

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

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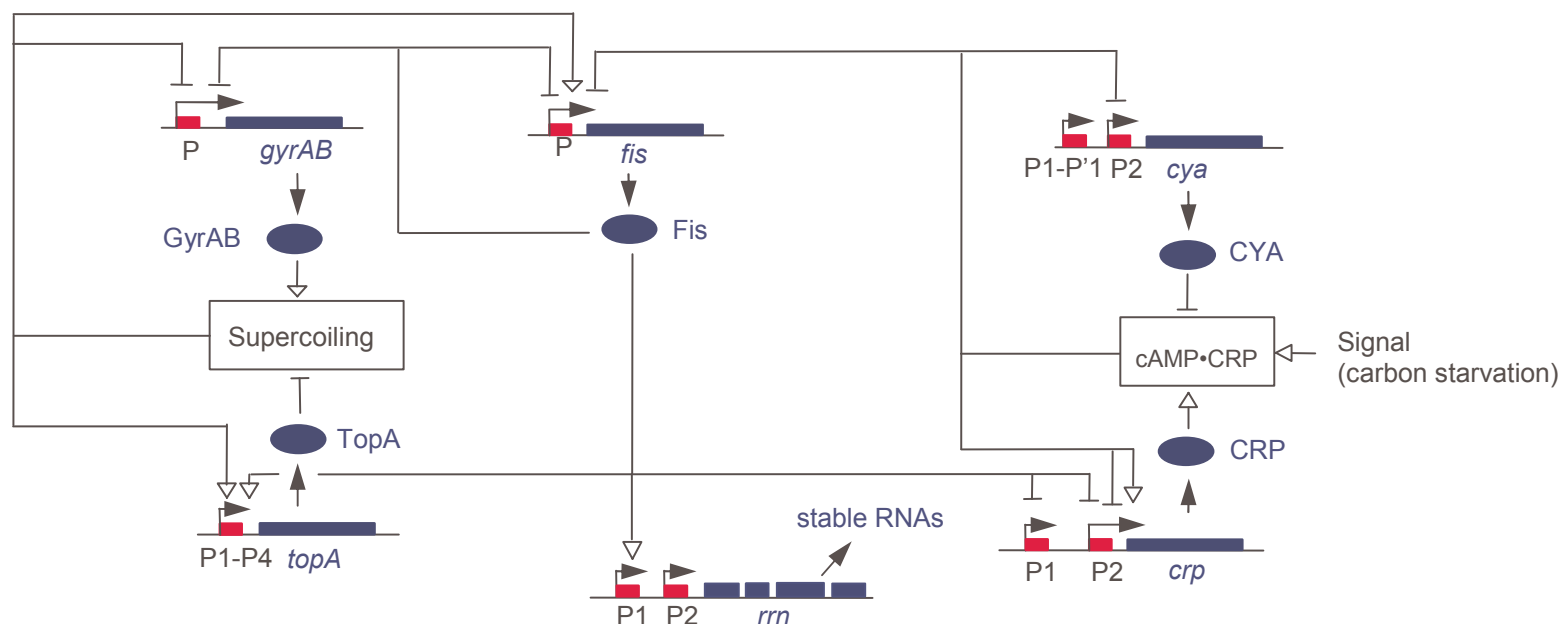
Overview

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

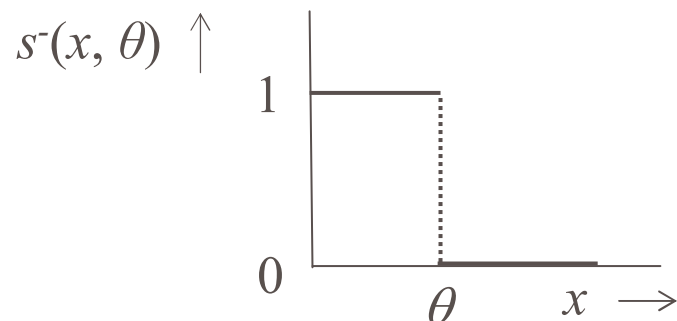
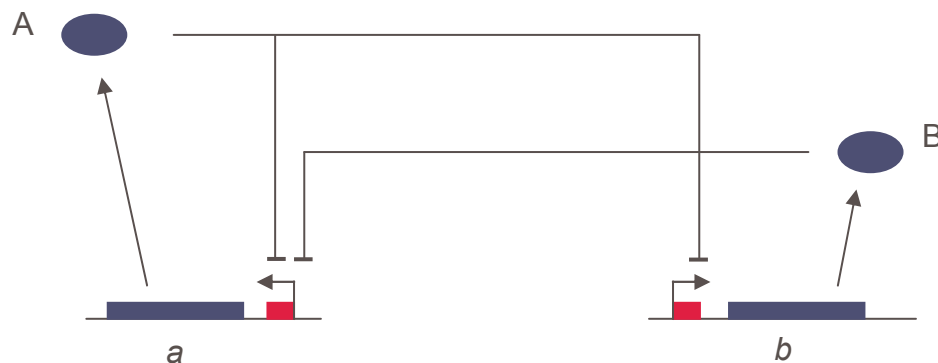
$$\dot{x}_a = \kappa_a s^-(x_a, \theta_{a2}) s^-(x_b, \theta_b) - \gamma_a x_a$$

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

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:
 - 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

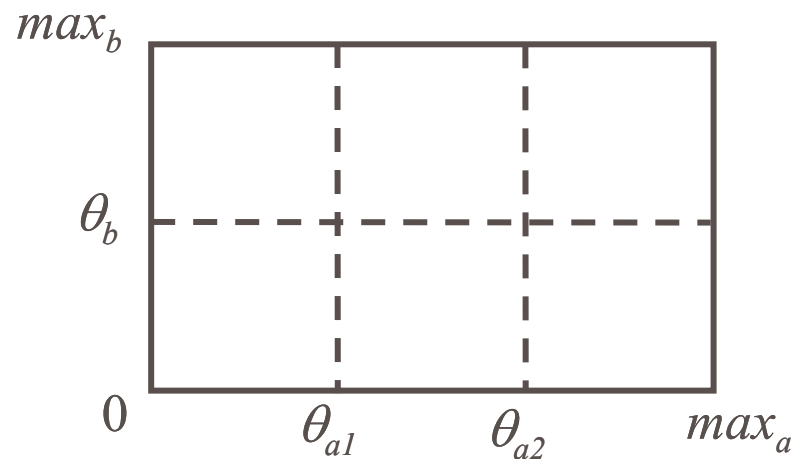
Gouzé and Sari, *Dyn. Syst.*, 03

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

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

Qualitative analysis of network dynamics

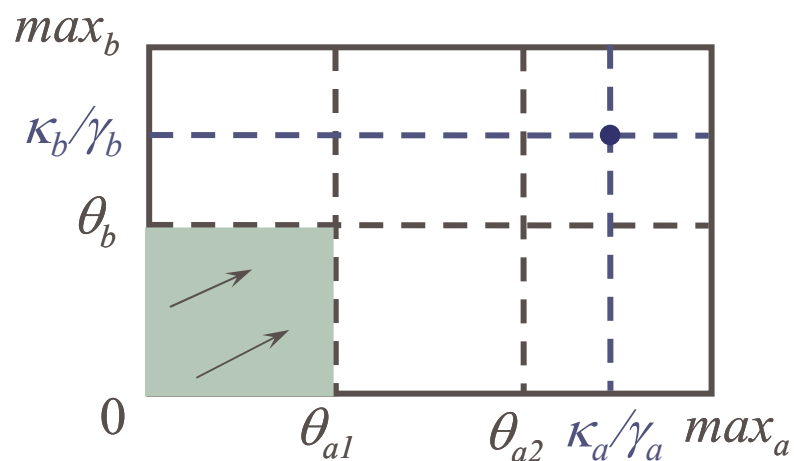
- ❖ Analysis of the dynamics in phase space



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

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

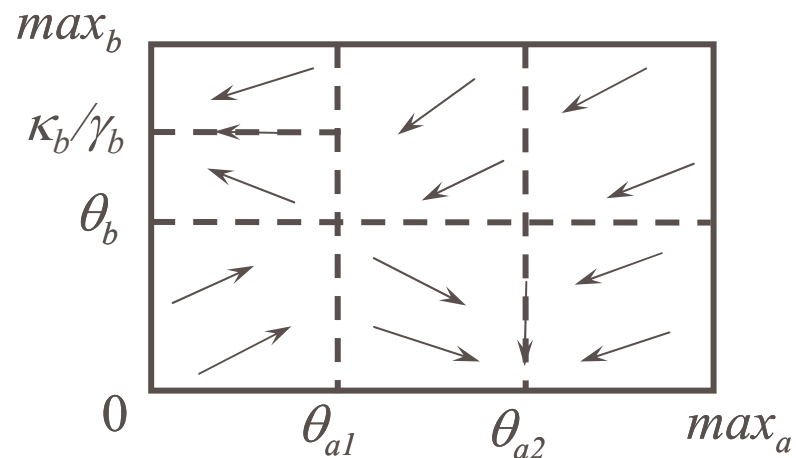
$$\dot{x}_b = \kappa_b - \gamma_b x_b$$

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

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

Qualitative analysis of network dynamics

- ❖ Analysis of the dynamics in phase space



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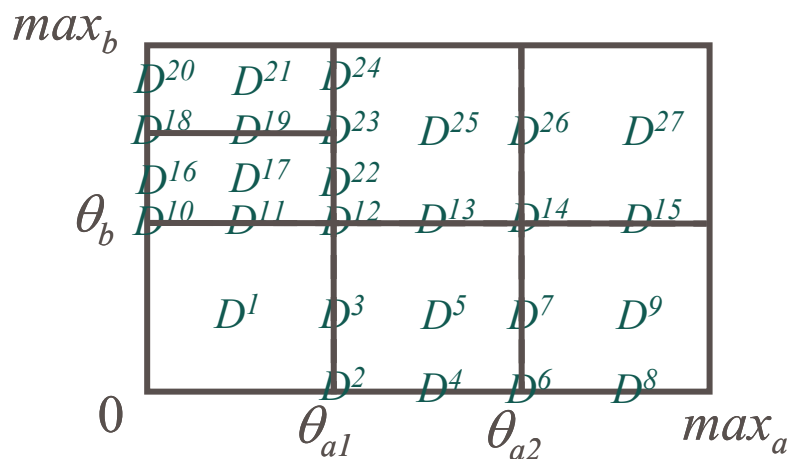
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Qualitative analysis of network dynamics

- ❖ Analysis of the dynamics in phase space
- ❖ Phase space partition: unique derivative sign pattern in domains



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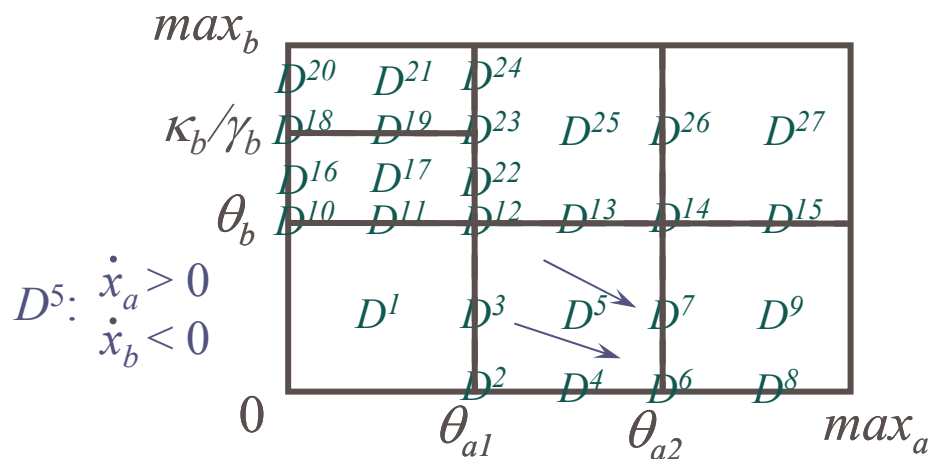
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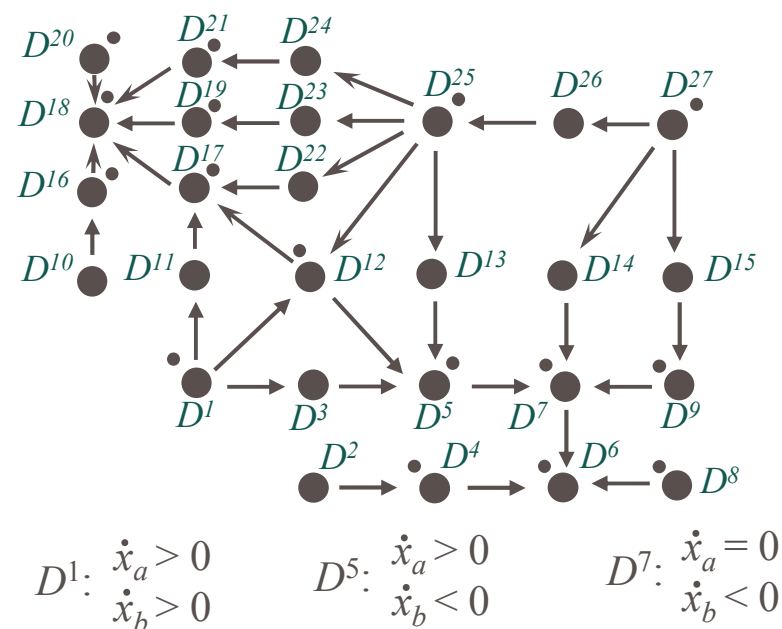
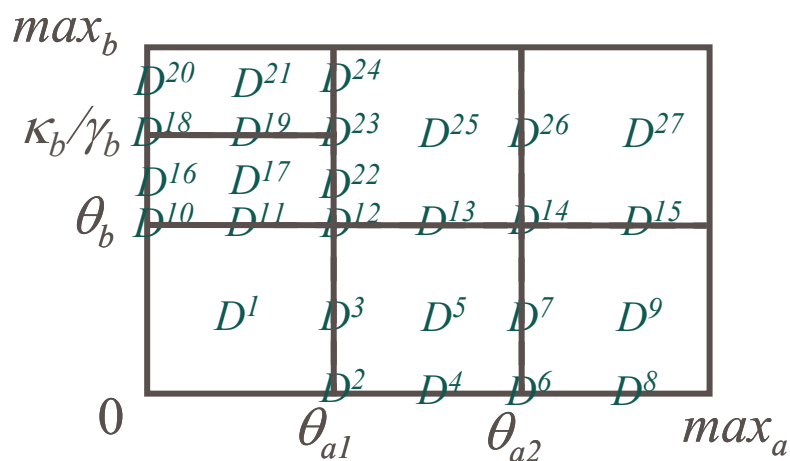
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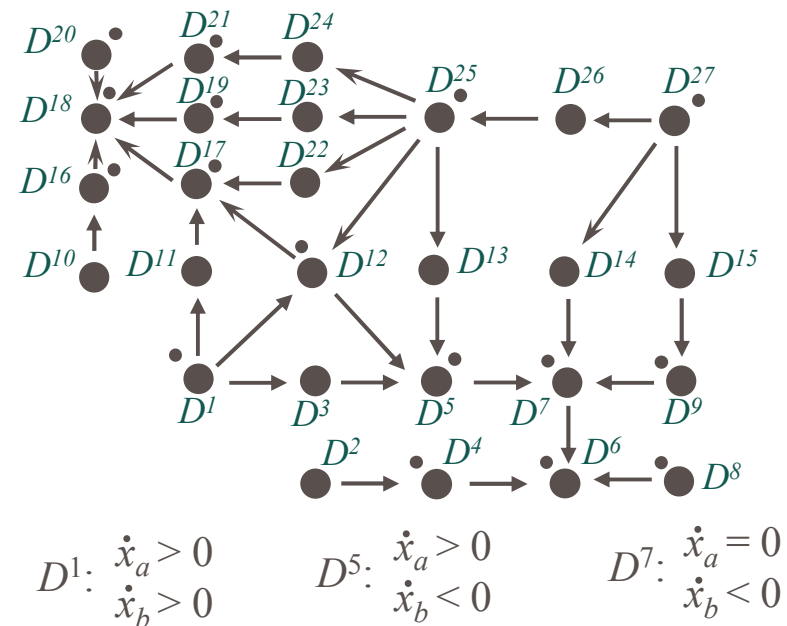
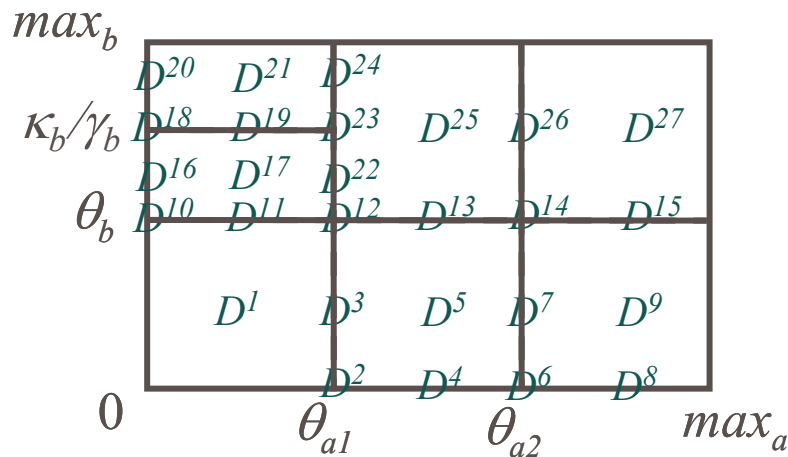
Qualitative analysis of network dynamics

- ❖ Analysis of the dynamics in phase space
- ❖ Phase space partition: unique derivative sign pattern in domains
- ❖ Qualitative abstraction yields discrete transition system



Qualitative analysis of network dynamics

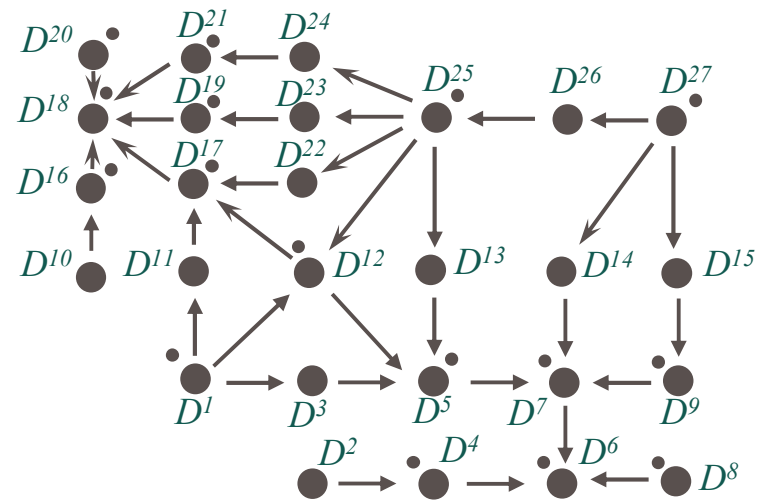
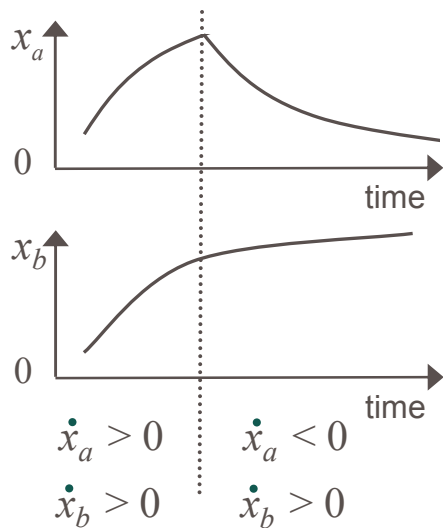
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- ❖ Abstraction preserves **unicity** of derivative sign pattern

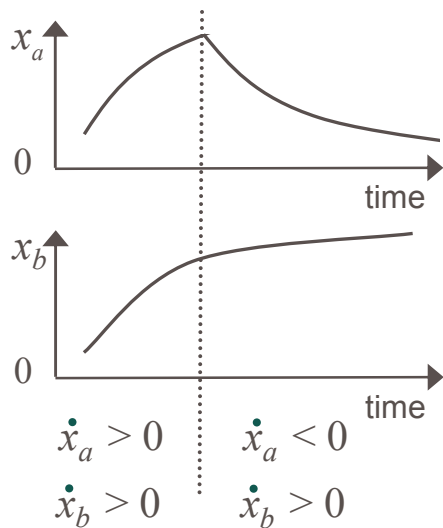
Validation of qualitative models

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

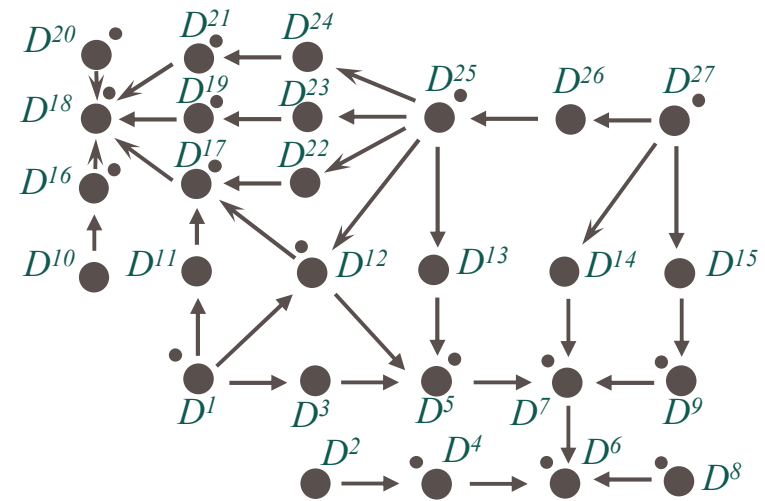


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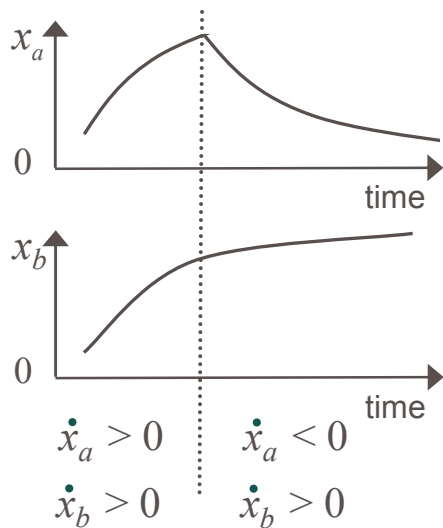
Consistency?



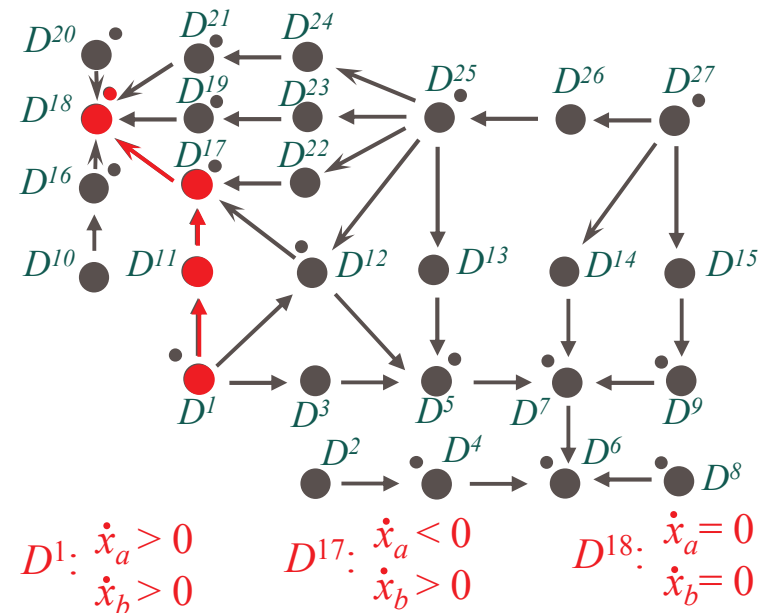
- ❖ Model validation: comparison of derivative sign patterns in observed and predicted behaviors

Validation of qualitative models

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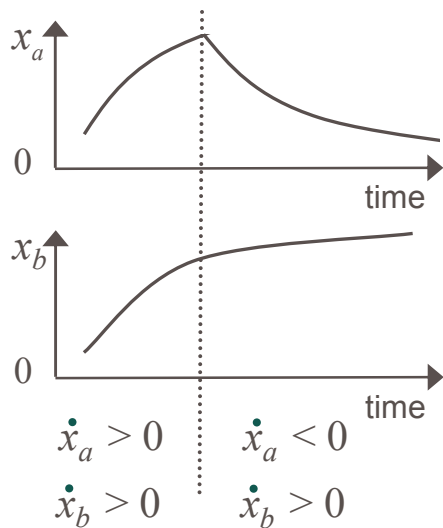
Consistency?
Yes



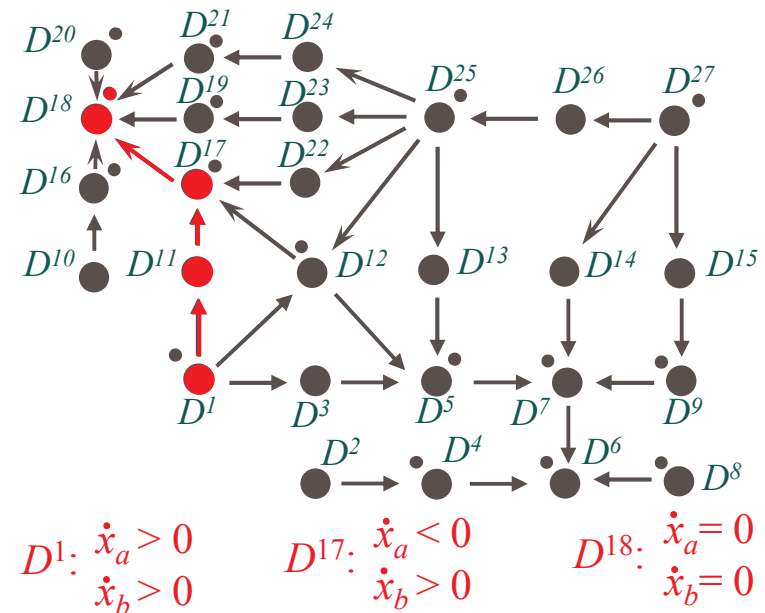
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Validation of qualitative models

- ❖ 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
- ❖ CTL model-checking framework:
 - set of **atomic propositions** AP
 - discrete transition system $KS = \langle S, R, L \rangle$,
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

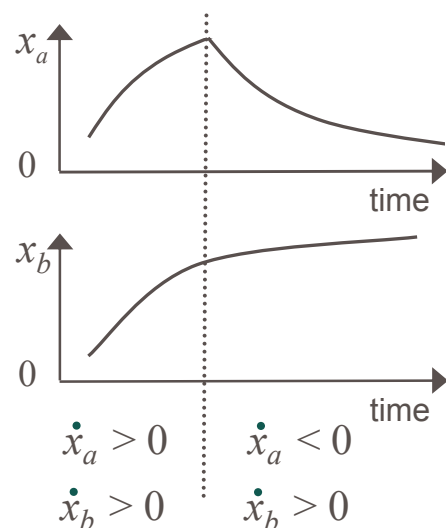
$$AP = \{x_a = 0, x_a < \theta_a^1, \dots, x_b < \max_b, \dot{x}_a < 0, \dot{x}_a = 0, \dots, \dot{x}_b > 0\}$$

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

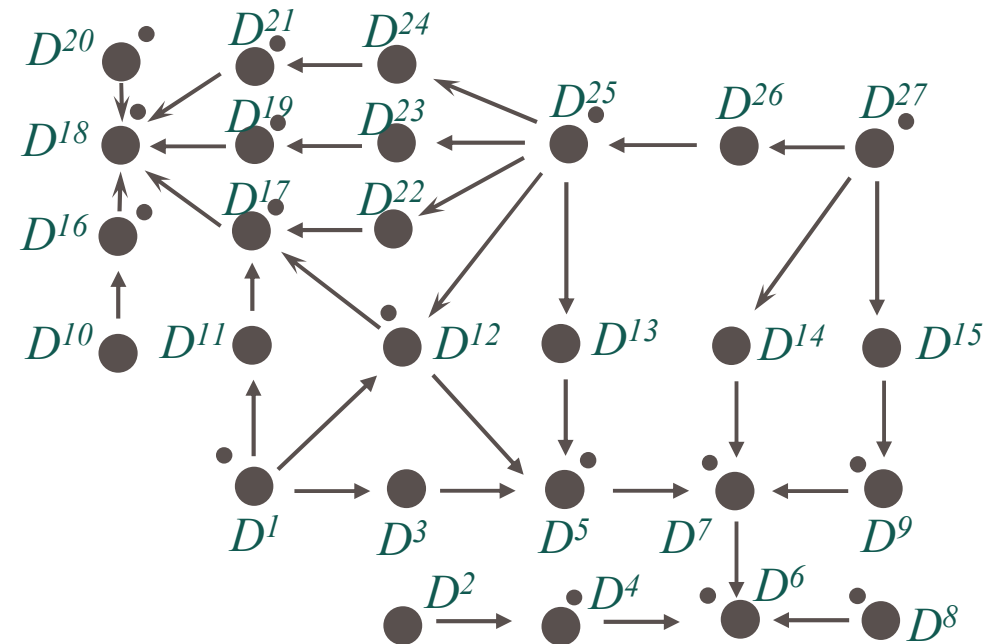
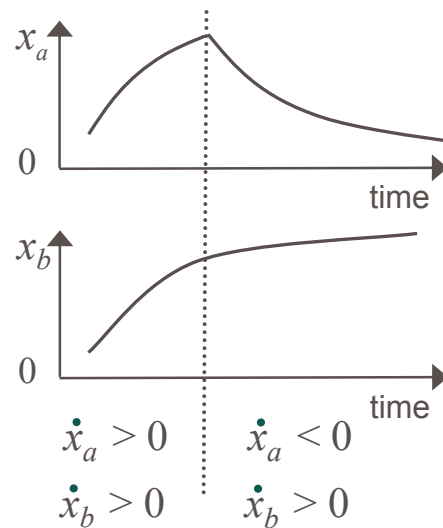


There *Exists* a *Future* state where $\dot{x}_a > 0$ and $\dot{x}_b > 0$
and from that state,
there *Exists* a *Future* state where $\dot{x}_a < 0$ and $\dot{x}_b > 0$

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

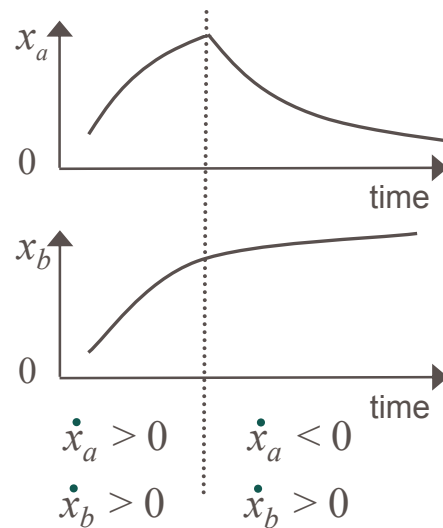
Validation using model checking

- ❖ Discrete transition system computed using qualitative simulation

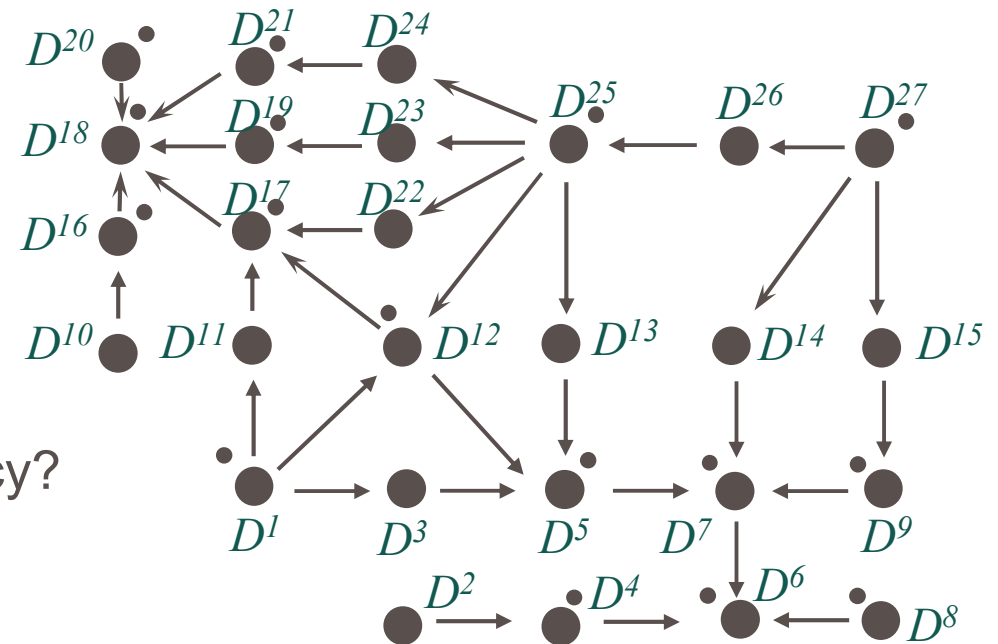


Validation using model checking

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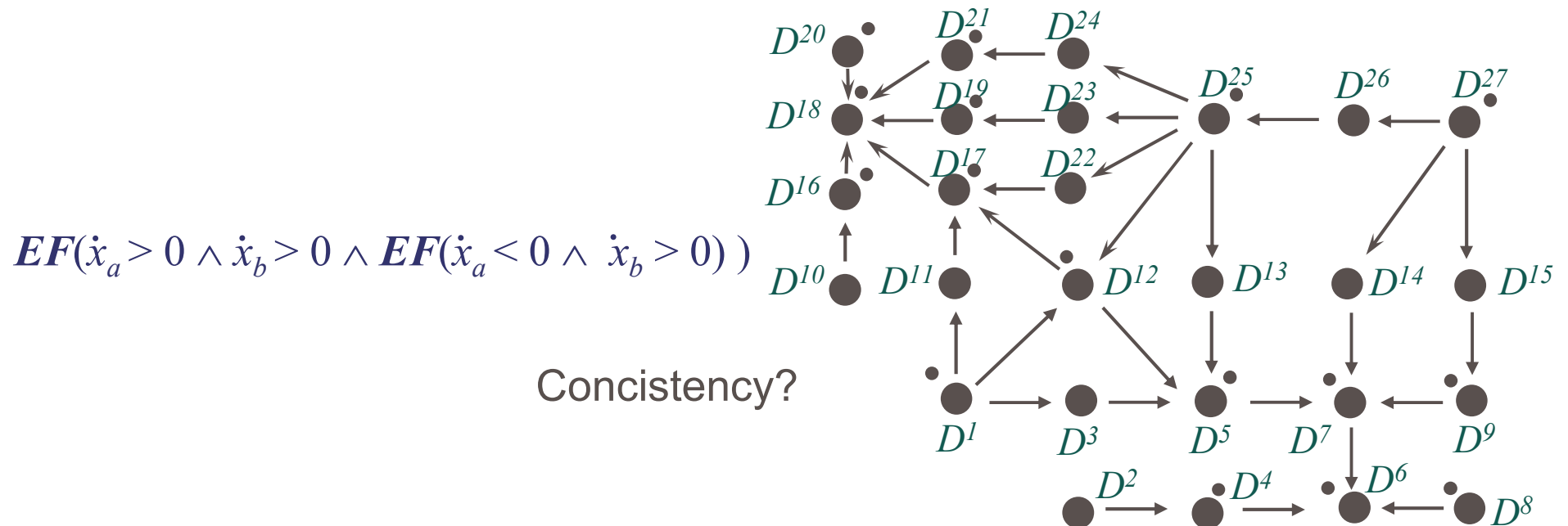


Consistency?



Validation using model checking

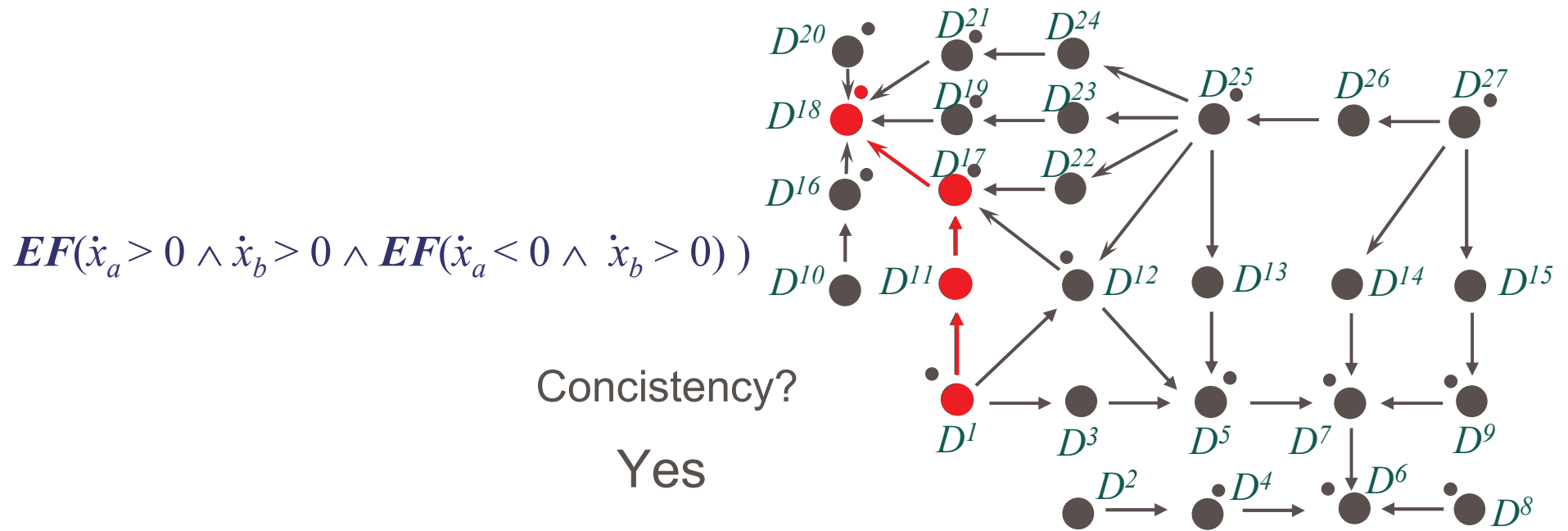
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- ❖ Use of model checkers to check consistency between experimental data and predictions

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

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

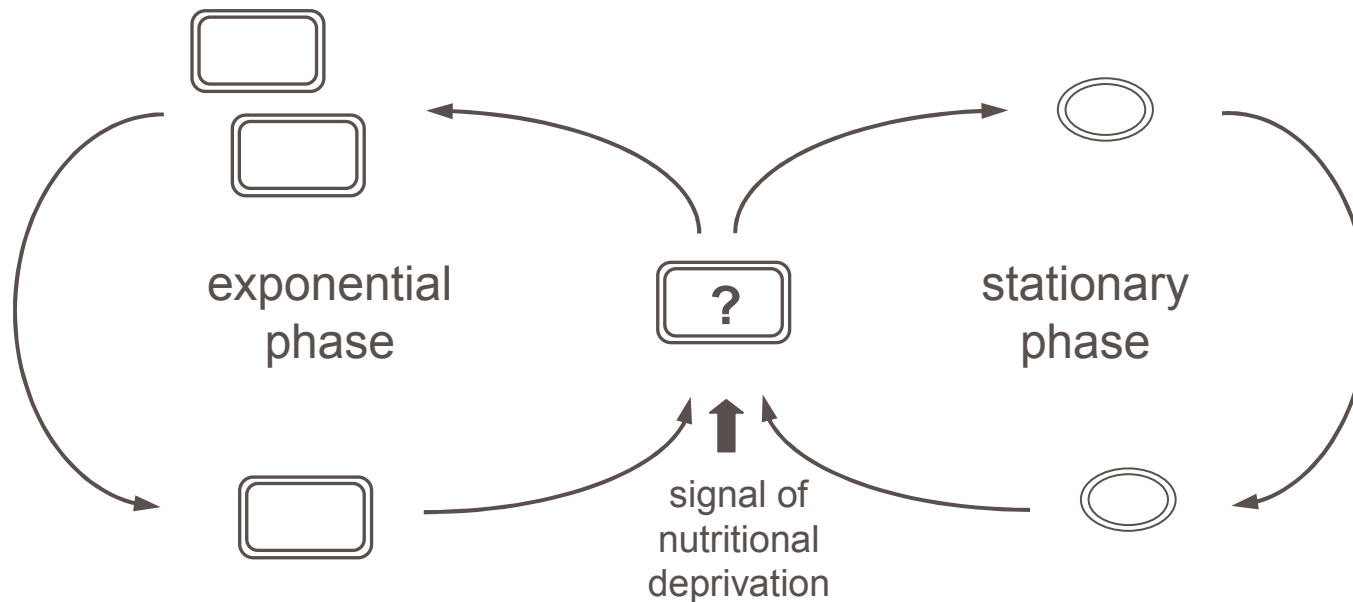
The screenshot displays the Visual GNA 6.0 interface for the model 'Transreg43-6_0.gna'. The main window shows a state transition graph titled 'Graph3 - Filter instantaneous states from Graph2'. The graph consists of numerous states (labeled s1 through s31) connected by directed edges, representing the transition system. A context menu is open over the graph, offering actions such as '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'.

The interface also includes several control panels:

- Variable Fis:** Name: Fis, Input variable: . Threshold parameters: z_Fis, t_Fis_1, t_Fis_2, t_Fis_3, t_Fis_4, t_Fis_5, max_Fis. Synthesis parameters: k_Fis_2, k_Fis_1.
- State equation:**
$$\frac{d}{dt} \text{Fis} = k_{\text{Fis}_1} * (1 - s + (\text{Crp}, t_{\text{Crp}_1}) * s + (\text{Cya}, t_{\text{Cya}_1}) * s + (\text{NoNut}, t_{\text{NoNut}_1}) * s + (\text{TopA}, t_{\text{TopA}_2}) * s + (\text{GyrAB}, t_{\text{GyrAB}_2}) * s - (\text{Fis}, t_{\text{Fis}_1}) * s - g_{\text{Fis}} * \text{Fis}$$
- Initial conditions:** myTransreg43-stat_HSCC05. A table lists variables and their bounds.
- Simulation with myTransreg43-stat_HSCC05:** Properties, Results, Search states. Initial conditions: myTransreg43-stat_HSCC05. Time-out: 60 s. Max. number of states to display: 600.
- Status:** Finished. Elapsed simulation time: 0.201. Number of reached states: 129. Number of states on stack: 13. Used memory: 7741 kb.

Nutritional stress response in *E. coli*

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



Validation of stress response model

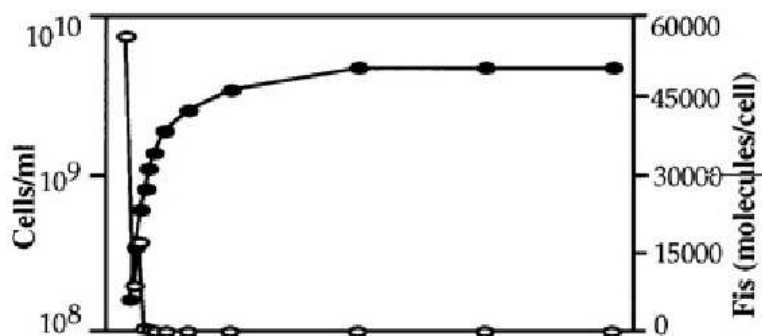
- ❖ Qualitative simulation of carbon starvation:
 - 66 reachable domains ($< 1s.$)
 - single attractor domain (asymptotically stable equilibrium point)

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

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

- “DNA supercoiling decreases during transition to stationary phase”

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

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

Validation of stress response model

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❖ **Model-driven experiments** for more extensive tests of validity

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

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
- ❖ Approach **implemented** 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
- ❖ Further work: integration of tailored model checker into GNA

❖ Thanks for your attention!