V15 Elementary Flux Modes / Minimal Reaction Cut Sets

Metabolic Pathway Analysis can be used to study e.g.

- metabolic network structure

- functionality of networks (including identification of futile cycles)

- robustness, fragility, flexibility/redundancy of networks

- to identify all (sub-) optimal pathways with respect to product/biomass yield

- rational strain design

Definition of Elementary Flux Modes (EFMs)

A pathway $P(\mathbf{v})$ is an **elementary flux mode** if it fulfills conditions C1 – C3.

(C1) **Pseudo steady-state.** $\mathbf{S} \cdot \mathbf{e} = 0$. This ensures that none of the metabolites is consumed or produced in the overall stoichiometry.

(C2) **Feasibility:** rate $e_i \geq 0$ if reaction is irreversible. This demands that only thermodynamically realizable fluxes are contained in $\mathbf{e}$.

(C3) **Non-decomposability:** there is no vector $\mathbf{v}$ (except the null vector and $\mathbf{e}$) fulfilling C1 and C2 and so that $P(\mathbf{v})$ is a proper subset of $P(\mathbf{e})$. This is the core characteristics for EFMs and EPs and provides the decomposition of the network into smallest units that are able to hold the network in steady state.

C3 is often called „genetic independence“ because it implies that the enzymes in one EFM or EP are not a subset of the enzymes from another EFM or EP.

Definition of Extreme Pathways (Eps)

The pathway $P(e)$ is an **extreme pathway** if it fulfills conditions C1 – C3 AND conditions C4 – C5.

(C4) **Network reconfiguration**: Each reaction must be classified either as exchange flux or as internal reaction. All reversible internal reactions must be split up into two separate, irreversible reactions (forward and backward reaction).

(C5) **Systemic independence**: the set of EPs in a network is the **minimal** set of EFM that can describe all feasible steady-state flux distributions.

The algorithms for computing EPs and EFM are quite similar. We will not cover the algorithmic differences here.

Comparison of EFMs and EPs

Reconfigured Network: split up R7

3 EFMs are not systemically independent:
EFM1 = EP4 + EP5
EFM2 = EP3 + EP5
EFM4 = EP2 + EP3
Property 1 of EFMs

The only difference in the set of EFMs emerging upon reconfiguration consists in the **two-cycles** that result from splitting up reversible reactions.

However, two-cycles are not considered as meaningful pathways.

Valid for any network: Property 1

Reconfiguring a network by **splitting up reversible reactions** leads to the same set of meaningful EFMs.
EFMs vs. EPs

What is the consequence when all exchange fluxes (and hence all reactions in the network) are made irreversible?

Table 1. Configurations of the example network (upper part N1 and N3; lower part N2 and N4), with corresponding elementary flux modes (EFM) and extreme pathways (EP) (see also Fig. 1)

<table>
<thead>
<tr>
<th>N1 (R2 and R7 reversible) N3 (as N1 but R2 irreversible)</th>
<th>N1 EFMs</th>
<th>N3 EFMs</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
<th>R9</th>
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<tr>
<td>EFMs</td>
<td>(\times)</td>
<td>(\times)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>-1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>EFM1</td>
<td>(\times)</td>
<td>(\times)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>EFM4</td>
<td>(\times)</td>
<td>(\times)</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>EFM5</td>
<td>(\times)</td>
<td>(\times)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>EFM6</td>
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<td>-1</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EFM7</td>
<td>1</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EFM8</td>
<td>(\times)</td>
<td>(\times)</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th>N2 (R2 reversible, R7 split up) N4 (as N2 but R2 irreversible)</th>
<th>N2 EFMs</th>
<th>N4 EFMs</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
<th>R9</th>
<th>R7b</th>
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</thead>
<tbody>
<tr>
<td>EFMs</td>
<td>(\times)</td>
<td>(\times)</td>
<td>(EP_1^\prime)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>EFM1</td>
<td>(\times)</td>
<td>(\times)</td>
<td>(EP_2^\prime)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EFM2</td>
<td>(\times)</td>
<td>(\times)</td>
<td>(EP_3)</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
<td>EFM3</td>
<td>(\times)</td>
<td>(\times)</td>
<td>(EP_4)</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>EFM4</td>
<td>(\times)</td>
<td>(\times)</td>
<td>(EP_5)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>EFM5</td>
<td>(\times)</td>
<td>(\times)</td>
<td>(EP_6)</td>
<td>1</td>
<td>-1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>EFM6</td>
<td>(\times)</td>
<td>(\times)</td>
<td>(EP_7)</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EFM7</td>
<td>(\times)</td>
<td>(\times)</td>
<td>(EP_8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Then EFMs and EPs always co-incide!
Property 2 of EFMs

Property 2
If all exchange reactions in a network are irreversible then the sets of meaningful EFMs (both in the original and in the reconfigured network) and EPs coincide.
3 EFMs are not systemically independent:
EFM1 = EP4 + EP5
EFM2 = EP3 + EP5
EFM4 = EP2 + EP3
Problem

Recognition of operational modes:
routes for converting exclusively A to P.

EFM (network N1)

Set of EPs does not contain all genetically independent routes, only EP1.

EP (network N2)

No EP leads directly from A to P via B.

Finding optimal routes

Problem

Finding all the optimal routes:

optimal pathways for synthesizing P during growth on A alone.

EFM (network N1)

One would only find the suboptimal EP1, not the optimal routes EFM1 and EFM2.

EP (network N2)

EFM1 and EFM2 are optimal because they yield one mole P per mole substrate A (i.e. R3/R1 = 1), whereas EFM3 and EFM4 are only suboptimal (R3/R1 = 0.5).


15. Lecture WS 2016/17
Network flexibility (structural robustness, redundancy)

Problem

Analysis of network flexibility: relative robustness of exclusive growth on A or B.

EFM (network N1)

4 pathways convert A to P (EFM1-EFM4), whereas for B only one route (EFM8) exists.

When one of the internal reactions (R4-R9) fails, 2 pathways will always "survive" for production of P from A.

By contrast, removing reaction R8 already stops the production of P from B alone.

EP (network N2)

Only 1 EP exists for producing P by substrate A alone (EP1), and 1 EP for synthesizing P by (only) substrate B (EP5).

This suggests that both substrates possess the same redundancy of pathways, but as shown by EFM analysis, growth on substrate A is much more flexible than on B.

Relative importance of single reactions

EFM (network N1)

R8 is essential for producing P by substrate B (EFM8), whereas for A there is no structurally „favored“ reaction (R4-R9 all occur twice in EFM1-EPM4).

However, considering the optimal modes EFM1, EFM2, one recognizes the importance of R8 also for growth on A.

EP (network N2)

Consider again biosynthesis of P from substrate A (EP1 only).

Because R8 is not involved in EP1 one might think that this reaction is not important for synthesizing P from A.

However, without this reaction, it is impossible to obtain optimal yields (1 P per A; EFM1 and EFM2).

Enzyme subsets and excluding reaction pairs:

suggest regulatory structures or rules.

EFM (network N1)

R6 and R9 are an enzyme subset.

By contrast, R6 and R9 never occur together with R8 in an EFM.

Thus (R6,R8) and (R8,R9) are excluding reaction pairs.

(In an arbitrary composable steady-state flux distribution they might occur together.)

EP (network N2)

The EPs pretend R4 and R8 to be an excluding reaction pair – but they are not (EFM2).

The enzyme subsets would be correctly identified in this case. However, one can construct simple examples where the EPs would also pretend wrong enzyme subsets (not shown).

Pathway length

Problem

Pathway length:
shortest/longest pathway for production of P from A.

EFM (network N1)
The shortest pathway from A to P needs 2 internal reactions (EFM2), the longest 4 (EFM4).

EP (network N2)
Both the shortest (EFM2) and the longest (EFM4) pathway from A to P are not contained in the set of EPs.

Removing a reaction and mutation studies: effect of deleting R7.

**Problem**

**Removing a reaction and mutation studies:** effect of deleting R7.

All EFMs not involving the specific reactions build up the complete set of EFMs in the new (smaller) sub-network.

If R7 is deleted, EFMs 2,3,6,8 „survive“. Hence the mutant is viable.

**EFM (network N1)**

**EP (network N2)**

Analyzing a subnetwork implies that the EPs must be newly computed.

E.g. when deleting R2, EFM2 would become an EP.

For this reason, mutation studies cannot be performed easily.

Software: FluxAnalyzer, based on Matlab

Steffen Klamt.

FluxAnalyzer has both EPs and EFMs implemented.

Allows convenient studies of metabolic systems.

Klamt et al.
Bioinformatics 19, 261 (2003)

Fig. 1. The network project of ‘SMALLNET’ constructed by the FluxAnalyzer. Left: Interactive flux map displaying a flux scenario (unknown rates are denoted by ‘###’). Right: network composer.

Fig. 2. Structural setup of the FluxAnalyzer.

Fig. 3. Concise graphical representation of the stoichiometric matrix (here: catabolic part of the network studied in Klamt et al., 2002)
Carotenoids (e.g. DPL and DPA) are light-harvesting pigments, UV-protecting compounds, regulators of membrane fluidity, and antioxidants.

They are used as nutrient supplements, pharmaceuticals, and food colorants.

**Aim**: increase carotenoid synthesis in *E.coli*
58 metabolic reactions,
22 reversible
36 irreversible
57 metabolites
29532 EFMs
In 5923 EFMs, the
production of biomass
and DPA are coupled.

Unrean et al. Metabol Eng 12, 112-122 (2010)

15. Lecture WS 2016/17
Results of virtual gene knockout calculations (counting number of EFMs and computing their yield from reaction stochiometries).

Select target genes where knockouts still maintain a maximum possible yield of carotenoid production, a reasonable yield of biomass while the largest number of EFMs is eliminated.

Unrean et al. Metabol Eng 12, 112-122 (2010)
Effect of single gene deletions

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total modes</th>
<th>Aerobic modes</th>
<th>Anaerobic modes</th>
<th>Predicted CRT yield*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>29,532</td>
<td>24,155</td>
<td>5377</td>
<td>0.0-426</td>
</tr>
<tr>
<td>ΔldhA</td>
<td>15,662</td>
<td>13,405</td>
<td>2257</td>
<td>0.0-426</td>
</tr>
<tr>
<td>ΔldhAΔfrdA</td>
<td>8573</td>
<td>7810</td>
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<td>0.0-426</td>
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<td>ΔldhAΔfrdAΔapoB</td>
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<td>6861</td>
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<td>571</td>
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<td>ΔldhAΔfrdAΔapoBΔptaΔadhE</td>
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<tr>
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<td>5</td>
<td>0</td>
<td>0.4-426</td>
</tr>
</tbody>
</table>

* Yield is in mg-diapolyocapndioic acid/g-glucose.

Optimal: 8 gene knockouts lead to predicted over-production of DPL and DPA.

After this deletion, only 5 EFMs remain.

Unrean et al. Metabol Eng 12, 112-122 (2010)
Remaining EFMs

Unrean et al. Metabol Eng 12, 112-122 (2010)  Bioinformatics III
15. Lecture WS 2016/17
Experimental verification: increased carotenoid yield

Mutant grows slower, but CRT production is increased 4 times.

Unrean et al. Metabol Eng 12, 112-122 (2010)
Complexity of finding and enumerating EFMs

**Theorem:** Given a stochiometric matrix $S$, an elementary mode can be found in polynomial time.

**Theorem:** In case all reactions in a metabolic network are reversible, the elementary modes can be enumerated in polynomial time.

The enumeration task becomes dramatically more difficult if the reactions are irreversible. In this case, the modes of the network form a cone, and the elementary modes are the rays of the cone.

**Theorem:** Given a flux cone and two coordinates $i$ and $j$, deciding if there exists an extreme ray of the cone that contains both $r_i$ and $r_j$ is NP-complete.

**Theorem:** Given a matrix $S$ and a number $k$, deciding whether an elementary mode exist that contains at most $k$ reactions is NP-complete.

It is an open question whether all elementary modes of a general network can be enumerated in polynomial time.

Summary EFM

EFMs are a robust method that offers great opportunities for studying functional and structural properties in metabolic networks.

The decomposition of a particular flux distribution (e.g. determined by experiment) as a linear combination of EFM is not unique.

Klamt & Stelling suggest that the term „elementary flux modes“ should be used whenever the sets of EFM and EPs are identical. In cases where they don’t, EPs are a subset of EFM.

It remains to be understood more thoroughly how much valuable information about the pathway structure is lost by using EPs.

Ongoing Challenges:
- study really large metabolic systems by subdividing them into sub-systems
- combine metabolic model with model of cellular regulation.

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 \( \text{obR} \) exporting synthesized metabolite X.

→ Characterize solution space by computing elementary flux modes.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
Elementary flux modes of NetEx

One finds 4 elementary flux modes for NetEx.

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

Klamt & Gilles, Bioinformatics 20, 226 (2004)
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.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
Cut set

A trivial cut set is the reaction itself: $C_0 = \{obR\}$.

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.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
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

Klamt & Gilles, Bioinformatics 20, 226 (2004)
Remarks

(1) An MCS always guarantees **dysfunction** as long as the assumed network structure is correct. 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**.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
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.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
Comparison with graph theory

Example: suppose 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.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
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.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
Algorithm for computing MCSs

**Necessary** condition for cut sets: they **interrupt** all possible balanced flux distributions involving the objective reaction,

Use the fact that any feasible steady-state flux distribution \( r \) in a given network can be represented by a non-negative **linear combination** of the \( N \) elementary modes:

\[
    r = \sum_{i=1}^{N} \alpha_i EM_i, \quad \alpha_i \geq 0
\]

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.
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 $MAX\_CUTSETS\_SIZE$
   (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

Klamt & Gilles, Bioinformatics 20, 226 (2004)

According to Acuna (2009) this algorithm is often very inefficient.
More efficient algorithms exist already and are still being developed.
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.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
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.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
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.

Besides the essential reaction R1, reaction R5 is most crucial for the objective reaction.
**Example: MCSs in the central metabolism of *E.coli***

<table>
<thead>
<tr>
<th></th>
<th>Acetate</th>
<th>Succinate</th>
<th>Glycerol</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of EMs with growth</td>
<td>363</td>
<td>3421</td>
<td>9479</td>
<td>21 592</td>
</tr>
<tr>
<td>No. of MCSs (objective reaction: growth)</td>
<td>245</td>
<td>1255</td>
<td>2970</td>
<td>4225</td>
</tr>
<tr>
<td>Maximal number of preliminary MCSs (during computation)</td>
<td>3563</td>
<td>69 628</td>
<td>344 196</td>
<td>902 769</td>
</tr>
<tr>
<td>Computation time (Intel Pentium, 1 MHZ; 4 GB RAM)</td>
<td>7 s</td>
<td>20 min</td>
<td>5.42 h</td>
<td>29.67 h</td>
</tr>
</tbody>
</table>

*F_i* values (in parentheses: size of the smallest MCS in which the reaction occurs)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Value (size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F16P-bisphosphatase</td>
<td>1 (1)</td>
</tr>
<tr>
<td>ATP-synthase</td>
<td>1 (1)</td>
</tr>
<tr>
<td>SuccCoA-synthetase</td>
<td>0.207 (2)</td>
</tr>
<tr>
<td>PEP-carboxylase</td>
<td>0.128 (2)</td>
</tr>
<tr>
<td>Malic enzyme</td>
<td>0.5 (2)</td>
</tr>
<tr>
<td>R15P-X5P (epimerase)</td>
<td>0.198 (2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Value (size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>0.783</td>
</tr>
</tbody>
</table>

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.

Klamt & Gilles, Bioinformatics 20, 226 (2004)
Conclusion - MCS

A MCS is an 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.

→ Computing MCSs and EMs becomes challenging in large networks.

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

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)