Biomolecular Feedback Systems

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Version 1.1a, June 24, 2025
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Chapter 8
Resource Competition[1]

This chapter expands on some of the topics described in Section 7.1 and will eventually replace the material in that section. The material in this chapter is not currently included in the printed version of the book.

Genetic circuits operate by virtue of the resources that the cellular host is providing, including resources for transcription, translation, protein folding, and more. So far in the book we have assumed that the level of such resources are constant parameters, which appear, for example, in the transcription and translation rate constants derived in Chapter 2. In this chapter, we revisit the form of those constants and take into account that the level of shared resources may change as a consequence of the demand applied by different gene expression modules.

8.1 Resource competition in bacterial genetic circuits

We are going to revisit the models of transcription and translation from Chapter 2 by considering now that RNAP and ribosomes are used by several genes concurrently. Let us consider genetic module i with mRNA m_i and protein p_i.

Transcription

We will write the differential equations corresponding to transcription as we did in Chapter 2 as follows:

$$\frac{dm_i}{dt} = u_i\left(D_{\text{tot},i}\frac{x/K_i}{1+x/K_i}\right) - \delta m_i,$$

in which $D_{\text{tot},i}$ is the total DNA in module i, and the term in parentheses represents the RNAP bound to the DNA of module i, with x representing the concentration of free RNAP, and u_i is a constant that captures the effective "catalytic rate constant" with which RNAP bound to DNA gives rise to an mRNA and encapsulates the speed at which the RNAP travels on the DNA. Here, K_i is the dissociation constant of RNAP from DNA and we approximated $x \ll K_i$, such that $D_{\text{tot},i}\frac{x/K_i}{1+x/K_i} \approx D_{\text{tot},i}\cdot x/K_i$. This is a reasonable approximation considering that the free RNAP in the cell is typically around 100-200nM, and that K_i are typically on

[1] Thanks to Nicholas Nolan for editing this chapter.

the order of 1,000nM [MJM+10]. With this approximation and letting x_{tot} represent the total RNAP in the system, we can write the conservation law, assuming we have two modules only:

$$x_{\text{tot}} = x + D_{\text{tot},1} \cdot x/K_1 + D_{\text{tot},2} \cdot x/K_2,$$

from which we obtain that the free level of RNAP is not constant but is affected by the presence of transcribed genes:

$$x = \frac{x_{\text{tot}}}{1 + D_{\text{tot},1}/K_1 + D_{\text{tot},2}/K_2}.$$

Given typical numbers, we estimate that $D_{\text{tot},i}/K_i \approx 0.1$, such that if we ask what the relative change is in free RNAP when we add module 2, then we have that it is given by:

$$\frac{D_{\text{tot},2}/K_2}{1 + D_{\text{tot},1}/K_1 + D_{\text{tot},2}/K_2} \approx 10\%,$$

which is negligible. So, based on this estimate, we do not expect that competition for RNAP leads to significant impact on the free level of RNAP in bacterial cells.

Translation

We captured translation through the following differential equation:

$$\frac{dp_i}{dt} = \kappa \frac{m_i y}{\bar{K}_i} - \gamma p_i,$$

in which $\bar{K}_i$ is the dissociation constant of the ribosome binding to the mRNA, with y representing the concentration of free ribosomes, and $m_i \cdot y/\bar{K}_i$ representing the concentration of ribosomes bound with mRNA m_i. Letting y_{tot} represent the total ribosome level, we have the conservation law:

$$y_{\text{tot}} = y + m_1 \cdot y/\bar{K}_1 + m_2 \cdot y/\bar{K}_2,$$

so that free ribosome levels can be calculated as:

$$y = \frac{y_{\text{tot}}}{1 + m_1/\bar{K}_1 + m_2/\bar{K}_2}.$$

Now consider that, when expressed from a low- to medium-copy plasmid, the mRNA level can hover around 100 copies, or 100nM; we further have that $\bar{K}_i$ can be designed to span a wide range and can certainly reach values of 100nM or lower [MJM+10]. Thus, we have that the relative change in free ribosome level due to the addition of module 2 with $m_2 \approx 200$nM and $m_1 \approx 100$nM is given by:

$$\frac{m_2/\bar{K}_2}{1 + m_1/\bar{K}_1 + m_2/\bar{K}_2} \approx 50\%,$$

which is non-negligible.

Overall gene expression

Pulling the above together to obtain how output protein level p_1 is affected when adding the DNA of module 2, we obtain that:

$$\frac{dp_1}{dt} = \kappa y_{\text{tot}} \frac{m_1/\bar{K}_1}{1 + m_1/\bar{K}_1 + m_2/\bar{K}_2} - \gamma p_1,$$

in which:

$$m_i = \frac{u_i}{\delta} \frac{D_{\text{tot},i} x}{K_i} \approx \frac{u_i}{\delta} \frac{D_{\text{tot},i} x_{\text{tot}}}{K_i},$$

which, together, give:

$$\frac{dp_1}{dt} = \kappa y_{\text{tot}} \frac{u_1}{\delta} \frac{D_{\text{tot},1} x_{\text{tot}}}{K_1 \bar{K}_1} \left(\frac{1}{1 + \frac{u_1}{\delta} \frac{D_{\text{tot},1} x_{\text{tot}}}{K_1 \bar{K}_1} + \frac{u_2}{\delta} \frac{D_{\text{tot},2} x_{\text{tot}}}{K_2 \bar{K}_2}} \right) - \gamma p_1,$$

in which the term in the parenthesis is a "correction factor" that appears when considering the conservation law for ribosomes across different gene expression modules. In Chapter 2, that factor was assumed to be 1.

The consequence of this expression is that, if u_1 goes from zero to $u_1' > 0$, then we have that p_1 goes from zero to $p_1' > 0$ and p_2 goes to $p_2' < p_2$, assuming $u_2 > 0$. Using the above expression, if one computes the ratio $\frac{p_2' - p_2}{p_1' - p_1}$, we obtain that this is a negative constant. In particular, there are positive constants c_1, c_2, c_3, c_4, such that:

$$\frac{p_2' - p_2}{p_1' - p_1} = -\frac{c_1 + c_2 \bar{K}_1}{c_3 + c_4 \bar{K}_2},$$

which tells us that any pair of p_1 and p_2 are constrained on a line: Note that a

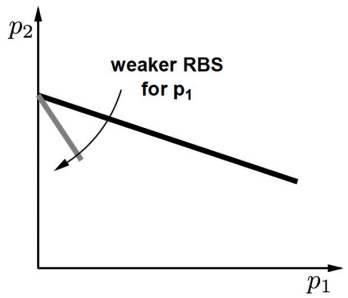

Figure 8.1: Isocost lines describe the "economy" of gene expression; the more p_1 present in the system, the lower the maximal level of p_2 can be. Having a weaker RBS for p_1 translates to having a larger $\bar{K}_1$.

weaker ribosome binding site for p_1 yields a larger $\bar{K}_1$, and increases the negative

slope because more ribosomes are required to achieve the same value of p_1. In summary, the isocost line is given by:

$$\alpha p_1 + \beta p_2 = y_{\text{tot}},$$

in which α and β are positive coefficients and y_{tot} is the budget. The coefficients α and β can be regarded as the prices of p_1 and p_2 and they depend on the ribosome binding sites strengths encoded by $1/\bar{K}_i$.

Transcriptional regulation

We next revisit the model of transcriptional regulation through Hill functions when we have resource sharing. In particular, going back to Chapter 2 (eq. 2.24), we had the following forms for transcriptional regulation for $p \to p_1$ or $p \dashv p_1$:

$$\frac{dm_1}{dt} = F(p) - \delta m_1,$$

in which $F(p)$ had the following forms, depending on whether p is a repressor or an activator. For a repressor, we had:

$$F(p) = u_1 D_{\text{tot},1} \frac{x/K}{1 + x/K} \left(\frac{1}{1 + \frac{p}{k_d(1+\frac{x}{K})}} \right),$$

which was derived from the competitive binding reaction scheme $\mathrm{x} + \mathrm{D}_1 \rightleftharpoons \mathrm{C}$ and $\mathrm{p} + \mathrm{D}_1 \rightleftharpoons \mathrm{C}_1$, in which D_1 is the DNA in gene expression module 1, such that the term $C = D_{\text{tot},1} \frac{x/K}{1+x/K} \left(\frac{1}{1+p/(k_d(1+\frac{x}{K}))} \right)$ is the amount of RNAP bound to the DNA. With the assumption we made that $x \ll K$, the above expression simplifies to $F(p) = u_1 D_{\text{tot},1} \frac{x}{K} \frac{1}{1+p/k_d}$, in which we call $\bar{F}(p) = \frac{1}{1+p/k_d}$. Similarly, for an activator, we have with this same assumption that

$$F(p) = u_1 D_{\text{tot},1} \frac{x}{K} \frac{p/k_d}{1 + p/k_d},$$

in which $C = D_{\text{tot},1} \frac{x}{K} \frac{p/k_d}{1+p/k_d}$ is the amount of RNAP bound to the DNA and it comes from a cooperative binding scheme: $\mathrm{p} + \mathrm{D}_1 \rightleftharpoons \mathrm{C}_1$ and $\mathrm{x} + \mathrm{C}_1 \rightleftharpoons \mathrm{C}$ (see Chapter 2). We also define $\bar{F}(p) = \frac{p/k_d}{1+p/k_d}$.

Now, we assume that we have two gene expression modules, one producing p_1 and transcriptionally regulated by p_j and the other producing p_2 and transcriptionally regulated by p_k, so we have:

$$p_j \to p_1, \text{ or } p_j \dashv p_1,$$

and

$$p_k \to p_2, \text{ or } p_k \dashv p_2.$$

We are then going to write the reaction rate equation describing each of p_1 and p_2. To this end, we use the quasi-steady state of the mRNA level for both modules to obtain

$$m_1 = \frac{u_1}{\delta} D_{\text{tot},1} \frac{x}{K_1} \bar{F}(p_j), \;\; m_2 = \frac{u_2}{\delta} D_{\text{tot},2} \frac{x}{K_2} \bar{F}(p_k)$$

in which $x_{\text{tot}} = x + D_{\text{tot},1}(x/K_1)\bar{F}_1(p_j) + D_{\text{tot},2}(x/K_2)\bar{F}_2(p_k)$, and since $x/K_i \ll 1$, we can approximate $x \approx x_{\text{tot}}$. We thus have

$$\frac{dp_i}{dt} = \kappa \frac{m_i \cdot y}{\bar{K}_i} - \gamma p_i,$$

in which y is given by the conservation law as before

$$y_{\text{tot}} = y + m_1 \cdot y/\bar{K}_1 + m_2 \cdot y/\bar{K}_2,$$

in which we can substitute the expressions of the mRNA levels m_1 and m_2 and solve for y to obtain

$$y = \frac{y_{\text{tot}}}{1 + (q_1/\bar{K}_1)\bar{F}_1(p_j) + (q_2/\bar{K}_2)\bar{F}_2(p_k)}, \;\; q_i = \frac{u_i}{\delta} D_{\text{tot},i} \frac{x}{K_i}.$$

By substituting this in the differential equation for p_1, we obtain

$$\frac{dp_1}{dt} = \kappa y_{\text{tot}} \frac{q_1/\bar{K}_1 \bar{F}_1(p_j)}{1 + (q_1/\bar{K}_1)\bar{F}_1(p_j) + (q_2/\bar{K}_2)\bar{F}_2(p_k)} - \gamma p_1,$$

or equivalently

$$\frac{dp_1}{dt} = \kappa y_{\text{tot}} \frac{u_1 x_{\text{tot}}}{\delta} \frac{D_{\text{tot},1}}{\bar{K}_1 K_1} \bar{F}(p_j) \left(\frac{1}{1 + \frac{u_1}{\delta} \frac{D_{\text{tot},1} x_{\text{tot}}}{K_1 \bar{K}_1} \bar{F}(p_j) + \frac{u_2}{\delta} \frac{D_{\text{tot},2} x_{\text{tot}}}{K_2 \bar{K}_2} \bar{F}(p_k)} \right) - \gamma p_1,$$

in which the term in parenthesis accounts for resource competition and was not present in the classical Hill function-based model. We call $J_i = \frac{u_i}{\delta} \frac{D_1 \cdot x_{\text{tot}}}{K_1 \bar{K}_i}$ the *resource demand coefficient* for module i. From this expression, we see that p_1 depends also on the concentration of regulators to protein p_2 through $\bar{F}(p_k)$ in the denominator of the bracketed expression. From this, it follows that if p_k is a repressor and hence $\bar{F}(p_k)$ is a decreasing function of p_k, then the bracketed term is an increasing function of p_k. That is, p_1 will increase as p_k increases. On the other hand, if p_k is an activator and hence $\bar{F}(p_k)$ is an increasing function of p_k, then the bracketed term is a decreasing function of p_k. That is, p_1 will decrease as p_k increases.

The consequence is that if p is a repressor, it becomes an effective activator for any gene not repressed directly by it. Qualitatively, this occurs because by repressing its target, it releases resources that become available to other genes. Similarly, if p is an activator, it becomes an effective repressor for any gene not activated by it. Qualitatively, this occurs because by activating its target, it sequesters resources from other genes. These relationships are summarized in Figure 8.2.

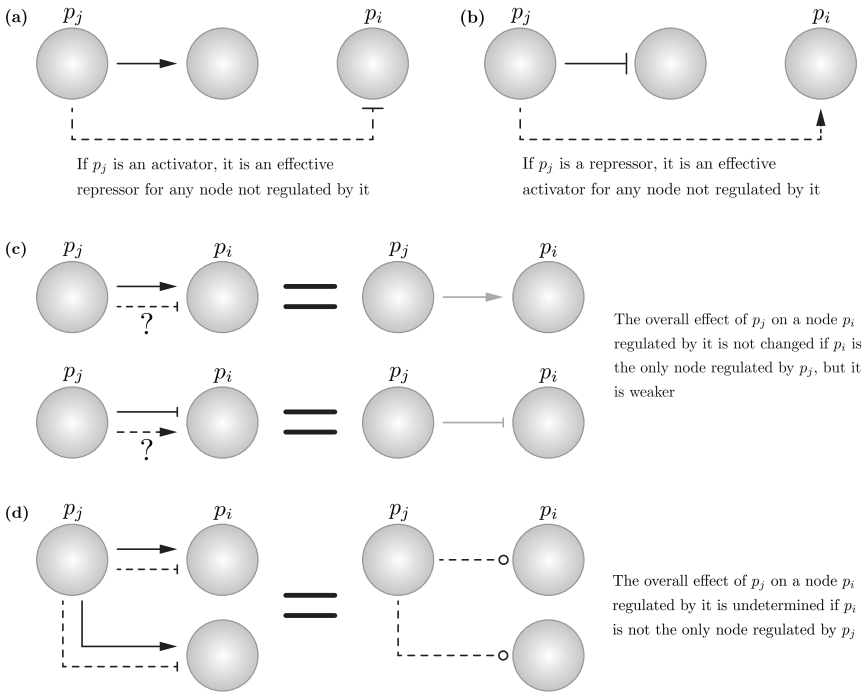

Figure 8.2: Solid black lines are intended transcriptional regulation interactions, while the dotted black lines represent interactions arising due to resource sharing. In (a) and (b), it can be seen that otherwise-uncoupled modules experience unintended interactions on account of resource sharing. (c) This effect attenuates, but does not fundamentally modify, the intended interaction, if that regulatory activity is exclusive to a single node. (d) The overall regulatory impact of a node cannot be determined if that node acts on more than one node, however.

8.2 Resource competition in mammalian genetic circuits

We are going to introduce a simplified model of transcriptional activation in mammalian cells and study how competition for gene expression resources affects gene expression regulation. We will focus on transcription as opposed to translation, since it was experimentally shown that, for mammalian systems, translation resources are not a bottleneck in the current design of synthetic genetic circuits, but transcription resources are [SJWDV21]. This provides a stark contrast to the previous section, in which the opposite was true for bacterial systems. A transcriptional activator A enables transcription by recruiting transcription co-activators to the DNA. One of these co-activators that has been studied in the literature is the *mediator*, which is a complex of proteins that, once recruited to DNA by an activator, stabilizes the binding of RNAP to the DNA [CKK$^+$05]. We will lump this mediator and similar co-activators that an activator protein binds and directs to DNA into a species M. The activator protein achieves this through an activation domain (AD),

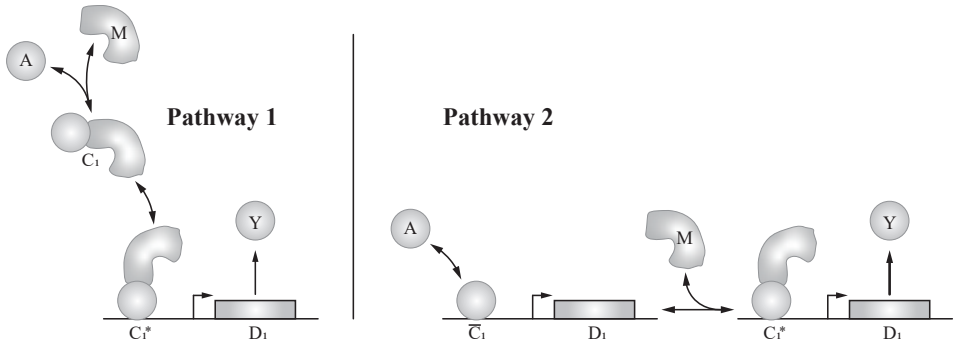

Figure 8.3: Two simplified models of the transcriptional activation of mammalian genes. In Pathway 1, the mediator M forms a complex C_1 with the activator protein A, which then goes on to form an active complex C_1^* by binding to the DNA binding domain. In Pathway 2, the activator protein binds to the DNA first, forming a complex $\bar{C}_1$ which cannot produce proteins. It is only when the mediator binds to $\bar{C}_1$ that the system becomes activated, producing Y.

which binds to the mediator and other co-activators. The DNA binding domain (DBD) of the activator protein instead enables recruitment of these resources to the DNA target [CKK+05]. We therefore model transcriptional activation as depicted in Fig. 8.3 through the reactions:

$$\text{Pathway 1}: \mathrm{A} + \mathrm{M} \underset{k_1}{\rightleftharpoons} \mathrm{C}_1, \ \mathrm{C}_1 + \mathrm{D}_1 \underset{k_1^*}{\rightleftharpoons} \mathrm{C}_1^* \xrightarrow{\alpha} \mathrm{Y} + \mathrm{D}_1 + \mathrm{C}_1,$$

$$\text{Pathway 2}: \mathrm{A} + \mathrm{D}_1 \underset{\bar{k}_1}{\rightleftharpoons} \bar{C}_1, \ \bar{C}_1 + \mathrm{M} \underset{\bar{k}_1^*}{\rightleftharpoons} \mathrm{C}_1^* \xrightarrow{\alpha} \mathrm{Y} + \bar{\mathrm{C}}_1 + \mathrm{M},$$

in which, for simplicity, we have lumped translation together with transcription. The constants k represent dissociation constants, that is, the ratios between the dissociation rate constant and the association rate constant for each reversible reaction depicted. The reaction rate equation for the protein output Y will be given by:

$$\frac{dY}{dt} = \alpha C_1^* - \gamma Y, \tag{8.1}$$

in which C_1^* is the transcriptionally active complex from which RNAP can lead to transcription. By setting the complexes to their quasi-steady state, we obtain that

$$C_1^* = \frac{\bar{C}_1 \cdot M}{\bar{k}_1^*} + \frac{C_1 \cdot D_1}{k_1^*}, \ \bar{C}_1 = \frac{A \cdot D_1}{\bar{k}_1}, \ C_1 = \frac{A \cdot M}{k_1}. \tag{8.2}$$

Also, from the conservation law of DNA and letting $D_{\text{tot},1}$ represent the total amount of DNA, we obtain that $D_{\text{tot},1} = D_1 + C_1^* + \bar{C}_1$, in which assuming that the activator

is in excess, we will have that most of the DNA is bound by the activator only, so that $C_1^* \ll \bar{C}_1$ and $D_{\text{tot},1} \approx D_1 + \bar{C}_1$. From this, it follows that

$$D_1 = \frac{D_{\text{tot},1}}{1 + A/\bar{k}_1}. \tag{8.3}$$

By substituting (8.2) and (8.3) into (8.1) and letting $\theta = \frac{1}{\bar{k}_1^* \bar{k}_1} + \frac{1}{k_1 k_1^*}$, we have:

$$\frac{dY}{dt} = \alpha D_{\text{tot},1} \theta \frac{AM}{1 + A/\bar{k}_1} - \gamma Y.$$

When M is a constant parameter, the production rate has the standard form of a Hill function for transcriptional activation (see Chapter 2).

In reality, however, M is not a constant — it is the free level of mediator and, as such, it may be affected by the presence of other modules that also require it for transcription activation. We will thus write the conservation law for the mediator. In this process, we will also assume that there is another transcriptional module which takes input activator A_0 total DNA D_0, produces output protein X, and has the following complexes containing the mediator:

$$C_0 = \frac{M \cdot A_0}{k_0}, \quad C_0^* = \frac{A_0 \cdot D_0 \cdot M}{\bar{k}_0^* \bar{k}_0} + \frac{A_0 \cdot D_0 \cdot M}{k_0 k_0^*}.$$

This module has the same structure as module 1 in Fig. 8.3, in which we add subscripts 0 to A and replace the 1 subscripts with 0. Letting M_{tot} represent the total concentration of mediator, we can therefore write the mediator conservation law as $M_{\text{tot}} = M + C_1^* + C_1 + C_0^* + C_0$, in which C_1 and C_0 represent the amount of mediator bound to either A or A_0, while C_1^* and C_0^* represent the amount of mediator bound to the DNA D_1 or D_0. At this point, we can reason about numbers and note that the amount of DNA — typically a few copies per cell — is much smaller than the amount of activator protein in the system [MJM+10]. Therefore, we can safely assume that $C_1^* \ll C_1$ and $C_0^* \ll C_0$. Therefore, we have $M_{\text{tot}} \approx C_1 + C_0$, in which using the expressions for $C_1 = \frac{A \cdot M}{k_1}$ and $C_0 = \frac{A_0 \cdot M}{k_0}$, we obtain that:

$$M = \frac{M_{\text{tot}}}{1 + A/k_1 + A_0/k_0}.$$

By substituting this into the expression of the rate of change of Y, we finally obtain:

$$\frac{dY}{dt} = \alpha D_{\text{tot},1} \theta M_{\text{tot}} \frac{A}{1 + A/\bar{k}_1} \left(\frac{1}{1 + A/k_1 + A_0/k_0} \right) - \gamma Y.$$

The term in the bracket is a factor that appears by considering the conservation law for the mediator and is absent in models that do not account for resource competition. The consequence of this factor on the steady state response of Y to A (independent of whether A_0 is present) is a phenomenon called *squelching*, wherein

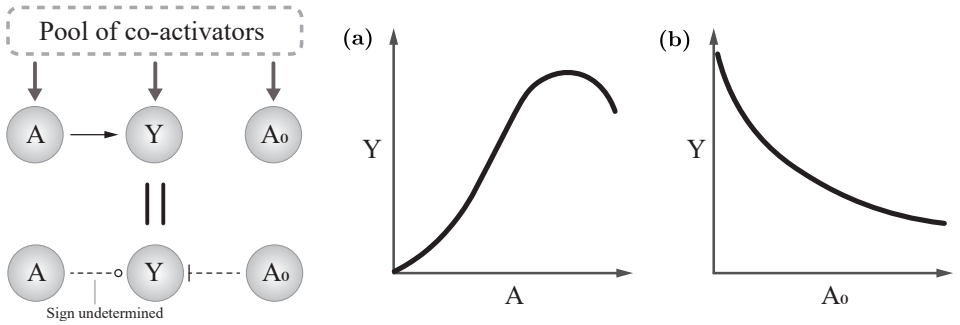

Figure 8.4: Summary of the effects of competition for transcriptional co-activators. (a) Squelching: after a point, increasing the level of activator in the system leads to the transcriptional repression of Y. (b) An orthogonal activator A_0 sequesters resources from Y.

increasing the activator level A increases the steady state level of Y up to a certain point, and after that further increasing the activator leads to complete target repression (Fig. 8.4(a)). Additionally, since A_0 appears in the denominator, we observe that the level Y decreases as the activator A_0 for the competitor module is increased (Fig. 8.4(b)). Squelching has been observed before and has to do with the fact that when the activator A is in excess compared to the mediator M and the DNA, then most of the mediator will bind to A "in solution" as opposed to on the DNA and hence the likelihood of M binding to DNA will decrease.

The extent of these effects depend on the binding constants of the activators to the mediator, which are dictated by the so called strength of the activation domain. Typical activation domains (in order of strength) include VPR, VP16, and VP64 [SJWDV21].

8.3 Feedforward control to mitigate the effects of resource sharing

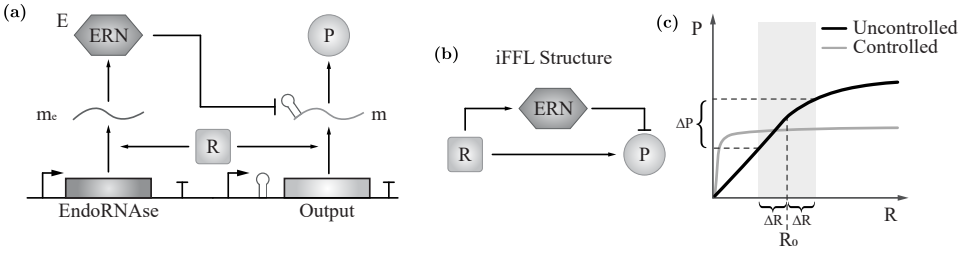

Figure 8.5: Feedforward controller to attenuate the effect of changes of transcriptional resource R on the output protein P (a) and corresponding incoherent iFFL diagram (b). We show in (c) the steady state response of the protein to a change ΔR with respect to a nominal value R of the resource level.

In the case of genetic circuits in the mammalian cell, we saw that gene expression modules become coupled with each other because they compete for transcriptional resources, and in particular, transcriptional co-activators such as the mediator complex. In particular, when a gene becomes activated through expression of an activator protein, transcriptional co-activators' free levels decrease as they are sequestered away by the activator. The lowering of the free level of co-activator causes a lowering of gene expression in any other module. Here, we are going to address the question of how to make the protein output level of one gene expression module be insensitive to changes in the free level of transcriptional co-activators. To this end, we propose a feedforward control approach by which we repress the output protein level by a molecule that also requires transcriptional co-activators in order to be expressed. This way, when the co-activator level drops, the drop in the level of this molecule causes a de-repression of the output protein, which ultimately compensates the initial decrease due to the drop in co-activator level. This design is depicted in Figure 8.5(a). Specifically, we choose to repress the output protein's mRNA m through an endoribonuclease (ERN), denoted by E, which is designed to bind a target sequence on the mRNA m of the output protein P. Letting R represent the transcriptional resource that is subject to fluctuations, then the effect on P of a drop of this resource level will be compensated by a decreased degradation of its mRNA m.

We model this system by transcription rates that are proportional to the free level of the resource R (see Chapter 2) and by capturing the action of E on m through the degradation enzymatic-like reaction:

$$\mathrm{E} + \mathrm{m} \underset{d}{\overset{a}{\rightleftharpoons}} \mathrm{C}, \quad \mathrm{C} \xrightarrow{k} \mathrm{E}.$$

We therefore write the reaction rate equations as:

$$\frac{dP}{dt} = \kappa m - \gamma P, \quad \frac{dm}{dt} = \alpha R - \delta m - am \cdot E + (d+k)C$$

$$\frac{dm_E}{dt} = \bar{\alpha} R - \delta m_E, \quad \frac{dE}{dt} = \bar{\kappa} m_E - \gamma E - am \cdot E + (d+k)C,$$

$$\frac{dC}{dt} = am \cdot E - (d+k)C - \gamma C \approx am \cdot E - (d+k)C,$$

in which we have accounted for the separation of time scales between decay and catalytic rates $\gamma \ll k$. After performing the QSSA for the complex C, we obtain that $C = m \cdot E/K_m$, in which K_m is the Michaelis-Menten constant of the enzymatic reaction and is given by $K_M = (d+k)/a$. After setting the mRNA levels to their quasi-steady state, we obtain:

$$m = \frac{\alpha R}{\delta + (k/K_m)R},$$

so that we obtain the reduced ODE model:

$$\frac{dE}{dt} = \bar{\kappa}\frac{\bar{\alpha}}{\delta}R - \gamma E, \quad \frac{dP}{dt} = \kappa\frac{\alpha R}{\delta + (k/K_m)R} - \gamma P.$$

We are going to study first the steady state behavior of the protein level P as a function of a perturbation of R about its nominal value R_0. After setting the derivatives to zero, we obtain that the steady state value of the protein level is given by

$$P = \frac{\kappa}{\gamma}\alpha\frac{R}{\delta + (k/K_m)\frac{\bar{\kappa}\bar{\alpha}}{\delta\gamma}R}.$$

From this expression, we observe that when

$$(k/K_m)\frac{\bar{\kappa}\bar{\alpha}}{\delta\gamma}R \gg \delta,$$

then the steady state protein level becomes independent of the resource level R:

$$P \approx \frac{\kappa}{\gamma}\alpha\frac{1}{(k/K_m)\frac{\bar{\kappa}\bar{\alpha}}{\delta\gamma}}.$$

This effect is shown in Figure 8.5(c): when there is no feedforward controller, a ΔR change of the free resource level with respect to its nominal level R_0 will transfer to a large change (depicted by the double arrow) in the level of P. By contrast, with the feedforward controller, the range of R values where the expression of P changes substantially with R is highly restricted to low values of R. As a consequence, the same ΔR change results in practically no change on the output protein level P.

In order to achieve this, we need to design our system to have a large $\bar{\kappa}$ and/or a large $\bar{\alpha}$, which implies large level of the endoribonuclease E since these parameters are the transcription and translation rate constants therefor. We can achieve the same effect by increasing k/K_m, that is, by making the degradation more effective. This can be achieved by improving the binding affinity of E to the target mRNA m, which makes K_m smaller.

Note that when we accomplish this, the level of P will also decrease. In order to keep the same level of P while at the same time mitigating the effect of a change in R, we can increase the promoter strength of the translation rate constant of P by increasing κ and/or α.

Finally, we observe that any time there is perfect adaptation — in this case, when we assume δ is negligible — there must be a hidden integral action in the system. This action can be observed in the full system of equations before approximating the mRNA to its quasi-steady state. Specifically, we take $m_E(t) = \frac{\bar{\alpha}R}{\delta}(1 - e^{-\delta t})$ and consider a candidate memory variable z, where $z = E/\beta - m\alpha$ with $\beta = \bar{\kappa}(\bar{\alpha}/\delta)$, we have that

$$\frac{dz}{dt} = E(m\frac{k}{\alpha K_m} - \frac{\gamma}{\beta}) - Re^{-\delta t}.$$

When t is large enough for the transient to die out, the above becomes an integrator. When the steady state is reached and z achieves a constant (one can check that the system's equilibrium is asymptotically stable), then we have that $dz/dt = 0$ implies $E(m\frac{k}{\alpha K_m} - \frac{\gamma}{\beta}) = 0$. Assuming $E \neq 0$, this implies that m (and therefore also P) will have a steady state value that is independent of R.

Remark. As an exercise one can check that if E were a protease for P, then the same adaptation result would hold. These adaptation results would hold also of the resource being perturbed were a translation resource. Instead, if E were a microRNA, one could check that translation resource variability would not be compensated for, but only transcriptional resource variability would be compensated for.

8.4 Feedback control to mitigate the effects of resource sharing

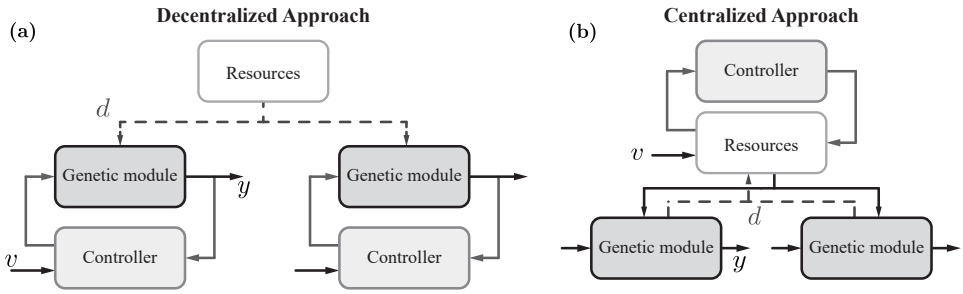

Figure 8.6: Feedback control for mitigating the effect of resource sharing. (a) Decentralized feedback control: the resource input to each module is subject to variability due to loading applied by other modules. As a consequence, we can regard the variation of resource as a disturbance d, which is applied to every module. Every module will have a "local" feedback controller around it to make the output y independent of d. (b) Centralized feedback control: Each module applies a load to the resources. The controller's objective is to keep the free resource level R constant independent of the load. As a consequence, each module's output will not be affected by the addition of other modules.

In order to mitigate the effects of resource competition through feedback control, there are two main approaches. The first, which we call a decentralized approach (Fig. 8.6(a)), considers the change in resource level applied to any given module due to the presence of other modules as a disturbance d to the module itself. In this case, each module will implement a controller, the decentralized controller, which aims to keep the module's output unchanged when a disturbance hits the system [HQDV18]. In this way, every module will have its own controller. Although each controller should in principle be designed independent of the others, in practice this is not quite possible [QDV21]. The second approach, which we call a centralized approach (Fig. 8.6(b)), considers the load applied by the modules to the cellular system, creating the resource as a disturbance to the "resource system".

As a consequence, within this view, the controller is designed around the resource system and aims at keeping the resource output level constant independent of the loads applied by the modules [DKJB18]. Here, we describe in detail two different decentralized control designs that have been implemented in bacterial [HQDV18] and mammalian [JQI+22] cells.

Before proceeding, we recall that integral control can be used for the following purpose. Letting x represent the internal state of the genetic module, such as the mRNA and protein levels, and the output y represent the protein level, we have the following general description for a quasi-integral feedback control, which accounts for the molecule dilution that is unavoidable in growing cells:

$$\frac{dx}{dt} = f(x,z,d), \;\; y = g(x), \quad \text{and} \quad \frac{dz}{dt} = k(u-y) - \gamma z,$$

in which z is the controller variable implementing the quasi-integral action, γz represents the decay rate of z and can be regarded as the integrator leakage, d is the disturbance, and u is a reference input. Without leakage (i.e., with $\gamma = 0$), if the system is asymptotically stable — that is, trajectories converge to an equilibrium point — then at steady state $dz/dt = 0$, which implies that $y = u$ independent of d. However, with leakage, this is no longer true. To make this statement approximately true with leakage, one can engineer k to be very large, that is, we can set $k = \bar{k}/\varepsilon$ with $0 < \varepsilon \ll 1$ and $\bar{k} > 0$. In this case, and under asymptotic stability assumptions, we have that at steady state $\bar{k}(u-y) - \varepsilon\gamma z = 0$, which leads to $y \approx u$, which is independent of the disturbance d. Therefore, we will implement biomolecular systems that can achieve the following quasi-integral control (QIC) structure:

$$\frac{dx}{dt} = f(x,z,d), \;\; y = g(x), \quad \text{and} \quad \frac{dz}{dt} = \frac{\bar{k}}{\varepsilon}(u-y) - \gamma z.$$

Therefore, if a system of ODEs has the above structure, in which the QIC is apparent, and the system's equilibrium is asymptotically stable, then we can immediately conclude that the output y will asymptotically, and approximately, approach a function of the reference input u and will be independent of the disturbance d. Note that in general it is difficult to prove global asymptotic stability of the equilibrium; therefore we will only request local asymptotic stability. In this case, the result will hold as long as the initial condition is sufficiently close to the equilibrium. Local asymptotic stability can be demonstrated by linearizing the system about the equilibrium and by showing that the eigenvalues all have strictly negative real component.

Bacterial feedback controller

For bacteria, we will consider a controller that can maintain the output protein of a genetic module at a constant level despite changes in free ribosome level, due to ribosome sequestration by other modules. Given that the perturbation is applied

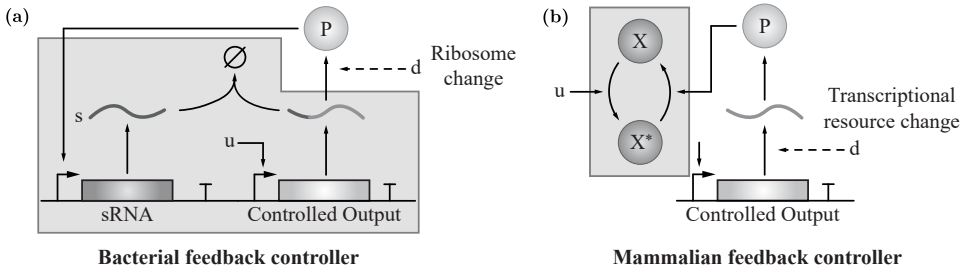

Figure 8.7: Implementations of feedback controllers to mitigate the effect of resource variability. (a) Post-transcriptional controller to mitigate variability in ribosome levels. (b) Transcriptional controller to mitigate variability in transcription co-factor levels.

to the translation process, we will be implementing a post-transcriptional feedback controller. Specifically, we use sRNA interference as a method to implement post-transcriptional actuation as illustrated in Fig. 8.7(a). To this end, we recall that the sRNA reaction is given by

$$\mathrm{s} + \mathrm{m} \xrightarrow{k} \emptyset,$$

in which s represents the sRNA and m its target RNA, where the target mRNA has a complementary sequence in the 5' region that is recognized by the sRNA. Upon binding, the resulting double-stranded RNA will be targeted by molecular processes that degrade both molecules together. We will then let the sRNA be activated by (a proxy for) the output protein P, such that the reaction rate equations describing the system are given by:

$$\frac{ds}{dt} = \beta F(P) - km \cdot s - \delta s, \quad \frac{dm}{dt} = \alpha - km \cdot s - \delta m, \quad \frac{dP}{dt} = \kappa(R)m - \gamma P,$$

in which $\kappa(R) = k_f R/K_m$ is the translation rate constant, which is proportional to the level of free ribosomes R, whose variation we can regard as a disturbance. Here $F(P)$ is an activating Hill function and we have "pulled out" the maximal expression rate constant β, which we will consider a tunable parameter through the promoter strength. Here, α is also a tunable parameter, which we can increase by increasing the promoter strength for the mRNA.

In order to determine whether $y = P$ is approximately independent of the variations in $d = R$, we must (a) find the memory variable z and (b) demonstrate that the system's equilibrium is asymptotically stable, at least locally, from the Jacobian of the system. For (a), we take as candidate memory variable $z = m - s$, such that

$$\frac{dz}{dt} = \alpha - \beta F(P) - \delta z,$$

and take both α and β to be large, which we represent by assuming that there is an

$\varepsilon \ll 1$ such that $\alpha = \bar{\alpha}/\varepsilon$ and $\bar{\beta} = \beta/\varepsilon$. We therefore obtain:

$$\frac{dz}{dt} = \frac{1}{\varepsilon}\left(\bar{\alpha} - \bar{\beta}F(P)\right) - \delta z,$$

which provides the QIC structure. At this point, we are left to determine whether the whole closed-loop system is asymptotically stable. By setting all derivatives to zero, one can find that there is a unique equilibrium point. Performing system linearization at this equilibrium results in a Jacobian matrix whose eigenvalues always have negative real component as long as $\partial F(P)/\partial P > 0$. Under these conditions, then we have that at steady state P will satisfy $\bar{\alpha} - \bar{\beta}F(P) \approx 0$, which results into a value of P that is approximately independent of R, the disturbance. For details on the rigorous proof for stability, the reader is referred to Lecture 10 (supplements to Chapter 3).

From a practical point of view, we can ensure that α and β are large by picking strong promoters for the sRNA and mRNA. Stability, not only requires that P is an activator for s, thereby implementing a *negative* feedback, but also that P does not saturate $F(P)$, which can occur if P is too large. In this case, a change in P will result in no change in $F(P)$, leading to a system that is practically open loop. Therefore, to ensure the feedback is active, it is necessary to ensure that P is not too large. Given that $F(P) = P/K_d/(a + P/K_d)$, assuming Hill coefficient equal to 1, we will need to ensure that $P < K_d$, in which K_d is the dissociation constant of the binding of P to the DNA promoter sites. Experimentally, we can ensure this by increasing K_d, which can be accomplished by introducing point mutations in the sequence of DNA binding sites to artificially weaken the binding of P to its target site.

Mammalian feedback controller

In the case of mammalian systems, we learned that the resource most commonly competed for is a transcriptional resource required for transcription initiation. One such transcription co-factor is best known as the mediator and it helps stabilize the binding of the RNAP to the promoter. It is recruited to the DNA by activators, which bind it through their activation domain (AD). Because then the perturbation hitting a genetic module when such a resource is sequestered occurs at the transcription level, we have a disturbance on the transcription rate. As a consequence, the same type of controller used for bacterial systems will not work, since it can only mitigate perturbations hitting the translation rate. To this end, we need to consider a controller that acts on transcription, not on translation (Fig 8.7(b)). In this design, we consider a covalent modification cycle as the biomolecular process to implement the feedback due to its ability to implement an integral controller (see Lecture 10 and supplements to Chapter 3).

Specifically, looking at Figure 8.7(b), we note that if d drops and causes a drop in the level of P, then the rate from X^* to X will also drop, thereby increasing the

level of X^*, which will increase the transcription rate of P, thereby compensating for the original drop in the level of P. We can write a reaction rate equation for this system describing the rate of change of P and of X^* as follows:

$$\frac{dP}{dt} = \alpha(R)\frac{X^*}{X^* + K_d} - \gamma P, \quad \frac{dX^*}{dt} = k_1 u \frac{X}{X + K_1} - k_2 P \frac{X^*}{X^* + K_2} - \delta X^*,$$

in which $\alpha(R)$ captures the expression rate as a function of the shared resources R, whose concentration is subject to perturbations, and the dynamics of X^* are taken from Chapter 2, where they were derived from the two-step enzymatic reactions of phosphorylation and dephosphorylation. The constants K_1 and K_2 are the Michaelis-Menten constants of the two enzymatic reactions forming the covalent modification cycle.

As before, we will (a) look for a QIC structure with a clear memory variable z, and (b) assess the stability of the system. For (a), we will assume that we can take $X \gg K_1$ and $X^* \gg K_2$, that is, that the enzymatic reactions are in the zero order regime. We will also account for the fact that the catalytic rate constants of the enzymatic reactions k_i are much larger than protein decay δ. With this, we can take as small parameter $\varepsilon = \delta/k_2 \ll 1$ and re-write the above ODEs as:

$$\frac{dX^*}{dt} = \frac{\delta}{\varepsilon}\left(\frac{k_1}{k_2} - P\right),$$

which is in the form of a QIC. In order to check (b), we can use the linearization matrix and check its eigenvalues. This analysis will reveal that the eigenvalues have negative real part as long as $\partial F/\partial X^* > 0$, in which $F(X^*) = X^*/(K_d + X^*)$. This requirement translates into a requirement on X^* being sufficiently small as to not saturate the F function. If it does, then the feedback loop breaks and the system becomes open loop and loses asymptotic stability. Therefore, we will ask that $X^* < K_d$, which gives two conflicting requirements in X^*, that is, $K_1 \ll X^* < K_d$. This is in practice difficult to reach. In fact, to satisfy the left-hand side, one would express X from a strong promoter to achieve a high X and X^* value and ensure we have sufficient amount of input u. With this level of X^*, to ensure the right-hand side inequality, one can increase K_d by performing single base pair mutations on the operator sequence. This system was designed this way in [JQI+22].

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