



Cells as Machines: Towards Deciphering Biochemical Programs in the Cell (keynote talk)

François Fages

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Cells as Machines: towards deciphering biochemical programs in the cell



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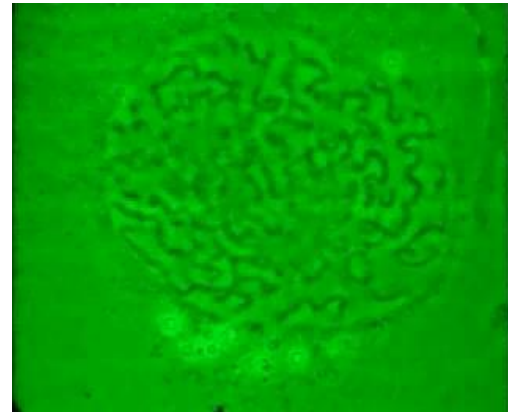
To tackle the complexity of biochemical reaction systems, investigate:

- Programming theory concepts
- Formal methods of circuit and program verification
- Artificial Intelligence techniques

Implemented in BIOCHAM v3.5 (Biochemical Abstract Machine)

Systems Biology Challenge

Gain system-level understanding of multi-scale biological processes in terms of their elementary interactions at the molecular level. [Kitano 1999]

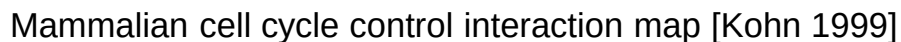
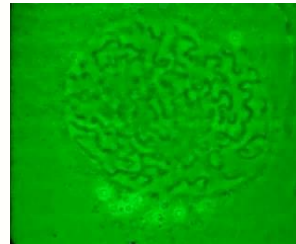


Cell mitosis videos [Lodish et al. 03]

Follow-up of Human Genome Project (90s)

- Beyond genomic data: [protein-protein interaction](#) databases, RNAs, ...
- [Model repositories of cell processes](#) : e.g. [biomodels.net](#) 1000 models
- Systems Biology Markup Language (SBML): model exchange format
- Modeling environments (Cell designer, Cytoscape, Copasi, [BIOCHAM](#),...)
- Simulation of a whole-cell *mycoplasma genitalium* [Karr Covert et al 12]

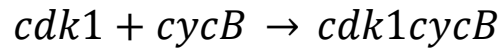
- 1) Models for representing knowledge : the more detailed the better
- 2) Models for answering questions : the more abstract the better



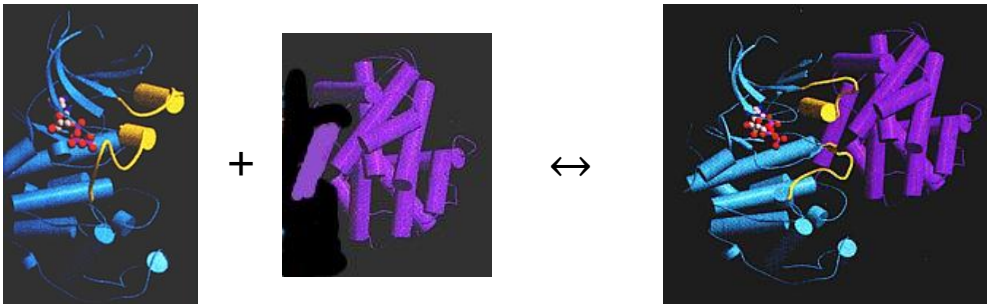
Simplified yeast cell cycle model [Tyson 1991]

Biochemical reactions

- Binding, complexation:

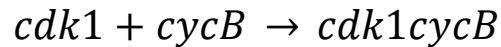


- Unbinding, decomplexation:



Biochemical reactions

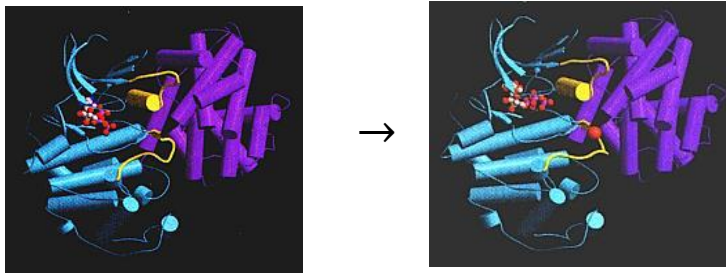
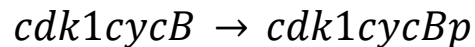
- Binding, complexation:



- Unbinding, decomplexation:

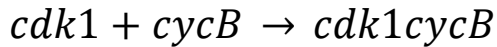


- Transformation, phosphorylation, transport: $A \rightarrow B$ $(A + K \rightarrow C \rightarrow B + K)$



Biochemical reactions

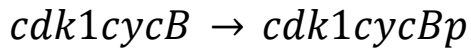
- $$A + B \rightarrow C$$



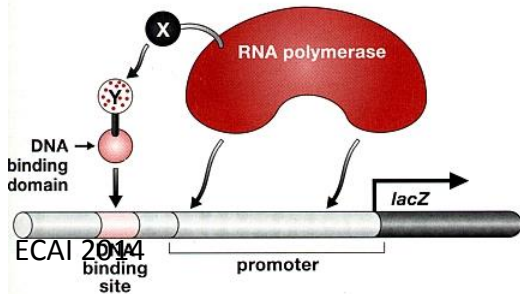
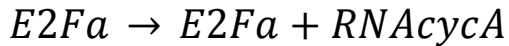
- $$A \rightarrow B + C$$



- $$A \rightarrow B \quad (A + K \rightarrow C \rightarrow B + K)$$



- $$A \rightarrow A + B$$



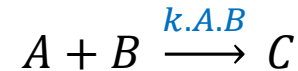
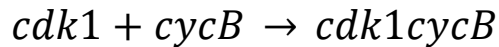
Biochemical reactions

- Binding, complexation: $A + B \rightarrow C$
 $cdk1 + cycB \rightarrow cdk1cycB$
- Unbinding, decomplexation: $A \rightarrow B + C$
- Transformation, phosphorylation, transport: $A \rightarrow B$ $(A + K \rightarrow C \rightarrow B + K)$
 $cdk1cycB \rightarrow cdk1cycBp$
- Gene expression, synthesis: $A \rightarrow A + B$
 $E2Fa \rightarrow E2Fa + RNAcycA$
- Degradation: $A \rightarrow _$

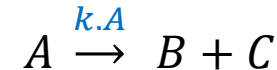
Biochemical reaction rates

Time matters

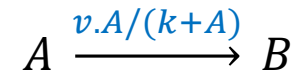
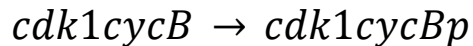
- Binding, complexation:



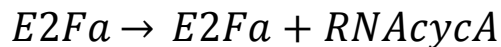
- Unbinding, decomplexation:



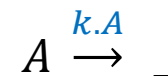
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- Gene expression, synthesis:



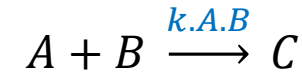
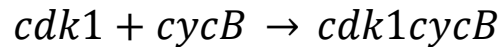
- Degradation:



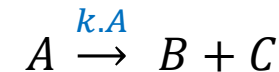
Biochemical reaction rates

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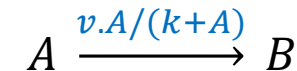
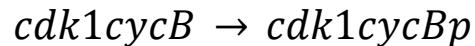
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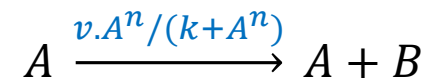
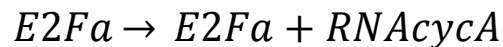
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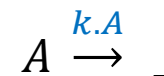
- Transformation, phosphorylation, transport:



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- Degradation:

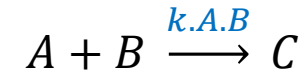
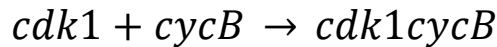


Mass action law kinetics

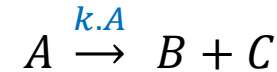
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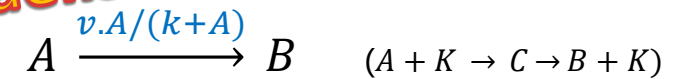
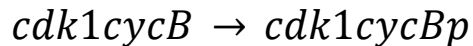
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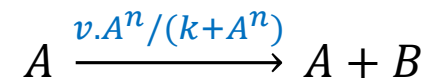
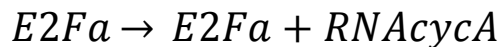
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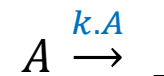
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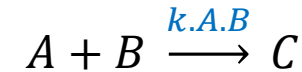
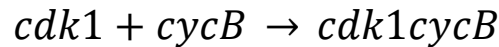
Mass action law kinetics

Michaelis Menten kinetics

Biochemical reaction rates

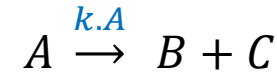
Time matters

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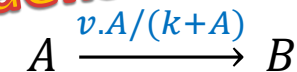
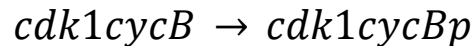
Mass action law kinetics

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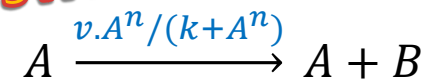
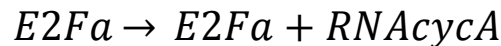
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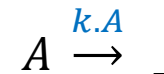


Hill sigmoid: switch like behavior

- Gene expression, synthesis:



- Degradation:



Semantics of Reactions $A+B \xrightarrow{f(A,B)} C$

Boolean Semantics: presence-absence of molecules $A \wedge B \rightarrow C \wedge A/\neg A \wedge B/\neg B$

Asynchronous Transition System

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Asynchronous Transition System

Petri Net Semantics: numbers of molecules

$A, B \rightarrow C++, A--, B--$

Multiset rewriting

CHAM [Berry Boudol 90] [Banatre Le Metayer 86]

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Stochastic Semantics: reaction probabilities, time of next reaction in a given state S_i

Continuous Time Markov Chain (CTMC)

$A, B \xrightarrow{p(S_i), t(S_i)} C++, A--, B--$

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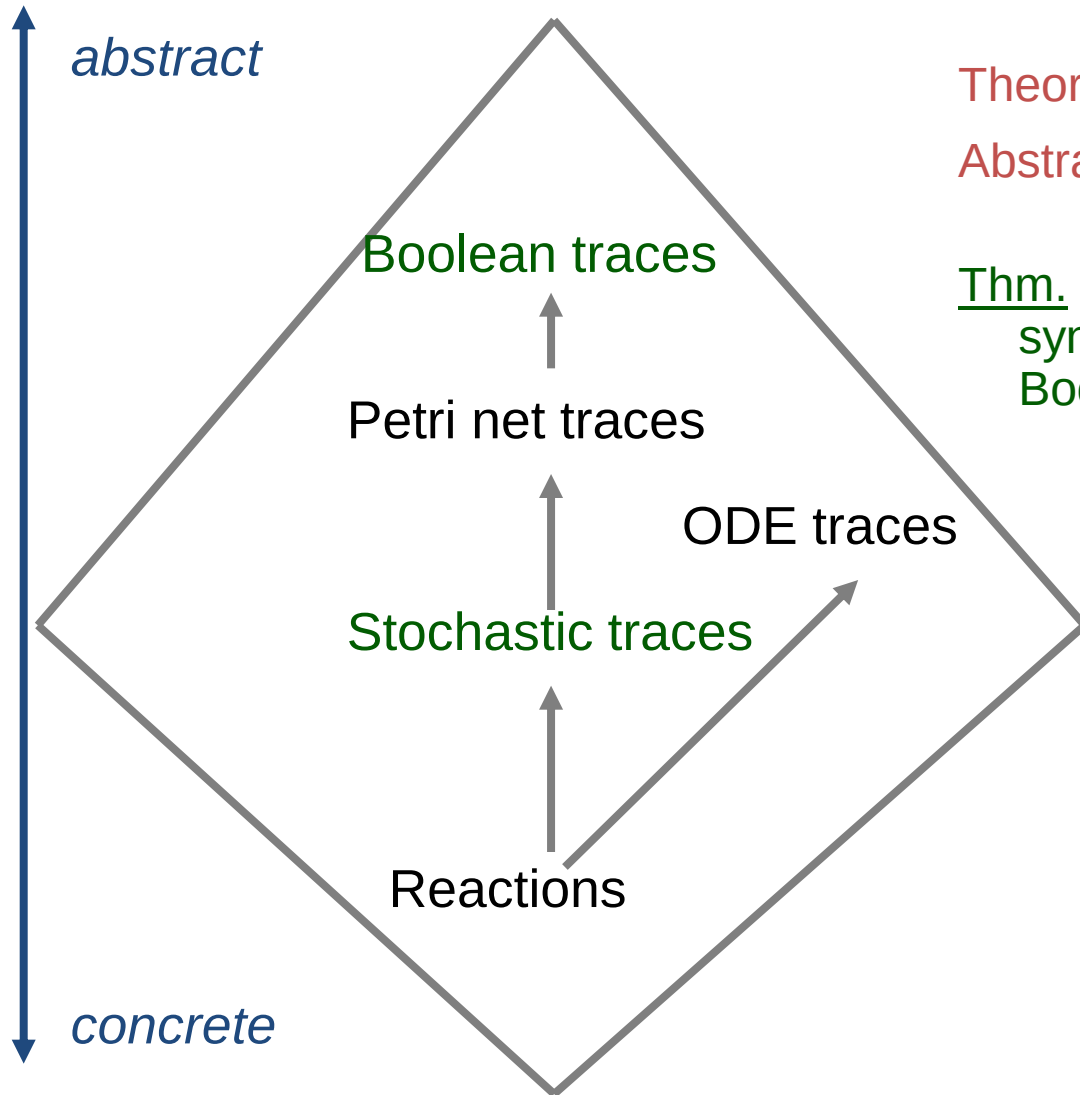
Real time

Continuous Semantics: concentrations, continuous evolution

Ordinary Differential Equations (ODE)

$$\dot{A}_i = \sum_{r=1}^n f_r \times \delta_r(A_i)$$

Abstraction Relationships



Theory of abstract Interpretation

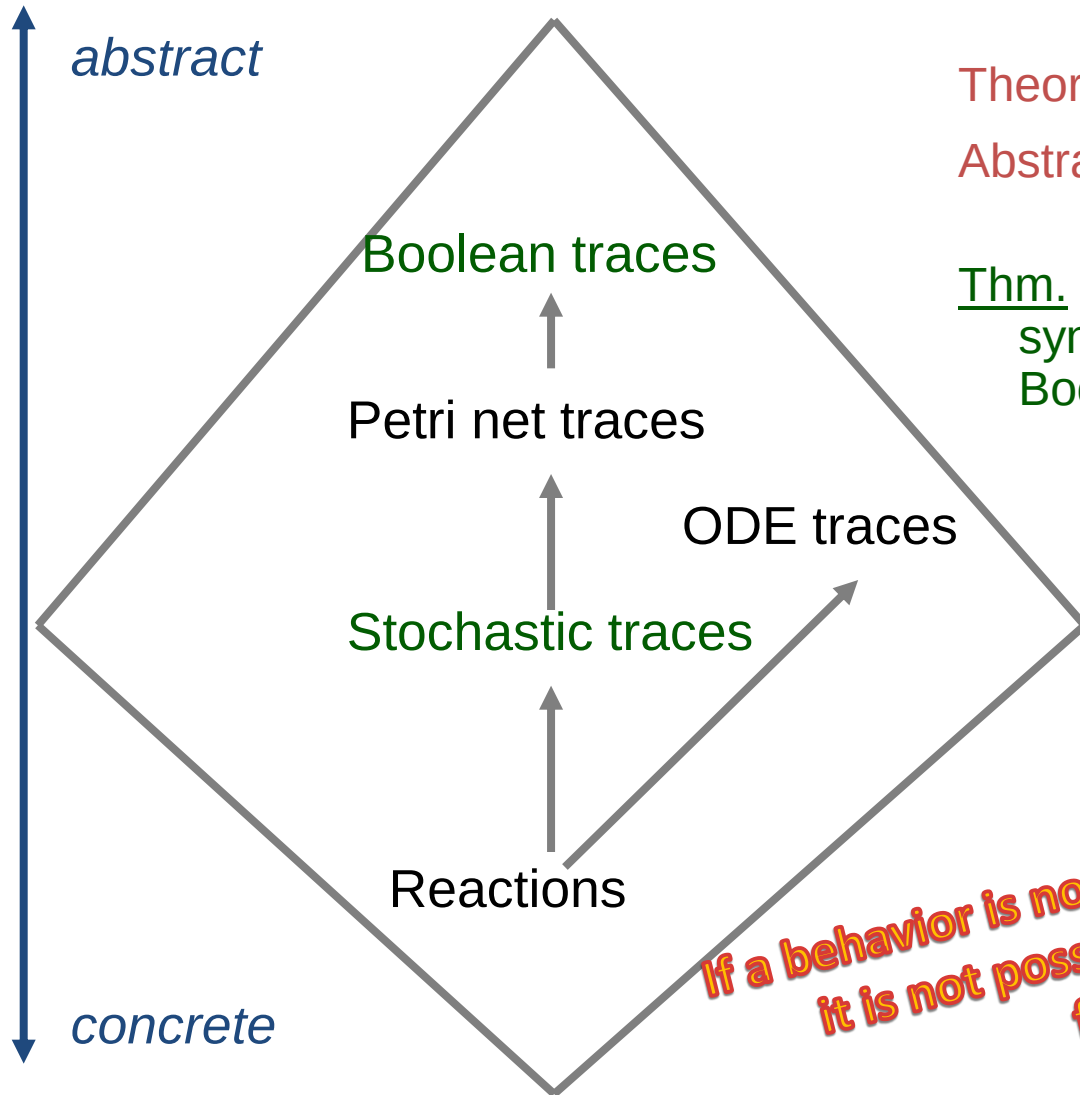
Abstractions as Galois connections

[Cousot Cousot POPL'77]

Thm. Galois connections between the
syntactical, stochastic, Petri Net and
Boolean semantics

[FF Soliman CMSB'06,TCS'08]

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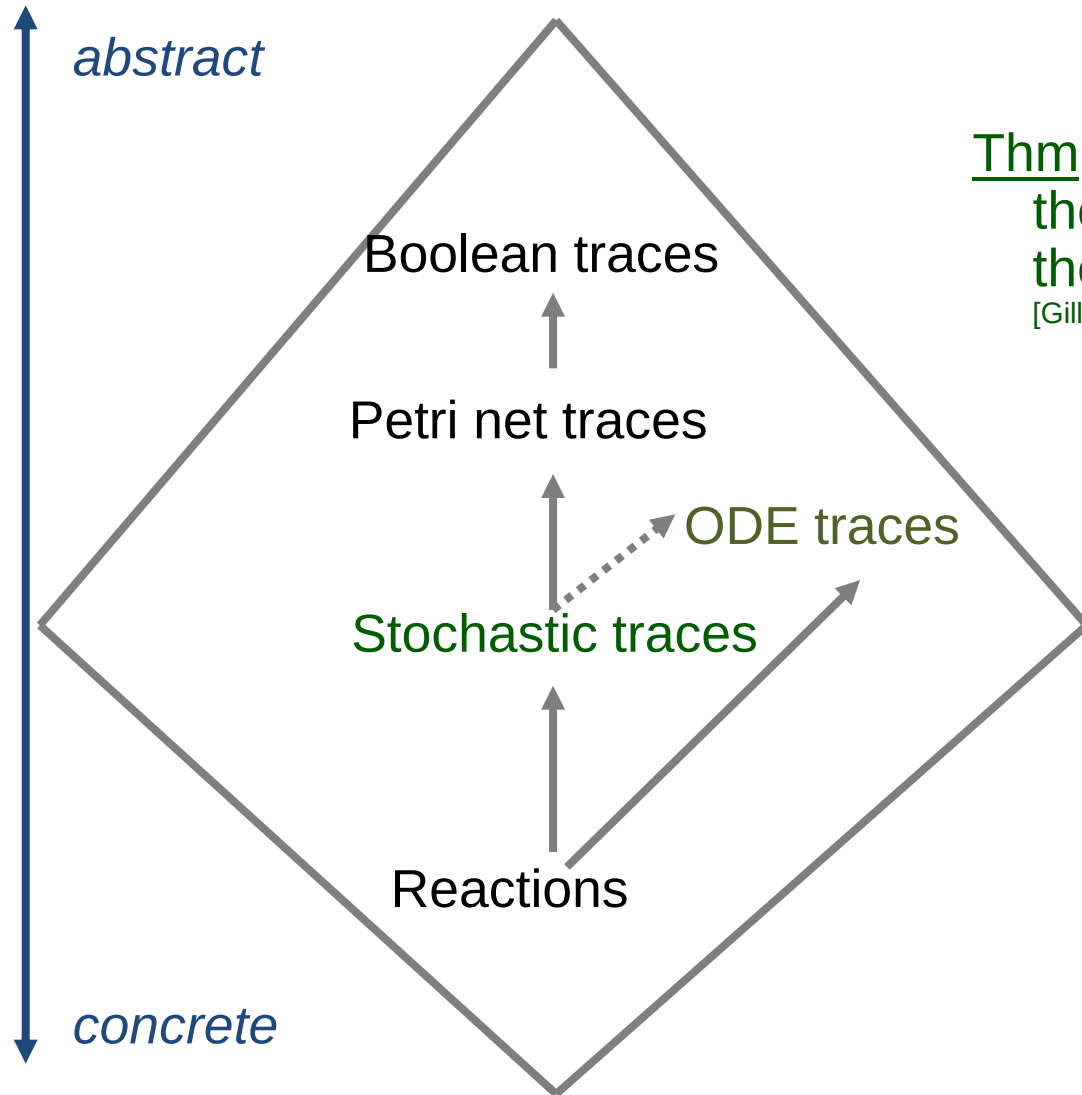
[Cousot Cousot POPL'77]

Thm. Galois connections between the
syntactical, stochastic, Petri Net and
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[FF Soliman CMSB'06,TCS'08]

**If a behavior is not possible in the Boolean semantics
it is not possible in the stochastic semantics
for any reaction rates**

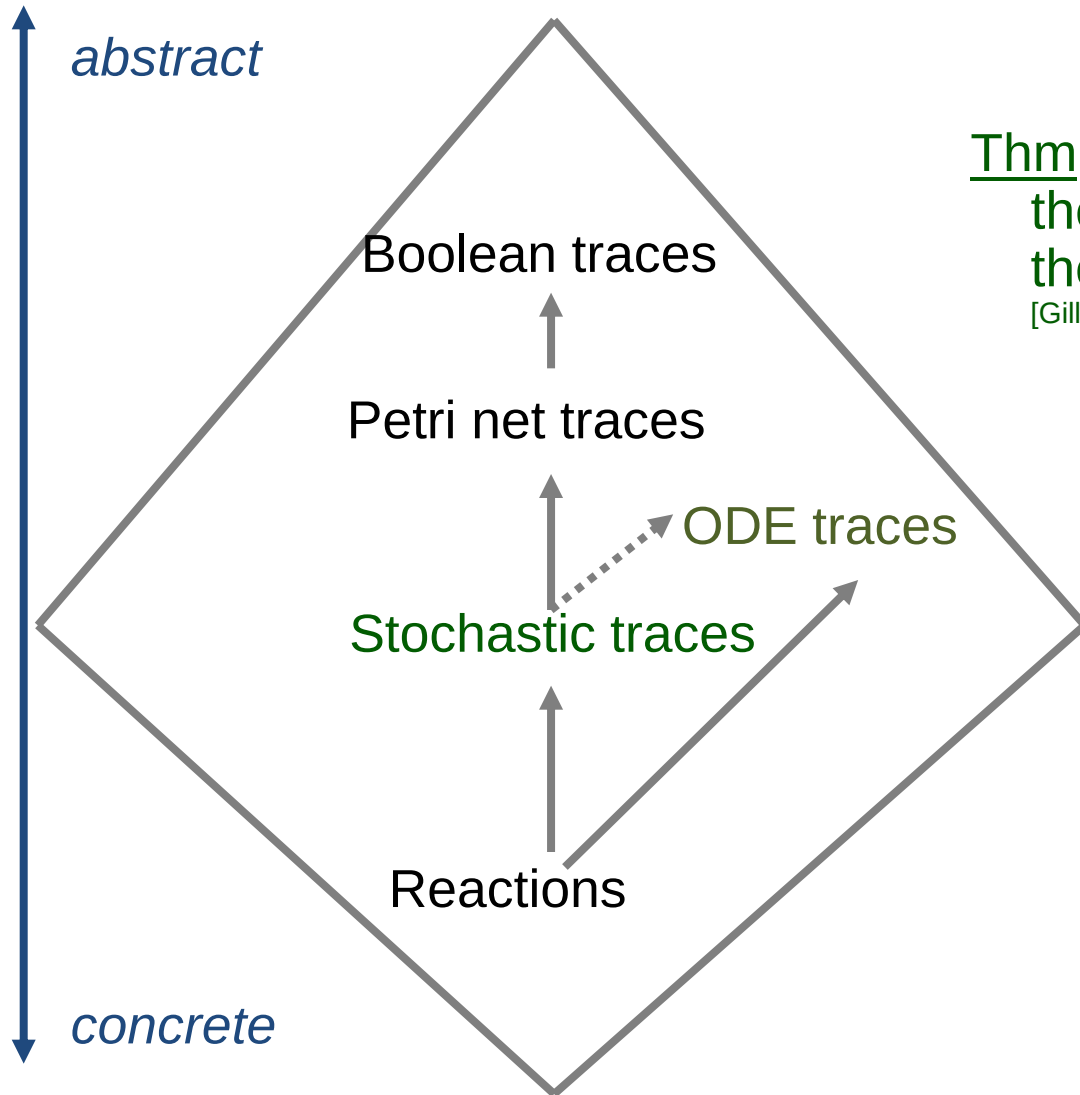
Abstraction Relationships



Thm. Under large number conditions
the ODE semantics approximates
the mean stochastic behavior

[Gillespie 71]

Abstraction Relationships

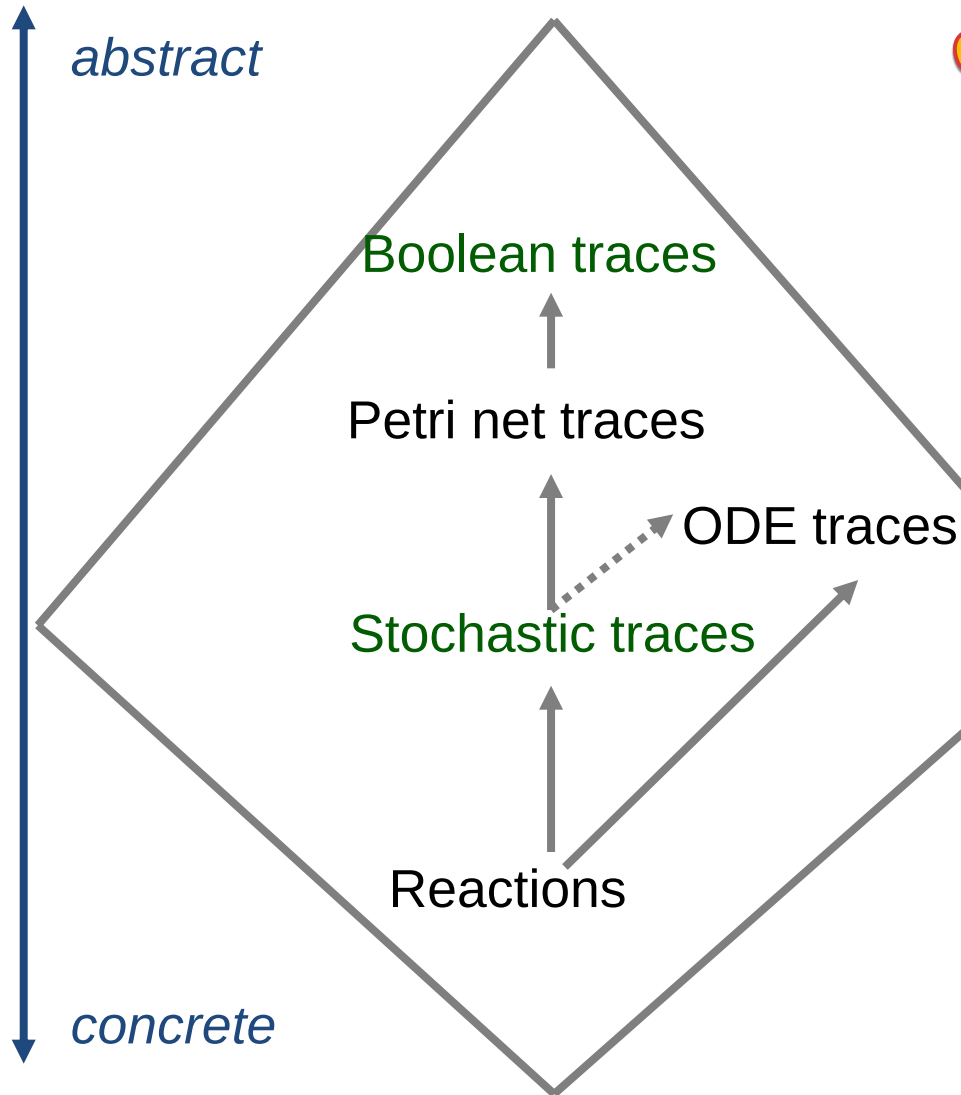


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[Gillespie 71]

Hot topic:
higher order moments
ODE for mean, variance,...

Hybrid Models

Crucial for model reduction and multi-scale models



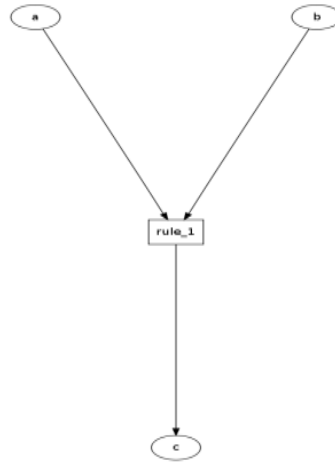
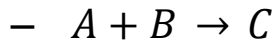
- Hybrid Boolean-continuous models (hybrid automata)
Boolean gene expression + continuous protein activation
- Hybrid stochastic-continuous models (CTMC+ODE)
Stochastic gene expression + continuous protein activation

Hybrid simulators for free with SBML's reactions+events

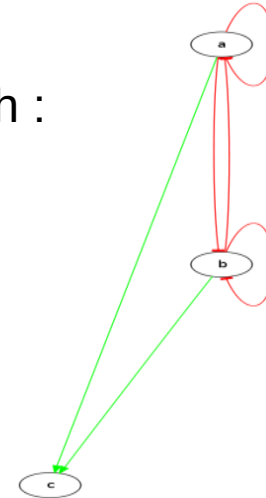
[Chiang FF Huang Soliman 13 cmsb]

Before going to simulations: structural analyses

- Reaction hypergraph :



- Influence graph :



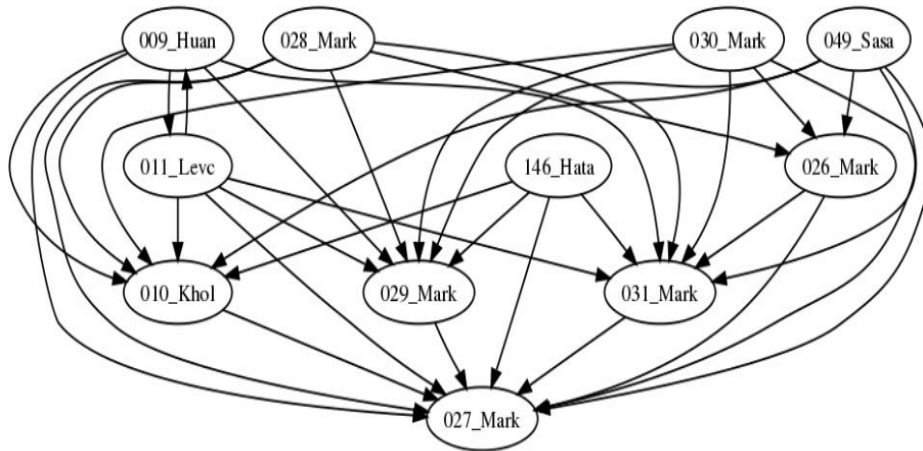
- Large independence of the influence graph from the kinetics [FF Soliman 08 fmsb]
- Existence of positive circuits in the influence graph
→ [necessary condition for multi-stationarity](#) (cell differentiation, homeostasis) in Boolean and ODE semantics [Soliman 13 bmb, Soule 03, Thomas 82]
- Existence of negative circuits in the influence graph
→ [necessary condition for oscillations](#) in ODE semantics [Snoussi 93, Thomas 82]

Structural analysis: reaction graph reduction

- Subgraph epimorphisms (SEPI) [Gay FF Martinez Soliman 13 dam]
 - Structural notion of model reduction by [deletion & merge of species & reactions](#) in reaction hypergraphs

Structural analysis: reaction graph reduction

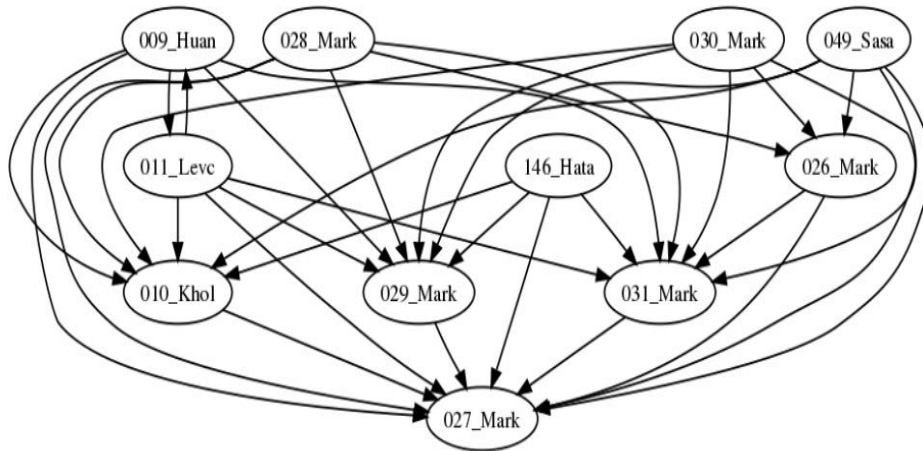
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- Sufficient to detect model reductions in biomodels.net
 - Reconstructed hierarchy of MAPK models in biomodels.net [Gay Soliman FF 10 bi]



Possibility to query model repositories by the structure of reaction networks

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Possibility to query model repositories by the structure of reaction networks

- The SEPI existence problem is NP-complete
- Quite efficient computation of SEPI by [CLP\(FD\) / SAT solvers](#) [Gay FF Santini Soliman 13 wcb]

Structural analysis: Petri net invariants

- P-invariants: linear invariants on the number of tokens in all reachable markings
 - linear conservation laws, elimination of variables in ODE

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The existence of a minimal siphon containing a given place is NP-complete

However, very efficient enumeration of all minimal siphons in all biomodels.net
by [CLP\(FD\)](#) and/or [SAT solvers](#) [Nabli Martinez FF Soliman 12 CP]

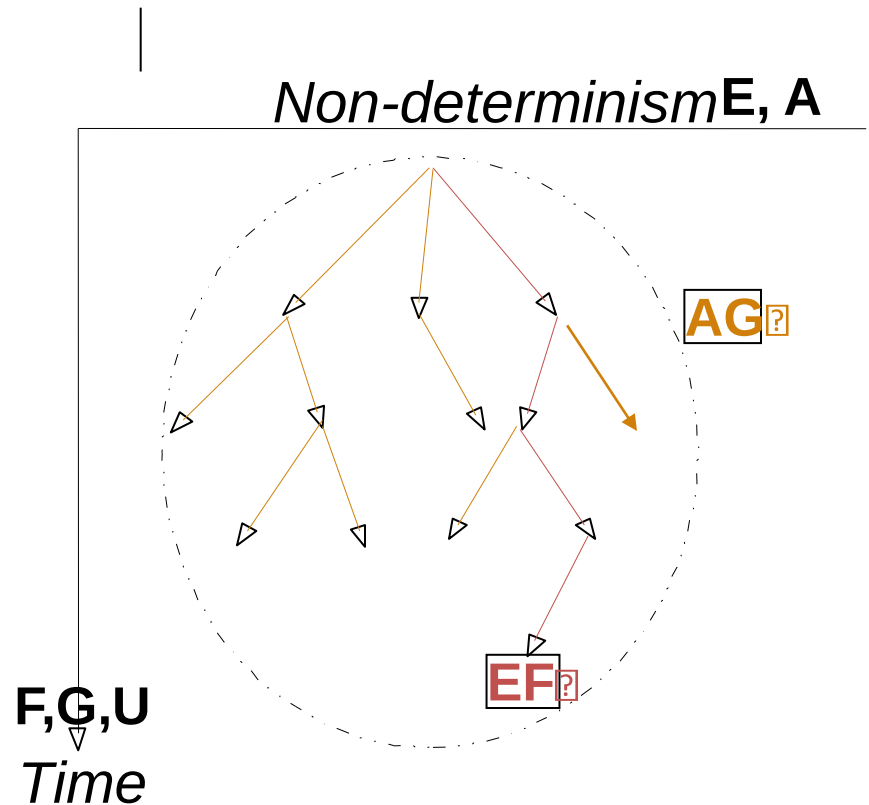
Structural analysis of dynamical behaviors

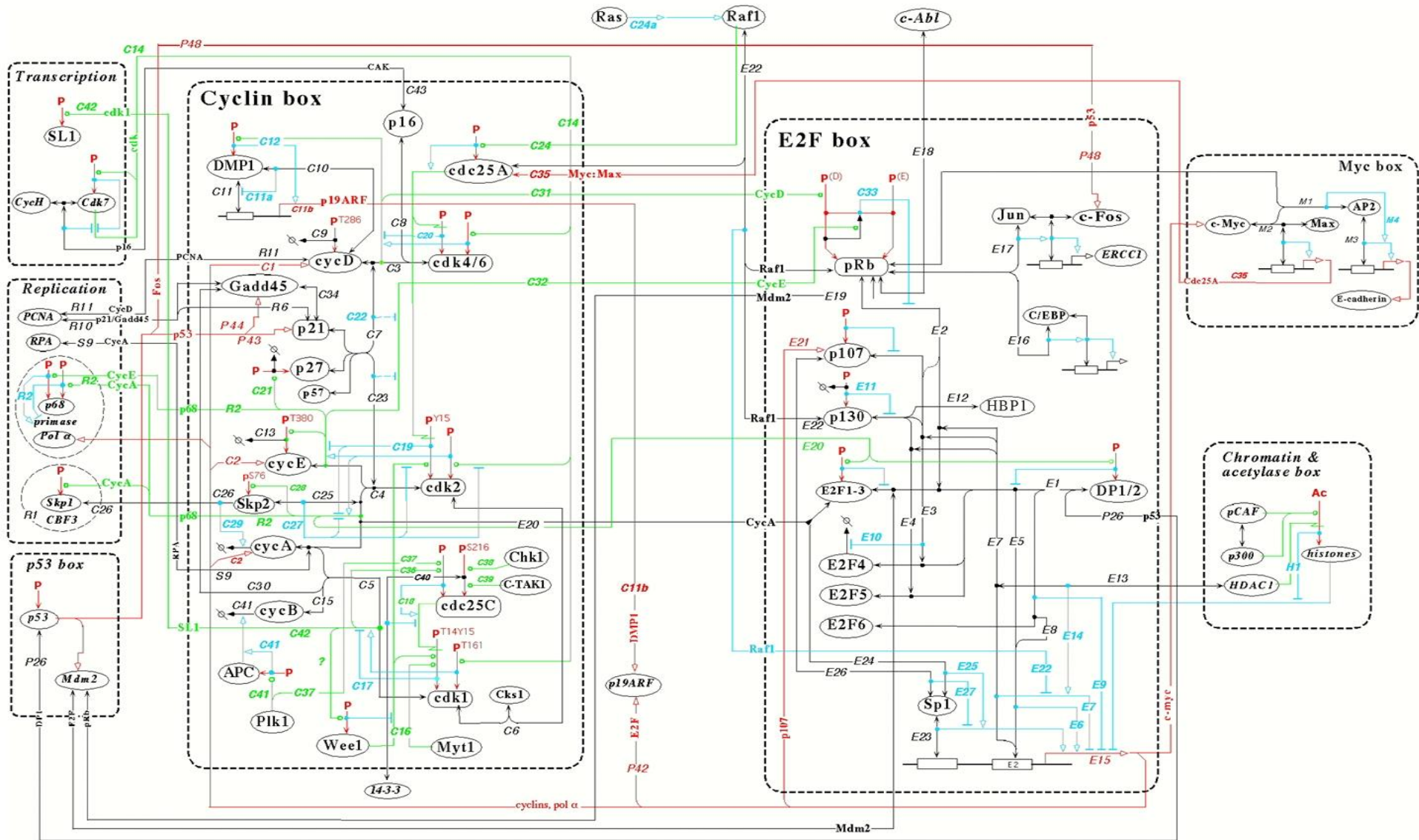
- Temporal logic FO patterns
 - Stability $\mathbf{G}\phi$
 - Reachability $\mathbf{F}\phi$, thresholds $\mathbf{F}([A]>0.1)$,
 - Peaks of concentration $\mathbf{F}([A]<V \wedge \mathbf{X}([A]=V \wedge \mathbf{X}([A]<V))$
 - Amplitude
 - Periods and phases as distance between peaks
 - ...
- More flexible than curve fitting, can deal with imprecise data
- Constraints for model validation/synthesis w.r.t. experimental observations
- Constraints for parameter search
 - Good fit $\rightarrow$ model-based predictions
 - No good fit $\rightarrow$ revisit structure $\rightarrow$ model-based contribution to biology

Boolean Semantics Queries

Computation Tree Logic CTL [Emerson Clarke 80]

Non-det. Time	E exists	A always
X next time	EX (φ)	AX (φ)
F finally	EF (φ) $\neg$ AG ($\neg \varphi$)	AF (φ) <i>liveness</i>
G globally	EG (φ) $\neg$ AF ($\neg \varphi$)	AG (φ) <i>safety</i>
U until	E (φ_1 U φ_2)	A (φ_1 U φ_2)



informatics mathematics
inria

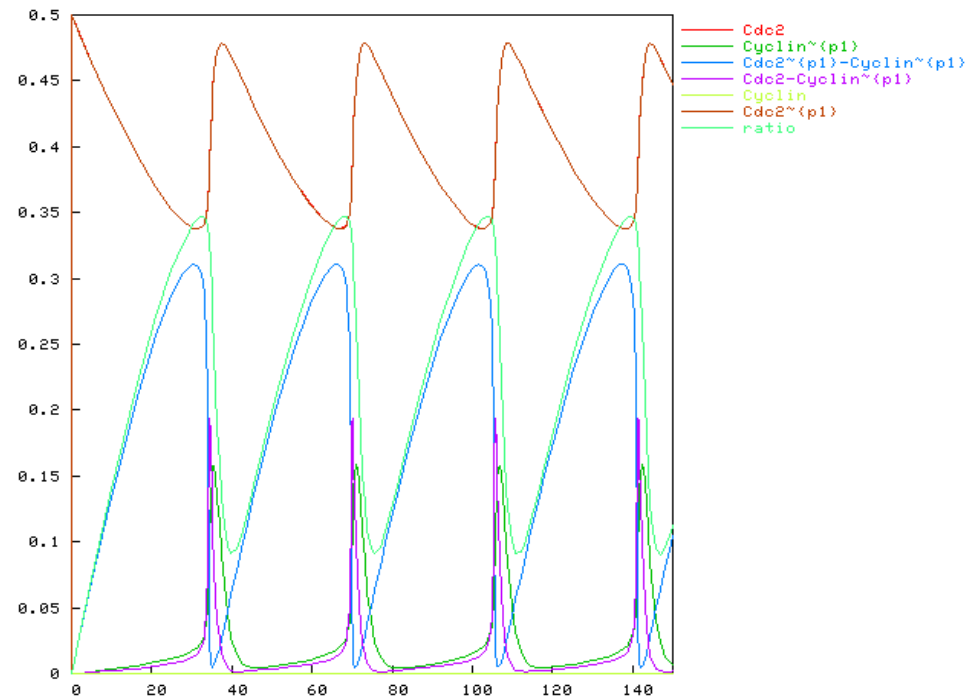
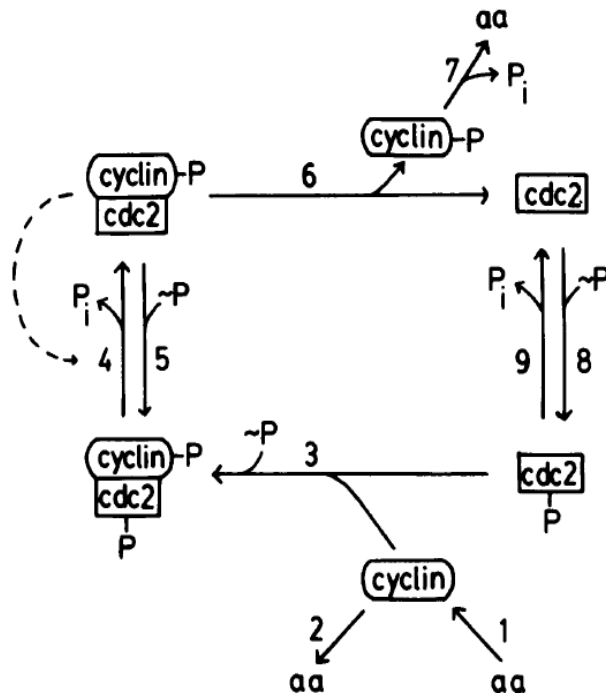
Kohn's Map Boolean Model-Checking

800 reactions, 165 proteins and genes, 500 variables, 2^{500} states.

Biocham NuSMV symbolic model-checker time in seconds [Chabrier et al. TCS 04]

Initial state G2	Query:	Time in sec.
	compiling	29
Reachability G1	EF CycE	2
Reachability G1	EF CycD	1.9
Reachability G1	EF PCNA-CycD	1.7
Checkpoint for mitosis complex	$\neg$ EF ($\neg$ Cdc25~{Nterm} U Cdk1~{Thr161}-CycB)	2.2
Oscillations CycA	EG ((EF $\neg$ CycA) & (EF CycA))	31.8
Oscillations CycB	EG ((EF $\neg$ CycB) & (EF CycB))	6

Dynamical Model of Cell Cycle Control [Tyson 91]



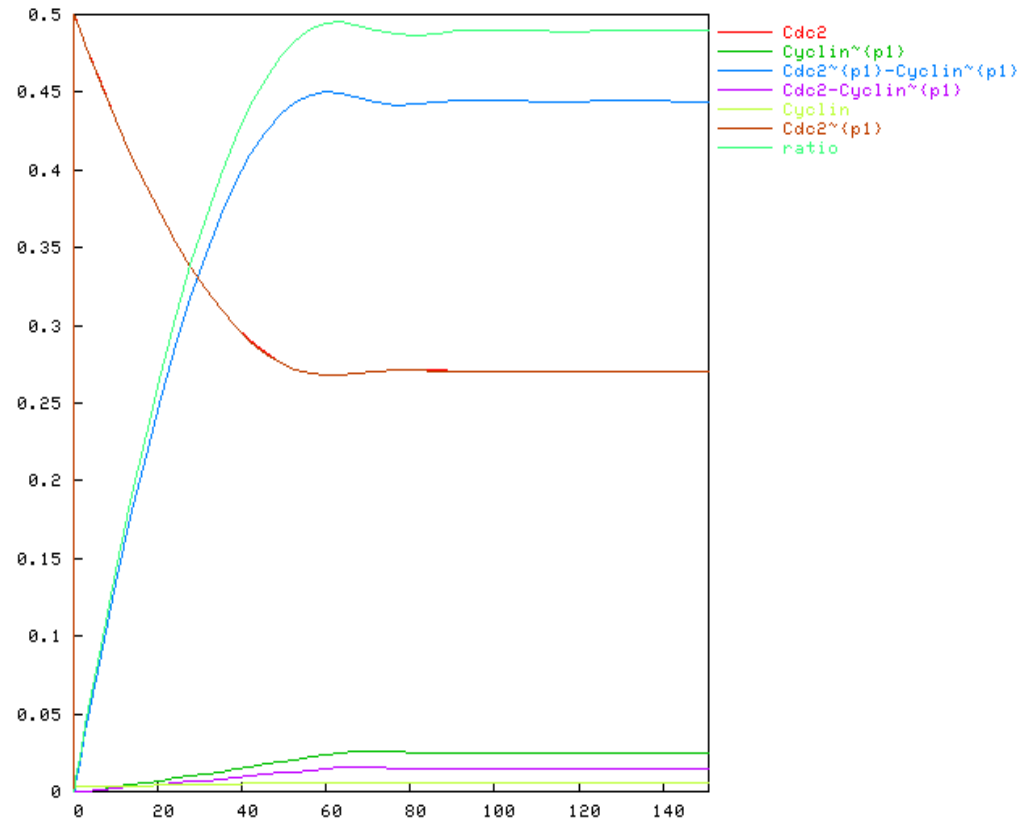
6 molecular species
8 reactions
8 kinetic parameters

ODE semantics

Parameter Search from LTL(R) Properties

biocham: search_parameter([k3,k4],[(0,200),(0,200)],20,
oscil(Cdc2-Cyclin~{p1},3),150).

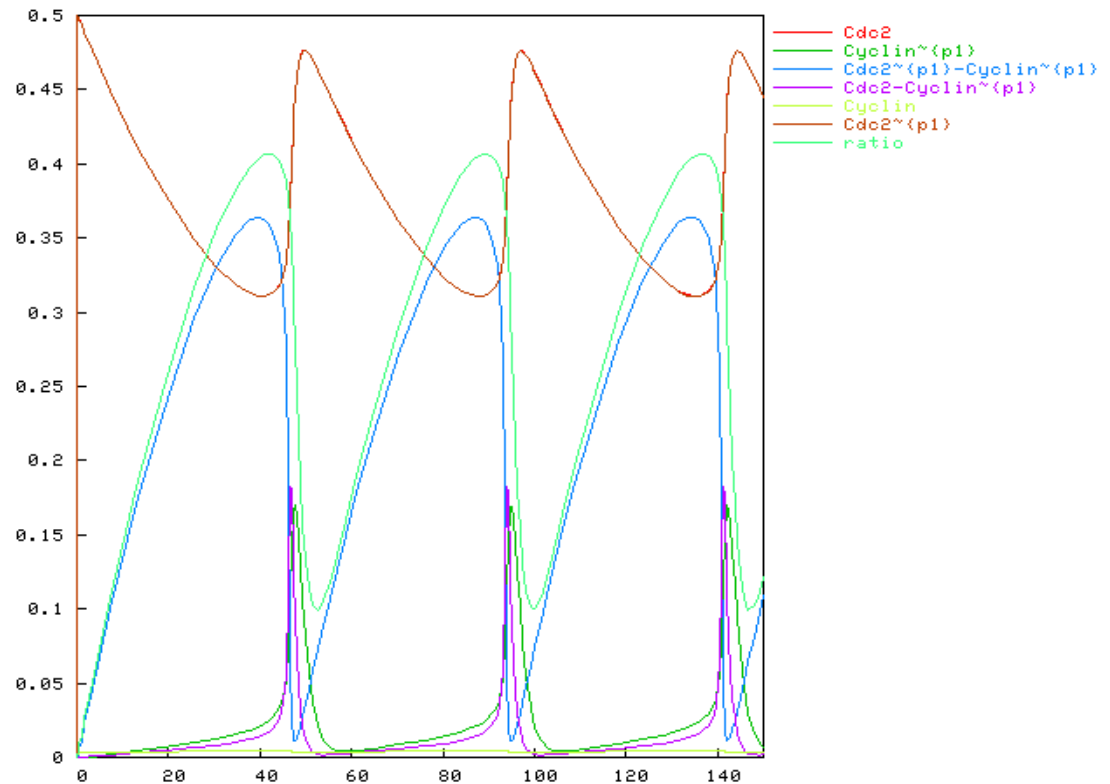
First values found :
parameter(k3,10).
parameter(k4,70).



Parameter Search from LTL(R) Properties

biocham: search_parameter([k3,k4],[(0,200),(0,200)],20,
oscil(Cdc2-Cyclin~{p1},3) & F([Cdc2-Cyclin~{p1}]>0.15), 150).

First values found :
parameter(k3,10).
parameter(k4,120).

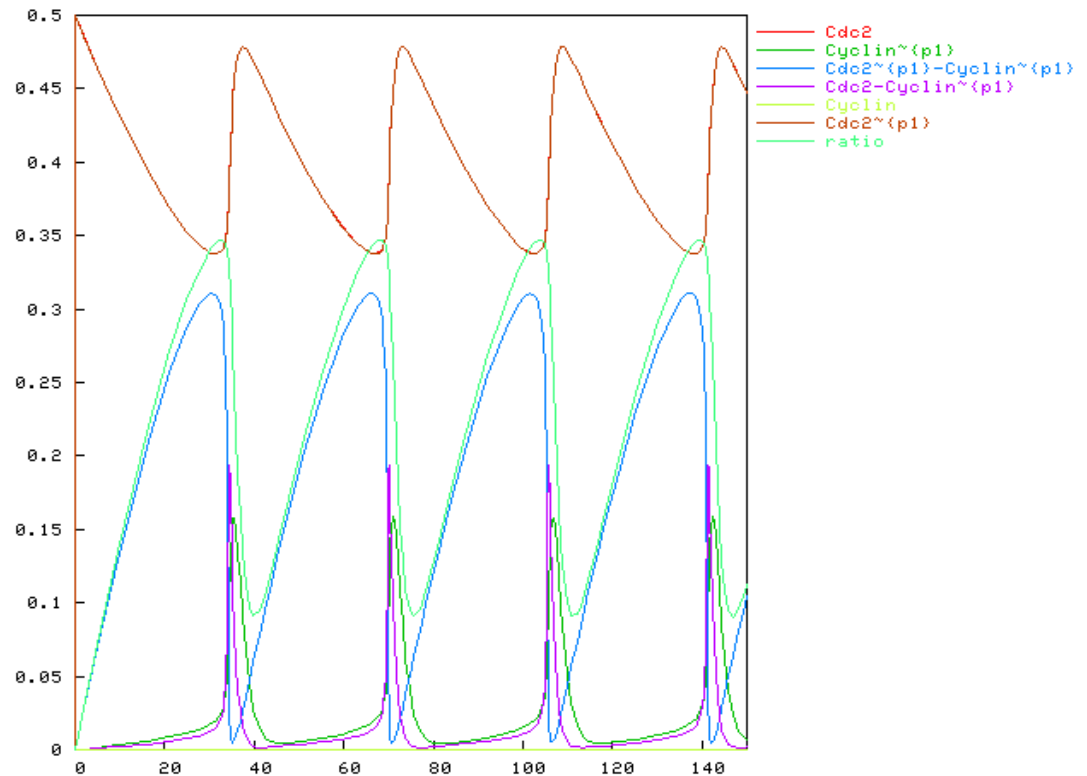


Parameter Search from LTL(R) Properties

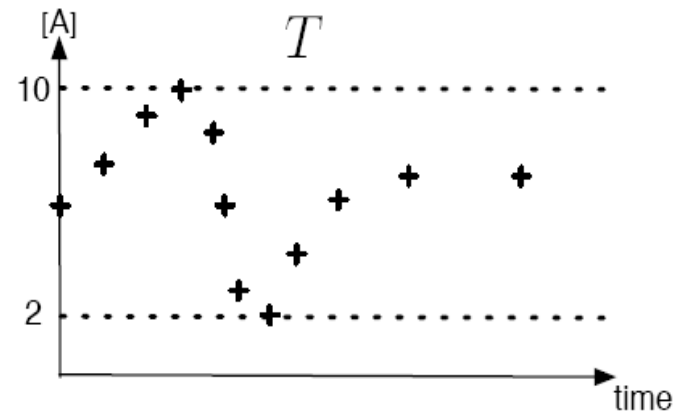
biocham: search_parameter([k3,k4],[(0,200),(0,200)],20,
period(Cdc2-Cyclin~{p1},35), 150).

First values found:
parameter(k3,10).
parameter(k4,280).

**But parameter scanning
does not scale up
beyond 3 parameters...**



Model-Checking Generalized to Constraint Solving



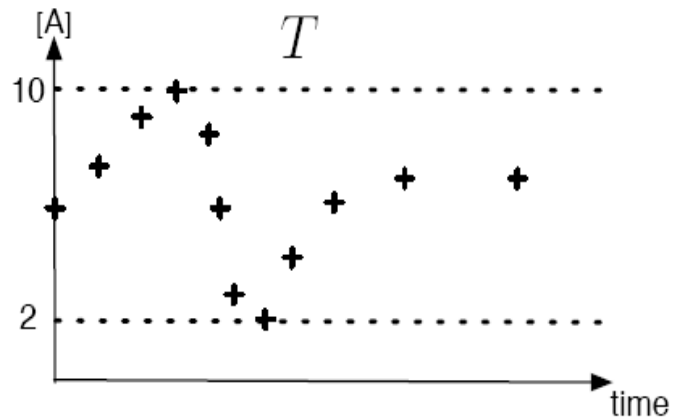
$LTL(\mathbb{R})$

$$\Phi = F([A] \geq 7) \wedge F([A] \leq 0)$$

Model-checking

the formula is false

Model-Checking Generalized to Constraint Solving



$LTL(\mathbb{R})$

$$\Phi = F([A] \geq 7) \wedge F([A] \leq 0)$$

Model-checking

the formula is false

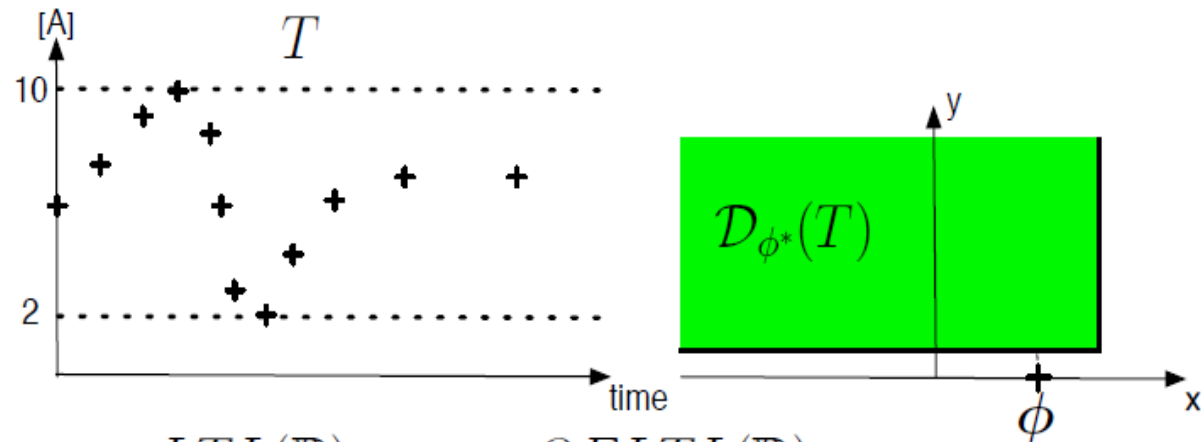
$QFLTL(\mathbb{R})$

$$\Phi^* = F([A] \geq x) \wedge F([A] \leq y)$$

Constraint solving

the formula is true for any
 $x \leq 10 \wedge y \geq 2$

Model-Checking Generalized to Constraint Solving



$LTL(\mathbb{R})$

$$\Phi = F([A] \geq 7 \wedge F([A] \leq 0))$$

Model-checking

the formula is false $vd=2$ $sd=1/3$

$QFLTL(\mathbb{R})$

$$\Phi^* = F([A] \geq x \wedge F([A] \leq y))$$

Constraint solving

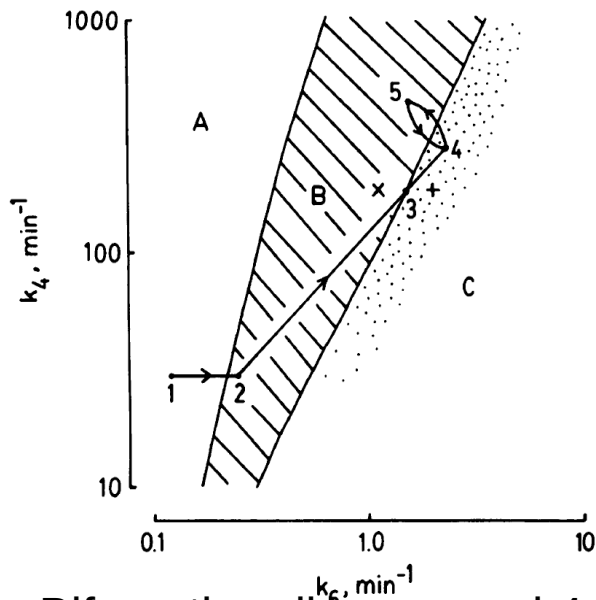
the formula is true for any
 $x \leq 10 \wedge y \geq 2$

Validity domain $\mathcal{D}_{\phi^*}(T)$ for the **free variables** in ϕ^* [Fages Rizk CMSB'07]

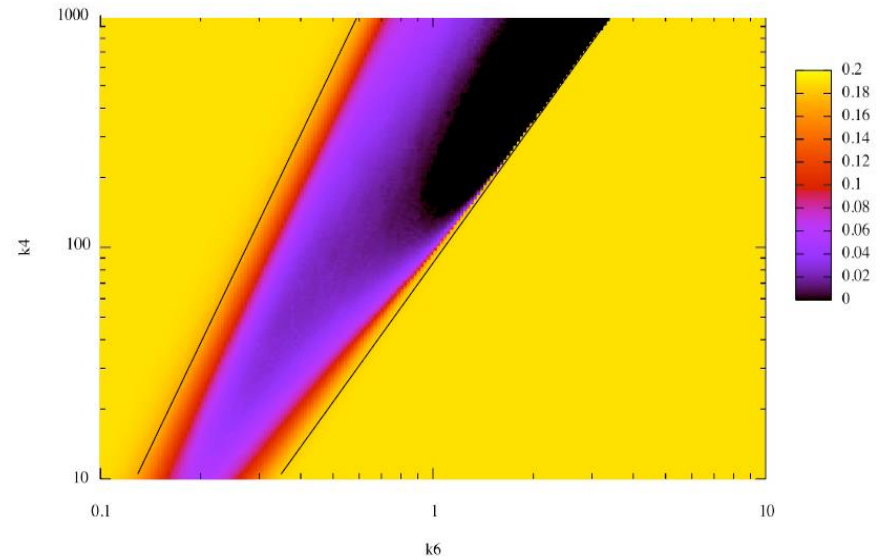
Violation degree $vd(T, \phi) = \text{distance}(\text{val}(\phi), \mathcal{D}_{\phi^*}(T))$

Satisfaction degree $sd(T, \phi) = \frac{1}{1+vd(T, \phi)} \in [0, 1]$

LTL(R) Continuous Satisfaction Degree in [0,1]



Bifurcation diagram on k_4 , k_6
[Tyson 91]

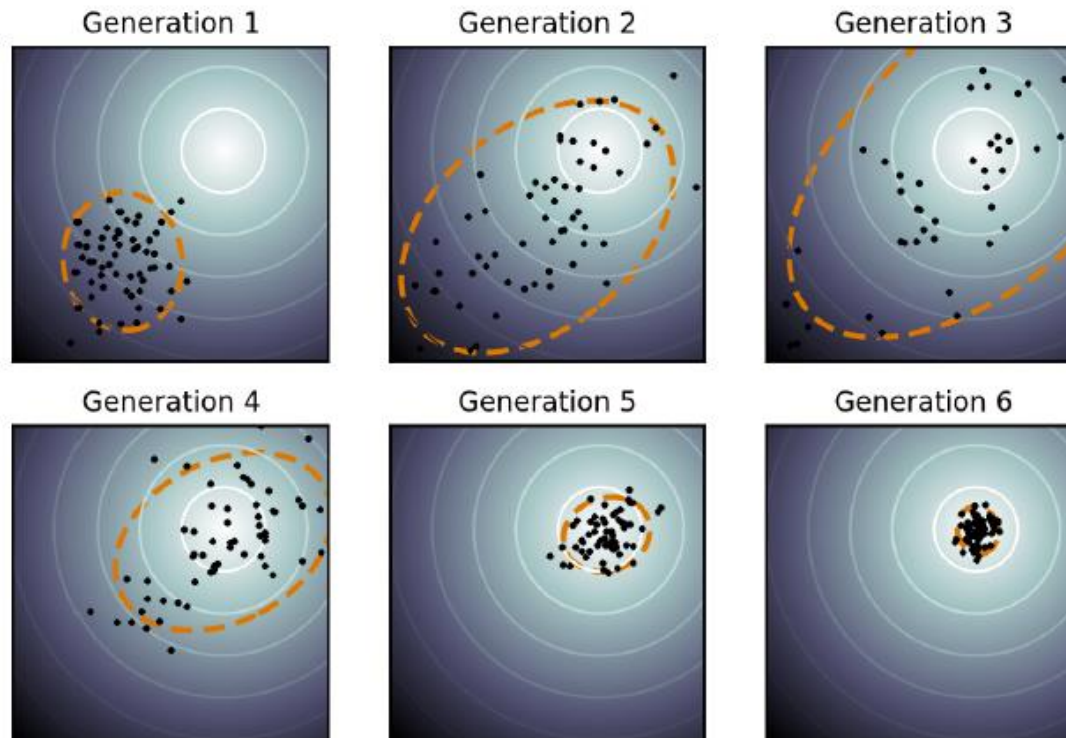


Continuous satisfaction degree in $[0,1]$
of the LTL(R) formula for oscillation
with amplitude constraint [Rizk Batt FF Soliman CMSB 08]

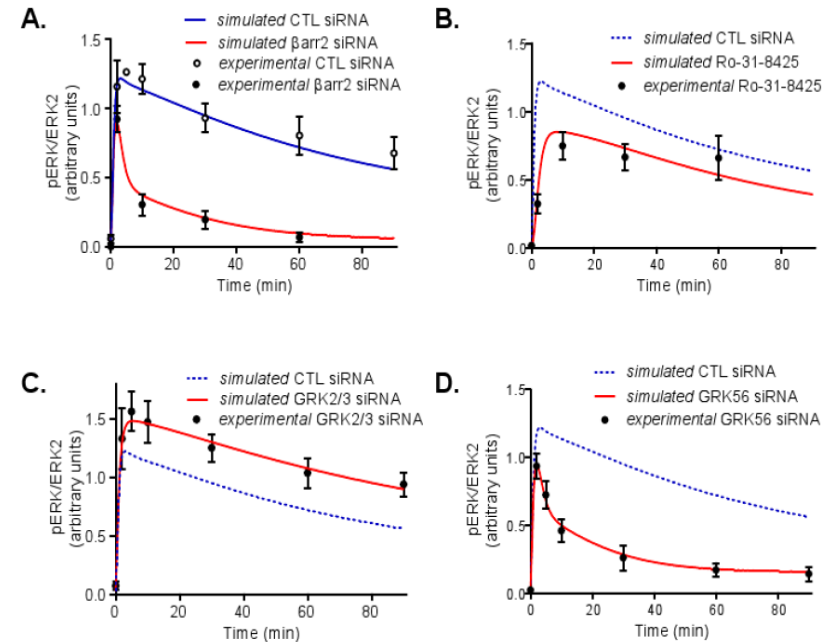
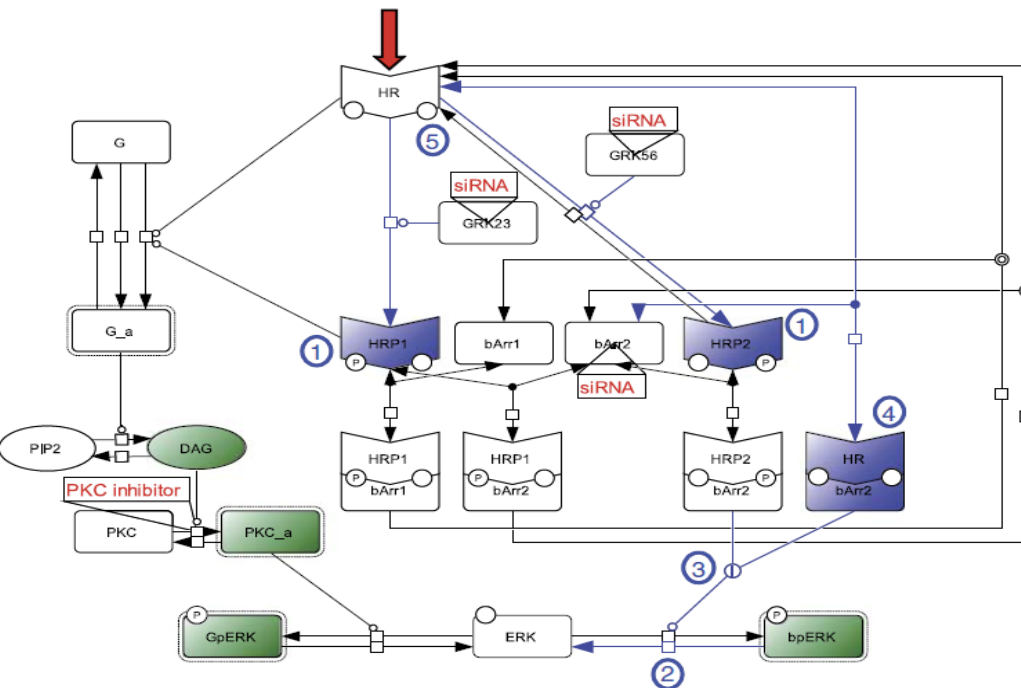
- **Parameter search** under LTL(R) constraints in high dimension (100 parameters) by continuous optimization (evolutionary algorithm CMA-ES)
- **Robustness** and **sensitivity** analyses w.r.t. LTL(R) specification

Covariance Matrix Adaptation Evolutionary Strategy

- CMA-ES maximizes a black box fitness function ($sd(\phi)$) in continuous domain ($k_i's$) [Hansen Osermeier 01, Hansen 08]
- CMA-ES uses a probabilistic neighborhood and updates information in covariance matrix at each move



Success Story in GPCR Signaling



Failure to find parameters fitting the data under perturbed conditions in BIOCHAM with CMAES-LTL(R) was the key to [revisit the structure](#) of the interactions

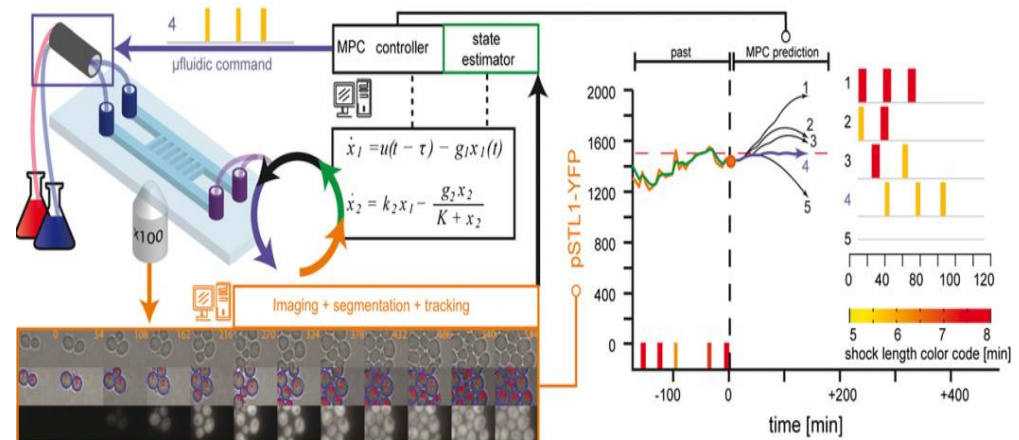
[D. Heitzler, ..., FF, R. Lefkowitz, E. Reiter 2012 *Molecular Systems Biology*]

Model-based control of gene expression in yeast

Perception - learning - action loop

1. Microscope, cell tracking
2. Model fitting (parameter opt.)
3. Osmotic pressure (parameter opt.)

[Uhlendorf ... Batt Hersen PNAS 109(35) 2012]



SM 5 : Real time yeast single cell control to a constant target profile

Long-term model predictive control of gene expression at the population and single-cell levels

J. Uhlendorf, A. Miermont, T. Delaveau, G. Charvin, F. Fages, S. Bottani, G. Batt* & P. Hersen*

pascal.hersen@univ-paris-diderot.fr http://tinyurl.com/TeamHersen
gregory.batt@inria.fr http://www-roq.inria.fr/~batt/



SM 7 : Real time yeast single cell control to a trapeze target profile

Long-term model predictive control of gene expression at the population and single-cell levels

J. Uhlendorf, A. Miermont, T. Delaveau, G. Charvin, F. Fages, S. Bottani, G. Batt* & P. Hersen*

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gregory.batt@inria.fr http://www-roq.inria.fr/~batt/



SM 6 : Real time yeast single cell control to a sine wave target profile

Long-term model predictive control of gene expression at the population and single-cell levels

J. Uhlendorf, A. Miermont, T. Delaveau, G. Charvin, F. Fages, S. Bottani, G. Batt* & P. Hersen*

pascal.hersen@univ-paris-diderot.fr http://tinyurl.com/TeamHersen
gregory.batt@inria.fr http://www-roq.inria.fr/~batt/



Wrap-up

- Chemical reaction network theory
 - Sufficient/necessary conditions for dynamical behaviors
 - Hybrid models and hybrid simulations

Crucial for reducing models

Wrap-up

- Chemical reaction network theory
 - Sufficient/necessary conditions for dynamical behaviors
 - Hybrid models and hybrid simulations
- Static analyzers of reaction programs
 - Structural invariants
 - Model reduction relationships
 - Efficient CLP(FD) and SAT solvers

Crucial for reducing models

What can be said from the structure of a reaction network ?

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- Dynamic analyzers of behaviors using temporal logic patterns
 - Parameter search for model building and control

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What can be said from the structure of a reaction network ?

The nerve of war in biological modeling

Wrap-up

- Chemical reaction network theory
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 - Structural invariants
 - Model reduction relationships
 - Efficient CLP(FD) and SAT solvers
- Dynamic analyzers of behaviors using temporal logic patterns
 - Parameter search for model building and control
- Biochemical programming
 - Synthetic biology: correct or implement new functions in the cell or in non living vesicles
 - Systems biology: elucidate natural circuits as optimized biochemical code

Crucial for reducing models

What can be said from the structure of a reaction network ?

The nerve of war in biological modeling

"What I cannot create I do not understand"
R. Feynman

Lifeware team at Inria



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