V16 Minimal cut sets in biochemical reaction networks

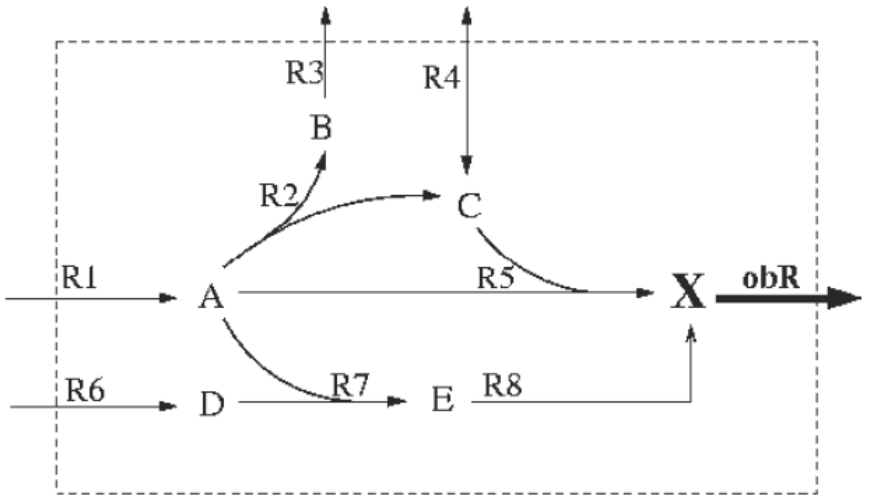
Concept of minimal cut sets (MCSs): smallest „failure modes“ in the network that render the correct functioning of a cellular reaction impossible.

Right: fictitious reaction network NetEx.

The only reversible reaction is R4.

We are particularly interested in the flux `obR` exporting synthesized metabolite X.

→ Characterize **solution space** by computing **elementary flux modes**.



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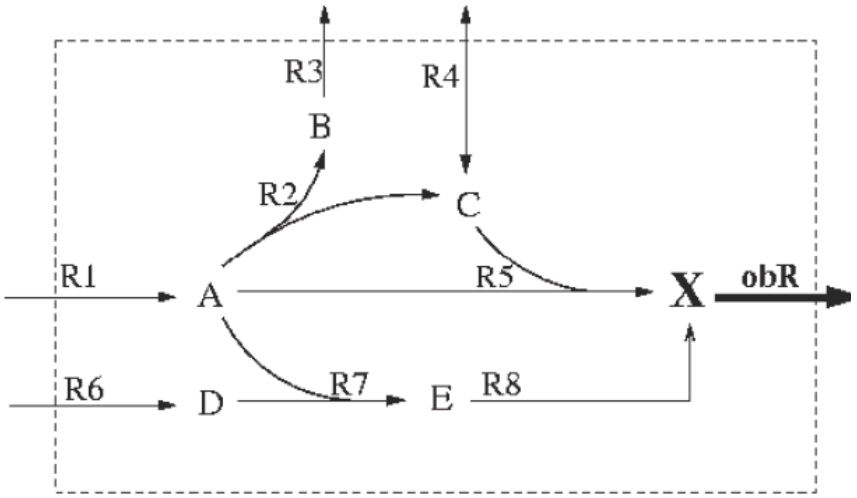
Elementary flux modes of NetEx



	R1	R2	R3	R4	R5	R6	R7	R8	obR
Elementary modes									
EM1	1	1	1	−1	0	0	0	0	0
EM2	1	0	0	0	0	1	1	1	1
EM3	2	1	1	0	1	0	0	0	1
EM4	1	0	0	1	1	0	0	0	1

One finds 4 elementary flux modes for NetEx.

3 of them (shaded) allow the production of metabolite X.

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Cut set

Now we want to prevent the production of metabolite X.

→ demand that there is no balanced flux distribution possible which involves `obR`.

Definition. A set of reactions is termed a **cut set** (with respect to a defined objective reaction)
if after the removal of these reactions from the network
no feasible balanced flux distribution involves the objective reaction.

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Cut set

A **trivial cut set** is the reaction itself: C0 = {`obR`}.

Another extreme case is the **removal of all reactions** except obR ..
This is very inefficient if this involves knocking out these genes or developing small molecule inhibitors!

Desirable solutions:

- From an engineering point of view, it might be desirable to cut reactions **at the beginning of a pathway**.

- The production of biomass is usually not coupled to a single gene or enzyme, and can therefore not be directly inactivated.

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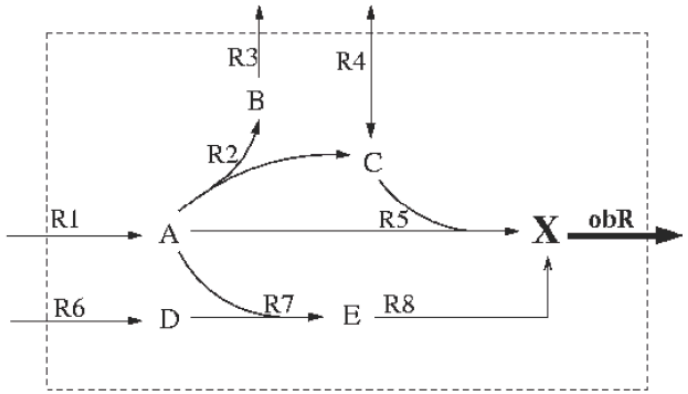
Cut set

C1 = {R5,R8} is a cut set already sufficient for preventing the production of X.

Removing R5 or R8 alone is not sufficient.

Definition. A cut set C (related to a defined objective reaction) is a **minimal cut set** (MCS) if no proper subset of C is a cut set.

→ C1 is a minimal cut set



	R1	R2	R3	R4	R5	R6	R7	R8	obR
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EM3	2	1	1	0	1	0	0	0	1
EM4	1	0	0	1	1	0	0	0	1
Minimal cut sets (objective reaction: obR)									
MCS0									×
MCS1	×								
MCS2					×	×			
MCS3					×		×		
MCS4					×			×	
MCS5		×		×		×			
MCS6			×	×		×			
MCS7		×		×			×		
MCS8			×	×			×		
MCS9		×		×				×	
MCS10			×	×				×	

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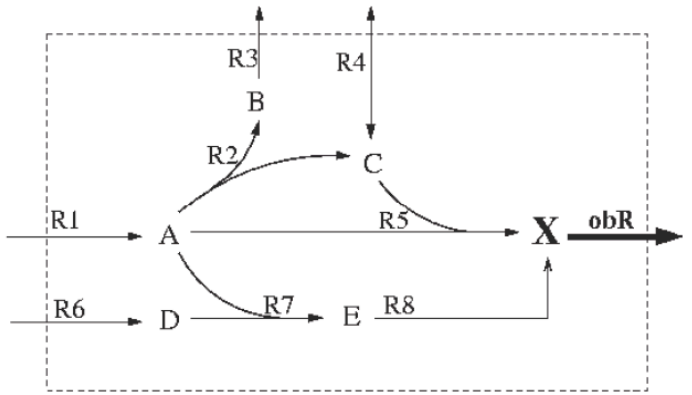
Remarks

(1) An MCS always guarantees dysfunction as long as the assumed network structure is currect. However, additional regulatory circuits or capacity restrictions may allow that even a proper subset of a MCS is a cut set.
The MCS analysis should always be seen from a purely structural point of view.

(2) After removing a complete MCS from the network, other pathways producing other metabolites may still be active.

(3) MCS4 = {R5,R8} clearly stops production of X.



What about MCS6 = {R3,R4,R6}?

Cannot X be still be produced via R1, R2, and R5?
However, this would lead to accumulation of B and is therefore physiologically impossible.

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Similar concepts

Graph theory:
we previously introduced a similar definition of minimal cut sets where they ensure a disconnectivity of a given graph.

However, these graph-theoretical concepts do not fit into the definition of MCSs as defined here and would, in general, lead to other results!

The reason is that metabolic networks use an explicit consideration of the hypergraphical nature of metabolic networks.

Hypergraphs: generalized graphs, where an edge (reaction) can link *k* nodes (reactants) with *l* nodes (products), whereas in graphs only 1:1 relations are allowed.

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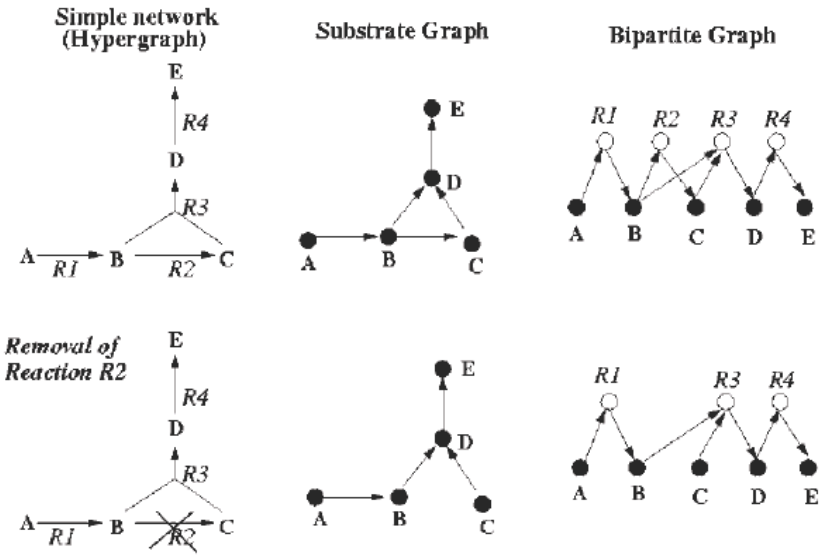
Comparison with graph theory

Example: we are interested in inhibiting the production of E.
Thus, R4 is our objective reaction.

If R2 is removed from the network, E can no longer be produced because C is required for driving reaction R3.

However, R2 would not be an MCS in terms of graph theory, neither in the substrate or in the bipartite graph representation because all metabolites are still connected after R2 is removed.



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Algorithm for computing MCSs

The MCSs for a given network and objective reaction are members of the power set of the set of reaction indices and are uniquely determined.

A systematic computation must ensure that the calculated MCSs are:

(1) cut sets („destroying“ all possible balanced flux distributions involving the objective reaction), and

(2) that the MCSs are really minimal, and

(3) that all MCSs are found.

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Algorithm for computing MCSs

(1) cut sets („destroying“ all possible balanced flux distributions involving the objective reaction),

→ any feasible steady-state flux distribution in a given network – expressed as vector **r** of the *q* net reaction rates – can be represented by a non-negative linear combination of the *N* elementary modes:

$$\mathrm{r} = \sum_{i=1}^{N} \alpha_i EM_i, \qquad \alpha_i \geq 0$$

To ensure that the rate r_k of the objective reaction is 0 in all **r**, each EM must contain 0 at the *k*-th place.

→ If C is a proper cut set the following cut set condition must hold:
For each EM involving the objective reaction (with a non-zero value),
there is at least one reaction in C also involved in this EM.

This guarantees that all EMs, in which the objective reaction participates,
will vanish when the reactions in the cut set are removed from the network.

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Algorithm

ALGORITHM:

(1) Calculate the EMs in the given network

(2) Define the objective reaction obR

(3) Choose all EMs where reaction obR is non-zero and store it in the binary array *em_obR* (*em_obR*[*i*][*j*]==1 means that reaction *j* is involved in EM *i*)

(4) Initialize arrays *mcs* and *precutsets* as follows (each array contains sets of reaction indices): append {*j*} to *mcs* if reaction *j* is essential (*em_obR*[*i*][*j*]=1 for each EM *i*), otherwise to *precutsets*

(5) FOR i=2 TO MAX_CUTSETSIZE

(5.1) *new_precutsets*=[ ];

(5.2) FOR *j* = 1 TO *q* (*q*: number of reactions)

(5.2.1) Remove all sets from *precutsets* where reaction *j* participates

(5.2.2) Find all sets of reactions in *precutsets* that do not cover at least one EM in *em_obR* where reaction *j* participates; combine each of these sets with reaction *j* and store the new preliminary cut sets in *temp_precutsets*

(5.2.3) Drop all *temp_precutsets* which are a superset of any of the already determined minimal cut sets stored in *mcs*

(5.2.4) Find all retained *temp_precutsets* which do now cover all EMs and append them to *mcs*; append all others to *new_precutsets*

ENDFOR

(5.3) If isempty(*new_precutsets*)

(5.3.1) Break

ELSE

(5.3.2) *precutsets*=*new_precutsets*

ENDIF

ENDFOR

(6) result: *mcs* contains the MCSs

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According to Acuna (2009) this algorithm is often very inefficient.

Applications of MCSs

Target identification and repression of cellular functions

A screening of all MCSs allows for the identification of the best suitable manipulation. For practical reasons, the following conditions should be fulfilled:

- usually, a small number of interventions is desirable (small size of MCS)

- other pathways in the network should only be weakly affected

- some of the cellular functions might be difficult to shut down genetically or by inhibition, e.g. if many isozymes exist for a reaction.

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Applications of MCSs

Network verification and mutant phenotype predictions

We expect that cutting away an MCS from the network is definitely intolerable for the cell with respect to certain cellular reactions/processes.

Such predictions, derived purely from network structure, are a useful strategy for verification of hypothetical or reconstructed networks.

If the outcome of prediction and experiments differ, this often indicates an incorrect or incomplete network structure.

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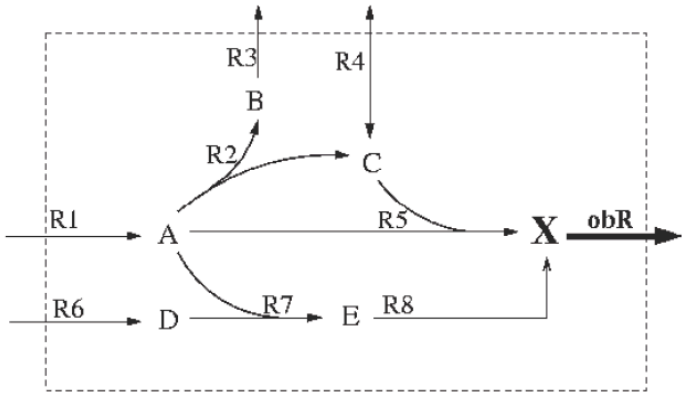
Structural fragility and robustness

If we assume that each reaction in a metabolic network has the same probability to fail, small MCSs are most probable to be responsible for a failing objective function.

Define a **fragility coefficient** F_i as the reciprocal of the average size of all MCSs in which reaction *i* participates.



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Minimal cut sets (objective reaction: obR)									
MCS0									×
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MCS2					×	×			
MCS3					×		×		
MCS4					×			×	
MCS5		×		×		×			
MCS6			×	×		×			
MCS7		×		×			×		
MCS8			×	×			×		
MCS9		×		×				×	
MCS10			×	×				×	
F_i	1	1/3	1/3	1/3	1/2	3/8	3/8	3/8	1

Besides the essential reaction R1, reaction R5 is most crucial for the objective reaction.

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Example: MCSs in the central metabolism of *E.coli*

objective reaction
„biomass synthesis“
Network: 110 reactions,
89 metabolites,
see Stelling et al. (2002)

Table 2. Overview on computed MCSs in the central metabolism of *E.coli* for growth on four different substrates

	Acetate	Succinate	Glycerol	Glucose
No. of EMs with growth	363	3421	9479	21 592
No. of MCSs (objective reaction: growth)	245	1255	2970	4225
Maximal number of preliminary MCSs (during computation)	3563	69 628	344 196	902 769
Computation time (Intel Pentium, 1 MHZ; 4 GB RAM)	7 s	20 min	5.42 h	29.67 h
F_i values (in parentheses: size of the smallest MCS in which the reaction occurs)				
F16P-bisphosphatase	1 (1)	1 (1)	1 (1)	0.102 (6)
ATP-synthase	1 (1)	0.325 (3)	0.141 (3)	0.149 (3)
SuccCoA-synthetase	0.207 (2)	0.145 (2)	0.125 (2)	0.131 (2)
PEP-carboxylase	0.128 (2)	0.117 (2)	0.120 (2)	0.143 (2)
Malic enzyme	0.5 (2)	0.5 (2)	0.114 (2)	0.123 (2)
R15P-X5P (epimerase)	0.198 (2)	0.135 (2)	0.128 (2)	0.148 (2)
F	0.783	0.718	0.699	0.643

The computation time does not involve the time needed for computing the elementary modes. F_i: fragility coefficient of reaction i; **F**: network (overall) fragility coefficient.

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Conclusion - MCS

An MCS is a irreducible combination of network elements whose simultaneous inactivation leads to a guaranteed dysfunction of certain cellular reactions or processes.

Theorem: Determining a reaction cut of minimum cardinality is NP-hard.

MCSs are inherent and uniquely determined structural features of metabolic networks similar to EMs.

The computation of MCSs and EMs becomes challenging in large networks.

Analyzing the MCSs gives deeper insights in the structural fragility of a given metabolic network and is useful for identifying target sets for an intended repression of network functions.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
Acuna et al. BioSystems 95, 51-60 (2009)

Current metabolomics

Review:

(1) recent work on metabolic networks required revising the picture of separate biochemical pathways into a **densely-woven metabolic network**

(2) **Connectivity** of substrates in this network follows a **power-law** (Yeong&Barabasi).

(3) **Constraint-based modeling approaches** (FBA) were successful in analyzing the **capabilities** of cellular **metabolism** including

- its capacity to predict **deletion phenotypes**
- the ability to calculate the **relative flux values** of metabolic reactions, and
- the capability to identify properties of **alternate optimal growth states** in a wide range of simulated environmental conditions

Open questions

- what parts of metabolism are involved in adaptation to environmental conditions?
- is there a central essential metabolic core?
- what role does transcriptional regulation play?

Application of elementary modes
Metabolic network structure of *E.coli* determines key aspects of functionality and regulation

Table 1 **Number and distribution of elementary flux modes.**

Selection*		Glucose	Acetate	Glycerol	Succinate	Sum
-	N	27,099	598	11,332	4,249	43,279
Growth only	$N(\mu, \neq ATP)$	73.1%	58.7%	78.6%	76.3%	74.6%
ATP only	$N(\neq \mu, ATP)$	3.2%	5.0%	2.4%	2.4%	3.0%
Growth and ATP	$N(\mu, ATP)$	6.6%	2.0%	5.1%	4.2%	5.9%
No growth/ATP	$N(\neq \mu, \neq ATP)$	17.1%	34.3%	13.9%	17.1%	16.5%
Aerobic growth	$N(\mu, O_2)$	73.1%	60.7%	83.6%	80.5%	76.4%
Anaerobic growth	$N(\mu, \neq O_2)$	6.6%	0.0%	0.0%	0.0%	4.1%

*We denote the number of elementary flux modes simultaneously meeting a set of conditions, $C_1, \ldots, C_n$, by $N(C_1, \ldots, C_n)$. These conditions include, for example, the situation where cells can grow, which is abbreviated by μ. Excess energy production in the form of ATP (ATP), the substrate metabolized (S_k for the k-th substrate) and oxygen uptake (O_2) are specified accordingly. The operator '$\neq$' indicates that certain fluxes must not occur. The total number of modes includes one futile cycle without substrate uptake.

Compute EFMs for central metabolism of *E.coli*.

Catabolic part: substrate uptake reactions, glycolysis, pentose phosphate pathway, TCA cycle, excretion of by-products (acetate, formate, lactate, ethanol)

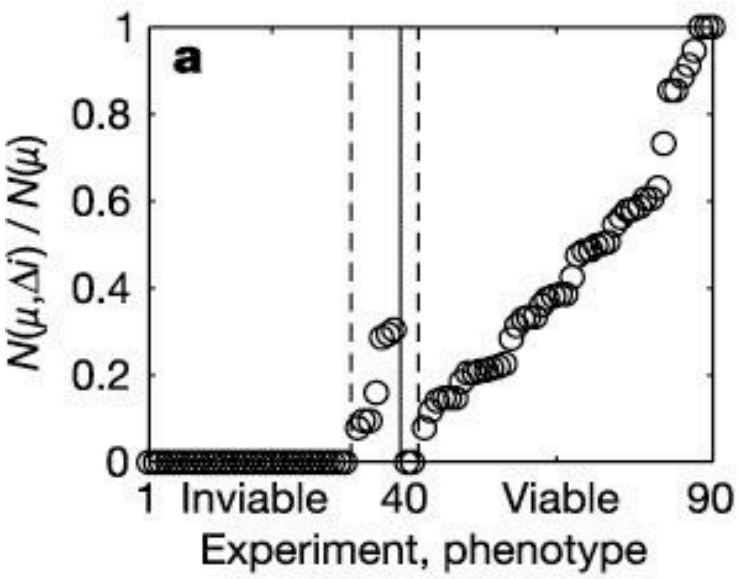
Anabolic part: conversions of precursors into building blocks like amino acids, to macromolecules, and to biomass.

Stelling et al. Nature 420, 190 (2002)

Metabolic network topology and phenotype

Idea:

Can the total number of EFMs for given conditions be used as quantitative measure of metabolic flexibility?



a, Relative number of EFMs *N* enabling deletion mutants of gene *i* (Δ *i*) in *E. coli* to grow (abbreviated by μ) for 90 different combinations of mutation and carbon source.

Shown are results for 90 deletions of different individual genes.

Answer: Yes, the # of EFMs for mutant strain allows correct prediction of growth phenotype in more than 90% of the cases.

Stelling et al. Nature 420, 190 (2002)

Relative yield

Robustness analysis

The # of EFMs qualitatively indicates whether a mutant is viable or not, but does not describe quantitatively how well a mutant grows.

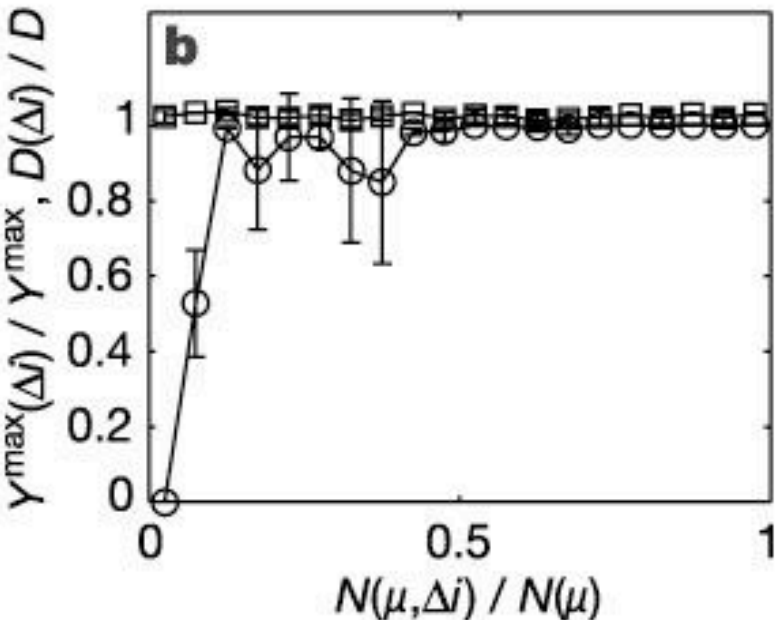
Define maximal biomass yield Y^{mass} as the optimum of:

$$Y_{i,X/S_i} = \frac{e_i^{\mu}}{e_i^{S_k}}$$

e_i is the single reaction rate (growth and substrate uptake) in EFM i selected for utilization of substrate S_k.

Stelling et al. Nature 420, 190 (2002)

Robustness Analysis



Dependency of the mutants' maximal growth yield $Y^{max}(\Delta i)$ (open circles) and the network diameter $D(\Delta i)$ (open squares) on the share of elementary modes operational in the mutants.

Stelling et al. Nature 420, 190 (2002)

→ Central metabolism of *E.coli* behaves in a highly robust manner because mutants with significantly reduced metabolic flexibility show a growth yield similar to wild type.

Distribution of fluxes in *E.coli*

Global organization of metabolic fluxes in the bacterium *Escherichia coli*

E. Almaas[1], B. Kovács[1,2], T. Vicsek[2], Z. N. Oltvai[3] & A.-L. Barabási[1]

[1]*Department of Physics, University of Notre Dame, Notre Dame, Indiana 46556, USA*
[2]*Biological Physics Department and Research Group of HAS, Eötvös University, H-1117 Budapest, Hungary*
[3]*Department of Pathology, Northwestern University, Chicago, Illinois 60611, USA*

Nature 427, 839 (2004)

Aim: understand principles that govern the use of individual reactions under different growth conditions.

Stoichiometric matrix for *E.coli* strain MG1655 containing 537 metabolites and 739 reactions taken from Palsson *et al*.

Apply flux balance analysis to characterize solution space (all possible flux states under a given condition).

$$\frac{d}{dt}[A_i] = \sum_j S_{ij} v_j = 0$$

v_j is the flux of reaction j and S_{ij} is the stoichiometric coefficient of reaction j.

Optimal states

Denote the **mass** carried by reaction *j* producing (consuming) metabolite *i* by

$$\hat{v}_{ij} = \left| S_{ij} \right| v_j$$

Observation:

Fluxes vary widely: e.g. dimensionless flux of succinyl coenzyme A synthetase reaction is 0.185, whereas the flux of the aspartate oxidase reaction is 10.000 times smaller, 2.2×10^{-5}.

Using linear programming and adapting constraints for each reaction flux v_i of the form $\beta_i^{min} \leq v_i \leq \beta_i^{max}$, the flux states were calculated that optimize cell growth on various substrates.

Plot the flux distribution for active (non-zero flux) reactions of *E.coli* grown in a glutamate- or succinate-rich substrate.

Overall flux organization of *E.coli* metabolic network

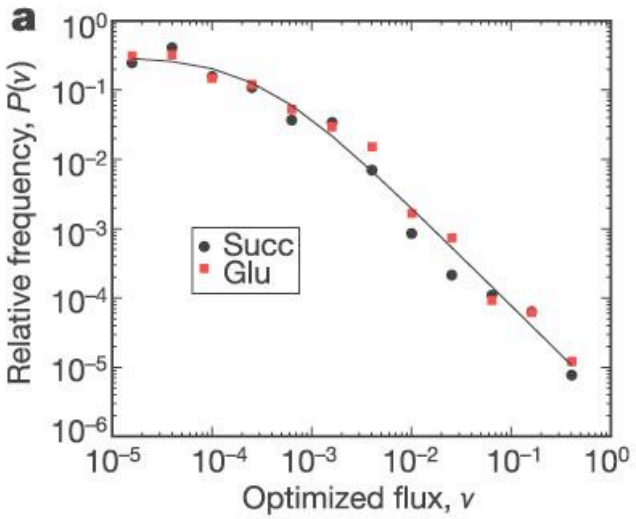
a, Flux distribution for optimized biomass production on succinate (black) and glutamate (red) substrates.

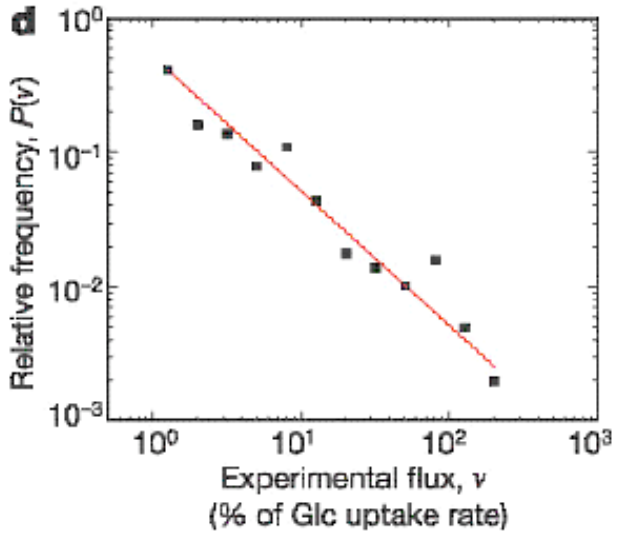
The solid line corresponds to the power-law fit that a reaction has flux v

$P(v) \propto (v + v_0)^{-\alpha}$, with $v_0 = 0.0003$ and $\alpha = 1.5$.

d, The distribution of experimentally determined fluxes from the central metabolism of *E. coli* shows power-law behaviour as well, with a best fit to $P(v) \propto v^{-\alpha}$ with $\alpha = 1$.

Both computed and experimental flux distribution show wide spectrum of fluxes.

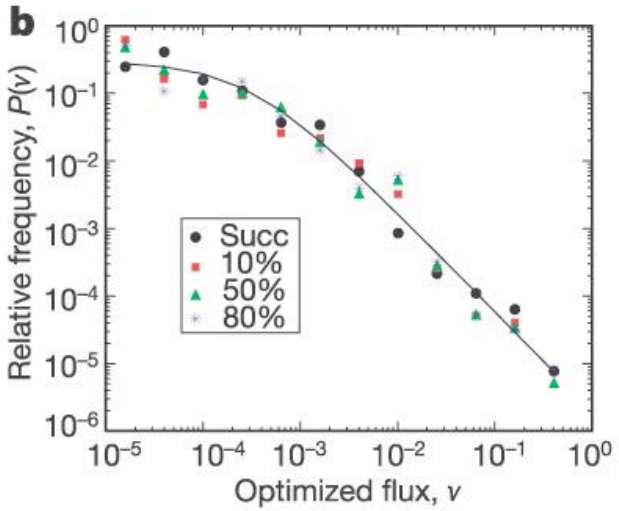




Almaar et al., Nature 427, 839 (2004)

Response to different environmental conditions

Is the flux distribution independent of environmental conditions?



b, Flux distribution for optimized biomass on succinate substrate (black) with an additional 10% (red), 50% (green) and 80% (blue) randomly chosen subsets of the 96 input channels (substrates) turned on.

The flux distribution was averaged over 5,000 independent random choices of uptake metabolites.

→ **Yes, the flux distribution is independent of the external conditions.**

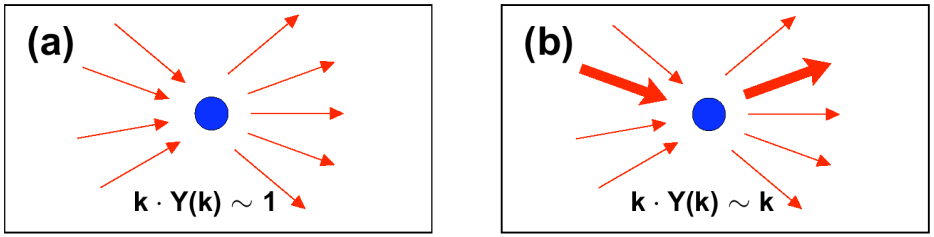
Almaar et al., Nature 427, 839 (2004)

Use scaling behavior to determine local connectivity

The observed flux distribution is compatible with two different potential local flux structures:

(a) a **homogenous local organization** would imply that all reactions producing (consuming) a given metabolite have comparable fluxes

(b) a more delocalized „**high-flux backbone** (HFB)“ is expected if the local flux organisation is heterogenous such that each metabolite has a dominant source (consuming) reaction.

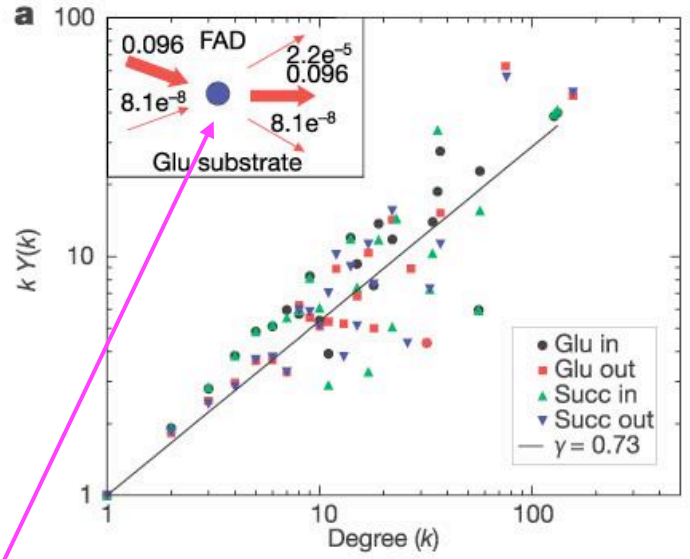


$$Y(k,i) = \sum_{j=1}^{k} \left[\frac{\hat{V}_{ij}}{\sum_{l=1}^{k} \hat{V}_{il}} \right]^2$$

Almaar et al., Nature 427, 839 (2004)

Characterizing the local inhomogeneity of the flux net

a, Measured $kY(k)$ shown as a function of k for incoming and outgoing reactions, averaged over all metabolites, indicates that $k \times Y(k) \propto k^{0.73}$. Inset shows non-zero mass flows, $v^{\wedge}_{ij}$, producing (consuming) FAD on a glutamate-rich substrate.



→ an **intermediate behavior** is found between the two extreme cases.

→ the large-scale inhomogeneity observed in the overall flux distribution is also increasingly valid at the level of the individual metabolites.

The more reactions that consume (produce) a given metabolite, the more likely it is that a single reaction carries most of the flux, see FAD.

Almaar et al., Nature 427, 839 (2004)

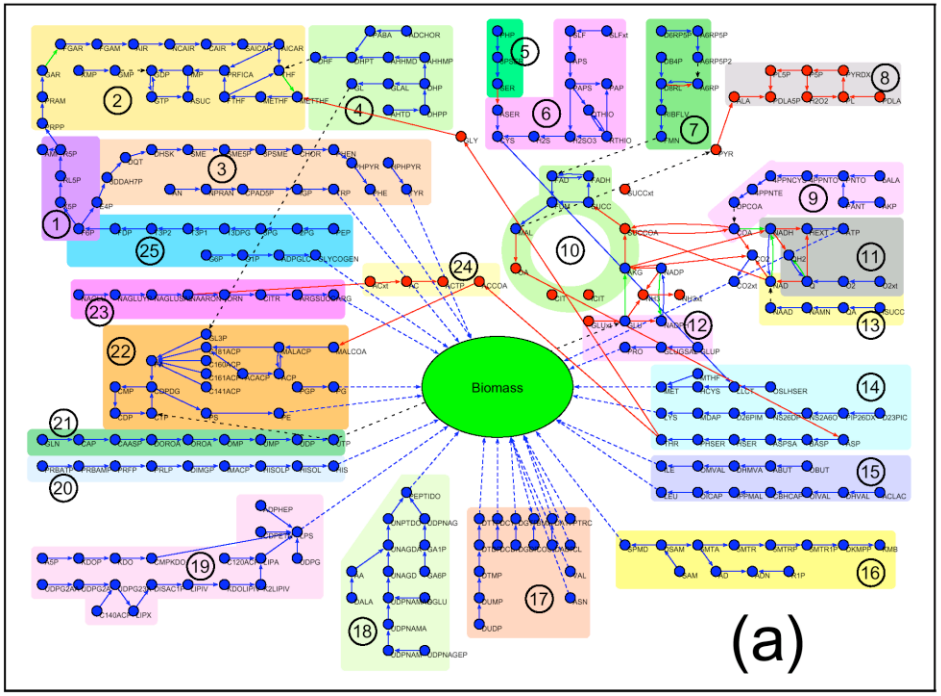
Clean up metabolic network

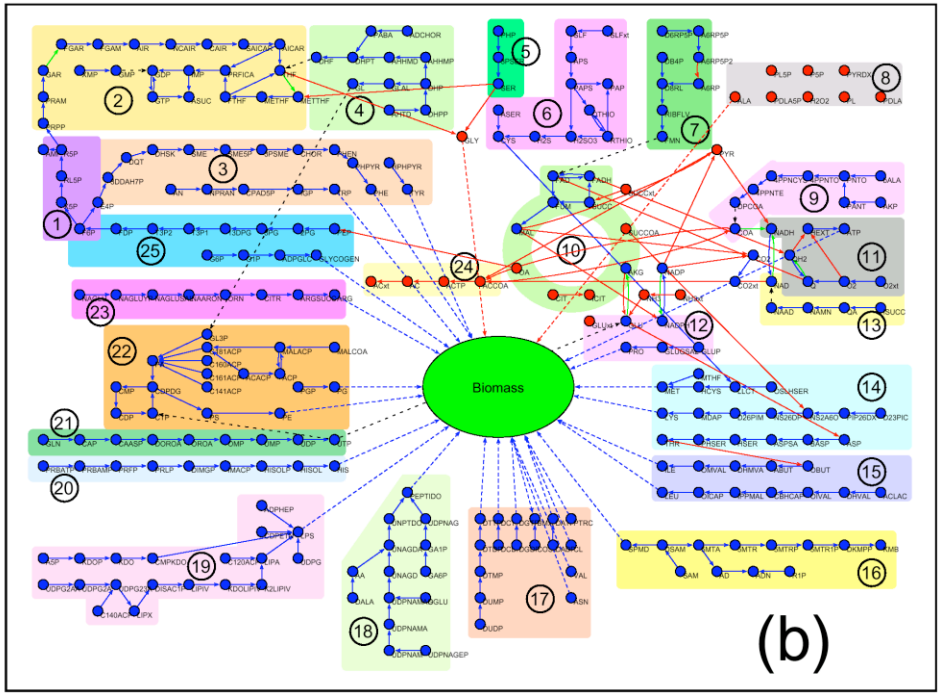
Use simple algorithm that removes for each metabolite systematically all reactions but the one providing the largest incoming (outgoing) flux distribution.

The algorithm uncovers the „**high-flux-backbone**“ of the metabolism, a distinct structure of linked reactions that form a giant component with a star-like topology.

Almaar et al., Nature 427, 839 (2004)

Maximal flow networks





glutamate rich

succinate rich substrates

Directed links: Two metabolites (e.g. A and B) are connected with a directed link pointing from A to B only if the reaction with maximal flux consuming A is the reaction with maximal flux producing B.

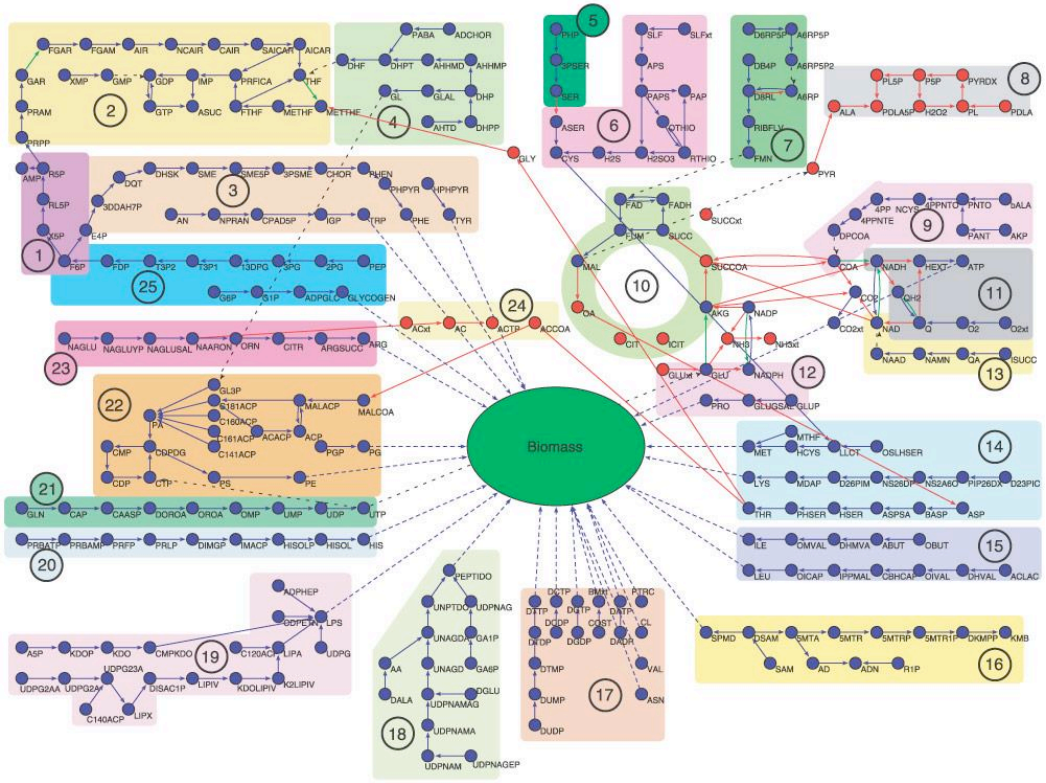
Shown are all metabolites that have at least one neighbour after completing this procedure.

The **background colours** denote different known biochemical pathways.

Almaar et al., Nature 427, 839 (2004)

FBA-optimized network on glutamate-rich substrate

High-flux backbone for FBA-optimized metabolic network of *E. coli* on a glutamate-rich substrate. **Metabolites** (vertices) coloured blue have at least one neighbour in common in glutamate- and succinate-rich substrates, and those coloured red have none. **Reactions** (lines) are coloured blue if they are identical in glutamate- and succinate-rich substrates, green if a different reaction connects the same neighbour pair, and red if this is a new neighbour pair. Black dotted lines indicate where the disconnected pathways, for example, folate biosynthesis, would connect to the cluster through a link that is not part of the HFB. Thus, the red nodes and links highlight the predicted changes in the HFB when shifting *E. coli* from glutamate- to succinate-rich media. Dashed lines indicate links to the biomass growth reaction.



(1) Pentose Phospate
(2) Purine Biosynthesis
(3) Aromatic Amino Acids
(4) Folate Biosynthesis
(5) Serine Biosynthesis
(6) Cysteine Biosynthesis
(7) Riboflavin Biosynthesis
(8) Vitamin B6 Biosynthesis
(9) Coenzyme A Biosynthesis
(10) TCA Cycle
(11) Respiration
(12) Glutamate Biosynthesis
(13) NAD Biosynthesis
(14) Threonine, Lysine and Methionine Biosynthesis
(15) Branched Chain Amino Acid Biosynthesis
(16) Spermidine Biosynthesis
(17) Salvage Pathways
(18) Murein Biosynthesis
(19) Cell Envelope Biosynthesis
(20) Histidine Biosynthesis
(21) Pyrimidine Biosynthesis
(22) Membrane Lipid Biosynthesis
(23) Arginine Biosynthesis
(24) Pyruvate Metabolism
(25) Glycolysis

Almaar et al., Nature 427, 839 (2004)

Interpretation

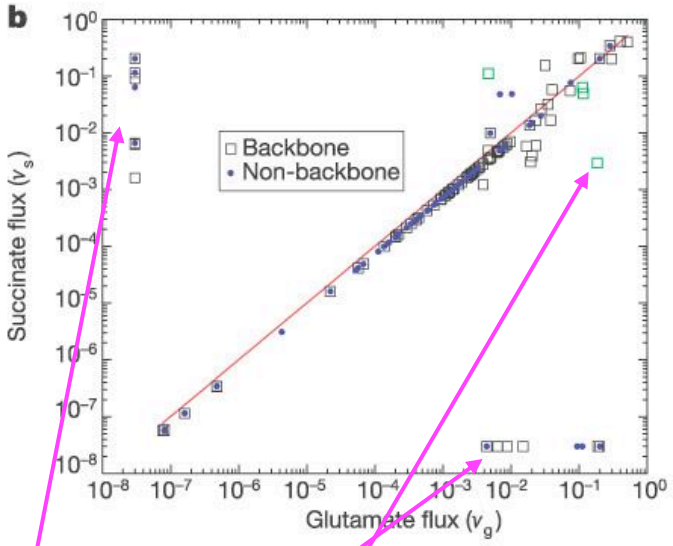
Only a few pathways appear disconnected indicating that although these pathways are part of the HFB, their end product is only the second-most important source for another HFB metabolite.

Groups of individual **HFB reactions largely overlap with traditional biochemical partitioning** of cellular metabolism.

Almaar et al., Nature 427, 839 (2004)

How sensitive is the HFB to changes in the environment?

b, Fluxes of individual reactions for glutamate-rich and succinate-rich conditions. Reactions with negligible flux changes follow the diagonal (solid line).
Some reactions are turned off in only one of the conditions (shown close to the coordinate axes). Reactions belonging to the HFB are indicated by black squares, the rest are indicated by blue dots. Reactions in which the direction of the flux is reversed are coloured green.



Only reactions in the high-flux territory undergo noticeable differences!

Type I: reactions turned on in one conditions and off in the other (symbols).
Type II: reactions remain active but show an orders-in-magnitude shift in flux under the two different growth conditions.

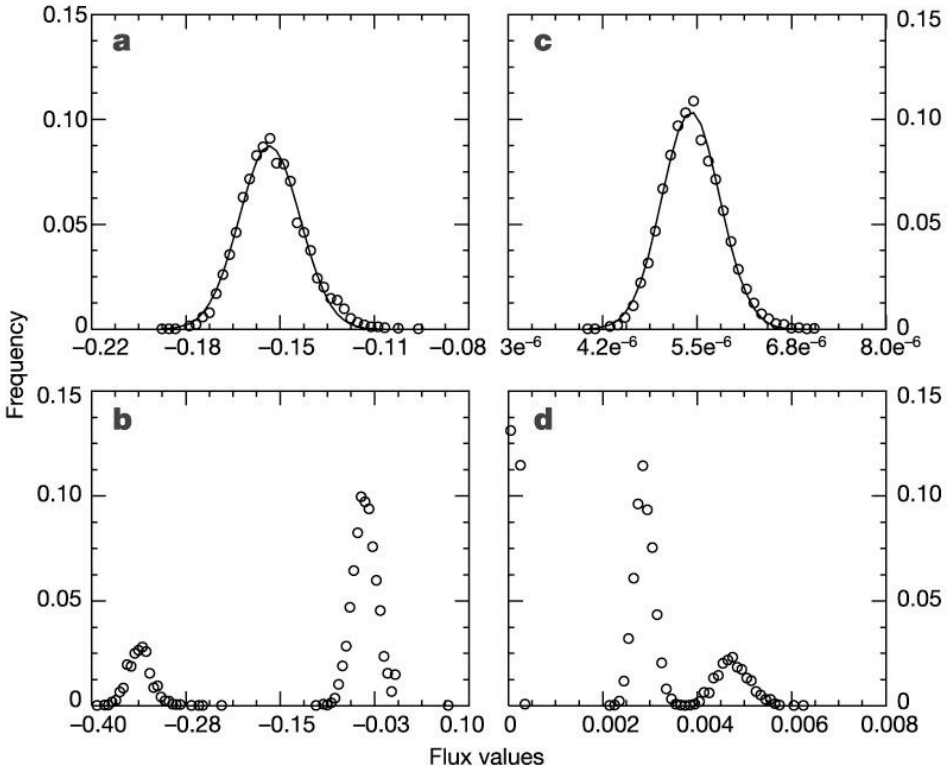
Almaar et al., Nature 427, 839 (2004)

Flux distributions for individual reactions

Shown is the flux distribution for four selected *E. coli* reactions in a 50% random environment.
a Triosphosphate isomerase;
b carbon dioxide transport;
c NAD kinase;
d guanosine kinase.

Reactions on the $\sigma \propto v$ curve (small fluxes) have **unimodal/gaussian distributions** (a and c). Shifts in growth-conditions only lead to small changes of their flux values.

Reactions off this curve have **multimodal distributions** (b and d), showing several discrete flux values under diverse conditions. Under different growth conditions they show several discrete and distinct flux values.



Almaar et al., Nature 427, 839 (2004)

Summary

Metabolic network use is highly uneven (power-law distribution) at the global level and at the level of the individual metabolites.

Whereas most metabolic reactions have low fluxes, the overall activity of the metabolism is dominated by several reactions with very high fluxes.

E. coli responds to changes in growth conditions by reorganizing the rates of selected fluxes predominantly within this high-flux backbone.
Apart from minor changes, the use of the other pathways remains unaltered.
These reorganizations result in large, discrete changes in the fluxes of the HFB reactions.

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The Activity Reaction Core and Plasticity of Metabolic Networks

Eivind Almaas[1,2], Zoltán N. Oltvai[3*], Albert-László Barabási[2,4]

PLoS Computational Biology | www.ploscompbiol.org 0557 December 2005 | Volume 1 | Issue 7 | e68

The same authors as before used FBA to examine utilization and relative flux rate of each metabolite in various simulated environmental conditions for *E.coli, H. pylori* and *S. cerevisae*:
For each system they considered 30.000 randomly chosen combinations where each uptake reaction is a assigned a random value between 0 and 20 mmol/g/h.

→ **adaptation** to different conditions occurs by 2 mechanisms:
(a) **flux plasticity**: changes in the fluxes of already active reactions.
E.g. changing from glucose- to succinate-rich conditions alters the flux of 264 E.coli reactions by more than 20%

(b) less commonly, adaptation includes **structural plasticity**, turning on previously zero-flux reactions or switching off active pathways.

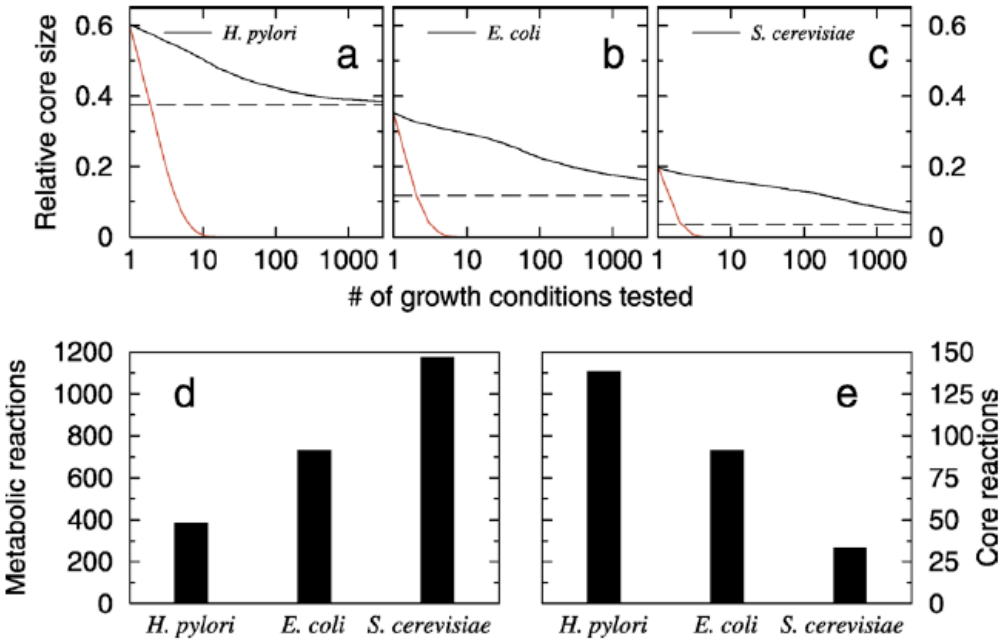
Emergence of the Metabolic Core

The two adaptation method mechanisms allow for the possibility of a group of reactions not subject to structural plasticity being active under all environmental conditions.

Assume that active reactions were randomly distributed.

If typically a q fraction of the metabolic reactions are active under a specific growth condition,
we expect for n distinct conditions an overlap of at least q^n reactions.
This converges quickly to 0.

Emergence of the Metabolic Core



(a–c) The average relative size of the number of reactions that are always active as a function of the number of sampled conditions (black line).

(d and e) The number of metabolic reactions (d) and the number of metabolic core reactions (e) in the three studied organisms.

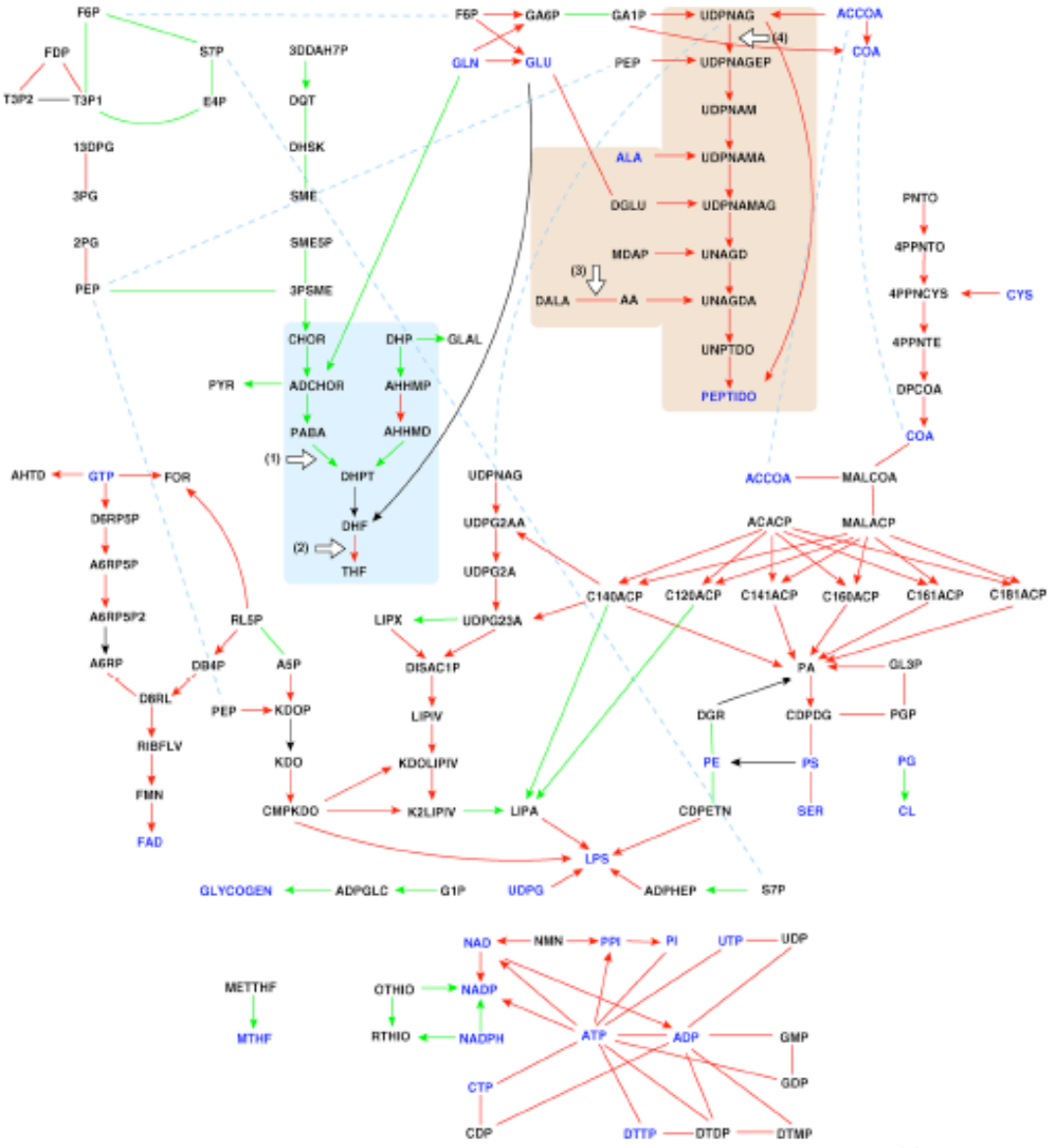
In a-c, as the number of conditions increases, the curve converges to a constant enoted by the dashed line, identifying the metabolic core of an organism.

Red line : number of reactions that are always active if activity is randomly distributed in the metabolic network. The fact that it converges to zero indicates that the real core represents a collective network effect, forcing a group of reactions to be active in all conditions.

Metabolic Core of *E.coli:* The constantly active reactions form a tightly connected cluster!

Shown are all reactions that are found to be active in each of the 30,000 investigated external conditions.
Blue: Metabolites that contribute directly to biomass formation,
Red (green): core reactions (links) catalyzed by essential (or nonessential) enzymes.
Black-colored links: enzymes with unknown deletion phenotype.
Blue dashed lines: multiple appearances of a metabolite,
Links with arrows: unidirectional reactions.
Note that 20 out of the 51 metabolites necessary for biomass synthesis are not present in the core, indicating that they are produced (or consumed) in a growth-condition-specific manner.
Blue and brown shading: folate and peptidoglycan biosynthesis pathways

White numbered arrows denote current antibiotic targets inhibited by: (1) sulfonamides, (2) trimethoprim, (3) cycloserine, and (4) fosfomycin.
A few reactions appear disconnected since we have omitted the drawing of cofactors.



Metabolic Core Reactions

The metabolic cores contain 2 types of reactions:

(a) reactions that are essential for biomass production under all environment conditions (81 of 90 in E.coli)

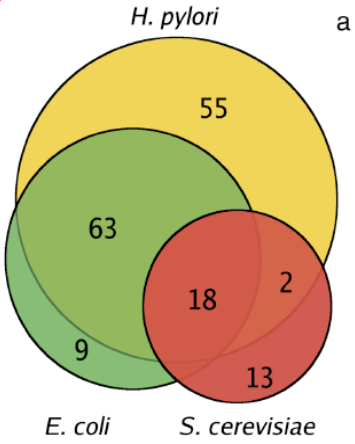
(b) reactions that assure optimal metabolic performance.

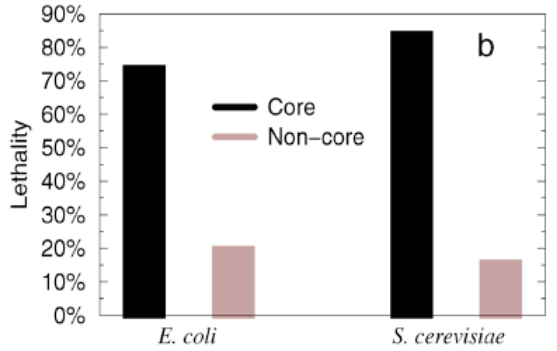
Characterizing the Metabolic Cores

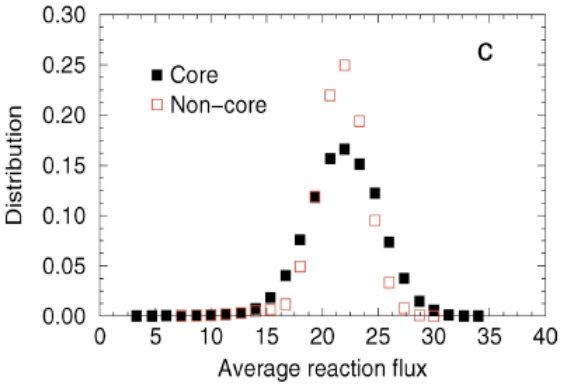
(A) The number of overlapping metabolic reactions in the metabolic core of *H. pylori, E. coli,* and *S. cerevisiae*.
The metabolic cores of simple organisms (*H. pylori* and *E.coli*) overlap to a large extent.
The largest organism (*S.cerevisae*) has a much larger reaction network that allows more flexbility → the relative size of the metabolic core is much lower.

(B) The fraction of metabolic reactions catalyzed by essential enzymes in the cores (black) and outside the core in *E. coli* and *S. cerevisiae*.
→ **Reactions of the metabolic core are mostly essential ones.**

(C) One could assume that the core represents a subset of high-flux reactions. This is apparently not the case.
The distributions of average metabolic fluxes for the core and the noncore reactions in *E. coli* are very similar.







Summary

- Adaptation to environmental conditions occurs via structural plasticity and/or flux plasticity.

Here: a surprisingly stable **metabolic core** of reactions was identified that are tightly connected to eachother.

- the reactions belonging to this core represent **potential targets** for antimicrobial intervention.