We explore the dynamics of network motifs, illustrated in Fig. 1 and provided as ordinary differential equations (ODEs) in the matching questions. For an on-step, the input signal $S(t) = 0$ for $t < 0$ and $S(t) > 0$ for $t \geq 0$. For an off-step, $S(t) > 0$ for $t < 0$ and $S(t) = 0$ for $t \geq 0$. At time 0, the system has steady-state values consistent with the input for $t < 0$.

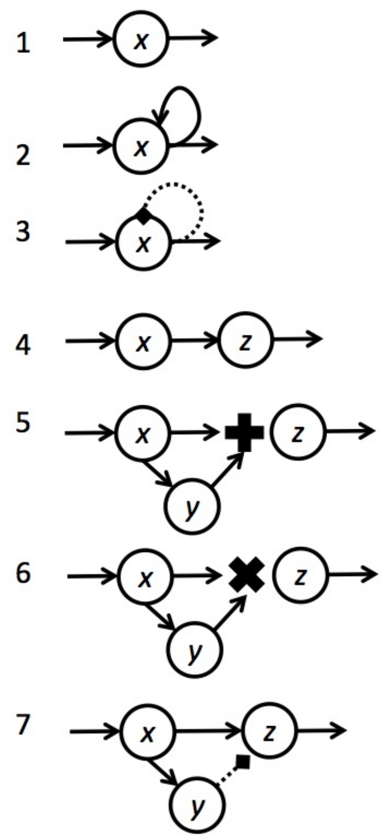


Figure 1: Network motifs illustrate ODEs in the problems below. Solid lines with arrows indicate positive regulation; dotted lines with blocks indicate negative regulation.

1. The standard two-state switch has ODE

$$\dot{x}(t) = \beta\Theta[S(t) > 0] - \alpha x(t).$$

For an on-step, provide the response $x(t)$ and the time $t_{1/2}$ when the response is half complete.
For $t > 0$, $\Theta[S(t) > 0] = 1$ because of the step input. We can find

$$x(t) = \frac{\beta}{\alpha}\left[1 - e^{-\alpha t}\right]$$

At steady state,

$$0 = \beta - \alpha x(t)$$
$$x(t) = x_{ss} = \frac{\beta}{\alpha}$$

And then calculate

$$x(t_{1/2}) = \frac{\beta}{2\alpha}$$
$$\frac{\beta}{\alpha}\left[1 - e^{-\alpha t_{1/2}}\right] = \frac{\beta}{2\alpha}$$
$$1 - e^{-\alpha t_{1/2}} = \frac{1}{2}$$
$$e^{-\alpha t_{1/2}} = \frac{1}{2}$$
$$t_{1/2} = -\frac{1}{\alpha}\ln\frac{1}{2}$$
$$= \frac{1}{\alpha}\ln 2$$
$$\approx \frac{0.69}{\alpha}$$

2. With weak positive feedback, the ODE is

$$\dot{x}(t) = \beta\Theta[S(t) > 0] + \beta_f x(t) - \alpha x(t).$$

(a) Consider the on-step. Provide the response $x(t)$ and the time $t_{1/2}$ when the response is half complete. Does positive feedback increase or decrease the response time? Does positive feedback increase or decrease the final steady-state value $\lim_{t\to\infty} x(t)$?

For $t > 0$,

$$\begin{aligned}
\dot{x}(t) =& \beta - (\alpha - \beta_f)x(t) \\
x(t) =& \frac{\beta}{\alpha - \beta_f}\left[1 - e^{-(\alpha-\beta_f)t}\right] \\
x_{ss} =& \frac{\beta}{\alpha - \beta_f} \\
x(t_{1/2}) =& \frac{\beta}{2(\alpha - \beta_f)} \\
\frac{\beta}{\alpha - \beta_f}\left[1 - e^{-(\alpha-\beta_f)t_{1/2}}\right] =& \frac{\beta}{2(\alpha - \beta_f)} \\
t_{1/2} =& \frac{\ln 2}{\alpha - \beta_f}
\end{aligned}$$

(b) At what value of the positive feedback parameter $\beta_f$ does $t_{1/2}$ become non-physical (for example, infinite or negative)?

$$\beta_f > \alpha$$

(c) With strong positive feedback, a better ODE is

$$\dot{x}(t) = \beta\Theta[S(t) > 0] + \beta_f\Theta[x(t) > K] - \alpha x(t).$$

Consider an input signal with duration $\tau$: $S(t) = 0$ for $t < 0$, $S(t) > 0$ for $0 \leq t < \tau$, and $S(t) = 0$ for $t > \tau$. What are the smallest values of $\beta$, $\beta_f$, and $\tau$ that permit the system to support its own production as $t \to \infty$?

In order for $x$ to support its own production, we first require that $x(\tau) > K$. While the signal is on and $x(t) < K$, our equation is

$$\begin{aligned}
\dot{x}(t) =& \beta - \alpha x(t) \\
x(t) =& \frac{\beta}{\alpha}(1 - e^{-\alpha t})
\end{aligned}$$

$x(\tau) > K$ then requires

$$\frac{\beta}{\alpha}(1 - e^{-\alpha\tau}) > K$$

which we can solve for $\beta$ or $\tau$:

$$\begin{aligned}\beta >& \frac{\alpha K}{1 - e^{-\alpha\tau}} \\ \tau >& -\frac{1}{\alpha}\ln\left(1 - \frac{K\alpha}{\beta}\right) \\ >& \frac{1}{\alpha}\ln\left(\frac{\beta}{\beta - K\alpha}\right)\end{aligned}$$

Second, we require that $\frac{\beta_f}{\alpha} > K$. If $x(\tau) < K$, then after $\tau$ there are no positive terms in the differential equation, and $x$ will decay. If $x(\tau) > K$ then after $\tau$, as long as $x(t)$ remains $> K$, the system will have a new steady state at $\frac{\beta_f}{\alpha}$. However, if that steady state is $< K$, then the system will first decay to $K$, then lose all production terms and decay to 0.

3. With negative autoregulation, the ODE is

$$\dot{x}(t) = \beta\Theta[S(t) > 0]\Theta[x(t) < K] - \alpha x(t),$$

and assume that $\beta/\alpha > K$. Provide the response $x(t)$ for $S(t) = 0$ for $t < 0$ and $S(t) > 0$ for $t > 0$. Calculate the time $t_{1/2}$ when the response is half complete. Is the response faster or slower than the system without negative feedback, Question 1? Just after the system turns on, we will have $S(t) > 0$ and $x(t) < K$. Until $x(t) > K$, we have the equation

$$\begin{aligned}\dot{x}(t) =& \beta - \alpha x(t) \\ x(t) =& \frac{\beta}{\alpha}[1 - e^{-\alpha t}]\end{aligned}$$

When $x(t) > K$, we have

$$\dot{x}(t) = -\alpha x(t)$$

Which implies $x(t)$ will immediately fall below $K$, returning us to the previous equation. So, the solution is that $x(t)$ rises according to the first equation until it reaches $K$, at which point it remains there. So,

$$\begin{aligned} x_{ss} &= K \\ x(t_{1/2}) &= \frac{K}{2} \\ \frac{\beta}{\alpha}[1-e^{-\alpha t_{1/2}}] &= \frac{K}{2} \\ e^{-\alpha t_{1/2}} &= 1-\frac{K\alpha}{2\beta} \\ t_{1/2} &= \frac{1}{\alpha}\ln\frac{1}{1-\frac{K\alpha}{2\beta}} \\ &= \frac{1}{\alpha}\ln\frac{\beta}{\beta-\frac{K\alpha}{2}} \end{aligned}$$

We can show that the response time of a system with negative feedback is faster by using the provided limitation on $K$

$$\begin{aligned} \frac{\beta}{\beta-\frac{K\alpha}{2}} &< \frac{\beta}{\beta-\frac{\beta}{2}} = 2 \\ \frac{1}{\alpha}\ln\frac{\beta}{\beta-\frac{K\alpha}{2}} &< \frac{1}{\alpha}\ln 2 \end{aligned}$$

4. A basic cascade has ODE

$$\begin{aligned} \dot{x}(t) &= \beta\Theta[S(t)>0]-\alpha x(t) \\ \dot{z}(t) &= \beta\Theta[x(t)>K]-\alpha z(t). \end{aligned}$$

Assume that $\beta/\alpha > K$, and note that $y$ is skipped; it will be added later as an intermediate component of the cascade.

(a) For the on-step, provide $x(t)$, $z(t)$. Provide $t_{1/2}$ for $x$ and $z$.

$$x(t) = \frac{\beta}{\alpha}[1-e^{-\alpha t}]$$

The variable $z$ remains at zero (no production) until $x(t)$ reaches threshold $K$ at time point $t_1$. At $t_1$ we have:

$$\begin{aligned} K =& \frac{\beta}{\alpha}[1 - e^{-\alpha t_1}] \\ t_1 =& -\frac{1}{\alpha}\ln(1 - \frac{\alpha K}{\beta}) \\ \approx& -\frac{1}{\alpha} * (-1) * \frac{\alpha K}{\beta} \\ t_1 =& \frac{K}{\beta} \end{aligned}$$

At times after $t_1$, we have production, and decay of $z$ with rates $\beta$ and $\alpha$. Thus:

$$z(t) = \begin{cases} \frac{\beta}{\alpha}[1 - e^{-\alpha(t-t_1)}], & t \geq t_1 \\ 0, & \text{otherwise} \end{cases}$$

Now with $x(t)$ and $z(t)$ available, we can readily find $t_{1/2}$ for each variable as follows. For $x(t)$:

$$\begin{aligned} x(t_{1/2}) =& \frac{\beta}{\alpha}[1 - e^{-\alpha t_{1/2}}] = \frac{\beta}{2 * \alpha} \\ e^{-\alpha t_{1/2}} =& 0.5 \\ t_{1/2} =& \frac{\ln 2}{\alpha} \end{aligned}$$

For $z(t)$:

$$\begin{aligned} z(t_{1/2}) =& \frac{\beta}{\alpha}[1 - e^{-\alpha(t_{1/2}-t_1)}] = \frac{\beta}{2 * \alpha} \\ e^{-\alpha(t_{1/2}-t_1)} =& 0.5 \\ t_{1/2} =& t_1 + \frac{\ln 2}{\alpha} \\ t_{1/2} =& \frac{K}{\beta} + \frac{\ln 2}{\alpha} \end{aligned}$$

(b) For the off-step, provide $x(t)$, $z(t)$. Provide $t_{1/2}$ for $x$ and $z$.
For the off-step, since $S(t)$ has been on for an infinitely long period of time, variables $x(t)$ and $z(t)$ have reached their steady state values for the on-step as predicted by part a. Therefore,

$$x(0^-) = \frac{\beta}{\alpha}, \text{ and } z(0^-) = \frac{\beta}{\alpha}$$

Starting from $t = 0$ the input signal $S(t)$ is turned off. So, the differential equation for x(t) changes to:

$$\dot{x}(t) = -\alpha x(t)$$

So, we will have:

$$x(t) = x(0)e^{-\alpha t} = \frac{\beta}{\alpha}e^{-\alpha t}$$

However, as long as $x(t)$ is above the threshold $K$, the differential equation for $z(t)$ stays the same as before, and $z$ stays at $\frac{\beta}{\alpha}$. This condition changes at $t^*$ when:

$$\begin{aligned} x(t) =& \frac{\beta}{\alpha}e^{-\alpha t^*} = K \\ -\alpha t^* =& \ln\frac{K\alpha}{\beta} \\ t^* =& \frac{1}{\alpha}\ln\frac{\beta}{K\alpha} \end{aligned}$$

At $t^*$, the differential equation for $z(t)$ changes to:

$$\dot{z}(t) = -\alpha z(t)$$

Therefore, $z(t)$ behaves as:

$$z(t) = \begin{cases} \frac{\beta}{\alpha}, & t \leq t^* \\ \frac{\beta}{\alpha}e^{-\alpha(t-t^*)}, & \text{otherwise} \end{cases}$$

where

$$t^* = \frac{1}{\alpha}\ln\frac{\beta}{K\alpha}$$

Now we can find $t_{1/2}$ for $x$ and $z$. For variable $x$:

$$\begin{aligned} x(t_{1/2}) =& \frac{\beta}{\alpha}e^{-\alpha t_{1/2}} = \frac{\beta}{2\alpha} \\ t_{1/2} =& \frac{\ln 2}{\alpha} \end{aligned}$$

For variable $z$:

$$\begin{aligned} z(t_{1/2}) =& \frac{\beta}{\alpha}e^{-\alpha(t_{1/2}-t^*)} = \frac{\beta}{2\alpha} \\ t_{1/2} =& t^* + \frac{\ln 2}{\alpha} \\ =& \frac{1}{\alpha}\ln\left(\frac{\beta}{K\alpha}\right) + \frac{\ln 2}{\alpha} \end{aligned}$$

5. Protein $y$ is also a transcription factor for protein $z$. If either $x$ or $y$ can activate $z$, for example by binding as homotetramers, the motif functions as an OR gate with ODE

$$\begin{aligned}\dot{x}(t) &= \beta\Theta[S(t) > 0] - \alpha x(t)\\ \dot{y}(t) &= \beta\Theta[x(t) > K] - \alpha y(t)\\ \dot{z}(t) &= \beta\Theta[(x(t) > K) \text{ or } (y(t) > K)] - \alpha z(t).\end{aligned}$$

Provide $t_{1/2}$ for $z$ for the on-step and the off-step. How do these differ from the simpler cascade, Question 4?

**On-step**

Making arguments similar to Question 4, one can start by solving the ODE for variable $x$.

$$x(t) = \frac{\beta}{\alpha}[1 - e^{-\alpha t}]$$

Starting at time $t_1 \approx K/\beta$ variable $x$ reaches threshold $K$, and we will have production of $y$ with rate $\beta$. Therefore,

$$y(t) = \begin{cases} 0, & t \le t_1 \approx \frac{K}{\beta} \\ \frac{\beta}{\alpha}[1 - e^{-\alpha(t-t_1)}], & \text{otherwise} \end{cases}$$

And at $t_1$, since $x$ exceeds the threshold $K$, the conditions for production of $z$ are satisfied. So,

$$z(t) = \begin{cases} 0, & t \le t_1 \approx \frac{K}{\beta} \\ \frac{\beta}{\alpha}[1 - e^{-\alpha(t-t_1)}], & \text{otherwise} \end{cases}$$

We can find $t_{1/2}$ as:

$$\begin{aligned} z(t_{1/2}) =& \frac{\beta}{\alpha}[1 - e^{-\alpha(t-t_1)}] = \frac{\beta}{2\alpha}\\ t_{1/2} =& t_1 + \frac{\ln 2}{\alpha}\\ t_{1/2} =& \frac{K}{\beta} + \frac{\ln 2}{\alpha}\end{aligned}$$

Which is the same as $t_{1/2}$ for the simpler cascade.

**Off-step**

The variables $x$, $y$, and $z$ start from their steady state values of $\frac{\beta}{\alpha}$ at time 0 (please see the answer to on-step). The differential equation for $x(t)$ changes instantaneously to:

$$\dot{x}(t) = -\alpha x(t)$$

So we will have:

$$x(t) = \frac{\beta}{\alpha} e^{-\alpha t}$$

However, as long as $x$ is above the threshold K, the argument of $\Theta[x(t) > K]$ , and $\Theta[(x(t) > K) \text{ or } (y(t) > K)]$ stays true. So, variables $y$, and $z$ stay at $\frac{\beta}{\alpha}$ until:

$$\begin{aligned} x(t_1) =& \frac{\beta}{\alpha} e^{-\alpha t} = K \\ t_1 =& \frac{1}{\alpha} \ln\left(\frac{\beta}{K\alpha}\right) \end{aligned}$$

And since production of $y$ stops at $t_1$,

$$y(t) = \begin{cases} \frac{\beta}{\alpha}, & t \le t_1 = \frac{1}{\alpha}\ln(\frac{\beta}{K\alpha}) \\ \frac{\beta}{\alpha} e^{-\alpha(t-t_1)}, & \text{otherwise} \end{cases}$$

Despite the decay in $x(t)$ and $y(t)$, $z$ will remain at its steady state value of $\frac{\beta}{\alpha}$ until $y$ falls below the threshold value of $K$ at $t_2$:

$$\begin{aligned} y(t_2) =& \frac{\beta}{\alpha} e^{-\alpha(t_2 - t_1)} = K \\ t_2 =& t_1 + \frac{1}{\alpha} \ln\left(\frac{\beta}{K\alpha}\right) \\ t_2 =& \frac{2}{\alpha} \ln\left(\frac{\beta}{K\alpha}\right) \end{aligned}$$

After $t_2$, the production of $z(t)$ stops, and it starts to decay exponentially:

$$z(t) = \begin{cases} \frac{\beta}{\alpha}, & t \le t_2 = \frac{2}{\alpha}\ln(\frac{\beta}{K\alpha}) \\ \frac{\beta}{\alpha} e^{-\alpha(t-t_2)}, & \text{otherwise} \end{cases}$$

We can find $t_{1/2}$ for $z$ similarly as:

$$\begin{aligned} z(t_{1/2}) =& \frac{\beta}{\alpha} e^{-\alpha(t-t_2)} = \frac{\beta}{2\alpha} \\ t_{1/2} =& \frac{2}{\alpha} \ln(\frac{\beta}{K\alpha}) + \frac{\ln(2)}{\alpha} \end{aligned}$$

Which is longer than $t_{1/2}$ for the simpler cascade.

6. If $x$ and $y$ bind as a heteromeric complex to activate $z$, the motif functions as an AND gate with ODE

$$\begin{aligned}\dot{x}(t) &= \beta\Theta[S(t) > 0] - \alpha x(t)\\ \dot{y}(t) &= \beta\Theta[x(t) > K] - \alpha y(t)\\ \dot{z}(t) &= \beta\Theta[(x(t) > K) \text{ and } (y(t) > K)] - \alpha z(t).\end{aligned}$$

Provide $t_{1/2}$ for $z$ for the on-step and the off-step. How do these differ from the simpler cascade, Question 4?

**On-step**

Making arguments similar to questions 4, and 5:

$$x(t) = \frac{\beta}{\alpha}[1 - e^{-\alpha t}]$$

and

$$y(t) = \begin{cases} 0, & t \leq t_1 \approx \frac{K}{\beta} \\ \frac{\beta}{\alpha}[1 - e^{-\alpha(t-t_1)}], & \text{otherwise} \end{cases}$$

The difference here lies in the fact that the production of $z$ does not start, until both $x$, and $y$ are above the threshold $K$. Variable $x$ exceeds the threshold at $t_1 = \frac{K}{\beta}$ and variable $y$ reaches threshold at $t_2 = \frac{2K}{\beta}$. Thus, we will have:

$$z(t) = \begin{cases} 0, & t \leq t_2 \approx \frac{2K}{\beta} \\ \frac{\beta}{\alpha}[1 - e^{-\alpha(t-t_2)}], & \text{otherwise} \end{cases}$$

and we will have:

$$t_{1/2} = \frac{\ln 2}{\alpha} + \frac{2K}{\beta}$$

Which is longer than $t_{1/2}$ for the simpler cascade.

**Off-step**

The behaviour of variables $x$ and $y$ is identical to what we had in question 5:

$$x(t) = \frac{\beta}{\alpha} e^{-\alpha t}$$

and

$$y(t) = \begin{cases} \frac{\beta}{\alpha}, & t \leq t_1 = \frac{1}{\alpha}\ln(\frac{\beta}{K\alpha}) \\ \frac{\beta}{\alpha} e^{-\alpha(t-t_1)}, & \text{otherwise} \end{cases}$$

However, here as soon as $x$ falls below the threshold $K$, the production of $z$ stops (AND operation). So,

$$z(t) = \begin{cases} \frac{\beta}{\alpha}, & t \leq t_1 = \frac{1}{\alpha}\ln(\frac{\beta}{K\alpha}) \\ \frac{\beta}{\alpha} e^{-\alpha(t-t_1)}, & \text{otherwise} \end{cases}$$

Therefore,

$$t_{1/2} = \frac{1}{\alpha}\ln(\frac{\beta}{K\alpha}) + \frac{\ln 2}{\alpha}$$

Which is the same as $t_{1/2}$ for the simpler cascade.

7. If the intermediate transcription factor $y$ bind as a repressor, the motif functions as a derivative operator or edge detector with ODE

$$\begin{aligned}
\dot{x}(t) &= \beta\Theta[S(t) > 0] - \alpha x(t) \\
\dot{y}(t) &= \beta\Theta[x(t) > K] - \alpha y(t) \\
\dot{z}(t) &= \beta\Theta[(x(t) > K) \text{ and } (y(t) < K)] - \alpha z(t).
\end{aligned}$$

For an on-step input, provide the output $z(t)$, the time $t_{\max}$ when $z$ has its maximum value, and the maximum value $z_{\max} = z(t_{\max})$. Provide the two times when $z(t) = z_{\max}/2$.

All variables start from initial rest condition ($x(0) = y(0) = z(0) = 0$). As the input signal $S(t)$ is turned on, the production of $x$ starts immediately, and it approaches its steady state value of $\frac{\beta}{\alpha}$.

$$x(t) = \frac{\beta}{\alpha}[1 - e^{-\alpha t}]$$

As $x$ passes threshold $K$ (at $t = t_1 \approx \frac{K}{\beta}$), the production of $y$ starts and it reaches its steady state value of $\frac{\beta}{\alpha}$.

$$y(t) = \begin{cases} 0, & t \le t_1 \approx \frac{K}{\beta} \\ \frac{\beta}{\alpha}[1 - e^{-\alpha(t-t_1)}], & \text{otherwise} \end{cases}$$

During the period between the start of production of $y$ and the point when it reaches threshold $K$, we will have production of $z$. In this period, $z$ starts to approach its steady state value of $\frac{\beta}{\alpha}$. However, as soon as $y$ exceeds the threshold $K$ (at $t_2 \approx \frac{2K}{\beta}$), the production of $z$ stops and we will have exponential decay of $z$ from that point on.

$$z(t) = \begin{cases} 0, & t \le t_1 \approx \frac{K}{\beta} \\ \frac{\beta}{\alpha}[1 - e^{-\alpha(t-t_1)}], & t_1 \le t \le t_2 \\ z(t_2)e^{-\alpha(t-t_2)}, & t_2 \ge t \end{cases}$$

The variable $z$ reaches its maximum value at:

$$\begin{aligned}
t_{max} &= t_2 \approx \frac{2K}{\beta} \\
z_{max} &= \frac{\beta}{\alpha}[1 - e^{-\alpha(t_2-t_1)}] \\
&= \frac{\beta}{\alpha}[1 - e^{-\alpha(\frac{K}{\beta})}]
\end{aligned}$$

At two time points, one in $t_1 \le t \le t_2$ and one in $t_2 \ge t$, $z$ assumes half its maximum value.

$$\begin{aligned} z(t^s_{1/2}) =& \frac{\beta}{\alpha}[1-e^{-\alpha(t^s_{1/2}-\frac{K}{\beta})}] = \frac{\beta}{2\alpha}[1-e^{-\alpha(\frac{K}{\beta})}] \\ t^s_{1/2} =& \frac{-1}{\alpha}\ln\left(\frac{1}{2}\left(1+e^{-\frac{\alpha K}{\beta}}\right)\right)+\frac{K}{\beta} \\ t^s_{1/2} =& \frac{\ln 2}{\alpha}+\frac{1}{\alpha}\ln\left(\frac{1}{1+e^{-\frac{\alpha K}{\beta}}}\right)+\frac{K}{\beta} \end{aligned}$$

For the larger $t$, when $z$ is half of its maximum value:

$$\begin{aligned} z(t^l_{1/2}) =& z(t_2)e^{-\alpha(t^l_{1/2}-t_2)} = \frac{1}{2}z(t_2) \\ t^l_{1/2} =& t_2+\frac{\ln 2}{\alpha} \\ t^l_{1/2} =& \frac{2K}{\beta}+\frac{\ln 2}{\alpha} \end{aligned}$$