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Noise Propagation in Series Enzymatic Cascades

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Abstract: Series enzymatic cascades are ubiquitously found in signaling networks and act as key signal amplifiers. During signal transduction process, cell-to-cell variability or noise travels along with the signal and strongly affects the fitting response that the cells exhibit. Modulation of noise propagation through the enzymatic cascades can play a strong role in regulating cellular response. We find the conditions under which the noise propagation through the cascades is bounded. Using global sensitivity analysis, we quantified the dependence of these conditions on the system parameters and estimated the parameter range in which the system when operated will result in attenuation of noise propagation through the cascade.

Keywords: Series enzymatic cascade, Noise propagation, Langevin technique, Global sensitivity analysis.

1. INTRODUCTION

Signal transduction through biological signalling networks often occurs via enzymatic cascades. Enzymatic cascades found ubiquitously in eukaryotic signalling networks (Widmann et al., 1999; Zhang and Dong, 2005) act as important signal amplifier for many cell-fate processes (Huang and Ferrell, 1996; Chang and Karin, 2001; Zhang and Dong, 2007, Dhanasekaran and Johnson, 2007) such as cell proliferation, apoptosis (Qi and Elion, 2005). Proteins involved in an enzymatic cascade such as MAPK cascade are therefore considered potential therapeutic targets for multiple diseases (Lee et al., 1999).

Cells are constantly exposed to inevitable fluctuations or noise. Noise in a system can be classified into two types, *viz.*, extrinsic and intrinsic noise, sources for which respectively are external and internal to the cells. These two types of noise can be correlated under certain conditions (Tanase-Nicola et al., 2006). Fluctuations have been observed during many cell-fate processes such as apoptosis (Spencer et al., 2009). Fluctuations or noise or cell-to-cell variability flows along with signal into the signalling networks. Therefore, signalling networks have to process these fluctuations for the cell's normal functioning and also to make faithful decisions when necessary (Raj and van Oudenaarden, 2008). Propagation of noise through signalling networks and its amplification is beneficial to cells in some situations (McDonnell and Ward, 2011; Paszek et al., 2010; Eldar and Elowitz, 2010) and deleterious in other cases (Barkai and Leibler, 2000).

Enzymatic cascades being a crucial signal processing module in signalling networks, attenuation or amplification of noise by such cascades can have significant impact on the cellular response to a certain cue. It is therefore important to understand how the cascades modulate noise and the conditions under which they may attenuate or amplify noise. Enzymatic cascades are typically made up of building blocks such as single-step, series and parallel cascades (Kholodenko, 2006 and references therein). A few recent attempts have been made to characterize noise propagation in single-step (Detwiler et al., 2000; Shibata and Fujimoto, 2005), two-step series (Dhananjaneyulu et al., 2012) and two-substrate parallel (Viswanathan et al., 2008) cascades. Thattai and van Oudenaarden (2000) identified conditions that guarantee attenuation of noise propagation through transcriptional cascade assuming first-order degradation and that all species decay at the same rate. In this study, we consider a series enzymatic cascade and find the conditions under which the noise propagation through the enzymatic cascade is bounded. Using global sensitivity analysis, we unravel the feasible range of system parameters, operating within which will guarantee an upper bound for noise propagation through series enzymatic cascades.

2. NOISE IN ENZYMATIC CASCADES

2.1 Model

Consider a series cascade consisting of N enzymatic phosphorylation-dephosphorylation steps (Fig. 1). The phosphorylation of the substrate S_i to S_i^* in the i^{th} cascade switches the substrate from inactive to active form and thereby facilitates signal transfer. On the contrary a dephosphorylation event switches the substrate from active to inactive form. While enzyme S_{i-1}^*, which is the phosphorylated substrate in the $(i\text{-}1)^{\text{th}}$ cascade acts as a kinase for the i^{th} cascade, another enzyme P_i acts as phosphatase for the dephosphorylation event. The kinase for the 1st cascade is S_0.

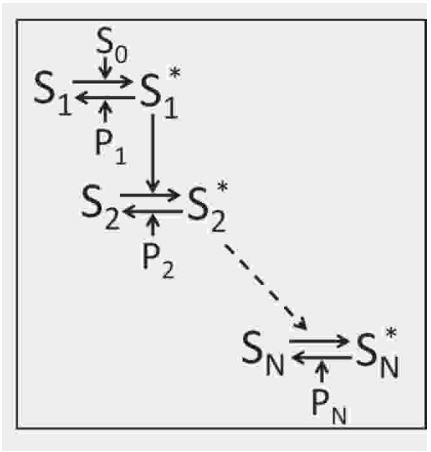

Figure 1: Schematic of an N step series enzymatic cascade.

Assuming that the concentration of P_i's is not affected by the enzymatic cascades, the biochemical reactions involved in the i^{th} cascade are

$$S_i + S_{i-1}^* \underset{k_{2i}}{\overset{k_{1i}}{\rightleftarrows}} S_i S_{i-1}^* \xrightarrow{k_{3i}} S_i^* + S_{i-1}^* \quad (1)$$

$$S_i^* + P_i \underset{k_{5i}}{\overset{k_{4i}}{\rightleftarrows}} S_i^* P_i \xrightarrow{k_{6i}} S_i + P_i \quad (2)$$

where $S_i S_{i-1}^*$ and $S_i^* P_i$ are the intermediates and k_{ji}, j = 1 to 6 are the rate constants of the biochemical reactions corresponding to the i^{th} cascade. Using the classical Michaelis-Menten formulation and Langevin technique (van Kampen, 1992), the stochastic differential equation (SDE) that capture the dynamics of i^{th} phosphorylated substrate is

$$\frac{dx_i^*}{dt} = \frac{k_{3i}\left(x_i^t - x_i^*\right)x_{i-1}^*}{K_{1i} + \left(x_i^t - x_i^*\right)} - \frac{k_{6i}x_i^* p_{i0}}{K_{2i} + x_i^*} + \eta_i(t) = R_i(x_i^*, x_{i-1}^*) + \eta_i(t) \quad (3)$$

where $x_i, x_i^*, x_i^t = x_i + x_i^*$, respectively are the number of unphosphorylated, phosphorylated, and total i^{th} substrate. $R_i(x_i^*, x_{i-1}^*)$ and p_{i0} are the reaction rate and total available number of i^{th} phosphatase, respectively. $K_{1i} = \left(k_{2i} + k_{3i}\right)/k_{1i}$ and $K_{2i} = \left(k_{5i} + k_{6i}\right)/k_{4i}$ are the Michaelis-Menten constants for the i^{th} cascade. $\eta_i(t)$ are the independent Gaussian white noise terms that have zero mean, that is, $\langle \eta_i(t) \rangle = 0$ and that satisfy

$$\left\langle \eta_i(t)\eta_i(t') \right\rangle = A_i \delta(t - t') \quad (4)$$

where $\delta(t-t')$ is the Dirac delta function, $A_i = \frac{k_{3i}\left(\bar{x}_i^t - \bar{x}_i^*\right)\bar{x}_{i-1}^*}{K_{1i} + \left(\bar{x}_i^t - \bar{x}_i^*\right)} + \frac{k_{6i}\bar{x}_i^* p_{i0}}{K_{2i} + \bar{x}_i^*}$ is the strength of the fluctuations which quantifies the total variance of the increment in the i^{th} phosphorylated substrate in the time $t - t'$ (Detwiler et al., 2000). The strength A_i is estimated at the mean number of species $(\bar{x}_i^*, \bar{x}_{i-1}^*)$ which are essentially the steady states of macroscopic rate equations, obtained by solving

$$\frac{dx_i^*}{dt} = R_i(x_i^*, x_{i-1}^*) \quad (5)$$

2.2 Extrinsic and intrinsic noise

Extrinsic and intrinsic noise are caused respectively by sources external and internal to the cells. The classical method of estimation of noise involves linearization of Langevin type stochastic differential equation (Eq. 3) around steady states. This method is simple and elegant as it is amenable to obtaining analytical expressions for noise in biological systems (Shibata and Fujimoto, 2005; Detwiler et al., 2000; Viswanathan et al., 2008; Elf and Ehrenberg, 2003). Linearizing the model (Eq. 3) around steady states $\bar{x}_i^*$, i=1 to N of the macroscopic rate equations (Eq. 5) leads to

$$\frac{d\Delta x_i^*}{dt} = \tau_i^{-1}\Delta x_i^* = \tau_i^{-1} g_i \Delta x_{i-1}^* + \eta_i(t) \quad (6)$$

where, $\Delta x_i^*, \tau_i, g_i$, respectively represent the perturbation from the steady states for the i^{th} phosphorylated substrate, relaxation time, gain for the i^{th} enzymatic cascade. The relaxation time and gain for the i^{th} cascade, respectively are given by

$$\tau_i^{-1} = \frac{k_{3i}K_{1i}x_{i-1}^*}{\left(K_{1i} + (x_i^t - x_i^*)\right)^2} + \frac{k_{6i}K_{2i}p_{i0}}{\left(K_{2i} + x_i^*\right)^2} \quad (7a)$$

and

$$g_i = \frac{\tau_i(x_i^t - x_i^*)k_{3i}}{K_{1i} + (x_i^t - x_t^*)} \quad (7b)$$

Note that although upstream kinases S_j^*, j = 1 to *i-2* do not directly participate in the phosphorylation of S_i, the fluctuations in all the upstream kinases propagate and affect noise in S_i^*.

Noise in the phosphorylated substrate S_i^* for the i^{th} cascade can be obtained by solving Eq. (6) simultaneously for all i = 1 to N using Fourier transforms. Square of the appropriate perturbation, ensemble averaged, is used as a measure of noise (Detwiler et al., 2000). The total noise in i^{th} substrate around the steady state is given by the sum of the intrinsic I_i and extrinsic E_i noise, assuming these two types of noise arise from independent sources. Assuming Poisson statistics for the birth and death processes (via a phosphorylation-dephosphorylation cycle) of the first kinase in the cascade S_0 with a corresponding time scale τ_0, the extrinsic noise in the phosphorylated substrate of the i^{th} cascade is given by

$$E_i = \frac{\left|\Delta x_i^*\right|^2}{\left|\Delta x_0\right|^2} = \left(\tau_0 \sum_{j=1}^{i} T_{i,j} \prod_{j=1}^{i} g_j\right)^2 \quad (8)$$

where, $\left|\Delta x_0\right|^2$ is the fluctuations around x_0^t, the total number of the kinase available for the first cascade and

$T_{i,j} = \tau_j^{2i-1} \Big/ \prod_{i=1, i\neq k}^{n} \left(\tau_i^2 - \tau_j^2\right)$ and the corresponding intrinsic noise is given by

$$I_i = \left|\Delta x_i^*\right|^2 = \sum_{j=1}^{i} A_j \left[\prod_{k=j+1}^{i} \left(\tau_k^{-1} g_k\right)^2\right] \left[\sum_{k=1}^{i} \left(2\tau_k^{-1} \prod_{\substack{m=1,\\ m\neq k}}^{i} \left(\tau_k^{-2} - \tau_m^{-2}\right)\right)^{-1}\right] \quad (9)$$

We assume that the extrinsic noise from all sources are represented in the fluctuations in the first kinase S_0.

3 ATTENUATION OF NOISE PROPAGATION

3.1 Noise propagation is bounded

We next look for conditions that ensure that the noise in the cascade will remain bounded. We follow the methodology proposed by Thattai and van Oudenaarden (2002) of expressing the noise in Fourier space as a recursive series and arriving at appropriate conditions for its boundedness. Using Eq. (4), the Fourier transformed total noise in the i^{th} cascade can be written as

$$\left|\Delta x_i^*(\omega)\right|^2 = c_i = a_i + b_i \left|\Delta x_{i-1}^*(\omega)\right|^2 = a_i + b_i c_{i-1} \quad (10)$$

where, $a_i = \dfrac{\tau_i^2 A_i}{1+\tau_i^2\omega^2}$ and $b_i = \dfrac{\tau_i^{-2} g_i^2}{\tau_i^{-2} + \omega^2} = \dfrac{g_i^2}{1+\tau_i^2\omega^2}$

Using Eq. (10) the total noise in N^{th} cascade can be written in the form of a recursive relation

$$\left|\Delta x_N^*(\omega)\right|^2 = c_N = a_N + b_{N-1}a_{N-1} + b_{N-2}b_{N-1}a_{N-2} + \ldots + b_{N-1} \ldots b_1 c_0 \quad (11)$$

where, $c_0 = \left|\Delta x_0(\omega)\right|^2$ is the noise in the first kinase. Conditions that guarantee an upper bound for the recursive relation in Eq. (11) (Thattai and van Oudenaarden, 2002) are:

1) $b = \sup(b_i) < 1$ (12)

2) $a = \sup(a_i)$ exists (13)

Imposition of these conditions on Eq. (11) results in the inequality

$$c_N < a(1 + b + b^2 + \ldots) + b^N c_0 \quad (14)$$

As $N \to \infty$, Eq (14) reduces to

$$c_\infty < a/(1-b) \quad (15)$$

Inverse Fourier transform of the RHS in Eq. (15) gives the upper bound on the noise in the cascade.

Condition 1 (Eq. 12) is valid iff $b_i < 1, \forall i$. Since $\omega^2 > 0$, for condition 1 (Eq. 12) to satisfy, it is sufficient if $\max(g_i) < 1$. Next, it remains to be shown that condition 2 (Eq. 13) is valid.

Suppose if the parameters of i^{th} and $(i+1)^{\text{th}}$ are same for some large *i*, then the steady state macroscopic rate equations (Eq. 5 with lhs set to zero) can be re-written in the form of a sequence

$$\bar{x}_i^* = F(\bar{x}_{i-1}^*) \quad (16)$$

where $F(\bar{x}_{i-1}^*)$ is a function in $\bar{x}_{i-1}^*$. Based on contraction mapping theorem, if

$$\frac{dF(\bar{x}_{i-1}^*)}{d\bar{x}_{i-1}^*} < 1 \quad (17)$$

in some neighbourhood of $\bar{x}_{i-1}^*$, then $F(\bar{x}_{i-1}^*)$ is a contraction and the sequence in Eq. (16) will converge to a unique fixed point for any set of parameters provided the same set is used for every cascade. Convergence of the sequence in Eq. (16) implies existence of an upper bound for $a_i = \tau_i^2 A_i \big/ \left(1 + \tau_i^2 \omega^2\right)$ as $\tau_i^2 A_i$ is a function of the steady state number of the phosphorylated substrates for the i^{th} and $(i-1)^{\text{th}}$ cascades.

Based on experimentally derived parameters (Table 1) for a two-step MAPK enzymatic cascade in mammalian systems (Fujioka et al., 2006) and the relaxation time scales (Santos et al., 2007; Ruf et al., 2007), we seek to estimate the range of $\bar{x}_i^*$ for which F is a contraction. The parameters obtained from literature were converted into numbers using the formula

$$N_S = \frac{\pi d^3 C_S N_A}{6} \quad (18)$$

where, N_S is the number of molecules, C_S is the molar concentration, d is the cell diameter and N_A is Avogadro's constant. Cells were assumed to be spherical in shape with a diameter of 10 μm. (Note that the cell diameter is in the same order of magnitude as that reported in Fujioka et al., 2006).

Parameter	Value
x_i^t	377
p_{i0}	32
k_{3i}	$0.18\ s^{-1}$
k_{6i}	$0.3\ s^{-1}$
K_{1i}	100
K_{2i}	22

Table 1: Parameter values used in the simulations (Fujioka et al., 2006).

In Fig. 2, we show the dependence of $d\,F(\bar{x}_i^*)/d\,\bar{x}_i^*$ on $\bar{x}_i^*$. The range for $\bar{x}_i^*$ was chosen based on the typical constitutive number of kinase molecules present in a cell. A wide range of $\bar{x}_i^*$ exists in which F will be a contraction (Fig. 2). This proves that for a certain set of parameters, an upper bound for the total noise in the cascade exists.

3.2 Parametric dependence of noise propagation

In the previous section, the validity of the two conditions (Eq. 12 and 13) was shown by assuming that all system parameters are same for downstream cascades. However, the parameters are known to differ across cascades. Moreover, it has been shown that the quasi-steady state assumption involved in Michaelis-Menten formulation is valid only

under certain conditions imposed on the total number of molecules of a species in the cell (Segal, 1980). Numerical values for most of the parameters for larger cascades have not been measured experimentally and therefore are unavailable.

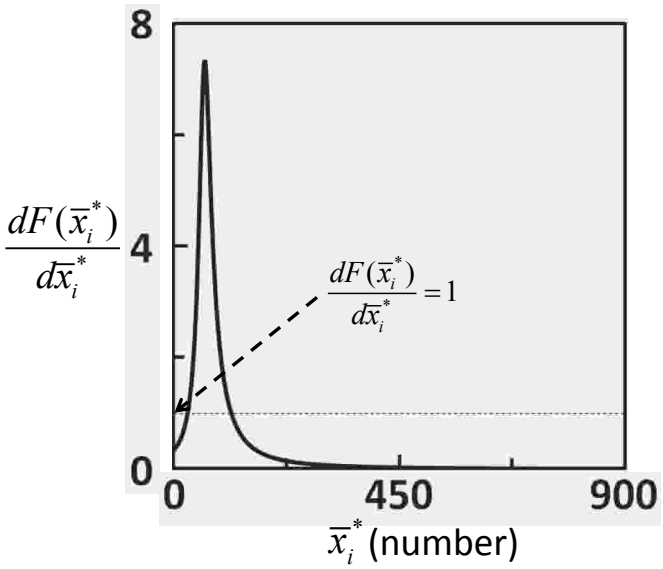

Figure 2: Dependence of $dF(\bar{x}_i^*)/d\bar{x}_i^*$ on $\bar{x}_i^*$ showing that the function F will be a contraction for a wide range of phosphorylated substrate concentrations. All parameters in the function F were assigned values reported in Table 1.

We consider a 5 step enzymatic cascade as an example and show that the noise estimates are bounded for a wide range of parameters. A global sensitivity analysis (GSA) (Jianfang et al., 2007; Dhananjaneyulu et al., 2012) using 1000 sets of parameters generated randomly by Latin Hypercube Sampling technique (Imam and Conover, 1980) was performed to measure the extent of dependence of gain factor g_5 and of $dF(\bar{x}_5^*)/d\bar{x}_5^*$ on various system parameters. Note that the gain factor in the 5th cascade and the derivative of the function in Eq. (17) depend on parameters of the upstream cascades. Therefore GSA was conducted by varying all 32 parameters associated with all 5 cascades considered. The samples for GSA were generated using uniform distribution for all the parameters with a deviation of $\pm 20\%$ from the nominal value. We then found those sample sets with gain factor $g_5^2 < 1$ that additionally satisfy the constraint in Eq. (17). From this set of samples, we then estimated the bound on these parameters that permit $g_5^2 < 1$ and Eq. (17). Note that the extrinsic (Eq. 8) and intrinsic (Eq. 9) noise are strong functions of the gain factor. Figure 3 shows the lower and upper bounds for the total number of substrate molecules of all 5 cascades.

Similar bounds for the remaining 27 parameters are presented in Appendix I. The bounds obtained for various parameters are indeed in the range estimated experimentally for a 2-step enzymatic cascade (Fujioka et al., 2006). Moreover, in the parameter range where noise is bounded, we found that the cascade was operating in the ultrasensitive regime. This suggests that there may be many combinations

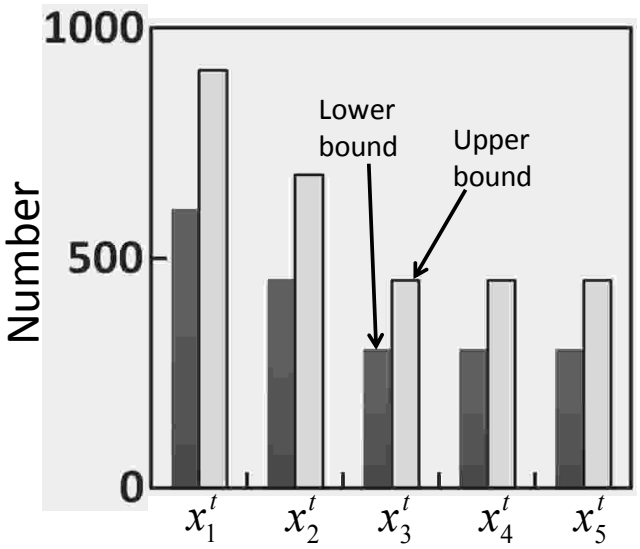

Figure 3: Lower and upper bounds on the total number of substrates of all five cascades for which the noise will be bounded. Bounds for other parameters of the cascade are in Appendix I.

of parameters for which attenuation of noise in series enzymatic cascades can be achieved while preserving the signal amplifying nature of the cascade.

4. CONCLUSIONS

Series enzymatic cascades are shown to have the ability to regulate noise propagation through them. In particular, using a combination of analytical conditions and global sensitivity analysis, we show that there exists a practical range of parameters for which an upper bound on the total noise can be obtained. The analytical conditions that mandate this upper bound was first arrived at without distinguishing the values of parameters across different cycles. Identification of the two conditions provided a rational constraint to characterize the ability of ultrasensitive MAPK cascades to attenuate noise propagation under feasible range of parameters. The hallmark of the method is that the analytical conditions provide pinpointed directions towards identifying the range of parameters where noise is bounded without considering the nature, that is, extent of monotonicity of noise propagation through the cascades.

Using global sensitivity analysis and these conditions as a constraint, we find the range for all the system parameters for which total noise in a 5 step series in enzymatic cascade will be bounded. This range of parameters falls in the regime where the cascade exhibits ultrasensitivity. As a result, under certain conditions, MAPK enzymatic cascades can simultaneously perform dual function of signal amplification and of attenuation of noise to protect the fidelity of signal. This ability of the MAPK cascades identified in this study requires thorough experimental validation.

We argue that these findings can now pave way for performing pinpointed experiments to arrive at strategies for capitalizing on this dual functionality of enzymatic cascades to tune signalling networks towards useful functions. For instance, experimental methods are available for independently altering the total enzyme concentration (Santos et al., 2007) or inhibiting them (Favata et al., 1988; English and Cobb, 2002). These methods could now be used to regulate the concentrations of various kinases in the cascade accordingly in the range where attenuation of noise is desirable in activated signalling networks. On the other hand, similar regulation could be employed to operate under the regime where amplification of noise is desirable to achieve a certain function.

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APPENDIX I: Lower and upper bounds for biochemical parameters

Parameter	Range	Parameter	Range
K_{11}	96.1 – 144	k_{61}	0.2 – 0.4 s^{-1}
K_{12}	88 – 132	k_{62}	0.2 – 0.4 s^{-1}
K_{13}	80 – 120	k_{63}	0.2 – 0.4 s^{-1}
K_{14}	80.1 – 120	k_{64}	0.2 – 0.4 s^{-1}
K_{15}	80.1 – 119.9	k_{65}	0.2 – 0.4 s^{-1}
K_{21}	17.6 – 26.4	p_{10}	25.6 – 38.4
K_{22}	17.6 – 26.4	p_{20}	25.6 – 38.4
K_{23}	17.6 – 26.4	p_{30}	25.6 – 38.4
K_{24}	17.6 – 26.4	p_{40}	25.6 – 38.4
K_{25}	17.6 – 26.4	p_{50}	25.6 – 38.4
k_{31}	0.1 – 0.2 s^{-1}	τ	80 – 119.9 s
k_{32}	0.2 – 0.3 s^{-1}	x_0	37.8 – 56.7
k_{33}	0.1 – 0.2 s^{-1}		
k_{34}	0.1 – 0.2 s^{-1}		
k_{35}	0.1 – 0.2 s^{-1}		

Table AI.1: Lower and upper for biochemical parameters and upstream kinase relaxation time scale and total number.