

Supporting Appendix 3: Modeling Subgraph Dynamics

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Scenarios of Regulation by Small Metabolites

In the following, we describe the biochemical reactions and differential equations used to model a single regulatory interaction (SRI), in which an initially inactive transcription factor (TF), once bound by an activating small molecule, binds to the operator (promoter) region of the regulated gene. However, the signal bound TF could also be a repressor, and there are also cases when the small molecule inhibits operator binding rather than activating it. Considering these possibilities, there are four possible scenarios of regulation. These four scenarios, activation, repression, deactivation and derepression, are described below.

Assuming that one metabolite molecule per TF protein is enough to activate or inactivate operator binding, the following four scenarios exist:

Metabolite	Protein (TF) state	Effect of signal
Inducer	Inactive activator	Activation
Effector	Active activator	Deactivation
Inducer	Active repressor	Derepression
Effector	Inactive repressor	Repression

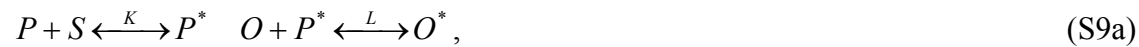
For each of these scenarios, we derive the transfer function, i.e., the ratio of operators that allow transcription O_A to the total operator concentration O_T :

$$f(S_X, P_X) = \frac{O_A}{O_T}. \quad (\text{S8})$$

The meaning of O_A depends on which scenario is discussed, because in the case of a repressor, unbound operators O allow for polymerase binding, while in the case of an activator, bound operators O^* will facilitate transcription initiation. We will denote the activator and repressor TF proteins unbound and bound by the signaling metabolite S by P and P^* , respectively. The equilibrium constant for metabolite-protein binding/unbinding is K , while the equilibrium constant for protein-operator binding/unbinding is L .

Next, we will deduce expressions for the transcription of genes which are not leaky (i.e., they are not transcribed without the activator bound or with the repressor bound), following a previously established modeling approach (1). The obtained expressions can be easily generalized to make genes leaky (see regulation by two TFs for an example).

Activation. The reactions in this case are:



for which the equilibrium constants are defined as:

$$K = \frac{P^*}{S P} \quad L = \frac{O^*}{P^* O}. \quad (\text{S10a})$$

The total operator concentration is:

$$O_T = O + O^* = O + LOP^* = O(1 + LP^*) \quad (\text{S11a})$$

From here, the ratio of unbound to total operator concentration can be found:

$$\frac{O}{O_T} = \frac{1}{1 + LP^*}$$

However, in this case, transcription initiation is facilitated by the bound operator, so the bound to total operator concentration is:

$$\frac{O^*}{O_T} = 1 - \frac{O}{O_T} = \frac{LP^*}{1 + LP^*}. \quad (\text{S12a})$$

The total protein concentration is (if we suppose that the number of protein bound to the operator is negligibly small):

$$P_T = P + P^* + O^* \approx P + P^* = P + KSP = P(1 + KS). \quad (\text{S13a})$$

From here, the free protein concentration is:

$$P \approx \frac{P_T}{1 + KS},$$

from which the ratio of metabolite-bound protein concentration is:

$$P^* = P_T - P \approx \frac{KS}{1 + KS} P_T. \quad (\text{S14a})$$

Substituting (S14a) into (S12a) yields:

$$f(S) = \frac{O^*}{O_T} = \frac{KLSP_T}{1 + KS(1 + LP_T)} \quad (\text{S15a})$$

Deactivation. The reactions in this case are:



for which the equilibrium constants are defined as:

$$K = \frac{P^*}{S P} \quad L = \frac{O^*}{P O}. \quad (\text{S10b})$$

The total operator concentration is:

$$O_T = O + O^* = O + LOP = O(1 + LP) \quad (\text{S11b})$$

From here, the ratio of unbound to total operator concentration can be found:

$$\frac{O}{O_T} = \frac{1}{1 + LP}$$

However, just as in the previous case, transcription initiation is facilitated by the bound operator, so the bound to total operator concentration is:

$$\frac{O^*}{O_T} = 1 - \frac{O}{O_T} = \frac{LP}{1 + LP}. \quad (\text{S12b})$$

The total protein concentration is (if we suppose that the number of protein bound to the operator is negligibly small):

$$P_T = P + P^* + O^* \approx P + P^* = P + KSP = P(1 + KS). \quad (\text{S13b})$$

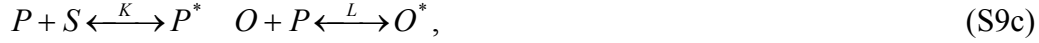
From here, the free protein concentration is:

$$P \approx \frac{P_T}{1 + KS}, \quad (\text{S14b})$$

Substituting (S14b) into (S12b) yields:

$$f(S) = \frac{O^*}{O_T} = \frac{LP_T}{1 + LP_T + KS} \quad (\text{S15b})$$

Derepression. The reactions in this case are:



for which the equilibrium constants are defined as:

$$K = \frac{P^*}{S P} \quad L = \frac{O^*}{P O}. \quad (\text{S10c})$$

The total operator concentration is:

$$O_T = O + O^* = O + LOP = O(1 + LP) \quad (\text{S11c})$$

In this case, transcription initiation is facilitated by the unbound operator O . The ratio of unbound to total operator concentration is from (S11C):

$$\frac{O}{O_T} = \frac{1}{1 + LP} \quad (\text{S12c})$$

The total protein concentration is (if we suppose that the number of proteins bound to the operator is negligibly small):

$$P_T = P + P^* + O^* \approx P + P^* = P + KSP = P(1 + KS). \quad (\text{S13c})$$

From here, the free protein concentration is:

$$P \approx \frac{P_T}{1 + KS}, \quad (\text{S14c})$$

Substituting (S14c) into (S12b) yields:

$$f(S) = \frac{O}{O_T} = \frac{1}{1 + LP_T + KS} \quad (\text{S15c})$$

Repression. The reactions in this case are:



for which the equilibrium constants are defined as:

$$K = \frac{P^*}{S P} \quad L = \frac{O^*}{P^* O}. \quad (\text{S10d})$$

The total operator concentration is:

$$O_T = O + O^* = O + LOP^* = O(1 + LP^*) \quad (\text{S11d})$$

From here, the ratio of unbound to total operator concentration can be found:

$$\frac{O}{O_T} = \frac{1}{1 + LP^*} \quad (\text{S12d})$$

The total protein concentration is (if we suppose that the number of proteins bound to the operator is negligibly small):

$$P_T = P + P^* + O^* \approx P + P^* = P + KSP = P(1 + KS). \quad (\text{S13d})$$

From here, the free protein concentration is:

$$P \approx \frac{P_T}{1 + KS},$$

from which the ratio of metabolite-bound protein concentration is:

$$P^* = P_T - P \approx \frac{KS}{1 + KS} P_T. \quad (\text{S14d})$$

Substituting (S14d) into (S12d) yields:

$$f(S) = \frac{O}{O_T} = \frac{1 + KS}{1 + KS(1 + LP_T)}. \quad (\text{S15d})$$

Sensitivity to External Signals

Next, we investigate the sensitivity, defined as the amplitude of concentration fluctuation of promoter available for transcription per amplitude of fluctuation in signal concentration ($\Delta f/\Delta S$), for each of the four scenarios of regulation listed above. For infinitesimally small signal fluctuations around the mean S , this quantity is equal to the derivative of the function $f(S)$ from formulas (S15a-d). After some calculations, we find the following expressions for sensitivity in the case of activation (S16a), deactivation (S16b), derepression (S16c) and repression (S16d):

$$\frac{\partial f}{\partial S} = \frac{KLP_T}{[1 + KS(1 + LP_T)]^2} \quad (\text{S16a})$$

$$\frac{\partial f}{\partial S} = -\frac{KLP_T}{(1 + LP_T + KS)^2} \quad (\text{S16b})$$

$$\frac{\partial f}{\partial S} = \frac{KLP_T}{(1 + LP_T + KS)^2} \quad (\text{S16c})$$

$$\frac{\partial f}{\partial S} = -\frac{KLP_T}{[1 + KS(1 + LP_T)]^2} \quad (\text{S16d})$$

Based on these formulas, the scenarios of activation and repression have the same sensitivity (if we do not regard the sign) to fluctuations of the signal S . Similarly, the scenarios of deactivation and derepression have the same sensitivity, if we ignore the sign. The different sign in the expression of sensitivity for activation and repression (and similarly, for deactivation and derepression) simply indicates that the outputs for these pairs of scenarios are inverted compared to each other.

Next, we group the four scenarios in two pairs: activation-repression and deactivation-derepression, and investigate how their sensitivity depends on the available sensor protein (TF) concentration. To this end, we calculate the derivative of sensitivity $\partial f/\partial S$ versus sensor protein concentration P_T , or $\partial^2 f/(\partial S \partial P_T)$. After some calculations, for the scenarios of activation and repression, we obtain:

$$\frac{\partial^2 f}{\partial S \partial P_T} = KL \frac{1 + KS(1 - LP_T)}{[1 + KS(1 + LP_T)]^3} \quad (\text{S17a})$$

from where the highest sensitivity to external signal fluctuations occurs at the optimal sensor protein concentration of

$$P_T^{OPT} = \frac{1 + KS}{KSL} = \frac{1}{L} \left(\frac{1}{KS} + 1 \right). \quad (\text{S18a})$$

Similarly, for the scenarios of deactivation and derepression, we obtain:

$$\frac{\partial^2 f}{\partial S \partial P_T} = KL \frac{1 + KS - LP_T}{[1 + KS + LP_T]^3}, \quad (\text{S17b})$$

from where the optimal sensor TF concentration, allowing for the highest sensitivity, is

$$P_T^{OPT} = \frac{1 + KS}{L}. \quad (\text{S18b})$$

Therefore, for every signal concentration, there is an optimal sensor TF concentration which maximizes the amplitude of fluctuations at the output. As input signal concentrations often

appear due to environmental changes, producing a jump from 0 to a finite concentration value, it is important to find the optimal sensor TF concentrations for vanishingly small average signal concentrations. Based on formulas (S18a) and (S18b) we find that these optimal sensor TF concentration values are infinite for activation-repression, and $1/L$ for deactivation-derepression. This indicates that for activation and repression, the higher the sensor TF concentration, the higher the sensitivity to signals of small amplitude. This is illustrated in Fig. 41 where we show the amplitude of fluctuations at the output of a SRI for several different average signal concentrations. For deactivation and derepression, there is an optimal value of the sensor concentration ($1/L$) above which the sensitivity decreases.

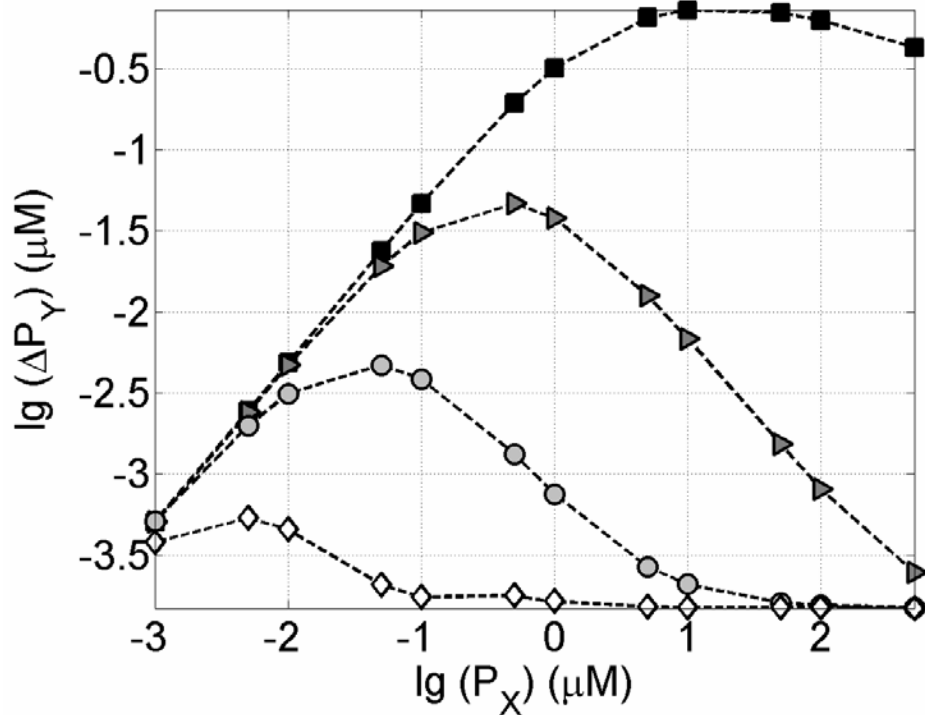


Fig. 41. Amplitude of fluctuations at the output of an SRI as a function of sensor TF concentration, for various average signal concentrations: 0.01 μM (black squares); 0.1 μM (dark gray triangles); 1 μM (light gray circles); 10 μM (white diamonds).

Regulation by Two Transcription Factors

To simulate CNVs (i.e., to model the combined action of two TFs converging on the same promoter), we used an approach similar to the one used for the SRI, but we augmented the set of reactions (S9-S15) as follows



In reactions (S19-S20), the small molecules S_X and S_Y bind and activate the sensor TF proteins P_X and P_Y into P_X^* and P_Y^* , respectively. In reactions (S21-S22), the activated proteins P_X^* and P_Y^* bind to the free operator O and form the complexes O_X and O_Y , respectively. Finally, in reactions (S23-S24), P_Y^* binds to O_X and P_X^* binds to O_Y to form the complexes O_{XY} and O_{YX} , respectively.

The total operator concentration is given by the sum

$$O_T = O + O_X + O_Y + O_{XY} + O_{YX}, \quad (\text{S25})$$

which, after using the definitions for the equilibrium coefficients L_X , L_Y , M_X and M_Y becomes

$$O_T = O[1 + L_X P_X^* + L_Y P_Y^* + P_X^* P_Y^* (L_X M_X + L_Y M_Y)]. \quad (\text{S26})$$

The total sensor protein concentration P_X is given by

$$P_X^T = P_X + P_X^* + O_X + O_{XY} + O_{YX}, \quad (\text{S27})$$

which, after assuming that the concentration of protein bound to the operator is small, simplifies to

$$P_X^T \approx P_X + P_X^* = P_X (1 + K_X S_X), \quad (\text{S28})$$

from where the active or inactive protein concentration can be calculated.

Depending on the combinatorial logic of transcription (2), an arbitrary subset of sensor TF-operator complexes from $(O, O_X, O_Y, O_{XY}, O_{YX})$ can be chosen to allow mRNA polymerase binding and transcription initiation. For example, the AND-type logic corresponds to the case when P_R only binds and initiates transcription from complexes O_{XY} and O_{YX} (see the next section). OR-type logic can be modeled by allowing P_R to bind and initiate transcription from O_{XY} , O_{YX} , O_X and O_Y but not to the free operator O . Binding of P_R to O_X or O_Y , but not to O , O_{XY} , or O_{YX} results in XOR logic, and so on. Intermediate logic can be modeled by associating various P_R binding and complex-opening reaction constants with various states of the operator (see below). Repression by one or both sensor TFs can be modeled similarly to activation. Below we list the formulas for operator concentration with various combinations of TFs bound to it, by listing the values Z_0 , Z_X , Z_Y , Z_{XY} , Z_{YX} , and Z , which are proportional to the concentration of O , O_X , O_Y , O_{XY} , O_{YX} and O_T , respectively.

Case 1. P_X^* and P_Y^* Bind to the Operator.

$$Z_0 = (1 + K_X S_X)(1 + K_Y S_Y); \quad (\text{S29a})$$

$$Z_X = K_X L_X S_X P_X^T (1 + K_Y S_Y); \quad (\text{S30a})$$

$$Z_Y = K_Y L_Y S_Y P_Y^T (1 + K_X S_X); \quad (\text{S31a})$$

$$Z_{XY} + Z_{YX} = K_X K_Y (M_X L_X + M_Y L_Y) S_X S_Y P_X^T P_Y^T; \quad (\text{S32a})$$

$$Z = Z_0 + Z_X + Z_Y + Z_{XY} + Z_{YX}. \quad (\text{S33a})$$

The ratio of operator concentration that allows transcription can be calculated according to the desired logic. For example, to obtain AND logic, the ratio of operator concentration that allows transcription is

$$f(S_X, S_Y) = \frac{O^*}{O_T} = \frac{O_{XY} + O_{YX}}{O_T} = \frac{Z_{XY} + Z_{YX}}{Z} = \frac{Z_{XY} + Z_{YX}}{Z_0 + Z_X + Z_Y + Z_{XY} + Z_{YX}} \quad (\text{S34a}).$$

The output of a CNV as a function of input signal concentrations S_X and S_Y can be seen in Fig. 42. We simulated signal combination by CNV subgraphs corresponding to genes with two regulators binding near their promoter. In previous studies highly simplified models of CNVs have been modeled as Boolean logic gates, implementing various logic functions, i.e., AND, OR, XOR, etc. (3). Yet, the combination of transcriptional inputs is often more complicated than allowed by simple Boolean logic gates (4). For example, transcription from the Y-node of a CNV (Fig. 42) with Boolean AND logic would be initiated only if the concentration of both TFs X_1 and X_2 are above certain thresholds, resulting in two plateaus when the concentration of Y is plotted as a function of the concentrations of TFs X_1 and X_2 . However, for the very few experimentally tested CNVs (4), four different plateaus appear when the homeostatic concentration of protein Y is plotted as a function of concentrations of TFs X_1 and X_2 , corresponding to both X_1 and X_2 absent, only X_1 , only X_2 , and both X_1 and X_2 present in high concentration, respectively. As the four plateaus in Fig. 42 indicate, our model is able to reproduce the non-Boolean nature of combined transcriptional inputs.

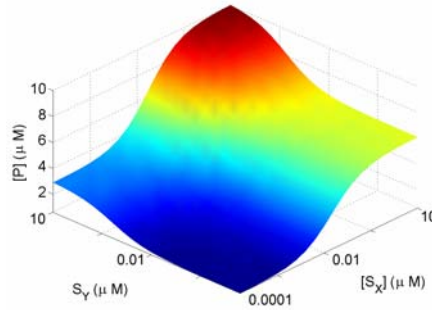


Fig. 42. Combination of two input signals by a CNV.

Case 2. P_X^* and P_Y Bind to the Operator.

$$Z_0 = (1 + K_X S_X)(1 + K_Y S_Y); \quad (\text{S29b})$$

$$Z_X = K_X L_X S_X P_X^T (1 + K_Y S_Y); \quad (\text{S30b})$$

$$Z_Y = L_Y P_Y^T (1 + K_X S_X); \quad (\text{S31b})$$

$$Z_{XY} + Z_{YX} = K_X (M_X L_X + M_Y L_Y) S_X P_X^T P_Y^T; \quad (\text{S32b})$$

$$Z = Z_0 + Z_X + Z_Y + Z_{XY} + Z_{YX}. \quad (\text{S33b})$$

Case 3. \mathbf{P}_X and \mathbf{P}_Y^* Bind to the Operator.

$$Z_0 = (1 + K_X S_X)(1 + K_Y S_Y); \quad (\text{S29c})$$

$$Z_X = L_X P_X^T (1 + K_Y S_Y); \quad (\text{S30c})$$

$$Z_Y = K_Y L_Y S_Y P_Y^T (1 + K_X S_X); \quad (\text{S31c})$$

$$Z_{XY} + Z_{YX} = K_Y (M_X L_X + M_Y L_Y) S_Y P_X^T P_Y^T; \quad (\text{S32c})$$

$$Z = Z_0 + Z_X + Z_Y + Z_{XY} + Z_{YX}. \quad (\text{S33c})$$

Case 4. \mathbf{P}_X and \mathbf{P}_Y Bind to the Operator.

$$Z_0 = (1 + K_X S_X)(1 + K_Y S_Y); \quad (\text{S29d})$$

$$Z_X = L_X P_X^T (1 + K_Y S_Y); \quad (\text{S30d})$$

$$Z_Y = L_Y P_Y^T (1 + K_X S_X); \quad (\text{S31d})$$

$$Z_{XY} + Z_{YX} = (M_X L_X + M_Y L_Y) P_X^T P_Y^T; \quad (\text{S32d})$$

$$Z = Z_0 + Z_X + Z_Y + Z_{XY} + Z_{YX}. \quad (\text{S33d})$$

Modeling Transcription

Empirically, the rate of transcription per promoter has been described (5), as:

$$V_R = \frac{1}{D_T} \frac{dR_+}{dt} = \frac{V_R^{MAX} P_R}{K_R + P_R}, \quad (S34)$$

where V_R^{MAX} is the maximum initiation rate, P_R is the concentration of free mRNA polymerase, D_T is the total promoter concentration available for transcription, and K_R is a promoter-specific constant. Next, we will approximate the empirical constants V_R^{MAX} and K_R by using a simple model for transcription.

Although more complicated models have been proposed (5-7), for the sake of simplicity we model transcription by using a two-step reaction scheme proposed by McClure (8,9), consisting of mRNA polymerase reversibly binding to the promoter region on the DNA, and the nearly irreversible opening of the polymerase-promoter complex to form an open complex which is available for transcription initiation. In the following, we consider three biochemical reactions to model transcription, corresponding to the following steps in transcription initiation:

1. The mRNA polymerase P_R binds to the DNA (D) and closed DNA - RNA polymerase complex D_C is formed:



The equilibrium constant K_{RC} associated with this reaction is defined as $K_{RC} = \frac{k_{RC}^+}{k_{RC}^-}$.

2. D_C is (nearly) irreversibly transformed into the open complex D_O :



3. Transcription is initiated and mRNA (R) is produced with a rate k_{RT} and delay τ_R :



The system of differential equations associated with reactions (S35-S37) is:

$$\frac{dD}{dt} = -k_{RC}^+ P_R D + k_{RC}^- D_C \quad (S38a)$$

$$\frac{dD_C}{dt} = k_{RC}^+ P_R D - k_{RC}^- D_C - k_{RO}^+ D_C + k_{RO}^- D_O \quad (S38b)$$

$$\frac{dD_O}{dt} = k_{RO}^+ D_C - k_{RO}^- D_O - k_{RT} D_O \quad (S38c)$$

$$\frac{dR}{dt} = k_{RT} D_O (t - \tau_R) e^{-\mu \tau_R} - \gamma R \quad (S38d)$$

From equation (S38c), by using the quasi-equilibrium approximation, we get:

$$D_O = \frac{k_{RO}^+}{k_{RT} + k_{RO}^-} D_C. \quad (S39)$$

Using the quasi-equilibrium approximation for equation (S38b), we obtain:

$$D_O (k_{RC}^- + k_{RO}^+) = k_{RC}^+ P_R (D_T - D_O - D_C) + k_{RO}^- D_O, \quad (S40)$$

where D_T is the total concentration of DNA available for transcription with the rates given in reactions (S35-S37). After rearranging the equation, and substituting for D_O , we obtain for the

concentration of closed complex:

$$D_C = \frac{k_{RC}^+ P_R D_T (k_{RT} + k_{RO}^-)}{(k_{RT} + k_{RO}^-)(k_{RC}^- + k_{RC}^+ P_R) + k_{RO}^+ (k_{RC}^+ P_R + k_{RT})}. \quad (\text{S41})$$

Using equation (S39), the concentration of open complex can be calculated:

$$D_O = \frac{k_{RC}^+ k_{RO}^+ P_R D_T}{(k_{RT} + k_{RO}^-)(k_{RC}^- + k_{RC}^+ P_R) + k_{RO}^+ (k_{RC}^+ P_R + k_{RT})}. \quad (\text{S42})$$

From here, the differential equation describing mRNA synthesis and decay is:

$$\frac{dR}{dt} = \frac{k_{RC}^+ k_{RO}^+ k_{RT} P_R D_T (t - \tau_R) e^{-\mu \tau_R}}{(k_{RT} + k_{RO}^-)(k_{RC}^- + k_{RC}^+ P_R) + k_{RO}^+ (k_{RC}^+ P_R + k_{RT})} - \gamma_R R. \quad (\text{S43})$$

From here, we can identify the empirical constants V_R^{MAX} and K_R from equation (S34) as:

$$V_R^{MAX} = \frac{k_{RO}^+ k_{RT}}{k_{RT} + k_{RO}^+ + k_{RO}^-}; \quad (\text{S44})$$

$$K_R = \frac{k_{RC}^- (k_{RT} + k_{RO}^-) + k_{RO}^+ k_{RT}}{k_{RC}^+ (k_{RT} + k_{RO}^- + k_{RO}^+)}. \quad (\text{S45})$$

According to the literature (8), the opening of the DNA-RNA polymerase complex is a nearly irreversible reaction. Also, the rate of transcription is higher than the rate of closed complex opening. Therefore, it is reasonable to assume:

$$k_{RT} \gg k_{RO}^+ \gg k_{RO}^-.$$

Based on these assumptions, the expressions for V_R^{MAX} and K_R are simplified to the following:

$$V_R^{MAX} \approx k_{RO}^+; \quad (\text{S46})$$

$$K_R = \frac{k_{RO}^+ + k_{RC}^-}{k_{RC}^+}. \quad (\text{S47})$$

As indicated in the literature, typically $k_{RO}^+ \ll k_{RC}^-$, and therefore the expressions for can be simplified once more:

$$V_R^{MAX} \approx k_{RO}^+; \quad (\text{S48})$$

$$K_R \approx \frac{k_{RC}^-}{k_{RC}^+} = \frac{1}{K_{RC}}. \quad (\text{S49})$$

Modeling Translation

Although translation is a complex process, involving many molecules and reaction steps (10-12), the rates of which are currently being determined (13,14), Draper describes translation kinetics in parallel with transcription kinetics (15). Therefore, to model translation, we use the differential equation:

$$\frac{dP}{dt} = \frac{V_p^{MAX} R P_p e^{-\mu \tau_p}}{K_p + P_p} - \gamma_p P, \quad (S50)$$

with V_p^{MAX} and K_p approximated as for translation:

$$V_p^{MAX} \approx k_{PO}^+; \quad (S51)$$

$$K_p \approx \frac{k_{PC}^-}{k_{PC}^+} = \frac{1}{K_{PC}}. \quad (S52)$$

Estimation of Parameters Used in the Simulations

General Constants.

E. coli cell volume

$$V = 8 \times 10^{-16} \text{ liters (16,17).}$$

Growth rate

$$\mu = 1.3 \times 10^{-2} \text{ min}^{-1} \text{ (16,17).}$$

Free mRNA polymerase concentration

$$P_R = 2.6 \text{ } \mu\text{M (16,17).}$$

Ribosomal subunit concentration

$$P_P = 2.9 \text{ } \mu\text{M (16,17).}$$

Length of *E. coli* ORFs (number of nucleotides in transcript)

$$N_R \approx 930 \pm 635 \text{ nt} = 1000 \text{ nt}$$

(Blattner et al., 1997; Gerdes et al., 2003)

Length of *E. coli* polypeptides

$$N_P = N_R/3 = 310 \pm 208 \text{ (18,19).}$$

Speed of transcription elongation

$$V_R = 3000 \text{ nt/min (20).}$$

Speed of translation elongation

$$V_P = 1000 \text{ aa/min} = 3000 \text{ nt/min (20).}$$

Time needed to transcribe an ORF

$$T_R = N_R/V_R = 1/3 \text{ min}$$

Time needed to translate the mRNA

$$T_P = N_P/V_P = 1/3 \text{ min}$$

Rate of mRNA degradation

$$\gamma_R = k_D D + \mu$$

$$k_D D = 0.6 \text{ min}^{-1} \text{ (0.5} \div \text{1 min}^{-1}\text{): degradation due to RNAses (16,17).}$$

Rate of protein degradation

$$\gamma_P = \gamma'_P + \mu$$

$$\gamma'_P = 0 \div 0.6 \text{ min}^{-1}\text{: degradation due to proteases (16,17).}$$

Constants Related to Signaling.

Signal concentration

$$S_A = 0.01 \div 10 \text{ } \mu\text{M} \text{ (16,17).}$$

Substrate-sensor association constant

$$K = 0.001 \div 1 \text{ } \mu\text{M} \text{ (16,17).}$$

Sensor-promoter association constant

$$L, M = 1,000 \div 100,000 \text{ } \mu\text{M} \text{ (16,17).}$$

Sensor protein concentration

$$P_S = 0.01 \div 10.0 \text{ } \mu\text{M} \text{ (16,17).}$$

Total operator concentration

$$O_T = 3.32e-3 \text{ } \mu\text{M} \text{ (16,17).}$$

Constants Related to Transcription Initiation.

Polymerase binding eq. coefficient

$$K_{RC} = 1 \div 1000 \text{ } \mu\text{M}^{-1} \text{min}^{-1} \text{ (5,8).}$$

Maximum speed of transcription

$$k_{RO}^+ = V_R^{MAX} = 6e-2 \div 60 \text{ min}^{-1} \text{ (5).}$$

Constants Related to Translation Initiation.

Ribosome binding eq. coefficient

$$K_P = 10 \div 100 \text{ } \mu\text{M}^{-1} \text{ (15).}$$

Maximum speed of translation initiation

$$k_{OP}^+ = V_P^{MAX} = 6 \div 20 \text{ min}^{-1} \text{ (15)}$$

Robustness of the Results to Simulation Parameters

We compared the amplitude of the fluctuations at the output node of the FFL to those at the output node of CAS, using the same set of parameters for both subgraphs. The parameters were randomly chosen from the ranges listed above. As Fig. 43 indicates, in most cases, the amplitude of fluctuations at the output node of the FFL were much larger than at the output node of CAS.

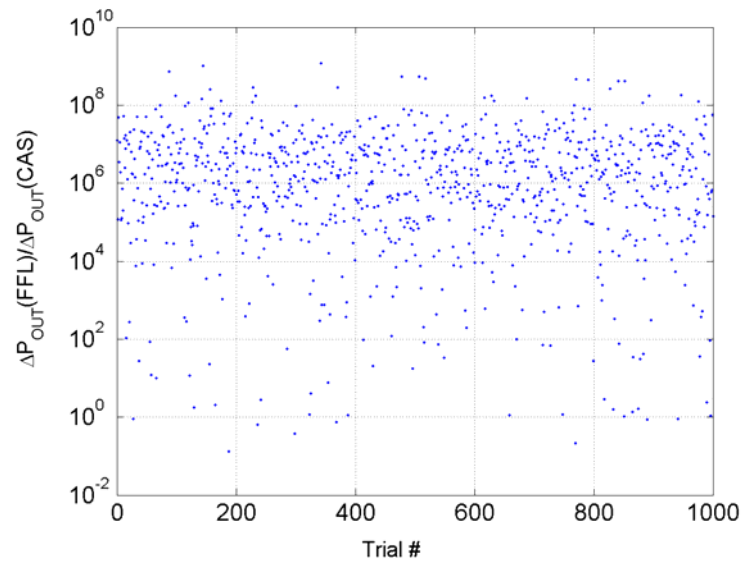


Fig. 43. CAS is a stronger low-pass filter than FFL (robustness to simulation parameters).

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