Continuous Valuations of Temporal Logic Specifications with applications to Parameter Optimization and Robustness Measures

François Fages

Inria Saclay,

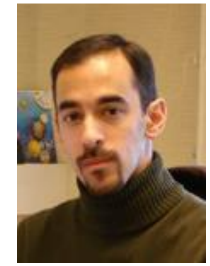
EPI Lifeware (formerly EPI Contraintes)

France

http://lifeware.inria.fr/

Implemented in the Biochemical Abstract Machine (BIOCHAM v3.7 next v4.0)

Lifeware Group

Research Scientists

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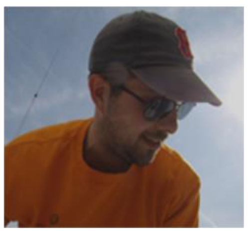
François Fages (Inria DR1, head)
Jakob Ruess (Inria CR2, sept. 2016)
Sylvain Soliman (Inria CR1)




Associates

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Lifeware: hardware-software of the living

- How to compute with biochemical reactions ?
  - Analog/digital computation
  - Compositionality and robustness of biochemical circuits
  - Programming artificial vesicles - Reprogramming living cells
- How to analyze natural cell processes as programs ?
  - Cell signaling, cell cycle, circadian clock, gene expression, …
  - Temporal logic specification of the behaviour, parameter inference, robustness
  - Beyond describing, understanding natural circuits and their evolution
- How to control cell processes?
  - Microfluidic platform in an image analysis-model calibration-action loop
  - Optimal experimental design
- How to reason with cell populations ?
  - Cell-to-cell variability analysis and control
  - Model of extrinsic/intrinsic noise

How to Compute with Biochemical Reactions ?

- Binding, complexation: $A + B \rightarrow C$

  $cdk1 + cycB \rightarrow cdk1cycB$

- Unbinding, decomplexation: $A \rightarrow B + C$

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

  $cdk1cycB \rightarrow cdk1cycBp$

- Gene expression, synthesis: $A \rightarrow A + B$

  $E2Fa \rightarrow E2Fa + RNAcycA$

- Degradation: $A \rightarrow \_$

How to Compute with Biochemical Reactions ?

- Binding, complexation: $A + B \xrightarrow{k.A.B} C$

  $cdk1 + cycB \rightarrow cdk1cycB$

Time matters

- Unbinding, decomplexation: $A \xrightarrow{k.A} B + C$

- Transformation, phosphorylation, transport: $A \xrightarrow{k.A} B$ or $A \xrightarrow{v.A/(k+A)} B$

  $cdk1cycB \rightarrow cdk1cycBp$

- Gene expression, synthesis: $A \xrightarrow{v.A^n/(k+A^n)} A + B$

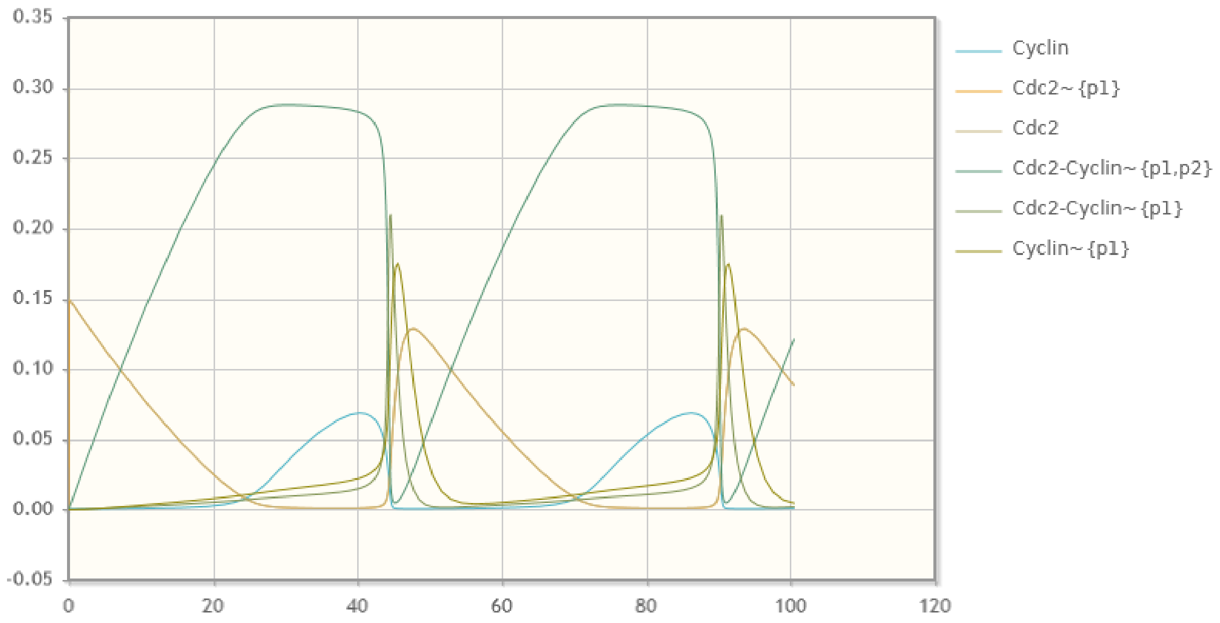
  $E2Fa \rightarrow E2Fa + RNAcycA$

- Degradation: $A \xrightarrow{k.A} \_$ or $A \xrightarrow{v.A/(k+A)} \_$

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

Continuous semantics: concentrations, continuous time evolution

Ordinary differential equations (ODE) $\frac{dAi}{dt} = \sum_{r=1}^{n} f_r \times \delta_r(Ai)$



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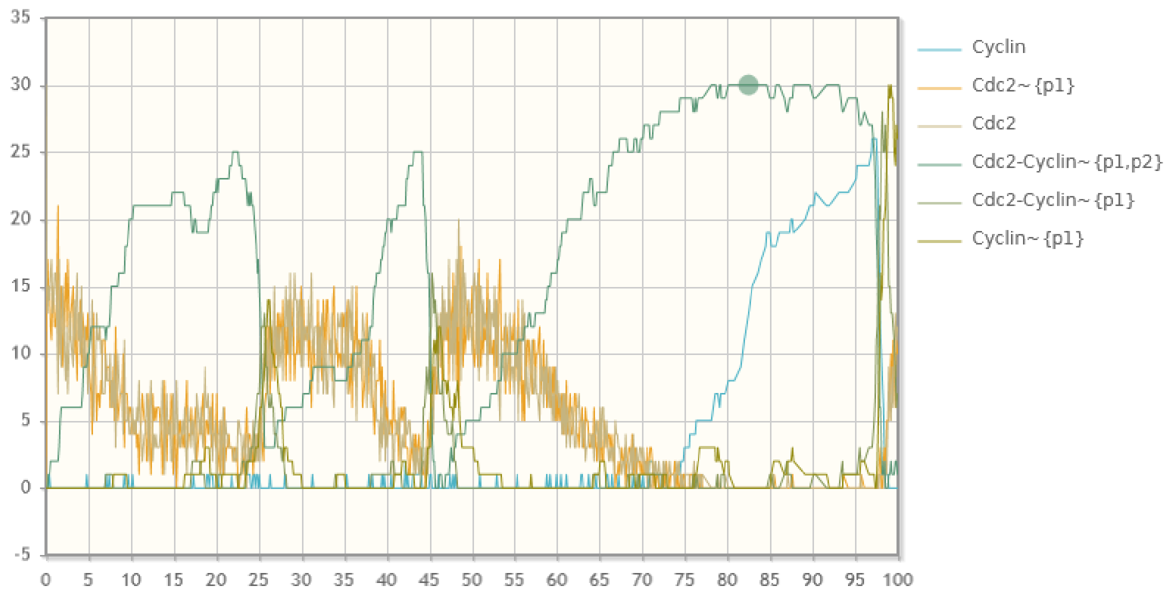
Stochastic semantics: numbers of molecules, probability and time of transition

Continuous Time Markov Chain (CTMC) $$A, B \xrightarrow{p(S_i),\, t(Si)} C{+}{+}, A{-}{-}, B{-}{-}$$



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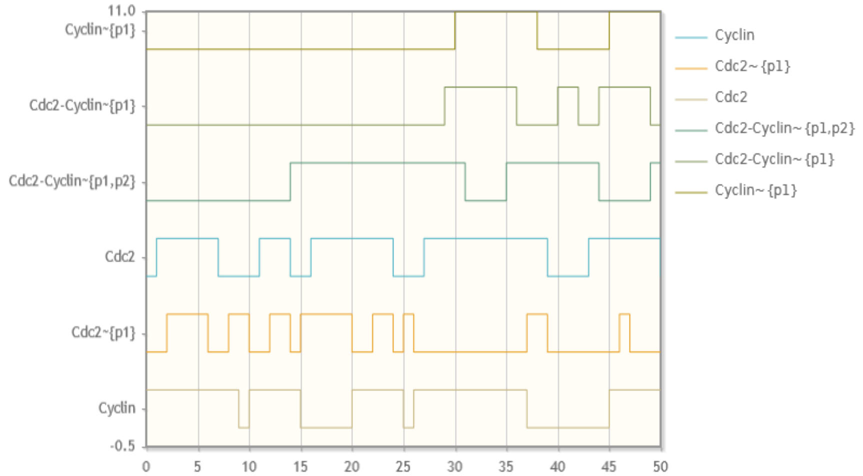
Petri net semantics: numbers of molecules $A , B \rightarrow C{+}{+}, A{-}{-}, B{-}{-}$

Multiset rewriting

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



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Petri net semantics: numbers of molecules $A , B \rightarrow C{+}{+},\ A{-}{-},\ B{-}{-}$

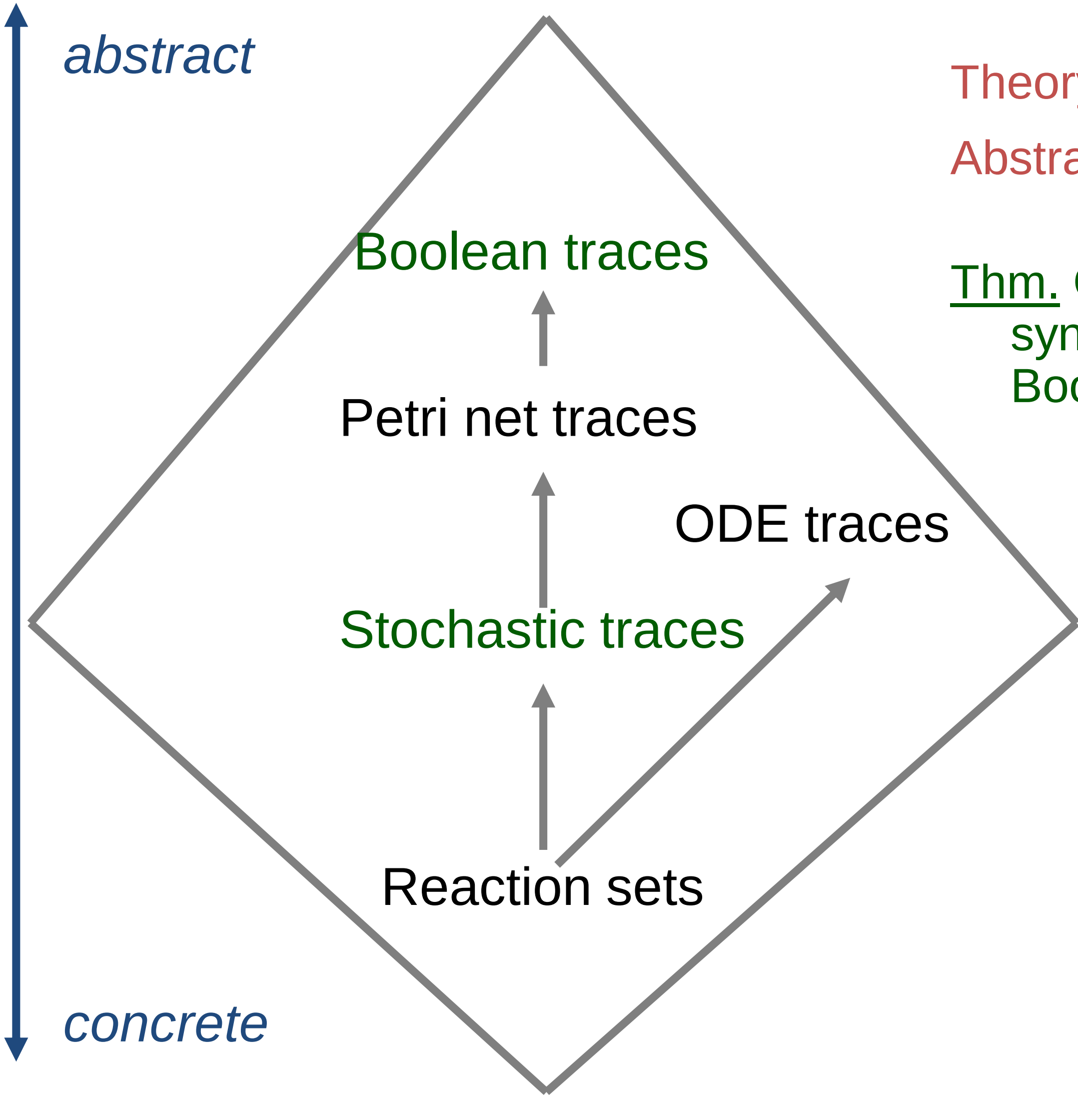
Multiset rewriting

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

Boolean semantics: presence/absence $A \wedge B \rightarrow C \wedge A/\neg A \wedge B/\neg B$

Asynchronous transition system

Abstraction Relationships



Theory of abstract Interpretation

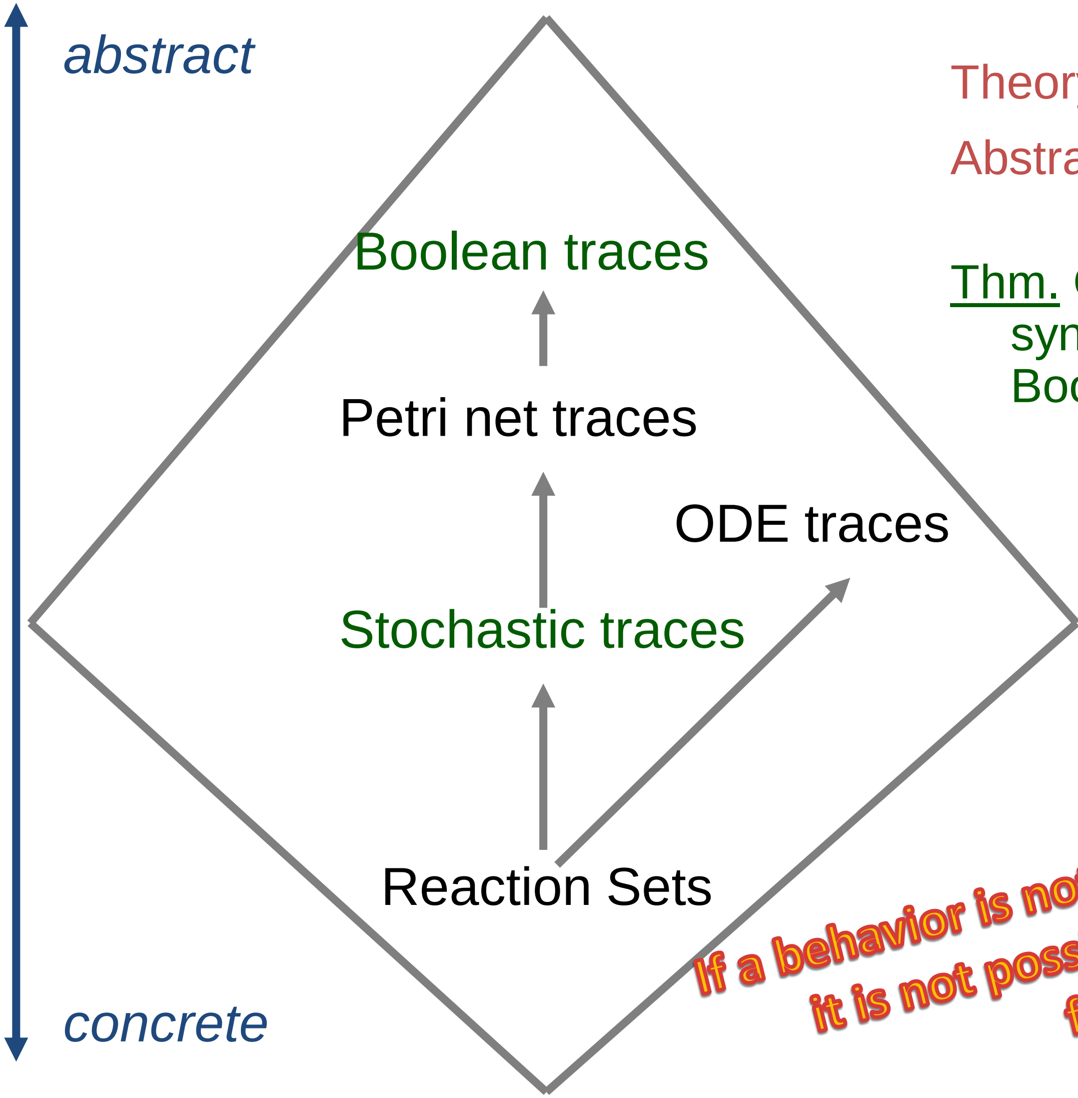
Abstractions as Galois connections

[Cousot Cousot POPL'77]

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

[FF Soliman CMSB'06,TCS'08]

Abstraction Relationships



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Abstractions as Galois connections

[Cousot Cousot POPL'77]

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

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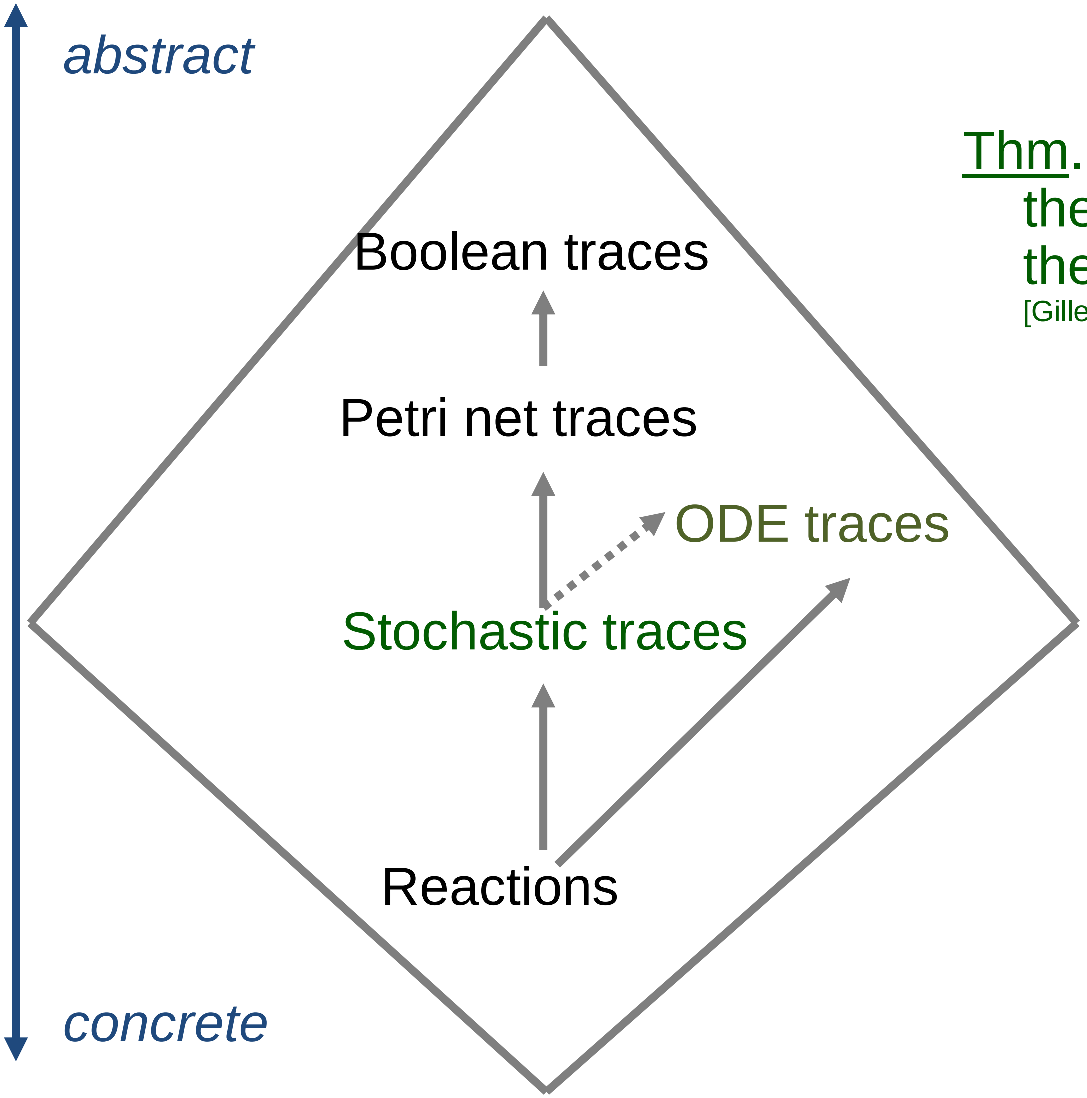
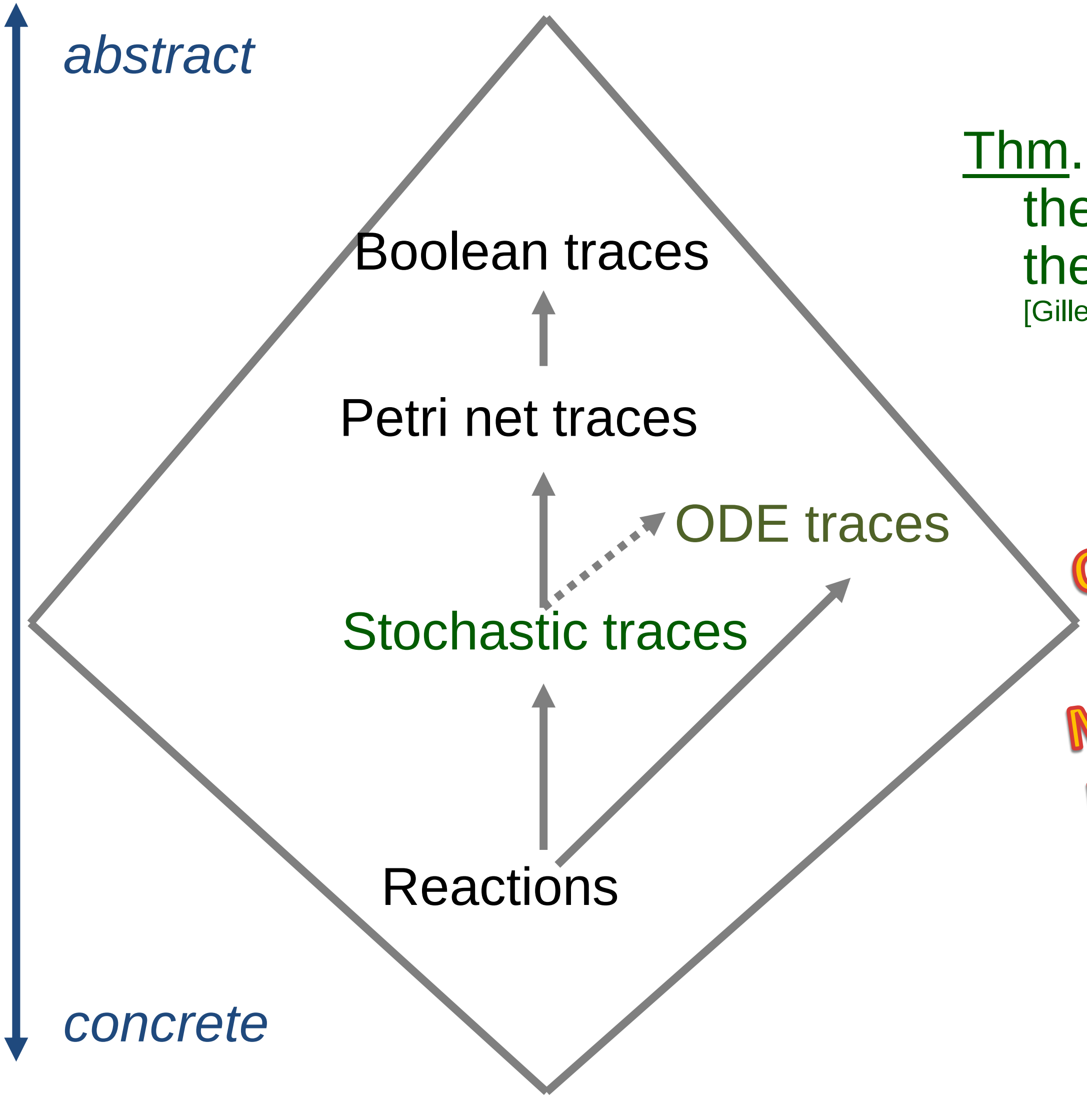
If a behavior is not possible in the Boolean semantics it is not possible in the stochastic semantics for any reaction rates

Abstraction Relationships



<u>Thm</u>. Under large number conditions the ODE semantics approximates the *mean* stochastic behavior [Gillespie 71]

Abstraction Relationships

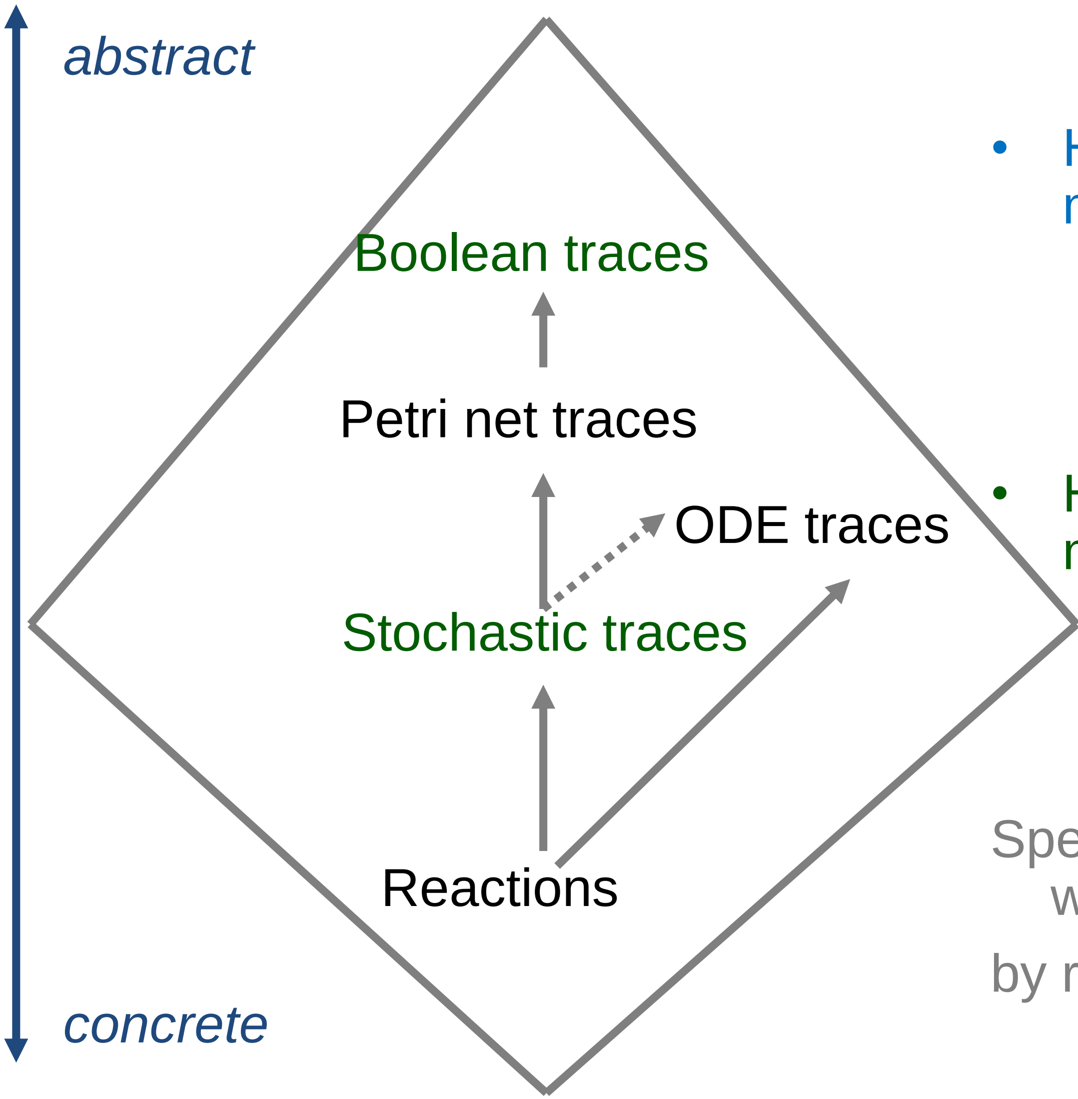


Thm. Under large number conditions the ODE semantics approximates the *mean* stochastic behavior [Gillespie 71]

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

Model cell-to-cell variability
Intrinsic and extrinsic noise

Hybrid Models and Hybrid Simulations



- Hybrid Boolean-continuous models (hybrid automata)

  Boolean gene expression + continuous protein activation

- Hybrid stochastic-continuous models (CTMC+ODE)

  Stochastic gene expression + continuous protein activation

Specification of hybrid simulators with *dynamic partitioning*

by reactions+events in SBML

[Chiang FF Huang Soliman 15 ACM TOMACS]

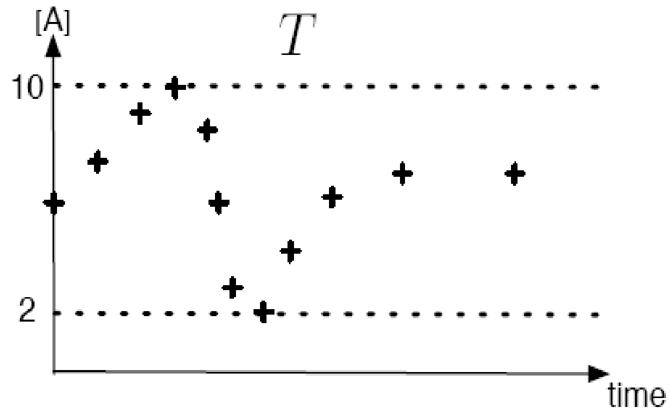
Quantitative Temporal Logic Specifications

- Formalization of (imprecise) behaviors observed experimentally
  - Quantitative temporal logic constraints FO-LTL(Rlin) [A. Rizk 2011 Thesis]
  - Stability **G**φ; Reachability **F**φ , thresholds **F**([A]>0.1),
  - Peaks of concentration ∃V **F**([A]<V ∧ **X**([A]=V ∧ **X**([A]<V))
  - Amplitude, periods and phases as distance between peaks [Traynard Fages Soliman 14 CMSB]

- Model verification
  - Boolean symbolic model-checking [Chabrier Chiaverini Danos FF Schachter 04 TCS]
  - FO-LTL(Rlin) constraint solving [FF Rizk 08 TCS]
  - Continuous satisfaction degree of FO-LTL formulae [Rizk Batt FF Soliman 11 TCS]
  - Parameter sensitivity, robustness measures [Rizk Batt FF Soliman 09 Bioinformatics]

- Model synthesis (parameter inference)
  - Evolutionary search algoritm CMA-ES [Hansen 01] maximize satisfaction degree FO-LTL
  - FO-LTL satisfaction → dynamical model → quantitative predictions, control
  - FO-LTL unsatisfaction → model structure revision → contributions to biology

Model-Checking Generalized to Constraint Solving



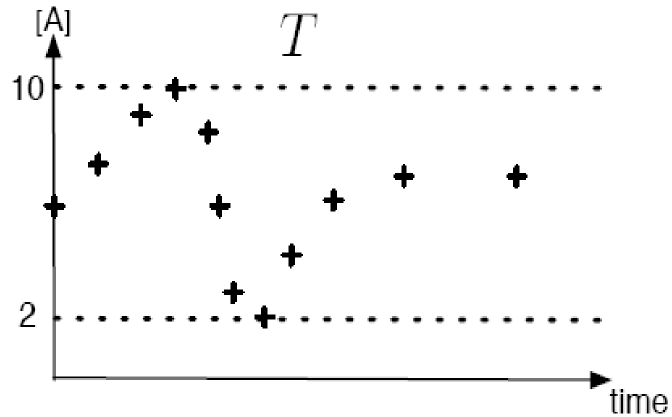
$LTL(\mathbb{R})$

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

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

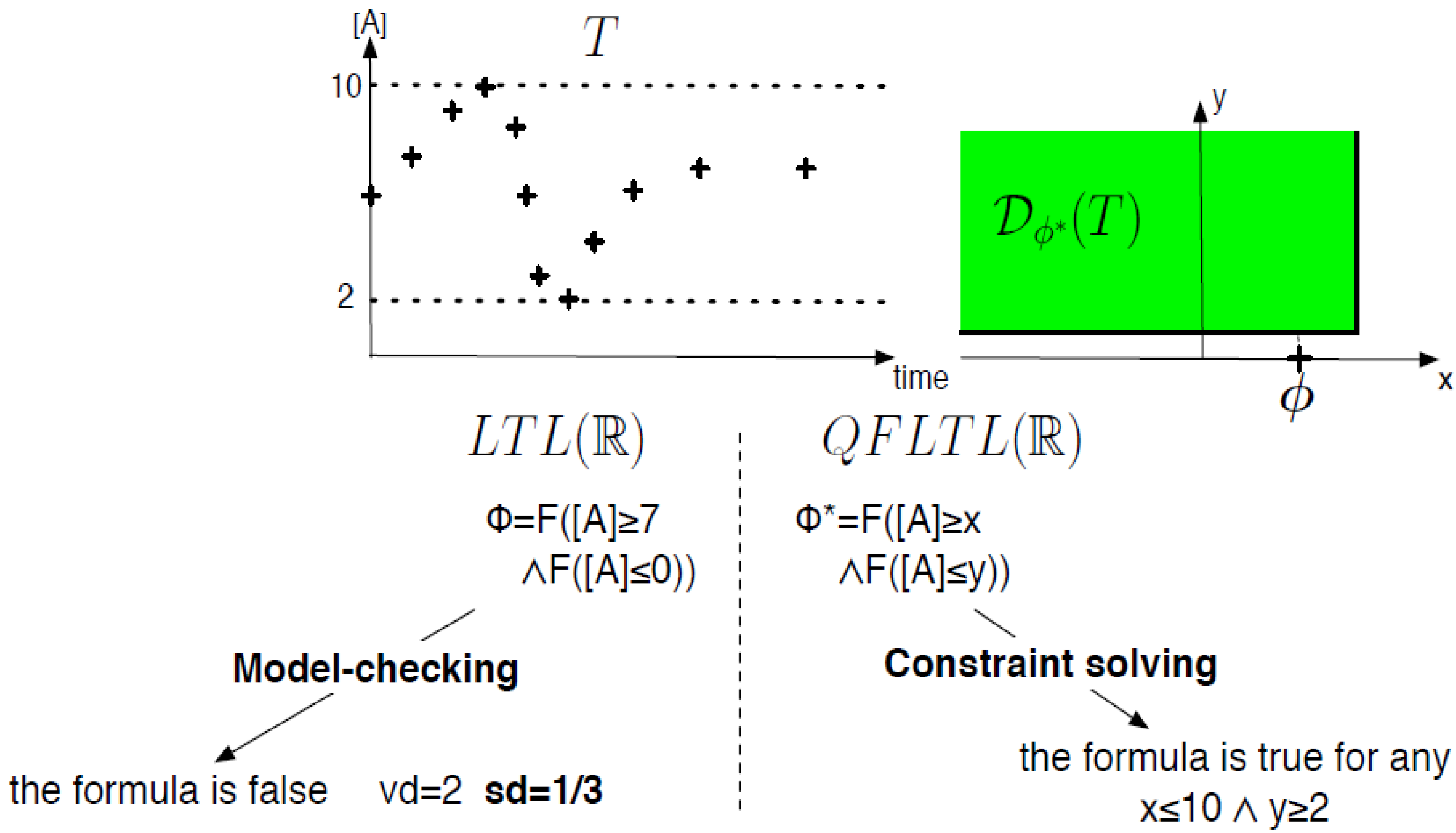
$QFLTL(\mathbb{R})$

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

Constraint solving

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

Model-Checking Generalized to Constraint Solving

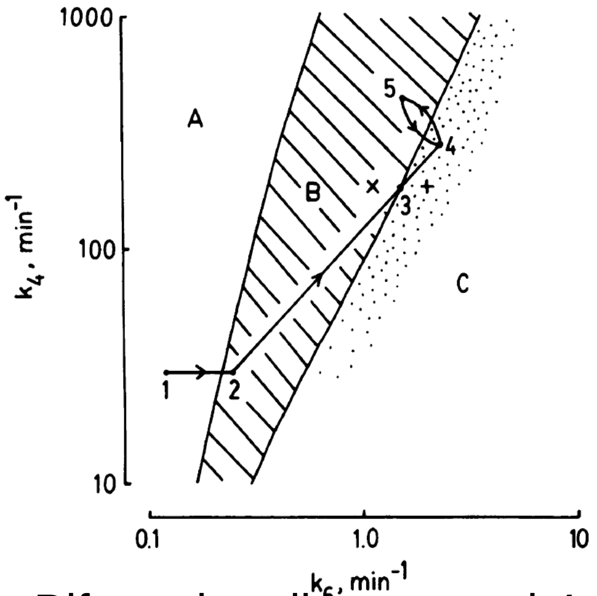


Validity domain $\mathcal{D}_{\phi^*}(T)$ for the **free variables** in ϕ^* [Fages Rizk CMSB'07]

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

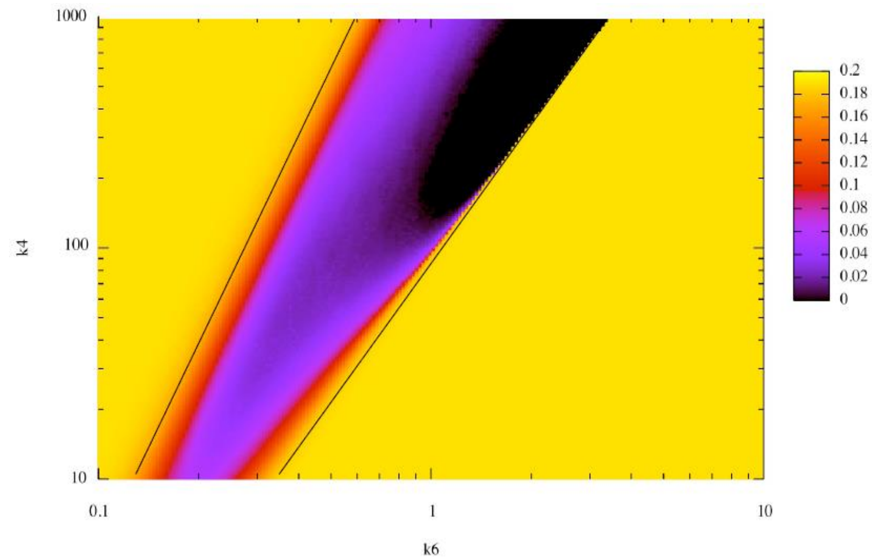
Satisfaction degree $sd(T,\phi) = \frac{1}{1+vd(T,\phi)} \in [0,1]$

FO-LTL(R_{lin}) Continuous Satisfaction Degree in [0,1]



Bifurcation diagram on k4, k6

[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

Robustness Measure Definition

Robustness defined with respect to :

- a biological system
- a functionality property D_a
- a set P of perturbations

- General notion of robustness proposed in [Kitano MSB 07]:

$$\mathcal{R}_{a,P} = \int_{p \in P} D_a(p)\ prob(p)\ dp$$

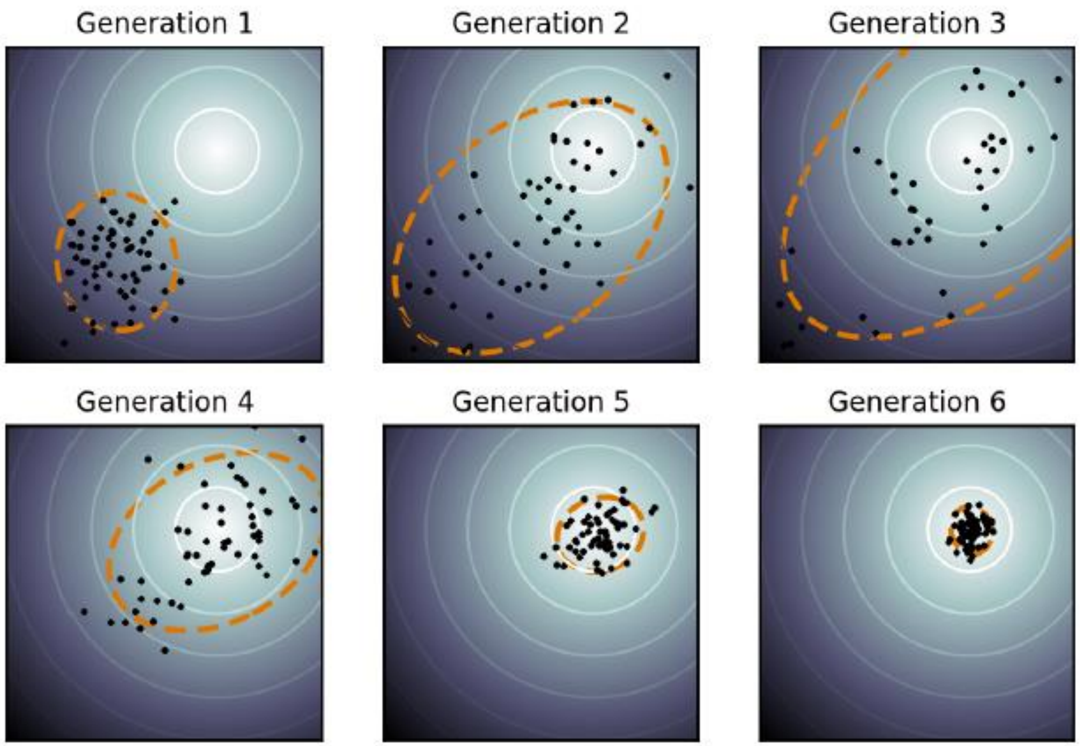
- Our computational measure of robustness w.r.t. LTL($\mathbb{R}$) spec: Given an ODE model with initial conditions, a TL formula ϕ and a set of perturbations P (on initial conditions or parameters),

$$\mathcal{R}_{\phi,P} = \sum_{p \in P} sd(T(p), \phi)\ prob(p)$$

where $T(p)$ is the trace obtained by numerical integration of the ODE for perturbation p

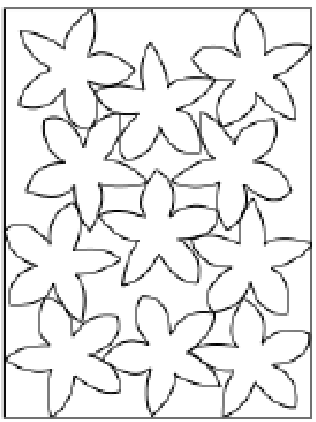
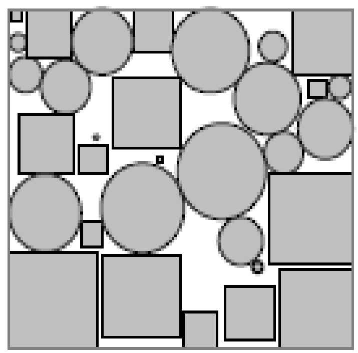
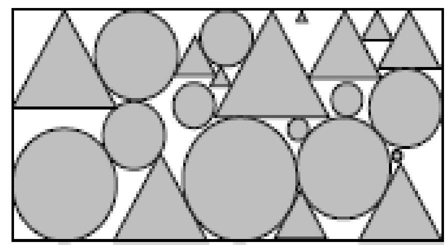
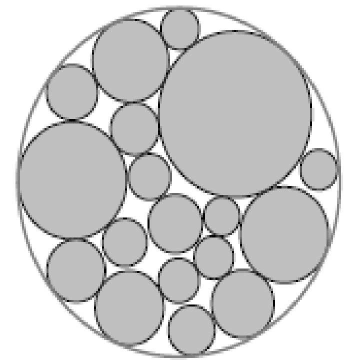
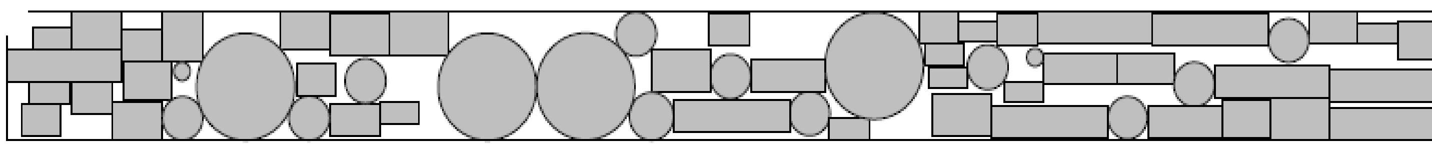
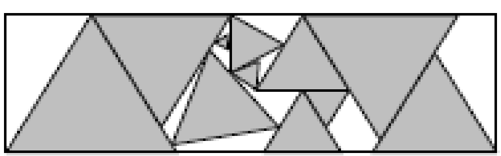
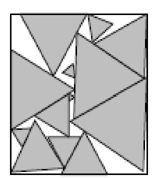
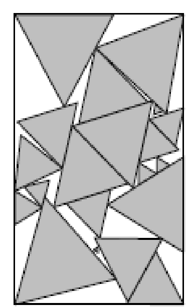
Covariance Matrix Adaptation Evolutionary Strategy

- 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

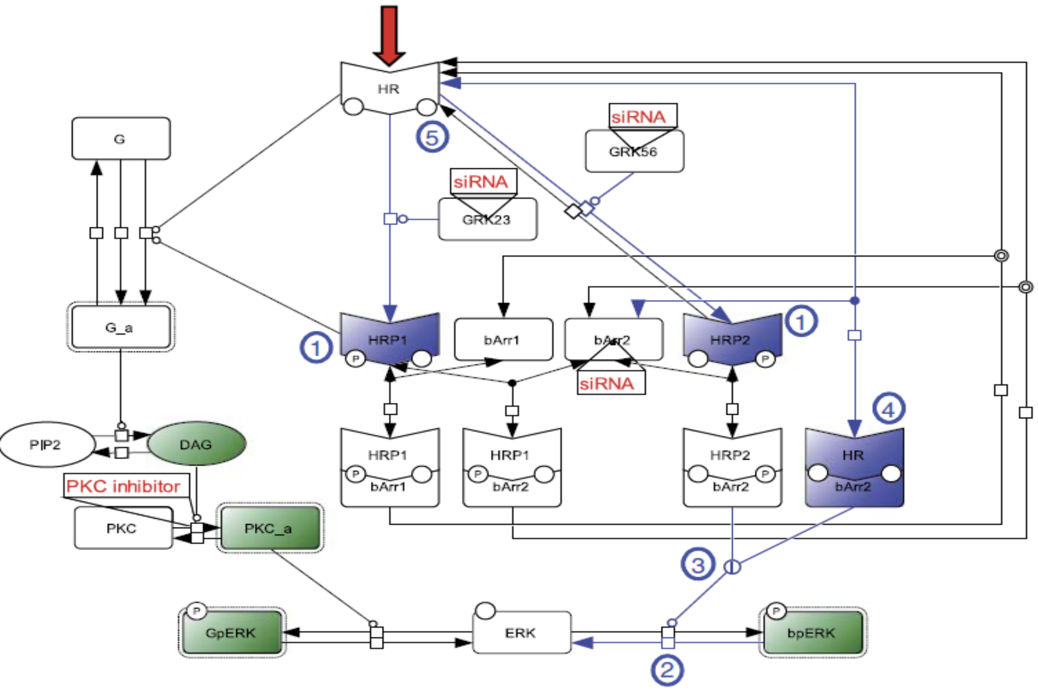


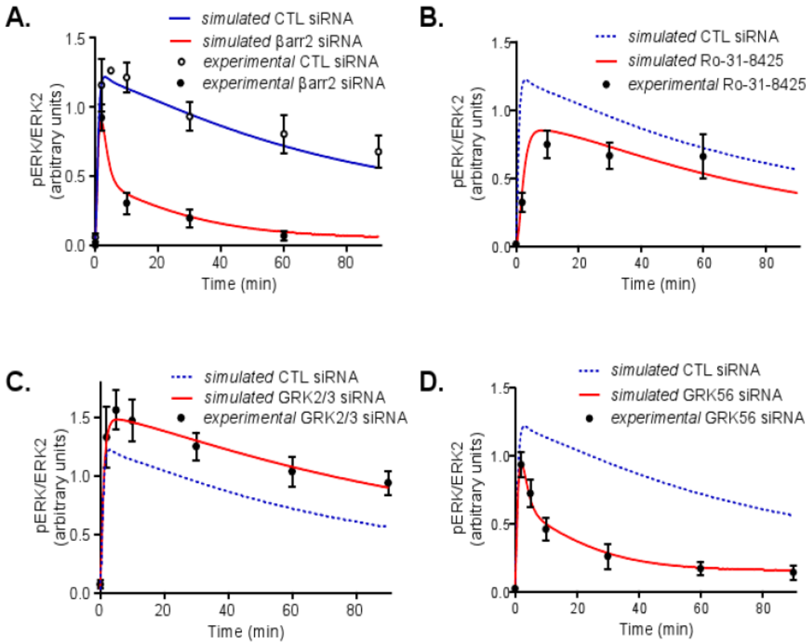
Packing of Complex Shapes with MiniZinc-CMAES

From simple shapes to continuous rotations and complex shapes defined by Bézier curves

Success Story in GPCR Signaling



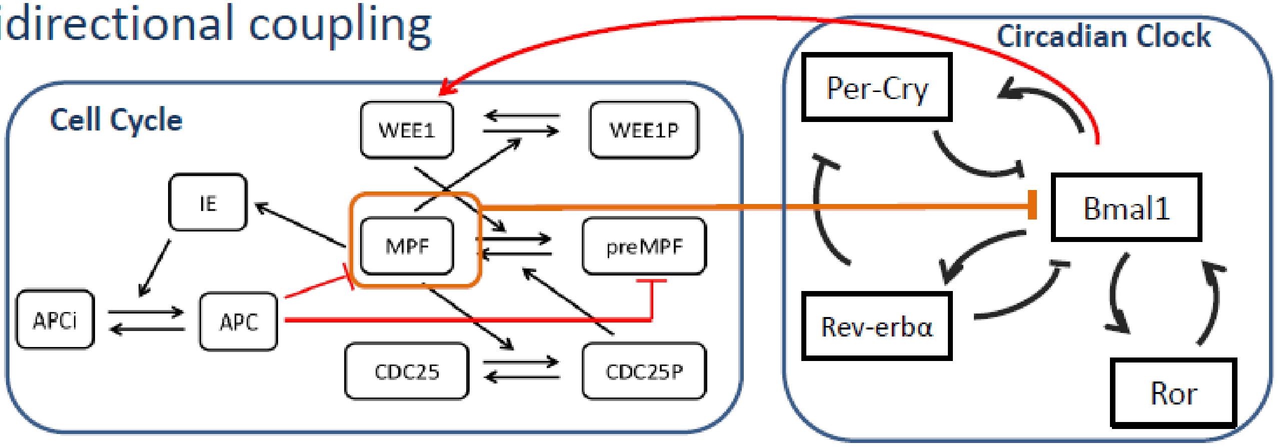


- Reduced model with 4 observables, 4 mutations, known interactions
- *Failure to find satisfying parameter values* using quantitative temporal logic in BIOCHAM
- Revision of the model structure for 3 interactions, experimentally verified *a posteriori*

[D. Heitzler, …, FF , R. Lefkowitz, E. Reiter 2012 *Molecular Systems Biology 8(590)*]

Cell Cycle and Circadian Clock Coupling

Bidirectional coupling



- Influence of circadian clock on cell cycle: time gating for Mitosis through Wee1
- Influence of cell cycle on circadian clock ?
  - Acceleration of the clock observed in fibroblasts in cells with fast cell cycle
  - Hypothesis of selective regulation of clock genes
  - Model-based prediction of up-regulation of RevErb around mitosis

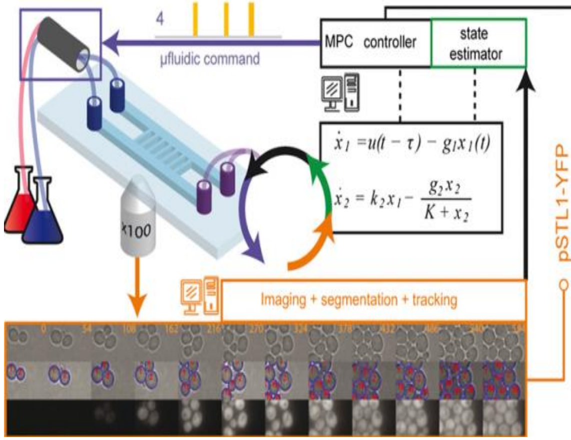
Parameters	First set	Second set
Synthesis coefficient for *Per*	0.66	2.40
Synthesis coefficient for *Cry*	2.30	0.67
Synthesis coefficient for *RevErb-α*	1.04	1.92
Synthesis coefficient for *Ror*	2.1	1.51
Synthesis coefficient for *Bmal1*	0	0.78
Duration	2.97h	2.81h

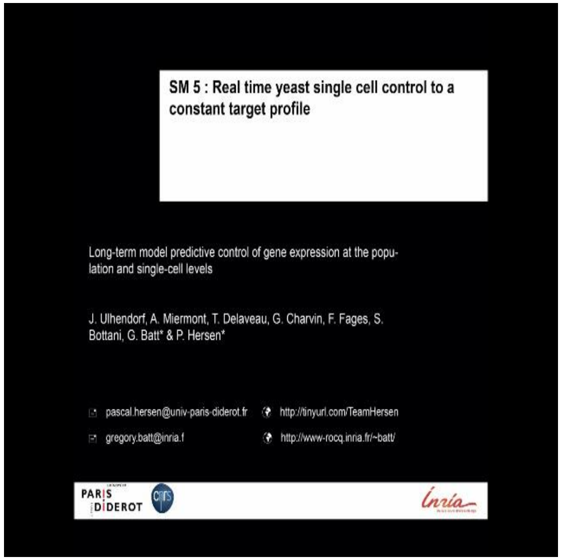
Model-based Control of Gene Expression in Yeast

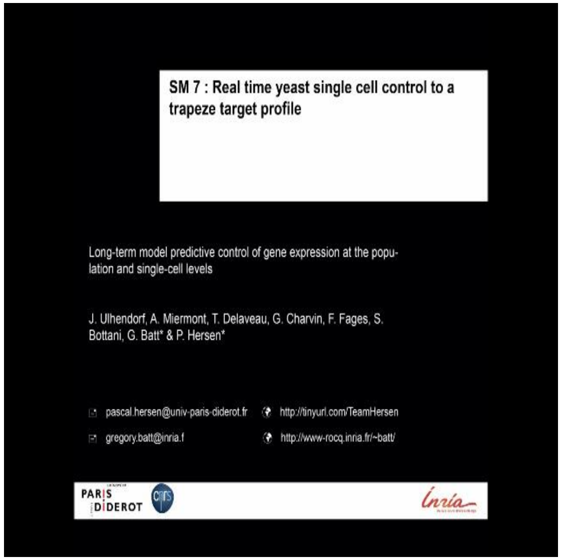
Perception – learning – action loop on a microfluidic device:

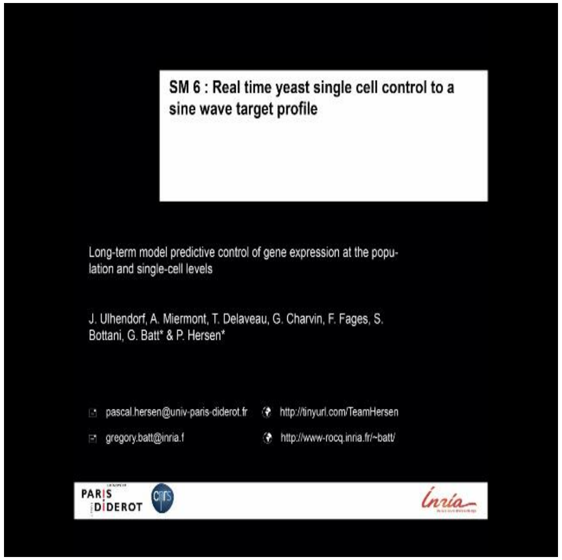
1. Microscope, image analysis (cell tracking or population)
2. Model calibration (kinetic parameter optimization)
3. Osmotic pressure control (parameter optimization)

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



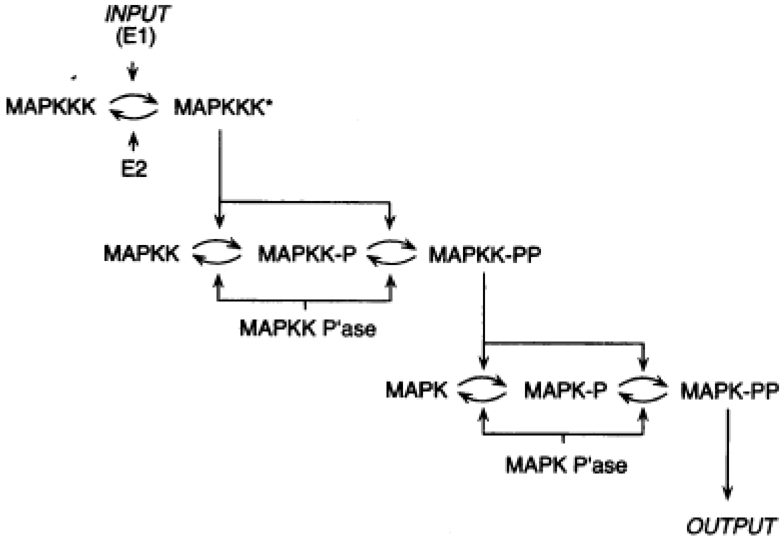


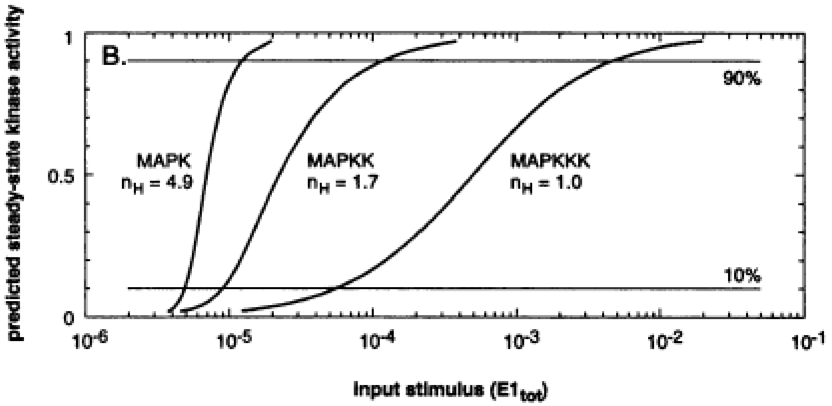




Beyond Describing Natural Circuits
Understanding them ? Why those structures ?

- Analog/Digital Computations
- MAPK signaling = Analog / Digital converter





- How to implement analog circuits with biochemical reactions ?
- How to program with biochemical reactions ?

Analog Arithmetic with Reactions ?

- Infering reaction systems from ODEs [FF Gay Soliman 15 TCS]
- Compute y=f(X)
  1. dy/dt = k*f(X) – k*y   at steady state we will have f(X)=y
  2. Two reactions: k*f(X) for X => X+y   k*y for y =>_
- Multiplication z=x*y
  1. x*y for x+y => x+y+z
  2. z for z => _
- Addition z=x+y
  1. x for x => x+z
  2. y for y => y+z
  3. z for z => _
- Integral z = ∫ x dt
  1. x for x => x+z

General Purpose Analog Computer (Shannon 41)

Logical Preconditions on Reactions?

- Conjunction $X \xrightarrow{A_\theta \wedge B_\theta} Y$

$$X + A + B \rightarrow Y + A + B$$

- Disjunction $X \xrightarrow{A_\theta \vee B_\theta} Y$

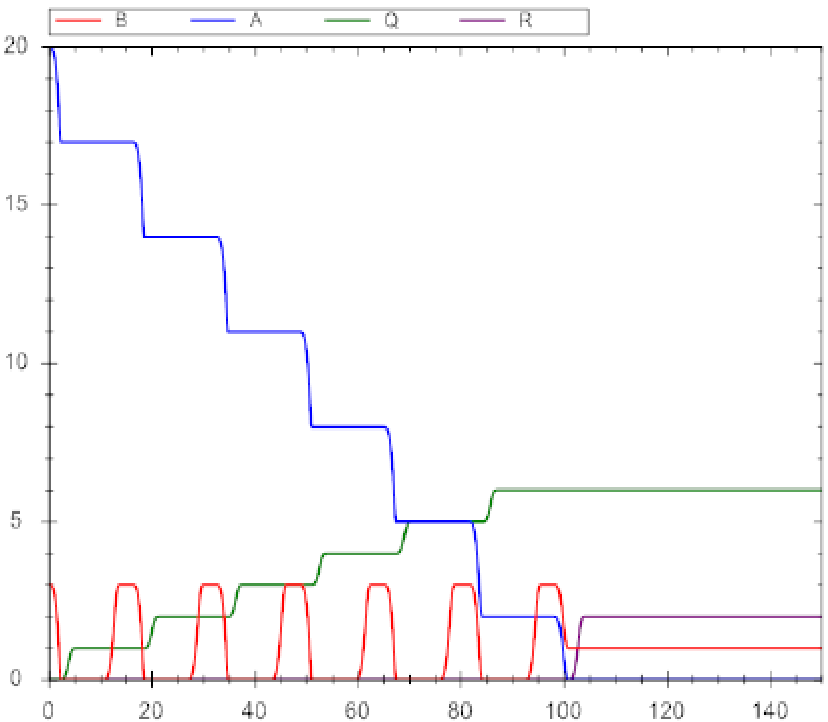
$$X + A \rightarrow Y + A$$
$$X + B \rightarrow Y + B$$

- Negation

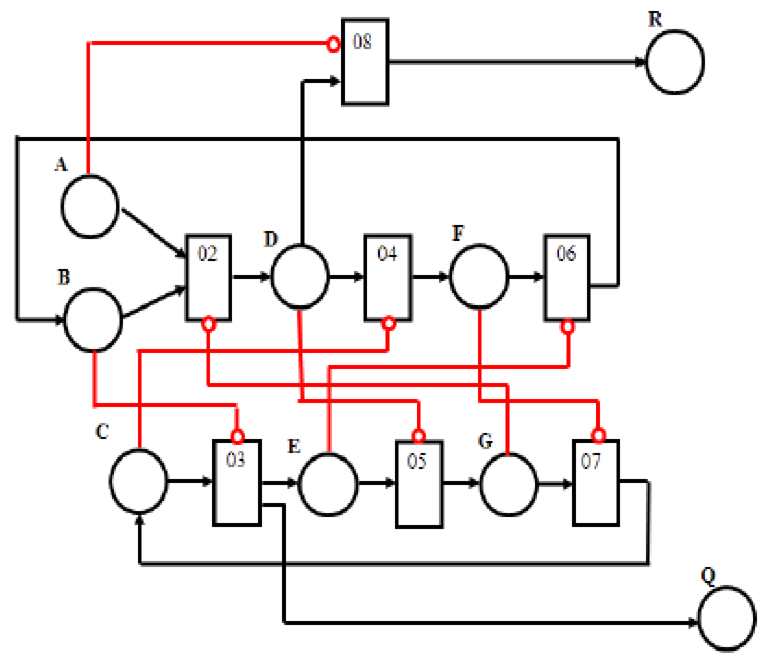
$$\emptyset \xrightarrow{r_s} A'$$
$$A + A' \xrightarrow{r_f} A$$
$$(2A' \xrightarrow{r_f} A')$$

C Compiler into Reactions [Jiang et al 2012, 2013]

$Division(A, B)$
begin
01 while $A \geq B$
02 $A := A - B$
03 $Q := Q + 1$
04 $R := A$
end

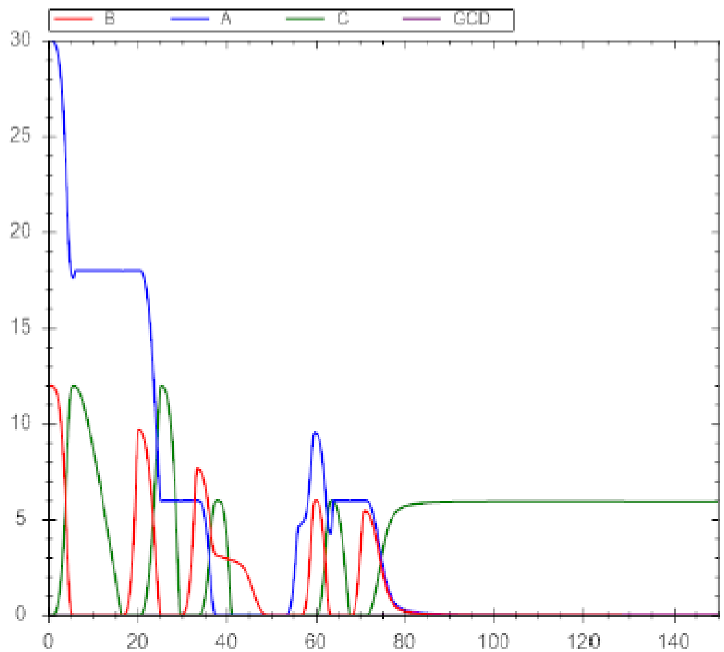


Main Reactions		Preconditions
01	while $[A] \geq [B]$	
02	$(A + B \to D)$	$\neg G_\theta$
03	$C \to Q + E$	$A_\theta \wedge \neg B_\theta$
04	$D \to F$	$\neg C_\theta$
05	$E \to G$	$\neg D_\theta$
06	$F \to B$	$\neg E_\theta$
07	$G \to C$	$\neg F_\theta$
08	$D \to R$	$\neg A_\theta$

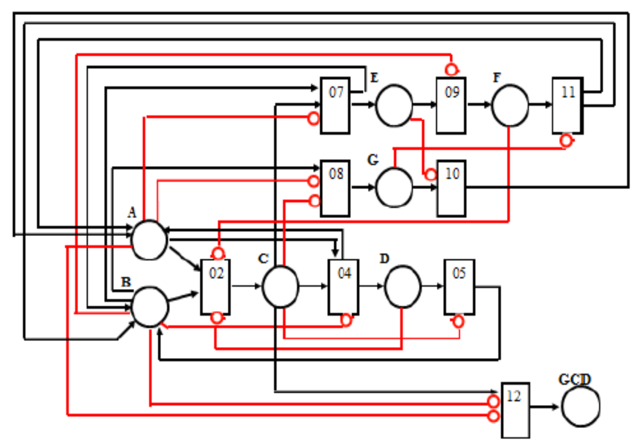


C Compiler into Reactions [Jiang et al 2012, 2013]

```
GreatestCommonDivisor(A, B)
begin
    while A ≠ B
        if A > B
            A := A − B
        else if B > A
            swap(A, B)
    GCD := A
end
```



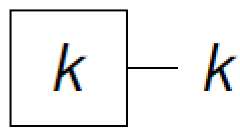
	Main Reactions	Preconditions
01	while $[A] \neq [B]$	
02	$(A + B \rightarrow C)$	$\neg D_\theta \wedge \neg F_\theta$
03	if $[A] > [B]$	
04	$C \rightarrow D$	$A_\theta \wedge \neg B_\theta$
05	$D \rightarrow B$	$\neg C_\theta$
06	else if $[B] > [A]$	
07	$C \rightarrow E$	$\neg A_\theta \wedge B_\theta$
08	$B \rightarrow G$	$\neg C_\theta \wedge \neg A_\theta$
09	$E \rightarrow F$	$\neg B_\theta$
10	$G \rightarrow A$	$\neg E_\theta$
11	$F \rightarrow A + B$	$\neg G_\theta$
12	$C \rightarrow GCD$	$\neg A_\theta \wedge \neg B_\theta$



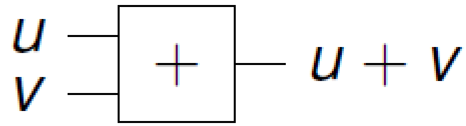
General Purpose Analog Computer [Shannon 41]

idealization of an analog computer: Differential Analyzer

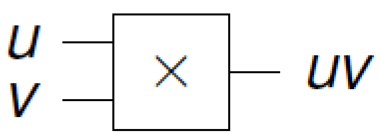
circuit built from:



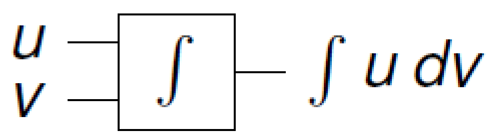
A constant unit



An adder unit



An multiplier unit



An integrator unit

Church-Turing Thesis for Analog Computation

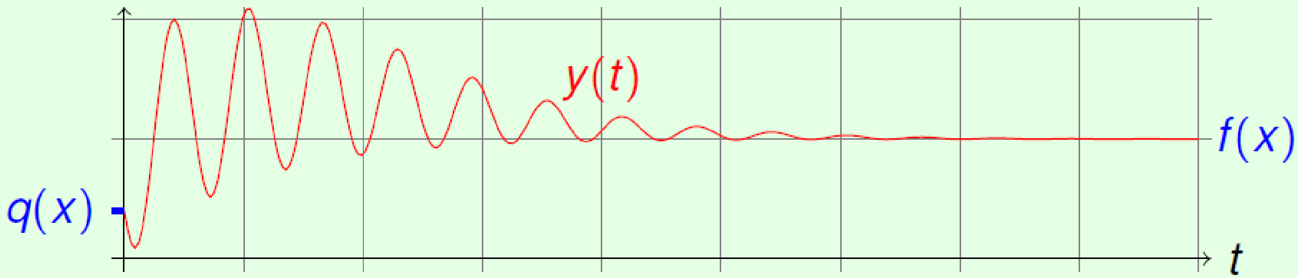
Definition

f is **computable** by a GPAC iff $\exists p, q$ polynomials s.t. $\forall x \in \mathbb{R}$, the solution $y = (y_1, \ldots, y_d)$ of:

$$\begin{cases} y' &= p(y) \\ y(t_0) &= q(x) \end{cases}$$

satisfies $f(x) = \lim_{t \to \infty} y_1(t)$.

Example



Theorem (Bournez, Campagnolo, Graça, Hainry)

f is GPAC-computable functions iff it is computable (in the sense of Computable Analysis).

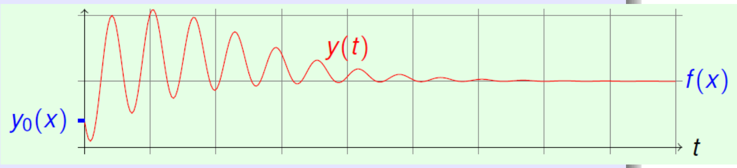
Purely Analog Characterization of Ptime !

[Pouly Bournez Graca 2015]

Definition

f is **poly-computable** by a GPAC iff $\exists p, q$ polynomials s.t. $\forall x \in \mathbb{R}$, the solution $y = (y_1, \ldots, y_d)$ of:

$$\begin{cases} y'(t) = p(y(t)) \\ y(t_0) = q(x) \end{cases}$$



satisfies that:

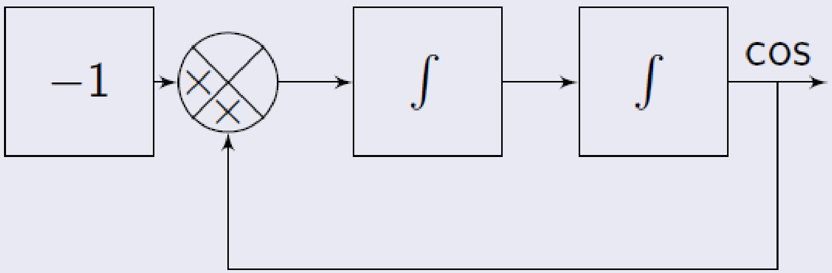
- $\|f(x) - y_1(t)\| \leqslant e^{-\mu}$ when $t \geqslant \text{poly}(\|x\|, \mu)$
- $\|y(t)\| \leqslant \text{poly}(\|x\|, t)$

Theorem

f is poly-computable if and only if it is computable in polytime in the sense of Computable Analysis.

Cosine Function Graph Generation

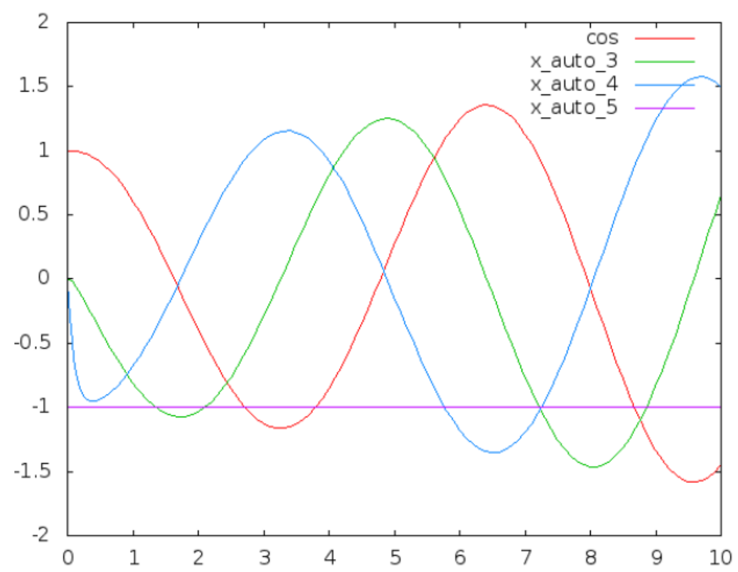
Blocks representation



Example in BIOCHAM

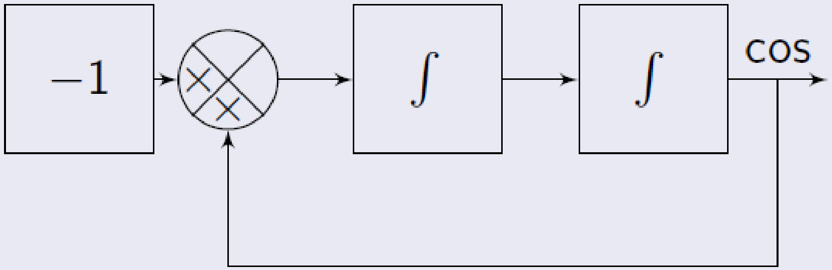
```
    compile_wgpac(cos10 ::
                  integral integral -1*cos10, 10).
    present(cos10).


[0] 10*[x_auto_2]*[cos]for _=[x_auto_2+cos]=>x_auto_1
[1] 10*[x_auto_1]for x_auto_1=>_
[2] _=[x_auto_1]=>x_auto_0
[3] _=[x_auto_0]=>cos
```



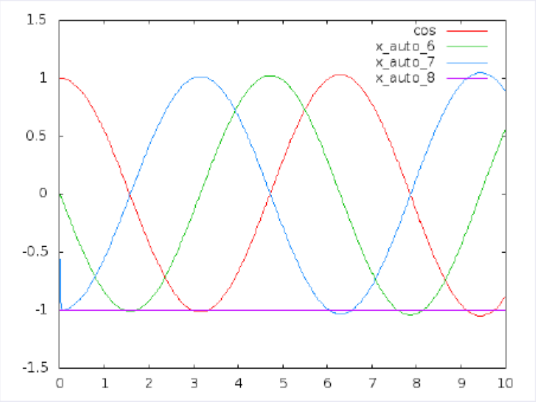
Cosine Function Graph Generation

Blocks representation



Example in BIOCHAM

```
compile_wgpac(cos100 ::
                  integral integral -1*cos100, 100).
present(cos100).
```



Linear Time Invariant Systems

Definition: Laplace transform

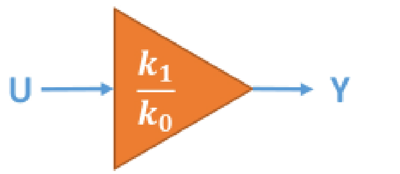
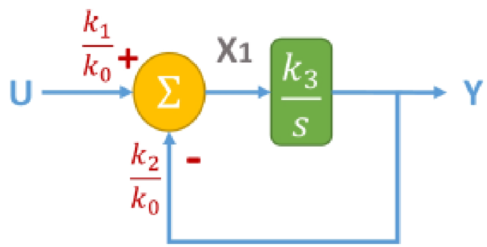
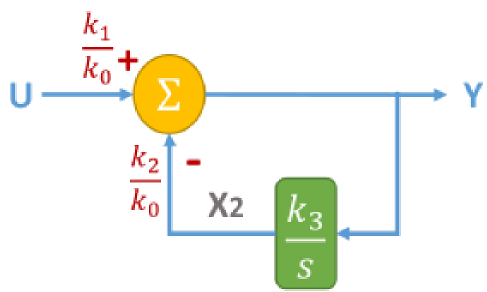
$$\forall s \in \mathbf{R}_+, \quad \mathcal{L}f(s) = \int_0^{+\infty} e^{-st} f(t) \mathrm{d}t$$

$\rightarrow$ Laplace transform of linear and time invariant systems are rational fractions of $\mathbf{R}(s)$.

Definition: Transfer function

The transfer function of a LTI system with input U and output Y is the rational fraction H such that $Y(s) = H(s)U(s)$.
It is said to be *strictly proper* when its degree is negative.

Transfer Function of Reaction Impl. [Jiang et al 2015]

Block Diagram	Normal Transfer Function	CRN Transfer Function
	$\frac{k_1}{k_0}$	$\frac{k_1}{s+k_0}$
	$\frac{k_1 k_3}{k_0 s + k_2 k_3}$	$\frac{k_1 k_3}{s^2 + k_0 s + k_2 k_3}$
	$\frac{k_1 s}{k_0 s + k_2 k_3}$	$\frac{k_1 s}{s^2 + k_0 s + k_2 k_3}$

Compiling Transfer Functions into Reactions

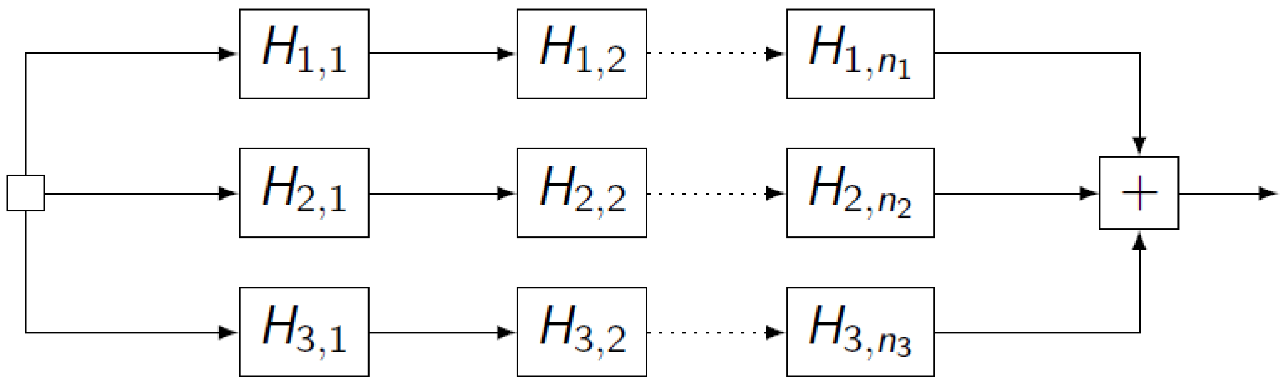
Compiler principle:

1. 'break' the transfer function into simple functions, this step is performed by computing the partial fraction expansion of H ;
2. each of these simple functions consist of a chemical network with input u and output y_i ;
3. recombine the individual outputs y_i to get y.

Compiling Transfer Functions into Reactions

- Write $H = \sum H_i$ where H_i are simple functions, that is either of the form $\frac{a}{(s+\alpha)^n}$ or $\frac{a}{(s^2+\beta s+\gamma)^m}$ or $\frac{bs}{(s^2+\beta s+\gamma)^m}$.
- Each of these functions can in turn be writen a product of elementary functions, that is either of the form $\frac{a}{s+\alpha}$ or $\frac{a}{s^2+\beta s+\gamma}$ or $\frac{bs}{s^2+\beta s+\gamma}$, denoted respectively by $(1,0)$, $(2,0)$ and $(2,1)$.
- Product corresponds to series composition of modules ;
- Sum is performed by parallel computation.

Final Summing Block



Remark: The summing node is chemically implemented through the reactions $y_i \xrightarrow{k} y_i + y$ for each local output y_i and $y \xrightarrow{k} \emptyset$.
Therefore one has

$$Y(s) = \frac{k}{s+k} \sum Y_i(s)$$

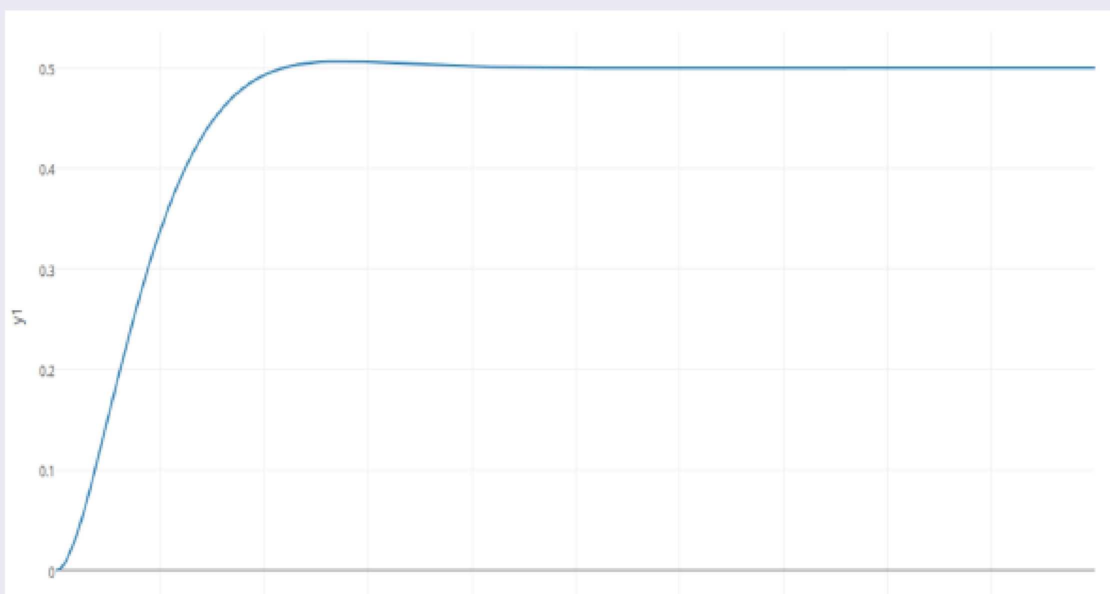
This unwanted factor can be compensated by computing $\hat{H} = \frac{s+k}{k} H$ instead of H.

Example: a first-order filter $\frac{1}{s+2}$, 'naive' implementation

Biocham

```
compile_wgpac([y1 :: integral x1,
               x1 :: u1 + (-2)*y1],
               10).
present(u1).
```

Illustration of time response: $y_1(t)$

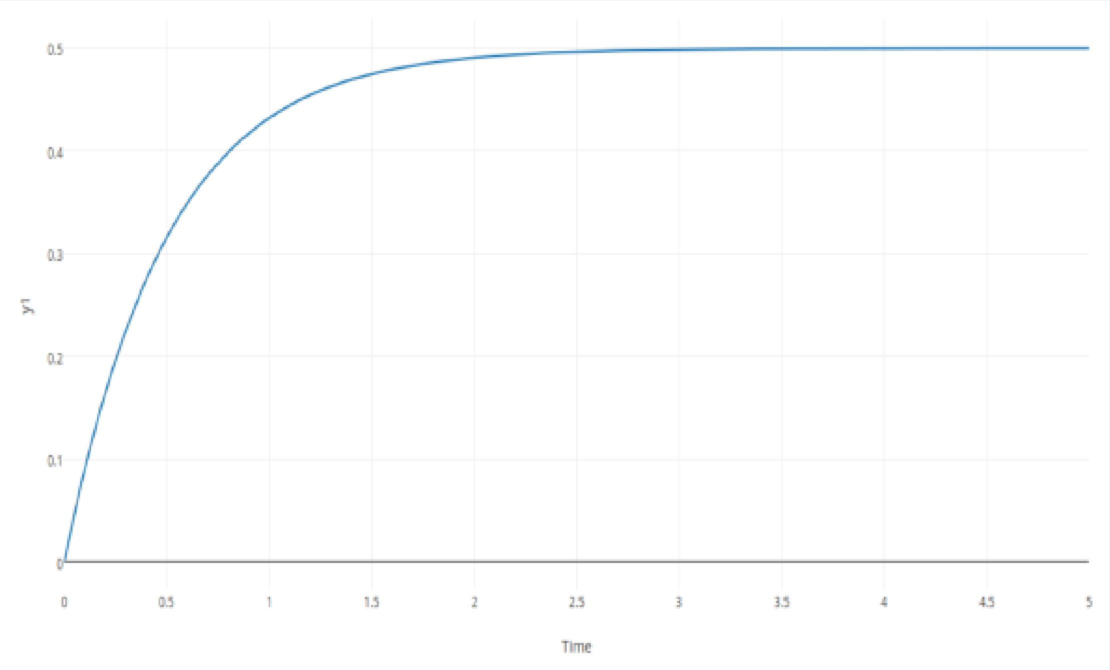


Example: a first-order filter $\frac{1}{s+2}$

Biocham

```
compile_transfer_function(1/(s+2), u1, y1).
present(u1).
```

Illustration of time response: $y_1(t)$



Comparison of the created systems

naive

```
[0] _=[x1]=>y1
[1] 10*[x_auto_4]*[y1]for _=[x_auto_4+y1]=>x_auto_3
[2] 10*[x_auto_3]for x_auto_3=>_
[3] 10*[u1]for _=[u1]=>x1
[4] 10*[x_auto_3]for _=[x_auto_3]=>x1
[5] 10*[x1]for x1=>_
```

transfer function specific

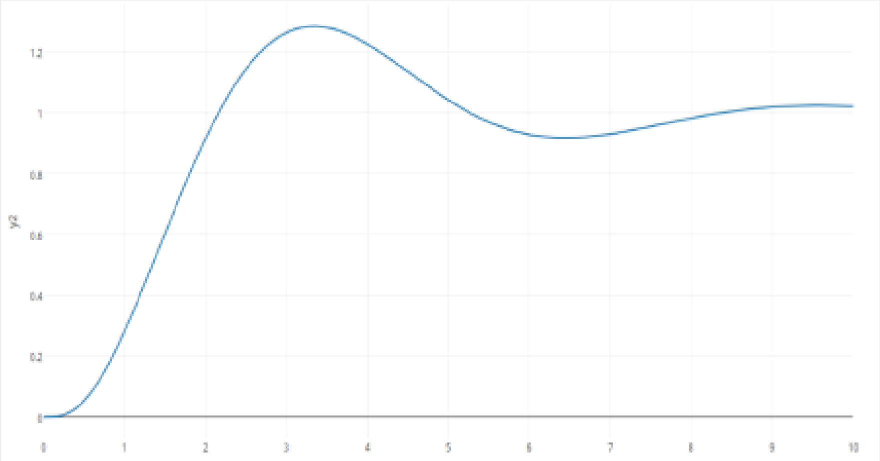
```
[0] _=[u1]=>y1
[1] 2*[y1]for y1=>_
```

Example: a second-order filter $\frac{1}{1+s+s^2}$, 'naive' implementation

Biocham

```
compile_wgpac([y1 :: integral x1, x1 :: u1 + (-1)*y1,
               y2 :: integral y1, u1 :: u2 + (-1)*y2],
               10).
present(u2).
```

Illustration of time response: $y_2(t)$

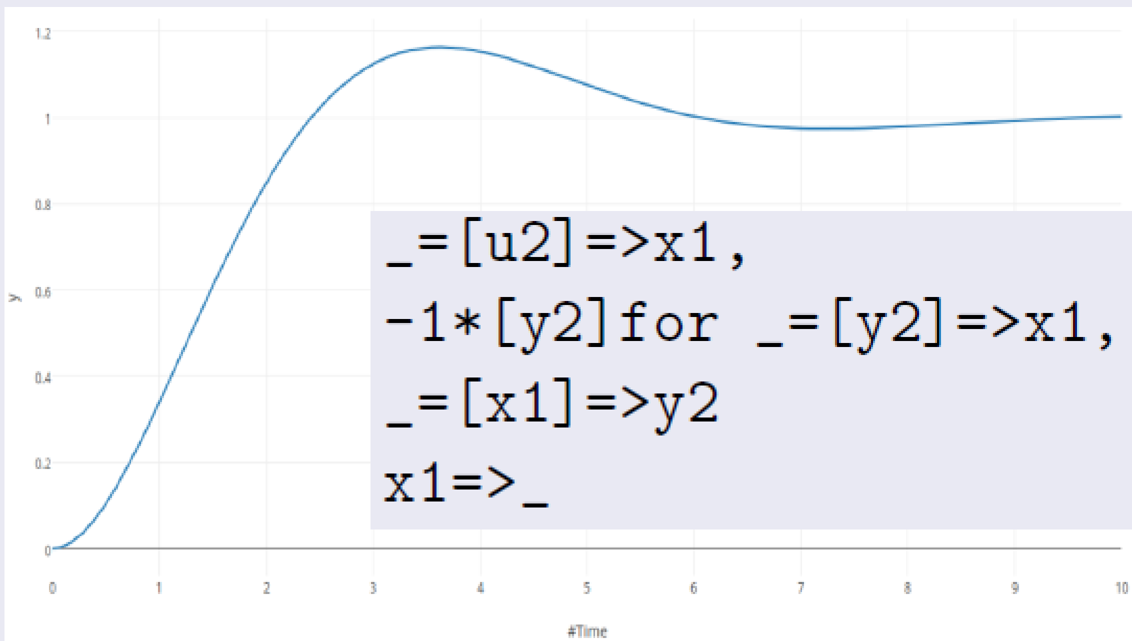


Example: a second-order filter $\frac{1}{1+s+s^2}$

Biocham

```
compile_transfer_function(1/(s*s+s+1), u2, y2).
present(u2).
```

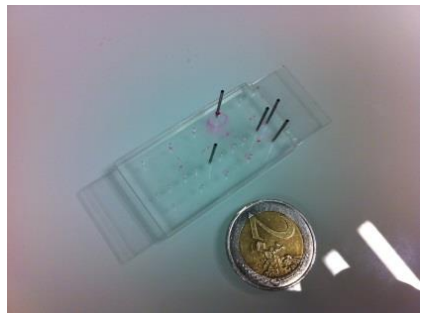
Illustration of time response: $y_2(t)$

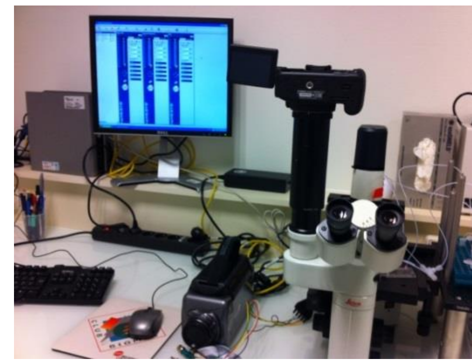



```
_=[u2]=>x1,
-1*[y2]for _=[y2]=>x1,
_=[x1]=>y2
x1=>_
```

Enzymatic Computation in Non-Living Vesicles

- Biosensor design and implementation in non-living vesicles
  [Franck Molina lab CNRS Sys2Diag Montpellier]





- Implementation of linear I/O systems, PI controllers and simple programs ?
  → Issue of approximation and compositionality
  → Issue of reaction code optimization (number of species and reactions)

- Comparison of synthetic programs with natural programs
  → Multiple functions of a circuit ?
  → Evolution history ? Evolution capacity ?

Thank you !

Et désolé si j'étais à l'ouest