

# Graph-based Modeling of Biological Regulatory Networks : Introduction of Singular States

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# Motivations

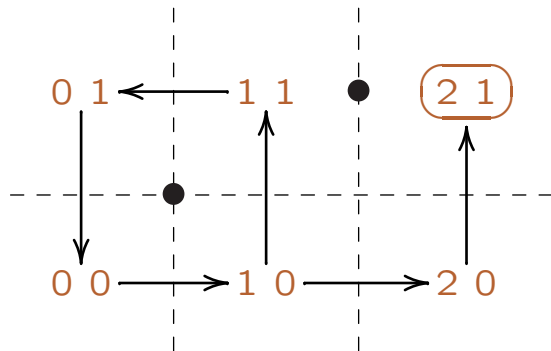
Dynamics of regulatory networks



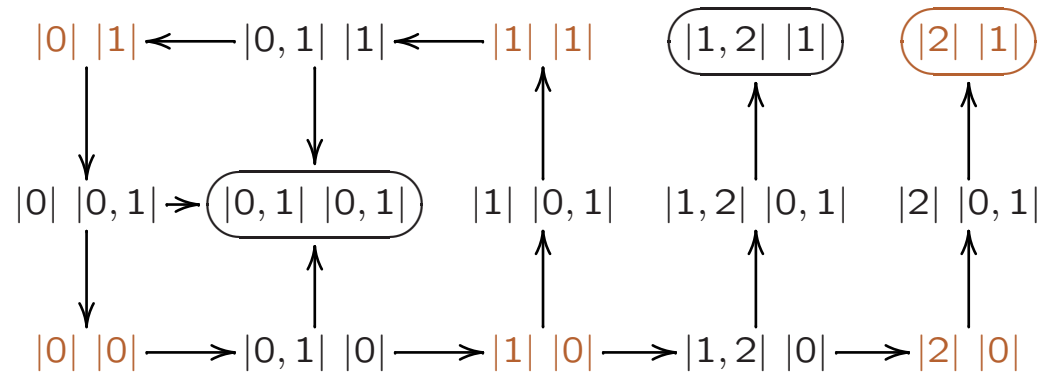
Differential systems



Discretization



Thomas' formalism



Introduction of singular states

The introduction of singular states in Thomas' formalism allows us to check by computer temporal properties concerning them

# Summary

1. **Dynamics of regulatory networks** with singular states
  - ▷ Quantitative description
  - ▷ Qualitative description
2. **Selection of models** : how find satisfactory models ?
  - ▷ Feedback circuit functionality
  - ▷ Temporal logic and Model checking
3. **SMBioNet** : a tool for the selection of models
4. **Conclusions**

# Biological regulatory networks

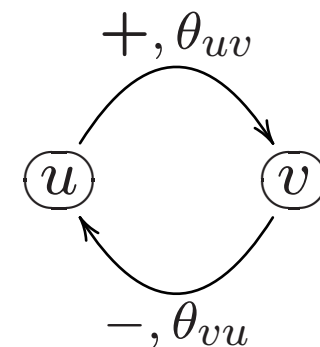
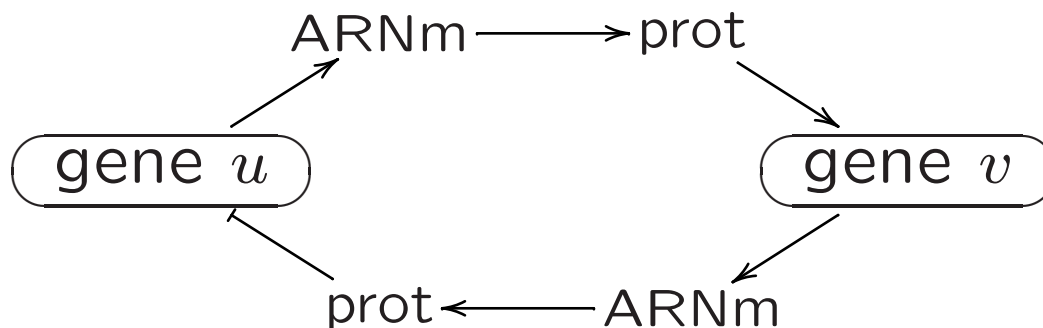
Regulatory networks  $\Rightarrow$  oriented labelled graphs :

▷ vertices  $\Rightarrow$  biological entities

▷ edges  $\Rightarrow$  regulations (activations/inhibitions) dependent on thresholds

A regulation  $u \rightarrow v$  is labelled by a sign  $\alpha_{uv}$  and a threshold  $\theta_{uv}$

High abstraction level :

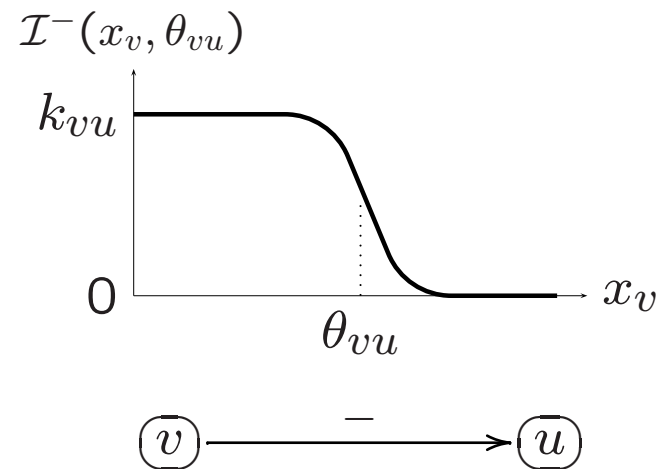
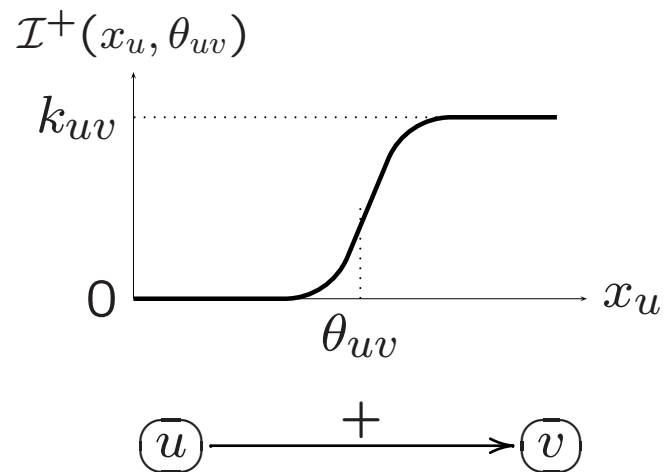


# Quantitative description

Differential equation systems :

$$\frac{dx_v}{dt} = \sum_{u \rightarrow v} \mathcal{I}^{\alpha_{uv}}(x_u, \theta_{uv}) - \lambda_v x_v \quad \text{for all } v$$

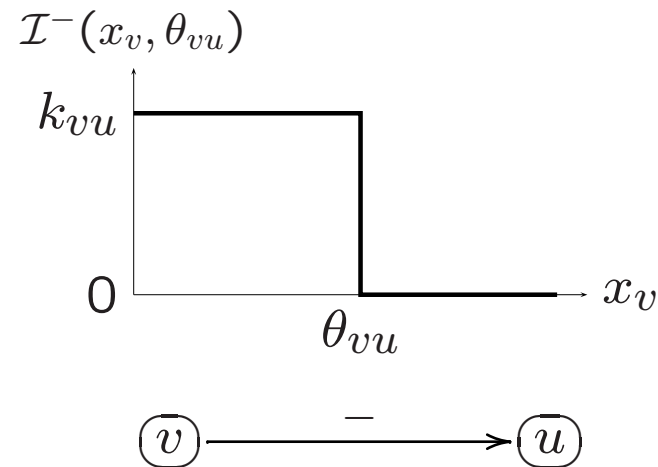
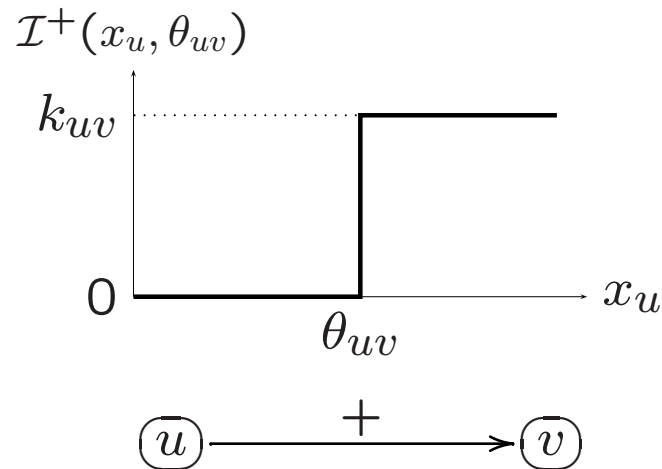
= synthesis rate – degradation rate



- ▷ No analytic solution
- ▷ Too many unknown parameters

# Quantitative description

Piecewise linear approximation :



- ▷ Uncertain effect on thresholds :  $\mathcal{I}^-(\theta_{uv}, \theta_{uv}) = ]0, k_{uv}[$
- ▷ On singular states, the equation system becomes an inclusion system :

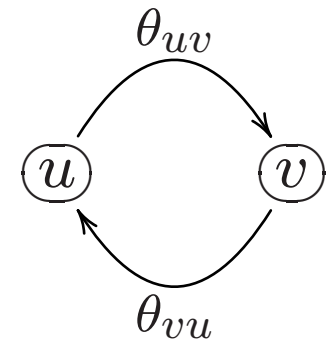
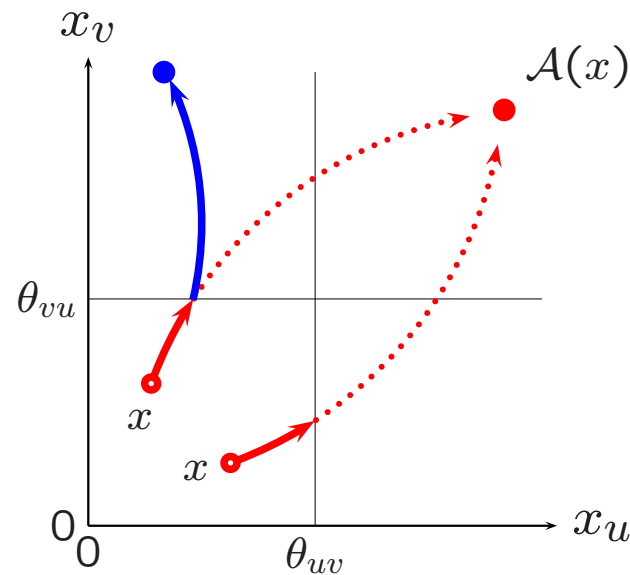
$$\frac{dx_v}{dt} \in \sum_{u \rightarrow v} \mathcal{I}^{\alpha_{uv}}(x_u, \theta_{uv}) - \lambda_v x_v, \quad \text{for all } v$$

# Quantitative description

Piecewise linear approximation :

- ▷ **Analytic solution** on each domain where the synthesis rates are constant

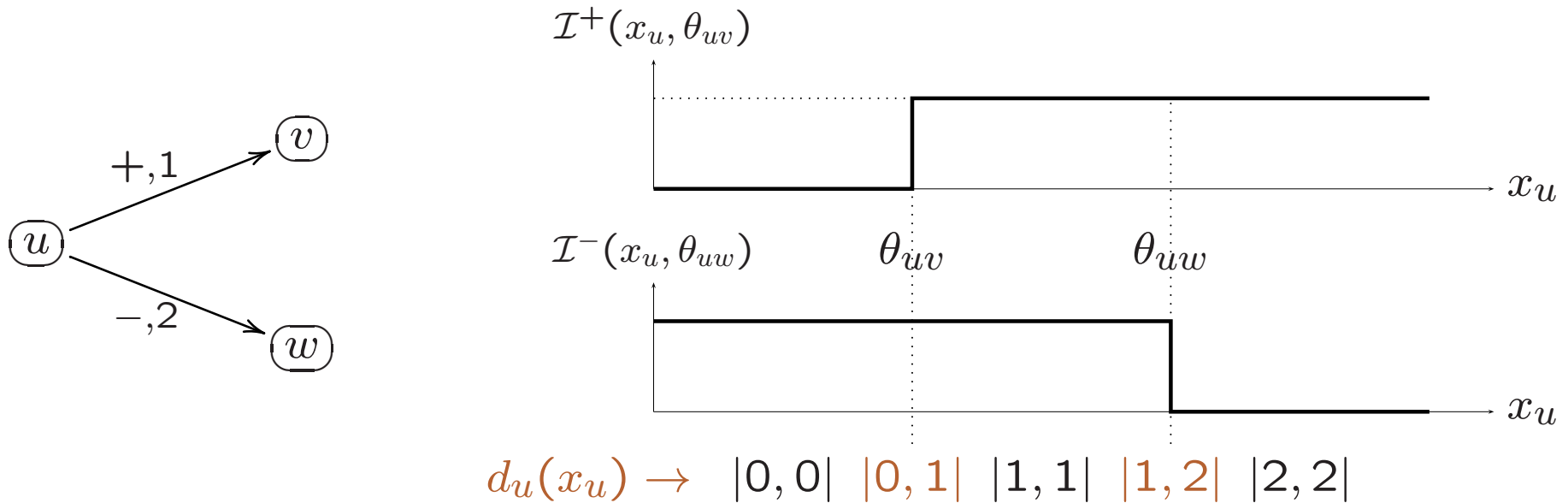
$$\mathcal{A}(x) = \lim_{t \rightarrow \infty} x(t)$$



- ▷ Towards a **qualitative description**

# Qualitative description : discretization

A gene  $u$  with  $n$  targets has  $2n - 1$  qualitative expression levels :



The qualitative variable  $x_u = d_u(x_u)$  can have two kinds of values :

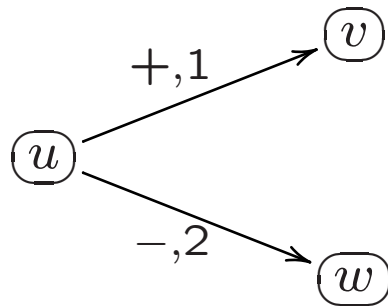
- ▷ **regular value** : if  $x_u = |q, q| = |q|$  then  $u$  regulates  $q$  of its targets
- ▷ **singular value** : if  $x_u = |q, q + 1|$  then  $u$  regulates the same  $q$  targets and regulates **uncertainly** an other one



# Qualitative description : resources

At the qualitative state  $x$  :

- ▷ the **regular resources**  $R_v(x)$  of a gene  $v$  are the genes which induce the increase of synthesis of  $v$
- ▷ the **singular resources**  $S_v(x)$  of a gene  $v$  are the genes which regulate  $v$  **uncertainly**



$$\begin{array}{l}
 R_v(x) = \\
 S_v(x) =
 \end{array}
 \begin{array}{|c|c|c|c|c|}
 \hline
 \emptyset & \emptyset & \{u\} & \{u\} & \{u\} \\
 \hline
 \emptyset & \{u\} & \emptyset & \emptyset & \emptyset \\
 \hline
 \end{array}$$

$$\begin{array}{l}
 R_w(x) = \\
 S_w(x) =
 \end{array}
 \begin{array}{|c|c|c|c|c|}
 \hline
 \{u\} & \{u\} & \{u\} & \emptyset & \emptyset \\
 \hline
 \emptyset & \emptyset & \emptyset & \{u\} & \emptyset \\
 \hline
 \end{array}$$

$$x_u = \begin{array}{|c|c|c|c|c|}
 \hline
 |0| & |0, 1| & |1| & |1, 2| & |2| \\
 \hline
 \end{array}$$

# Qualitative description : attractors

At the state  $x$ , the variable  $x_v$  evolves towards the **attractor**  $A_v(x)$  according to its regular and singular resources :

$$A_v(x) = | K_{v,R_v(x)} , K_{v,R_v(x) \cup S_v(x)} |$$

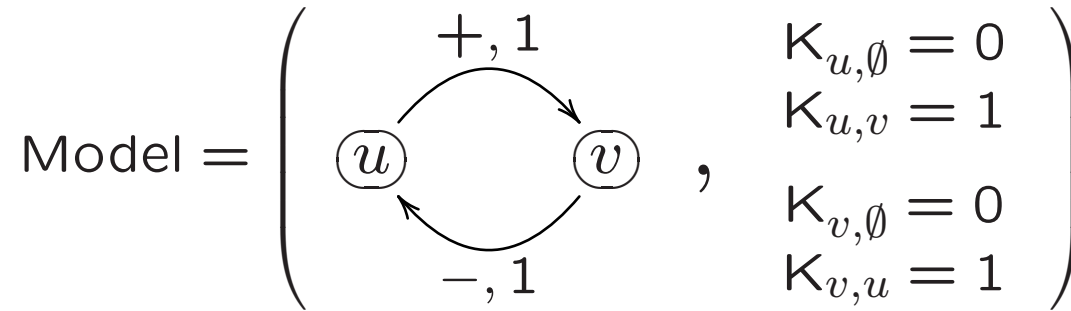
Where the **qualitative parameters** associated  $v$  are such that :

- ▷ if  $\omega_1 \subseteq \omega_2$  then  $K_{v,\omega_1} \leq K_{v,\omega_2}$
- ▷  $K_{v,\omega} \in \{0, \dots, n\}$  if  $v$  has  $n$  targets

$A_v(x)$  gives the **tendency** of  $x_v$  :

- ▷ if  $x_v < A_v(x)$  then  $x_v$  tends to **increase** ( $\nearrow$ )
- ▷ if  $x_v > A_v(x)$  then  $x_v$  tends to **decrease** ( $\searrow$ )
- ▷ if  $x_v \subseteq A_v(x)$  then  $x_v$  is **steady** ( $\rightsquigarrow$ )

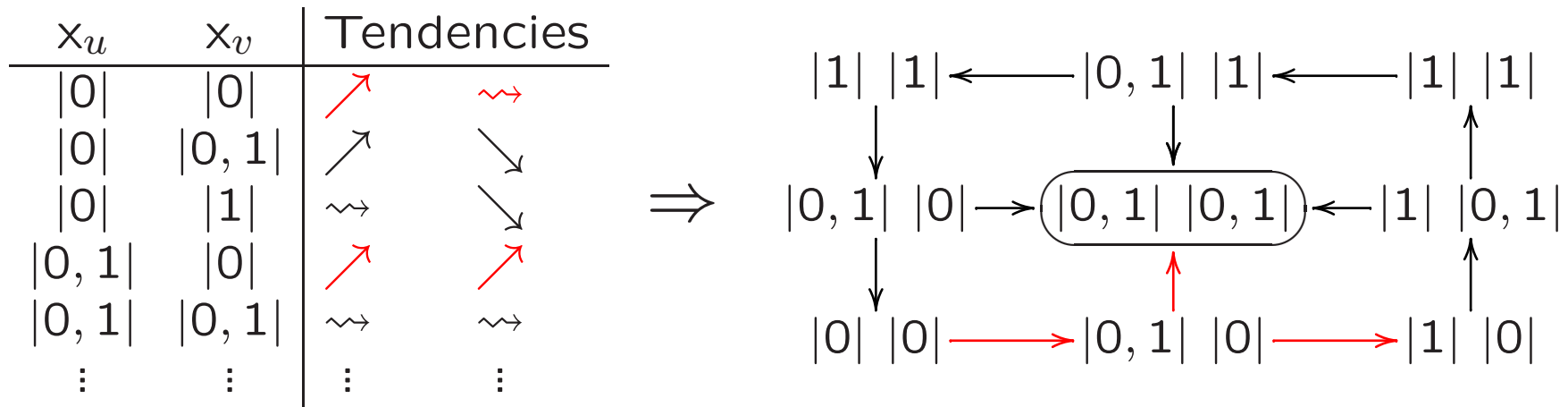
# Qualitative description : example



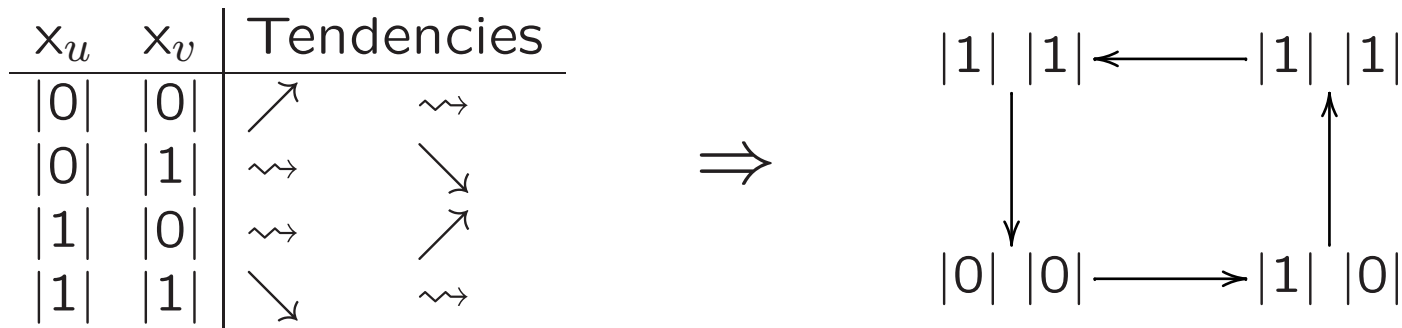
$x_u$	$x_v$	$A_u(x)$	$A_v(x)$	$A_u(x)$	$A_v(x)$	Tendencies of $x_u$ of $x_v$	
0	0	$K_{u,v}$	$K_{v,\emptyset}$	1	0	$\nearrow$	$\rightsquigarrow$
0	0, 1	$K_{u,\emptyset}, K_{u,v}$	$K_{v,\emptyset}$	0, 1	0	$\nearrow$	$\searrow$
0	1	$K_{u,\emptyset}$	$K_{v,\emptyset}$	0	0	$\rightsquigarrow$	$\searrow$
0, 1	0	$K_{u,v}$	$K_{v,\emptyset}, K_{v,u}$	1	0, 1	$\nearrow$	$\nearrow$
0, 1	0, 1	$K_{u,\emptyset}, K_{u,v}$	$K_{v,\emptyset}, K_{v,u}$	0, 1	0, 1	$\rightsquigarrow$	$\rightsquigarrow$
0, 1	1	$K_{u,\emptyset}$	$K_{v,\emptyset}, K_{v,u}$	0	0, 1	$\searrow$	$\searrow$
1	0	$K_{u,v}$	$K_{v,u}$	1	1	$\rightsquigarrow$	$\nearrow$
1	0, 1	$K_{u,\emptyset}, K_{u,v}$	$K_{v,u}$	0, 1	1	$\searrow$	$\nearrow$
1	1	$K_{u,\emptyset}$	$K_{v,u}$	0	1	$\searrow$	$\rightsquigarrow$

# Qualitative description : example

Tendencies  $\Rightarrow$  asynchronous state graph :

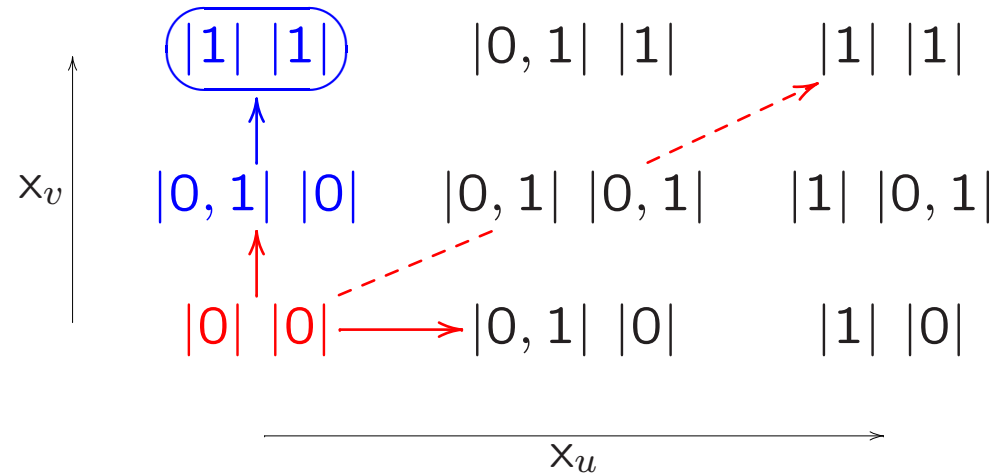
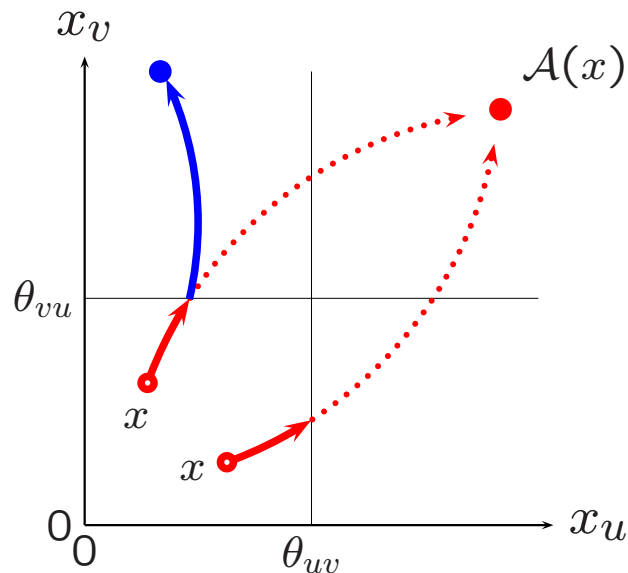


Without singular states  $\Rightarrow$  Thomas' formalism :



# Qualitative description : properties

For a given network, if for all  $K_{v,\omega}$  we have  $K_{v,\omega} = d_v(\sum_{u \in \omega} \frac{k_{uv}}{\lambda_v})$  then for all state  $x$  such that  $d(x) = x$  we have  $A(x) = d(A(x))$



Consequently :

- ▷ The qualitative description extracts the **essential qualitative features** of the continuous one
- ▷ **each continuous steady state corresponds to a qualitative steady state**

# Qualitative description : properties

- ▷ Increase in the number of states but not in the number of qualitative parameters
- ▷ The qualitative parameters are unknown but they can take a **finite** number of possible values
- ▷ We can use an **exhaustive approach** to model the dynamics of a network :
  - Generate all the models associated to a network in the aim to select those which give a dynamics coherent with the experimental knowledge
- ▷ Three approaches :
  - **feedback circuit functionality = steadyness of singular states**
  - **temporal logic**
  - **model checking**

# Selection of models : circuit functionality

Feedback circuits play a major role :

- ▷ a **positive** circuit is a necessary condition for **multistationarity**
- ▷ a **negative** circuit is a necessary condition for **stable cycle**

Some **singular states** are **circuit-characteristic** states and when they are **steady**, the corresponding circuits are **functional** :

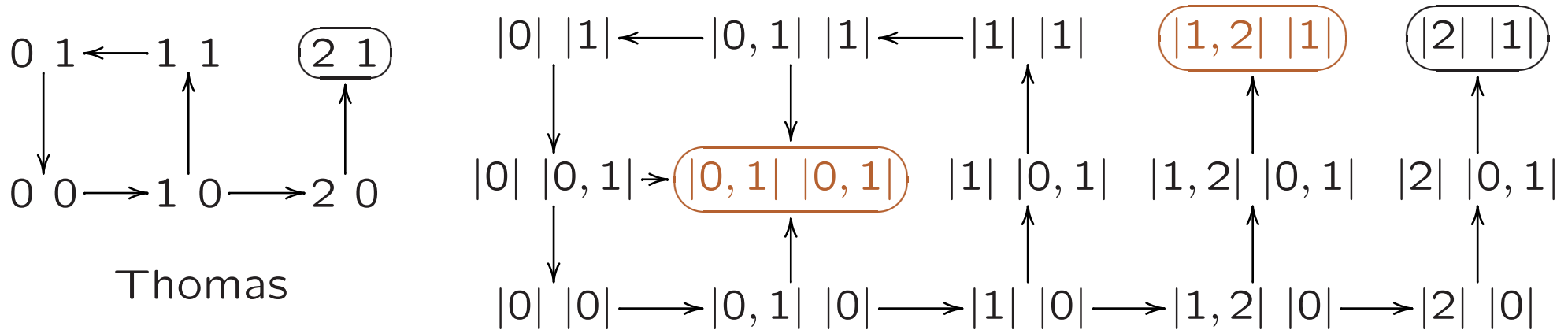
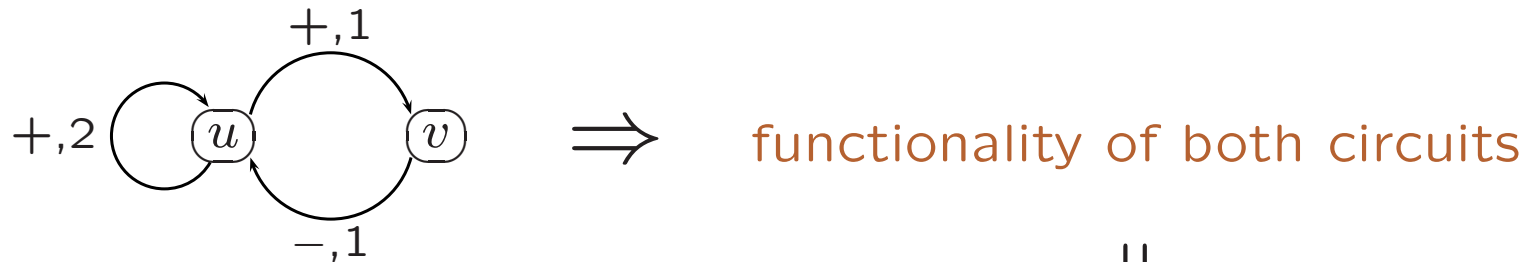
- ▷ a positive circuit **generates** multistationarity (differentiation)
- ▷ a negative circuit **generates** stable cycle (homeostasis)

Differentiation and homeostasis are experimentally measurable



**constraints on  $K$**  for the steadiness of circuit-characteristic states

# Selection of models : circuit functionality



With the **singular states** the multistationarity and homeostasis induced by the circuit functionality are **more explicit**



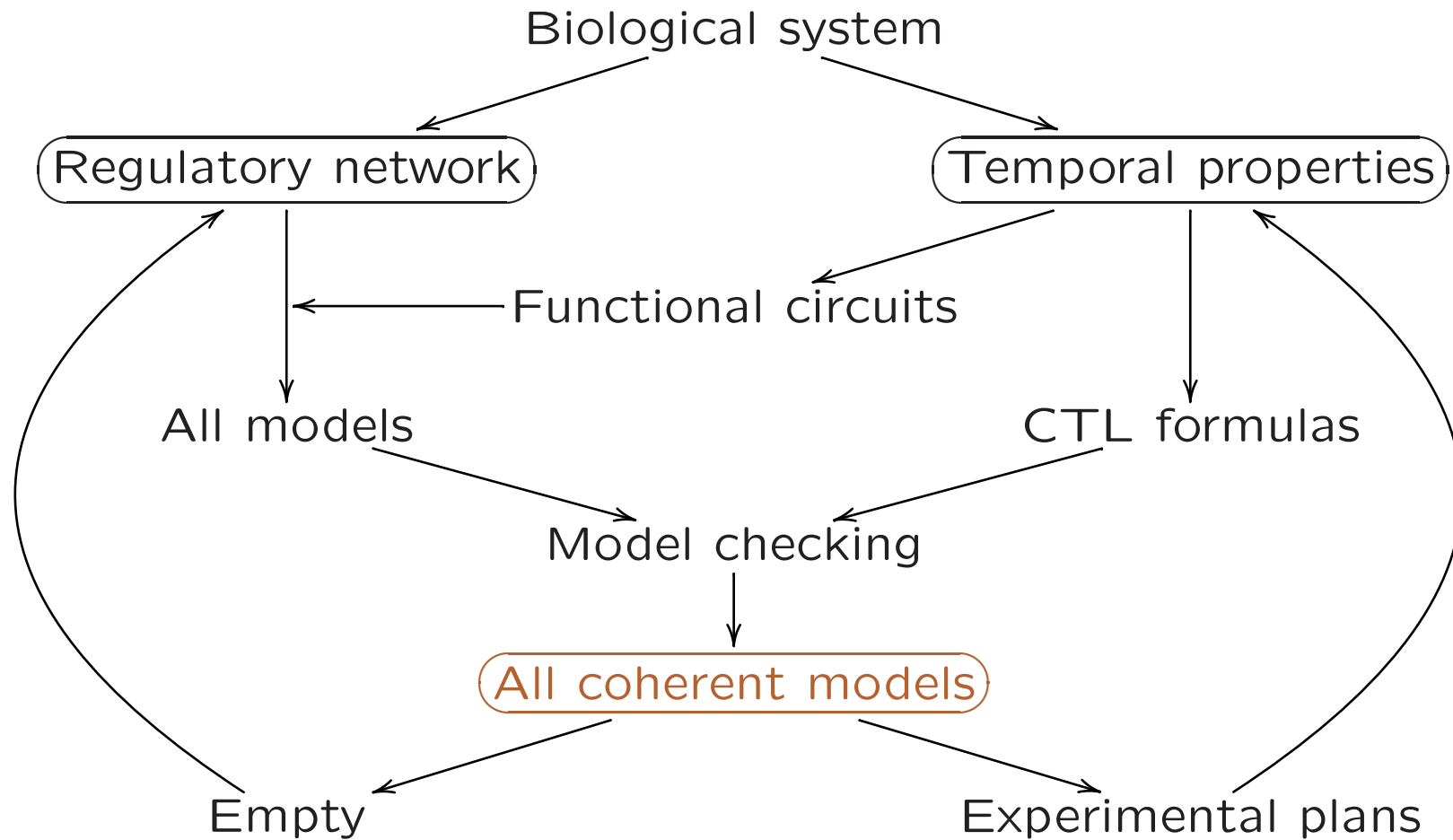
# Selection of models : model checking

Temporal logic and model checking can be used as an indirect criterion to constrain parameters  $K$

Undeterminism in biology :

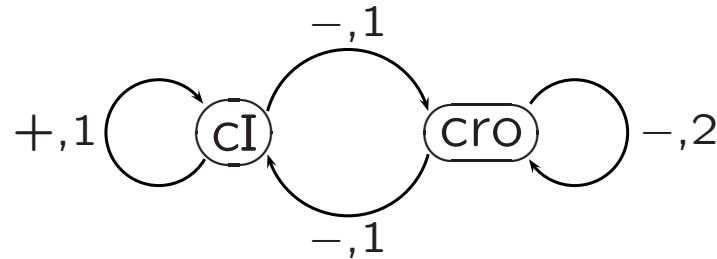
- ▷ temporal logic : Computation Tree Logic
- ▷ model checker : NuSMV

# SMBioNet : input/output



# SMBioNet : example

Network controlling the immunity of bacteriophage  $\lambda$  :



▷ 1296 models

▷ CTL formulas :

initial state	$(cI=0 \ \& \ cro=0) \rightarrow EF \ AG(cI=1 \ \& \ cro=0)$	lysogeny
	$(cI=0 \ \& \ cro=0) \rightarrow EF \ AG(cI=0 \ \& \ cro>0)$	lyse

▷ **Functional circuit** : steadyness of  $|0| \ |1, 2|$  (functionality of the negative circuit on cro during the lytic way)

# SMBioNet : example

SMBioNet

Network Constraints Filter Result CHECK

CTL formula  $(cI=0 \ \& \ cro=0) \rightarrow (EX \ AG(cI=1 \ \& \ cro=0)) \ \& \ (EX \ AG(cI=0 \ \& \ cro>0))$

Steady States 2-0

Network & Circuits States & Parameters Model Filter Result

/home/moi/PRESENTATION\_CMSB/lambda.ne

```
graph LR; cI((cI)) -- "1+" --> cI; cI -- "1-" --> cro((cro)); cro -- "2-" --> cro; cro -- "1-" --> cI;
```

Circuit 2 : cro-->cro (-)

id	characteristic state		0	1
----- -----				
id	min adj regular state		2	5
id	max adj regular state		1	4

2/48 (4%) models are selected in 02 s

# SMBioNet : example

The screenshot shows the SMBioNet software interface. At the top, the window title is "SMBioNet". Below the title bar, there are tabs for "Network", "Constraints", "Filter", and "Result", with a "CHECK" button to the right. The "Filter" tab is active, showing a CTL formula:  $(cI=0 \ \& \ cro=0) \rightarrow (EX \ AG(cI=1 \ \& \ cro=0)) \ \& \ (EX \ AG(cI=0 \ \& \ cro>0))$ . Below the formula, there is a checkbox for "Steady States" which is checked, with the value "2-0" entered. The main area is divided into four tabs: "Network & Circuits", "States & Parameters", "Model Filter", and "Result". The "States & Parameters" tab is active, displaying a table of states and parameters. The "Result" tab is also active, showing a table of parameter ranges for each state.

id	State	cI	cro	KcI	Kcro
0		0	0	$KcI\{cro\}$	$Kcro\{cI, cro\}$
1		0	1	$KcI\{\}$	$Kcro\{cI, cro\}$
2		0	2	$KcI\{\}$	$Kcro\{cI\}$
3		1	0	$KcI\{cI, cro\}$	$Kcro\{cro\}$
4		1	1	$KcI\{cI\}$	$Kcro\{cro\}$
5		1	2	$KcI\{cI\}$	$Kcro\{\}$

min	KcI	max
0	$KcI\{cI, cro\}$	1
0	$KcI\{cro\}$	1
0	$KcI\{cI\}$	1
0	$KcI\{\}$	1

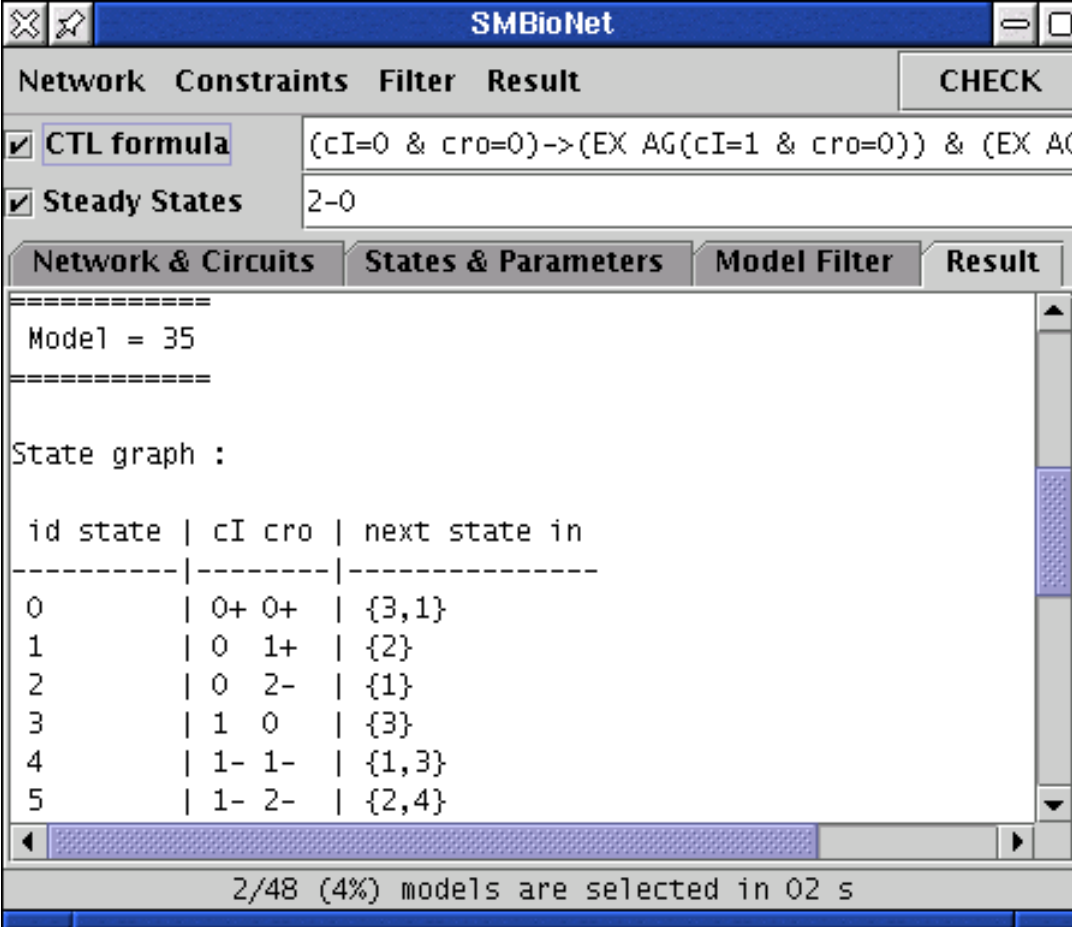
  

min	Kcro	max
0	$Kcro\{cI, cro\}$	2
0	$Kcro\{cro\}$	2
1	$Kcro\{cI\}$	2
1	$Kcro\{\}$	2

2/48 (4%) models are selected in 02 s

# SMBioNet : example

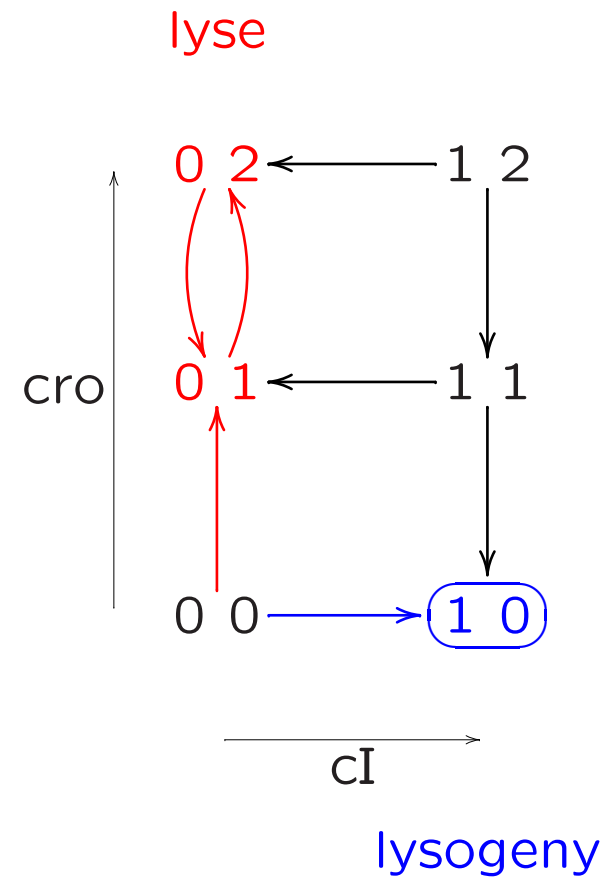
SMBioNet generates the 48 models which make the state  $|0\rangle$   $|1, 2\rangle$  steady and selects the 2 models which fulfil the CTL formulas



The screenshot shows the SMBioNet application window. The 'Filter' tab is active, displaying a CTL formula:  $(cI=0 \ \& \ cro=0) \rightarrow (EX \ AG(cI=1 \ \& \ cro=0)) \ \& \ (EX \ AG(cI=0 \ \& \ cro=0))$ . The 'Steady States' filter is set to '2-0'. The 'Result' tab shows 'Model = 35' and a 'State graph' table. The table lists 6 states with their corresponding cI and cro values and the next states they can reach.

id	state	cI	cro	next state in
0	0+ 0+	0+	0+	{3,1}
1	0 1+	0	1+	{2}
2	0 2-	0	2-	{1}
3	1 0	1	0	{3}
4	1- 1-	1-	1-	{1,3}
5	1- 2-	1-	2-	{2,4}

At the bottom of the window, it indicates '2/48 (4%) models are selected in 02 s'.



# Conclusions

## Introduction of singular states :

- ▷ refinement of Thomas' formalism
- ▷ representation of all the steady states of the piecewise linear equation system
- ▷ explicit representation of the properties induced by the circuit functionality
- ▷ possibility to check temporal properties concerning singular states

## Perspectives :

- ▷ implementation of the new formalism in SMBioNet
- ▷ using of NuSMV to learn the networks which can have a dynamics satisfying a given set of CTL formulas

Thank you !

[www.smbionet.lami.univ-evry.fr](http://www.smbionet.lami.univ-evry.fr)