Supporting Text S1- Calculations for the Feedback Gain & Overshoot.

Feedback Gain
Suppose a simple feedback system consisting of an mRNA molecule (X) that encodes a transcriptional repressor (Y), able to repress transcription from its own promoter.

Let G be the feedback gain, defined as the ratio between the steady-states of the negative feedback (\(X_{0b}\)) and the open loop (\(X_0\)).

The dynamics of the negative feedback follow

\[
\begin{align*}
\frac{dx}{dt} &= \frac{\lambda_1 k^n x^n}{k^n + (y)^n} - \beta_1 x_t \\
\frac{dy}{dt} &= \lambda_2 x_t - \beta_2 y_t
\end{align*}
\]

Eq 1.

Where x is the RNA concentration, Y is the protein concentration , \(\lambda\) stands for production rates, \(\beta\) represents degradation/dilution rates, \(k\) is the feedback constant, and \(n\) is the cooperativity of the system (non-linearity of the feedback). In these condition the steady-state of the system is
\[ \begin{aligned}
\frac{dx}{dt} &= \frac{\lambda_1 k^n}{k^{n+1} y^n} - \beta_1 x_t = 0 \\
\frac{dy}{dt} &= \lambda_2 x_t - \beta_2 y_t = 0
\end{aligned} \]

In order to simplify the calculations we replace \( k^n = K \) and the steady-state for strong repression (\( K + y_{ss} \approx y_m \)) for \( X \) follows

\[ X_{ss} = \left( \frac{\lambda_1 K}{\beta_1} \right)^{\frac{1}{n+1}} \left( \frac{\beta_2}{\lambda_2} \right)^{\frac{1}{n+1}} \]

Eq 2.

Similarly the equations for the open loop

\[ \begin{aligned}
\frac{dx}{dt} &= \lambda_1 - \beta_1 x_t \\
\frac{dy}{dt} &= \lambda_2 x_t - \beta_2 y_t
\end{aligned} \]

yield in steady-state

\[ X_0 = \frac{\lambda_1}{\beta_1} \]

Therefore the feedback gain equals

\[ G = \frac{X_m}{X_{ss}} = \left( \frac{\lambda_1 K}{\beta_1 \beta_2} \right)^{\frac{n}{n+1}} \left( \frac{1}{K} \right)^{\frac{1}{n+1}} \left( \frac{\beta_2}{\lambda_2} \right)^{\frac{1}{n+1}} \]

\[ G = \left( \frac{\lambda_1 K}{\beta_1 \beta_2 K} \right)^{\frac{n}{n+1}} \]

Eq 4.

This expressions yields the following limits:

\[ \text{Lim}_{k \to 0} \left( \frac{X_m}{X_{ss}} \right) = \left( \frac{\lambda_1}{\beta_1 \beta_2} \right)^{\frac{n}{n+1}} \left( \frac{1}{K} \right)^{\frac{1}{n+1}} = \left( \frac{\lambda_1}{\beta_1 \beta_2 K} \right)^{\frac{n}{n+1}} = \infty \]

Eq 5.

\[ \text{Lim}_{n \to \infty} \left( \frac{X_m}{X_{ss}} \right) = \left( \frac{\lambda_1}{\beta_1 \beta_2} \right)^{\frac{n}{n+1}} \left( \frac{1}{K} \right)^{\frac{1}{n+1}} = \left( \frac{\lambda_1}{\beta_1 \beta_2 \beta_3 K} \right)^{\frac{n}{n+1}} = \left( \frac{\lambda_1}{\beta_1 \beta_2} \right)^{\frac{n}{n+1}} = \frac{\lambda_1}{\beta_1 \beta_2} K \]

Eq 6.

This indicates that while decreasing \( k \) (increasing the affinity of the repressor for its cognate site) increases indefinitely the feedback gain, increasing \( n \) reaches a limit that equals the intrinsic promoter strength \( \left( \frac{\lambda_1}{\beta_1 \beta_2} \right) \) times the inverse of the half-repression constant \( (k) \).

The gain in \( y \) is calculated in the same way, yielding

\[ G = \frac{Y_m}{Y_{ss}} = \left( \frac{\lambda_1 K}{\beta_1 \beta_2} \right)^{\frac{n}{n+1}} \left( \frac{1}{K} \right)^{\frac{1}{n+1}} \left( \frac{\beta_2}{\lambda_2} \right)^{\frac{1}{n+1}} \]

\[ G = \left( \frac{\lambda_1 K}{\beta_1 \beta_2 K} \right)^{\frac{n}{n+1}} \]

Which indicates that the feedback gain is equivalent for values of \( x \) and \( y \)
Feedback Overshoot existence

System of Eq. 1 produces a transient overshoot whenever \(x\) and \(y\) reach a maximum that is higher than the steady state value. A max. in \(x\) is reached if

\[
x' = \frac{dx}{dt} = 0 \quad \text{and} \quad x'' = \frac{d^2x}{dt^2} < 0
\]

\[
\frac{dx}{dt} = \frac{\lambda_1 K}{K + (y)^n} - \beta_1 x_{\text{max}} = 0
\]

\[
x_{\text{max}} = \frac{\lambda_1 K}{\beta_1(K + (y)^n)} - \frac{x}{\beta_1} = \frac{\lambda_1 K}{\beta_1(K + (y)^n)}
\]

\[
x'' = \lambda_1 K \frac{-n y^{n-1} y'}{(K+y)^2} = \lambda_1 K \frac{-n y^{n-1} y'}{(K+y)^2}
\]

\[
x'' = \lambda_1 K \frac{-n y^{n-1} y'}{(K+y)^2} < 0
\]

\(-n < 0\) since there cannot be negative cooperativity

\(y^{n+1} > 0\) since \(y\) cannot take negative values
\(\lambda_1 K\) are always positive

then \(x'' < 0 \iff y' > 0\)

From this follows that \(y\) at \(x = x_{\text{max}}\) \(y\) is always smaller than its steady state value

\[
y' = \lambda_2 x - \beta_2 y
\]

\[
y' = \lambda_2 x - \beta_2 y_{\text{ss}}
\]

\[
\frac{\lambda_1 \lambda_2 K}{\beta_1 \rho} > y_{\text{max}}^n + 1
\]

Since \(y_{\text{ss}} = \left(\frac{\lambda_1 \lambda_2 K}{\beta_1 \rho}\right)^{\frac{1}{n+1}}\)

\[
\left(\frac{\lambda_1 \lambda_2 K}{\beta_1 \rho}\right)^{\frac{1}{n+1}} > y_{\text{ss}} > y_{\text{max}}
\]

Equivalent reasoning yields that \(x' < 0\) at \(y = y_{\text{max}}\) and \(x_{\text{ss}} < x_{\text{max}}\)

Gain-Overshoot relationship

The overshoot (O) is the ratio between the maximal value of \(X\) or \(Y\) and its steady-state value. In the case of an RNA

\[
O = \frac{X_{\text{max}}}{X_{\text{ss}}} = \frac{X_{\text{max}}}{\left(\frac{\lambda_1 \lambda_2 K}{\beta_1 \rho}\right)^{\frac{1}{n+1}}} = \frac{X_{\text{max}}}{\left(\frac{\lambda_1 \lambda_2 K}{\beta_1 \rho}\right)^{\frac{1}{n+1}}} \quad \text{Eq. 7}
\]

The highly non-linear nature of the ODE system prevents the calculation of \(X_{\text{max}}\), but in the case of strong self-repression \((K+y \approx y)\) and high cooperativity we can linearize the system, approximating \(X_{\text{max}}\) to the equivalent
value for the open loop when \( t = t_{\text{max}} \)

\[
\frac{dx}{dt} = \lambda_1 - \beta_1 x_t \quad \text{from} \quad t=0 \rightarrow t = t_{\text{max}} \\
\frac{dx}{dt} = \frac{\lambda_1 K}{K + (y)^p} - \beta_1 x_t \quad \text{from} \quad t = t_{\text{max}} \rightarrow t = \infty
\]

This allows direct integration of \( X_{\text{max}} \)

\[
\int \frac{dx}{dt} \, dt = \int_0^{t_{\text{max}}} (\lambda_1 - \beta_1 x) \, dt = \frac{\lambda_1}{\beta_1} \left( 1 - e^{-\beta_1 t_{\text{max}}} \right)
\]

This approximation holds for highly-nonlinear systems, where the high cooperativity index \((n)\) acts as an effective delay between the accumulation of the repressor \((y)\) and the onset of repression. Therefore

\[
O_x = \frac{X_{\text{max}}}{X_{\text{ss}}} \approx \frac{\lambda_1}{\beta_1} \left( 1 - e^{-\beta_1 t_{\text{max}}} \right) \approx \frac{\lambda_1 \lambda_2}{\beta_1 \beta_2} \left( \frac{1}{K} \right)^{1/n} \left( 1 - e^{-\beta_1 t_{\text{max}}} \right)
\]

\[
O_x \approx \left( 1 - e^{-\beta_1 t_{\text{max}}} \right) G
\]

To calculate the overshoot for \( y \) we follow the same reasoning. Linearizing \( x \) until the onset of the negative feedback, we can approximate the value of \( Y_{\text{max}} \)

\[
O_x = \frac{Y_{\text{max}}}{Y_{\text{ss}}}
\]

\[
\frac{dy}{dt} = \lambda_2 x_t - \beta_2 y_t \quad \text{and} \quad x_t \approx \frac{\lambda_1}{\beta_1} \left( 1 - e^{-\beta_1 t} \right) \\
\frac{dy}{dt} \approx \frac{\lambda_1 \lambda_2}{\beta_1 \beta_2} \left( 1 - e^{-\beta_1 t} \right) - \beta_2 y_t
\]

\[
O_y \approx \left( \frac{\lambda_1 \lambda_2}{\beta_1 \beta_2} \right)^{1/n} \left( \frac{1}{K} \right)^{1/n} \left( \frac{\beta_1 (1-e^{-\beta_1 t}) - \beta_2 (1-e^{-\beta_2 t})}{\beta_1 - \beta_2} \right)
\]

\[
O_y \approx \left( \frac{\beta_1 (1-e^{-\beta_1 t}) - \beta_2 (1-e^{-\beta_2 t})}{\beta_1 - \beta_2} \right) G
\]

This indicates that the \( O_y \neq O_x \), and

\[
O_y = O_x \left( \frac{\beta_1 - \beta_2}{\beta_1 - \beta_2} \right)
\]

Simulations indicate that these approximation holds for highly-nonlinear systems (Figure 4, main text). This condition can be met by systems where the repressor exhibits high cooperativity to its cognate binding site or where the repressor dimer/multimerization is required for binding.

Effect of multimerization

The following system includes a step of repressor dimerization

\((k^a = K)\)
\[
\begin{align*}
\frac{dx}{dt} &= \frac{\lambda_1 K}{K+(y)} - \beta_1 x_t \\
\frac{dy}{dt} &= \lambda_2 x_t - \beta_2 y_t \\
\frac{dz}{dt} &= c_d y - c_d z
\end{align*}
\]

And in steady-state

\[
\begin{align*}
\frac{dx}{dt} &= c_d y - c_d z \\
z &= \frac{c_d}{c_d} y
\end{align*}
\]

Therefore

\[
\begin{align*}
\frac{dx}{dt} &= \frac{\lambda_1 K}{K+(y)} - \beta_1 x_{ss} = \frac{\lambda_1 K}{K+(y)} - \beta_1 x_{ss} = 0
\end{align*}
\]

This indicates that at steady state the dimer system is formally identical to the monomeric system with the only difference of \(c_d/c_d\) multiplying \(y\), which reflects the steady-state of the dimerization dynamics. Similarly, the gain in the dimer system can be expressed as

\[
\mathcal{G} = \frac{x_t}{x_{ss}} = \left( \frac{\lambda_1 \lambda_2 K}{\beta_1 \beta_2 k} \right)^{\frac{y_{ss}}{1+1}}
\]