

III A Genetic Switch in Lamba Phage

References:

1. J. Hasty, J. Pradines, M. Dolnik, and J. J. Collins. Noise-based switches and amplifiers for gene expression. *PNAS* **97**, 2075-2080 (2000).

The goal of this Section is to apply our knowledge of reaction kinetics and equilibrium binding to a real biological problem: the lysis-lysogeny decision of the bacterial phage lambda. The first goal is to derive the kinetic equation [7] in Hasty's paper.

Figure 5 schematically depicts the genetic regulation of the P_{RM} λ promoter. The gene of this promoter, called *cI*, encodes for a repressor protein, which in turn dimerizes and binds to the DNA as a transcription factor. In this model Hasty *et al.* assume that binding to OR2 enhances transcription whereas binding to OR3 switches off transcription. Note that the wild-type lambda operon has three binding sites.

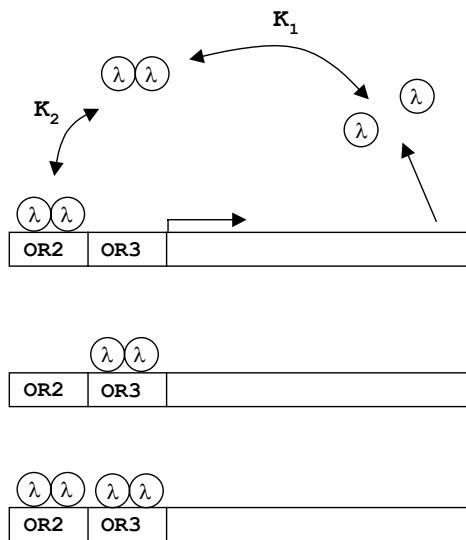
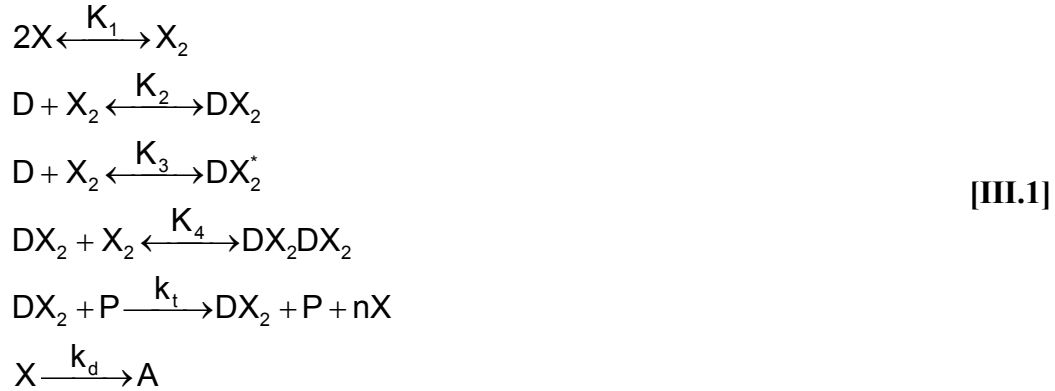


Figure 5. Possible binding states for the lambda promoter considered by Hasty *et al.* In the unbound state the gene is switched off.



The system of reactions [III.1] reflects the reaction depicted in Fig. 5. The first four equations are fast reversible reactions since DNA binding and unbinding of the repressors dimers and the dimerization itself occur within seconds, whereas the synthesis (transcription, translation, folding) and degradation (e.g dilution through cell growth) of monomers takes minutes to sometimes an hour. Therefore, the first four reactions are in equilibrium and the steady state concentrations are given in terms of the equilibrium constants. This separation of time scales is an important step in simplifying the model. Since the first four reactions in [III.1] are in equilibrium compared to protein synthesis and degradation, the concentrations of the ‘fast’ variables are ($y=[X_2]$; $d=[D]$; $u=[DX_2]$; $v=[DX_2^*]$; $z=[DX_2X_2]$):

$$\begin{aligned}
y &= K_1 x^2 \\
u &= K_2 dy = K_1 K_2 dx^2 \\
v &= \sigma_1 K_2 dy = \sigma_1 K_1 K_2 dx^2 \\
z &= \sigma_2 K_2 uy = \sigma_2 (K_1 K_2)^2 dx^4
\end{aligned}
\tag{III.2}$$

Where:

$$\begin{aligned}
\sigma_1 &= \frac{K_3}{K_2} \\
\sigma_2 &= \frac{K_4}{K_2}
\end{aligned}
\tag{III.3}$$

The only slow variable is the concentration of cI monomers $x=[X]$. The rate of synthesis of repressor monomer is given by:

$$\frac{dx}{dt} = nk_t p_o u - k_d x + r
\tag{III.4}$$

where p_o is the concentration of RNA polymerase (assumed to be constant). A basal synthesis rate is modeled by r . In other words the promoter is never fully off, but leaks at a rate r . Now realizing that the total amount of lambda promoter sites d_T is conserved:

$$d_T = d + u + v + z \quad \text{[III.5]}$$

This, together with [III.2] gives:

$$d_T = d[1 + (1 + \sigma_1)K_1K_2x^2 + \sigma_2K_1^2K_2^2x^4] \quad \text{[III.6]}$$

Using this result [III.4] becomes:

$$\frac{dx}{dt} = \frac{nk_t p_o d_T K_1 K_2 x^2}{1 + (1 + \sigma_1)K_1 K_2 x^2 + \sigma_2 K_1^2 K_2^2 x^4} - k_d x + r \quad \text{[III.7]}$$

Now the goal is to ‘clean up’ the ugly looking [III.7]. This is done by normalizing the variables x and t . Since the product $K_1 K_2$ always appears before x^2 it is logical to introduce a new variable:

$$x = x\sqrt{K_1 K_2} \quad \text{[III.8]}$$

Since the units of K_1 and K_2 are [1/concentration], x is dimensionless. Using this new variable [III.7] reduces to:

$$\frac{1}{\sqrt{K_1 K_2}} \frac{dx}{dt} = \frac{nk_t p_o d_T x^2}{1 + (1 + \sigma_1)x^2 + \sigma_2 x^4} - \frac{k_d}{\sqrt{K_1 K_2}} x + r \quad \text{[III.9]}$$

An additional ‘clean-up’ arises when the time is normalized into a dimensionless form:

$$t = t(r\sqrt{K_1 K_2}) \quad \text{[III.10]}$$

Using this, gives:

$$r \frac{dx}{dt} = \frac{nk_t p_o d_T x^2}{1 + (1 + \sigma_1)x^2 + \sigma_2 x^4} - \frac{k_d}{\sqrt{K_1 K_2}} x + r \quad \text{[III.11]}$$

Or,

$$\begin{aligned} \frac{dx}{dt} &= \frac{\alpha x^2}{1 + (1 + \sigma_1)x^2 + \sigma_2 x^4} - \gamma x + 1 \\ \alpha &= \frac{nk_t p_o d_T}{r} \\ \gamma &= \frac{k_d}{r\sqrt{K_1 K_2}} \end{aligned} \quad \text{[III.12]}$$

The dimensionless parameter α is effectively a measure of the synthesis rate of monomer relative to the basal level of expression. The parameter γ reflects the ratio of degradation of monomer relative to the basal level. Matlab code 2 solves the kinetic equation [III.12]. Note that dependent on the initial conditions the system can have different steady state solutions.

MATLAB Code 2: Solution of equation [III.12]

```
% filename: hasty.m

alpha=50;
gamma=20;
sigma1=1;
sigma2=5;
options=[];

[t1 y1]=ode23('hastyfunc',[0 10],[0],options,alpha,gamma,sigma1,
sigma2);
[t2 y2]=ode23('hastyfunc',[0 10],[1],options,alpha,gamma,sigma1,
sigma2);
plot(t1,y1(:,1),'b',t2,y2(:,1),'r');
```

```
% filename: hastyfunc.m

function dydt = f(t,y,flag,alpha,gamma,sigma1,sigma2)
% [x] = y(1)

dydt = [alpha*y(1)^2/(1+(1+sigma1)*y(1)^2+sigma2*y(1)^4) -
gamma*y(1)+1];
```

Matlab code 2 shows that dependent on the initial conditions the steady state value that is reached can be different. In this specific example the system has two stable state and is therefore bistable. This multistability is analyzed by doing a stability analysis. Equation [III.12] can be rewritten as:

$$\frac{dx}{dt} = f(x) - g(x)$$

$$f(x) = \frac{\alpha x^2}{1 + (1 + \sigma_1)x^2 + \sigma_2 x^4} + 1 \quad \text{[III.13]}$$

$$g(x) = -\gamma x$$

The functions $f(x)$ and $g(x)$ represent the creation and destruction terms of cI monomer. In steady-state $f(x)=g(x)$ and this relation is graphically solved (Fig. 6). For small γ there is only one intersection point between $f(x)$ and $g(x)$ reflecting one solution in the steady-state (this point is called a fixed point). This is a stable fixed point since a deviation to a higher value of x results in a larger destruction rate than creation ($g(x)>f(x)$). This means that there will be a net decrease in x , pushing the deviation back to the fixed point. The opposite happens for a deviation of a lower x value ($g(x)<f(x)$). At intermediate γ there are 3 fixed points. Using the same strategy one finds that the outer fixed points are stable and the middle fixed point is unstable. A small deviation to larger x from the middle fixed point leads to an increased x until it reaches the upper fixed point. A small deviation to a smaller x from the middle fixed point results in a decrease in x until it reaches the lower fixed point.

Further reading on phage lambda and multistability

M. Ptashne. A genetic switch: gene control and phage lambda (Cell Press, 1987)

J. D. Murray. Mathematical Biology (Springer-Verlag, 1989)

A. Arkin, J. Ross, and H. H. McAdams. Stochastic kinetic analysis of developmental pathway bifurcation in phage lambda-infected Escherichia coli cells. *Genetics* **149**, 1633-1648 (1998).

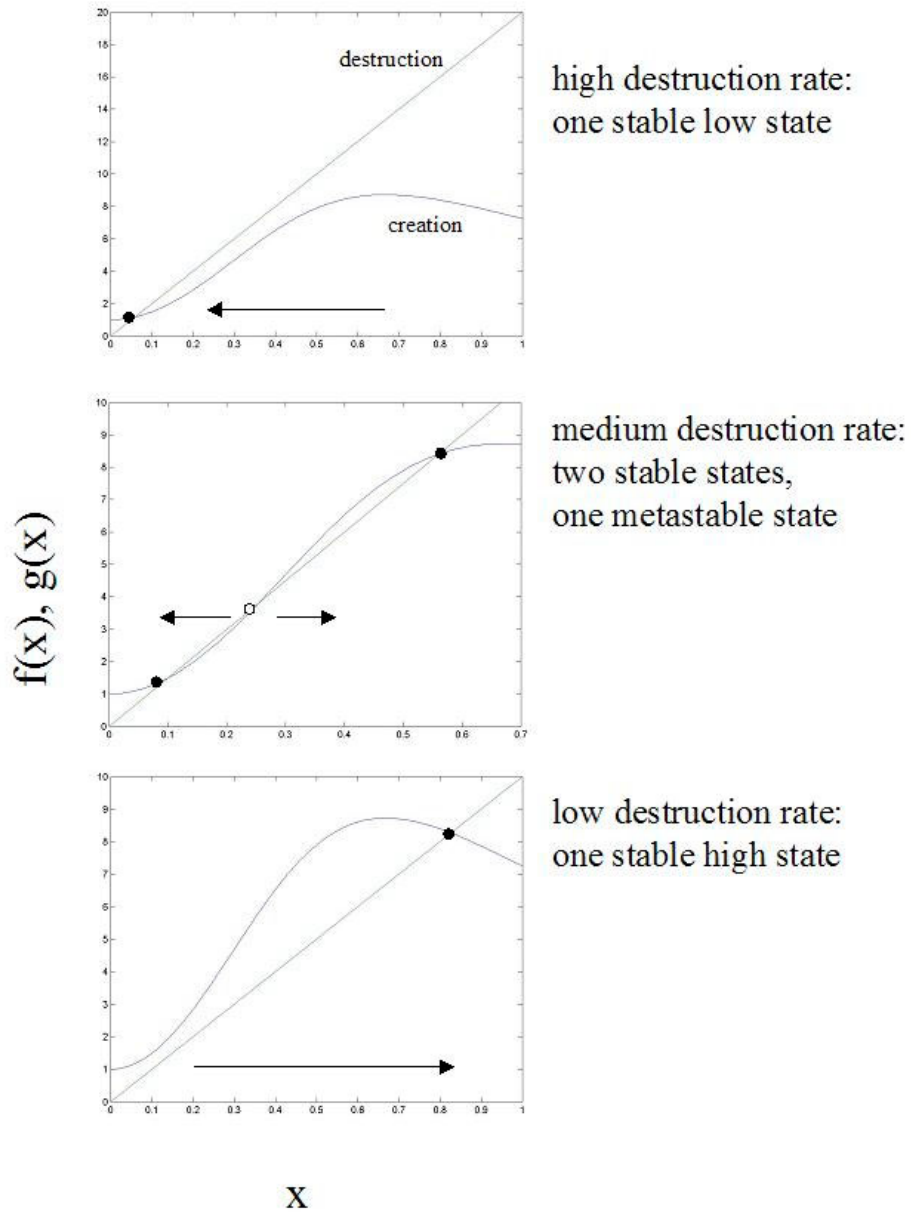


Figure 6. Creation terms and destruction term in [III.12] as a function of time. For high destruction rates (unstable cI protein) only one steady-state solution is found at low values of x . At intermediate destruction rates three solutions in the steady state are possible: two stable and one unstable solution. For low destruction rates (stable cI protein) one steady state solution is found at a large x value.