Supplementary information

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Appendix A: Some remarks on the model construction

In the main text, we establish a stochastic model describing the dynamics of the levels of proteins X and Y. The model can be represented by the following two-dimensional system of stochastic differential equations (SDE):

$$\begin{aligned} \dot{x} &= -\alpha(x - F(a, y)) + \sqrt{2\epsilon}\xi_x, \\ \dot{y} &= -\beta(y - G(x)) + \sqrt{2\eta}\xi_y, \end{aligned} \tag{1}$$

where $F(a, y)$ describes the activation of protein X by inducer A and protein Y, and $G(x)$ describes the activation of protein Y by protein X. We also point out that in natural bacterial systems, the most common expression of $F(a, G(x))$ has the following form:

$$F(a, G(x)) = \frac{\gamma x^n}{K + x^n} + (\mu a + \delta). \tag{2}$$

In this section, we shall derive this expression based on the underlying biochemical reactions and the law of mass action.

We first derive the specific expressions of $F(a, y)$ and $G(x)$. To this end, we list the possible reactions involved in the double-positive feedback regulatory network as

$$\mathrm{X} \to \varnothing, \quad \mathrm{Y} \to \varnothing, \tag{3}$$

$$\mathrm{Y} + \cdots + \mathrm{Y} \leftrightarrow \mathrm{Y}^*, \tag{4}$$

$$\text{gene } X \to \text{gene } X + \mathrm{X}, \tag{5}$$

$$\text{gene } X + Y^* \leftrightarrow (\text{gene } X)^* \to \text{gene } X + \mathrm{X}, \tag{6}$$

$$\mathrm{A} \dashrightarrow \mathrm{X}, \tag{7}$$

$$\mathrm{X} \dashrightarrow \mathrm{Y}. \tag{8}$$

Here, Equation (3) describes the degradation of proteins X and Y. In many cases, the transcription factor Y is inactive. To be active, protein Y needs to be present as a multimer or a complex [1]. Equation (4) describes the process of multimerization of protein Y or cooperativity of protein Y with other proteins. Equation (5) describes a basal expression of gene X independent of the activation of protein Y. Equation (6) describes the expression of gene X with the binding of the transcription factor Y. In addition, Equations (7) and (8) describe the activation of protein X by inducer A and the activation of protein Y by protein X, respectively. Such activation effects can be direct or indirect.

Based on the above discussion, $F(a, y)$ has the following from:

$$F(a, y) = \frac{\gamma_0 y^n}{K_0 + y^n} + (f(a) + \delta). \tag{9}$$

Here, the Hill function describes the activation of gene X by protein Y. The Hill coefficient n comes from the multimerization of Y or the cooperativity of protein Y with other proteins. $f(a)$ describes the activation of protein X by inducer A. δ describes the basal expression of gene X. Since the specific mechanisms of the activation effects can be very complex and can be different from systems to systems, $f(a)$ and $G(x)$ may have various different forms. To simplify the discussion, we use linear functions to approximate $f(a)$ and $G(x)$.

Based on Equation (9) and the simplification of using linear functions to approximate $f(a)$ and $G(x)$, we obtain a most common expression of $F(a, G(x))$ with the following explicit form:

$$F(a, G(x)) = \frac{\gamma x^n}{K + x^n} + (\mu a + \delta). \tag{10}$$

Appendix B: Derivation of the steady-state probability density

We denote the steady-state probability density of the stochastic system (1) by $p_s(a, x, y)$. To calculate the expression of $p_s(a, x, y)$, we make an assumption that that the dynamics of the variable y is much faster than that of the variable x. In order words, we assume that $\alpha \ll \beta$. Under this assumption, we see that x is a slow variable and y is a fast variable. In order to study the dynamics of the fast variable y, we can think that the slow variable x is frozen. Thus the steady-state probability density $p_s(a, x, y)$ of the stochastic system can be represented as

$$p_s(a, x, y) = p_s(y|a, x)p_s(a, x), \tag{11}$$

where $p_s(y|a, x)$ is the steady-state probability density of the the fast variable y given a and x, and $p_s(a, x)$ is the steady-state probability density of the slow variable x.

Note that the dynamics of the fast variable y described by the second equality of Equation (1) is an Ornstein-Uhlenbeck (OU) process if we froze the slow variable x. Thus the steady-state distribution of y given a and x follows the normal distribution $N(G(x), \eta/\beta)$ with probability density

$$p_s(y|a, x) = \sqrt{\frac{\beta}{2\pi\eta}} \exp\left(-\frac{\beta}{2\eta}(y - G(x))^2\right). \tag{12}$$

In order to study the dynamics of the slow variable x, we can think that the fast variable y is always in a quasi-steady state. This indicates that the fast variable y in the first equality of Equation (1) can be averaged out as

$$\dot{x} = \int_R \left(-\alpha(x - F(a, y)) + \sqrt{2\epsilon}\xi_x\right) p_s(y|a, x)dy. \tag{13}$$

Since the noise level η is small, according to the Laplace method, Equation (1) can be further approximated as

$$\begin{aligned}
\dot{x} &= \sqrt{\frac{\beta}{2\pi\eta}} \int_R \left(-\alpha(x - F(a, y)) + \sqrt{2\epsilon}\xi_x\right) \exp\left(-\frac{\beta}{2\eta}(y - G(x))^2\right) dy \\
&\doteq \sqrt{\frac{\beta}{2\pi\eta}} \sqrt{\frac{2\pi\eta}{\beta}} \left(-\alpha(x - F(a, G(x))) + \sqrt{2\epsilon}\xi_x\right) \\
&= -\alpha(x - F(a, G(x))) + \sqrt{2\epsilon}\xi_x.
\end{aligned} \tag{14}$$

This shows that the dynamics of the slow variable x can be approximated by the following one-dimensional SDE:

$$\dot{x} = -\alpha(x - F(a, G(x))) + \sqrt{2\epsilon}\xi_x = -\partial_x U(a, x) + \sqrt{2\epsilon}\xi_x, \tag{15}$$

where

$$U(a, x) = \alpha \int_0^x (u - F(a, G(u)))du. \tag{16}$$

Let $p(a, x, t)$ denote the probability density of the slow variable x at time t. Then the dynamics of $p(a, x, t)$ is governed by the following Fokker-Planck equation:

$$\partial_t p = \partial_x(\partial_x U p + \epsilon \partial_x p). \tag{17}$$

By solving the Fokker-Planck equation, the steady-state probability density $p_s(a, x)$ of the slow variable x has the form of

$$p_s(a, x) = \frac{1}{Z_x} \exp\left(-\frac{1}{\epsilon} U(a, x)\right), \tag{18}$$

where Z_x is a normalization constant.

Combining Equations (12) and (20), we obtain the approximate steady-state probability density $p_s(a, x, y)$ in the whole phase space:

$$p_s(a, x, y) = \frac{1}{Z} \exp\left\{-\frac{1}{\epsilon} U(a, x, y)\right\}, \tag{19}$$

where Z is a normalization constant and $U(a, x, y)$ is an appropriate (global) potential of the stochastic system (1) defined as

$$U(a, x, y) = \frac{\epsilon\beta}{2\eta}(y - G(x))^2 + \alpha \int_0^x (u - F(a, G(u))du. \tag{20}$$

Appendix C: Derivation of the mean escape times

The approach here to derive the mean escape times is very similar to the reaction rate theory established by Kramers [2]. Readers who are familiar with the reaction rate theory can skip this part. We only discuss the mean escape time $\langle T_L \rangle$ from the low-expression state, that is, the mean time needed for a cell to switch from the low- to the high-expression state. The derivation of the mean escape time $\langle T_H \rangle$ from the high-expression state is quite similar.

We denote by X_t the level of protein X at time t. For any x, we define

$$\tau_x = \inf\{t \geq 0 : X_t = x\}. \tag{21}$$

Intuitively, τ_x is the time needed for X_t to arrive at x for the first time. Mathematically, the time τ_x is a random time and is named as the first-passage time for x. Under this notation, the mean escape time $\langle T_L \rangle$ from the low-expression state should be

$$\langle T_L \rangle = E_{x_L} \tau_{x_H} = E(\tau_{x_H} | X_0 = x_L), \tag{22}$$

that is, the mean time needed for X_t which starts from x_L to arrive at x_H for the first time.

We rewrite the one-dimensional system (16) in Ito's differential form as

$$dX_t = -U'(X_t)dt + \sqrt{2\epsilon}dW_t, \tag{23}$$

where $U'(x) = \partial_x U(a,x)$. According to Ito's formula in the theory of SDE, for any function f whose second derivative is continuous, we have

$$\begin{aligned} df(X_t) &= f'(X_t)dX_t + \frac{1}{2}f''(X_t)dX_t^2 \\ &= f'(X_t)(-U'(X_t)dt + \sqrt{2\epsilon}dW_t) + \frac{1}{2}f''(X_t)2\epsilon dt \\ &= (\epsilon f''(X_t) - U'(X_t)f'(X_t))dt + \sqrt{2\epsilon}f'(X_t)dW_t. \end{aligned} \tag{24}$$

We rewrite the above equation as the integration form as

$$f(X_t) - f(X_0) = \int_0^t (\epsilon f'' - U'f')(X_s)ds + \sqrt{2\epsilon}\int_0^t f'(X_s)dW_s. \tag{25}$$

This shows that

$$f(X_{t\wedge\tau_{x_H}}) - f(X_0) - \int_0^{t\wedge\tau_{x_H}} (\epsilon f'' - U'f')(X_s)ds \tag{26}$$

is a martingale. Taking expectation in the above equation and letting $t \to \infty$, we obtain that

$$E_{x_L} f(X_{\tau_{x_H}}) - f(x_L) = E_{x_L}\int_0^{\tau_{x_H}} (\epsilon f'' - U'f')(X_s)ds. \tag{27}$$

If we can find a function f so that

$$\epsilon f'' - U'f' \equiv 1, \tag{28}$$

then the above equation reduces to

$$E_{x_L}\tau_{x_H} = E_{x_L} f(X_{\tau_{x_H}}) - f(x_L) = f(x_H) - f(x_L). \tag{29}$$

Thus the remaining question is to solve the second-order ODE (28).

Recall that the steady-state probability density of the one-dimensional system is

$$p_s(a,x) = \frac{1}{Z_x} e^{-\frac{1}{\epsilon}U(a,x)}. \tag{30}$$

Thus we obtain that

$$p_s' = -\frac{1}{\epsilon}U'p_s. \tag{31}$$

Multiplying Equation (28) by p_s, we obtain that

$$\epsilon f''p_s - U'f'p_s = p_s. \tag{32}$$

Applying Equation (31), we obtain that

$$\epsilon(f'p_s)' = \epsilon f''p_s + \epsilon f'p_s' = p_s. \tag{33}$$

Integrating the above equation, we obtain that

$$f'(y)p_s(y) = \frac{1}{\epsilon}\int_{-\infty}^{y} p_s(z)dz. \tag{34}$$

Thus

$$f'(y) = \frac{1}{\epsilon p_s(y)}\int_{-\infty}^{y} p_s(z)dz. \tag{35}$$

Integrating the above equation, we obtain that

$$f(x) - f(x_L) = \frac{1}{\epsilon}\int_{x_L}^{x} \frac{1}{p_s(y)}dy \int_{-\infty}^{y} p_s(z)dz. \tag{36}$$

By Equation (29), we obtain that

$$\begin{aligned} E_{x_L}\tau_{x_H} = f(x_H) - f(x_L) &= \frac{1}{\epsilon}\int_{x_L}^{x_H}\frac{1}{p_s(y)}dy\int_{-\infty}^{y}p_s(z)dz \\ &= \frac{1}{\epsilon}\int_{x_L}^{x_H}e^{\frac{1}{\epsilon}U(a,y)}dy\int_{-\infty}^{y}e^{-\frac{1}{\epsilon}U(a,z)}dz. \end{aligned} \tag{37}$$

Applying the Laplace method, we obtain that

$$\begin{aligned} E_{x_L}\tau_{x_H} &\doteq \frac{1}{\epsilon}\int_{x_L}^{x_H}e^{\frac{1}{\epsilon}U(a,y)}dy\int_{-\infty}^{x_M}e^{-\frac{1}{\epsilon}U(a,z)}dz \\ &\doteq \frac{1}{\epsilon}\sqrt{\frac{2\pi\epsilon}{\kappa_M}}e^{\frac{1}{\epsilon}U(a,x_M)}\sqrt{\frac{2\pi\epsilon}{\kappa_L}}e^{-\frac{1}{\epsilon}U(a,x_L)} \\ &= \frac{2\pi}{\sqrt{\kappa_L\kappa_M}}e^{\frac{1}{\epsilon}(U(a,x_M)-U(a,x_L))}, \end{aligned} \tag{38}$$

where $\kappa_L = \partial_x^2(a, x_L)$ and $\kappa_M = -\partial_x^2(a, x_M)$ are the curvatures of $U(a, x)$ at x_L and x_M, respectively. Thus we finally obtain that

$$\langle T_L\rangle = E_{x_L}\tau_{x_H} \doteq \frac{2\pi}{\sqrt{\kappa_L\kappa_M}}e^{\frac{1}{\epsilon}(U(a,x_M)-U(a,x_L))}. \tag{39}$$

Similarly, we can obtain that

$$\langle T_H\rangle = E_{x_H}\tau_{x_L} \doteq \frac{2\pi}{\sqrt{\kappa_H\kappa_M}}e^{\frac{1}{\epsilon}(U(a,x_M)-U(a,x_H))}, \tag{40}$$

where $\kappa_H = \partial_x^2(a, x_H)$ is the curvature of $U(a, x)$ at x_H. Thus the transition rate from the low- to the high-expression state is

$$k_{LH} = 1/\langle T_L\rangle = \frac{\sqrt{\kappa_L\kappa_M}}{2\pi}e^{\frac{1}{\epsilon}(U(a,x_L)-U(a,x_M))}, \tag{41}$$

and the transition rate from the high- to the low-expression state is

$$k_{HL} = 1/\langle T_H\rangle = \frac{\sqrt{\kappa_H\kappa_M}}{2\pi}e^{\frac{1}{\epsilon}(U(a,x_H)-U(a,x_M))}. \tag{42}$$

Thus we can derive the steady-state proportion p_L of low-expressing cells and the steady-state proportion p_H of high-expressing cells as

$$p_L = \frac{k_{HL}}{k_{LH}+k_{HL}} = \frac{\sqrt{\frac{1}{\kappa_L}}e^{-\frac{1}{\epsilon}U(a,x_L)}}{\sqrt{\frac{1}{\kappa_L}}e^{-\frac{1}{\epsilon}U(a,x_L)}+\sqrt{\frac{1}{\kappa_H}}e^{-\frac{1}{\epsilon}U(a,x_H)}}, \tag{43}$$

and

$$p_H = \frac{k_{LH}}{k_{LH}+k_{HL}} = \frac{\sqrt{\frac{1}{\kappa_H}}e^{-\frac{1}{\epsilon}U(a,x_H)}}{\sqrt{\frac{1}{\kappa_L}}e^{-\frac{1}{\epsilon}U(a,x_L)}+\sqrt{\frac{1}{\kappa_H}}e^{-\frac{1}{\epsilon}U(a,x_H)}}. \tag{44}$$

Appendix D: Derivation of the variance function

According to the main text, the variance function $D(x)$ is defined as

$$D(x) = \text{Var}(X_h|X_0 = x) = E_x(X_h - E_xX_h)^2. \tag{45}$$

To calculate $D(x)$, we have to first gain some knowledge about the distribution of X_h. To this end, we define a random time

$$\tau = \tau_{x_L} \wedge \tau_{x_H} = \min\{\tau_{x_L}, \tau_{x_H}\}. \tag{46}$$

Clearly, τ is the time needed for the X_t to arrive at either x_L or x_H for the first time. We can decompose X_h into

$$X_h = X_\tau + (X_h - X_\tau). \tag{47}$$

Thus we have

$$D(x) = \text{Var}_x(X_\tau) + \text{Var}_x(X_h - X_\tau) + 2\text{Cov}_x(X_\tau, X_h - X_\tau), \tag{48}$$

where $\text{Var}_x(\cdot) = \text{Var}(\cdot|X_0 = x)$. We next define

$$p_L(x) = P_x(X_\tau = x_L) \tag{49}$$

and

$$p_H(x) = P_x(X_\tau = x_H). \tag{50}$$

Obviously, $p_L(x) + p_H(x) = 1$. Note that X_τ is a random variable which takes the value of x_L with probability $p_L(x)$ and takes the value of x_H with probability $p_H(x)$. Thus

$$\begin{aligned}
\text{Var}_x(X_\tau) &= E_x(X_\tau - E_x X_\tau)^2 \\
&= E_x((X_\tau - E_x X_\tau)^2 | X_\tau = x_L)p_L(x) + E_x((X_\tau - E_x X_\tau)^2 | X_\tau = x_H)p_H(x) \\
&= (x_L - E_x X_\tau)^2 p_L(x) + (x_H - E_x X_\tau)^2 p_H(x).
\end{aligned} \tag{51}$$

Note that

$$E_x X_\tau = x_L p_L(x) + x_H p_H(x). \tag{52}$$

Thus we obtain that

$$\begin{aligned}
\text{Var}_x(X_\tau) &= ((x_L - x_H)p_H(x))^2 p_L(x) + ((x_H - x_L)p_L(x))^2 p_H(x) \\
&= (x_H - x_L)^2 p_L(x) p_H(x)(p_L(x) + p_H(x)) \\
&= (x_H - x_L)^2 p_L(x) p_H(x).
\end{aligned} \tag{53}$$

Note further that once X_t arrives at x_L or x_H, it will maintain around x_L or x_H before stochastic phenotype switching. In this case, $U'(x)$ can be first-order approximated by

$$U'(x) \doteq U'(x_L) + \kappa_L(x - x_L) = \kappa_L(x - x_L) \tag{54}$$

or

$$U'(x) \doteq U'(x_H) + \kappa_H(x - x_H) = \kappa_H(x - x_H). \tag{55}$$

Thus conditioned on $\{X_\tau = x_L\}$, the one-dimensional system can be approximated by the OU process

$$\dot{x} = -\kappa_L(x - x_L) + \sqrt{2\epsilon}\xi_x, \tag{56}$$

whose steady-state distribution is the normal distribution $N(x_L, \epsilon/\kappa_L)$, and conditioned on $\{X_\tau = x_H\}$, the one-dimensional system can be approximated by another OU process

$$\dot{x} = -\kappa_H(x - x_H) + \sqrt{2\epsilon}\xi_x, \tag{57}$$

whose steady-state distribution is another normal distribution $N(x_H, \epsilon/\kappa_H)$. Since we have assumed that the time scale of the interval h is much shorter than the time scale of stochastic phenotype switching,

we can use the steady-state distribution of the OU process to approximate the distribution of $X_h - X_\tau$. Thus we have

$$\begin{aligned}\text{Var}_x(X_h - X_\tau) &\doteq \text{Var}_x(X_h - X_\tau | X_\tau = x_L)p_L(x) + \text{Var}_x(X_h - X_\tau | X_\tau = x_H)p_H(x) \\ &\doteq \frac{\epsilon}{\kappa_L}p_L(x) + \frac{\epsilon}{\kappa_H}p_H(x) \\ &= \epsilon\left(\frac{p_L(x)}{\kappa_L} + \frac{p_H(x)}{\kappa_H}\right).\end{aligned} \tag{58}$$

Moreover, by the above OU approximation, we have

$$\begin{aligned}\text{Cov}_x(X_\tau, X_h - X_\tau) &= E_x(X_\tau - E_x X_\tau)(X_h - X_\tau - E_x(X_h - X_\tau)) \\ &= E_x(X_\tau - E_x X_\tau)E_x(X_h - X_\tau - E_x(X_h - X_\tau)|X_\tau) \\ &\doteq 0.\end{aligned} \tag{59}$$

Combining Equations (53), (58), and (59), the variance function $D(x)$ can be represented as

$$D(x) \doteq (x_H - x_L)^2 p_L(x)p_H(x) + \epsilon\left(\frac{p_L(x)}{\kappa_L} + \frac{p_H(x)}{\kappa_H}\right). \tag{60}$$

Thus the remaining question is to calculate $p_L(x)$ and $p_H(x)$.

By Equation (25), we see that

$$f(X_{t\wedge\tau}) - f(X_0) - \int_0^{t\wedge\tau} (\epsilon f'' - U'f')(X_s)ds \tag{61}$$

is a martingale. Taking expectation in the above equation and letting $t \to \infty$, we obtain that

$$E_x f(X_\tau) - f(x) = E_x \int_0^\tau (\epsilon f'' - U'f')(X_s)ds. \tag{62}$$

If we can find a function f so that

$$\epsilon f'' - U'f' \equiv 0, \tag{63}$$

then the above equation reduces to

$$E_x f(X_\tau) = f(x). \tag{64}$$

In other words,

$$f(x) = f(x_L)p_L(x) + f(x_H)p_H(x). \tag{65}$$

Noting that $p_L(x) + p_H(x) = 1$, we obtain that

$$p_H(x) = \frac{f(x) - f(x_L)}{f(x_H) - f(x_L)}. \tag{66}$$

We now solve the second-order ODE (63). Multiplying Equation (63) by p_s, we obtain that

$$\epsilon f'' p_s - U'f'p_s = 0. \tag{67}$$

Applying Equation (31), we obtain that

$$\epsilon(f'p_s)' = \epsilon f'' p_s + \epsilon f' p_s' = 0. \tag{68}$$

Thus we can choose f so that

$$f'(y)p_s(y) \equiv 1. \tag{69}$$

Thus we have

$$f'(y) = \frac{1}{p_s(y)}. \tag{70}$$

Integrating the above equation, we obtain that

$$f(x) - f(x_L) = \int_{x_L}^{x} \frac{1}{p_s(y)} dy. \tag{71}$$

Substituting this result for Equation (66), we obtain that

$$p_H(x) = \frac{\int_{x_L}^{x} \frac{1}{p_s(y)} dy}{\int_{x_L}^{x_H} \frac{1}{p_s(y)} dy} = \frac{\int_{x_L}^{x} e^{\frac{1}{\epsilon}U(a,y)} dy}{\int_{x_L}^{x_H} e^{\frac{1}{\epsilon}U(a,y)} dy}. \tag{72}$$

From Equation (60), we easily see that $D(x)$ attains its maximum at a point x^* satisfying

$$p_H(x^*) = \frac{1}{2} + \frac{(\kappa_L - \kappa_H)\epsilon}{2(x_H - x_L)^2 \kappa_L \kappa_H}. \tag{73}$$

Applying the Laplace method, we see that

$$p_H(x) \to \begin{cases} 0 & \text{if } x < x_M; \\ 1 & \text{if } x > x_M, \end{cases} \tag{74}$$

as $\epsilon \to 0$. This shows that the maximum point x^* of $D(x)$ tends to x_M as $\epsilon \to 0$, that is,

$$\lim_{\epsilon \to 0} x^* = x_M. \tag{75}$$

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