Solution of Exercise 1:

1.1 A change in production rate. A gene Y with simple regulation is produced at a constant rate $\beta_1$. The production rate suddenly shifts to a different rate $\beta_2$.

(a) Calculate and plot $Y(t)$.

(b) What is the response time (time to reach halfway between the steady-states)?

Solution:

(a): Lets mark the time when the shift occurs as $t = 0$. Before the shift, $Y$ reaches steady state at a level $Y(t = 0) = Y_{st} = \beta_1 / \alpha$. After the shift,

$$\frac{dY}{dt} = \beta_2 - \alpha Y.$$

The solution of such an equation is generally $Y = \beta_2 / \alpha + C_1 \exp(-\alpha t)$, where the constant $C_1$ need to be determined so that $Y(t = 0) = \beta_1 / \alpha$. This yields the following solution

$$Y(t) = \frac{\beta_1}{\alpha} \exp(-\alpha t) + \frac{\beta_2}{\alpha} (1 - \exp(-\alpha t))$$

One can see that the initial condition $\beta_1 / \alpha$ decays exponentially at the same rate as the new steady state $\beta_2 / \alpha$ increases.

(b) To find the response time we have to solve the equation:

$$\frac{\beta_1}{\alpha} \exp(-\alpha \tau_{1/2}) + \frac{\beta_2}{\alpha} (1 - \exp(-\alpha \tau_{1/2})) = \frac{1}{2} \left( \frac{\beta_1}{\alpha} + \frac{\beta_2}{\alpha} \right)$$

After some algebra the response time is found to be $\log(2) / \alpha$. 
1.2 Cascades. Consider a cascade of three activators, $X \rightarrow Y \rightarrow Z$. Protein $X$ is initially present in the cell in its inactive form. The input signal of $X$, $S_x$, appears at time $t = 0$. As a result, $X$ rapidly becomes active and binds the promoter of gene $Y$, so that protein $Y$ starts to be produced at rate $\beta$. When $Y$ levels exceed a threshold $K_y$, gene $Z$ begins to be transcribed. All proteins have the same degradation/dilution rate $\alpha$. What is the concentration of protein $Z$ as a function of time? What is its response time with respect to the time of addition of $S_x$? What about a cascade of three repressors? Compare your solution to the experiments shown in Rosenfeld and Alon, 2003.

Solution:

We will assume all proteins have the same $\alpha$. After induction, $Y$ is produced at rate $\beta_y$ and degraded/diluted at rate $\alpha$:

$$\frac{dY}{dt} = \beta_y - \alpha Y$$

yielding the familiar exponential approach to steady-state:

$$Y(t) = \frac{\beta_y}{\alpha} \left(1 - \exp(-\alpha t)\right)$$

Assuming a step function for the activation of gene $Z$ by $Y$ (logic input function), transcription of gene $Z$ starts at time $\tau_{yz}$ when $Y(\tau_{yz}) = K_y$:

$$Y(\tau_{yz}) = \frac{\beta_y}{\alpha} \left(1 - \exp(-\alpha \tau_{yz})\right) = K_y \Rightarrow \tau_{yz} = \frac{1}{\alpha} \log \left(\frac{Y_{st}}{Y_{st} - K_y}\right)$$

where $Y_{st} = \beta_y / \alpha$. Just for extra clarity, let’s consider the limits of this equation to see if this makes sense. When $K_y \ll Y_{st}$, $Y_{st} - K_y \rightarrow Y_{st}$ and $\tau_{yz} \rightarrow 0$. In this case the threshold for $Z$ activation is low, and $Y$ levels cross it very fast. Conversely, if the activation threshold $K_y$ is very high, approaching $Y_{st}$, $Z$ is never activated because $Y_{st} - K_y \rightarrow 0$ and $\tau_{yz} \rightarrow \infty$.

Production of $Z$ starts after time $t = \tau_{yz}$ at a constant rate of $\beta_z$:

$$\frac{dZ}{dt} = \begin{cases} 
0 & t < \tau_{yz} \\
\beta_z - \alpha Z & t > \tau_{yz} 
\end{cases}$$

Solving we get

$$Z(t) = \begin{cases} 
0 & t < \tau_{yz} \\
\frac{\beta_z}{\alpha} \left(1 - \exp\left(-\alpha(t - \tau_{yz})\right)\right) & t > \tau_{yz} 
\end{cases}$$

Solving for the response time, the time to reach half of the steady state of $Z$:
\[
\frac{\beta_z}{\alpha} \left( 1 - \exp \left( -\alpha (t_{1/2} - \tau_{yz}) \right) \right) = \frac{1}{2} \frac{\beta_z}{\alpha} \Rightarrow t_{1/2} = \tau_{yz} + \log(2) / \alpha 
\]

Hence, there is an extra delay of \( \tau_{yz} \) in the response time of gene Z relative to simple regulation with no cascade. If Z activates a third gene W when it crosses a threshold \( K_Z \), this will occur at a time of \( \tau_{ZW} \) found from:

\[
\frac{\beta_z}{\alpha} \left( 1 - e^{-a(\tau_{ZW}-\tau_{yz})} \right) = Z_{St} \left( 1 - e^{-a(\tau_{ZW}-\tau_{yz})} \right) = K_Z
\]

solving for \( \tau_{ZW} \) we obtain:

\[
\tau_{ZW} = \tau_{yz} + \frac{1}{\alpha} \log \left( \frac{Z_{st}}{Z_{st}-K_Z} \right)
\]

We can generalize this result: each step in a cascade, where a gene X activates a downstream gene after crossing a threshold \( K_X \) adds a delay of:

\[
\tau_{delay} = \frac{1}{\alpha} \log \left( \frac{X_{st}}{X_{st}-K_X} \right)
\]

In the special case in which the activation threshold is half the steady-state level (this can be shown to be in some cases an optimal value), the delay is \( \tau_{delay} = \log(2) / \alpha \). In summary, since \( 1/\alpha \) is often on the scale of a cell generation, a transcriptional cascade can be a slow process.
1.3 Fan-out: Transcription factor X regulates two genes $Y_1$ and $Y_2$. Draw the resulting network, termed a fan-out with two target genes. The activation thresholds for these genes are $K_1$ and $K_2$. The activator X begins to be produced at time $t=0$ at rate $\beta$, and is degraded/diluted at rate $\alpha$, and its signal $S_X$ is present throughout. What are the times at which $Y_1$ and $Y_2$ reach halfway to their maximal expression? Design a fan-out with three target genes in which the genes are activated with equal temporal spacing.

Solution:

Based on the previous problem:

\[
\begin{align*}
\tau_1 &= \frac{1}{\alpha} \log \left( \frac{X_{st}}{X_{st} - K_1} \right) \\
\tau_2 &= \frac{1}{\alpha} \log \left( \frac{X_{st}}{X_{st} - K_2} \right)
\end{align*}
\]

After the corresponding delays in gene activation, denoted $\tau_1$ and $\tau_2$, production of $Y_1$ and $Y_2$ starts at a constant rate reaching half the steady state after $\log(2)/\alpha$. The time to reach half maximum is therefore: $\tau_{1/2} = t_i + \log(2)/\alpha$ (i=1,2), where i=1,2 for $Y_1$ and $Y_2$ respectively.

For three target genes, we require $\tau_2 - \tau_1 = \tau_3 - \tau_2$, or $\tau_2 = \frac{1}{2} (\tau_1 + \tau_3)$. This amounts to the following requirements on the thresholds,

\[
\frac{1}{\alpha} \log \left( \frac{X_{st}}{X_{st} - K_2} \right) = \frac{1}{2} \left( \frac{1}{\alpha} \log \left( \frac{X_{st}}{X_{st} - K_1} \right) + \frac{1}{\alpha} \log \left( \frac{X_{st}}{X_{st} - K_3} \right) \right) \Rightarrow
\]

\[
X_{st} - K_2 = \sqrt{(X_{st} - K_1)(X_{st} - K_3)}
\]

namely that the difference between $X_{st}$ and the thresholds are arranged according to a geometric mean.
1.4. Positive feedback. What is the effect of positive auto-regulation on the response time? Use as a model the following linear equation:

\[ \frac{dX}{dt} = \beta + \beta_1 X - \alpha X \]

Explain each term and solve for the response time. When might such a design be biologically useful?

Solution: The basal production rate is \( \beta \), the positive effect of \( X \) on its own production (positive auto-regulation - PAR) is described in this model by the linear term \( \beta_1 X \), and degradation/dilution is represented as usual by \(- \alpha \, X\). Let's group the terms that multiply \( X \) in this linear model:

\[ \frac{dX}{dt} = \beta - (\alpha - \beta_1) \, X \]

We see that the degradation/dilution rate is effectively reduced by positive auto-regulation, to an effective rate \( \alpha' = \alpha - \beta_1 \). Assuming that the auto-regulation is not too strong, that is that \( \beta_1 < \alpha \), the term multiplying \( X \) is negative and we get an approach to a stable steady-state:

\[ X(t) = X_{st} \left( 1 - \exp(-\alpha' \, t) \right) \]

Where \( \alpha' = \alpha - \beta_1 \). The response time is defined as the time to reach half of the steady state: \( T_{1/2} (PAR) = \log(2) / \alpha' \). The response time is longer than that for simple regulation due to the reduced effective degradation/dilution rate

\[ T_{1/2}(PAR) = \log(2) / (\alpha - \beta_1) > \log(2) / \alpha = T_{1/2}(simple) \]

Thus positive auto-regulation has an effect that is opposite to that of negative auto-regulation. The former slows response time, whereas the latter speeds response times. Note that strong auto-regulation, in which \( \beta_1 > \alpha \), can lead to instability and unchecked growth of \( X \) in the model. In real systems, this instability will be limited by other factors (such as saturation of the input function), locking \( Y \) in an ON state of high expression even after its activating input \( \beta \) vanishes. Hence, strong positive feedback creates a bistable system, in which \( X \) is either at a low or at a high fixed point. This is useful for commitment-type biological decisions, such as those made in development. Positive feedback characterizes developmental systems that make a switch that is either OFF or is locked ON (e.g., a cell commits to become a muscle cell rather than, say, a blood cell, by means of positive feedback loops on key transcription factors).

A different biological example is found in some regulatory systems that govern the transcription of protein parts of multi-protein structures that are assembled slowly. An example is the bacterial flagellum described in Chapter 6 that can take two cell generations to be completed. Such slow processes can benefit from weak positive auto-regulation to slow down responses and prolong delays (Kalir et al., 2005)