Online Supplementary Material

Derivation of the LNA

This section demonstrates how the size expansion method (van Kampen 1997) can be applied to a general multidimensional reaction system.

Define a new random variable, ξ_i , from the relation

$$X_i \equiv \Omega \phi_i + \Omega^{1/2} \xi_i \, , \tag{A1}$$

X_i is the copy number of component i and ϕ_i is its macroscopic concentration defined from $\lim_{\Omega\to\infty} X_i / \Omega = \phi_i; i = 1, 2 \ldots N$, where Ω is the system volume (Eq. (23) in main text).

The probability distribution $P(\mathbf{X},t)$ for $\mathbf{X} = \left(X_1 \cdots X_N \right)^T = \Omega \mathbf{x}$ is related to the probability distribution $\Pi(\boldsymbol{\xi}, t)$ for $\boldsymbol{\xi} = \left(\xi_1 \quad \cdots \quad \xi_N \right)$ through

$$P(\mathbf{X}, t) = P(\Omega \boldsymbol{\varphi} + \Omega^{1/2} \boldsymbol{\xi}, t) = \Pi(\boldsymbol{\xi}, t) \tag{A2}$$

Differentiation with respect to time of $\Pi(\boldsymbol{\xi}, t)$ at constant molecule numbers gives

$$\frac{\partial P(\mathbf{X},t)}{\partial t} = \frac{\partial \Pi(\boldsymbol{\xi},t)}{\partial t} + \sum_{i=1}^{N} \frac{\partial \xi_i}{\partial t} \frac{\partial \Pi(\boldsymbol{\xi},t)}{\partial \xi_i} = \frac{\partial \Pi(\boldsymbol{\xi},t)}{\partial t} - \Omega^{1/2} \sum_{i=1}^{N} \frac{\partial \phi_i}{\partial t} \frac{\partial \Pi(\boldsymbol{\xi},t)}{\partial \xi_i}, \tag{A3}$$

since $\partial X_i / \partial t = 0$ implies that $\partial \xi_i / \partial t = -\Omega^{1/2} \, \partial \phi_i / \partial t$.

Taylor expansion of the transition rates, $\tilde{f}_j(\mathbf{x})$ around the macroscopic value $f_j(\boldsymbol{\varphi})$ gives

$$\tilde{f}_j(\mathbf{x}) = \tilde{f}_j(\boldsymbol{\varphi} + \Omega^{-1/2}\boldsymbol{\xi}) = f_j(\boldsymbol{\varphi}) + \Omega^{-1/2} \sum_{i=1}^{N} \frac{\partial f_j(\boldsymbol{\varphi})}{\partial \phi_i} \xi_i + O\left(\Omega^{-1}\right) \tag{A4}$$

The mesoscopic transition rates $\tilde{f}_j(\mathbf{x})$ differ from the macroscopic rates $f_j(\boldsymbol{\varphi})$ by a term of order Ω^{-1}, which corresponds to the order of single molecules. The difference is therefore absorbed in $O\left(\Omega^{-1}\right)$ in Eq. (A4).

The step operator in the master equation (Eq. (25)) is approximated by a differential operator according

$$E^k f\left(x\right) = f\left(\phi + \Omega^{-1/2}\left(\xi + \Omega^{-1/2}k\right)\right) = \left[1 + \Omega^{-1/2} k \frac{\partial}{\partial \xi} + \frac{\Omega^{-1}k^2}{2}\frac{\partial^2}{\partial\xi\partial\xi} + O(\Omega^{-3/2})\right] f(x) \qquad \text{(A5)}$$

For jumps in several dimensions one gets

$$E^{-S_{\cdot j}} = \prod_{i=1}^{N} E^{-S_{ij}} \approx 1 - \Omega^{-1/2}\sum_i S_{ij}\frac{\partial}{\partial\xi_i} + \frac{\Omega^{-1}}{2}\sum_i\sum_k S_{ij}S_{kj}\frac{\partial^2}{\partial\xi_i\partial\xi_k} + O(\Omega^{-3/2}) \qquad \text{(A6)}$$

Insert Eqs. A2, A3, A4 and A6 into the master equation, Eq. (25). This gives

$$\frac{\partial\Pi(\xi,t)}{\partial t} - \Omega^{1/2}\sum_{i=1}^{N}\frac{\partial\phi_i}{\partial t}\frac{\partial\Pi(\xi,t)}{\partial\xi_i} =$$

$$\Omega\sum_{j=1}^{R}\left(-\Omega^{-1/2}\sum_i S_{ij}\frac{\partial}{\partial\xi_i} + \frac{\Omega^{-1}}{2}\sum_i\sum_k S_{ij}S_{kj}\frac{\partial^2}{\partial\xi_i\partial\xi_k} + O(\Omega^{-3/2})\right)\left(f_j(\boldsymbol{\varphi}) + \Omega^{-1/2}\sum_i\frac{\partial f_j(\boldsymbol{\varphi})}{\partial\phi_i}\xi_i + O\left(\Omega^{-1}\right)\right)\Pi(\xi,t)$$

(A7)

Identify terms of order $\Omega^{1/2}$

$$\Omega^{1/2}: \qquad \sum_{i=1}^{N}\frac{\partial\phi_i}{\partial t}\frac{\partial\Pi(\xi,t)}{\partial\xi_i} = \sum_{i=1}^{N}\sum_{j=1}^{R} S_{ij}f_j(\boldsymbol{\varphi})\frac{\partial\Pi(\xi,t)}{\partial\xi_j} \qquad \text{(A8)}$$

These $\Omega^{1/2}$-terms cancel, since ϕ_i follow the macroscopic equation $\frac{\partial\phi_i}{\partial t} = \sum_{j=1}^{R} S_{ij}f_j(\boldsymbol{\varphi})$, Eq. (24).

Identify terms of order Ω^0

$$\begin{aligned}\Omega^0: \qquad \frac{\partial\Pi(\xi,t)}{\partial \mathrm{t}} &= \sum_j\left[\sum_{i,k} -S_{ij}\frac{\partial f_j}{\partial\phi_k}\frac{\partial\left(\xi_k\Pi(\xi,t)\right)}{\partial\xi_i} + \frac{1}{2}f_j\sum_{i,k}S_{ij}S_{kj}\frac{\partial^2\Pi(\xi,t)}{\partial\xi_i\partial\xi_k}\right] \\ &= -\sum_{i,k}A_{ik}\frac{\partial\left(\xi_k\Pi\right)}{\partial\xi_i} + \frac{1}{2}\sum_{i,k}\left[\mathbf{BB}^T\right]_{ik}\frac{\partial^2\Pi}{\partial\xi_i\partial\xi_k}\end{aligned} \qquad \text{(A9)}$$

with $f_i = f_i(\boldsymbol{\varphi})$, $A_{ik} = \sum_{j=1}^{R} S_{ij}\frac{\partial f_j}{\partial\phi_k} = \frac{\partial\left(\mathbf{S}_{i}.\mathbf{f}\right)}{\partial\phi_k}$ and $\left[\mathbf{BB}^T\right]_{ik} = \sum_{j=1}^{R} S_{ij}S_{kj}f_j(\boldsymbol{\varphi}) = \left[\mathbf{S}\ diag(f(\boldsymbol{\varphi}))\ \mathbf{S}^T\right]_{ik}$.

Eq. (A9) is the linear Fokker-Plank equation (Risken 1984) with coefficient matrices **A** and $\mathbf{BB}^T$ that depend on time through the deterministic rate vector $\mathbf{f}\left(\boldsymbol{\varphi}(t)\right)$.

Higher orders than Ω^0 are not included in the linear noise approximation.

The stationary solution of Eq. (A9) follows from

$$0 = -\sum_{ik} \mathbf{A}_{ik} \frac{\partial}{\partial \xi_i} \xi_k \Pi(\boldsymbol{\xi}) + \frac{1}{2} \sum_{ik} \left[\mathbf{B}\mathbf{B}^T \right]_{ik} \frac{\partial^2 \Pi(\boldsymbol{\xi})}{\partial \xi_i \partial \xi_k}, \tag{A10}$$

where the Jacobian matrix $\mathbf{A}$ and the diffusion matrix $\mathbf{B}\mathbf{B}^T$ are evaluated in the macroscopic stationary state $\overline{\boldsymbol{\varphi}}$ (Eq. (26)).

The stationary solution (Gardiner 1985; Risken 1984) of the linear Fokker-Planck equation Eq. (A10) is the multidimensional Normal distribution

$$P(\boldsymbol{\xi}) = \left((2\pi)^{N/2} \sqrt{\det \boldsymbol{\Xi}} \right)^{-1} \exp\left(-\boldsymbol{\xi}^T \boldsymbol{\Xi} \boldsymbol{\xi} / 2 \right) \tag{A11}$$

It has zero average vector and the covariance matrix $\boldsymbol{\Xi} = \left\langle \boldsymbol{\xi}\boldsymbol{\xi}^T \right\rangle$ that follows from the Lyapunov equation $\mathbf{A}\boldsymbol{\Xi} + \boldsymbol{\Xi}\mathbf{A}^T + \mathbf{B}\mathbf{B}^T = 0$. The correlation matrix of the stationary process is $\left\langle \boldsymbol{\xi}(t)\boldsymbol{\xi}^T(s) \right\rangle = \boldsymbol{\Xi} \exp\left(\mathbf{A} |t - s| \right)$. By using $\langle \mathbf{X} \rangle = \Omega \boldsymbol{\varphi}$ in Eq. (A1) we note that the covariance matrix $\mathbf{C} = \left\langle \left(\mathbf{X} - \langle \mathbf{X} \rangle \right) \left(\mathbf{X} - \langle \mathbf{X} \rangle \right)^T \right\rangle$ is given by $\mathbf{C} = \Omega \boldsymbol{\Xi}$.

The stochastic differential equation

The linear Fokker-Planck equation Eq. (A9) describes an Ornstein-Uhlenbeck process which can equivalently be formulated (Gardiner 1985) as (Ito) a stochastic differential equation (SDE)

$$d\boldsymbol{\xi} = \mathbf{A}\boldsymbol{\xi} dt + \mathbf{B} d\mathbf{W}(t), \tag{A12}$$

Here, $d\mathbf{W}(t)$ is a Wiener process in R dimensions and, as before, $\mathbf{B} = \mathbf{S}\sqrt{diag\left(\mathbf{f}(\overline{\mathbf{x}}) \right)}$. The SDE (A12) reformulates the Fokker-Planck description of internal fluctuations in terms of a deterministic dynamical system with added external noise. This transformation is useful for comparisons of the behavior of chemical networks with the performance of technical feedback systems subjected to external noise (Glad and Ljung 2000).

Example 1 with general stochiometries and general kinetic order

Consider a system with two elementary reactions and one chemical component. In the first reaction (er_1) the number X_1 of molecules is increased by m at the time with constant intensity k_1. In the second (er_2), X_1 is reduced by n molecules at the time in an elementary reaction with rate constant k_2 that is of order ℓ in the concentration of X_1:

$$\varnothing \xrightarrow{k_1} m\mathrm{X}_1 \qquad (er_1)$$

$$nX_1 \xrightarrow{k_2(\ell)} \varnothing \qquad (er_2)$$

Since X_1 is increased by m in er_1 and decreased by n in er_2, $S_{11} = m$ and $S_{12} = -n$. The transition rates are, respectively,

$$\begin{aligned} \tilde{f}_1 &= k_1 \\ \tilde{f}_2 &= k_2 \frac{X_1+n}{\Omega}\frac{X_1+n-1}{\Omega}\cdots\frac{X_1+n-\ell+1}{\Omega} \end{aligned} \tag{A13}$$

Their macroscopic counterparts are obtained in the limit where both X_1 and Ω go to infinity while X_1/Ω, n and ℓ remain finite and are given by

$$\begin{aligned} f_1 &= k_1 \\ f_2 &= k_2\phi_1^{\ell} \end{aligned} \tag{A14}$$

The macroscopic differential equation (compare Eq.(24)) is with $\kappa_1 = mk_1; \kappa_2 = nk_2$

$$\frac{d\phi_1}{dt} = \kappa_1 - \kappa_2\phi_1^{\ell}, \tag{A15}$$

and the asymptotically stable steady-state solution is $\bar{\phi}_1 = \sqrt[\ell]{\kappa_1/\kappa_2}$.

The master equation is given by (compare Eq. (25))

$$\frac{dP(X_1,t)}{dt} = \Omega\left[\tilde{f}_1 P(X_1 - m,t) + \tilde{f}_2(X_1 + n,\Omega) P(X_1 + n,t) - \left[\tilde{f}_1 + \tilde{f}_2(X_1,\Omega) \right] P(X_1,t) \right] \tag{A16}$$

When $\ell = n$ *er₂* has simple mass action kinetics, but cases with $\ell \neq n$ can be implemented as elementary complex reactions carried out by enzymes. For example, when $\ell \geq n$ the exit reaction in *er₂* could be catalyzed by an unsaturated enzyme that rapidly equilibrates with its substrates and can remove *n* X molecules from the system in a single event, provided that it is in complex with ℓ X molecules. When $\ell \leq n$ this master equation can arise for an unsaturated enzyme which initially is in rapid equilibrium with its substrates. When ℓ molecules are bound it can change to a hyper-active conformation which rapidly removes *n* X molecules from the system.

Macroscopically the stoichiometries *m* and *n* play trivial roles since they can be hidden in the compounded parameters κ_1 and κ_2 but their roles in the master equation are irreducible. Therefore, linear noise approximations must be based on macroscopic descriptions that distinguish between stochiometries and rate laws, as done in Eq. (24).

Insertion of the macroscopic rate laws, $f_1(\phi) = k_1$ and $f_2(\phi) = k_2\phi^\ell$ *i.e.* Eq. (A14), and the stochiometric coefficients, $S_{11} = m$ and $S_{12} = -n$ in Eq. (5) gives the LNA for $\langle \delta X_1^2 \rangle$ as

$$C_{11} = \frac{\Omega\left[n^2 k_2 \bar{\phi}_1^{\ell} + m^2 k_1 \right]}{2\left[n\ell k_2 \bar{\phi}_1^{\ell-1} \right]} = \Omega \frac{n^2 k_1 + mnk_1}{2m\ell k_2} \left(\frac{nk_1}{mk_2} \right)^{\frac{1-\ell}{\ell}} = \Omega \frac{n+m}{2\ell} \left(\frac{\kappa_1}{\kappa_2} \right)^{\frac{1}{\ell}}, \tag{A17}$$

with $\bar{\phi} = \sqrt[\ell]{\kappa_1/\kappa_2}$.

The Fano-factor is given by (Paulsson 2000)

$$F_1 = \frac{C_{11}}{\Omega \bar{\phi}_1} = \frac{(n+m)}{2\ell}. \tag{A18}$$

It can increase with increasing values of *m* and *n* although the macroscopic dynamics might be virtually unchanged. All positive real values of ℓ can be implemented by carefully designed elementary complex reactions. When $\ell \rightarrow 0$ the system approaches zero order kinetics which makes the fluctuations very large.

This example clarifies the relation between the logarithmic gain and the Fano factor. The logarithmic gain for the system is

$$a_{\phi_1\kappa_1} = \left.\frac{d\phi_1/\phi_1}{d\kappa_1/\kappa_1}\right|_{\bar{\phi}} = \ell^{-1}. \tag{A19}$$

which is equal to the Fano-factor only when n=m=1, see (Elf et al. 2003b; Paulsson and Ehrenberg 2001), but otherwise smaller.

When one molecule at the time is synthesized (m=1) and later eliminated in a first order reaction $(n = \ell = 1)$, then the stationary process has Poissoninan fluctuations and the Fano-factor Eq. (A18) evaluates to 1.

Example 3: Coupled fluctuations in a two component systems

To illustrate use of the LNA in multi component systems we start with a scheme where the two molecules A and B are synthesized independently and then removed together from the system through a bimolecular reaction according to

$$\begin{array}{l} \varnothing \xrightarrow{f_1} \mathrm{A} \quad \varnothing \xrightarrow{f_2} \mathrm{B} \\ \mathrm{A} \xrightarrow{f_3} \varnothing \quad \mathrm{B} \xrightarrow{f_4} \varnothing \\ A + B \xrightarrow{f_5} \mathrm{AB} \end{array} \tag{A20}$$

The concentration vector $\mathbf{x}$, the transition rate vector $\tilde{\mathbf{f}}(\mathbf{x})$ and the stoichiometric matrix $\mathbf{S}$ are

$$\mathbf{x} = \begin{pmatrix} [A] \\ [B] \end{pmatrix} = \begin{pmatrix} x_1 \\ x_2 \end{pmatrix}, \quad \tilde{\mathbf{f}}(\mathbf{x}) = \begin{pmatrix} k_1 \\ k_2 \\ k_3 x_1 \\ k_4 x_2 \\ k_5 x_1 x_2 \end{pmatrix}, \mathbf{S} = \begin{pmatrix} 1 & 0 & -1 & 0 & -1 \\ 0 & 1 & 0 & -1 & -1 \end{pmatrix} \tag{A21}$$

In the macroscopic limit we retain the rate laws $\mathbf{f}(\boldsymbol{\varphi}) = \tilde{\mathbf{f}}(\mathbf{x} = \boldsymbol{\varphi})$. The master equation for this system follows directly from Eq.(25) and analytical solutions are available in special cases (Elf et al. 2003b). For the LNA we need the Jacobian (**A**) and the diffusion matrix $\mathbf{BB}^T$ according to Eqs. **(28)** and **(31)**, respectively:

$$\mathbf{A}_{ik} = \partial\left(\mathbf{S}_{i\cdot}\mathbf{f}\right)/\partial\phi_k\Big|_{\boldsymbol{\varphi}=\overline{\boldsymbol{\varphi}}} \Rightarrow \mathbf{A} = -\begin{bmatrix} k_3 + k_5\phi_2 & k_5\phi_1 \\ k_5\phi_2 & k_4 + k_5\phi_1 \end{bmatrix}_{\boldsymbol{\varphi}=\overline{\boldsymbol{\varphi}}} \tag{A22}$$

$$\mathbf{BB}^T = \mathbf{S}\, diag\left(\mathbf{f}(\overline{\boldsymbol{\varphi}})\right)\, \mathbf{S}^T = \begin{bmatrix} k_1 + k_3\phi_1 + k_5\phi_1\phi_2 & k_5\phi_1\phi_2 \\ k_5\phi_1\phi_2 & k_2 + k_4\phi_2 + k_5\phi_1\phi_2 \end{bmatrix}_{\boldsymbol{\varphi}=\overline{\boldsymbol{\varphi}}} \tag{A23}$$

In figure 2a the average stationary concentration of A molecules is plotted as a function of their rate k_1 of synthesis for different degradation rate constants $k_3 = k_4 \equiv \mu$.

The LNA estimate of the covariance matrix **C** can be calculated numerically directly from Eq. (35) using **A** and $\mathbf{BB}^T$ (Eqs. A22 and A23). **C** was then used to calculate the Fano-factor ($F_1 \approx C_{11}/\left(\Omega\overline{\phi}_1\right)$) which is plotted in figure 2b for parameters as in figure 2a. There is again good agreement between the LNA and results from Gillespie simulations.

When the degradation rate constants k_3 and k_4 are small, the Jacobian **A** is nearly singular at the point where k_1=k_2. This results in large anti-correlated fluctuations in X_1 and X_2, as seen in the Gillespie simulation in figure 2c. The reason for this behavior is zero order kinetics in several dimensions where a change in one concentration can be compensated by a change in the other leaving the overall flow unchanged (Elf et al. 2003b).

To derive an analytical expression for the size of the fluctuations at the point where **A** is near singular, we assume that $k_1 = k_2 \equiv \kappa$ and that $k_3 = k_4 \equiv \mu$. This makes **A** in Eq. (A22) symmetric at steady-state, where $\bar{\phi}_1 = \bar{\phi}_2 \equiv \bar{\phi}$. The eigenvectors of **A** can then be chosen to be orthonormal and

$$\mathbf{Q} = \frac{1}{\sqrt{2}}\begin{bmatrix} -1 & 1 \\ 1 & 1 \end{bmatrix} = \mathbf{Q}^{-1} = \mathbf{Q}^T , \tag{A23}$$

with the eigenvalues

$$\boldsymbol{\lambda} = \begin{bmatrix} \text{-}\mu & \text{-}2k_5\bar{\phi}\text{ - }\mu \end{bmatrix}. \tag{A24}$$

The transformed stochastic variables are

$$\tilde{\mathbf{X}} = \mathbf{Q}^{-1}\mathbf{X} \Rightarrow \begin{pmatrix} \tilde{X}_1 \\ \tilde{X}_2 \end{pmatrix} = \frac{1}{\sqrt{2}}\begin{pmatrix} X_2 - X_1 \\ X_1 + X_2 \end{pmatrix}. \tag{A25}$$

$\tilde{X}_1$ is thus proportional to the difference between X_2 and X_1 and $\tilde{X}_2$ is proportional to the sum of X_2 and X_1. In these new variables the reaction stochiometric matrix is

$$\tilde{\mathbf{S}} = \mathbf{Q}^{-1}\mathbf{S} = \frac{1}{\sqrt{2}}\begin{pmatrix} -1 & 1 \\ 1 & 1 \end{pmatrix}\begin{pmatrix} 1 & 0 & -1 & 0 & -1 \\ 0 & 1 & 0 & -1 & -1 \end{pmatrix} = \frac{1}{\sqrt{2}}\begin{pmatrix} -1 & 1 & 1 & -1 & 0 \\ 1 & 1 & -1 & -1 & -2 \end{pmatrix} \tag{A26}$$

Note that the dimerization reaction with rate f_5 does not change the difference variable $\tilde{X}_1$ and therefore $\tilde{S}_{15} = 0$. To use the analytical solution for the covariance matrix from Eq. (35) we also need the transformed diffusion matrix

$$\tilde{\mathbf{B}}\tilde{\mathbf{B}}^T = \tilde{\mathbf{S}}\; diag\left(\mathbf{f}(\bar{\mathbf{x}})\right)\; \tilde{\mathbf{S}}^T =$$

$$\frac{1}{2}\begin{pmatrix} \left[k_1 + k_2 + k_3\bar{\phi}_1 + k_4\bar{\phi}_2\right] & \left[-k_1 + k_2 - k_3\bar{\phi}_1 + k_4\bar{\phi}_2\right] \\ \left[-k_1 + k_2 - k_3\bar{\phi}_1 + k_4\bar{\phi}_2\right] & \left[k_1 + k_2 + k_3\bar{\phi}_1 + k_4\bar{\phi}_2 + 4k_5\bar{\phi}_1\bar{\phi}_2\right] \end{pmatrix} = \begin{pmatrix} \left[\kappa + \mu\bar{\phi}\right] & 0 \\ 0 & \left[\kappa + \mu\bar{\phi} + 4k_5\bar{\phi}^2\right] \end{pmatrix} \tag{A27}$$

The covariance matrix can now be calculated analytically by insertion in Eq. (35)

$$\tilde{C}_{ik} = \Omega\frac{\left(\tilde{B}\tilde{B}^*\right)_{ik}}{\lambda_i + \lambda_k} \Rightarrow \tilde{\mathbf{C}} = \Omega\begin{pmatrix} \frac{1}{2}\left(\frac{\kappa}{\mu} + \bar{\phi}\right) & 0 \\ 0 & \frac{\kappa + \mu\bar{\phi} + 4k_5\bar{\phi}^2}{2\left(\mu + 2\bar{\phi}k_5\right)} \end{pmatrix}, \tag{A28}$$

showing that $\tilde{X}_1$ and $\tilde{X}_2$ are uncorrelated ($\tilde{C}_{12} = \tilde{C}_{21} = 0$).

Next we compare the size of the stationary fluctuations in $\tilde{X}_1 = (X_2 - X_1)/\sqrt{2}$ and $\tilde{X}_2 = (X_2 + X_1)/\sqrt{2}$ to the size of the individual pool, i.e. $\Omega\bar{\phi}$. This measure, which is similar to a Fano-factor, is in this case:

$$\tilde{F}_1 = \frac{\tilde{C}_{11}}{\Omega\bar{\phi}} = \frac{1}{2}\left(\frac{\kappa}{\bar{\phi}}\frac{1}{\mu} + 1\right) \tag{A29}$$

$$\tilde{F}_2 = \frac{\tilde{C}_{22}}{\Omega\bar{\phi}} = \frac{\kappa + \mu\bar{\phi} + 4k_5\bar{\phi}^2}{2\bar{\phi}\left(\mu + 2\bar{\phi}k_5\right)} \tag{A30}$$

In Fig. 2d the modified Fano-factor Eq. (A29) is compared to Gillepie simulations.
In the limit where the rate of degradation is much smaller than the turnover rate of either of the substrate pools ($\mu \ll \kappa/\bar{\phi}$), the synthesis rates κ equals the stationary rate of dimerization $k_5\bar{\phi}^2$ and the modified Fano-factors approach

$$\tilde{F}_1 \to \frac{\kappa}{\bar{\phi}}\frac{1}{\mu} \tag{A31}$$

$$\tilde{F}_2 \to \frac{5}{4} \tag{A32}$$

In this limit, the Fano-factor $\tilde{F}_1$ for $\tilde{X}_1 = (X_2 - X_1)/\sqrt{2}$ is large. It equals the ratio between the turnover rate $\kappa/\bar{\phi}_1$ of either one of the X₁ and X₂ pools and the effective degradation rate constant μ . The modified Fano-factor $\tilde{F}_2$ for $\tilde{X}_2 = (X_2 + X_1)/\sqrt{2}$ is, however, small. These results explain the very large and highly correlated fluctuations seen in Figs. 2c and 2b for $k_1 = k_2$.

Example 4: Elimination of fast variables in a four-component system

To exemplify the technique of elimination of fast variables we analyze an extended version of the probability density function in *Example 3*:

$$\begin{array}{ll} \mathrm{E_A} \xrightarrow{k_1 e_\mathrm{A}} \mathrm{E_A} + \mathrm{A} & \mathrm{E_B} \xrightarrow{k_4 e_\mathrm{B}} \mathrm{E_B} + \mathrm{B} \\ \mathrm{A} + \mathrm{E_A} \underset{k_3 (e_\mathrm{A}^{tot} - e_\mathrm{A})}{\overset{k_2 a e_\mathrm{A}}{\rightleftharpoons}} \mathrm{AE_A} & \mathrm{B} + \mathrm{E_B} \underset{k_6 (e_\mathrm{B}^{tot} - e_\mathrm{B})}{\overset{k_5 b e_\mathrm{B}}{\rightleftharpoons}} \mathrm{BE_B} \\ \mathrm{A} + \mathrm{B} \xrightarrow{k_7 ab} \mathrm{AB} & \end{array} \tag{A33}$$

A and B molecules are synthesized by enzymes E$_\mathrm{A}$ and E$_\mathrm{B}$, respectively. A and B can bind E$_\mathrm{A}$ and E$_\mathrm{B}$ and thereby inhibit their own synthesis. A and B can also bind each other irreversibly. These seven reactions are described by the following transitions rates and stochichiometric matrix

$$\mathbf{x} = \begin{pmatrix} [E_A] \\ [E_B] \\ [A] \\ [B] \end{pmatrix} = \begin{pmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \end{pmatrix}, \quad \tilde{\mathbf{f}}(\mathbf{x}) = \begin{pmatrix} k_1 x_1 \\ k_2 x_1 x_3 \\ k_3 (x_1^{tot} - x_1) \\ k_4 x_2 \\ k_5 x_2 x_4 \\ k_6 (x_2^{tot} - x_2) \\ k_7 x_3 x_4 \end{pmatrix}, \quad \mathbf{S} = \begin{pmatrix} 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 1 & 0 \\ 1 & -1 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 1 & -1 & 1 & -1 \end{pmatrix} \tag{A34}$$

The macroscopic rate laws and mesoscopic transition rates are the same also in this case, i.e. $\mathbf{f}(\boldsymbol{\varphi}) = \tilde{\mathbf{f}}(\mathbf{x} = \boldsymbol{\varphi})$. The steady-state equation Eq. (26) is solved to get $\overline{\boldsymbol{\varphi}}$, which is used in Eqs (28) and (31) to calculate **A** and $\mathbf{BB}^T$. These are used in the Liapunov equation Eq. (32) to get the covariance matrix **C** from Eq. (35), which defines the normal distribution for ***X*** centered around $\Omega\overline{\boldsymbol{\varphi}}$. This LNA works well except near the critical point where $k_1 \overline{\phi}_1 = k_4 \overline{\phi}_2$ as shown in Fig. 3. Here, the marginal distributions for B molecules estimated by Gillespie simulations (*gray line*) of the full system (A33) has similar mean and variance as the Gaussian resulting from the LNA, but the shape of the latter (*black line*) is very different from the more accurate distribution obtained from Gillespie simulations. However, this failure of directly applying the LNA can be overcome by elimination of fast variables. For the parameters used in Fig. 3 the smallest eigenvalue of the Jacobian (λ_1) is 1000 times smaller then the second smallest (λ_2) and the variance $\tilde{C}_{11}$ is 1000 times larger than $\tilde{C}_{22}$. The slow variable is chosen as $\tilde{x}_1 = \left[\mathbf{Q}^{-1}\right]_{1\bullet}\mathbf{x} = 0.895x_1 - 0.822x_2 - 0.914x_3 + 0.913x_4$ (*j*=1 in Eq. (39)) and all other variables are in a pseudo-stationary state $\bar{\tilde{\phi}}_2 \ldots \bar{\tilde{\phi}}_4$, conditional on the value of $\tilde{x}_1 = \Omega^{-1}\tilde{X}_1$. The probability distribution for $\tilde{X}_1$ is taken to be the normal distribution $P_1\left(\tilde{X}_1\right) = N(mean = \Omega\bar{\tilde{\phi}}_1, \sigma^2 = \tilde{C}_{11})$. Next we calculate $\tilde{P}\left(\tilde{\mathbf{X}}\right)$ from Eq.(40), using the steady state relations in Eq. (39) to get the values of $\tilde{X}_2\ \tilde{X}_3\ \tilde{X}_4$ for each value of $\tilde{X}_1$. The non-linearly transformed probability distribution can be expressed in the original variables, using Eq. (41), and the marginal distributions are given by Eq. (42). Fig. 3 shows that (*dashed line*) initial elimination of fast variables and application of the LNA gives a much better representation of P(***X***) than direct use of the LNA.