A new qualitative control strategy for the genetic Toggle Switch

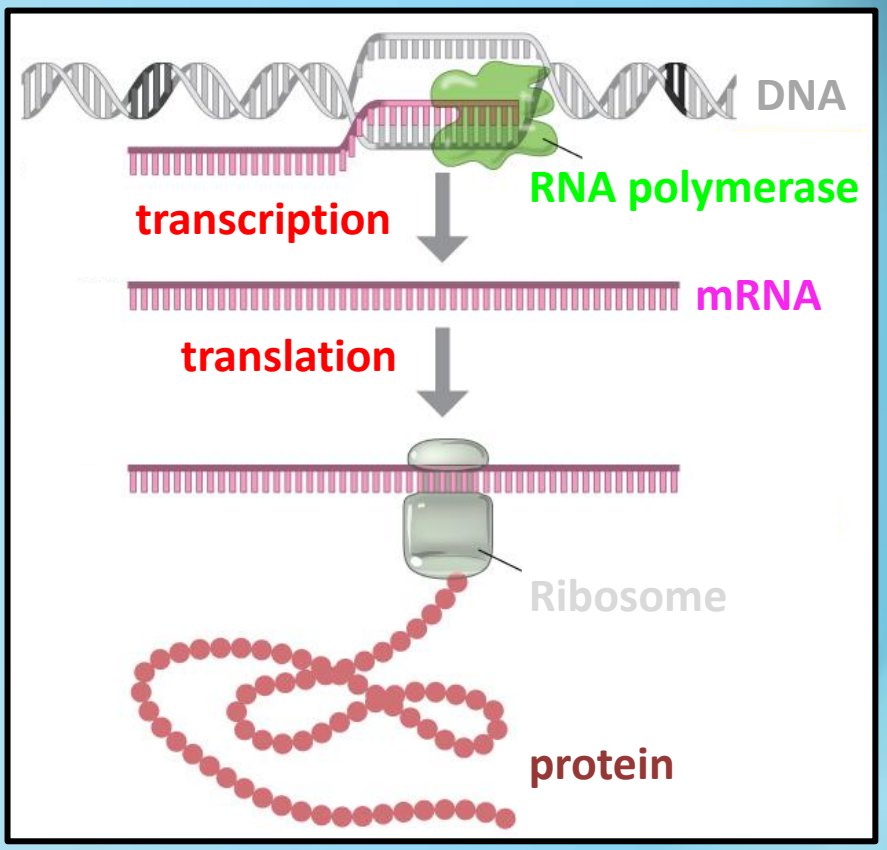
Phd: Lucie Chambon
Supervisor: Jean-Luc Gouzé

0. **General background**
   a. Genetics: transcription and translation
   b. Transcription factors
   c. Gene regulatory networks
1. **Genetic Toggle Switch**
   a. Genetic motif
   b. Biological behaviour
   c. Biological control
2. **A new quantized control strategy**
   a. ODE System
   b. PWC control
   c. Global results
   d. Application to the genetic Toggle Switch
3. **Conclusion & future work**

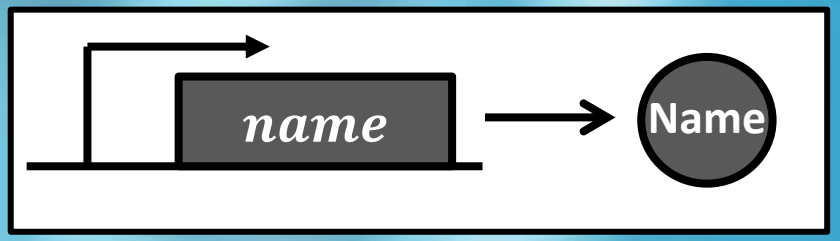
0. General background

0.a. Genetics: transcription and translation

- A DNA sequence forms a gene.
- The **transcription** of a gene through **RNA polymerase** forms **mRNA**.
- The **translation** of RNA through the **Ribosome** forms a **protein**.



Schematic representation:



Role of proteins:

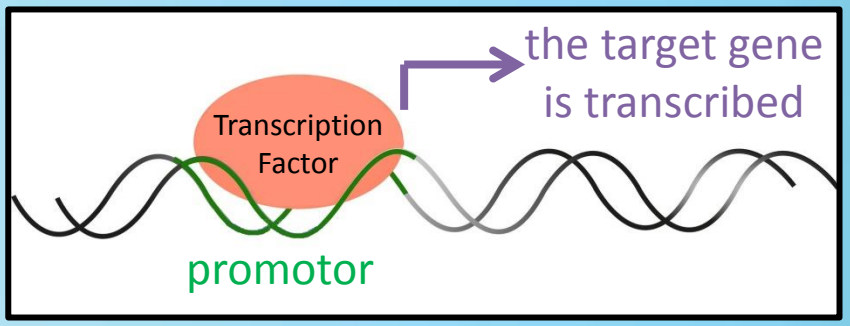
- Transport
- Cell structure
- **Regulation**

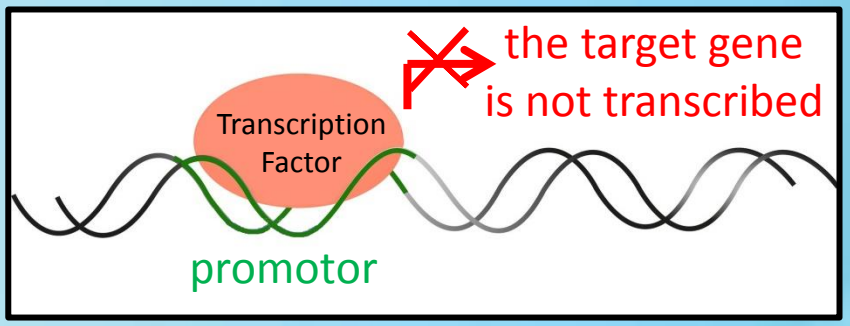
0.b. Transcription factors

Some proteins called **transcriptions factors (TF)** are able to, either:

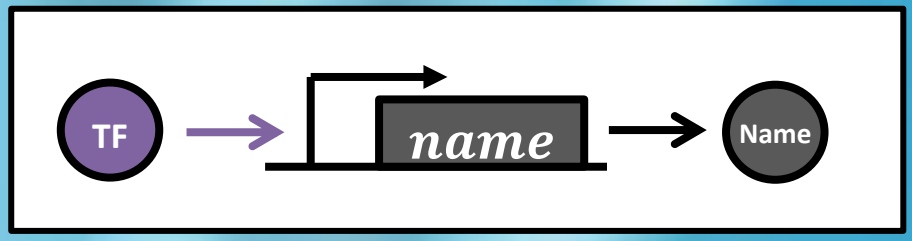
- **activate** the transcription of a target gene,
- or **inhibit** the transcription of a target gene,

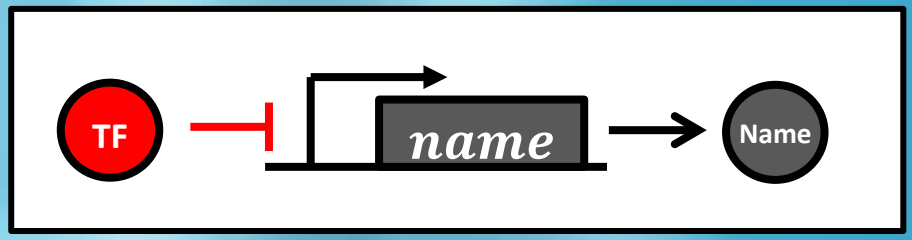
by binding to its promotor.





Schematic representation:

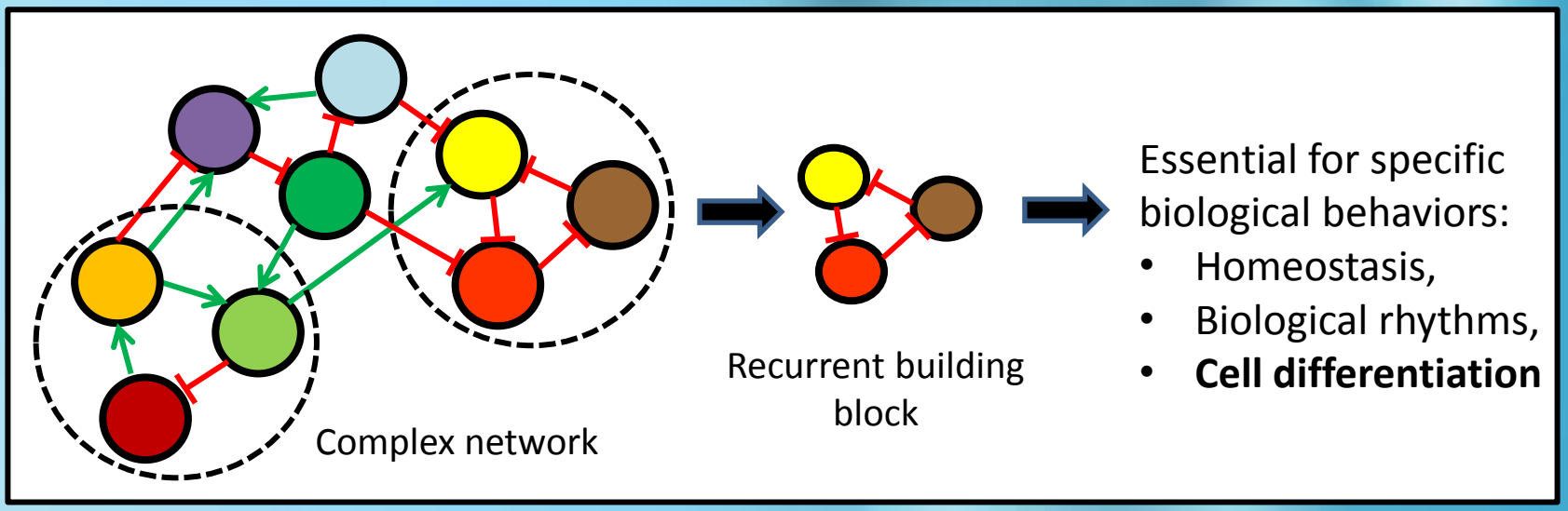




0.c. Gene regulatory networks

Genes influence each other through the proteins for which they code:

- Construction of a **network** of indirect influence between proteins
- Detection of building blocks, or key motifs



It is really important to:

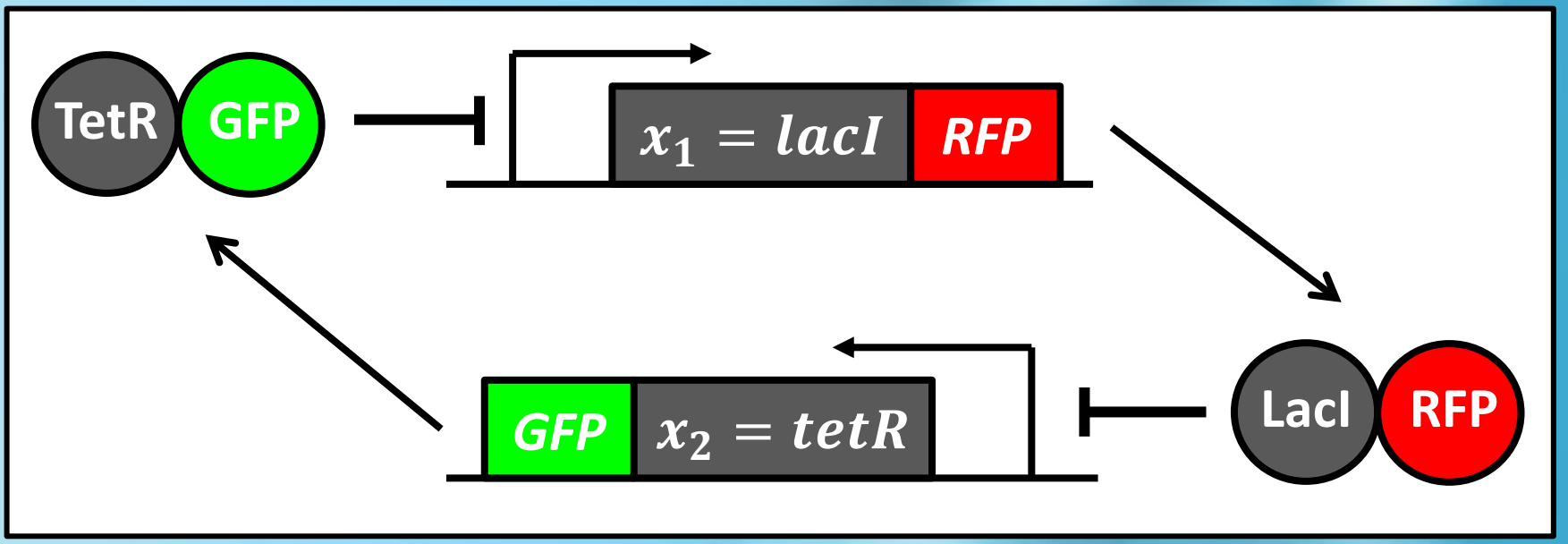
- **Understand** these building blocks **dynamics**
- Be able to **control** them

1. Genetic Toggle Switch

1.a. Genetic motif

Toggle Switch [1]:

- Two genes: $\boldsymbol{lacI}$ and $\boldsymbol{tetR}$
- Two transcription factors: **LacI** and **TetR**
- Two fluorescent proteins: **GFP** and **RFP**

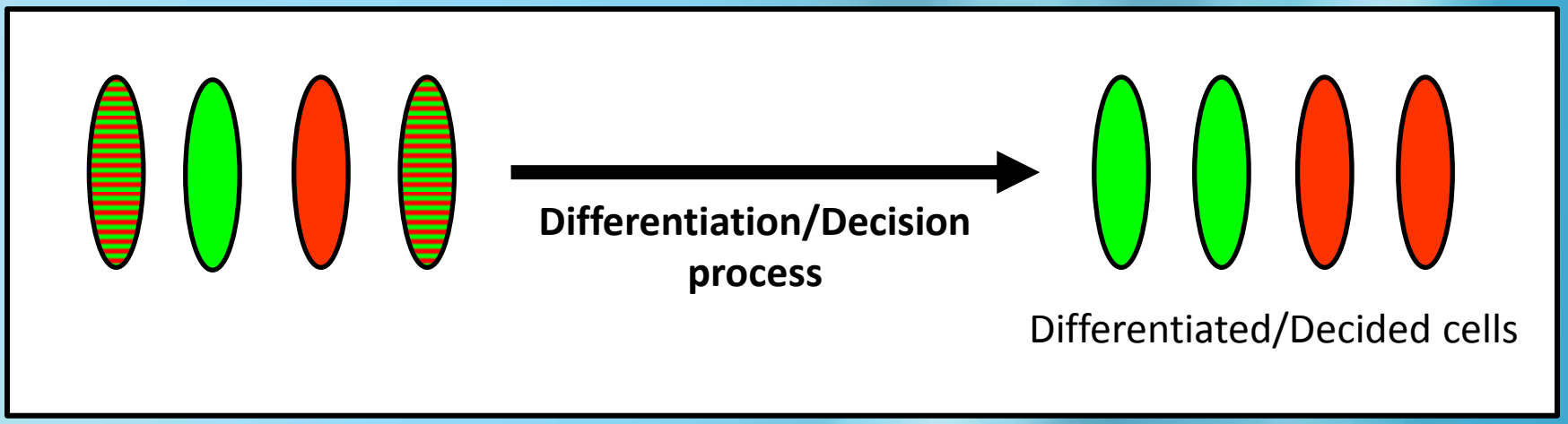


[1] T.S. Gardner, C.R. Cantor, J.J. Collins, 2000

1.b. Biological behaviour

Bistable switch:

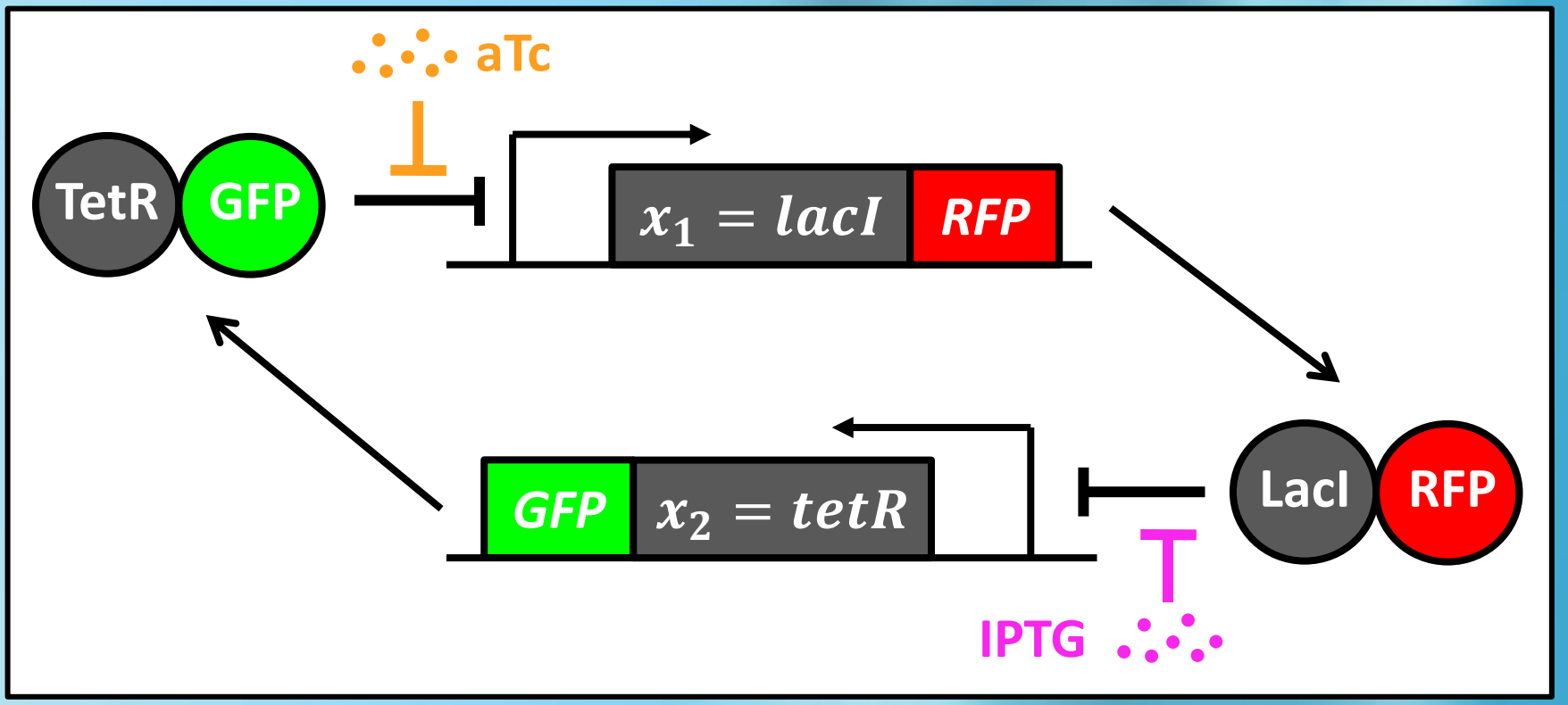
- **Stable state 1: TetR** fully expressed (green cells)
- **Stable state 2: LacI** fully expressed (red cells)
- **Unstable state**: **TetR** and **LacI** expressed (green/red cells)



1.c. Biological control (1/3)

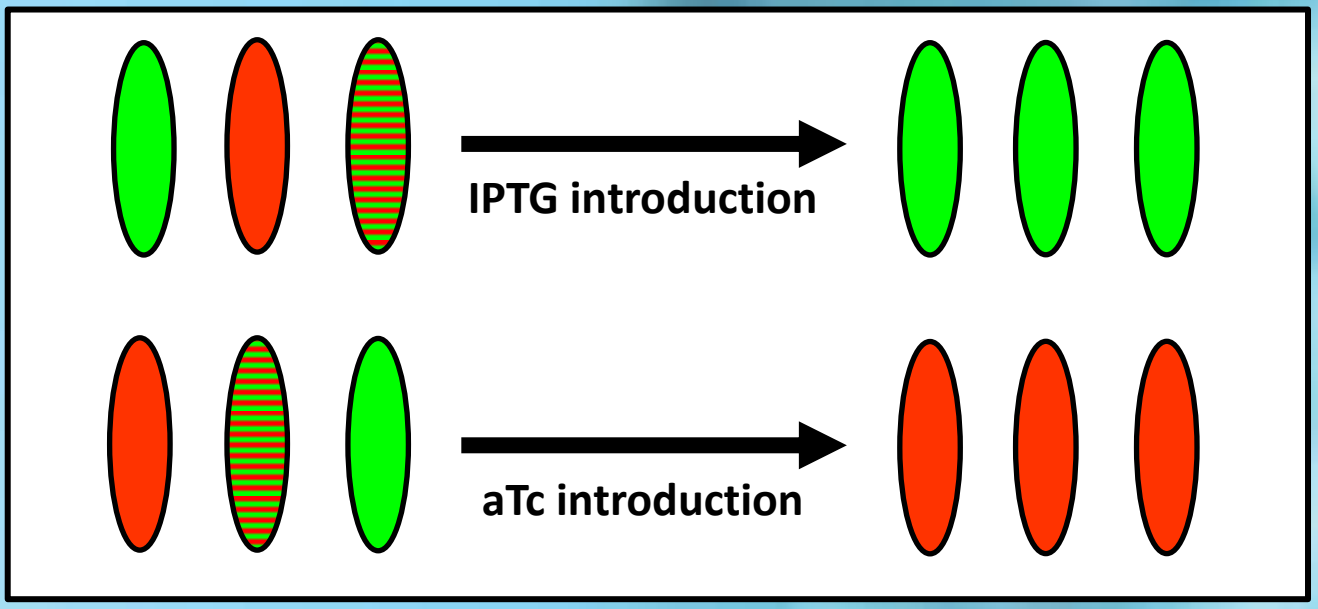
Inducer molecules control:

- **aTc** represses the repression of **TetR**
- **IPTG** represses the repression of **LacI**

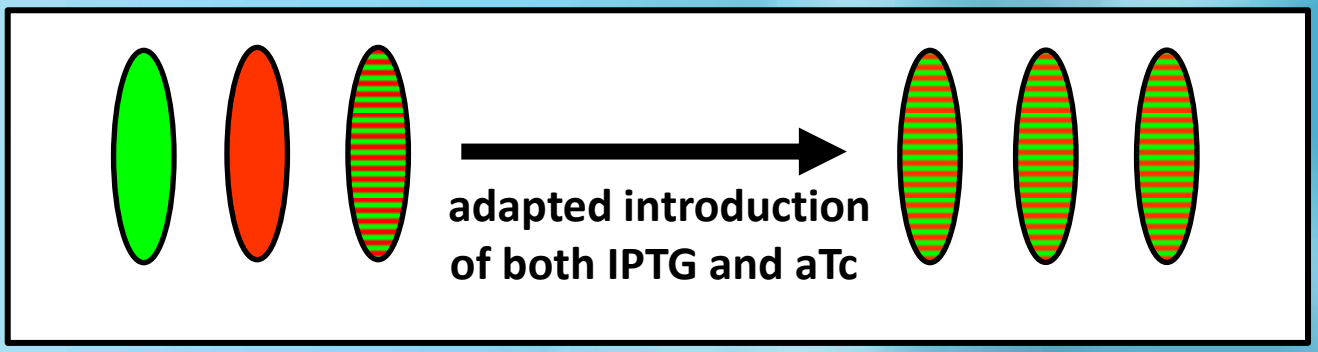


1.c. Biological control (2/3)

Global stabilization of one of the two stable state:



Global stabilization of the undecided/undifferentiated state [2]:



[2] J.B. Lugagne et. al, 2017

1.c. Biological control (3/3)

Application: Dedifferentiation processes: stem cells

Drawbacks: both genes $lacI$ and $tetR$ are measured and controlled

- From a mathematical point of view: this system is always controllable. It is a lot **more challenging to control and measure only one gene.**
- From a biological point of view: **reduce measurements** devices by using a unique fluorescent protein and **facilitate control implementation** by introducing a single inducer molecule.

Goal: achieve the same convergence objective by measuring and controlling a unique gene within the loop.
Biological interest: block a cell in an undifferentiated state (applications: stem cells)

2. A new quantized control strategy

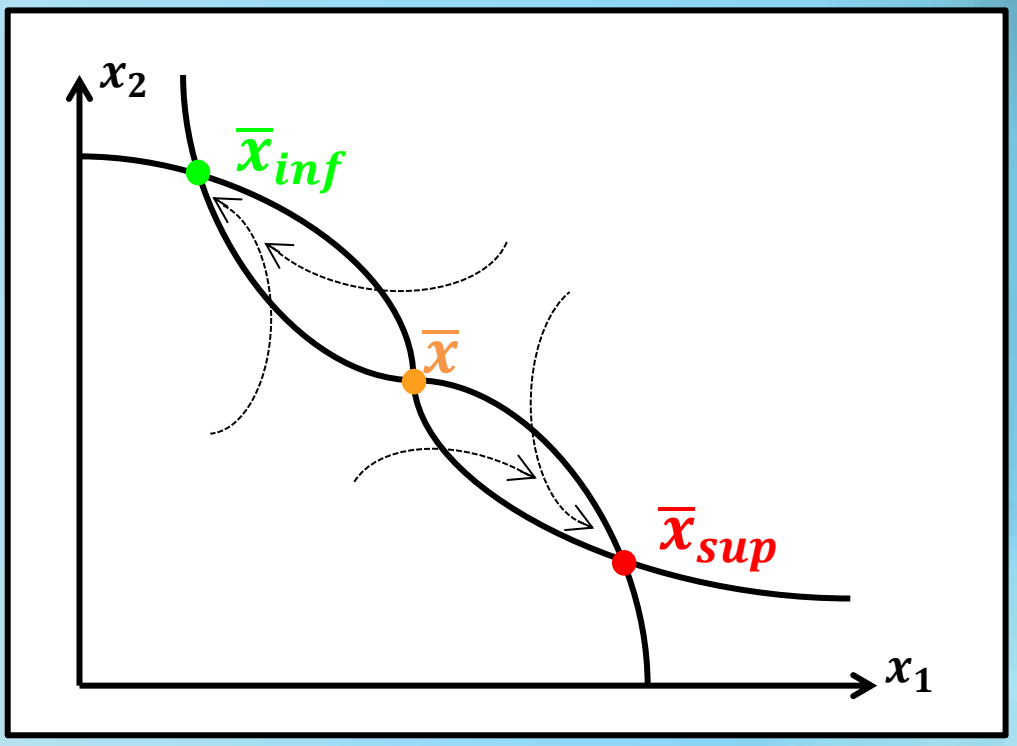
2.a. ODE system

$$\dot{x}_1 = k_{01} + k_1 h^-(x_2, \theta_2, n_2) - \gamma_1 x_1$$
$$\dot{x}_2 = k_{02} + k_2 h^-(x_1, \theta_1, n_1) - \gamma_2 x_2$$

basal rate — influence of the other gene — decay

Hill Functions:

$$h^-(x, \theta, n) = \frac{\theta^n}{\theta^n + x^n}$$



For appropriate parameters:

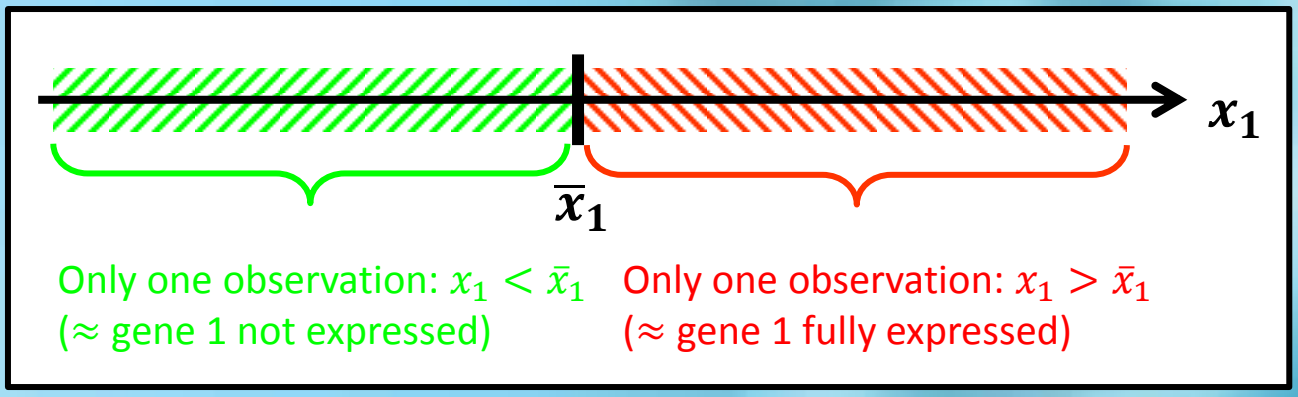
- **Stable fixed point 1: $\overline{x}_{inf}$**
  low x_1, high x_2.
- **Stable fixed point 2: $\overline{x}_{sup}$**
  low x_2, high x_1.
- **Unstable fixed point: $\overline{x}$**
  both x_1 and x_2.

Goal: global convergence towards $\overline{x}$.

2.b. PWC control (1/2)

Biological constraints for the control law choice:

- **Quantized measurements:** due to fluorescent microscopy, smooth and continuous observations of the variables are not available.



- **Biological control techniques with constant inputs:**
  - ➢ Introduction of specific doses of inducer molecules.

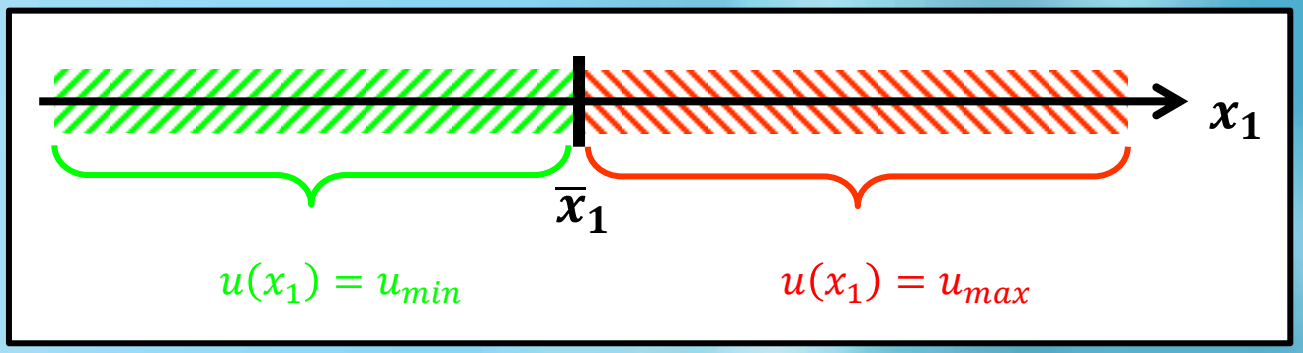
2.b. PWC control (2/2)

New quantized control strategy: Control and measurement of a unique gene in order to stabilize $\bar{x}$.

$$\dot{x}_1 = k_{01} + k_1 h^-(u(x_1)x_2, \theta_2, n_2) - \gamma_1 x_1$$
$$\dot{x}_2 = k_{02} + k_2 h^-(x_1, \theta_1, n_1) - \gamma_2 x_2$$

where:

$$u(x_1) = u_{max} > 1 \text{ if } x_1 \geq \bar{x}_1$$
$$u(x_1) = u_{min} < 1 \text{ if } x_1 \leq \bar{x}_1$$



2.c. Global results (1/5)

Theorem: With

- $u_{min} \leq \frac{\bar{x}_2}{\bar{x}_2+\beta_2}$
- $u_{max} \geq \frac{\bar{x}_2}{\bar{x}_2-\alpha_2}$

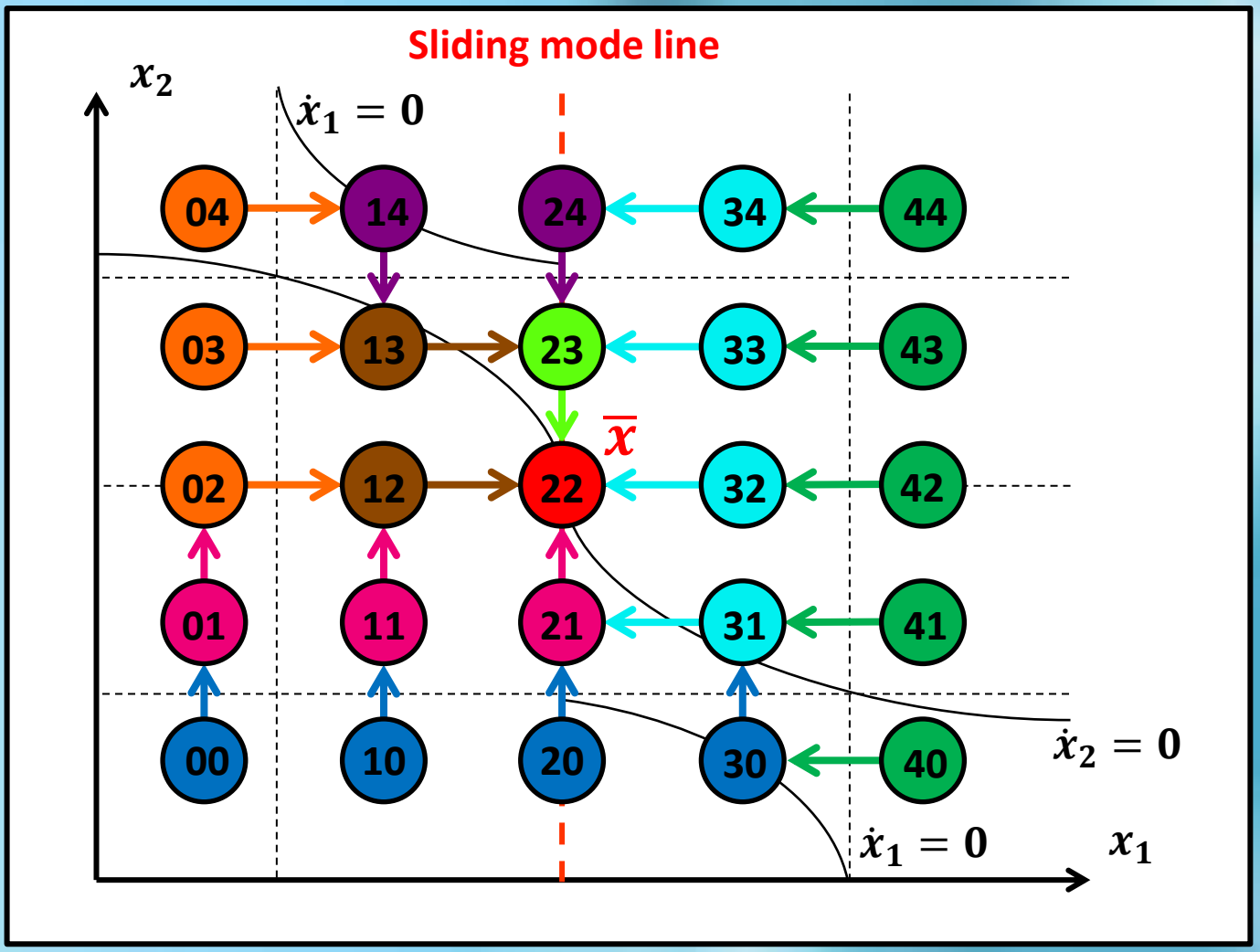
$\bar{x}$ becomes globally asymptotically stable.
The trajectories **converge** towards $\bar{x}$ through a sliding mode on the line $x_1 = \bar{x}_1$, and the system is **robust** to small perturbations.

where:

- $\beta_1 = \frac{k_{01}+k_1}{\gamma_1} - \bar{x}_1,$
- $\alpha_2 = \bar{x}_2 - \frac{k_{02}+k_2 h^-(\bar{x}_1+\beta_1,\theta_1,n_1)}{\gamma_1},$
- $\alpha_1 = \bar{x}_1 - \frac{k_{01}}{\gamma_1},$
- $\beta_2 = \frac{k_{02}+k_2 h^-(\bar{x}_1-\alpha_1,\theta_1,n_1)}{\gamma_1} - \bar{x}_2.$

2.c. Global results (2/5)

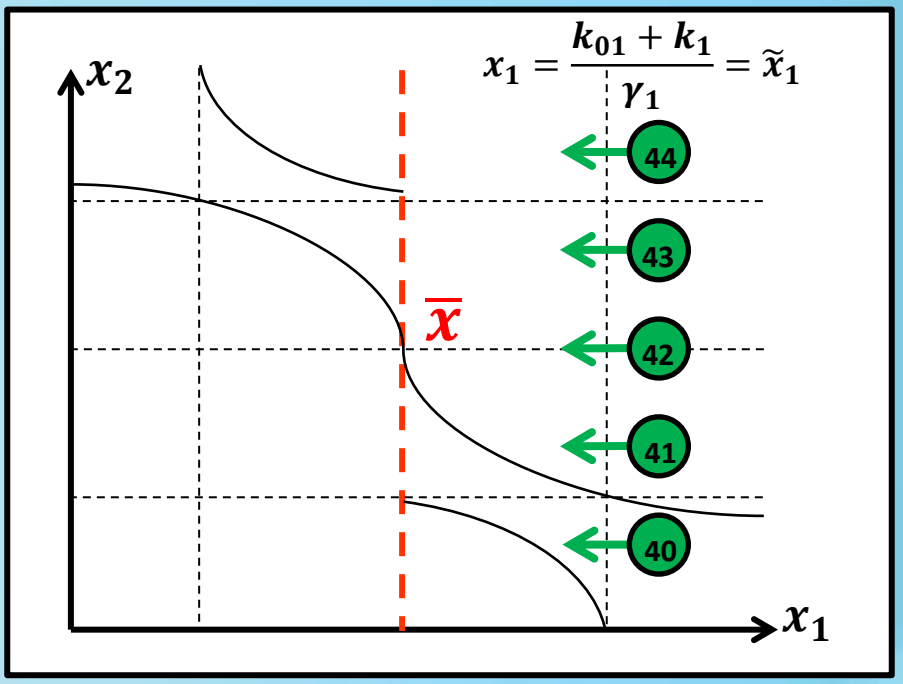
Global attractivity: successive repulsive regions and sliding mode.



2.c. Global results (3/5)

Illustration: The green region defined by $x_1 > \frac{k_{01}+k_1}{\gamma_1}$ is repulsive

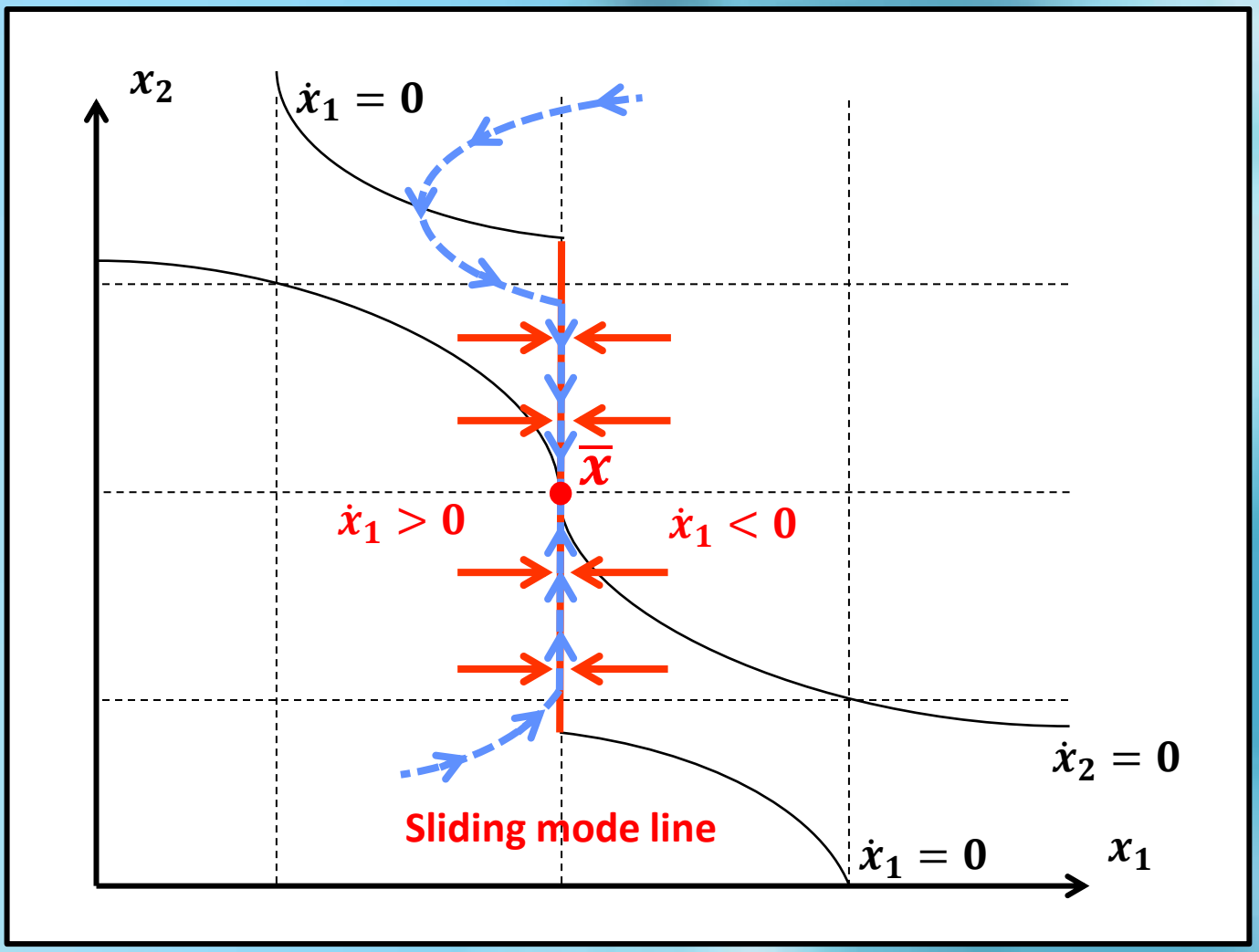
$$\leftrightarrow \dot{x}_1(x_1, x_2) < 0 \quad \forall x_1 > \frac{k_{01}+k_1}{\gamma_1} \quad \text{and} \quad x_2 \geq 0.$$



- As $\frac{k_{01}+k_1}{\gamma_1} > \bar{x}_1$, $u(x_1) = u_{max}$:
  $\dot{x}_1 = k_{01} + k_1 h^-(u_{max} x_2, \theta_2, n_2) - \gamma_1 x_1$.
- On the line $x_1 = \frac{k_{01}+k_1}{\gamma_1} = \tilde{x}_1$:
  $\dot{x}_1(\tilde{x}_1, x_2) = k_1[h^-(u_{max} x_2, \theta_2, n_2) - 1]$.
- As $h^-(u_{max} x_2, \theta_2, n_2) \in]0,1]$ then
  $\dot{x}_1(\tilde{x}_1, x_2) \leq 0 \; \forall \; x_2 \geq 0$.
- $\forall \; x_1 > \tilde{x}_1$, for x_2 fixed, the linear term $-\gamma_1 x_1$ gives: $\dot{x}_1(\tilde{x}_1, x_2) > \dot{x}_1(x_1, x_2)$.
- Then $\dot{x}_1(x_1, x_2) < 0 \; \forall \; x_2 \geq 0$ and $\forall \; x_1 \geq \tilde{x}_1$.

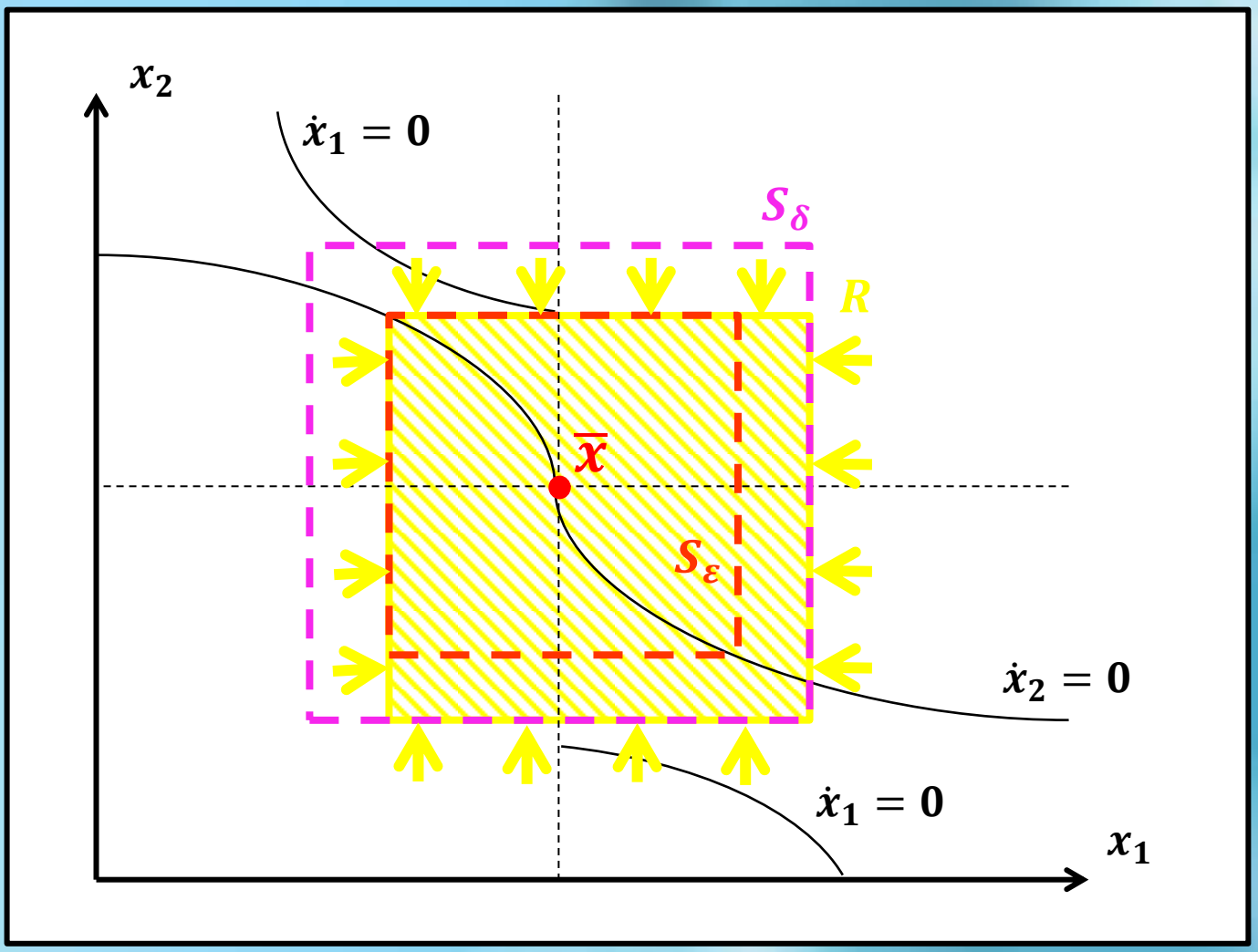
2.c. Global results (4/5)

Sliding mode on the line $x_1 = \overline{x}_1$: The x_1 vector field points in opposite direction on both sides of this line.



2.c. Global results (5/5)

Lyapunov stability: for any square S_δ of length δ, we can construct an invariant rectangle R s.t. $R \in S_\delta$, and its biggest embedded square S_ε of length ε.

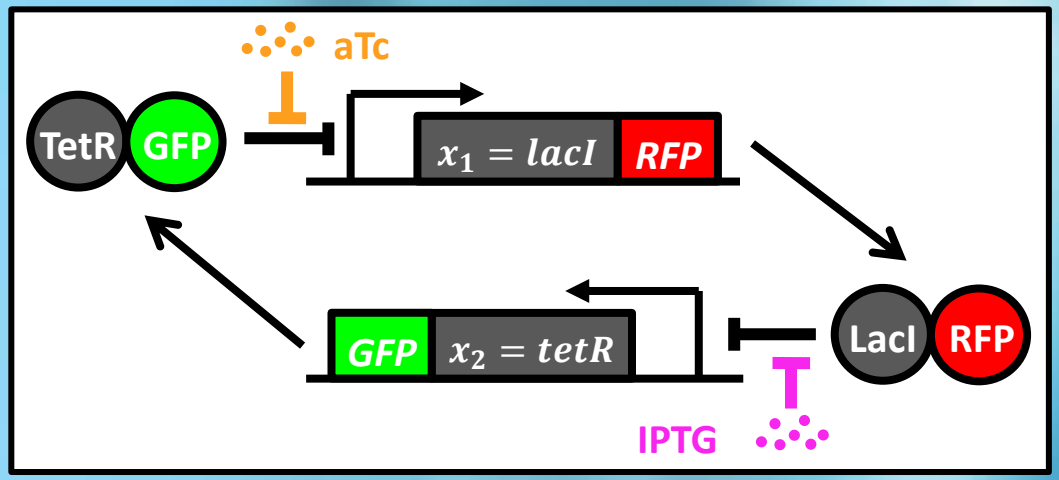


2.d. Application to the genetic Toggle Switch (1/2)

Comparison of the two strategies: Implementation simplification

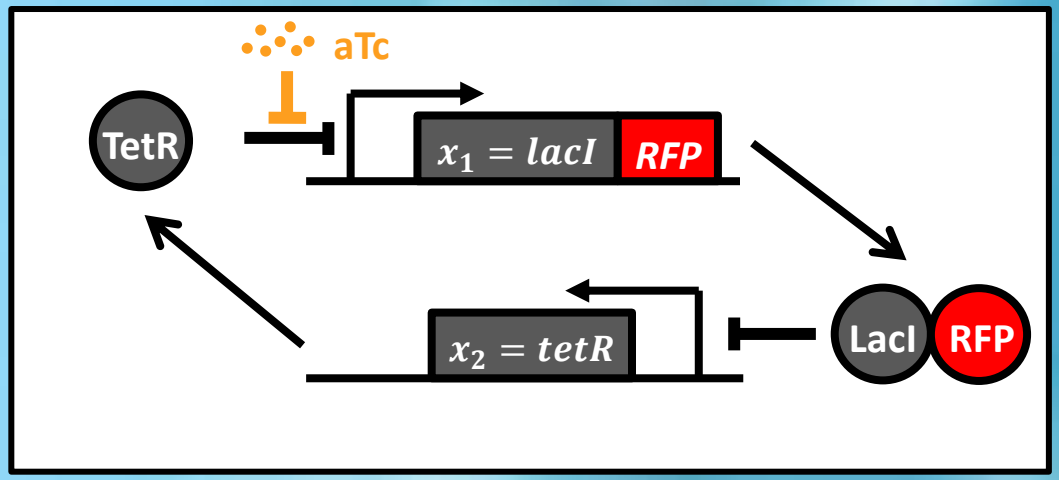
First strategy in [2]:

- Two measurements **(RFP and GFP)**
- Two inducer molecules **(aTc and IPTG)**

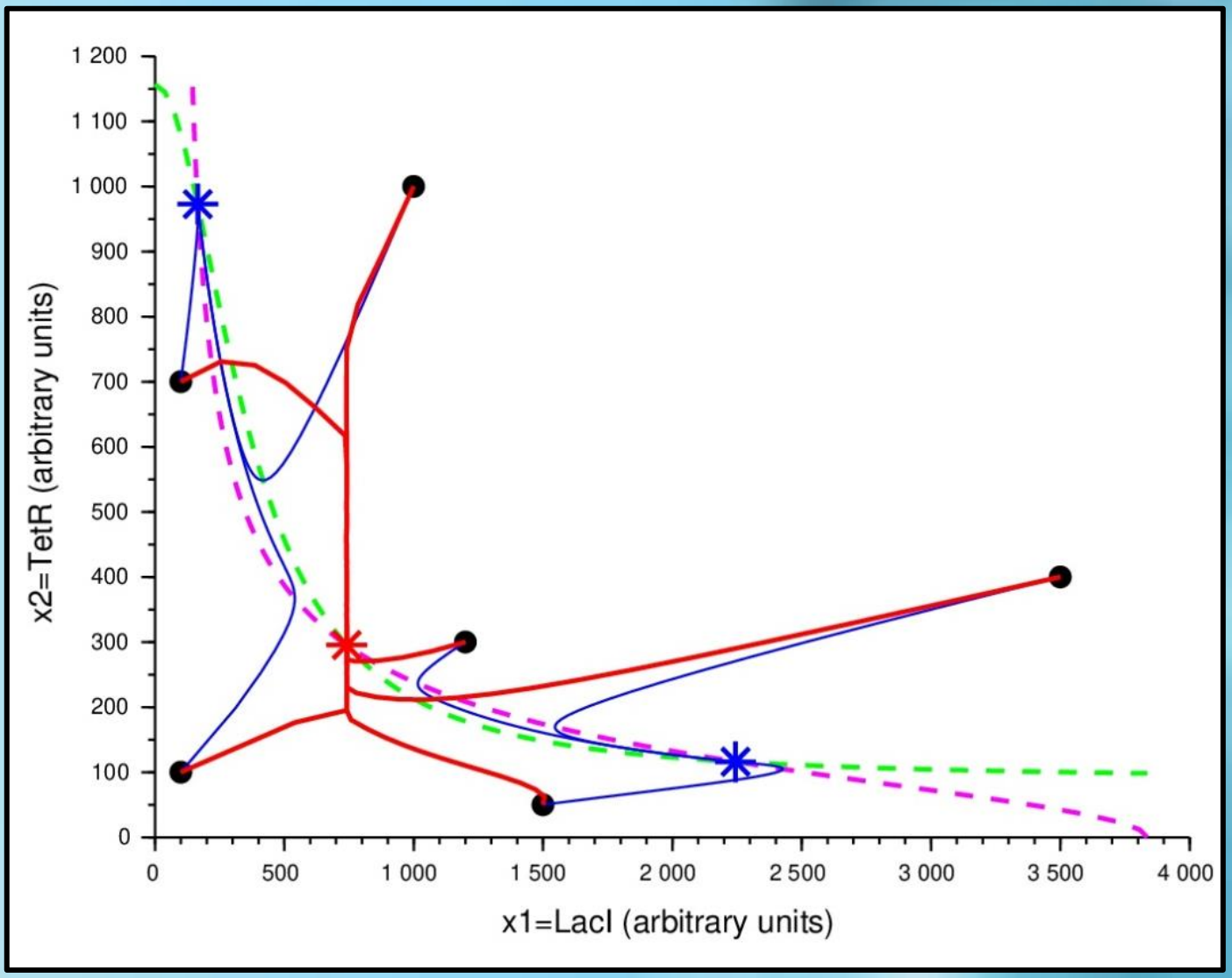


New strategy:

- One measurement **(RFP)**
- One inducer molecule **(aTc)**



2.d. Application to the genetic Toggle Switch (2/2)



Dashed lines: nullclines;
Blue stars: stable fixed points;
Red star: unstable fixed point;
Blue lines: trajectories without control.
Red lines: global convergence towards $\bar{x}$ with a **switch between $aTc = 6.5ng.ml^{-1}$ and $aTc = 42.9ng.ml^{-1}$.**

3. Conclusion & future work

Major **advantages**:

- Apparent **simplification** of biological implementation
- Allows **fluctuations** in the control law

Major **drawbacks**:

- Needs **fast switch**: not realistic with inducer molecules:
  - **Optogenetics**: make the homodimer of TetR photosensitive,
  - Introduce a **delay** in the system.

Thanks !
Any questions ? ☺