Identification of dynamical models of genetic networks

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Outline

- The problem of genetic network identification
- A traditional approach: Boolean networks
- Identification of Ordinary Differential Equation (ODE) models
  - The general problem
  - Linearization methods (steady-state, time series)
  - Boolean-like methods (time series)
- Identification of stochastic models: A quick view
- Conclusions

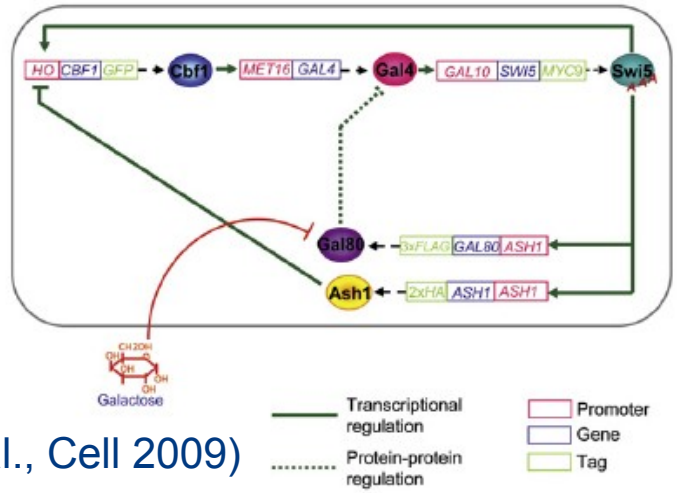
Myself

- Formation: Computer Engineering (Laurea), Automation (Ph.D.) (University of Padova, Italy)
- Post-Doc on identification of stochastic models of biological systems (and other stuff, ETH Zurich, Switzerland)
- Since November 2009, Research Scientist at INRIA (IBIS team, Grenoble – Rhône-Alpes)
  - Identification of nutrients stress response regulatory network in bacterium *Escherichia coli*
  - Methods for identification of genetic network dynamics

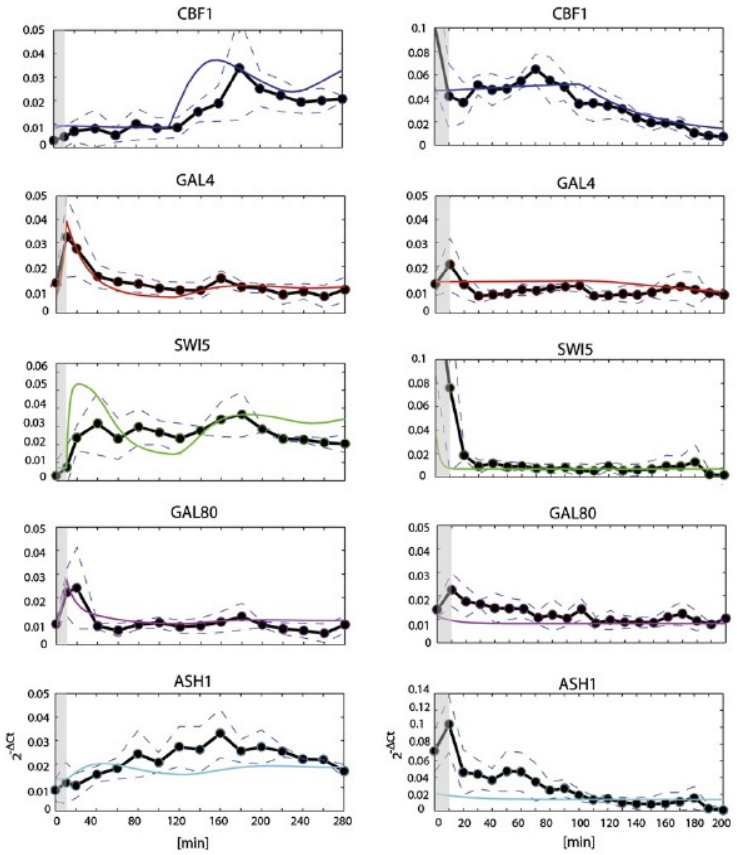
The problem of genetic network identification

Objective

- Determine a mathematical description of the structure and behavior of a network of genes
  - Structure: genes and their interconnection
  - Behavior: inhibition vs. activation, dynamics

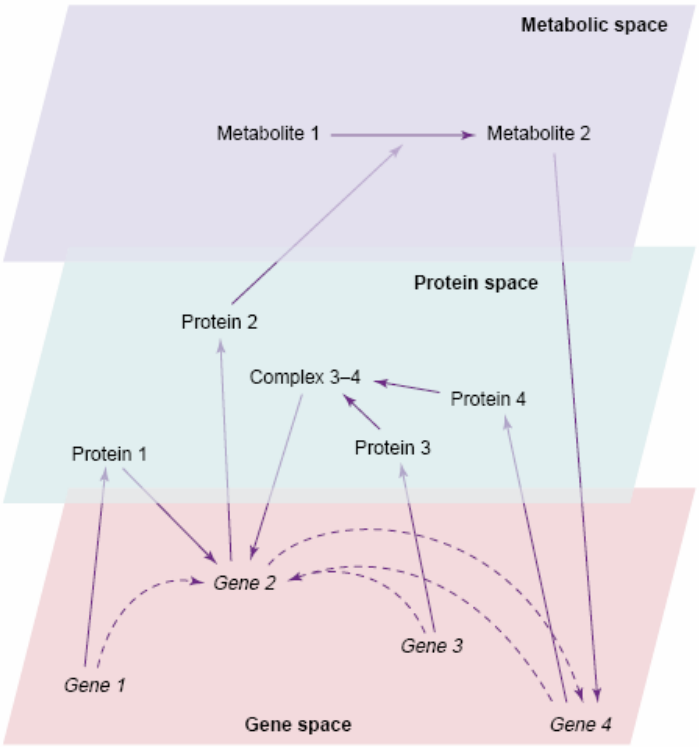


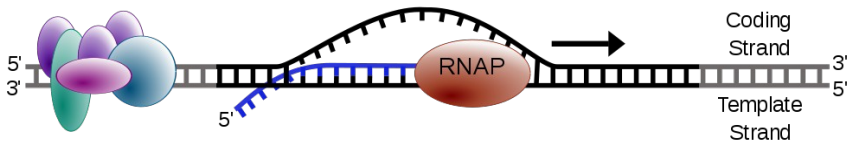
(Cantone et al., Cell 2009)



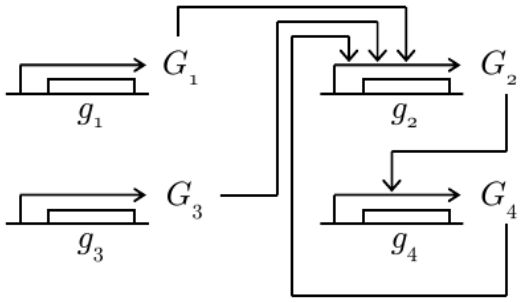
Scale

- Different levels of detail:
  - genes, but also mRNA, transcription factors, protein complexes...
  - expression: binding, DNA unfolding, transcription, translation, ...





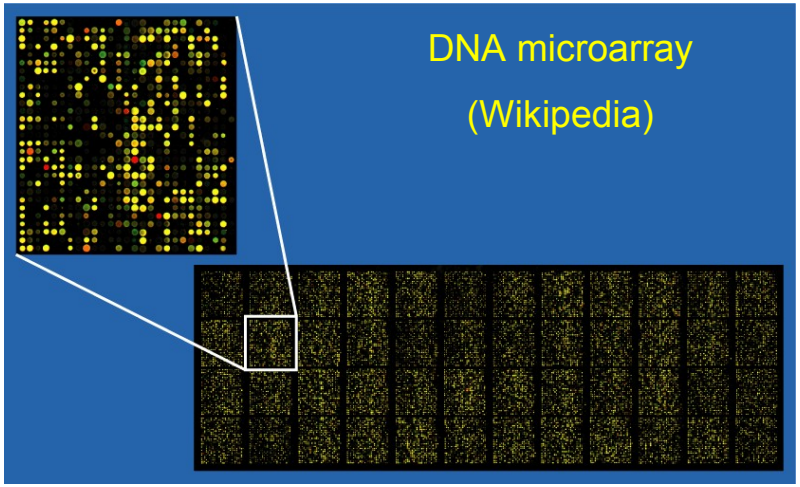
(Wikipedia)

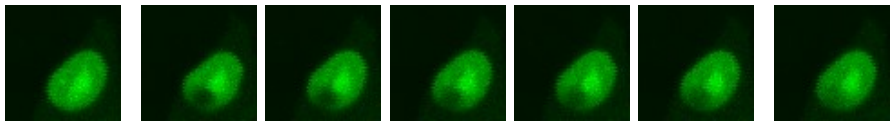


(Brazhnik et al., 2002)

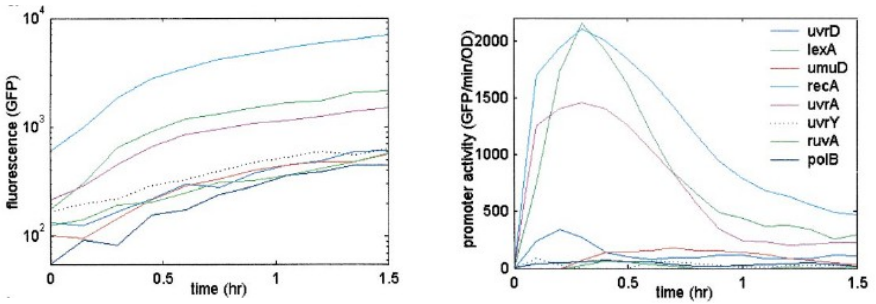
Information content

- Modelling framework depends on available data...
  - Type, quality, quantity
  - System excitation, experimental conditions





GFP fusions (courtesy of Z.Lygerou)

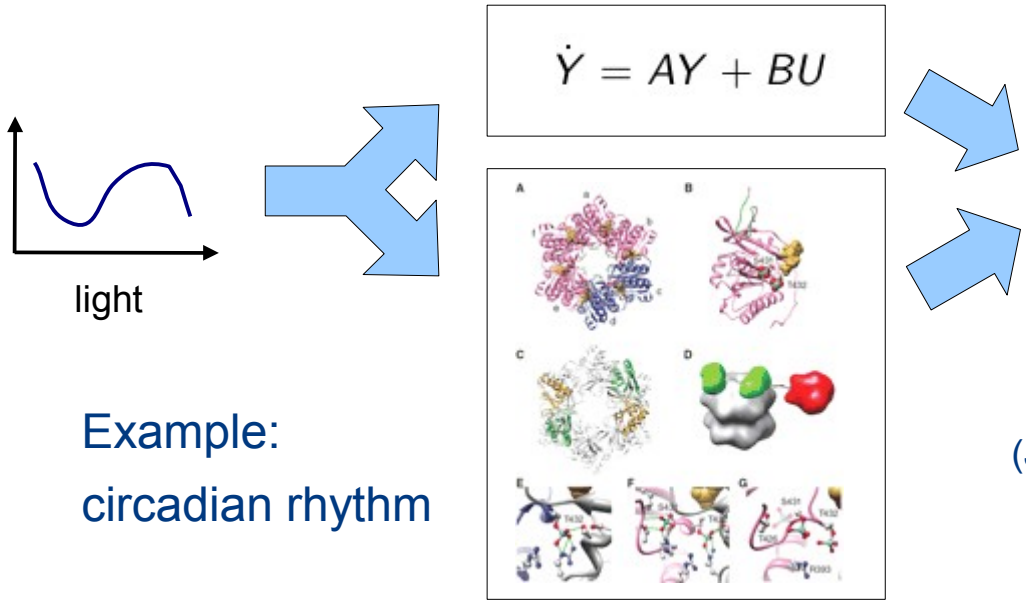


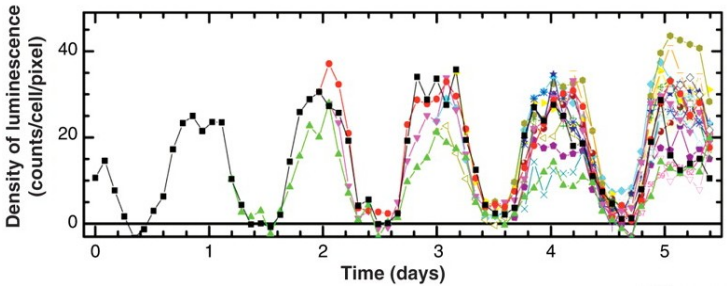
Gene reporter systems (Ronen et al, PNAS 2002)

- ... and on the use of the model
  - Understanding the functioning of a biological system
  - Prediction (response of an organism to perturbations/stimuli)
  - Control (industrial exploitation, targeted chemicals for medical therapies...)

Modelling: A world of tradeoffs

- Qualitative vs. quantitative
- Mechanistic vs. phenomenological
- Fitting accuracy vs. predictive power (overfitting!)
- Complexity vs. identifiability
- Static vs. dynamic
- Black-box vs. grey-box vs. white-box



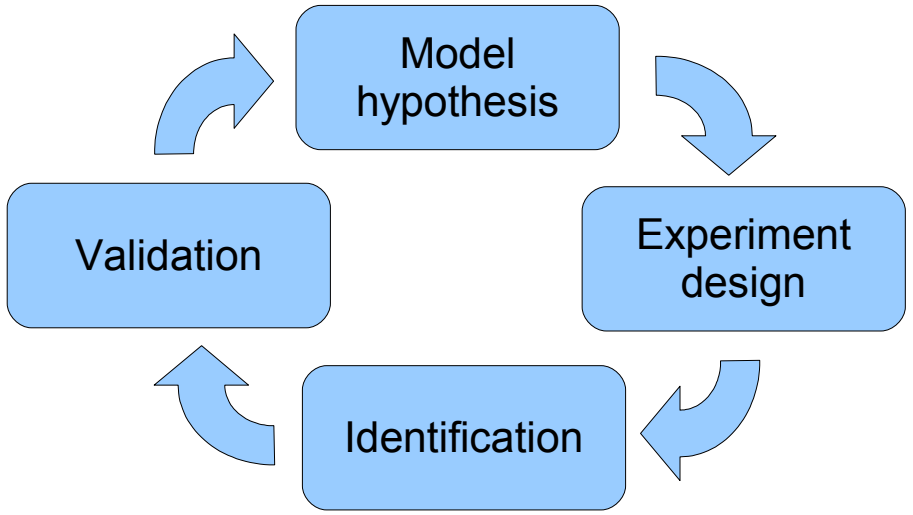


Example:
circadian rhythm

(Johnson et al, *Science,* 2008)

The identification circle

- Model hypothesis:
  - Choice of modelling framework
  - Application of first principles
  - Use of a priori knowledge
- Experiment design:
  - Address unknown model parts
  - Excite system in conditions appropriate for later use
- Identification
  - Collect data via experiment
  - Find model(s) that explains data
- Validation
  - Determine confidence level
  - Test model against new data



Today's focus: formal statement of gene network inference problems and solution with selected methods

A traditional approach: Boolean networks

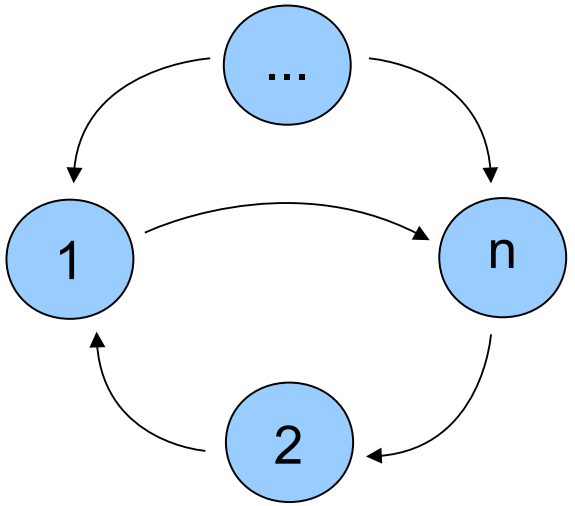
Boolean models

- N Boolean variables representing n genes

$$(X_1, X_2 \ldots, X_n) \in \{0,1\}^n$$

$X_i = 0$ gene not expressed

$X_i = 1$ gene expressed



- Boolean regulation function

$$X_i \text{ expressed iff } b_i(X) = 1$$

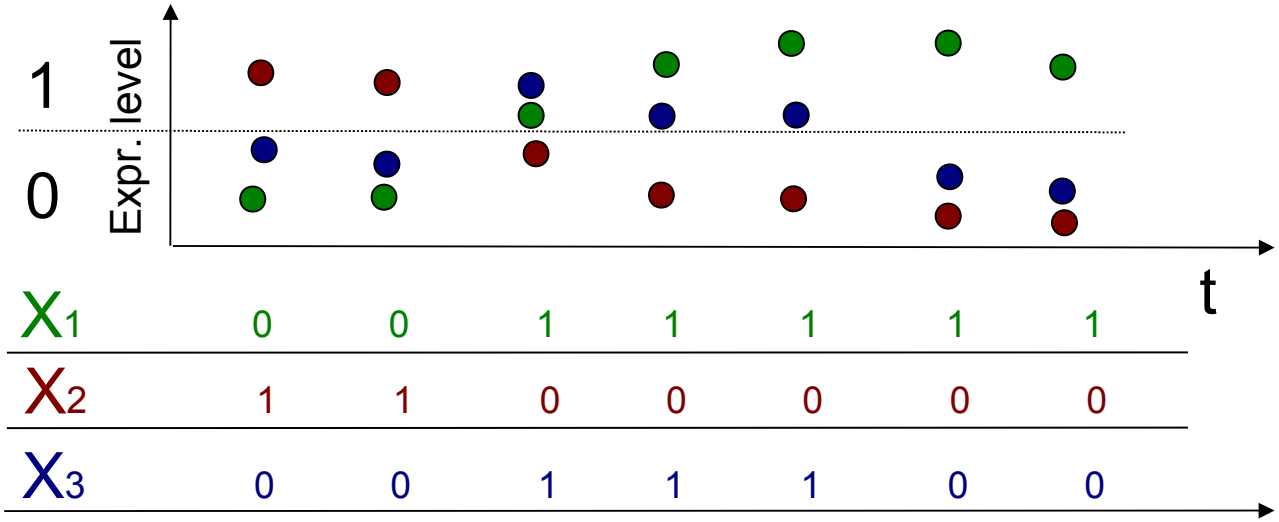
- Dynamic Boolean networks (discrete time):

$$X_i(t+1) = b_i(X(t)) \qquad i = 1, \ldots, n \qquad t = 0, 1, 2, \ldots$$

- Can associate regulatory interaction graph
  - n nodes (genes), arcs (incoming arcs of node i = effective inputs of b_i)

Identification

- Description of qualitative gene expression data



- Approximation of quantitative data
- Discrete math & graph theory for analysis of stability, oscillations, ...
- Learning of regulation rules from transitions observed in the data

REVerse Engineering ALgorithm

(Liang et al, 1998)

- Based on information-theoretic concepts

$X_1, \ldots, X_n$ random variables

$H(X_i)$ entropy ("variability") of X_i

$M(X_i, X_j)$ mutual information of X_i and X_j

generalizations to sets of variables

$$\frac{M(X_i, X_j)}{H(X_i)} \in (0, 1)$$

$0 = X_i$ is independent of X_j

$1 = X_i$ is fully determined by X_j

  - Functions of probability distribution of X
  - Estimated from the observed trajectories of X
  - Used to determine the effective inputs of a Boolean update map, e.g.

$$\text{If } \frac{M\big(X_1(t+1), [X_2(t), X_3(t)]\big)}{H\big(X_1(t+1)\big)} = 1 \text{ then } X_1(t+1) = b_1\big(X_2(t), X_3(t)\big)$$

  - Specific form of update map determined from the observed transitions
- May cope with noise (measurement error)
- Worst case: evaluation of all possible combinations of inputs
  - Bound complexity with maximum allowable number of inputs

Simulation example

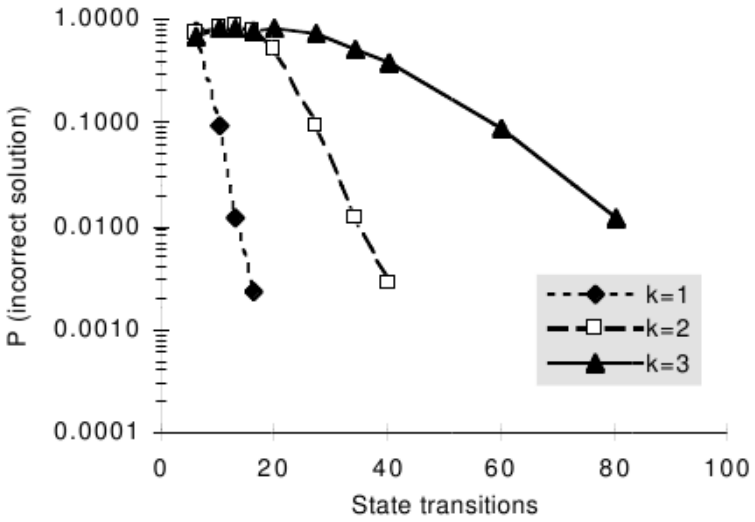
a

c

input			output		
A	B	C	A'	B'	C'
0	0	0	0	0	0
0	0	1	0	1	0
0	1	0	1	0	0
0	1	1	1	1	1
1	0	0	0	1	0
1	0	1	0	1	1
1	1	0	1	1	1
1	1	1	1	1	1

b

A' = B
B' = A or C
C' = (A and B) or (B and C) or (A and C)



Input entropies

H(A)	1.00
H(B)	1.00
H(C)	1.00
H(A,B)	2.00
H(B,C)	2.00
H(A,C)	2.00
H(A,B,C)	3.00

$$H(X) = -\sum p(x) \log p(x)$$
$$H(X,Y) = -\sum p(x,y) \log p(x,y)$$
$$M(X,Y) = H(X) + H(Y) - H(X,Y)$$
$$M(X,[Y,Z]) = H(X) + H(Y,Z) - H(X,Y,Z)$$

Determination of inputs for element A ①

H(A')	1.00				
H(A',A)	2.00	M(A',A)	0.00	M(A',A) / H(A')	0.00
H(A',B)	1.00	M(A',B)	1.00	**M(A',B) / H(A')**	**1.00**
H(A',C)	2.00	M(A',C)	0.00	M(A',C) / H(A')	0.00

② **Rule table for A**
rule no. 2

input	output
B	A'
0	0
1	1

Determination of inputs for element B ③

H(B')	0.81				
H(B',A)	1.50	M(B',A)	0.31	M(B',A) / H(B')	0.38
H(B',B)	1.81	M(B',B)	0.00	M(B',B) / H(B')	0.00
H(B',C)	1.50	M(B',C)	0.31	M(B',C) / H(B')	0.38
H(B',[A,B])	2.50	M(B',[A,B])	0.31	M(B',[A,B]) / H(B')	0.38
H(B',[B,C])	2.50	M(B',[B,C])	0.31	M(B',[B,C]) / H(B')	0.38
H(B',[A,C])	2.00	M(B',[A,C])	0.81	**M(B',[A,C]) / H(B')**	**1.00**

④ **Rule table for B**
rule no. 14

input		output
A	C	B'
0	0	0
0	1	1
1	0	1
1	1	1

Determination of inputs for element C ⑤

H(C')	1.00				
H(C',A)	1.81	M(C',A)	0.19	M(C',A) / H(C')	0.19
H(C',B)	1.81	M(C',B)	0.19	M(C',B) / H(C')	0.19
H(C',C)	1.81	M(C',C)	0.19	M(C',C) / H(C')	0.19
H(C',[A,B])	2.50	M(C',[A,B])	0.50	M(C',[A,B]) / H(C')	0.50
H(C',[B,C])	2.50	M(C',[B,C])	0.50	M(C',[B,C]) / H(C')	0.50
H(C',[A,C])	2.50	M(C',[A,C])	0.50	M(C',[A,C]) / H(C')	0.50
H(C',[A,B,C])	3.00	M(C',[A,B,C])	1.00	**M(C',[A,B,C]) / H(C')**	**1.00**

⑥ **Rule table for C**
rule no. 170

input			output
A	B	C	C'
0	0	0	0
0	0	1	0
0	1	0	0
0	1	1	1
1	0	0	0
1	0	1	1
1	1	0	1
1	1	1	1

Discussion

- Well established analysis/identification methods
- Large understanding of dynamic effects of Boolean maps
- Effective network reconstruction for qualitative data
- Wasteful use of quantitative data due to discrete approximation:
  New experimental techniques allow for more!

Identification of ODE models

The model family

- Vector of concentrations: $x = (x_1, \ldots, x_n) \in \mathbb{R}^n_{\geq 0}$
- ODE model: $\dot{x}_i = f_i(x, u, \theta) - \Gamma_i(x, u, \theta)$

$f_i \geq 0$ synthesis rate functions

$\Gamma_i \geq 0$ degradation rate functions

$\theta \in \Theta$ unknown parameters (and structure)

$u(t)$ perturbation input

- Depending on the identification approach and on the data, specific (parametric) form for rate functions
- Common choice: unregulated degradation

$$\Gamma_i(x_i) = \gamma_i x_i$$

Model family: examples

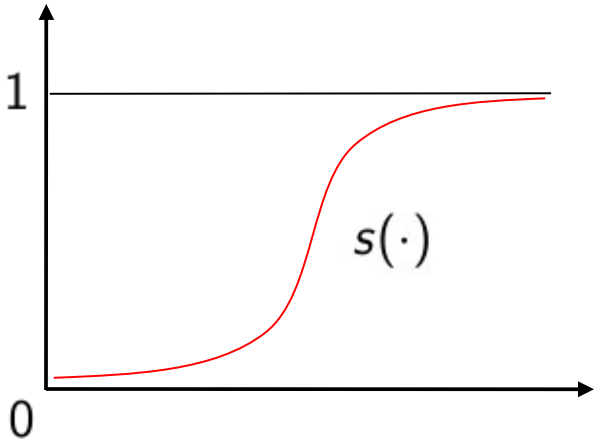
- Linear model plus saturation (Jaeger et al, Nature 2004):

$$f_i = s_i\left(\sum_j A_{i,j} x_j + b_i\right)$$

s_i sigmoidal functions

$A_{i,j}$ gene connectivity matrix

b_i basal expression rate



- Piecewise affine models (Glass & Kauffman, 1973, de Jong, ...):

$$f_i = \kappa_i^j, \quad x \in \Delta_j$$

$\{\Delta_j\}$ hyperrectangular partitioning of $\mathbb{R}^n$

The data

- Measurement model

$$y_i(t) = h_i\big(x_i(t), e\big), \qquad \begin{cases} h_i & \text{output function} \\ e & \text{(random) measurement noise} \end{cases}$$

(not always used in full detail)

- Data set

$$\mathcal{D} = \{y^m(t_k) : \ k = 1, \ldots, K, \ m = 1, \ldots, M\}$$

K measurement times

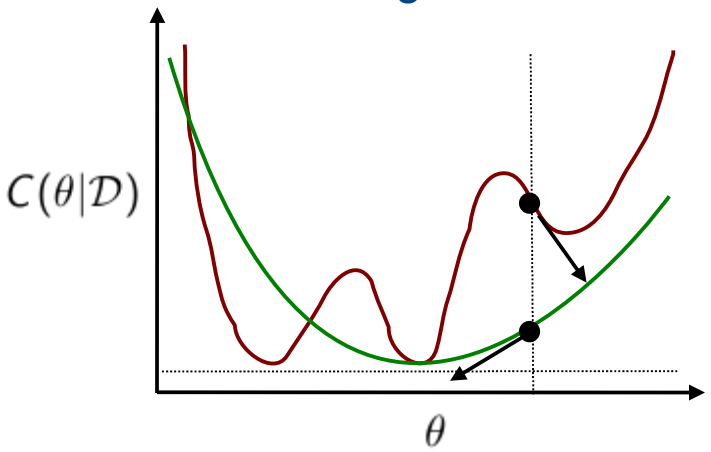
M time series (possibly different inputs)

The problem

- Identification: find “the best” model of the data in a family of alternatives
- Typical formulation: optimization of a (problem-dependent) cost function

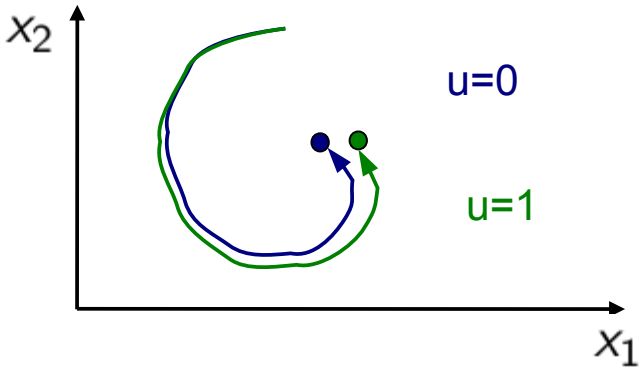
$$\text{minimize } C(\theta|\mathcal{D}) \text{ with respect to } \theta \in \Theta$$

- Cost function describes the ability of a model to explain the data
  - Minimization of the data fitting error
  - Penalization of overly complicated models to avoid overfitting
- In general, cost function is non-convex
  - Non-uniqueness of the solution
  - Optimization heuristics are needed



Linearization methods: steady state

- Working assumption:
  - all concentrations converge to an equilibrium
  - small, fixed perturbations modify the system equilibrium
  - perturbations are known, equilibria can be measured



- What perturbations ?
  - Changes in concentration of chemicals in the medium
  - Gene knockout/overexpression

- Idea: infer local dynamics around unperturbed equilibrium from several known perturbations of the system

Linearized dynamics

- True dynamics without perturbation

$$\dot{x} = \phi(x, u), \qquad u(t) \equiv 0 \text{ implies } x(t) \to x^*$$

- Linearization about equilibrium

$$\frac{d}{dt}(x - x^*) = \phi(x, u) = D_x\phi(x^*)(x - x^*) + D_u\phi(x^*)u + \text{h.o.t.}$$

- Perturbed equilibria

$$u(t) \equiv \bar{u} \text{ implies } x(t) \to x^* + \bar{x}, \text{ where}$$

$$0 = D_x\phi(x^*)(x^* + \bar{x} - x^*) + D_u\phi(x^*)\bar{u} + h.o.t \simeq A\bar{x} + B\bar{u}$$

Identification of linearized model

- Perform repeated perturbation experiments until equilibrium

$$u(t) \equiv \bar{u}_m \text{ implies } x(t) \to \bar{x}_m, \quad m = 1, \ldots, M$$

- Collect observed results in data matrices

$$U = [\bar{u}_1, \ldots, \bar{u}_M], \quad Y = [\bar{y}_1, \ldots, \bar{y}_M], \text{ where } \bar{y}_m = \bar{x}_m + e_m$$

- Solve the least-squares problem

$$\text{minimize} \quad ||AY + BU|| \quad \text{with respect to } A$$

- Solution well defined if B known and M large enough

Discussion

- A is network regulation matrix, B is (known?) perturbation effect

  $A_{i,j} > 0$ gene j induces expression of gene i $(x_j \uparrow \Longrightarrow x_i \uparrow)$

  $A_{i,j} < 0$ gene j inhibits expression of gene i $(x_j \uparrow \Longrightarrow x_i \downarrow)$

  $A_{i,j} = 0$ gene j is not affected by gene i (x_j indep. of x_i)

- Explicit solution (Frobenius norm):

$$\widehat{A} = BUY^T(YY^T)^{-1}$$

  warning: no zero elements (Overfitting !)

- Penalization of complexity: several semi-empirical strategies

$$\begin{aligned} \min \quad & ||AY + BU|| \\ \text{s.t.} \quad & \sum_j \mathbf{1}(A_{i,j} \neq 0) \leq n_{max} \ \forall i \end{aligned}$$

$$\begin{aligned} \min \quad & \sum_{i,j} |A_{i,j}| \\ \text{s.t.} \quad & ||AY + BU|| \leq \epsilon \end{aligned}$$

Linearization methods: Time Series Network Identification

- Assumes linear dynamics (system evolving near equilibrium)

$$\frac{d}{dt}(x - x^*) = A(x - x^*) + Bu$$

- Allows for time-dependent (small) perturbation experiments
- Attempts to solve the equation

$$\dot{Y} = AY + BU$$

with the following time-course data (from a single experiment)

$$Y = [y(t_1), \ldots y(t_K)], U = [u(t_1), \ldots, u(t_K)], \quad y(t_k) = x(t_k) - x^* + e_k$$

- In practice derivatives not known, resort to discretized dynamics

Identification from time-series

- Discretized linear dynamics (equidistant measurement samples)

$$x(t_{k+1}) = A^d x(t_k) + B^d u(t_k)$$

- Solution of the approximate equality

$$Y^+ = [A^d \; B^d] \begin{bmatrix} Y^- \\ U \end{bmatrix},$$

$$\begin{aligned} Y^+ &= [y(t_2), \ldots y(t_K)], \\ Y^- &= [y(t_1), \ldots y(t_{K-1})], \\ U &= [u(t_1), \ldots, u(t_{K-1})] \end{aligned}$$

- Also identifies perturbation matrix
- Regularized solution via Principal Component Analysis (PCA)
- Conversion to continuous-time network parameters

Principal Component Analysis

- Singular Value Decomposition (SVD) of a matrix

$$M \in \mathbb{R}^{p \times q}, \quad p < q$$

$$M = USV^T = \begin{bmatrix} U_1 & \cdots & U_p \end{bmatrix} \left[\begin{array}{ccc|c} s_1 & & & \\ & \ddots & & 0 \\ & & s_p & \end{array}\right] \begin{bmatrix} V_1^T \\ \vdots \\ V_q^T \end{bmatrix}$$

U, V orthogonal matrices, $s_1 \geq s_2 \geq \ldots \geq s_p \geq 0$

- PCA principle: eliminate contributions from smallest singular values

$$M = \sum_{i=1}^{p} s_i U_i V_i^T \simeq \sum_{i=1}^{r} s_i U_i V_i^T, \quad r < p$$

- i=1 , ... , r are called the principal components of M

PCA in linear regression

- Problem: find combination H of rows of M that is closest to Y^+:

$$\text{minimize} \quad ||Y^+ - HM||, \qquad H = [A^d \; B^d], \quad M = \begin{bmatrix} Y^- \\ U \end{bmatrix}$$

- Idea: exploit PCA to project Y^+ on the approximate row space of M
- Define:

$$H = Y^+ V S^\dagger U^T, \quad S^\dagger = \begin{bmatrix} s_1^{-1} & & & & \\ & \ddots & & & \\ & & s_r^{-1} & & \\ & & & 0 & \\ & & & & \ddots \\ & & & & & 0 \\ 0 & & & & & \end{bmatrix} \quad \text{s.t.} \; S^\dagger S = \begin{bmatrix} 1 & & & & \\ & \ddots & & & \\ & & 1 & & \\ & & & 0 & \\ & & & & \ddots \\ & & & & & 0 \\ 0 & & & & & \end{bmatrix}$$

Then:

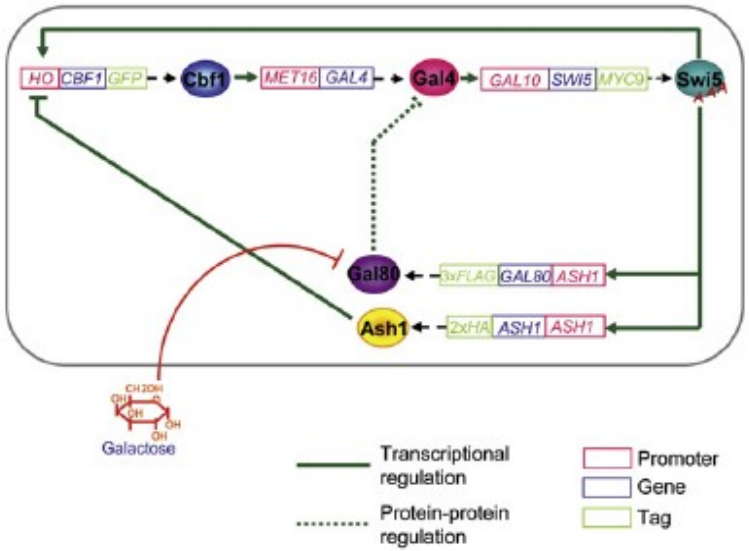
$$H \cdot M = (Y^+ V S^\dagger U^T) \cdot (U S V^T) = Y^+ V S^\dagger S V^T = \sum_{i=1}^{r} (Y^+ \cdot V_i) \cdot V_i^T$$

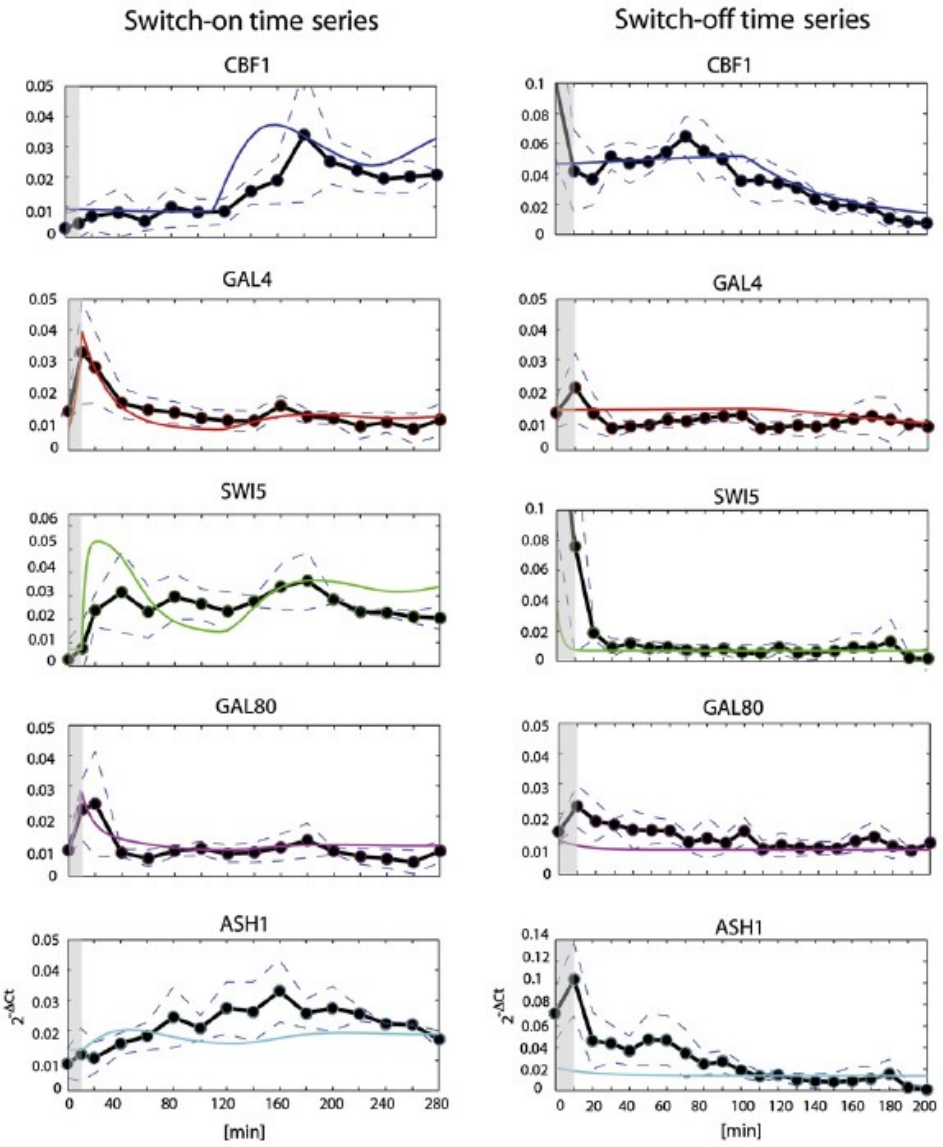
- Low-rank solution, elimination of noise (non-principal components)

Experiment

Synthetic gene regulatory network in Yeast

(Cantone et al., Cell 2009)





Results

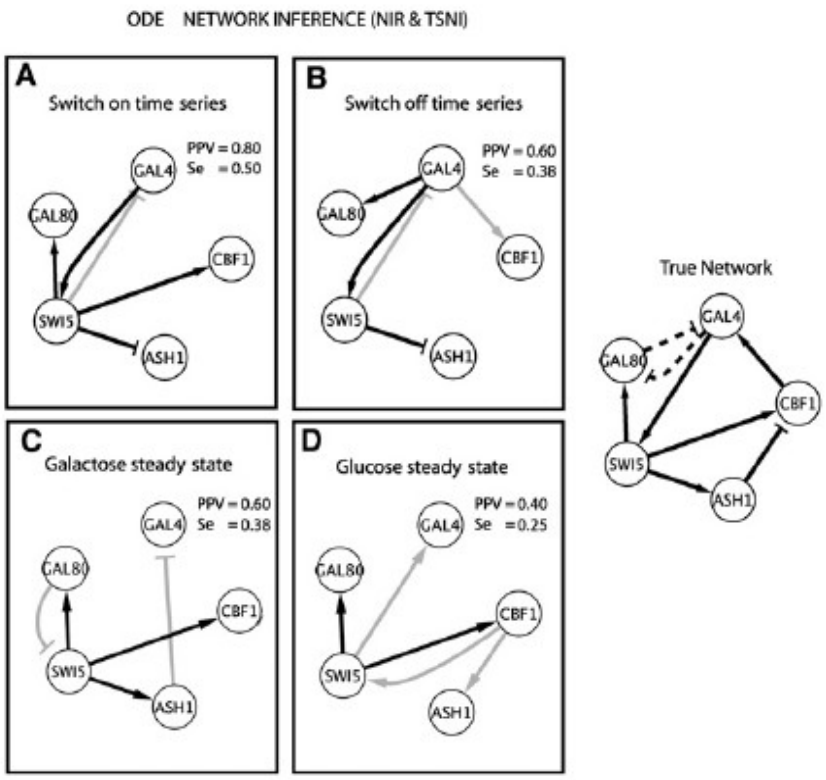


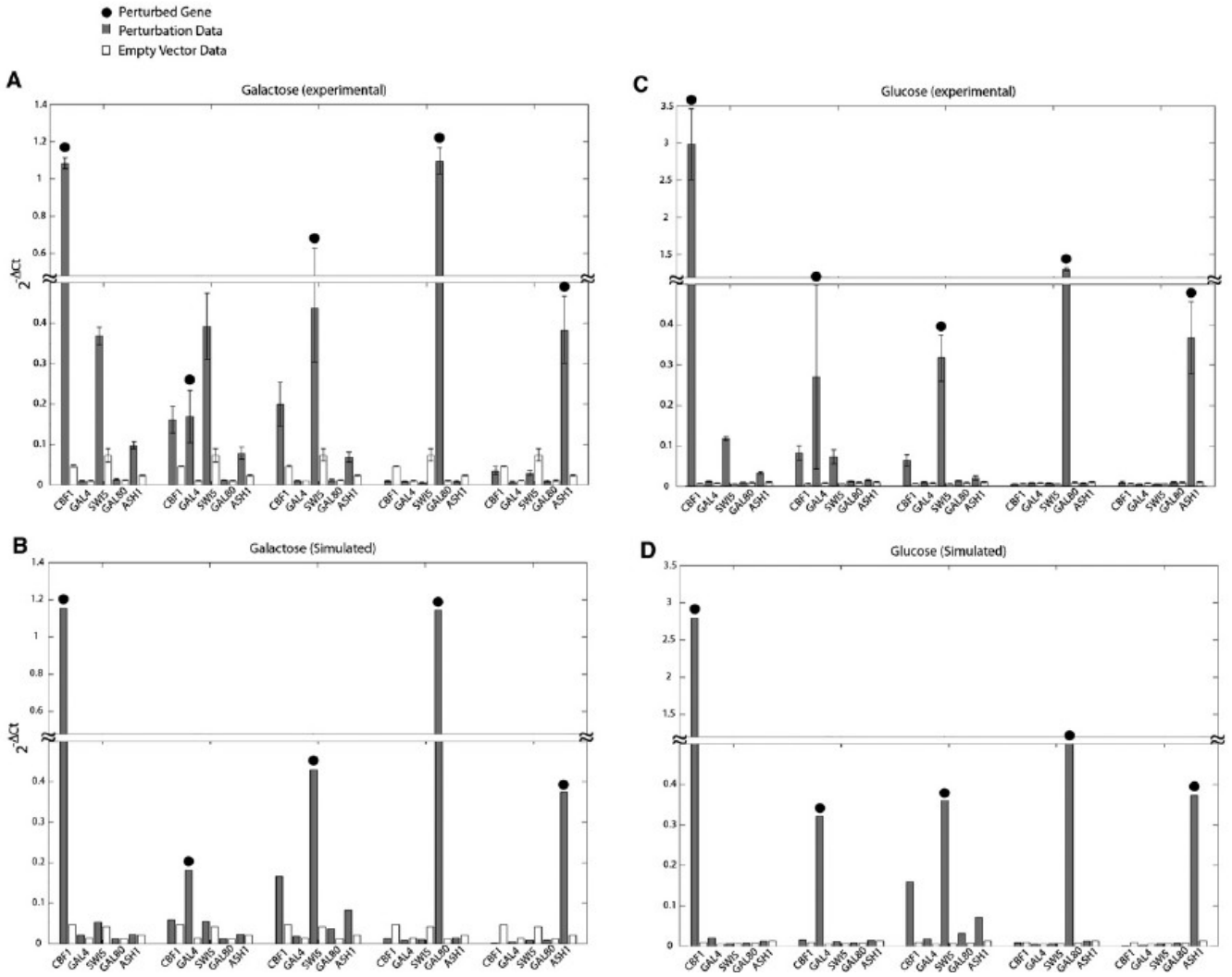
Figure 5. Reverse Engineering of the IRMA Gene Network from Steady-State and Time Series Experimental Data Using the ODE-Based Approach

The true network shows the regulatory interactions among genes in IRMA. Dashed lines represent protein-protein interactions. Directed edges with an arrow end represent activation, whereas a dash end represents inhibition.

(A and B) Inferred network using the TSNI reverse-engineering algorithm and the switch-on and switch-off time series experiments. Solid gray lines represent inferred interactions that are not present in the real network, or that have the wrong direction (FP, false positive). PPV [Positive Predictive Value = TP/(TP + FP)] and Se [Sensitivity = TP/(TP + FN)] values show the performance of the algorithm for an unsigned directed graph. TP, true positive; FN, false negative. The random PPV for the unsigned directed graph is equal to 0.40.

(C and D) Inferred network using the NIR reverse-engineering algorithm and the steady-state experimental data from network gene overexpression in cells grown in galactose or glucose medium, respectively.

Qualitative validation



Boolean-like methods

- Recall Boolean update map:

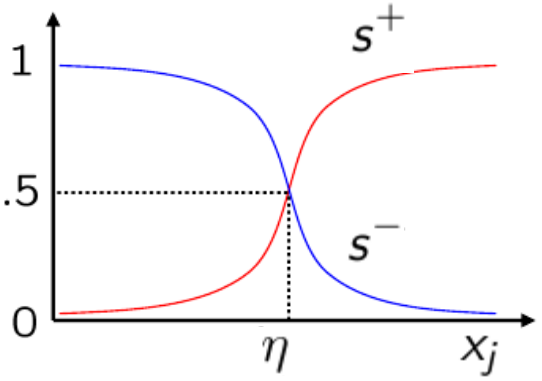
$$X_i^+ = b_i(X), \quad \text{where } b_i = \bigvee_l \bigwedge_j X'_{l,j}, \quad X'_{l,j} \in \{X_j, \neg X_j\}$$

- Algebraic equivalent (Plahte et al, 1998): apply the transformation

$$\begin{aligned} X_j &\rightarrow \sigma^+(x_j) \\ \neg\, expr(X) &\rightarrow 1 - expr(x) \\ expr(X) \wedge expr'(X) &\rightarrow expr(x) \cdot expr'(x) \end{aligned}$$

$$s^+(x_j) = \frac{x_j^d}{x_j^d + \eta^d}$$

$$s^-(x_j) = 1 - s^+(x_j)$$

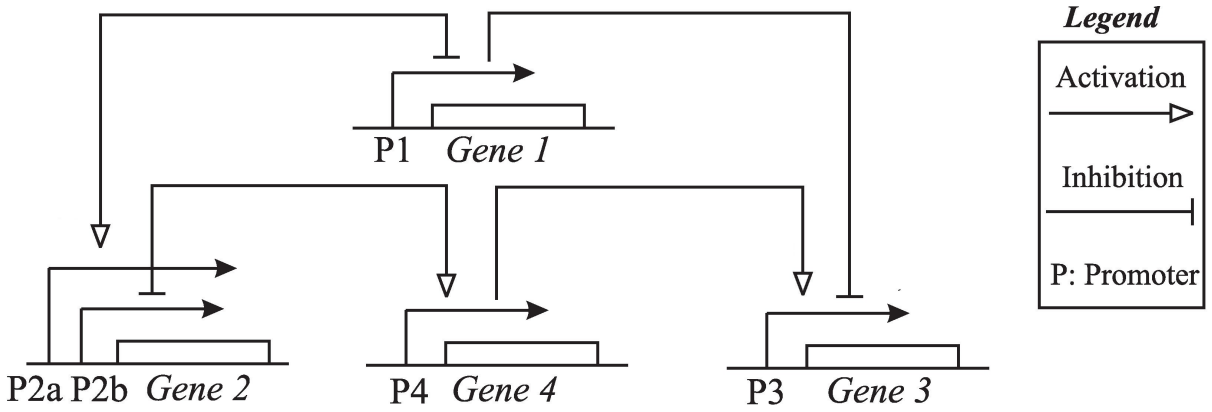


- Boolean-like model: define ODE

$$\dot{x}_i = \kappa_{0,i} + \kappa_{1,i} b_i(x) - \gamma_i x_i$$

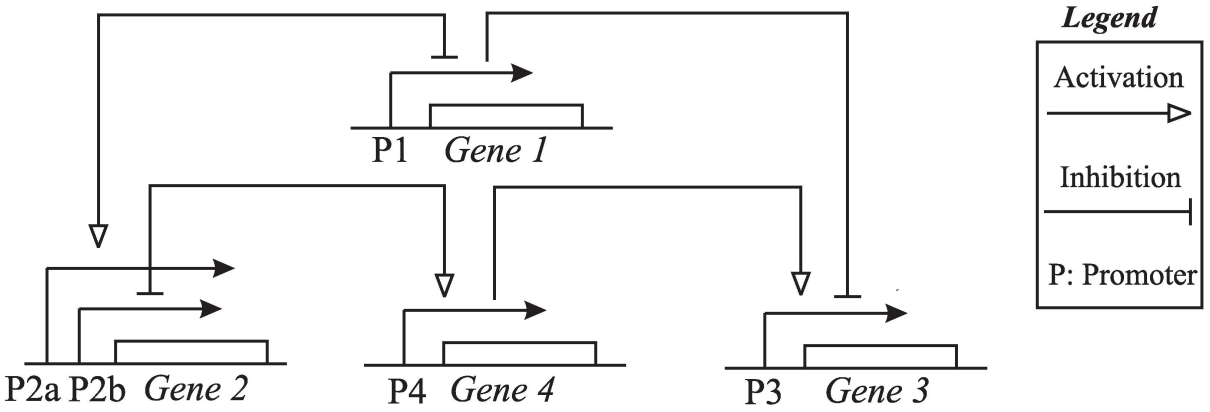
$b_i(x)$ algebraic equivalent of $b_i(X)$

Example (Boolean model)



Gene	Expressed when	Boolean model
1	G2 not expressed	$b_1(X) = \neg X_2$
2	G1 expressed or G4 not expressed	$b_2(X) = X_1 \vee \neg X_4$
3	G4 expressed and G1 not expressed	$b_3(X) = X_4 \wedge \neg X_1$
4	G2 expressed	$b_4(X) = X_2$

Example (Boolean-like ODE)



Gene	More active when
1	G2 low
2	G1 high or G4 low
3	G4 high and G1 low
4	G2 high

ODE model

$$b_1(x) = s^-(x_2)$$
$$b_2(x) = 1 - s^-(x_1) \cdot s^+(x_4)$$
$$b_3(x) = s^+(x_4) \cdot s^-(x_1)$$
$$b_4(x) = s^+(x_2)$$

Plausibility ?

- Experimental evidence that *often* (Gjuvsland et al, 2007)
  - Transcription factors combine into Boolean-like input functions
  - Sigmoidal functions relate transcription factor concentrations and transcription rates
  - Post-transcriptional, transport, (and reaction) processes at equilibrium can be described by sigmoidal functions
- Still a phenomenological framework, but ...
  - Easy to interpret biologically
  - Accurate and flexible

Tractability ?

- General Boolean-like model:

$$\dot{x}_i = \kappa_i^1 + \kappa_i^2 b_i(x) - \gamma_i x_i, \quad \text{where } b_i = \sum_l \prod_j s^{\pm}(x_j|\theta_{l,j})$$

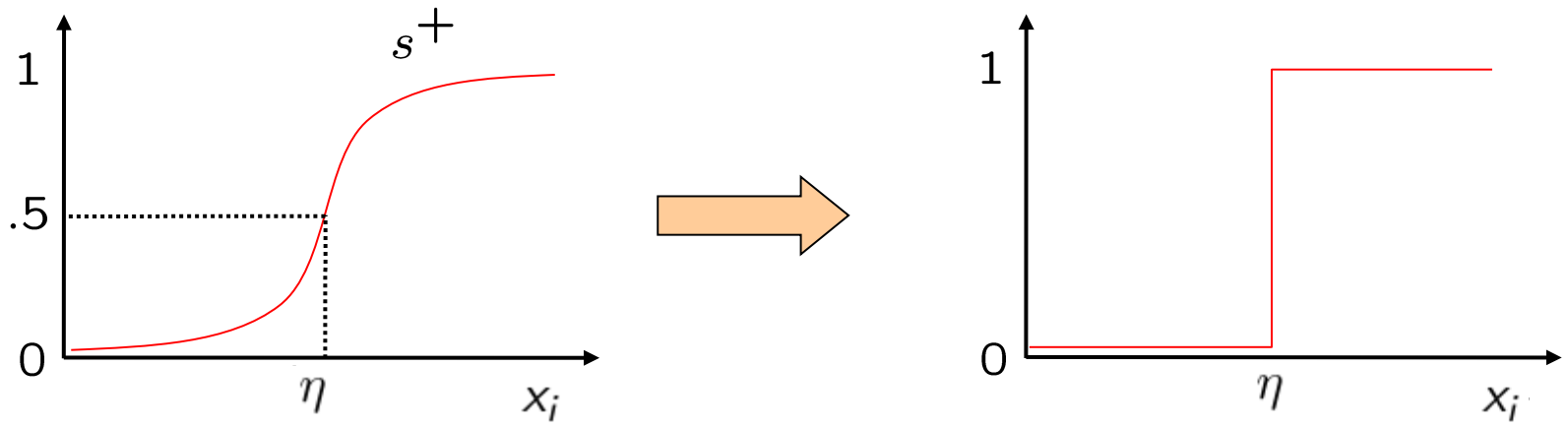
- Structure identification: based on data, decide
  - The number of summands
  - The sigmoids in each product
  - The signs of the sigmoids

... combinatorial explosion and identifiability issues !!

- Parameter identification: paramaters of each sigmoid, rates
- Intractable problem. But, good starting point
  - Structured expression
  - Reduction to specific families of Boolean-like functions
  - Approximation

Piecewise Affine models

- Simple idea: abstract nonlinearities by switches

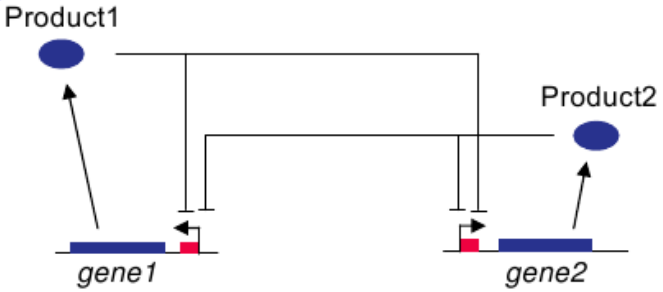


- Dynamical models with Boolean-type events
- Coarse approximation, but ...
- Powerful analysis (de Jong et al. 2004) & identification (Porreca et al, 2009) tools!

Example: double-inhibition network

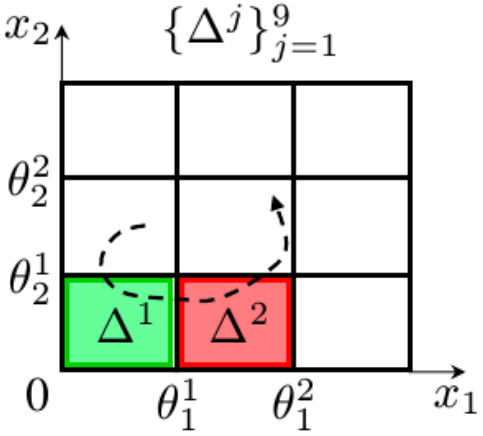
Courtesy of G.Ferrari-Trecate
(apologies for notational changes...)

Double inhibition network



$$\dot{x}_1 = \alpha_{11} b_{11}(x) - \gamma_1 x_1$$
$$\dot{x}_2 = \alpha_{21} b_{21}(x) - \gamma_2 x_2$$
$$b_{11}(x) = s^-(x_1, \theta_1^1) s^-(x_2, \theta_2^1)$$
$$b_{21}(x) = s^-(x_1, \theta_1^2) s^-(x_2, \theta_2^2)$$

Domains and affine dynamics



$$\begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \end{bmatrix} = \begin{cases} \begin{bmatrix} \alpha_{11} \\ \alpha_{21} \end{bmatrix} - \begin{bmatrix} \gamma_1 & 0 \\ 0 & \gamma_2 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix}, & \text{if } x \in \Delta^1 \\ \begin{bmatrix} 0 \\ \alpha_{21} \end{bmatrix} - \begin{bmatrix} \gamma_1 & 0 \\ 0 & \gamma_2 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix}, & \text{if } x \in \Delta^2 \\ \vdots \end{cases}$$

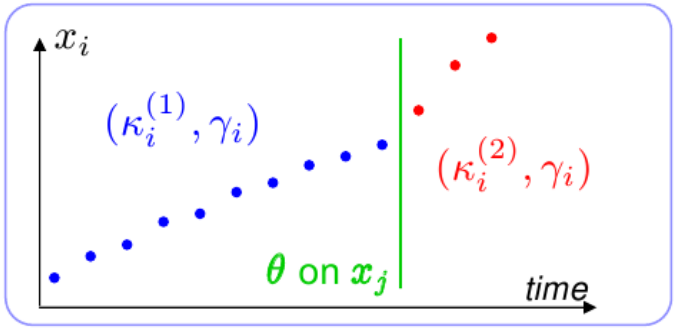
PWA models: key features

- thresholds split Ω into **hyperrectangular domains** $\Delta^1, \Delta^2, \ldots$:

$$\dot{x} = \begin{bmatrix} \kappa_1^j \\ \kappa_2^j \\ \vdots \\ \kappa_n^j \end{bmatrix} - \begin{bmatrix} \gamma_1 & 0 & \cdots & 0 \\ 0 & \gamma_2 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & \gamma_n \end{bmatrix} x \quad \text{if } x \in \Delta^j$$

→ system of n decoupled affine equations

- switching thresholds and rate parameters define the interactions

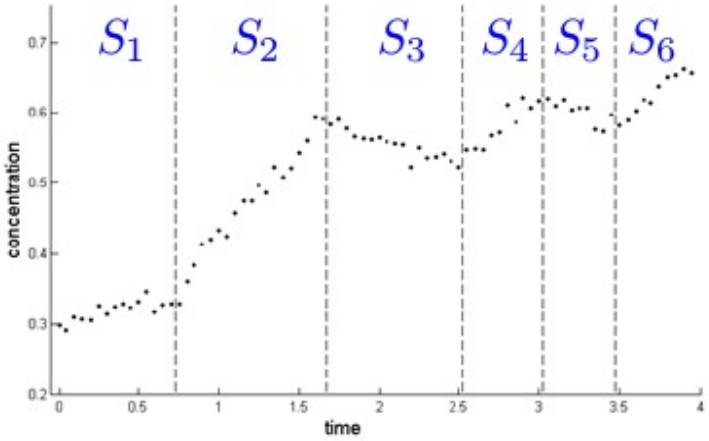


- gene *j* acts on of gene *i*
- interaction: activation/inhibition based on changes in κ_i

PWA models: key features cont'd

decoupling $\Rightarrow$ local 1st order dynamics for each concentration: if no switches occur over $[t_{k_0}, t_{k_1}]$ there exist $\kappa \geq 0, \gamma > 0$ such that

$$x_i(t_{k_1}) = \frac{\kappa}{\gamma} - \left(\frac{\kappa}{\gamma} - x_i(t_{k_0})\right) e^{-\gamma(t_{k_1} - t_{k_0})}$$

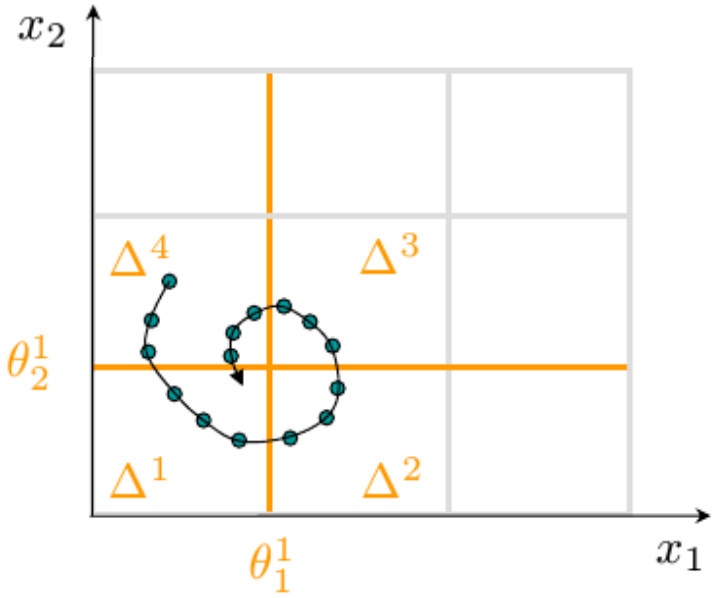


Data can be split in *segments* S_j generated by rate parameters (κ^j, γ)

PWA model identification

Goal: reconstruct from data

- **number of submodels**
  (excited during the experiment)
- **switching thresholds**
  (defining the domains)
- **rate parameters**
  (on the reconstructed domains)



Identification algorithm

Data segmentation

↓

Data classification

↓

Threshold reconstruction

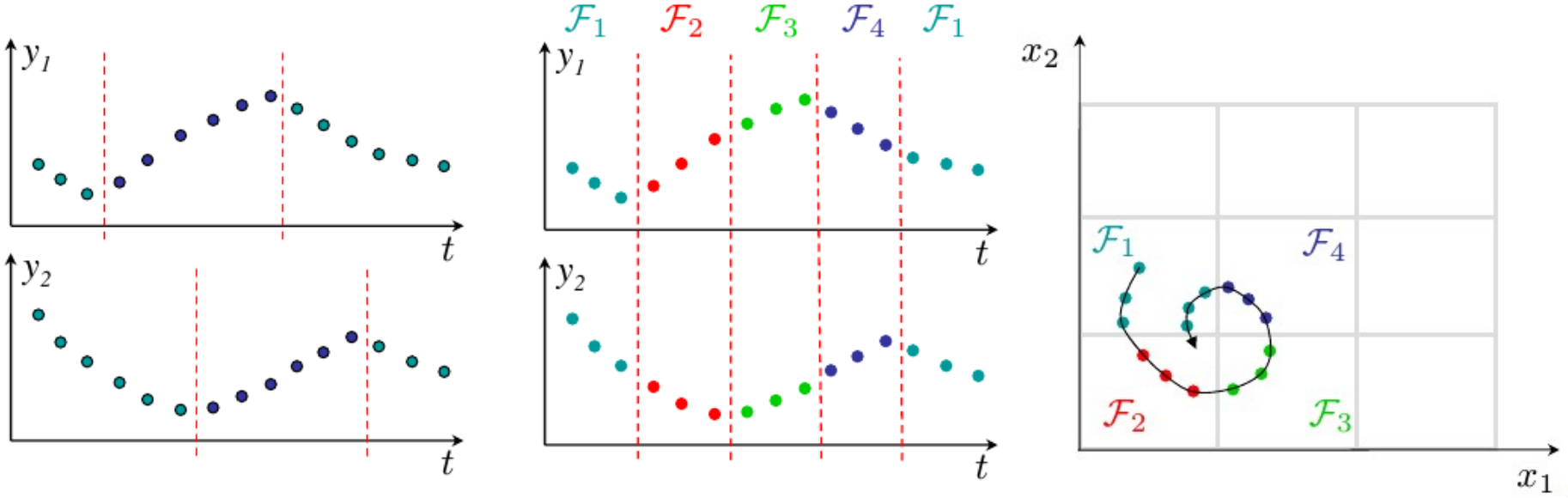
$$\begin{bmatrix}\dot{x}_1\\ \dot{x}_2\end{bmatrix} = \begin{cases} \begin{bmatrix}\alpha_{11}\\ \alpha_{21}\end{bmatrix} - \begin{bmatrix}\gamma_1 & 0\\ 0 & \gamma_2\end{bmatrix}\begin{bmatrix}x_1\\ x_2\end{bmatrix}, & \text{if } x \in \Delta^1 \\ \begin{bmatrix}0\\ \alpha_{21}\end{bmatrix} - \begin{bmatrix}\gamma_1 & 0\\ 0 & \gamma_2\end{bmatrix}\begin{bmatrix}x_1\\ x_2\end{bmatrix}, & \text{if } x \in \Delta^2 \\ \vdots & \end{cases}$$

Data segmentation and classification

- Given one time series
  - Variable sampling time
  - Extends to multiple time series

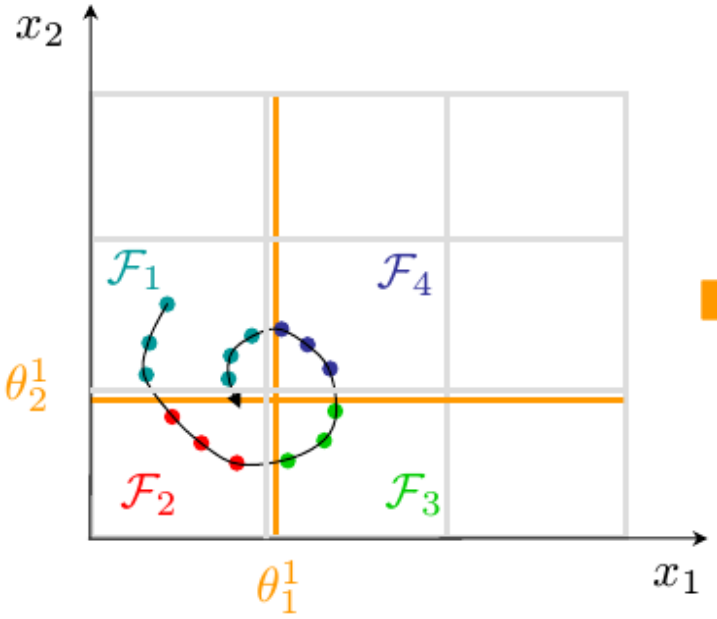
$$y_i(t_k) = x_i(t_k) + e_k, \qquad i = 1, \ldots, n$$
$$e_k \sim \mathcal{N}(0, \sigma^2) \qquad k = 1, \ldots, K$$

- Use statistical procedures to
  - Find segments with exponential behavior in each concentration profile (fit parameters and check that fitting residuals are compatible with noise)
  - Partition data into sets with the same exponential model



Threshold reconstruction

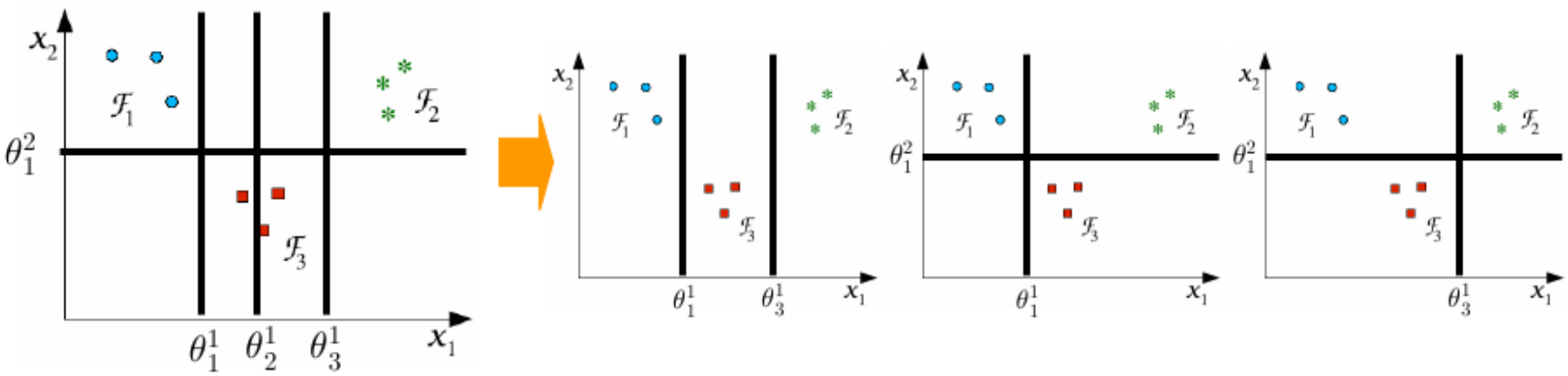
- Find *minimal* sets of thresholds that separate data clusters (multicuts)
  - Find all thresholds that separate two clusters
  - Define and exploit partial order relations among multicuts to find the minimal ones
  - Combinatorial number of multicuts: exploit branch-and-bound optimization techniques to avoid exploring all possibilities



- Two cuts
- Only one multicut = only one possible GRN

Optimal models

- Search of minimal multicuts: complexity reduction
- Identifiability issues:
  - Cannot discriminate certain models on the basis of the data
    (pool of equivalent models providing alternative biological hypotheses)
  - Cannot fix thresholds, only bounds can be established

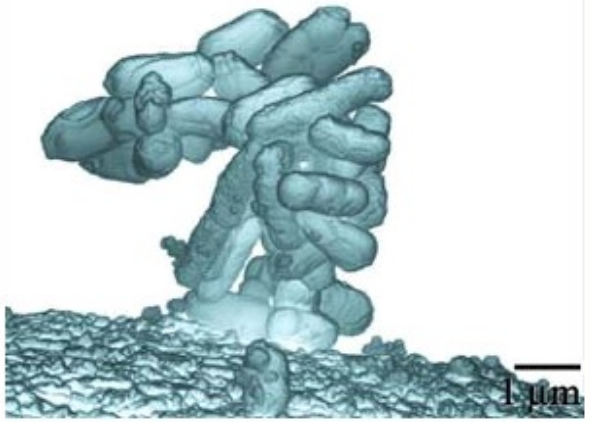


Three minimal multicuts = three possible GRNs

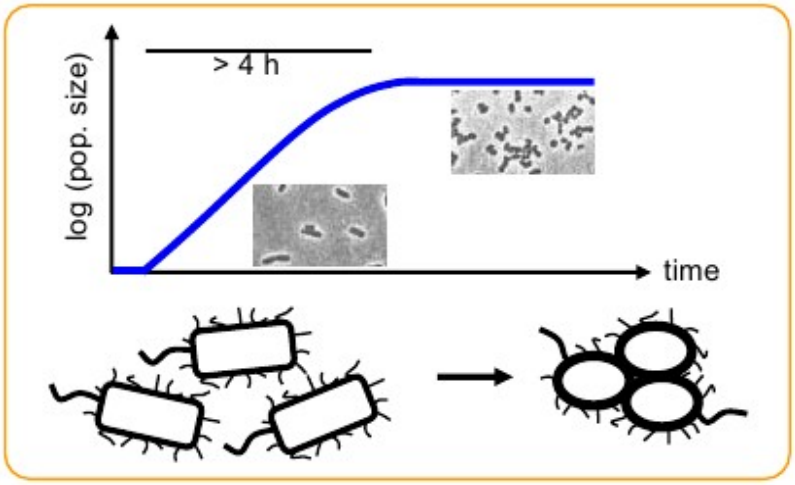
Example: carbon starvation in E.coli

Nutritional stress → Transition from exponential to stationary phase → Changes in:
- morphology,
- metabolism,
- gene expression,
- …

Escherichia coli



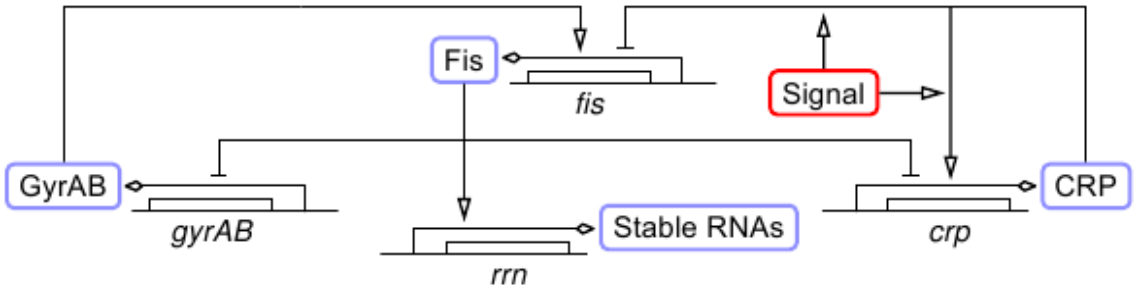
Low-temperature electron micrograph of a cluster of *E. coli* bacteria. Photo by Eric Erbe, digital colorization by Christopher Pooley, both of USDA, ARS, EMU.



Model and simulation

(Ropers et al., *Biosystems*, 2006)

Simplified model



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

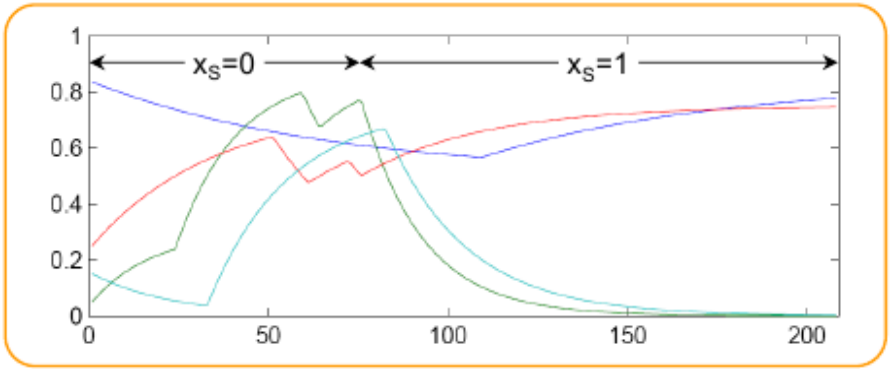
$$\dot{x}_{Fis} = \kappa^1_{Fis}\,(1 - s^+(x_{CRP}, \theta^1_{CRP})\, s^+(x_S, \theta_S)) + \kappa^2_{Fis}\, s^+(x_{GyrAB}, \theta_{GyrAB})\,(1 - s^+(x_{CRP}, \theta^1_{CRP})\, s^+(x_S, \theta_S)) - \gamma_{Fis}\, x_{Fis}$$

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

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

non identifiable interactions

simulation given x_0, x_S



Identification from simulated data

Cut #	Variable	Threshold value	Interaction	Correct? (Y/N)
1	CRP	0.61	activator of the synthesis of Fis	N
2	CRP	0.64	activator of the synthesis of Fis	N
3	CRP	0.71	inhibitor of the synthesis of Stable RNAs	N
4	CRP	0.74	inhibitor of the synthesis of Fis	N
5	Fis	0.09	inhibitor of the synthesis of CRP	Y
6	Fis	0.23	activator of the synthesis of Fis	N
7	Fis	0.49	activator of the synthesis of Stable RNAs	Y
8	Fis	0.75	inhibitor of the synthesis of GyrAB	Y
9	GyrAB	0.48	activator of the synthesis of Fis	N
10	GyrAB	0.50	activator of the synthesis of Fis	Y
11	GyrAB	0.55	inhibitor of the synthesis of Stable RNAs	N
12	GyrAB	0.67	activator of the synthesis of CRP	N
13	Stable RNAs	0.04	inhibitor of the synthesis of Fis, activator of the synthesis of Stable RNAs	N
14	Stable RNAs	0.18	inhibitor of the synthesis of CRP	N
15	Stable RNAs	0.53	inhibitor of the synthesis of Fis	N
16	Stable RNAs	0.55	activator of the synthesis of GyrAB	N
17	Stable RNAs	0.64	inhibitor of the synthesis of Fis, activator of the synthesis of Stable RNAs	N
18	Signal	0.50	inhibitor of the synthesis of Fis	Y

Minimal multicuts found

Multicut composed of cuts #:	Correct? (Y/N)
1, 5, 7, 8, 10	N, Y, Y, Y, Y
1, 7, 8, 10, 12	N, Y, Y, Y, N
5, 7, 8, 10, 18	Y, Y, Y, Y, Y
7, 8, 10, 12, 18	Y, Y, Y, N, Y
7, 8, 10, 14, 18	Y, Y, Y, N, Y

the best minimal multicut captures all interactions that are identifiable from the data

Models with unate structure

- Unate functions: Boolean rules monotone in each input variable
  - Transcription factors with unambiguous role (activator XOR repressor)
  - Arguably, the experimentally observable rules ? (↔ identifiability)
  - Includes most of the known gene activation rules
- Boolean-like ODE model: preserves monotonicity properties
  - Model:

$$b_i(x) = \prod_{l=1}^{n_i} \tau_l, \quad \tau_l = 1 - \prod_{j \in J_l} (1 - s^{\pm}(x_j)) \quad \text{where} \quad s^{\pm}(x_j) = \begin{cases} s^+(x_j), \\ s^-(x_j), \end{cases} \quad \text{or}$$

  - Sign pattern:

$$p = (p_1, \ldots p_n), \qquad p_j = \begin{cases} 1, & \text{if } s^{\pm}(x_j) = s^+(x_j), \\ -1 & \text{if } s^{\pm}(x_j) = s^-(x_j), \\ 0 & \text{if } j \notin J_l \ \forall l \end{cases} \quad j = 1, \ldots, n$$

Example: $p = (-1, 1)$: $s^-(x_1)s^+(x_2)$, $1 - s^+(x_1)s^-(x_2)$, $s^-(x_1)s^+(x_2) + \frac{1}{2}s^+(x_2)$, ...

$b(x)$ is nondecreasing (resp. nonincreasing) in x_j if $p_j = 1$ (resp. $p_j = -1$)

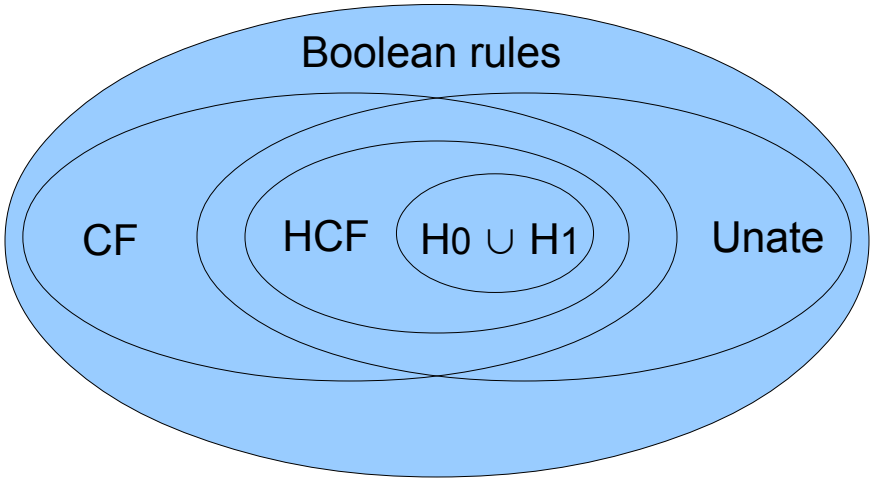
... and so is any synthesis rate $g_i(x) = \kappa_{0,i} + \kappa_{1,i} b_i(x)$, provided $\kappa_{0,i}, \kappa_{1,i} \geq 0$

Case study: unate models with canalizing structure

- Goal: use a priori knowledge to reduce the family of network structures
- Intuition: many Boolean expression rules are unlikely/uncommon
- Evidence: (Szallasi et al 1998, Kauffman et al 2004, ...)

  out of 139 gene activation rules analyzed in (Harris et al., 2002), 99% are "Canalizing Functions", 95% are "Hierarchically Canalizing Functions", 90% are "$H_0 \cup H_1$"

  - CFs: at least one (canalizing) value of at least one (canalizing) variable determines the value of the function
  - HCFs: when the canalizing variable takes its non-canalizing value, a second variable is canalizing, etc.



We focus on H_0 U H_1

The class $H_0 \cup H_1$

- Class H_0: $b_i(X) = X'_{j_1} \wedge X'_{j_2} \wedge \cdots \wedge X'_{j_\ell}$ $\qquad X'_{l,j} \in \{X_j, \neg X_j\}$

- Class H_1: $b_i(X) = X'_{j_1} \wedge X'_{j_2} \wedge \cdots \wedge X'_{j_{\ell-2}} \wedge (X'_{j_{\ell-1}} \vee X'_{j_\ell})$

- Boolean-like ODE model with $H_0 \cup H_1$-structure:

$$\dot{x}_i = \kappa_i^1 + \kappa_i^2 b_i(x) - \gamma_i x_i$$

$$b_i(x) = \begin{cases} s^{\pm}(x_{j_1}) \cdot s^{\pm}(x_{j_2}) \cdots s^{\pm}(x_{j_\ell}) \\ s^{\pm}(x_{j_1}) \cdot s^{\pm}(x_{j_2}) \cdots s^{\pm}(x_{j_{\ell-2}}) \big(1 - s^{\mp}(x_{j_{\ell-1}}) \cdot s^{\mp}(x_{j_\ell})\big) \end{cases}$$

Structure: $\ell,\ (j_1, j_2, \ldots, j_\ell),\ H_0$ vs. H_1

Parameters: $\kappa_i^1,\ \kappa_i^2,$ sigmoids'parameters (threshold, cooperativity)

Identification of H_0 U H_1 models

- Given concentration and synthesis rate measurements

$$y_i(t_k) = x_i(t_k)(1 + e_{i,k}) \qquad z_i(t_k) = f_i\big(x(t_k)\big)(1 + \epsilon_{i,k}) \qquad i = 1, \ldots, n$$

$$e_{i,k} \sim \mathcal{N}(0, \sigma_e^2) \qquad \epsilon_{i,k} \sim \mathcal{N}(0, \sigma_\epsilon^2) \qquad k = 1, \ldots, K$$

  - For known degradation rate, can compute synthesis rates from x:

$$f_i(x) = \kappa_i^1 + \kappa_i^2 b_i(x) = \dot{x}_i + \gamma_i x_i$$

(Ronen et al 2002, Brown et al 2008,...)

- Estimate
  - Structure: $\ell,\ (j_1, j_2, \ldots, j_\ell),\ H_0$ vs. H_1
  - Parameters: $\kappa_i^1,\ \kappa_i^2,\ \theta_j$ (possibly depending on i)

Mixed-Integer Parametrization

$$s_j^{\pm}(x_j) = s^{\pm}(x_j|\theta_j)$$
$$\theta_j \subseteq \{\eta_j, d_j\}$$

"AND" part

$$b_i(x) = \left[\prod_{j=1}^{n}\left[(1-\alpha_j) + \alpha_j\left(\beta_j s_j^+(x_j) + (1-\beta_j)s_j^-(x_j)\right)\right]\right] \times$$

$$\left[1 - \prod_{j=1}^{n}\left[(1-\alpha_j^*) + \alpha_j^*\left(\beta_j(1-s_j^+(x_j)) + (1-\beta_j)(1-s_j^-(x_j))\right)\right] + \gamma\right]$$

"OR" part

Constraints

$$\alpha_j, \alpha_j^*, \beta_j \in \{0,1\}$$
$$\alpha_j + \alpha_j^* \leq 1$$
$$\alpha_1^* + \ldots + \alpha_n^* \leq 2$$
$$\gamma = (1-\alpha_1^*) \cdot \ldots \cdot (1-\alpha_n^*)$$

Sigmoid $s_j^{\pm}(x_j)$ can be

- absent: $\alpha_j = \alpha_j^* = 0$
- present at most once
- increasing ($\beta_j = 1$) or decreasing ($\beta_j = 0$)

Identification via MI optimization

$$\text{minimize} \quad C(p) \times \sum_{k=1}^{K} w_k \left(z_i(t_k) - \left(\kappa_i^1 + \kappa_i^2 b_i(y(t_k)) \right) \right)^2$$

$$\text{with respect to} \quad \alpha_j,\ \alpha_j^*, \beta_j,\ j = 1, \ldots, n$$

$$\kappa_i^1, \kappa_i^2, \theta_j \text{ for all effective } j$$

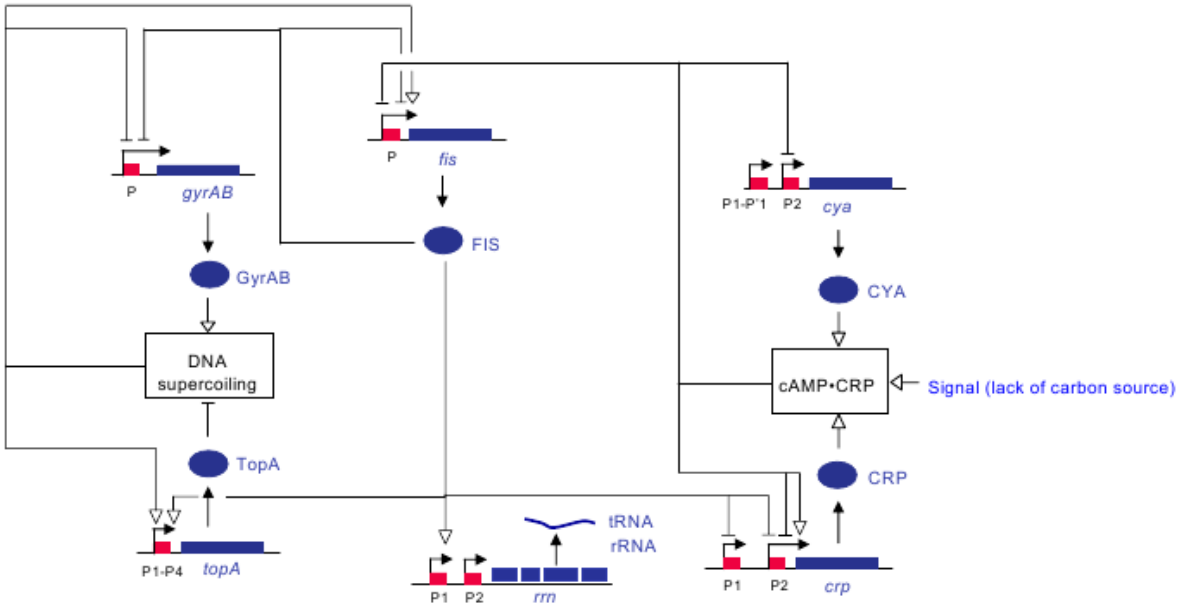
$$\text{subject to} \quad \text{constraints in the previous slide}$$

$$\text{positiveness of rate/threshold parameters}$$

- Weights w_k compensate for variable measurement accuracy
- Complexity penalization C(p), p number of effective parameters
  - Several statistical criteria (FPE, MDL, ...)
- Mixed Integer (nonlinear) programming: effective heuristics
- Highly non-convex:
  - For fixed structure parameters, cannot guarantee optimality of solution
  - Post-processing for the correction of artifacts (local minima)

Identification example

- 6-gene E.coli carbon starvation response network
- Model in exponential growth phase:



$$\dot{x}_1 = \kappa_1^1 + \kappa_1^2 \ - \gamma_1 x_1$$

$$\dot{x}_2 = \kappa_2^1 + \kappa_2^3 s^-(x_3, \theta_3^1) - \gamma_2 x_2$$

$$\dot{x}_3 = \kappa_3^1 s^-(x_3, \theta_3^5) + \kappa_3^2 \ s^+(x_4, \theta_4^1)\, s^-(x_5, \theta_5^2)\, s^-(x_3, \theta_3^5) - \gamma_3 \ x_3$$

$$\dot{x}_4 = \kappa_4 (1 - s^+(x_4, \theta_4^2)\, s^-(x_5, \theta_5^1)) \ s^-(x_3, \theta_3^4) - \gamma_4 x_4$$

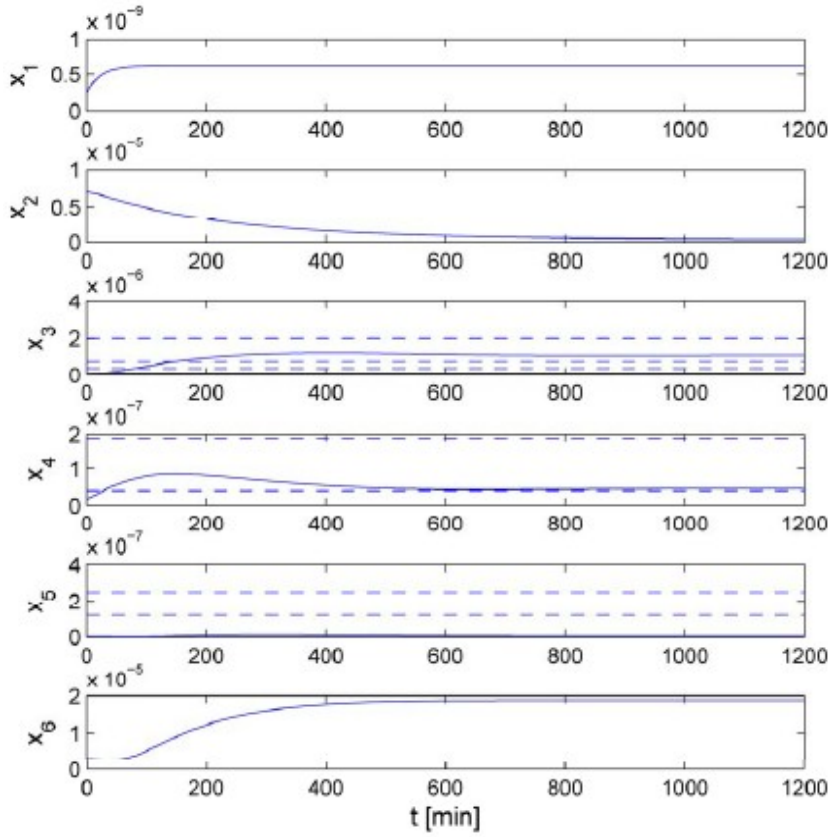
$$\dot{x}_5 = \kappa_5 \ s^+(x_4, \theta_4^2)\, s^-(x_5, \theta_5^1)\, s^+(x_3, \theta_3^4) - \gamma_5 \ x_5$$

$$\dot{x}_6 = \kappa_6^1 + \kappa_6^2 s^+(x_3, \theta_3^3) - \gamma_6 x_6$$

(Ropers et al, 2006)

- Observation: All but third equation have H_0 u H_1-structure

Identification scenario

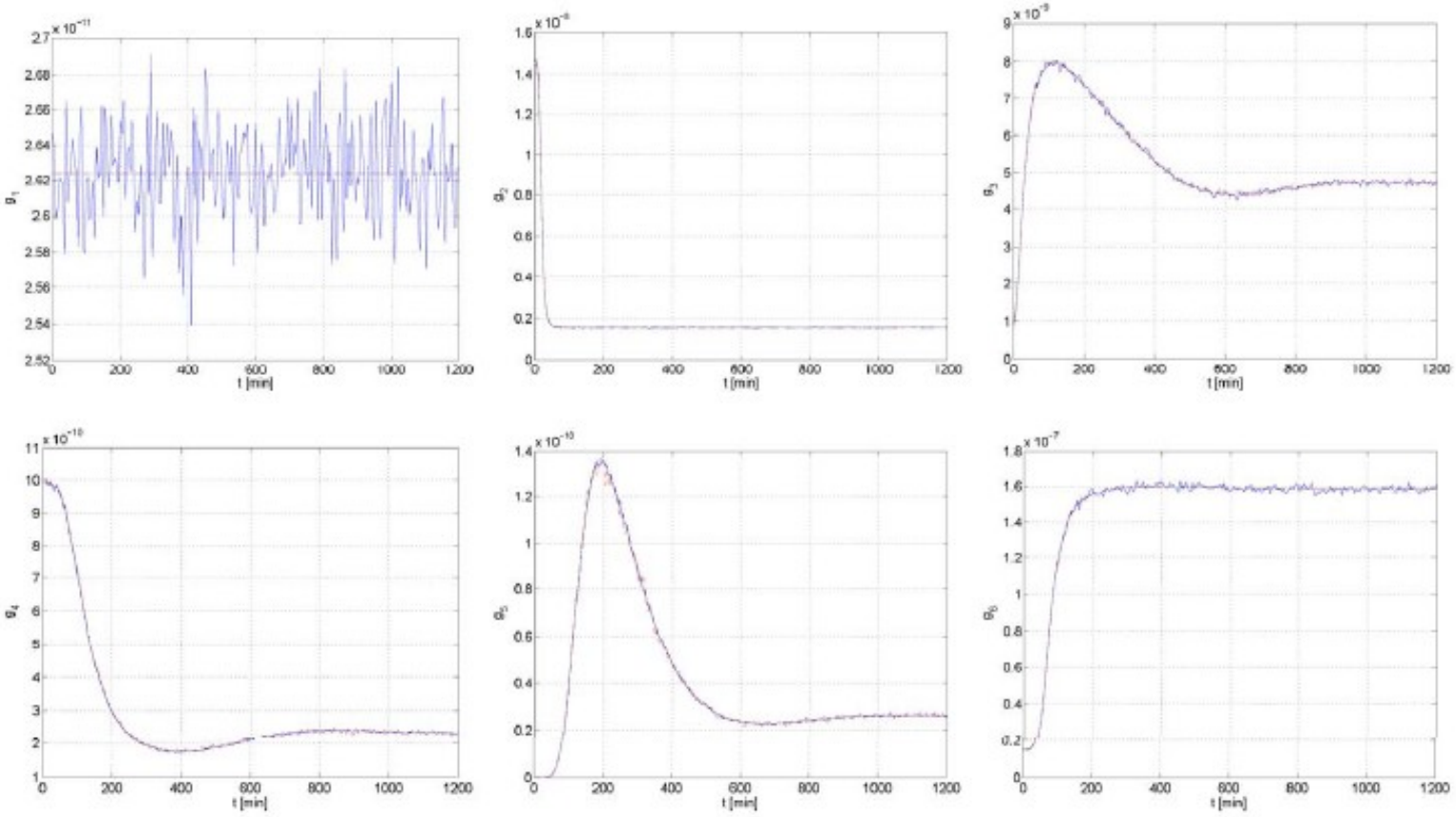


- Simulated data
- Samples every 5 min over 1200 min
- 5% noise
- Realistic parameters and initial cond.
- Dynamics excited in the experiment:

$$
\begin{aligned}
f_1 &= \kappa_1^1 + \kappa_1^2 \\
f_2 &= \kappa_2^1 + \kappa_2^2 s^-(x_3, \eta_{2,3}) \\
f_3 &\simeq \tilde{\kappa}_3^1 + \kappa_3^2 s^+(x_4, \eta_{3,4}) s^-(x_3, \eta_{3,3}) \\
f_4 &\simeq \kappa_4^2 s^-(x_4, \eta_{4,4}) s^-(x_3, \eta_{4,3}) \\
f_5 &\simeq \kappa_5^2 s^+(x_4, \eta_{5,4}) s^+(x_3, \eta_{5,3}) \\
f_6 &= \kappa_6^1 + \kappa_6^2 s^+(x_3, \eta_{6,3})
\end{aligned}
$$

- All equations have $H_0 \cup H_1$-structure!

Results: data fitting



Results, noise on synthesis rates

$$
\begin{aligned}
\hat{f}_1 &= \hat{\kappa}_1^1 + \hat{\kappa}_1^2 \\
\hat{f}_2 &= \hat{\kappa}_2^1 + \hat{\kappa}_2^2 s^-(x_3, \hat{\eta}_{2,3}) \\
\hat{f}_3 &= \hat{\kappa}_3^1 + \hat{\kappa}_3^2 s^-(x_3, \hat{\eta}_{3,3}) s^+(x_4, \hat{\eta}_{3,4}) \\
\hat{f}_4 &= \hat{\kappa}_4^1 + \hat{\kappa}_4^2 s^-(x_3, \hat{\eta}_{4,3}) s^-(x_4, \hat{\eta}_{4,4}) \\
\hat{f}_5 &= \hat{\kappa}_5^1 + \hat{\kappa}_5^2 s^+(x_3, \hat{\eta}_{5,3}) s^+(x_4, \hat{\eta}_{5,4}) \times \\
&\quad \times s^-(x_6, \hat{\eta}_{5,6}) \\
\hat{f}_6 &= \hat{\kappa}_6^1 + \hat{\kappa}_6^2 s^+(x_3, \hat{\eta}_{6,3})
\end{aligned}
$$

Gene	Param	Estimate	True	Sigmoid span
1	κ_1^1	$2.477_{10^{-11}}$	$3.034_{10^{-12}}$	
	κ_1^2	$1.476_{10^{-12}}$	$2.317_{10^{-11}}$	
2	κ_2^1	$1.552_{10^{-9}}$	$1.553_{10^{-9}}$	
	κ_2^2	$1.32_{10^{-8}}$	$1.322_{10^{-8}}$	
	$\eta_{2,3}$	$4.005_{10^{-8}}$	$3.991_{10^{-8}}$	[0.000, 0.998]
3	κ_3^1	$3.502_{10^{-10}}$	$3.404_{10^{-10}}$	
	κ_3^2	$8.682_{10^{-9}}$	$8.668_{10^{-9}}$	
	$\eta_{3,3}$	$1.991_{10^{-6}}$	$2.020_{10^{-6}}$	[0.807, 1.000]
	$\eta_{3,4}$	$4.02_{10^{-8}}$	$3.991_{10^{-8}}$	[0.061, 0.910]
4	κ_4^1	0	0	
	κ_4^2	$9.954_{10^{-10}}$	$9.938_{10^{-10}}$	
	$\eta_{4,3}$	$7.469_{10^{-7}}$	$7.472_{10^{-7}}$	[0.181, 1.000]
	$\eta_{4,4}$	$1.883_{10^{-7}}$	$1.888_{10^{-7}}$	[0.911, 0.999]
5	κ_5^1	0	0	
	κ_5^2	$1.809_{10^{-9}}$	$2.548_{10^{-9}}$	
	$\eta_{5,3}$	$7.684_{10^{-7}}$	$7.472_{10^{-7}}$	[0.000, 0.806]
	$\eta_{5,4}$	$1.63_{10^{-7}}$	$1.888_{10^{-7}}$	[0.001, 0.131]
	$\eta_{5,6}$	$4.418_{10^{-5}}$	–	[0.929, 1.000]
6	κ_6^1	$1.496_{10^{-8}}$	$1.506_{10^{-8}}$	
	κ_6^2	$1.488_{10^{-7}}$	$1.488_{10^{-7}}$	
	$\eta_{6,3}$	$3.669_{10^{-7}}$	$3.663_{10^{-7}}$	[0.000, 0.974]

- Just one spurious sigmoid

Results, noise on rates and concentrations

$$
\begin{aligned}
\hat{f}_1 &= \hat{\kappa}_1^1 + \hat{\kappa}_1^2 \\
\hat{f}_2 &= \hat{\kappa}_2^1 + \hat{\kappa}_2^2 s^+(x_2, \hat{\eta}_{2,2}) s^-(x_3, \hat{\eta}_{2,3}) \\
\hat{f}_3 &= \hat{\kappa}_3^1 + \hat{\kappa}_3^2 s^+(x_1, \hat{\eta}_{3,1}) s^-(x_3, \hat{\eta}_{3,3}) \times \\
&\quad \times s^+(x_4, \hat{\eta}_{3,4}) s^-(x_6, \hat{\eta}_{3,6}) \\
\hat{f}_4 &= \hat{\kappa}_4^1 + \hat{\kappa}_4^2 s^-(x_4, \hat{\eta}_{4,4}) s^-(x_5, \hat{\eta}_{4,5}) \times \\
&\quad \times \left(1 - s^-(x_2, \hat{\eta}_{4,2}) s^+(x_3, \hat{\eta}_{4,3})\right) \\
\hat{f}_5 &= \hat{\kappa}_5^1 + \hat{\kappa}_5^2 s^+(x_3, \hat{\eta}_{5,3}) s^+(x_4, \hat{\eta}_{5,4}) \\
\hat{f}_6 &= \hat{\kappa}_6^1 + \hat{\kappa}_6^2 s^+(x_3, \hat{\eta}_{6,3})
\end{aligned}
$$

Gene	Param	Estimate	True	Sigmoid span
1	κ_1^1	$2.477_{10^{-11}}$	$3.034_{10^{-12}}$	
	κ_1^2	$1.476_{10^{-12}}$	$2.317_{10^{-11}}$	
2	κ_2^1	$1.554_{10^{-9}}$	$1.553_{10^{-9}}$	
	κ_2^2	$9.894_{10^{-7}}$	$1.322_{10^{-8}}$	
	$\eta_{2,2}$	$2.955_{10^{-5}}$	–	$[0.000, 0.014]$
	$\eta_{2,3}$	$4.359_{10^{-8}}$	$3.991_{10^{-8}}$	$[0.000, 0.998]$
3	κ_3^1	$5.781_{10^{-10}}$	$3.404_{10^{-10}}$	
	κ_3^2	$8.797_{10^{-9}}$	$8.668_{10^{-9}}$	
	$\eta_{3,1}$	$2.313_{10^{-10}}$	–	$[0.529, 0.954]$
	$\eta_{3,3}$	$2.116_{10^{-6}}$	$2.020_{10^{-6}}$	$[0.831, 1.000]$
	$\eta_{3,4}$	$3.929_{10^{-8}}$	$3.991_{10^{-8}}$	$[0.065, 0.920]$
	$\eta_{3,6}$	$4.449_{10^{-5}}$	–	$[0.926, 1.000]$
4	κ_4^1	$3.967_{10^{-11}}$	0	
	κ_4^2	$9.634_{10^{-10}}$	$9.938_{10^{-10}}$	
	$\eta_{4,2}$	$8.638_{10^{-6}}$	–	$[0.644, 1]$
	$\eta_{4,3}$	$7.018_{10^{-7}}$	$7.472_{10^{-7}}$	$[0, 0.848]$
	$\eta_{4,4}$	$1.613_{10^{-7}}$	$1.888_{10^{-7}}$	$[0.858, 0.999]$
	$\eta_{4,5}$	$2.516_{10^{-8}}$	$(1.221_{10^{-7}})$	$[0.879, 1.000]$
5	κ_5^1	0	0	
	κ_5^2	$2.252_{10^{-9}}$	$2.548_{10^{-9}}$	
	$\eta_{5,3}$	$7.444_{10^{-7}}$	$7.472_{10^{-7}}$	$[0.000, 0.823]$
	$\eta_{5,4}$	$1.81_{10^{-7}}$	$1.888_{10^{-7}}$	$[0.001, 0.105]$
6	κ_6^1	$1.494_{10^{-8}}$	$1.506_{10^{-8}}$	
	κ_6^2	$1.487_{10^{-7}}$	$1.488_{10^{-7}}$	
	$\eta_{6,3}$	$3.657_{10^{-7}}$	$3.663_{10^{-7}}$	$[0.000, 0.975]$

- Several spurious sigmoids:
  - Least squares do not account for noise on concentrations !

Discussion

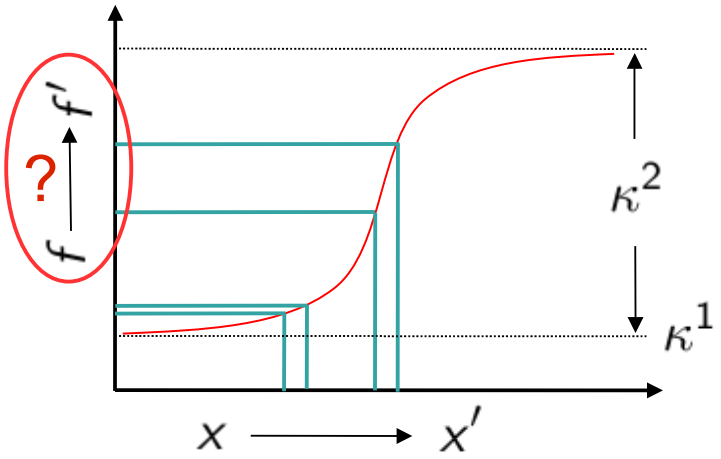
- Computational complexity still high, need to reduce model family
  - A priori: use general and system-specific biological knowledge
  - Via preprocessing: certain model structures are falsified by the data, e.g.:

Given (x, f) and (x', f'), with $x < x'$ and $f > f'$.

Then $f(x) \neq \kappa^1 + \kappa^2 \frac{x^d}{x^d + \eta^d}$ for any $\eta, d, \kappa^1, \kappa^2 \geq 0$.

- Explicit account of all noise sources
  - Existing solutions (Total Least Squares) are computationally intensive
  - Development of ad-hoc statistical tests

$$f(x + e) \simeq f(x) + Df(x) \cdot e$$



Identification via sign patterns: rationale

- Given: protein concentrations & synthesis rates (recall $\dot{x}_i = g_i(x) - \gamma_i(x)$)
- Step 1: Exploit monotonicity properties

  to <u>invalidate</u> sign patterns

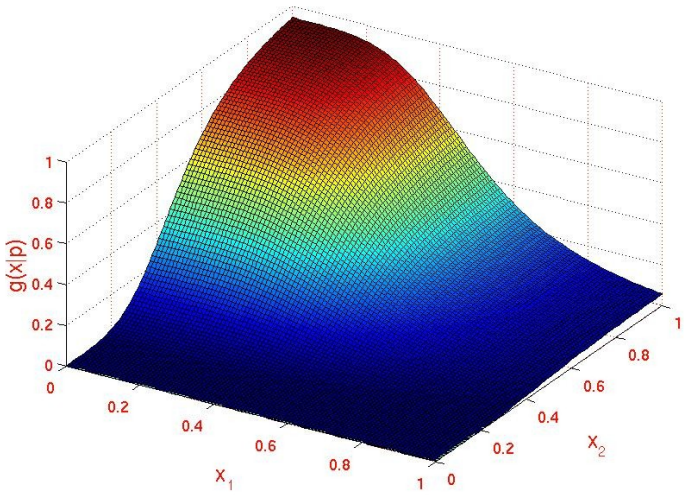
Example. $g(x|p)$, $x = (x_1, x_2)$, unknown $p = (p_1, p_2)$.

Given (x, g_i), (x', g_i') with $x_1 > x_1'$, $x_2 < x_2'$, $g_i > g_i'$.

Can exclude: $p = (-1, 1) = \big(\text{sign}(x_1' - x_1), \text{sign}(x_2' - x_2)\big)$.

Can also exclude: $p = (0, 1)$, $p = (-1, 0)$, $p = (0, 0)$.

Note: Parameter values play no role here!



- Step 2: Search best fitting model structure with <u>valid</u> sign pattern
  - Enumerate valid sign patterns of increasing level of complexity
  - Fit model structures with valid sign pattern to the data
    - *Parametrization of model structures S(p) with sign pattern p*
    - *Prior knowledge embedded in the definition of S(p)*
  - Evaluate fitted models based on a statistical test on the fitting errors

Sign patterns: definitions and properties

- Given data pairs: $(x^1, g^1), \ldots, (x^m, g^m)$, with $g^k = g(x^k|p)$
- Definition: p is *inconsistent* if the property

$$p_j(x_j^k - x_j^l) \geq 0,\ j = 1, \ldots, n \implies g(x^k|p) - g(x^l|p) \geq 0$$

  is falsified for some k,l
- Definition: subpattern and superpattern

		Complexity
Superpatterns	1 1 -1 1 1 1 -1 -1 1 -1 -1 1 1 -1 -1 -1 1 1 -1 0 1 -1 -1 0	4 3
Pattern	1 0 -1 0	2
Subpatterns	1 0 0 0 0 0 -1 0 0 0 0 0	1 0

- Subpatterns of inconsistent patterns are also inconsistent
- Superpatterns of consistent patterns are also consistent
- Minimal consistent and maximal inconsistent patterns exist

Algorithm 1: original version (full data)

- Protein concentrations & synthesis rates
- Time-course noisy data, known variance:

$$\tilde{x}_i^k = x_i^k + e_i^k \qquad \tilde{g}_i^k = g_i^k + \epsilon_i^k$$

$$x_i^k = x_i(t_k) \qquad g_i^k = g\big(x(t_k)\big)$$

with $k = 1, \ldots, m$ and zero-mean Gaussian noise

$$v_e(x_i^k) = \mathrm{var}(e_i^k) \qquad v_\epsilon(g_i^k) = \mathrm{var}(\epsilon_i^k)$$

Computation of $\bar{P}$: set $\bar{P} = \emptyset$. For all indices $k, l \in \{1, \ldots, m\}$:

(I) If $g^k - g^l < 0$, define the sign pattern $\bar{p} = (\bar{p}_1, \ldots, \bar{p}_n)$ by setting $\bar{p}_j = \mathrm{sign}(x_j^k - x_j^l)$, with $j = 1, \ldots, n$, and include $\bar{p}$ in $\bar{P}$.

Computation of P^*: define $\bar{\ell} = \max\{C(\bar{p}) : \bar{p} \in \bar{P}\}$. Initialize $P^* = \emptyset$. For increasing values of complexity $\ell = 0, \ldots, \min\{n, \bar{\ell} + 1\}$:

(II) Generate all patterns p of complexity ℓ. For each such p,

(III) Check if p is consistent by verifying that there is no $\bar{p} \in \bar{P}$ such that $p \sqsubseteq \bar{p}$. If this is the case,

(IV) Check if p is minimal consistent by verifying that there is no $p^* \in P^*$ such that $p^* \sqsubseteq p$. If this is the case, include p in P^*.

Algorithm 1 Two-step identification.

Step 1. (Selection of consistent model structures)

I. Set $\bar{P} = \emptyset$. For all indices $k, l \in \{1, \ldots, m\}$, if $\tilde{g}_i^k - \tilde{g}_i^l < -N\sigma_{g_i}^{k,l}$ then define $\bar{p} = (\bar{p}_1, \ldots, \bar{p}_n)$ by

$$\bar{p}_j = \begin{cases} -1, & \text{if } \tilde{x}_j^k - \tilde{x}_j^l \le -N\sigma_{x_j}^{k,l}, \\ 1, & \text{if } \tilde{x}_j^k - \tilde{x}_j^l \ge N\sigma_{x_j}^{k,l}, \\ 0, & \text{otherwise,} \end{cases} \qquad j = 1, \ldots, n,$$

and include $\bar{p}$ in $\bar{P}$.

II–IV. Execute the computation of P^* from the resulting $\bar{P}$, as described in Section 2.2.

Step 2. (Identification of best consistent models) Set $\mathscr{P} = \emptyset$. Define $\ell^* = \min\{C(p^*) : p^* \in P^*\}$. For $\ell = \ell^*$ to n:

V. Generate patterns p such that $C(p) = \ell$ and $p^* \sqsubseteq p$ for some $p^* \in P^*$. For each such p, execute VI.

VI. For all $s \in S(p)$, fit the model $g_i(\cdot)$ with sign pattern p and structure s by solving the nonlinear regression problem

$$\delta = \min_\theta \sum_{k=1}^{m} w_k \big(\tilde{g}_i^k - g_i(\tilde{x}^k)\big)^2. \qquad (8)$$

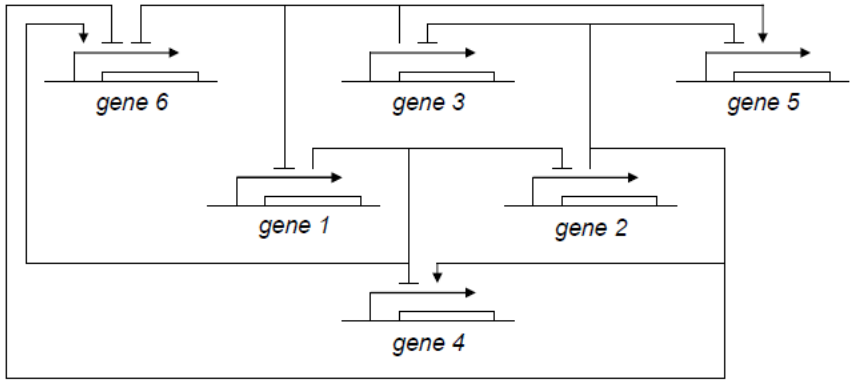
If $\delta < \tau(\alpha)$, include the fitted model in $\mathscr{P}$.

VII. If $\mathscr{P} \neq \emptyset$ return $\mathscr{P}$ and exit.

Comments

- Separate identification of regulation function of each gene
- Hierarchical search of model structures of increasing complexity
  - Stops when a good model is found (statistical test on the model residuals)
  - Favors simple over complicated models
  - Returns pool of biological alternatives
- What is a statistically good model?
  - Under the null hypothesis that the estimated model is correct, the fitting residual is distributed as $\chi^2(m)$
  - Use this property to define confidence levels (threshold on the fitting residuals) on the model estimate
- Limitations: Nonconvex parameter fitting, Data requirements

Test on a repressilator system



$$\dot{x}_1 = \kappa_{0,1} + \kappa_{1,1}\sigma^-(x_3) - \gamma_1 x_1,$$

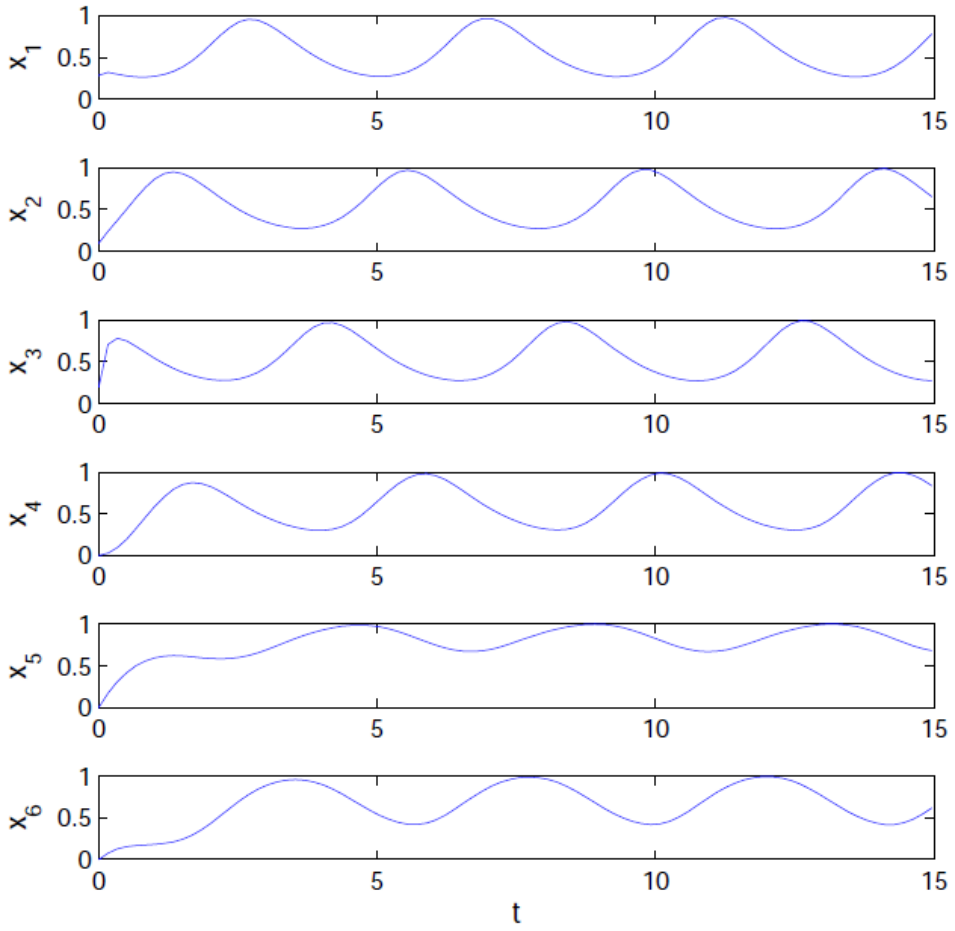
$$\dot{x}_2 = \kappa_{0,2} + \kappa_{1,2}\sigma^-(x_1) - \gamma_2 x_2,$$

$$\dot{x}_3 = \kappa_{0,3} + \kappa_{1,3}\sigma^-(x_2) - \gamma_3 x_3,$$

$$\dot{x}_4 = \kappa_{0,4} + \kappa_{1,4}\sigma^-(x_1)\sigma^+(x_2) - \gamma_4 x_4,$$

$$\dot{x}_5 = \kappa_{0,5} + \kappa_{1,5}[1 - \sigma^+(x_2)\sigma^-(x_3)] - \gamma_5 x_5,$$

$$\dot{x}_6 = \kappa_{0,6} + \kappa_{1,6}[1 - \sigma^+(x_2)\sigma^+(x_3)]\sigma^+(x_1) - \gamma_6 x_6.$$



Performance results

We attempted identification of this system with 90 equally spaced data points over a time interval such that the product concentrations of the core genes complete three full oscillations. Measurements $\tilde{x}_i^k$ and $\tilde{g}_i^k$ were artificially corrupted by Gaussian noise samples according to the observation model (7), with $v_e(x_i^k) = (\sigma_e x_i^k)^2$ and $v_\epsilon(g_i^k) = (\sigma_\epsilon g_i^k)^2$, for the different noise levels $\sigma_e = \sigma_\epsilon = 0.01, 0.03, 0.05, 0.07$. This corresponds to noise roughly within 3%, 10%, 15% and 20% of the actual values of x_i^k and g_i^k. The performance of Algorithm 1 (with $N=6$ and $\alpha=0.95$) for the various noise levels and all genes is conveyed by the scores on the performance indices R, S, A and D (Table 1). These were computed as described in Section 2.3.4 on the basis of $M=100$ identification runs with the same system evolution, but with different random outcomes of the noise. Each run (MATLAB V.7 R.14) took on an average roughly 5 min on a Windows XP workstation with Pentium 3.20 GHz processor and 2.00 GB RAM. Computational time ranged from ~2 s for the identification of g_3 to ~4 min for the identification of g_6. Step 1 always performs very reliably, i.e. index R is constantly

		$\sigma_e, \sigma_\epsilon$	0.01	0.03	0.05	0.07
Gene 1	Step 1	R	1	1	1	1
		S	0.92	0.92	0.92	0.91
	Step 2	A	0.90	0.92	0.91	0.89
		D	1	1	1	1
Gene 2	Step 1	R	1	1	1	1
		S	0.92	0.92	0.92	0.91
	Step 2	A	0.93	0.92	0.89	0.89
		D	1	1	1	1
Gene 3	Step 1	R	1	1	1	1
		S	0.92	0.92	0.92	0.92
	Step 2	A	0.93	0.93	0.93	0.92
		D	1	1	1	1
Gene 4	Step 1	R	1	1	1	1
		S	0.94	0.92	0.87	0.65
	Step 2	A	0.94	0.94	0.93	0.89
		D	1	1	1.02	1.44
Gene 5	Step 1	R	1	1	1	1
		S	0.94	0.74	0.53	0.48
	Step 2	A	0.95	0.94	0.91	0.83
		D	1	1	1.79	4
Gene 6	Step 1	R	1	1	1	1
		S	0.79	0.65	0.57	0.43
	Step 2	A	0.89	0.92	0.85	0.42
		D	1	1.02	2.76	2.74

	Index	Range	Description
Step 1	R eliability	[0,1]	Probability that the true p is deemed consistent
	S electivity	[0,1]	Percentage of sign patterns eliminated from the search in Step 2
Step 2	A ccuracy	[0,1]	Probability that the true structure is In the pool of identified models
	D ispersion	≥1	Average number of models in the pool

Simulated identification on *E.coli* model

- 6-gene carbon starvation response network
- Model in exponential growth phase
- All but third equation have $H_0 \cup H_1$-structure (all have unate structure)

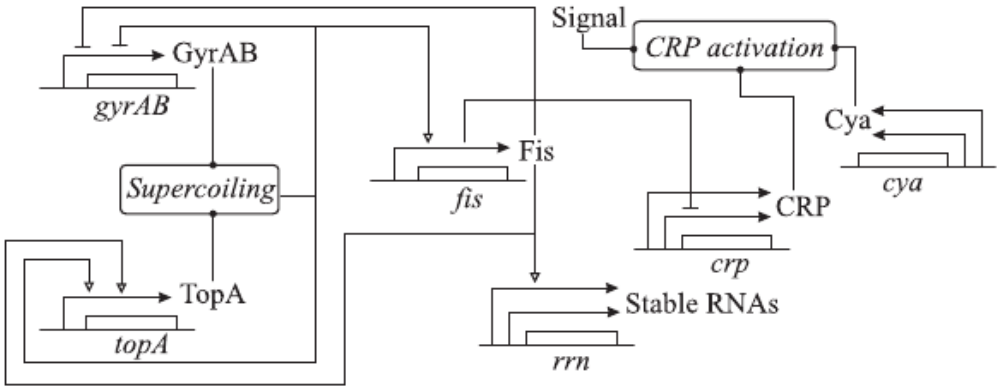


FIGURE 1. Key global regulators and regulatory interactions taking place during the transition from stationary to exponential growth phase in *E.Coli*.

(Ropers et al, Biosystems 2006)

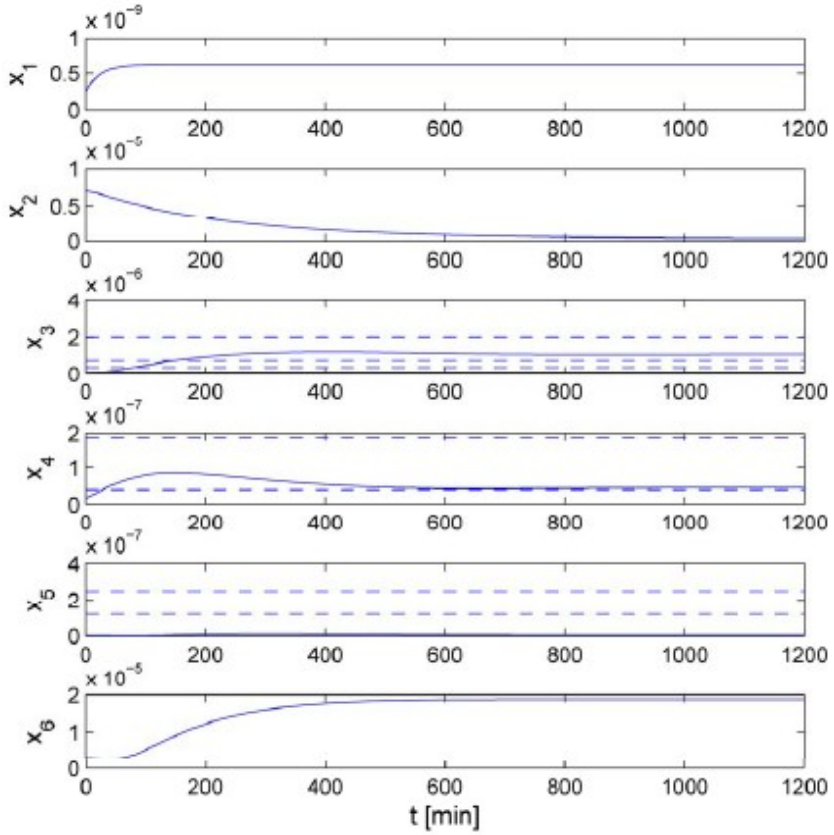
$$
\begin{aligned}
\dot{x}_1 &= \kappa_1^1 + \kappa_1^2 \ - \gamma_1 \ x_1 \\
\dot{x}_2 &= \kappa_2^1 + \ \kappa_2^3 \ \sigma^-(x_3) - \gamma_2 \ x_2 \\
\dot{x}_3 &= \kappa_3^1 \ \sigma^-(x_3) \ + \ \kappa_3^2 \ \sigma^+(x_4)\, \sigma^-(x_5)\, \sigma^-(x_3) \ - \gamma_3 \ x_3 \\
\dot{x}_4 &= \kappa_4 \ (1 - \sigma^+(x_4)\, \sigma^-(x_5)) \ \sigma^-(x_3) - \gamma_4 \ x_4 \\
\dot{x}_5 &= \kappa_5 \ \sigma^+(x_4)\, \sigma^-(x_5)\, \sigma^+(x_3) - \gamma_5 \ x_5 \\
\dot{x}_6 &= \kappa_6^1 \ \sigma^+(x_3) + \kappa_6^2 - \gamma_6 \ x_6
\end{aligned}
$$

$x_1, \ x_2, \ x_3, \ x_4, \ x_5, \ x_6 =$

Cya, CRP, Fis, GyrAB, TopA

Stable RNAs

Identification scenario



- Simulated data collected every 10 min
- Measurements over 1200 min
- Various noise levels
- Performance from 100 simulated runs
- Realistic parameters and initial cond.
- Dynamics excited in the experiment:

$$g_1 = \kappa_{0,1}, \qquad g_4 \simeq \kappa_{1,4}\sigma^-(x_4)\sigma^-(x_3),$$
$$g_2 = \kappa_{0,2} + \kappa_{1,2}\sigma^-(x_3), \qquad g_5 \simeq \kappa_{1,5}\sigma^+(x_4)\sigma^+(x_3),$$
$$g_3 \simeq \kappa_{0,3} + \kappa_{1,3}\sigma^+(x_4)\sigma^-(x_3), \quad g_6 = \kappa_{0,6} + \kappa_{1,6}\sigma^+(x_3).$$

- All excited dynamics have $H_0 \cup H_1$-structure

  Use this as a “reference” model

Results on *E.coli*

Note that the expression of gene 1 obeys trivial dynamics. Correspondingly, a constant model for g_1 is returned by the preprocessing Step 0 in roughly 95% of the runs. This is summarized by the accuracy index A. In the remaining runs the algorithm rules out the constant model, i.e. the true pattern is not in the patterns deemed consistent and a model with correct structure cannot be found in Step 2. For the remaining genes, the values of reliability R and selectivity S witness that Step 1 is still very effective and robust to noise. Step 2 includes the correct model structure in a small pool of identified models in all cases, with a moderate performance decay at increased noise levels. For gene 4 only, this decay is abrupt when the noise level raises above 5% ($\sigma_e = \sigma_\epsilon > 0.01$), possibly due to a limited excitation of the expression dynamics. Finally, for gene 5, the limited accuracy of Step 2 ($A = 0.14$) at the lowest noise level is due to convergence to local minima in the solution of the nonconvex optimization (8). With low noise, the local minima are more pronounced and the solver currently used cannot escape them. This limitation could be ameliorated by a randomized optimization strategy ([28]). To conclude we mention that, whenever the identifiable model structure was estimated correctly, the corresponding parameter estimates were generally accurate (best accuracy being obtained with lowest noise, results not shown).

		$\sigma_e, \sigma_\epsilon$	0.01	0.03	0.05	0.07
Gene 1	Step 1	R	–	–	–	–
		S	–	–	–	–
	Step 2	A	0.95	0.95	0.96	0.95
		D	–	–	–	–
Gene 2	Step 1	R	1	1	1	1
		S	0.75	0.58	0.56	0.50
	Step 2	A	0.98	0.97	0.95	0.94
		D	1	1	1	1
Gene 3	Step 1	R	1	1	1	1
		S	0.81	0.58	0.54	0.50
	Step 2	A	0.95	0.93	0.87	0.58
		D	1	1.39	2.47	2.84
Gene 4	Step 1	R	1	1	1	1
		S	0.60	0.50	0.44	0.37
	Step 2	A	0.93	0.16	0	0
		D	1.24	4.31	–	–
Gene 5	Step 1	R	1	1	1	1
		S	0.73	0.66	0.61	0.54
	Step 2	A	0.14	0.84	0.88	0.79
		D	1	1	1	1
Gene 6	Step 1	R	1	1	1	1
		S	0.75	0.67	0.64	0.55
	Step 2	A	0.93	0.93	0.93	0.88
		D	1	1	1	1.01

Algorithm 2: extension to partial data

- Assuming only protein concentrations are available:
  1. Reconstruct missing information (synthesis rates, variances)
  2. Apply Algorithm 1 (unchanged)
- Option 1: Deconvolution

$$\dot{x}_i(t) = -\gamma_i x_i(t) + g_i(t), \quad g_i(t) = \kappa_{0,i} + \kappa_{1,i} b_i\big(x(t)\big) \text{ is a forcing input}$$

  - Well established (Bayesian) methods for regularized estimates
  - Severe over- and under-smoothing observed in practice
- Option 2 (our choice): Data fitting + Bootstrapping

Choose basis functions for $x_i(\cdot)$, e.g. cubic splines

Compute estimate $\hat{x}_i$ by fitting data $\tilde{x}_i^k$, and $\hat{\dot{x}}_i = \dot{\hat{x}}_i$ by explicit differentiation

Reconstruct the synthesis rates $\tilde{g}_i^k = \dot{\hat{x}}_i(t_k) + \gamma_i \tilde{x}_i^k$

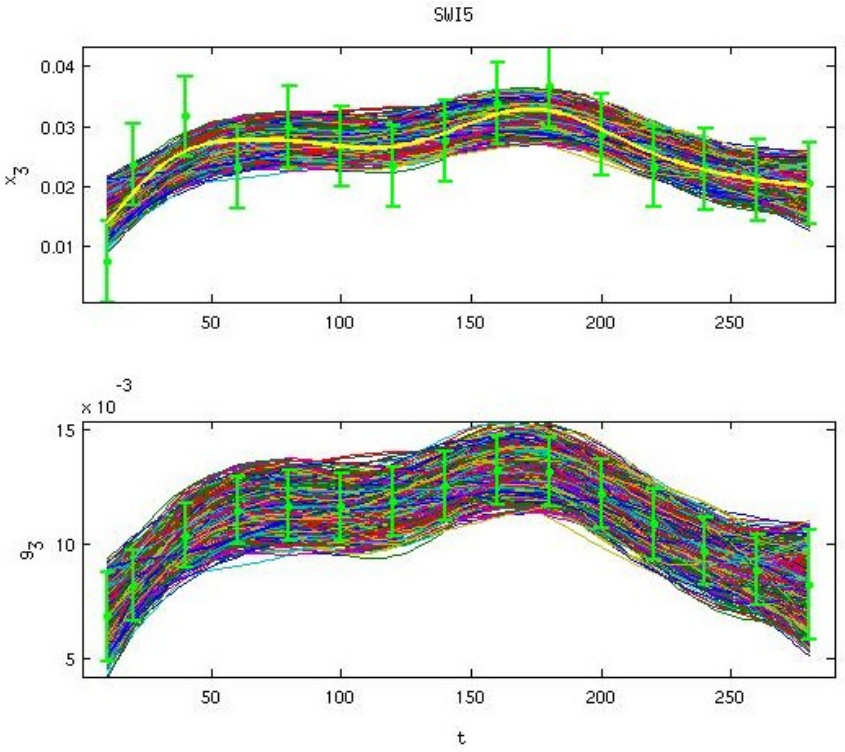
Utilize the fitting errors $\tilde{x}_i^k - \hat{x}_i(t_k)$ to reproduce the noise statistics

Residual resampling

- Randomized procedure to infer statistics of any functional of the regression curve
- Applicable to any type of regression curve (But sensitive to this choice!)
- Our implementation computes statistics of protein concentration and synthesis rate measurements from a single protein concentration dataset.

Algorithm 2 Bootstrap spline-based resampling.

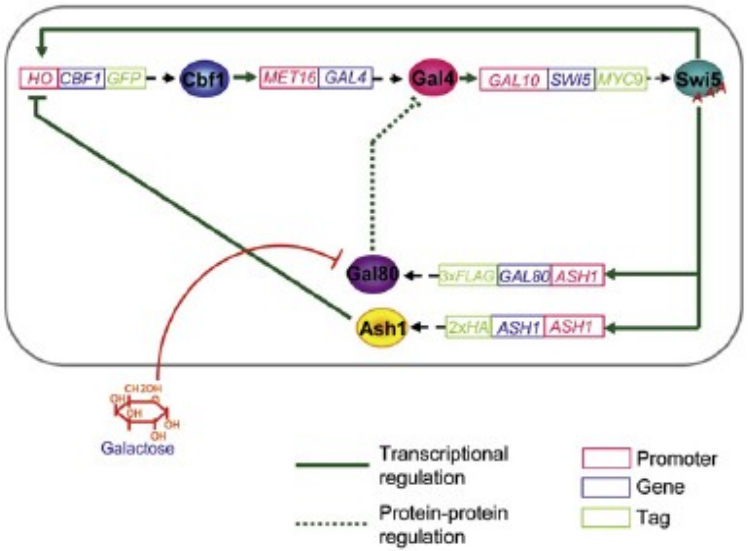
1: compute the spline $\hat{x}_i(t)$ from $\{\tilde{x}_i^k\}$ using weights $\{w^k\}$
2: let $R = \{w^k(\tilde{x}_i^k - \hat{x}_i(t_k)), k = 1, \ldots, m\}$
3: **for** $r = 1$ to N_r **do**
4: extract with replacement m residuals $\{\varepsilon^k\}$ from R
5: let $\tilde{x}_i^{k(r)} = \hat{x}_i(t_k) + \varepsilon^k / w^k, k = 1, \ldots, m$
6: compute the spline $\hat{x}_i^{(r)}(t)$ from $\{\tilde{x}_i^{k(r)}\}$ using weights $\{w^k\}$
7: let $\hat{g}_i^{k(r)} = \dot{\hat{x}}_i^{(r)}(t_k) + \gamma_i \hat{x}_i^{(r)}(t_k), k = 1, \ldots, m$
8: **end for**
9: let $\hat{g}_i^k = \frac{1}{N_r} \sum_r \hat{g}_i^{k(r)}$, $\hat{v}_\epsilon(g_i^k) = \frac{1}{N_r - 1} \sum_r (\hat{g}_i^k - \hat{g}_i^{k(r)})^2$ and $\hat{v}_\epsilon(x_i^k) = \frac{1}{m-1} \sum_{\varepsilon \in R} (\varepsilon / w^k)^2$

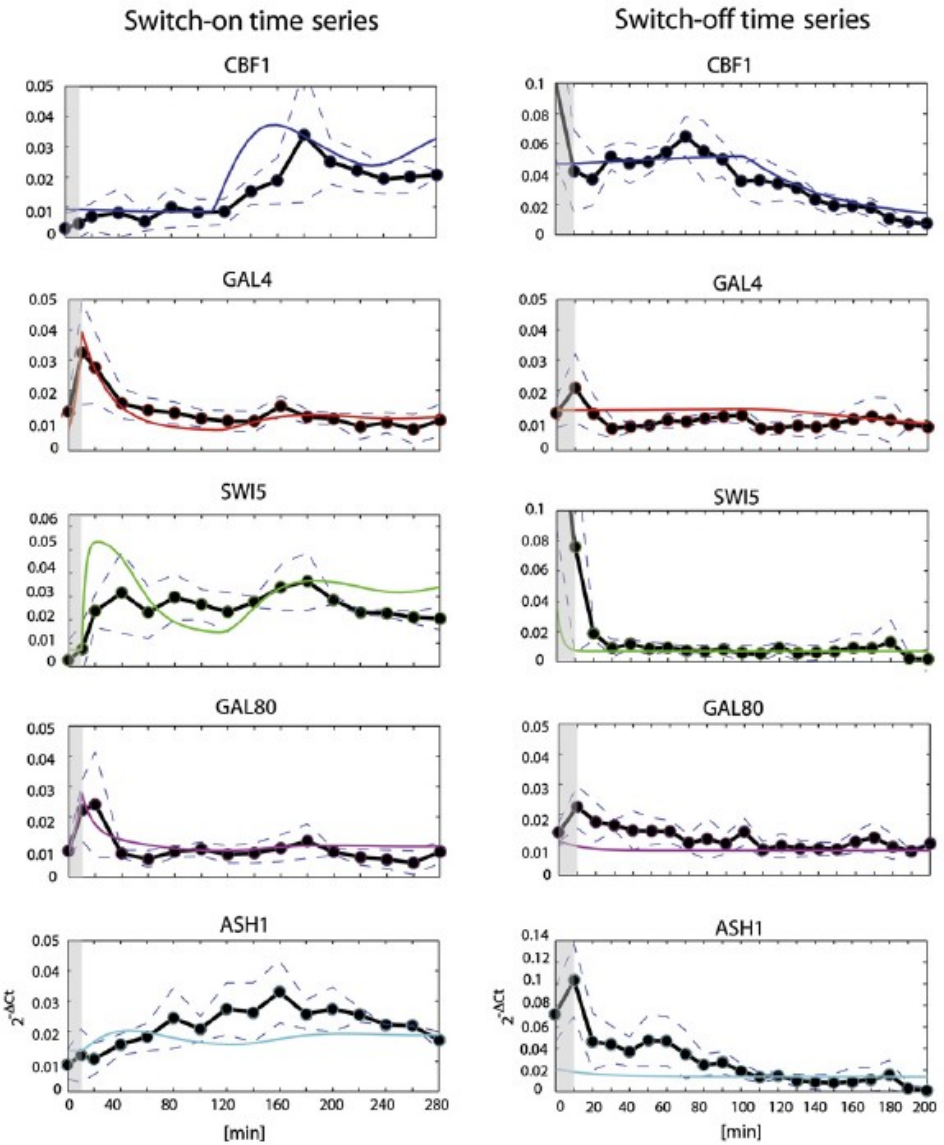


Experiment on IRMA

Synthetic gene network

in Yeast (Cantone et al., Cell 2009)





Mathematical model

(Cantone *et al.*, Cell 2009)

Letting $[CBF1] = x_1$; $[GAL4] = x_2$; $[SWI5] = x_3$; $[GAL80] = x_4$; $[ASH1] = x_5$, the evolution of the mRNAs concentrations were modelled as follows:

$$\frac{dx_1}{dt} = \alpha_1 + v_1 \left(\frac{x_3^{h_1}(t-\tau)}{(k_1^{h_1} + x_3^{h_1}(t-\tau)) \cdot \left(1 + \frac{x_5^{h_2}}{k_2^{h_2}}\right)} \right) - d_1 x_1, \quad (1)$$

$$\frac{dx_2}{dt} = \alpha_2 + v_2 \left(\frac{x_1^{h_3}}{k_3^{h_3} + x_1^{h_3}} \right) - (d_2 - \Delta(\beta_1)) x_2, \quad (2)$$

$$\frac{dx_3}{dt} = \alpha_3 + \widehat{v_3} \left(\frac{x_2^{h_4}}{\widehat{k_4}^{h_4} + x_2^{h_4}(1 + \frac{x_4^4}{\widehat{\gamma}^4})} \right) - d_3 x_3, \quad (3)$$

$$\frac{dx_4}{dt} = \alpha_4 + v_4 \left(\frac{x_3^{h_5}}{k_5^{h_5} + x_3^{h_5}} \right) - (d_4 - \Delta(\beta_2)) x_4, \quad (4)$$

$$\frac{dx_5}{dt} = \alpha_5 + v_5 \left(\frac{x_3^{h_6}}{k_6^{h_6} + x_3^{h_6}} \right) - d_5 x_5, \quad (5)$$

- We attempt identification in the class of models with $H_0 \cup H_1$-structure
  - Different but similar analytical form
  - Test for flexibility of the approach
  - Known delays can be accounted for

Results: full data

- Comparison with TSNI (Cantone *et al.*, Cell 2009)
- True protein concentrations (very few data points)
- Rates simulated from the model ("what-if" performance test)
- Evaluation of network reconstruction performance, but not of parameter fit
- PPV=TD/TD+FD and Se=TD/TD+FU (T=True, D=Detected, U=Undetected edges)

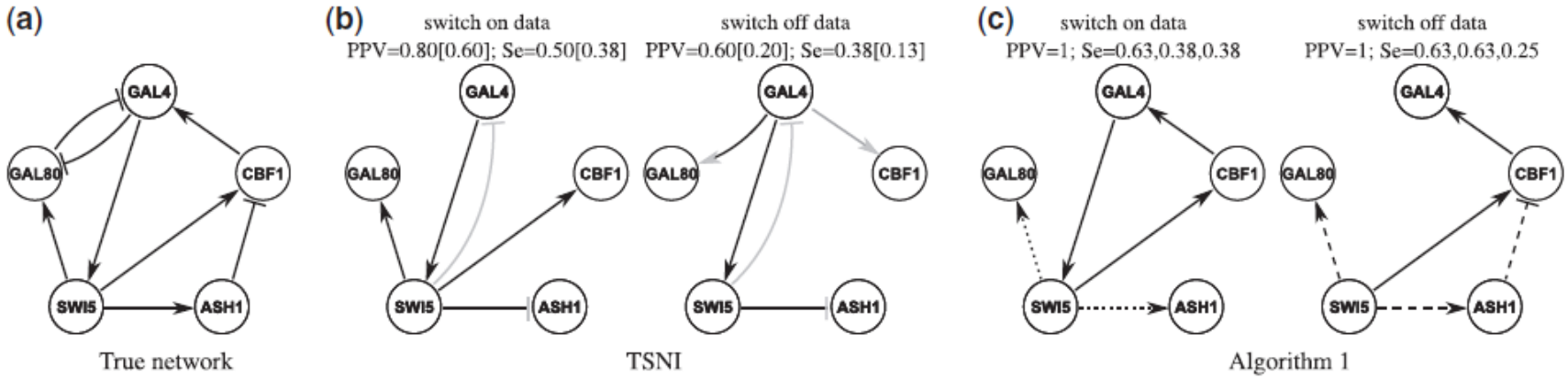


Fig. 1. (a) True network of interactions in IRMA. Results obtained by (b) the TSNI algorithm (Cantone *et al.*, 2009) and by (c) Algorithm 1. Grey arcs (respectively, grey-end markers) denote incorrect direction (respectively, sign) of the inferred interactions. Values of PPV and Se for the signed directed graph, when different from the unsigned case, appear in square brackets. The three values of Se in (c) refer to increasing noise levels, while dashed and dotted arcs denote interactions inferred only for $\sigma_\epsilon < 0.3$ and $\sigma_\epsilon < 0.1$, respectively.

Porreca *et al,* Bioinformatics 2010

Results: partial data

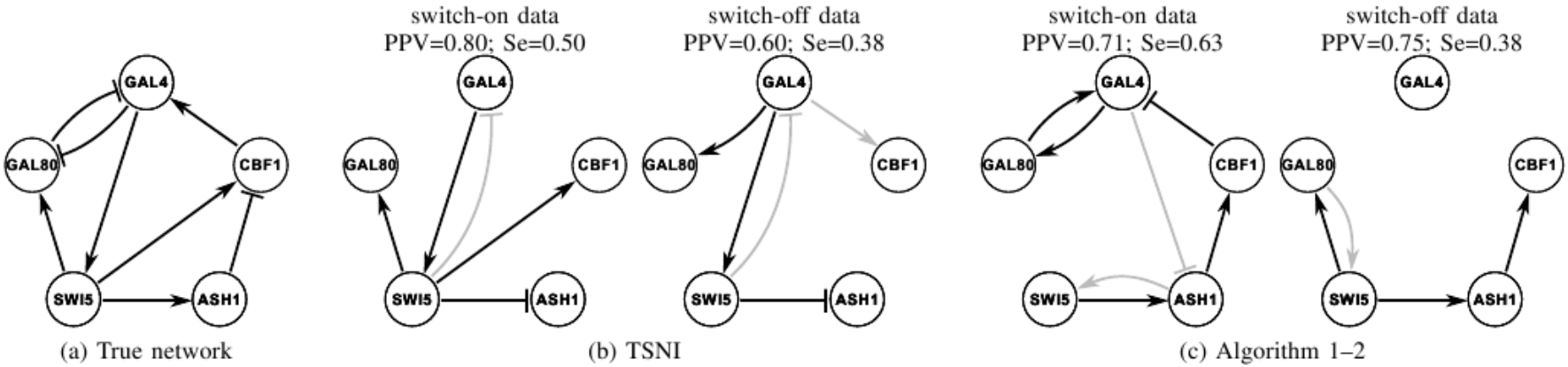
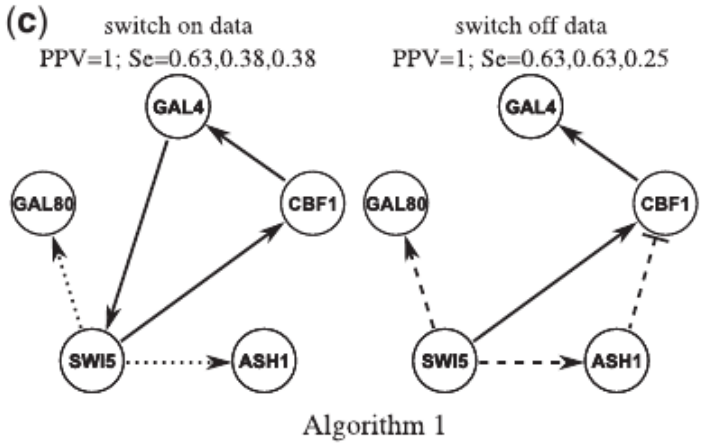


Fig. 1: (a) True network of interactions in IRMA. Results obtained by (b) the TSNI algorithm [27] and by (c) Algorithms 1 and 2. Gray edges denote incorrect direction of the inferred interactions.

- Additional assumptions (no self-regulation)
- Loss of accuracy
  - Parameter estimates (when applicable, not shown)
  - Sign of interaction (possibly due to low data quality)
  - Direction of regulation (bad!)
- Still better than TSNI...

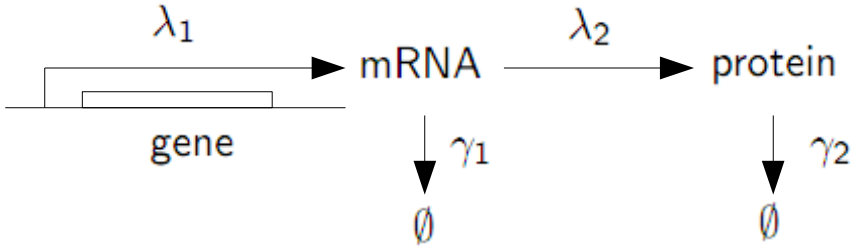
To be compared with...



Identification of stochastic models: A quick view

Introduction: stochastic gene expression

- At the cell level, protein synthesis depends on *random* events
  - Binding/unbinding of activators/repressors and RNApol to DNA, ...
  - Environmental conditions (temperature, availability of free RNAP,...)
- Classical stochastic gene expression model:
  - Describes the formation and degradation of single molecules
  - Time resolution, no spatial resolution (homogeneous reaction volume)



$\mathbf{x}_1$ = number of mRNA molecules

$\mathbf{x}_2$ = number of protein molecules

λ_1, λ_2 = prob. of molecule formation per unit time

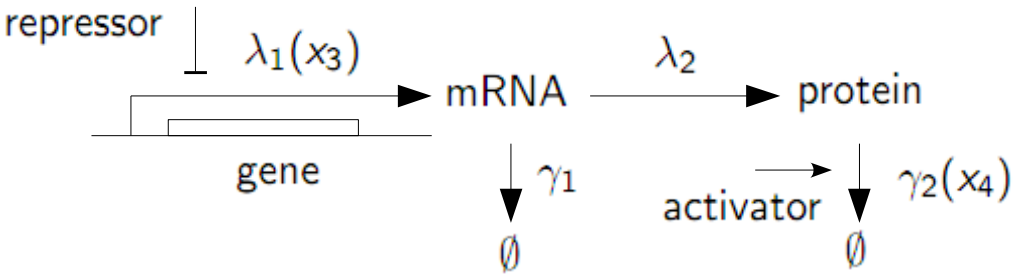
γ_1, γ_2 = prob. of molecule degradation per unit time

$$p(\mathbf{x}_1 = x_1; t+\delta) = p(\mathbf{x}_1 = x_1 - 1; t) \cdot \lambda_1\delta + p(\mathbf{x}_1 = x_1 + 1; t) \cdot \gamma_1\delta + p(\mathbf{x}_1 = x_1; t) \cdot \lambda_2\delta + p(\mathbf{x}_1 = x_1; t) \cdot (1 - \lambda_1\delta - \gamma_1\delta - \lambda_2\delta)$$

$$p(\mathbf{x}_2 = x_2; t+\delta) = p(\mathbf{x}_2 = x_2 - 1; t) \cdot \lambda_2\delta + p(\mathbf{x}_2 = x_2 + 1; t) \cdot \gamma_2\delta + p(\mathbf{x}_2 = x_2; t) \cdot (1 - \lambda_2\delta - \gamma_2\delta)$$

Regulation and noise

- Example: regulated gene expression and protein degradation



x_3 = number of repressor molecules

x_4 = number of activator molecules

$\lambda_1(x_3) = \kappa_1 \cdot 1/(1 + x_3)$ (*e.g.*)

$\gamma_2(x_4) = \kappa_2 \cdot x_4/(1 + x_4)$ (*e.g.*)

- This modelling framework describes the random nature of the events *internal* to the gene expression mechanism (*intrinsic noise*)
- Random fluctuations of the event rates, due to changes *external* to the gene expression mechanism, are not modelled (*extrinsic noise*)

[Many contributors: Paulsson, Elowitz, Alon, Arkin, ...]

Network modeling: Chemical Master Equation

- Generalization of the stochastic modelling framework seen before to any biochemical (regulatory) network

$$\dot{p}(\mathbf{x};t) = \sum_{\mu=1}^{M} p(\mathbf{x}-s_\mu;t)a_\mu(\mathbf{x}-s_\mu) - p(\mathbf{x};t)\cdot\sum_{\mu=1}^{M} a_\mu(\mathbf{x})$$

$\mathbf{x}$ = random vector of the number of molecules of every species, one per entry

μ = reaction index (from 1 to M possible reactions)

s_μ = state change associated to the μ-th reaction

a_μ = propensity (prob. per unit time) of μ-th reaction (state dependent)

- Infinite-dimensional linear equation in the probabilities p
- No closed-form solution, but finite-complexity approximations

[Recent references: Gillespie, Khammash, ...]

Experimental measurement of p

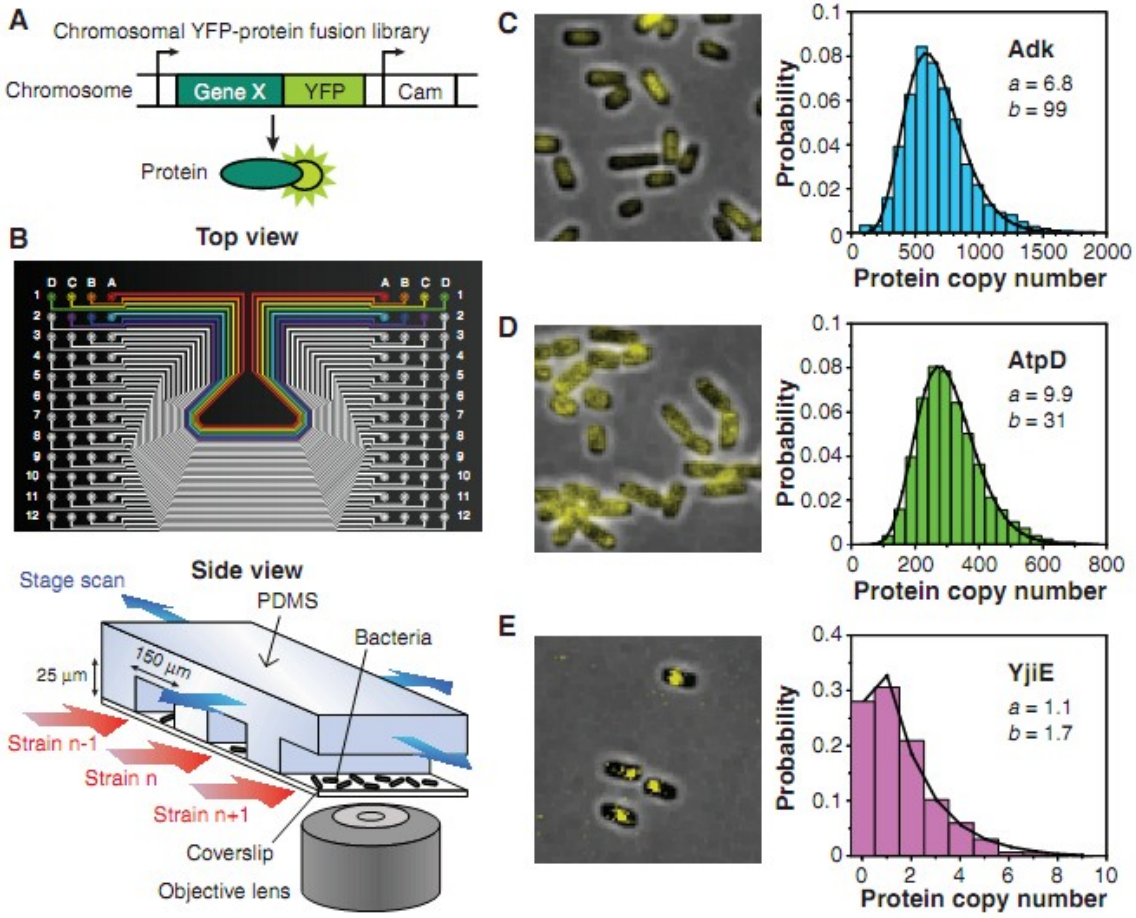


Fig. 1. Quantitative imaging of a YFP-fusion library. (**A**) Each library strain has a YFP translationally fused to the C terminus of a protein in its native chromosomal position. (**B**) A poly(dimethylsiloxane) (PDMS) microfluidic chip is used for imaging 96 library strains. *E. coli* cells of each strain are injected into separate lanes and immobilized on a polylysine-coated coverslip for automated fluorescence imaging with single-molecule sensitivity. (**C** to **E**) Representative fluorescence images overlaid on phase-contrast images of three library strains, with respective single-cell–protein level histograms that are fit to gamma distributions with parameters *a* and *b*. Protein levels are determined by deconvolution (*18*). The protein copy number per average cell volume, or the concentration, was determined as described in the main text and the SOM (*18*). (C) The cytoplasmic protein Adk uniformly distributed intracellularly. (D) The membrane protein AtpD distributed on the cell periphery. (E) The predicted DNA-binding protein YjiE with clear intercellular localization. Single YjiE-YFPs can be visualized because they are localized. Note that, unlike (C) and (D), the gamma distribution asymmetrically peaks near zero if *a* is close to or less than unity.

[Taniguchi *et al.*, Science 329, 533 (2010)]

Identification: Finite State Projection method

- Form a vector p* with the probabilities of most likely states X*
- Approximate the CME with the linear equation

$$\dot{p}^*(t) = A^* p^*(t), \; p^*(0) = p_0^*, \qquad p^*(t) = \exp(A^* t) p_0^*$$

- For any t and any x* in X*, p*(t) is an approximation of p(x*;t) (theoretical guarantees for “smart” choice of X*)
- Solve the optimization problem

$$\widehat{A}^* = \arg \min_{A^*} \sum_k ||y_k - p^*(t_k)||$$

where y_k are empirical measurements of p* at times t_k

(histograms from measurements of x* over many cells)

[Finite State Projection: Munsky and Khammash, J. Chem. Phys 124 (2006)]
[Use in identification: Munsky et al, Mol Syst Biol 5:318 (2009)]

Identification: Other methods

- Moment matching: [e.g. work by J.Hespana]
  - Instead of probabilities, consider vector of all moments z and a truncation z*

$$z(t) = \begin{bmatrix} Ex(t) & Ex(t)^T x(t) & \dots \end{bmatrix}^T, \qquad z^*(t) = \begin{bmatrix} Ex(t) & Ex(t)^T x(t) \end{bmatrix}^T$$

  evolving according to the equations depending on the model parameters

$$\dot{z}(t) = Bz(t), \qquad \dot{z}^*(t) \simeq B^* z^*(t)$$

  and fit the equation for z* to the corresp. empirical statistics from many cells

- At stochastic steady state: [Taniguchi *et al.*, Science 329, 533 (2010)]
  - System evolves until stochastic equilibrium where p does not change
  - Use asymptotic approximation with a Gamma distribution

$$p(x;t) \to d(x) \text{ for } t \to +\infty$$

  to fit (combinations of the) model parameters

Determinism vs. randomness

- Population-level dynamics vs. cell-level phenomena
- Need to explain multiple equilibria (multimodality)
- Effects of noise not always negligible
- Particle-level stochastic model of biochemical reaction networks (Chemical Master Equation)

$$\dot{p}(\mathbf{x};t) = -p(\mathbf{x};t)\sum_{\mu=1}^{M} a_\mu(\mathbf{x}) + \sum_{\mu=1}^{M} p(\mathbf{x}-s_\mu;t)a_\mu(\mathbf{x}-s_\mu)$$

is a quite complex tool and may be overly detailed

Stochastic hybrid modelling

Aim: convenient tradeoff b/w accuracy and tractability

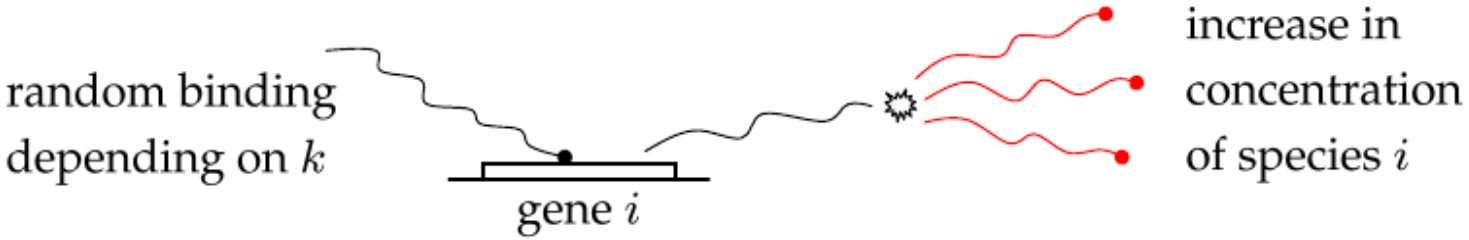
- Gene expression depends on a molecular complex binding/unbinding to a promoter site

  Particle-level event

- Expression of one gene leads to production of several molecules of the same protein

  Concentration-level dynamics

- Idea: model gene expression as a random event with state-dependent stochastic laws

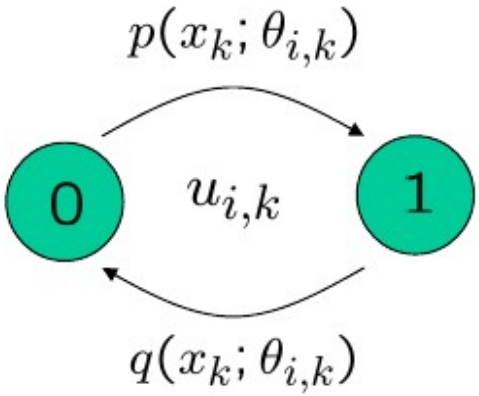


Piecewise deterministic model

- Concentration dynamics: $x_i(t+1) = \lambda_i x_i(t) + g_i(t)$
- Synthesis rate: combination of random binary processes

$$g_i(t) = \sum_j b_i^j \prod_{k \in \ell(i,j)} u_{i,k}(t)$$

- Sigmoidal transition probabilities:



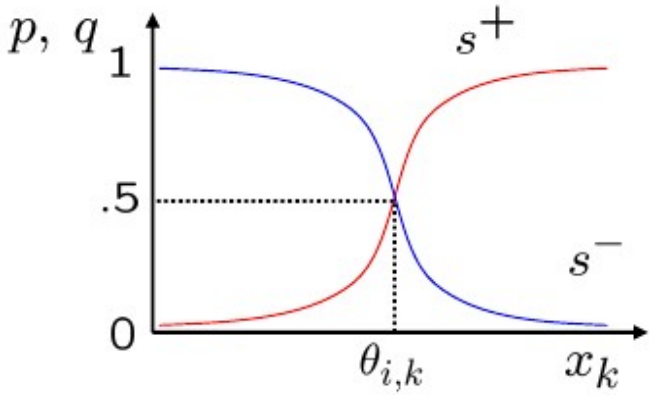
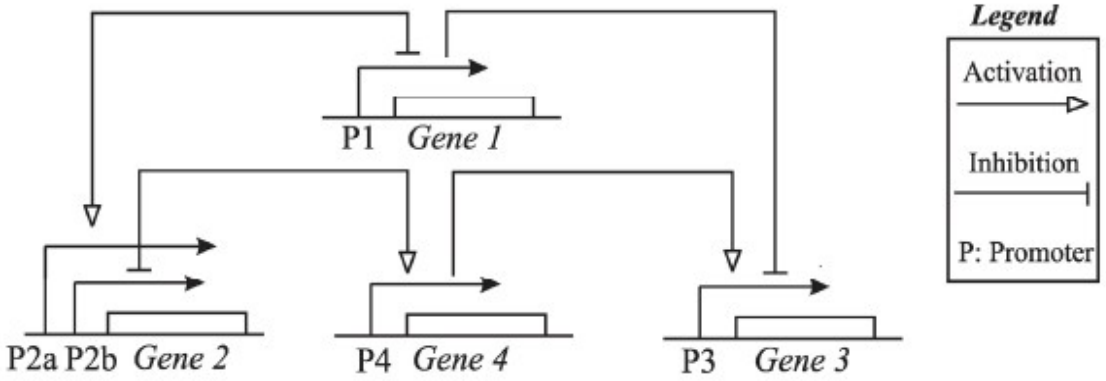
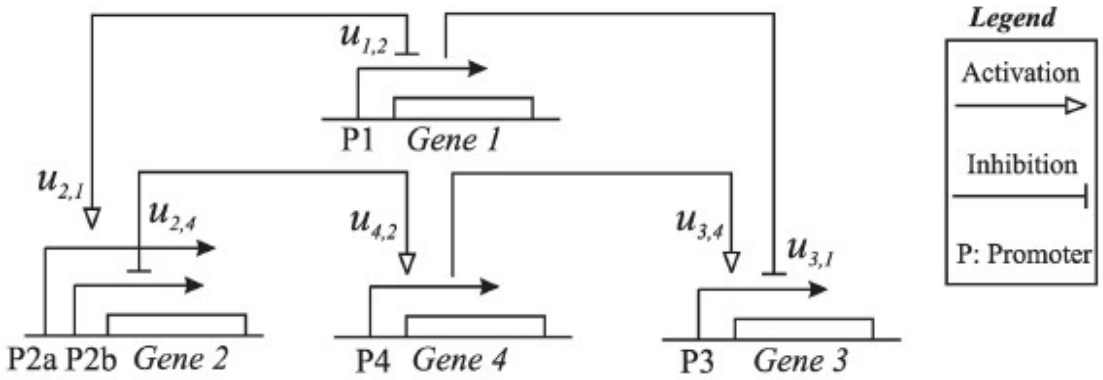


Illustration (Boolean model)



Gene	Expressed when	Boolean model
1	G2 not expressed	$b_1(x) = \neg x_2$
2	G1 expressed or G4 not expressed	$b_2(x) = x_1 \vee \neg x_4$
3	G4 expressed and G1 not expressed	$b_3(x) = x_4 \wedge \neg x_1$
4	G2 expressed	$b_4(x) = x_2$

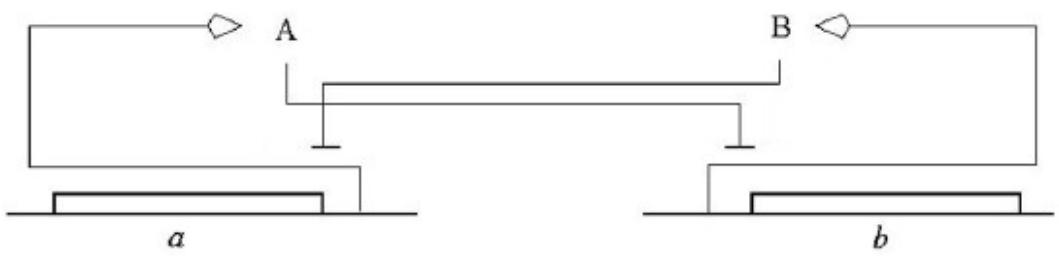
Illustration (piecewise deterministic model)



Gene	Expressed when	Synthesis rate model
1	TF2 not on P1	$g_1(t) = b_1^1\big(1 - u_{1,2}(t)\big)$
2	TF1 on P2a ∨ TF4 not on P2b	$g_2(t) = b_2^1 u_{2,1}(t) + b_2^2\big(1 - u_{2,4}(t)\big)$
3	TF4 on P3 ∧ TF1 not on P3	$g_3(t) = b_3^1 u_{3,4}(t)\big(1 - u_{3,1}(t)\big)$
4	TF2 on P4	$g_4(t) = b_4^1 u_{4,2}(t)$

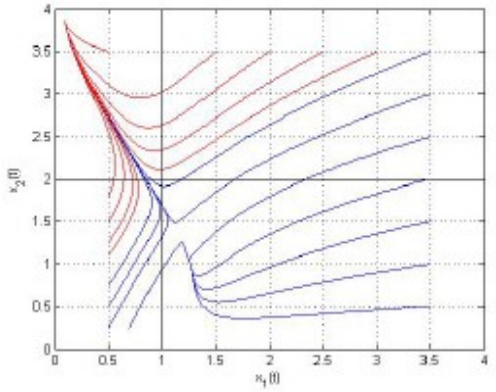
Example: bistable switch

- Network that admits two distinct stable equilibria

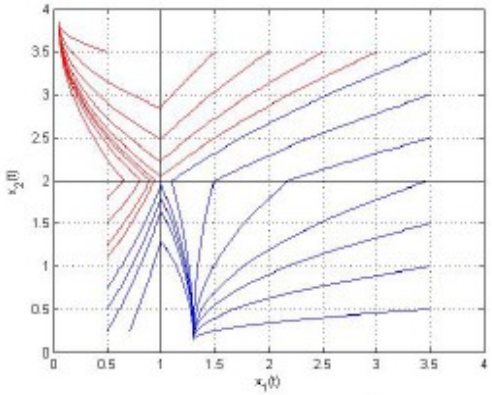


A	B	A^+	B^+
Lo	Lo	Hi	Hi
Lo	Hi	Lo	Hi
Hi	Lo	Hi	Lo
Hi	Hi	Lo	Lo

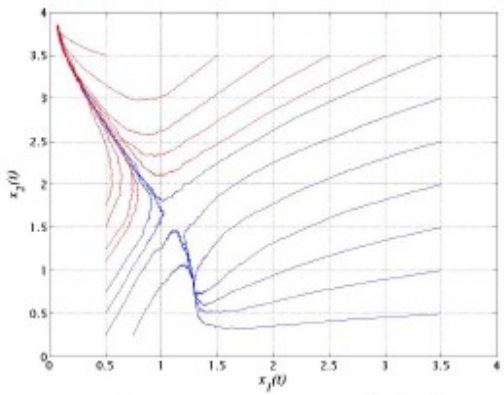
- State determined by dynamics (initial state/perturbations)



Deterministic, sigmoids



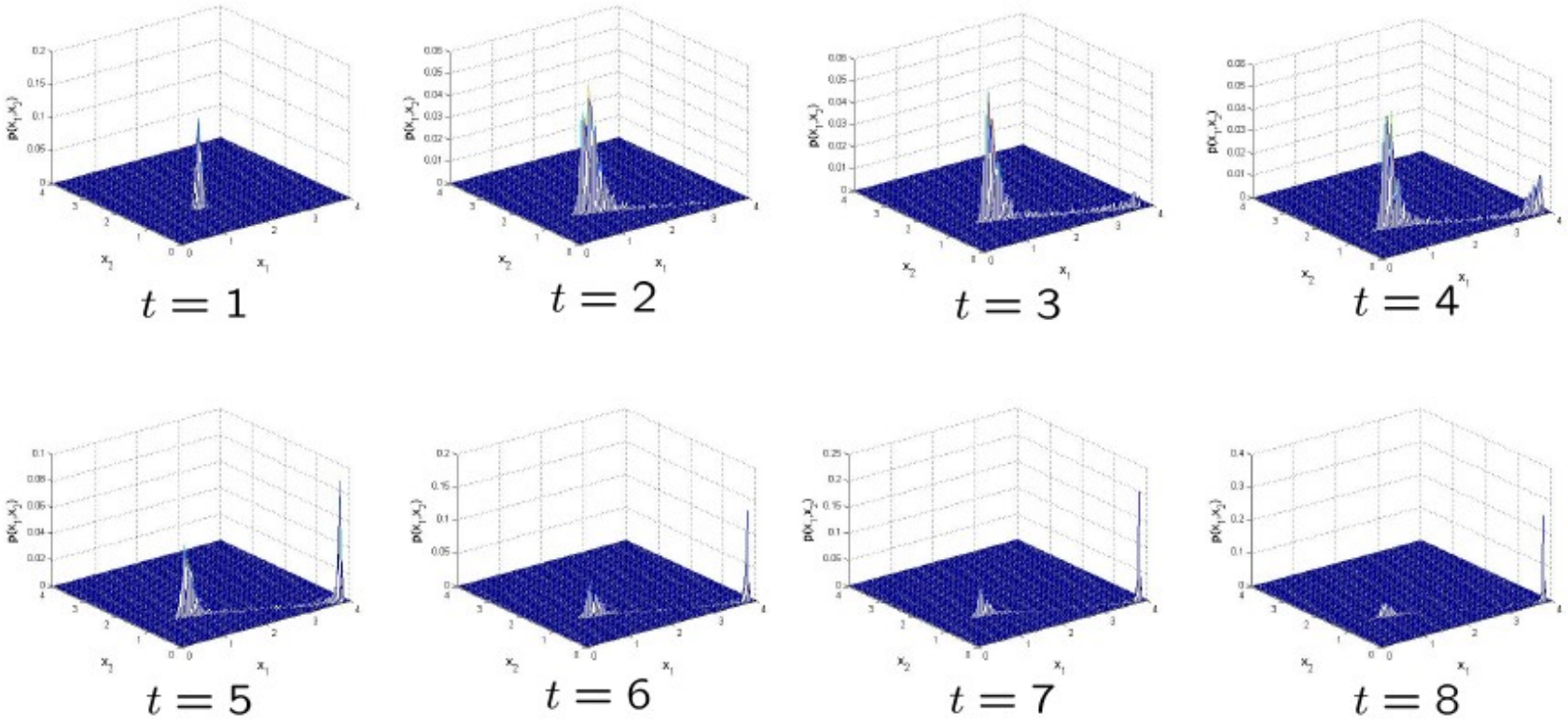
Deterministic, step functions



Stochastic, sigmoidal laws

Probability density of the state

(Stochastic model, fixed initial concentrations)



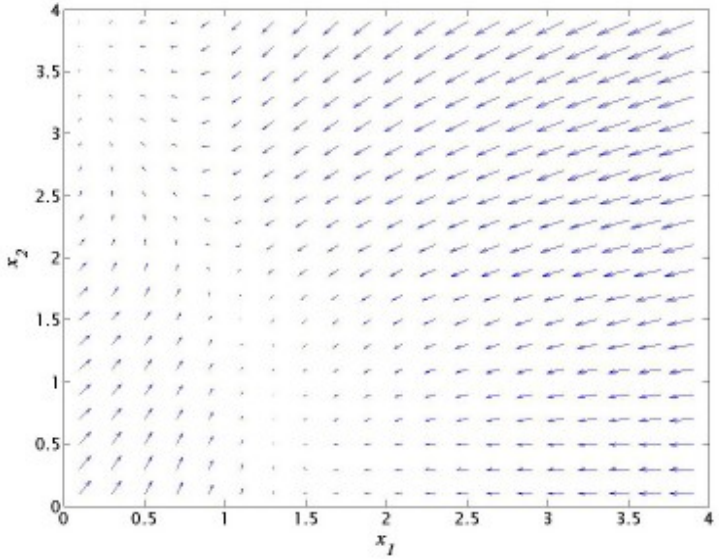
(Convergence to different equilibria with different probabilities)

Piecewise deterministic model vs. ...

… *ODE models:*

Vector field recovered in terms of expected state transitions:

$$\mathbb{E}[x(t+1)|x(t) = (x_1, x_2)]$$



… *Chemical Master Equation:* approximation of slow modes with average dynamics leads to stochastic hybrid models

… *Continuous-time SHS:* common forms of discrete-event propensities lead to sigmoidal stationary probabilities

Parameter identification

- Assumption: the interaction network is known
- Data: noisy measurements $y_i(\tau)$ of $x_i(\tau)$
  - Zero-mean measurement noise
  - Sparse sampling ($\tau \in \{\tau_0, \tau_1, \ldots, \tau_l, \ldots\}$)
  - Multiple asynchronous experiments ($m = 1, 2, \ldots$)
- Aim: estimate
  - Parameters of the sigmoidal switching probabilities (thresholds, steepness, …)
  - Protein synthesis and decay rates

Prediction error identification method

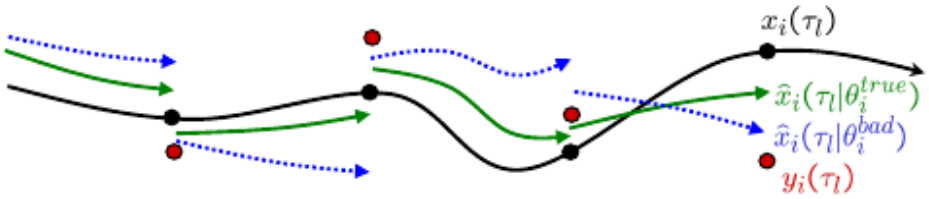
- Consider: prediction of $x_i(t)$ given data up to τ_l

$$\mathscr{Y}_i(\tau_l) = \{y_{\ell(i)}(\tau_h), y_i(\tau_h) : \ h = 1, \ldots, l\}$$

$$\widehat{x}_i(t, \tau_l | \theta_i) \triangleq \mathbb{E}[x_i(t) | \mathscr{Y}_i(\tau_l), \theta_i]$$

- Rationale: minimization of prediction errors

$$\min_{\theta_i} \ \sum_m \sum_l \left(y_i^{(m)}(\tau_l) - \widehat{x}_i^{(m)}(\tau_l, \tau_{l-1} | \theta_i) \right)^2$$



- Wishes:
  - Explicit expression of the predictor as a function of data
  - Numerical solution of separate optimization problems, one for each gene: $i = 1, \ldots, n$

Computation of the predictor

Hypothesis: $p(u|x,u^-) \simeq p(u|x)$

(validated by numerical results)

Result: recursive predictor update

$$\widehat{x}_i(t+T,\tau_l|\theta_i) = \lambda_i \widehat{x}_i(t,\tau_l|\theta_i) + \mathbb{E}[\bar{g}\Big(x_{\ell(i)}(t)\Big)|\mathscr{Y}_i(\tau_l),\theta_i]$$

$$\bar{g}\Big(x_{\ell(i)}(t)\Big) = \sum_j b_i^j \prod_{k\in\ell(i,j)} s^{\pm}(x_k(t);\theta_{i,k})$$

Two approximations:

- For a suitable $x^*_{\ell(i)}$ inferred from data,

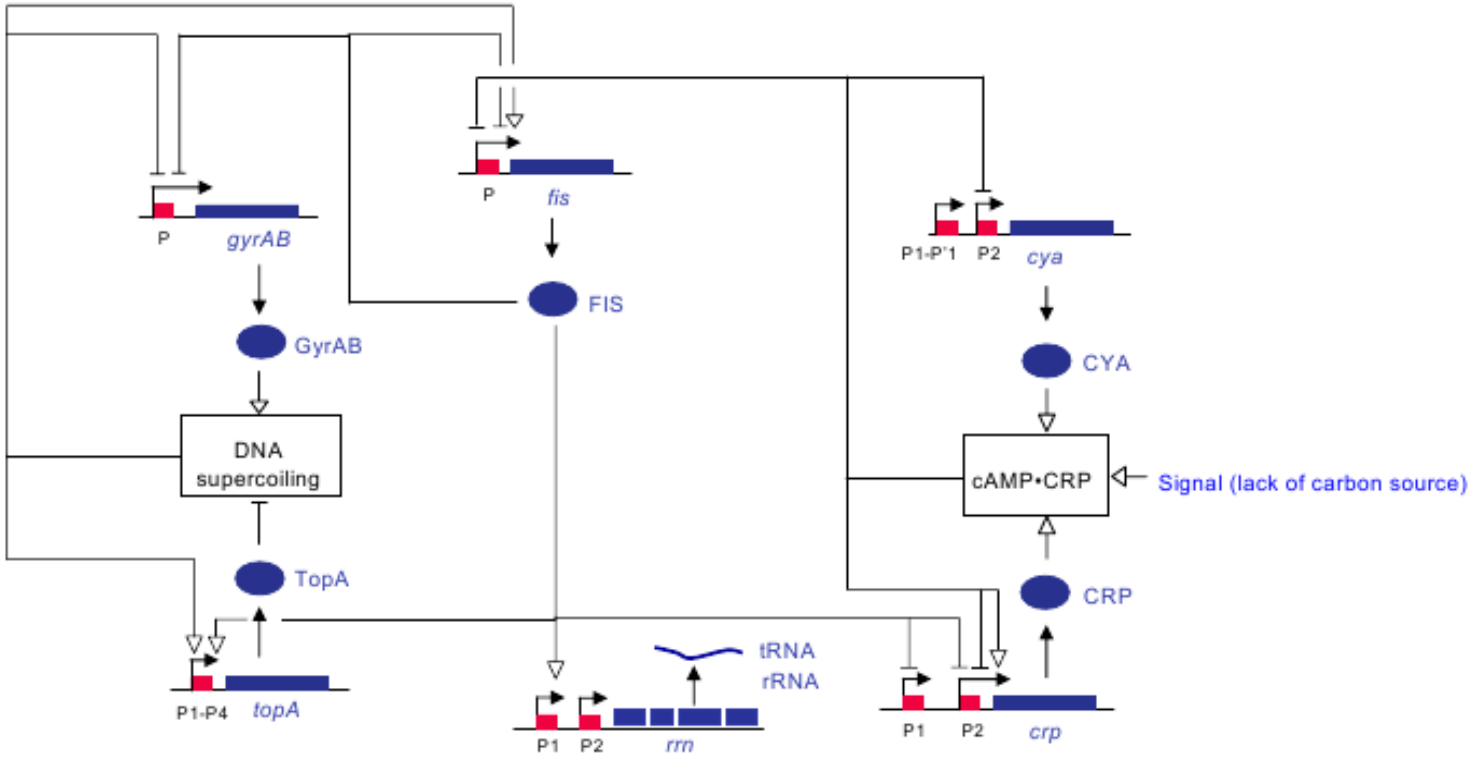
$$\mathbb{E}[\bar{g}\Big(x_{\ell(i)}(t)\Big)|\mathscr{Y}_i(\tau_l),\theta_i] \simeq \bar{g}\Big(x^*_{\ell(i)}\Big),$$

- Initialization of the recursion:

$$\widehat{x}_i(\tau_l,\tau_l|\theta_i) \simeq \gamma\, y_i(\tau_l) + (1-\gamma)\, \widehat{x}_i(\tau_l,\tau_{l-1}|\theta_i)$$

Case study: E.coli carbon starvation response

- Recall 6 genes interaction network (Ropers et al, 2006)



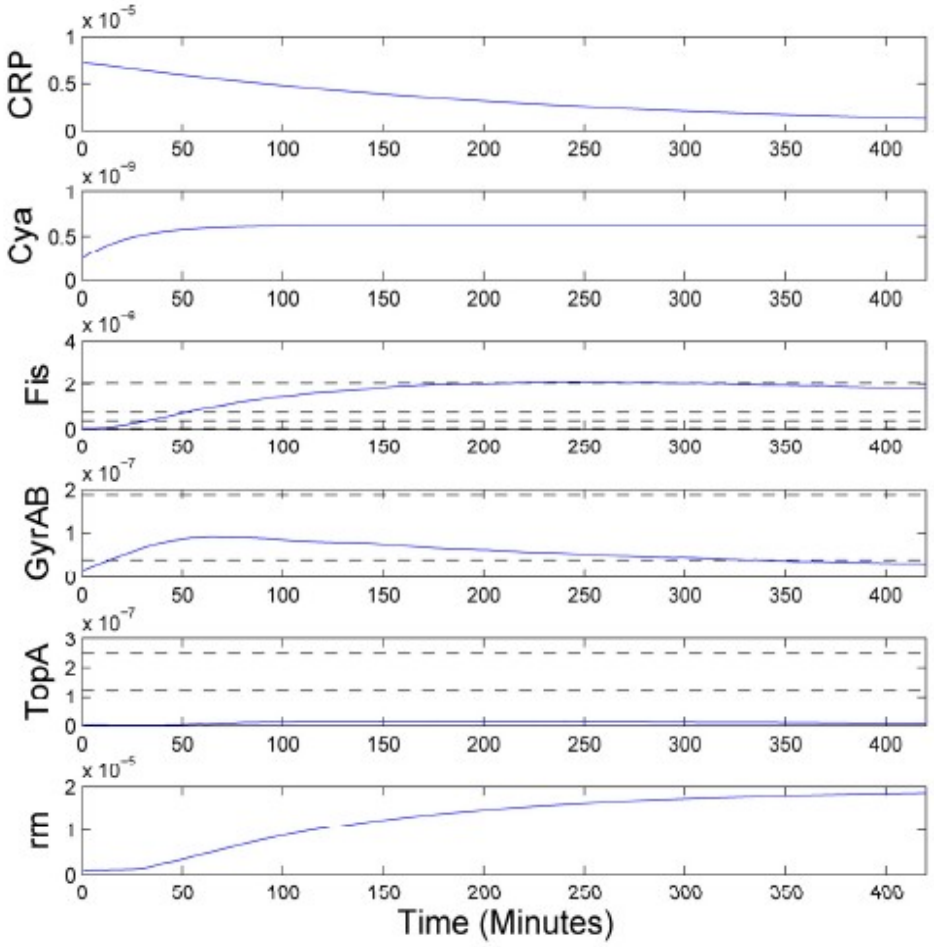
Piecewise deterministic version

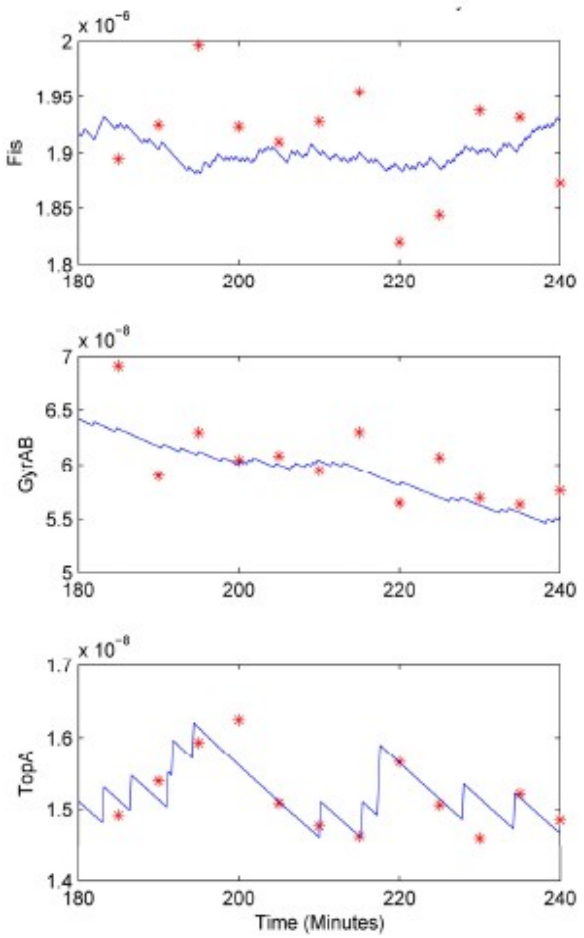
$$
\begin{aligned}
x_y^+ &= \lambda_y x_y + b_y(1 - u_{yc}u_{yy}u_s) + \bar{b}_y \\
x_c^+ &= \lambda_c x_c + b_c u_{cf} u_{cc} u_{cy} u_s + b'_c u'_{cf} + \bar{b}_c \\
x_f^+ &= \lambda_f x_f + b_f(1 - u_{fc}u_{fy}u_s)u_{ff} + b'_f u_{fa}u_{ft}u_{ff} \times (1 - u_{fc}u_{fy}u_s) + \bar{b}_f \\
x_a^+ &= \lambda_a x_a + b_a(1 - u_{aa}u_{at})u_{af} + \bar{b}_a \\
x_t^+ &= \lambda_t x_t + b_t u_{ta} u_{tt} u_{tf} + \bar{b}_t \\
x_n^+ &= \lambda_n x_n + b_n u_{nf} + \bar{b}_n
\end{aligned}
$$

$$
\begin{aligned}
p_{yc} &= s^+(x_c, \theta_{yc}) \\
p_{yy} &= s^+(x_y, \theta_{yy}) \\
p_{cf} &= s^-(x_f, \theta_{cf}) \\
p_{cc} &= s^+(x_c, \theta_{cc}) \\
p_{cy} &= s^+(x_y, \theta_{cy}) \\
p'_{cf} &= s^-(x_f, \theta'_{cf})
\end{aligned}
\qquad
\begin{aligned}
p_{fc} &= s^+(x_c, \theta_{fc}) \\
p_{fy} &= s^+(x_y, \theta_{fy}) \\
p_{ff} &= s^-(x_f, \theta_{ff}) \\
p_{fa} &= s^+(x_a, \theta_{fa}) \\
p_{ft} &= s^-(x_t, \theta_{ft})
\end{aligned}
\qquad
\begin{aligned}
p_{aa} &= s^+(x_a, \theta_{aa}) \\
p_{at} &= s^-(x_t, \theta_{at}) \\
p_{af} &= s^+(x_f, \theta_{af}) \\
p_{ta} &= s^+(x_a, \theta_{ta}) \\
p_{tt} &= s^-(x_t, \theta_{tt}) \\
p_{tf} &= s^+(x_f, \theta_{tf}) \\
p_{nf} &= s^+(x_f, \theta_{nf})
\end{aligned}
$$

- We focus on reentry into exponential growth ($u_s \longrightarrow 0$)

Simulated example





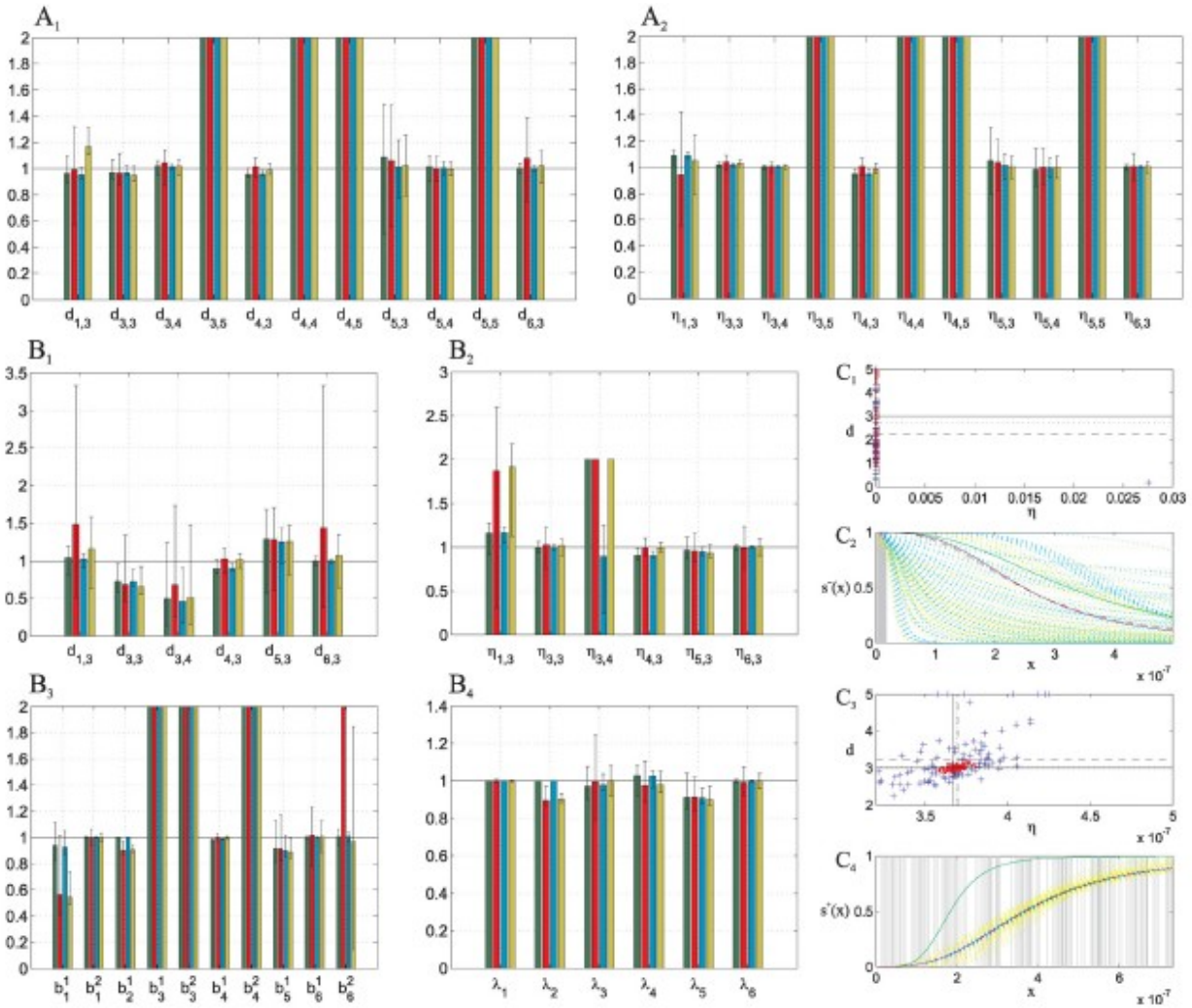
Identification experiment

- Data simulated from *E.coli* stochastic model with realistic parameter values
- 50 data points sampled every 5 minutes
- With and without noise
- 25 or 100 repeated experiments
- Two scenarios:

  A. Known synthesis/degradation rates, estimation of all sigmoidal parameters

  B. Unobservable sigmoidal parameters fixed to their true values, estimation of all the remaining parameters

Numerical results



Legend	25 trajs	100 trajs
No noise	Green	Blue
With noise	Red	Yellow

Structure identification

- Problem: infer network structure from data
- Complex (intractable ?) problem, happy with gene interconnectivity
- Aim: extend steady-state linearization methods to stochastic models
  - Assumptions require small but several perturbations
  - Only one data point per experiment used
  - Randomness is inherent perturbation that is not exploited
- Idea: Work in “stochastic steady state” (stationarity), account for intrinsic noise to infer local dynamics

Local dynamics from local statistics

- Piecewise deterministic model

$$x^+ = \Lambda x + g(u), \qquad p(u|x, u^-) = p(u|x)$$

- Undersampled measurement model

$$y(Nt) = x(Nt) + n(Nt), \qquad n \sim \mathcal{N}(0, R) \text{ i.i.d.}$$

- Idea: stationary 2nd order moment approx around "equilibrium"

$$(\bar{x}, \bar{u}) \triangleq \mathbb{E}(\bar{x}, \bar{u})$$

- Result: for $\mathbb{A}_{\bar{x},\bar{u}} = \Lambda + Dg(\bar{u})Dp(\bar{x})$,

$$x^+ \simeq \mathbb{A}_{\bar{x},\bar{u}} x + w$$

$$\Sigma_y(\ell + 1) \simeq \mathbb{A}_{\bar{x},\bar{u}}(\Sigma_y(\ell) - \delta(\ell)R) \qquad \Sigma_y(\ell) \triangleq \mathbb{E}\, y(t+\ell)y(t)^T$$

Optimization problem

- Remove empirical mean from data
- Compute estimates

$$\widehat{\Sigma}_y(\ell) \propto \sum y(t+\ell)y(t)^T$$

- Set

$$\widehat{\Sigma}_+ = [\widehat{\Sigma}(2N) \cdots \widehat{\Sigma}(LN)]$$

$$\widehat{\Sigma}_- = [\widehat{\Sigma}_y(N) - R \quad \widehat{\Sigma}_y(2N) \cdots \widehat{\Sigma}_y(LN-N)]$$

- Solve the two-step problem:

1. convex formulation of the constrained optimization

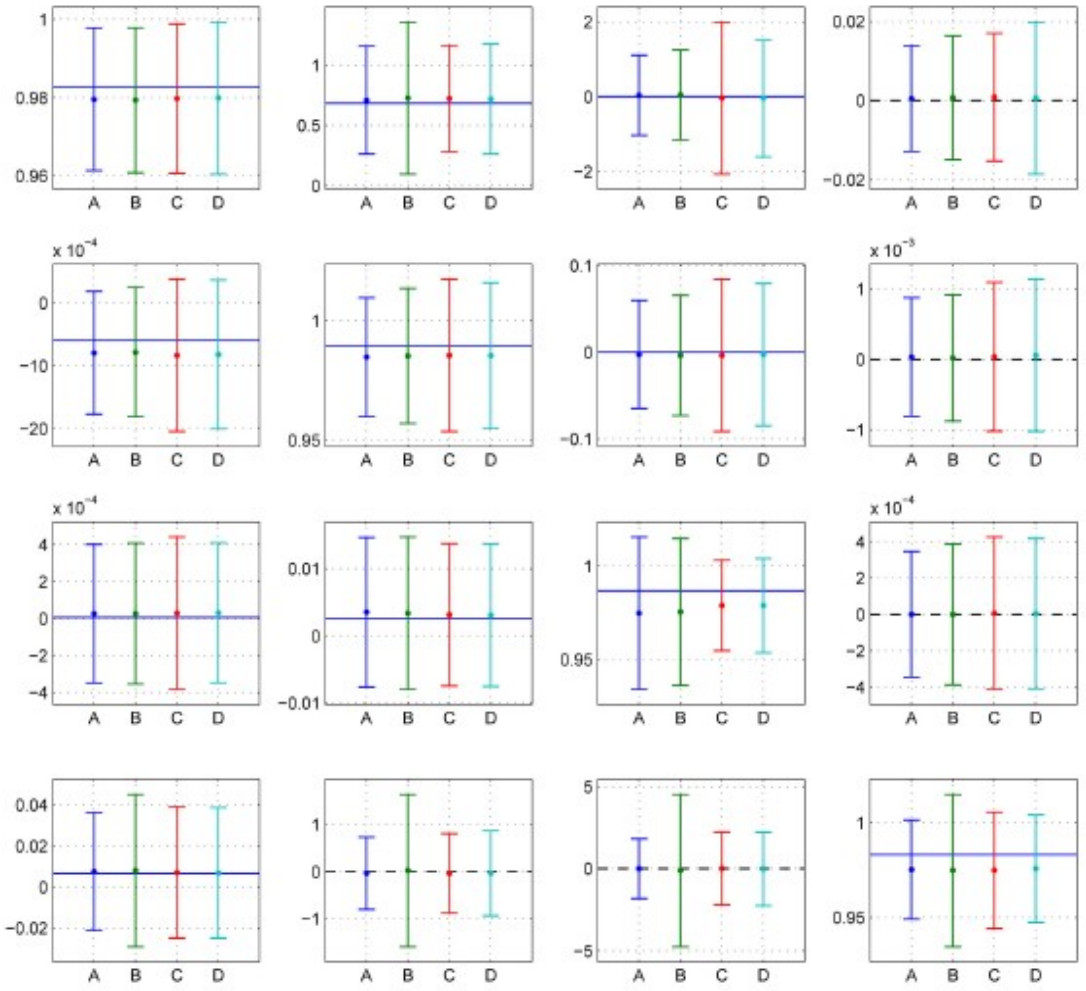
$$\min_{Z \in \mathscr{Z}} ||\widehat{\Sigma}_+ - Z\widehat{\Sigma}_-||, \qquad \mathscr{Z} = \{...(\text{stable})\}$$

2. take the N-th principal matrix root

$$\widehat{\mathbb{A}} = \widehat{Z}^{1/N}$$

Results from simulation

Legend	N=1	N=10
No noise	A	C
3% noise	B	D



Discussion

- Evidence for fundamental role of intrinsic and extrinsic noise

  (e.g. Elowitz et al, *Science*, 2002)

- Identification of stochastic models of genetic networks still in its infancy, first results on problem analysis and solution methods

  (Munsky, Khammash et al 2009)

- Theory and tools from stochastic linear system identification

- Great interest for near-future experimental techniques

Conclusions

- Masses of data wait for being processed. Automated processing unavoidable
- Modern experimental techniques enable inference of quantitative dynamic models at population and (sometimes) single cell level, even more to come
- Numerous applications in medicine, (bio)chemical industry etc.
- A lot of work in progress for model identification methods
- Intriguing mathematical problems
- Nonstandard identification problems: a lot to use, a lot to invent
- Exciting interdisciplinary activity
- Opportunities for internships & research projects !

... Thank you!

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