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Minimal Cut Sets as Computational Tool in Metabolic Engineering

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Minimal Cut Sets as Computational Tool in Metabolic Engineering

Novel Theoretical Results and Their Applications

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Stoichiometric Approaches for Metabolic Engineering and Rational Strain Optimization

FBA-based optimization:

OptKnock (Maranas et al.) and variants: identify minimal set of knockouts that lead to coupling between biomass and product synthesis

→ bi-level optmization problems solved by Mixed Integer Programming

→ one solution

Elementary-modes-based optimization:

Minimal metabolic functionality (Trinh, Srienc et al.): identify knockout set that keeps only a small number of (optimal) elementary modes functional

→ one solution

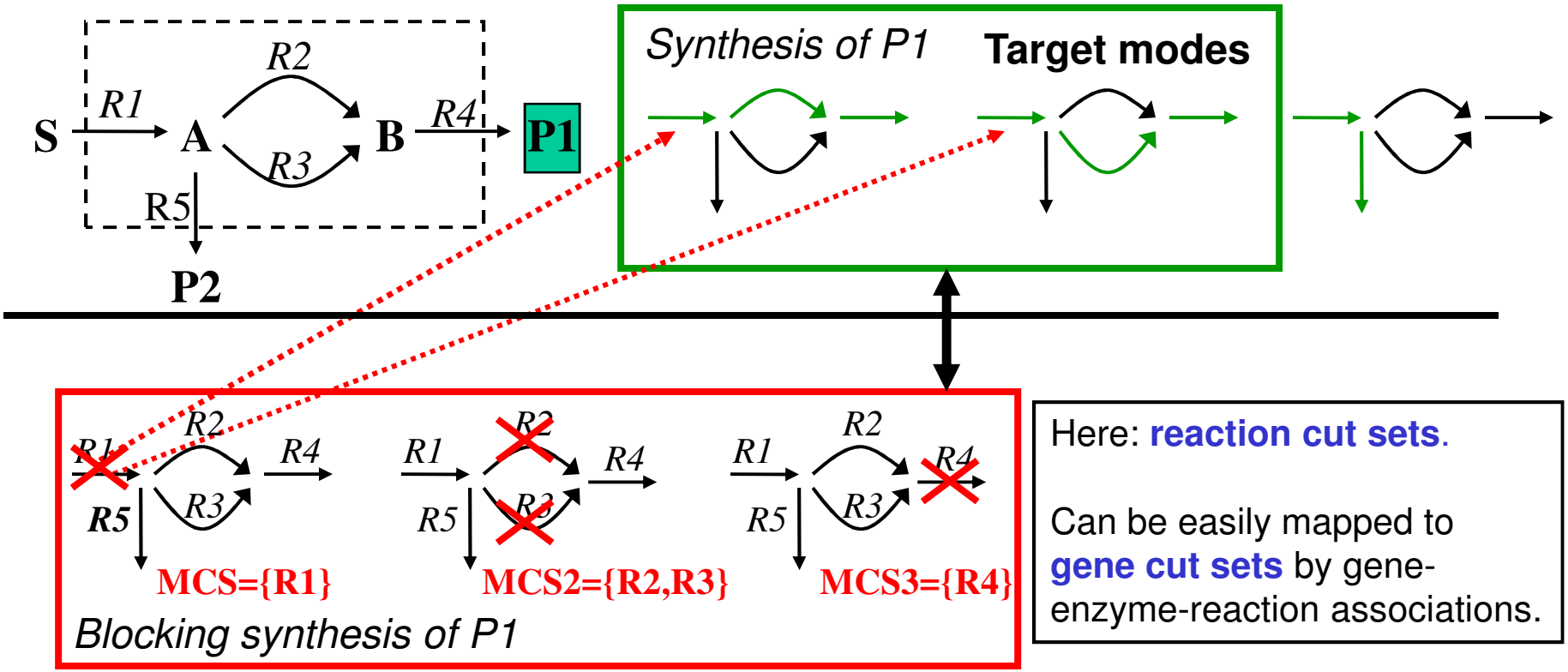
(Constrained) Minimal Cut Sets: enumeration of all minimal knockout sets that block *undesired* and keep *desired* behaviors.

→ based on elementary modes

→ all knockout solutions are generated

From Elementary Modes to Minimal Cut Sets

Elementary Modes: minimal functional units (pathways) that can operate in steady state



Here: **reaction cut sets**.

Can be easily mapped to **gene cut sets** by gene-enzyme-reaction associations.

Minimal Cut Set (MCS): minimal set of cuts (knockouts) blocking certain functions in steady state

***MCSs** are the minimal hitting sets of the corresponding EMs*

Klamt S: BioSystems 2006, 83:233-237.

From Elementary Modes to Minimal Cut Sets

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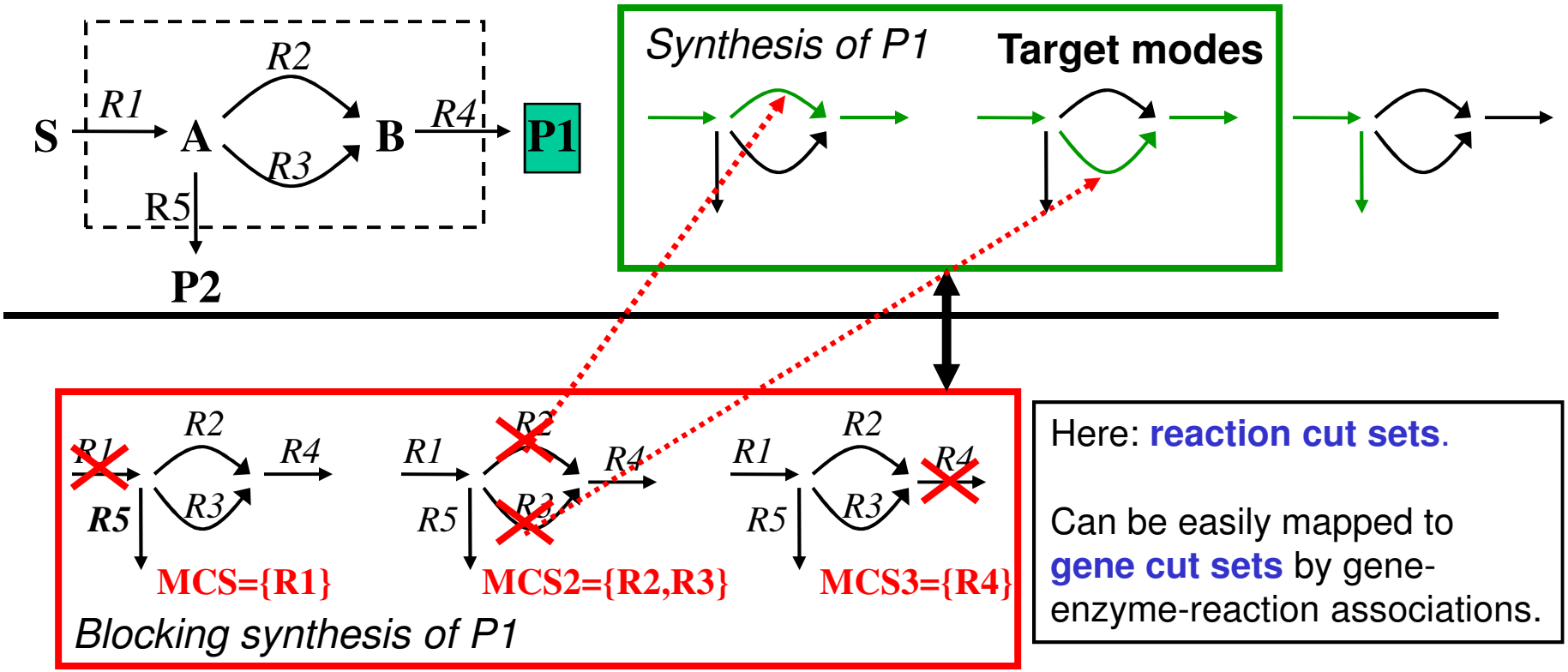

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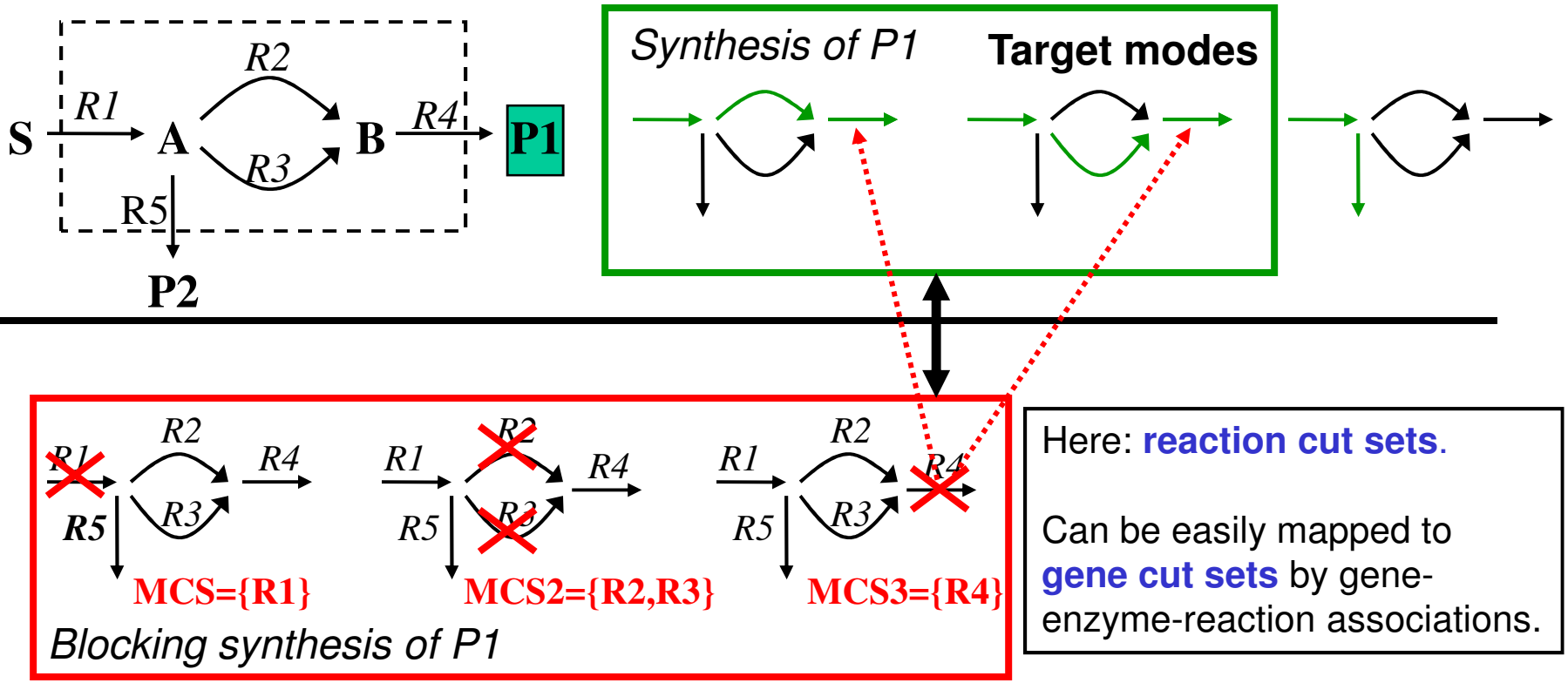
From Elementary Modes to Minimal Cut Sets

Elementary Modes (EM)*:* minimal functional units that can operate in steady state



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Elementary Modes, Minimal Cut Sets and Minimal Hitting Sets

Computation of Minimal Hitting Sets (here: for a given set of Target Modes):

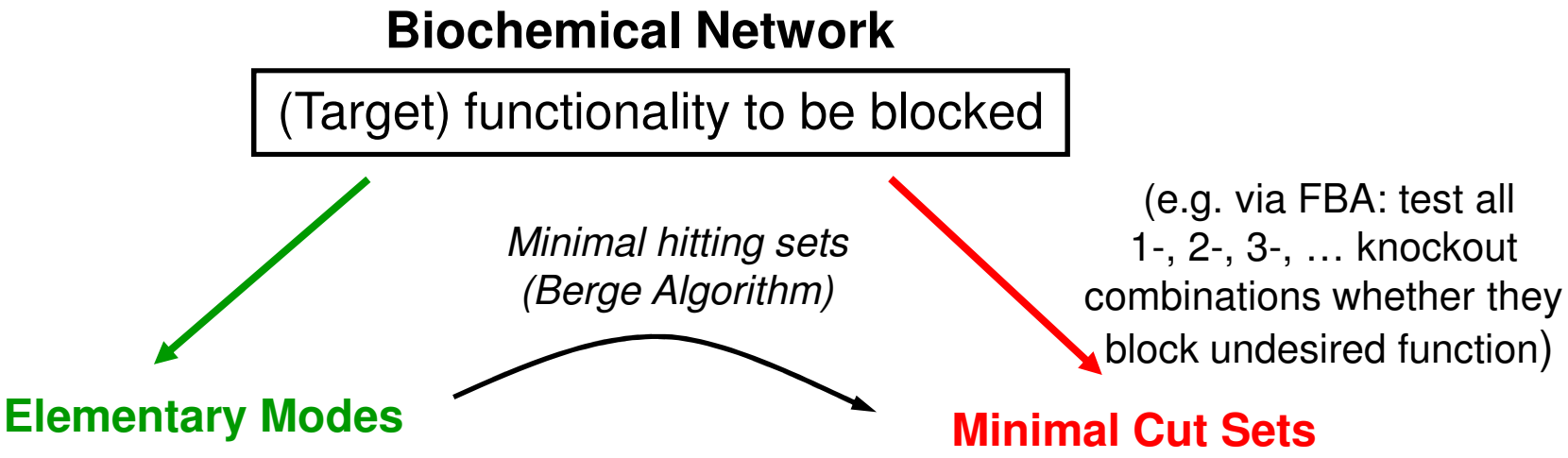
Many algorithms are known from hypergraph theory.

Algorithm of Claude Berge [1] performs well for metabolic networks [2].

[1] Claude Berge. (1989): Hypergraphs. Combinatorics of finite sets.(North-Holland, Amsterdam)

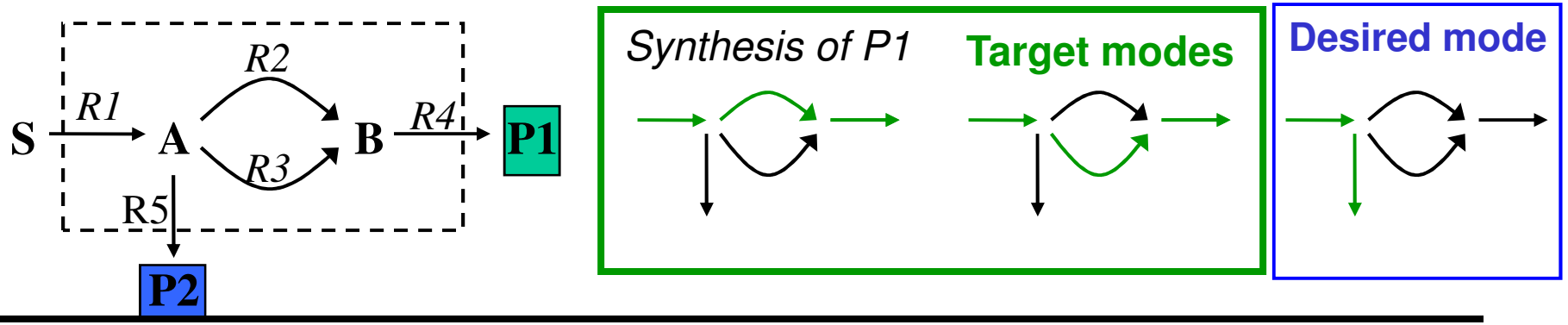
[2] Haus U, Klamt S, Stephen T. (2008) Computing knockout strategies in metabolic networks. J Comp Biol 15: 259-268.

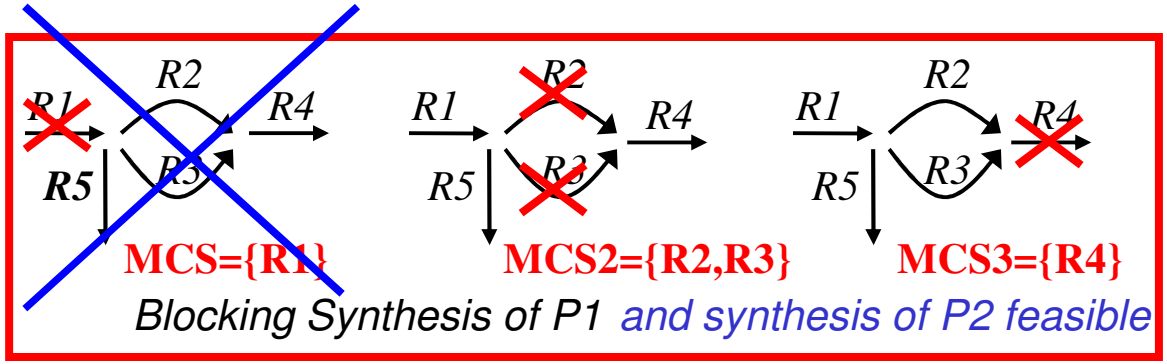
Elementary Modes, Minimal Cut Sets and Minimal Hitting Sets



(Constrained) Minimal Cut Sets for Rational Redesign of Metabolic Networks

Elementary Modes: minimal functional units (pathways) that can operate in steady state





Constrained MCSs:
- specify „desired modes“ (must not be hit by MCSs)

Constrained Minimal Cut Set (MCS): minimal cut sets blocking undesired while keeping desired metabolic behaviors.

Hädicke and Klamt (2011): Computing Complex Metabolic Intervention Stratgeies using Constrained Minimal Cut Sets. Metabolic Engineering, 13:204-213.

Generalization: Constrained MCSs

Constrained Minimal Cut Set (cMCSs) problem:

- set $\mathcal{T}$ of target EMs (to be blocked)
- set $\mathcal{D}$ of desired EMs
- n: minimal number of desired EMs in $\mathcal{D}$ that must not be hit by the MCSs

→ A constrained MCS **C** fulfills: $\mathbf{C} \cap \mathbf{T} \neq \emptyset \ \forall \mathbf{T} \in \mathcal{T}; \quad |\{\mathbf{D} \in \mathcal{D} \mid \mathbf{C} \cap \mathbf{D} = \emptyset\}| \geq n$

- algorithm for minimal hitting set calculation adapted for cMCSs:
  a) check on-the-fly whether MCS candidates keep desired modes (often faster)
  b) identify constrained MCSs from unconstrained MCSs during post-processing

- large variety of complex intervention problems can be conveniently formulated and solved by cMCSs

- many other methods (including OptKnock, Minimal Metabolic Functionality) can be reformulated as special cMCS problems

- all possible knockout strategies (also with higher cardinalities) can be found
  (... if the network is not too large)

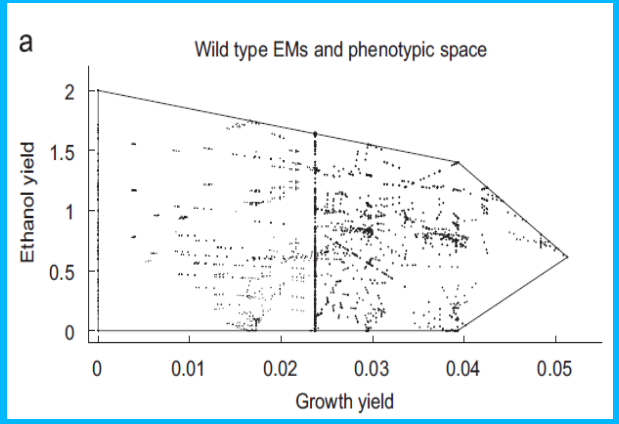
Hädicke and Klamt (2011): Computing Complex Metabolic Intervention Stratgeies using Constrained Minimal Cut Sets. Metabolic Engineering, 13:204-213.

Example: Coupled Biomass and Ethanol Synthesis in *E.coli*

Goal: search for interventions that lead to high (anaerobic) ethanol synthesis still enabling some formation of biomass

1) Compute elementary modes and inspect phenotypic space

(each dot = one mode)

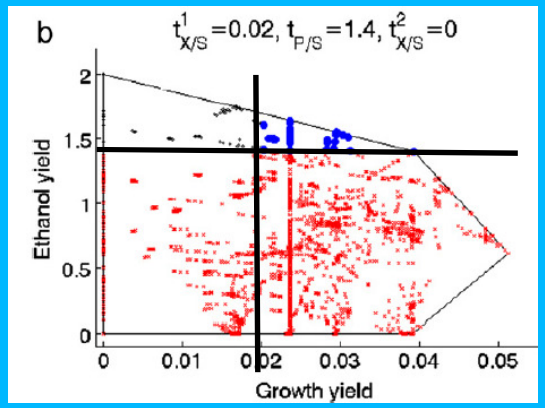


2) Specify target and desired modes

Red dots = target modes

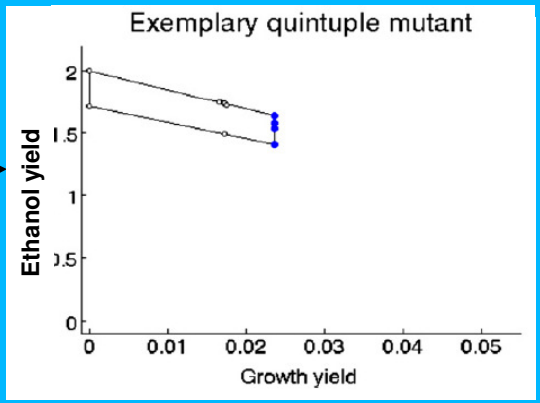
Blue dots = desired modes

Black dots = „don‘t care“



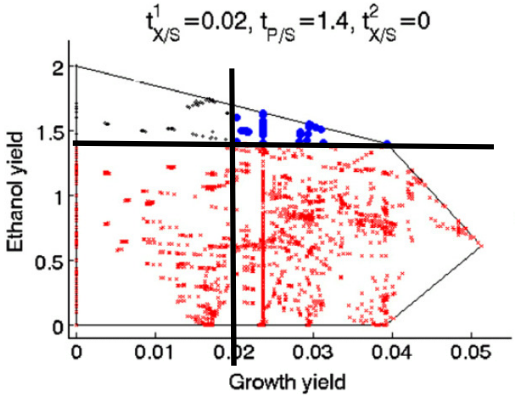
3) Compute constrained MCSs and implement the most practical

with n=1

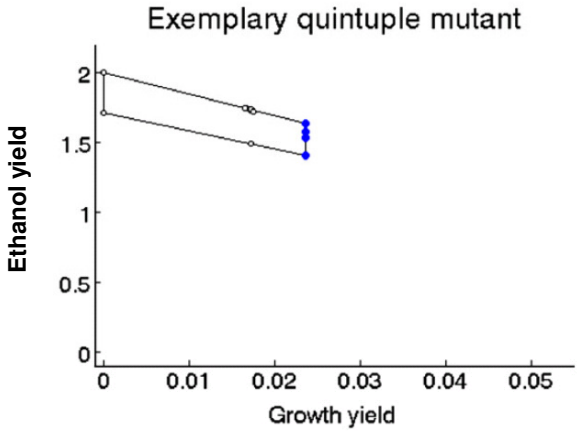


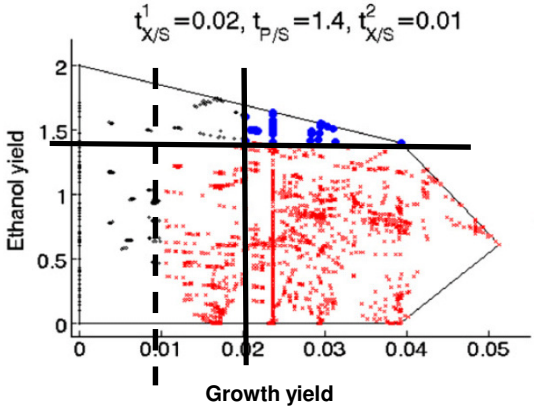
Example: Coupled Biomass and Ethanol Synthesis in *E.coli*

Testing different scenarios



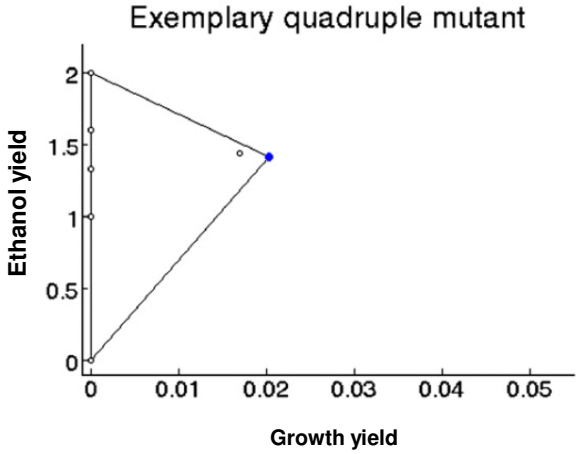
At least 5 knock-outs necessary





At least 4 knock-outs necessary

… smaller set of target modes
→ less knockouts required
→ but modes with low or no ethanol production exist (at low growth rates)



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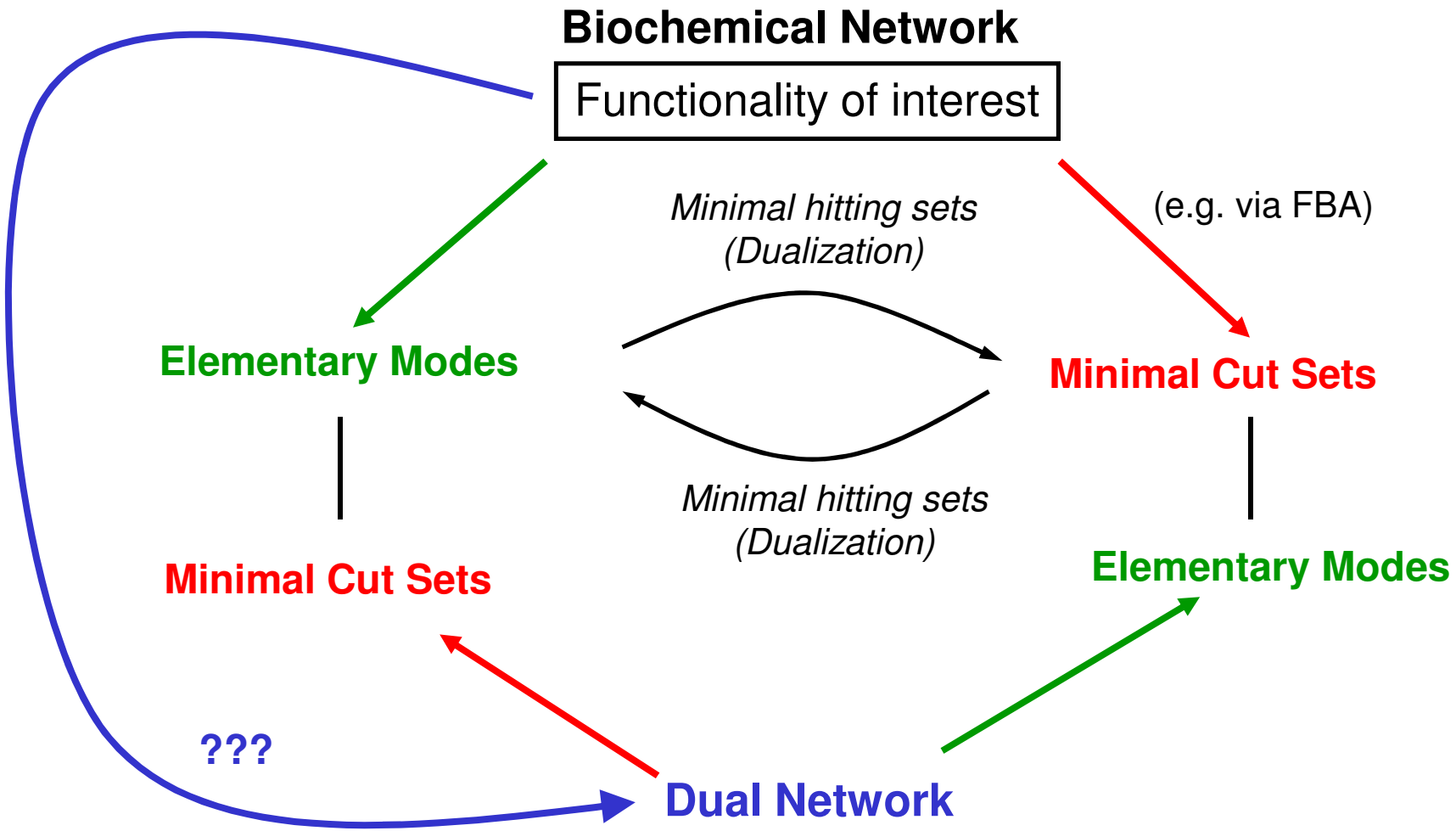
Duality: Elementary Modes and Minimal Cut Sets



How to Get the Dual Network

- specify the set $\boldsymbol{T}$ of target flux vectors (to be blocked by MCS) by a vector $\mathbf{t}$:

$$\boldsymbol{T} = \{\mathbf{r}:\ \mathbf{Nr} = \mathbf{0},\ \mathbf{r}_{Irrev} \geq \mathbf{0},\ \mathbf{t}^T\mathbf{r} > 0\ \}$$

(e.g. $\mathbf{t}^T$=(0,0,0,1,0) if the 4-th reaction produces undesired product)

Define Inconsistent System S

$$\mathbf{Nr} = \mathbf{0}$$

$$\mathbf{I}_{Irrev}\mathbf{r}_{Irrev} \geq \mathbf{0}$$

$$\mathbf{t}^T\mathbf{r} \geq 1$$

$$\mathbf{Ir} = \mathbf{0}$$

Farkas Lemma
Theory of IISs
(Ryan et al.)

Consider System D

$$(\mathbf{I}\ -\mathbf{t}\ -\mathbf{I}^T_{irrev}\ \mathbf{N}^T)\begin{pmatrix}\mathbf{v}\\ w\\ \mathbf{z}\\ \mathbf{u}\end{pmatrix} = \mathbf{0};\quad \mathbf{z} \geq \mathbf{0}; w \geq 0$$

$\mathbf{N}_{dual}$ $\mathbf{r}_{dual}$

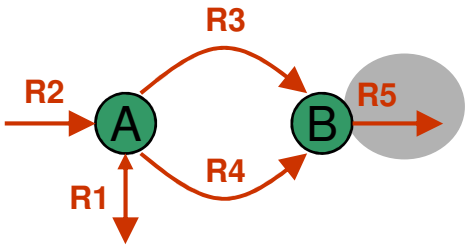
Minimal cut sets are the *irreducible inconsistent subsystems* (IISs) of **S** keeping the system inconsistent with a minimal subset of the equations **Ir**=**0**.

The IISs of **S** (corresponding to the MCSs) are the elementary modes of system D which are minimal in **v** and have support in *w*.

Ballerstein K, von KampA, Klamt S and Haus UU. (2012) Minimal cut sets in a metabolic network are elementary modes in a dual network. Bioinformatics 18: 381-387.

How to Get the Dual Network: Example

Primal Network



$$\mathbf{N} = \begin{array}{c} \begin{matrix} \mathbf{R1} & \mathbf{R2} & \mathbf{R3} & \mathbf{R4} & \mathbf{R5} \end{matrix} \\ \begin{pmatrix} -1 & 1 & -1 & -1 & 0 \\ 0 & 0 & 1 & 1 & -1 \end{pmatrix} \begin{matrix} \mathbf{A} \\ \mathbf{B} \end{matrix} \end{array}$$

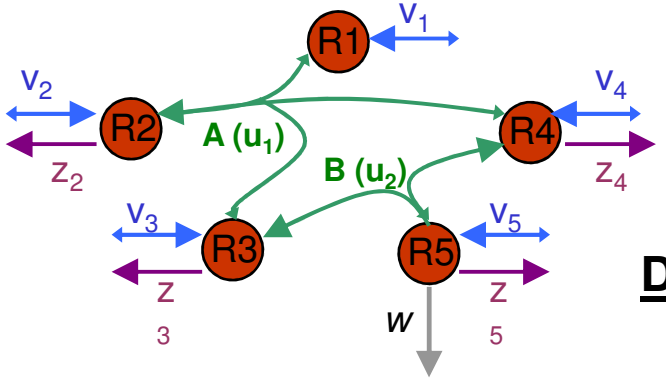
Reversible reaction: R1

Target reaction: R5

$\mathbf{t}^T\mathbf{r} = (0,0,0,0,1)\,\mathbf{r} > 0$

Target EMs (with R5):
- EM1={R1,R3,R5}
- EM2={R1,R4,R5}
- EM3={R2,R3,R5}
- EM4={R2,R4,R5}

MCSs for target reaction R5
- MCS1={R1,R2}
- MCS2={R3,R4}
- MCS3={R5}



Dual Network

$$\mathbf{N}_{dual} = (\mathbf{I}_{MCS} \; -\mathbf{t} \; -\mathbf{I}_{irrev} \; \mathbf{N}^T) =$$

	$\mathbf{I}_{MCS}$					$-\mathbf{t}$	$-\mathbf{I}^T_{irrev}$				$\mathbf{N}^T_A$		$\mathbf{N}^T_B$	
	v_1	v_2	v_3	v_4	v_5	w	z_2	z_3	z_4	z_5	u_1		u_2	
R1	1	0	0	0	0	0	0	0	0	0	−1		0	R1
R2	0	1	0	0	0	0	−1	0	0	0	1		0	R2
R3	0	0	1	0	0	0	0	−1	0	0	−1		1	R3
R4	0	0	0	1	0	0	0	0	−1	0	−1		1	R4
R5	0	0	0	0	1	−1	0	0	0	−1	0		−1	R5

Reversible reactions: $v_1 \ldots v_5$ and u_1, u_2 (former metabolites A and B in $\mathbf{N}^T$)

EMs with support in *w* and minimal support w.r.t reactions $v_1 \ldots v_5$:
- EM1={v_1,v_2,u_1,u_2,w}
- EM2={v_3,v_4,u_2,w}
- EM3={v_5,w}

MCSs hitting the three EMs above by cutting only $v_1,\ldots,v_5$:
- MCS1={v_1,v_3,v_5}
- MCS2={v_1,v_4,v_5}
- MCS3={v_2,v_3,v_5}
- MCS4={v_2,v_4,v_5}

*Ballerstein K, von Kamp A, Klamt S, Haus UU (2012): Minimal cut sets in a metabolic network are elementary modes in a dual network. Bioinformatics, **28**:381-387.*

Duality: Elementary Modes and Minimal Cut Sets

Implementation details:
- compress matrix **N** before dualization
- exploit that "**z**" reactions are parallel to "**v**" reactions
- the **u** reactions does not need to be processed
  (only interested in minimality of **v** reactions)

First results (α version):
- computation time similar to EM + Minimal Hitting Set calculation
  - but: still room for improvements

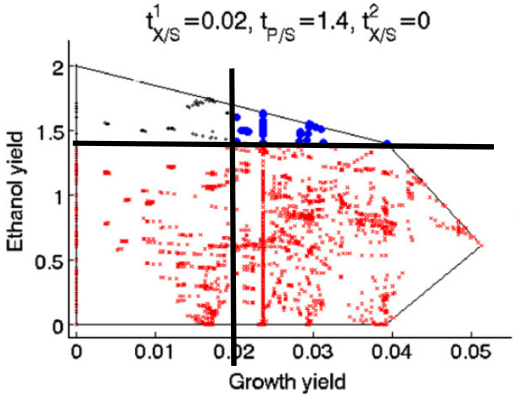
Generalization:
- incorporation of inhomogeneous constraints straightforward …

*Ballerstein K, von Kamp A, Klamt S, Haus UU (2012): Minimal cut sets in a metabolic network are elementary modes in a dual network. Bioinformatics, **28**:381-387.*

Coupled Biomass and Ethanol Synthesis in *E.coli* with Inhomogeneous Constraints

Homogeneous system:

Nr = 0; $r_{Irrev} \geq 0$



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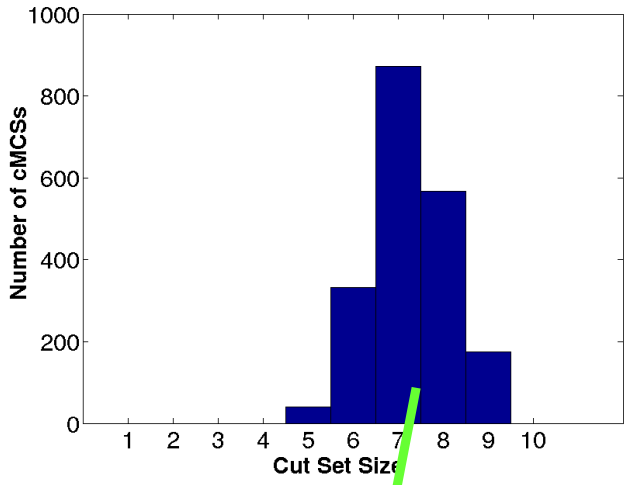
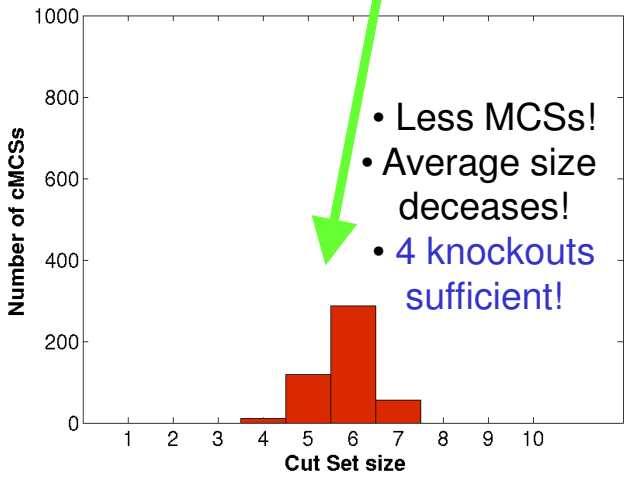


Inhomogeneous system (considering ATP maintenance)

Nr = 0;
$r_{Irrev} \geq 0$
$0 \leq r_{Gluc\_up} \leq 10$ mmol/(gDW*h);
$r_{ATPmaintenance} \geq 8.4$ mmol/(gDW*h)

(+ same thresholds for desired/target modes as above)



Computation of EMs / MCSs in Genome-Scale Networks ?!

- EM / MCSs calculation only possible in networks with moderate size
  - → useful for models of central metabolism
  - → for many products: suitable interventions lie often in central metabolism

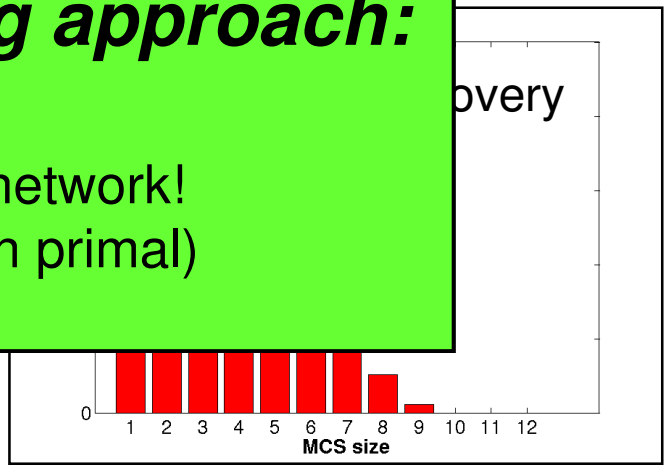
- possible solution for large-scale applications: EM / MCS sampling

  - → approximate combinatorial properties of EMs by computing a re… …leta)
  - → co…

First test … …overy
sufficient t…
smaller siz…
h…

Alternative sampling approach:

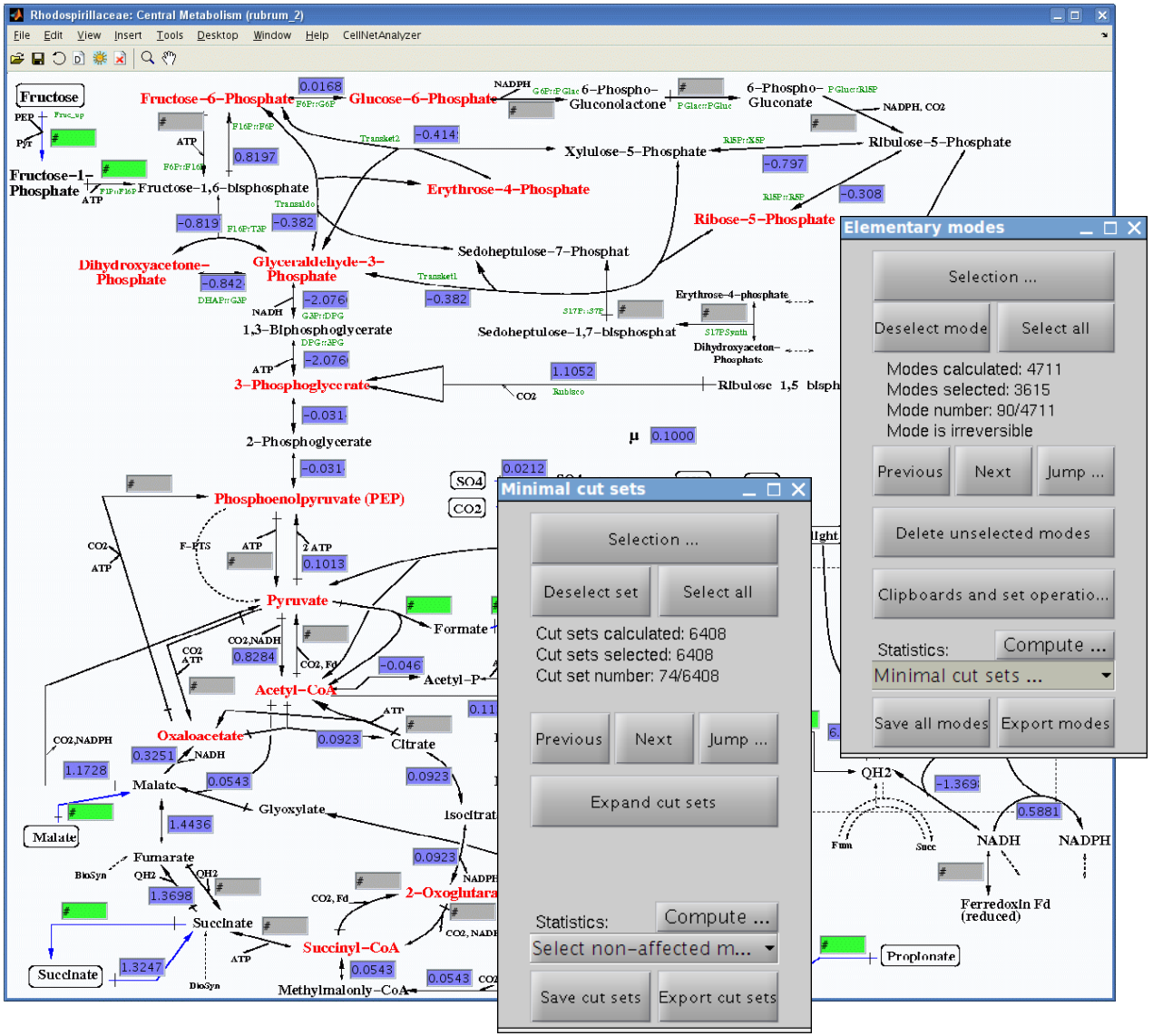
Sample EMs in dual network!
(= sample of MCSs in primal)



CellNetAnalyzer

Large variety of methods for computing and analysing EMs and (c)MCSs.

Experimental work based on cMCSs engineering strategies underway.



Conclusions

- **Constrained Minimal Cut Sets**: *blocking undesired* while *keeping desired* metabolic behaviors.
  → very flexible and convenient approach for enumerating intervention strategies for metabolic engineering.

- **Duality** between EMs and MCSs offers new *computational* and *conceptual* perspectives for studying functions and (re)design strategies in metabolic networks.

- **Inhomogeneous constraints** can be considered.

- **Sampling approaches** for large-scale networks.

- Algorithms for (c)MCSs computation and analysis implemented in ***CellNetAnalyzer***.

- Similar approach for regulatory/signaling networks: **Minimal Intervention Sets**.

Samaga R, von Kamp A and Klamt S (2010) Computing combinatorial intervention strategies and failure modes in signaling networks. *Journal of Computational Biology* **17**:39-53.

Acknowledgements

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Magdeburg Centre for
Systems Biology (by BMBF)

CellNetAnalyzer

http://www.mpi-magdeburg.mpg.de/projects/cna/cna.html


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THANK YOU!


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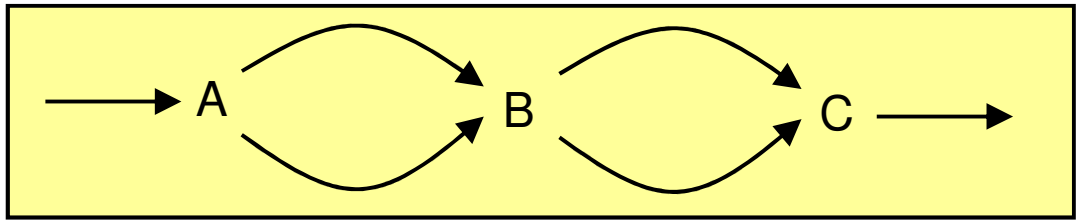
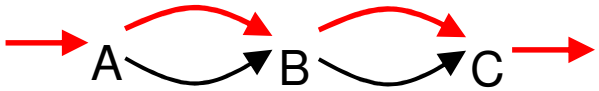
Collection

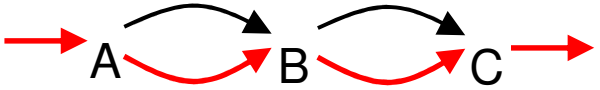
Elementary-Modes Sampling for Computing MCS in Large Networks



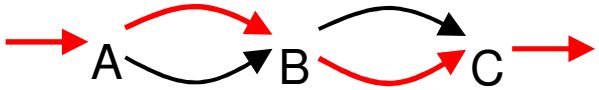
EM1



EM2



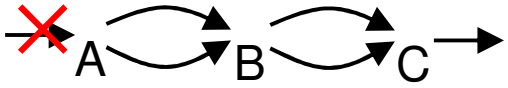
EM3

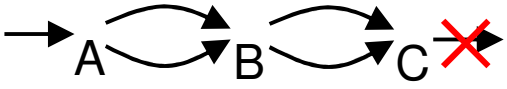


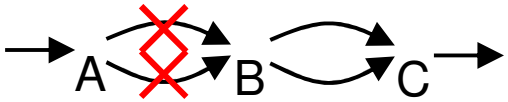
EM4

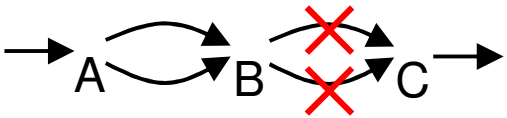
MCSs for intervention goal; block synthesis of C (via minimal hitting sets)!

4 MCSs

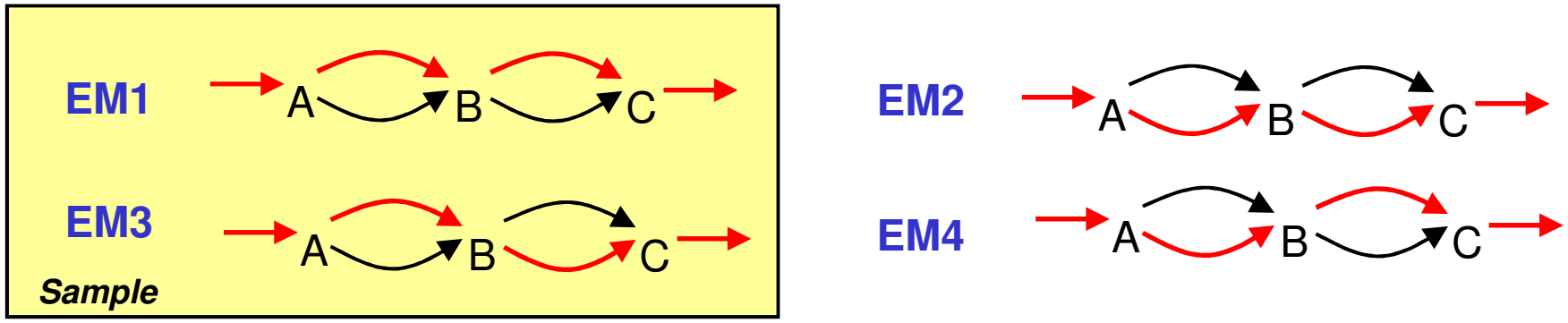




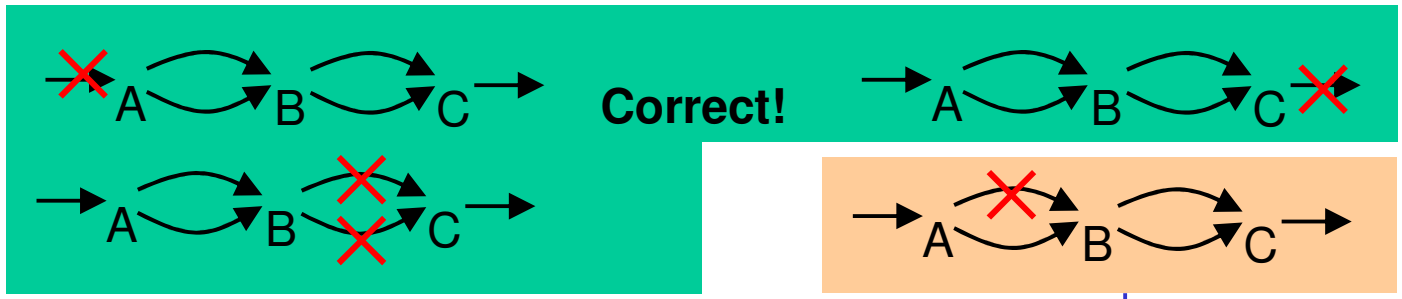




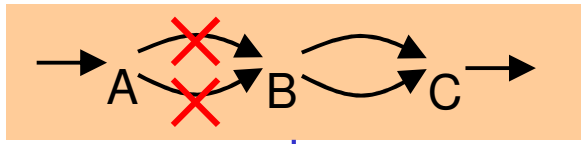
Elementary-Modes Sampling for Computing MCS in Large Networks



MCSs for Sample



Not a correct (full) MCSs!
… but can easily be excluded by FBA in postprocessing!



Missed MCSs!
Cannot be found by this sample!

Example for EM / MCS Sampling: Compute MCSs that Block Growth in *E. coli*

Via full enumeration of EMs

1) Compute all EMs (21592 EMs)

↓

2) Compute MCSs (via hitting set algorithm)

Via a sample of EMs (10%)

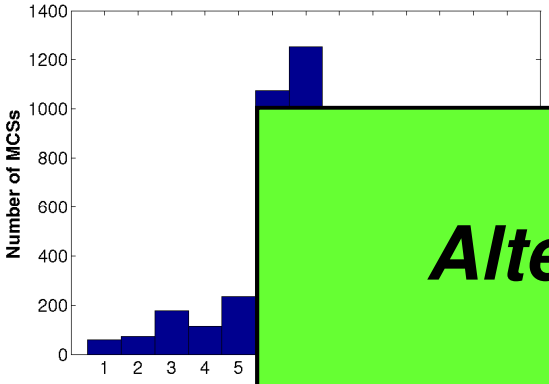
1) Sample EMs (2160 EMs = 10%)

↓ (e.g. EFMEnumerator; Kaleta et al.)

2) Compute MCSs (via hitting set algorithm)

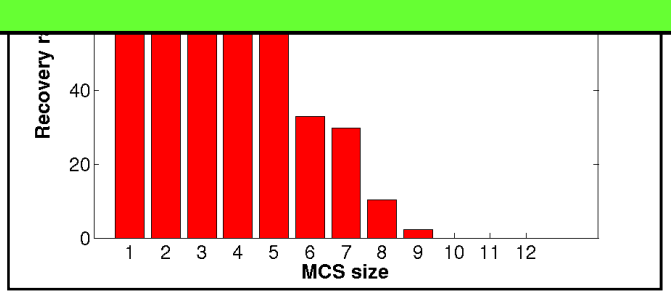
↓

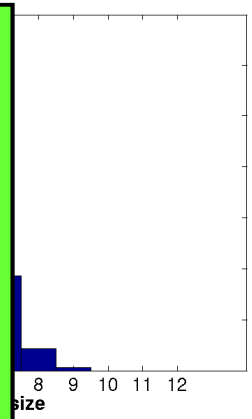
3) Postprocessing: discard false MCSs via FBA



Alternative approach:

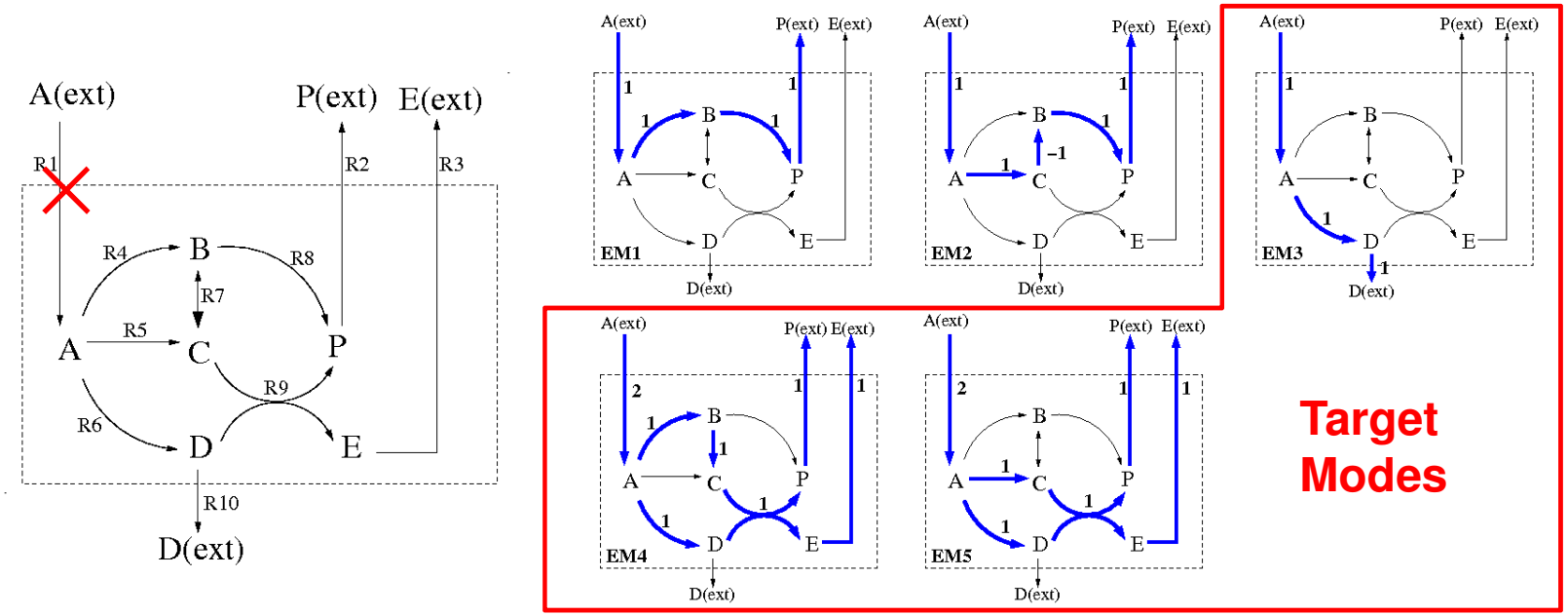
Sample EMs in dual network!
(= sample of MCSs in primal)





Constrained Minimal Cut Sets: Limitation of "Simple" Minimal Cut Sets

Intervention goal: repress non-optimal production routes for P



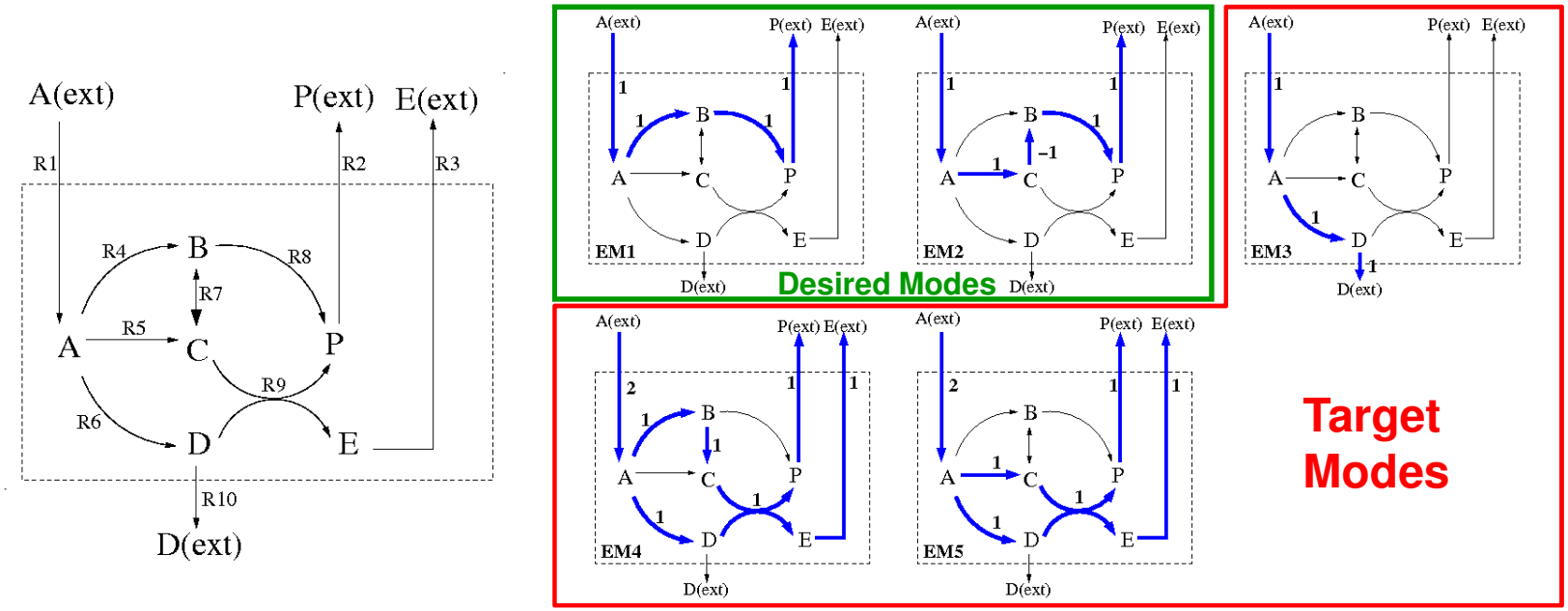
MCS1={R6}, MCS2={R1}, MCS3={R2, R10}, MCS4={R9,R10}, MCS5={R3,R10}, MCS6={R4,R5,R10}, MCS7={R5,R7,R10}

→ **Some MCSs (e.g. {R1}) induce side effects and disable functions we want to keep!**

→ **Constrained MCSs (cMCSs):** define set **T** of target modes *and* set **D** of desired modes and specify minimum number of modes in **D** to be preserved.

Generalization: Constrained MCSs

Intervention goal: repress non-optimal production routes for P



Target modes: T={EM3,EM4,EM5} (all non-optimal pathways for synthesis of P)

Desired modes: D={EM1,EM2} (all optimal pathways for synthesis of P}

Minimum number of desired modes to be saved: *n*=1

→ **MCS1={R6}, MCS2={R9,R10}, MCS3={R3,R10}, MCS4{R5,R7,R10}**

Minimum number of desired modes to be saved: *n*=2

→ **MCS1={R6}, MCS2={R9,R10}, MCS3={R3,R10}**

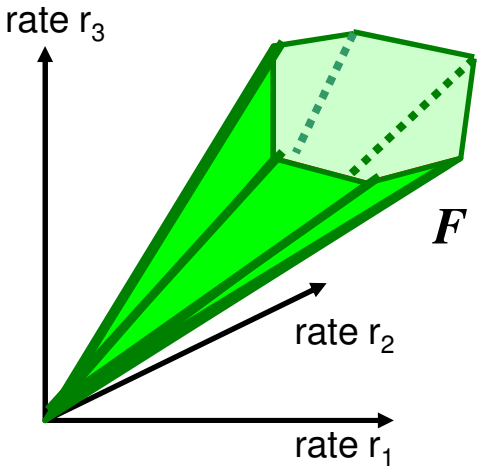
Computation of EMs / MCSs in Genome-Scale Networks ?!

- EM / MCSs calculation only possible in networks with moderate size

  → typically used in models of central metabolism
  → for many products: suitable interventions lie often in central metabolism (as also suggested/found by genome-scale methods)

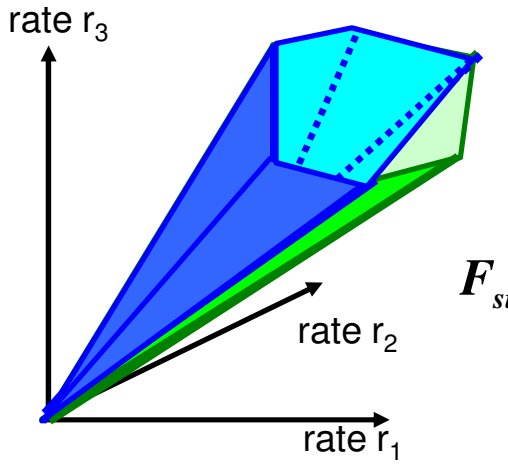
- possible solution for other applications: EM / MCS sampling …

Flux cone *F* spanned by set *E* of (all) EMs:



$$\boldsymbol{F} = \sum_{e_i \in \boldsymbol{E}} \alpha_i \mathbf{e}_i$$

Sub-cone spanned by (sampled) subset *S* of EMs:



$$\boldsymbol{S} \subset \boldsymbol{E}$$

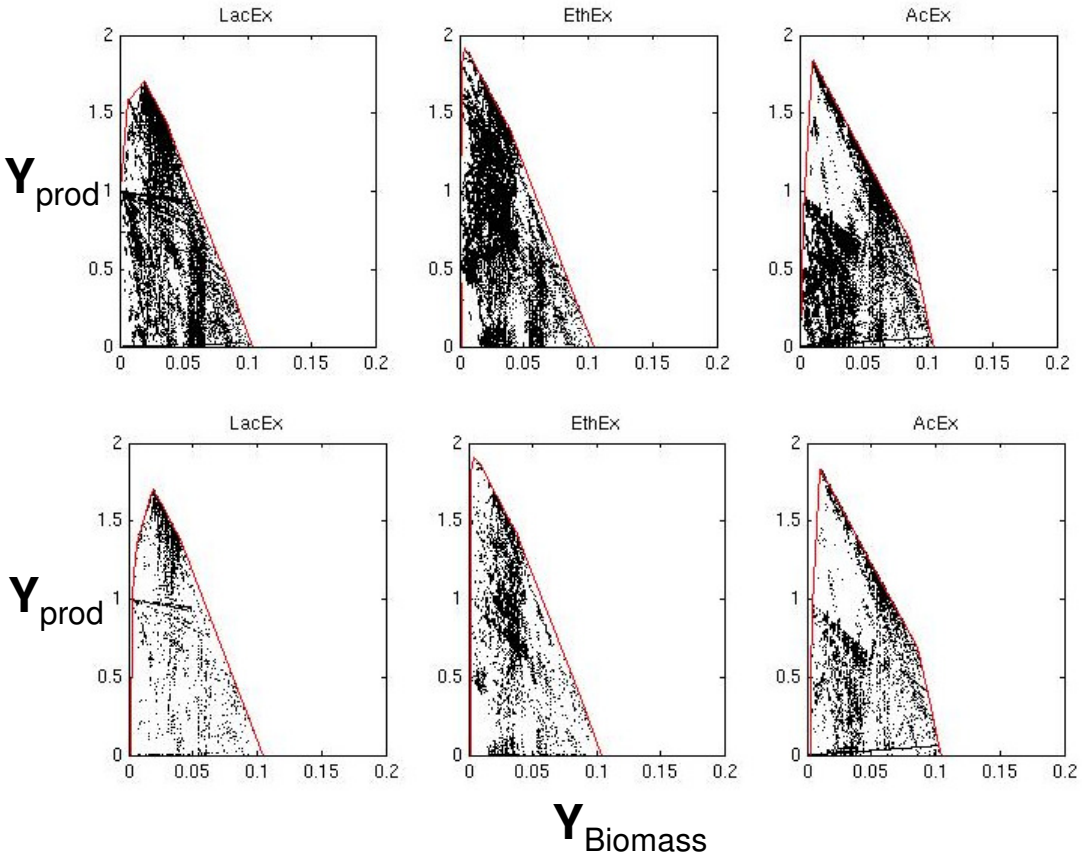
$$\boldsymbol{F}_{sub} = \sum_{e_i \in \boldsymbol{S}} \alpha_i \mathbf{e}_i$$

Elementary-Modes Sampling for MCSs Calculation

Example: 2D-yield space of EMs for central metabolism of E.coli
(with **EFMEvolver**; Kaleta et al.)

Full enumeration

Sampling (20%)

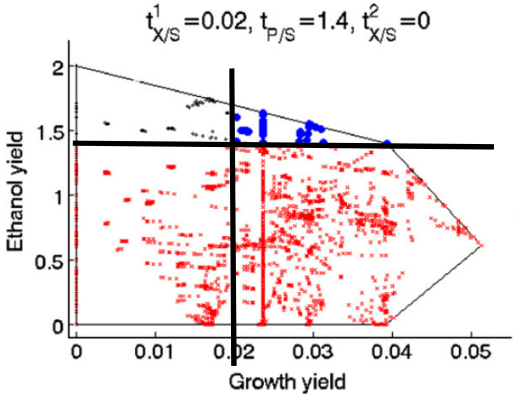


→ CASOP for genome-scale networks (**CASOP-GS**) underway (Kaleta group)

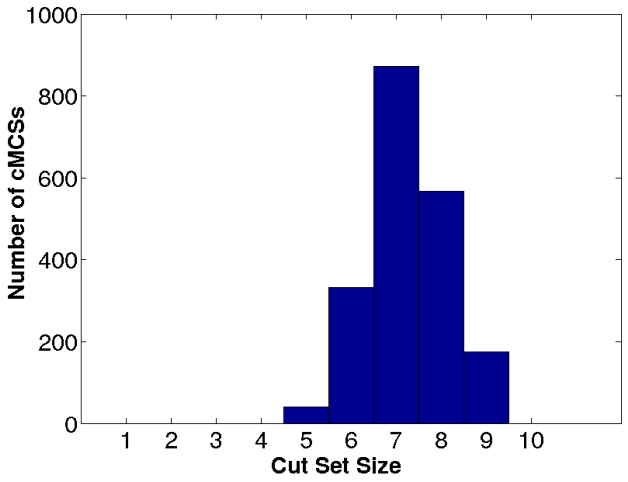
Inhomogenous Constraints: Coupled Biomass and Ethanol Synthesis in *E.coli*

Homogeneous system:

Nr = 0; $r_{Irrev} \geq 0$



At least 5 knockouts necessary



Inhomogeneous sys… nce)

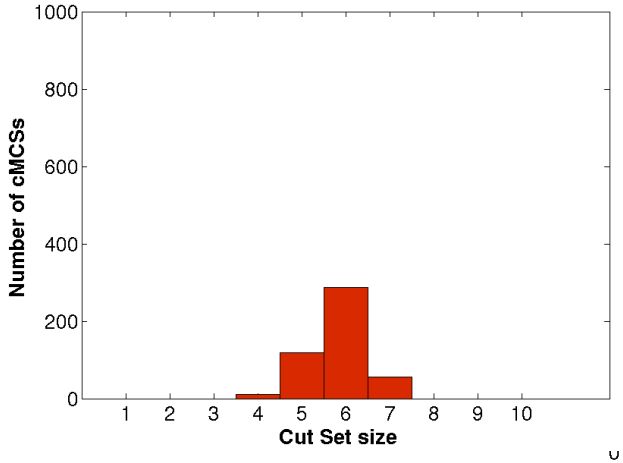
Shift!

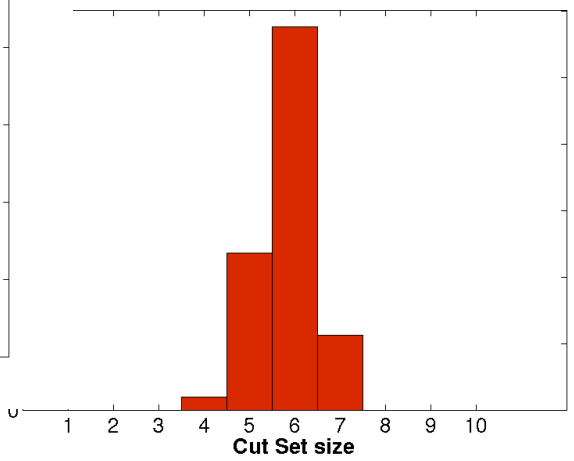
Nr = 0;

$r_{Irrev} \geq 0$

$0 \leq r_{Gluc\_up} \leq 10$ mmol/(g

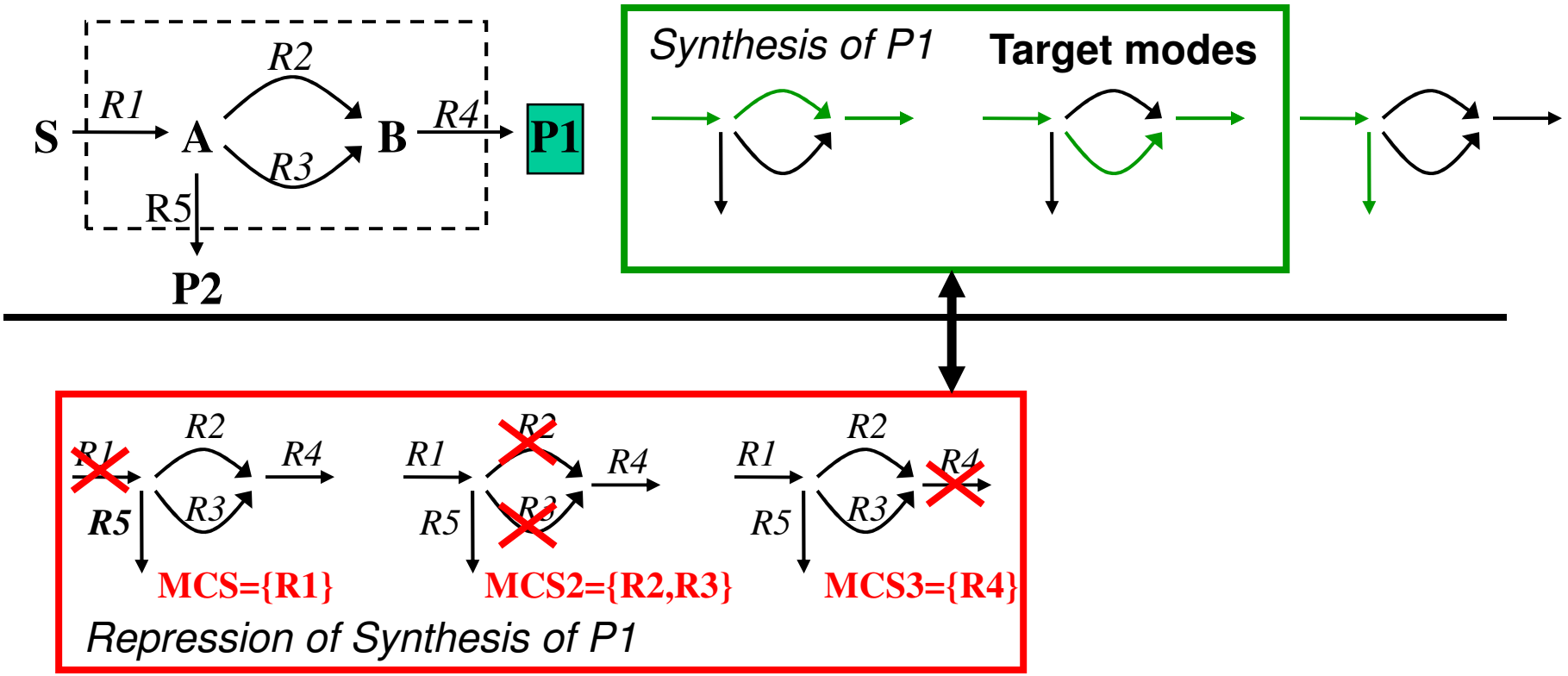
$r_{ATPmaintenance} \geq 8.4$ mmo





From Elementary Modes to Minimal Cut Sets

Elementary Modes: minimal functional units (pathways) that can operate in steady state



Minimal Cut Set (MCS): minimal set of cuts (knockouts) blocking a certain network function in steady state

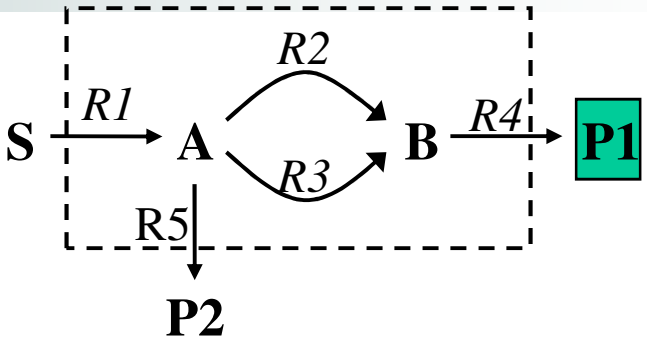
***MCSs** are the minimal hitting sets of the corresponding target EMs*

Klamt S: BioSystems 2006, 83:233-237.

Elementary Modes, Minimal Cut Sets and Minimal Hitting Sets

For now we identify an EM **e** (a vector) by its support **E** (which is a set):

E := supp(**e**) = {i: $e_i \neq 0$}.

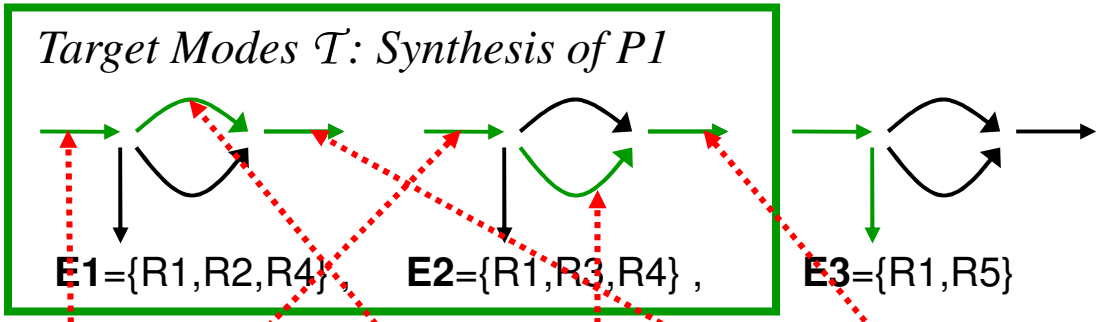


Set $\mathcal{T}$ of target modes:

$\mathcal{T}$ = {**E1**,**E2**}

= {{R1,R2,R4} , {R1,R3,R4}}

→ $\mathcal{T}$ is a family of (reaction) subsets (= undirected hypergraph)

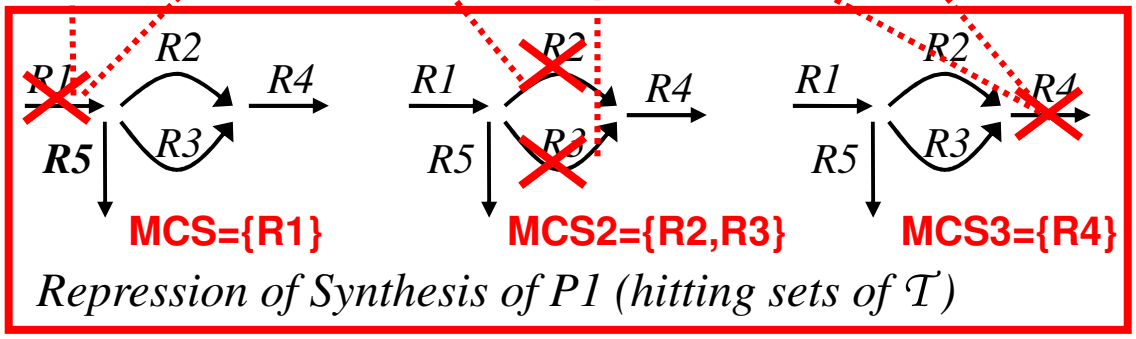


A minimal hitting set **C** of $\mathcal{T}$ hits all subsets (EMs) of $\mathcal{T}$, i.e.

$\mathbf{C} \cap \mathbf{E} \neq \emptyset$, $\forall\ \mathbf{E} \in \mathcal{T}$

and no subset of **C** does so.

A minimal hitting set hits all target EMs and knocking it out thus yields an MCS blocking all target EMs.



Klamt S: BioSystems 2006, 83:233-237.

Generalization: Constrained MCSs

Constrained Minimal Cut Set (cMCSs) problem:

- set $\mathcal{T}$ of target EMs (to be blocked)
- set $\mathcal{D}$ of desired EMs
- n: minimal number of desired EMs in $\mathcal{D}$ that must not be hit by the MCSs

→ A constrained MCS **C** fulfills: $\mathbf{C} \cap \mathbf{T} \neq \emptyset \; \forall \mathbf{T} \in \mathcal{T}; \quad |\{\mathbf{D} \in \mathcal{D} \mid \mathbf{C} \cap \mathbf{D} = \emptyset\}| \geq n$

Generalized Version:

→ set $\mathcal{T}$ of target EMs (to be blocked)
→ sets $\mathcal{D}_1 \ldots \mathcal{D}_k$ of desired EMs
→ $n_1 \ldots n_k$: minimal number of desired EMs in $\mathcal{D}_1 \ldots \mathcal{D}_k$ that must not be hit by the MCSs

→ A constrained MCS **C** fulfills: $\mathbf{C} \cap \mathbf{T} \neq \emptyset \; \forall \mathbf{T} \in \mathcal{T}; \quad |\{\mathbf{D} \in \mathcal{D}_i : \mathbf{C} \cap \mathbf{D} = \emptyset\}| \geq n_i \;\; \forall i$

Hädicke and Klamt (2011): Computing Complex Metabolic Intervention Stratgeies using Constrained Minimal Cut Sets. Metabolic Engineering, 13:204-213.

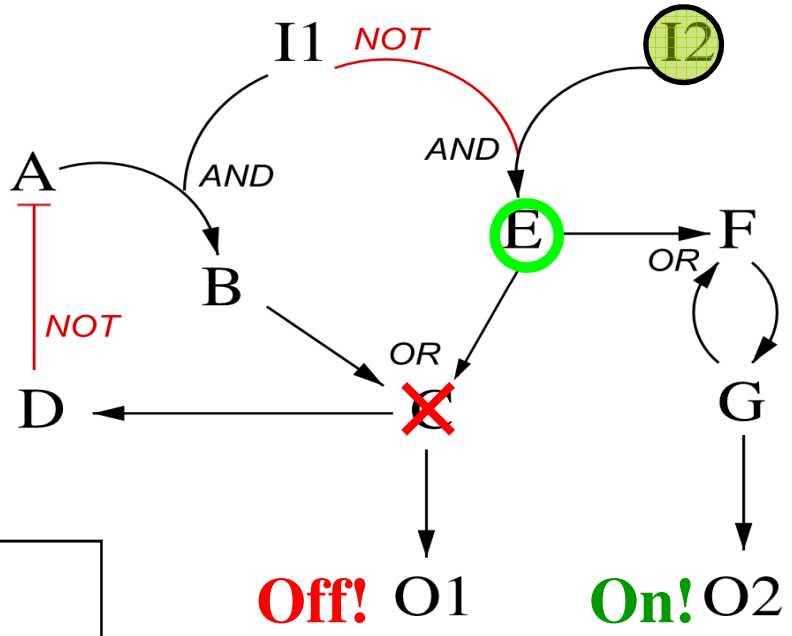
Counterpart of MCSs in Regulatory / Signaling Networks: Minimal Intervention Sets

Minimal intervention set (MIS): minimal combination of knockouts (off) and constitutive activations (on) that induce a desired response (defined by intervention goal)

Intervention Goal:
Inactivation of O1 (0)
Activation of O2 (1)
Side Constraint:
I2 is active (1)

MIS1={**I1=off, C=off**}
MIS2={**C=off**, **E=on**}

Example



Applications:
- target identification
- fragile points in the network
- diagnosis: what causes an observed effect

Samaga R, von Kamp A and Klamt S (2010) Computing combinatorial intervention strategies and failure modes in signaling networks. *Journal of Computational Biology* **17**:39-53.

CASOP:

Computational Approach for Strain Optimization Aiming at High Productivity

CASOP: Computational Approach for Strain Optimization Aiming at High Productivity

Motivation:

- existing approaches consider only knockouts, not overexpression candidates
- existing approaches for metabolic engineering aim at optimizing **product yield**:

$$Y^{P/S} \; [mol\ Product / mol\ Substrate]$$

- However, in practice, the (specific) **productivity** r_P is often the relevant performance parameter to be optimized:

$$\text{Specific Productivity}: \quad r_P = Y^{P/S} \cdot r_S \quad [mol\ Product\ /\ (gDW \cdot h)]$$

$$r_S: \text{ Substrate uptake rate } [mmol\ Substrate\ /\ (gDW \cdot h)]$$

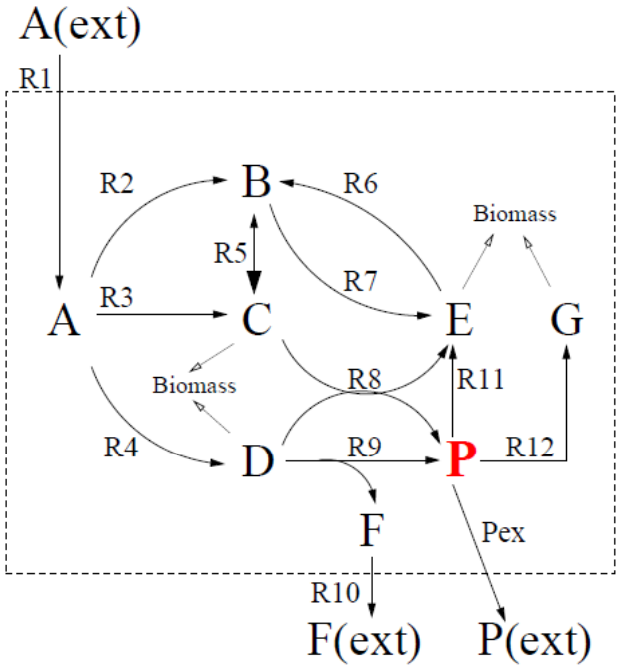
Optimizing productivity: search for optimal trade-off between high yield and high capacity (low-yield pathways may have a high capacity!)

→ CASOP: *stoichiometric* approach based on elementary-modes analysis suggesting knockout + overexpression candidates for optmizing productivity

CASOP: Computational Approach for Strain Optimization Aiming at High Productivity

Example network

P is the product of interest



R1 : Aext => A
R2 : A => B
R3 : A => C
R4 : A => D
R5 : B <=> C
R6 : E => B
R7 : B => E
R8 : C + D => E + P
R9 : 3 D => 2 F + P
R10: F => F(ext)
R11: P => E
R12: P => G
Pex: P => P(ext)
μ : 4 C + D + 4 E + G => Biomass

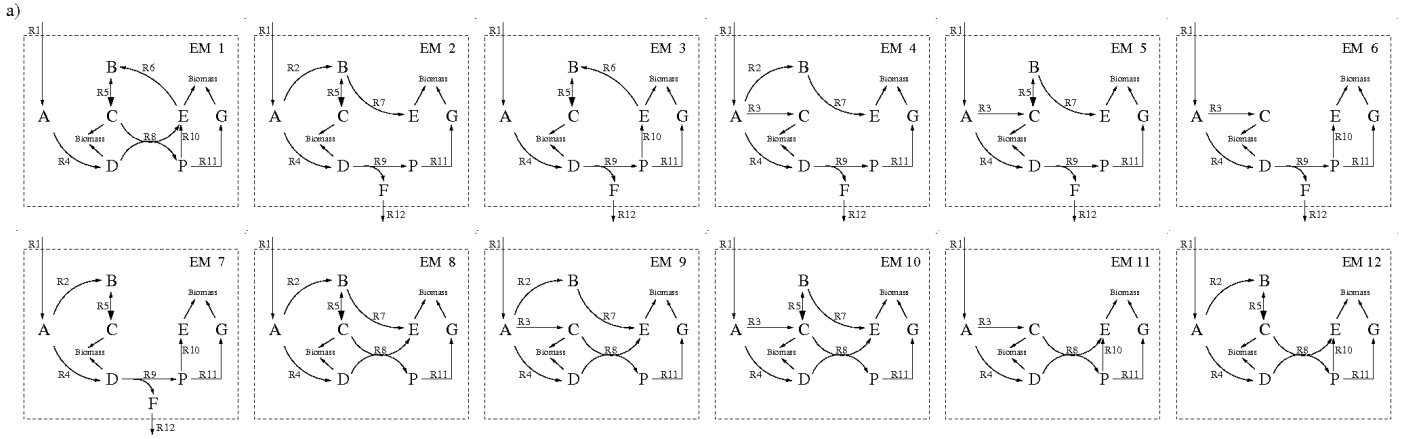
First step: add a pseudo-reaction *Rx* „consuming“ *product* P(ext) and *biomass:*

$$Rx : (1-\gamma)\, Biomass + \gamma\, \alpha_P P(ext) \rightarrow X \quad , \quad \gamma \in [0,1]$$ („proportion factor“)

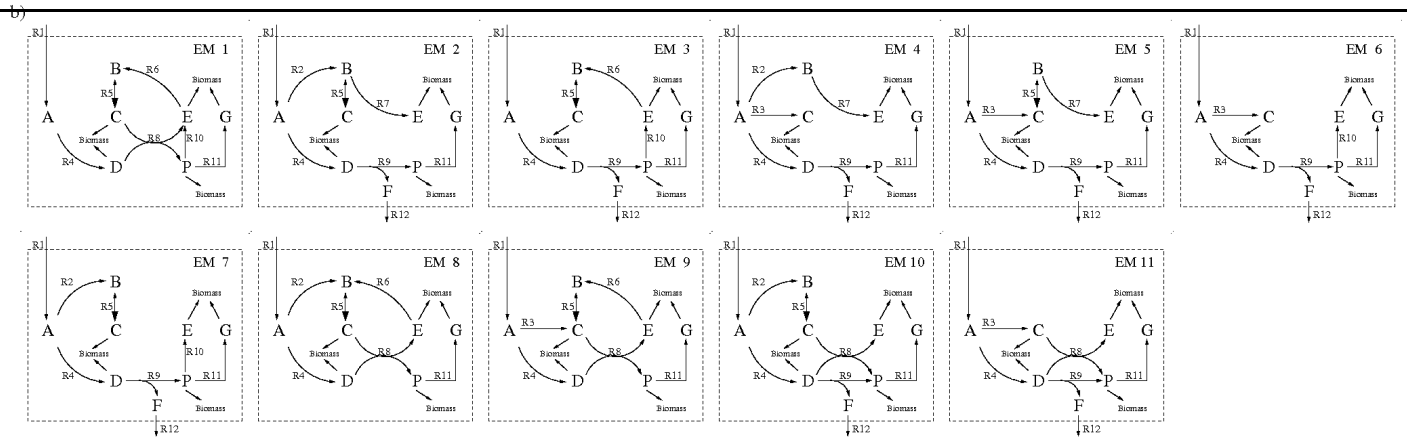
Increasing γ simulates the change in stoichiometric precursor demand from pure biomass production (γ = 0) via combined production of biomass and product (e.g. γ = 0.5) to exclusive production of P (γ = 1).

Compute Elementary Modes (EMs) for Different γ

γ = 0



γ = 0.9 (biomass synthesis still possible)



→ different spectra of EMs (pathways) for different **γ**
→ analyze these differences for each reaction …

Elementary-Modes Weights, Reaction Importances and Reaction Rankings

EM weight for EM *i* in scenario γ:

$$\nu_{i,\gamma} = \frac{(Y_i^{X/S})^k}{\sum_{i=1}^{n(\gamma)} (Y_i^{X/S})^k}$$

- relates the yield of an EM to sum of yields of all EMs
- adjustable parameter *k*: large *k* weight yield-optimal and EMs stronger; *k*=0→ each EM has same weight)
- sum of all weights is 1

Reaction importance measure for reaction *j* in a given scenario γ:

$$\omega_\gamma(r_j) = \sum_{i \in EM(r_j)} \nu_{i,\gamma}$$

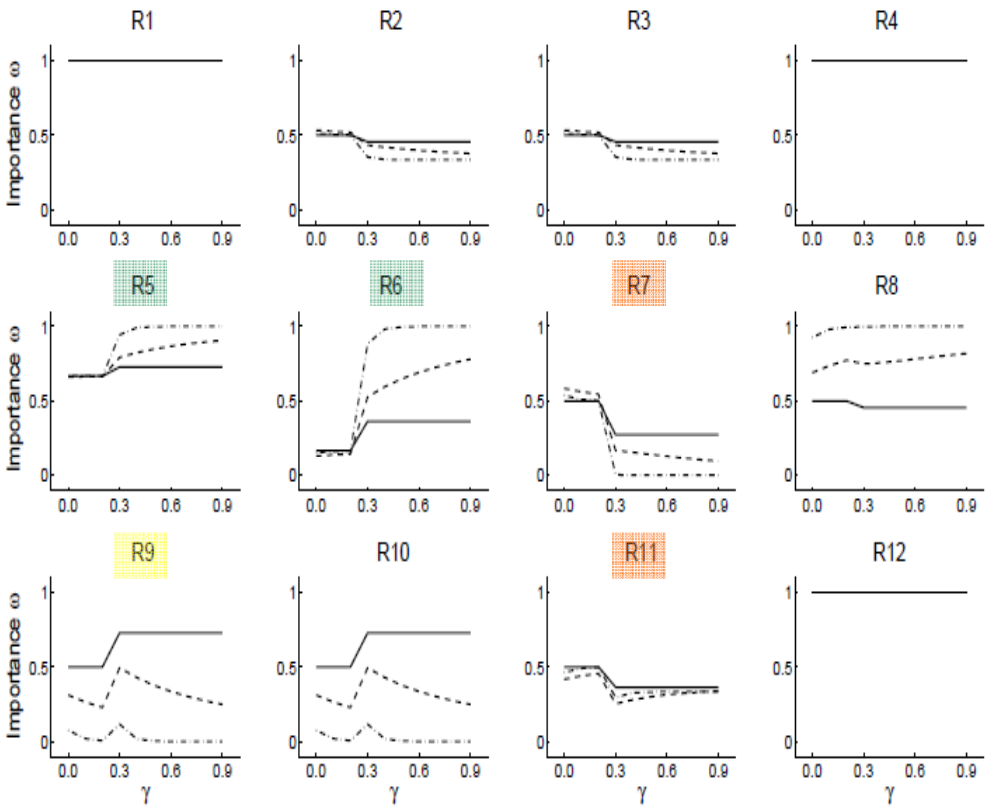
- sum of all EM weights in which reaction *j* partcipates
  → considers importance of *j* with respect to yield (weight) + flexibility/capacity
- in the range of [0,1]; e.g. 1 for essential reactions

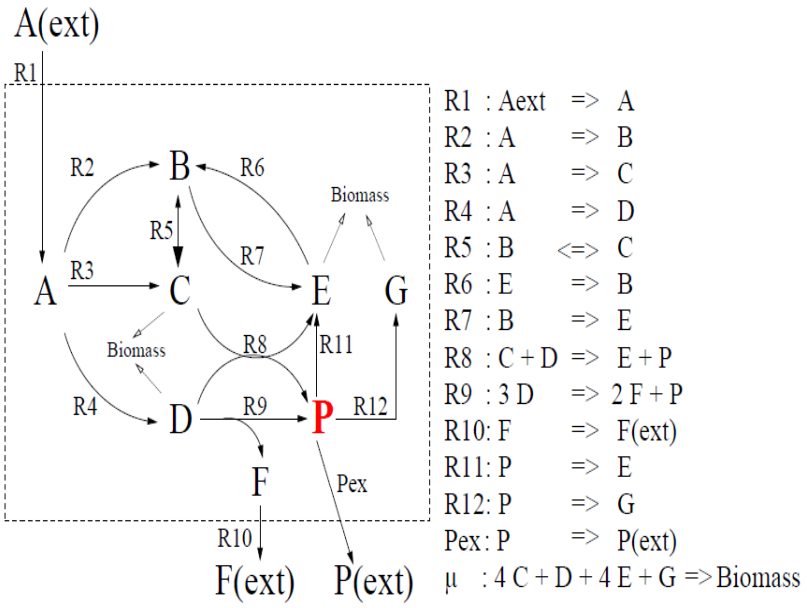
Rank reactions based on the importances:

Relative to wild type: $Z(r_j) = \omega_{0.9}(r_j) - \omega_0(r_j)$ $\quad Z \in [-1,1]$

high Z-score: overexpression candidate
low Z-score: knockout (KO) candidate

Example Network





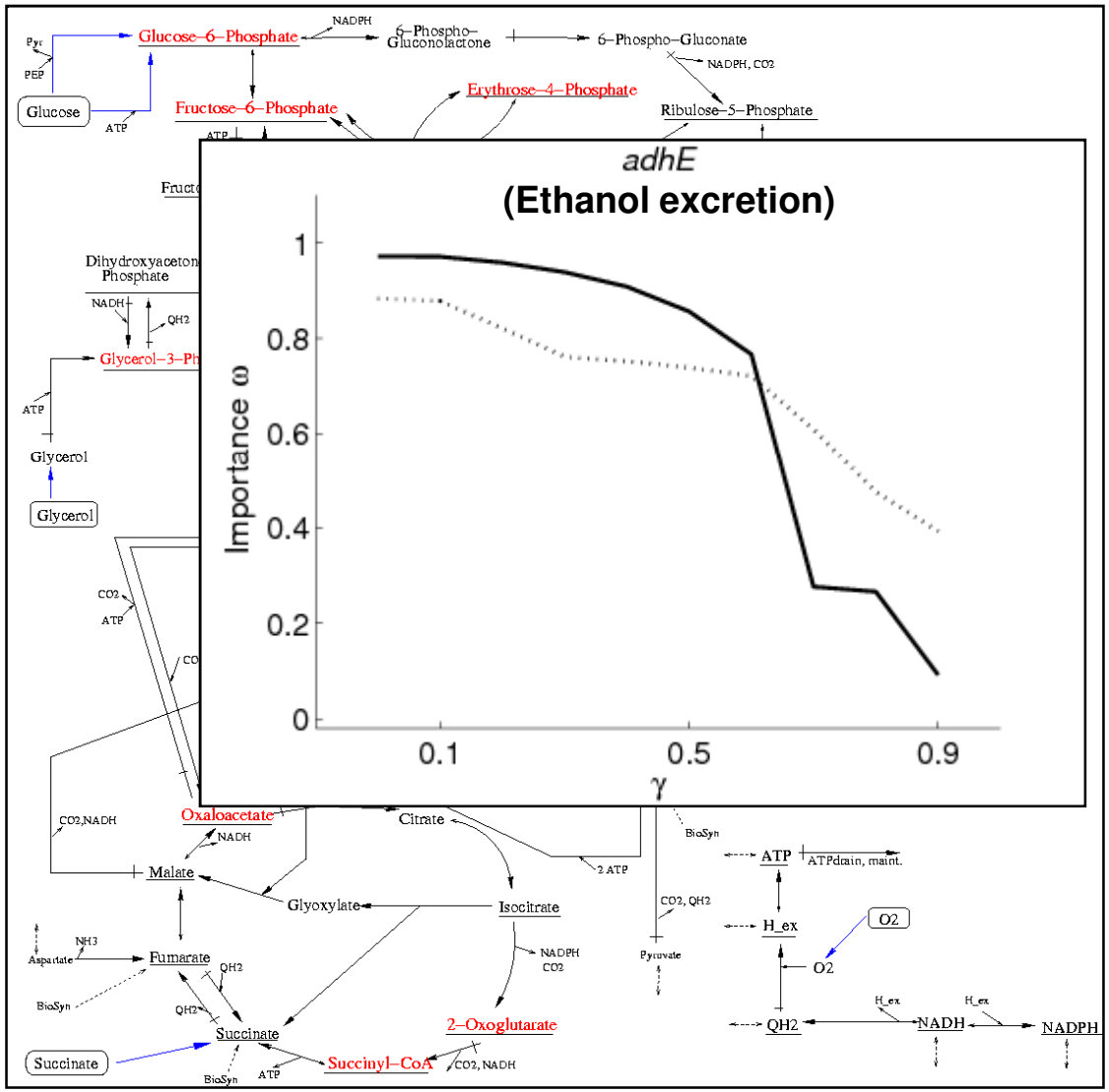
k=0

k=2

k=10

- R7, R11: for all k: ω deacreses when increasing γ → KO candidates
- R6: for all k: ω increases when increasing γ → overexpression candidates
- R9: overexpression candidate for low *k*, KO candidate for high *k*

Realistic Example: Succinate Production with *E.coli*



- model of central metabolism
- substrate: glucose; anaerobic conditions (fermentation)

Highest ranked KO candidates:

- Ethanol excretion
- Acetate excretion
- Lactate excretion
- Glucose PTS
- Pyruvate kinase
- Malic enzyme
- Succinate dehydrogenase
- Pyruvate formiate lyase

Highest ranked overexpression candidates

- G6P-kinase
- fumarate reductase
- PEP synthase
- Malate Dehydrogenase
- PEP carboxylase
- „CO2 uptake“
- glyoxylate shunt

Applied in practice

Identification of Potential Excess or Undersupply of Co-factors and Small Metabolites

- cofactors: NADH, ATP, CoA ... (also applicable to other (small) metabolites: CO2, ...)
- for a cofactor/metabolite of interest:

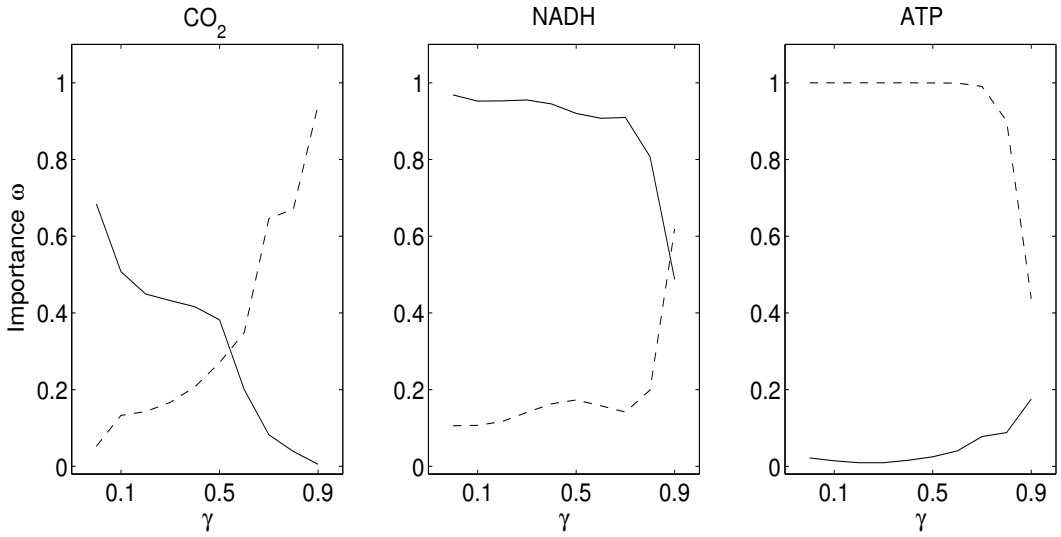
  insert (separately) artifical reactions consuming/supplying the cofactor/metabolite

  NADH → NAD (electron sink) / NAD → NADH (electron source)

  ATP → ADP + Pi (energy sink) / ADP + Pi → ATP (energy source)

- apply CASOP and check the importances of the artificial reactions!

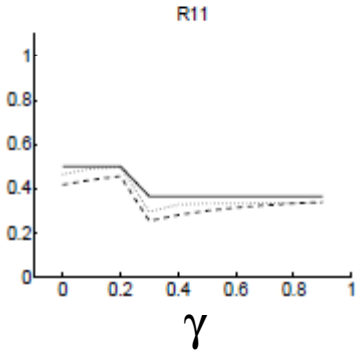
Anaerobic succinate production in *E.coli* (*k*=2)



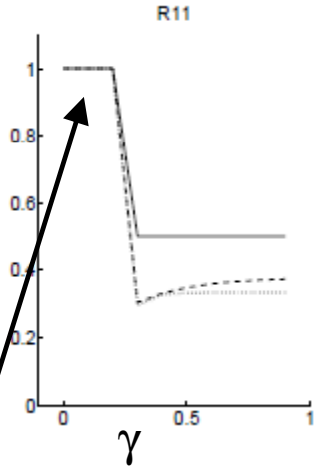
Multiple Interventions

- Select intervention based on reaction ranking (e.g. Knockout)
- Select all EMs where the reaction knocked-out is not used and recalculate elementary-modes weights, reaction importances + ranking
- Select next intervention

k=0
k=2
k=10



Knockout of R7



R11 is a promising KO-candidate in the next iteration: Knocking out of R11 will lead to coupling of biomass synthesis and product formation

How to Get the Dual Network

- define intervention goal (target reactions / target flux vectors) by $\mathbf{t}^T\mathbf{r} > 0$

- wanted: MCSs *C* such that for all **r** satisfying

$$(1)\ \mathbf{Nr} = \mathbf{0} \quad (2)\ \mathbf{r}_{Irrev} \geq \mathbf{0} \quad (3)\ r_i = 0,\ i \in C$$

it holds that $\mathbf{t}^T\mathbf{r} = 0$

Define Inconsistent System **S**

$$\mathbf{Nr} = \mathbf{0}$$
$$\mathbf{r}_{Irrev} \geq \mathbf{0}$$
$$\mathbf{t}^T\mathbf{r} \geq 1$$
$$\mathbf{Ir} = \mathbf{0}$$

Farkas Lemma
Theory of IISs
(Ryan et al.)

Consider System **D**

$$(\mathbf{I}\ \ -\mathbf{t}\ \ -\mathbf{I}_{irrev}\ \ \mathbf{N}^T)\begin{pmatrix}\mathbf{v}\\ w\\ \mathbf{z}\\ \mathbf{u}\end{pmatrix} = \mathbf{0};\quad \mathbf{z} \geq \mathbf{0}; w \geq 0$$

$\mathbf{N}_{dual}$ $\mathbf{r}_{dual}$

We are interested in *irreducible inconsistent subsystems* (IISs) of **S** keeping the system inconsistent with a minimal subset of equations from **Ir=0** (i.e. with a minimal (cut) set of reactions set to zero).

The IISs of **S** (corresponding to the MCSs) are the elementary modes of system D which are minimal in **v** and have support in *w*.

Ballerstein, von Kamp, Klamt, Haus: Minimal cut sets in a metabolic network are elementary modes in a dual network. Submitted.