Development and Experimental Validation of Piecewise Affine Models of Carbon Starvation Response in *Escherichia coli*

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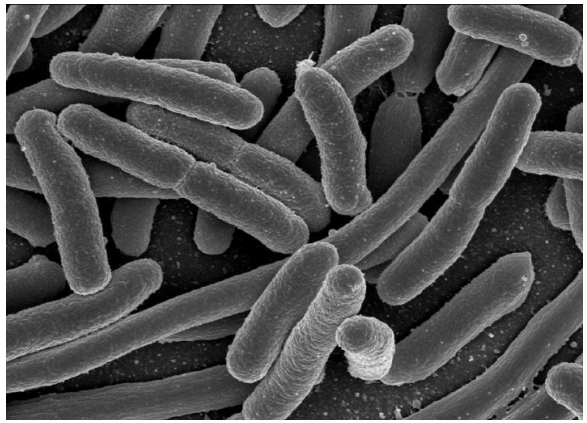
Overview

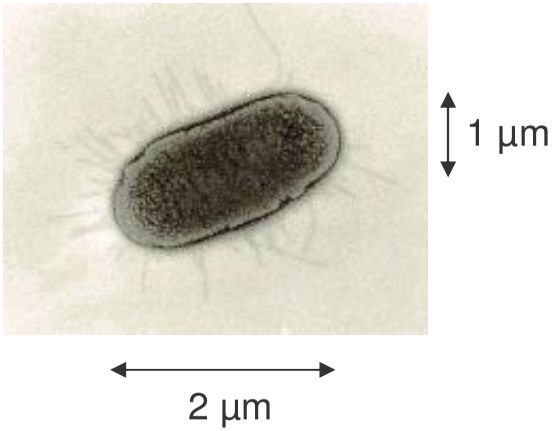
1. Carbon starvation response of *Escherichia coli*
2. Modeling and simulation: objective and constraints
3. Qualitative modeling and simulation of carbon starvation
4. Experimental validation of carbon starvation model
5. Conclusions

Escherichia coli

- The average **human gut** contains about **1 kg of bacteria**
  - Normally, approximatively 0.1% are *E. coli*



Rocky Mountain Laboratories, NIAID, NIH

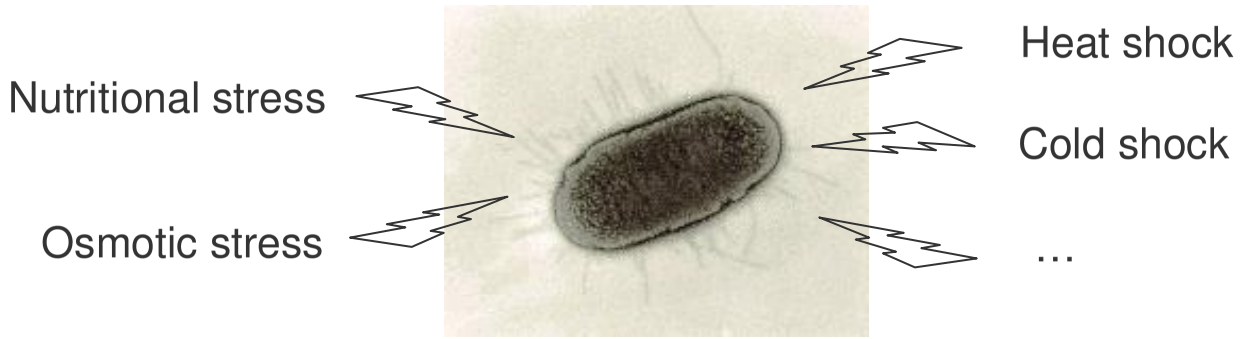


  - *E. coli*, along with other enterobacteria, synthesize **vitamins** which are absorbed by our body (*e.g.*, vitamin K, B-complex vitamins)

Escherichia coli stress responses

- *E. coli* is able to adapt and respond to a variety of stresses from its environment

Storz and Hengge-Aronis (2000), *Bacterial Stress Responses*, ASM Press

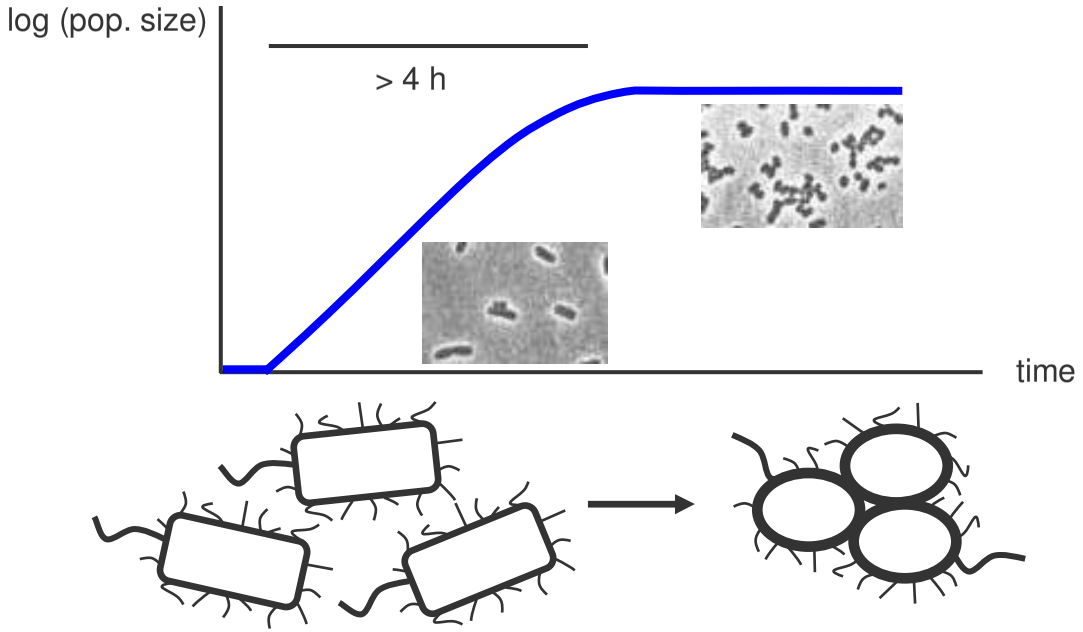


- Model organism for understanding adaptation of pathogenic bacteria to their host

Nutritional stress response in *E. coli*

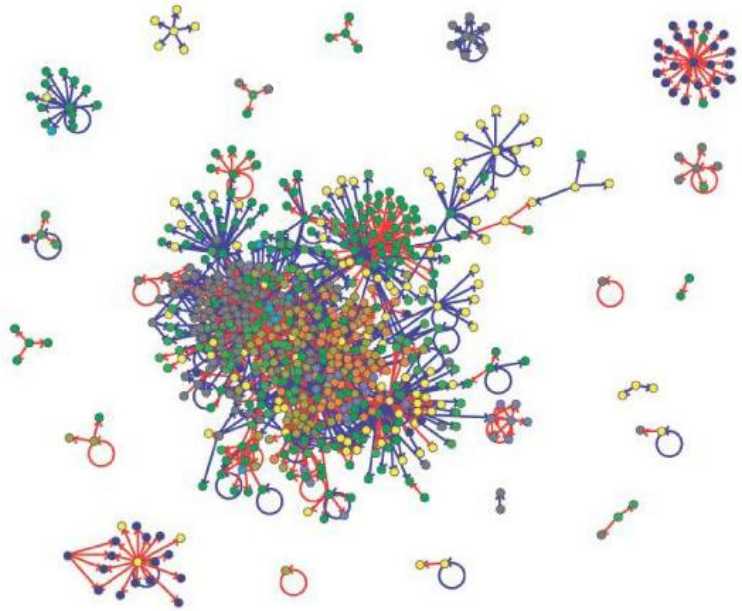
- Response of *E. coli* to nutritional stress conditions: transition from **exponential phase** to **stationary phase**

  Changes in morphology, metabolism, gene expression, …



Network controlling stress response

- Response of *E. coli* to nutritional stress conditions controlled by large and complex **genetic regulatory network**



Cases et de Lorenzo (2005),
Nat. Microbiol. Rev., 3(2):105-118

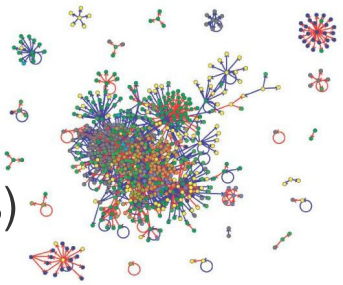
- **No global view of functioning** of network available, despite abundant knowledge on network components

Analysis of carbon starvation response

- **Modeling** and **experimental** studies directed at understanding how network controls carbon starvation response

- Which network components and which interactions to take into account?
  - Impossible to model the whole network

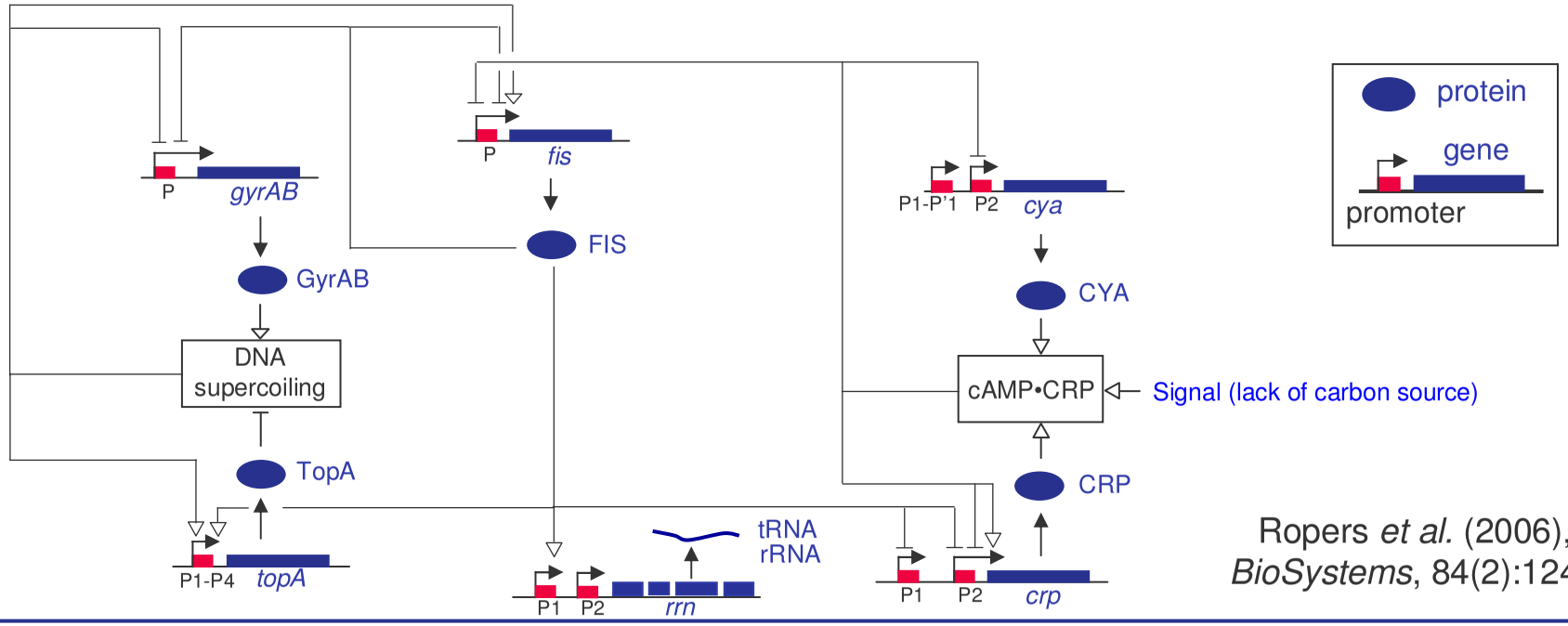
    E. coli genome: ~4500 genes (~150 transcription factor genes)



  - Start with the **simplest possible representation** of the carbon starvation response in *E. coli*

Analysis of carbon starvation response

- **Modeling** and **experimental** studies directed at understanding how network controls carbon starvation response
- Bottom-up strategy:
  1) Initial model of carbon starvation response



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

Analysis of carbon starvation response

- **Modeling** and **experimental** studies directed at understanding how network controls carbon starvation response

- Bottom-up strategy:

  1) Initial model of the carbon starvation response

     Search and curate data available in the literature and databases

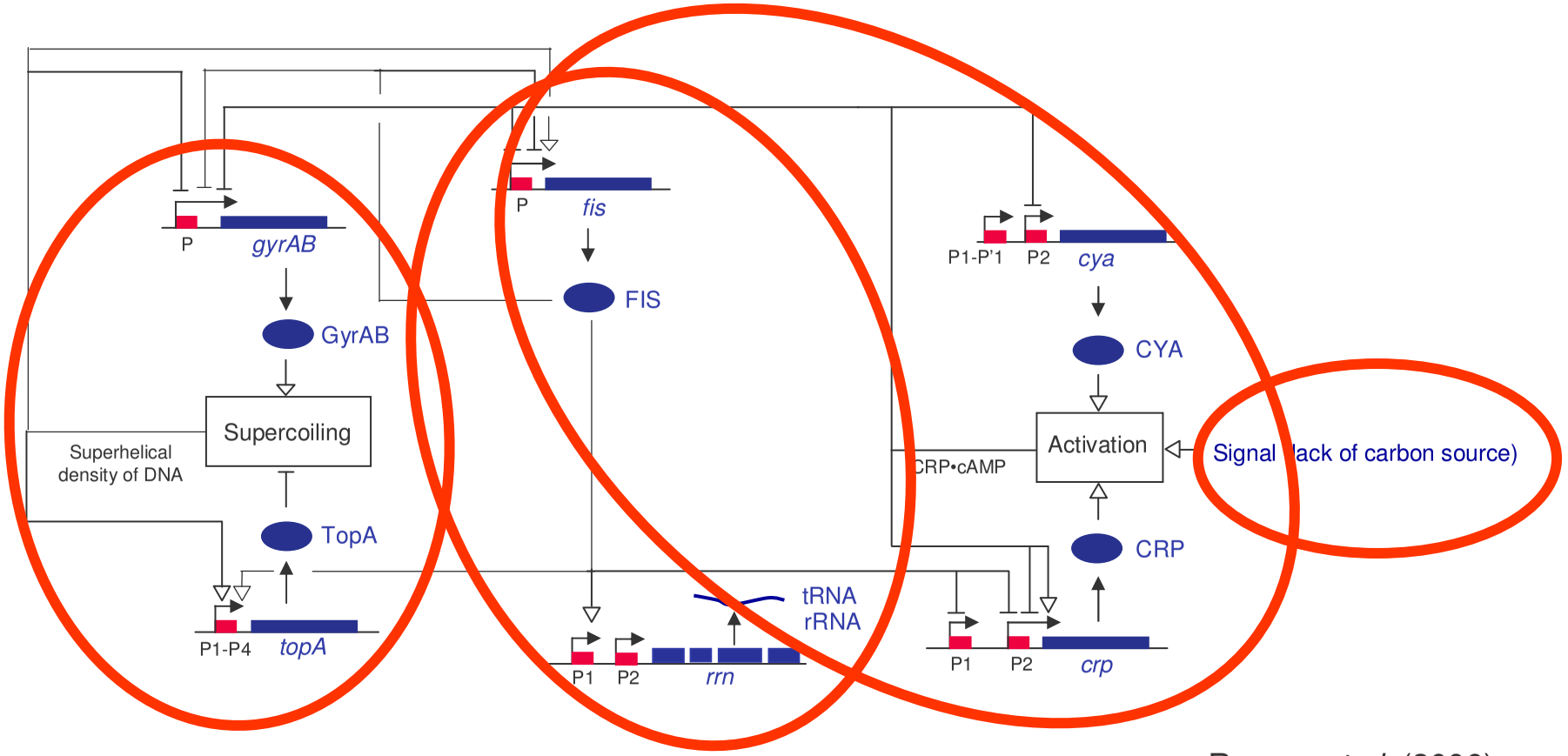
  2) Experimental verification of model predictions

  3) Extension of model to take into account wrong predictions

     Additional global regulators: IHF, HNS, ppGpp, FNR, LRP, ArcA, …

Modeling of carbon starvation network

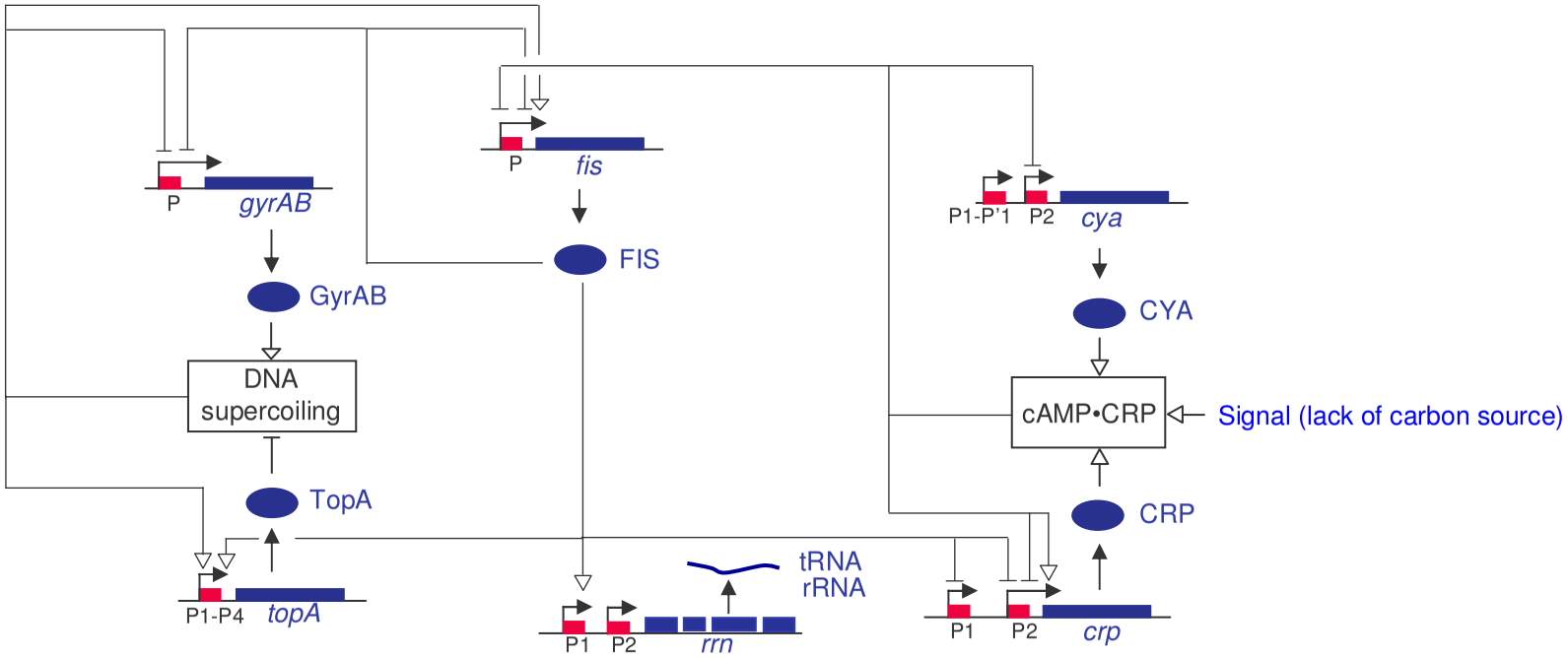
- Modular structure of carbon starvation network



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

Modeling of carbon starvation network

- Can the initial model explain the carbon starvation response of *E. coli* cells?



- Translation of biological data into a mathematical model

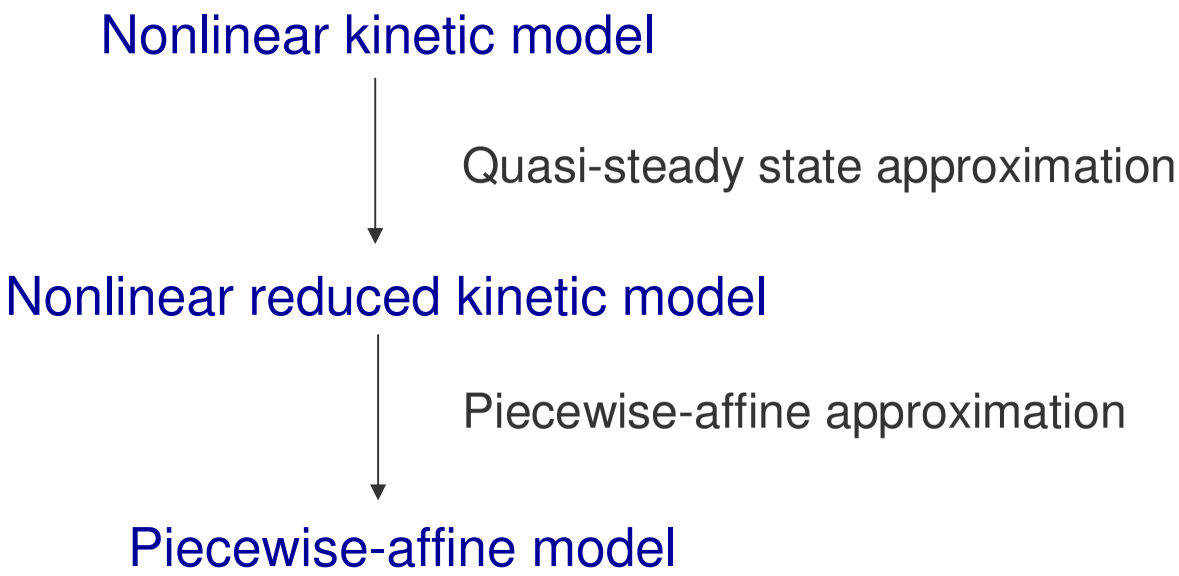
Constraints on modeling and simulation

- Current **constraints** on modeling and simulation:
  - Knowledge on molecular mechanisms rare
  - Quantitative information on kinetic parameters and molecular concentrations absent
- Possible strategies to overcome the constraints
  - Parameter estimation from experimental data
  - Parameter sensitivity analysis
  - Model simplifications
- Intuition: essential properties of system dynamics **robust** against moderate changes in kinetic parameters and rate laws

Stelling *et al.* (2004), *Cell*, 118(6):675-86

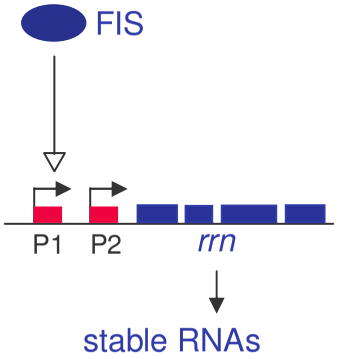
From nonlinear kinetic model to PA model

- Modeling process consists of reducing classical nonlinear kinetic model to PA model



Nonlinear kinetic model

- Nonlinear kinetic ODE model of 12 variables and 46 parameters
  - Regulation of gene expression (Hill)



$$\dot{x}_n \quad = \quad \kappa_n^1 \, h^+(x_f, \theta_f, m_f) + \kappa_n^2 - \gamma_n \, x_n$$

R. Heinrich and S. Schuster (1996), *The regulation of cellular systems*, Chapman & Hall, New York.

Nonlinear kinetic model

- Nonlinear kinetic ODE model of 12 variables and 46 parameters
  - Regulation of gene expression (Hill)
  - Enzymatic reactions (Michaelis-Menten or mass action)

$$\text{ATP} + \text{CYA}^* \overset{K_1}{\rightleftharpoons} \text{CYA}^*\bullet\text{ATP} \overset{k_2}{\longrightarrow} \text{CYA}^* + \text{cAMP} \overset{k_3}{\longrightarrow} \text{degradation/export}$$

$$\begin{aligned}
\dot{x}_{y\sim p} &= k_1\, x_{y\sim}\, x_p - (k_{-1} + k_2\, h^+(u_s, \theta_s, m_s) + \gamma_y)\, x_{y\sim p} \\
\dot{x}_{m\sim} &= k_2\, h^+(u_s, \theta_s, m_s)\, x_{y\sim p} + k_{-4}\, x_{c\sim m} - k_3\, x_{m\sim} - k_4\, x_{c\sim}\, x_{m\sim}
\end{aligned}$$

R. Heinrich and S. Schuster (1996), *The regulation of cellular systems*, Chapman & Hall, New York.

Nonlinear kinetic model

- Nonlinear kinetic ODE model of 12 variables and 46 parameters
  - Regulation of gene expression (Hill)
  - Enzymatic reactions (Michaelis-Menten or mass action)
  - Formation of biochemical complexes (mass action)

$$\text{cAMP} + \text{CRP} \underset{}{\overset{K_4}{\rightleftharpoons}} \text{CRP•cAMP}$$

$$\dot{x}_{c\sim m} = k_4\, x_{c\sim}\, x_{m\sim} - (k_{-4} + \gamma_c)\, x_{c\sim m}$$

R. Heinrich and S. Schuster (1996), *The regulation of cellular systems*, Chapman & Hall, New York.

Modeling of carbon starvation network

Ordinary differential equations :

$$\dot{u}_s = 0 \tag{1}$$

$$\dot{x}_{y\sim} = \kappa_y^1 + \kappa_y^2\, h^-(x_{c\sim m}, \theta^3_{c\sim m}, m_{c\sim m}) - \gamma_y\, x_{y\sim} + (k_{-1} + k_2\, h^-(u_s, \theta_s, m_s))\, x_{y\sim p} - k_1\, x_{y\sim}\, x_p \tag{2}$$

$$\dot{x}_{y\sim p} = k_1\, x_{y\sim}\, x_p - (k_{-1} + k_2\, h^+(u_s, \theta_s, m_s) + \gamma_y)\, x_{y\sim p} \tag{3}$$

$$\dot{x}_{c\sim} = \kappa_c^1 + \kappa_c^2\, h^-(x_f, \theta_f^2, m_f)\; h^+(x_{c\sim m}, \theta^1_{c\sim m}, m_{c\sim m}) + \kappa_c^3\, h^-(x_f, \theta_f^1, m_f) - \gamma_c\, x_{c\sim} + k_{-4}\, x_{c\sim m} - k_4\, x_{c\sim}\, x_{m\sim} \tag{4}$$

$$\dot{x}_{c\sim m} = k_4\, x_{c\sim}\, x_{m\sim} - (k_{-4} + \gamma_c)\, x_{c\sim m} \tag{5}$$

$$\dot{x}_{m\sim} = k_2\, h^+(u_s, \theta_s, m_s)\, x_{y\sim p} + k_{-4}\, x_{c\sim m} - k_3\, x_{m\sim} - k_4\, x_{c\sim}\, x_{m\sim} \tag{6}$$

$$\dot{x}_f = \kappa_f^1\, h^-(x_{c\sim m}, \theta^1_{c\sim m}, m_{c\sim m})\, h^-(x_f, \theta_f^5, m_f) + \kappa_f^2\, h^+(SC, \theta^1_{SC}, m_{SC}) \times h^-(x_{c\sim m}, \theta^1_{c\sim m}, m_{c\sim m})\, h^-(x_f, \theta_f^5, m_f) - \gamma_f\, x_f \tag{7}$$

with $SC = a + b\,\dfrac{x_a}{x_t}$

$$\dot{x}_a = \kappa_a\, h^-(SC, \theta^2_{SC}, m_{SC})\, h^-(x_f, \theta_f^4, m_f) - \gamma_a\, x_a \tag{8}$$

$$\dot{x}_t = \kappa_t\, h^+(SC, \theta^2_{SC}, m_{SC})\, h^+(x_f, \theta_f^4, m_f) - \gamma_t\, x_t \tag{9}$$

$$\dot{x}_n = \kappa_n^1\, h^+(x_f, \theta_f^3, m_f) + \kappa_n^2 - \gamma_n\, x_n \tag{10}$$

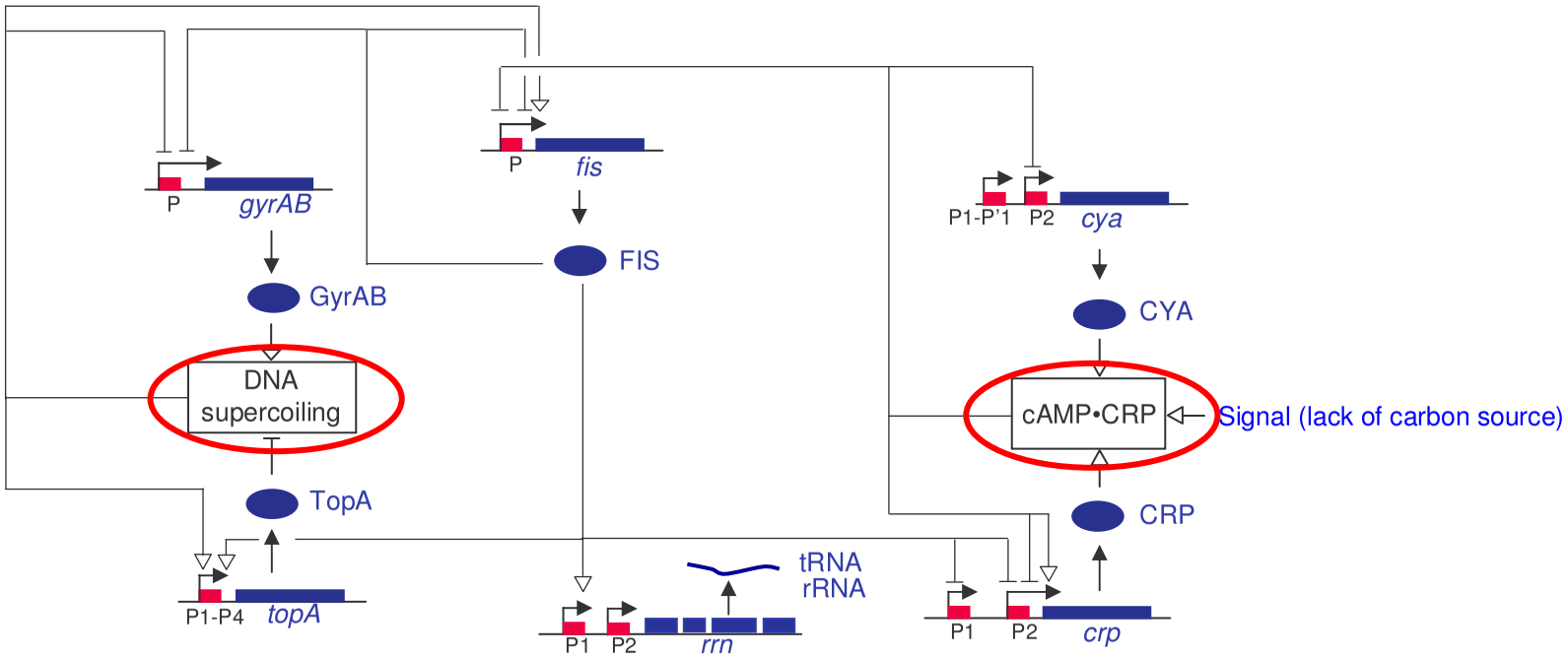
Conservation relations :

$$x_y = x_{y\sim} + x_{y\sim p} \tag{11}$$

$$x_c = x_{c\sim} + x_{c\sim m} \tag{12}$$

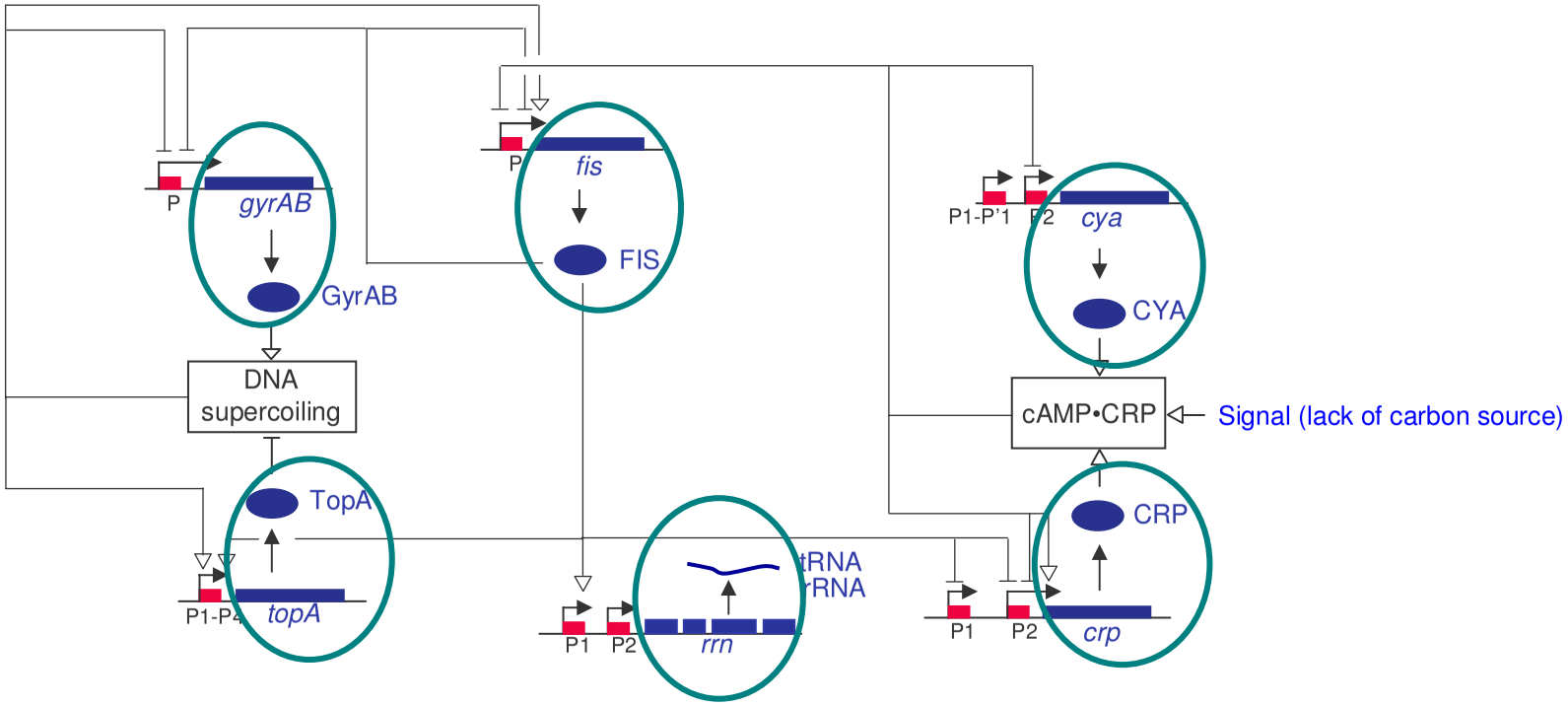
Quasi steady state approximation

- Identification of slow and **fast** processes in network



Quasi steady state approximation

- Identification of **slow** and fast processes in network



- Change of variables and quasi steady-state approximation

Heinrich and Schuster, 1996

Nonlinear reduced model

- QSSA model of 7 variables and 46 parameters

$$\dot{u}_s = 0 \tag{1}$$

$$\dot{x}_y = \kappa_y^1 + \kappa_y^2\, h^-(x_{c\sim m}, \theta^2_{c\sim m}, m_{c\sim m}) - \gamma_y\, x_y \tag{2}$$

$$\dot{x}_c = \kappa_c^1 + \kappa_c^2\, h^-(x_f, \theta^2_f, m_f)\; h^+(x_{c\sim m}, \theta^1_{c\sim m}, m_{c\sim m}) + \kappa_c^3\, h^-(x_f, \theta^1_f, m_f) - \gamma_c\, x_c \tag{3}$$

$$\dot{x}_f = \kappa_f^1\, h^-(x_{c\sim m}, \theta^1_{c\sim m}, m_{c\sim m})\, h^-(x_f, \theta^5_f, m_f) + \kappa_f^2\, h^+(SC, \theta^1_{SC}, m_{SC}) \times h^-(x_{c\sim m}, \theta^1_{c\sim m}, m_{c\sim m})\, h^-(x_f, \theta^5_f, m_f) - \gamma_f\, x_f \tag{4}$$

$$\dot{x}_a = \kappa_a\, h^-(SC, \theta^2_{SC}, m_{SC})\, h^-(x_f, \theta^4_f, m_f) - \gamma_a\, x_a \tag{5}$$

$$\dot{x}_t = \kappa_t\, h^+(SC, \theta^2_{SC}, m_{SC})\, h^+(x_f, \theta^4_f, m_f) - \gamma_t\, x_t \tag{6}$$

$$\dot{x}_n = \kappa_n^1\, h^+(x_f, \theta^3_f, m_f) + \kappa_n^2 - \gamma_n\, x_n \tag{7}$$

with

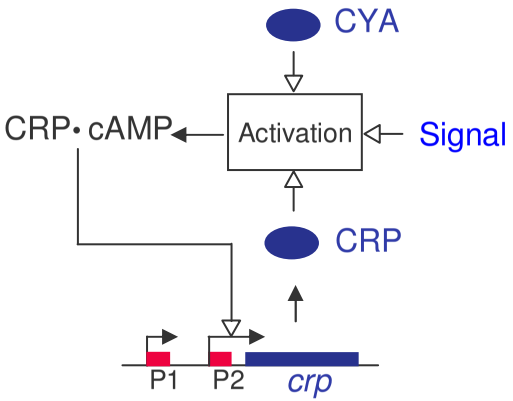
$$x_{y\sim p} = \frac{k_1\, x_y\, x_p}{k_1\, x_p + k_{-1} + k_2\, h^+(u_s, \theta_s, m_s)}$$

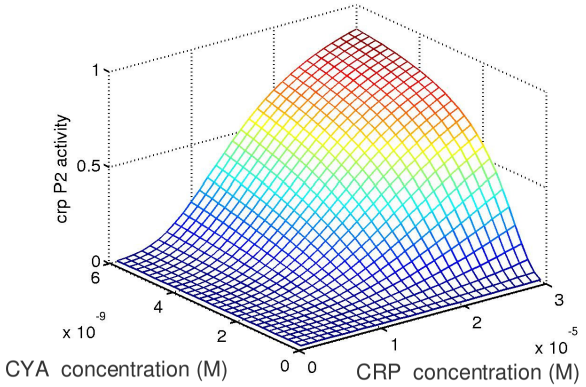
$$x_{c\sim m} = \frac{k_2\, h^+(u_s, \theta_s, m_s)\, x_c\, x_y}{K_4\, k_3 + k_2\, h^+(u_s, \theta_s, m_s)\, x_y}$$

$$SC = a + b\,\frac{x_a}{x_t}$$

Piecewise-affine approximation

- Approximation of Hill function with step function
- Approximation of sigmoidal surfaces with product of step functions





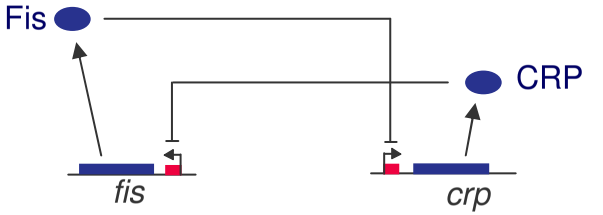
$$s^{+}(x_{CYA}, \theta_{CYA}{}^{1})\, s^{+}(x_{CRP}, \theta_{CRP}{}^{1})\, s^{+}(x_{SIGNAL}, \theta_{SIGNAL})$$

Model of carbon starvation network

- PADE model of 7 variables and 36 parameter inequalities

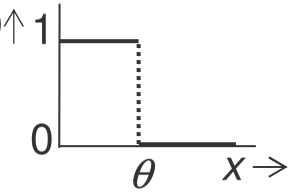
$$
\begin{aligned}
\dot{u}_s &= 0 && (1)\\
\dot{x}_y &= \kappa_y^1 + \kappa_y^2\ (1 - s^+(x_c, \theta_c^3)\ s^+(x_y, \theta_y^3)\ s^+(u_s, \theta_s)) - \gamma_y\ x_y && (2)\\
\dot{x}_c &= \kappa_c^1 + \kappa_c^2\ s^-(x_f, \theta_f^2)\ s^+(x_c, \theta_c^1)\ s^+(x_y, \theta_y^1)\ s^+(u_s, \theta_s) + \kappa_c^3\ s^-(x_f, \theta_f^1) - \gamma_c\ x_c && (3)\\
\dot{x}_f &= \kappa_f^1\ (1 - s^+(x_c, \theta_c^1)\ s^+(x_y, \theta_y^1)\ s^+(u_s, \theta_s))\ s^-(x_f, \theta_f^5)\\
&\quad + \kappa_f^2\ s^+(x_a, \theta_a^1)\ s^-(x_t, \theta_t^2)\ s^-(x_f, \theta_f^5)\\
&\quad \times (1 - s^+(x_c, \theta_c^1)\ s^+(x_y, \theta_y^1)\ s^+(u_s, \theta_s)) - \gamma_f\ x_f && (4)\\
\dot{x}_a &= \kappa_a\ (1 - s^+(x_a, \theta_a^2)\ s^-(x_t, \theta_t^1))\ s^-(x_f, \theta_f^4) - \gamma_a\ x_a && (5)\\
\dot{x}_t &= \kappa_t\ s^+(x_a, \theta_a^2)\ s^-(x_t, \theta_t^1)\ s^+(x_f, \theta_f^4) - \gamma_t\ x_t && (6)\\
\dot{x}_n &= \kappa_n^1\ s^+(x_f, \theta_f^3) + \kappa_n^2 - \gamma_n\ x_n && (7)
\end{aligned}
$$

Outline of qualitative simulation

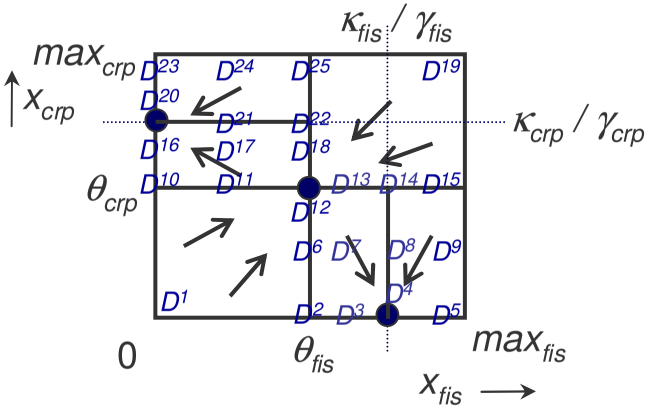


$$\dot{x}_{fis} = \kappa_{fis}\, s^{-}(x_{crp}, \theta_{crp}) - \gamma_{fis}\, x_{fis}$$

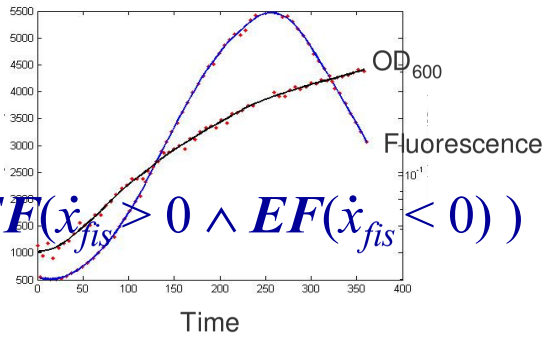
$$\dot{x}_{crp} = \kappa_{crp}\, s^{-}(x_{fis}, \theta_{fis}) - \gamma_{crp}\, x_{crp}$$



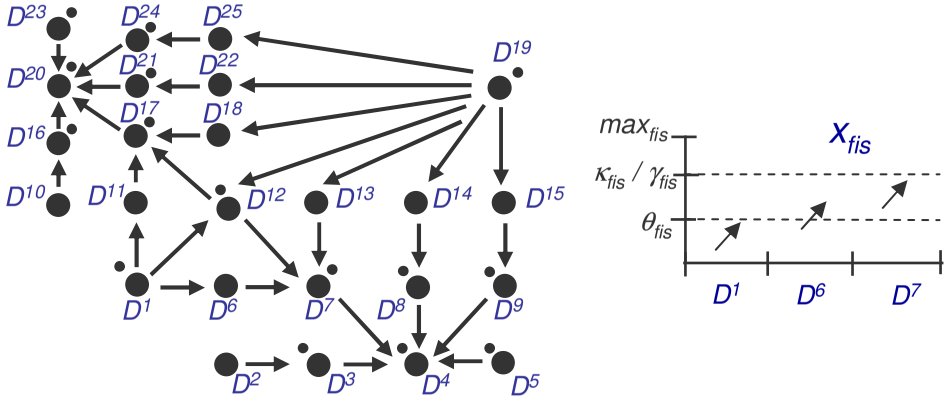
Models using step functions



Network dynamics easy to analyze



Model-checking for verification of model predictions



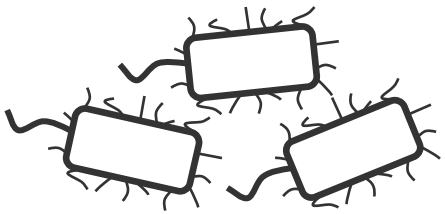
Qualitative predictions of dynamics, robust for large variations in parameter values

Attractors of stress response network

❖ Analysis of attractors of PA model: two **steady states**

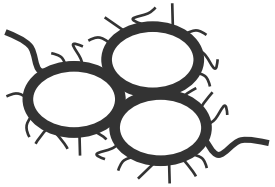
- **Stable** steady state, corresponding to exponential-phase conditions

```
Crp = k_Crp_1/g_Crp
Cya = (k_Cya_1+k_Cya_2)/g_Cya
Fis = t_Fis_4
GyrAB = t_GyrAB_1
z_Signal <= Signal < t_Signal
TopA = z_TopA
rrn = (k_rrn_2+k_rrn_1)/g_rrn
```



- **Stable** steady state, corresponding to stationary-phase conditions

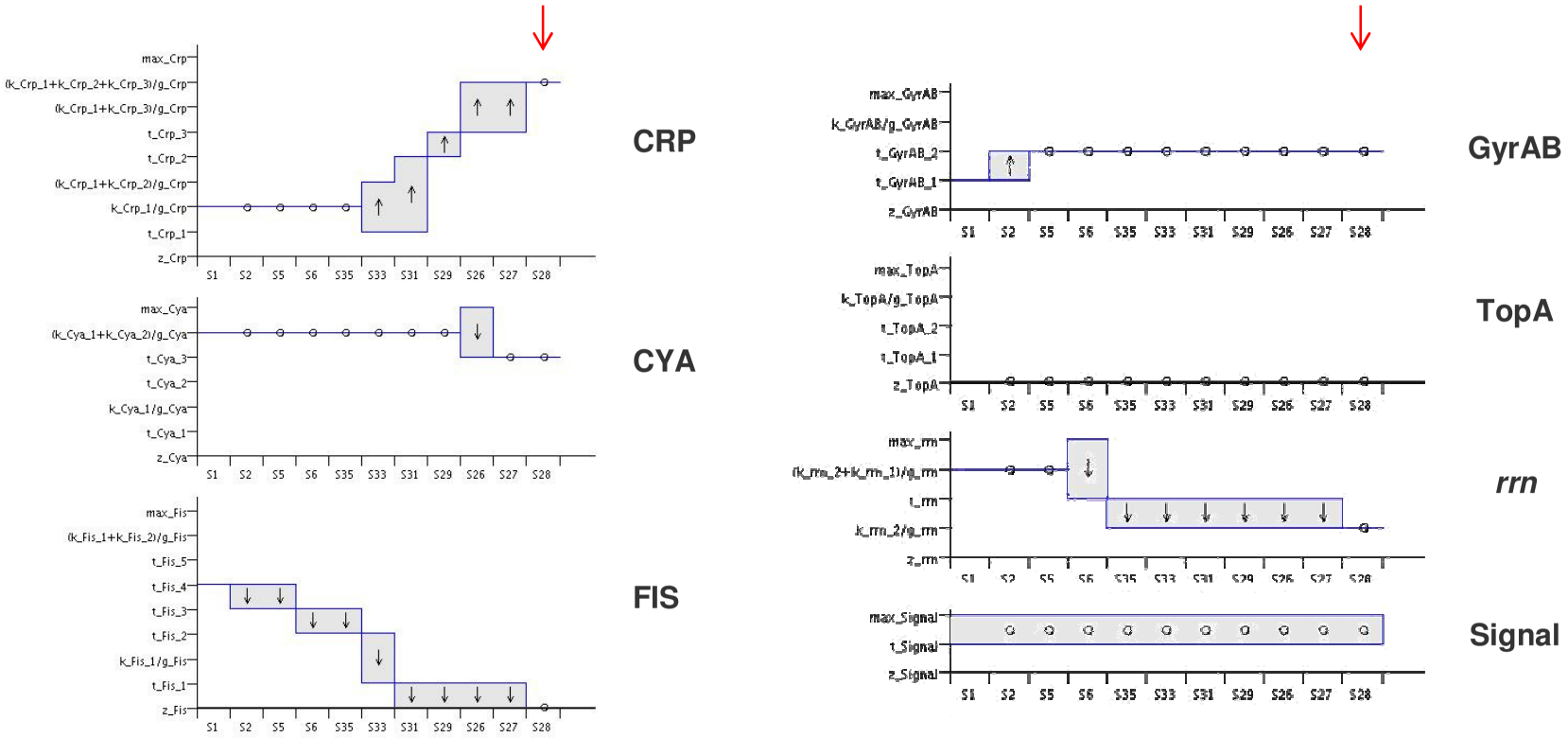
```
Crp = (k_Crp_1+k_Crp_2+k_Crp_3)/g_Crp
Cya = t_Cya_3
Fis = z_Fis
GyrAB = t_GyrAB_2
t_Signal < Signal <= max_Signal
TopA = z_TopA
rrn = k_rrn_2/g_rrn
```



Simulation of stress response network

- Simulation of transition from exponential to stationary phase

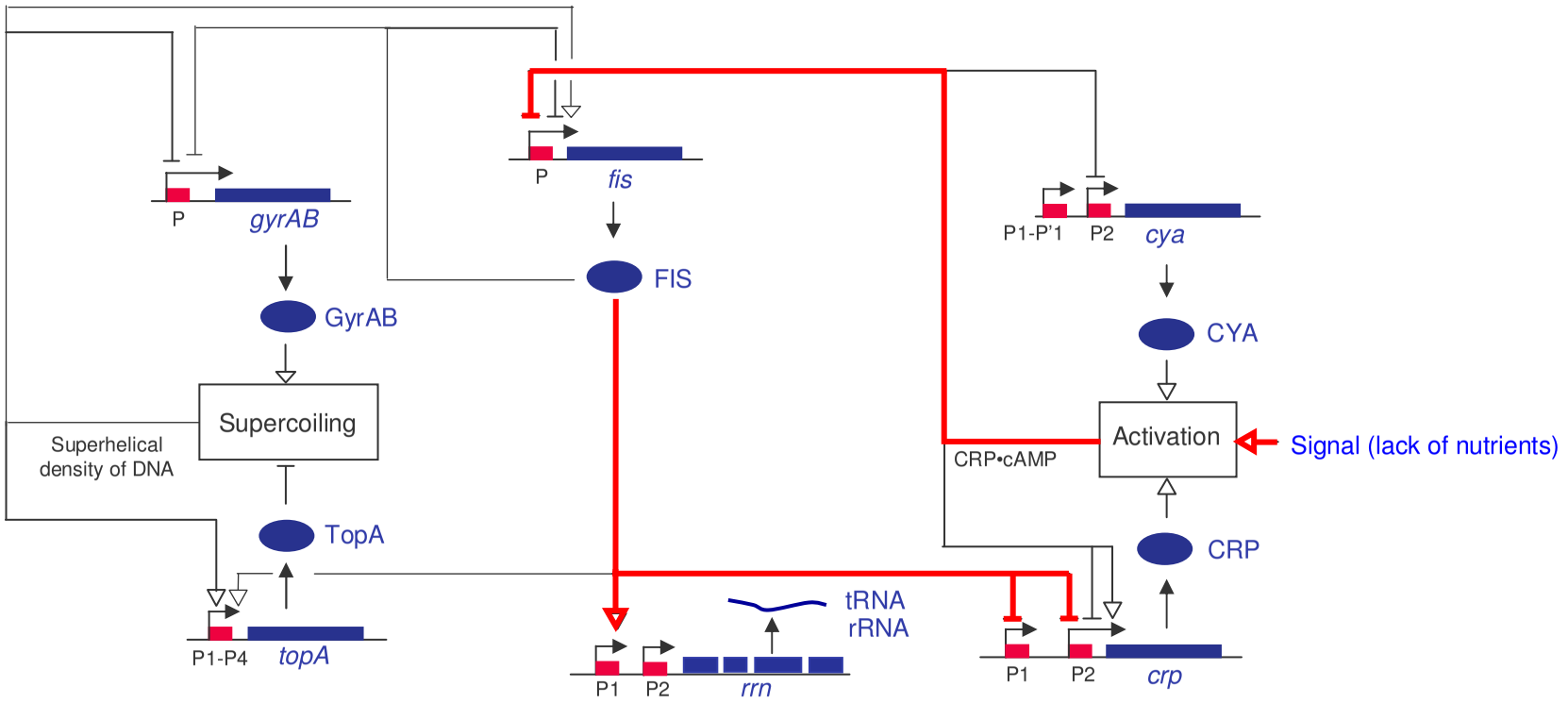
  State transition graph with 27 states, 1 stable steady state



Insight into nutritional stress response

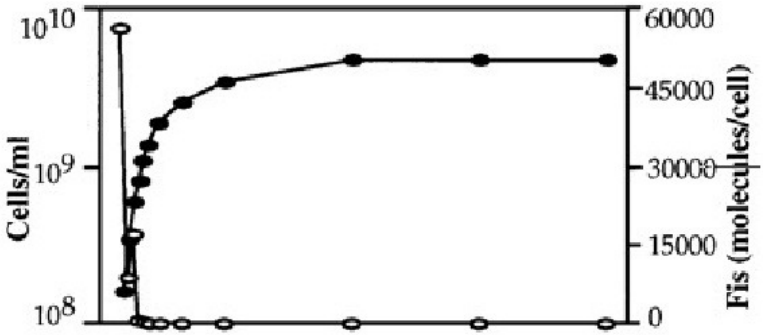
- Sequence of **qualitative events** leading to adjustment of growth of cell after nutritional stress signal

Role of the mutual inhibition of Fis and CRP•cAMP



Validation of carbon starvation response model

- Validation of model using model checking
  - "Fis concentration decreases and becomes steady in stationary phase"



Ali Azam *et al.* (1999), *J. Bacteriol.*, 181(20):6361-6370

$$\boldsymbol{EF}(\dot{x}_{fis} < 0 \wedge \boldsymbol{EF}(\dot{x}_{fis} = 0 \wedge x_{rrn} < \theta_{rrn}))$$

True

  - *"cya* transcription is negatively regulated by the complex cAMP-CRP"

Kawamukai *et al.* (1985), *J. Bacteriol.*, 164(2):872-877

$$\boldsymbol{AG}(x_{crp} > \theta^{3}_{crp} \wedge x_{cya} > \theta^{3}_{cya} \wedge x_s > \theta_s \rightarrow \boldsymbol{EF}\, \dot{x}_{cya} < 0)$$ True

  - "DNA supercoiling decreases during transition to stationary phase"

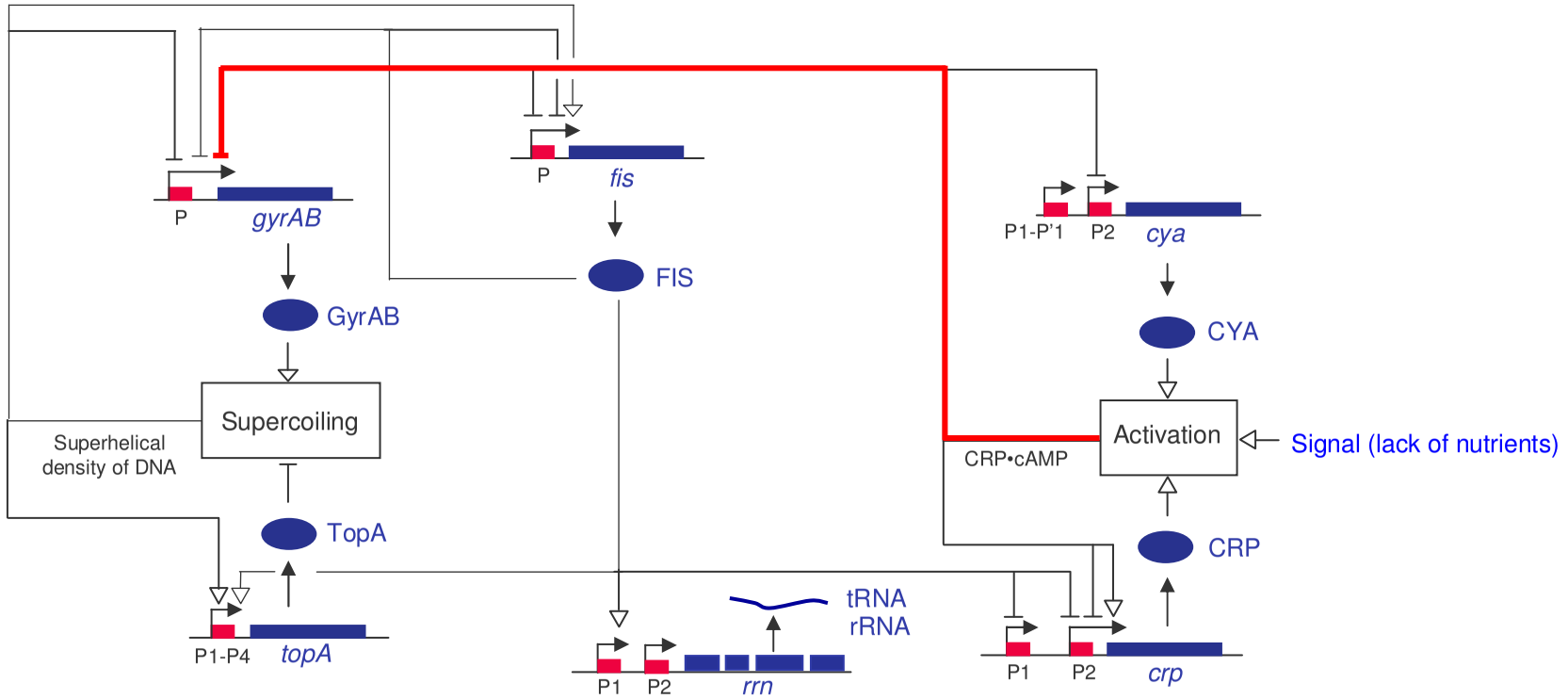
Balke, Gralla (1987), *J. Bacteriol.*, 169(10):4499-4506

$$\boldsymbol{EF}((\dot{x}_{gyrAB} < 0 \vee \dot{x}_{topA} > 0) \wedge x_{rrn} < \theta_{rrn})$$ False

Suggestion of missing interaction

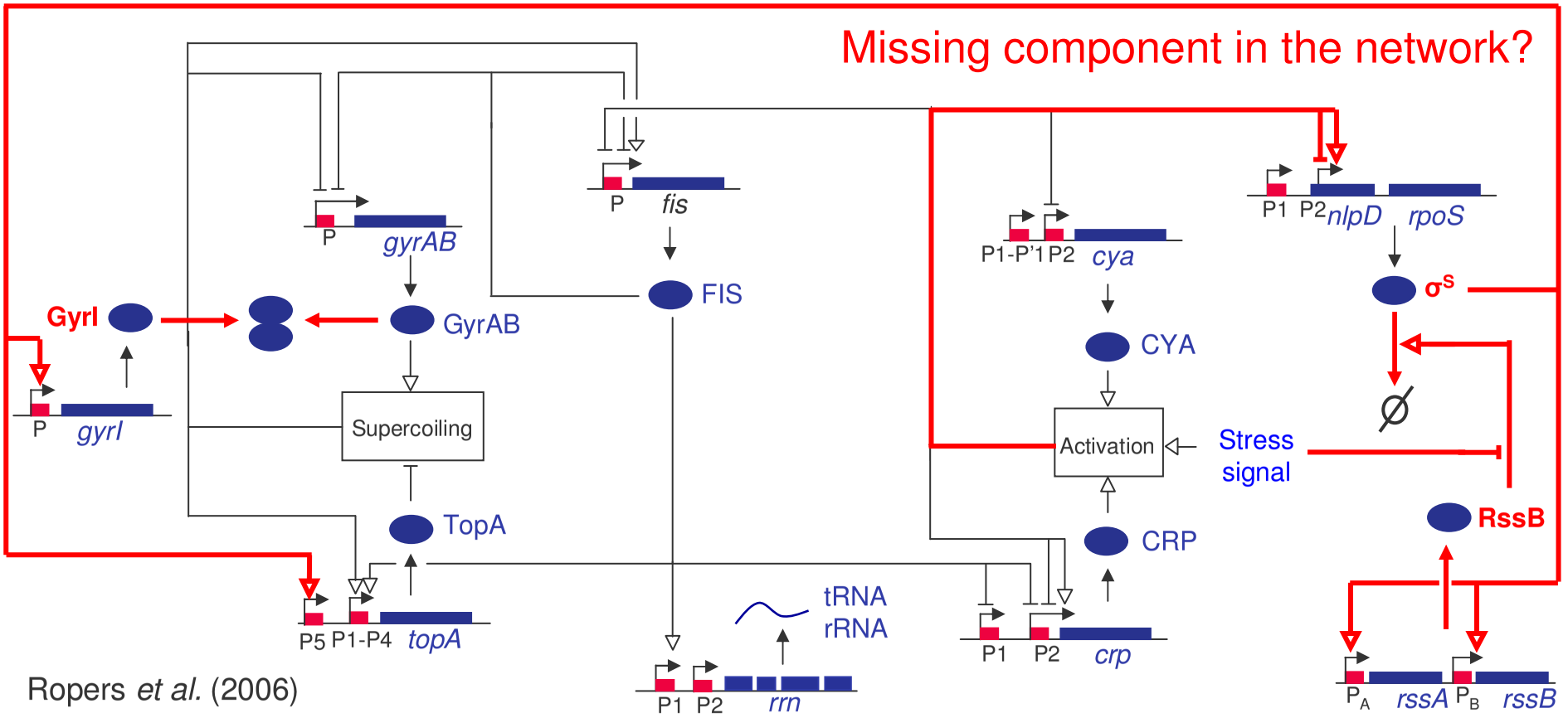
- Model does not reproduce observed downregulation of negative supercoiling

Missing interaction in the network?



Extension of stress response network

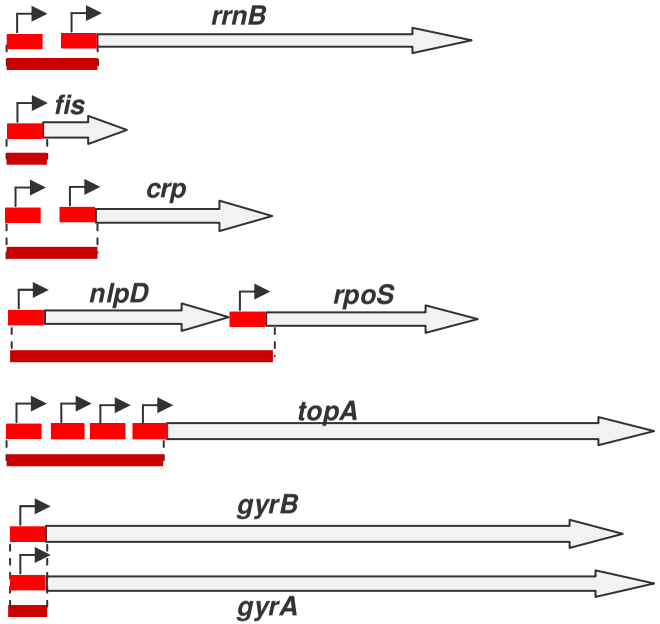
- Model does not reproduce observed downregulation of negative supercoiling



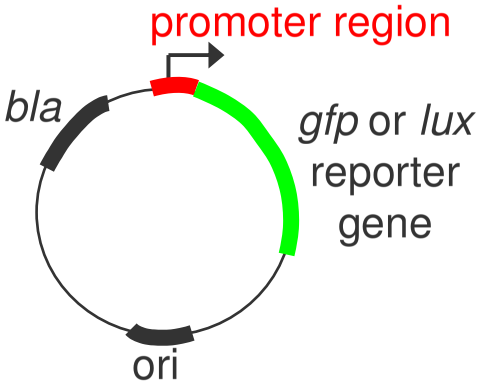
Ropers *et al.* (2006)

Reporter gene systems

- Simulations yield predictions that cannot be verified with currently avaliable experimental data
- Use of **reporter gene systems** to monitor gene expression

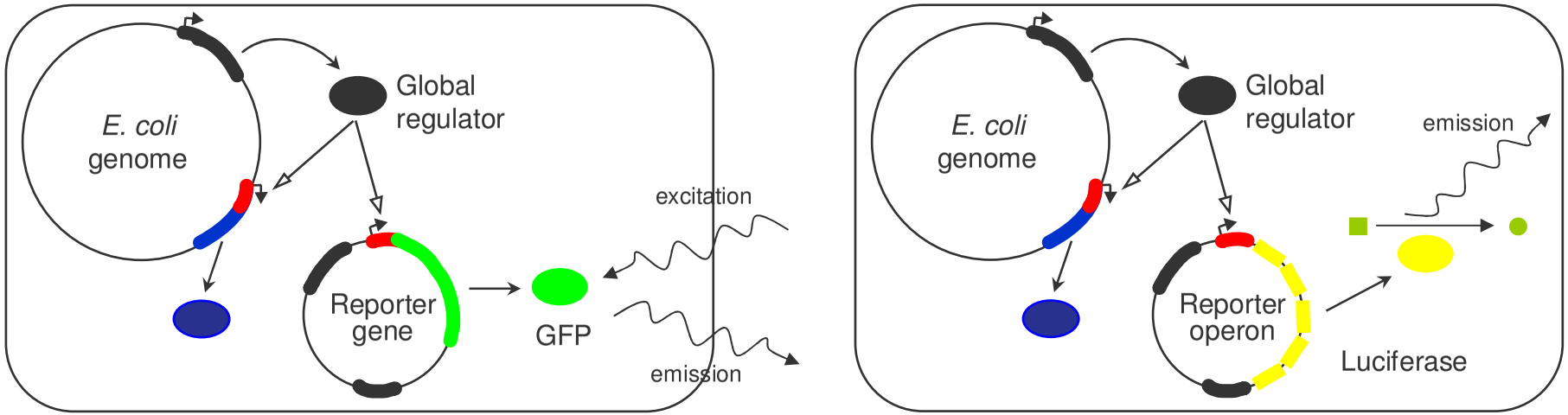


cloning promoter regions on plasmid



Monitoring of gene expression

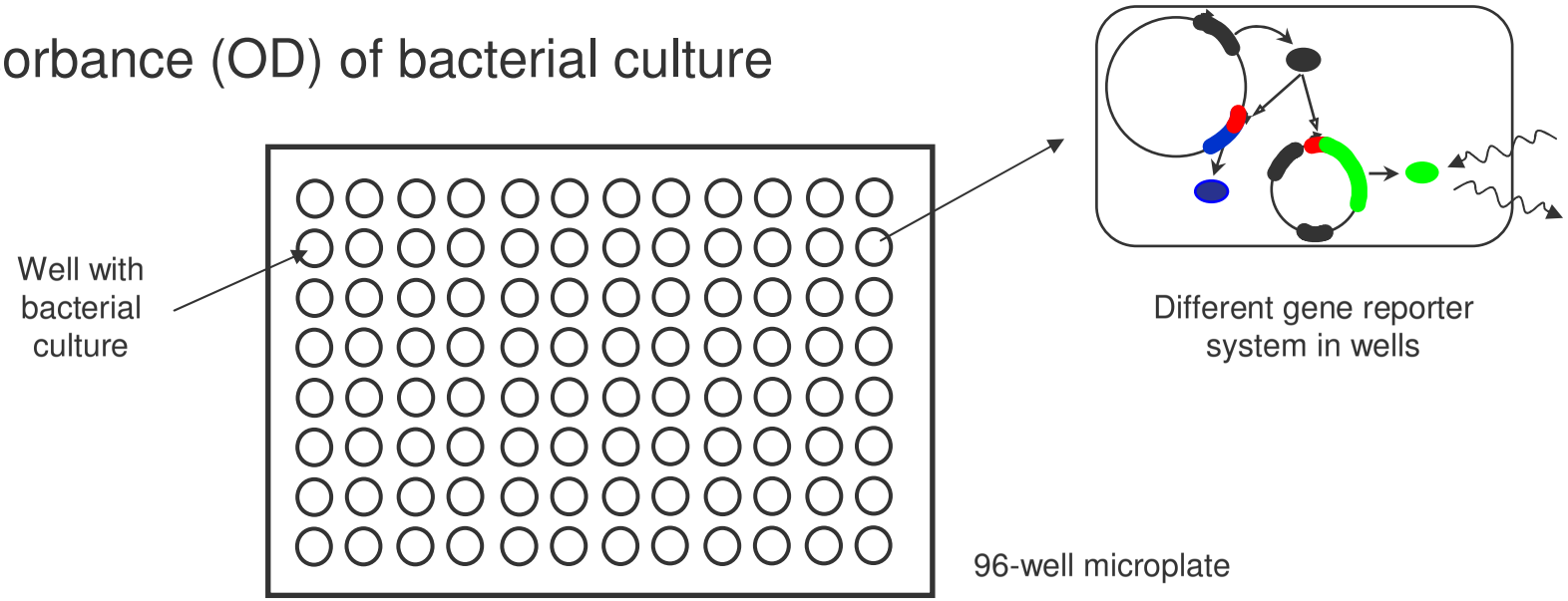
- Integration of fluorescent or luminescent reporter gene systems into bacterial cell



- Expression of reporter gene reflects expression of host gene of interest

Real-time monitoring: microplate reader

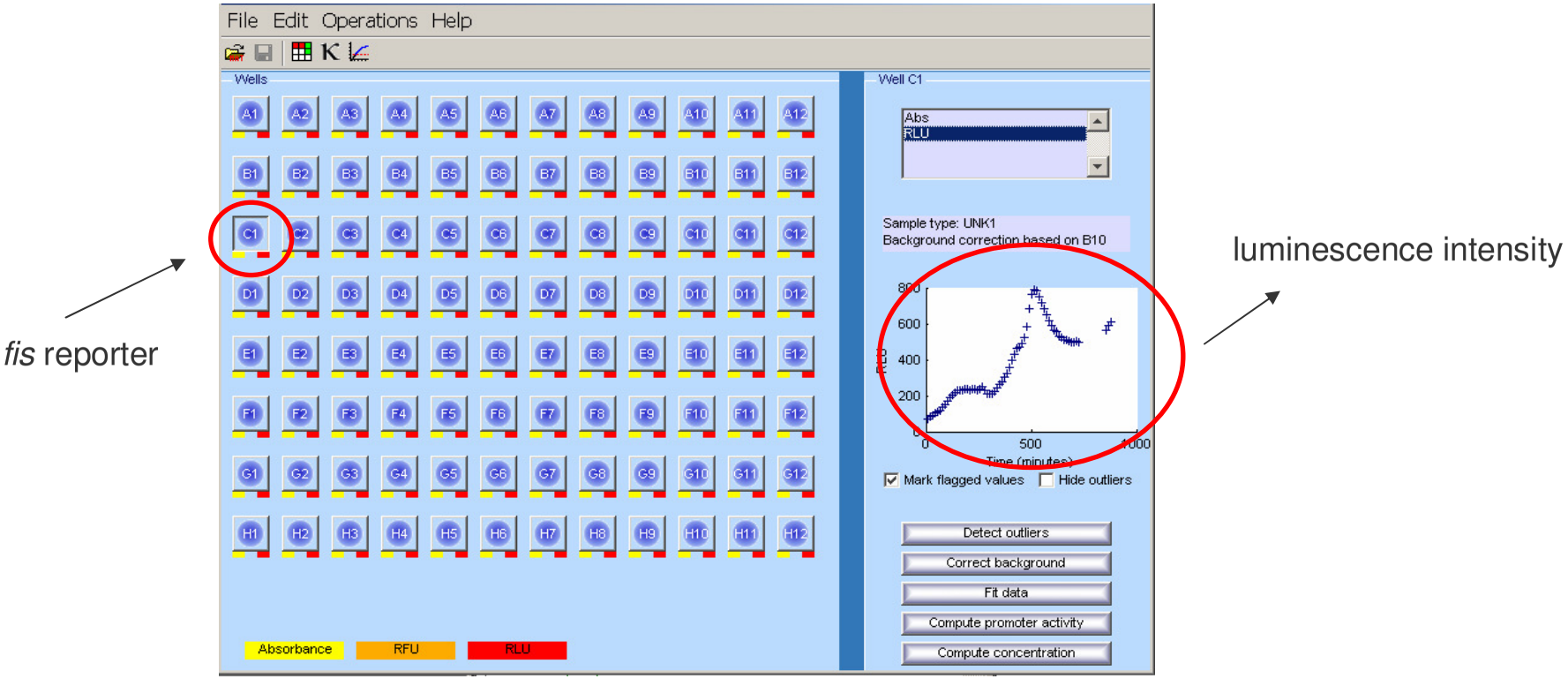
- Use of automated microplate reader to monitor in parallel in single experiment expression of different reporter genes
  - fluorescence/luminescent intensity
  - absorbance (OD) of bacterial culture



- Upshift experiments in M9/glucose medium

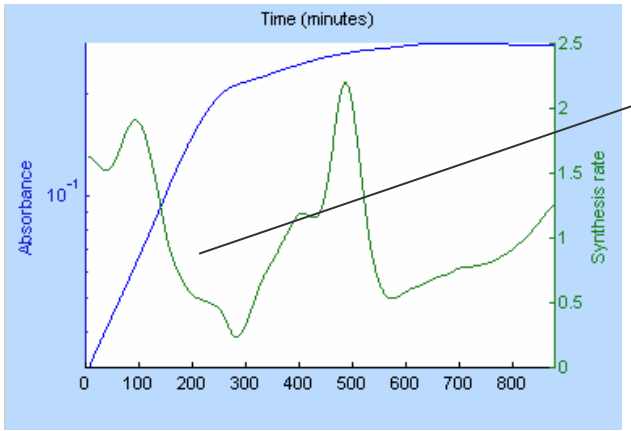
Analysis of reporter gene expression data

- **Wellreader**: Matlab program for analysis of reporter gene expression data



Data analysis issues

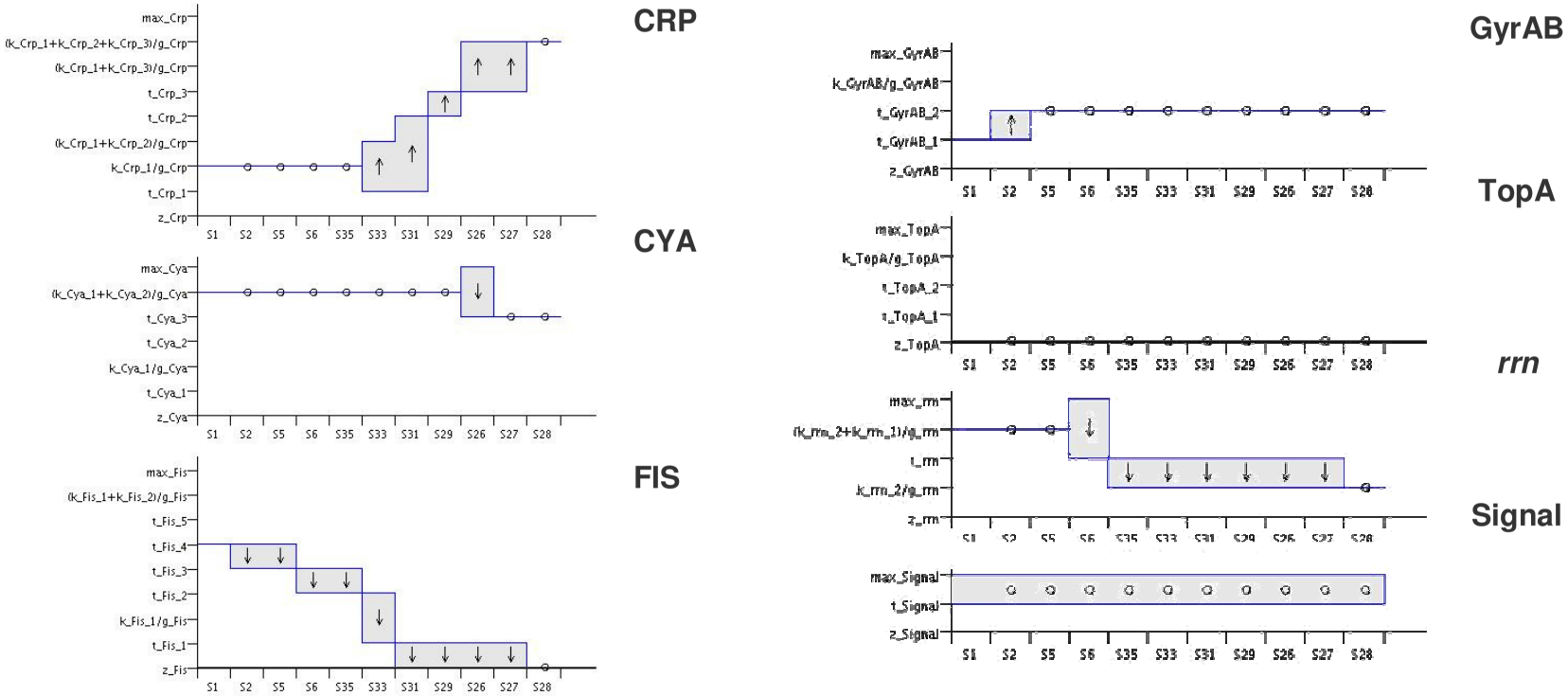
- Outlier detection and background substraction
- Data smoothing and interpolation by means of cubic smoothing splines
- Computation of reporter concentration, promoter activity, host protein concentration
- Computation of confidence intervals



fis promoter activity

Validation of carbon starvation model

- Model predictions verified by experimental data?



- Preliminary results: partly yes, partly no

Perspectives

- Refining model validation by monitoring gene expression in single cells

  Collaboration with Irina Mihalcescu, Université Joseph Fourier

- Inference of regulatory networks from gene expression data

  Use hybrid system identification methods adapted to PA models

  Drulhe *et al.* (2006), *Hybrid Systems: Computation and Control*, LNCS 3927, 184-99

- Composite models of *E. coli* stress response on genetic and metabolic level

  Collaboration with Daniel Kahn, INRA

Conclusions

- Understanding of functioning and development of living organisms requires **analysis of genetic regulatory networks**

  From structure to behavior of networks

- Need for **mathematical methods** and **computer tools well-adapted** to **available** experimental data

  Coarse-grained models and qualitative analysis of dynamics

- **Biological relevance** attained through **integration** of modeling and experiments

  Models guide experiments, and experiments stimulate models

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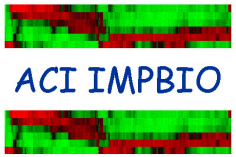

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