Supporting Information
Ensemble Bayesian Analysis of Bistability in a Synthetic Transcriptional Switch

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Monte Carlo Bayesian inference

Using the mathematical model described in Box 1, we chose to explore the parameter space in the vicinity of the best fit to data through Monte Carlo Bayesian inference, as outlined in [1]. The experimental data shown in Figure 8A is used as the training set; other experimental data are reserved as the test set. The cost function is calculated as

$$E = \sum_{n=1}^{N} ([\mathrm{rA}]_{\mathrm{exp}}^{\mathrm{final},n} - [\mathrm{rA}]_{\mathrm{sim}}^{\mathrm{final},n})^2,$$

where the concentration of rA is measured at 210 min in μM scale both for the experiment and simulation (n: experiment number). The training set contained fifty experimentally measured [rA] values ($N = 50$). To estimate errors in experimental measurements, most experimental conditions were measured in duplicate. For certain experimental conditions — e.g. in the transition region of blue curve in Figure 8A — the duplicate measurements differed as much as 0.65 μM. We chose $\sigma = 0.25$ μM as a reasonable estimate of the standard deviation of repeated experimental measurements.

The kinetic model has a total of 11 parameters: five hybridization parameters (k_{TA}, k_{AI}, k_{rAI}, k_{TAI}, and k_{AIrA}), and six enzyme parameters ($K_{\mathrm{M,ON}}$, $K_{\mathrm{M,OFF}}$, $k_{\mathrm{cat,ON}}$, $k_{\mathrm{cat,OFF}}$, $K_{\mathrm{M,H}}$, and $k_{\mathrm{cat,H}}$). We used pre-specified bounds for these parameters as shown in Box 1. In order to minimize the effect of these widely separated scales and avoid numerical issues, we deal with the logarithms of the parameters for all our calculations — i.e., $\vec{\theta}$ is a vector of natural logs of rate constants.

Following the approaches of [1, 2], we want to obtain an ensemble parameter set to explore the model's behavior when all parameter combinations consistent with the available data are considered. First, consider the conditional probability $P(D|M(\vec{\theta}))$ that our model with parameter $\vec{\theta}$ would generate the observed data $D = \{Y_n\}$ given the initial condition $I_1, I_2, ..., I_p$. If we model repeatability variance as Gaussian random measurement errors σ, for each experimental time course Y_n, $P(Y_n|M(\vec{\theta})) = \frac{1}{\sqrt{2\pi}\sigma} exp\left(-\frac{(y(t_n,\vec{\theta})-Y_n)^2}{2\sigma^2}\right)$. Also, if we assume that each experiment is independent, by converting the product to a sum and substituting for our cost function, we find

$$\begin{aligned} P(D|M(\vec{\theta})) &= \prod_n \frac{1}{\sqrt{2\pi}\sigma} exp\left(-\frac{(y(t_n,\vec{\theta}) - Y_n)^2}{2\sigma^2}\right), \\ &\propto exp\left(-\frac{\sum_n (y(t_n,\vec{\theta}) - Y_n)^2}{2\sigma^2}\right), \\ &\propto exp\left(-\frac{E}{2\sigma^2}\right), \end{aligned}$$

where $Y_n = [\mathrm{rA}]_{\mathrm{exp}}^{\mathrm{final},n}$ and $y(t_n,\vec{\theta}) = [\mathrm{rA}]_{\mathrm{sim}}^{\mathrm{final},n}$. We can identify the formula above with a Boltzmann distribution with energy E and temperature $T = 2\sigma^2$ in units where Boltzmann's constant is unity.

Next, we would like to estimate the conditional probability $P(M(\vec{\theta})|D)$ of our model with parameter $\vec{\theta}$ given the observed data, using Bayesian inference. Since we assumed a uniform *a priori* distribution for model parameters, we find the same Boltzmann distribution as above for $P(M(\vec{\theta})|D)$.

$$\begin{aligned} P(M(\vec{\theta})|D) &= \frac{P(M(\vec{\theta})) \cdot P(D|M(\vec{\theta}))}{P(D)} \\ &\propto P(D|M(\vec{\theta})) \\ &\propto exp\left(-\frac{E}{2\sigma^2}\right) \end{aligned}$$

Thus, our task is to generate a thermal ensemble of parameter sets that is consistent with the Boltzmann distribution above using Metropolis criteria. We initiated the parameter set $\vec{\theta}$ as a random 11×1 vector within the prescribed bounds. For each iteration, we generated a random 11×1 vector of −1, 0, or +1 (with equal probabilities)

to decide whether to decrease, remain, or increase each parameter in the next step. The step size for each parameter was set as 1/100th of the range for that parameter in a log scale. If the simultaneous updates of 11 parameters resulted in lower cost function (i.e., $\Delta E < 0$), the updates are accepted. On the other hand, if the simultaneous updates of parameters resulted in higher cost function (i.e., $\Delta E > 0$), the updates are accepted with a probability of $P = e^{-\Delta E/T}$ following Metropolis criteria. Parameter values beyond the pre-specified bounds are considered as high energy states (i.e., $\Delta E \gg 0$) such that the updates resulting in parameter values exceeding bounds are not accepted. For physical plausibility, two additional bounds were implemented analogous to pre-specified bounds: $K_{\mathrm{M,ON}} < K_{\mathrm{M,OFF}}$ and $k_{\mathrm{cat,ON}} > k_{\mathrm{cat,OFF}}$. Time is incremented without regard to acceptance or rejection of parameter updates. This sampling procedure ensures that the number of neighboring states are the same even when some parameter values are at the upper or lower bounds. To summarize, the probability of moving to parameter space i from parameter space j is as follows.

$$\begin{aligned} P(i \leftarrow j) &= e^{-(E_i - E_j)/T} \text{ if } \Delta E = E_i - E_j > 0 \\ P(i \leftarrow j) &= 1 \quad \text{otherwise} \end{aligned}$$

At equilibrium, detailed balance requires $P(i \leftarrow j) \cdot P(j) = P(j \leftarrow i) \cdot P(i)$ such that

$$\frac{P(i)}{P(j)} = \frac{P(i \leftarrow j)}{P(j \leftarrow i)} = e^{-(E_i - E_j)/T}.$$

The Boltzmann distribution $P(i) = \frac{1}{Z} e^{-E_i/T}$ with $Z = \sum_i e^{-E_i/T}$ satisfies the detailed balance requirement at equilibrium. Therefore, accepting or rejecting the parameter updates based on the Metropolis criteria with $T = 0.125$ $(= 2\sigma^2)$ would ensure that our sample parameters should result, when equilibrium is established, in the Boltzmann distribution.

Following these sampling procedures, 440,000 iterations were performed for trajectories 1 through 12 starting from 12 random initial conditions. This took about two weeks of simulation using 12 cores on a personal computer. The time evolution of cost function associated with each sampling trajectory is shown (Figure S1). Cost functions associated with all trajectories converge to a low-error basin ($E = 2$ to 3 $\mu\mathrm{M}^2$) prior to 50,000 iterations. To assess whether there are multiple attractor basins for parameter values, we plotted parameter values for all 12 trajectories (excluding the initial 50,000 transient iterations) as histograms (Figure 9A). The parameter distributions for all 11 parameters for individual trajectories achieved similar ranges and shapes irrespective of their initial values (data not shown). Therefore, we conclude that the Monte Carlo sampling with Metropolis criteria has converged for these trajectories.

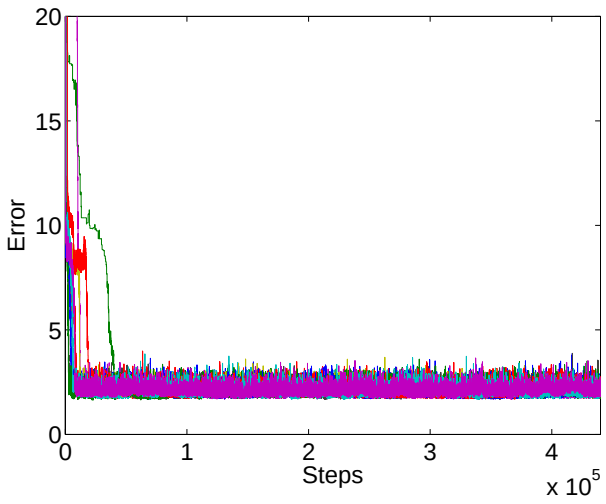

Figure S1: Error history for the 12 Monte Carlo sampling trajectories. For clarity, the initial transients with errors higher than 20 are not shown. Sampling has apparently reached equilibrium within 50,000 steps.

Our next goal is choosing an ensemble of parameter sets, $\{\vec{\theta}_j\}_{j=1}^{S}$, in such a way that they are independent of each other. These parameter sets cannot be randomly chosen from the above sampling trajectories because the

parameters at neighboring iterations may be highly correlated. To estimate the decorrelation time of parameter values so as to obtain independent samples, we analyzed the autocorrelation functions of parameter values between the 100,000th and 300,000th iteration steps averaged over 12 trajectories (Figure S2). If a random process X_t has time-independent mean and variance, the autocorrelation function is defined as follows:

$$R(\tau) = \frac{E[(X_t - E(X))(X_{t+\tau} - E(X))]}{Var(X)}.$$

To estimate autocorrelation function from sampling trajectories X_n of length L $(X_1, X_2, ..., X_L)$, we compute the following:

$$\hat{R}(l) = \sum_{n=1}^{L-l}(X_n - E(X))(X_{n+l} - E(X)),$$

for $l = 0, 1, 2, ..., L-1$, which is further normalized to have maximum value 1 at zero lag. Note that $\hat{R}(-l) = \hat{R}(l)$. For many parameters, the autocorrelation function quickly decays to zero — e.g., $K_{\mathrm{M,H}}$, $k_{\mathrm{cat,H}}$, and k_{TAI}. On the other hand, for some parameters such as $K_{\mathrm{M,ON}}$ and k_{AIrA}, for which the parameter values were poorly constrained in the histogram analysis, the averaged autocorrelation functions did not completely decay to zero even after 50,000 steps; typically, slight negative autocorrelations were observed for time lags longer than 50,000 steps possibly due to numerical undersampling issues. Nevertheless, all the autocorrelation functions cross zero at time lags of less than 50,000 steps. Thus, we used 50,000 iterations as the decorrelation time.

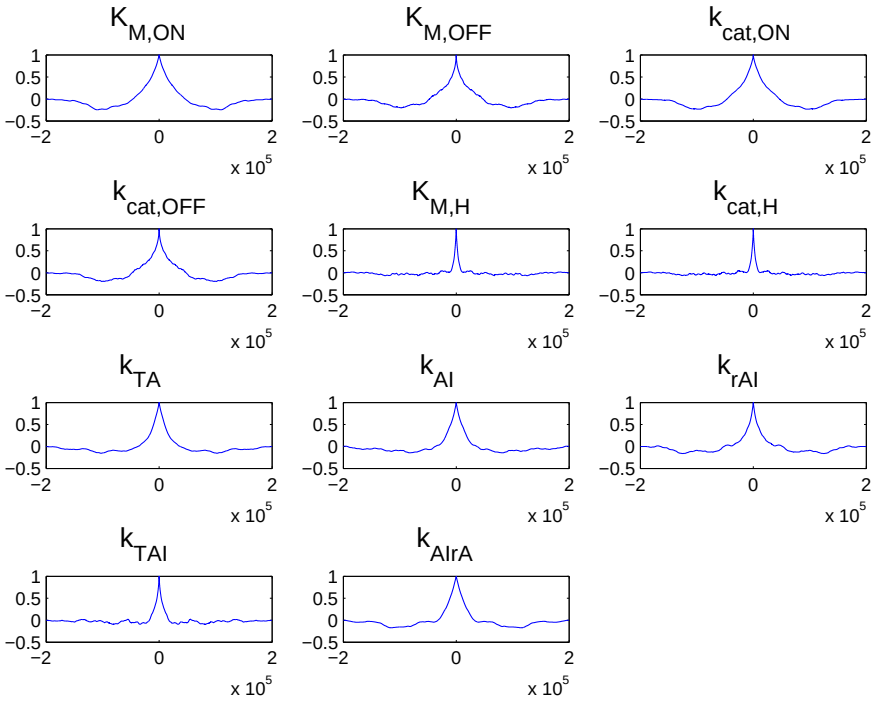

Figure S2: Autocorrelation functions for Monte Carlo sampling trajectories. The log-scale parameter values ($\vec{\theta}$) between the 100,000th and 300,000th iteration steps are plotted as autocorrelation functions averaged over 12 trajectories. Decorrelation time for each parameter can be estimated by measuring lag times beyond which the autocorrelation function is close to zero.

For sampling trajectories 1 through 12 (with 440,000 iterations each), we discarded the first 50,000 iterations (the transients) because not all the trajectories have reached low energy basin before 50,000 iterations; therefore, 390,000 iterations remain "usable" after removing the transients. One parameter set was selected per 50,000 iterations, resulting in 8 parameter sets per trajectory. This collection of parameter sets — 96 in total ($S = 96$) with E ranging from 2.03 to 2.43 $\mu\mathrm{M}^2$ — was used for simulation and compared with the experimental results (Figure 8).

To identify important parameter combinations that can explain the variability of training data and parameter inference, following the procedures outlined in previous studies [1, 2], one can start by constructing an empirical covariance matrix $\mathbf{\Theta}$ from the ensemble of parameters $\{\vec{\theta}_j\}_{j=1}^{S}$,

$$\mathbf{\Theta} = \langle (\vec{\theta} - \langle\vec{\theta}\rangle)(\vec{\theta} - \langle\vec{\theta}\rangle)^T \rangle,$$

where the angle brackets denote ensemble average. Consider $\vec{\theta}$ as Gaussian random vector of size 11×1, then its joint probability density function is given by:

$$P(\vec{\theta}) = \frac{1}{(2\pi)^{\frac{11}{2}}|\mathbf{\Theta}|^{\frac{1}{2}}} exp\left(-\frac{1}{2}(\vec{\theta} - \langle\vec{\theta}\rangle)^T \mathbf{\Theta}^{-1}(\vec{\theta} - \langle\vec{\theta}\rangle)\right),$$

where we used the empirical covariance matrix $\mathbf{\Theta}$. Also recall that $\vec{\theta}$ was sampled according to a Boltzmann distribution as follows:

$$P(\vec{\theta}) \quad = \quad P(M(\vec{\theta})|D) \propto exp\left(-\frac{E}{T}\right),$$

with $T = 2\sigma^2$. If we assume that the ensemble average $\langle\vec{\theta}\rangle$ corresponds to the minimum of the cost function E/T and that E/T should be approximately quadratic in the vicinity of the minimum, the Hessian H defined as the second derivatives of E/T ($H_{ij} = \frac{\partial^2(E/T)}{\partial\theta_i\partial\theta_j}$) can be used to approximate the shape of the cost surface:

$$\frac{E}{T} = \frac{E_{min}}{T} + \frac{1}{2}(\vec{\theta} - \langle\vec{\theta}\rangle)^T H(\vec{\theta} - \langle\vec{\theta}\rangle).$$

Comparing terms in $P(\vec{\theta})$, we can see that in this approximation $H = \mathbf{\Theta}^{-1}$ (cf. [3]). An eigenvalue decomposition of the inverse of the covariance matrix $\mathbf{\Theta}$ (called principal component analysis (PCA)) can be used to obtain information about soft and stiff modes analogous to using the Hessian H — i.e., eigenvectors corresponding to large eigenvalues of H (or small eigenvalues of $\mathbf{\Theta}$) indicate stiffness (little variability) of such parameter combinations. Mode spectrum and eigenvector projections are shown with eigenvalue-eigenvector correspondence indicated by the numbers 1–11 (Figure 9B).

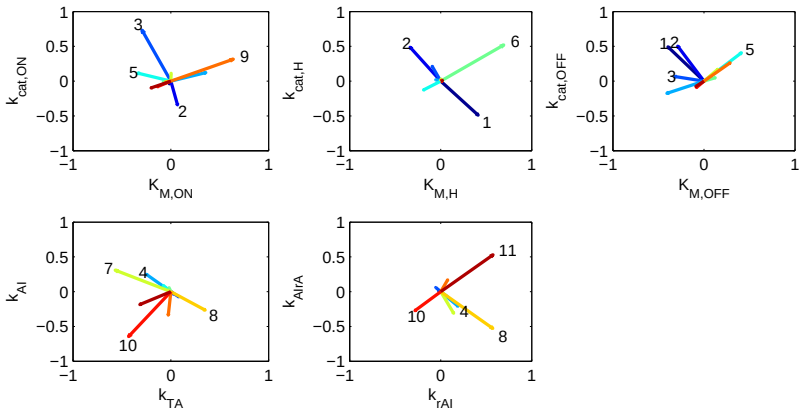

Figure S3: Eigenvectors from PCA of the inverse of the covariance matrix $\mathbf{\Theta}$ projected on paired parameter planes (cf. Figure 9B). Eigenvector-eigenvalue correspondence is indicated by the numbers 1–11: small numbers correspond to large eigenvalues (stiff modes) and large numbers correspond to small eigenvalues (soft modes). Note that zeros correspond to $\langle\vec{\theta}\rangle$ in the parameter space.

Here, we chose to explore the projections of eigenvectors on paired parameter planes (Figure S3); these parameter pairs are chosen based on whether their corresponding weights on eigenvectors are correlated. First, we note that planes of paired enzyme parameters typically contain small numbers (stiff modes) as compared to planes of paired hybridization parameters, indicating that enzyme parameters contribute to stiff modes significantly. Second, for any

given paired parameters, eigenvector projections with smaller numbers appear in the second and fourth quadrants as compared to projections with larger numbers that appear in the first and third quadrants — these parameter pairs change in opposite directions for stiff modes, while they change in the same direction for soft modes (cf. Figure 9D). More discussions on stiff and soft modes are found in the Results section.

References

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