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Identification Procedure for PWA Models of Genetic Regulatory Networks

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Overview

- Genetic regulatory networks in brief
- Identification for PWA models of genetic regulatory networks
- PWA system identification
- Description of our approach
- Reconstruction of switching thresholds
- A case study: carbon starvation response of *E. coli*
- Conclusions

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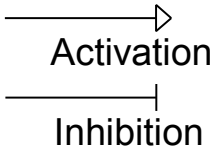
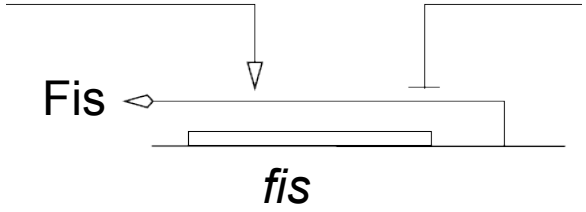
Genetic regulatory networks

- **Genetic regulatory networks** underlie functioning and development of living organisms
  Components: genes, proteins, metabolites, and their mutual regulatory interactions

Genes



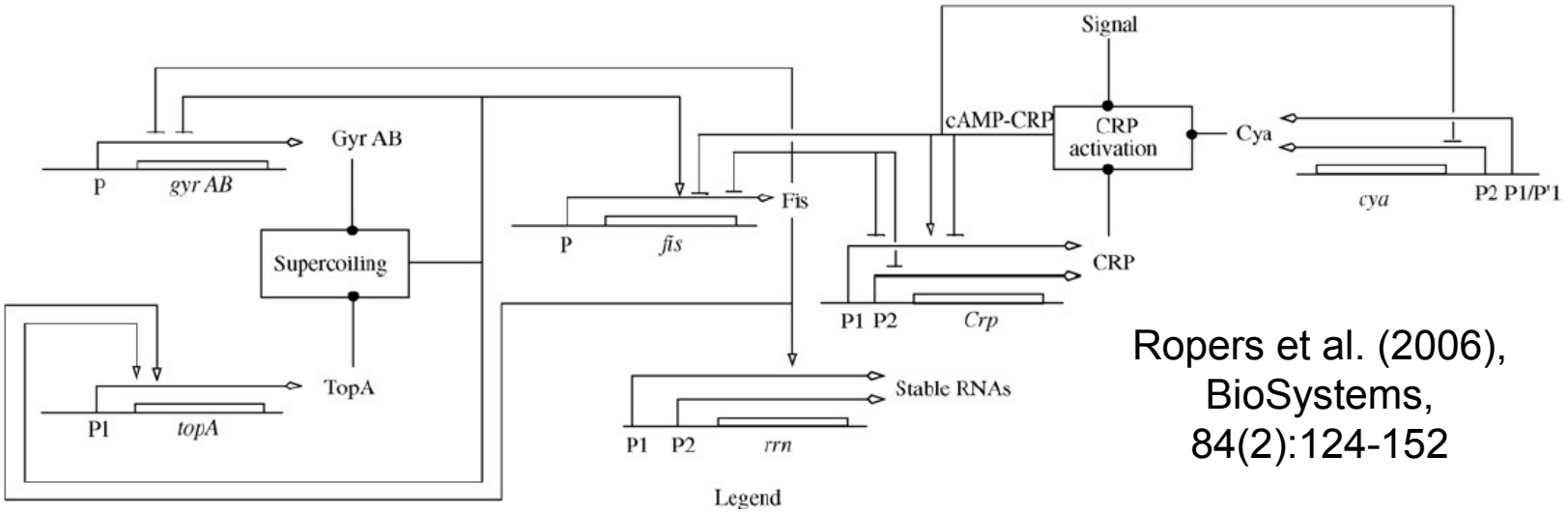


- Gene: dynamical system coding for a molecule (e.g. a protein)
- Genes are regulated by the concentration of proteins present in the cell
  - Genes can be turned on and off

Genetic regulatory networks

- GRN are usually **large** (many genes) and **complex** (feedback loops)

GRN governing *E. coli* carbon starvation response

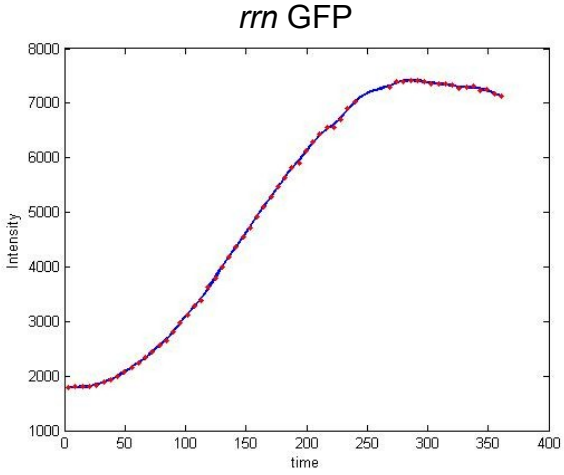


Ropers et al. (2006), BioSystems, 84(2):124-152

Expression data

Experimental techniques in biology have led to the production of enormous amount of data on the dynamics of gene expression:

- DNA microarrays
- Gene reporter systems



Time-series measurement of fluorescence or luminescence

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Data-driven modeling of GRNs

System identification problem: derive a model of the regulatory interactions according to measurements and model structure

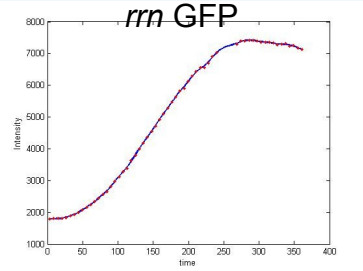
List of:

- genes
- proteins
- small molecules

List of:

- genetic interactions
- dynamical parameters

Expression data



Model structure

$$\frac{dx}{dt} = f(x) - \gamma(x)x$$

State of the art

Classes of dynamical models that were used for biological network identification:

x : a vector of protein concentrations

f, γ : functions that map interactions

$$\frac{dx}{dt} = f(x) - \gamma(x)x$$

synthesis rate ≥ 0 degradation rate > 0

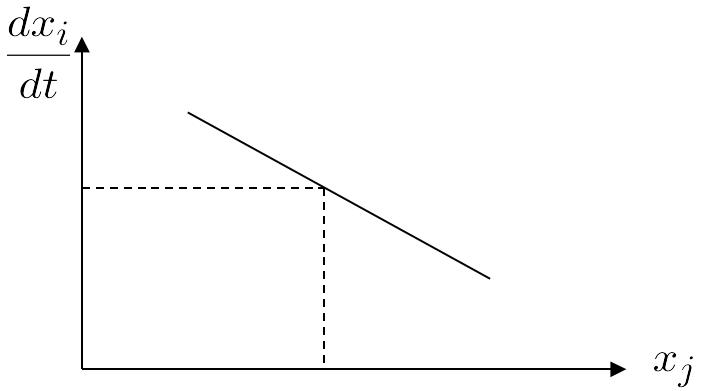
State of the art

Classes of dynamical models that were used for biological network identification:

- Linear (Gardner et al., Science 301 (2003) 102–105)
  → only valid near an equilibrium point

$$\frac{dx_i}{dt} = f_i(x) - \gamma_i x_i$$

affine



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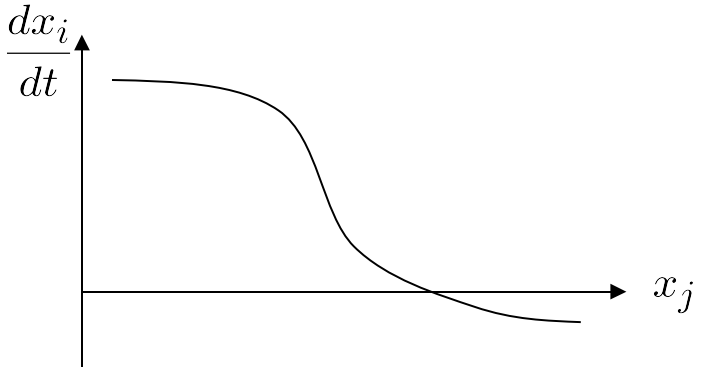
- Nonlinear (Jaeger et al., Nature 430 (2004) 368–371)
  → more adequate description but difficult to use for identification

$$\frac{dx_i}{dt} = f_i(x) - \gamma_i(x)x_i$$



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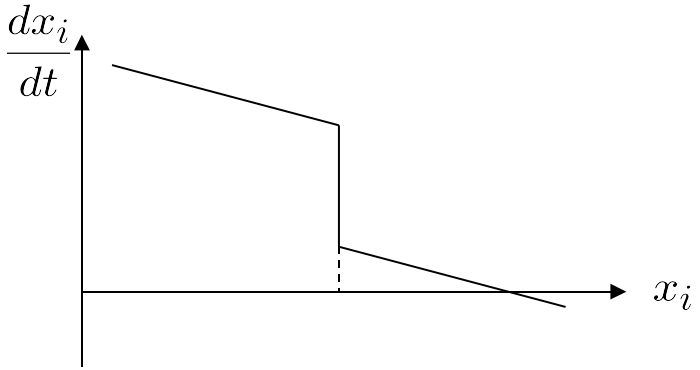
  → only valid near an equilibrium point
- Nonlinear (Jaeger et al., Nature 430 (2004) 368–371)
  → more adequate description but difficult to use for identification
- Piecewise Affine
  → compromise between linear and non-linear

$$\frac{dx_i}{dt} = f_i(x) - \gamma_i(x)x_i$$

piecewise constant



State of the art

Introduced by Glass and Kauffman in the 1970s

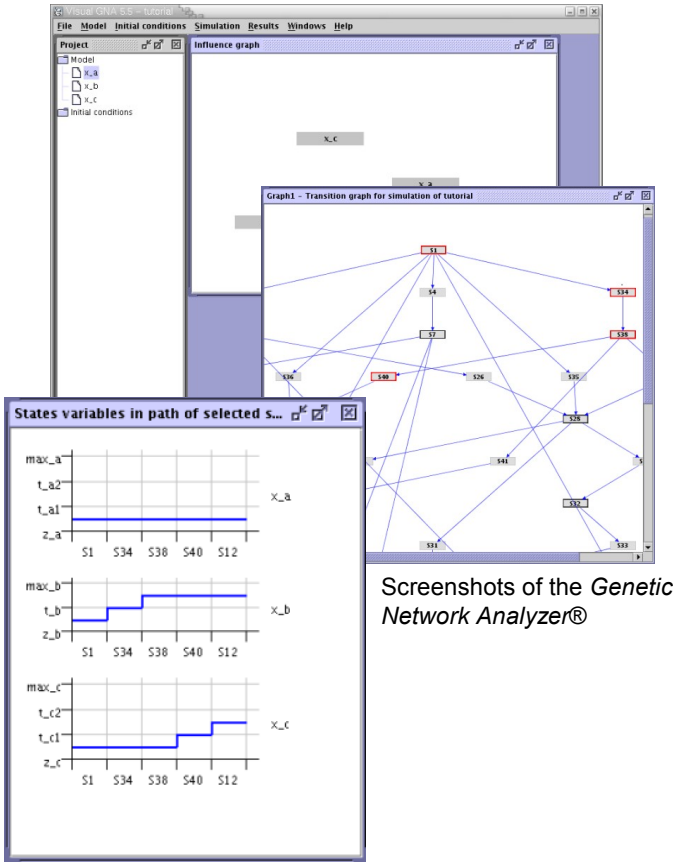
Piecewise Affine

→ compromise between linear and non-linear

- deJong et al., Bull. Math. Biol. 66 (2004) 301–340
- Belta et al., HSCC04, Vol. 2993 of LNCS (2004) 111-125
- Ghosh and Tomlin, Syst. Biol. 1 (2004) 170–183
- Batt et al., HSCC05, Vol. 3414 of LNCS (2005) 134–150

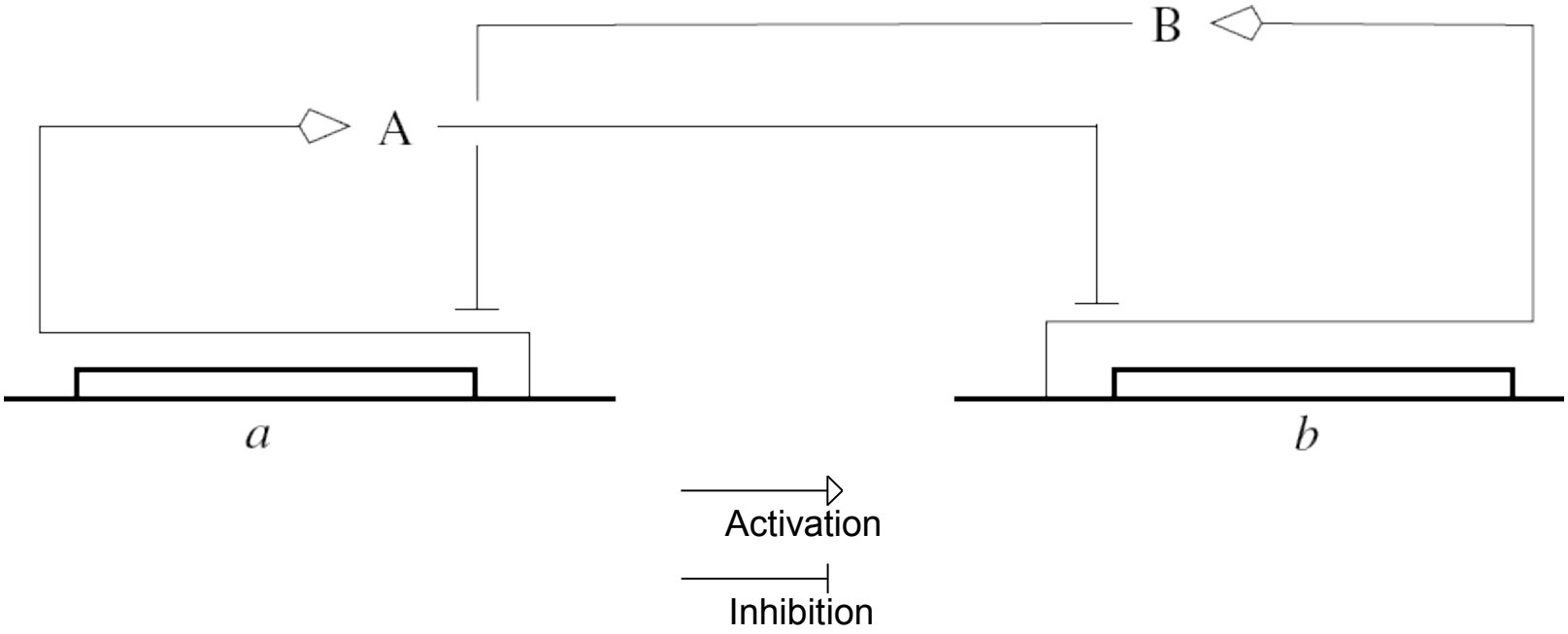
→ abstraction techniques available

→ identification methods for PWA systems available



Screenshots of the *Genetic Network Analyzer®*

PWA models: a simple example.



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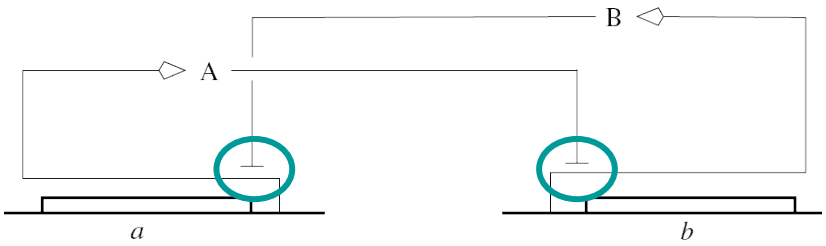
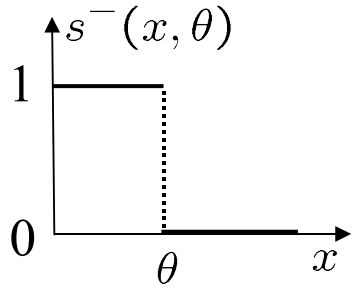


$$\dot{x}_1 = \kappa_1 s^-(x_2, \theta_2) - \gamma_1 x_1$$
$$\dot{x}_2 = \kappa_2 s^-(x_1, \theta_1) - \gamma_2 x_2$$



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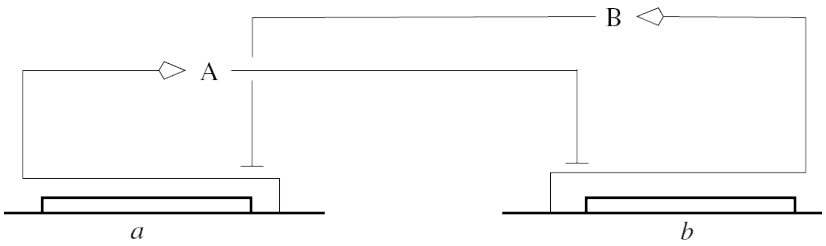
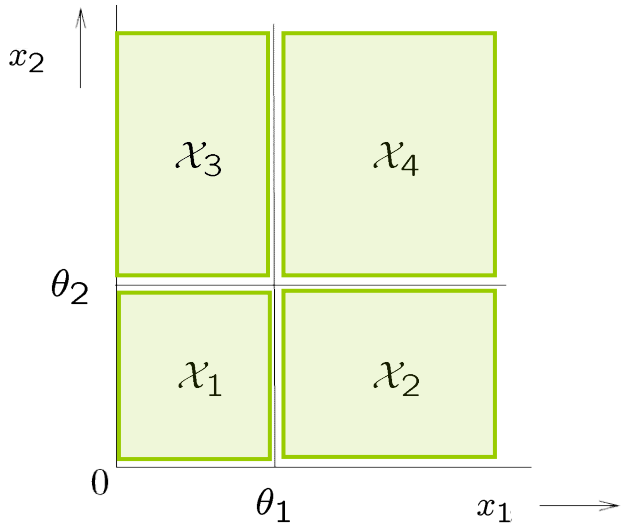


$$\dot{x}_1 = \kappa_1 \, s^-(x_2, \theta_2) - \gamma_1 \, x_1$$
$$\dot{x}_2 = \kappa_2 \, s^-(x_1, \theta_1) - \gamma_2 \, x_2$$



$$\dot{x} = \begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \end{bmatrix} = \begin{cases} \begin{bmatrix} \kappa_1 \\ \kappa_2 \end{bmatrix} - \begin{bmatrix} \gamma_1 & 0 \\ 0 & \gamma_2 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix}, & \text{if } x \in \mathcal{X}_1 \\ \begin{bmatrix} \kappa_1 \\ 0 \end{bmatrix} - \begin{bmatrix} \gamma_1 & 0 \\ 0 & \gamma_2 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix}, & \text{if } x \in \mathcal{X}_2 \\ \begin{bmatrix} 0 \\ \kappa_2 \end{bmatrix} - \begin{bmatrix} \gamma_1 & 0 \\ 0 & \gamma_2 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix}, & \text{if } x \in \mathcal{X}_3 \\ - \begin{bmatrix} \gamma_1 & 0 \\ 0 & \gamma_2 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix}, & \text{if } x \in \mathcal{X}_4 \end{cases}$$

Data model

Dynamics of the *i*-th molecule concentration:

$$\dot{x}_i(t) = \kappa_i^j - \gamma_i^j x_i(t) \text{ if } x(t) \in \mathcal{X}_j$$

Discrete-time model for the *i*-th molecule concentration:

$$x_i(k+1) = \tilde{\kappa}_i^j - \tilde{\gamma}_i^j x_i(k) + \eta_i(k) \text{ if } x(k) \in \mathcal{X}_j$$
$$y_i(k) = x_i(k) + \xi_i(k)$$

- T : sampling time step
- rate parameters: $\tilde{\kappa}_i^j = (\kappa_i^j/\gamma_i^j)(1 - e^{-\gamma_i^j T}) \quad ; \; \tilde{\gamma}_i^j = -e^{-\gamma_i^j T}$
- additive noise: η_i, ξ_i

Common data models:

- PieceWise Autoregressive eXogenous (PWARX): $\xi_i = 0$
- PWA Output-Error (PWA-OE): $\eta_i = 0$

PWA GRNs identification

Model:

$$\forall i \in \{1,\dots,n\}, \forall k \in \{1,\dots,N-1\}$$
$$x_i(k+1) = \tilde{\kappa}_i^j - \tilde{\gamma}_i^j x_i(k) + \eta_i(k) \text{ if } x(k) \in \mathcal{X}_j$$
$$y_i(k) = x_i(k) + \xi_i(k)$$

Identification problem: reconstruct

- the number of modes ← Given $x(0)$, how many $\mathcal{X}$ are reached ?
- all rate parameters ← For all of them, estimate $\tilde{\kappa}_i^j, \tilde{\gamma}_i^j$
- all switching thresholds ← Could some gene interactions cause the switch ?

from the noisy dataset $\mathcal{N} = \{y(k)\}_{k=0}^{N}$

Simple example

« biologocal » model ?

$$\dot{x}_1 = \kappa_1 s^-(x_2, \theta_2) - \gamma_1 x_1$$
$$\dot{x}_2 = \kappa_2 s^-(x_1, \theta_1) - \gamma_2 x_2$$
on $\mathcal{X}_1, \mathcal{X}_2, \mathcal{X}_3, \mathcal{X}_4$

identifiable model

$$\dot{x}_1 = \kappa_1 s^-(x_2, \theta) - \gamma_1 x_1$$
$$\dot{x}_2 = \kappa_2 - \gamma_2 x_2$$
on $\mathcal{X}_1, \mathcal{X}_3$ given $x(0)$

features to identify

- 2 modes
- kinetic parameters: $\tilde{\kappa}^1, \tilde{\gamma}^1, \tilde{\kappa}^2, \tilde{\gamma}^2$
- switching threshold: $\tilde{\theta}$

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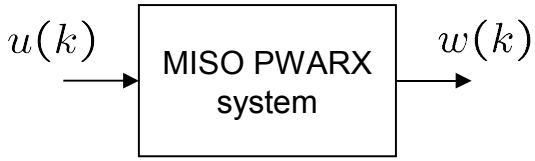
PWA identification methods

PWARX / PWA-OE models considered in hybrid identification:



$$z(k+1) = \pi^j [r(k)'1]' + \eta(k) \text{ if } r(k) \in \mathcal{X}_j$$
$$w(k) = z(k) + \xi(k)$$

$r(k) = [u'(k) \dots u'(k-n_a)\ z'(k) \dots z'(k-n_b)]'$ is the regressor vector.

$\{\mathcal{X}_j\}_{j=1}^{\tilde{s}}$ is a polyhedral partition of $\mathcal{X}$.

Dataset = noisy samples

$\mathcal{N} = \{ (r(k), y(k)) \}_{k=1}^{N}$

Common assumptions:

4. Known model orders
5. Known regressor set $\mathcal{X} \subset \Re^n$

Estimate:

2. The number $\tilde{s}$ of modes
3. The parameter vectors $\{\pi^j\}_{j=1}^{\tilde{s}}$
4. The regions $\{\mathcal{X}_j\}_{j=1}^{\tilde{s}}$

Identification in hybrid systems

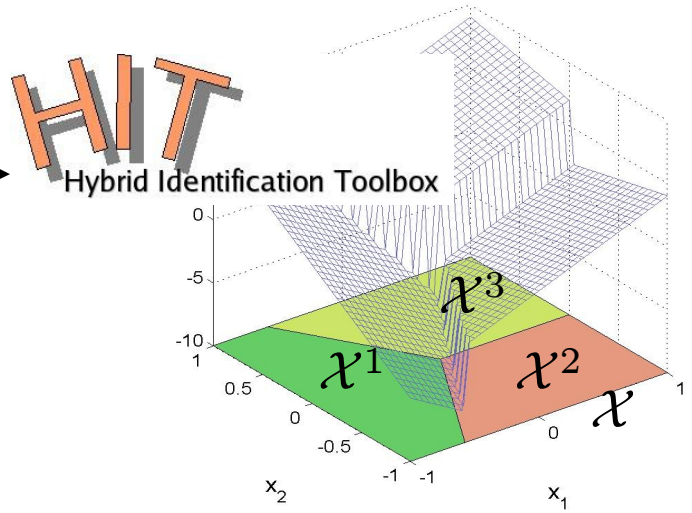
Some methods :

PWARX system identification:

- Ferrari-Trecate et al., 2003
- Bemporad et al., 2005
- Vidal et al., 2005
- Juloski et al., 2005

PWA-OE system identification:

- Juloski & Weiland, 2006
- Rosenqvist & Karlström, 2006

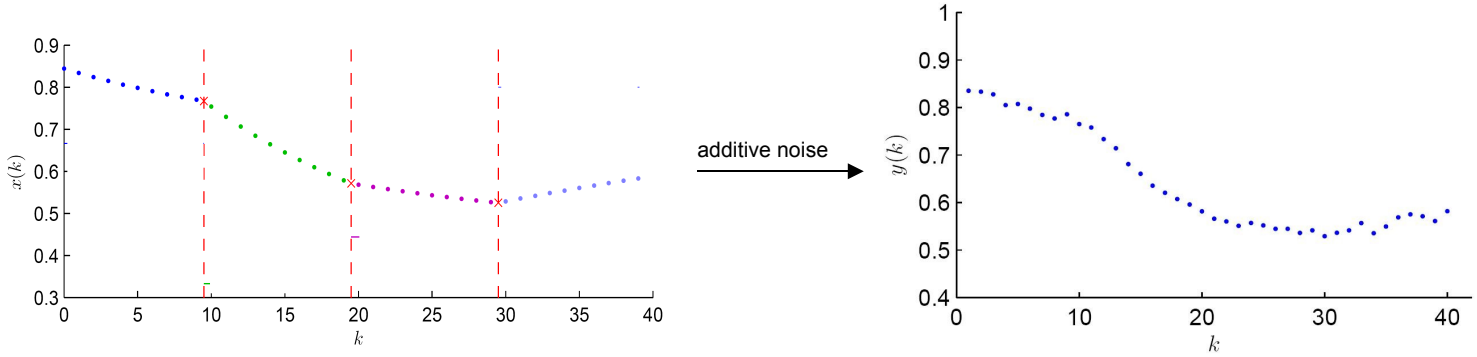


PWA models for a single molecule concentration fall within this class…

Hybrid identification pitfalls

- Existing identification methods are generic in nature and do not exploit features of PWA models of GRNs

Example 1: Switch detection from noisy measurements



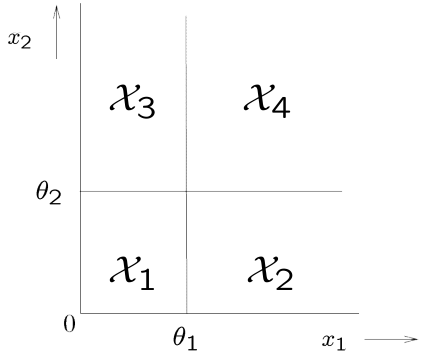
- Very challenging problem for general PWARX / PWA-OE models
- Much easier for PWA models of GRNs

Hybrid identification pitfalls

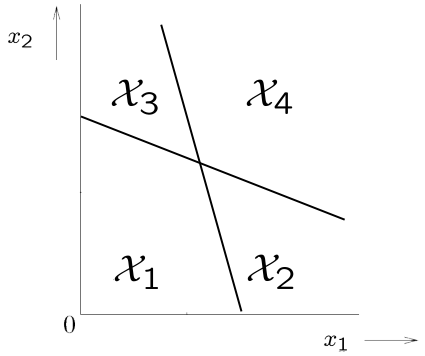
- Existing identification methods are generic in nature and do not exploit features of PWA models of GRNs
- Existing identification methods do not take into account constraints of PWA models of GRNs

Example 2: switching thresholds $\Rightarrow$ hyperrectangular domains

"true" domains



identified domains



The concept of threshold associated to a concentration variable is lost.

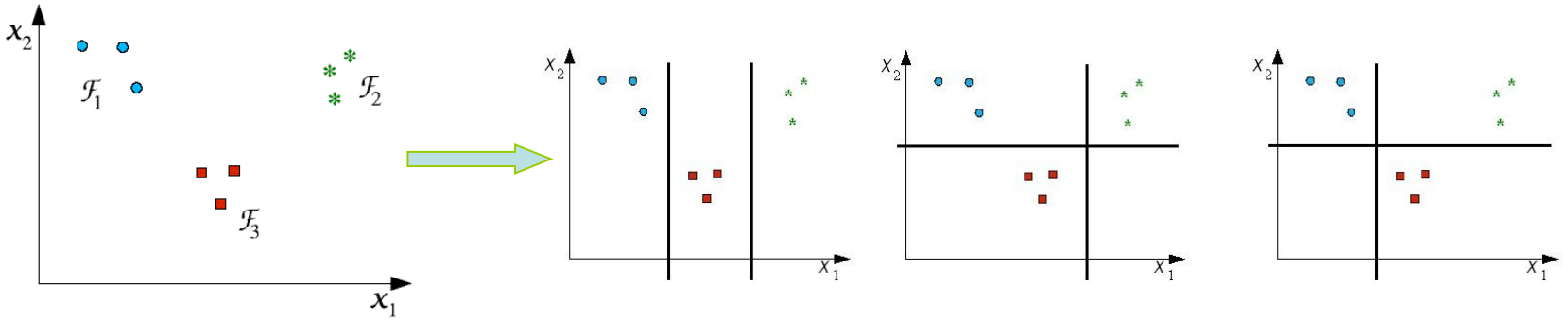
Hybrid identification pitfalls

- Existing identification methods are generic in nature and do not exploit features of PWA models of GRNs
- Existing identification methods do not take into account constraints of PWA models of GRNs
- Existing hybrid identification methods produce a single result but data are often scarce and multiple models might be plausible

Example 3: thresholds reconstruction

3 identified modes

reconstructed thresholds: all sets are sufficient to explain the modes



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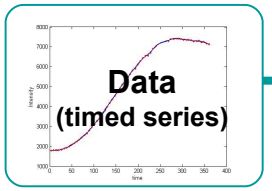
Identification of PWA models of GRNs



Switch detection

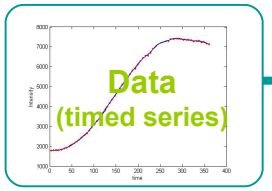
Mode estimation

Thresholds reconstruction

Kinetic parameters inference

Our approach:
« gray-box » identification.

Identification of PWA models of GRNs



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Mode estimation

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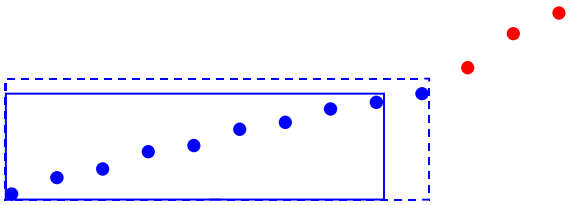
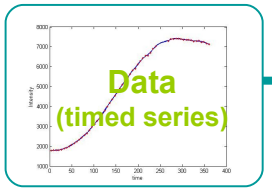
1) Detection of switches in gene expression data



(Porreca et al., HSCC 2006)

Identification of PWA models of GRNs



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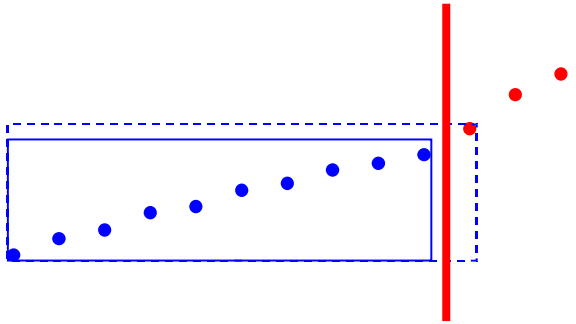

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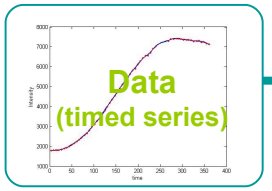


Switch detection

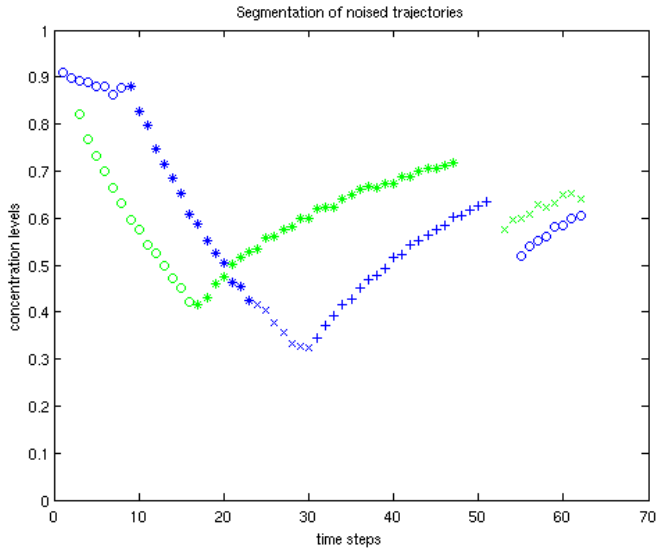
Mode estimation

Thresholds reconstruction

Kinetic parameters inference

2) Estimation of the number of modes and attribution of the measurements to mode data sets

Simple example



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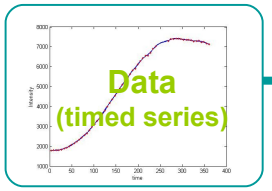

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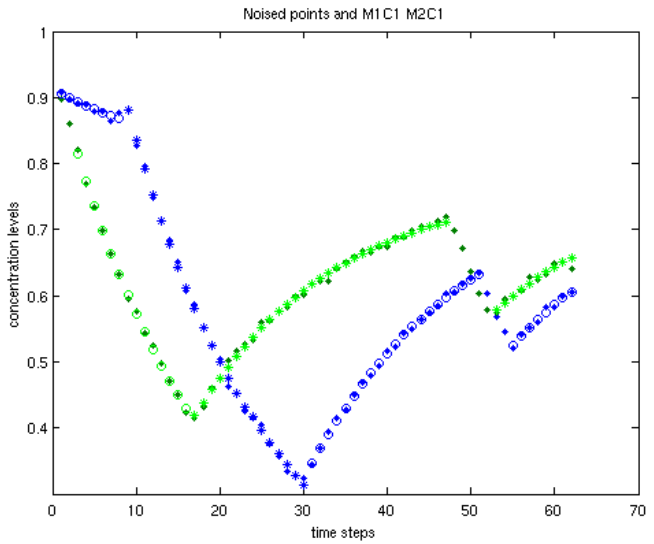

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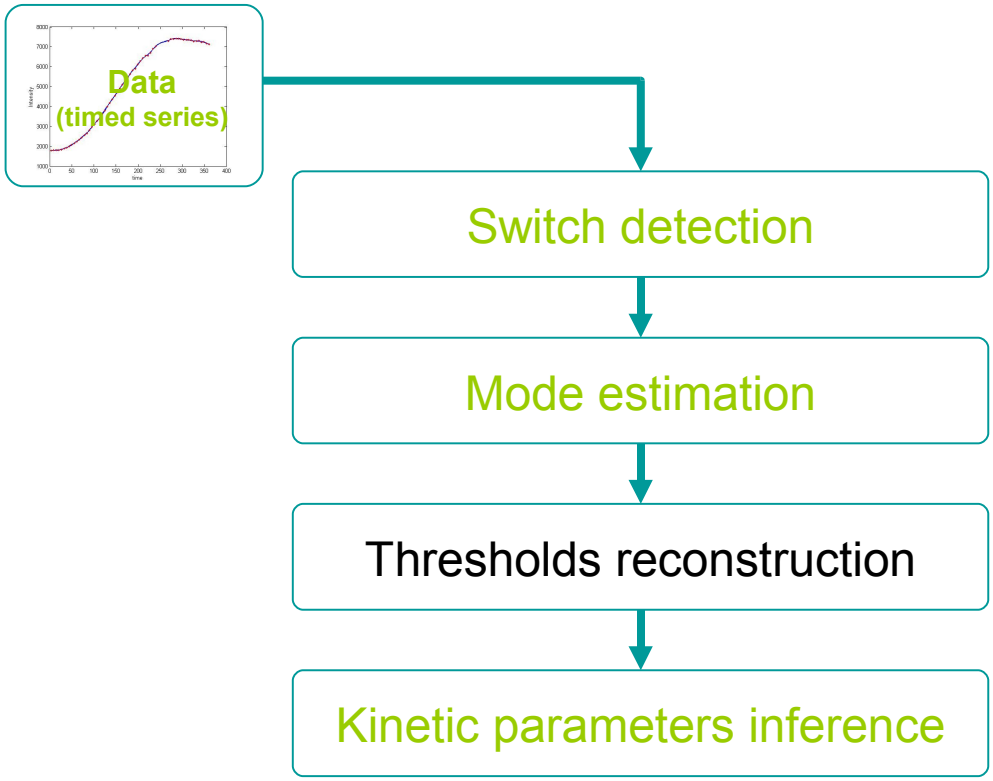
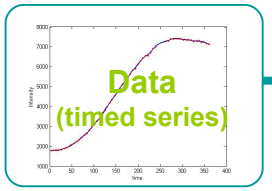


3) Reconstruction of

- thresholds on concentration variables
- all “minimal” combinations of thresholds consistent with the data

(Drulhe et al., HSCC 2005)

Identification of PWA models of GRNs



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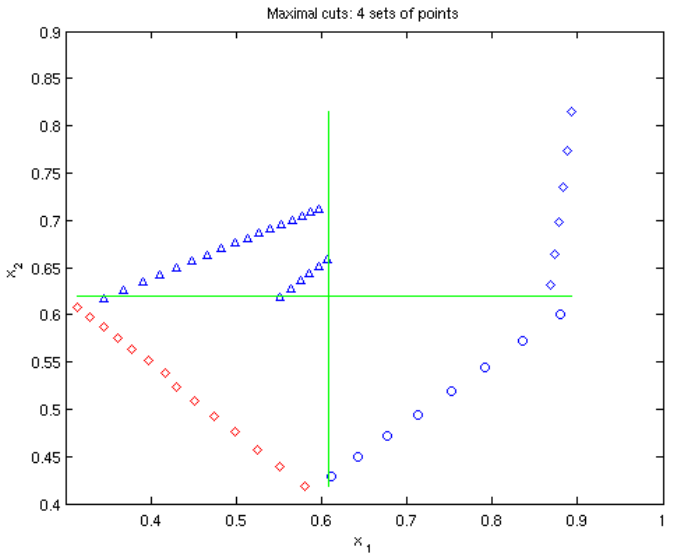

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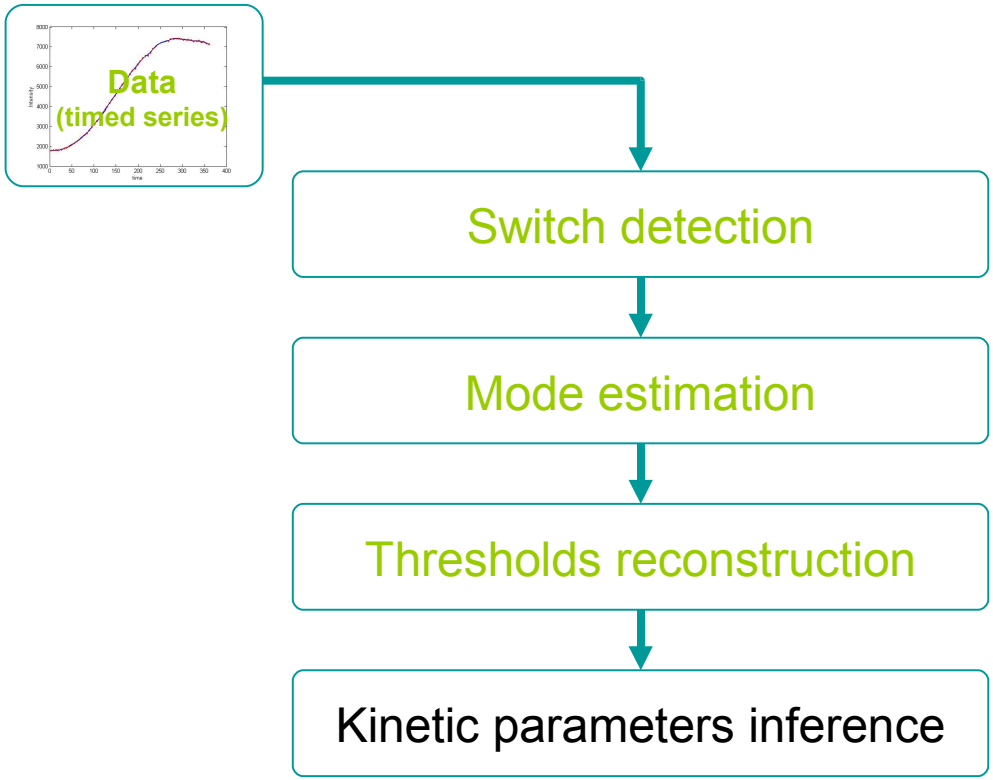

4) Estimation of kinetic parameters for all models generated at previous stage

It is easy: LS on each mode data set.

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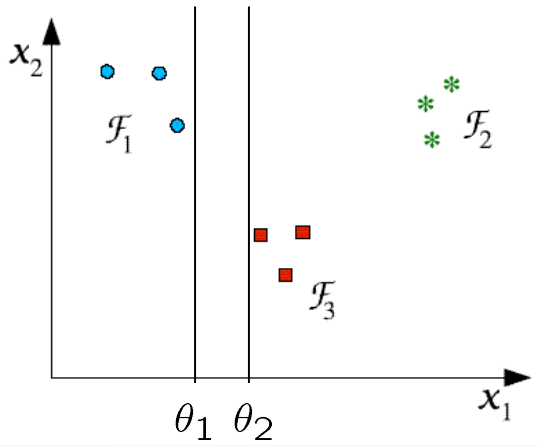
Switching thresholds as ap-hyperplanes

- An ap-hyperplane has a supporting vector parallel to one axis.
  - The label of the axis is the direction of the ap-hyperplane.
- The separation power $S(\theta)$ of an ap-hyperplane θ describes the separated data sets.
- Two ap-hyperplanes with a same direction and a same separation power are equivalent (thus defining equivalence classes of ap-hyperplanes).

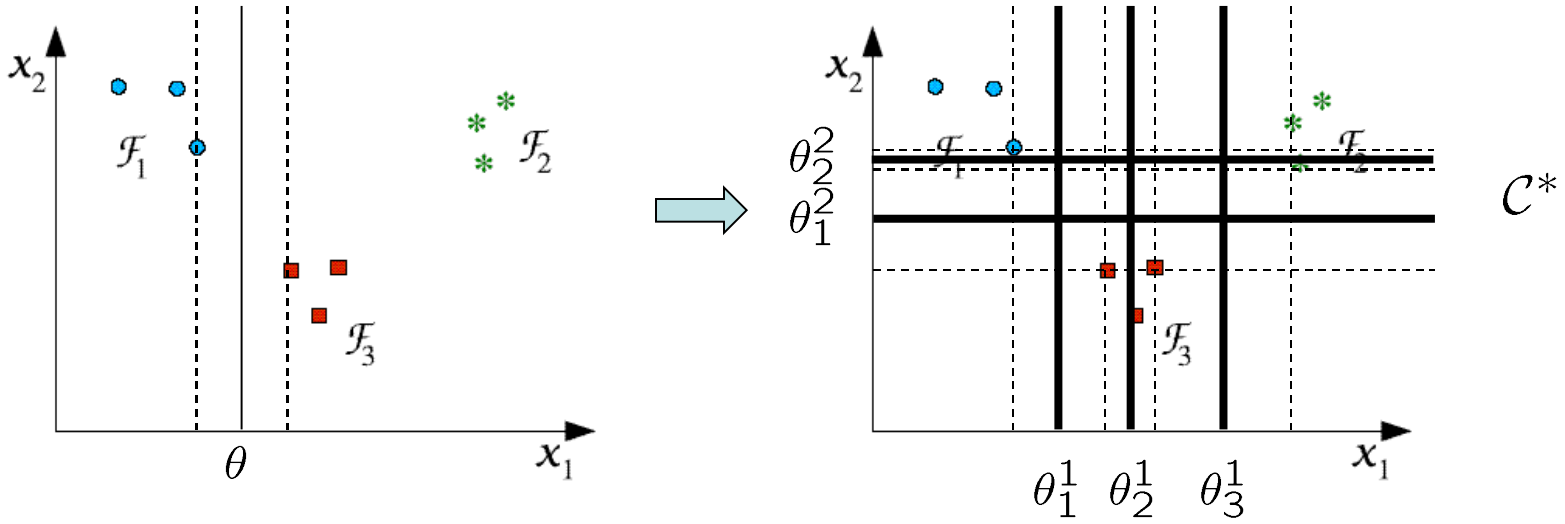


$$\begin{cases} S(\theta_1) = S(\theta_2) = \{(1,2),(1,3)\} \\ dir(\theta_1) = dir(\theta_2) = 1 \end{cases}$$

$$\Rightarrow \theta_1 \sim \theta_2$$

Switching thresholds and cuts

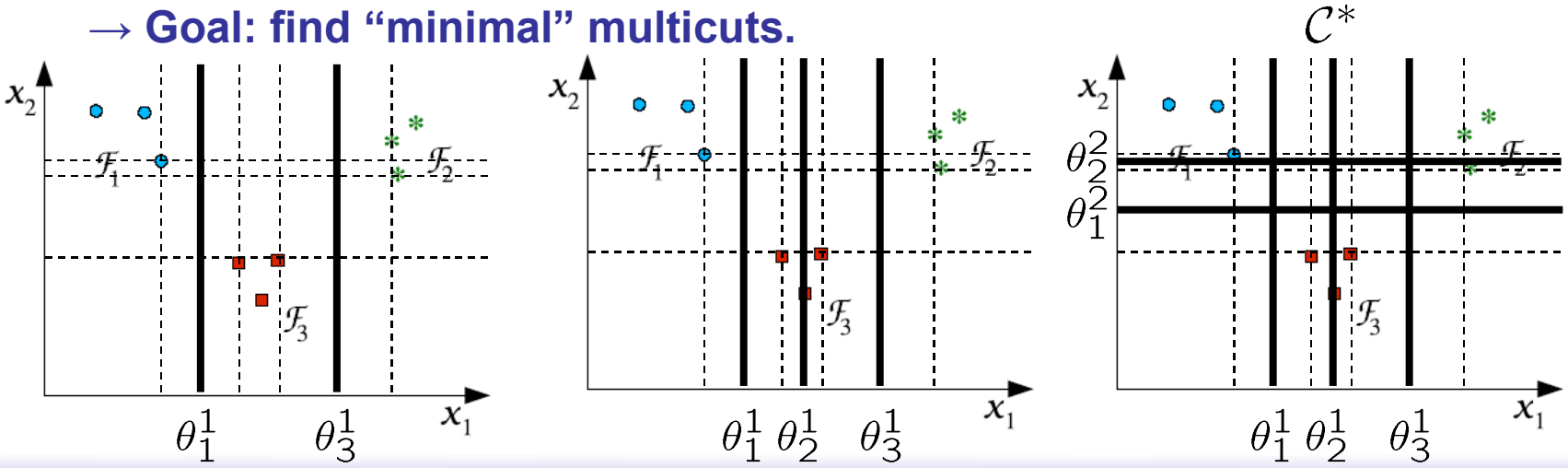
- For each class of equivalence, the ap-hyperplane that minimizes the empirical risk (i.e. that lies in the middle of the equivalence class) is a cut.
- The collection of all cuts $\mathcal{C}^*$ can be easily computed.
- Standing assumption: all pairs of sets are separated by at least one cut in $\mathcal{C}^*$



Switching thresholds and multicuts

- A collection of cuts such that any couple of data sets is separated by at least one of them is called a multicut.
- Any multicut is a collection of cuts that are sufficient to explain the observed dynamics. It provides a possible model of the genetic regulatory network.
- We are interested in finding models with a minimal number of interactions

→ **Goal: find "minimal" multicuts.**



Minimal multicuts

Assuming one can enumerate all possible multicuts from $\mathcal{C}^*$, a multicut is minimal if it has the *minimal cardinality*.

- Computation:
  - Rough idea: find all minimal multicuts by enumerating all multicuts ➔ combinatorial explosion!
  - Combinatorial optimization: one need a strategy to bound the search

- Mathematical ideas:
  - A cut is required if it is the only one to separate at least two mode data sets.
  - A cut is superfluous if all the required cuts separate at least all the same data sets as it does.

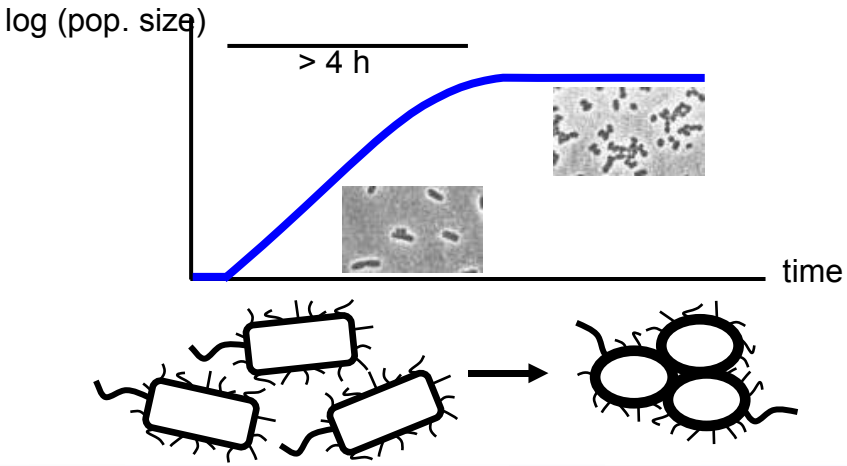
Overview

- Genetic regulatory networks in brief
- Identification for PWA models of genetic regulatory networks
- PWA system identification
- Description of our approach
- Reconstruction of switching thresholds
- A case study: carbon starvation response of *E. coli*
- Conclusions

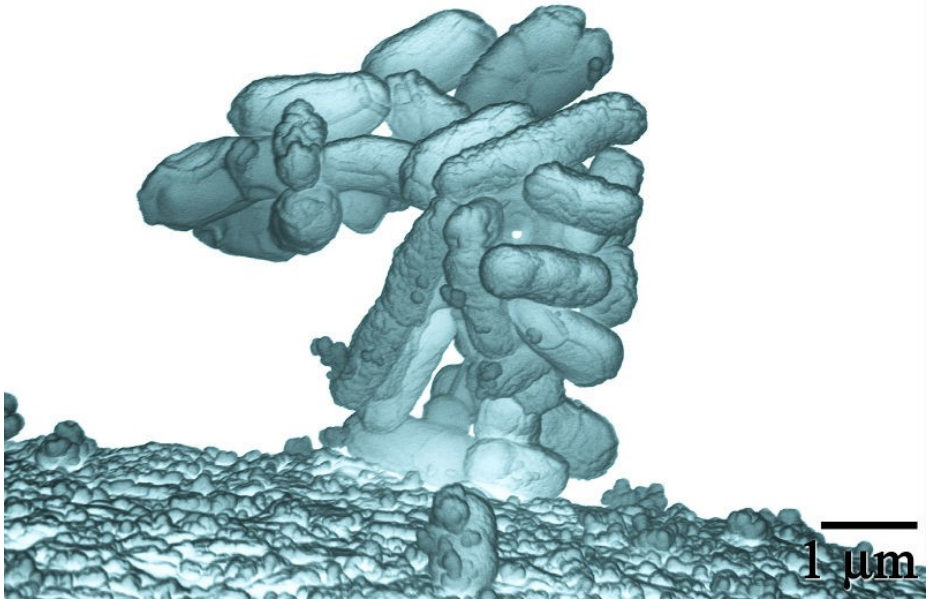
Application to *E.coli* carbon starvation response

Transitions from exponential to stationary phase involve observable changes in:

- morphology,
- metabolism,
- gene expression, …



Escherichia coli



Low-temperature electron micrograph of a cluster of E. coli bacteria, magnified 10,000 times. Photo by Eric Erbe, digital colorization by Christopher Pooley, both of USDA, ARS, EMU.

Application to *E.coli* carbon starvation response

Simplified PWA model of the response of *E. coli* to nutritional stress:

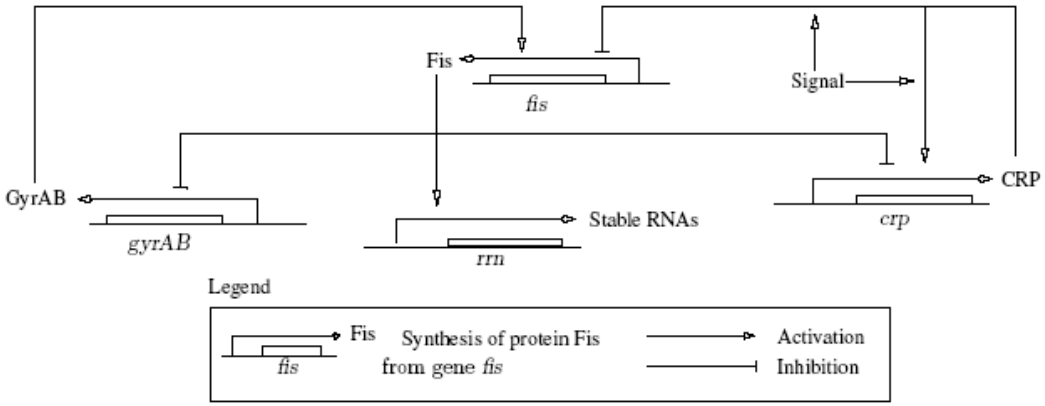
$$\dot{x}_{CRP} = \kappa^0_{CRP} + \kappa^1_{CRP}\, s^-(x_{Fis}, \theta^1_{Fis})\, s^+(x_{CRP}, \theta^1_{CRP})\, s^+(x_S, \theta_S) - \gamma_{CRP}\, x_{CRP}$$

$$\dot{x}_{Fis} = \kappa^1_{Fis}\,(1 - s^+(x_{CRP}, \theta^1_{CRP})\, s^+(x_S, \theta_S)) + \kappa^2_{Fis}\, s^+(x_{GyrAB}, \theta_{GyrAB})\,(1 - s^+(x_{CRP}, \theta^1_{CRP})\, s^+(x_S, \theta_S)) - \gamma_{Fis}\, x_{Fis}$$

$$\dot{x}_{GyrAB} = \kappa_{GyrAB}\, s^-(x_{Fis}, \theta^3_{Fis}) - \gamma_{GyrAB}\, x_{GyrAB}$$

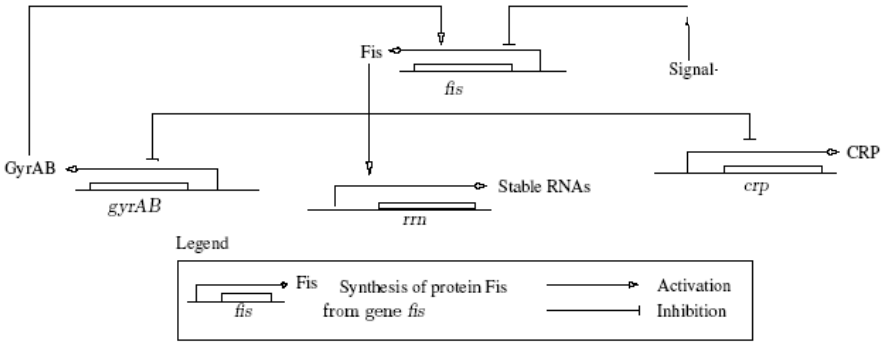
$$\dot{x}_{rrn} = \kappa_{rrn}\, s^+(x_{Fis}, \theta^2_{Fis}) - \gamma_{rrn}\, x_{rrn}$$

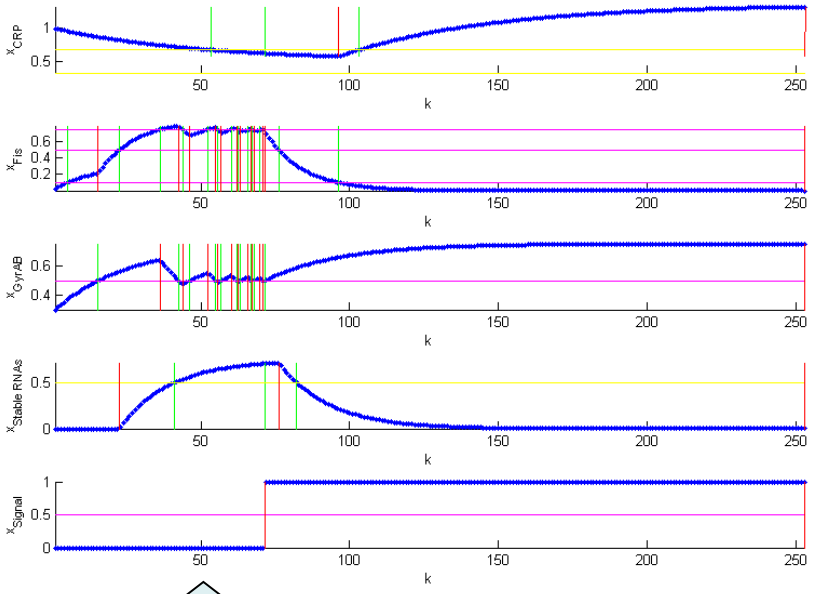
$$\dot{x}_S = 0$$



Application to *E.coli* carbon starvation response

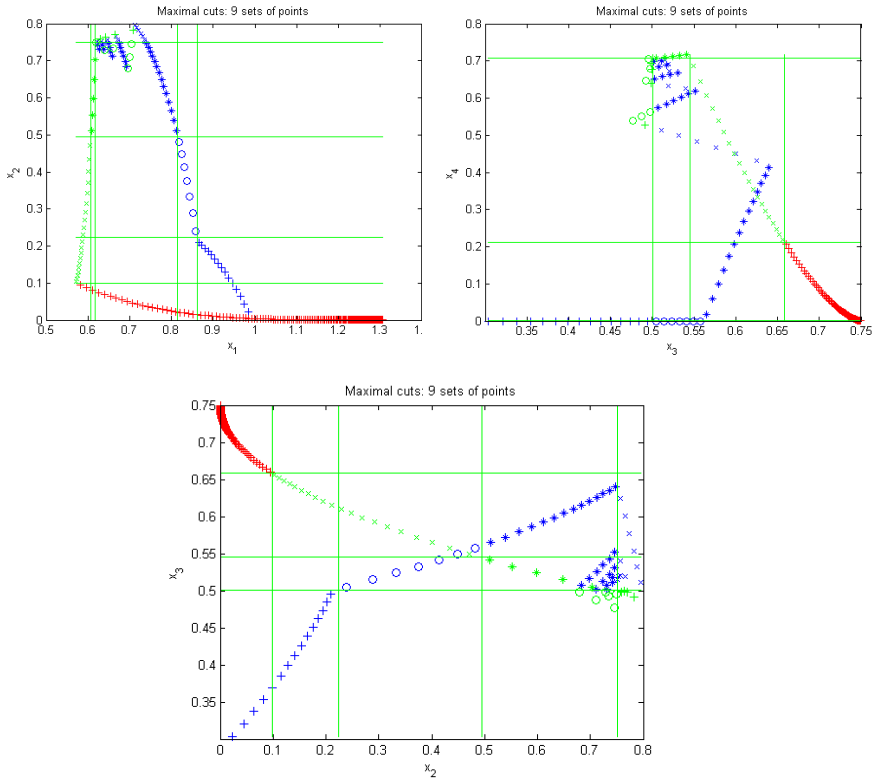
→ reconstruction of the interactions from simulated data.





Switching threshold reconstruction

The simulated data correspond to transitions exp→stat and stat→exp.



Cut	Variable	Threshold value	Interaction	Correct? (Y/N)
#1	CRP	0.60	activator of the synthesis of Stable RNAs	
#2	CRP	0.61	activator of the synthesis of Fis	
#3	CRP	0.81	inhibitor of the synthesis of Stable RNAs	
#4	CRP	0.86	inhibitor of the synthesis of Fis	
#5	Fis	0.10	inhibitor of the synthesis of CRP	Y
#6	Fis	0.22	activator of the synthesis of Fis	
#7	Fis	0.49	activator of the synthesis of Stable RNAs	Y
#8	Fis	0.75	inhibitor of the synthesis of GyrAB, activator and inhibitor of the synthesis of Fis	Y
#9	GyrAB	0.50	activator and inhibitor of the synthesis of Fis and GyrAB	Y
#10	GyrAB	0.54	inhibitor of the synthesis of Stable RNAs	
#11	GyrAB	0.65	activator of the synthesis of CRP	
#12	Stable RNAs	8.8e-6	activator of the synthesis of Stable RNAs	
#13	Stable RNAs	0.21	inhibitor of the synthesis of CRP	
#14	Stable RNAs	0.70	inhibitor of the synthesis of Fis, activator of the synthesis of Stable RNAs	
#15	Signal	0.50	Inhibitor of the synthesis of Fis	Y

Switching threshold reconstruction

The simulated data correspond to transitions exp→stat and stat→exp.

Multicut composed of cuts #:	Correct? (Y/N)
2, 5, 7, 8, 9	N, Y, Y, Y, Y
2, 7, 8, 9, 11	N, Y, Y, Y, N
5, 7, 8, 9, 15	Y, Y, Y, Y, Y
7, 8, 9, 11, 15	Y, Y, Y, N, Y
7, 8, 9, 13, 15	Y, Y, Y, N, Y

For the best globally minimal multicuts, the multicut approach has inferred all the identifiable interactions from the data.

Cut	Variable	Threshold value	Interaction	Correct? (Y/N)
#1	CRP	0.60	activator of the synthesis of Stable RNAs	
#2	CRP	0.61	activator of the synthesis of Fis	
#3	CRP	0.81	inhibitor of the synthesis of Stable RNAs	
#4	CRP	0.86	inhibitor of the synthesis of Fis	
#5	Fis	0.10	inhibitor of the synthesis of CRP	Y
#6	Fis	0.22	activator of the synthesis of Fis	
#7	Fis	0.49	activator of the synthesis of Stable RNAs	Y
#8	Fis	0.75	inhibitor of the synthesis of GyrAB, activator and inhibitor of the synthesis of Fis	Y
#9	GyrAB	0.50	activator and inhibitor of the synthesis of Fis and GyrAB	Y
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#11	GyrAB	0.65	activator of the synthesis of CRP	
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#13	Stable RNAs	0.21	inhibitor of the synthesis of CRP	
#14	Stable RNAs	0.70	inhibitor of the synthesis of Fis, activator of the synthesis of Stable RNAs	
#15	Signal	0.50	Inhibitor of the synthesis of Fis	Y

Conclusions

- Reconstruction of the switching thresholds consistent with the data
- Computation of different minimal models that are sufficient to explain the observed switches
- Example demonstrates applicability of this approach

Work in progress:

- Evaluation of the performance of the processing chain
- Validate the results on the full network of *E.Coli* with gene expression data (reporter genes)
- Optimization

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