Flexibility is in the Core Part of Metabolism

^{13}C Flux analysis in 35 of the 46 ‚flexible' yeast mutants:

- 9 alternative pathway
- 4 duplicate & alternative
- 22 duplicate genes (isoenzymes)



Blank, Küpfer & Sauer unpublished

Genetic Network Robustness: Alternative Pathways or Duplicates in Metabolism?

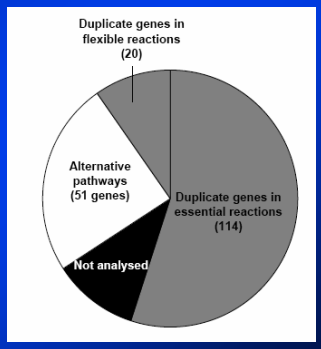
207 knockout mutants in reactions that are

a) active in wild type

b) viable on glucose

Experimental data:

- growth phenotypes of duplicates
- ^{13}C based fluxes



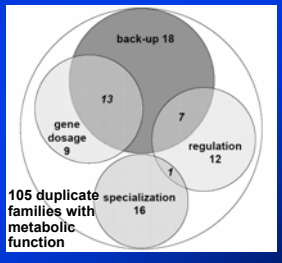
Blank, Küpfer & Sauer unpublished

Function of Duplicate Genes ?

Discussed functions:

- back up(robustness)
- gene dosage
- functional divergence
- evolutionary playground
- .....

Mechanistic answers are possible for duplicates with metabolic function:



Blank, Küpfer & Sauer Poster U-P03

Computation - Experiment Iterations in Systems Biology:

- Start from many, quantitative data to construct a detailed parameterized model and make very ‚educated' predictions from there.
- Start from very few data to construct a ‚coarse-grained' (for example constrained-based stoichiometic) model and reduce experimental effort by identifying obvious results!

THANKS .....

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ETH

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J. Stelling

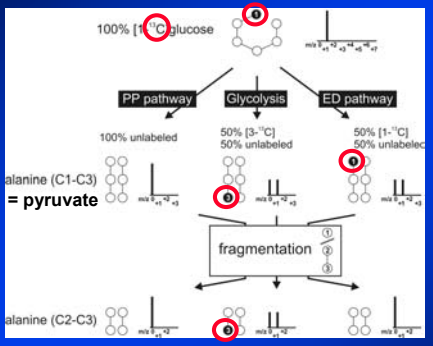
ETH
M. Peter

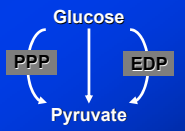
INRA Paris
S. Aymerich

THANKS .....

Eliane Fischer
Nicola Zamboni
Lars Küpfer
Lars Blank
Annik Perrenoud

Principle of MS-Detected Isotope Pattern





Molecules in metabolism

Parts of molecules detected in MS