Intrinsic phenotypic stability of a bi-stable auto regulatory gene

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Even under homogenous conditions clonal cells can assume different distinct states for generations to follow, also known as epigenetic inheritance. Such long periods of different phenotypic states can be formed due to the existence of more than one stable state in the molecule concentration, where the different states are explored through molecular fluctuations. By formulating a single reaction variable representing the birth and death of molecules, including transcription, translation and decay, we calculate the escape time from the phenotypic states attained from autocatalytic synthesis through a Fokker-Planck formulation and integration of an effective pseudo-potential. We calculate the stability of the phenotypic states both for cooperative binding feedback and dimer binding feedback, resulting in non-linear decay.

The cellular machinery often work with few molecules at hand. A gene may exist as one single copy on the DNA and the copy number of the messenger RNA and following protein are typically very low\(^1\). Chemical reactions are probabilistic – before a reaction can take place the participating molecules first need to find each other through random motion, chemical bonds form at random. Molecular decay is also random. Combining low copy number and random birth and death of molecules make biochemical reaction networks noisy, even if we eliminate all external variations in the environment of a given biochemical process. Two clonal cells placed in the same environment may therefore at times exhibit very different behaviour. Fluctuations generated from birth and death of molecules in a reaction network are commonly referred to as intrinsic fluctuations whereas extrinsic fluctuations are fluctuations of the environment that the studied reaction network are embedded in, i.e. variations in the reaction rates of the reaction network\(^2,3\). The magnitude of the molecular fluctuations can be altered by a change in the reaction rates and by regulatory processes. Negative autoregulation is shown both to reduce noise\(^4\) and to amplify fluctuations if the feedback mechanism is delayed\(^5–7\) or if the feedback is strong\(^8\). The fidelity of regulatory control is also limited by the molecular noise associated with the process of transmitting biochemical signals\(^8\) and by finding the regulatory sites\(^10–12\). However, there are no a priori reasons to assume molecular fluctuations only being detrimental for an organism. In theory noise could be exploited for enhancing the sensitivity of regulatory control processes\(^13\). Moreover, there are many situations where noise is an essential component of a process. Overcoming potential barriers by thermal fluctuations are what drives chemical reactions in the first place and in are this respect of course essential, but potential wells and multi-stable states can also be formed by the dynamics of molecule synthesis, e.g. if the synthesis is enhanced by its own presence. The consequence of such feedback is that there are two or more stable concentrations that the different molecule species of the reaction network may attain. Autocatalytic protein synthesis is shown both theoretically and experimentally to generate meta stable states and the consequence of such seemingly simple positive feedback is that genetically identical individuals may even at identical homogenous conditions attain different phenotypic states\(^14–18\). The states are referred to as phenotypic states, since the protein concentration may increase (or decrease) by orders of magnitude and last for generations. E.g. the spontaneous exit of the lysogenic state for the lambda phage is only about \(10^{-7}–10^{-5}\) per cell and generation\(^18,19\) showing that dynamic stability can be on par with the stability of DNA\(^20,21\). External perturbations, e.g. by sudden changes in the reaction rates, can push the biochemical reaction network from one meta stable state to another. Random switching from one stable state to another may occur from extrinsic fluctuation or perturbations but also as a consequence of the intrinsic noise generated from the birth and death of molecules, without any external signal preceding the escape of the state. Random switching is exploited in bacteria as a stochastic survival strategy to blindly anticipate variations in the environment, where different rates of switching is resulting in different fitness\(^22\). Note that the random switching mechanism display an intrinsic time scale which is not triggered by changes in the environment. Random switching is also observed to induce antibiotic resistance in otherwise identical cells\(^23\).
The biochemical reaction schemes are illustrated in Fig. 1. In Fig. 1a, b cooperative binding feedback and dimer binding feedback is illustrated, respectively. Protein synthesis is modeled as a two-step process with mRNA synthesis and decay and a noise term $B(x)$, originating from the small perturbations of birth and death of molecules. The drift term is calculated in the limit of large molecules whereas the noise term is given by the relative perturbation at finite molecule numbers. When we have expressed $\Phi(x)$ and $B(x)$ we can through a pseudo-potential $\Psi(x) = -\int_{0}^{x} \Phi(x') dx'$, where $\theta(x) = \frac{B(x)}{2}$, calculate the average escape time as

$$
\int_{0}^{x(t)} \Phi(x') dx' = \Psi(x) = -\int_{0}^{x(t)} \Phi(x') dx'.
$$
\[
\Gamma_{LH,HL} = \frac{2\pi e^{\Delta \Psi_{LH,HL}}}{\theta(x_{L,H}) \left(\frac{d^2}{dx^2} \Psi + \ln(\theta) \right)_{x_{L,H}} \left(\frac{d^2}{dx^2} \Psi \right)_{x_{L,H}}},
\]

where \( \Delta \Psi_{LH,HL} = \Psi(x_{LH}) - \Psi(x_{L,H}) \). The indices 1 and 2 denotes Low and High stable concentration which are separated by the potential peak at the Middle unstable point, \( \Psi \). The derivation is given in the Methods Section. Thus we need to choose our variable \( x \), calculate \( \Phi(x) \) and \( \theta(x) \) and then integrate the pseudo potential \( \Psi(x) \) and insert into equation 2.

**Concentration drift and stability.** The concentrations of the molecules illustrated in Fig. 1 will in the large molecule limit (for dimer binding feedback) evolve according to the following set of ordinary differential equations (ODE)

\[
\begin{align*}
\frac{dm}{dt} &= k_{m,0} + k_{m,a} \left( \frac{p_2}{K_2 + p_2} \right) - \gamma_m m \\
\frac{dp}{dt} &= k_p m + 2k_p p_2 - 2k_p p^2 - \gamma_p p \\
\frac{dp_2}{dt} &= k_p p^2 - k_p p_2 - \gamma_p p_2
\end{align*}
\]

Typically, the protein life-time is large compared to the mRNA life-time and the equilibration of monomer and dimer concentrations. Under such conditions we can formulate an equation for the concentration drift \( \Phi(\rho_T) \) in the total scaled protein concentration, \( \rho_T = \rho + 2\rho_2 \) where \( \rho_1 = \frac{\rho}{2} \) as

\[
\Phi(\rho_T) \equiv \frac{d\rho_T}{d\tau} = S_0 + S_a \left( \frac{\rho^2}{1 + \rho^2} \right) - (\rho + \alpha \rho^3),
\]

\[
\rho = \left( \frac{K_1}{\gamma} \right) \left( \frac{\rho_1 + K_1}{8K} - \frac{K_2}{4K} \right)
\]

giving us the effective synthesis rate constants \( S_0 = \frac{k_{nas}}{K_1}, S_a = \frac{k_{nas} k_2}{K_1 \gamma^2} \) and the scaled dimer decay rate constant \( \alpha = \frac{2 \gamma^2 K}{k_p} \) and the dissociation constants \( K^2 = K_1 K_2 \). Time is normalized by the protein life-time as \( \tau = \gamma \tau_p \).

The equations describing cooperative binding feedback, illustrated in Fig. 1a, can be obtained by letting \( K_i \to \infty \) making \( \alpha = 0 \). Moreover, since \( \rho_1 = \rho + 2\rho_2 \) and \( \rho_2 = \rho_2 K/K \) we can formulate equation 4 closed in terms of any of the concentrations \( \rho, \rho_1 \) or \( \rho_2 \). For the rate parameters where the system displays bi-stability there are always two stable stationary solutions separated by an unstable one and the region of bi-stability is given by the interval \( (\rho_1, \rho_2) \) where both endpoints are solutions of \( \Phi(\rho_1) = 0 \) and \( \Phi(\rho_2) = 0 \). The region of bi-stability is given by

\[
S_a(\rho_1) = \frac{1 + 2\alpha \rho_1 (1 + \rho_1^2)}{2\rho_1},
\]

\[
S_a(\rho_2) = \rho_2 + \alpha \rho_2^2 - \frac{\rho_2}{2} (1 + 2\alpha \rho_2 (1 + \rho_2^2)),
\]

where \( \rho_a \) are the monomer concentrations separating the bi-stable region. The region of bi-stability is displayed in Fig. 2a. In the limit of \( \alpha \to 0 \) the dimer binding feedback give the same region of bi-stability as cooperative binding. In the limit of having large (scaled) activated synthesis rate, \( S_a \), the limit for having bi-stability is given by \( S_a = 1/4S_c \). Thus a large feedback activation need to be accompanied by a low basal synthesis rate for bi-stability to occur. In Fig. 2b we display the potential function, given by the primitive function of the concentration drift \( \Phi(\rho_T) \), at the three marked points in Fig. 2a.

**Intrinsic noise.** We will now derive an expression for the intrinsic noise of the total concentration of proteins, \( B(\rho_T) \), assuming that mRNA turnover and protein dimerization equilibration are both fast compared to protein turnover. One species, total protein copy number, \( P_T \), and three reactions,

\[
\begin{align*}
\text{Synthesis, } P_T &\to P_T + q, \quad a_1 = \frac{k_p \Phi(\rho_T)}{\gamma_m q}, \quad v_1 = q \\
\text{Monomer decay, } P_T &\to P_T - 1, \quad a_2 = \gamma_p P_2, \quad v_2 = -1 \\
\text{Dimer decay, } P_T &\to P_T - 2, \quad a_3 = \gamma_p P_2, \quad v_3 = -2
\end{align*}
\]

The function \( \Phi(\rho_T) \) in the propensity of the synthesis is given by the two first terms in the first row of equation 3, i.e. the synthesis rate of proteins. However, we have scaled the propensity of synthesis with the average protein synthesised per mRNA and the stoichiometric correction \( q \). The idea is that the average synthesis rate, which is the propensity times the stoichiometry, is not dependent on \( q \) and that \( q \) gives a measure of the burst rate of the...
protein synthesis in the full reaction system. Increasing $q$ implies that more proteins are produced per synthesis event but that the synthesis events appear less frequently. We can calculate the variance $C$ of the total protein concentration using the fluctuation dissipation relation (see Methods section), $C = -D/2A$, where

$$A = \left[ \frac{k_p}{\gamma_m} \frac{dF(p_T)}{dp_T} - \gamma_p \left( 1 + 2\beta \frac{\partial p_T}{\partial p_T} \right) \right]_{(p_T, p_T')}$$

(7)

letting $\beta = \frac{\gamma_m}{\gamma_p} - 1$ and where the diffusion component is

$$D = \left[ \frac{\gamma_p}{\Omega} (2p_T + (3\beta + 1)p_2) + (q - 1)(p_T + 2\beta p_2) \right]_{(p_T, p_T')}$$

(8)

By matching the variance of the total protein concentration for the full system, calculated in the same way as above using fluctuation dissipation relation, we obtain the effective stoichiometry for the reduced system 6 to be $q = 1 + 2b$ when mRNA decay is assumed to be rapid compared to protein decay (calculations of $q$ is presented in the supplementary information). The parameter $b = \frac{k_m}{\gamma_m}$ is the number of proteins translated per mRNA. We then obtain the noise component for the dimer binding feedback by inserting the calculated $q$ in equation 8.
\[ \theta(\rho_T) = \frac{D}{2K^2 \gamma_p} = \frac{1}{K\Omega} \left( (1 + b)\rho_T + (1 + 3b + 2\beta b) \frac{K_1}{K} \sqrt{\frac{K_1}{2K} \left( \rho_T + \frac{K_1}{8K} \right) - \frac{K_1}{4K} } \right) \]  

where we have re-written \( \rho_2 \) in terms of \( \rho_T \). For cooperative binding feedback we get \( \theta(\rho_T) = \frac{1}{K\Omega} (1 + b)\rho_T \). Note that \( \theta(\rho_T) \) is only valid at the two stable fixed points \( \rho_T = \rho_2 \) and \( \rho_T = \rho_0 \), but we now assume that the dependence on \( \rho_T \) can be approximated to hold for all concentrations.

**Escaping the stable states.** Now we know both \( \Phi(\rho_T) \) and \( \theta(\rho_T) \) are ready to calculate the escape time of the phenotypic states by integrating the quasi-potential \( \Psi(\rho_T) \). The integration of \( \Psi \) is presented in the Supplementary Information and for brevity we just state the results here. For dimer binding feedback we obtain

\[
\Psi(\rho_T) = \frac{K\Omega}{1 + b} \left[ \rho_T + \ln \left( \frac{(\rho_T - \delta_1)^{\alpha_1}}{(\rho_T - \delta_2)^{\alpha_2}} \right) \right] + n_T \tan^{-1} \left( \frac{\rho_T - \delta_1}{\frac{1}{2}\delta_2 \frac{1}{2} \delta_2} \right) + n_T \sqrt{\rho_T}
\]

where \( \rho_T = \rho_T + \frac{K}{2K} \). The parameters \( \delta_1 \) and \( n \) are presented in the Supplementary Information. For cooperative binding feedback

\[
\Psi(\rho_T) = \frac{K\Omega}{1 + b} \left[ \rho_T - S_n \ln(\rho_T) - \frac{S_n}{2} \ln(1 + \rho_T^2) \right]
\]

We note that the pseudo potential difference between the stable states and the unstable peak, \( \Delta \Psi_{LH,HL} = \Psi(x_{LH}) - \Psi(x_{HL}) \), scale to first order as \( \Delta \Psi_{LH,HL} = \frac{P_{TM} - P_{THL}}{1 + b} \) for dimer binding feedback \( \Delta \Psi_{LH,HL} = \frac{P_{TM} - P_{THL}}{1 + b} \) for cooperative binding feedback.

We have plotted both the noise component \( \theta \) and the quasi potential \( \Psi \) in Fig. 2b,c. The escape time is obtained by insertion of \( \Psi(\rho_T) \) and \( \theta(\rho_T) \) in equation 2 with \( x = \rho_T \). In Fig. 3a-c we display escape properties for cooperative binding feedback and in panels 3(d)–(f) for dimer binding feedback. The accuracy of the escape time is displayed in Fig. 3a,b,d,e where we as a reference also display the escape time calculated for the constant noise approximation (dashed line). The constant noise approximation is performed by approximating to the noise level at the stationary points occur with a time-scale much shorter than the time scale of switching from one phenotypic state to another, implying that the actual jump from one phenotypic state to the another it fast compared to the time spent in either of the phenotypic states, it is reasonable to assume that the switching process is memoryless an can be approximated as a one-step process (similar to a regular chemical reaction). Under such assumptions the time between switching events can be described by the exponential distribution, with the average rate \( m_T^{-1} \) of switching as the only parameter describing the distribution. We test this assumption by measuring the probability of escaping from the low state to the high state during the time interval \([0, t]\) from stochastic simulations (Figure 3c and 3f, diamonds and circles), that is the cumulative distribution \( P(\leq t) \). The cumulative probability of the exponential distribution is given by \( P(\leq t) = 1 - e^{-\Gamma_{THL} t} \), where we have inserted our calculated mean time of switching \( m_T = \Gamma_{THL} \). The small inlets display the probability density function \( P(t) = \Gamma_{THL} e^{-\Gamma_{THL} t} \).

**Discussion**

We have presented a method for calculating the switching kinetics between phenotypic states, formed by autocatalytic synthesis, where the switching is driven by intrinsic fluctuations. The method relies on deriving a single reaction parameter by a separation of timescales and formulating an effective stoichiometric parameter such that both the concentration drift and fluctuations represent what is observed in the full system. Here we have applied the results on transcription factors binding to its own promoter and enhancing its own synthesis. Such feedback is the most common transcriptional feedback motif in bacteria with the possibility of generating meta-stable states. The feedback need to be sufficiently sensitive to generate metastable states and this can be achieved either by forming multimeric complexes before binding to the promoter or having multiple and cooperative binding sites on the promoter. Typically, transcription factors form multimeric complexes, as dimers or tetramers,
and most likely it is not only due to improving the feedback sensitivity but also due to symmetry in the molecule’s binding to DNA. The formation of a multimeric complex of the feedback molecule gives that the molecule can bind in several orientations which makes the search time to the specific binding site shorter, since the binding (and re-binding after dissociation) becomes more probable. Assuming all other reactions parameters fixed, faster search kinetics of the transcription factor will increase the equilibrium binding constant. From the method in this paper, one may directly investigate such effects since both the pre-exponential factor and the exponential rate dependence is directly expressed from the kinetic parameters of the reaction networks. We have also incorporated another effect of forming multimeric complexes in the method, the effect of having non-linear decay since more than one molecule may be removed at one instant, e.g. by spontaneous decay of a multimer, or by a multimer being consumed in some biochemical pathway. Since the time scale of the stability may change orders of magnitude between feedback from dimer binding and cooperative binding with otherwise similar reaction parameters we conclude that to understand phenotypic switching we need to be careful when formulating a model of an existing reaction network.

Methods

Escape rate calculation. Assume that we have a chemical species whose concentration is described by the variable $x$. Assume furthermore that the species are displaying two stable concentrations $x_L$ and $x_H$, a low and high stable concentration, respectively. The two stable concentrations are separated by the middle unstable concentration $x_M$. We want to calculate the intrinsic stability of the two stable phenotypic states $x_L$ and $x_H$, that is, for how long will the system stay (on average) in a stable state before exit to the other stable state due to the intrinsic fluctuations generated from synthesis and decay of molecules. Individual trajectories $x(t)$ evolve according to the stochastic differential equation

$$dx(t) = \Phi(x)dt + B(x)dW(t)$$

The term $\Phi(x)$ gives the average drift in concentration and $B(x)$ the fluctuations, where $W(t)$ is a Wiener Process. Notably, both the drift and the fluctuations depend on the concentration since both the drift and fluctuations are generated from the biochemical reactions of the system (which typically depends on the concentrations). Since the system is time-homogenous, i.e. the conditional probabilities satisfying $\Pi(x', t | x, 0) = \Pi(x', 0 | x, -t)$, the

Figure 3. The accuracy of the escape time from the phenotypic low states $L$ and high states $H$ with increasing number of molecules. Upper panels show escape properties for cooperative feedback binding and lower panels for dimer binding feedback. The rate parameters are given from the yellow and blue points in Fig. 2a for cooperative and dimer feedback, respectively. In addition we set the monomer-to-dimer decay rate ratio to 10 for dimer binding feedback, making the lifetime of dimers ten times longer than monomers. Errors of the stochastic simulations are smaller than the symbols. The panels (a, b, d, e) show the mean escape rate, where $\Gamma_{LH}$ denotes escape from Low to High state and vice versa. In the rightmost panels we display the probability of escaping the low state to the high state as a function of increasing time where the lines are the theoretical expected probability assuming an cumulative probability of the exponential distribution, $P_{LH}(\leq t) = 1 - e^{-\Gamma_{LH}t}$. The small insets show the probability density function of escaping assuming an exponential distribution, $P_{LH} = \Gamma_{LH}e^{-\Gamma_{LH}t}$. Errors are smaller that symbols and circles are for the low state having 80 proteins and diamonds with the low state having 160 molecules.
backward Kolmogorov (or Fokker-Planck) equation corresponding to the stochastic process described in equation 12 can be written

$$\frac{\partial \Pi(x', t|x, 0)}{\partial t} = \Phi(x) \frac{\partial \Pi(x', t|x, 0)}{\partial x} + \theta(x) \frac{\partial^2 \Pi(x', t|x, 0)}{\partial x^2},$$

where $\theta(x) = \frac{\Phi(x)}{2}$. The probability that the concentration remains in the region $R$ at time $t$ starting from some concentration $x$ inside $R$ is $G(x, t) = \int R \Pi(x', t|x, 0) dx'$. By integration of the backward equation 13 over $R$

$$\int R \Phi(x) \frac{\partial G(x, t)}{\partial x} + \theta(x) \frac{\partial^2 G(x, t)}{\partial x^2} \text{d}x = 0,$$

Now, the average time of escaping $R$ starting in $x$ is given by $\Gamma(x) = -\int_0^\infty \frac{\partial G(x, t)}{\partial t} \text{d}t = \int_0^\infty G(x, t) \text{d}t$, is therefore obeying the equation

$$\Phi(x) \frac{d\Gamma(x)}{dx} + \theta(x) \frac{d^2\Gamma(x)}{dx^2} = G(x, \infty) - G(x, 0) = -1$$

Introducing the quasi-potential $\Psi(x)$ where $\frac{d\Psi(x)}{dx} = -\Phi(x)$ gives the solution by the integrating factor $e^{-\Psi}$. The escape time from the low state to the high state, $\Gamma_{LH}$, is obtained by integrating twice, from 0 to $x'$ with boundary condition $\frac{d\Gamma(x)}{dx} = 0$, and from the low stable concentration, $x_L$, to the high stable concentration, $x_H$, with boundary condition $\Gamma(x_H) = 0$ gives $\Gamma_{LH} = \int_{x_L}^{x_H} e^{\Psi} \text{d}x' \int_0^{x'} e^{-\Psi - \ln(\theta)} \text{d}x''$. In the same manner we obtain the escape time from the high state to the low state, $\Gamma_{HL}$. Integrating from $\infty$ to $x'$ with boundary condition $\frac{d\Gamma(x)}{dx} = 0$, and from $x_H$ to $x_L$ with boundary condition $\Gamma(x_L) = 0$ gives $\Gamma_{HL} = \int_{x_H}^{x_L} e^{\Psi} \text{d}x' \int_0^{\infty} e^{-\Psi - \ln(\theta)} \text{d}x''$. In these equations for the escape time, the external integrands get their main contribution near the middle unstable stationary point, $s_H$, and the internal integrands get their main contribution near the low stable stationary point $x_L$ for $\Gamma_{LH}$, and near high stable stationary point $x_H$ for $\Gamma_{HL}$. Therefore, a parabolic approximation of the integrand functions around the dominant points gives the average escape time as

$$\Gamma_{LH,HL} = \frac{2\pi e^{-\Delta\Psi_{LH,HL}}}{\theta(x_L, \theta) \sqrt{\frac{d^2}{dx^2} \left( \Psi + \ln(\theta) \right)_{s_H}}} \left[ \frac{d^2}{dx^2} \left( \Psi \right)_{s_H} \right],$$

where $\Delta\Psi_{LH,HL} = \Psi(x_H) - \Psi(x_L)$.

**System size scaling.** Since we are interested in analysing the intrinsic, finite-molecule, stochastic properties of the bistable system it is convenient if the reaction network can be scaled from a system of few molecules to a large number of molecules without affecting the bi-stability. This can be achieved by introducing a system size parameter $\Omega$36. In this setting we can view the scaled variables as concentrations, e.g. the protein monomer size parameter $\Omega$36. The quasi-potential $\Psi(x)$, where $\frac{d\Psi(x)}{dx} = -\Phi(x)$, gives the solution by the integrating factor $e^{-\Psi}$. The escape time from the low state to the high state, $\Gamma_{LH}$, is obtained by integrating twice, from 0 to $x'$ with boundary condition $\frac{d\Gamma(x)}{dx} = 0$, and from the low stable concentration, $x_L$, to the high stable concentration, $x_H$, with boundary condition $\Gamma(x_H) = 0$ gives $\Gamma_{LH} = \int_{x_L}^{x_H} e^{\Psi} \text{d}x' \int_0^{x'} e^{-\Psi - \ln(\theta)} \text{d}x''$. In the same manner we obtain the escape time from the high state to the low state, $\Gamma_{HL}$. Integrating from $\infty$ to $x'$ with boundary condition $\frac{d\Gamma(x)}{dx} = 0$, and from $x_H$ to $x_L$ with boundary condition $\Gamma(x_L) = 0$ gives $\Gamma_{HL} = \int_{x_H}^{x_L} e^{\Psi} \text{d}x' \int_0^{\infty} e^{-\Psi - \ln(\theta)} \text{d}x''$. In these equations for the escape time, the external integrands get their main contribution near the middle unstable stationary point, $s_H$, and the internal integrands get their main contribution near the low stable stationary point $x_L$ for $\Gamma_{LH}$, and near high stable stationary point $x_H$ for $\Gamma_{HL}$. Therefore, a parabolic approximation of the integrand functions around the dominant points gives the average escape time as

$$\Gamma_{LH,HL} = \frac{2\pi e^{-\Delta\Psi_{LH,HL}}}{\theta(x_L, \theta) \sqrt{\frac{d^2}{dx^2} \left( \Psi + \ln(\theta) \right)_{s_H}}} \left[ \frac{d^2}{dx^2} \left( \Psi \right)_{s_H} \right],$$

where $\Delta\Psi_{LH,HL} = \Psi(x_H) - \Psi(x_L)$.

**Fluctuation dissipation relation.** For a multivariate Ornstein–Uhlenbeck process defined by the stochastic differential equation $dx(t) = -Ax(t)dt + B\text{d}W(t)$, where $A$ and $B$ are constant matrices, the covariance matrix $C$ at stationary conditions satisfies a fluctuation-dissipation relation

$$AC + CA^T - BB^T = 0$$

If $x$ is sufficiently close to a stationary point $x$, we can for a more general system $dx(t) = -f(x(t))dt + B\text{d}W(t)$ linearise the drift $f(x(t))$. The matrix $A$ is then the Jacobian matrix evaluated at the stationary point $x$, $A_{ij} = \frac{\partial f_i}{\partial x_j}$. For a reaction network, the diffusion matrix is given by $D_i = \left( BB^T \right)_{ij} = \frac{1}{\theta} \sum_k q_k v_{ik} v_{jk}$ where $q_k$ is the propensity of reaction $k$ and $v_{ij}$ the stoichiometry of species $i$ in reaction $k$, evaluated at the stationary concentration $x^{eq}_i$. Once $A$ and $D$ are calculated we can compute the covariance matrix $C$ through the fluctuation dissipation theorem 17. For a single variable process the variance is $C = -\frac{\Delta}{2\theta}$.

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