Mechanisms of Stochastic Focusing and Defocusing in Biological Reaction Networks: Insight from Accurate Chemical Master Equation (ACME) Solutions

Accepted, Conf Proc IEEE Eng Med Biol Soc. 2016.

Gamze Gu¨rsoy[1], Anna Terebus[1],Youfang Cao[2] and Jie Liang[1]

***Abstract*— Stochasticity plays important roles in regulation of biochemical reaction networks when the copy numbers of molecular species are small. Studies based on Stochastic Simulation Algorithm (SSA) has shown that a basic reaction system can display stochastic focusing (SF) by increasing the sensitivity of the network as a result of the signal noise. Although SSA has been widely used to study stochastic networks, it is ineffective in examining rare events and this becomes a significant issue when the tails of probability distributions are relevant as is the case of SF. Here we use the ACME method to solve the exact solution of the discrete Chemical Master Equations and to study a network where SF was reported. We showed that the level of SF depends on the degree of the fluctuations of signal molecule. We discovered that signaling noise under certain conditions in the same reaction network can lead to a decrease in the system sensitivities, thus the network can experience stochastic defocusing. These results highlight the fundamental role of stochasticity in biological reaction networks and the need for exact computation of probability landscape of the molecules in the system.**

I. INTRODUCTION

Understanding how biochemical reaction networks function is essential to investigate important cellular processes. Reaction networks are often stochastic due to random thermal fluctuations, when the copy numbers of molecular species are small [1], [2]. For example, stochasticity plays critical roles in determining cellular fate, as in the examples of stochastic switch between the lysogenic state and the lytic state in phage lambda [10] and the transition into and from competence in the *Bacillus subtilis* [3].

In an enzymatic reaction system with a few copy number of enzyme molecules, the reactions of the system can exhibit the behavior of stochastic focusing (SF) when responding to the fluctuating signal molecules [4]. SF is observed when the fold change of the product molecules is more than the fold change the signal molecules. The discovery of SF was based on extensive simulations using the Stochastic Simulation Algorithm (SSA). However, it was assumed that enzyme molecules do not experience fluctuation prior to the onset of alterations in the number of signal molecules, which models the changes in the environment. This assumption is unrealistic in low-copy enzymatic reactions. Milias-Argeitis et. al suggested a possible noise suppression mechanism for the same enzymatic reaction, when there is a small and fluctuating number of active enzymes [5]. The underlying SF phenomena was estimated by approximating the mean and standard deviation of the steady state probability landscape of molecules. This approach, however, is problematic when probability landscape exhibits multistability, where the mean of the overall landscape does not describe the most probable states of the system.

Although stochastic simulation algorithm has been widely used to study the behavior of biochemical reaction networks [6], it is ineffective in examining rare events. For example, the convergence of such simulations is difficult to determine [13], and the errors in the sampled steady state probability landscape are unknown. The discrete Chemical Master Equation (dCME) provides a general framework for modeling of such networks [7], [8]. Solving dCME is challenging, since analytical solution is not possible and computational solutions are challenged with the problem of large state space.

Here we used the recently developed Accurate Chemical Master Equation (ACME) method [9]–[12] to study the phenomenon of stochastic focusing, which allows optimum enumeration of the state space according to predefined error tolerance, and can directly calculate the solution of the dCME for the enzymatic reaction system described in [4]. We investigated the behavior of the reaction system under different distributions of noise of the signaling molecules. Our results showed that the phenomenon of SF is diminished if the birth and death of signaling molecule follows a Poison process both before and after changing the conditions instead of following a Poisson process only after changing the conditions. Furthermore, we used a bi-stable Shlögl model for the synthesis and degradation of signaling molecule and report the discovery the phenomenon of stochastic defocusing in the same system under certain conditions.

*This work was supported by NIH Grants GM079804, GM086145 and NSF Grants DBI 1062328 and DMS-0800257. This work was also funded by the Chicago Biomedical Consortium with support from the Searle Funds at The Chicago Community Trust

[1]G. Gürsoy is with the Bioinformatics Program, Richard and Loan Hill Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, USA ggurso2@uic.edu

[1]A. Terebus is with the Bioinformatics Program, Richard and Loan Hill Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, USA atereb2@uic.edu

[2]Y. Cao is Postdoctoral Research Associate with the Theoretical Biology and Biophysics and Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, NM, USA youfangcao@gmail.com

[1]J. Liang is on Faculty of Richard and Loan Hill Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, USA jliang@uic.edu

II. Models and Methods

A. Discrete Chemical Master Equation

Consider a well-mixed biochemical system with constant volume and temperature contains n molecular species X_i, which participate in m reactions R_k with reaction rate constants r_k. At time t, the microstate of the system is represented by the non-negative integer column vector of copy numbers of each molecular species: $\boldsymbol{x}(t) = (x_1(t), x_2(t), \cdots, x_n(t))^T$, where ^{T} is the transpose. The general form for a reaction R_k ($k = 1, 2, \cdots, m$) with intrinsic rate r_k is

$$c_{1k}X_1 + \cdots + c_{nk}X_n \xrightarrow{r_k} c'_{1k}X_1 + \cdots + c'_{nk}X_n,$$

which transitions the system from a microstate $\boldsymbol{x}_i$ to $\boldsymbol{x}_j$. The difference between $\boldsymbol{x}_i$ and $\boldsymbol{x}_j$ is the stoichiometry vector $\boldsymbol{s}_k$ of the reaction R_k: $\boldsymbol{s}_k = \boldsymbol{x}_j - \boldsymbol{x}_i = (s_{1k}, s_{2k}, \cdots, s_{nk})^T = (c'_{1k} - c_{1k}, c'_{2k} - c_{2k}, \cdots, c'_{nk} - c_{nk})^T \in \mathbb{Z}^n$. The stoichiometry matrix $\boldsymbol{S}$ for all the reactions in the network is defined as: $\boldsymbol{S} = (\boldsymbol{s}_1, \boldsymbol{s}_2, \cdots, \boldsymbol{s}_m) \in \mathbb{Z}^{n\times m}$, where each column represents a single reaction. The rate $A_k(\boldsymbol{x}_i, \boldsymbol{x}_j)$ of reaction R_k that brings microstate from $\boldsymbol{x}_i$ to $\boldsymbol{x}_j$ is determined by the intrinsic rate constant r_k and the combination of the reactants in the current microstate $\boldsymbol{x}_i$: $A_k(\boldsymbol{x}_i, \boldsymbol{x}_j) = A_k(\boldsymbol{x}_i) = r_k \prod_{l=1}^{n} \begin{pmatrix} x_l \\ c_{lk} \end{pmatrix}$.

The state space $\mathcal{S}$ is the set of all possible microstates that the system can visit from a given initial condition over time t: $\mathcal{S} = \{\boldsymbol{x}(t) | \boldsymbol{x}(0), t \in (0, \theta)\}$. The probability of each microstate at time t is $p(\boldsymbol{x}(t))$, and the probability distribution at time t over the whole state space is $\boldsymbol{p}(t) = \{(p(\boldsymbol{x}(t)) | \boldsymbol{x}(t) \in \mathcal{S})\}$. $\boldsymbol{p}(t)$ is also called the *probability landscape* of the network [10].

The dCME of a microstate $\boldsymbol{x} = \boldsymbol{x}(t)$ is defined as:

$$\frac{dp(\boldsymbol{x})}{dt} = \sum_{\boldsymbol{x}'} [A(\boldsymbol{x}', \boldsymbol{x})p(\boldsymbol{x}') - A(\boldsymbol{x}, \boldsymbol{x}')p(\boldsymbol{x})]$$

where $\boldsymbol{x}' \neq \boldsymbol{x}$. This equation can be further represented in matrix form: $\frac{d\boldsymbol{p}(t)}{dt} = \boldsymbol{A}^T\boldsymbol{p}(t)$ For any $\boldsymbol{x}_i$, $\boldsymbol{x}_j \in \mathcal{S}$, where $\boldsymbol{A} \in \mathbb{R}^{|\mathcal{S}|\times|\mathcal{S}|}$ is called the transition rate matrix formed by the collection of all $A(\boldsymbol{x}_i, \boldsymbol{x}_j)$:

$$A = \|A(\boldsymbol{x}_i, \boldsymbol{x}_j)\| = \begin{cases} -\sum\limits_{\substack{\boldsymbol{x}'\in\mathcal{S}, \\ \boldsymbol{x}'\neq\boldsymbol{x}_i}} A_k(\boldsymbol{x}_i, \boldsymbol{x}'), & \boldsymbol{x}_i = \boldsymbol{x}_j, \\ A_k(\boldsymbol{x}_i, \boldsymbol{x}_j), & \boldsymbol{x}_i \neq \boldsymbol{x}_j. \end{cases}$$

B. ACME Method

The ACME method has been recently developed to optimally enumerate state space of an arbitrary biological network. It can be used to compute the probability landscape of the dCME, for any given initial condition [9]–[12]. When the network is an open system, *i.e.*, containing synthesis and degradation reactions, reactions are partitioned into independent groups. Each group shares common molecular species and is equipped with a finite buffer for efficient state enumeration, with the same limit of the total copy number of species in the state space as that of the conventional hypercube methods. Details can be found in [9]–[12].

III. Results

A. Stochastic Focusing (SF) in enzymatic reaction system

Here we study a simple network of three molecular species, where the degradation rate of the I (intermediate) molecule is determined by the copy number of the S (signal) molecule. The basic enzymatic reaction system is taken from [4]. The molecular species and reactions are shown below:

$$\emptyset \underset{k_d}{\overset{k_s}{\leftrightarrows}} S, \quad \emptyset \underset{k_d}{\overset{k_a[S]}{\leftrightarrows}} I \xrightarrow{k_p} P \xrightarrow{k_t} \emptyset$$

This network consists of three molecular species: enzyme S, intermediate I, and product P. Following Paulsonn et. al [4], we take the synthesis rate of S as $k_s = 10k_d$. After the system reaches the steady state, we shift the synthesis rate of S to $k_s = 5k_d$. Here we analyze how changes in the S molecule affects changes in the P molecule by calculating the sensitivity of the system, which is the ratio between the fold change in P molecule and the fold change in S molecule. In contrast to previous work [4], the network is modeled as a stochastic system both before and after shifting the synthesis rate of the signaling molecule S. For a deterministic system, the sensitivity is expected to be 1. When SF is observed, the sensitivity of the system is greater than 1. When stochastic defocusing is observed, the sensitivity of the system is less than 1. Due to the nature of the network, the direction of change is always opposite in direction for S and P molecules, as S molecules catalyze the degradation of I molecules, from which P molecules are synthesized.

We first reproduced previously observed sensitivity [4] by solving the ODE solution of the steady state of the system for the synthesis rate of S as $k_s = 10k_d$ and the dCME solution of the steady state of system when the shifting of the synthesis rate of S to $k_s = 5k_d$. The corresponding ODE system is defined as following:

$$\frac{d[S]}{dt} = k_s - [S]k_d, \quad \frac{d[I]}{dt} = k_1 - [S][I]k_a, \quad \frac{d[P]}{dt} = [I]k_p - [P]k_a.$$

The steady state solution of ODE and the probability landscape for the S and P molecules before and after the shift are shown in Fig. 1. The $2\times$ fold change in S molecule results in $2.75\times$ fold change in the P molecule. This gives a sensitivity value of 1.38, which is similar to the value of $\sim$1.40 observed in [4].

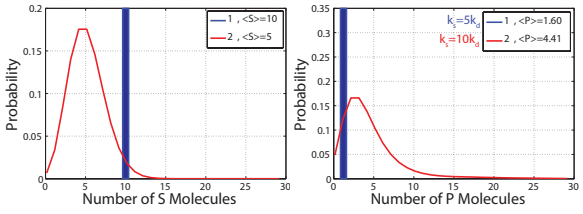

Fig. 1. The steady state probability landscape and ODE solutions of the S and P molecules.

We then examined the reaction system by modeling it stochastically both before and after the shift. The computed

TABLE I

THE REACTION SCHEMES THAT ARE USED FOR SYNTHESIS AND DEGRADATION OF S MOLECULE

Scheme 1:	Scheme 2:
$R_1 : \emptyset \xrightarrow{k_s} 2S$	$R_1 : \emptyset \xrightarrow{k_s} S$
$R_2 : S \xrightarrow{k_d} \emptyset$	$R_2 : 3S \xrightarrow{k_d} \emptyset$
Scheme 3:	Scheme 4:
$R_1 : \emptyset \xrightarrow{k_s} S$	$R_1 : \emptyset \xrightarrow{k_s} S$
$R_2 : 5S \xrightarrow{k_d} \emptyset$	$R_2 : 7S \xrightarrow{k_d} \emptyset$
Scheme 5:	Scheme 6:
$R_1 : \emptyset \xrightarrow{k_s} S$	$R_1 : \emptyset \xrightarrow{k_s} S$
$R_2 : 2S \xrightarrow{k_d} \emptyset$	$R_2 : 4S \xrightarrow{k_d} \emptyset$
Scheme 7:	Scheme 8:
$R_1 : \emptyset \xrightarrow{k_s} S$	$R_1 : \emptyset \xrightarrow{k_s} S$
$R_2 : 6S \xrightarrow{k_d} \emptyset$	$R_2 : 8S \xrightarrow{k_d} \emptyset$

time evolution and the steady state probability landscape for the S and P molecules are shown in Fig. 2. The $2\times$ fold change in S molecule results in $2.35\times$ fold change in the P molecule. This gives a sensitivity value of 1.18, which is far less than the value of$\sim$1.40 observed when the system is assumed to be deterministic before the shift [4].

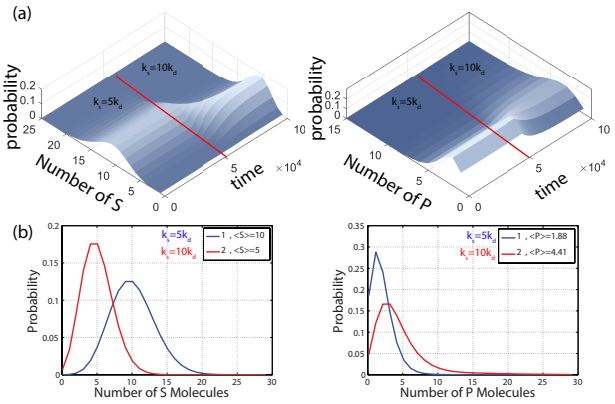

Fig. 2. Probability landscape of the S and P molecules. (a) Time evolution probability landscapes of the S and the P molecules. (b) The steady-state probability landscapes of S and P molecules.

B. The sensitivity and the degree of the SF of the system is determined by S molecule distribution

We further examined the reaction systems when the synthesis and the degradation rates of the S molecules are different to study SF under different signaling noise. We calculated the steady-state probability landscape of S and P molecules, as well as the sensitivity of the system for each of different reaction schemes (Table 1):

We found that Schemes 3, 4, 7 and 8 do not lead to any increase in the sensitivity of the system, hence the SF is not observed (Fig. 3). However, Schemes 1 and 5 exhibit strong SF, Schemes 2 and 6 exhibit moderate SF. These results demonstrated that, in this network, the distribution of the signal noise determines quantitatively the degree of stochastic focusing.

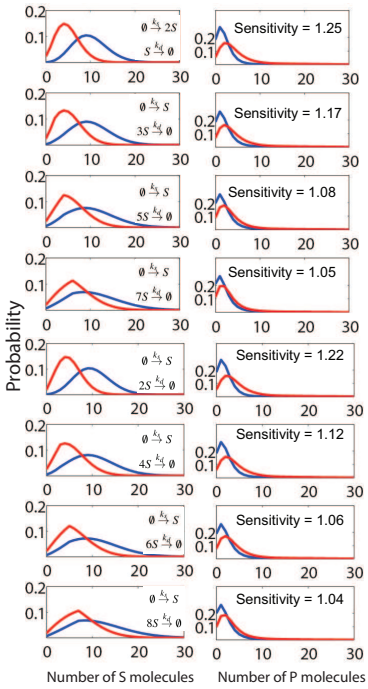

Fig. 3. Steady state probability landscape for molecules S and P before and after shift, under different reaction schemes for synthesis and degradation of S molecule. The sensitivity of the system for each scheme is also shown.

C. Stochastic Defocusing is observed when S molecule is synthesized under Schölgl model

We then introduced the Schölgl model as the system controlling the synthesis and the degradation of the S molecule to understand the behavior of system when the signal molecules exhibit bi-stability. We altered the mean number of S molecules by changing the reaction rates and calculated how it affects the P molecule. The reaction system that S molecule undergoes is modeled as the following:

$$R_1 : A + 2S \xrightarrow{k_1} 3S, \quad R_2 : 3S \xrightarrow{k_2} A + 2S,$$

$$R_3 : B \xrightarrow{k_3} S, \quad R_4 : S \xrightarrow{k_4} B.$$

We found that when the altered number of the S molecules decreases $3.91\times$ fold, the number of the P molecules increases only $2.35\times$ fold. This indicates that the system experiences stochastic defocusing, its sensitivity is only $2.35/3.91 = 0.60$ (Fig 4.).

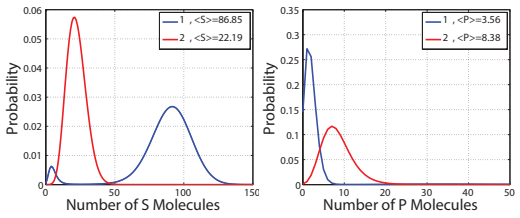

Fig. 4. Steady state probability landscape for molecules S and P. The number of S molecules is determined by Schölgl model

D. Noise induced bi-stability and stochastic focusing

We then expanded our investigation and studied the following reactions scheme for the synthesis and degradation of the S molecule:

$$R_1 : \emptyset \xrightarrow{k_s} 2S, \quad R_2 : 2S \xrightarrow{k_d} \emptyset.$$

The distribution of the P molecule is monostable. However, when the mean number of the S molecule is altered by changing the reaction rates, the distribution of the P molecule exhibits bi-stability (Fig. 5). In this case, the reaction system experiences an enormous stochastic focusing with a sensitivity of 5.43 if the reaction system shifts to the second peak. If the reaction system shifts to the first peak, the system does not experience much stochastic focusing with a sensitivity of 1.08.

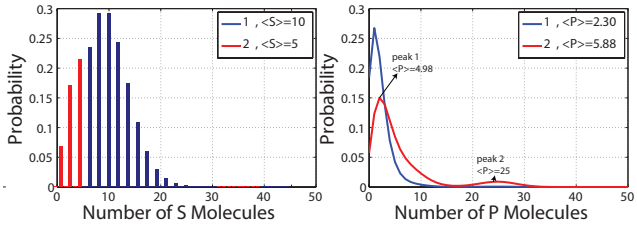

Fig. 5. Steady state probability landscape for molecules S and P when the distribution of P molecule experience a bistable behavior due to the shift in the mean number of S molecules.

IV. Conclusion

We studied the phenomenon of Stochastic Focusing based on the results of exact calculation of the probability landscape of the underlying enzymatic reaction system, where signal molecules follow different noise distributions. We showed that when the distribution of the number of the signalling molecules in the system shift from a deterministic process to a noisy Poissonian process, previously observed [4] stochastic focusing is at play. However, the assumption of system with a deterministic process before the shift is unrealistic as the low copy number of the molecules leads to non-negligible stochasticity before the shift in the system. We showed that, in this case, stochastic focusing is diminished.

We also studied the phenomenon of SF under different signalling molecule distributions. We showed that the level of the SF greatly depends on the distribution of signal molecule, as SF is diminished under certain conditions. We also studied system when the signal molecule follow a bi-stable Schölgl model. We showed that the system can experience strong Stochastic Defocusing with a sensitivity smaller than 1. This finding is the first discovery of the Stochastic Defocusing in this enzymatic reaction system.

We also found that under certain distributions, shift in the S molecule causes a monostable to bistable shift in the P molecule. In this case, the sensitivity of the system, hence the stochastic focusing, depends on which peak the P molecule is located. This finding cannot be observed by using methods that calculates mean and standard deviation of the steady state distribution of P molecule, as the mean behavior of the system does not correspond to the high probability states. This observation is also not possible by SSA, as it cannot capture the low probability events and the system will likely be stuck at the highest probability peak, when there is no Stochastic Focusing.

Overall, our results suggest that stochastic behavior such focusing and defocusing is dictated by multiple factors including signal molecule S distributions before and after the alteration, the nature of these distributions (i.e, different distributions for synthesis and degradation of signal molecule, monostable to bistable switches) and the number of stable states that the product molecule P can have.

V. Acknowledgments

Support from NIH Grants GM079804, DMS0800257 and the Chicago Biomedical Consortium are gratefully acknowledged.

References

[1] Stewart-Ornstein J, El-Samad H (2012) Stochastic modeling of cellular networks. *Methods Cell Biol.*, **110**:111–137.

[2] Qian H (2012) Cooperativity in cellular biochemical processes: noise-enhanced sensitivity, fluctuating enzyme, bistability with nonlinear feedback, and other mechanisms for sigmoidal responses. *Annu Rev Biophys.*, **41**:179–204.

[3] Schultz D, Jacob E-B, Onuchic J. N. and Wolynes G (2007) Molecular level stochastic model for competence cycles in *Bacillus subtilis*. *Proceedings of the National Academy of Sciences of the United States of America*, **30**(1):17582–17587.

[4] Paulsson J, Berg O. G., Ehrenberg M (2000) Stochastic focusing: fluctuation-enhanced sensitivity of intracellular regulation. *Proceedings of the National Academy of Sciences of the United States of America*, **97**(13):7148–7153.

[5] Milias-Argeitis A, Engblom S, Bauer P, Khammash M (2015) Stochastic focusing coupled with negative feedback enables robust regulation in biochemical reaction networks. *J R Soc Interface.*, **12**(113), 20150831.

[6] Gillespie D. T. (1977) Exact stochastic simulation of coupled chemical reactions. *J. Phys. Chem.*, **81**(25):2340-2361.

[7] Van Kampen N. G. (2007) Stochastic Processes in Physics and Chemistry. *North Holland*, **3rd edition**

[8] Liang J and Qian H (2010) Computational cellular dynamics based on the chemical master equation: A challange for understanding complexity. *Journal of Computer Science and Technology*, **25**(1):154–168.

[9] Cao Y, and Liang J (2008) Optimal enumeration of state space of finitely buffered stochastic molecular networks and exact computation of steady state landscape probability. *BMC Systems Biology*, **2**(1):30.

[10] Cao Y, Lu H-M and Liang J (2010) Probability landscape of heritable and robust epigeneric state of lysogeny in phage lambda. *Proceedings of the National Academy of Sciences of the United States of America*, **107**(43):18445–18450.

[11] Cao Y, Terebus A and Liang J (2016) State space truncation with quantified errors for accurate solutions to discrete Chemical Master Equation *Bulletin of Mathematical Biology*, **78**(4):617–661.

[12] Cao Y, Terebus A and Liang J (2016) Accurate Chemical Master Equation solution using multi-finite buffers SIAM Multiscale Modeling and Simulation. *SIAM Multiscale Modeling and Simulation*, **Accepted**

[13] Cao Y, and Liang J (2013) Adaptively Biased Sequential Importance Sampling for Rare Events in Reaction Networks with Comparison with Exact Solutions from Finite Buffer dCME Method. *J. Chem. Phys.*, **139**(2):025101.