Constraint-based modeling of cellular networks
WS 2023/2024

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Last lecture

Flux variability analysis

feasible or operational ranges

Flux variability and classification of reactions

blocked reactions

Flux variability to generate alternative optima

Flux sampling

Flux sampling and reaction classification

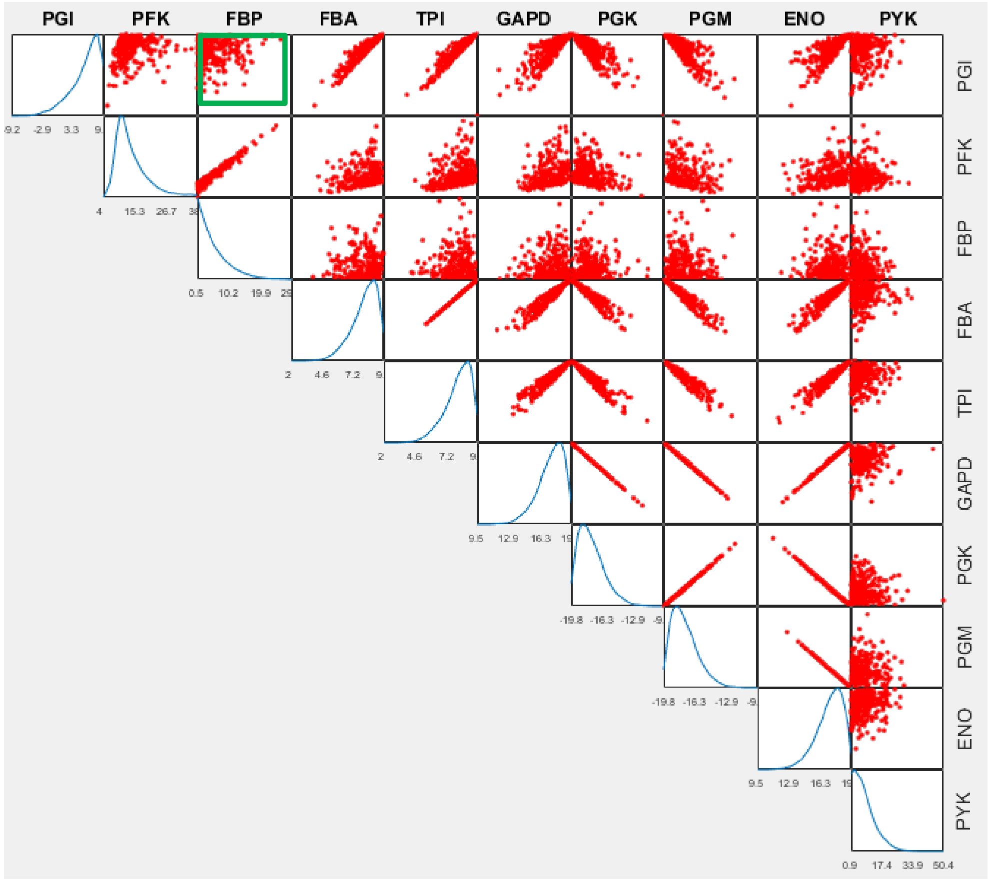
shape probability distribution

correlation between sampled fluxes

Flux sampling and classification of reactions

Pairs of reactions can be classified based on the correlation between their respective fluxes

Scatter plot



Correlation of 1

Correlation of -1

Moderate correlation

No signifnicant or

small correlation

Flux sampling and classification of reactions

Flux sampling is costly

three algorithms for sampling

(could be affected by bias, depending on implementation)

Questions

1. Is there a more efficient way to determine reactions whose fluxes show correlation of 1?

   do we have to sample or one LP is sufficient?

2. Can we specify how the structure of the metabolic network leads to dependencies between reaction fluxes?

3. What are the biological implications of such dependencies?

Flux coupling analysis (FCA) questions

Questions

1. Is there a more efficient way to determine reactions whose fluxes show correlation of 1?

   do we have to sample or one LP is sufficient?

2. Can we specify how the structure of the metabolic network leads to dependencies between reaction fluxes?

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These questions can be answered by

Flux Coupling Analysis (FCA)

Flux coupling analysis (FCA)

Main goal

Classify reaction pairs based on their dependencies.

The dependencies between reactions are called **reaction couplings**.

We will show that this classification can be achieved by a formulation which resembles Flux Variability Analysis

Flux coupling analysis (FCA)

Main goal

Classify reaction pairs based on their couplings.

Three types of reaction couplings

Directional coupling vi != 0 => vj !=0

Partial coupling vi != 0 => vj !=0, with vi/vj != const

Full coupling vi/vj = vij, s.t. for every v, Nv=0

Reaction pairs which are not directionally, partially, or fully coupled will be called **uncoupled**.

Directional coupling

Definition

A reaction R_i is directionally coupled to reaction R_j if for every steady-state flux distribution non-zero steady-state flux for R_i implies a non-zero steady-state flux for R_j but necessarily the reverse.

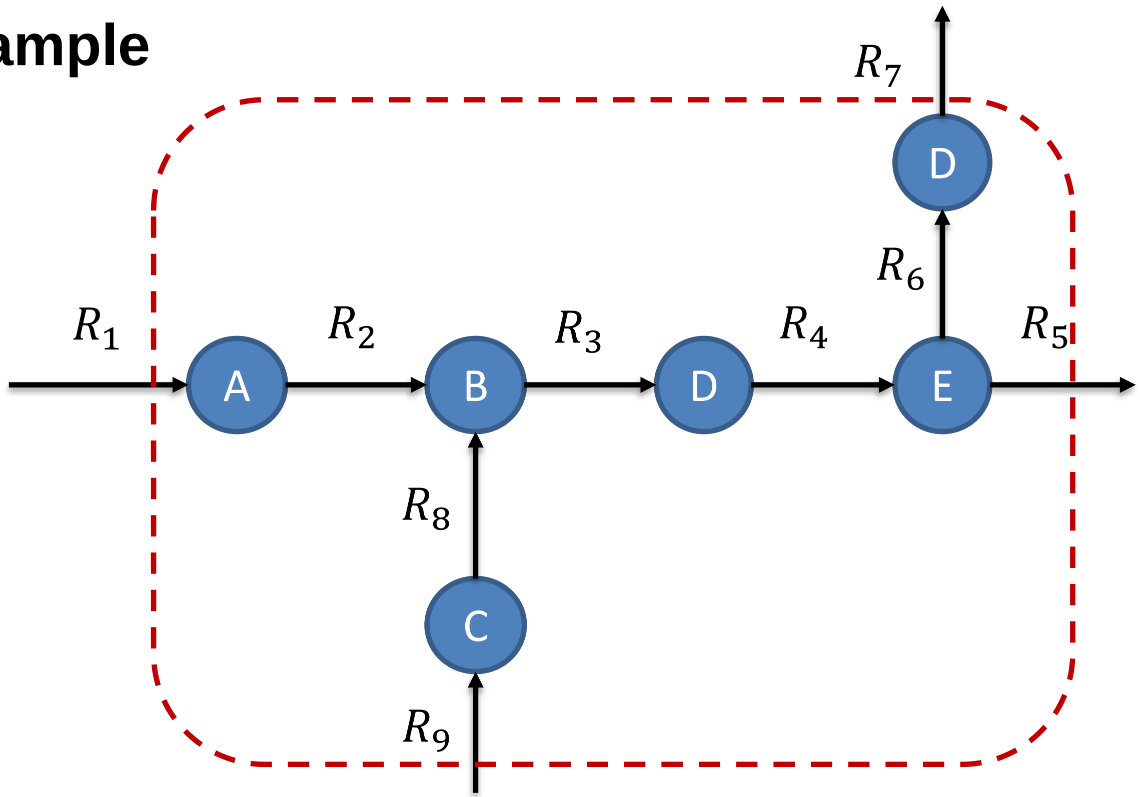
Mathematical

A reaction R_i is directionally coupled to reaction R_j if for every v with $Nv = 0, v_i \neq 0$ implies $v_j \neq 0$, but not the reverse.

Notation $R_i \rightarrow R_j$ or $i \rightarrow j$

Directional coupling

Example

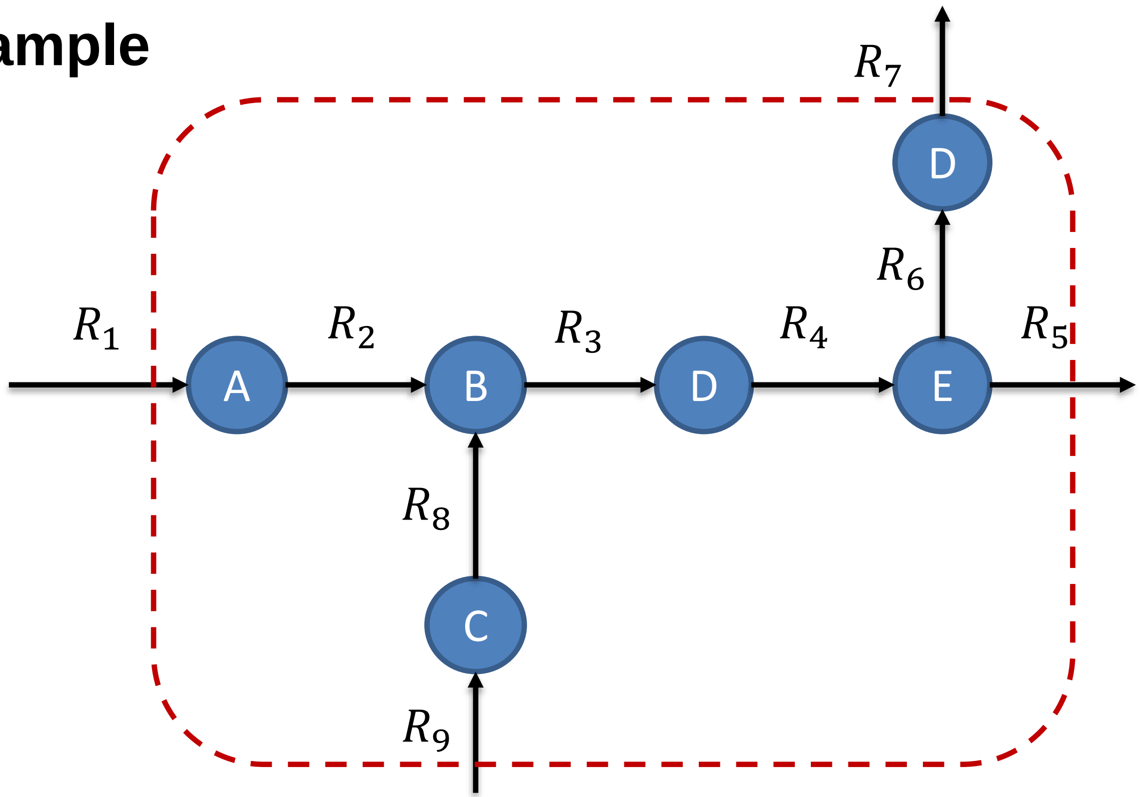


Reaction R_2 is directionally coupled to R_3

Denoted by $R_2 \rightarrow R_3$

Directional coupling

Example



$$\frac{dc_B}{dt} = v_2 + v_8 - v_3$$

$$v_2 + v_8 - v_3 = 0$$

If $v_2 \neq 0$, then $v_3 \neq 0$
(observe the signs)

Reaction R_2 is directionally coupled to R_3
Denoted by $R_2 \rightarrow R_3$

Partial coupling

Definition

A reaction R_i is partially coupled to reaction R_j if non-zero flux for R_i implies a non-zero, though variable, flux for R_j and vice versa.

Mathematical

A reaction R_i is partially coupled to reaction R_j if for every v with $Nv = 0, v_i \neq 0$ implies $v_j \neq 0$, with $v_i/v_j \neq const$ and vice versa.

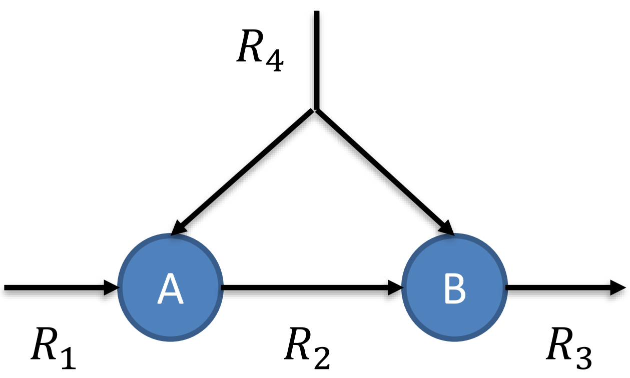
Notation $R_i \leftrightarrow R_j$ or $i \leftrightarrow j$

Flux coupling analysis (FCA)

Example

It turns out that arriving at an example for partially coupled reactions is a non-trivial task

Consider the following:



$$\frac{dc_A}{dt} = v_1 + v_4 - v_2$$

$$\frac{dc_B}{dt} = v_2 + v_4 - v_3$$

Flux coupling analysis (FCA)

Example

It turns out that arriving at an example for partially coupled reactions is a non-trivial task

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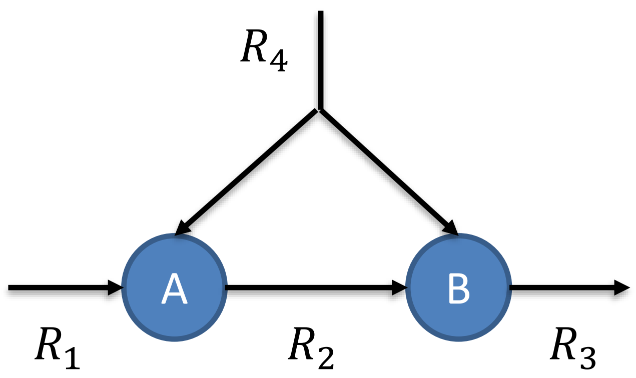


$v_1 + v_4 - v_2 = 0$ (1)

$v_2 + v_4 - v_3 = 0$ (2)

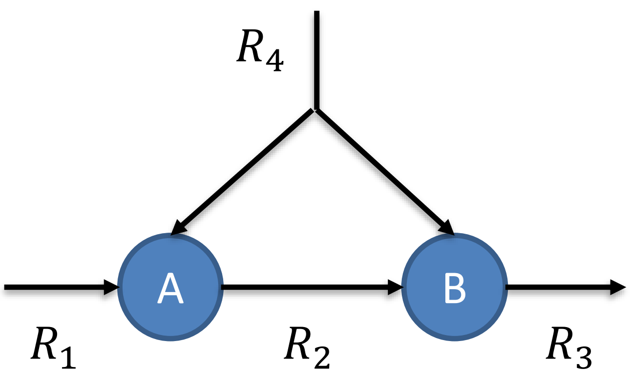
If $v_2 \neq 0$ then $v_3 \neq 0$ (from 2)

Flux coupling analysis (FCA)

Example

It turns out that arriving at an example for partially coupled reactions is a non-trivial task

Consider the following:



$v_1 + v_4 - v_2 = 0$ (1)

$v_2 + v_4 - v_3 = 0$ (2)

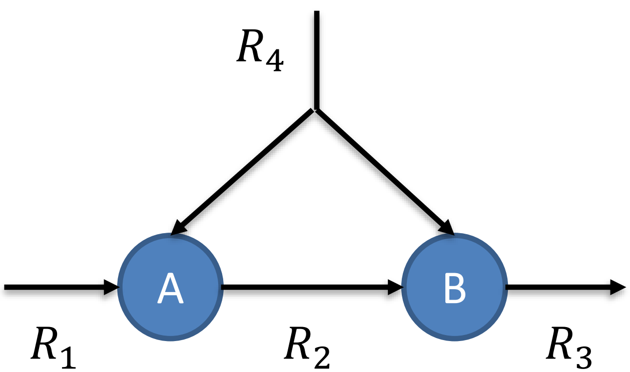
If $v_3 \neq 0$ then either $v_2 \neq 0$ or $v_4 \neq 0$ (from 2)
If $v_2 \neq 0$, done!
If $v_4 \neq 0$, then $v_2 \neq 0$ (from 1)

Flux coupling analysis (FCA)

Example

It turns out that arriving at an example for partially coupled reactions is a non-trivial task

Consider the following:



$v_3 \neq 0$ if and only if $v_2 \neq 0$

Note that partial coupling arises as a result of the network as a whole

Emergent property

Emergent property

Property of which holds as a result of the network as a whole, rather than some characteristics of a subnetwork or a component itself

Partial coupling is an emergent property

Is partial coupling requiring multisubstrate reactions (like R_4 in the previous example)?

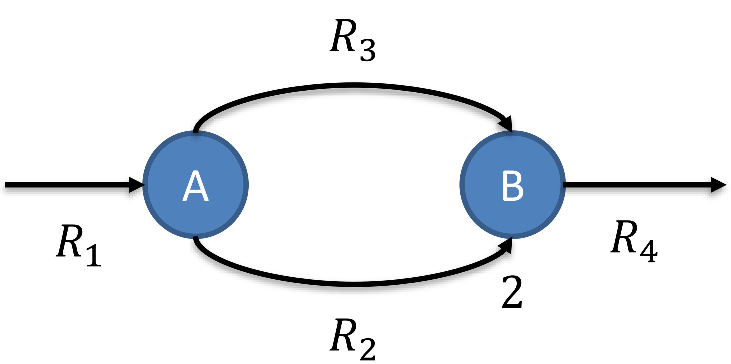
Partial coupling and multisubstrate reactions

Does partial coupling require multisubstrate reactions (like R_4 in the previous example)?

Consider the following:

$$\frac{dc_A}{dt} = v_1 - v_2 - v_3$$

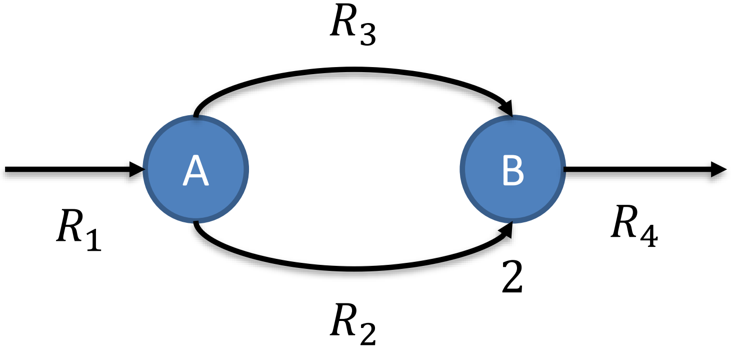
$$\frac{dc_B}{dt} = v_3 + 2v_2 - v_4$$



Partial coupling and multisubstrate reactions

Does partial coupling require multisubstrate reactions (like R_4 in the previous example)?

There is a subtle network difference which we will tackle in the next lectures that distinguishes the two cases.



$$\frac{dc_A}{dt} = v_1 - v_2 - v_3$$

$$\frac{dc_B}{dt} = v_3 + 2v_2 - v_4$$

$v_1 \neq 0$ if and only if $v_4 \neq 0$

Full coupling

Definition

A reaction R_i is fully coupled to reaction R_j if non-zero flux for R_i implies not only a non-zero but also a fixed flux for R_j and vice versa.

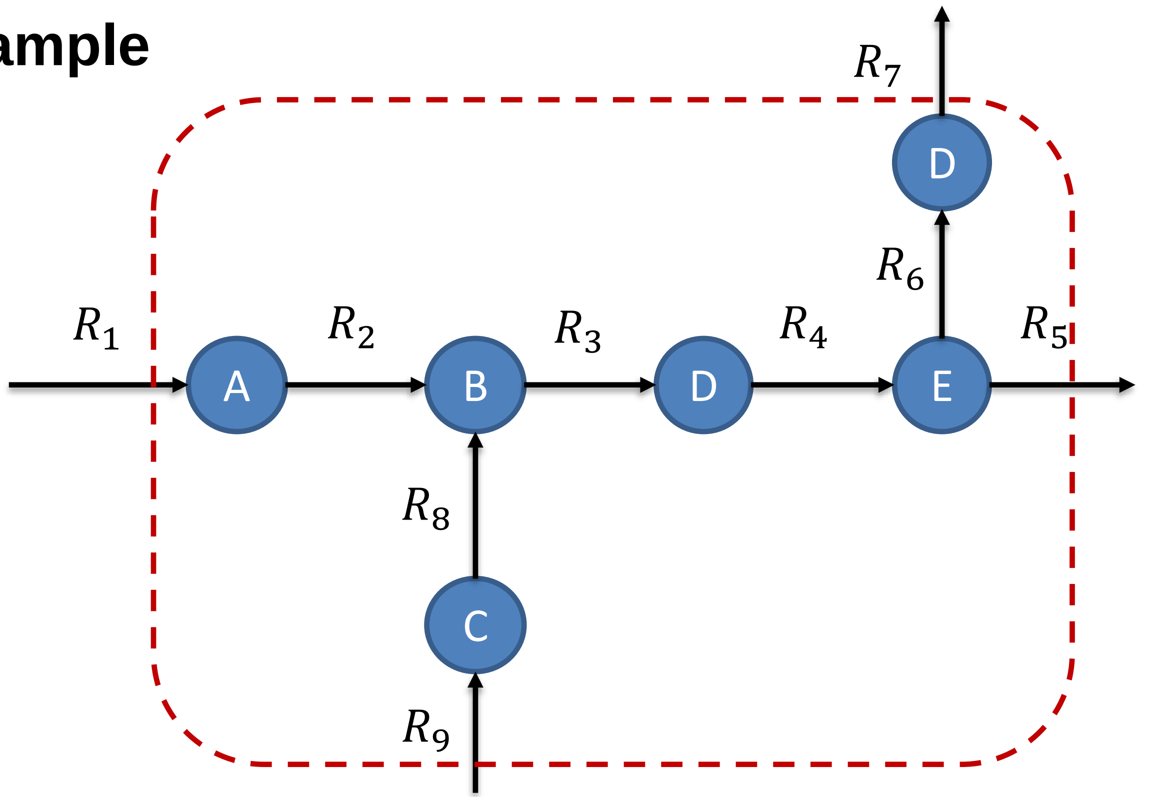
Mathematical

A reaction R_i is directionally coupled to reaction R_j if for every v with $Nv = 0, v_i \neq 0$ implies $v_j \neq 0$, with $\frac{v_i}{v_j} = const$ and vice versa.

Notation $R_i \Leftrightarrow R_j$ or $i \Leftrightarrow j$

Full coupling

Example

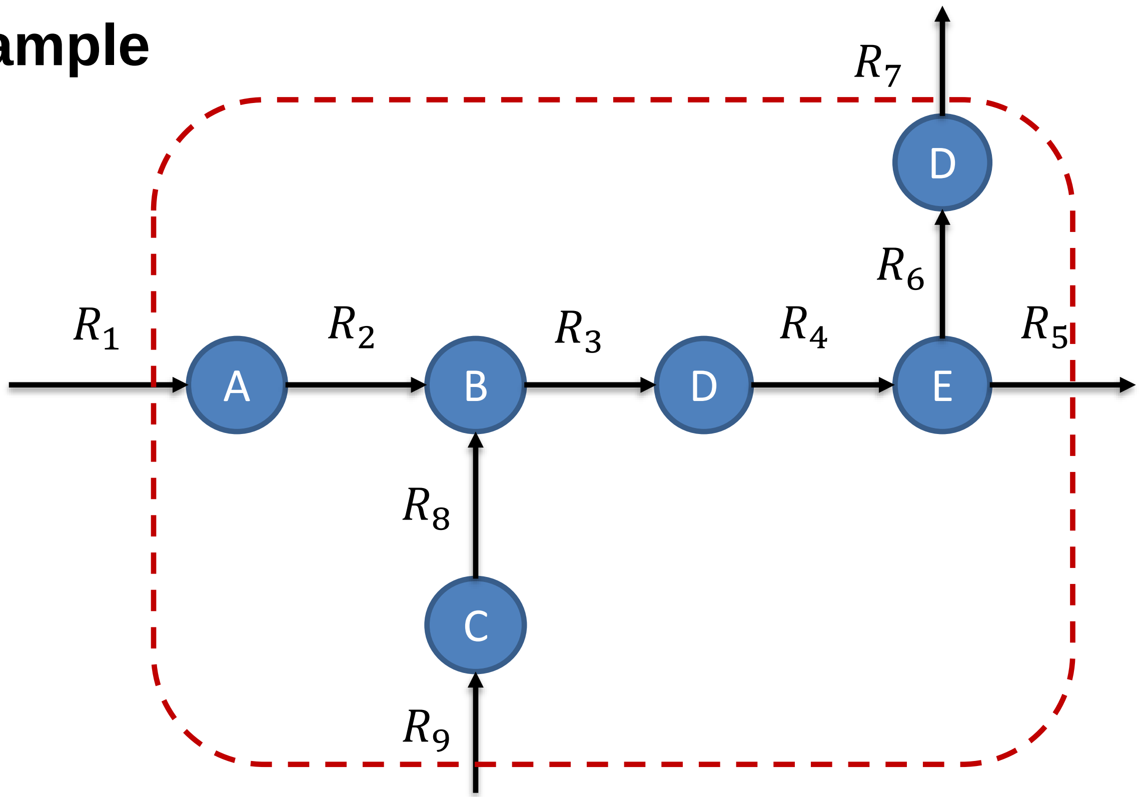


Reaction R_3 is fully coupled to R_4

Denoted by $R_3 \Leftrightarrow R_4$

Full coupling

Example



$$\frac{dc_D}{dt} = v_3 - v_4$$

$$v_3 - v_4 = 0$$

Reaction R_3 is fully coupled to R_4

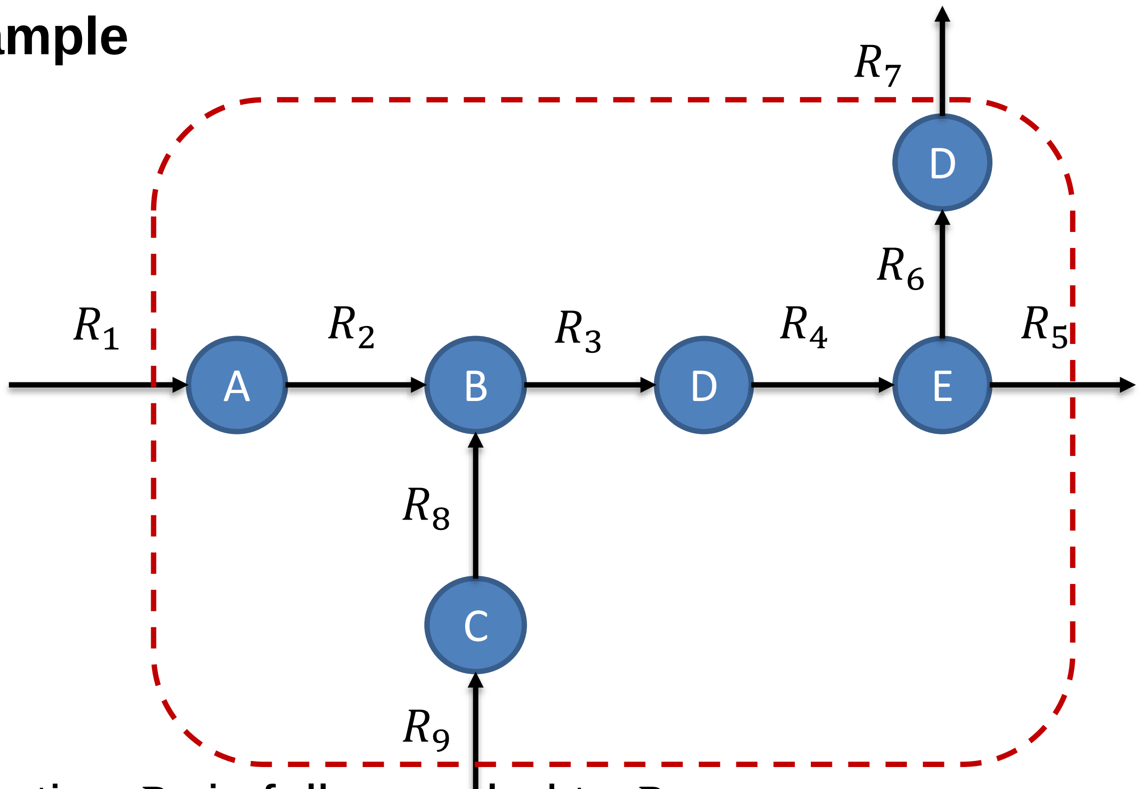
Denoted by $R_3 \Leftrightarrow R_4$

Hence

$\frac{v_3}{v_4} = 1$ (fixed constant)

Full coupling

Example



$$\frac{dc_D}{dt} = v_3 - v_4$$

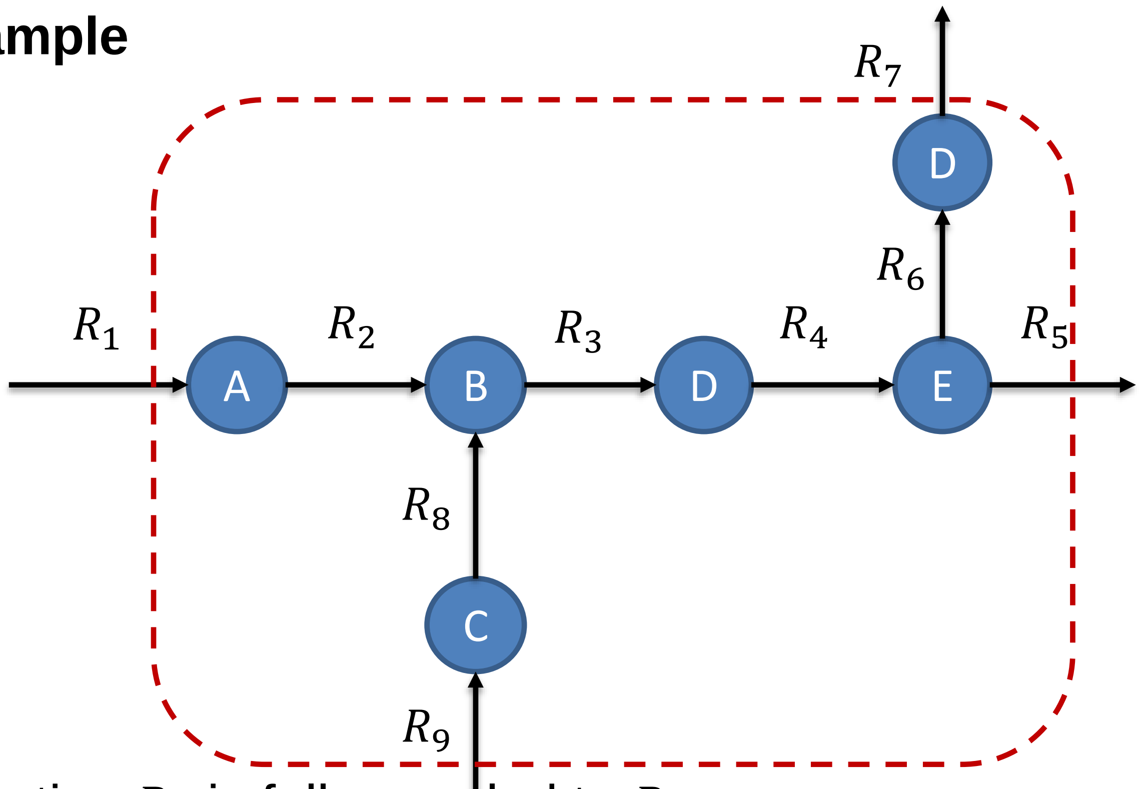
$$v_3 - v_4 = 0$$

Reaction R_3 is fully coupled to R_4
Denoted by $R_3 \Leftrightarrow R_4$
Other fully coupled pairs?

Hence
$\frac{v_3}{v_4} = 1$ (fixed constant)

Full coupling

Example



$$\frac{dc_D}{dt} = v_3 - v_4$$

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Reaction R_3 is fully coupled to R_4
Denoted by $R_3 \Leftrightarrow R_4$
Other fully coupled pairs?

Hence
$\frac{v_3}{v_4} = 1$ (fixed constant)

Flux coupling analysis (FCA)

Main goal

Classify reaction pairs based on their couplings.

Three types of reaction couplings

Directional coupling vi != 0 => vj !=0

Partial coupling vi != 0 => vj !=0, with vi/vj != const

Full coupling vi/vj = vij, s.t. for every v, Nv=0

Reaction pairs which are not directionally, partially, or fully coupled will be called **uncoupled**.

FCA questions

Questions

1. Is there a more efficient way to determine reactions whose fluxes show correlation of 1? corr 1을 보기 위해 샘플링을 하는것말곤 방법이 없는가?
싱글 LP로 끝낼 순 없을까?

do we have to sample or one LP is sufficient?

2. Can we specify how the structure of the metabolic network leads to dependencies between reaction fluxes?

3. What are the biological implications of such dependencies?

These questions can be answered by

Flux Coupling Analysis (FCA)

Computational ways to determine reaction couplings

vi != 0 => vj !=0

vi != 0 => vj !=0, with vi/vj != const

vi/vj = vij(const), s.t. for every v, Nv=0

Consider the following program

$$\max_{v} (\min) \frac{v_i}{v_j}$$ ratio를 본다.

s.t.

$$Nv = 0$$ *steady state*

$$\forall i, 1 \leq i \leq m, v_i^{min} \leq v_i \leq v_i^{max}$$ *capacity constraints*

The objective is not linear function but a fraction of linear functions

this is referred to as **linear fractional program**

Computational ways to determine reaction couplings

이미 min이 0이라는것을 알고,
max는 fluxrange()로 구할 수 있다.
네가티브는 없다.

What are the possible outcomes for $\max_{v}\,(\min)\,\frac{v_i}{v_j}$ assuming that all reactions in the network are irreversible?

Note: reversible reactions can be divided into two irreversible reactions (forward and backward direction)

Clearly $\frac{v_i}{v_j}$ lies in the interval $[0, \infty)$!

There are five possibilities

Computational ways to determine reaction couplings

c는 항상 양수이다.

There are five possibilities

all reactions are necessaarily irreversable

1. $0 \leq \frac{v_i}{v_j} \leq c$ for some constant $c > 0$
2. $c_1 \leq \frac{v_i}{v_j} \leq c_2$ for some constant $c_1 > 0$ and $c_2 > 0, c_1 \neq c_2$
3. $\frac{v_i}{v_j} = c$ for some constant $c > 0$
4. $c \leq \frac{v_j}{v_i}$ for some constant $c > 0$, without an upper bound
5. $0 \leq \frac{v_i}{v_j}$ without an upper bound

Computational ways to determine reaction couplings

There are five possibilities

1. $0 \leq \frac{v_i}{v_j} \leq c$ for some constant $c > 0$
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3. $\frac{v_i}{v_j} = c$ for some constant $c > 0$ **full coupling** $\boldsymbol{i \Leftrightarrow j}$
4. $c \leq \frac{v_j}{v_i}$ for some constant $c > 0$, without an upper bound
5. $0 \leq \frac{v_i}{v_j}$ without an upper bound

Computational ways to determine reaction couplings

There are five possibilities

vj가 0이 아니라는것을 의미 1번의 경우 ub를 가짐

1. $0 \le \frac{v_i}{v_j} \le c$ for some constant $c > 0$ **directional coupling $\boldsymbol{i \to j}$**

2. $c_1 \le \frac{v_i}{v_j} \le c_2$ for some constant $c_1 > 0$ and $c_2 > 0, c_1 \ne c_2$

3. $\frac{v_i}{v_j} = c$ for some constant $c > 0$ **full coupling $\boldsymbol{i \Leftrightarrow j}$**

4. $c \le \frac{v_j}{v_i}$ for some constant $c > 0$, without an upper bound

directional coupling $\boldsymbol{j \to i}$ 4번의 경우 lb를 가진다

5. $0 \le \frac{v_i}{v_j}$ without an upper bound

Computational ways to determine reaction couplings

There are five possibilities

1. $0 \le \frac{v_i}{v_j} \le c$ for some constant $c > 0$ **directional coupling** $\boldsymbol{i \to j}$
2. $c_1 \le \frac{v_i}{v_j} \le c_2$ for some constant $c_1 > 0$ and $c_2 > 0, c_1 \ne c_2$
ub와 lb를 모두 가지는 경우
partial coupling $\boldsymbol{i \leftrightarrow j}$
3. $\frac{v_i}{v_j} = c$ for some constant $c > 0$ **full coupling** $\boldsymbol{i \Leftrightarrow j}$
4. $c \le \frac{v_j}{v_i}$ for some constant $c > 0$, without an upper bound
directional coupling $\boldsymbol{j \to i}$
5. $0 \le \frac{v_i}{v_j}$ without an upper bound

Computational ways to determine reaction couplings

There are five possibilities

1. $0 \leq \frac{v_i}{v_j} \leq c$ for some constant $c > 0$ **directional coupling $\boldsymbol{i \rightarrow j}$**

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3. $\frac{v_i}{v_j} = c$ for some constant $c > 0$ **full coupling $\boldsymbol{i \Leftrightarrow j}$**

4. $c \leq \frac{v_j}{v_i}$ for some constant $c > 0$, without an upper bound **directional coupling $\boldsymbol{j \rightarrow i}$**

5. $0 \leq \frac{v_i}{v_j}$ without an upper bound **uncoupled**

Computational ways to determine reaction couplings

Solving the linear fractional program (LFP)

$$\max_{v} (\min) \frac{v_i}{v_j}$$

s.t.

$$Nv = 0 \text{ *steady state*}$$

$$\forall i, 1 \leq i \leq m, 0 \leq v_i \leq v_i^{max} \text{ *capacity constraints*}$$

provides the means to either classify each pair of reactions into the introduced coupling types or to state that it is uncoupled

However, we do not know how to solve LFPs

Solving Linear Fractional Program

To solve the linear fractional program (LFP), let us multiply numerator, denominator and all constraints by some positive t

특정 값 t를 곱해서, t에대한 스케일링을 한다

$$\max_{vt}\ (\min) \frac{v_i t}{v_j t}$$

위아래에 t를 곱해도 objective function은 변하지 않는다.

즉, 정답이 변하지는 않음!

s.t.

t를 곱함으로써 스케일된 flux distribution또한 steady state일것이 분명함!

$$N(vt) = 0 \ \textit{steady state}$$

$$\forall i, 1 \le i \le m, 0 \le v_i t \le v_i^{max} t \ \textit{capacity constraints}$$

vit는 flux distribution

$$t \ge 0$$

vmint <= vt <= vmaxt

+ v' 가 스케일된 distribution vt라고 할때, vj'은?

+ vj' = vjt = vj * 1/vj = 1

+ 즉, vit/vjt = vit = vi'

If $v_j > 0$, then we can take $t = 1/v_j$

t = 1/vj라고 가정해보자. 왜냐면 t는 0보다 큰 어떤 값이든 될 수 있으니까!

Solving Linear Fractional Program

To solve the linear fractional program (LFP), let us multiply numerator, denominator and all constraints by some positive t

fluxrange가 min/max Vi'를 구한다면,
s.t. Nv'=0, Vj' = 1
0<= V' < Vmax , t > 0
d이 조건의 LP는 풀 수 있다.

$$\max_{vt} (\min) \frac{v_i t}{v_j t}$$

s.t.

$$N(vt) = 0 \text{ \textit{steady state}}$$

$$\forall i, 1 \leq i \leq m, 0 \leq v_i t \leq v_i^{max} t \text{ \textit{capacity constraints}}$$

$$t \geq 0$$

For the vector $v' = vt$ it then holds that $v'_j = 1$, so the LFP can be transformed into

Solving Linear Fractional Program

To solve the linear fractional program (LFP), let us multiply numerator, denominator and all constraints by some positive t
이게 가능하다면, V1+V2/(V3+V4)도 구할 수 있지 않을까?

$$\max_{v'} (\min)\ v'_i$$

s.t.

$$Nv' = 0 \text{ steady state}$$

$$\forall i, 1 \le i \ne j \le m, 0 \le v'_i \le v_i^{max} t \text{ capacity constraints}$$

$$v'_j = 1$$ t = 1/Vj

$$t \ge 0$$

This is a linear program which we encountered in FVA

The transformation is known as Charnes-Cooper transformation!

Solving Linear Fractional Program

To solve the linear fractional program (LFP), let us multiply numerator, denominator and all constraints by some positive t

$$\max_{v'} (\min) \, v'_i$$

s.t.

$$Nv' = 0 \text{ *steady state*}$$

$$\forall i, 1 \leq i \neq j \leq m, 0 \leq v'_i \leq v_i^{max} t \text{ *capacity constraints*}$$

$$v'_j = 1$$

$$t \geq 0$$

Conclusion: For one reaction, we need to solve $2(m-1)$ linear programs to determine the couplings to the other $(m-1)$ reactions

For all reactions – we need to solve $2m(m-1)$ LPs

모든 커플링을 조사하려면 굉장히 많은 수의 계산이 필요하다.. 너무 낭비임.

Solving Linear Fractional Program

The Charnes-Cooper transformation

예를 들어서,
cT = [1,1,0,0,0]
dT = [0,0,1,1,0]
alpha = 0
beta = 0

$$\max_x \frac{c^T x + \alpha}{d^T x + \beta}$$

s.t.

$$Ax \leq b$$

vi/vj = i( ), s.t. for every v, Nv=0

Assumption:

The feasible region is such that $d^T x + \beta > 0$

Solving Linear Fractional Program

The Charnes-Cooper transformation

두개로 나눌 수 있다

$$\max_x \frac{c^T x + \alpha}{d^T x + \beta} = \max_x \frac{c^T}{d^T x + \beta} x + \frac{\alpha}{d^T x + \beta}$$

dTx + beta는 Vj이고, t는 1/Vj라고 가정해보자.

s.t.

$$Ax \leq b$$

Introduce the substitution

$y = \frac{1}{d^T x + \beta} x$ and $t = \frac{1}{d^T x + \beta}$

dTx + beta = dTy + beta*t = 1..? 이건 좀더 공부해보자

이 둘을 이렇게 정의해보고 써보자.

Solving Linear Fractional Program

The Charnes-Cooper transformation

cT가 이라면? 그냥 0이다.

$$\max_{x}\ c^T y + \alpha t$$

s.t.

$$Ay \leq bt$$
$$d^T y + \beta t = 1$$
$$t \geq 0$$

Introduce the following

$y = \frac{1}{d^T x + \beta} x$ and $t = \frac{1}{d^T x + \beta}$

Solving Linear Fractional Program

For all reactions – we need to solve $m(m-1)$ LPs (expensive!)

For instance, calculation of coupling in the large-scale network of E. coli (iJR904) would take more than 24 hours

Recent methods and tools are based on assessing **feasibility programs, rather than linear programs**

계산이 훨씬 적고, 저기하다.

This allows calculations in a matter of few hours for the same network

FCA questions

Questions

1. Is there a more efficient way to determine reactions whose fluxes show correlation of 1?

    do we have to sample or one LP is sufficient?

2. Can we specify how the structure of the metabolic network leads to dependencies between reaction fluxes?

3. What are the biological implications of such dependencies?

These questions can be answered by

Flux Coupling Analysis (FCA)

Couplings in real-world networks

All coupling types can be found in metabolic network reconstructions across all kingdoms of life

Reaction couplings can be identified in well-studied pathways

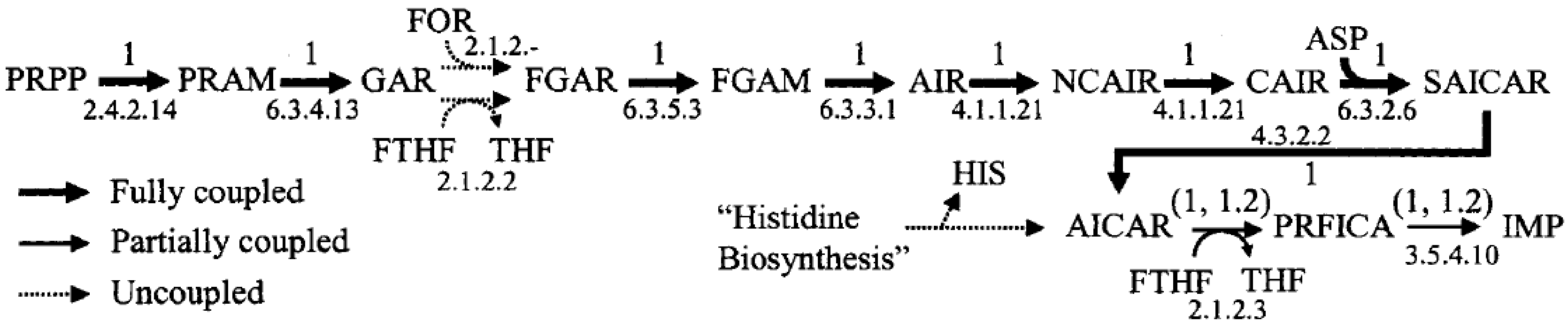


Figure 6 Coupled reaction set identified for purine biosynthesis in *E. coli* on a glucose-minimal medium, assuming a constant biomass composition. The numbers indicate the relative values or range of values for each flux in any particular flux distribution for given growth condition. Secondary metabolites and cofactors are omitted for simplicity.

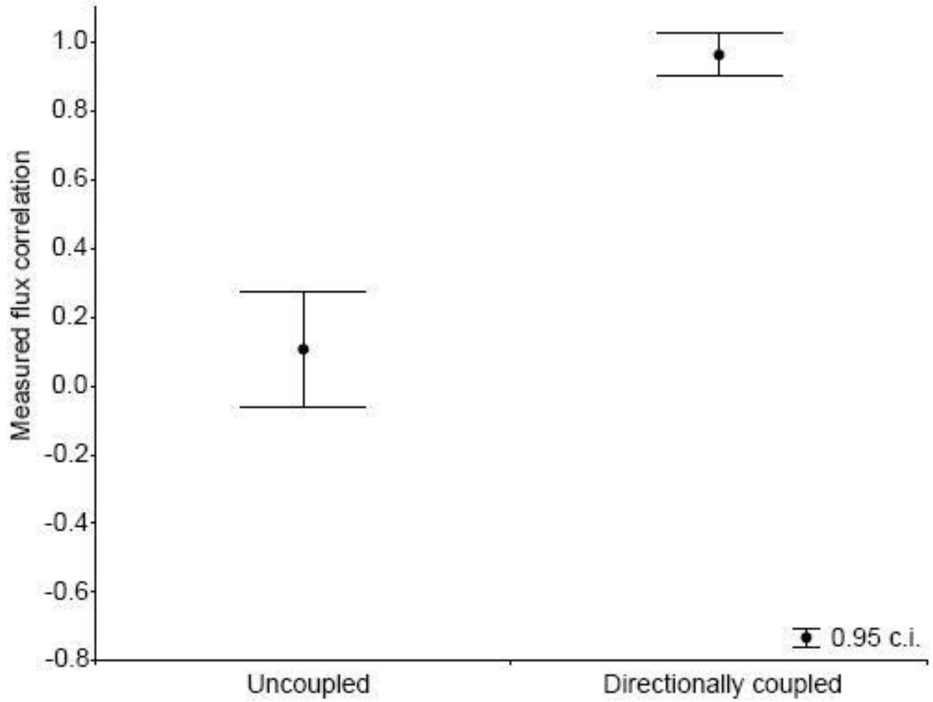
Burgard et al. (2004) *Genome Research*

Couplings and flux data

Do directionally coupled reactions exhibit higher correlation of their fluxes in comparison to uncoupled reactions based on measured flux distributions?

Couplings and flux data

Do directionally coupled reactions exhibit higher correlation of their fluxes in comparison to uncoupled reactions based on measured flux distributions?



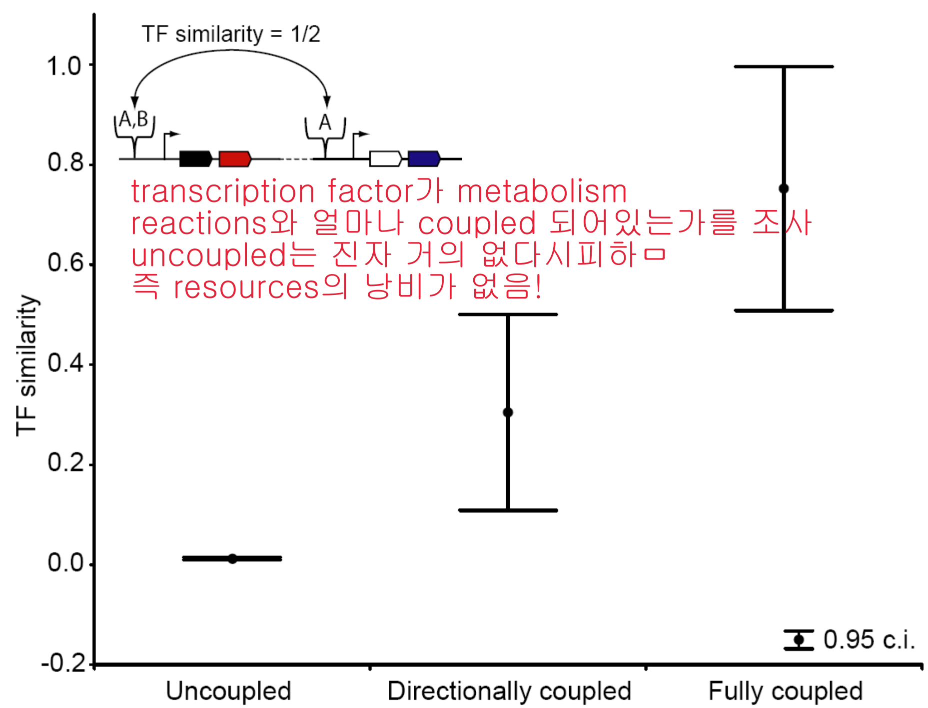
This holds!

(p < 10^{-14})

Couplings and gene regulation

Does flux coupling relate to transcriptional co-regulation of genes?

For fully coupled fluxes, this would imply that transcriptional regulation is organized in a way that no resources are wasted!



transcription factor가 metabolism
reactions와 얼마나 coupled 되어있는가를 조사
uncoupled는 진자 거의 없다시피하ㅁ
즉 resources의 낭비가 없음!

Notebaart et al. (2007)

PloS Comp. Biol.

Couplings and evolution

Some genes are lost during evolution
(for instance, if they have a deleterious effect on fitness)

Pal et al. (2005) *Nature Genetics* found that genes associated to reactions with coupled fluxes are more likely lost together over evolution!

Coupling type	Event	#Events	OR* (95% c.i.)
Fully coupled	Transfer	59	64.6 (24.2-168.8)
Fully coupled	Loss	1,624	50.0 (41.8-59.6)
Directionally coupled	Transfer	78	60.3 (24.3-147.2)
Directionally coupled	Loss	2,833	9.6 (8.3-11.1)

Odds-ratio

진화 과정중에 굉장히 많은양의 genes이 소실되었다.

Recap

Definitions of three coupling types

- directional
- partial
- full

Computational ways to determine reaction couplings

- linear fractional program (LFP)
- Charnes-Cooper transformation to LP

Type text here

Implications of reactions couplings

- flux correlations
- co-regulation of fully coupled reactions
- gene gain or loss in evolution