Qualitative Analysis and Verification of Hybrid Models of Genetic Regulatory Networks: Nutritional Stress Response in *E. coli*

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Overview1. Analysis of genetic regulatory networks

2. Problem for experimental validation of models

3. Refined analysis of genetic regulatory networks

   Partition refinement

   Qualitative abstraction

   Symbolic computation

4. Nutritional stress response in *Escherichia coli*

5. Conclusion

2

Genetic regulatory networks

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

   Genes, proteins, small molecules, and their regulatory interactions

❖ Due to switch-like character of network dynamics, **hybrid models** of genetic regulatory networks have been proposed

   Antoniotti et al., Theor. Comput. Sci., 2004; Belta et al., HSCC, 2004; de Jong et al., HSCC, 2003; Ghosh and Tomlin, Syst. Biol., 2004; Hu et al., HSCC, 2004; …

❖ Specific constraints for genetic regulatory network analysis:

   • lack of quantitative information on kinetic constants and molecular concentrations

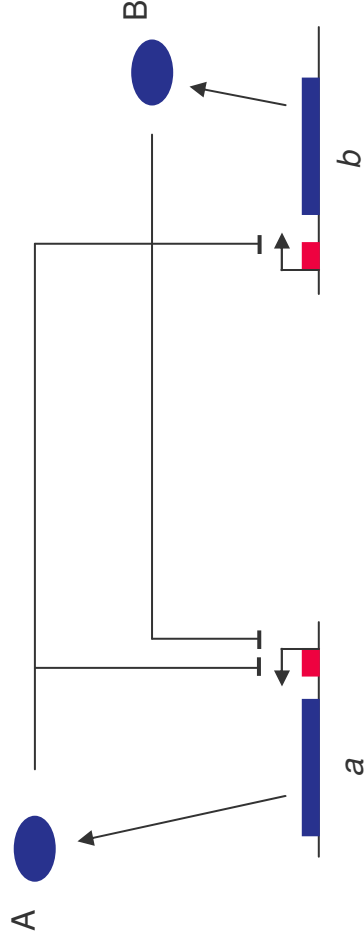   • size of networks and complexity of dynamics

PA models of genetic regulatory networks

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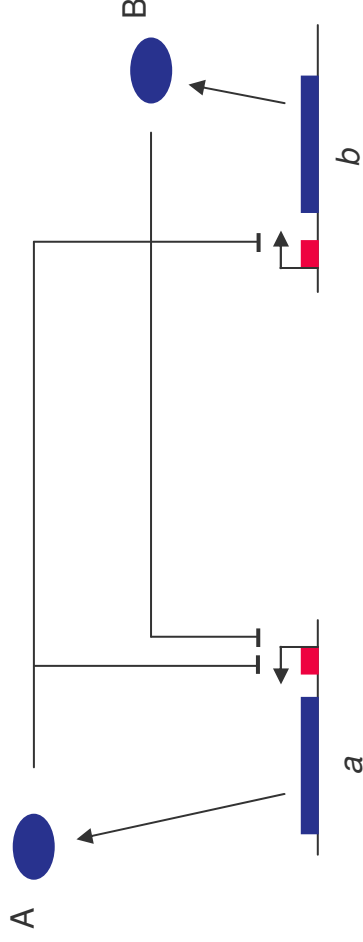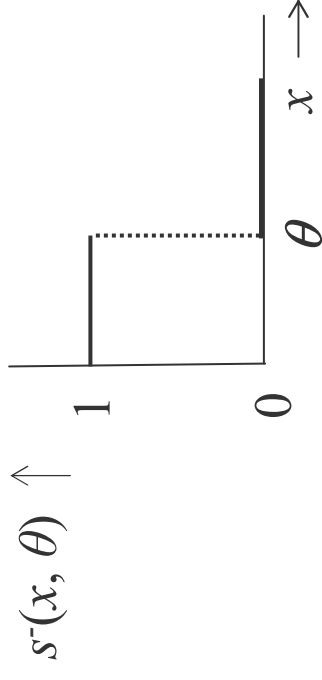
PA models of genetic regulatory networks

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

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

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

$s^-(x, \theta)$

1

0

θ $x \rightarrow$

x : protein concentration
θ : threshold concentration
κ, γ: rate constants

A

B

a

b

❖ Differential equation models of regulatory networks are **piecewise-affine (PA)**

Glass and Kauffman, *J. Theor. Biol.*, 1973

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 **discrete** or **qualitative abstraction** 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 initiation of sporulation in *B. subtilis*
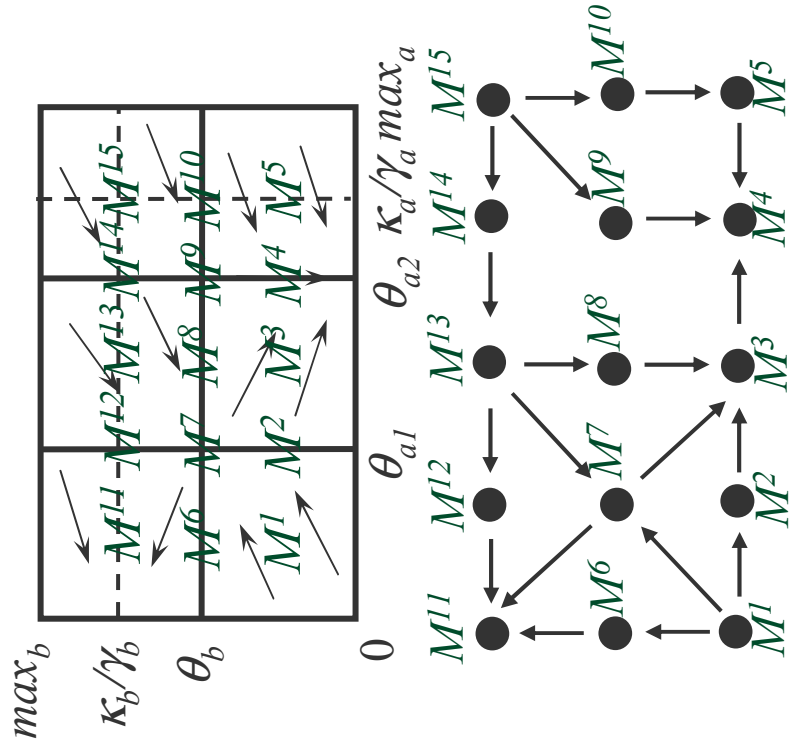
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Problem for model validation

❖ **Model validation** using gene expression data: observation of changes in derivative signs

x_a

0 · · · · · · · · · · · · ·

time

x_b

0

time

- $\dot{x}_a > 0$ · $\dot{x}_a < 0$
- $\dot{x}_b > 0$ · $\dot{x}_b < 0$
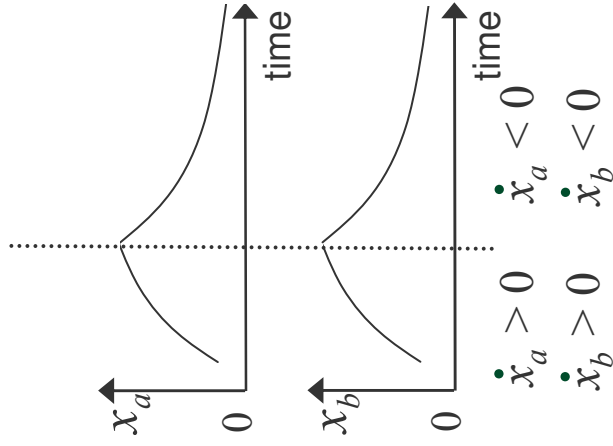
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Problem for model validation

❖ **Model validation** using gene expression data: observation of changes in derivative signs
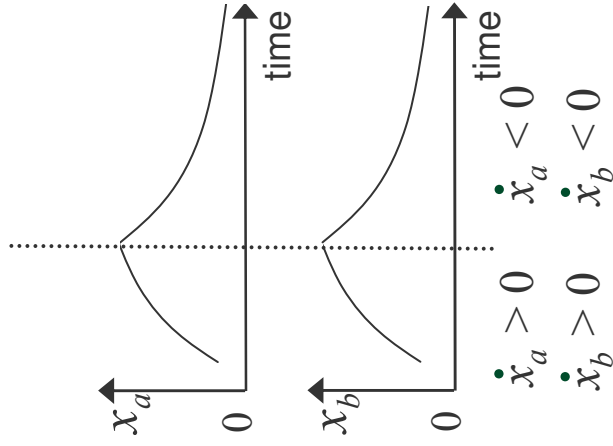

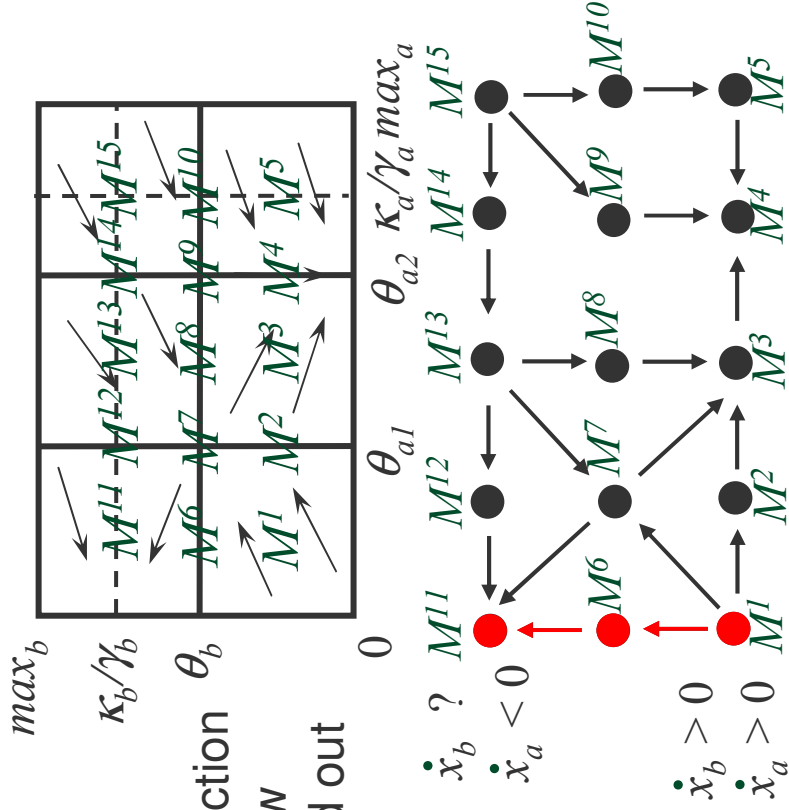$x_a > 0$ $\dot{x}_a < 0$

$\dot{x}_b > 0$ $\dot{x}_b < 0$
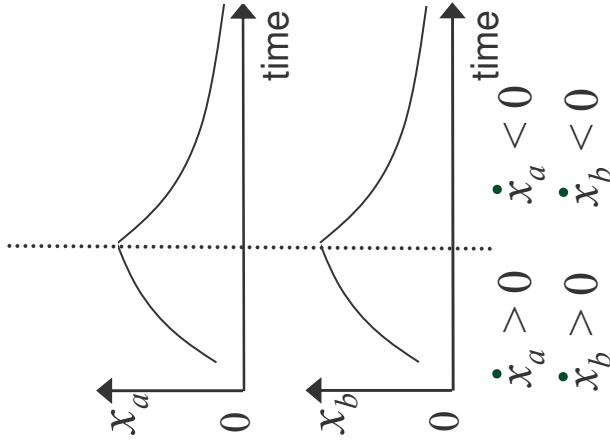
Problem for model validation

❖ **Model validation** using gene expression data: observation of changes in derivative signs

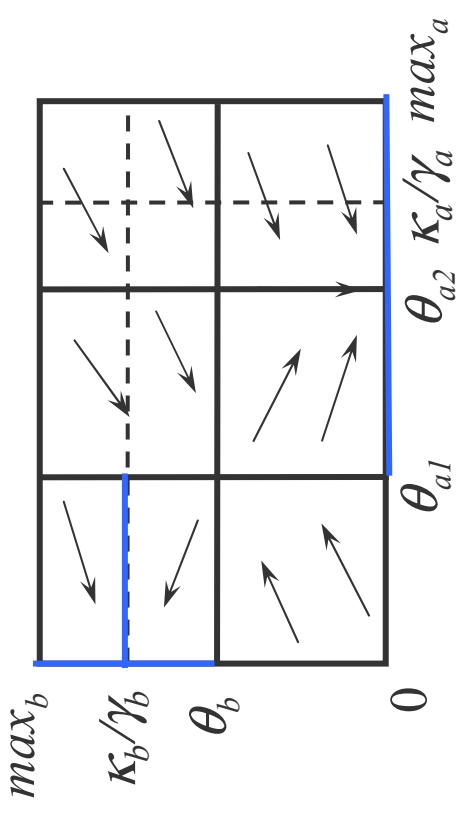Qualitative abstraction does not allow model to be ruled out
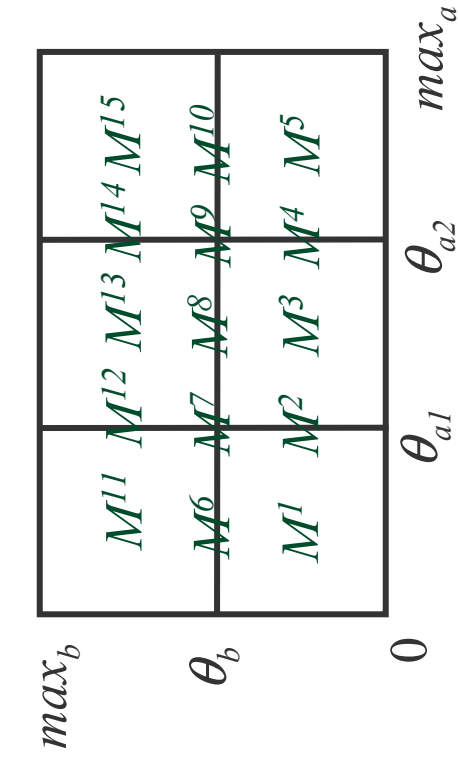
❖ Partition of phase space too coarse: need for finer-grained partition with unique derivative sign pattern in each domain

Refinement of phase-space partition

❖ Refinement of partition of phase space: from **mode domains** to **flow domains**
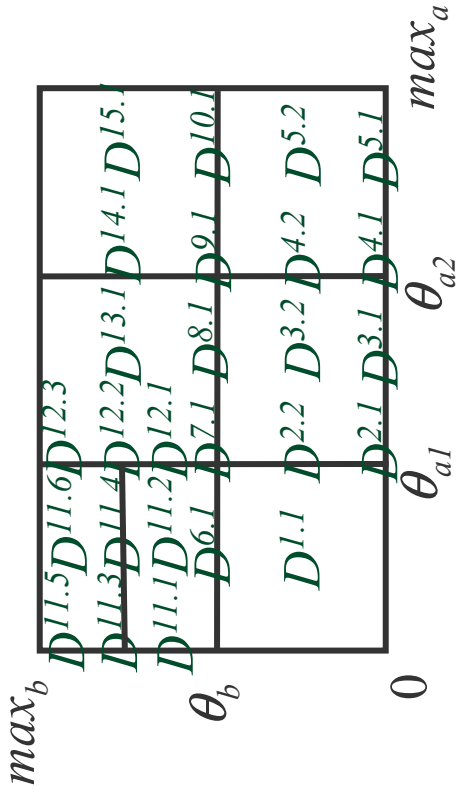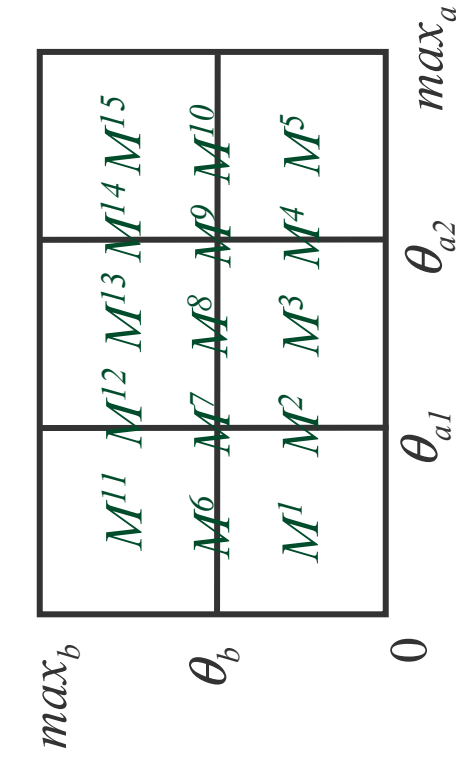
Repartitioning of mode domains by means of nullcline planes

Refinement of phase-space partition

❖ Refinement of partition of phase space: from **mode domains** to **flow domains**

Repartitioning of mode domains by means of nullcline planes

❖ New partition preserves derivative sign pattern and thus forms basis for more adequate qualitative abstraction

Unique derivative sign pattern

❖ PA differential equation generalized to differential inclusion in order to deal with discontinuities

$\dot{x} \in H(x),\ x \in \Omega;$ Ξ set of solutions of differential inclusion

Non-unicity of solutions of differential inclusion

❖ Derivative sign pattern at $x \in D$:

$$S(x) = \{\ \mathbf{sign}(\dot{\xi}(t_x))\ |\ \xi \in \Xi \text{ in } D,\ \xi(t_x) = x,\ \dot{\xi}(t_x) \in H(\xi(t_x))\ \}$$

❖ Unique sign pattern in every flow domain: $\forall x, x' \in D,\ S(x) = S(x')$
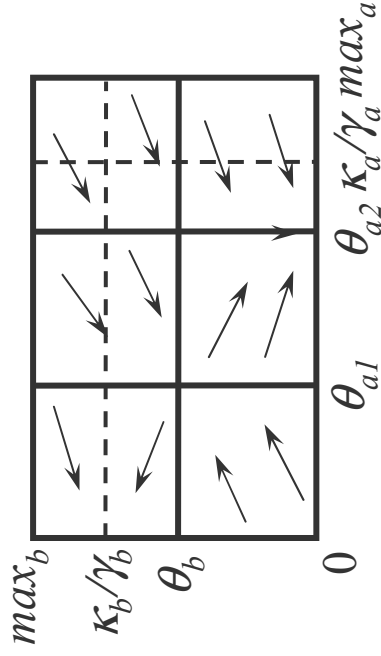
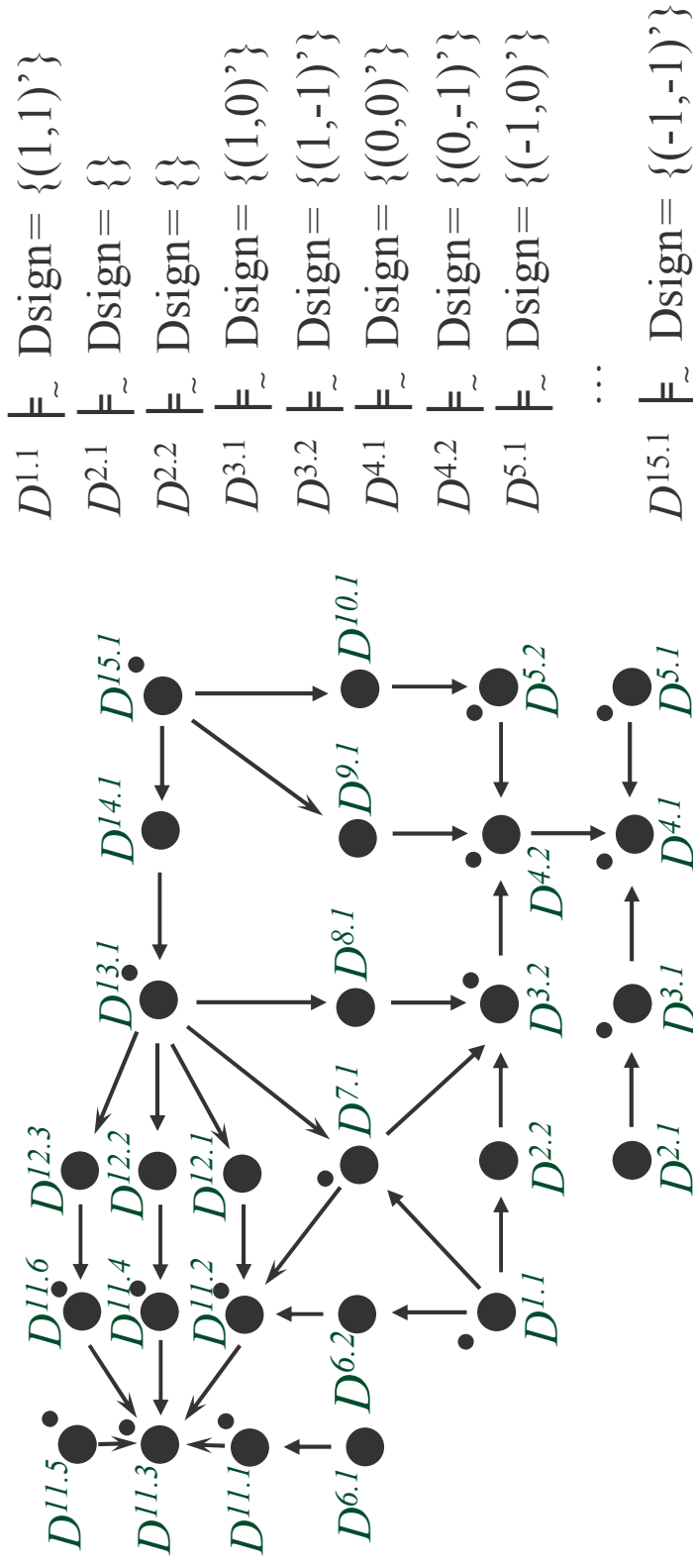$S(x) = \{(-1,1)'\},\ x \in D^{11.2}$

$S(x) = \{(-1,0)'\},\ x \in D^{11.4}$

Qualitative abstraction

❖ Continuous PA transition system $\Sigma\text{-}TS = (\Omega, \rightarrow, \models)$, where $\models$ associates to x the derivative sign pattern of solutions at x

❖ Equivalence relation $\sim$ induced by refined partition into flow domains

❖ Discrete or qualitative PA transition system $\Sigma\text{-}QTS = (\mathcal{D}, \rightarrow_\sim, \models_\sim)$, defined as quotient transition system of $\Sigma\text{-}TS$ given $\sim$

Alur et al., Proc. IEEE, 2000

❖ $\Sigma\text{-}QTS$ is a **simulation** of $\Sigma\text{-}TS$: conservative approximation

❖ $\Sigma\text{-}QTS$ provides qualitative description of the dynamics in phase space well-adapted to model validation

Changes in derivative sign pattern over time

Qualitative abstraction

$$D^{1.1} \models_\sim \text{Dsign} = \{(1,1)'\}$$
$$D^{2.1} \models_\sim \text{Dsign} = \{\}$$
$$D^{2.2} \models_\sim \text{Dsign} = \{\}$$
$$D^{3.1} \models_\sim \text{Dsign} = \{(1,0)'\}$$
$$D^{3.2} \models_\sim \text{Dsign} = \{(1,-1)'\}$$
$$D^{4.1} \models_\sim \text{Dsign} = \{(0,0)'\}$$
$$D^{4.2} \models_\sim \text{Dsign} = \{(0,-1)'\}$$
$$D^{5.1} \models_\sim \text{Dsign} = \{(-1,0)'\}$$
$$\cdots$$
$$D^{15.1} \models_\sim \text{Dsign} = \{(-1,-1)'\}$$

Qualitative abstraction
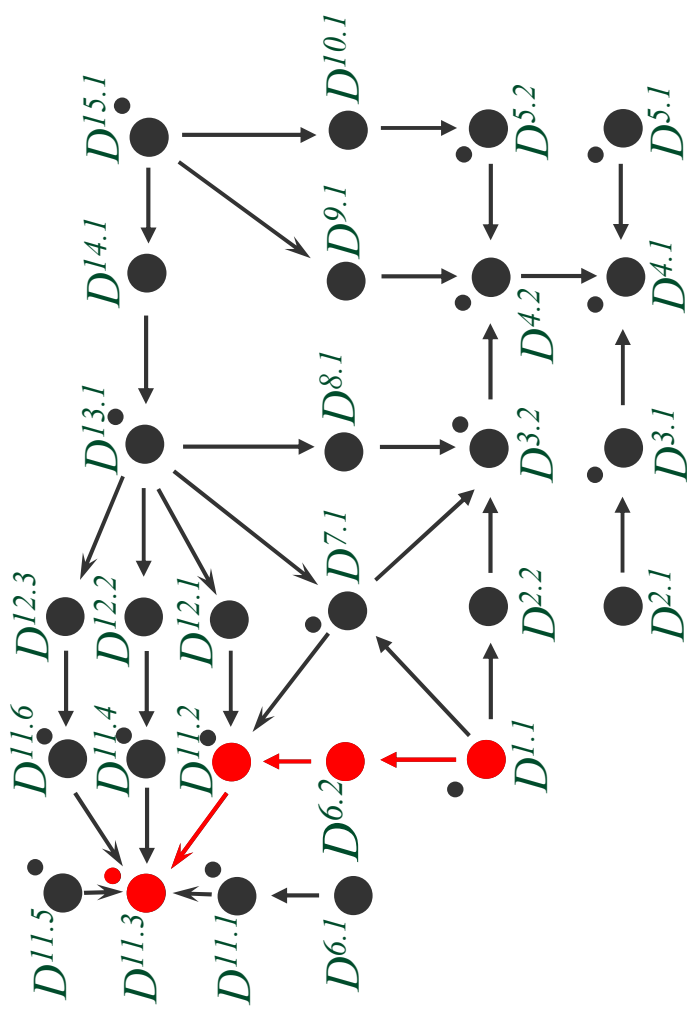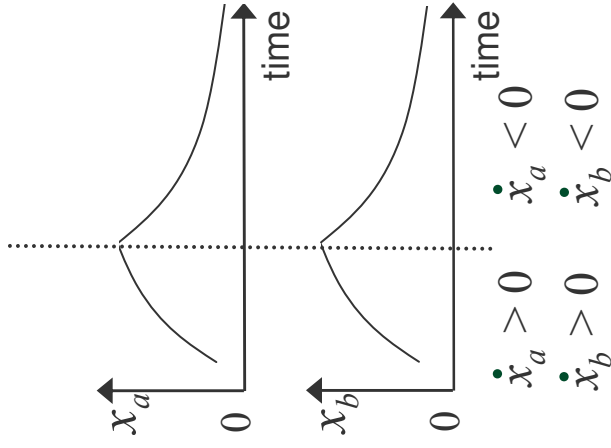
$D^{1.1} \models_\sim \text{Dsign}= \{(1,1)^?\}; \quad D^{6.1} \models_\sim \text{Dsign}= \{\}$

$D^{11.2} \models_\sim \text{Dsign}= \{(-1,1)^?\}; \quad D^{11.3} \models_\sim \text{Dsign}= \{(0,0)^?\}$

❖ Using fined-grained partition, the model can be safely ruled out

Genetic Network Analyzer (GNA)

❖ Tailored algorithms for symbolic computation of Σ-QTS implemented in new version of GNA

❖ Export functionalities to model checkers (NuSMV, CADP)
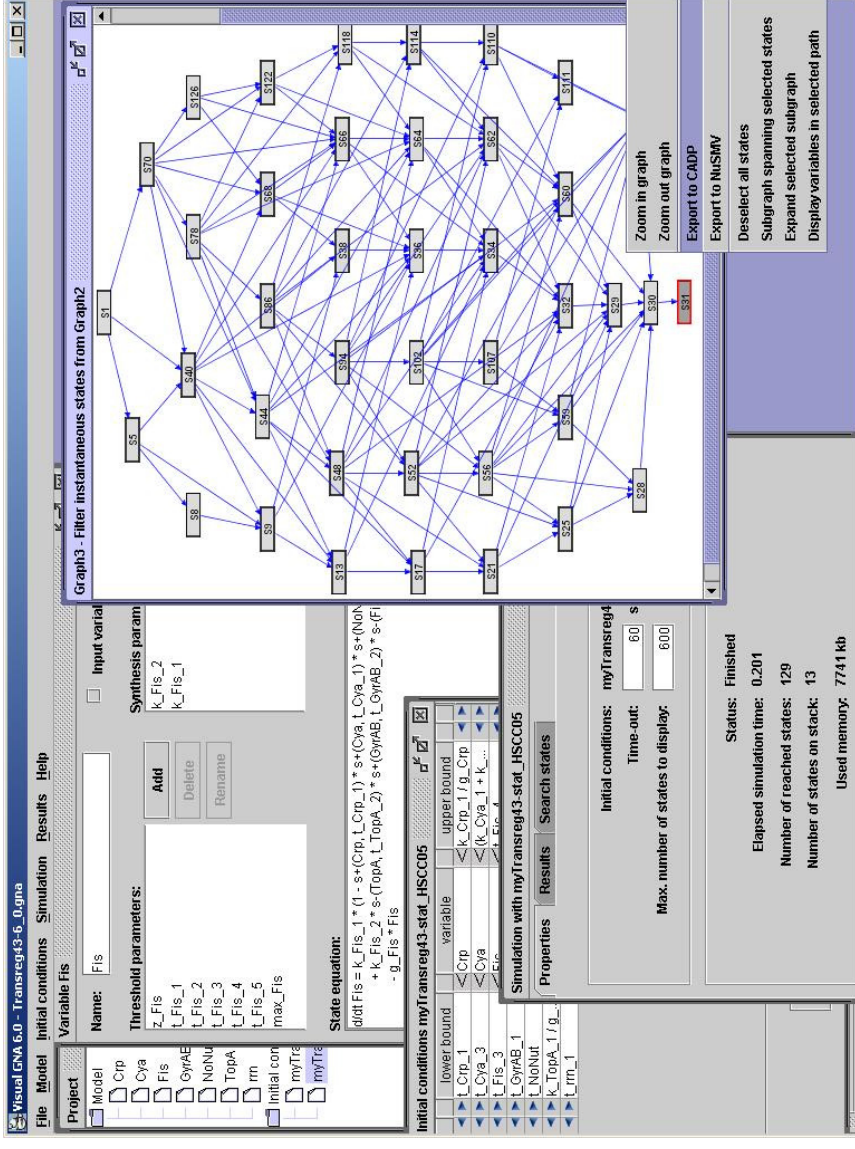
Nutritional stress response in *E. coli*

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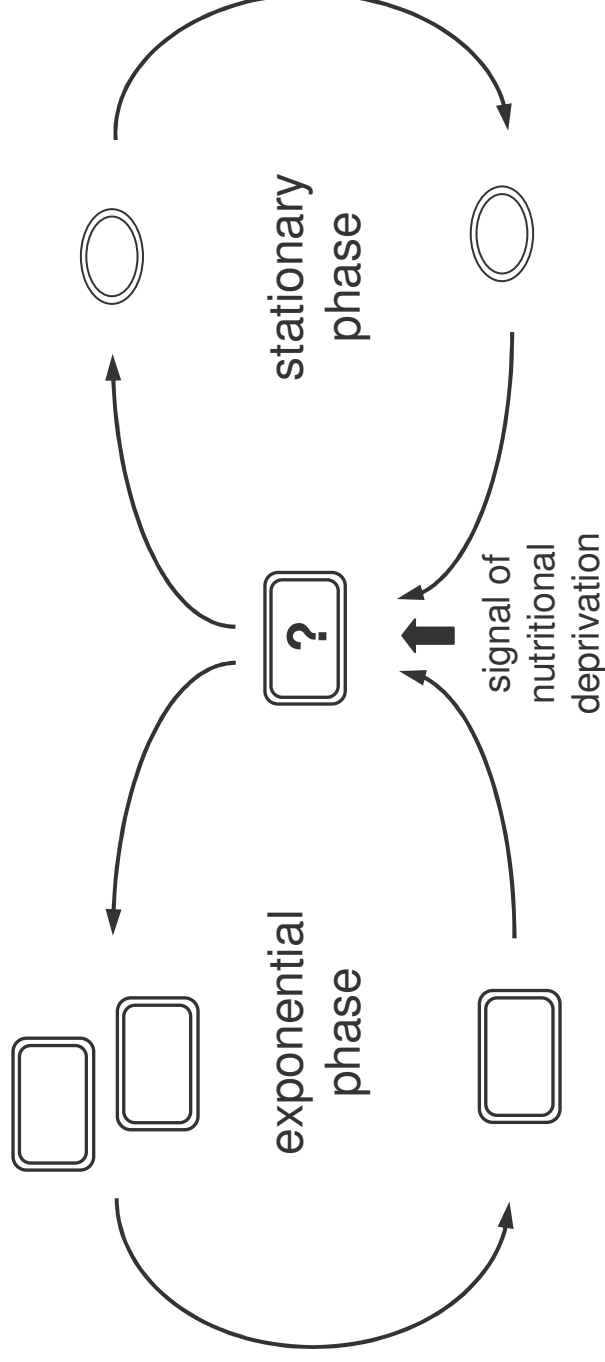
Nutritional stress response in *E. coli*

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

Ropers *et al.*, 2005

❖ Decision to abandon or continue growth is controlled by complex genetic regulatory network

Results of reachability analysis

❖ PA model of nutritional stress response network

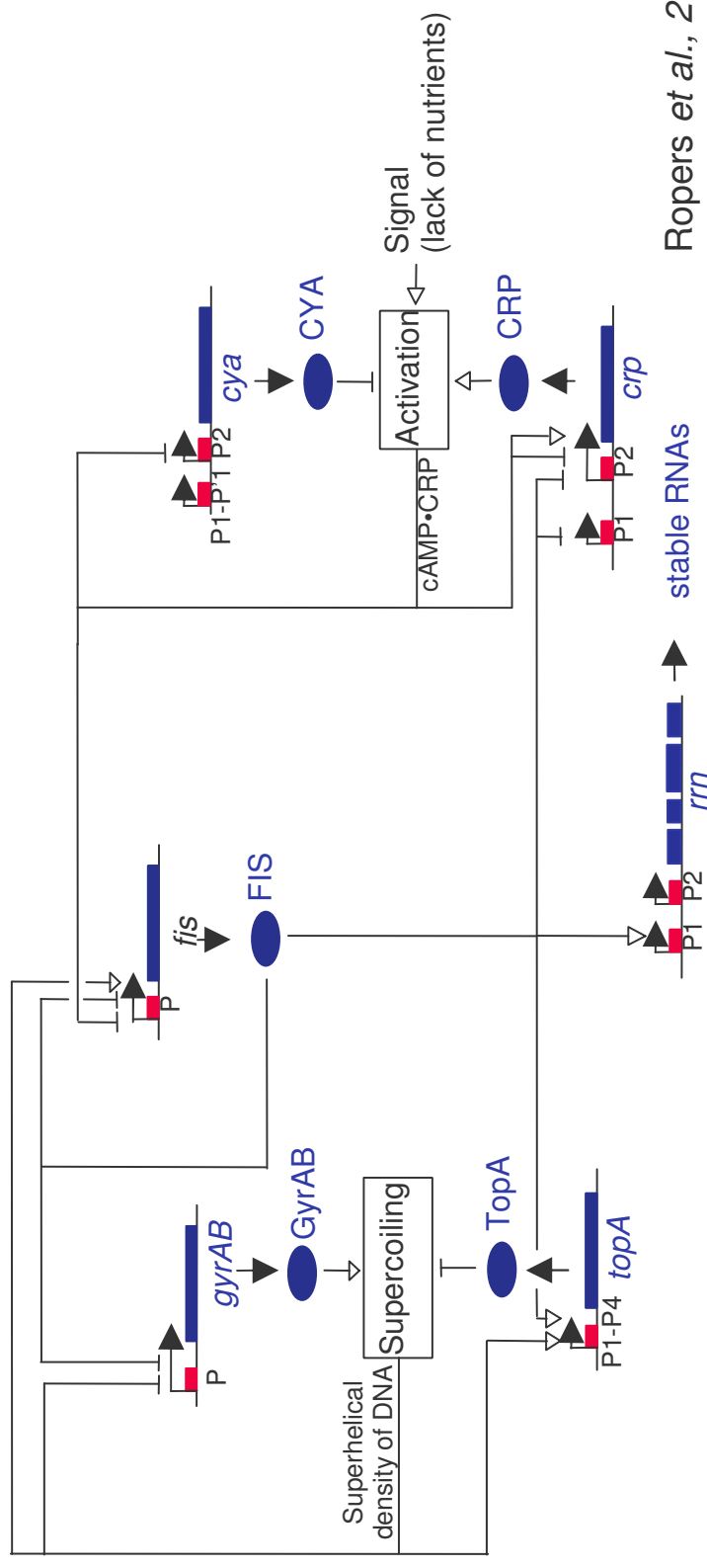 7 PA differential equations, 40 parameters and 54 inequality constraints

❖ Analysis of entry into stationary phase (nutrient deprivation)

  • 712 reachable domains, < 1 s. on average PC

  • single attractor domain (asymptotically stable equilibrium point)

  • mostly consistent with available experimental data on temporal evolution
    of protein concentrations

❖ Analysis of re-entry into exponential phase (nutrient upshift) has
  given rise to unexpected predictions: occurrence of oscillations
  in some protein concentrations

  Predictions currently tested in laboratory

Utility of refined method
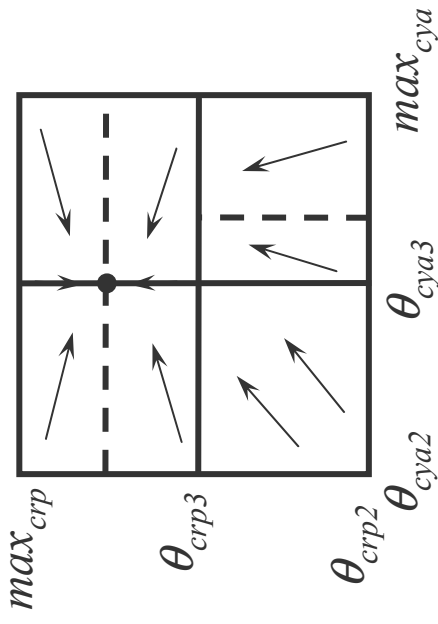
❖ Fine-grained analysis of reachability properties

Utility of refined method

❖ Fine-grained analysis of reachability properties

max_{crp}

θ_{crp3}

θ_{crp2} $\quad \theta_{cya2}$ $\qquad \theta_{cya3}$ $\qquad max_{cya}$

M^{30} $\quad M^{29}$

M^{32}

M^{61}

$\dot{x}_{crp} > 0$ $\qquad$ $\dot{x}_{crp}$? $\qquad$ $\dot{x}_{crp}$? $\qquad$ $\dot{x}_{crp}$?

M^{61} $\longrightarrow$ M^{32} $\longrightarrow$ M^{29} $\longrightarrow$ M^{30}

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Utility of refined method

❖ Fine-grained analysis of reachability properties

max_{crp}
θ_{crp3}
θ_{crp2} θ_{cya2}
θ_{cya3}
max_{cya}

$D^{30.2}$ $D^{30.1}$ $D^{29.1}$ $D^{32.1}$ $D^{61.1}$

$\dot{x}_{crp} > 0$ $\dot{x}_{crp} > 0$ $\dot{x}_{crp} > 0$ $\dot{x}_{crp} = 0$

$D^{61.1} \rightarrow D^{32.1} \rightarrow D^{29.1} \rightarrow D^{30.1} \rightarrow D^{30.2}$

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Utility of refined method

❖ Fine-grained analysis of reachability properties

$$\dot{x}_{crp}>0 \quad \dot{x}_{crp}>0 \quad \dot{x}_{crp}>0 \quad \dot{x}_{crp}=0$$

$$D^{61.1} \longrightarrow D^{32.1} \longrightarrow D^{29.1} \longrightarrow D^{30.1} \longrightarrow D^{30.2}$$

max_{crp}, θ_{crp3}, θ_{crp2}, θ_{cya2}, θ_{cya3}, max_{cya}

$D^{30.2}$, $D^{30.1}$, $D^{29.1}$, $D^{32.1}$, $D^{61.1}$

❖ Refined partition leads to moderate increase of number of reachable domains

Analysis of models of initiation of sporulation in *B. subtilis* (9 variables)
de Jong et al., *Bull. Math. Biol.*, 2004

B. subtilis strain	coarse grained	fine grained	ratios
wild type	1363 dom. (20s)	7752 dom. (124s)	**5.7**
ΔSigH	255 dom. (3.2s)	564 dom. (4.5s)	**2.2**
ΔSinI	675 dom. (5.3s)	700 dom. (3.8s)	**1.0**
ΔSpo0A	18 dom. (0.2s)	55 dom. (0.4s)	**3.1**

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Conclusions

❖ Method for the qualitative analysis and verification of hybrid models of genetic regulatory networks

- Method deals with discontinuities in PA models

- Method based on discrete abstraction preserving sign of derivatives

- Method applied to analysis of nutritional stress response in *E. coli*

❖ Trade-off between precision and upscalability

- Restricted vs more general classes of models

- Approximate vs exact reachability analysis

- Tailored vs general-purpose tools for reachability analysis

❖ Analysis of large models by model checking

❖ Thanks for your attention!