Part 2

Simple network, complex function

Synthetic switch: more complex network with bistability

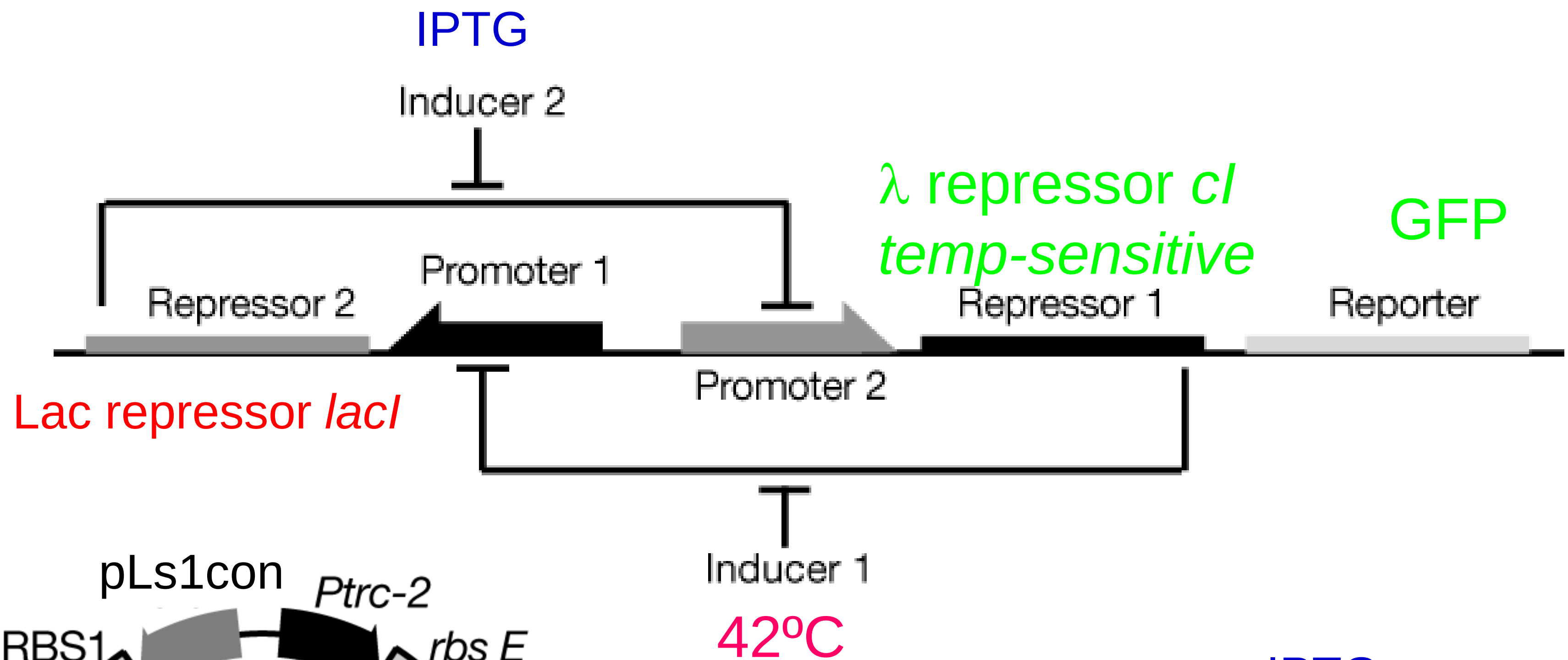
Week 3, 3/5-7/2019

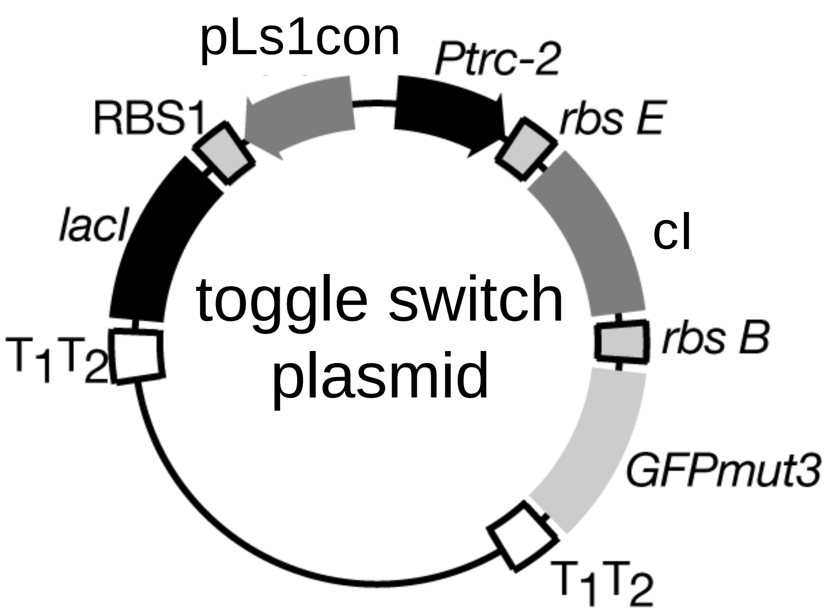
Synthetic bistability: a more complex network

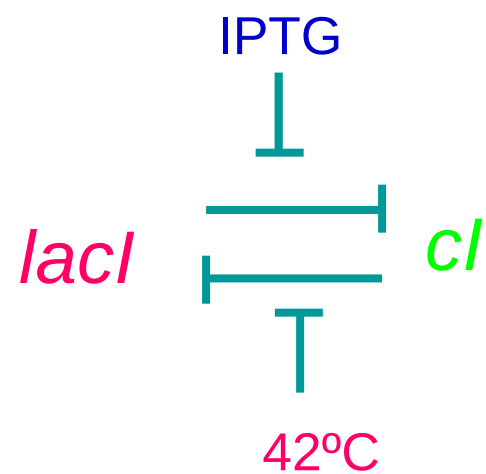
T. S. Gardner, C. R. Cantor, and J. J. Collins. Construction of a genetic toggle switch in *Escherichia coli*. *Nature* **403**, 339-342 (2000).

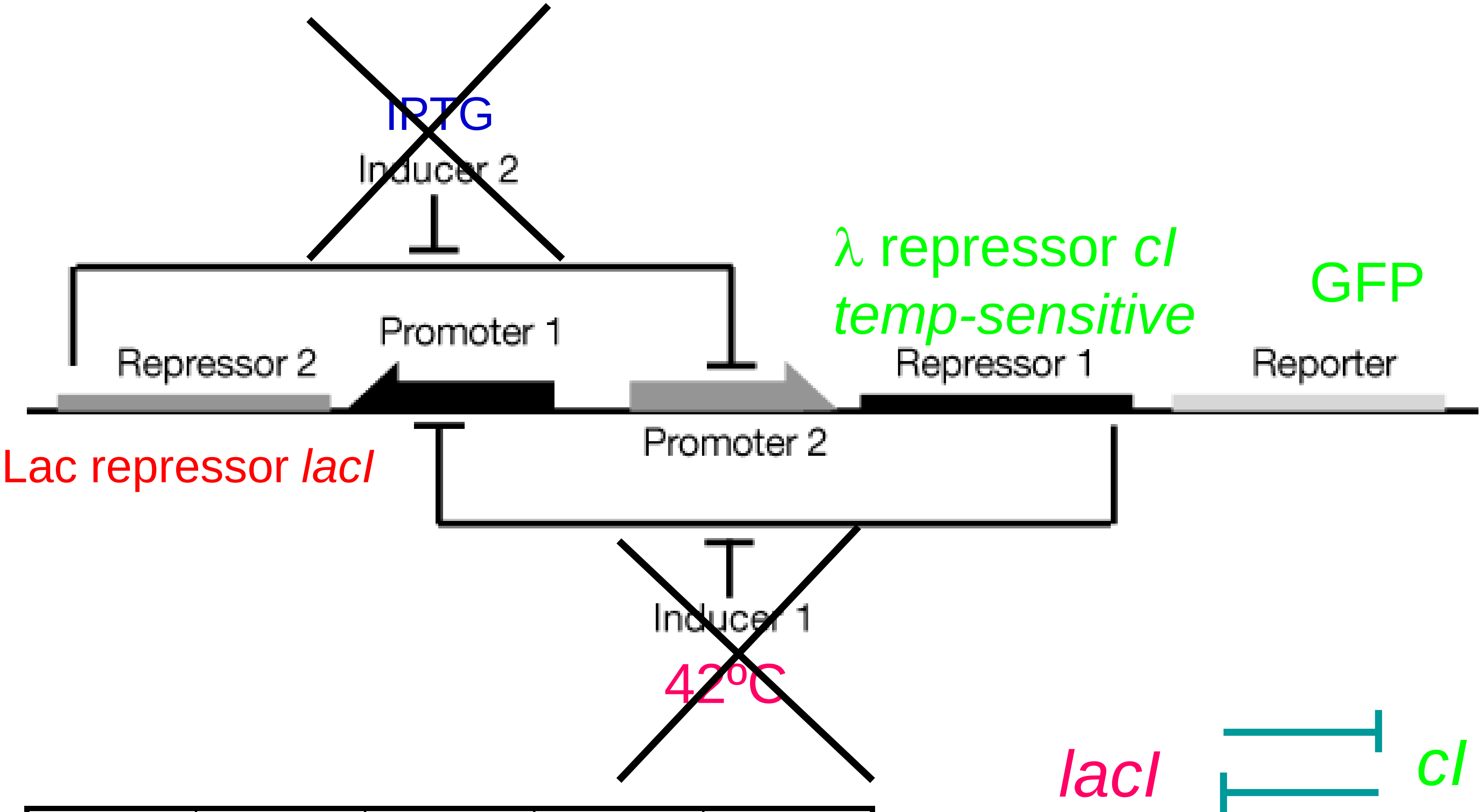
- why did they do this?

- they are engineers, who want to create new things from known
- toggle switch (拨动开关）is simple, but possibly useful
- know the mathematic principles
- have the tools and bioparts
- results might be as predicted, or might not, let’s take a look!


IPTG
Inducer 2
λ repressor *cI*
temp-sensitive
GFP
Repressor 2
Promoter 1
Repressor 1
Reporter
Promoter 2
Lac repressor *lacI*
Inducer 1
42ºC


pLs1con
Ptrc-2
RBS1
rbs E
lacI
cI
toggle switch
plasmid
T_1T_2
rbs B
GFPmut3
T_1T_2


IPTG
lacI
cI
42ºC



IPTG	42ºC	*lacI*	*cI*	GFP
Off	Off	Off ON	On Off	On Off

Bistability
History dependent

Derivation of the mathematic model

$$P_1 + R_2^{\beta} \xleftrightarrow{K_1} P_1R_2^{\beta}$$

$$P_2 + R_1^{\gamma} \xleftrightarrow{K_2} P_2R_1^{\gamma}$$

$$\gamma R_1 \xleftrightarrow{K_3} R_1^{\gamma}$$

$$\beta R_2 \xleftrightarrow{K_4} R_2^{\beta}$$

Fast events

$$P_1 \xrightarrow{k_1} P_1 + R_1 \qquad R_1 \xrightarrow{\lambda_1} \phi$$

$$P_2 \xrightarrow{k_2} P_2 + R_2 \qquad R_2 \xrightarrow{\lambda_2} \phi$$

slow events

$$[P^T] = [P_1^T] = [P_1] + [P_1R_2^{\beta}] = [P_2^T] = [P_2] + [P_2R_1^{\gamma}]$$

Derivation of the mathematic model

Now the rate of synthesis of repressor 1 and 2 can be written as:

$$R_{gen1} = k_1[P^T]\frac{[P_1]}{[P_1]+[P_1R_2^{\beta}]} = k_1[P^T]\frac{1}{1+K_1[R_2^{\beta}]} = \frac{k_1[P^T]}{1+K_1K_4[R_2]^{\beta}}$$

$$R_{gen2} = k_2[P^T]\frac{[P_2]}{[P_2]+[P_2R_1^{\gamma}]} = k_2[P^T]\frac{1}{1+K_2[R_1^{\gamma}]} = \frac{k_2[P^T]}{1+K_2K_3[R_1]^{\gamma}}$$

The rates k_1 and k_2 are the effective synthesis rates

Derivation of the mathematic model

Assuming a first order decay process, the kinetic equations are

$$\frac{d[R_1]}{dt} = \frac{k_1[P^T]}{1 + K_1 K_4 [R_2]^{\beta}} - \lambda_1 [R_1]$$

$$\frac{d[R_2]}{dt} = \frac{k_2[P^T]}{1 + K_2 K_3 [R_1]^{\gamma}} - \lambda_2 [R_2]$$

The equations can be eventually simplified as

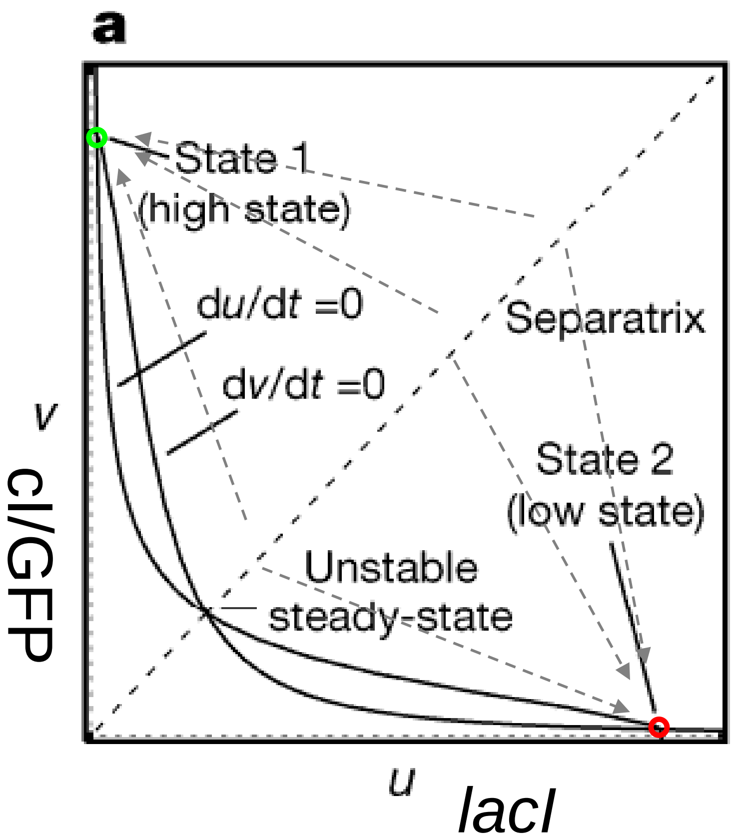
$$\boxed{\begin{aligned} \frac{dU}{dt} &= \frac{\alpha_1}{1 + V^{\beta}} - \lambda_1 U \\ \frac{dV}{dt} &= \frac{\alpha_2}{1 + U^{\gamma}} - \lambda_2 V \end{aligned}}$$

$$\frac{dU}{dt} = \frac{\alpha_1}{1+V^{\beta}} - \lambda_1 U$$
$$\frac{dV}{dt} = \frac{\alpha_2}{1+U^{\gamma}} - \lambda_2 V$$

稳态解：

$$\frac{dU}{dt} = \frac{\alpha_1}{1+V^{\beta}} - \lambda_1 U \equiv 0$$
$$\frac{dV}{dt} = \frac{\alpha_2}{1+U^{\gamma}} - \lambda_2 V \equiv 0$$

Bistability 双稳态



dU/dt=0 represent a line $U = \frac{\alpha_1}{\lambda_1(1+V^{\beta})}$

dV/dt=0 represent a line $V = \frac{\alpha_2}{\lambda_2(1+U^{\gamma})}$

The two lines are called nullclines, the intersections are called fixed point, or steady states.

What does this mean?

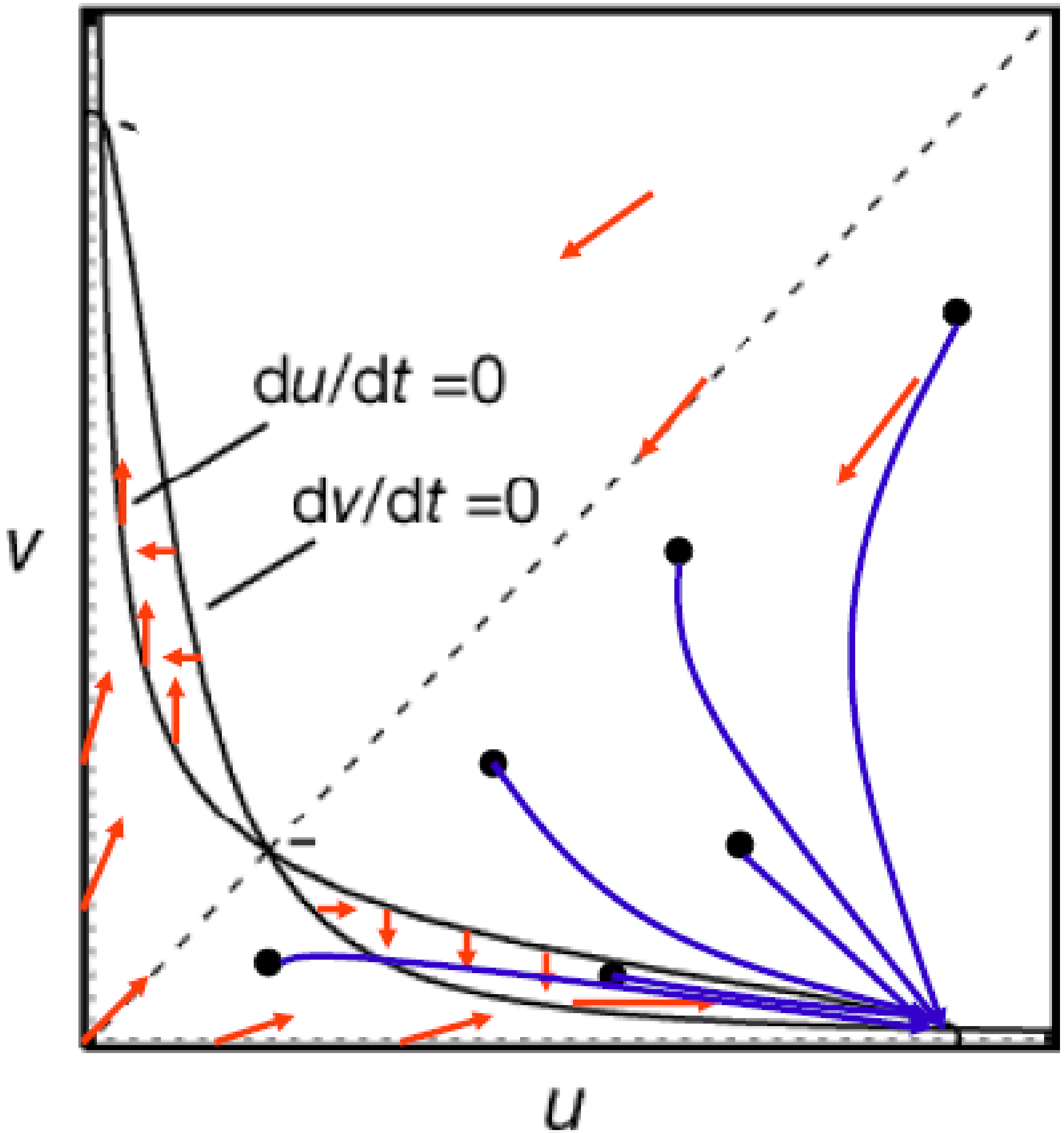
Analysis of this type of diagram without solving the ODE is called stability analysis.

Let's use the Matlab for graphical illustration of stability analysis. To simplify the equations, we set $\lambda 1$ and $\lambda 2$ to 1.

```
% function gardnerfunc.m define ODE equation
function dydt = f(t,y,flag,a1,a2,beta,gamma)
% [u] = y(1) [v]=y(2)
dydt = [a1/(1+y(2)^beta)-y(1);  a2/(1+y(1)^gamma)-y(2)];
```

```matlab
% function gardner1.m stability analysis for bistability
clear;hold off;options=[];
a1=10;b=2;
a2=10;g=2;
% draw nullcline
y1=0:0.01:30;x2=0:0.01:30;
x1=a1./(1+y1.^b); y2=a2./(1+x2.^g);
figure(1); plot(x1,y1);hold on; plot(x2,y2,'r-');plot([0 30],[0 30],'k--');
legend('dU/dt=0','dV/dt=0');
axis([-0.5 12 -0.5 12]);xlabel('U, cI');ylabel('V, LacI');title('IPTG off, 42^oC off');

% calculate the dynamic process from any initial value
button =0;
while button~=3 % right click stop the program
[x1,y1,button]=ginput(1); % get the initial value from mouse left click
x0=x1;y0=y1;
[t1 yt]=ode45('gardnerfunc',[0 10],[x0 y0],options,a1,a2,b,g);
    if x0>y0
        plot(yt(:,1),yt(:,2),'m.-');% lower half
    else
        plot(yt(:,1),yt(:,2),'c.-'); % upper half
    end
end
```



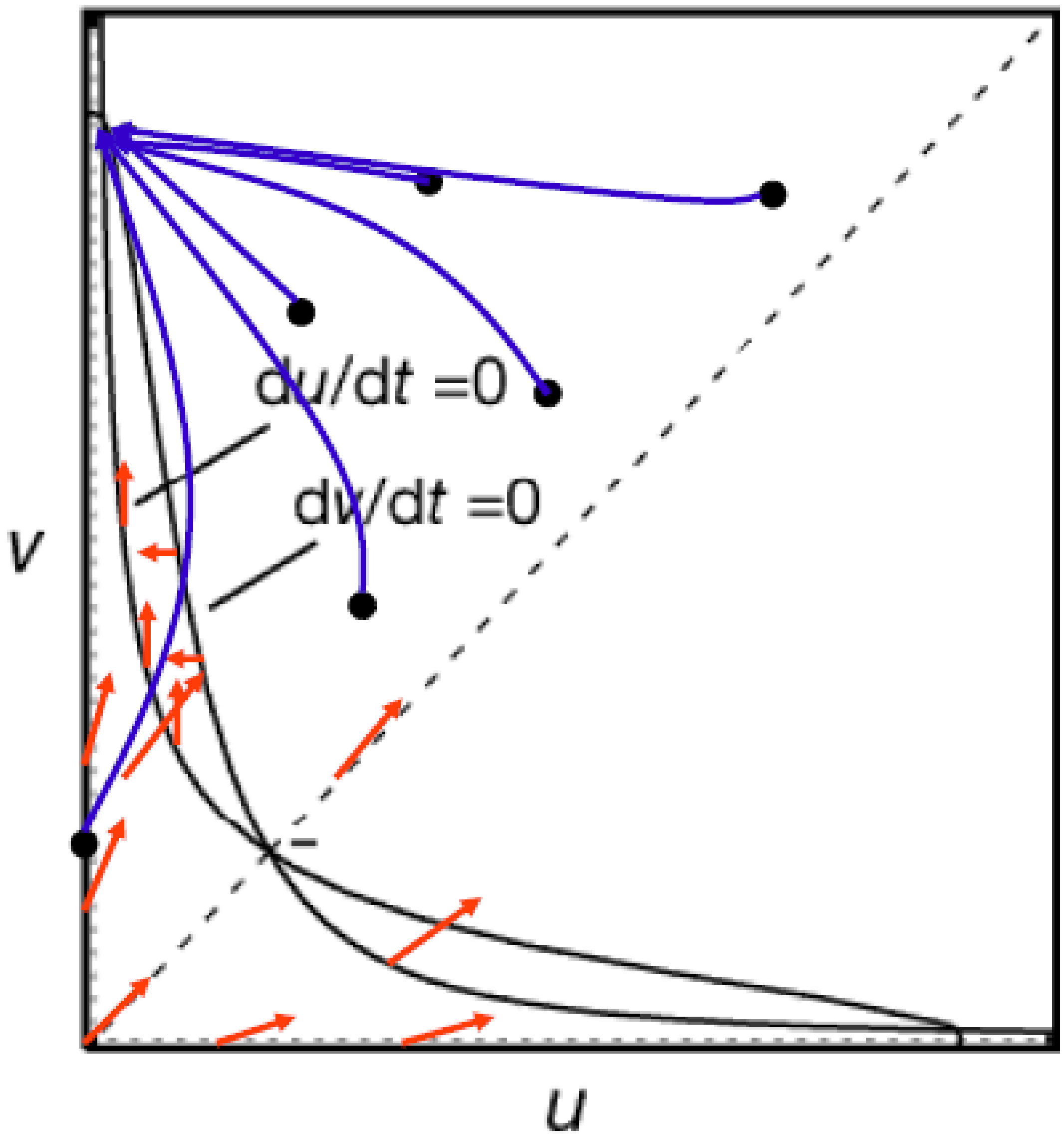
nullclines:

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

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

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

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



nullclines:

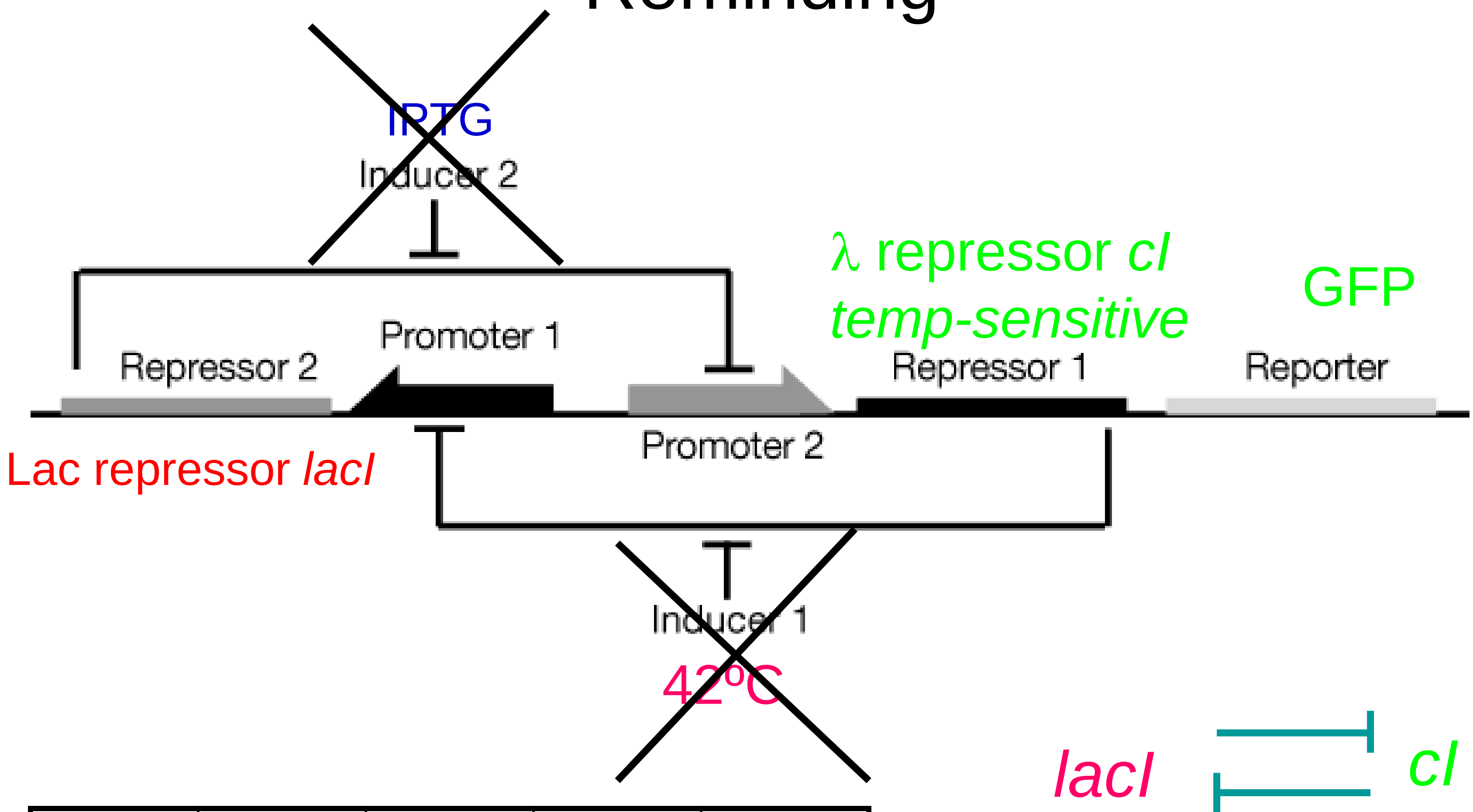
$$u = \frac{\alpha_1}{1+v^\beta}$$

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

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

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

Reminding



IPTG	42ºC	*lacI*	*cI*	GFP
Off	Off	Off ON	On Off	On Off

Bistability
History dependent

lacI ⊣ ⊢ *cI*

Bistability 双稳态

$$\boxed{\begin{aligned}\frac{dU}{dt} &= \frac{\alpha_1}{1+V^{\beta}} - \lambda_1 U \\ \frac{dV}{dt} &= \frac{\alpha_2}{1+U^{\gamma}} - \lambda_2 V\end{aligned}}$$

稳态解：

$$\frac{dU}{dt} = \frac{\alpha_1}{1+V^{\beta}} - \lambda_1 U \equiv 0$$

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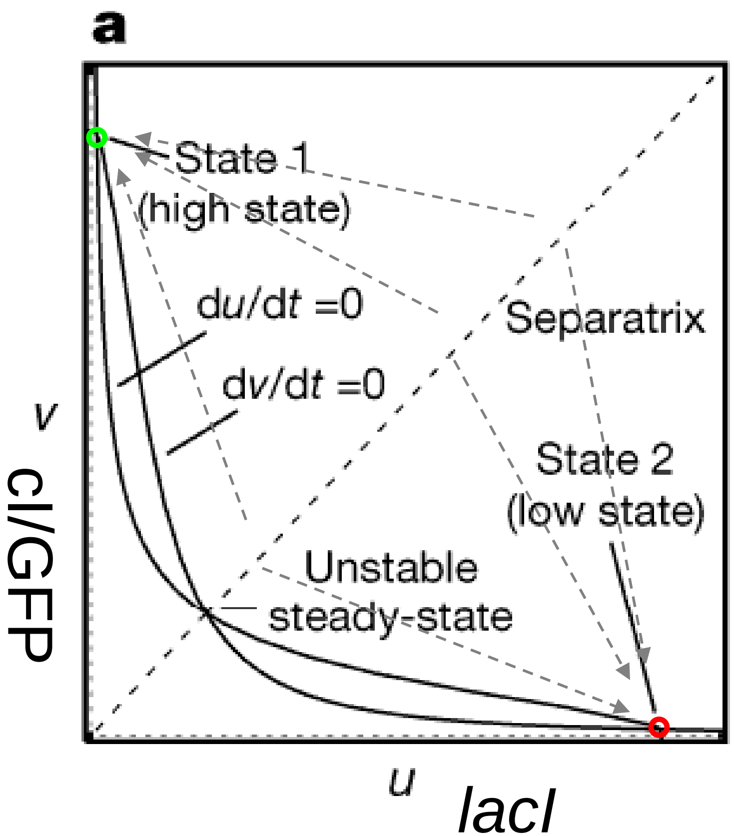

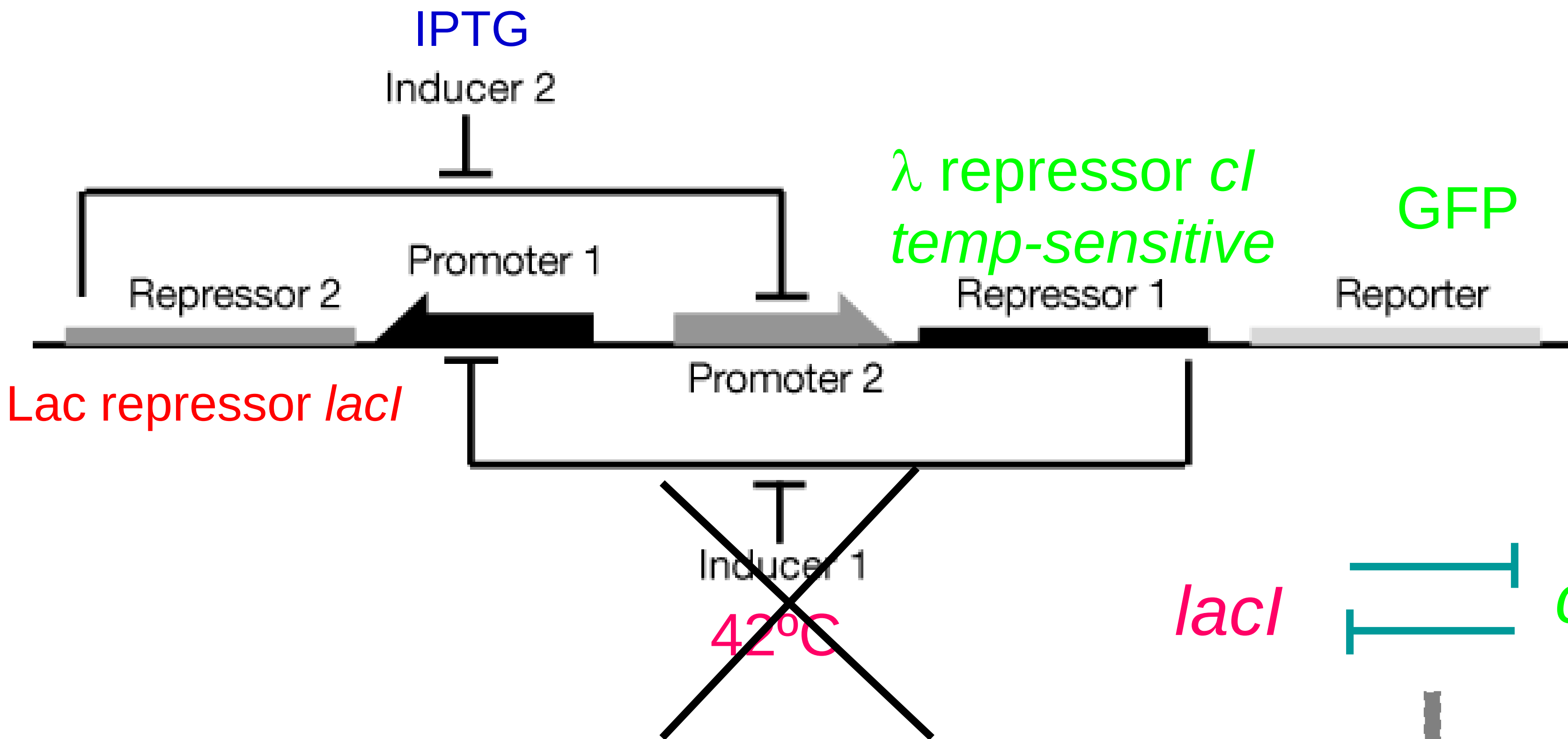

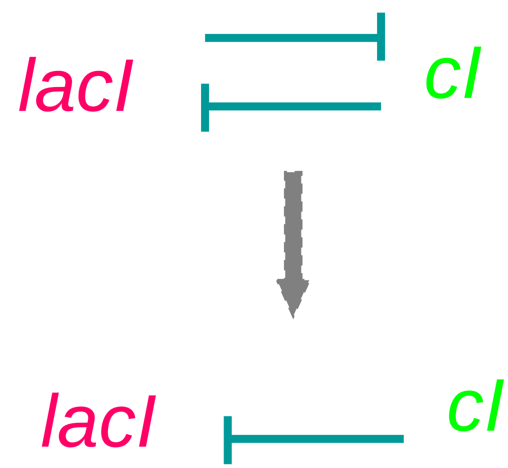

IPTG	42ºC	*lacI*	*cI*	GFP
off > on	Off	Off	On	On

GFP turned on

Bistability 双稳态

$$\boxed{\begin{aligned}\frac{dU}{dt}&=\frac{\alpha_1}{1+V^{\beta}}-\lambda_1 U\\ \frac{dV}{dt}&=\frac{\alpha_2}{1+U^{\gamma}}-\lambda_2 V\end{aligned}}$$

稳态解：

$$\frac{dU}{dt}=\frac{\alpha_1}{1+V^{\beta}}-\lambda_1 U\equiv 0$$

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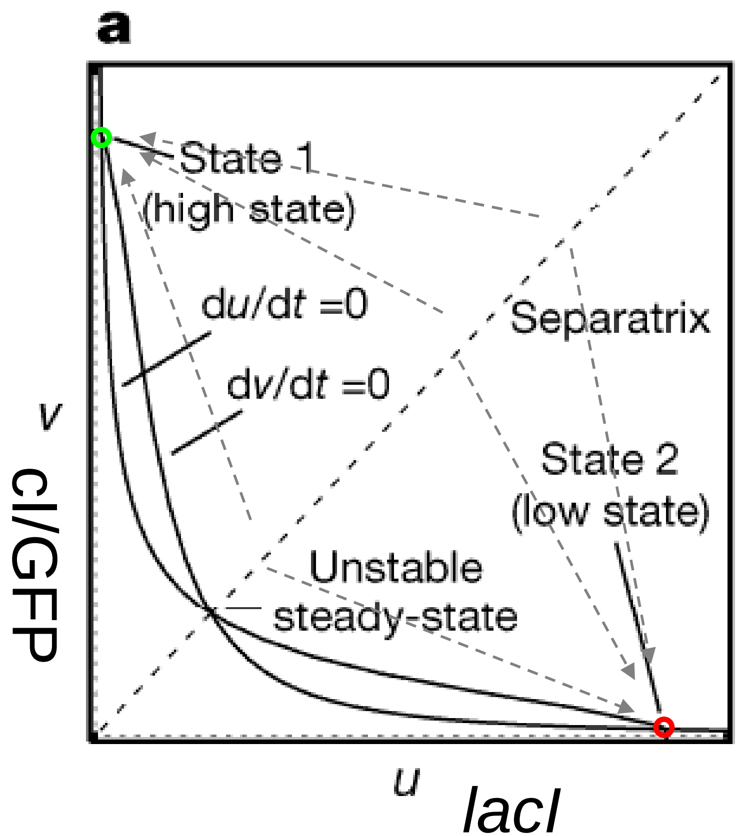
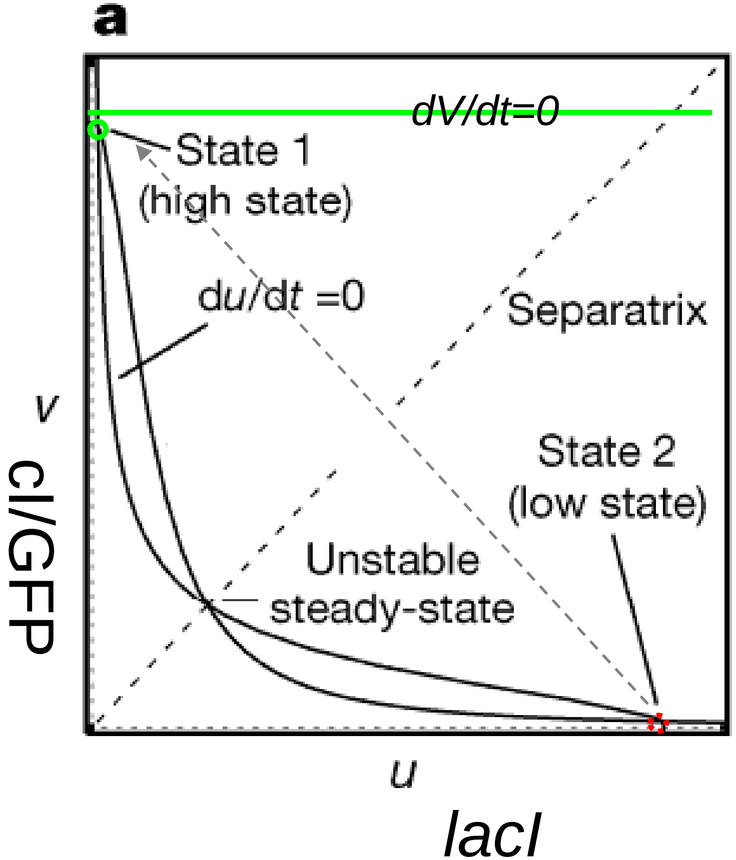
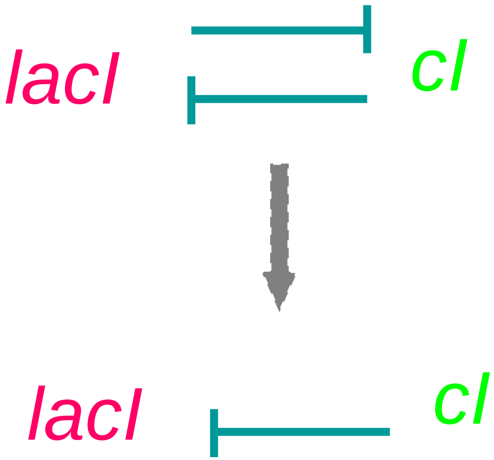
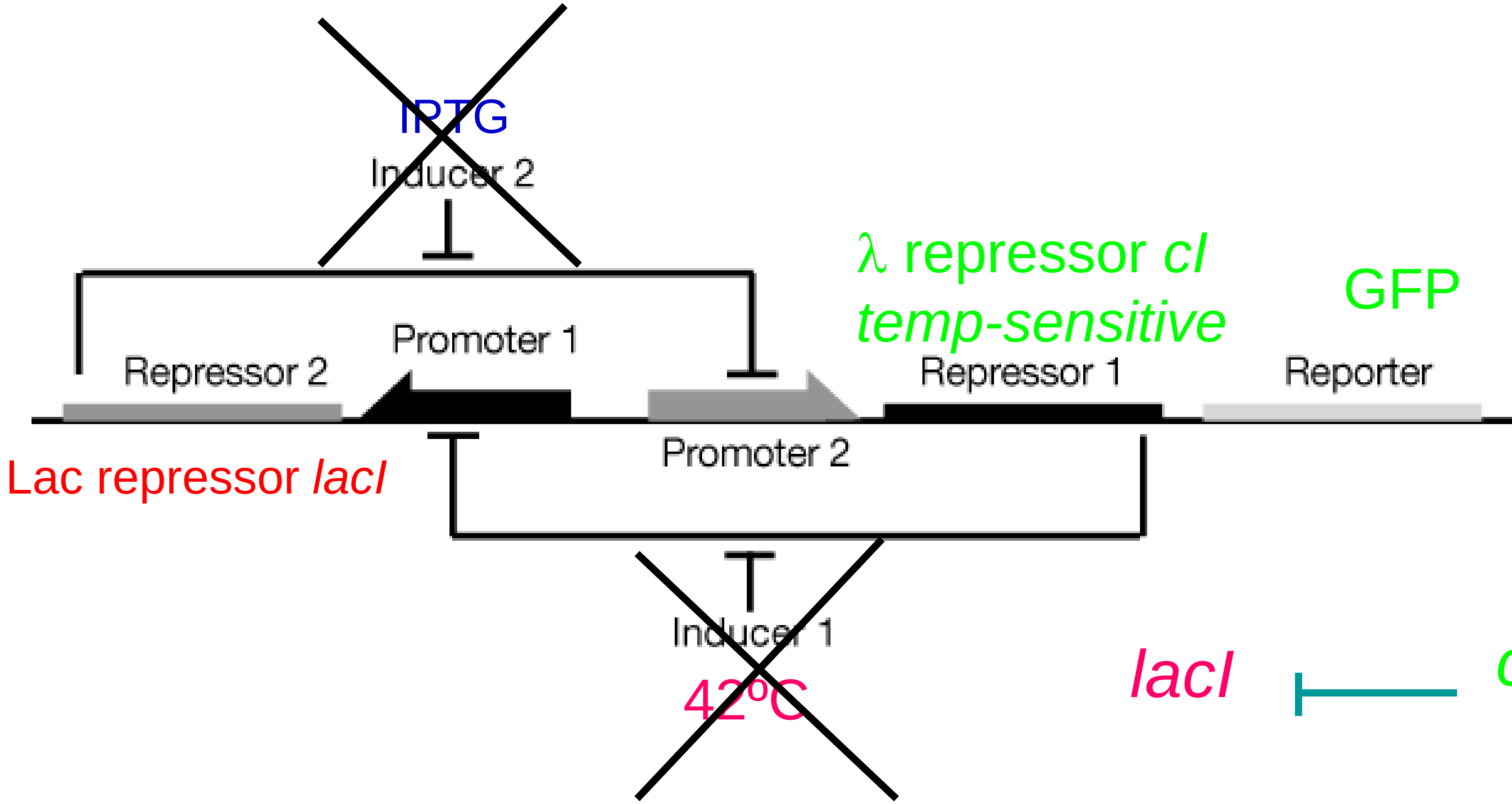


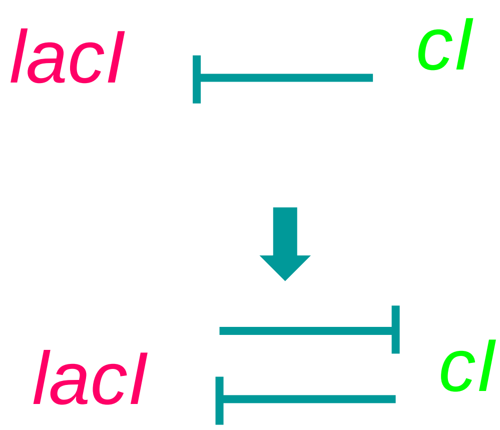
IPTG off>on

new null cline, one state:GFP on







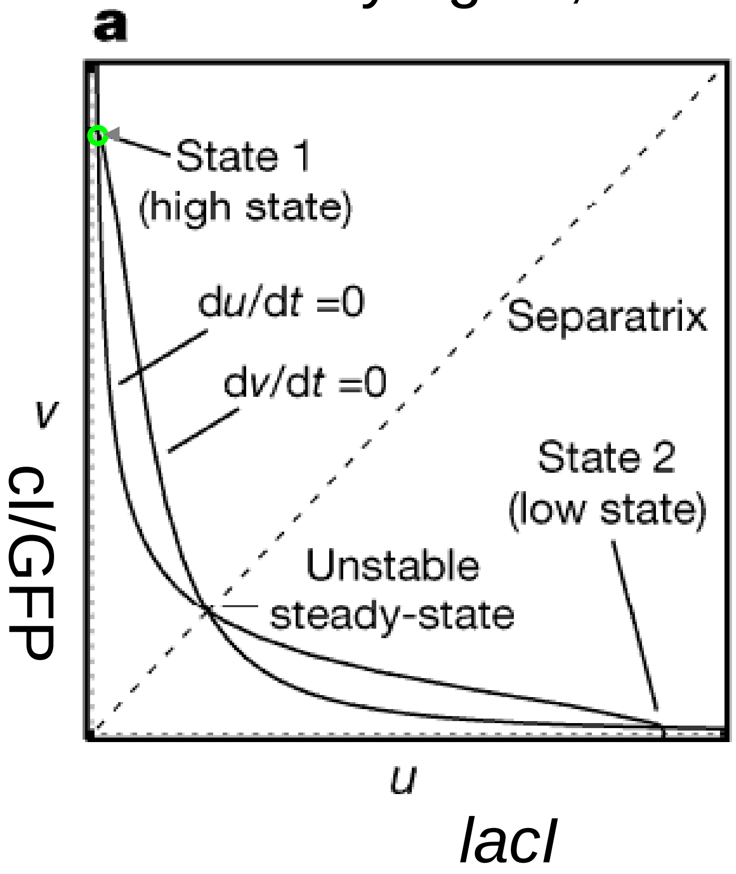


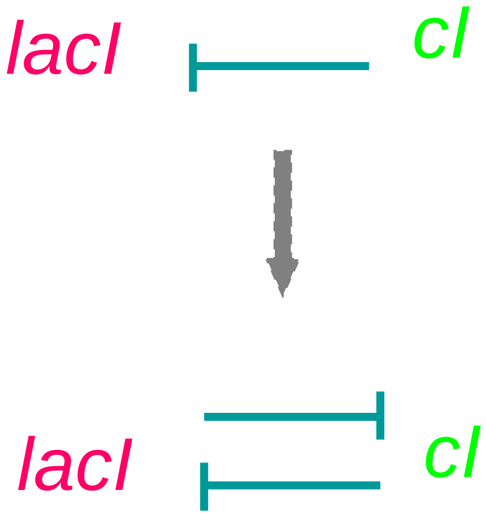
IPTG	42ºC	*lacI*	*cI*	GFP
On >off	Off	Off	On	On

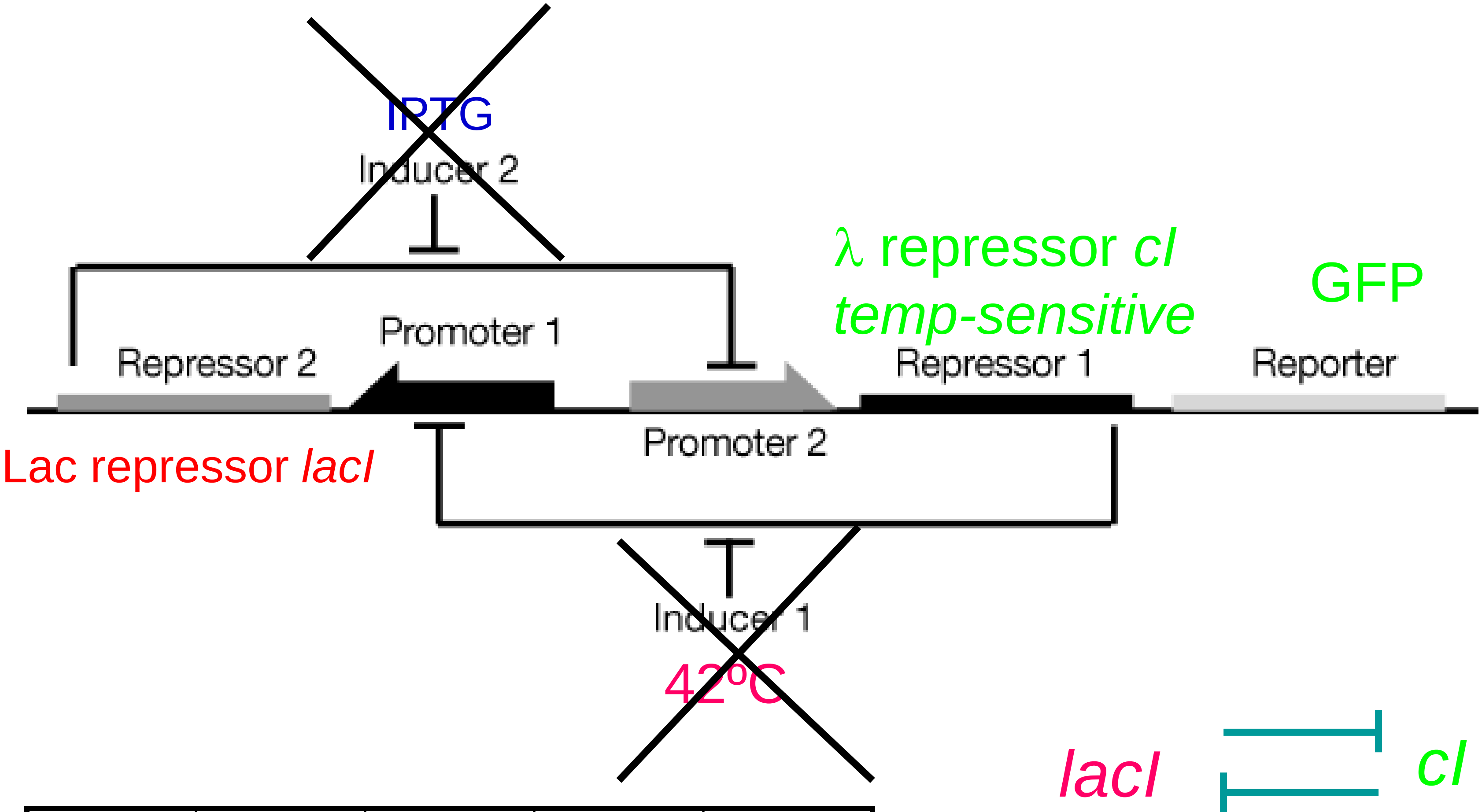
GFP kept on

IPTG on>off

Bistability again, but GFP on state is reachable

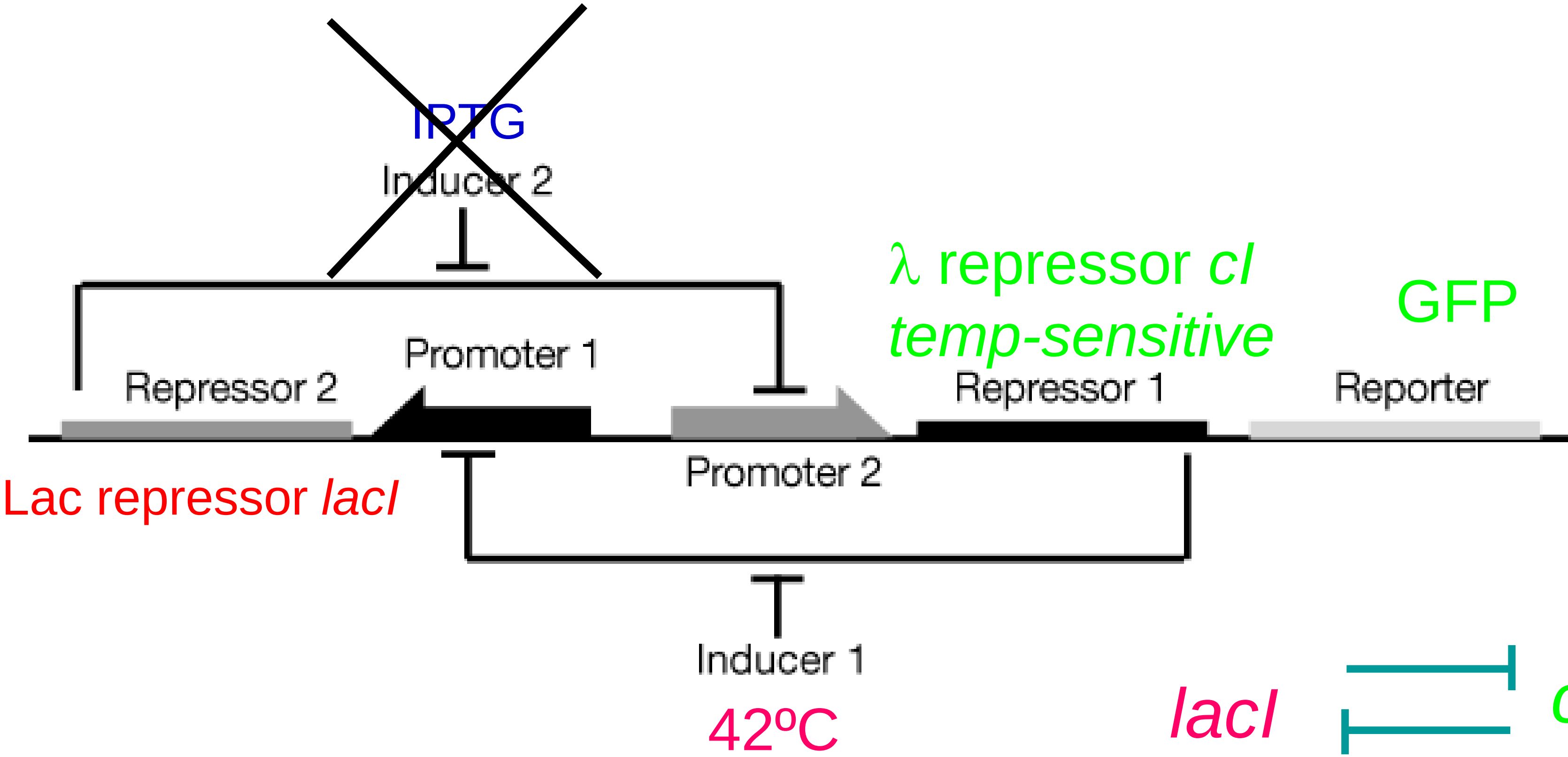


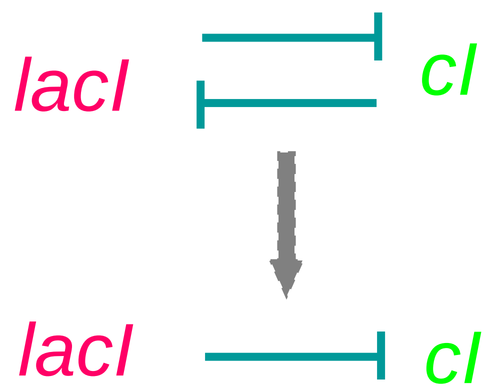




IPTG	42°C	*lacI*	*cI*	GFP
Off	Off	Off ON	On Off	On Off

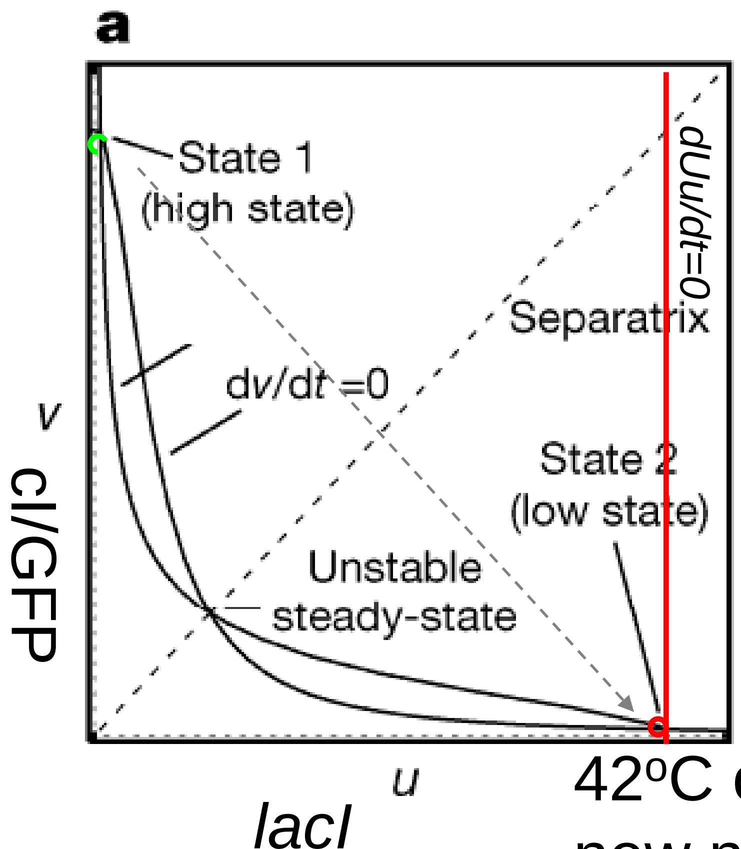
Bistability
History dependent

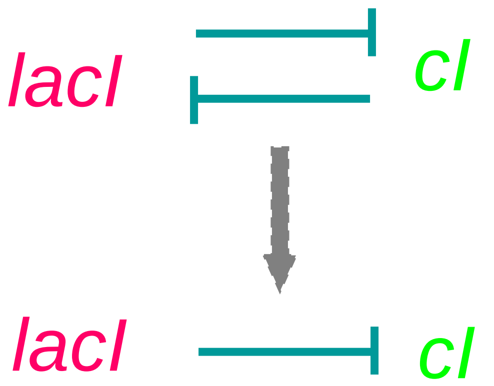




IPTG	42ºC	*lacI*	*cI*	GFP
Off	off>on	On	Off	Off

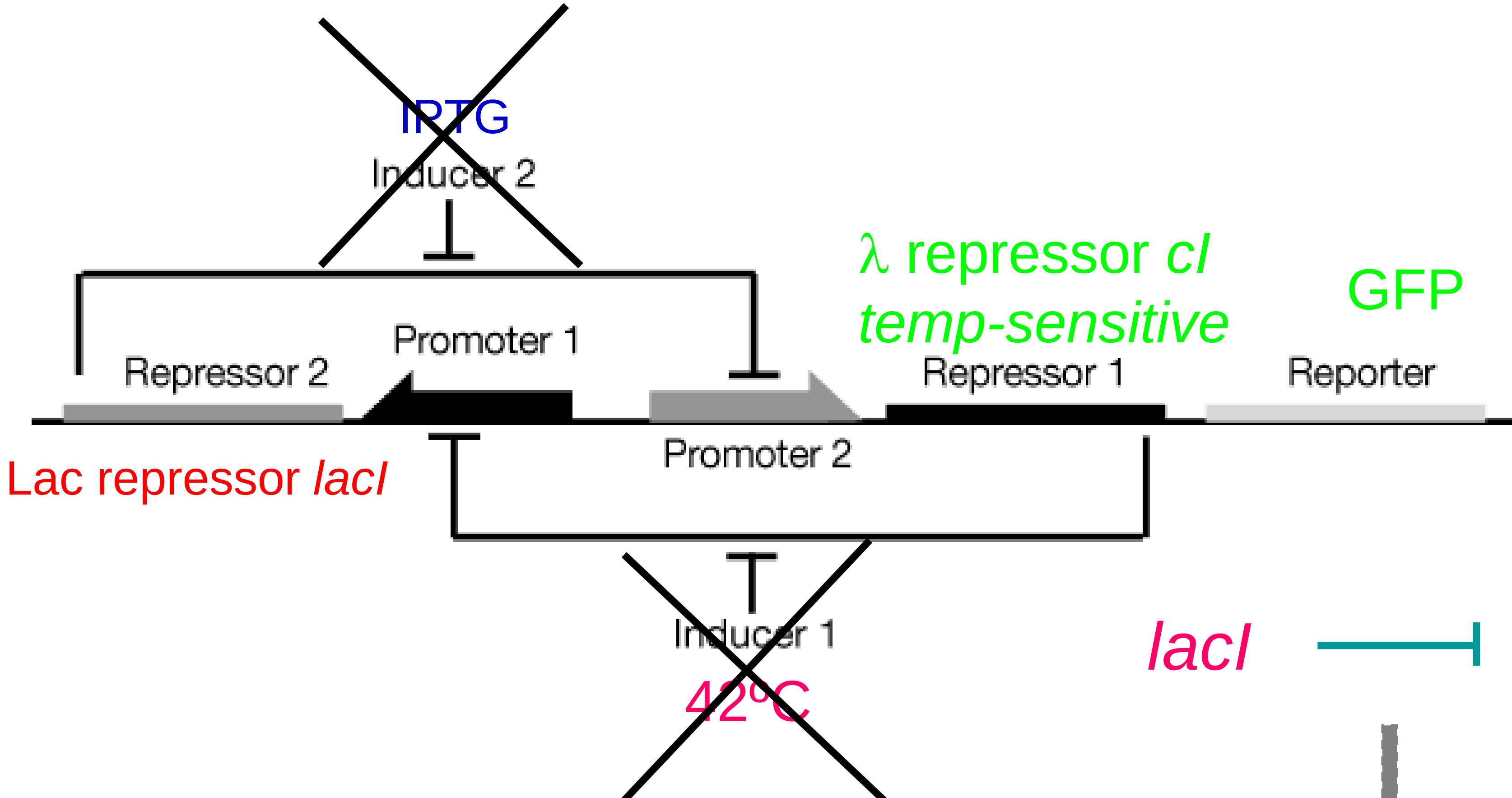
GFP turned off

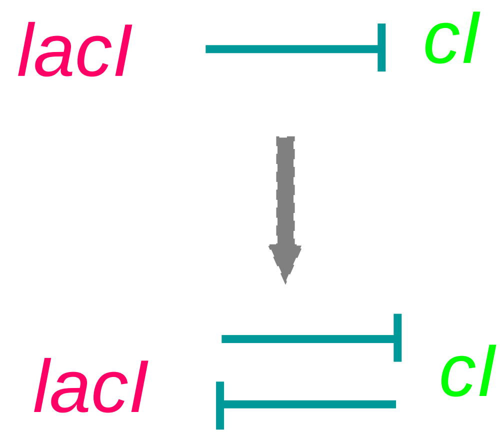




42^{o}C off>on

new null cline, one state:GFP off



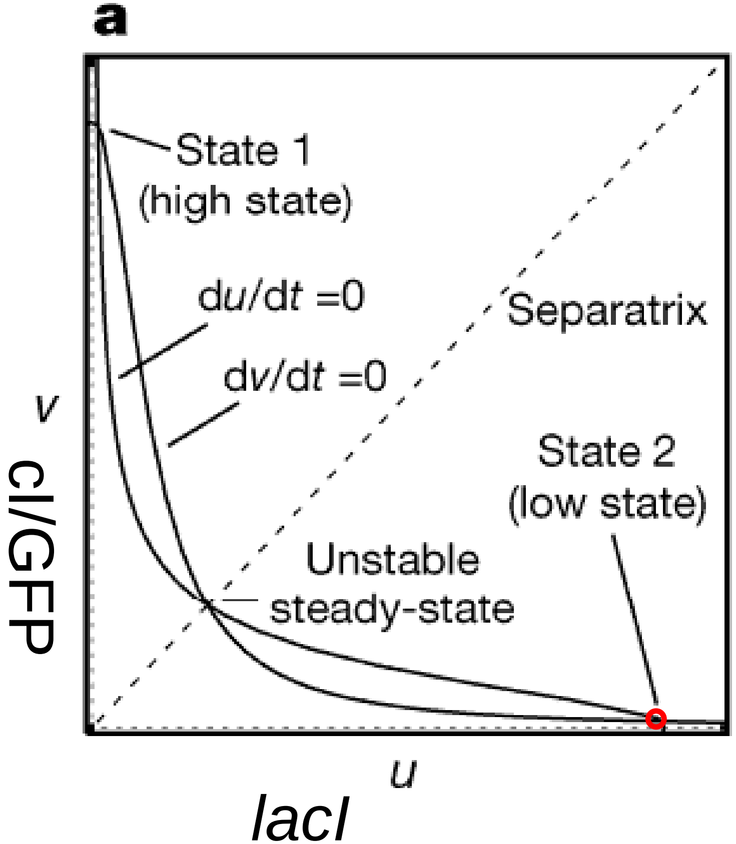


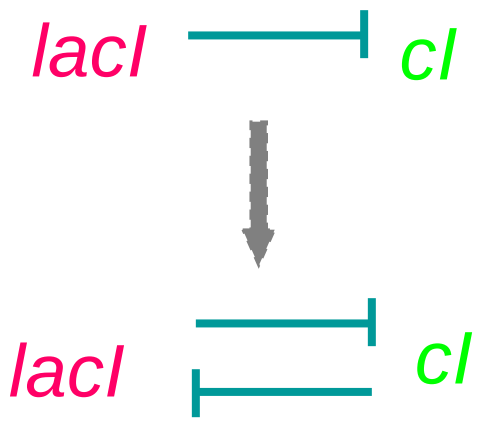
IPTG	42ºC	*lacI*	*cI*	GFP
Off	on>off	On	Off	Off

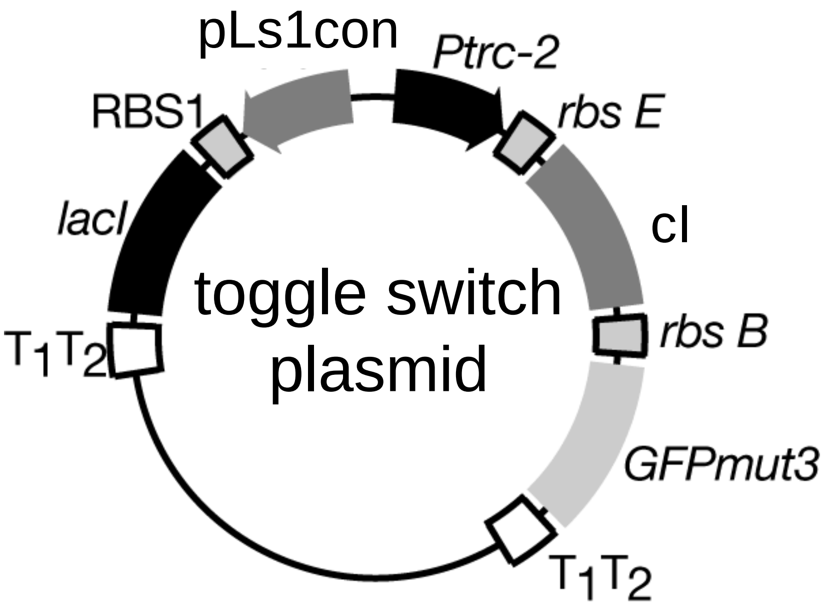
GFP kept off

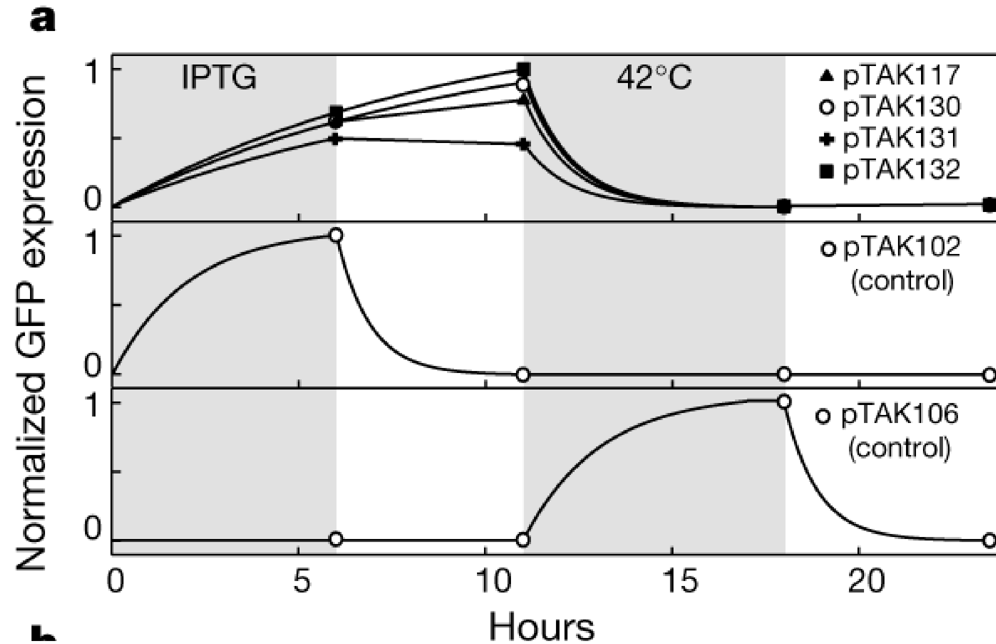
42^{o}C on>off

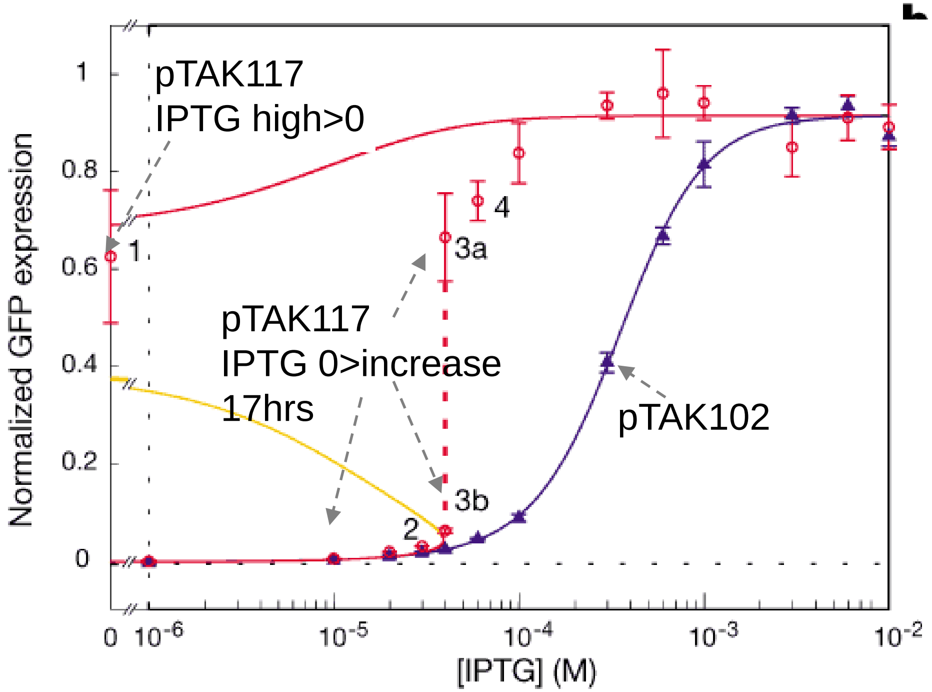
Bistability again, but only GFP off state isreachable

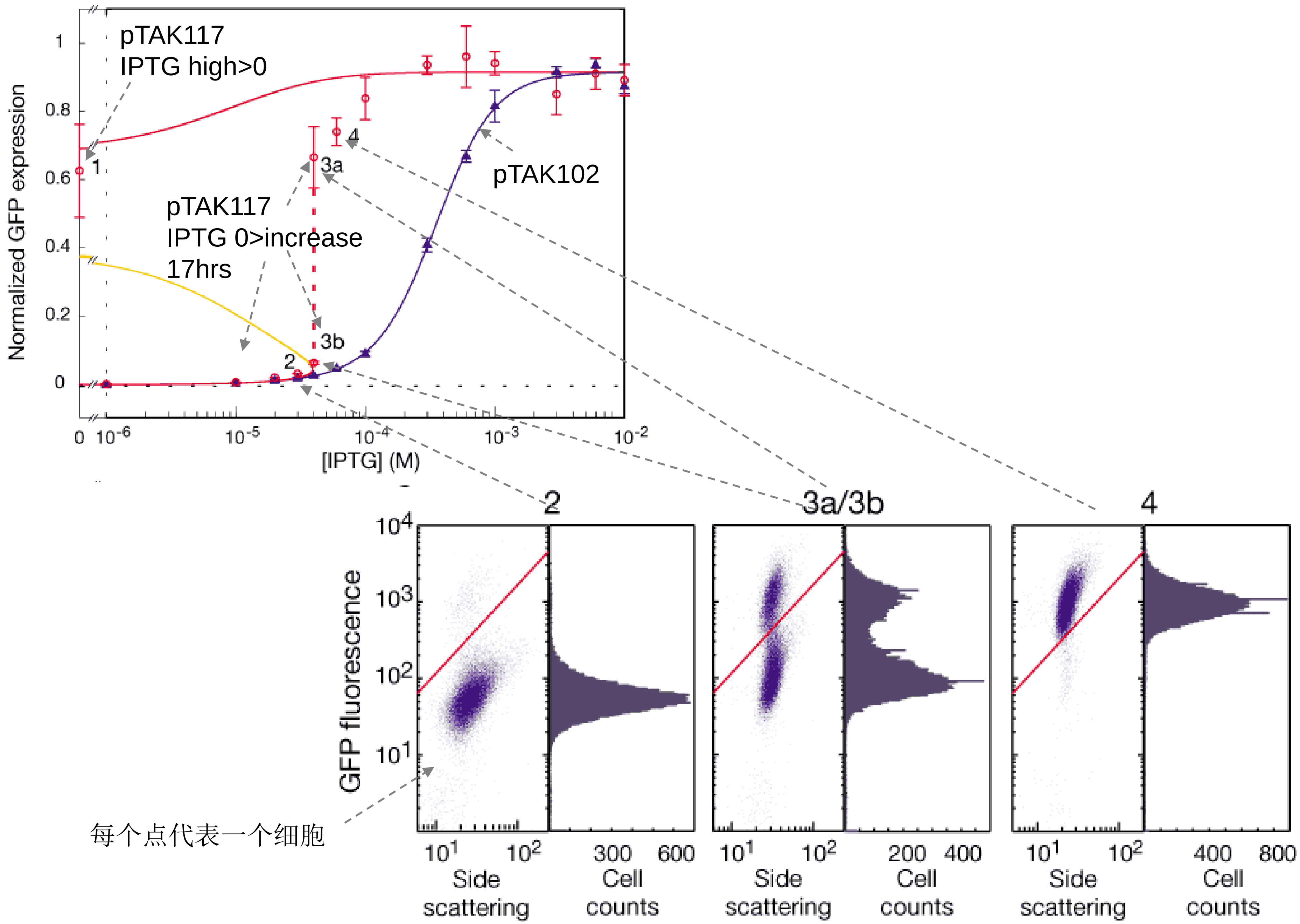





pLs1con
Ptrc-2
RBS1
rbs E
lacI
cI
toggle switch plasmid
rbs B
T_1T_2
GFPmut3
T_1T_2


a
IPTG
42°C
pTAK117
pTAK130
pTAK131
pTAK132
pTAK102 (control)
pTAK106 (control)
Normalized GFP expression
1
0
0
5
10
15
20
Hours

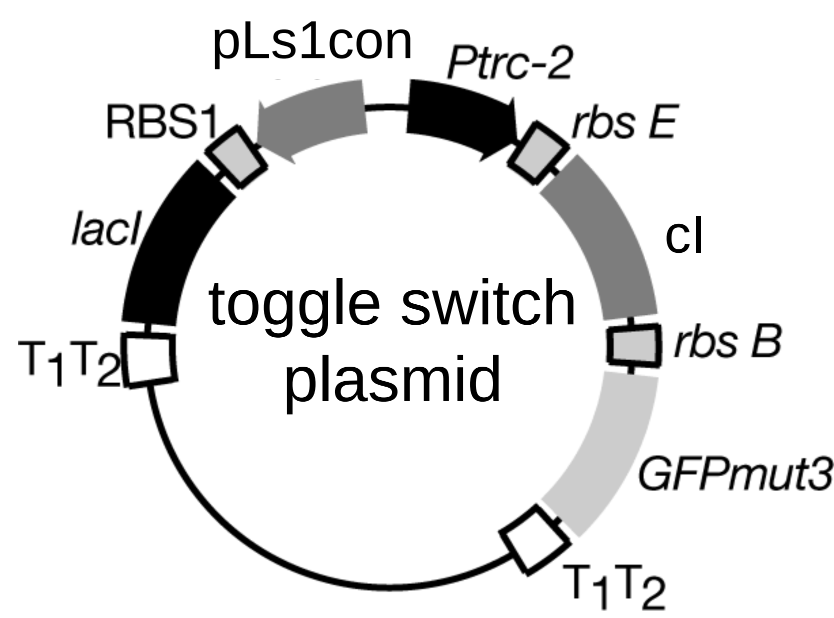

pTAK117
IPTG high>0
pTAK117
IPTG 0>increase
17hrs
pTAK102
1
2
3a
3b
4
Normalized GFP expression
0
0.2
0.4
0.6
0.8
1
0
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
[IPTG] (M)



pTAK117
IPTG high>0
pTAK117
IPTG 0>increase
17hrs
pTAK102
1
2
3a
3b
4
Normalized GFP expression
[IPTG] (M)
2
3a/3b
4
GFP fluorescence
Side scattering
Cell counts
每个点代表一个细胞

Lessons:

1. Quantitation is critical: they used a number of repressor, promoter and rbs, some don't work.
2. Coopertivity of repressor is critical, know from mathematics.
3. Mathematics mostly works
4. Single cell data is different from their mathematics
5. Noises in gene expressions



Analytic stability analysis (not required)

Considering the following two coupled differential equations:

$$\dot{x} = f(x, y)$$

$$\dot{y} = g(x, y)$$

The nullclines are defined as:

$$\dot{x} = 0 \rightarrow f(x_o, y_o) = 0$$

$$\dot{y} = 0 \rightarrow g(x_o, y_o) = 0$$

In order to solve the above eq. we linearize around the fixed points (x0,y0):

$$\tilde{x} \equiv x - x_o$$

$$\tilde{y} \equiv y - y_o$$

Analytic stability analysis (not required)

If f(x,y) and g(x,y) are approximated by a first order Taylor expansion, The previous eq. can be written as:

$$\dot{\vec{X}} = A\vec{X} \qquad \dot{\vec{X}} = \begin{bmatrix} \dot{x} \\ \dot{y} \end{bmatrix}$$

$$A = \begin{bmatrix} a & b \\ c & d \end{bmatrix} \qquad \vec{X} = \begin{bmatrix} \tilde{x} \\ \tilde{y} \end{bmatrix}$$

The matrix A is characterized by its trace and the determinant

$$\tau = trace(A) = a + d$$

$$\Delta = \det(A) = ad - bc$$

Analytic stability analysis (not required)

Let's try to find a solution of the convenient form:

$$\dot{\vec{v}} = \lambda \vec{v} = A \vec{v}$$

This vector is called the eigenvector, λ is the corresponding eigenvalue. The eq. about can be solved by:

$$\det \begin{bmatrix} a - \lambda & b \\ c & d - \lambda \end{bmatrix} = 0$$

Leading to:

$$\lambda_1 = \frac{\tau + \sqrt{\tau^2 - 4\Delta}}{2} \qquad \Delta = \lambda_1 \lambda_2$$

$$\lambda_2 = \frac{\tau - \sqrt{\tau^2 - 4\Delta}}{2} \qquad \tau = \lambda_1 + \lambda_2$$

Analytic stability analysis (not required)

For a stable fixed point both λ1 and λ2 should be negative. Therefore a stable fixed point:

$$\Delta > 0$$

$$\tau < 0$$

Analytic stability analysis (not required)

Now let's evaluate the stability of the toggle switch

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

$$\dot{v} = g(u,v) = \frac{\alpha_2}{1+u^{\gamma}} - v$$

The fixed points are:

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

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

Analytic stability analysis (not required)

The matrix A is given by:

$$A = \begin{bmatrix} -1 & \dfrac{-\alpha_1 \beta v^{\beta-1}}{(1+v^\beta)^2} \\ \dfrac{-\alpha_2 \gamma u^{\gamma-1}}{(1+u^\gamma)^2} & -1 \end{bmatrix}$$

The trace is always negative. The only requirement for stability is $\Delta>0$. let's focus on $\Delta=0$, which define the boundary in parameter space that separate bistable from monostable region, which is

$$\frac{\alpha_1 \beta v^{\beta-1}}{(1+v^\beta)^2} \frac{\alpha_2 \gamma u^{\gamma-1}}{(1+u^\gamma)^2} = 1$$

Analytic stability analysis (not required)

Assuming the ratio between ON and Off states is large, the phase diagram can be estimated