

Validation of Qualitative Models of Genetic Regulatory Networks by Model Checking: Nutritional Stress Response in *E. coli*

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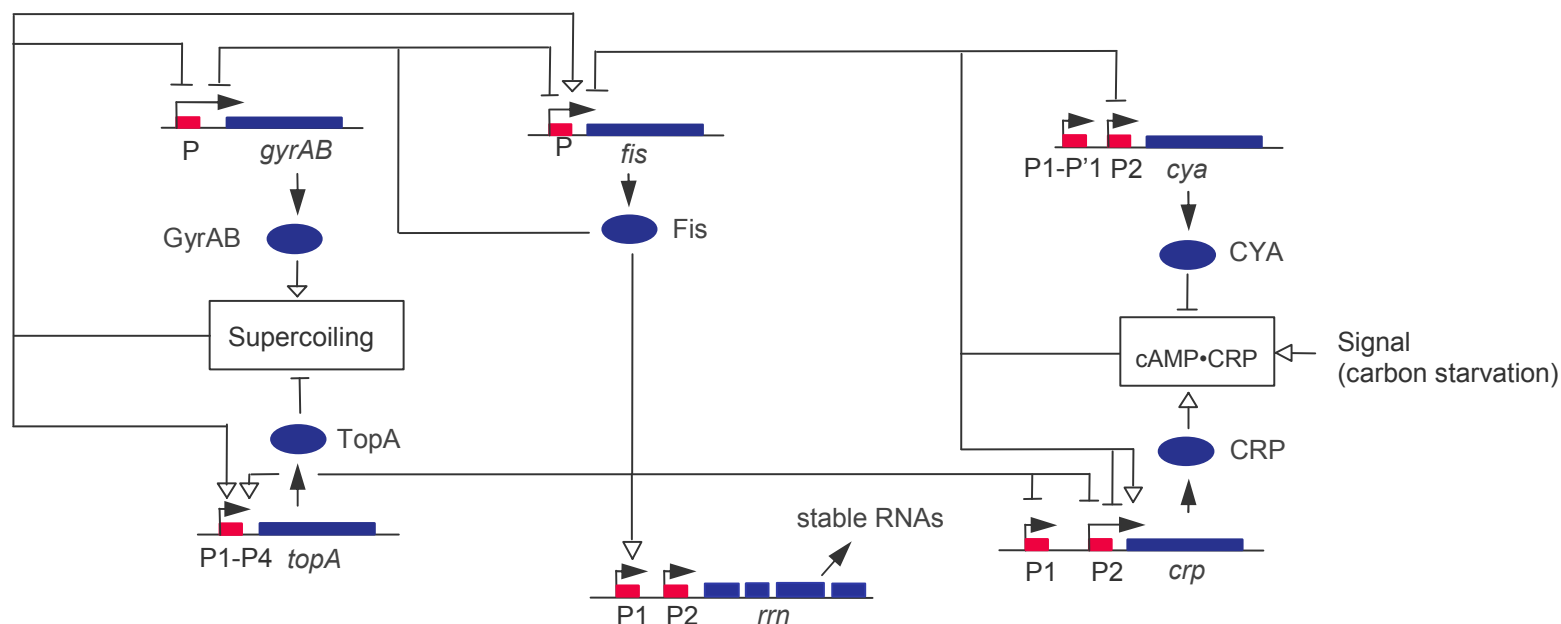
Overview

1. Introduction
2. Qualitative modeling and simulation method
3. Validation with model-checking techniques
4. Application to nutritional stress response in *E. coli*
5. Conclusions

Genetic regulatory networks

- ❖ **Genetic regulatory networks** underlie functioning and development of living organisms

Genes, proteins, small molecules, and their regulatory interactions



- ❖ Genetic regulatory networks are **large** and **complex**

Validation of genetic network models

- ❖ Mathematical methods and computational tools exist for analysis of genetic networks through modeling and simulation
- ❖ Problem of **model validation**: check consistency between experimental data and predictions
- ❖ Major constraints for model validation:
 - predictions **suitable for comparison** with available experimental data
 - **automatic** and **efficient** comparison between experimental data and predictions
- ❖ Approach:
 - use of **qualitative modeling and simulation** method
 - combination with **model-checking** techniques

PL differential equation models

- ❖ Genetic networks modeled by class of differential equations using **step functions** to describe regulatory interactions

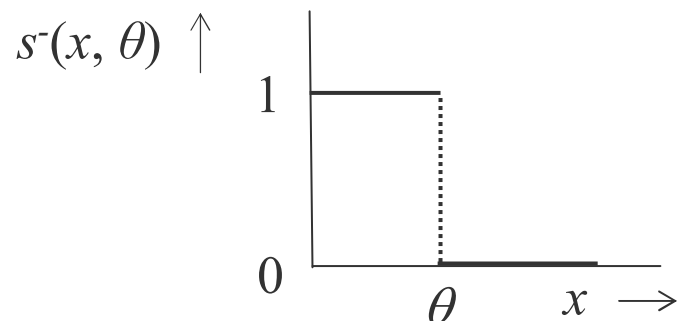
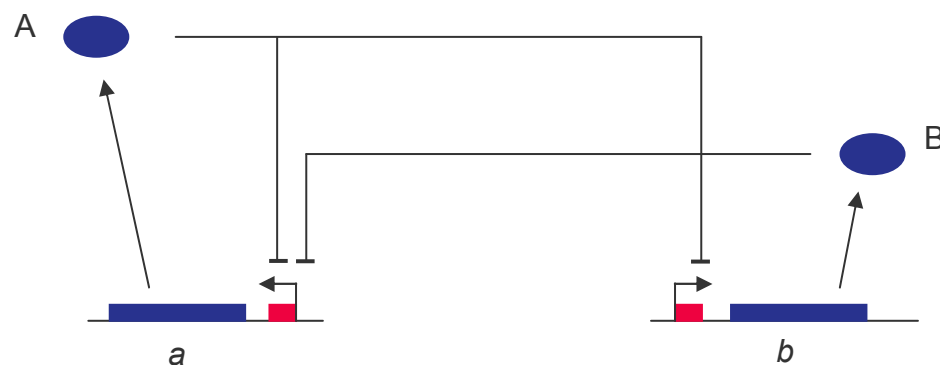
$$\dot{x}_a = \kappa_a s^-(x_a, \theta_{a2}) s^-(x_b, \theta_b) - \gamma_a x_a$$

$$\dot{x}_b = \kappa_b s^-(x_a, \theta_{a1}) - \gamma_b x_b$$

x : protein concentration

θ : threshold concentration

κ, γ : rate constants



- ❖ Differential equation models of regulatory networks are **piecewise-linear (PL)**

Glass and Kauffman, *J. Theor. Biol.*, 73

Qualitative analysis of network dynamics

- ❖ Method for **qualitative analysis** of dynamics of genetic regulatory networks:
 - treatment of **discontinuities** in step functions by generalizing differential equations to differential inclusions (Filippov-like approach)
 - **discrete transition system** obtained by **qualitative abstraction** of the dynamics, based on hyperrectangular partition of phase space
 - **inequality constraints** define regions in parameter space yielding the same discrete transition system
 - **symbolic computation** of transition system using inequality constraints and **tailored algorithms** (upscalability)
 - implementation in the computer tool **Genetic Network Analyzer (GNA)**
 - **application** to several bacterial systems

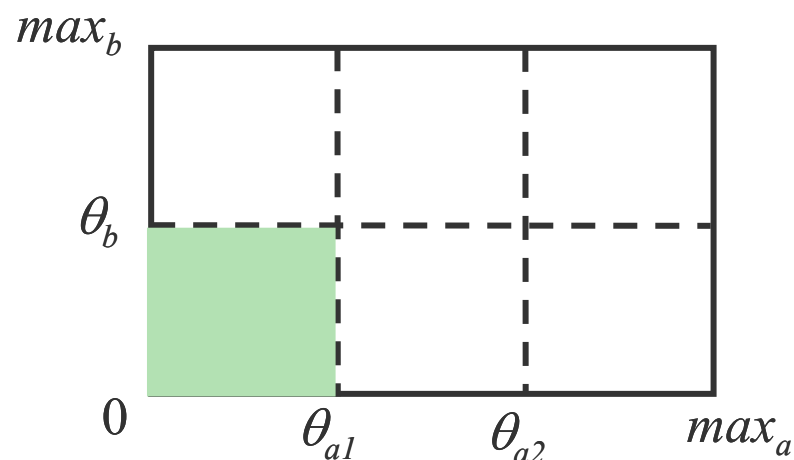
Gouzé and Sari, *Dyn. Syst.*, 03

de Jong *et al.*, *Bull. Math. Biol.*, 04

de Jong *et al.*, *Bioinformatics*, 03

Qualitative analysis of network dynamics

❖ Analysis of the dynamics in phase space



$$\dot{x}_a = \kappa_a - \gamma_a x_a$$

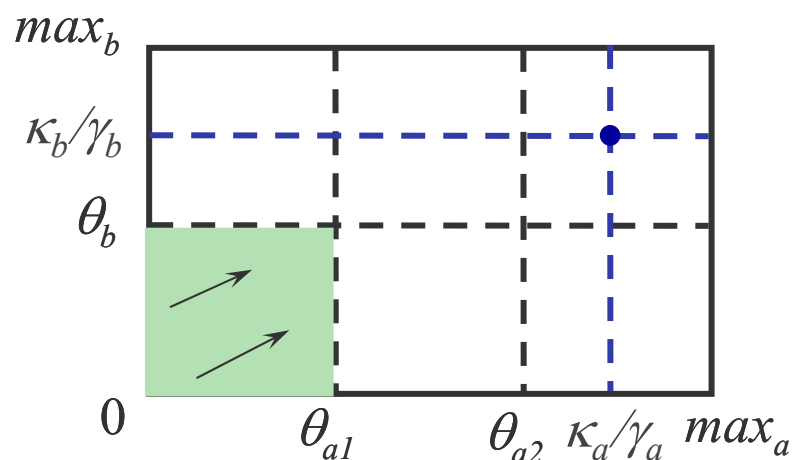
$$\dot{x}_b = \kappa_b - \gamma_b x_b$$

$$0 < \theta_{a1} < \theta_{a2} < \kappa_a / \gamma_a < \max_a$$

$$0 < \theta_b < \kappa_b / \gamma_b < \max_b$$

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❖ Analysis of the dynamics in phase space



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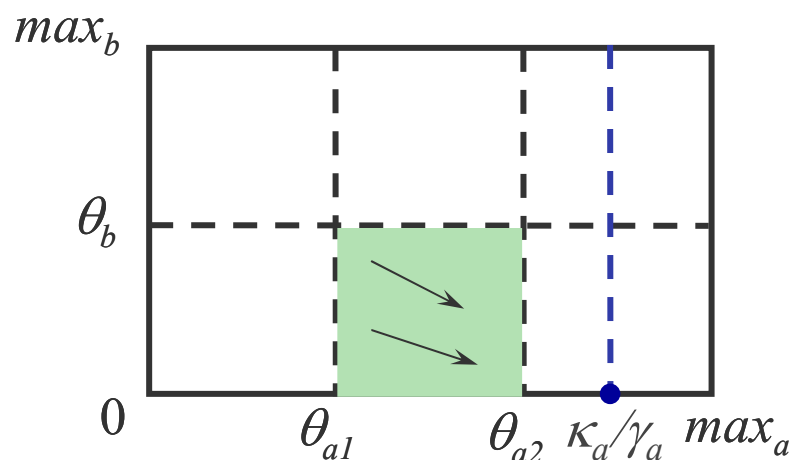
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Qualitative analysis of network dynamics

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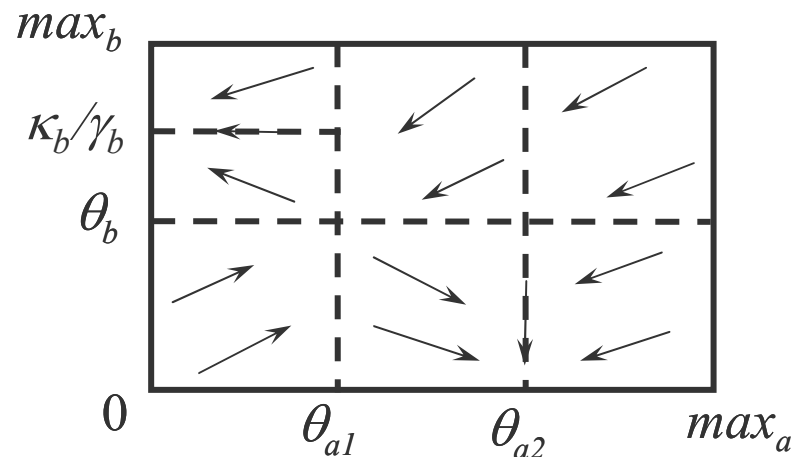
$$\dot{x}_b = -\gamma_b x_b$$

$$0 < \theta_{a1} < \theta_{a2} < \kappa_a/\gamma_a < \max_a$$

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Qualitative analysis of network dynamics

❖ Analysis of the dynamics in phase space



$$\dot{x}_a = \kappa_a s^-(x_a, \theta_{a2}) s^-(x_b, \theta_b) - \gamma_a x_a$$

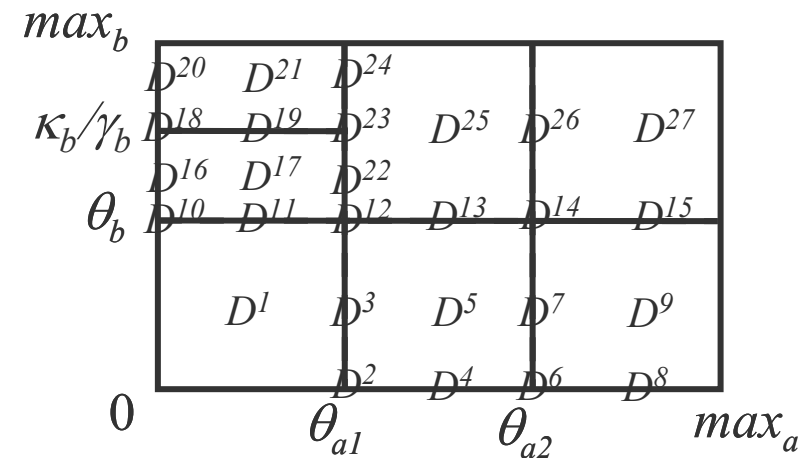
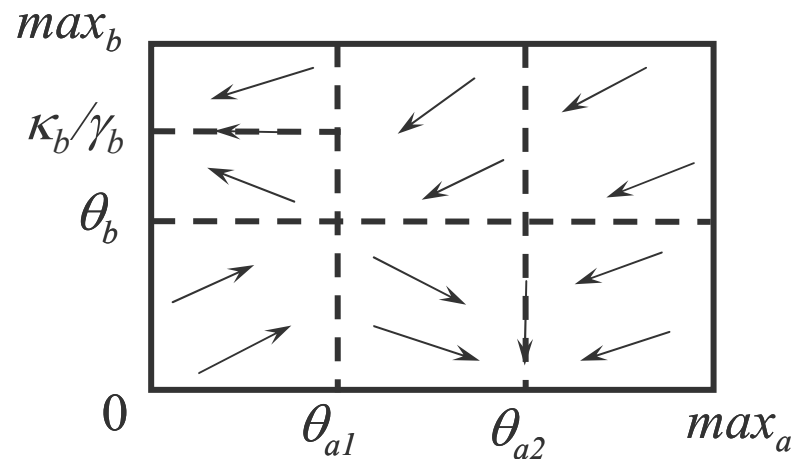
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Qualitative analysis of network dynamics

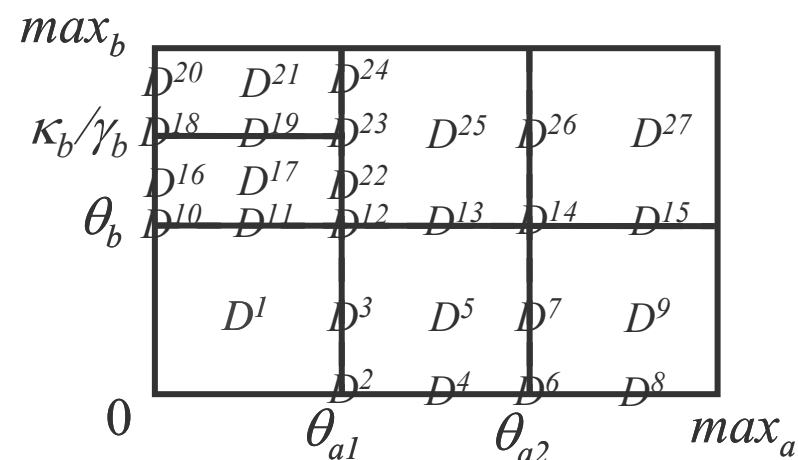
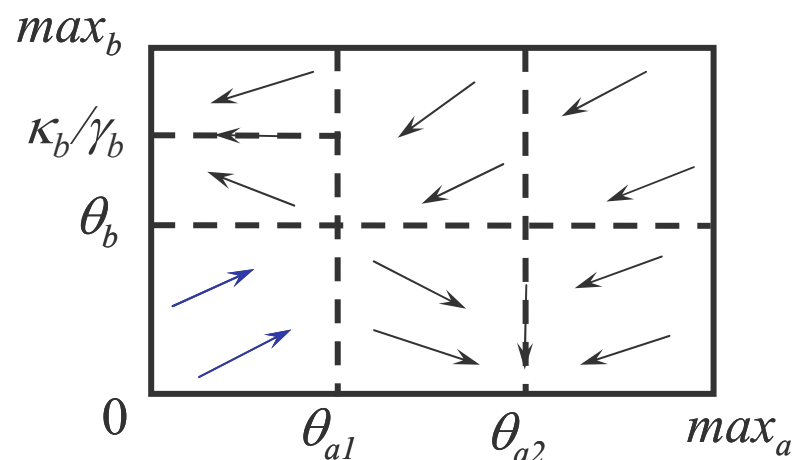
❖ Analysis of the dynamics in phase space



❖ Partition of phase space into domains

Qualitative analysis of network dynamics

❖ Analysis of the dynamics in phase space



$$\forall x \in D^1: \dot{x}_a > 0, \dot{x}_b > 0$$

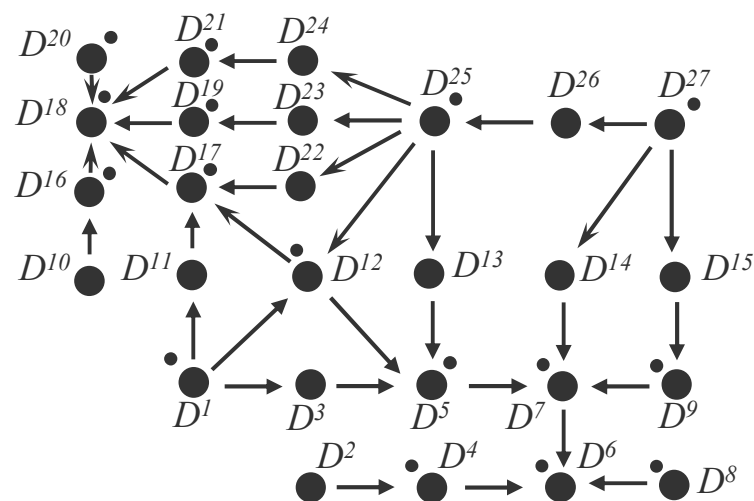
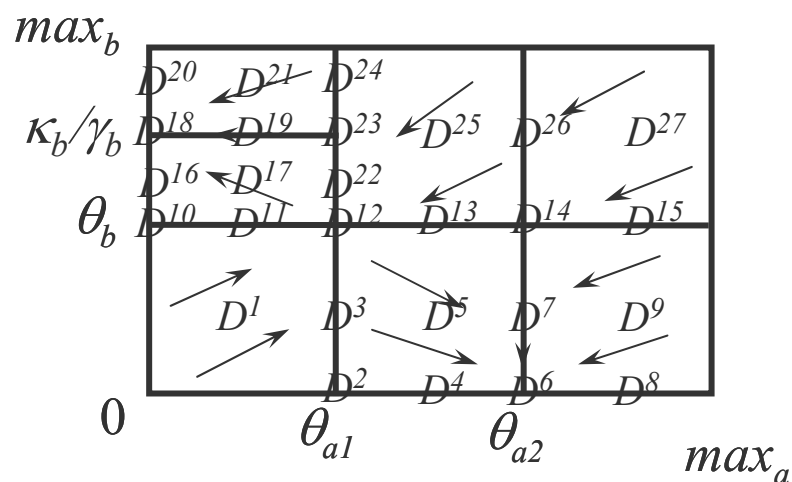
❖ Partition of phase space into domains

Unicity of derivative sign pattern in domains

Qualitative analysis of network dynamics

❖ Qualitative abstraction yields **discrete transition system**

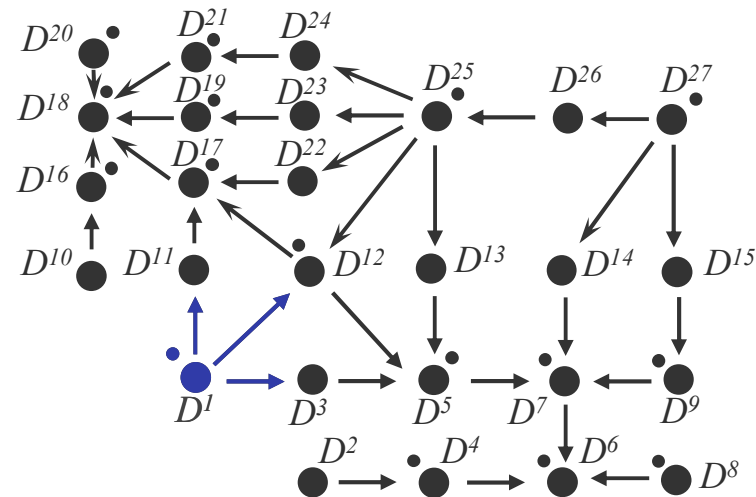
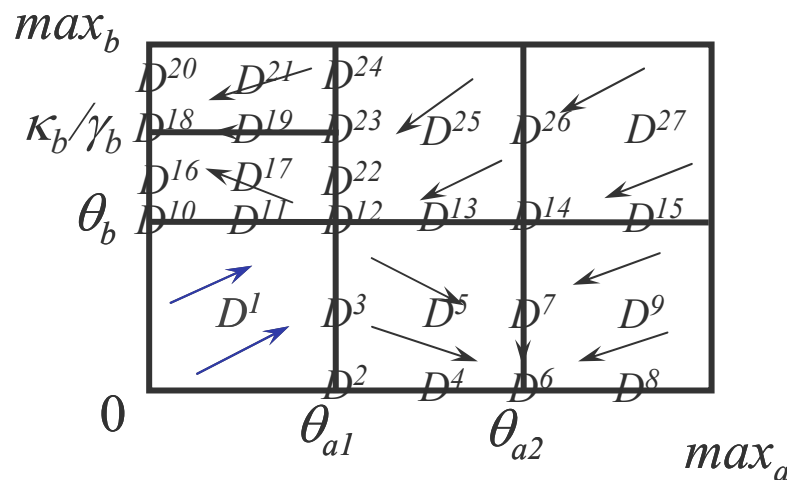
$DTS = \langle D, \rightarrow, L \rangle$, where D is the set of domains, \rightarrow the transition relation between domains and L a labeling function describing domain properties



Qualitative analysis of network dynamics

❖ Qualitative abstraction yields **discrete transition system**

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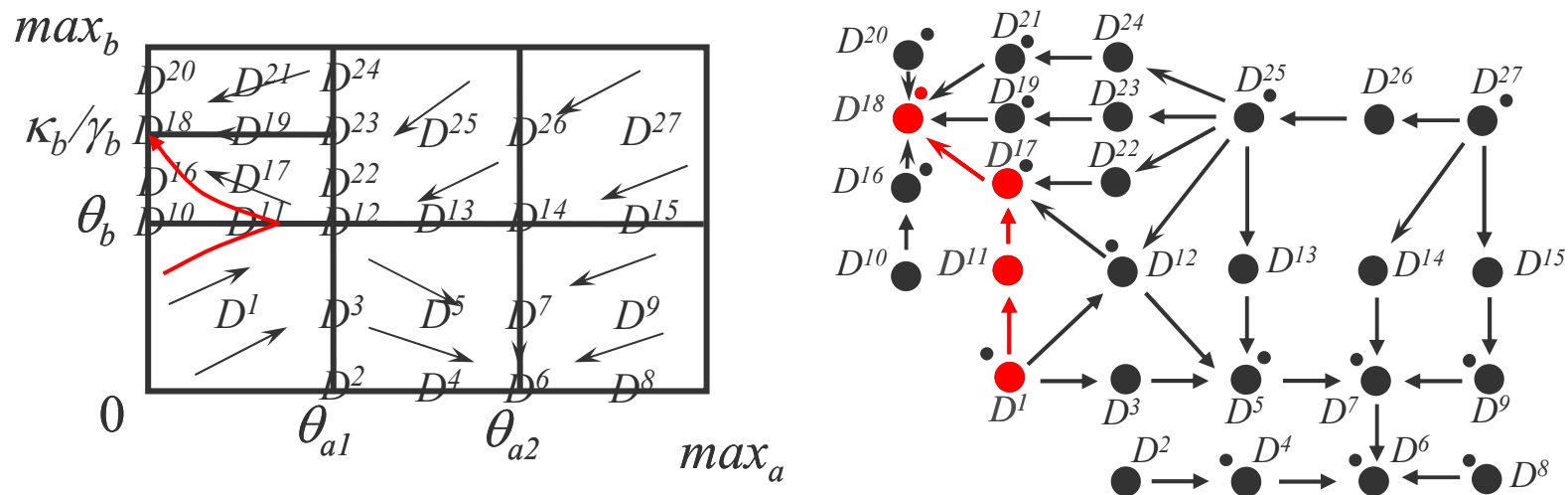


$$D^1 \in D \quad (D^1, D^3), (D^1, D^{11}), (D^1, D^{12}) \in \rightarrow \quad L(D^1) = \{x_a < \theta_a^1, x_b < \theta_b, \dot{x}_a > 0, \dot{x}_b > 0\}$$

Qualitative analysis of network dynamics

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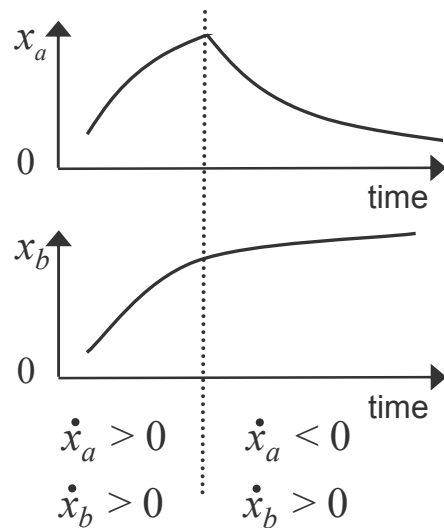


❖ Discrete transition system is a **conservative approximation**

Every solution corresponds to a path in the discrete transition system

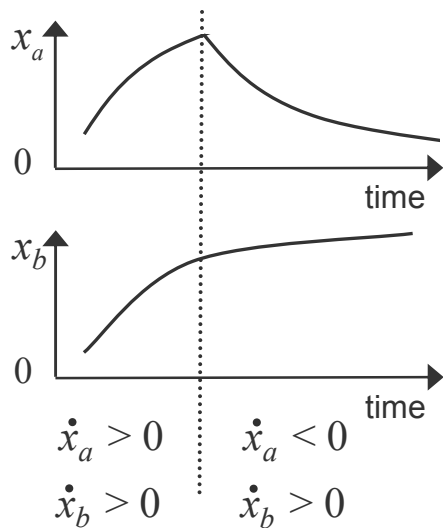
Validation of qualitative models

- ❖ Predictions well adapted to comparison with available experimental data: **changes of derivative sign patterns**

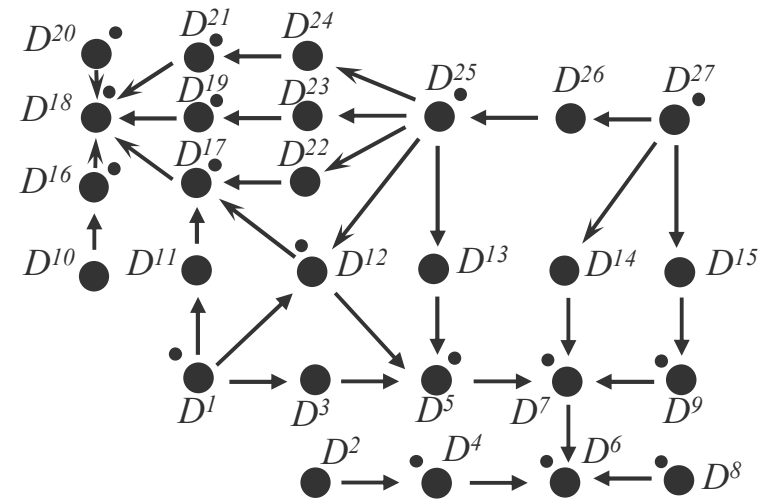


Validation of qualitative models

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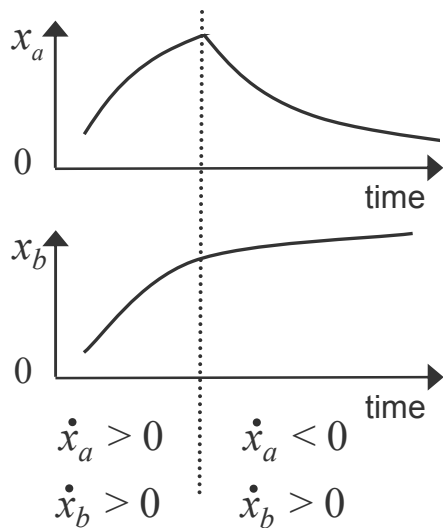
Consistency?



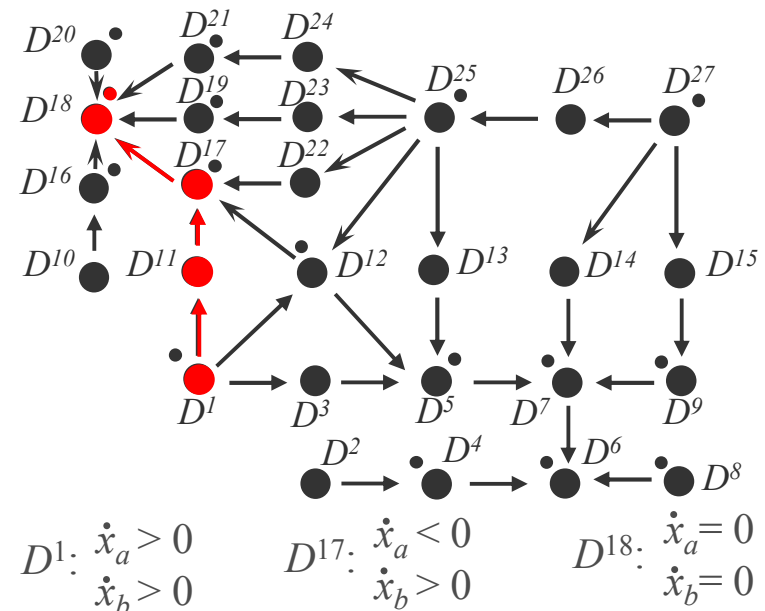
- ❖ Model validation: comparison of derivative sign patterns in observed and predicted behaviors

Validation of qualitative models

- ❖ Predictions well adapted to comparison with available experimental data: **changes of derivative sign patterns**



Consistency?
Yes



- ❖ Model validation: comparison of derivative sign patterns in observed and predicted behaviors
- ❖ Need for **automatic** and **efficient** comparison

Model-checking approach

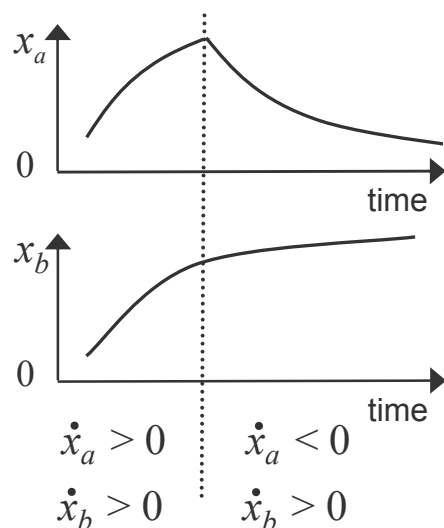
- ❖ **Model checking** is automated technique for verifying that discrete transition system satisfies certain temporal properties
- ❖ Computation tree logic model-checking framework:
 - set of **atomic propositions** AP
 - discrete transition system is **Kripke structure** $KS = \langle S, R, L \rangle$,
where S set of states, R transition relation, L labeling function over AP
 - temporal properties expressed in **Computation Tree Logic (CTL)**
 $p, \neg f_1, f_1 \wedge f_2, f_1 \vee f_2, f_1 \rightarrow f_2, EXf_1, AXf_1, EFf_1, AFf_1, EGf_1, AGf_1, Ef_1Uf_2, Af_1Uf_2$,
where $p \in AP$ and f_1, f_2 CTL formulas
- ❖ Computer tools are available to perform efficient and reliable model checking (e.g., NuSMV, SPIN, CADP)

Validation using model checking

❖ Atomic propositions

$$AP = \{x_a = 0, x_a < \theta_a^1, \dots, x_b < \max_b, \dot{x}_a < 0, \dot{x}_a = 0, \dots, \dot{x}_b > 0\}$$

❖ Observed property expressed in CTL

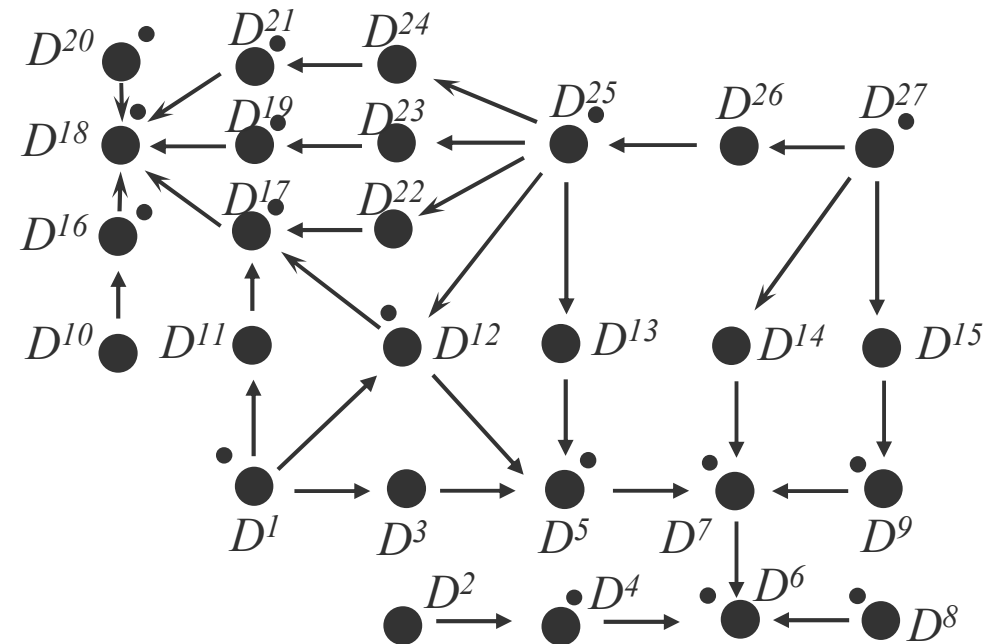


There *Exists* a *Future* state where $\dot{x}_a > 0$ and $\dot{x}_b > 0$
and from that state,
there *Exists* a *Future* state where $\dot{x}_a < 0$ and $\dot{x}_b > 0$

$$EF(\dot{x}_a > 0 \wedge \dot{x}_b > 0 \wedge EF(\dot{x}_a < 0 \wedge \dot{x}_b > 0))$$

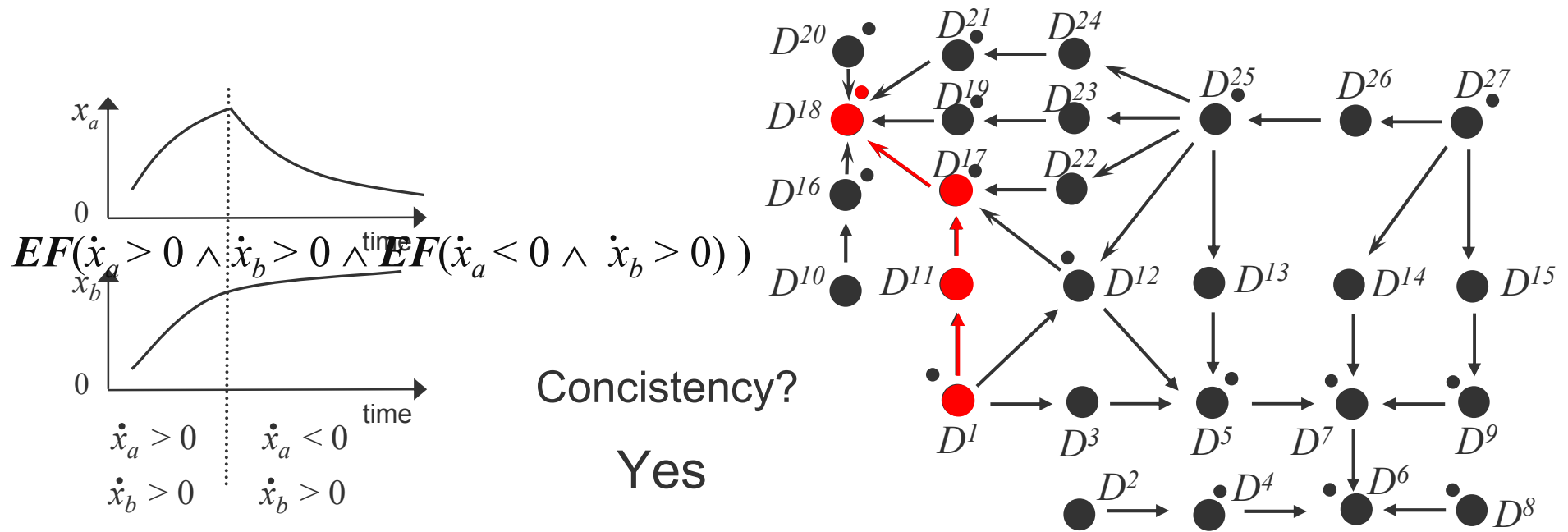
Validation using model checking

- ❖ Discrete transition system computed using qualitative simulation



Validation using model checking

- ❖ Discrete transition system computed using qualitative simulation



- ❖ Use of model checkers to check consistency between experimental data and predictions

Genetic Network Analyzer

❖ Model validation approach implemented in **new version** of GNA

Batt *et al.*, *Bioinformatics*, 05

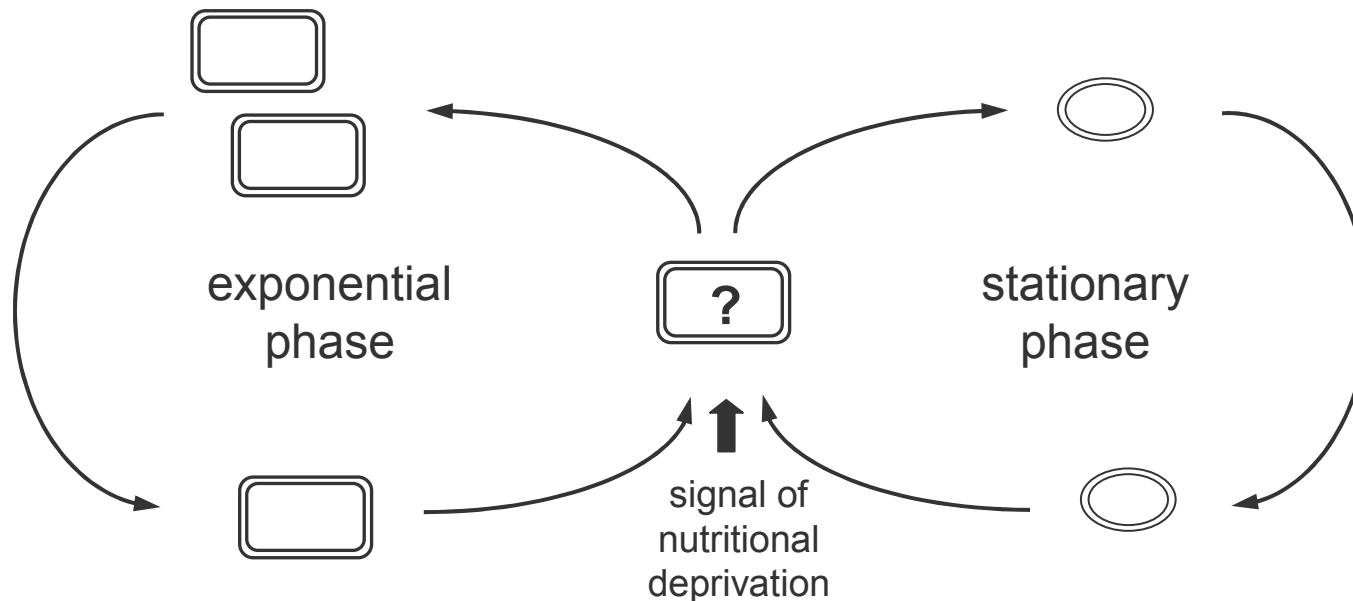
- Tailored algorithms for symbolic computation of transition system
- Export functionalities to model checkers (NuSMV, CADP)

The screenshot displays the Visual GNA 6.0 interface for a model named 'Transreg43-6_0.gna'. The main window is divided into several panels:

- Project Panel:** Lists model components like Crp, Cya, Fis, GyrAB, NoNut, TopA, rm, and initial conditions.
- Variable Fis Panel:** Shows the variable name 'Fis' and its threshold parameters: z_Fis , t_Fis_1 , t_Fis_2 , t_Fis_3 , t_Fis_4 , t_Fis_5 , and max_Fis .
- State equation Panel:** Contains the differential equation:
$$\frac{d}{dt} Fis = k_Fis_1 * (1 - s + (Crp, t_Crp_1) * s + (Cya, t_Cya_1) * s + (NoNut, t_NoNut) * s + (TopA, t_TopA_2) * s + (GyrAB, t_GyrAB_2) * s - (Fis, t_Fis) * Fis$$
- Initial conditions Panel:** A table for 'myTransreg43-stat_HSCC05' with columns for lower bound, variable, and upper bound.
- Simulation Panel:** Shows simulation settings for 'myTransreg43-stat_HSCC05', including 'Time-out: 60 s' and 'Max. number of states to display: 600'.
- Results Panel:** Displays simulation statistics: 'Status: Finished', 'Elapsed simulation time: 0.201', 'Number of reached states: 129', 'Number of states on stack: 13', and 'Used memory: 7741 kb'.
- Graph Panel (Graph3):** A state transition graph titled 'Filter instantaneous states from Graph2'. It shows a complex network of states (s1 to s31) connected by transitions. A context menu is visible over the graph with options: 'Zoom in graph', 'Zoom out graph', 'Export to CADP', 'Export to NuSMV', 'Deselect all states', 'Subgraph spanning selected states', 'Expand selected subgraph', and 'Display variables in selected path'.

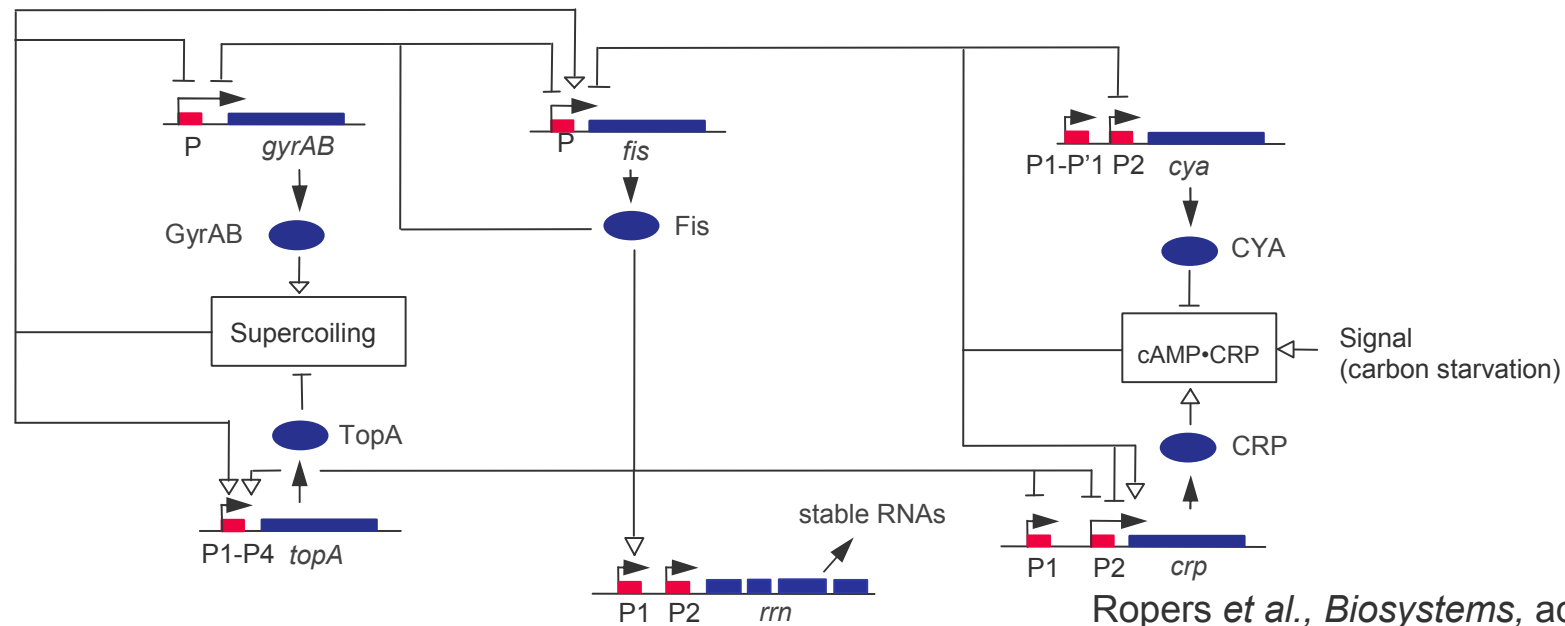
Nutritional stress response in *E. coli*

- ❖ In case of nutritional stress, *E. coli* population abandons growth and enters stationary phase



Nutritional stress response in *E. coli*

- ❖ In case of nutritional stress, *E. coli* population abandons growth and enters stationary phase



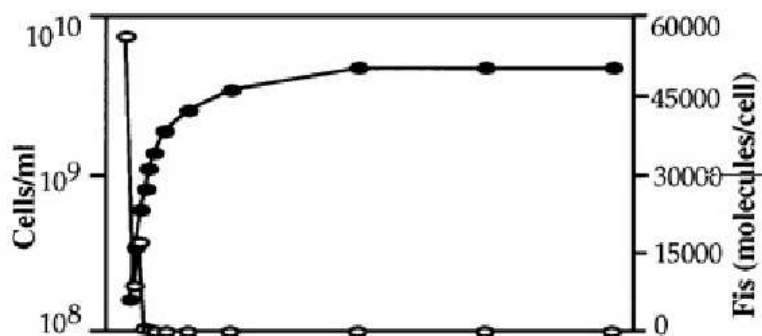
- ❖ Decision to abandon or continue growth is controlled by complex genetic regulatory network
- ❖ Model: 7 PLDEs, 40 parameters and 54 inequality constraints

Validation of stress response model

❖ Qualitative simulation of carbon starvation:

- 66 reachable domains (< 1s.)
- single attractor domain (asymptotically stable equilibrium point)

❖ Experimental data on Fis:



“Fis concentration decreases and becomes steady in stationary phase”

Ali Azam *et al.*, *J. Bacteriol.*, 99

CTL formulation:

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

Model checking with NuSMV: property true (< 1s.)

Validation of stress response model

❖ Other properties:

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

Kawamukai *et al.*, *J. Bacteriol.*, 85

$$AG(x_{crp} > \theta_{crp} \wedge x_{cya} > \theta_{cya} \wedge x_s > \theta_s \rightarrow EF \dot{x}_{cya} < 0) \quad \text{True (<1s)}$$

- “DNA supercoiling decreases during transition to stationary phase”

Balke and Gralla, *J. Bacteriol.*, 87

$$EF(\dot{x}_{gyrAB} < 0 \vee \dot{x}_{topA} > 0) \wedge x_{rrn} < \theta_{rrn}) \quad \text{False (<1s)}$$

❖ Inconsistency between observation and prediction calls for model revision or model extension

Nutritional stress response model extended with global regulator RpoS

Novel prediction of stress response model

❖ Qualitative simulation of carbon **upshift** response:

- discrete transition system of 1143 reachable domains (< 2s)
- one of the strongly connected components is an attractor:

$$AG(\text{statesInSCC} \rightarrow AG \text{ statesInSCC})$$

- attractor corresponds to damped oscillations towards stable equilibrium point

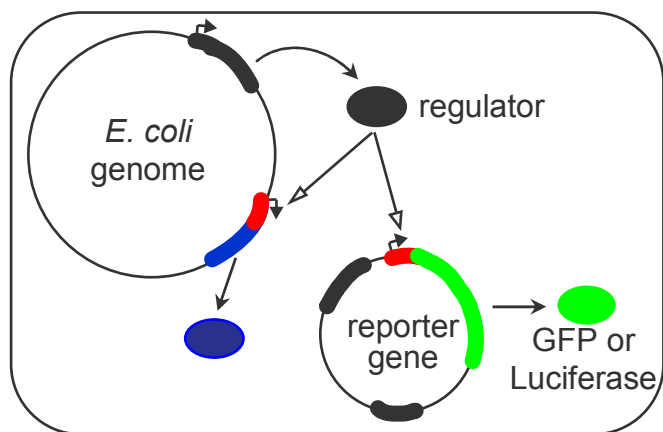
❖ Occurrence of damped oscillations in *E. coli* carbon upshift response is **unexpected** prediction

❖ **Experimental verification** of model predictions

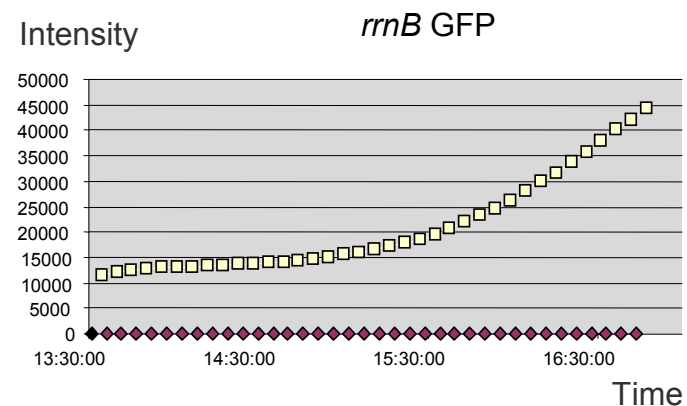
Comparison with observed qualitative evolution of protein concentrations

Experimental validation of predictions

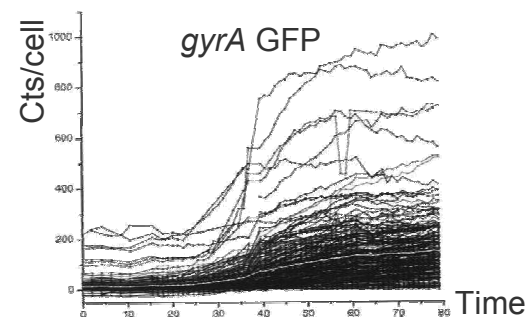
- ❖ Time-series measurements of protein concentrations in parallel and at high sampling rate using gene reporter system



in bacterial population



in individual bacteria



in collaboration with I. Mihalcescu

Conclusions

- ❖ Use of qualitative modeling and simulation method yielding predictions **suitable for comparison** with experimental data
- ❖ Combination with model-checking techniques to achieve **automatic** and **efficient** comparison
- ❖ **Tailored** approach **implemented** in new version of GNA and **applied** to nutritional stress response in *E. coli*
- ❖ Model-checking used in combination with different formalisms
 - generalized logical models Bernot *et al.*, *J. Theor. Biol.*, 04
 - concurrent systems Chabrier *et al.*, *Theor. Comput. Sci.*, 04; Eker *et al.*, *PSB*, 02
 - hybrid automata Ghosh *et al.*, *HSCC*, 03; Antoniotti *et al.*, *Theor. Comput. Sci.*, 04
- ❖ Further work: integration of tailored model checker into GNA

Acknowledgements

❖ Thanks for your attention!

❖ Work in collaboration with:

- Hidde de Jong
- Johannes Geiselman
- Jean-Luc Gouzé
- Radu Mateescu
- Michel Page
- Delphine Ropers
- Tewfik Sari
- Dominique Schneider