Noise propagation and signaling sensitivity in biological networks: A role for positive feedback loops

Supplementary Text

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I. GENERAL EXPRESSION FOR SUSCEPTIBILITY

We consider a network that is composed of $N$ nodes that respond to an upstream input signal $n_0$. The system of equations that describe the kinetic response of the network is given by:

$$\frac{dn_i}{dt} = J^+_i(n_0, n_1, \ldots, n_N) - J^-_i(n_0, n_1, \ldots, n_N), \quad (1)$$

where $J^+_i$ and $J^-_i$ are the total fluxes of production and elimination of $n_i$. At a stable steady state the equations given in (1) equal zero, hence

$$\langle J^+_i \rangle = \langle J^-_i \rangle = \langle J_i \rangle. \quad (2)$$

The triangular brackets denote the steady state average.

The susceptibility of component $j$ to changes in the input $n_0$ is

$$s_j = \frac{\langle n_0 \rangle}{\langle n_j \rangle} \frac{d \langle n_j \rangle}{d \langle n_0 \rangle} = \frac{d \ln \langle n_j \rangle}{d \ln \langle n_0 \rangle}. \quad (3)$$

Note that in the definition of susceptibility the derivatives are full derivatives, describing the changes in $\langle n_j \rangle$ after all the components have adjusted to the new steady state. Using the chain rule we differentiate Equation (1) at the steady state with respect to $n_0$, and then multiply by $\langle n_0 \rangle / \langle J_i \rangle$:

$$\langle n_0 \rangle \left( \frac{\partial \langle J^+_i \rangle}{\partial \langle n_0 \rangle} - \frac{\partial \langle J^-_i \rangle}{\partial \langle n_0 \rangle} \right) +$$

$$\langle n_1 \rangle \left( \frac{\partial \langle J^+_i \rangle}{\partial \langle n_1 \rangle} - \frac{\partial \langle J^-_i \rangle}{\partial \langle n_1 \rangle} \right) \langle n_0 \rangle \frac{d \langle n_1 \rangle}{\langle n_1 \rangle} + \ldots$$

$$\langle n_N \rangle \left( \frac{\partial \langle J^+_i \rangle}{\partial \langle n_N \rangle} - \frac{\partial \langle J^-_i \rangle}{\partial \langle n_N \rangle} \right) \langle n_0 \rangle \frac{d \langle n_N \rangle}{\langle n_N \rangle} = 0. \quad (4)$$

Finally using Equation (2), we obtain

$$H_{i0} + H_{i1}s_1 + \ldots + H_{iN}s_N = 0, \quad (5)$$

where the $s_j$ terms are the susceptibility of each individual component in the network (Equation (3)) and the $H_{ij}$ terms are the reaction flux elasticities, as defined by Paulsson [8, 9]

$$H_{ij} = -\frac{\langle n_j \rangle}{\langle J_i \rangle} \left( \frac{\partial \langle J^+_i \rangle}{\partial \langle n_j \rangle} - \frac{\partial \langle J^-_i \rangle}{\partial \langle n_j \rangle} \right)$$

$$= \frac{\partial \ln \langle J^+_i \rangle / \langle J^-_i \rangle}{\partial \ln \langle n_j \rangle}. \quad (6)$$

In matrix form:

$$H\vec{s} = -\vec{k}, \quad (7)$$

where the terms of $H$ are $H_{ij}$ and the terms of $\vec{k}$ are $H_{i0}$.

II. EXPRESSION FOR NOISE AMPLIFICATION

We derived an analytical expression for noise amplification in a three component system using Paulsson’s FDT-based approach [9]. Using this method the normalized (standard deviation over mean) noise components are given by the matrix equation

$$M\eta + \eta M^T + D = 0, \quad (8)$$

where the matrix $\eta$ contains the normalized noise terms.
\[ \eta = \begin{pmatrix} \eta_0^2 & \eta_{01} & \eta_{02} \\ \eta_{10} & \eta_1^2 & \eta_{12} \\ \eta_{20} & \eta_{21} & \eta_2^2 \end{pmatrix}. \]  
(9)

The matrix \( M \) describes the effect that each component exerts on its neighbours

\[ M = \begin{pmatrix} -1/\tau_0 & 0 & 0 \\ -H_{10}/\tau_1 & -H_{11}/\tau_1 & -H_{12}/\tau_1 \\ -H_{20}/\tau_2 & -H_{21}/\tau_2 & -H_{22}/\tau_2 \end{pmatrix}. \]  
(10)

The \( H_{ij} \) terms are the elasticities (Equation (6)) and the \( \tau_i \) parameters are the degradation time scales for each component. Note that the first row of \( M \) contains only one non-zero term, because components 1 and 2 do not affect the input.

The matrix \( D \) is composed of the noise sources. We assume that most of the noise originates from the input \( n_0 \), and hence \( D \) has only one non-zero term:

\[ D = \begin{pmatrix} 2\eta_0^2/\tau_0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}. \]  
(11)

Equation (8) was solved symbolically for \( \eta_2^2 \) using Maple (Maplesoft). The solution was used to substitute different values of \( H_{ij} \) and characterize the susceptibility-noise amplification relation for each network architecture.

**III. CRITERIA FOR STABILITY**

In this section we derive criteria for stability of a system with one input node, \( n_0 \), and two other components \( n_1, n_2 \). The differential equations for this system are

\[ \frac{dn_1}{dt} = J_1^+(n_1, n_2|n_0) - J_1^-(n_1, n_2|n_0), \]  
(12a)

and

\[ \frac{dn_2}{dt} = J_2^+(n_1, n_2|n_0) - J_2^-(n_1, n_2|n_0). \]  
(12b)

We distinguish \( n_0 \) from \( n_1 \) and \( n_2 \) because it can affect \( n_1 \) and \( n_2 \) but is not affected by them, and is therefore treated as a parameter of the system. The Jacobian for this system of equations is given by

\[ A = \begin{pmatrix} \frac{\partial J_1^+}{\partial n_1} & \frac{\partial J_1^-}{\partial n_1} \\ \frac{\partial J_2^+}{\partial n_1} & \frac{\partial J_2^-}{\partial n_1} \\ \frac{\partial J_1^+}{\partial n_2} & \frac{\partial J_1^-}{\partial n_2} \\ \frac{\partial J_2^+}{\partial n_2} & \frac{\partial J_2^-}{\partial n_2} \end{pmatrix}. \]  
(13)

and the eigenvalues of the Jacobian conform to

\[ (A_{11} - \lambda)(A_{22} - \lambda) - A_{12}A_{21} = 0. \]  
(14)

For the system to have a stable solution the eigenvalues must be negative. Therefore, the components of the Jacobian must satisfy

\[ A_{11} + A_{22} < 0, \]  
(15a)

and

\[ A_{11}A_{22} - A_{12}A_{21} > 0. \]  
(15b)

The Jacobian is related to the elasticities through

\[ A_{ij} = H_{ij}/n_j \]  
(Equation (6)). With this, the criteria for stability becomes

\[ H_{11}/\tau_1 + H_{22}/\tau_2 > 0, \]  
(16a)

and

\[ H_{11}H_{22} - H_{12}H_{21} > 0. \]  
(16b)

with the \( \tau_i \) terms defined as the time scales of the reactions \( \tau_i = n_i/J_i^0 \).

**IV. DESIGN OF PARAMETER SCREEN**

Each network is defined by the connections between its nodes and by the sign of the connections, i.e. activation or repression. We require a connection from \( n_0 \) to \( n_1 \) and from \( n_1 \) to \( n_2 \). Each of these connections can either be repressing or activating. There are four additional possible connections in the network (\( n_0 \rightarrow n_2, n_1 \rightarrow n_2, n_1 \rightarrow n_1, n_2 \rightarrow n_2 \)) which can be repressing, activating or non-existing. Thus we investigated \( 2^2 \times 3^4 = 324 \) specific circuits.

If node \( i \) is affected by node \( j \) then their interaction is captured by the equation

\[ \frac{dn_i}{dt} = J_i^+(\ldots, n_j, \ldots) - J_i^- (\ldots, n_j, \ldots). \]  
(17)

To calculate the susceptibility and noise properties of a specific network some information must be provided on the magnitude of the fluxes \( J_i^+ \) and \( J_i^- \) near the steady state, specifically their elasticities (see Sections I and II, and Equation (6)).

For the purposes of the screen we assume that the components undergo first order degradation, \( \partial \ln (J_i^-)/\partial \ln (n_j) \). With this assumption the effect of one component on the other is captured by the synthesis elasticity, defined as the relative change in the steady state transcription rate of \( n_i \) due to a small percent change in the level of \( n_j \).
FIG. 1: All three-node circuits we investigated were sorted into groups according to decreasing fraction of stable parameter sets out of all parameters sets (gray line). Within each group the networks were sorted according to the fraction of highly sensitive low-noise parameter sets out of all stable sets (blue line). The results are shown for a sampling range (A) \(0 < |S_{ij}| < 2\) and (B) \(0 < |S_{ij}| < 10\).

The synthesis elasticities are related to the overall reaction elasticities through
\[
H_{ii} = \frac{1}{S_{ii}} \quad \text{and} \quad H_{ij} = S_{ij}.
\]

We chose to work with the synthesis elasticities because a positive \(S_{ij}\) would always indicate that \(n_i\) enhances \(n_j\) (even when \(i = j\)) and vice versa (assuming first order degradation). In this framework the sensitivity of the interaction between \(i\) and \(j\) is determined through the absolute value \(|S_{ij}|\) and the nature of the interaction (activation or repression) is described by the sign of the interaction arrow of the network. This makes the sampling procedure of the parameter space much simpler. Furthermore, the synthesis elasticities have an intuitive physical meaning – they are proportional to the Hill coefficient of transcription factor binding and they decrease as the saturation increases.

To assess the susceptibility-noise amplification behavior of each network we considered many (20,000) positive values for \(S_{ij}\). The sampling distribution of \(S_{ij}\) could, in principle, have an effect on the results. To generate Fig. 2 of the main text we sampled \(S_{ij}\) from a uniform distribution between zero and four (chosen arbitrarily). Here we test the effect of the sampling range on the results. Supplementary Fig. 1 presents the fraction of stable parameter sets and the fraction of high-susceptibility low-noise parameters sets, for each network in the screen. The figure is presented for two sampling ranges, \(0 < |S_{ij}| < 2\) and \(0 < |S_{ij}| < 10\). The sampling range affects the stability as well as the fraction of high-susceptibility low-noise parameter sets. Nevertheless, regardless of the sampling range, all circuits with perfect stability do not display high susceptibility and low noise. Furthermore, the networks that exhibit the best noise properties (highest fraction of parameter sets with low noise amplification at a given susceptibility) contain only positive feedback loops (a combination of a coherent feed-forward element in addition to three positive feedback loops is also possible in the sampling range \(0 < |S_{ij}| < 10\), possibly because the noise buffering capacity of the three positive feedback loops overwhelms the slight increase in noise due to the feed-forward). Hence, changing the sampling range of the parameter screen does not impact the qualitative conclusions from the screen.

In the design of the screen we also set the time scales of all components of the networks equal to one. A choice of different values for the time scales alters the slope of the linear relation between noise amplification and susceptibility for linear networks (Supplementary Fig. 2a). Nonetheless, the fraction of parameter sets that lie above or below this linear line is virtually unaffected by the choice of the time constants (Supplementary Fig. 2b).
V. DERIVATION OF NOISE AND SUSCEPTIBILITY FOR A TWO COMPONENT SYSTEM

The noise properties of a two component circuit were derived by Paulsson [8, 9] using Fluctuation Dissipation Theorem [3]. For the sake of completeness we bring a similar derivation based on Frequency Domain Analysis [7, 12].

A. Solution neglecting intrinsic noise

We consider the simplest gene network: a two component system with an input $n_0$ and output $n_1$. We derive the susceptibility of $n_1$ to changes in $n_0$, and the magnitude of noise that propagates from $n_0$ to $n_1$. The system is described by a single differential equation

$$\frac{dn_1}{dt} = J^+(n_0, n_1) - J^-(n_0, n_1), \tag{19}$$

where $J^+$ is flux of generation of $n_1$, and $J^-$ is the flux of elimination. Typically, the elimination term will follow first order kinetics $J^-(n_0, n_1) = \text{const} \times n_1$, but we will consider the more general case. The average steady state levels of $n_0$ and $n_1$ are given by $\langle n_0 \rangle$ and $\langle n_1 \rangle$. To find the change in $n_1$ steady state levels due to a small change in $n_0$, we repeat the steps in Section I, and differentiate Equation (19) with respect to $n_0$ at the steady state:

$$\left( \frac{\partial J^+}{\partial \langle n_0 \rangle} - \frac{\partial J^-}{\partial \langle n_0 \rangle} \right) + \left( \frac{\partial J^+}{\partial \langle n_1 \rangle} - \frac{\partial J^-}{\partial \langle n_1 \rangle} \right) \frac{d}{dn_0} \langle n_1 \rangle = 0. \tag{20}$$

Recall that at steady state $\langle J^+ \rangle = \langle J^- \rangle = \langle J \rangle$. To obtain an expression for the susceptibility $s_1 = \frac{\langle n_0 \rangle}{\langle n_1 \rangle} \frac{d\langle n_1 \rangle}{d\langle n_0 \rangle}$ we multiply Equation (20) by $\langle n_0 \rangle / \langle J \rangle$ and rearrange to get

$$s_1 = \frac{-H_{10}}{H_{11}}. \tag{21}$$

The elasticities $H_{10}$ and $H_{11}$ are defined by

$$H_{10} = \frac{\partial \ln \langle J^- \rangle / \langle J^+ \rangle}{\partial \ln \langle n_0 \rangle}, \quad \tag{22a}$$

and

$$H_{11} = \frac{\partial \ln \langle J^- \rangle / \langle J^+ \rangle}{\partial \ln \langle n_1 \rangle}. \quad \tag{22b}$$

When $H_{10} > 0$ then $n_0$ downregulates $n_1$ and vice versa. First-order degradation of $n_1$ with no feedback implies $H_{11} = 1$. Positive feedback of $n_1$ on itself is characterized by $H_{11} < 1$, and negative feedback results in $H_{11} > 1$.

Now we turn to derive the level of noise that is propagated from $n_0$ to $n_1$. The approach we use for this purpose is frequency domain analysis (see [7, 12] and the supplementary of [1, 10]). We linearize Equation (19), and rewrite it in terms of fluctuations from the steady state $\Delta n_j(t) = n_j(t) - \langle n_j \rangle$:

$$\frac{d\Delta n_1}{dt} = \left( \frac{\partial \langle J^+ \rangle}{\partial \langle n_0 \rangle} - \frac{\partial \langle J^- \rangle}{\partial \langle n_0 \rangle} \right) \Delta n_0 + \left( \frac{\partial \langle J^+ \rangle}{\partial \langle n_1 \rangle} - \frac{\partial \langle J^- \rangle}{\partial \langle n_1 \rangle} \right) \Delta n_1. \tag{23}$$

Normalizing the equation through division by $\langle J \rangle$ we arrive at

$$\frac{\langle n_0 \rangle}{\langle J \rangle} \frac{dx_0}{dt} = \left( \frac{\partial \langle J^+ \rangle}{\partial \langle n_0 \rangle} - \frac{\partial \langle J^- \rangle}{\partial \langle n_0 \rangle} \right) x_0 + \left( \frac{\partial \langle J^+ \rangle}{\partial \langle n_1 \rangle} - \frac{\partial \langle J^- \rangle}{\partial \langle n_1 \rangle} \right) x_1. \tag{24}$$

FIG. 2: (A) The relation between susceptibility and noise amplification for linear networks, when the input noise autocorrelation time $\tau_0$ is varied. (B) Fraction of parameter sets that show low noise amplification at constant susceptibility (compared to the linear networks), for each network in the screen. Different colors represent different $\tau_0$ as in (A).
The $x_j$ variables are the normalized deviation from the steady state,

$$x_j(t) = \frac{n_j(t) - \langle n_j \rangle}{\langle n_j \rangle} \quad j = \{0, 1\}. \quad (23)$$

In terms of elasticities we get

$$-\tau_1 \frac{dx_1}{dt} = H_{10} x_0 + H_{11} x_1, \quad (24)$$

with $\tau_1$ describing the time constant of $n_1$ turnover, and it is defined as

$$\tau_1(\langle n_0 \rangle, \langle n_1 \rangle) = \frac{\langle n_1 \rangle}{\langle J \rangle}. \quad (25)$$

We apply the Fourier transform to Equation (24) and move from the time domain to the frequency domain:

$$\hat{x}_j(\omega) = \int_{-\infty}^{\infty} x_j(t)e^{-i\omega t} dt \quad j = \{0, 1\}, \quad (26)$$

resulting in the relation

$$\hat{x}_1(\omega) = -\frac{H_{10}}{H_{11} \tau_{mod} \omega + 1} \hat{x}_0(\omega), \quad (27)$$

where $\tau_{mod}$ is the time constant modified by the feedback $\tau_{mod} = \tau_1/H_{11}$. The function $H_{10}/H_{11} (i \tau_{mod} \omega + 1)^{-1}$ in Equation (26) is sometimes referred to as the transfer function [2] or the impulse response function [7], because it relates the frequency response of $x_1$ to that of the input. To describe the noise in $x_0$ and $x_1$ we utilize the concept of the autocorrelation function $\langle x_j(t_0)x_j(t_0 + t') \rangle$.

We assume the fluctuations $x_0$ can be represented by an exponentially decreasing autocorrelation function, with magnitude $\eta_0^2$,

$$\langle x_0(t_0)x_0(t_0 + t') \rangle = \eta_0^2 e^{-t'/\tau_0}. \quad (28)$$

The Fourier transform of the autocorrelation function of $x_0$ (which is termed the power spectrum of $x_0$) is

$$\langle \hat{x}_0(\omega) \hat{x}_0^*(\omega) \rangle = 2\eta_0^2 \frac{\tau_0}{\tau_0 \omega^2 + 1}. \quad (29)$$

The asterisk denotes the complex conjugate. The power spectrum of $x_1$ can be derived by multiplying $\hat{x}_1(\omega)$ from Equation (26) by its conjugate and taking the ensemble average [2, 7]. Then, using Equation (28)

$$\langle \hat{x}_1(\omega) \hat{x}_1^*(\omega) \rangle = \frac{H_{10}^2}{H_{11}} \frac{1}{(\tau_{mod} \omega)^2 + 1} \langle \hat{x}_0(\omega) \hat{x}_0^*(\omega) \rangle$$

and similarly for $f(t)$

$$\langle f(t_0)f(t_0 + t') \rangle = q_0 \delta(t' - t_0). \quad (30)$$

To find the magnitude of the noise $\eta_1$ we return to the time domain and substitute $t' = 0$ in the autocorrelation function:

$$\eta_1^2 = \left. \frac{1}{2\pi} \int_{-\infty}^{\infty} \langle \hat{x}_1(\omega) \hat{x}_1^*(\omega) \rangle e^{i\omega t'} d\omega \right|_{t' = 0}$$

$$= \frac{1}{2\pi} \int_{-\infty}^{\infty} \langle \hat{x}_1(\omega) \hat{x}_1^*(\omega) \rangle d\omega. \quad (31)$$

In the case of a loop-free cascade and first order degradation $H_{11} = 1$. If there is a negative feedback on $n_1$ then $H_{11} > 1$ and the noise is amplified beyond that of the linear cascade. However, when the feedback is positive and $0 < H_{11} < 1$ (which may still allow for the existence of a stable steady state) then the noise amplification can be much lower than that of the linear cascade, for a given susceptibility.

### B. Inclusion of Intrinsic Noise

The analysis above of a system composed of a single input and a single output did not take into account intrinsic noise that arises from translational bursts [6, 13] or other sources [8, 11]. In this section we incorporate, following [6, 13], noise that arises from the production of low-copy short-lived mRNA, denoted by $m$:

$$\frac{dm}{dt} = \beta_m(n_0, n_1) - m/\tau_m + f_m(t). \quad (32a)$$

The protein $n_1$ is translated from the mRNA:

$$\frac{dn_1}{dt} = \beta_n - n_1/\tau_1 + f(t). \quad (32b)$$

The terms $\beta_m$, $\beta$, $\tau_{m}^{-1}$ and $\tau_{1}^{-1}$ are the production and degradation rates of the mRNA and the protein, respectively. $\beta_m$ depends on $n_0$ and $n_1$ because these proteins may in general modulate the transcription rate of $n_1$. The functions $f_m(t)$ and $f(t)$ are white noise terms that have a mean zero and a very short autocorrelation time:

$$\langle f_m(t) \rangle = 0, \quad (33a)$$

$$\langle f_m(t) f_m(t + t') \rangle = q_m \delta(t' - t_0). \quad (33b)$$

and similarly for $f(t)$

$$\langle f(t) f(t + t') \rangle = q \delta(t' - t_0). \quad (33c)$$
The prefactors $q_m$ and $q$ define the magnitude of the autocorrelation for the fluctuations in the mRNA and in $n_1$, respectively. They are determined by the molecular interactions [3]. Typically the noise originates from a random birth-death process, hence its magnitude is the sum of the mean reaction fluxes [3, 6, 14]:

$$q_m = \beta_m (\langle n_0 \rangle, \langle n_1 \rangle) + \langle m \rangle / \tau_m = 2 \langle m \rangle / \tau_m,$$  \hspace{1cm} (34a)

and

$$q = \beta \langle m \rangle + \langle n_1 \rangle / \tau_1 = 2 \langle n_1 \rangle / \tau_1.$$  \hspace{1cm} (34b)

As before, we linearize Equations (32a) and (32b) near the mean steady state and divide by the steady state fluxes $\beta_m = \langle m \rangle / \tau_m$ and $\beta = \langle n_1 \rangle / \tau_1$ to obtain

$$\tau_m \frac{dx_m}{dt} = -H_m x_1 - H_m x_0 - x_m + \tau_m \frac{f_m(t)}{\langle m \rangle},$$  \hspace{1cm} (35a)

and

$$\tau_1 \frac{dx_1}{dt} = x_m - x_1 + \frac{\tau_1 f(t)}{\langle n_1 \rangle}.$$  \hspace{1cm} (35b)

The elasticities $H_{mj}$ for $j = \{0, 1\}$ are given by

$$H_{mj} = -\frac{\langle n_j \rangle}{\beta_m} \frac{\partial \beta_m}{\partial \langle n_j \rangle},$$

and the normalized deviations from the steady state are defined as $x_j = (n_j - \langle n_j \rangle) / \langle n_j \rangle$ for $j = \{0, 1, m\}$. Performing the Fourier transform on Equations (35a) and (35b) we arrive at

$$(i \tau_m \omega + 1) \hat{x}_m = -H_m \hat{x}_1 - H_m \hat{x}_0 + \tau_m \frac{\hat{f}_m(\omega)}{\langle m \rangle},$$  \hspace{1cm} (37a)

and

$$(i \tau_1 \omega + 1) \hat{x}_1 = \hat{x}_m + \frac{\tau_1 \hat{f}(\omega)}{\langle n_1 \rangle}.$$  \hspace{1cm} (37b)

If we are interested in correlation times that are much longer than the mRNA degradation time, $t' \gg \tau_m$, then $\tau_m \omega \ll 1$. In this case the solution for $\hat{x}_1(\omega)$, which can be found from (37a) and (37b), reduces to

$$\hat{x}_1(\omega) = -\frac{H_m}{i \tau_1 \omega + H_1} \hat{x}_0 + \frac{\tau_m \frac{\hat{f}_m(\omega)}{(i \tau_1 \omega + H_1)} \langle m \rangle}{\tau_1 \frac{\hat{f}(\omega)}{(i \tau_1 \omega + H_1)} \langle n_1 \rangle},$$

with the definition $H_1 = 1 + H_m$. Equation (38) describes how fluctuations are transferred from each noise source to $x_1$. We multiply $\hat{x}_1(\omega)$ in equation (38) by its conjugate and then perform an ensemble average [2, 7]. Because all noise sources are uncorrelated their cross-correlation terms equal zero. The power spectrum of $f_m(\omega)$ and $f(\omega)$ can be derived from the Fourier transform of Equations (33c), (34a) and (34b). The resulting expression for the power spectrum of $x_1$ is

$$\langle x_1(\omega) x_1^*(\omega) \rangle = \frac{H_m^2}{\tau_1 \omega^2 + H_1} \langle \hat{x}_0(\omega) \hat{x}_0^*(\omega) \rangle + \frac{2\tau_m}{(\tau_1^2 \omega^2 + H_1^2) \langle m \rangle} + \frac{2\tau_1}{(\tau_1^2 \omega^2 + H_1^2) \langle n_1 \rangle}.$$  \hspace{1cm} (39)

As explained in Equations (27) and (28) in the previous section, we assume that the autocorrelation of $x_0$ decreases exponentially with a time constant $\tau_0$. Hence

$$\langle \hat{x}_0(\omega) \hat{x}_0^*(\omega) \rangle = 2\eta_0^2 \frac{\tau_0}{\tau_0^2 \omega^2 + 1}.$$  \hspace{1cm} (40)

The normalized noise in $n_1$ can be found from the power spectrum of $x_1$ as explained in Equation (30) of the previous section:

$$\eta_1^2 = \frac{1}{2\pi} \int_{-\infty}^{\infty} \langle x_1(\omega) x_1^*(\omega) \rangle d\omega = \frac{H_m^2}{H_1^2} \frac{\tau_0}{\tau_0 + \tau_1 / H_1} \frac{\eta_0^2 + \frac{\tau_m}{\tau_1 H_1} \langle m \rangle}{\tau_1 H_1 \langle n_1 \rangle} + \frac{1}{H_1 \langle n_1 \rangle}.$$  \hspace{1cm} (41)

We recall that the susceptibility is $s_1 = -H_m / H_1$, and define the burst coefficient as the average number of proteins that are produced from a single mRNA molecule, $b = \beta \tau_m = \tau_m \langle n_1 \rangle / \langle m \rangle$. Finally we arrive at

$$\eta_1^2 = \frac{1 + b}{H_1 \langle n_1 \rangle} + s_1^2 \frac{\tau_0}{\tau_0 + \tau_1 / H_1} \frac{\eta_0^2}{\langle n_1 \rangle}.$$  \hspace{1cm} (41)

The contribution of the intrinsic noise is captured by the term $(1 + b) / (H_1 \langle n_1 \rangle)$.

VI. BIOLOGICAL MECHANISMS THAT FILTER NOISE

Positive feedback that increases susceptibility and time averaging occurs at intermediate elasticities, i.e. $0 < H_1 < 1$ in Equation (31). To preserve this behavior over a large range of input levels a constant value of $H_1$ must be maintained. One possible biological mechanism that can retain $0 < H_1 < 1$ is positive feedback that involves negative cooperativity (where one subunit of the protein inhibits the binding of a second subunit, leading to Hill coefficients lower than one). Although negative cooperativity was found in signal transduction pathways...
In this section we suggest a class of transcriptional networks that can provide high sensitivity and low noise amplification over a large range of input levels. These networks include positive feedback combined with competitive inhibition-based negative feedback. The competitive inhibition effectively decreases the binding affinity and generates effective negative cooperativity. The proposed mechanisms are shown in Supplementary Fig. 3. Before we analyze each case separately, we derive a general formulae for the susceptibility and noise amplification of the systems in Supplementary Fig. 3.

As derived in Section I the susceptibility vector is given by

\[ \mathbf{H} \mathbf{s} = -\mathbf{k}, \]  

(42)

where the components of \( \mathbf{H} \) are the elasticities \( H_{ij} \) and the terms of \( \mathbf{k} \) are \( H_{i0} \). For the class of three component systems at hand

\[ \mathbf{H} = \begin{pmatrix} H_{11} & H_{12} \\ H_{21} & H_{22} \end{pmatrix}, \]  

(43a)

and

\[ \mathbf{k} = \begin{pmatrix} H_{10} \\ 0 \end{pmatrix}. \]  

(43b)

The susceptibility of component \( n_1 \) to changes in the input \( n_0 \) will be

\[ s_1 = -\frac{H_{10}}{H_{11} - H_{12}H_{21}/H_{22}}. \]  

(44)

To find the noise amplification we use Paulsson’s FDT-based approach ([9] and Section II). Using this method the matrix of normalized noise components, \( \eta_i \), is given by

\[ \mathbf{M} \eta + \eta \mathbf{M}^T + \mathbf{D} = 0, \]  

(45)

where

\[ \mathbf{M} = \begin{pmatrix} -\alpha_0 & 0 & 0 \\ -\alpha_1 H_{10} & -\alpha_1 H_{11} & -\alpha_1 H_{12} \\ 0 & -\alpha_2 H_{21} & -\alpha_2 H_{22} \end{pmatrix}, \]  

(46a)

and

\[ \mathbf{D} = \begin{pmatrix} 2\alpha_0 \eta_0^2 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}, \]  

(46b)

where the \( \alpha_i \) terms are the degradation rates of each component. After some algebra the noise amplification is given by

\[ \frac{\eta_1^2}{\eta_0} = s_1^2 \times \frac{\alpha_1 (H_{11} - H_{12}H_{21}/H_{22})}{\alpha_0 + \alpha_1 (H_{11} - H_{12}H_{21}/H_{22})} \times \frac{\alpha_0 + \alpha_1 (H_{11} - H_{12}H_{21}/H_{22})}{\alpha_0 + \alpha_2 H_{22}H_{12}/H_{22}} \times \left( 1 - \frac{\alpha_0 + \alpha_2 H_{22}H_{12}/H_{22}}{\alpha_0 + \alpha_2 H_{22} \alpha_1 H_{11} + \alpha_2 H_{22}/H_{22}} \right). \]  

(47)

The first two terms in the equation are the susceptibility and “ideal” time averaging (compare these terms to Equation (31)). The elasticity \( H_{11} \) is modified by a factor \( H_{12}H_{21}/H_{22} \). This captures the effects of inhibition through \( n_2 \). The last two terms of the equation are correction terms that arise because the initiation of \( n_2 \) is not immediate. These terms approach one when the response time of the intermediate component \( n_2 \) is very short. From this analysis we immediately note that in order to have good noise properties two requirements must
The differential equations that describe this system are:

\[ 0 < H_{11} - H_{12}H_{21}/H_{22} < 1, \quad (48) \]

and second, that component \( n_2 \) will respond quickly

\[ \alpha_2 H_{22} \gg \alpha_1 H_{11}. \quad (49) \]

We now discuss each of the specific examples in Supplementary Fig. 3 separately.

A. Mechanism 1 – Self positive feedback and activation of a repressor

In the first mechanism of Supplementary Fig. 3a, an input signal, labeled 0, activates the output gene, labeled 1. Gene 1 encodes for a transcription factor that can directly enhance its own transcription. Transcription factor 1 also activates a repressor 2, that can bind to the same promoters as 1, thereby competitively inhibiting it. The differential equations that describe this system are:

\[
\frac{dn_1}{dt} = \beta_1 n_0 \frac{n_1/K_{11}}{1 + n_1/K_{11} + n_2/K_{12}} + l - \alpha_1 n_1, \quad (50a)
\]

\[
\frac{dn_2}{dt} = \beta_2 \frac{n_1/K_{21}}{1 + n_1/K_{21} + n_2/K_{22}} - \alpha_2 n_2. \quad (50b)
\]

where the \( \beta_i \) terms denote the transcription rate coefficients and the \( K_{ij} \) terms are the Michaelis-Menten binding constants. \( l \) is some low-level basal transcription which is needed to avoid the solution \( n_1 = n_2 = 0 \). In the next derivations we neglect \( l \). From these equations the elasticities are given by

\[
H_{10} = -1, \quad (51a)
\]

\[
H_{11} = 1 - \frac{1 + n_2/K_{12}}{1 + n_1/K_{11} + n_2/K_{12}}, \quad (51b)
\]

\[
H_{12} = \frac{n_2/K_{12}}{1 + n_1/K_{11} + n_2/K_{12}}, \quad (51c)
\]

\[
H_{21} = -\frac{1 + n_2/K_{22}}{1 + n_1/K_{21} + n_2/K_{22}}, \quad (51d)
\]

and

\[
H_{22} = 1 - \frac{n_2/K_{22}}{1 + n_1/K_{21} + n_2/K_{22}}. \quad (51e)
\]

Note that all Hill coefficients in this specific example equal one. Different Hill coefficients can be used just as long as the requirement in Equation (48) is fulfilled. In Fig. 3 of the main text, for example, the repressor binds its own promoter with a Hill coefficient of 2, whereas all other Hill coefficients equal 1.

Another requirement from the positive feedback, such that it can buffer noise effectively, is that it will not saturate over a large range of input signals, otherwise the susceptibility will vanish. This necessitates strong repression

\[
\frac{n_2}{K_{12}} \gg \frac{n_1}{K_{11}}, \quad (52a)
\]

\[
\frac{n_2}{K_{22}} \gg \frac{n_1}{K_{21}}, \quad (52b)
\]

\[
\frac{n_2}{K_{12}} \gg 1, \quad (52c)
\]

and

\[
\frac{n_2}{K_{22}} \gg 1. \quad (52d)
\]

Using (52a), (52b), (52c) and (52d) in (50a) and (50b) at the steady-state, we find that

\[
n_1 \approx \left( \frac{\beta_1 K_{12}}{\alpha_1 K_{11}} \right)^2 n_0^2, \quad (53a)
\]

and

\[
n_2 \approx \left( \frac{\beta_1 K_{12}}{\alpha_1 K_{11}} \right) n_0. \quad (53b)
\]

The upper bound on \( n_0 \), above which the system saturates, is given by substituting (53a) and (53b) back into the conditions in (52a) and (52b):

\[
\left( \frac{\beta_1}{\alpha_2} \frac{K_{12}}{K_{11}} \right) n_0 \ll 1. \quad (54a)
\]

and

\[
\left( \frac{\beta_1}{\alpha_2} \frac{K_{21}}{K_{22}} \right)^2 n_0 \ll 1. \quad (54b)
\]

The lower bound follows from Equations (52c) and (52d), but the system will probably be limited by intrinsic noise when the number of molecules is too low.

The expressions in (54a) and (54b) simply mean that in order to maintain strong repression over a large range of input signals, the transcription level of the repressor \( n_2 \) and its affinity to the promoters should be large compared to that of transcription factor \( n_1 \). This is the third condition, beyond Equations (48) and (49), that would make this system a good noise filter.
B. Mechanism 2 – Indirect positive feedback through a self-repressing component

Mechanism 2 is presented in Supplementary Fig. 3. The input signal \( n_0 \) activates the transcription of the output protein \( n_1 \). The protein \( n_1 \) can activate the transcription of \( n_2 \), which has two functions: it can enhance the transcription of \( n_1 \), thus establishing the positive feedback loop, and it can repress its own transcription through competitive binding, thereby reducing the effect of the positive feedback. The kinetic equations for this system are

\[
\frac{dn_1}{dt} = \beta_1 n_0 \frac{n_2}{K_{12}} - \alpha_1 n_1, \quad (55a)
\]

\[
\frac{dn_2}{dt} = \beta_2 \frac{n_1}{1 + n_1/K_{21} + n_2/K_{22}} - \alpha_2 n_2. \quad (55b)
\]

The corresponding elasticities are

\[
H_{10} = -1, \quad (56a)
\]

\[
H_{11} = 1, \quad (56b)
\]

\[
H_{12} = -\frac{1}{1 + n_2/K_{12}}, \quad (56c)
\]

\[
H_{21} = -\frac{1 + n_2/K_{22}}{1 + n_1/K_{21} + n_2/K_{22}}, \quad (56d)
\]

and

\[
H_{22} = 1 + \frac{n_2/K_{22}}{1 + n_1/K_{21} + n_2/K_{22}}. \quad (56e)
\]

The values of the elasticities can be manipulated by varying the Hill coefficients (which were taken to be 1 in this example), just as long as the relation in Equation (48) is kept.

Using similar arguments as in Section VIA, we arrive at the scaling of \( n_1 \) and \( n_2 \) when the system is far from saturation:

\[
n_1 \approx \left( \frac{\beta_1/\alpha_1}{K_{12}} \right)^2 \frac{\beta_2/\alpha_2 K_{22}}{K_{21}} n_0^2, \quad (57a)
\]

and

\[
n_2 \approx \left( \frac{\beta_1/\alpha_1}{K_{12}} \frac{\beta_2/\alpha_2 K_{22}}{K_{21}} \right) n_0. \quad (57b)
\]

Consequently, the upper bounds on \( n_0 \) to maintain an unsaturated response are

\[
\left( \frac{\beta_1/\alpha_1 K_{22}}{K_{12} K_{21}} \right) n_0 \ll 1, \quad (58a)
\]

and

\[
\left( \frac{\beta_1 \beta_2}{\alpha_1 \alpha_2 K_{12}^2 K_{21}} \right) n_0 \ll 1. \quad (58b)
\]

Simulations have shown that this mechanism can indeed buffer noise when compared to a linear cascade with the same susceptibility (not shown).

VII. SIMULATION PARAMETERS

The reactions and parameters that were simulated in the figures of the main text are given below.
<table>
<thead>
<tr>
<th>Description</th>
<th>Reaction</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generate mRNA</td>
<td>( \phi \rightarrow m_0 )</td>
<td>( \beta_{m0} )</td>
</tr>
<tr>
<td>Degrade mRNA</td>
<td>( m_0 \rightarrow \phi )</td>
<td>( \alpha_{m0} )</td>
</tr>
<tr>
<td>Generate ( n_0 )</td>
<td>( \phi \rightarrow n_0 )</td>
<td>( \beta_{n0} )</td>
</tr>
<tr>
<td>Degrade ( n_0 )</td>
<td>( n_0 \rightarrow \phi )</td>
<td>( \alpha_{n0} )</td>
</tr>
<tr>
<td>Generate ( n_1 )</td>
<td>( \phi \rightarrow n_1 )</td>
<td>( \beta_{n1} )</td>
</tr>
<tr>
<td>Degrade ( n_1 )</td>
<td>( n_1 \rightarrow \phi )</td>
<td>( \alpha_{n1} )</td>
</tr>
</tbody>
</table>

\( n_1 \) include feedback: \( \phi \rightarrow n_1 \) \( \beta_{n1} \frac{n_0}{K_{n0} + n_0} (K_{h1} + n_1)^{-1} \)

\( n_1 \) no feedback: \( \phi \rightarrow n_1 \) \( \beta_{n1} \frac{n_0}{K_{n0} + n_0} \)

"TABLE I: Reactions simulated in Fig. 1 of the main text"

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No feedback</th>
<th>Feedback</th>
</tr>
</thead>
<tbody>
<tr>
<td>( h )</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>( \alpha_{m0} )</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>( \alpha_0 )</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>( \alpha_1 )</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>( \beta_{m0} )</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>( \beta_0 )</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>( \beta_1 )</td>
<td>120</td>
<td>( 1.92 \times 10^4 )</td>
</tr>
<tr>
<td>( K )</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>( K_1 )</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>( n_0 )</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

"TABLE II: Parameters for the simulation in Fig. 1c of the main text"

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( h )</td>
<td>0</td>
</tr>
<tr>
<td>( \alpha_{m0} )</td>
<td>5</td>
</tr>
<tr>
<td>( \alpha_0 )</td>
<td>0.5</td>
</tr>
<tr>
<td>( \alpha_1 )</td>
<td>0.1</td>
</tr>
<tr>
<td>( \beta_{m0} )</td>
<td>5</td>
</tr>
<tr>
<td>( \beta_0 )</td>
<td>Adjusted according to ( n_0 )</td>
</tr>
<tr>
<td>( \beta_1 )</td>
<td>( 2.25 \times 10^4 )</td>
</tr>
<tr>
<td>( K )</td>
<td>1000</td>
</tr>
<tr>
<td>( K_1 )</td>
<td>10</td>
</tr>
<tr>
<td>( n_0 )</td>
<td>Mean from 125 to 8000</td>
</tr>
</tbody>
</table>

"TABLE III: Parameters for the simulation in Fig. 1d of the main text"
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_{m0}$</td>
<td>5</td>
</tr>
<tr>
<td>$\alpha_0$</td>
<td>0.5</td>
</tr>
<tr>
<td>$\alpha_1$</td>
<td>0.1</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>0.1</td>
</tr>
<tr>
<td>$\beta_{m0}$</td>
<td>0.45</td>
</tr>
<tr>
<td>$\beta_0$</td>
<td>Adjusted according to $n_0$</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>200</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>$8 \times 10^7$</td>
</tr>
<tr>
<td>$K_{11}$</td>
<td>$10^4$</td>
</tr>
<tr>
<td>$K_{12}$</td>
<td>10</td>
</tr>
<tr>
<td>$K_{21}$</td>
<td>$10^4$</td>
</tr>
<tr>
<td>$K_{22}$</td>
<td>10</td>
</tr>
<tr>
<td>$l$</td>
<td>0.1</td>
</tr>
<tr>
<td>$n_0$</td>
<td>Mean value from 100 to 1260</td>
</tr>
</tbody>
</table>

$r, c$ Adjusted to the steady state and susceptibility of the system with the feedback

TABLE V: Parameters for the simulation in Fig. 3 of the main text
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