4.3 Un interrupteur génétique à 2 gènes: exemple de la biologie synthétique

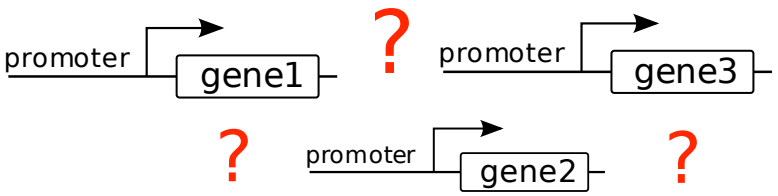
Construction of a genetic toggle switch in *Escherichia coli*

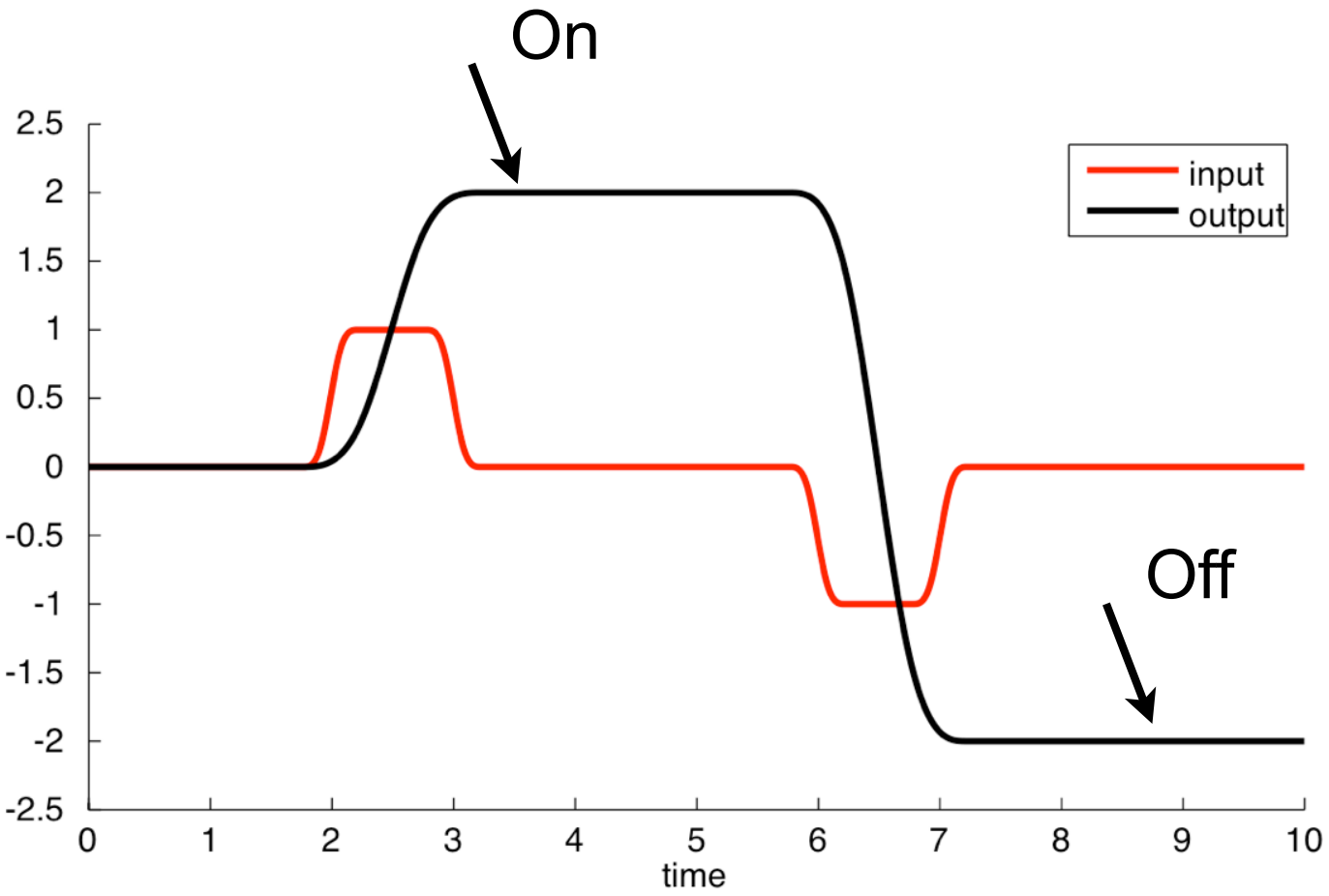
Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

* *Department of Biomedical Engineering,* † *Center for BioDynamics and* ‡ *Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*

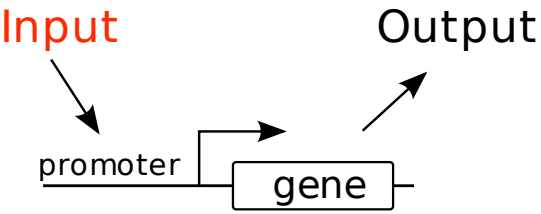
NATURE | VOL 403 | 20 JANUARY 2000 | www.nature.com

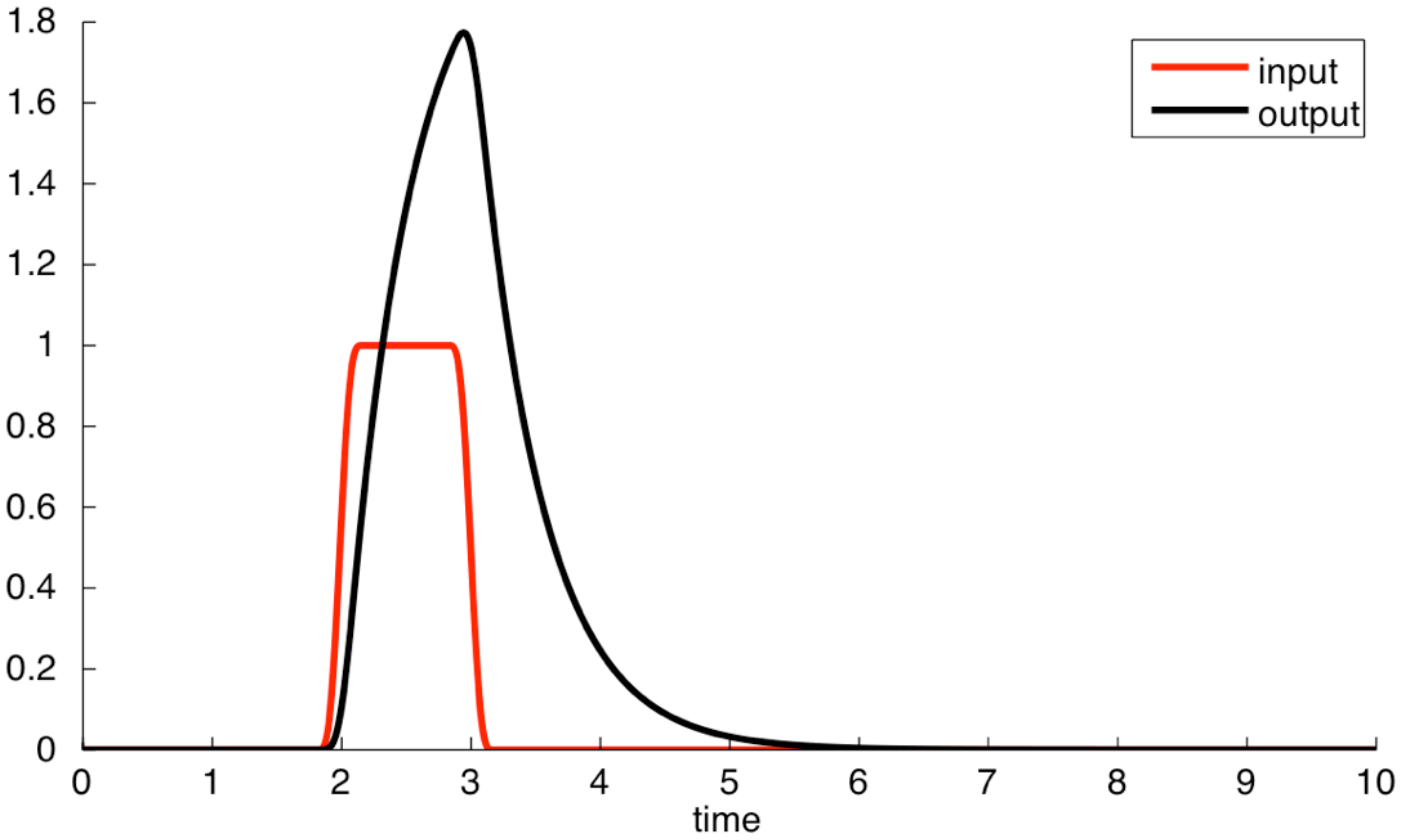
Toggle switch ?





Non-Toggle switch ?

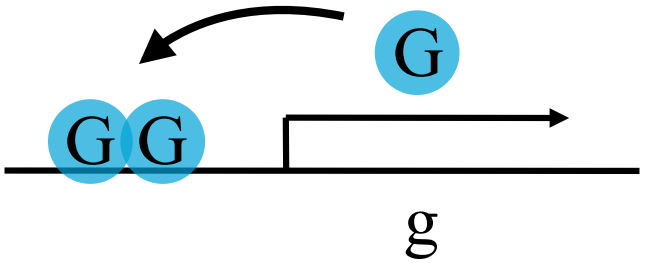




Rappel 1D, chapitre 2

Gene bistable

$$\frac{dg}{d\tau} = -f(g) + h(g)$$



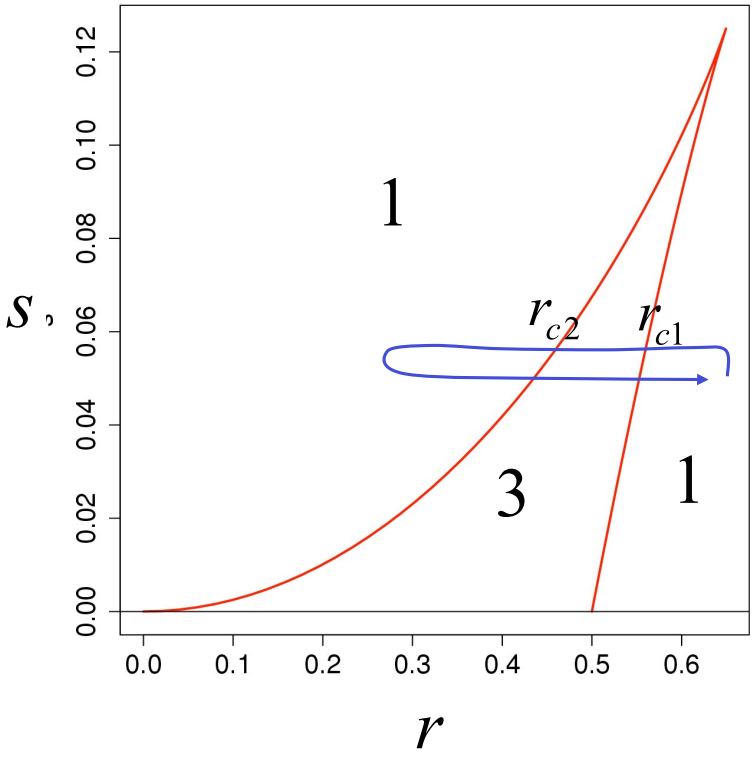
$f(g) = -s + rg$ leakage + degradation

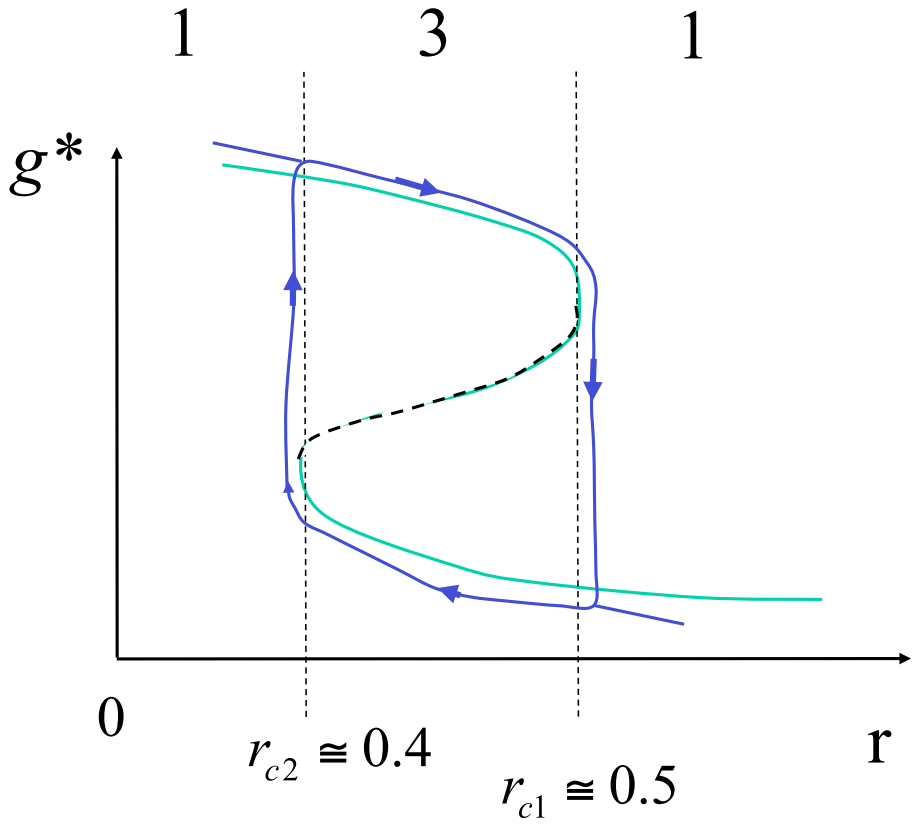
$h(g) = \frac{g^2}{1+g^2}$ positive feedback, from protein-DNA binding

Rappel 1D

Bistability, memory and hysteresis

s fixed ~0.05





Although bistability is theoretically possible with a single, autocatalytic promoter, it would be less robust and more difficult to tune experimentally.

page 1. col. 1

cf. chap. 2

Idée : Utiliser deux répresseurs mutuels

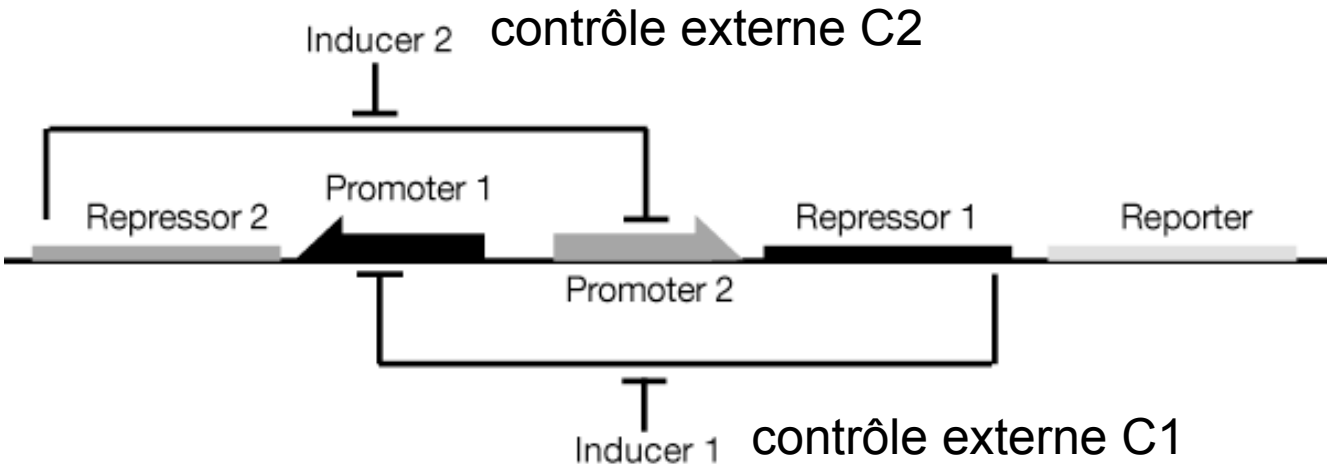


Fig. 1

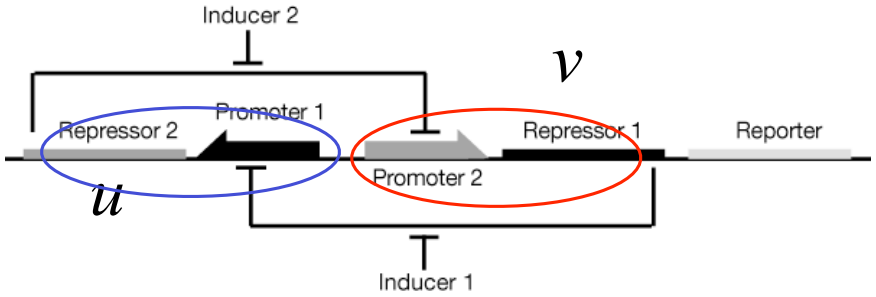
The toggle switch is composed of two repressors and two constitutive promoters (Fig. 1). Each promoter is inhibited by the repressor that is transcribed by the opposing promoter. We selected this design for the toggle switch because it requires the fewest genes and *cis*-regulatory elements to achieve robust bistable behaviour. By robust, we mean that the toggle exhibits bistability over a wide range of parameter values and that the two states are tolerant of the fluctuations inherent in gene expression (the toggle switch will not flip randomly between states).

Analyse mathématique

Analyse mathématique

$$\dot{u} = \alpha_1 \frac{1}{1 + v^{\beta}} - u$$

$$\dot{v} = \alpha_2 \frac{1}{1 + u^{\gamma}} - v$$



Premier terme:
taux d'expression est proportionnel à la fraction libre du promoteur (u et v sont des répresseurs)

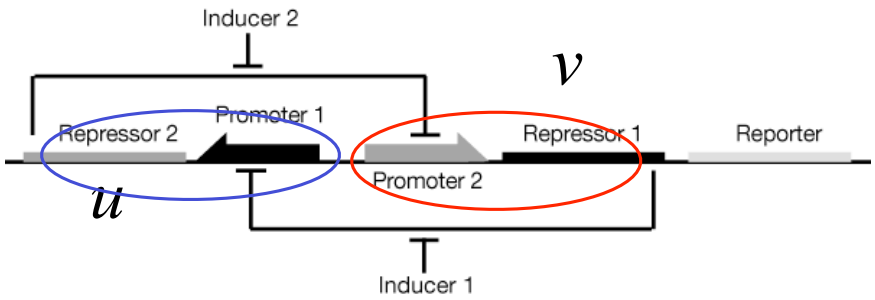
Deuxième terme:
dégradation de la protéine (identique pour les 2 protéines, égale à 1)

Exposants: cooperativité, il y a β sites pour *v* dans le promoteur de *u* et γ sites pour *u* dans le promoteur de *v*

Analyse mathématique

$$\dot{u} = \alpha_1 \frac{1}{1 + v^{\beta}} - u$$

$$\dot{v} = \alpha_2 \frac{1}{1 + u^{\gamma}} - v$$



- Isoclines
- Points fixes
- Stabilité des points fixes
- Portrait de phase

‘Portrait de phase’ (Fig. 2)

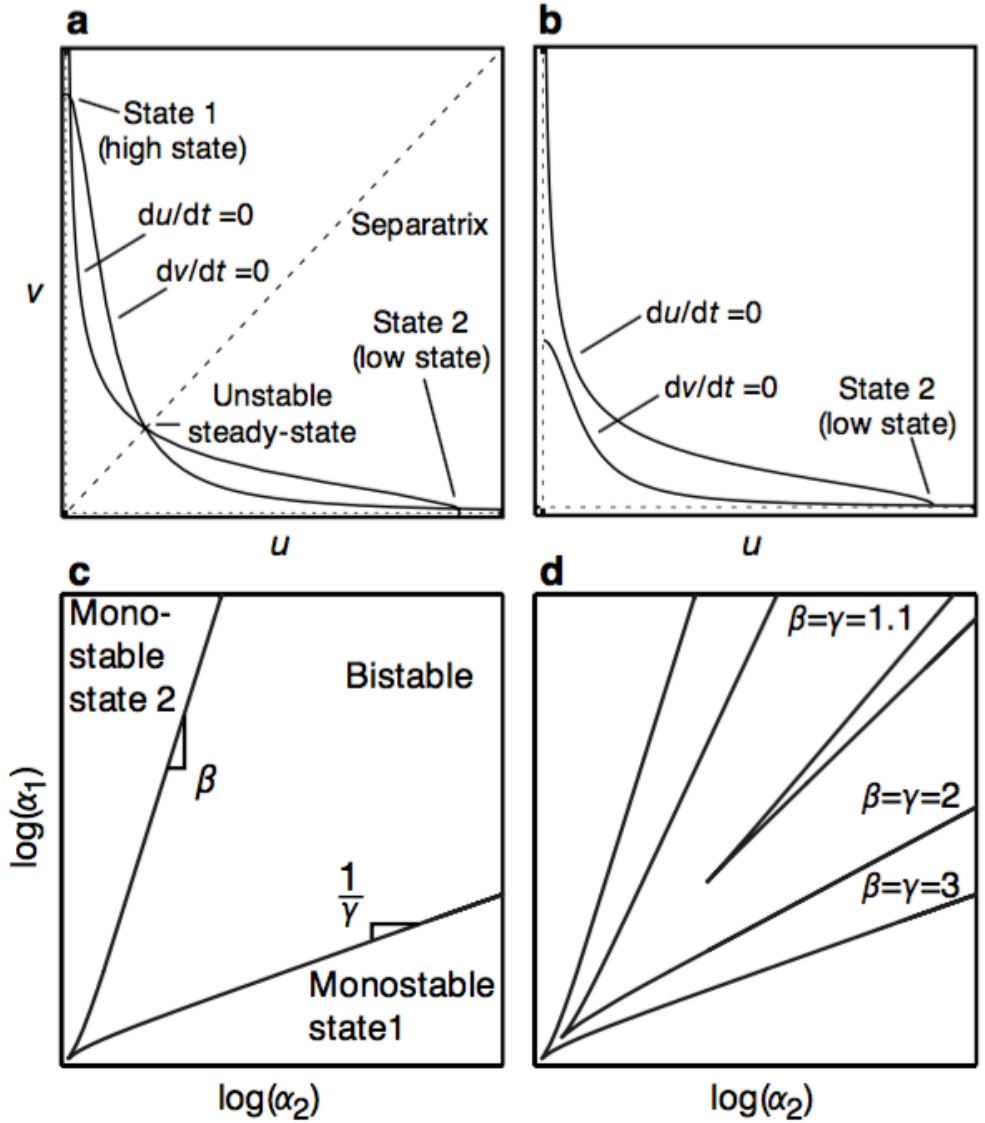
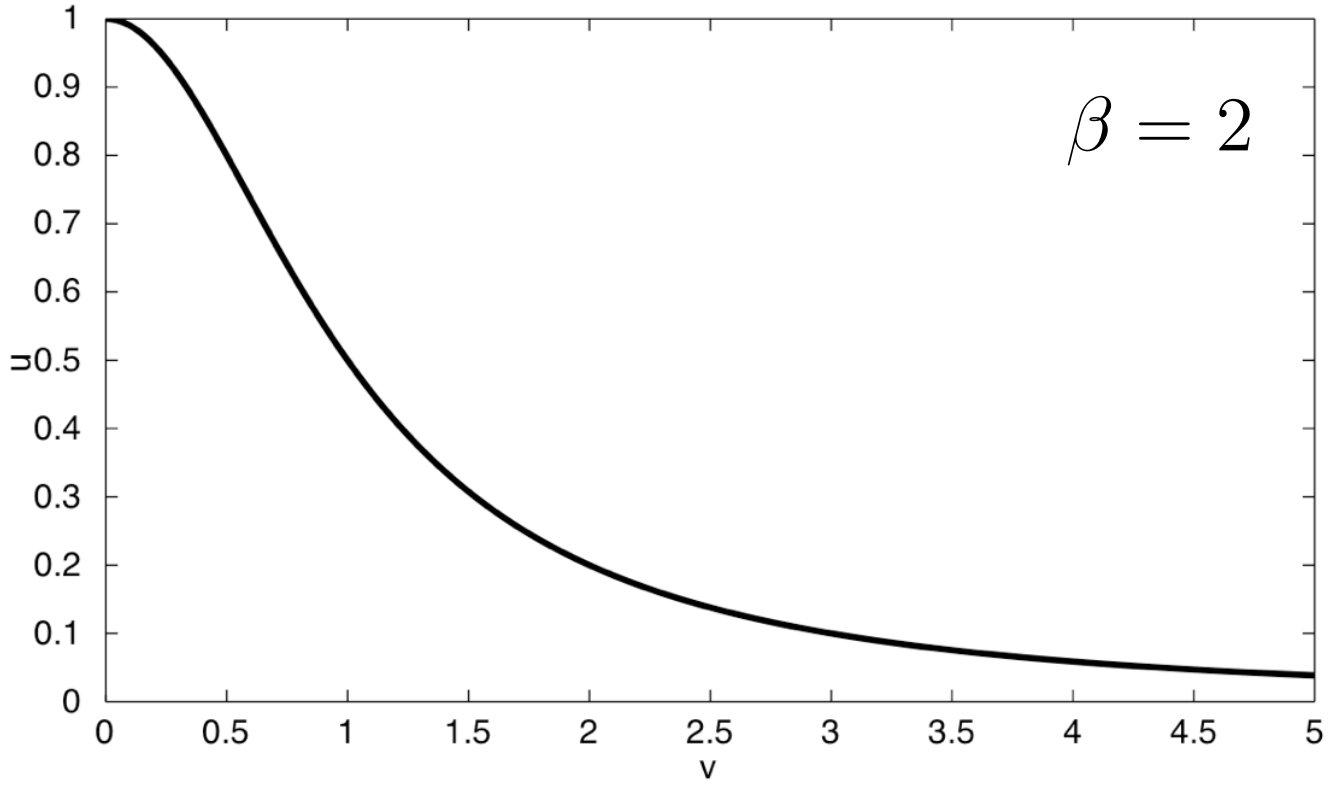


Figure 2 Geometric structure of the toggle equations. **a**, A bistable toggle network with balanced promoter strengths. **b**, A monostable toggle network with imbalanced promoter strengths. **c**, The bistable region. The lines mark the transition (bifurcation) between bistability and monostability. The slopes of the bifurcation lines are determined by the exponents β and γ for large α_1 and α_2. **d**, Reducing the cooperativity of repression (β and γ) reduces the size of the bistable region. Bifurcation lines are illustrated for three different values of β and γ. The bistable region lies inside of each pair of curves.

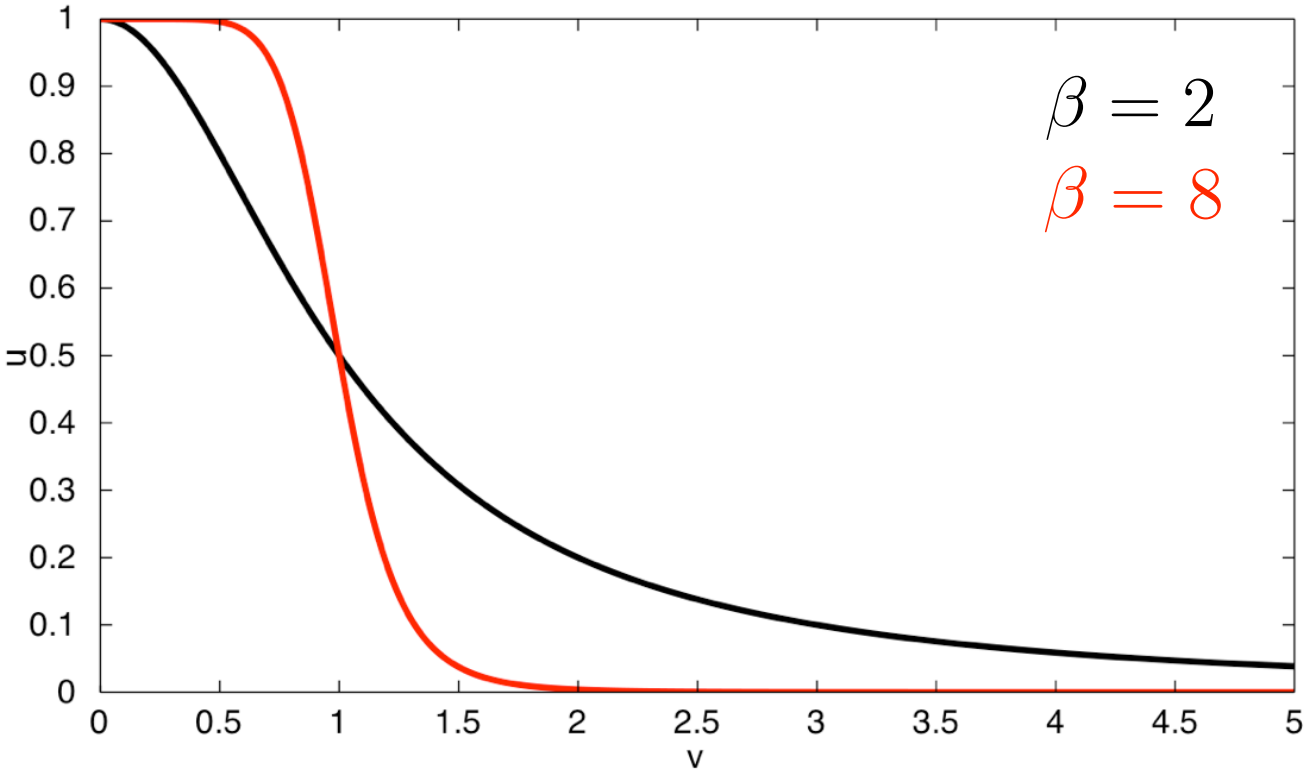
‘Portrait de phase’(Fig. 2)

Isoclines: $\dot{u} = \frac{\alpha_1}{1+v^\beta} - u = 0 \quad \Rightarrow u = \frac{\alpha_1}{1+v^\beta}$



Portrait de phase analyse géométrique (Fig. 2)

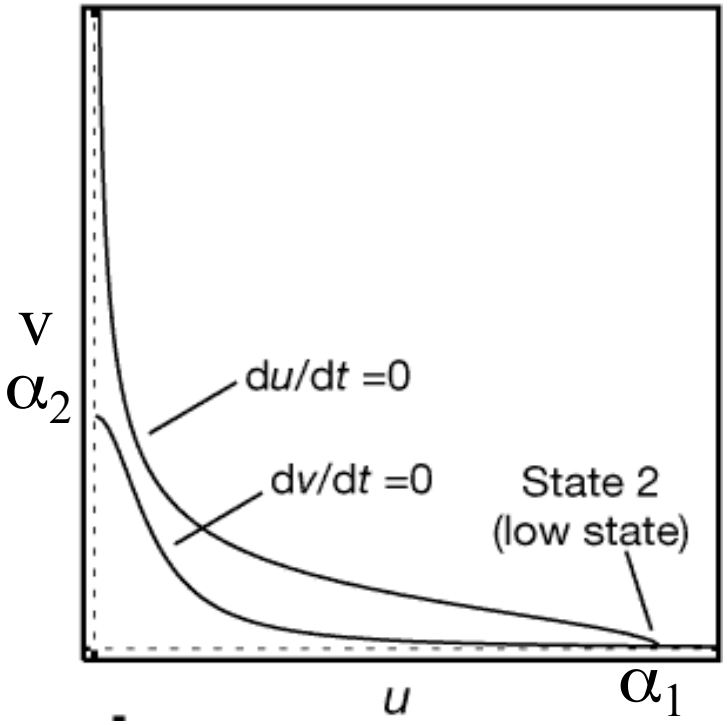
Isoclines: $\dot{u} = \frac{\alpha_1}{1+v^\beta} - u = 0 \qquad \Rightarrow u = \frac{\alpha_1}{1+v^\beta}$



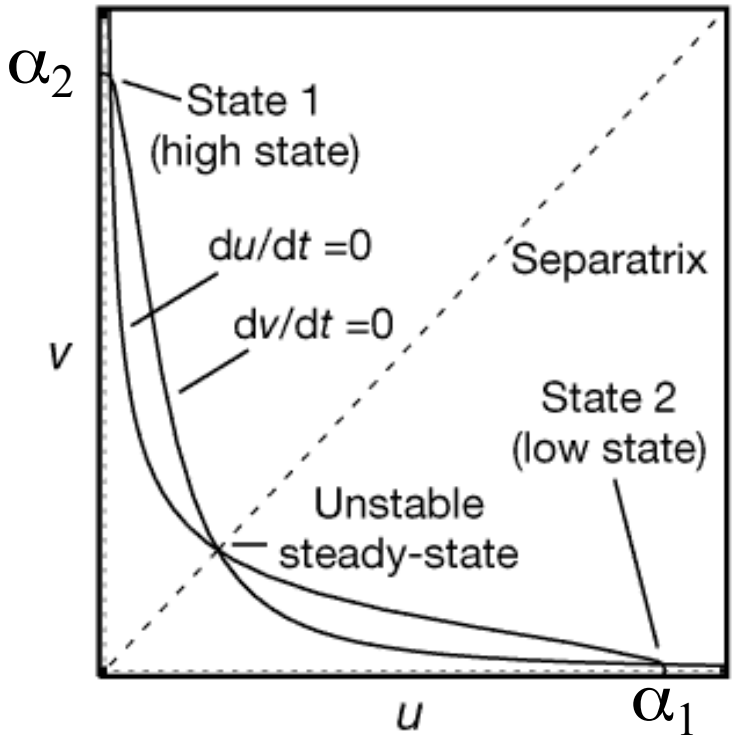
Portrait de phase (Fig. 2)

Isoclines: $u = \frac{\alpha_1}{1+v^{\beta}}, \quad v = \frac{\alpha_2}{1+u^{\gamma}}$

Cas A (1 croisement)



cas B (3 croisements)



mono-stabilité

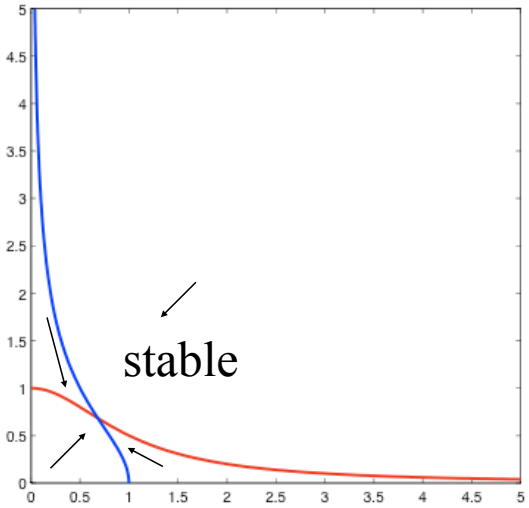
cas A

bistabilité

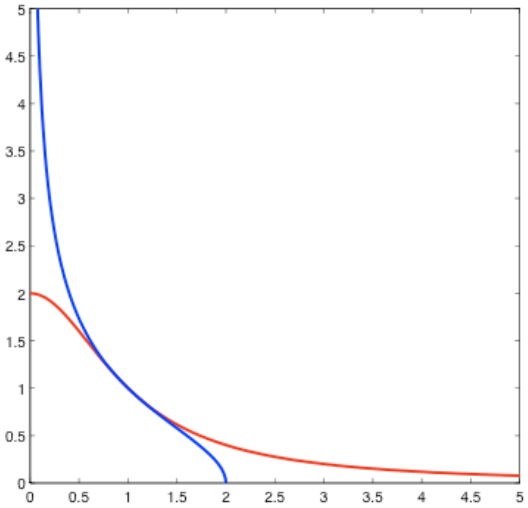
cas B

ici $\beta=\gamma=2$

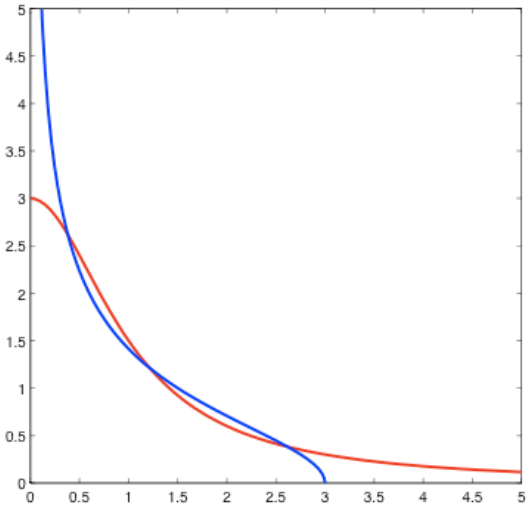
$\alpha 1=1$, $\alpha 2=1$



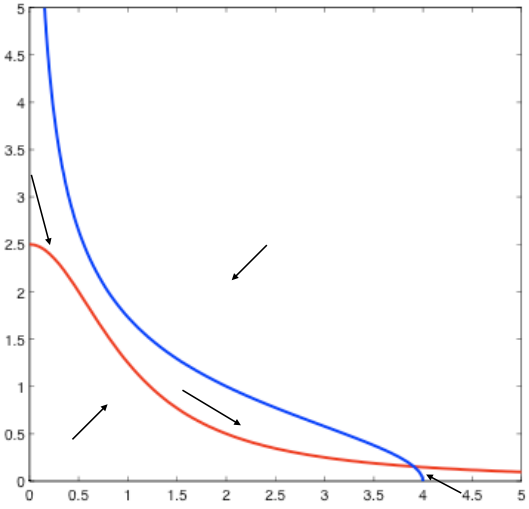
$\alpha 1=2$, $\alpha 2=2$



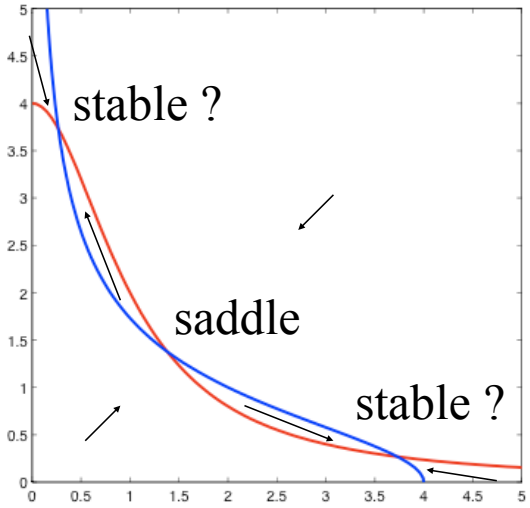
$\alpha 1=3$, $\alpha 2=3$



$\alpha 1=4$, $\alpha 2=2.5$



$\alpha 1=4$, $\alpha 2=4$



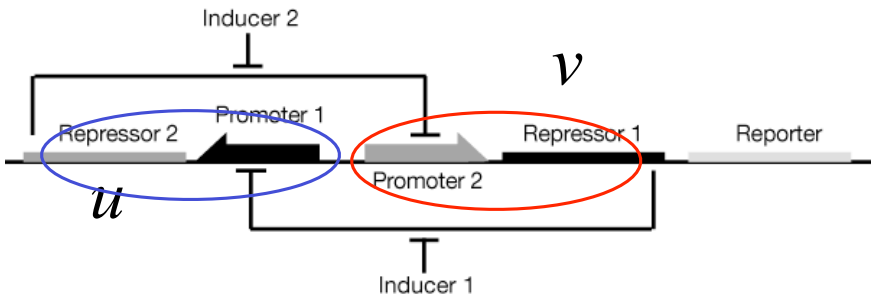
u nullcline

v nullcline

Analyse mathématique

$$\dot{u} = \alpha_1 \frac{1}{1+v^\beta} - u$$

$$\dot{v} = \alpha_2 \frac{1}{1+u^\gamma} - v$$



- Isoclines Ok
- Points fixes Ok
- Stabilité des points fixes → Etudier le Jacobien aux points fixes
- Portrait de phase

PF stable avec ou sans spirale ?

- étudier le Jacobien aux points fixes
- calcul exact des PFs n'est pas possible (équation de 4ème degré si $\beta=\gamma=2$)
- numérique (cf. isoclines.m)

cas A

$\alpha 1=1, \alpha 2=1$

$(u^*,v^*)=(0.6823,0.6823)$
$\tau = -2$
$\Delta = 0.5963$
$\tau^2-4\Delta = 1.6146>0$
PF stable

cas B

$\alpha 1=4, \alpha 2=4$

- **PF1**

$(u^*,v^*)=(1.3788,1.3788)$
$\tau = -2$
$\Delta = -0.7177$
$\tau^2-4\Delta = 6.8707>0$
PF selle

- **PF2,3**

$(u^*,v^*)=(3.7321,0.2679)$
$\tau = -2$
$\Delta = 0.75$
$\tau^2-4\Delta = 1>0$
PF stable

Preuve de l'absence de spirales (ici pour $\beta=\gamma=2$)

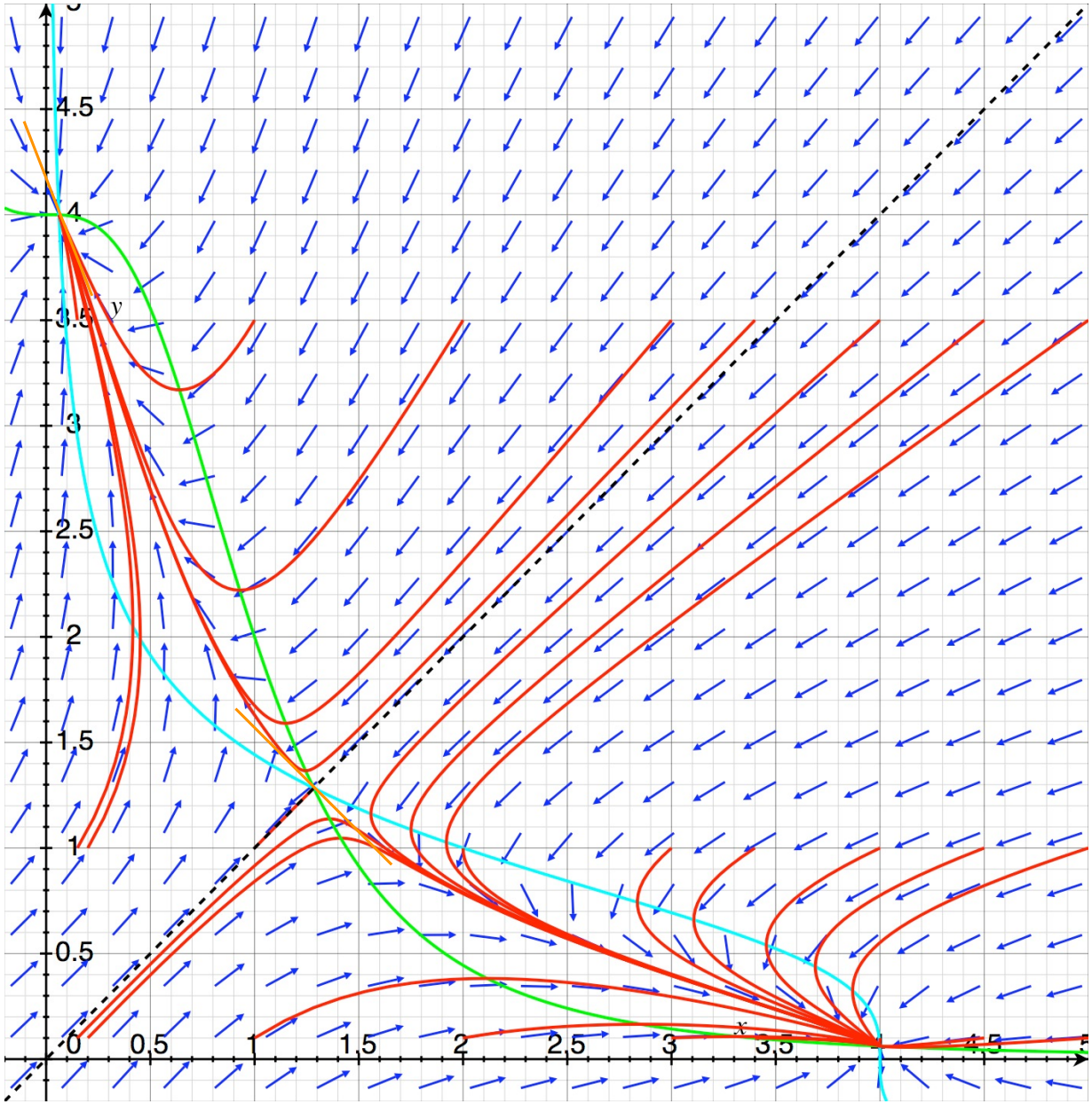
$$J = \begin{pmatrix} -1 & -2\,\alpha_1 v/(1+v^2)^2 \\ -2\,\alpha_2 u/(1+u^2)^2 & -1 \end{pmatrix}$$

$$\tau^2 - 4\Delta = 4 - 4\left(1 - \frac{4\,\alpha_1\alpha_2\,uv}{(1+u^2)^2(1+v^2)^2}\right) > 0 \qquad \text{si} \quad u, v > 0$$

Portraits de phase $\alpha1=\alpha2=4$, $\beta=\gamma=3$ bistabilité

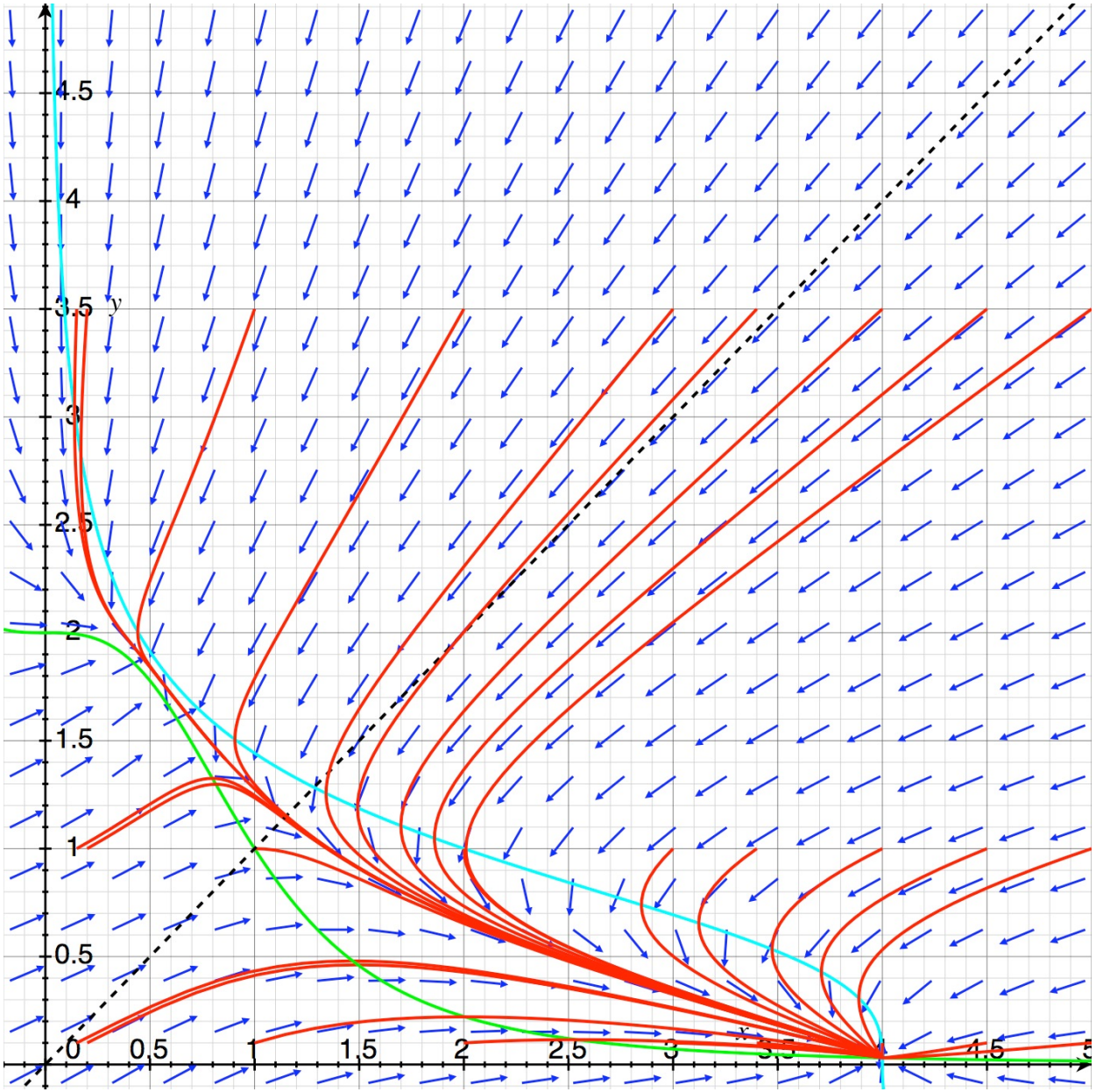
orange: direction lente

orange: direction instable



vert et bleu clair: isoclines

Portraits de phase $\alpha1=4, \alpha2=2, \beta=\gamma=3$ mono-stabilité



Commentaires des auteurs

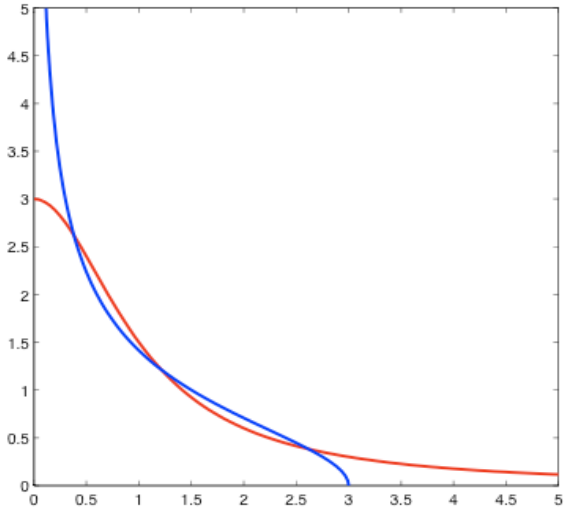
page 1. col. 2

The geometric structure of equation (1), illustrated in Fig. 2a and b, reveals the origin of the bistability: the nullclines ($du/dt = 0$ and $dv/dt = 0$ in Fig. 2) intersect at three points, producing one unstable and two stable steady states. From Fig. 2a and b, three key features of the system become apparent. First, the nullclines intersect three times because of their sigmoidal shape, which arises for $\beta, \gamma > 1$. Thus, the bistability of the system depends on the cooperative repression of transcription. Second, the rates of synthesis of the two repressors must be balanced. If the rates are not balanced, the nullclines will intersect only once, producing a single stable steady state. This situation arises in plasmid pIKE105. Third, the structure of the toggle network creates two basins of attraction. Thus, a toggle with an initial condition anywhere above the separatrix will ultimately settle to state 1, whereas a toggle starting below the separatrix will settle to state 2.

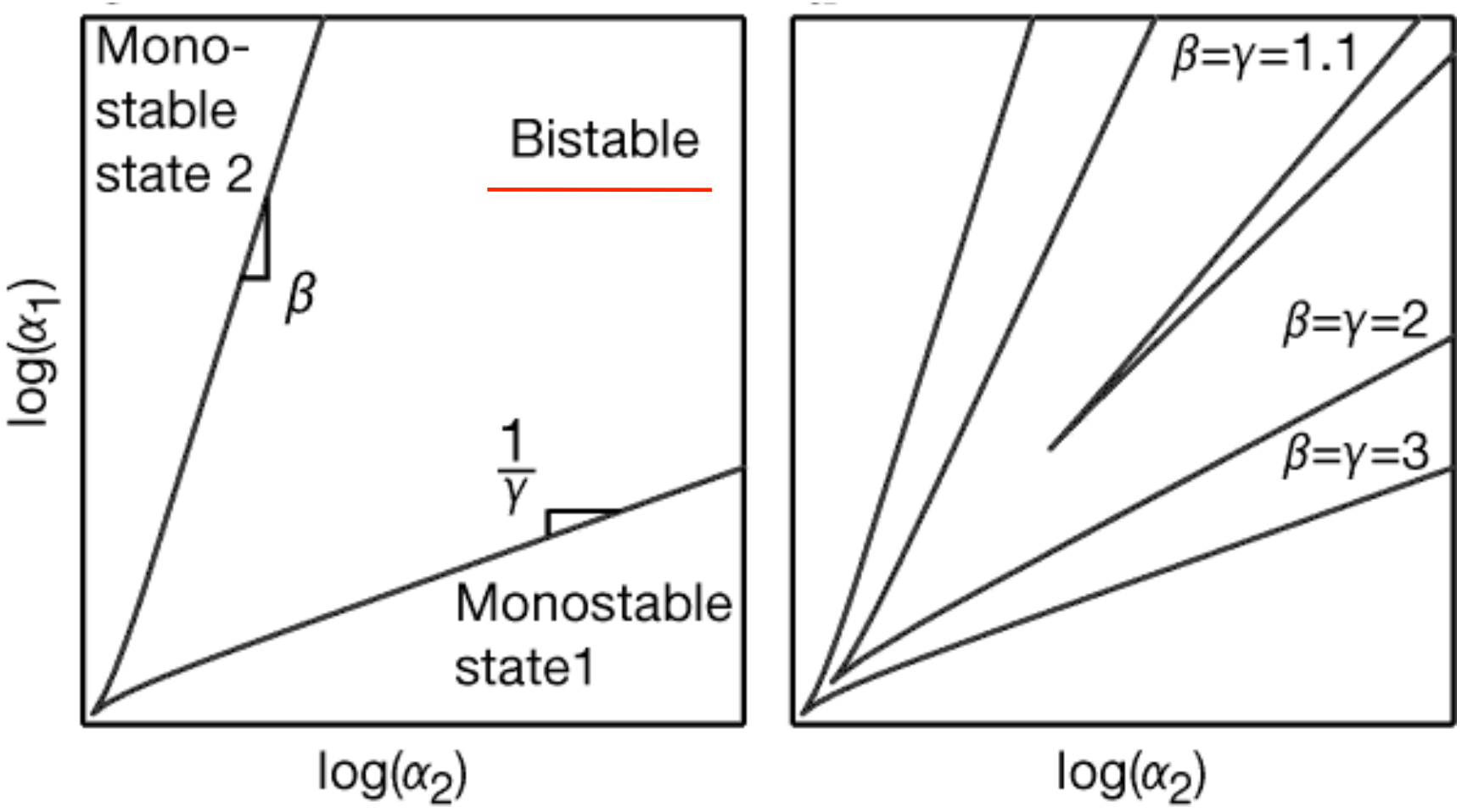
Conditions de bistabilité

- pour $\alpha 1$ et $\alpha 2$ suffisamment grands et équilibrés, le système est <u>bistable</u>

  <u>Corollaire:</u> pas de bistabilité si les promoteurs sont trop faibles
- la répression doit être coopérative, càd $\beta,\gamma>1$. plus β,γ plus il est aisé d'obtenir bistabilité



Stabilité des points fixes (Fig. 2b,c)

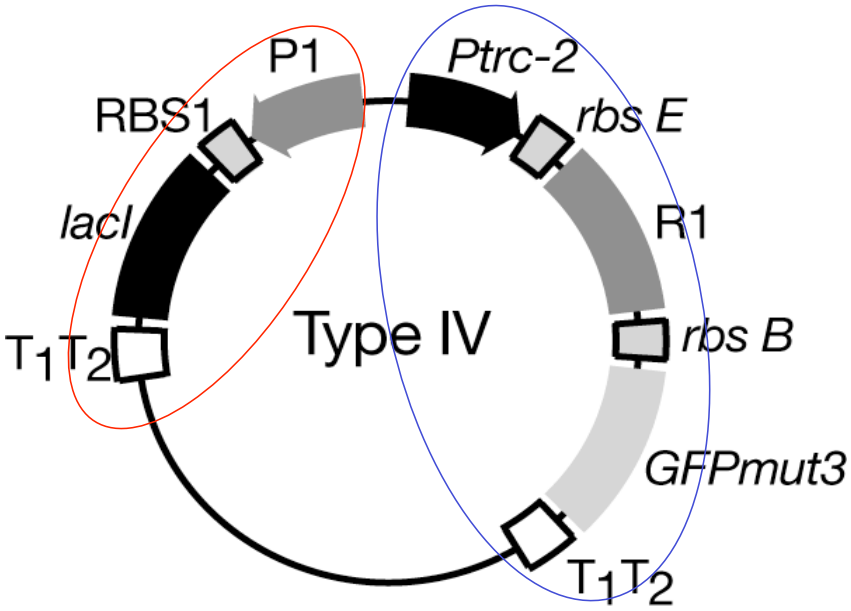


Note: la bistabilité est facilitée en présence de grands exposants et requiert une coopérativité>1

Implémentation expérimentale

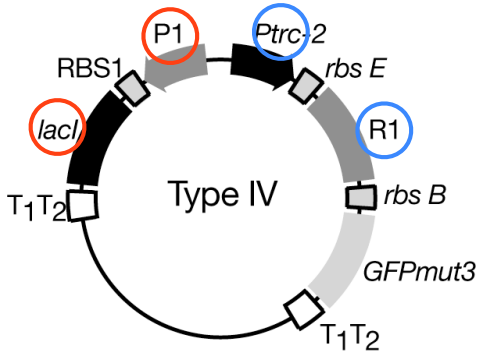
Implémentation expérimentale

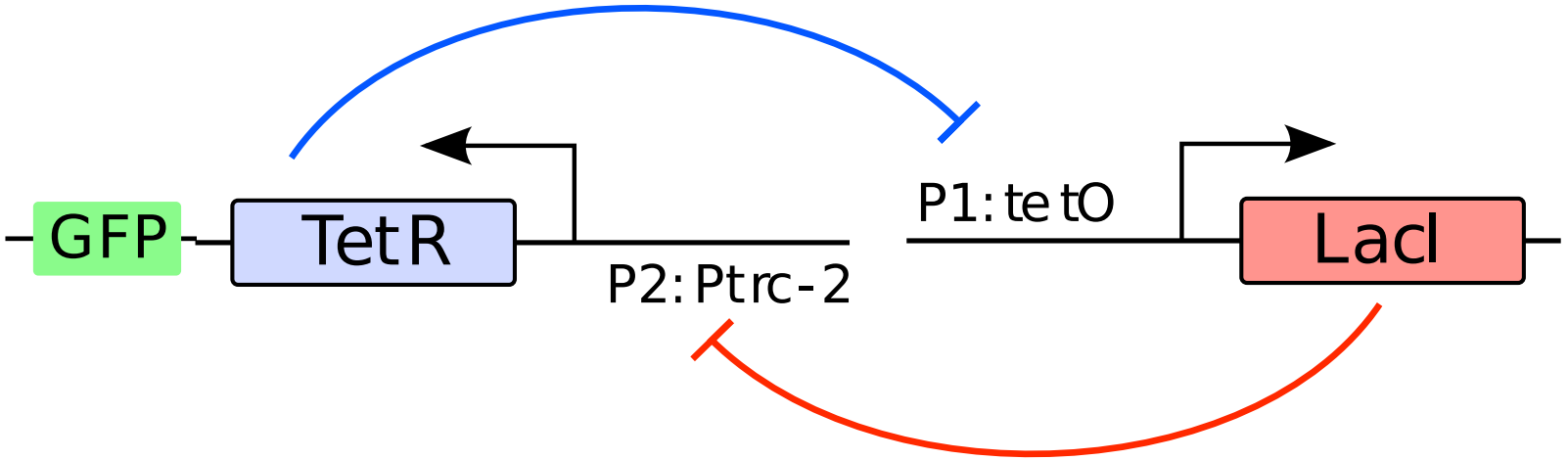
- utiliser des plasmides synthétiques dans E. coli



Ingrédients de base: lacI, Tet and lambda repressors

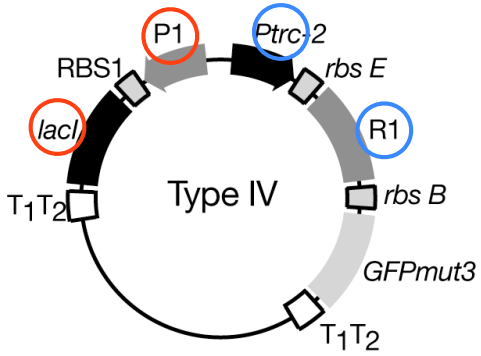
pIKE

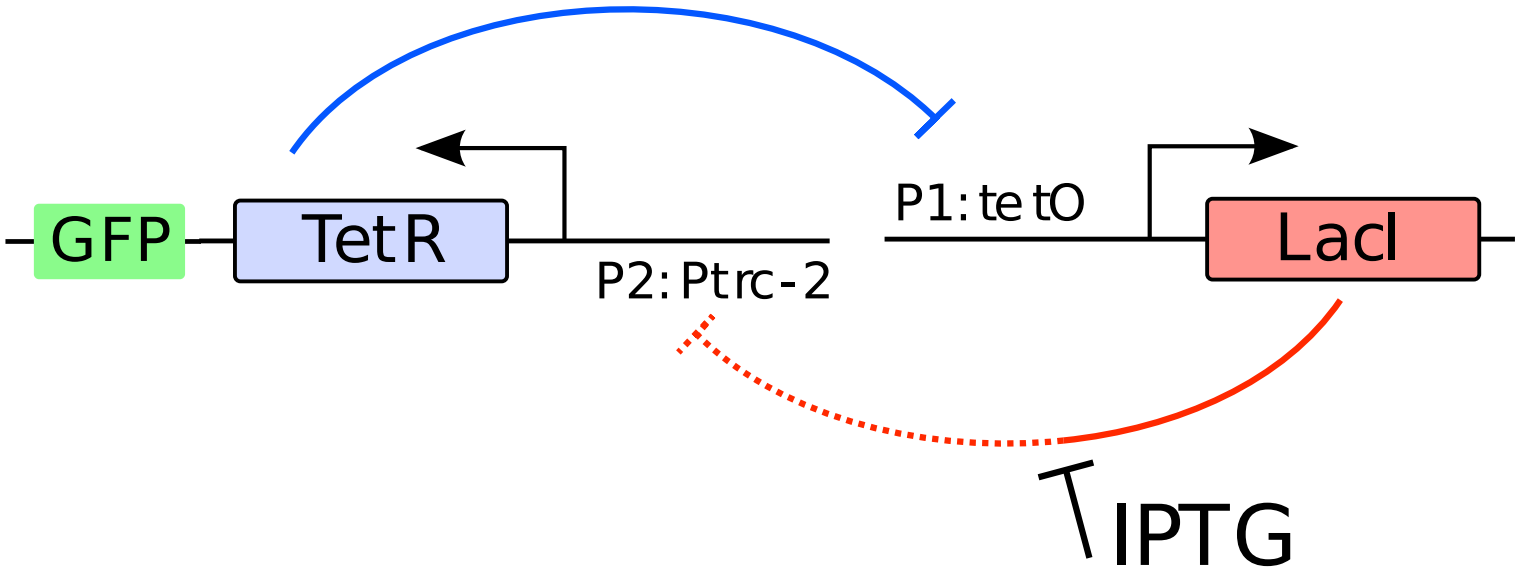




Ingrédients de base: lacI, tet and lambda repressors

pIKE



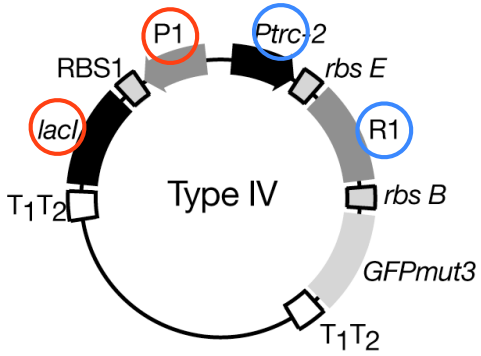


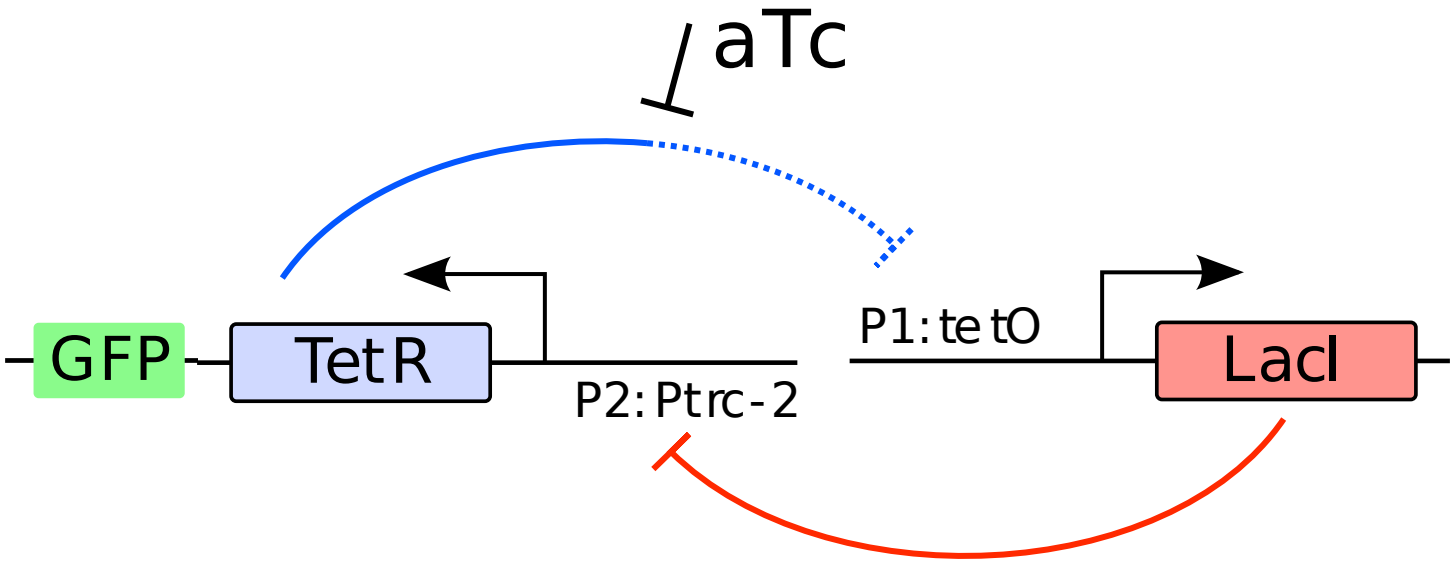
IPTG (~fake lactose) :
empêche lacI de réprimer le promoteur Ptrc-2

⟶ Active TetR

Ingrédients de base: lacI, tet and lambda repressors

pIKE



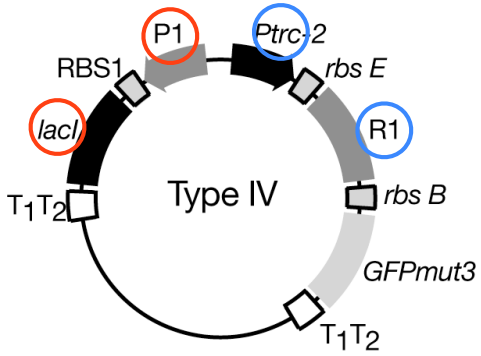


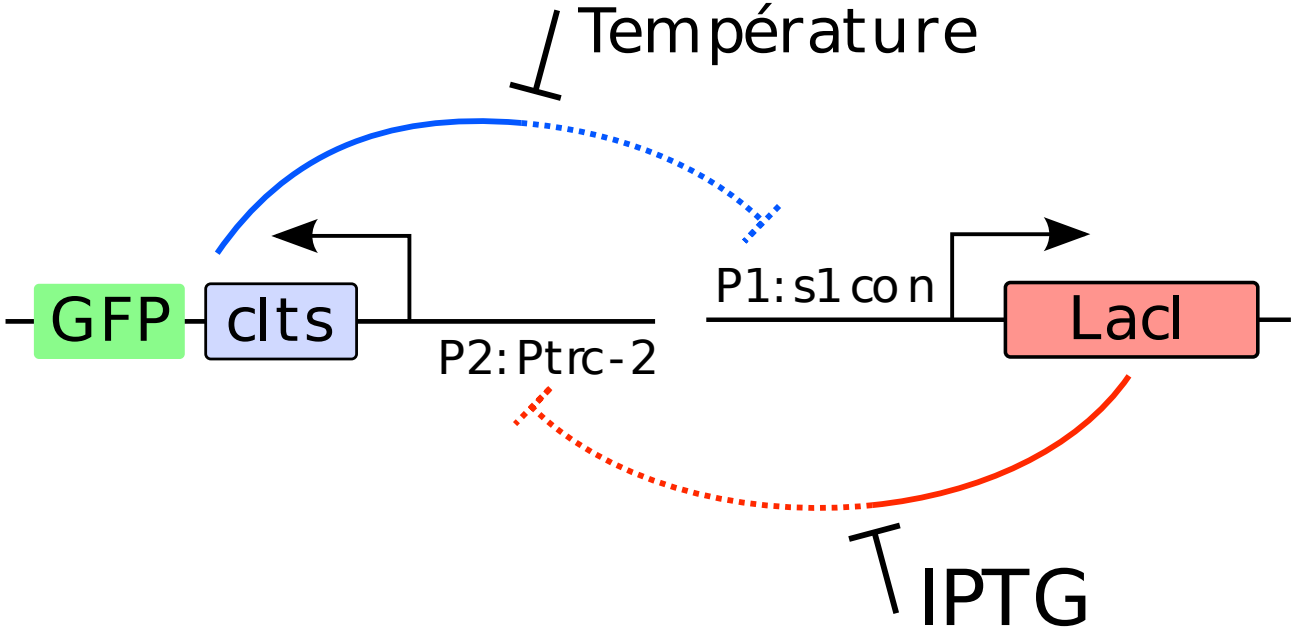
aTc empêche TetR de réprimer $P_L$tetO

⟶ Active LacI

Ingrédients de base: lacI, tet and lambda repressors

pTAK





- la température déstabilise la protéine cIts et ainsi empêche la répression du promoteur P_Ls1con

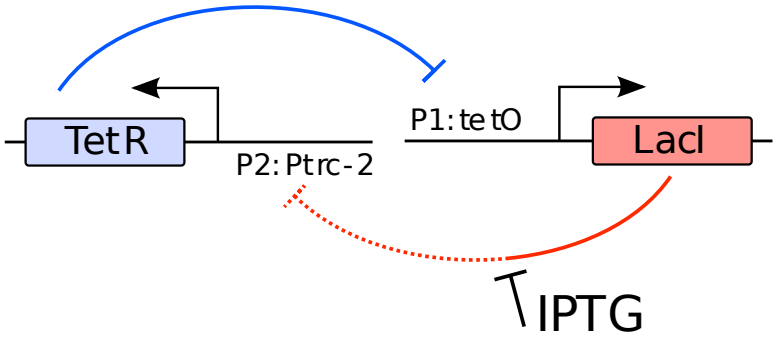
Contrôles externes

<u>switching signals</u> (IPTG, aTC, ou pulse de température)

- IPTG empêche lacI de réprimer le promoteur Ptrc-2

$$\frac{d}{dt}\mathrm{TetR} = \frac{\alpha_1}{1+(\mathrm{lacI}/\mathrm{IPTG})^\beta} - \mathrm{TetR}$$

$$\dot{u} = \alpha_1 \frac{1}{1+(v/I)^\beta} - u$$

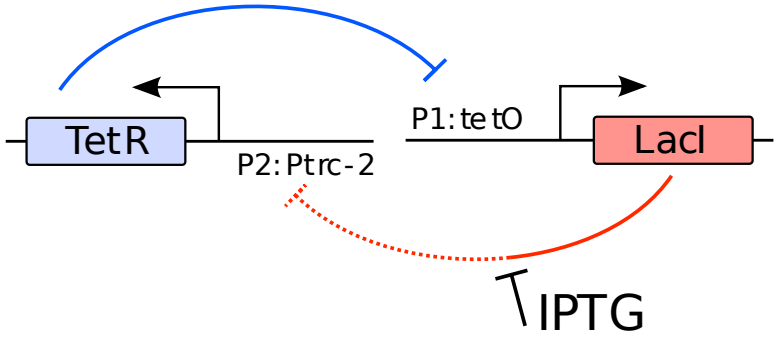


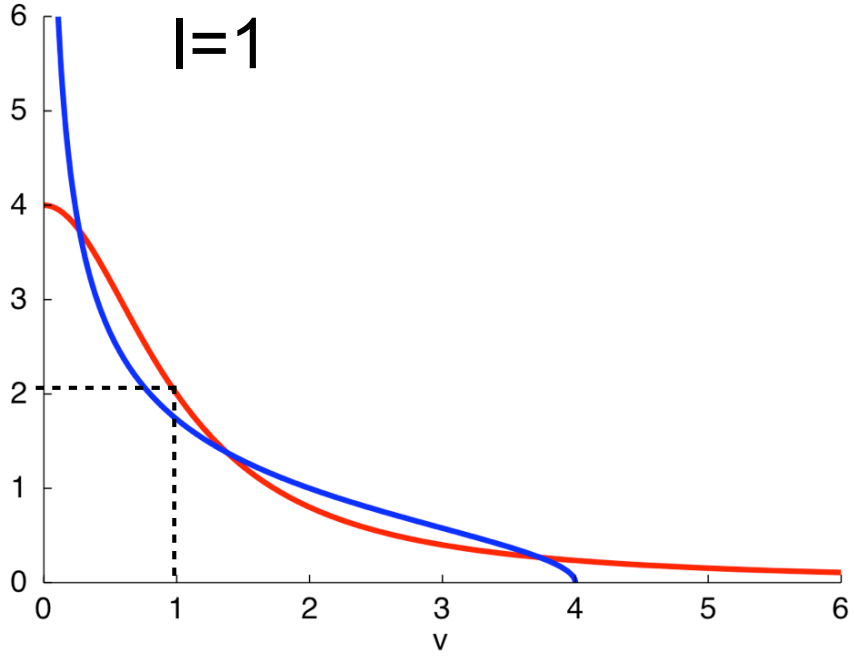
Effet sur les isoclines ?

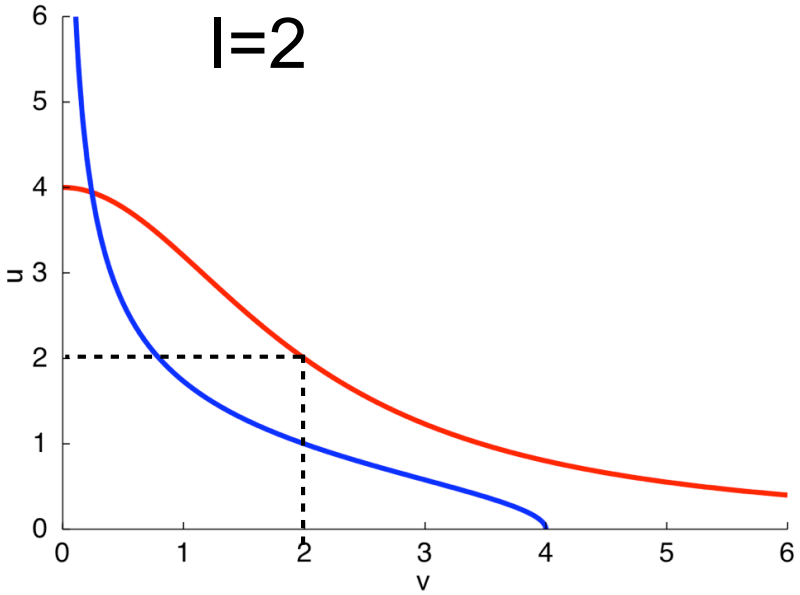
Contrôles externes

switching signals (IPTG, aTC, ou pulse de température)

$$\dot{u} = \alpha_1 \frac{1}{1 + (v/I)^\beta} - u$$







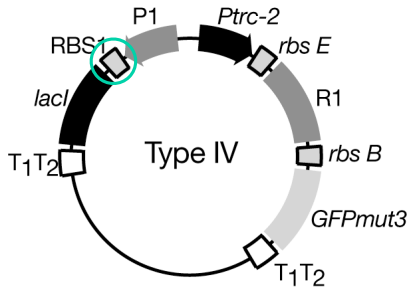
Contrôles des paramètres α

(taux d'expression)

changer les RBS (ribosomal binding sites)

régulation post-transcriptionelle

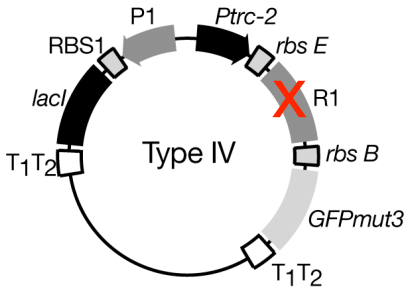
alternative: faire des mutations aux promoteurs



- Plasmides contrôles

  (sans feedback)

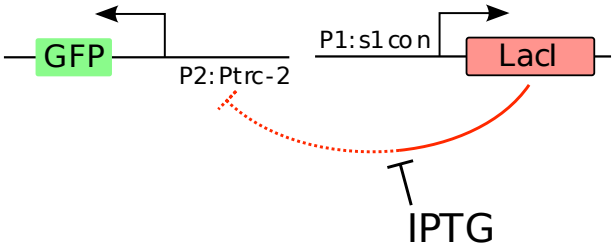
IPTG-inducible (pTAK102)



connexion R1-P1 manque

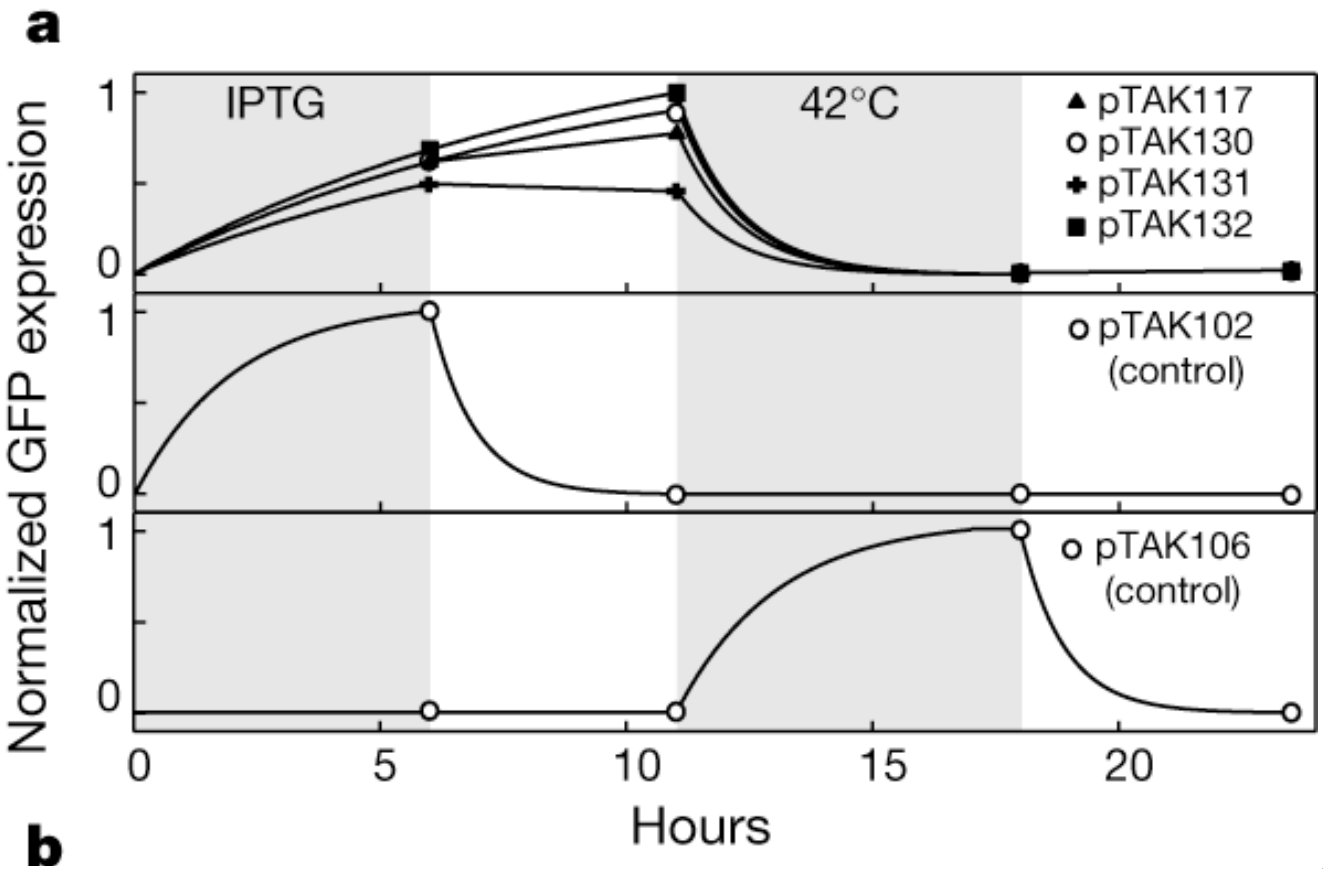
thermally-inducible (pTAK106)

aTc-inducible (pIKE108)



Résultats

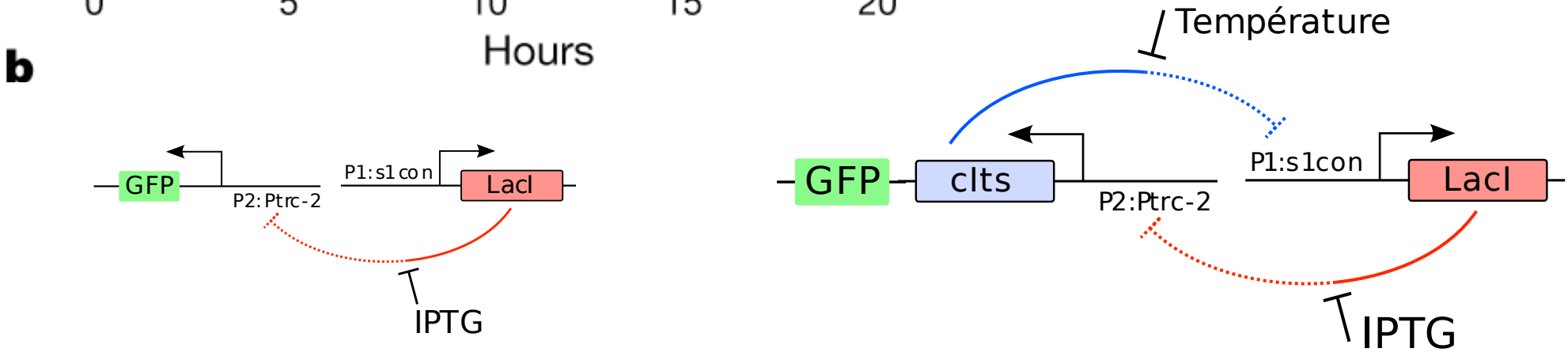
Expérience (pTAKs) 4x 6 heures (Fig. 4)



Bistable

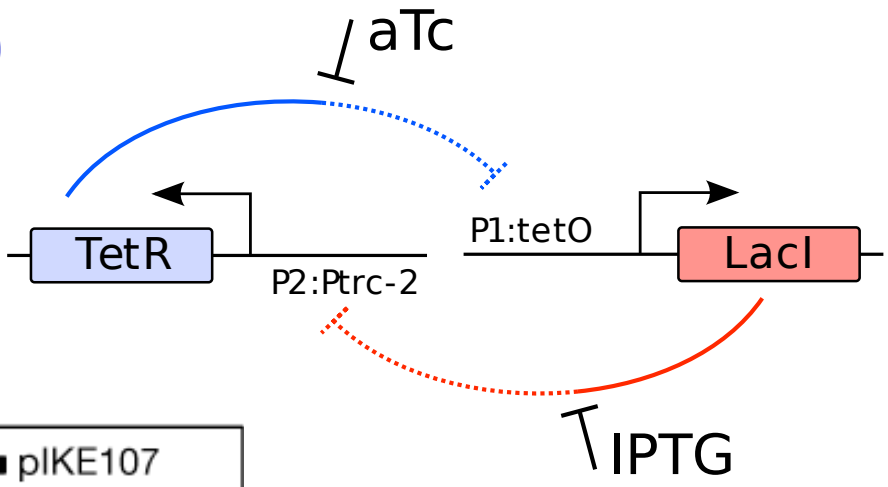
IPTG-inducible

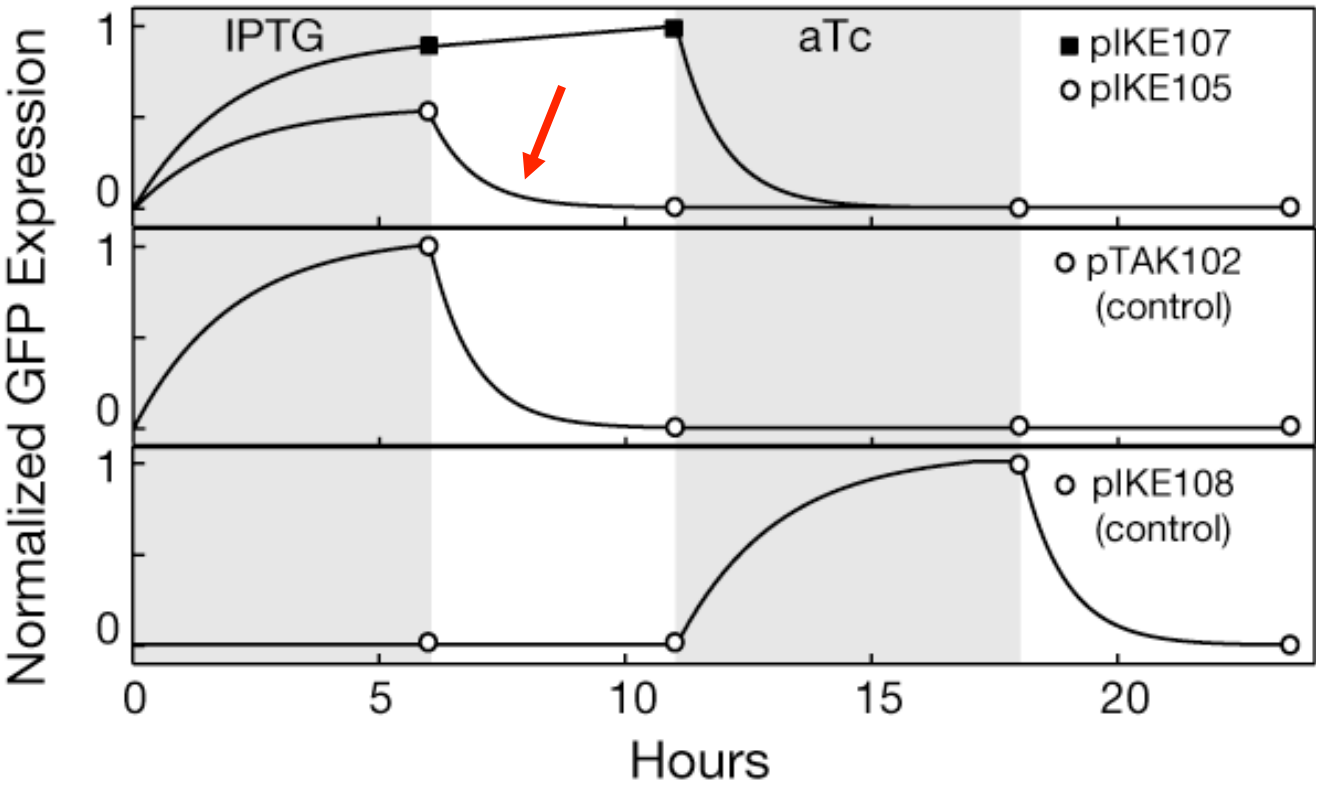
Temp-inducible



Tous les plasmids pTAKs sont bistables (sauf les contrôles)

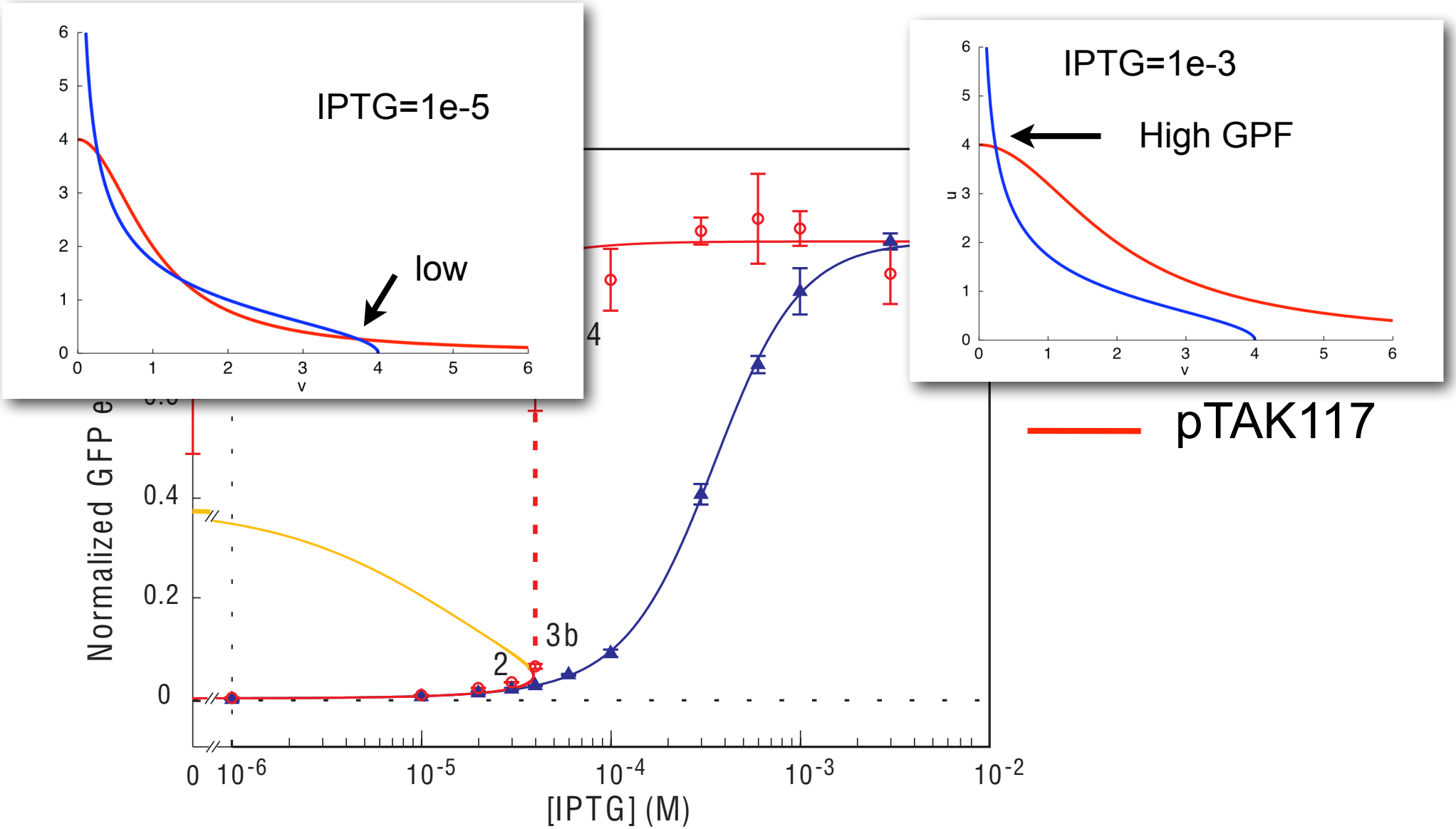
Expérience (pIKE) (Fig. 4)



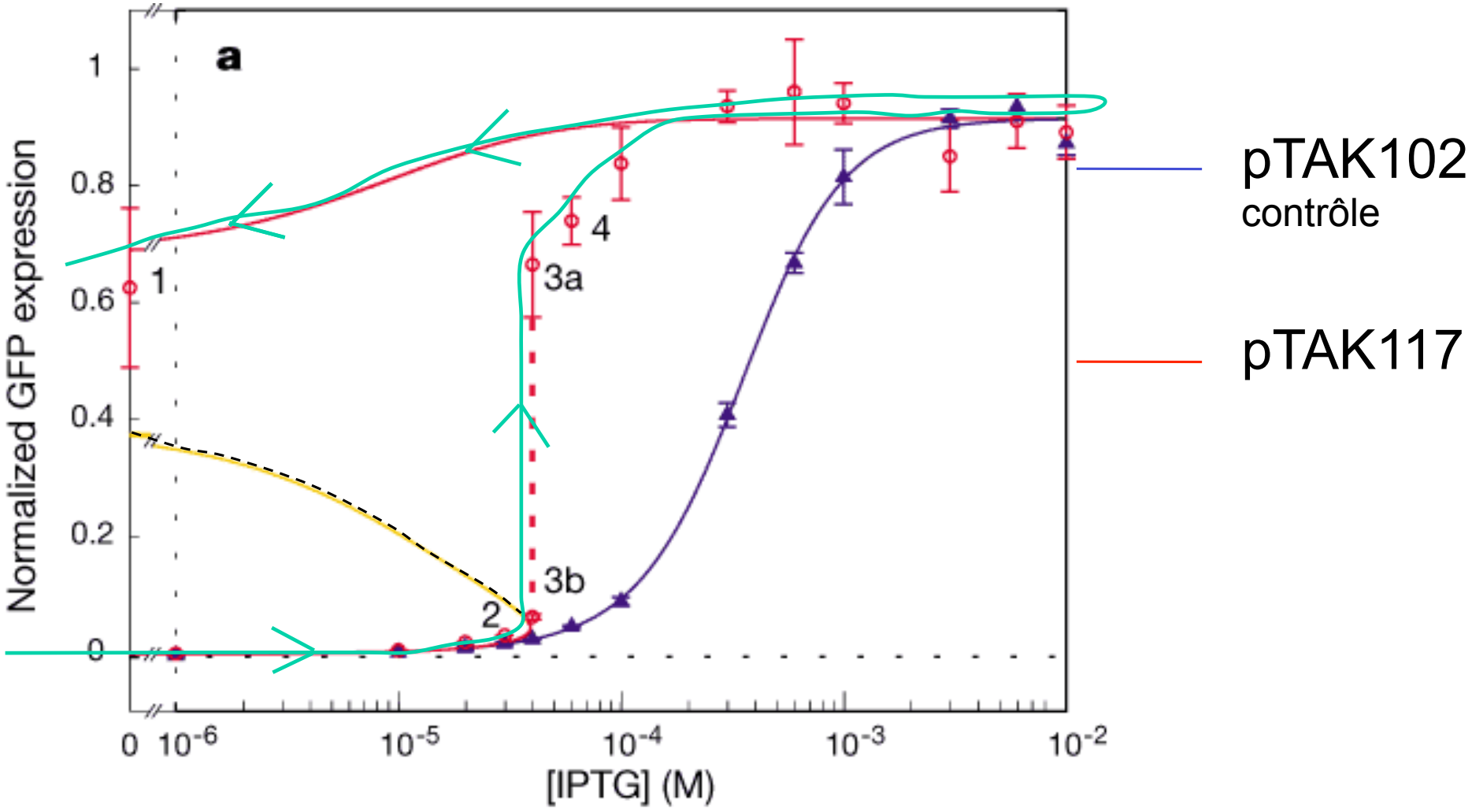


the rates of synthesis of the two repressors must be balanced. If the rates are not balanced, the nullclines will intersect only once, producing a single stable steady state. This situation arises in plasmid pIKE105. Third,

Bistabilité et seuil critique



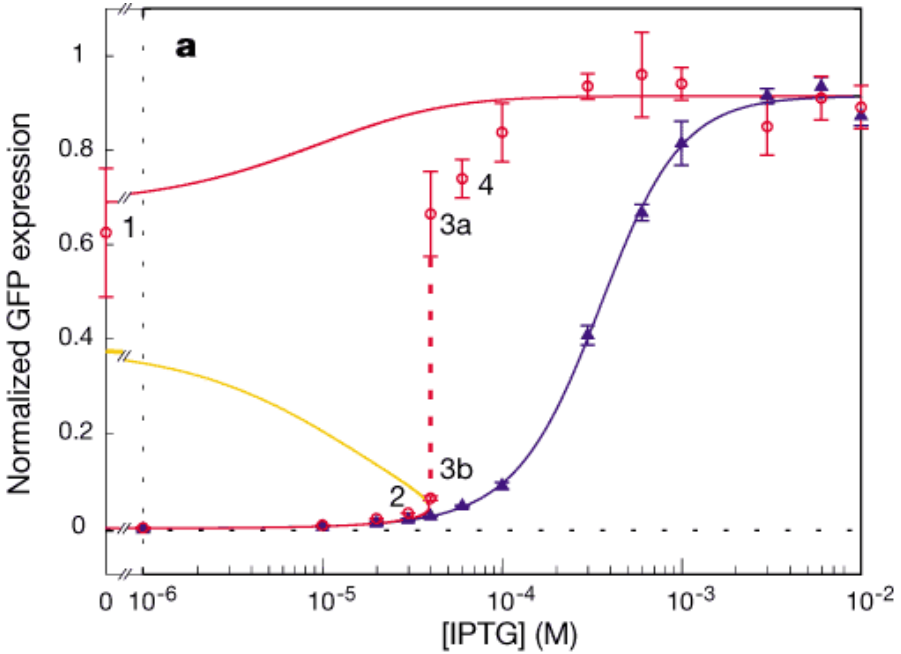
Bistabilité et seuil de transition (cf. cas 1D, hystérèse)



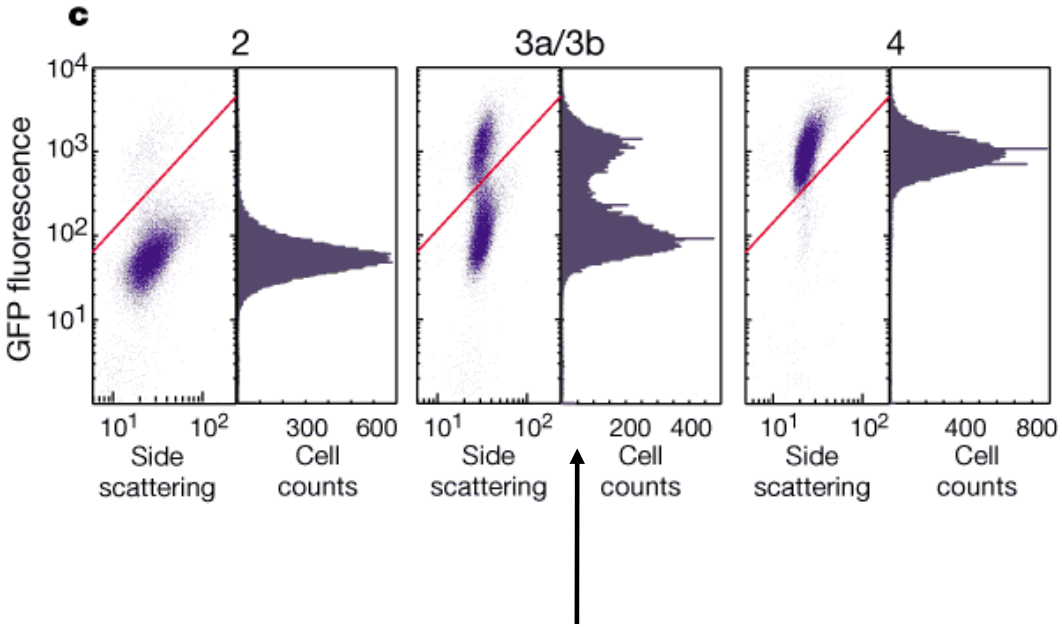
Note: analogie avec le chapitre 2.

Bimodalité au seuil de la transition (bruit)

Moyenne de population



Signal dans les cellules individuelles



fluctuations naturelles (bruit)
au point critique, coexistence des
2 états dans la population

Conclusions

works. As a practical device, the toggle switch, which requires only transient rather than sustained induction, may find applications in gene therapy and biotechnology. Finally, as a cellular memory unit, the toggle forms the basis for 'genetic applets'—self-contained, programmable, synthetic gene circuits for the control of cell function. □

applications futures:
mémoires cellulaires
biotechnologie

- L'analyse mathématique du modèle a permis de guider la construction du circuit génétique
- Elle permet de comprendre le comportement du réseau synthétique (bistabilité) de manière simple