IV Positive feedback and multistability

IV. 1. The lysis-lysogeny swich in phage lambda

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).
2. F. J. Isaacs, J. Hasty, C. R. Cantor, and J. J. Collins. Prediction and measurement of an autoregulatory genetic module. *PNAS* **100**, 7714-7719 (2003).

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 the Hasty *et al.* paper (Ref. 1).

Figure 10 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.

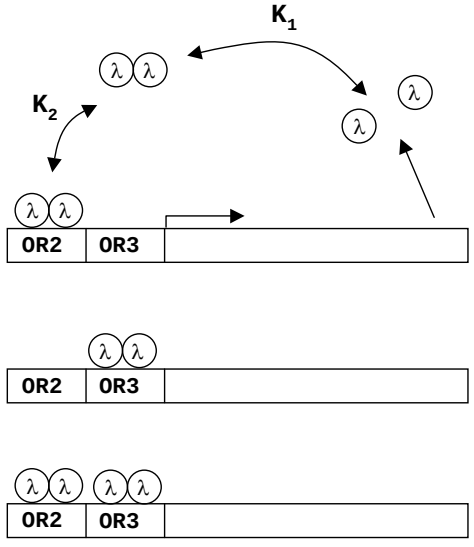

Figure 10. Possible binding states for the lambda promoter considered by Hasty *et al.* In the unbound state the gene is switched off.

$$
\begin{aligned}
&2X \xleftrightarrow{K_1} X_2 \\
&D + X_2 \xleftrightarrow{K_2} DX_2 \\
&D + X_2 \xleftrightarrow{K_3} DX_2^* \\
&DX_2 + X_2 \xleftrightarrow{K_4} DX_2DX_2 \\
&DX_2 + P \xrightarrow{k_t} DX_2 + P + nX \\
&X \xrightarrow{k_d} A
\end{aligned}
\qquad \textbf{[IV.1]}
$$

The system of reactions [IV.1] reflects the reaction depicted in Fig. 10. 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. Note that an explicit equation for the binding between the dimer X_2 and single-bound DNA state DX_2^* is missing. Since we are already know the equilibrium constants K_2, K_3 and K_4 and there is a detailed balance, the equilibrium constant K_5 of the missing reaction, $DX_2^* + X_2 \xleftrightarrow{K_5} DX_2^* X_2$, is just a combination of the known equilibrium constants: $K_5 = (K_2K_4)/K_3$. Therefore this equation does not contain any extra information. Since the first four reactions in [IV.1] are in equilibrium compared to protein synthesis and degradation, the concentrations of the 'fast' variables are ($y=[X_2]$; $d=[D]$; $u=[DX_2]$; $v=[DX_2^*]$; $z=[DX_2X_2]$):

$$
\begin{aligned}
y &= K_1 x^2 \\
u &= K_2 dy = K_1 K_2 dx^2 \\
v &= \sigma_1 K_2 dy = \sigma_1 K_1 K_2 dx^2 \\
z &= \sigma_2 K_2 uy = \sigma_2 (K_1 K_2)^2 dx^4
\end{aligned}
\qquad \textbf{[IV.2]}
$$

Where:

$$\sigma_1 = \frac{K_3}{K_2}$$
$$\sigma_2 = \frac{K_4}{K_2} \qquad \textbf{[IV.3]}$$

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

$$\frac{dx}{dt} = nk_t p_o u - k_d x + r \qquad \textbf{[IV.4]}$$

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:

$$d_T = d + u + v + z \qquad \textbf{[IV.5]}$$

This, together with [IV.2] gives:

$$d_T = d\left[1 + (1+\sigma_1)K_1K_2x^2 + \sigma_2 K_1^2 K_2^2 x^4\right] \qquad \textbf{[IV.6]}$$

Using this result [IV.4] becomes:

$$\frac{dx}{dt} = \frac{nk_t p_o d_T K_1 K_2 x^2}{1 + (1+\sigma_1)K_1K_2x^2 + \sigma_2 K_1^2 K_2^2 x^4} - k_d x + r \qquad \textbf{[IV.7]}$$

Now the goal is to 'clean up' the ugly looking [IV.7]. This is done by normalizing the variables x and t. Since the product K_1K_2 always appears before x^2 it is logical to introduce a new variable:

$$\bar{x} = x\sqrt{K_1K_2} \qquad \textbf{[IV.8]}$$

Since the units of K_1 and K_2 are [1/concentration], $\bar{x}$ is dimensionless. Using this new variable [IV.7] reduces to:

$$\frac{1}{\sqrt{K_1K_2}}\frac{d\bar{x}}{dt} = \frac{nk_t p_o d_T \bar{x}^2}{1 + (1+\sigma_1)\bar{x}^2 + \sigma_2 \bar{x}^4} - \frac{k_d}{\sqrt{K_1K_2}}\bar{x} + r \qquad \textbf{[IV.9]}$$

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

$$\bar{t} = t\left(r\sqrt{K_1K_2}\right) \qquad \textbf{[IV.10]}$$

Using this, gives:

$$r\frac{d\bar{x}}{d\bar{t}} = \frac{nk_t p_o d_T \bar{x}^2}{1+(1+\sigma_1)\bar{x}^2+\sigma_2\bar{x}^4} - \frac{k_d}{\sqrt{K_1K_2}}x + r \qquad \textbf{[IV.11]}$$

Or,

$$\frac{dx}{dt} = \frac{\alpha x^2}{1+(1+\sigma_1)x^2+\sigma_2 x^4} - \gamma x + 1$$
$$\alpha = \frac{nk_t p_o d_T}{r} \qquad \textbf{[IV.12]}$$
$$\gamma = \frac{k_d}{r\sqrt{K_1K_2}}$$

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 [IV.12]. Note that dependent on the initial conditions the system can have different steady state solutions.

MATLAB Code 2: Solution of equation [IV.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 [IV.12] can be rewritten as:

$$\frac{dx}{dt} = f(x) - g(x)$$
$$f(x) = \frac{\alpha x^2}{1 + (1 + \sigma_1)x^2 + \sigma_2 x^4} + 1 \qquad \textbf{[IV.13]}$$
$$g(x) = -\gamma x$$

The functions f(x) and g(x) represent the creation and destruction terms of cI monomer. In steady-state f(x) equals g(x) and this relation can be graphically solved (Fig. 11). 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 rate (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 to 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).

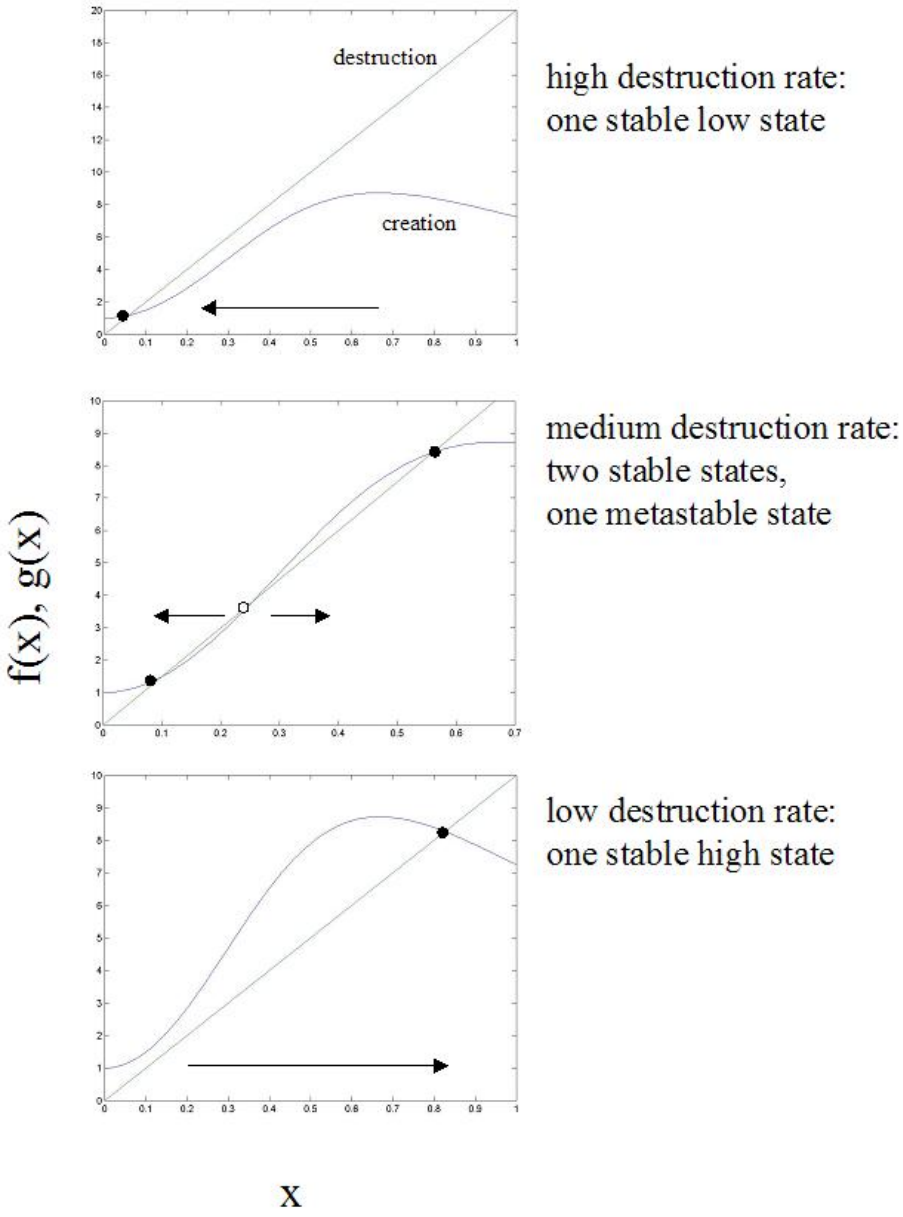

Figure 11. Creation terms and destruction term in [IV.12] as a function of x. For high destruction rates (unstable cI protein) only one steady-state solution is found at low values of x. At intermediate destruction rates three solutions are possible: two stable and one unstable solution. For low destruction rates (stable cI protein) one steady state solution is found at a large x