True/False valuation of temporal logic formulae

The **True/False** valuation of temporal logic formulae is **not well adapted** to several problems :

- parameter search, optimization and control of continuous models
- quantitative estimation of robustness
- sensitivity analyses

True/False valuation of temporal logic formulae

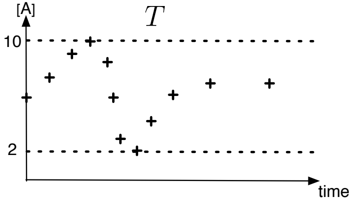
The **True/False** valuation of temporal logic formulae is **not well adapted** to several problems :

- parameter search, optimization and control of continuous models
- quantitative estimation of robustness
- sensitivity analyses

$\rightarrow$ need for a continuous degree of satisfaction of temporal logic formulae

How far is the system from verifying the specification ?

Model-Checking Generalized to Constraint Solving



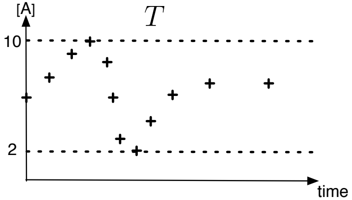
$LTL(\mathbb{R})$

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

Model-checking

the formula is false

Model-Checking Generalized to Constraint Solving



$LTL(\mathbb{R})$

Φ=F([A]≥7
∧F([A]≤0))

Model-checking

the formula is false

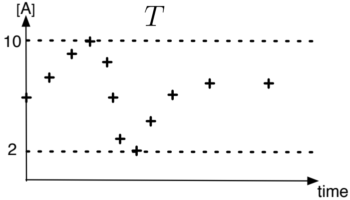
$QFLTL(\mathbb{R})$

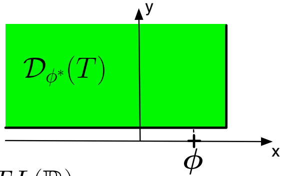
Φ*=F([A]≥x
∧F([A]≤y))

Constraint solving

the formula is true for any
x≤10 ∧ y≥2

Model-Checking Generalized to Constraint Solving

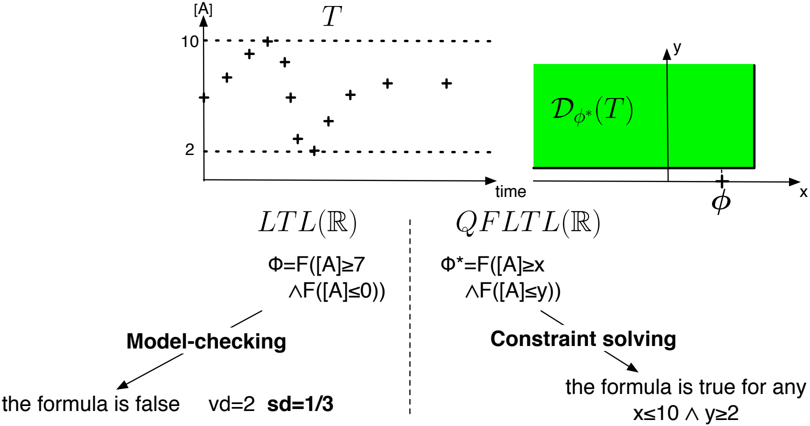




$LTL(\mathbb{R})$	$QFLTL(\mathbb{R})$
Φ=F([A]≥7 ∧F([A]≤0))	Φ*=F([A]≥x ∧F([A]≤y))
Model-checking	**Constraint solving**
the formula is false	the formula is true for any x≤10 ∧ y≥2

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

Model-Checking Generalized to Constraint Solving



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

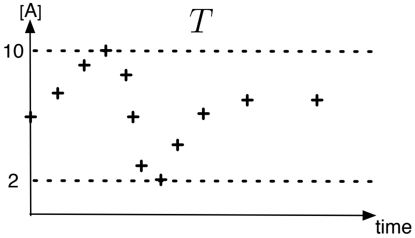
Violation degree $vd(T,\phi) = \text{distance}(val(\phi), D_{\phi^*}(T))$

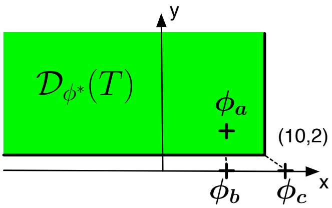
Satisfaction degree $sd(T,\phi) = \frac{1}{1+vd(T,\phi)} \in [0,1]$

Violation degree of an LTL formula

Definition of violation degree $vd(T,\phi)$ and satisfaction degree $sd(T,\phi)$

In the variable space of ϕ^*, original formula ϕ is single point $var(\phi)$.
$vd(T,\phi) = min_{v \in D_{\phi^*}(T)} d(v, var(\phi))$ $sd(T,\phi) = \frac{1}{1+vd(T,\phi)} \in [0,1]$





ϕ^*(x,y)= F([A]≥x ∧ F([A]≤y))

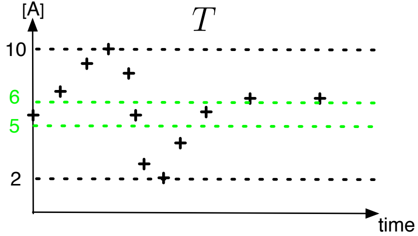
ϕ_a = F([A]≥6 ∧ F([A]≤5))	$\phi^*(6,5)$	vd=0	(✓)
ϕ_b = F([A]≥6 ∧ F([A]≤0))	$\phi^*(6,0)$	vd=2	(✕)
ϕ_c = F([A]≥12 ∧ F([A]≤0))	$\phi^*(12,0)$	vd=2√2	(✕)

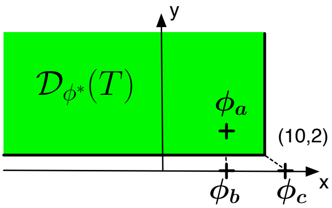
Violation degree of an LTL formula

Definition of violation degree $vd(T,\phi)$ and satisfaction degree $sd(T,\phi)$

In the variable space of ϕ^*, original formula ϕ is single point $var(\phi)$.

$vd(T,\phi) = min_{v \in D_{\phi^*}(T)} d(v, var(\phi))$ $\qquad$ $sd(T,\phi) = \frac{1}{1+vd(T,\phi)} \in [0,1]$





ϕ^*(x,y)= F([A]≥x ∧ F([A]≤y))

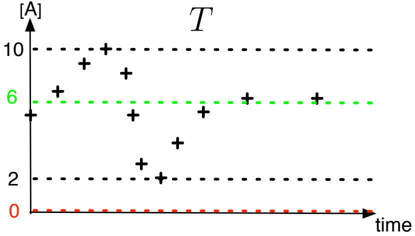
ϕ_a = F([A]≥6 ∧ F([A]≤5))	$\phi^*_{(6,5)}$	*vd*=0	(✓)
ϕ_b = F([A]≥6 ∧ F([A]≤0))	$\phi^*_{(6,0)}$	*vd*=2	(✕)
ϕ_c = F([A]≥12 ∧ F([A]≤0))	$\phi^*_{(12,0)}$	*vd*=2√2	(✕)

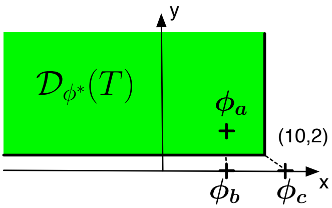
Violation degree of an LTL formula

Definition of violation degree $vd(T,\phi)$ and satisfaction degree $sd(T,\phi)$

In the variable space of ϕ^*, original formula ϕ is single point $var(\phi)$.

$vd(T,\phi) = min_{v \in D_{\phi^*}(T)} d(v, var(\phi))$ $\qquad$ $sd(T,\phi) = \frac{1}{1+vd(T,\phi)} \in [0,1]$





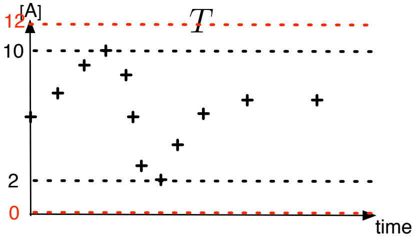
$\phi^*(x,y)$= F([A]≥x ∧ F([A]≤y))

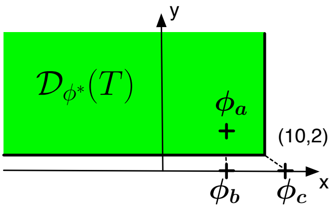
ϕ_a = F([A]≥6 ∧ F([A]≤5))	$\phi^*(6,5)$	*vd*=0	(✓)
ϕ_b = F([A]≥6 ∧ F([A]≤0))	$\phi^*(6,0)$	*vd*=2	(✕)
ϕ_c = F([A]≥12 ∧ F([A]≤0))	$\phi^*(12,0)$	*vd*=2√2	(✕)

Violation degree of an LTL formula

Definition of violation degree $vd(T,\phi)$ and satisfaction degree $sd(T,\phi)$

In the variable space of ϕ^*, original formula ϕ is single point $var(\phi)$.
$vd(T,\phi) = min_{v \in D_{\phi^*}(T)} d(v, var(\phi))$ $\quad$ $sd(T,\phi) = \frac{1}{1+vd(T,\phi)} \in [0,1]$



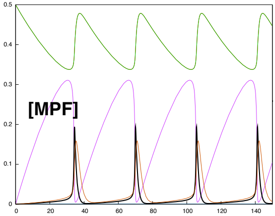


ϕ^*(x,y)= F([A]≥x ∧ F([A]≤y))

ϕ_a = F([A]≥6 ∧ F([A]≤5))	$\phi^*_{(6,5)}$	*vd*=0	(✓)
ϕ_b = F([A]≥6 ∧ F([A]≤0))	$\phi^*_{(6,0)}$	*vd*=2	(✗)
ϕ_c = F([A]≥12 ∧ F([A]≤0))	$\phi^*_{(12,0)}$	*vd*=2√2	(✗)

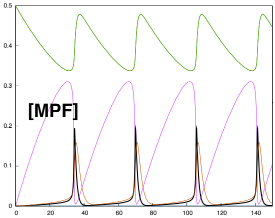
Learning kinetic parameter values from LTL specifications

- simple model of the yeast cell cycle from [Tyson PNAS 91]
- models Cdc2 and Cyclin interactions (6 variables, 8 kinetic parameters)



Learning kinetic parameter values from LTL specifications

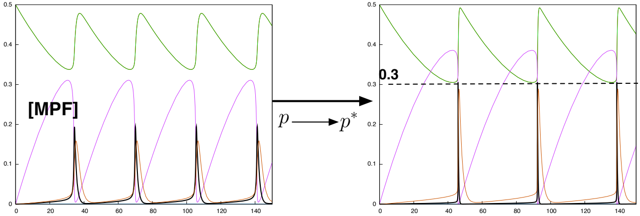
- simple model of the yeast cell cycle from [Tyson PNAS 91]
- models Cdc2 and Cyclin interactions (6 variables, 8 kinetic parameters)



- Pb : find values of 8 parameters such that amplitude is ≥ 0.3
  ϕ^*: **F**([A]>x $\wedge$ **F**([A]<y))
  amplitude z=x-y
  goal : z = 0.3

Learning kinetic parameter values from LTL specifications

- simple model of the yeast cell cycle from [Tyson PNAS 91]
- models Cdc2 and Cyclin interactions (6 variables, 8 kinetic parameters)



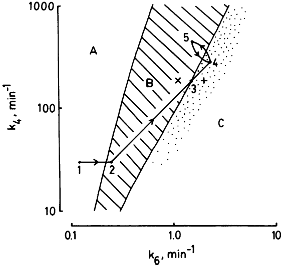
- Pb : find values of 8 parameters such that amplitude is $\geq$ 0.3
  ϕ^*: **F**([A]>x $\wedge$ **F**([A]<y))
  amplitude z=x-y
  goal : z = 0.3
- $\rightarrow$ solution found after 30s (100 calls to the fitness function)

LTL Continuous Satisfaction Diagram

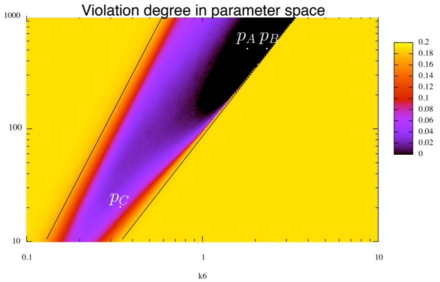
Example with :

- yeast cell cycle model [Tyson PNAS 91]
- oscillation of at least 0.3

$$\phi^*\text{: } \mathbf{F}(\ [A]>x \wedge \mathbf{F}([A]<y)\ );\ \text{amplitude } x\text{-}y \geq 0.3$$



Bifurcation diagram



LTL satisfaction diagram

Black-box Randomized Non-linear Optimization Method

- Use existing non-linear optimization toolbox for kinetic parameter search using satisfaction degree as fitness function

Black-box Randomized Non-linear Optimization Method

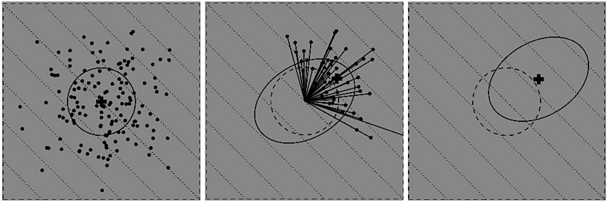
- Use existing non-linear optimization toolbox for kinetic parameter search using satisfaction degree as fitness function
- We use the state-of-the-art Covariance Matrix Adaptation Evolution Strategy (CMA-ES) [Hansen Osermeier 01, Hansen 08]

Black-box Randomized Non-linear Optimization Method

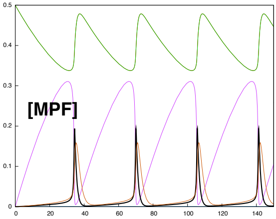
- Use existing non-linear optimization toolbox for kinetic parameter search using satisfaction degree as fitness function
- We use the state-of-the-art Covariance Matrix Adaptation Evolution Strategy (CMA-ES) [Hansen Osermeier 01, Hansen 08]
- CMA-ES maximizes an objective function in continuous domain in a black box scenario

Black-box Randomized Non-linear Optimization Method

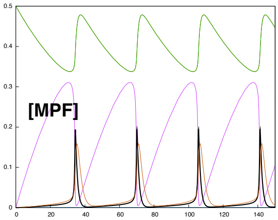
- Use existing non-linear optimization toolbox for kinetic parameter search using satisfaction degree as fitness function
- We use the state-of-the-art Covariance Matrix Adaptation Evolution Strategy (CMA-ES) [Hansen Osermeier 01, Hansen 08]
- CMA-ES maximizes an objective function in continuous domain in a black box scenario
- CMA-ES uses a probabilistic neighborhood and updates information in covariance matrix at each move



Learning Parameter Values from Period Constraints in LTL



Learning Parameter Values from Period Constraints in LTL



- Pb : find values of 8 parameters such that period is 20

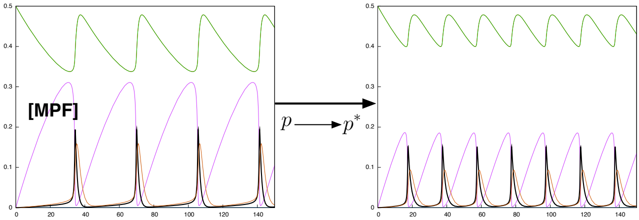
  ϕ^*:**F**(MPF$_{localmaximum}$ $\wedge$Time=t1$\wedge$ **F**(MPF$_{localmaximum}$ $\wedge$Time=t2))

  (with MPF$_{localmaximum}$: d([MPF])/dt>0 $\wedge$ **X**(d([MPF])/dt<0))

  period z=t2-t1

  goal z=20

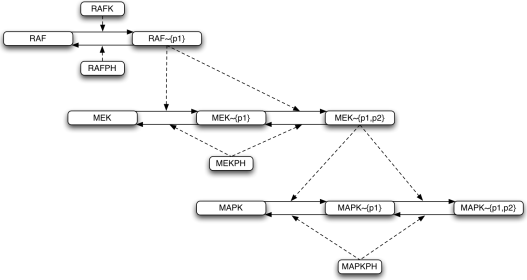
Learning Parameter Values from Period Constraints in LTL



- Pb : find values of 8 parameters such that period is 20
  ϕ^*:**F**(MPF$_{localmaximum}$ $\wedge$Time=t1$\wedge$ **F**(MPF$_{localmaximum}$ $\wedge$Time=t2))
  (with MPF$_{localmaximum}$: d([MPF])/dt>0 $\wedge$ **X**(d([MPF])/dt<0))
  period z=t2-t1
  goal z=20
- $\rightarrow$ Solution found after 60s (200 calls to the fitness function)

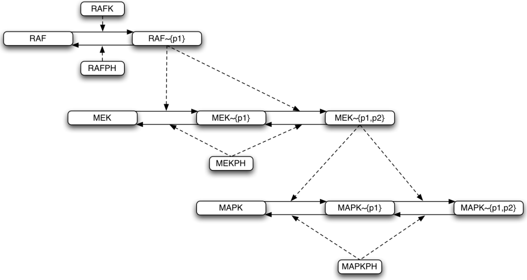
Oscillations in MAPK signal transduction cascade

- **MAPK** signaling model [Huang Ferrel PNAS 96]



Oscillations in MAPK signal transduction cascade

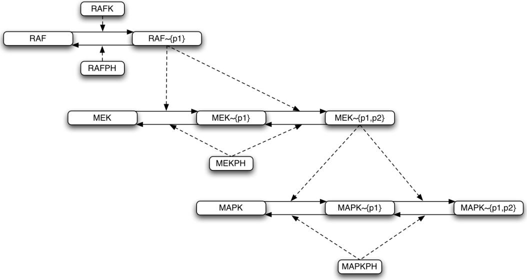
- **MAPK** signaling model [Huang Ferrel PNAS 96]



- **search for oscillations** in **37 dimensions** (30 parameters and 7 initial conditions)
  $\rightarrow$ solution found after 3 min (200 calls to the fitness function)
  Oscillations already observed by simulation [Qiao et al. 07]

Oscillations in MAPK signal transduction cascade

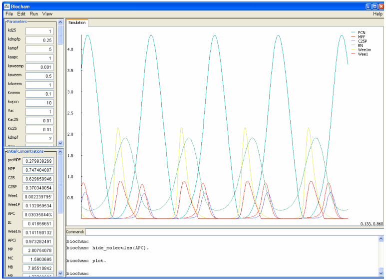
- **MAPK** signaling model [Huang Ferrel PNAS 96]



- **search for oscillations** in **37 dimensions** (30 parameters and 7 initial conditions)
  $\rightarrow$ solution found after 3 min (200 calls to the fitness function)
  Oscillations already observed by simulation [Qiao et al. 07]
- No negative feedback in the **reaction graph**, but negative circuits in the **influence graph** [Fages Soliman FMSB'08, CMSB'06]

Coupled Models of Cell Cycle, Circadian Clock, DNA repair

- Context of colorectal cancer chronotherapies
  EU FP6 TEMPO, EraSysBio C5Sys, coord. F. Lévi INSERM Villejuif
- Coupled model of the cell cycle [Tyson Novak 04][Gerard Goldbeter 09] and the circadian clock [Leloup Goldbeter 99] with condition of entrainment in period [Calzone Soliman 06]



- Coupled model with DNA repair system p53/Mdm2 [Cilberto et al.04], metabolism of irinotecan, and drug administration optimization [De Maria Soliman Fages 09 CMSB]

Basis of Operators of LTL(R)

Atomic propositions: arithmetic expressions with $<, \leq, =, \geq, >$ over the state variables (closed by negation)

Duality: $\neg \mathbf{X}\phi = \mathbf{X}\neg\phi$, $\neg \mathbf{F}\phi = \mathbf{G}\neg\phi$, $\neg \mathbf{G}\phi = \mathbf{F}\neg\phi$,
$\neg(\phi \ \mathbf{U} \ \psi) = (\neg\psi \ \mathbf{W} \ \neg\phi)$, $\neg(\phi \ \mathbf{W} \ \psi) = (\neg\psi \ \mathbf{U} \ \neg\phi)$,

Properties: $\mathbf{F}\phi = \text{true} \ \mathbf{U} \ \phi$, $\mathbf{G}\phi = \phi \ \mathbf{W} \ \text{false}$, $\phi \mathbf{W} \psi = \mathbf{G}\phi \vee (\phi \mathbf{U} (\phi \wedge \psi))$

Negation free formulae: expressed with $\wedge$, $\vee$, **F**, **G**, **U**, **X** with negations eliminated down to atomic propositions.

LTL(R) model-checking

Given a finite trace T and an LTL(R) formula ϕ

1. label each state with the atomic sub-formulae of ϕ that are true at this state;
2. add sub-formulae of the form $\phi_1\ U\ \phi_2$ to the states labeled by ϕ_2 and to the predecessors of states labeled with ϕ_2 as long as they are labeled by ϕ_1;
3. add sub-formulae of the form $\phi_1\ \mathbf{W}\ \phi_2$ to the last state if it is labeled by ϕ_1, and to the states labeled by ϕ_1 and ϕ_2, and to their predecessors as long as they are labeled by ϕ_1;
4. add sub-formulae of the form $\mathbf{X}\phi$ to the last state if it is labeled by ϕ and to the immediate predecessors of states labeled by ϕ;
5. return the vertices labeled by ϕ.

QFLTL(R) Formulae with Variables

Quantifier free LTL formulae, noted $\phi(\mathbf{y})$ with free variables $\mathbf{y}$

The *satisfaction domain* of $\phi(\mathbf{y})$ in a trace T is the set of $\mathbf{y}$ values for which $\phi(\mathbf{y})$ holds:

$$\mathcal{D}_{T,\phi(\mathbf{y})} = \{\mathbf{y} \in \mathbb{R}^q \mid T \models \phi(\mathbf{y})\} \tag{1}$$

For linear constraints over R, satisfaction domains can be computed with polyhedral libraries.

Biocham uses the Parma Polyhedral Library PPL

QFLTL(R) constraint solving

The satisfaction domains of QFLTL formulae satisfy the equations:

- $\mathcal{D}_{T,\phi(\mathbf{y})} = \mathcal{D}_{s_0,\phi(\mathbf{y})}$,
- $\mathcal{D}_{s_i,\pi(\mathbf{y})} = \{\mathbf{y} \in \mathbb{R}^m \mid s_i \models_{\mathcal{R}} \pi(\mathbf{y})\}$,
- $\mathcal{D}_{s_i,\phi(\mathbf{y})\wedge\psi(\mathbf{y})} = \mathcal{D}_{s_i,\phi(\mathbf{y})} \cap \mathcal{D}_{s_i,\psi(\mathbf{y})}$,
- $\mathcal{D}_{s_i,\phi(\mathbf{y})\vee\psi(\mathbf{y})} = \mathcal{D}_{s_i,\phi(\mathbf{y})} \cup \mathcal{D}_{s_i,\psi(\mathbf{y})}$,
- $\mathcal{D}_{s_i,\mathbf{F}\phi(\mathbf{y})} = \cup_{j\in[i,n]}\mathcal{D}_{s_j,\phi(\mathbf{y})}$,
- $\mathcal{D}_{s_i,\mathbf{G}\phi(\mathbf{y})} = \cap_{j\in[i,n]}\mathcal{D}_{s_j,\phi(\mathbf{y})}$,
- $\mathcal{D}_{s_i,\phi(\mathbf{y})\mathbf{U}\psi(\mathbf{y})} = \cup_{j\in[i,n]}(\mathcal{D}_{s_j,\psi(\mathbf{y})} \cap \cap_{k\in[i,j-1]}\mathcal{D}_{s_k,\phi(\mathbf{y})})$,
- $\mathcal{D}_{s_i,\mathbf{X}\phi(\mathbf{y})} = \begin{cases} \mathcal{D}_{s_{i+1},\phi(\mathbf{y})}, & \text{if } i < n, \\ \mathcal{D}_{s_i,\phi(\mathbf{y})}, & \text{if } i = n, \end{cases}$

Complexity with bound constraints $x > b$, $x < b$

Bound constraints define boxes $\mathcal{R}_i \in \mathbb{R}^v$.

Let the size of a union of boxes be the least integer k such that $\mathcal{D} = \bigcup_{i=1}^{k} \mathcal{R}_i$.

Proposition (complexity of the solution domain)

The validity domain of a QFLTL formula of size f containing v variables on a trace of length n is a union of boxes of size less than $(nf)^{2v}$.

The maximum number of bounds for a variable x is $n \times f$
E.g; $\mathbf{F}([A] = u \vee [A] + 1 = u \vee \cdots \vee [A] + f = u)$

$\mathbf{F}([A_1] = X_1 \vee [A_1] + 1 = X_1 \vee ... \vee [A_1] + f = X_1) \ \wedge \ ...$
$\wedge \ \mathbf{F}([A_v] = X_v \vee [A_v] + 1 = X_v \vee ... \vee [A_v] + f = X_v)$
has a solution domain of size $(nf)^v$ on a trace of n values with $[A_i] + k$ all different for $1 \leq i \leq v$, $0 \leq k \leq f$.

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]:

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

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]:

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

- Computational measure of robustness w.r.t. LTL($\mathbb{R}$) spec:

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

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

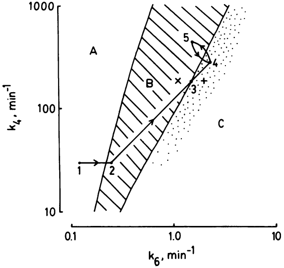
Robustness analysis w.r.t parameter perturbations

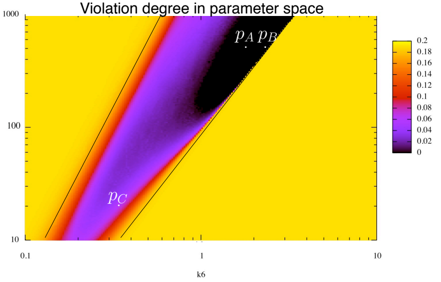
Example with :

- cell cycle model [Tyson PNAS 91]
- oscillation of amplitude at least 0.2

$$\phi^*\text{: } \mathbf{F}(\ [A]>x \wedge \mathbf{F}([A]<y)\ );\ \text{amplitude x-y} \geq 0.2$$

- parameters normally distributed, $\mu = p_{ref}$, CV=0.2

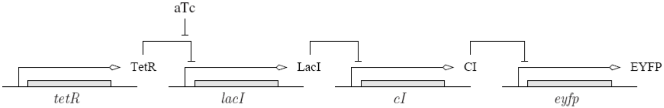


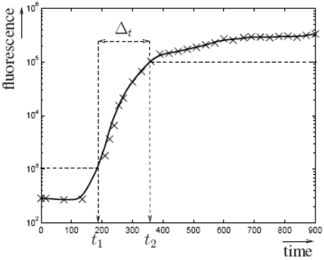


$$\mathcal{R}_{\phi,p_A} = 0.83,\ \mathcal{R}_{\phi,p_B} = 0.43,\ \mathcal{R}_{\phi,p_C} = 0.49$$

Application to Synthetic Biology in *E. Coli*

Cascade of transcriptional inhibitions added to *E.coli* [Weiss et al PNAS 05]
input small molecule aTc **output** protein EYFP





Specification: EYFP has to remain below 10^3 for at least 150 min., then exceeds 10^5 after at most 450 min., and switches from low to high levels in less than 150 min.

Specifying the expected behavior in QFLTL($\mathbb{R}$)

The timing specifications can be formalized in temporal logic as follows:

$$\begin{aligned}\phi(t_1, t_2) = \quad & \mathbf{G}(\mathit{time} < t_1 \rightarrow [\mathrm{EYFP}] < 10^3) \\ \wedge \quad & \mathbf{G}(\mathit{time} > t_2 \rightarrow [\mathrm{EYFP}] > 10^5) \\ \wedge \quad & t_1 > 150 \wedge t_2 < 450 \wedge t_2 - t_1 < 150\end{aligned}$$

which is abstracted into

$$\begin{aligned}\phi(t_1, t_2, b_1, b_2, b_3) = \quad & \mathbf{G}(\mathit{time} < t_1 \rightarrow [\mathrm{EYFP}] < 10^3) \\ \wedge \quad & \mathbf{G}(\mathit{time} > t_2 \rightarrow [\mathrm{EYFP}] > 10^5) \\ \wedge \quad & t_1 > b1 \wedge t_2 < b_2 \wedge t_2 - t_1 < b_3\end{aligned}$$

for computing validity domains for b_1, b_2, b_3

with the objective $b_1 = 150, b_2 = 450, b_3 = 150$
for computing the satisfaction degree in a given trace.

Improving robustness

Variance-based global sensitivity indices

$$S_i = \frac{Var(E(R|P_i))}{Var(R)} \in [0, 1]$$

S_γ	20.2 %	$S_{\kappa_{eyfp},\gamma}$	8.7 %
$S_{\kappa_{eyfp}}$	7.4 %	$S_{\kappa_{cl},\gamma}$	6.2 %
$S_{\kappa_{cl}}$	6.1 %	$S_{\kappa^0_{cl},\gamma}$	5.0 %
$S_{\kappa^0_{lacI}}$	3.3 %	$S_{\kappa^0_{cl},\kappa_{eyfp}}$	2.8 %
$S_{\kappa^0_{cl}}$	2.0 %	$S_{\kappa_{cl},\kappa_{eyfp}}$	1.8 %
$S_{\kappa_{lacI}}$	1.5 %	$S_{\kappa^0_{eyfp},\gamma}$	1.5 %
$S_{\kappa^0_{eyfp}}$	0.9 %	$S_{\kappa^0_{cl},\kappa_{cl}}$	1.1 %
$S_{u_{aTc}}$	0.4 %	$S_{\kappa^0_{cl},\kappa_{lacI}}$	0.5 %
total first order	40.7 %	total second order	31.2 %

degradation factor γ has the strongest impact on the cascade.

Improving robustness

Variance-based global sensitivity indices

$$S_i = \frac{Var(E(R|P_i))}{Var(R)} \in [0, 1]$$

S_γ	20.2 %	$S_{\kappa_{eyfp},\gamma}$	8.7 %
$S_{\kappa_{eyfp}}$	7.4 %	$S_{\kappa_{cl},\gamma}$	6.2 %
$S_{\kappa_{cl}}$	6.1 %	$S_{\kappa^0_{cl},\gamma}$	5.0 %
$S_{\kappa^0_{lacl}}$	3.3 %	$S_{\kappa^0_{cl},\kappa_{eyfp}}$	2.8 %
$S_{\kappa^0_{cl}}$	2.0 %	$S_{\kappa_{cl},\kappa_{eyfp}}$	1.8 %
$S_{\kappa_{lacl}}$	1.5 %	$S_{\kappa^0_{eyfp},\gamma}$	1.5 %
$S_{\kappa^0_{eyfp}}$	0.9 %	$S_{\kappa^0_{cl},\kappa_{cl}}$	1.1 %
$S_{u_{aTc}}$	0.4 %	$S_{\kappa^0_{cl},\kappa_{lacl}}$	0.5 %
total first order	40.7 %	total second order	31.2 %

degradation factor γ has the strongest impact on the cascade.

aTc variations have a very low impact

Improving robustness

Variance-based global sensitivity indices

$$S_i = \frac{Var(E(R|P_i))}{Var(R)} \in [0, 1]$$

S_γ	20.2 %	$S_{\kappa_{eyfp},\gamma}$	8.7 %
$S_{\kappa_{eyfp}}$	7.4 %	$S_{\kappa_{cl},\gamma}$	6.2 %
$S_{\kappa_{cl}}$	6.1 %	$S_{\kappa^0_{cl},\gamma}$	5.0 %
$S_{\kappa^0_{lacI}}$	3.3 %	$S_{\kappa^0_{cl},\kappa_{eyfp}}$	2.8 %
$S_{\kappa^0_{cl}}$	2.0 %	$S_{\kappa_{cl},\kappa_{eyfp}}$	1.8 %
$S_{\kappa_{lacI}}$	1.5 %	$S_{\kappa^0_{eyfp},\gamma}$	1.5 %
$S_{\kappa^0_{eyfp}}$	0.9 %	$S_{\kappa^0_{cl},\kappa_{cl}}$	1.1 %
$S_{u_{aTc}}$	0.4 %	$S_{\kappa^0_{cl},\kappa_{lacI}}$	0.5 %
total first order	40.7 %	total second order	31.2 %

degradation factor γ has the strongest impact on the cascade.

aTc variations have a very low impact

different importance of the basal κ^0_{eyfp} and regulated κ_{eyfp} EYFP production rates

Improving robustness

Variance-based global sensitivity indices

$$S_i = \frac{Var(E(R|P_i))}{Var(R)} \in [0,1]$$

S_γ	20.2 %	$S_{\kappa_{eyfp},\gamma}$	8.7 %
$S_{\kappa_{eyfp}}$	7.4 %	$S_{\kappa_{cl},\gamma}$	6.2 %
$S_{\kappa_{cl}}$	6.1 %	$S_{\kappa^0_{cl},\gamma}$	5.0 %
$S_{\kappa^0_{lacl}}$	3.3 %	$S_{\kappa^0_{cl},\kappa_{eyfp}}$	2.8 %
$S_{\kappa^0_{cl}}$	2.0 %	$S_{\kappa_{cl},\kappa_{eyfp}}$	1.8 %
$S_{\kappa_{lacl}}$	1.5 %	$S_{\kappa^0_{eyfp},\gamma}$	1.5 %
$S_{\kappa^0_{eyfp}}$	0.9 %	$S_{\kappa^0_{cl},\kappa_{cl}}$	1.1 %
$S_{u_{aTc}}$	0.4 %	$S_{\kappa^0_{cl},\kappa_{lacl}}$	0.5 %
total first order	40.7 %	total second order	31.2 %

degradation factor γ has the strongest impact on the cascade.

aTc variations have a very low impact

different importance of the basal κ^0_{eyfp} and regulated κ_{eyfp} EYFP production rates

the basal production of EYFP is due to an incomplete repression of the promoter by CI (high effect of κ_{cl}) rather than a constitutive leakage of the promoter (low effect of κ^0_{eyfp}).