Chapter 8

Dynamics of Network Motifs

Network motifs

None of the cellular processes happens in isolation. Take the example of gene expression. A handful of molecules may control the expression of the gene of our interest. In turn, this gene may also control the function of another set of molecules.

Therefore, we can imagine a molecular network where molecules are connected through some control or interactions. You must have seen such network diagrams in textbooks. We have networks for metabolism, cell signaling, and also gene regulatory networks.

However, diagrams provide qualitative information only. Suppose we want to study the dynamical properties of a large network. That would be a daunting task, both experimentally and mathematically.

Even using the latest high-throughput techniques, capturing thousands of molecules' temporal behavior in a signaling or transcriptional network is challenging. Further, we have to accept that we may be ignorant of many molecules involved in the processes, and our experimental design may fail to capture the dynamics of those molecules.

Now imagine you are trying to build an ODE-based model for a network with thousands of molecules. Even if we create the model, we will not have the numerical values for most of the model parameters. Further, considering model complexity, it would be tough to analyze it, even numerically.

Would it not be better if we break the whole network into small parts and study those separately? That is how they analyze electronic circuits. A large and complicated electronic circuit comprises standard components like resistor, capacitor, diode, etc. Each of these components has specific functions.

Suppose you zoom in to a large circuit. In that case, you will find certain smaller circuits that are used repeatedly, like an amplifier, filter, integrator. Each of these small circuits has specific input-output behavior. We can experimentally check the input-output behavior of each of these circuits. We can mathematically model the same. We can design them with specific input-output behavior. The arrangement of these smaller circuits decides the overall behavior of the complete circuit. .

A large molecular network can also be broken down into such standard sub-networks called network motifs. Network motifs were first described for gene regulatory networks. Large gene regulatory networks contain a small set of recurring regulation patterns or motif. For example, in *E. coli*, a common motif is negative autoregulation (NAR). In a NAR, a gene inhibits its expression. NAR has a peculiar dynamical behavior – it reduces response time and reduces cell-cell variability in gene expression.

Take another common network motif – input signal S activates A, A activates B, B activates C, and C inhibits A. That is a negative feedback motif. Negative feedback can generate a transient response, oscillation and can reduce cell-to-cell variability.

Note that a network motif is not defined by molecular details, like the number and identity of molecules of a particular motif. Instead, a motif is defined by its architecture/topology and flow of information/regulation through it. Two negative feedback motifs with the same architecture, and similar dynamics can have completely different molecular components.

A few common network motifs are shown in Figure 1. You can find these motifs in signaling networks, transcriptional networks, and metabolic networks. Not just that, many man-made networks also have some of these motifs.

We describe these motifs in terms of one, two, or three molecules. However, in reality, a particular motif could be made of more than three molecules. Even then, we club multiple molecules together to create a reduced model. For example, Suppose, A activates B, B activates C, and C activates D. This is a linear path. D will activate only when A triggers the process. The intermediates, B and C, merely add some time delay in the process.

In our model, we will remove B and C and connect D directly to A. This is a reduced model, but it still retains the logic of control of D by A. However, we have to choose the correct functions and parameters so that the observed temporal dynamics of D is captured adequately.

In this chapter, we will study some common network motifs of cell signaling and gene regulation. Our goal is to understand the generalized dynamical properties and input-output behavior of these motifs.

We will use ODE-based models. However, we must be cautious. The dynamical properties of a system depend upon model parameters. Two motifs of the same group may behave differently due to differences in parameter values. For example, an incoherent feedforward motif may act as an adaptive motif, but only for certain parameter values.

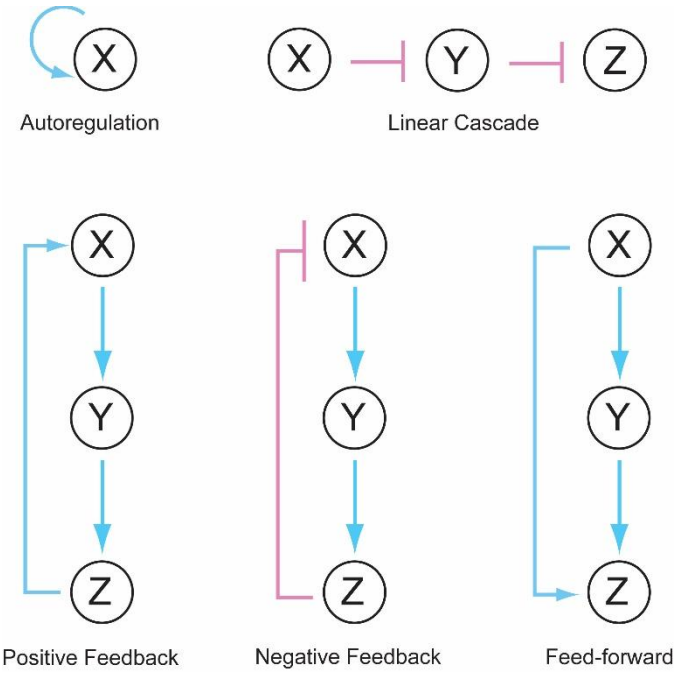
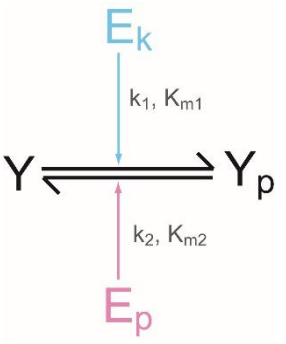


Figure 1: Some common network motifs. One representative of each of the groups is shown here. Some of these groups can have subgroups. For example, the feed-forward motif could be of two types– coherent and incoherent. Further, these motifs have variants with different topologies/architectures but with the same control logic. For example, take a motif where X activates Y, Y inhibits Z and Z activates X. This is negative feedback – activation of X eventually sends an inhibitory signal back to X.

An ultrasensitive switch

Suppose E_k is a kinase that phosphorylates Y to Y_p. E_p is a phosphatase that dephosphorylates Y_p. Both the reactions follow Michaelis-Menten kinetics.



This reversible process is not a network motif in itself but appears in many motifs. We model this using the following ODE,

$$\frac{dY_p}{dt} = \frac{k_1 E_k Y}{K_{m1} + Y} - \frac{k_2 E_p Y_p}{K_{m2} + Y_p} \tag{1}$$

Assuming that the total amount of Y remains constant ($Y_T = Y + Y_p$), we re-write Equation 1,

$$\frac{dY_p}{dt} = \frac{k_1 E_k \left(Y_T - Y_p\right)}{K_{m1} + \left(Y_T - Y_p\right)} - \frac{k_2 E_p Y_p}{K_{m2} + Y_p} \tag{2}$$

At steady state,

$$\frac{dY_p}{dt} = 0$$

$$\therefore \quad \frac{k_1 E_k \left(Y_T - Y_p\right)}{K_{m1} + \left(Y_T - Y_p\right)} - \frac{k_2 E_p Y_p}{K_{m2} + Y_p} = 0$$

Rearranging terms, we get

$$\frac{k_1 E_k \left(1 - \frac{Y_p}{Y_T}\right)}{\frac{K_{m1}}{Y_T} + \left(1 - \frac{Y_p}{Y_T}\right)} = \frac{k_2 E_p \frac{Y_p}{Y_T}}{\frac{K_{m2}}{Y_T} + \frac{Y_p}{Y_T}} \tag{3}$$

Let us define $y = \frac{Y_p}{Y_T}$, $v_1 = k_1 E_k$, $v_2 = k_2 E_p$, $J_1 = \frac{K_{m1}}{Y_T}$ and $J_2 = \frac{K_{m2}}{Y_T}$.

Now, we can rewrite Equation 3 as,

$$\frac{v_1(1-y)}{J_1+(1-y)} = \frac{v_2 y}{J_2+y} \tag{4}$$

Equation 4 gives a quadratic equation for *y*,

$$(v_2 - v_1)y^2 - (v_2 - v_1 + v_1 J_2 + v_2 J_1)y + v_1 j_2 = 0 \tag{5}$$

In general, we can represent *y* as a function of the rest of the terms in Equation 4: $y = G(v_1, v_2, \mathrm{J}_1, \mathrm{J}_2)$. This function is called the Goldbeter-Khosland Function.

Note that v_1 includes the concentration of the kinase, E_{k}, and *y* is a surrogate measure of Phosphorylated *Y*. Suppose we want to know how E_{k} affects the steady state of Y_{p}. The relation between *v* and *y* will answer that.

Rearrange Equation 4 and express v_1 in terms of *y*,

$$v_1 = \frac{v_2 y}{J_2 + y} \cdot \frac{J_1 + (1-y)}{(1-y)} \tag{6}$$

Figure 2a shows the behavior of this equation, when $v_2 = J_1 = J_2 = 1$. By our definition $y = \frac{Y_p}{Y_T}$. So *y* represents the fraction of Y that is phosphorylated, and *y* cannot be greater than 1. Therefore, we will discard the pink curve in Figure 2 from our analysis. The blue curve behaves like a rectangular hyperbola. *y* increases smoothly with v_1 and $y \to 1$ as v_1 gets very large.

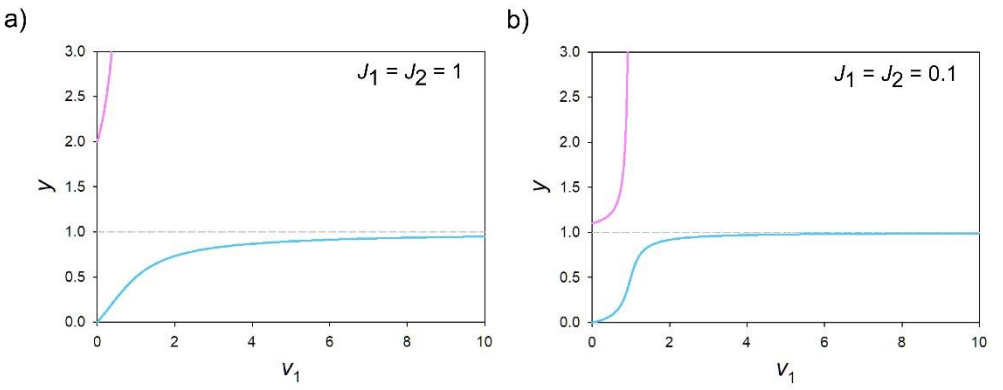


Figure 2: Behaviour of the Goldbeter-Khosland Function for different values of J_1 and J_2. In both the figures, v_2 = 1. Here, $y = \frac{Y_p}{Y_T}$, $v_1 = k_1 E_k$, $J_1 = \frac{K_{m1}}{Y_T}$ and $J_2 = \frac{K_{m2}}{Y_T}$.

However, when $J_1 < 1$ and $J_2 < 1$, this curve is sigmoidal (Figure 2b). Both J_1 and J_2 are the ratios of Michaelis-Menten constants to total Y. When total Y is much bigger than the Michaelis-Menten constants of the kinase and phosphatase, v_1 *vs.* y will have a sigmoidal behavior.

In fact, for $J_1 \ll 1$ and $J_2 \ll 1$, v_1 *vs.* y curve behaves like a sharp ON-OFF switch (Figure 3). Before the pink region (Figure 3), y remains very shallow and changes slowly with v_1. However, in the pink region, a small increase in v_1, causes a large increase in y. This behavior is called ultrasensitivity.

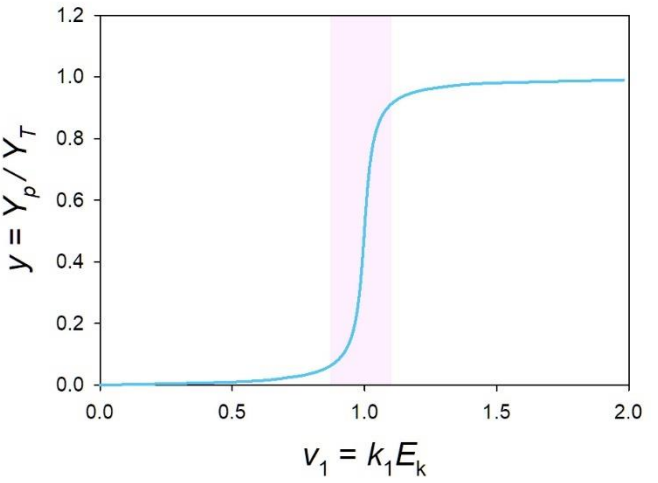


Figure 3: Ultrasensitive behavior of the kinase-phosphatase system. The Goldbeter-Khosland function is plotted for $v_2 = 1$ and $J_1 = J_2 = 0.01$. The pink region is the ultrasensitive region.

Imagine this reversible system as a molecular switch. E_k is the input for this switch. E_k phosphorylates Y to Y_p. So Y_p is the output of this switch. When the Michaelis-Menten constants are close to Y_T, this switch works like a rheostat – output increases smoothly with input.

However, when $K_{m1} \ll Y_T$ and $K_{m2} \ll Y_T$, this molecular switch works like an ultrasensitive ON-OFF switch. Up to a threshold value of the input, Yp remains very low. That is the OFF state. Beyond the threshold, Yp increases rapidly to the highest possible value and reaches the ON state.

Such ultrasensitive behavior is frequently observed in cell signaling. You treated cells with different doses of a signaling molecule (say EGF) and measured the extent of phosphorylation of a target molecule by Western Blot. You may observe that up to a certain dose the target protein remains largely unphosphorylated. However, for doses higher than a threshold, the target protein gets highly phosphorylated. This behavior can arise out of the molecular switch that we discussed here.

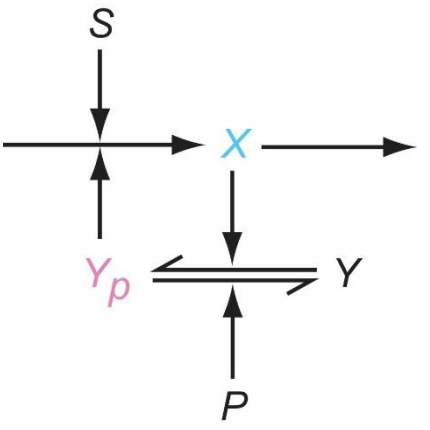
The threshold behavior observed in your Western Blot may disappear when you perform the same experiment in another cellular system. Suppose, in this case, you have observed a smooth increase in phosphorylation with an increase in the dose of the signaling molecule. Our molecular switch can also explain this observation. The expression of a protein varies from one cell type to another. Possibly, in this particular cell, the total

amount of the target protein is lesser and close to the Michaelis-Menten constants for the kinase and phosphatase. So, the switch is working like a rheostat in this particular cell type.

A positive feedback

The molecular system shown in question number 4 in the previous chapter's exercise is a system with positive feedback. Here I add a little modification to that architecture. X is a kinase. It phosphorylates Y to Yp. Yp is an active transcription factor, and it induces further production of X. This forms the feedback loop.

S is an external signal that also induces the expression of X. P is a phosphatase, and its concentration is fixed. Phosphorylation and dephosphorylation of Y follow Michaelis-Menten kinetics.



We use the following system of ODEs to model this positive feedback,

$$\frac{dX}{dt} = k_1 \frac{S^m}{K_s{}^m + S^m} + k_2 \frac{Y_p{}^n}{K_y{}^n + Y_p{}^n} - k_3 X \tag{7}$$

$$\frac{dY_p}{dt} = \frac{k_4 X (Y_T - Y_p)}{K_{m1} + (Y_T - Y_p)} - \frac{k_5 P Y_p}{K_{m2} + Y_p} \tag{8}$$

Here the total amount of Y is conserved, $Y_T = Y_p + Y$.

You must have noticed that the interconversion of Y and Y_p is the molecular switch that we discussed above. Therefore, the nullcline of Yp would be a Goldbeter-Khosland Function.

Nullcline plot for different values of S (S = 0, 0.25, and 0.5) is shown in Figure 4. We have considered Y_T = 1. The assumed Michaelis-Menten constantans (K_{m1} = K_{m2} = 0.05) are small with respect to Y_T. Therefore, Yp nullcline is sigmoidal.

Yp nullcline is independent of S. However, S affects the X nullcline. X nullcline is also sigmoidal, but it shifts to the right with an increase in S.

When S = 0, X and Yp nullclines intersect at only one point, and the system has only one steady state at (0, 0). This steady state is stable. When S is high, say S = 0.5, the system again has a stable steady but higher X and Yp values. However, at an intermediate value of S, S = 0.25, the nullclines intersect three times, giving rise to three steady states – two nodal sinks and one saddle.

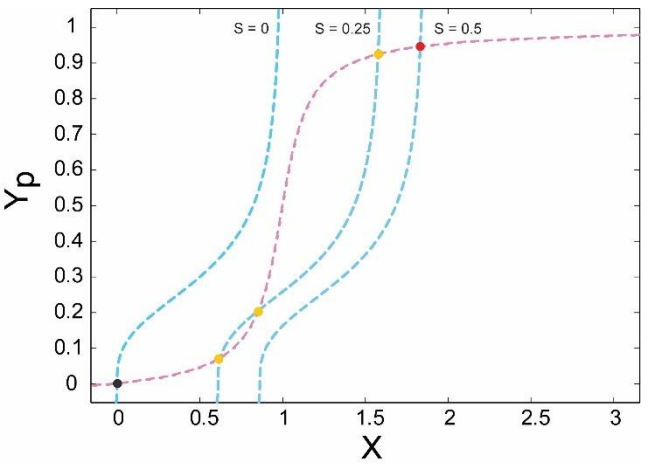


Figure 4: Effect of the input S on the nullclines of the positive feedback system. The pink dotted line is the Yp nullcline. The blue dotted lines are X nullclines for different values of S. Intersection of X and Yp nullclines are shown by coloured dots. Here, k_1 = k_2 = k_3 = k_4 = k_5 = P = Y_T = 1; K_s = 0.2; K_y = 0.3; K_{m1} = K_{m2} = 0.05; m = 2; n = 3.

The complete bifurcation diagram of this positive feedback is shown in Figure 5. This system has two saddle-node bifurcation points– SN1 and SN2. When S is low, the system is monostable, with a low steady state value of Yp. Similarly, beyond SN2, the system is monostable, with a very high steady state value for Yp. Between SN1 and SN2, this system is bistable. Therefore, we expect to observe hysteresis and bimodality in this system.

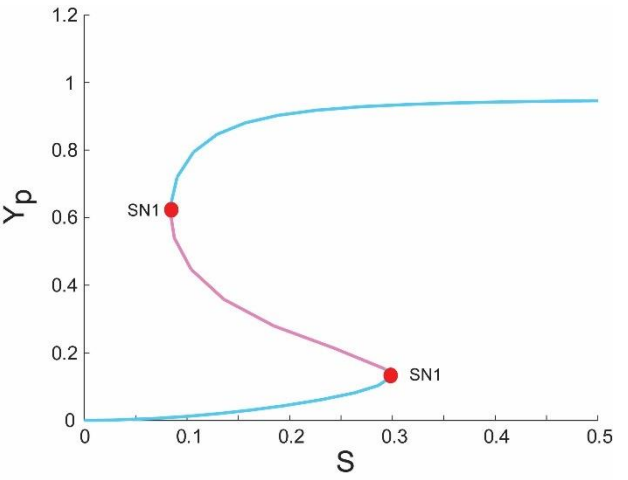


Figure 5: The bifurcation diagram for the positive feedback. Blue lines are for stable steady states, and the pink line is unstable. SN1 and SN2 are two bifurcation points.

Bistability is a characteristic property of positive feedback. For correct parameter values, various types of positive feedback motifs show bistability. The cell cycle model and the gene expression model in Chapter 5 have bistability. Both of those models have of mutual inhibition. Mutual inhibition or double-negative feedback is another form of positive feedback.

Take the cell cycle model in Chapter 5. APC degrades the cyclin. The cyclin degrades APC too. The negation of negation is an affirmation. When cyclin increases, it degrades more APC, which in turn reduces the degradation of the cyclin. That increases the level of cyclin. So this is, in a way, a positive feedback loop.

Double-positive feedback

X and Y are two transcription factors that induce each other's expression (Figure 6a). This is a motif of mutual activation or a double-positive feedback loop. Z is an inducer that can induce the expression of both X and Y.

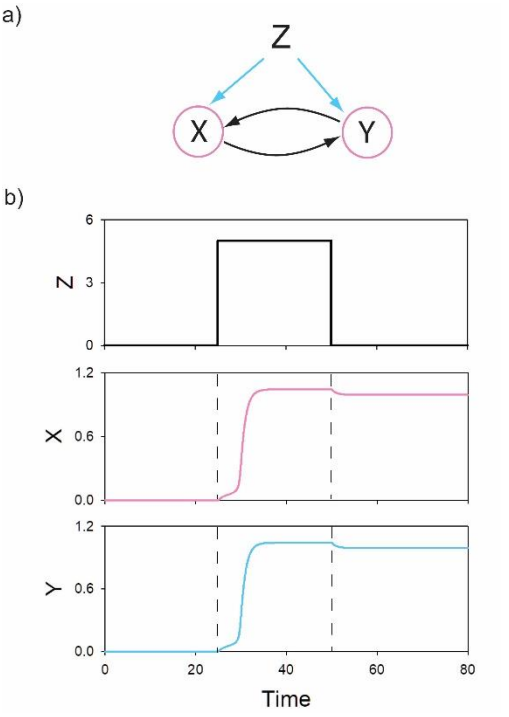


Figure 6: A double-positive feedback loop. a) Shows the architecture of the motif. b) Temporal dynamics of the system. Initially, $Z = X = Y = 0$. The input pulse of $Z = 5$ is given at $t = 25$. Z stays at 5 for $\Delta t = 25$ and then goes back to zero. Even after the decay of the input, both X and Y do not return to the basal level but stay at higher steady states.

We use the following system of ODEs to model the system,

$$\frac{dX}{dt} = k_1 Z + k_2 \frac{Y^n}{K^n + Y^n} - k_3 X \tag{9}$$

$$\frac{dY}{dt} = k_4 Z + k_5 \frac{X^n}{K^n + X^n} - k_6 Y \tag{10}$$

Consider Z as an input signal to this motif and X or Y as the output. Initially, there is no input, and both X and Y are at the basal level, $X = Y = 0$. Then at t = 25, cells receive a pulse of $Z = 5$ for a duration of $\Delta t = 25$ (Figure 6b).

With the pulse of Z, both X and Y reach steady states of high values. Interestingly, when Z returns to zero, X and Y do not return to their basal levels (Figure 6b). Instead, both X and Y settle for higher steady states and stays at those high values, even though there is no input signal. As if the switch has got stuck. Alternatively, you can imagine as if the system has remembered the input even though the input has died.

We explain this phenomenon using the bifurcation diagram of this motif (Figure 7). This system has a saddle-node bifurcation point near Z ≈ 3.5. For any value of Z, the system has a stable steady state with high X and Y. However, below the bifurcation point, there is another stable steady state close to $X = 0$, $Y = 0$. In this region, the stable steady states are separated by a saddle. When Z is increased beyond the bifurcation point, both X and Y move to the steady state close to X = Y = 1. When Z returns to zero, the system has two stable steady states. However, as it was near the higher steady state, the system moves to the higher steady state. Therefore, even when the input signal has disappeared, both X and Y stay near X = 1, Y = 1.

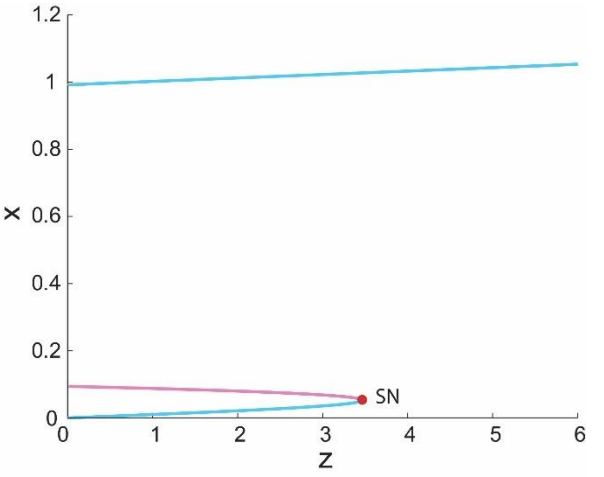


Figure 7: Bifurcation diagram of the double-positive motif. Both X and Y have identical bifurcation diagrams. SN is the bifurcation point. The blue lines are for stable steady states, and the pink one is for unstable steady states.

Feed-forward loop

In a feed-forward loop (FFL), an upstream molecule activates a downstream one through two parallel paths – one direct and another indirect. FFLs are of two types – coherent and

incoherent. In a coherent FFL, both the paths either activate or inhibit the downstream molecule. In that sense, the signs of control of both the paths are the same.

Take the example in Figure 8a. X activates Y. Y activates Z. So, the path X → Y → Z is an activating path. The direct path from X to Z also activates Z. Therefore, this loop is a coherent feed-forward.

However, the loop in Figure 8b is an incoherent FF (IFF). In this motif, X activates Y. Y activates Z. So, the path X → Y → Z is an activating path. However, the direct path from X to Z inhibits Z. Therefore, the sign of control of these two paths are opposite or incoherent.

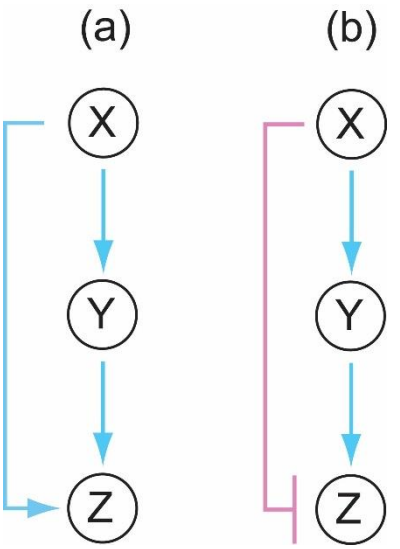


Figure 8: Feed-forward loops. (a) Coherent and (b) incoherent feed-forward loops.

These two motifs are abundant in biological networks and can have several architectures. Incoherent FFLs have two interesting properties. They can generate a pulsed or transient output for a persistent signal, and they can work as adaptive motifs. We discuss these two properties in this section—first, the generation of a short output pulse.

A small transcriptional network involving three genes are shown in Figure 9. The input signal S induces X. X induces Y's expression. Both X and Y are transcription factors but having opposing behavior. X induces the expression of Z and Y inhibits the expression. Therefore, this network is an IFF.

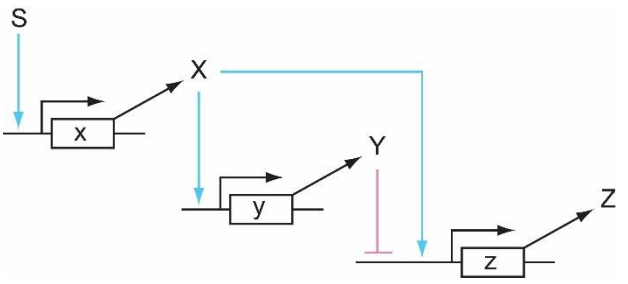


Figure 9: A transcriptional circuit with incoherent feed-forward. S is an input signal. Blue arrows indicate induction of expression, and pink hammer-head represents inhibition of gene expression.

When two transcription factors work simultaneously, there can be two types of control – AND-type and OR-type. These are equivalent to the logic gates of electronic circuits. In AND-type control, both the transcription factors are required for control of expression of the gene. Suppose two transcription factors A and B, activate the expression of a gene. However, the expression happens only when both A and B are bound to the promoter. This is AND-type control. In OR-type, as the name suggests, either of the transcription factors can work independently. So, expression of the gene will happen, even when either A or B is engaged with the promoter.

In the IFF of Figure 9, the control of expression of Z is of AND-type. Here, X activates, and Y inhibits the expression. Z is expressed only when X is present, and Y is absent or low.

We use the following ODEs to model the IFF.

$$\frac{dX}{dt} = k_1 \frac{S^n}{H_1^{\,n} + S^n} - k_2 X \tag{11}$$

$$\frac{dY}{dt} = k_3 \frac{X^m}{H_2^{\,m} + X^m} - k_4 Y \tag{12}$$

$$\frac{dZ}{dt} = k_5 \frac{X^q}{H_3^{\,q} + X^q} \cdot \frac{H_4^{\,r}}{H_4^{\,r} + y^r} - k_6 Z \tag{13}$$

The AND logic is reflected in Equation 13. The expression of Z is regulated through a composite function,

$$H(X,Y) = \frac{X^q}{H_3^q + X^q} \cdot \frac{H_4^r}{H_4^r + y^r}$$

This composite function is a product of a Hill function and an inverse Hill function. The behavior of $H(X, Y)$ is shown in Figure 10. The maximum expression of Z will happen when X is very high and Y is zero (Figure 10). The expression drops for high Y and low X.

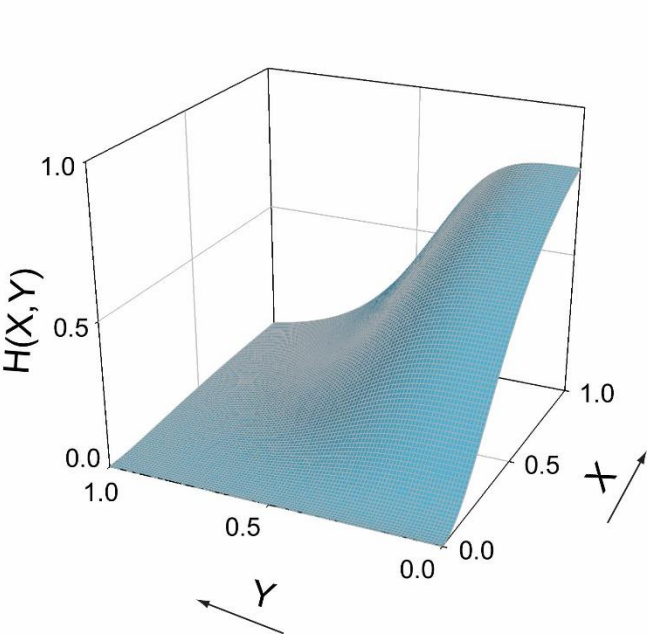


Figure 10: Behavior of the composite function used for modeling the AND-logic in the control of expression of Z by inducer X and inhibitor Y.

We simulated this model numerically. The JSim code for the same is given below. Figure 11 shows the result of our simulation. Initially, $X = Y = Z = 0$ and there is no input signal. At t = 10, an input signal S = 1 is given. S induces expression of X, and X rises rapidly.

As X induces expression of Y, Y also rises but slowly with a time delay. Z's expression is controlled by both X and Y. Initially, Y was very low, but X is high. So, the net effect is the expression of Z. So Z rises with time. However, after some time, Y reaches a considerable level, and its inhibitory effect starts showing. The rate of expression of Z drops. Eventually, both X and Y reach 1, and the composite function H(X, Y) becomes very small. The rate of expression of Z gets very low, and the system reaches a steady state with low Z.

```
math pulse
{     realDomain t ;
      t.min=0;t.delta=0.1;t.max=100;
      real x(t), y(t), Z(t);
      extern real s(t);
      real k1 =1.25;
      real k2 = 1;
      real k3 = 2;
      real k4 = 1;
      real k5 = 1;
      real k6 = 1;
      real H1 = 0.5;
      real H2 = 1;
      real H3 = 0.5;
      real H4 = 0.5;
      real n = 2;
      real m = 2;
      real q = 2;
      real r = 4;
when (t=t.min){x=0;y=0;Z=0;}
x:t =  k1*((s^n)/(H1^n+s^n))-k2*x;
y:t =  k3*((x^m)/(H2^m+x^m))-k4*y;
Z:t =  k5*((x^q)/(H3^q+x^q))*(H4^r/(H4^r+y^r))- k6*Z;
}
```


The ability to generate a transient response for a sustained input allows an IFF to work as an adaptive motif. By adaptive we mean the ability of a system to respond transiently to a change in the input signal and return to the earlier steady state. In a way an adaptive system, maintains a steady state in the presence of a changing input signal.

The voltage stabilizer that we use for electronic devices is an adaptive system. It provides constant voltage current and saves the device from voltage fluctuations. A cell also lives in a fluctuating environment. An input signal can change all of a sudden. However, the cell needs to maintain some sort of steady state or homeostasis for most of its functions.

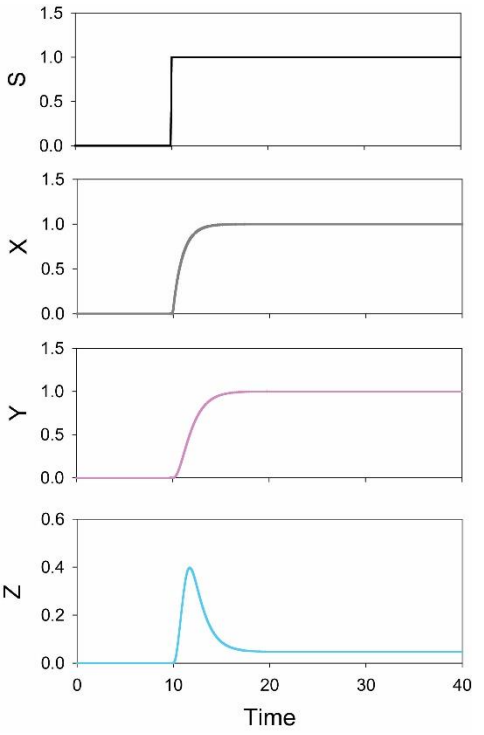


Figure 11: Transient response for sustained input. The results of numerical simulation of the IFF is shown here. Parameter values used for this simulation are in the JSim code of the model.

Adaptive network motifs provide stability in a fluctuating environment. Adaptive motifs are ubiquitous in cell signaling and transcriptional networks. Both negative feedback and an incoherent feed-forward loop can act as an adaptive motif. However, adaptive property depends on the architecture/topology and parameter values of negative feedback or IFF.

Here, we will analyze the adaptive behavior of an IFF. This motif comprises three proteins – X, Y, and Z. An input signal phosphorylates X to X_p. X_p is an active kinase. It phosphorylates Y and Z to Y_p and Z_p, respectively. Phosphorylated Y is an active phosphatase. It dephosphorylates Z_p.

P_1 and P_2 are two constitutive phosphatases that dephosphorylate X_p and Y_p.

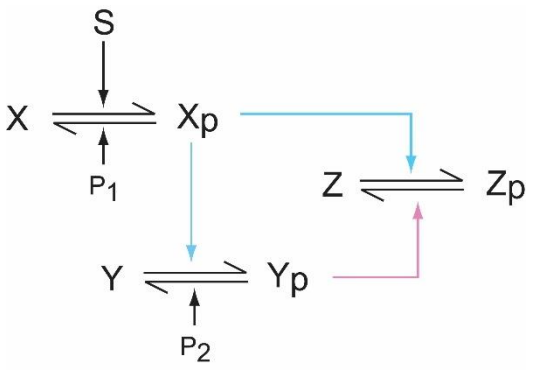


Figure 12: An incoherent feed-forward loop involving three proteins. Blue arrows indicate positive paths, and the red arrow represents the negative path.

We use the following system of ODEs to model this IFF. The total concentration of each protein (phosphorylated and unphosphorylated) is normalized to 1. So x_p, y_p, and z_p are fractional amounts of phosphorylated X, Y, and Z.

$$\frac{dx_p}{dt} = \frac{Sk_1(1-x_p)}{K_{m1}+(1-x_p)} - \frac{P_1k_2x_p}{K_{m2}+x_p} \tag{14}$$

$$\frac{dy_p}{dt} = \frac{x_pk_3(1-y_p)}{K_{m3}+(1-y_p)} - \frac{P_2k_4y_p}{K_{m4}+y_p} \tag{15}$$

$$\frac{dz_p}{dt} = \frac{x_pk_5(1-z_p)}{K_{m5}+(1-z_p)} - \frac{y_pk_6z_p}{K_{m6}+z_p} \tag{16}$$

We have considered that all the reactions follow Michaelis-Menten kinetics. K_{m1} to K_{m6} are the Michaelis-Menten constants.

Consider a special case – $K_{m3} \ll (1 - y_p)$, and $y_p \ll K_{m4}$. That means the kinase, X_p is working in the saturated region, and the phosphatase P_2 is working in the linear region.

With these two assumptions, we re-write Equation 15 as,

$$\frac{dy_p}{dt} = x_p k_3 - \frac{P_2 k_4 y_p}{K_{m4}} \tag{17}$$

Therefore, at steady state,

$$y_p = \frac{k_3 K_{m4}}{P_2 k_4} x_p = a x_p \tag{18}$$

Here, $a = \frac{k_3 K_{m4}}{P_2 k_4}$ is a constant.

Use this steady state value of y_p to calculate the steady state value of z_p. Following Equation 16, at steady state,

$$\frac{x_p k_5 (1 - z_p)}{K_{m5} + (1 - z_p)} - \frac{y_p k_6 z_p}{K_{m6} + z_p} = 0$$

Replace y_p by ax_p,

$$\frac{x_p k_5 (1 - z_p)}{K_{m5} + (1 - z_p)} - \frac{a x_p k_6 z_p}{K_{m6} + z_p} = 0$$

$$x_p \left(\frac{k_5 (1 - z_p)}{K_{m5} + (1 - z_p)} - \frac{a k_6 z_p}{K_{m6} + z_p} \right) = 0$$

If, $x_p \neq 0$,

$$\frac{k_5 (1 - z_p)}{K_{m5} + (1 - z_p)} - \frac{a k_6 z_p}{K_{m6} + z_p} = 0 \tag{19}$$

By rearranging the terms of Equation 19, we get a quadratic equation for z_p. Like the Goldbeter-Khoshland function, the solution of z_p is,

$$z_p = f\left(k_5, k_6, K_{m5}, K_{m6}, a\right) \tag{20}$$

Equation 20 gives the steady state value of z_p. Note that the function on the right-hand side is independent of S, x_p, and y_p. Therefore, the steady state of Z_p is independent of the

input signal S. This property makes this enzymatic circuit an adaptive motif. As the steady state of Z_p is independent of S, change in the input signal does not affect the steady state of Z_p.

However, the time evolution of Zp will be transiently affected by the change in the input signal. To understand the transient response and steady state behavior, we will take the help of the phase portrait and nullclines.

I have considered specific numerical values for phase plane analysis. Those parameter values are provided in the legend of Figure 13.

The phase portrait of the system when $S = 1$ is shown in Figure 13a. On this figure, I have overlaid the y_p and z_p nullclines for $S = 0.1$. These nullclines are shown by thin pink and blue lines. The green dot marks the intersection of these two nullclines. That is the steady state of the system when $S = 0.1$.

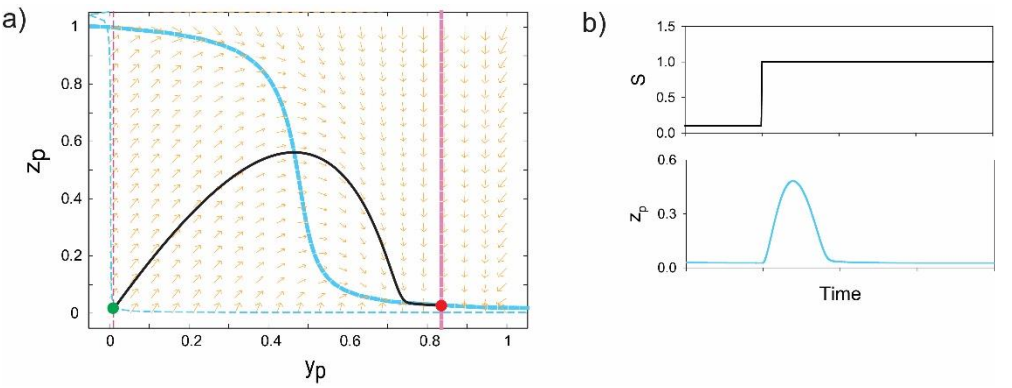


Figure 13: Phase portrait and temporal behaviour of the adaptive IFF. a) The phase portrait of the system for $S = 1$. The thick blue and pink lines are the z_p and y_p nullclines, respectively. The corresponding nullclines for $S = 0.1$ are shown by thin blue and pink lines. The red dot is the steady state for $S = 1$. The green dot is the steady state for $S = 0.1$. The black line is the trajectory of the system starting from the green dot for S = 1. b) numerical simulation for the system with change of S from 0.1 to 1. The parameter values are: $k_1 = k_2 = k_5 = k_6 = P_1 = P_2 = 1$, $k_3 = 0.5$, $k_4 = 30$, $K_{m1} = K_{m2} = 0.1$, $K_{m3} = 0.001$, $K_{m4} = 100$, $K_{m5} = 0.05$, $K_{m6} = 0.02$.

When S = 1, both the nullclines shift right (the thick pink and blue lines). The new steady state is the red dot. Notice that though the steady state value of y_p has increased, the steady state of z_p remains the same. So the change in S is not affecting the steady state of z_p.

The arrows in the phase portrait give the direction of the time evolution of z_p and y_p when $S = 1$. When S = 0.1, the system was at the steady state marked by the green dot. When S changes to 1, the system follows the black trajectory and reaches the red dot, a sink node. Notice the shape of this trajectory. Initially, z_p increases, and then it drops and reaches the steady state.

This transient rise in z_p and subsequent adaptation to the basal steady state is shown in Figure 13b.

Negative autoregulation

Negative autoregulation or NAR is a widespread motif in the transcriptional networks of different organisms. In a NAR, the gene itself inhibits its expression (Figure 14). We will use a simplified model to understand NAR dynamics and compare it with the constitutive expression of a protein.

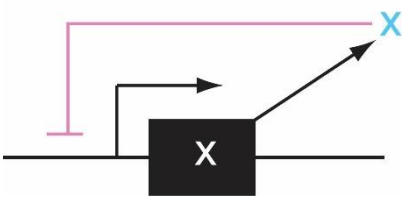


Figure 14: Schematic representation of negative autoregulation. The pink hammer-head represents inhibition of expression.

Let us start with the constitutive expression of a protein. We consider gene expression as a single step process happening at a constant rate β_c. The protein degrades by a first-order process. So, our model is,

$$\frac{dx}{dt} = \beta_c - \alpha x \tag{21}$$

Get the solution of this ODE by integration. Considering the initial condition, $x = 0$ at $t = 0$,

$$\int_0^x \frac{dx}{\beta_c - \alpha x} = \int_0^t dt$$

$$\Rightarrow x = \frac{\beta_c}{\alpha}\left(1 - e^{-\alpha t}\right) \tag{22}$$

Following Equation 22, x will increase with time and as $t \to \infty$, x reaches the steady state $x = \frac{\beta_c}{\alpha}$.

Let us define this system's response time as the time required to reach half of the steady state value. Following Equation 22,

$$x = \frac{1}{2}\left(\frac{\beta_c}{\alpha}\right) = \frac{\beta_c}{\alpha}\left(1 - e^{-\alpha \tau_c}\right)$$

So, the response time for this constitutive system,

$$\tau_c = \frac{\ln(2)}{\alpha} \tag{23}$$

We will now check the behavior of a NAR. We assume that the inhibition by the protein is nonlinear and can be modeled using an inverse Hill function. To keep the model simple, we will use an inverse Hill function with Hill coefficient n = 1.

$$\frac{dx}{dt} = \beta \frac{K}{K + x} - \alpha x \tag{24}$$

Assume that the protein has a very high affinity for the promoter. That means K is very small, and we can neglect it in the inverse Hill function's denominator. Re-writing Equation 24, with this assumption, we get,

$$\frac{dx}{dt} = \beta \frac{K}{x} - \alpha x \tag{25}$$

The solution of this ODE for the initial condition, x = 0 at t = 0, is,

$$x = \sqrt{\tfrac{\beta K}{\alpha}(1 - e^{-2\alpha t})} \tag{26}$$

Here, also x will increase with time and as $t \to \infty$, x reaches the steady state $x = \sqrt{\frac{\beta K}{\alpha}}$.

Using Equation 26, we calculate the response time τ for this NAR,

$$x = \frac{1}{2}\sqrt{\frac{\beta K}{\alpha}} = \sqrt{\frac{\beta K}{\alpha}(1 - e^{-2\alpha\tau})}$$

$$\Rightarrow \quad \tau = \frac{\ln\left(\frac{4}{3}\right)}{2\alpha} \tag{27}$$

Comparing Equation 23 with Equation 27, we get that the response time for the NAR is shorter than the response time of the constitutive system,

$$\tau = 0.21\tau_c$$

The difference in the response times for these two systems is shown in Figure 15. Though quite unintuitive, this behavior has been observed in experiments.

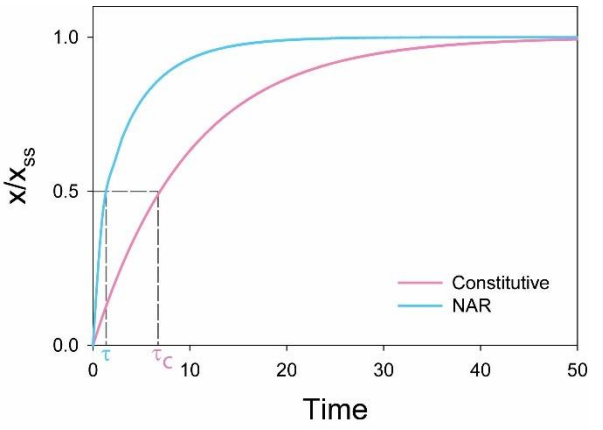


Figure 15: Temporal behaviors of a NAR and a constitutive system. x_{ss} is the steady state value of x. τ and τ_c are the response times for the NAR and constitutive system, respectively.

You may wonder why NAR is so ubiquitous in transcriptional networks. It seems futile to have negative autoregulation. In fact, the steady state level of a protein under the control of a strong constitutive promoter will be higher than that of a NAR.

However, a high steady state may not be useful for a cell, as it has an energetic cost. Instead, a cell needs a system that switches ON very fast. A NAR fits that criteria. With a balance between the strength of the negative feedback and protein stability, a NAR can achieve a fast response time with an adequate, steady state level of the protein.

Negative Feedback

In a way, a NAR is a negative feedback motif, but with only one molecule. In negative feedback, the inhibition is executed through intermediates. For example, consider this motif – X activates Y, Y activates Z, and Z inhibits X. This is a negative feedback (NF).

Unlike positive feedback that gives bistability, negative feedback makes the system monostable. Therefore, like an IFF, negative feedback can generate a transient response to a sustained input and act as an adaptive motif. Positive feedback amplifies the stochastic noise in gene expression and leads to bimodal or multimodal gene expression. Being monostable, negative feedback in a transcriptional circuit reduces cell-to-cell variability in gene expression.

However, negative feedback has unique characteristics that differentiate it from other motifs discussed here. A negative feedback loop can generate oscillation.

Oscillation is observed at every level of biology. Signaling and gene expression show oscillation. For example, cyclins have oscillation in their expression that controls the cell cycle. At an organism level, we have circadian rhythm and oscillation in the levels of certain hormones. All these processes involve some sort of negative feedback.

NAR is a reduced negative feedback. Can we expect oscillation in a NAR? Consider the NAR in Figure 14. Suppose, initially, X was very low; so is its inhibitory effect. Therefore, initially, the rate of production of X will be high. However, after some time, a considerable amount of X will accumulate and exert the inhibitory effect. The rate of production of X will drop, and the level of X will fall. Once X has dropped below a threshold, its inhibitory effect will disappear, and the production rate will increase. So there should be an oscillation.

However, the fact is that a NAR can not generate oscillation. You can understand that from the ODE that we have used to model the NAR. Equation 24 is a one-dimensional autonomous system. Therefore, dx/dt is independent of time, and the slopes of the arrows in its direction field do not change with time. So, this system can not show oscillation.

To make the system oscillatory, we have to introduce a time delay. You can understand that from another perspective. A NAR or an NF involves three competing processes – production, inhibition, and degradation. When these three processes balance each other, the system reaches a steady state and can not have oscillation. However, sufficient delay in the inhibition can disturb this steady state and trigger oscillation.

Time-delay can be introduced through different means. For example, there may be a time delay in transporting molecules, like mRNA movement from the nucleus to the cytoplasm. A long chain of intermediates will also introduce a time delay.

We will follow this second approach and add two intermediates in the NAR. That will create a negative feedback loop (Figure 16). After translation, X is modified to Y. Y is further modified through a post-translational modification to Z. Z is an active transcription factor. It inhibits the expression of X. This model is similar to the well known Goodwin model.

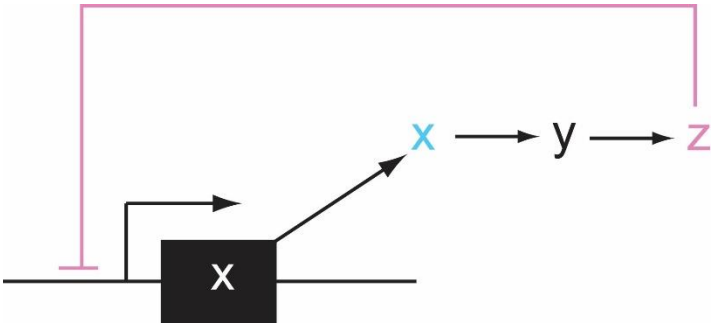


Figure 16: A negative feedback loop. The pink hammer-head represents transcriptional inhibition. This motif is a modification of NAR with two additional molecules, Y and Z. X does not inhibit itself directly but inhibits indirectly through Y and Z.

Accordingly, we modify Equation 24,

$$\frac{dx}{dt} = \beta \frac{K^n}{K^n + z^n} - \alpha x \tag{28}$$

$$\frac{dy}{dt} = k_1 x - k_2 y - k_3 y \tag{29}$$

$$\frac{dz}{dt} = k_2 y - k_3 z \tag{30}$$

Here, k_1 and k_2 are the rate constants for post-translational modifications, and k_3 is the rate constant for degradation.

We have analyzed this system numerically. Figure 17 shows the system dynamics when the Hill coefficient in Equation 28 is low (n = 3). When n is low, the system shows damped oscillation.

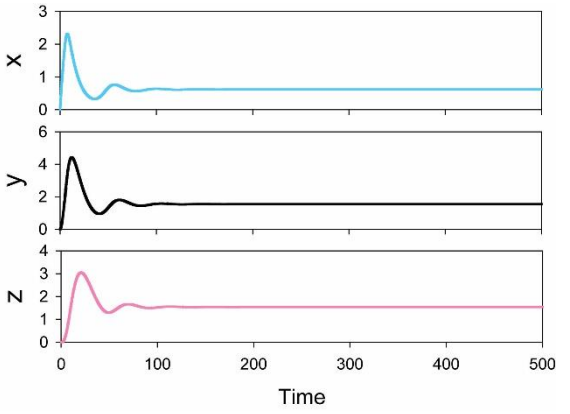


Figure 17: Dynamics of the negative feedback for Hill coefficient, n = 3. Other parameters used for this simulation are: β = 0.5, K =0.8, α = 0.1, k_1 = 0.5; k_2 = 0.1; k_3 = 0.1.

However, the same system with a high Hill coefficient (n = 20) shows oscillation (Figure 18a). High Hill coefficient means a sharp switch-like behavior of the inverse hill function. That means to achieve oscillation the inhibition should work like an ON-OFF switch. Below a threshold the inhibition should be none or very week, and beyond the threshold, it should strongly inhibit the production. A slow but consistently rising inhibitory effect would counterbalance X's production and lead to a steady state.

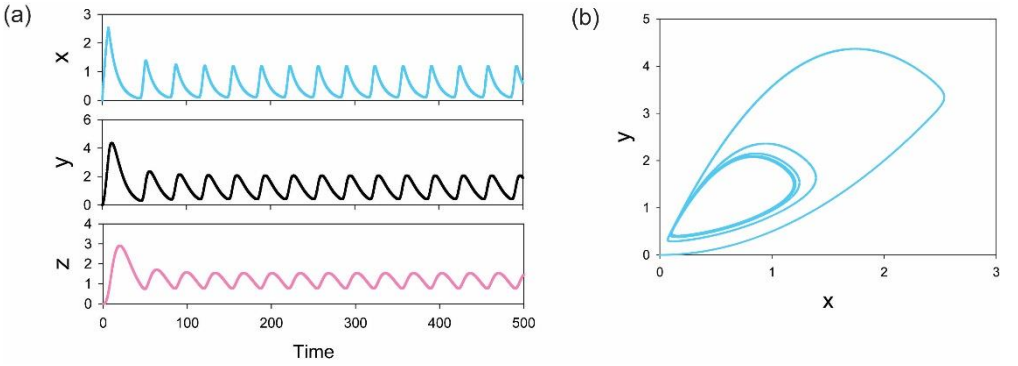


Figure 18: Dynamics of the negative feedback for high Hill coefficient, n = 20. a) shows the temporal behaviour of three molecules and b) is the corresponding trajectory in the x-y phase plane. Other parameters used for this simulation are: β =0.5, K =0.8, α = 0.1, k_1 = 0.5; k_2 = 0.1; k_3 = 0.1.

You must have noticed the pattern of oscillation in Figure 18a. Initially, the amplitude was high and varying. However, after some time, the oscillations settle for periodic oscillations with fixed amplitudes. This behavior is evident in Figure 18b, where a trajectory is shown on the phase plane. Starting from the initial position (0, 0), the trajectory spirals in and then settles on a closed orbit.

This type of oscillation is called limit cycle oscillation. A limit cycle is a closed trajectory in phase space such that at least one other trajectory spirals into it as the time approaches positive or negative infinity. When all neighboring trajectories approach the limit cycle with time, it is called a stable or attractive limit cycle. Just like a stable steady state, a stable limit cycle is stable to perturbation. Limit cycles are primarily found in two-dimensional dynamical systems but are also observed in some higher-dimensional systems.

We have achieved to generate oscillation in our negative feedback motif. However, you must be wondering whether such a high Hill coefficient is realistic. Hill coefficient represents the extent of co-operativity, and in most real transcriptional systems, Hill coefficient varies from 1 to 4. However, a sharp switch-like be behavior of the inhibitor can be achieved in ultrasensitive enzymatic systems.

We will now explore another negative feedback where the oscillation is observed even when the Hill coefficient has a reasonable value. This network motif involves three molecules. These molecules work sequentially – X inhibits Y, Y inhibits Z, and Z inhibits X. This motif is called a repressilator.

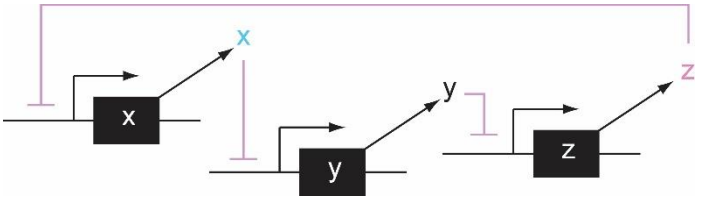


Figure 19: A repressilator. The pink hammer-heads represent transcriptional inhibition.

Real repressilators are created by genetic engineering, and those repressilators show oscillation. Here, we will model this system using a system of ODEs and investigate the oscillatory behavior. For simplicity, we will consider symmetric equations for these molecules. We will consider transcription and translation as separate processes. That increases the number of dependent variables but makes the model more realistic. Also,

remember that increase in intermediates gives rise to the time delay that is essential for oscillation.

We use the following system of ODEs,

$$\frac{dm_x}{dt} = k_0 + k_1 \frac{H^n}{H^n + z^n} - k_2 m_x \tag{31}$$

$$\frac{dm_y}{dt} = k_0 + k_1 \frac{H^n}{H^n + x^n} - k_2 m_y \tag{32}$$

$$\frac{dm_z}{dt} = k_0 + k_1 \frac{H^n}{H^n + y^n} - k_2 m_z \tag{33}$$

$$\frac{dx}{dt} = k_3 m_x - k_4 x \tag{34}$$

$$\frac{dy}{dt} = k_3 m_y - k_4 y \tag{35}$$

$$\frac{dz}{dt} = k_3 m_z - k_4 z \tag{36}$$

Here, m_x, m_y, and m_z are the mRNAs for proteins x, y, and z, respectively. k_o is the basal rate of transcription. We use the inverse Hill function for inhibition and use a reasonable value of the Hill coefficient.

We analyze this model numerically. The result of numerical solution of the system for the initial condition, $m_x = m_y = m_z = x = y = z = 0$ at $t = 0$ is shown in Figure 20. For this initial condition, the system does not show oscillation but reaches a stable steady state. As we have considered a symmetrical system with an identical initial condition for all molecules, the opposing processes cancel each other. This symmetry can be broken if we start with one molecule having a different amount.

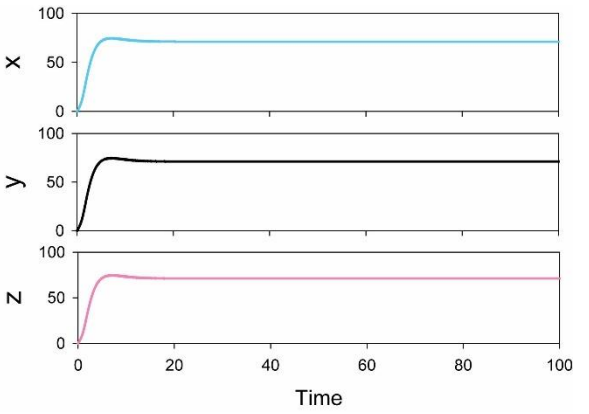


Figure 20: Temporal dynamics of the repressilator when at $t = 0$, $m_x = m_y = m_z = x = y = z = 0$. Parameters used for this simulation are: $k_0 = 0.03$, $k_1 = 30$, $H = 40$, $n = 3$, $k_2 = 0.8$, $k_3 = 1$, $k_4 = 0.08$.

Figure 21a shows the result of simulation for the initial condition, $m_x = m_y = m_z = y = z = 0$, and $x = 1$ at $t = 0$. A little increase in x, disturb the balance in opposing processes and triggers oscillation. Here also the system has limit cycle oscillation (Figure 21b).

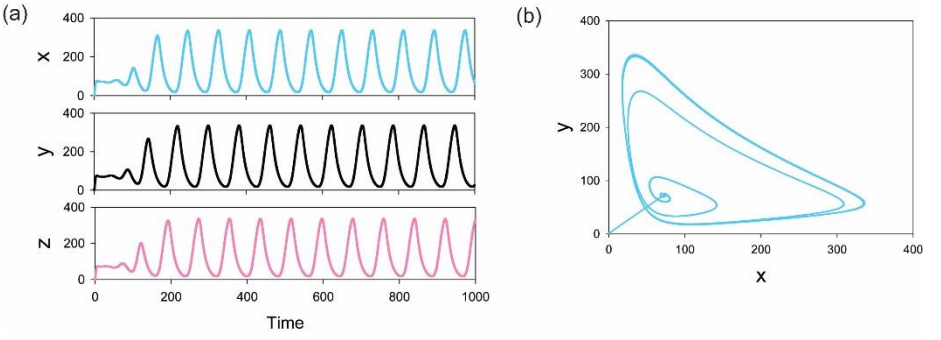


Figure 21: Temporal dynamics of the repressilator when at $t = 0$, $m_x = m_y = m_z = y = z = 0$ and $x = 1$. a) shows the temporal behaviour of three molecules and b) is the corresponding trajectory in the x-y phase plane. Parameters used for this simulation are: $k_0 = 0.03$, $k_1 = 30$, $H = 40$, $n = 3$, $k_2 = 0.8$, $k_3 = 1$, $k_4 = 0.08$.

The existence of a limit cycle oscillation depends upon several parameters of this system. Obviously, the Hill coefficient affects the dynamics of the system. The oscillation is lost when the Hill coefficient is low (Figure 22a). Therefore, cooperativity in transcriptional inhibition is required to achieve stable oscillation.

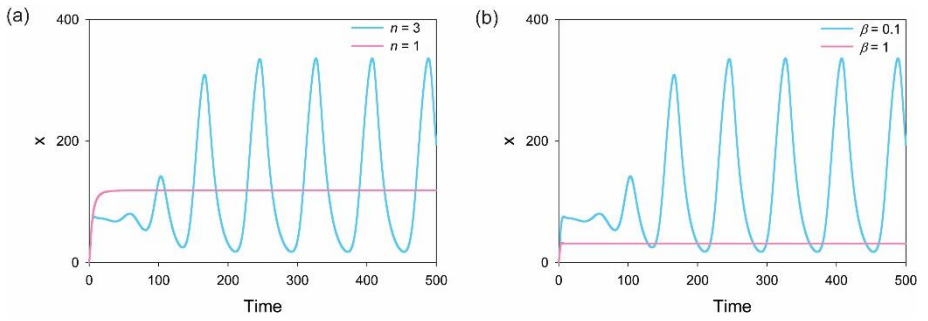


Figure 22: Effect of Hill coefficient and relative stability of mRNA to protein on the dynamics of the repressilator. a) shows the dynamics of x for n = 3 (blue) and n =1 (pink). b) shows the dynamics of x for β = 0.1 (blue) and β = 1 (pink).

The relative stability of mRNA to protein is also an important parameter. Let's define $\beta = k_4/k_2$. So, β is the ratio of the average lifetime of mRNA to that of the protein. In our simulation (Figure 21), β = 0.1. So the proteins are more stable than the mRNAs. Now we make them equally stable. Consider $\beta = 0.8/0.8 = 1$. As shown in Figure 22b, a decrease in protein stability leads the system to a stable steady state.

You must have realized that this system has bifurcation. Value of n or β affect the phase portrait of the system. The system can have either a stable steady state or a stable limit cycle oscillation depending upon the values of these parameters. You may try to explore this phenomenon further, but we will leave this here.

Exercise

1. We have a motif with two molecules – X and Y. Both are activated by input S. Y inactivates X. We model this motif using the following system of ODEs,

$$\frac{dx^*}{dt} = k_1 S\left(1 - x^*\right) - k_2 x^* y$$

$$\frac{dy^*}{dt} = k_3 S \frac{\left(1 - y^*\right)}{K_m + \left(1 - y^*\right)} - k_4 y^*$$

Here, x^* and y^* are the active X and Y. Find the condition for which x^* will show an adaptive behaviour.

2. Simulate the system of question 1 using JSim and show the adaptive behavior diagrammatically. Set S = 0. Let the system reach a steady state. Then after sometime, increase S to 1 using a step function. Let the system reach a steady state and then increase S to 2 using a step function. Plot the temporal behavior of S, x^* and y^*. You can use the inbuilt function generator of JSim.

3. Numerically nvestigate the effect the leaky expression of the oscillatory behavior of the repressilator discussed in this chapter.

4. Create a negative feedback involving two molecule that can show stable oscilation. Your model must be two-dimensional. Can a pure negative feedback with two molecule generate stable oscillation? Or we need to add other features like positive feedback or autocatalysis?

5. Dynamics of a small biochemical circuit is modelled using the following system of ODEs.

$$\frac{dx}{dt} = 1 - x\frac{y^2}{1+y^2}; \quad \frac{dy}{dt} = (1.02 - y)\frac{x^2}{1+x^2} - y$$

What type of network motif is this? The steady state of the of the system is x = 5, y = 0.5. What sort of dynamics do you expect for this system.

6. A negative feedback motif is shown here. Create a mathematical model similar to the one that we used for the IFF in Figure 12. Find the condition under which Z_p will show an adaptive behavior.