

Analysis and Verification of Qualitative Models of Genetic Regulatory Networks: A Model-Checking Approach

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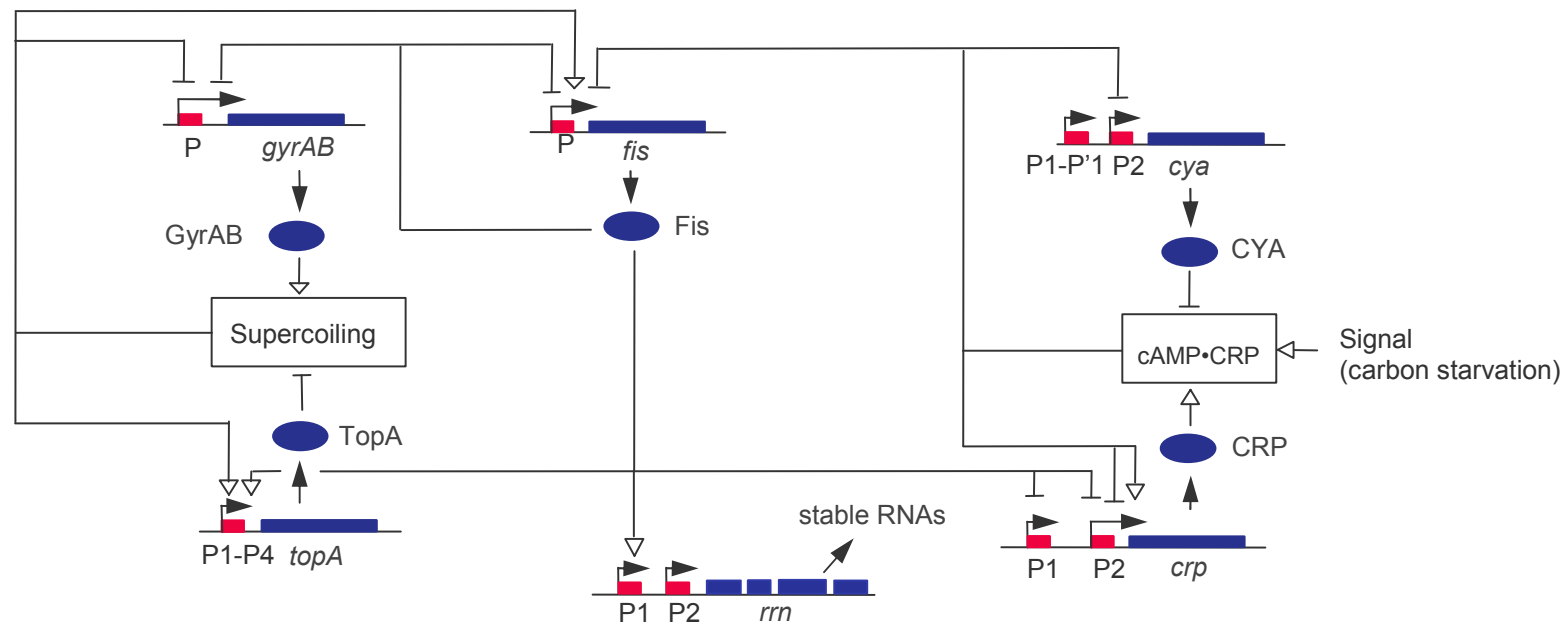
Overview

1. Introduction
2. Qualitative modeling and simulation method
3. Verification 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**

Qualitative analysis of network dynamics

- ❖ Computer support indispensable for dynamical analysis of genetic regulatory systems: **modeling** and **simulation**
 - precise and unambiguous description of network
 - systematic derivation of behavior predictions
- ❖ **Quantitative information** on kinetic parameters and molecular concentrations usually not available
- ❖ **Qualitative simulation** method based on class of **piecewise-linear (PL) differential equation** models

Gouzé and Sari, *Dyn. Syst.*, 03 de Jong et al., *Bull. Math. Biol.*, 04
- ❖ Method implemented in the tool **Genetic Network Analyzer** (GNA) and **applied** to several bacterial systems

de Jong et al., *Bioinformatics*, 03

Bottleneck: analysis of simulation results

- ❖ Qualitative simulation results in a state transition graph consisting of qualitative states and transitions between states
- ❖ **Bottleneck** of qualitative simulation: visual inspection of large state transition graphs

Need for an automatic method to analyze simulation results

- ❖ **Approach**: combination of qualitative simulation and model checking to efficiently analyze large state transition graphs

Brajnik and Clancy, *Ann. Math. Artif. Intell.*, 98 Shults and Kuipers, *Artif. Intell.*, 97

- ❖ Approach applied to analysis of genetic regulatory networks

Method **tailored** to PL models to favor **upscalability**

PL differential equation models

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

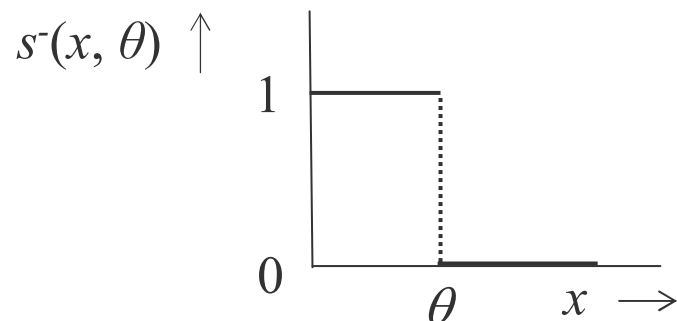
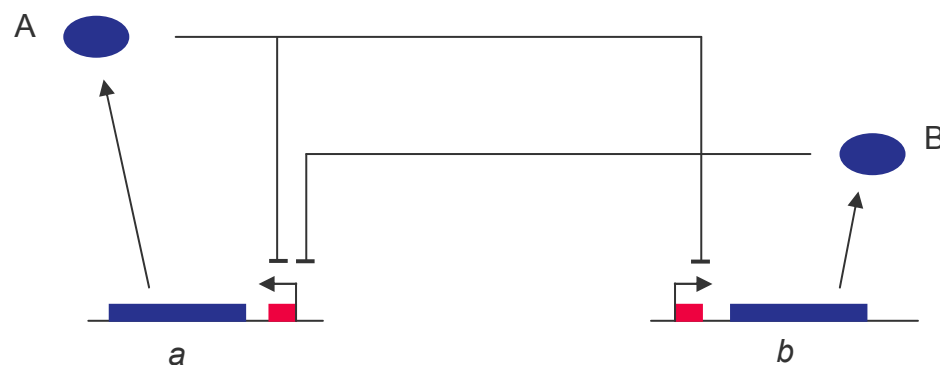
$$\dot{x}_a = \kappa_a s^-(x_a, \theta_{a2}) s^-(x_b, \theta_b) - \gamma_a x_a$$

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

x : protein concentration

θ : threshold concentration

κ, γ : rate constants

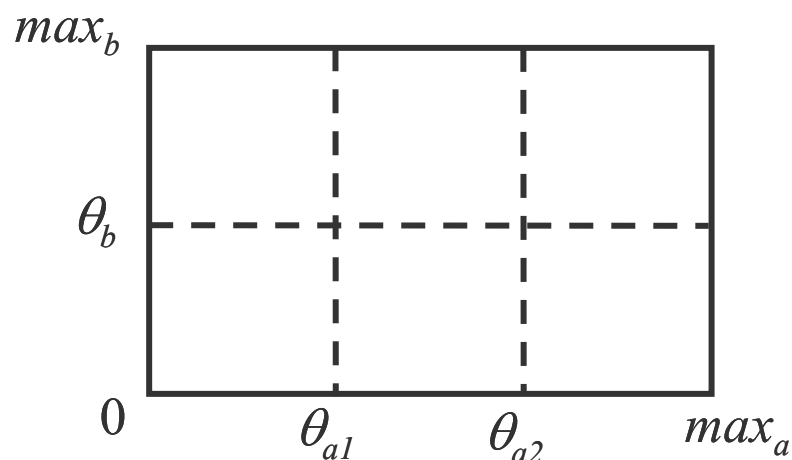


- ❖ Differential equation models of regulatory networks are **piecewise-linear**

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

Qualitative analysis of network dynamics

❖ Analysis of the dynamics in phase space

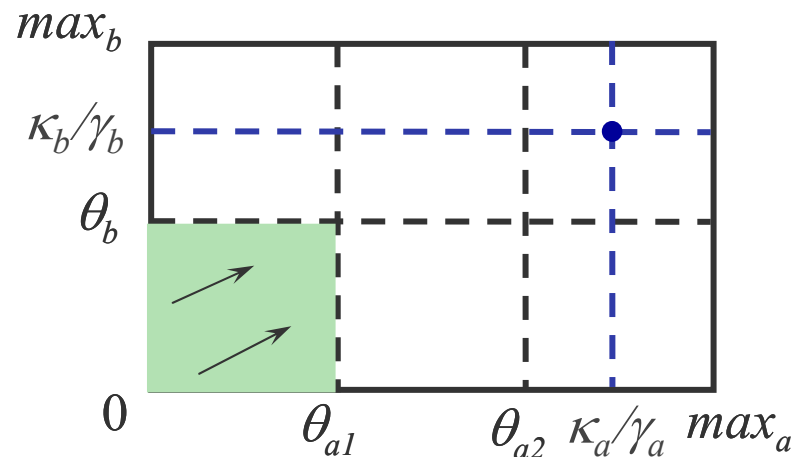


$$\dot{x}_a = \kappa_a s^-(x_a, \theta_{a2}) s^-(x_b, \theta_b) - \gamma_a x_a$$

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Qualitative analysis of network dynamics

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

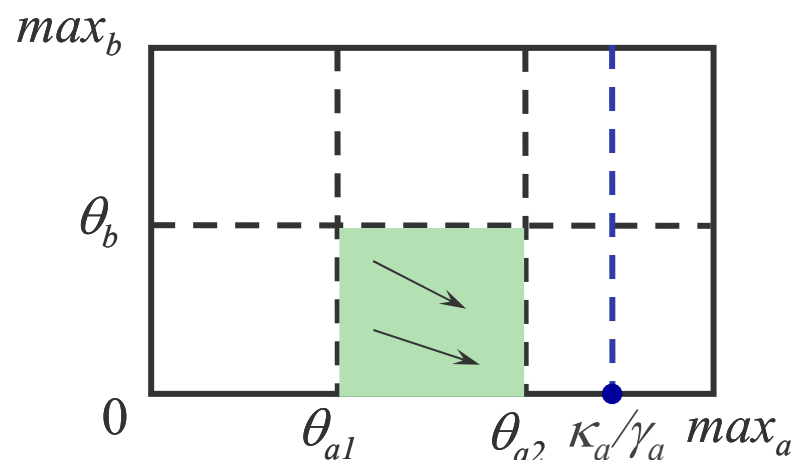
$$\dot{x}_b = \kappa_b - \gamma_b x_b$$

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

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

Qualitative analysis of network dynamics

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

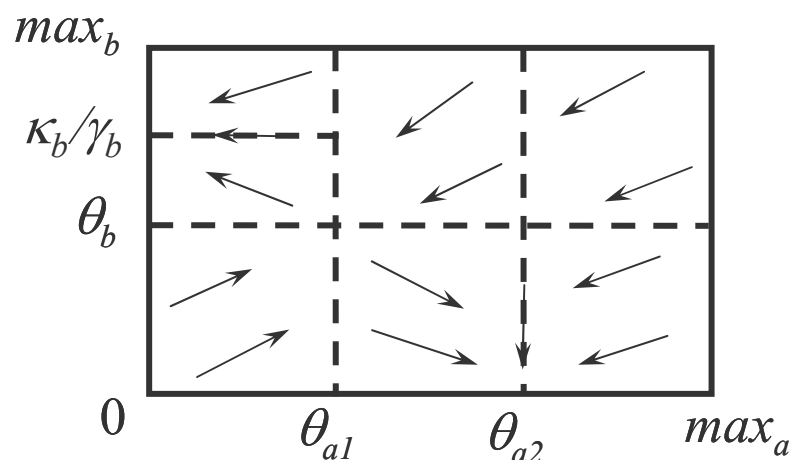
$$\dot{x}_b = -\gamma_b x_b$$

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

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Qualitative analysis of network dynamics

❖ Analysis of the dynamics in phase space



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

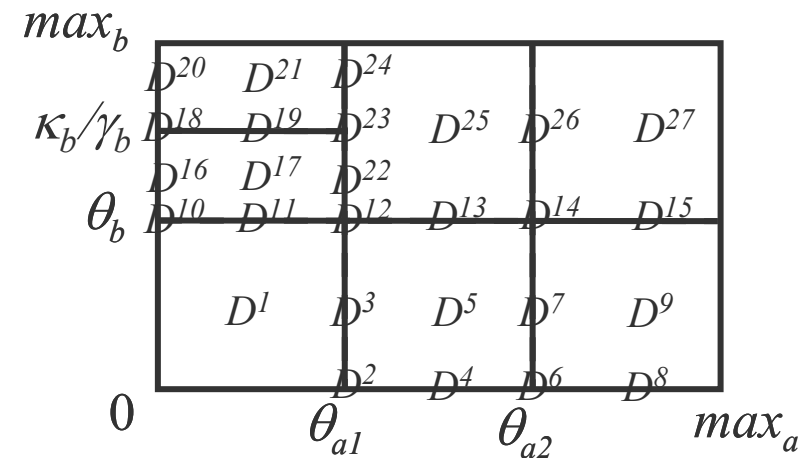
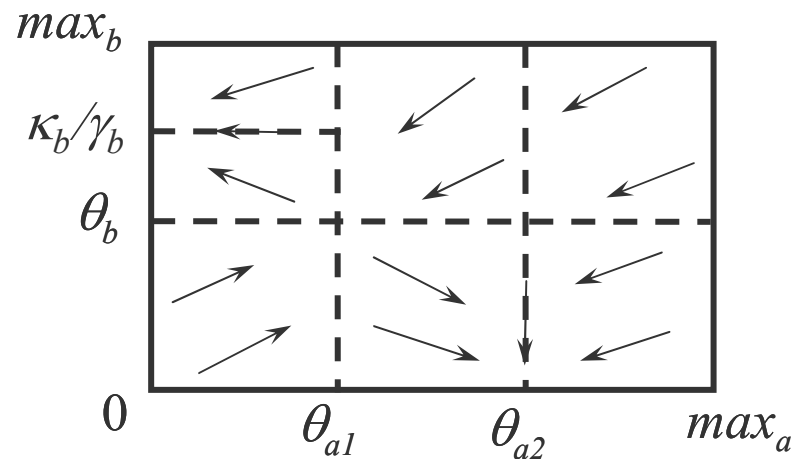
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Qualitative analysis of network dynamics

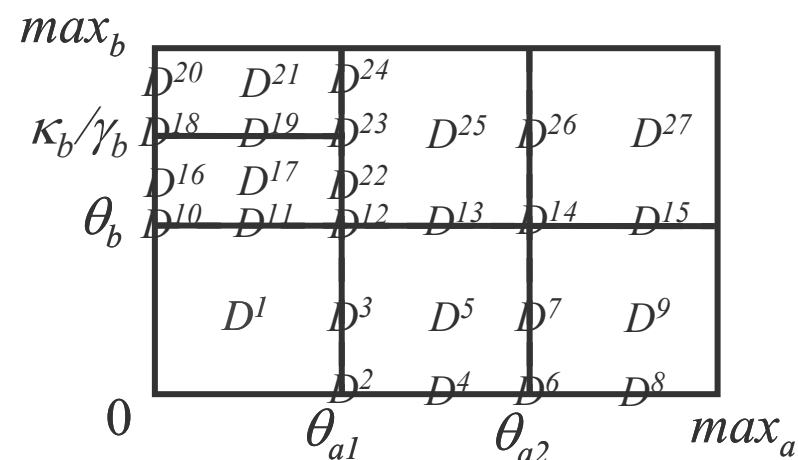
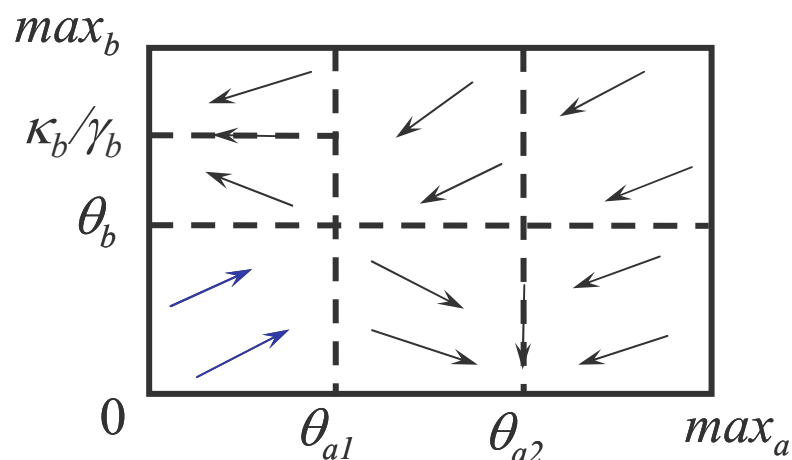
❖ Analysis of the dynamics in phase space



❖ Partition of phase space into domains

Qualitative analysis of network dynamics

❖ Analysis of the dynamics in phase space



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

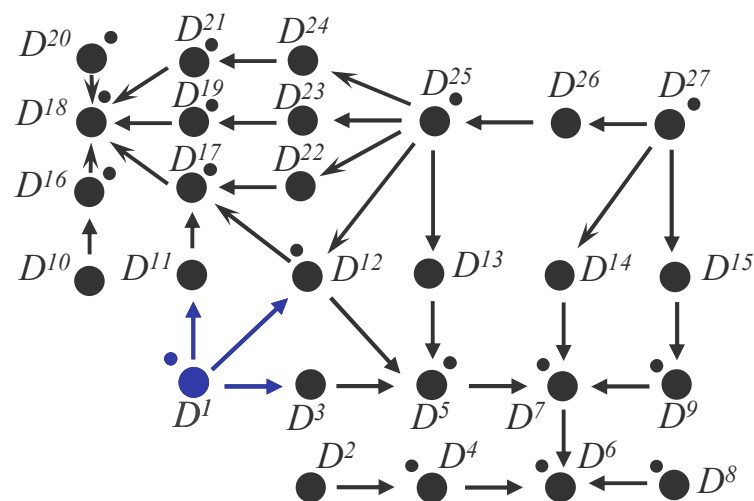
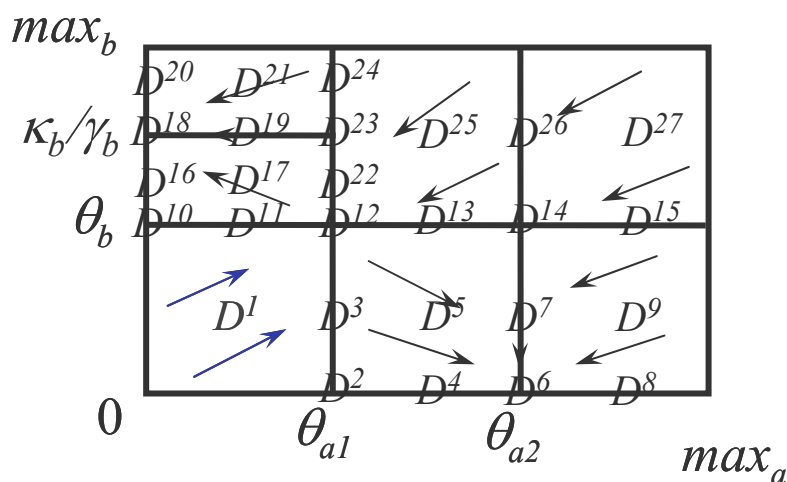
❖ Partition of phase space into domains

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

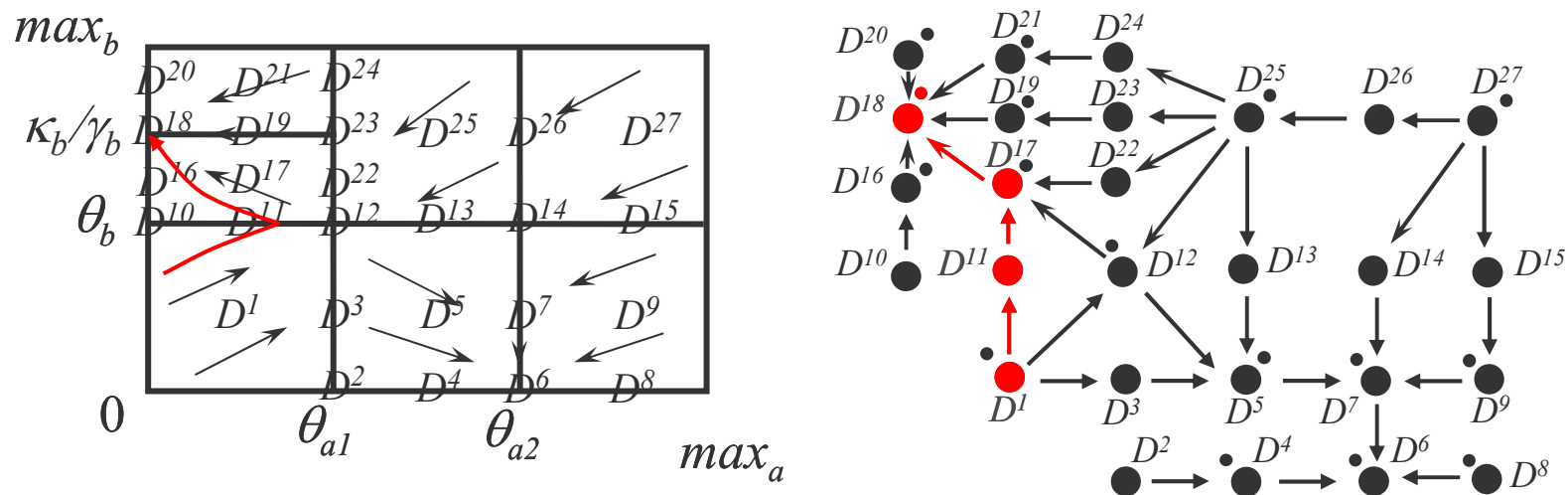


$$D^1 \in D \quad (D^1, D^3), (D^1, D^{11}), (D^1, D^{12}) \in \rightarrow \quad L(D^1) = \{x_a < \theta_a^1, x_b < \theta_b, \dot{x}_a > 0, \dot{x}_b > 0\}$$

Qualitative analysis of network dynamics

❖ Qualitative abstraction yields **discrete transition system**

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

Every solution corresponds to a path in the discrete transition system

Model-checking approach

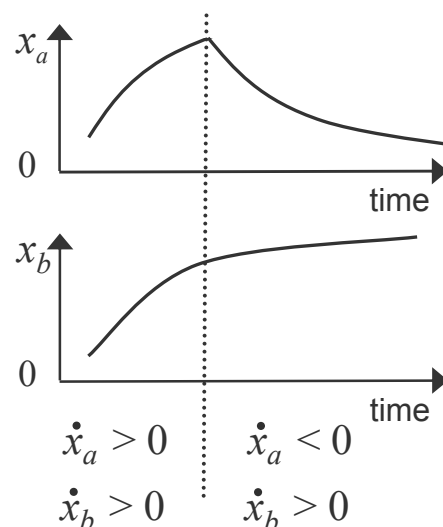
- ❖ **Model checking** is automated technique for verifying that discrete transition system satisfies certain temporal properties
- ❖ Computation Tree Logic (CTL) model-checking:
 - set of **atomic propositions** AP
 - discrete transition system is **Kripke structure** $KS = \langle S, R, L \rangle$, where S set of states, R transition relation, L labeling function over AP
 - temporal properties expressed in temporal 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 are CTL formulas
- ❖ Computer tools are available to perform efficient and reliable model checking (e.g., NuSMV, SPIN, CADP)

Verification using model checking

- ❖ Atomic propositions describing the state of the network

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

- ❖ Dynamical property expressed in temporal logic CTL

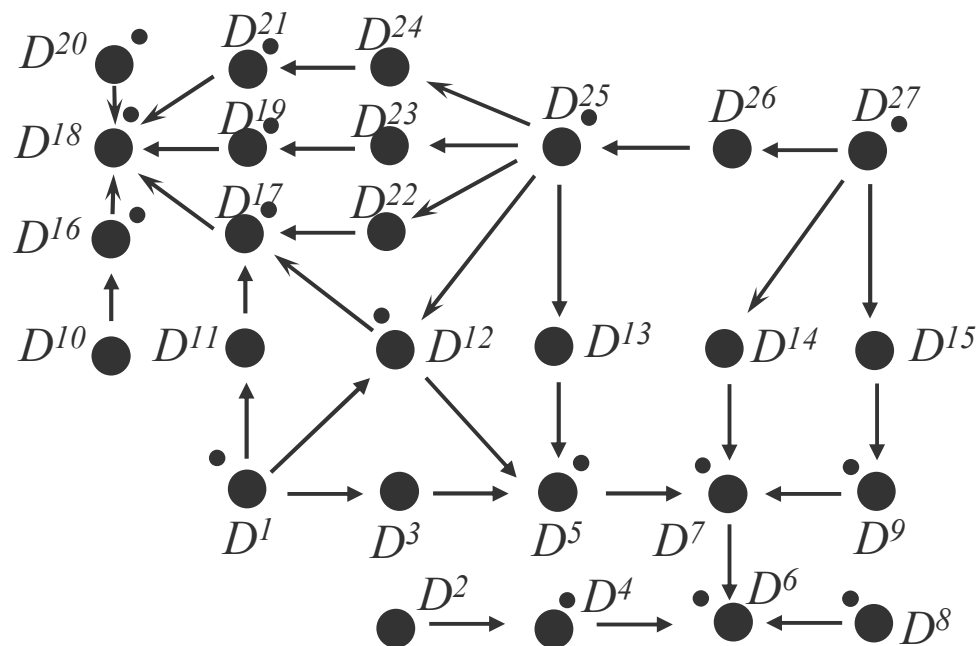
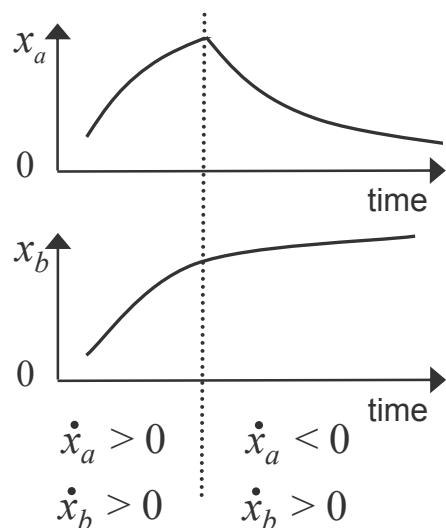


There *Exists* a *Future* state where $\dot{x}_a > 0$ and $\dot{x}_b > 0$
and from that state,
there *Exists* a *Future* state where $\dot{x}_a < 0$ and $\dot{x}_b > 0$

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

Verification using model checking

- ❖ Discrete transition system computed using qualitative simulation is Kripke structure
- ❖ Use of model checkers to check whether predictions satisfy dynamical property



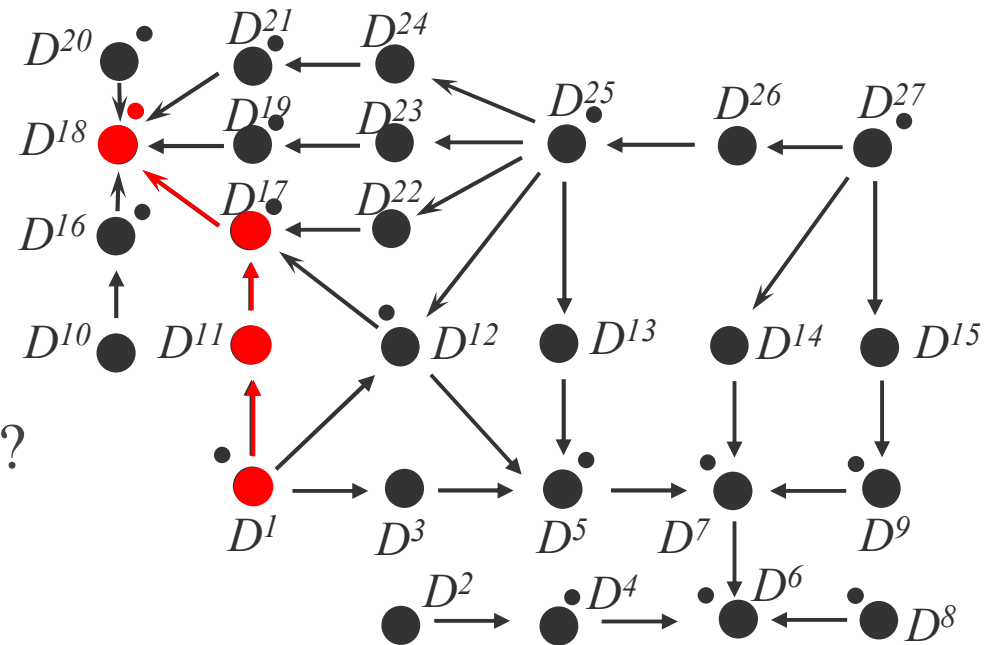
Verification using model checking

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

$DTS \models f ?$

Yes



Genetic Network Analyzer

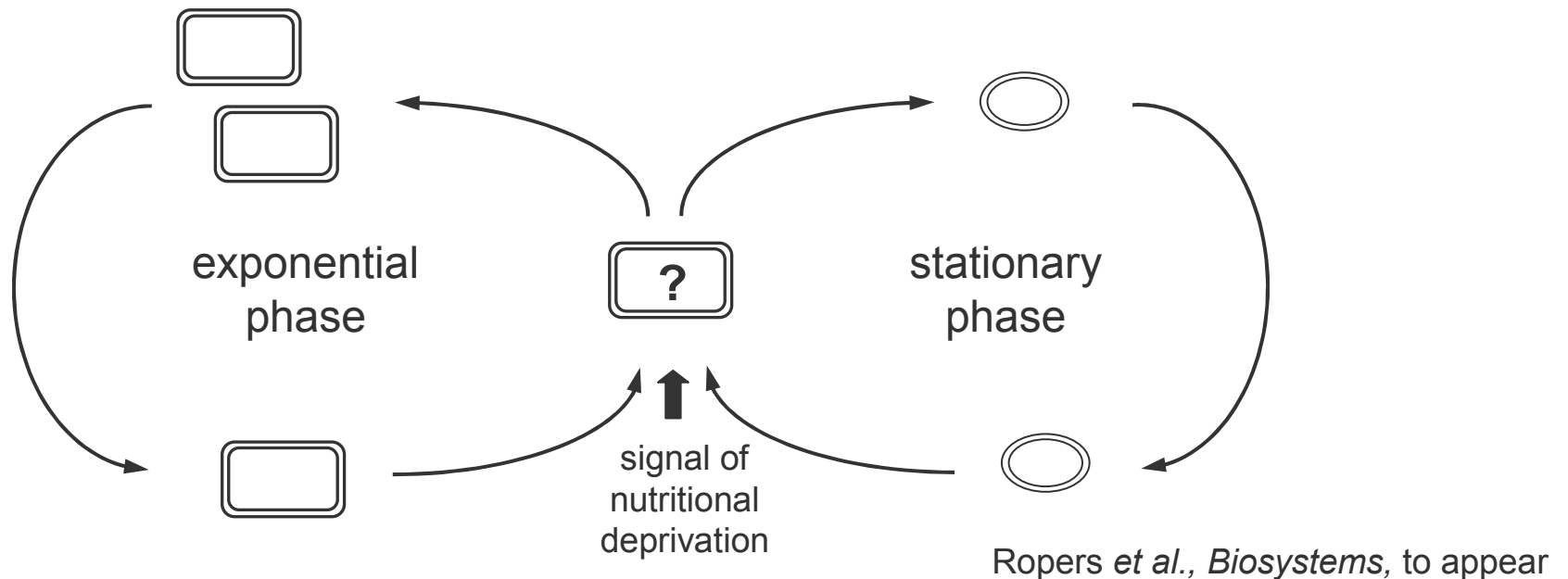
❖ Model verification method implemented in **new version** of GNA

- Tailored algorithms for qualitative simulation
- 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 shows a state transition graph with nodes labeled s1 through s31 and directed edges representing transitions. A context menu is open over the graph, offering options like 'Zoom in graph', 'Zoom out graph', 'Export to CADP', and 'Export to NuSMV'. The left sidebar shows a project tree with folders for 'Model', 'Initial conditions', and 'myTra'. The 'Variable Fis' panel includes a list of variables (z_Fis, t_Fis_1 to t_Fis_5, max_Fis) and a 'State equation' field containing a differential equation. The 'Simulation with myTransreg43-stat_HSCC05' panel shows simulation parameters such as 'Time-out: 60 s' and 'Max. number of states to display: 600'. The status bar at the bottom indicates 'Status: Finished', 'Elapsed simulation time: 0.201', 'Number of reached states: 129', 'Number of states on stack: 13', and 'Used memory: 7741 kb'.

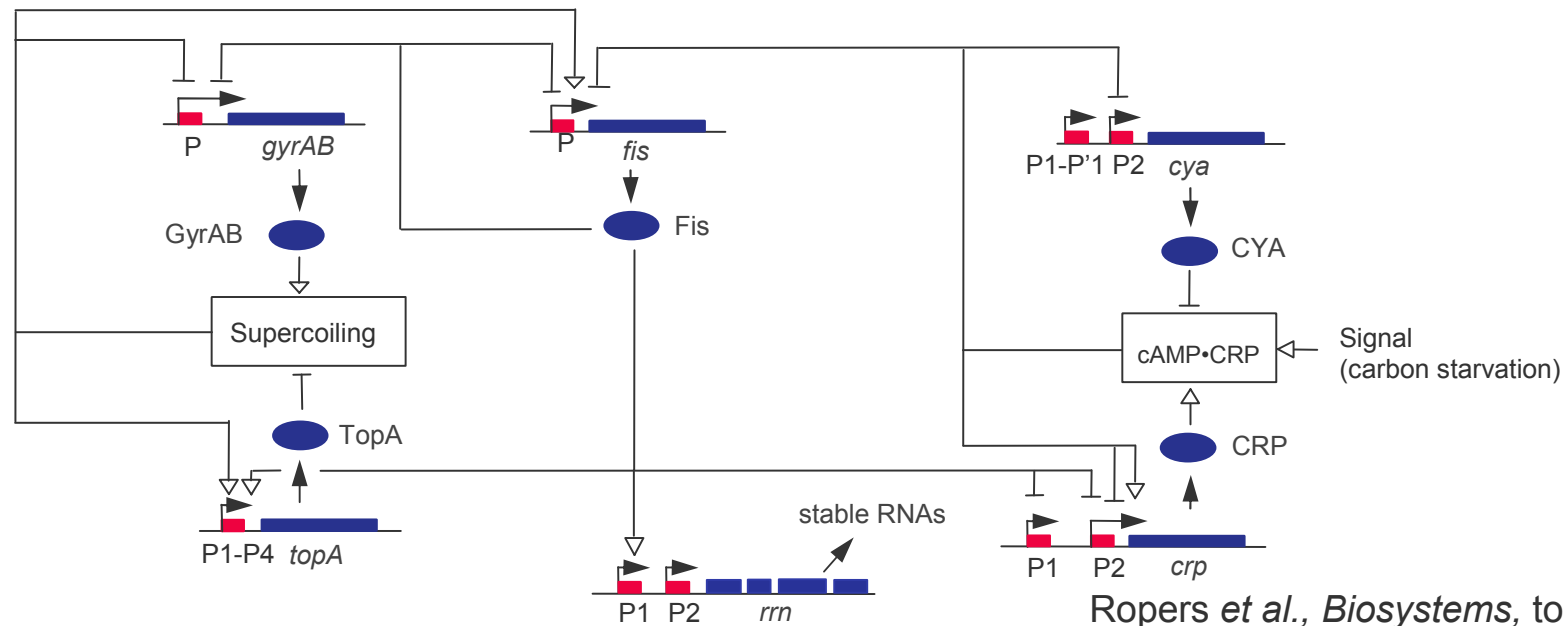
Nutritional stress response in *E. coli*

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



Nutritional stress response in *E. coli*

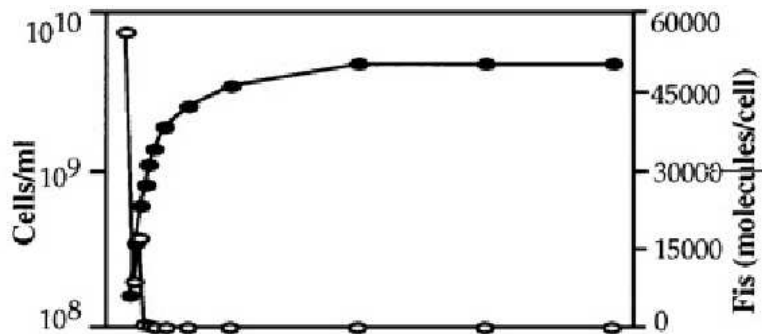
- ❖ In case of nutritional stress, *E. coli* population abandons growth and enters stationary phase



- ❖ 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** response:
 - discrete transition system of 66 reachable domains (<1s)
 - single attractor domain (asymptotically stable equilibrium point)
- ❖ **Model validation** by comparison with experimental data



“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:

$$AG(statesInSCC \rightarrow AG\ statesInSCC)$$

- 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

Time-series measurements of protein concentrations in parallel and at high sampling rate

Conclusions

- ❖ Combination of qualitative simulation with model-checking for verification of genetic regulatory network models
 - Method tailored to PL models to favor upscalability
- ❖ Approach implemented in new version of GNA and applied to nutritional stress response in *E. coli*
- ❖ Model-checking used in combination with different modeling formalisms for analysis of biological networks
 - 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

❖ Thanks for your attention!