3

Dynamics of regulatory links

3.1 Regulating a piece of DNA

3.1.1 *Consider a titration experiment that varies R concentration with a fixed operator concentration in order to probe the first-order chemical reaction* $\mathrm{R} + \mathrm{O} \leftrightarrow \mathrm{RO}$. *Compare the fractions of bound [RO] to [O] as a function of* $[\mathrm{R_T}]$, *for* $[\mathrm{O_T}] = 10\,K$ *and* $K/10$.

Answer As explained in the main text:

$$\frac{[\mathrm{CIO}]}{[O_\mathrm{t}]} = \frac{[\mathrm{CI}]}{K + [\mathrm{CI}]} \tag{3.1}$$

where K is the CI concentration at which [O] is half occupied. Here the free concentrations [CI] is less than the total concentrations, as

$$[\mathrm{CI_{total}}] = [\mathrm{CI}] + [\mathrm{CIO}] \tag{3.2}$$

The equation where we ignore the operator's effect on free concentration of CI is:

$$\frac{[\mathrm{CIO}]}{[\mathrm{O_t}]} = \frac{[\mathrm{CI_{total}}]}{K + [\mathrm{CI_{total}}]}$$

In contrast, the complete equation is:

$$K = \frac{([CI_{total}] - [CIO])([O_t] - [CIO])}{[CIO]} \Rightarrow$$

$$[CIO] = \frac{[CI_{total}] + [O_t] + K}{2} - \sqrt{\frac{([CI_{total}] + [O_t] + K)^2}{4} - [CI_{total}][O_t]}$$

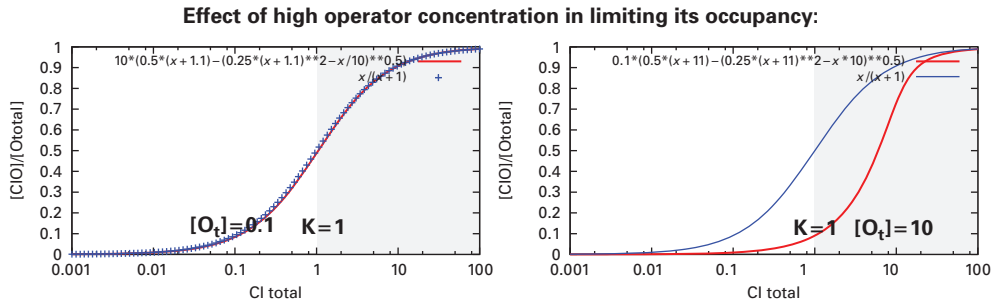

Figure 3.1 Comparison between a case where there is a lot of DNA, and the case with little operator DNA using $K = 1$.

which we investigate in Fig. 3.1 for the case where $K = 1$ and $[\mathrm{O_t}] = 0.1$, and $[\mathrm{O_t}] = 10$, plotting in both cases $[\mathrm{ClO}]/[\mathrm{O_t}]$.

3.2 Transcription regulation

3.2.1 *Simulate the three different regulatory systems in Fig. 3.5 by integrating the temporal evolution. Hint: for simple repression this amounts to* $C(t + \mathrm{d}t) = C(t) + (\frac{1}{1+R} - C) \cdot \mathrm{d}t$, *with* $\mathrm{d}t = 0.01$ *from* $t = 0$ *to* $t = 10$. *Start by using* $R = 0.1$ *and initialize* C *at its steady-state value* $C = \tau/(R + 1)$, $C = 1/(R+1/\tau)$ *and* $C = \tau R/(R+1)$. *At time* $t = 10$ *increase* R *by a factor of 100 to inspect the relaxation dynamics to the new steady state (integrating from* $t = 10$ *to* $t = 20$*). What parameter is important for determining the characteristic time of the relaxation, and what parameters define the steady state of* C*?*

Answer Results as shown in the figure in main text.
(a) Steady state is determined by setting $\mathrm{dC/dt} = 0 \Rightarrow \frac{1}{1+R} = C/\tau$, which implies that steady state $C = \frac{\tau}{1+R}$ with a prefactor that will be given by promoter strength and number of proteins per mRNA. The response time, on the other hand, is simply equal to τ, as $C \propto \exp(-t/\tau)$
(b) Steady state is determined from $C = 1/(R+1/\tau)$ with the prefactor given by overall promoter strength and a rescaling of the "R" factor proportional to the strength of the proteolytic degradation. The relaxation to new steady

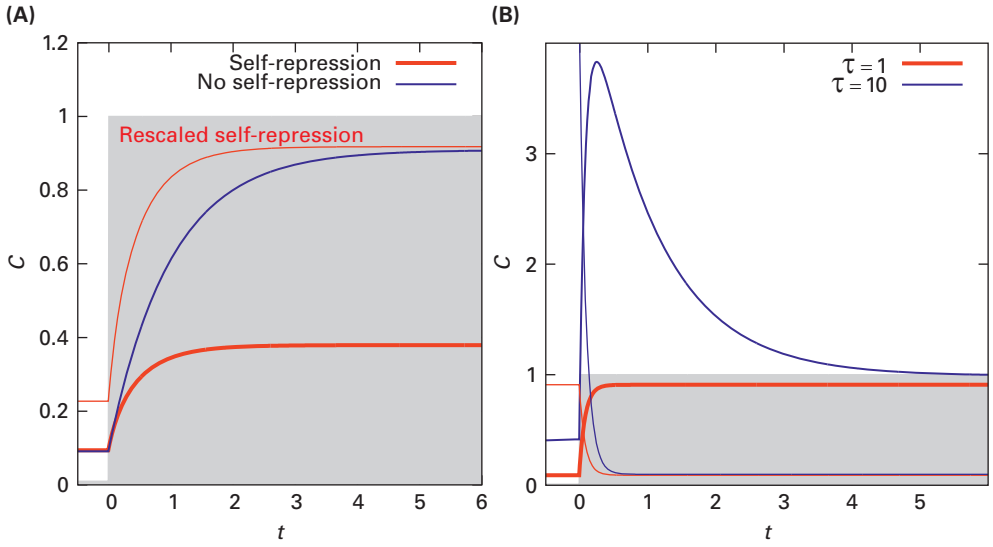

Figure 3.2 Self repression and response using a reservoir as a buffer. Gray area marks the value of $R/10$.

state is given by $C \propto \exp(-(R + \frac{1}{\tau}) \cdot t)$, and thus becomes very fast if the activity of R is large.
(c) Steady state is $C = \frac{\tau R}{R+1}$ with relaxation given by $C \propto \exp(-t/\tau)$.

3.2.2 *Simulate the two regulatory systems in Fig. 3.6. For the right-hand panel use* $\tau = 1$*, and investigate also* $\tau = 10$.

Answer The simulations are conducted in discrete time steps of size, say, $\mathrm{d}t = 0.01$, with $= 0.01$ for $t < 0$ and $R = 10$ for $t > 0$. Initially, the system is started at t $= -10$ and will reach steady state long before the switch in R. The result is shown in Fig. 3.2.
(A) Simulation of $\mathrm{d}C/\mathrm{d}t = (R/R+1) \cdot (2/(10C+1) - C$ (red) and $\mathrm{d}C/\mathrm{d}t = (R/R+1) - C$ (blue). Notice the thin red curve, which shows the behavior of the self-repressed system (red) when rescaled to the final steady-state value at $R = 10$. The system is seen to respond faster because it initially responds without sensing its final repressed value.
(B) Notice the overshoot of C, reflecting a fast conversion of a reservoir of m. This type of response is part of the unfolded protein response, where a passive mRNA is activated through cleaving by the protein Ire1.

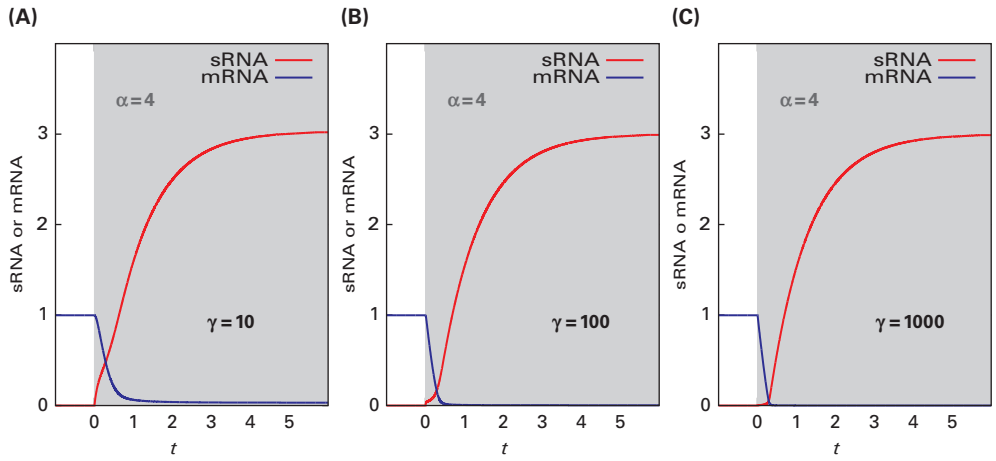

Figure 3.3 Simualtion of activation of a small RNA on the expression of its target mRNA. The gray area marks value of α.

3.3 Post-transcription regulation

3.3.1 *Consider the sRNA regulations from Eqs. (3.21) and (3.22) with parameter $\gamma = 10$, $\gamma = 100$, respectively $\gamma = 1000$. Calculate the steady-state concentration mRNA at $\alpha = 0.1$, then shift to $\alpha = 4$ and simulate the response. Use $\tau = 1$.*

Answer Simulate the equations:

$$\begin{aligned} \mathrm{d}s &= \mathrm{d}t \cdot (\alpha - \gamma \cdot s \cdot m - s) \\ \mathrm{d}m &= \mathrm{d}t \cdot (1 - \gamma \cdot s \cdot m - m) \end{aligned}$$

with $\mathrm{d}t < \gamma\alpha$, for example use $\mathrm{d}t = 0.0001$. Switch from $\alpha = 0.1$ at $t < 0$ to $\alpha = 4$ at larger times. The result is shown in Fig. 3.3

3.3.2 *Simulate activation and de-activation of a small RNA on the expression of its target mRNA with $\alpha = 0.1 \to \alpha = 4 \to \alpha = 0.1$, and $\gamma = 100$, $\tau = 1$. Repeat the simulation for $\alpha = 0.1 \to \alpha = 40 \to \alpha = 0.1$ and $\gamma = 100$, $\tau = 1$.*

Answer Simulate the equations:

$$\begin{aligned} \mathrm{d}s &= \mathrm{d}t \cdot (\alpha - \gamma \cdot s \cdot m - s) \\ \mathrm{d}m &= \mathrm{d}t \cdot (1 - \gamma \cdot s \cdot m - m) \end{aligned}$$

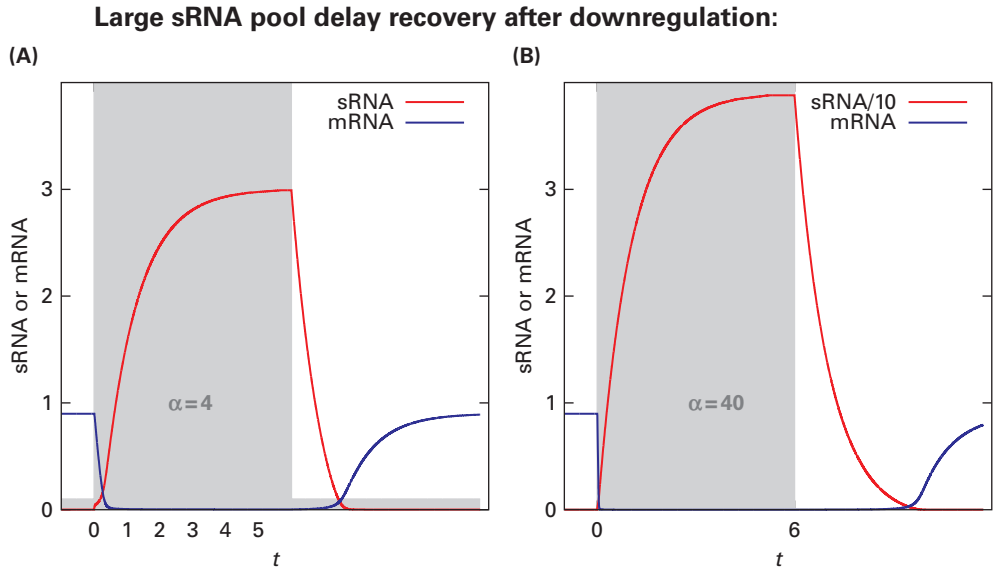

Figure 3.4 Simulation of activation and deactivation of a small RNA on the expression of its target mRNA. Gray area marks value of α.

with $\mathrm{d}t < \gamma\alpha$, for example use $\mathrm{d}t = 0.0001$. The result is shown in Fig. 3.4. Notice that large intermediate production of α makes recovery slow.

3.3.3 *Examine the indirect regulation caused by a protein R that sequesters a transcriptional repressor C as a function of the concentration of R with fixed $C = 1$. Assume that C only represses the promoter in its free form. This can be done by plotting the activity of the repressed promoter as one varies R around the critical value $C = 1$, for different values of K_o and K, with $K \cdot K_\mathrm{o} = 10^{-4}$ being fixed.*

Answer Plot as a function of R, the function:

$$\mathrm{Activity}(R) = \frac{1}{1 + C_\mathrm{f}/K_\mathrm{o}}$$

$$C_\mathrm{f} = \frac{1}{2} \cdot (1 - R - K) + \sqrt{\frac{1}{4} \cdot (1 + R + K)^2 - R}$$

for the different values of K and K_o, see Fig. 3.5. As R increases, the repressor C is sequestered and therefore its inhibition is removed. As an result the regulator R effectively acts as an activator. Importantly, when binding

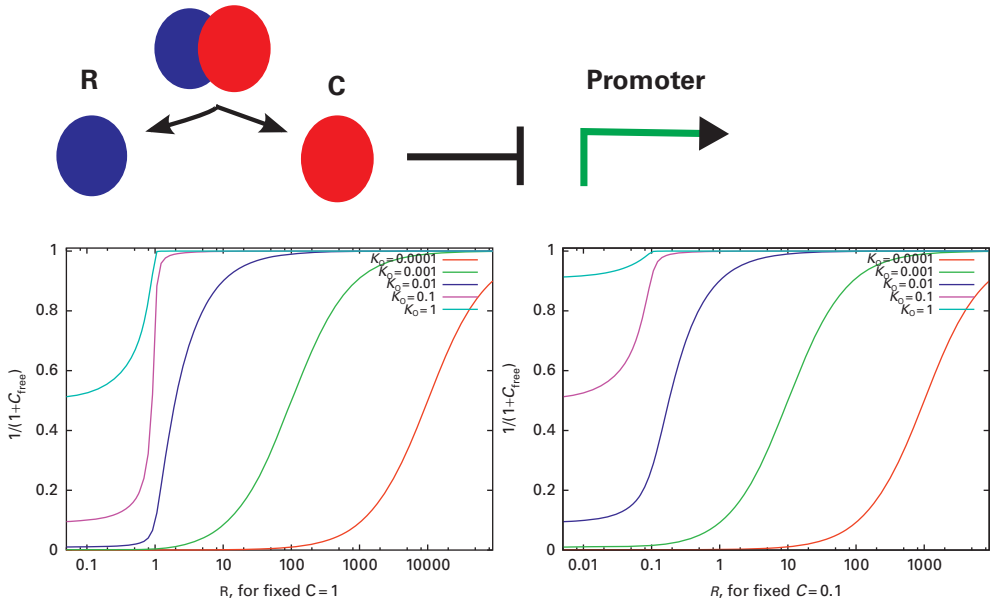

Figure 3.5 Activity of a promoter that is repressed by a protein, which in turn is sequestered by another protein.

between R and C is strong, the activation function exhibits a high Hill coefficient.

Noticeably, one may alternatively vary the repressor concentration C for a fixed value of $R = 1$. This amounts to plotting:

$$\text{Activity}(C) = \frac{1}{1 + C_\text{f}/K_\text{o}}$$

$$C_\text{f} = \frac{1}{2} \cdot (C - 1 - K) + \sqrt{\frac{1}{4} \cdot (C + 1 + K)^2 - C}$$

a dependence shown in Fig. 3.6. Importantly, when binding between R and C is strong, the activation function exhibits a high Hill coefficient.

3.3.4 *Consider a simplified toxin–anti-toxin system of two proteins T and A, where free $A(A_\text{f})$ is a repressor of T:*

$$\frac{\text{d}T}{\text{d}t} = \frac{1}{1 + A_\text{f}/K_\text{o}} - T/10 \tag{3.3}$$

where A_f is given by the total toxin amount T with an A:T binding constant $K = 0.01$. Set $K_\text{o} = 0.01$ and investigate fixed points for the above equation

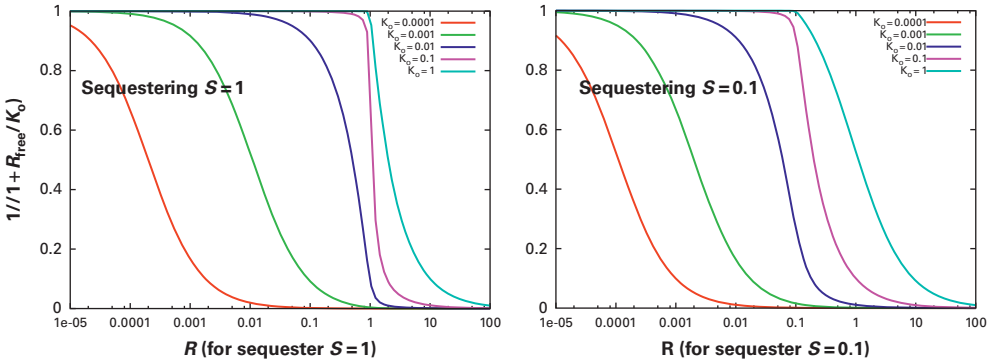

Figure 3.6 Activity of a promoter that is repressed by a protein, that in turn is sequestered by another protein.

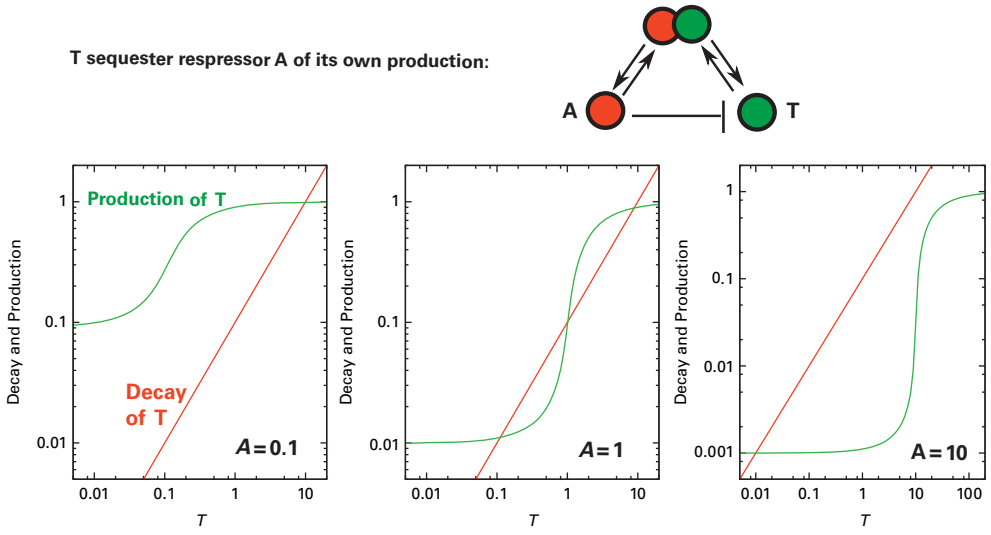

Figure 3.7 Production and decay of a toxin T as function of a fixed amount of antitoxin A. In the real toxin–anti-toxin systems, the toxins are short-lived, whereas anti-toxins are long-lived. Thus one may investigate fluctuations in T around fixed points set by the slowly varying antitoxin A (Kenn Gerdes, private communication)

when total $A = 0.1$, $A = 1$ *and* $A = 10$. *For literature on TA systems see [127, 128].*

Answer

$$\frac{\mathrm{d}T}{\mathrm{d}t} = \frac{1}{1 + A_\mathrm{f}/K_\mathrm{o}} - T/10 = 0 \Rightarrow$$
$$K_\mathrm{o}/A = K_\mathrm{o} + A_\mathrm{f}$$

where the concentraton of free A, A_f, is calculated from the part of A that is not sequestered by T, giving:

$$\text{Production of } T = \frac{K_\mathrm{o}}{K_\mathrm{o} + \frac{1}{2}\cdot(A - T - K) + \sqrt{\frac{1}{4}\cdot(A + T + K)^2 - A\cdot T}}$$
$$\text{Decay of } T = T/10$$

which is plotted for $A = 0.1$, $A = 1$ and $A = 10$ in Fig. 3.7. Intersections define fixed points. With three intersections there are two stable and one unstable fixed point in the middle. The two stable fixed points correspond to a T-dominated state and an A-dominated states, respectively. For literature on real TA systems see:

K. Gerdes and E. Maisonneve, Bacterial persistance and toxin-antitoxin loci. *Annu. Rev. Microbiol.* 66: 103–123 2012.

I. Cataudella K. Sneppen, K. Gerdes and N. Mitarai, Conditional cooperativity in toxin-antitoxin regulation prevents random toxin activation and promotes fast translational recovery. *Nucl. Acids Res.* 40:6424, 2012.