Stochastic Modeling and Statistical Inference of Intrinsic Noise in Gene Regulation System via Chemical Master Equation

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Abstract

Intrinsic noise, the stochastic cell-to-cell fluctuations in mRNAs and proteins, have been observed and proved to play important roles in cellular systems. Due to the recent development in single-cell-level measurement technology, the studies on intrinsic noise are becoming increasingly popular among scholars. The chemical master equation (CME) has been used to model the evolutions of complex chemical and biological systems since 1940, and are often served as the standard tool for modeling intrinsic noise in gene regulation system. A CME-based model can capture the discrete, stochastic, and dynamical nature of gene regulation system, and may offer casual and physical explanation of the observed data at single-cell level. Nonetheless, the complexity of CME also pose serious challenge for researchers in proposing practical modeling and inference frameworks. In this article, we will review the existing works on the modelings and inference of intrinsic noise in gene regulation system within the framework of CME model. We will explore the principles in constructing a CME model for studying gene regulation system and discuss the popular approximations of CME. Then we will study the simulation simulation methods as well as the analytical and numerical approaches that can be used to obtain solution to a CME model. Finally we will summary the exiting statistical methods that can be used to infer the unknown parameters or structures in CME model using single-cell-level gene expression data.

1 Introduction

In his influential book “What is Life”, physicist Erwin Schrodinger suggested that macroscopic physical laws often rise as a result of chaos on micro-scale, which was dubbed as “order-from-disorder” (Schrodinger, 1944). Schrodinger’s thought had left a strong impact on the development

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of modern molecular genetics (Dronamraju, 1999). And the manifestation of “order-from-disorder” can also be easily spotted in biological system. Both common sense and scientific inquisition all demonstrate that the living organisms are capable of extracting instruction from genome and carrying out complex tasks with great precision. However, all these tasks are firstly accomplished within individual cells, and are inevitably subject to various noises that are inherent at microscopic scale. Traditionally, due to the seemingly deterministic nature of biological processes at macroscopic levels, as well as the limitation of experimental techniques, inquires of cellular system are often made based on the data representing the average properties of large ensembles of cells (there are quite a few notable exceptions, such as the work on the induction of the bacterial *E.coli*, Novick and Weiner,1957). Correspondingly, the models used to describe biological system are deterministic by nature. The stochastic elements, if used, usually only represent the noises introduced by external factors.

In recent years, the development of microscopic measurement technology has enabled the scientists to study the biological processes at single cell or single molecular level. Notable approaches include: single molecule fluorescence in situ hybridization (smFISH), an imaging-based methods in which multiple oligonucleotides are used as probes to visualize the quantities and locations of individual RNA molecules within single cell (Raj, et al., 2008); flow cytometry and mass cytometry, in which fluorescence dyes or heavy metal-conjugated antibodies are used as markers so that multiple types of proteins (18 in flow cytometry and more than 36 in mass cytometry) can be measured simultaneously in the same cell (Chattopadhyay, et al., 2006, Bandura, et al., 2009, Bendall, et al., 2012); Single-cell RNA sequencing (scRNA-seq), in which isolated single cells are analyzed using next generation sequencing technology. This approach, although subjected to larger errors, allows whole genome measurements of copy-number distributions of mRNA species in individual cells (Tang, et al., 2010, Grun and Oudenaarden, 2015, Bacher and Kendziorski, 2016). Despite of difference in resolution and the number of species that can be tracked simultaneously, all these approaches provide direct experimental evidence that, at single cell level, the expressions of gene fluctuate significantly.

As such “noise” exists in genetically identical cells under same control condition, their sources must be intrinsic rather than extrinsic. Generally speaking, the root of “intrinsic noise” lies in the numerous chemical reactions responsible for the productions and degradations of mRNA and protein molecules. As all chemical reactions are driven by the collisions of discrete molecules in constant random motions (McQuarrie,1967), the inherent stochasticity in molecular kinetic would eventually manifest as observable fluctuation in gene expressions. In experiments carried at ensemble level, intrinsic noise is usually insignificant by the law of large number. Such is not the case in the single cell environment, however. First, there are usually only one or two copies of each gene within a single cell. Second, the copy number of each mRNA specie is also small, partly due to the relatively short time span of mRNA molecules (Bernstein, et al., 2008). Finally, the protein molecules, although often in relatively
high abundance, can still exhibit great variability in the copy number due to the fluctuation in mRNA (Ozbudak, et al., 2002; Cai, Friedman, and Xie, 2006). And certain important protein species that serve as transcription factors may have very low copy number as well (Xie, et al., 2008). Although cells within the same tissue do exchange particles through intracellular communication, such exchange must overcome the hindrance of membranes and is far from free. Consequently, this so-called “low-copy number” effect would ensure that the fluctuations at single cell are relatively significant, and could lead to important biological consequences.

Intrinsic noise, rather than serves as a nuisance factor, functions as an integrated part of the control system within cell. It is commonly know that gene regulatory system employ complex control mechanisms to fulfill many essential biological functions. While certain mechanisms serve the purpose of suppressing intrinsic noise to increase stability (Becskei and Serrano, 2000), many other mechanisms can only be realized by utilizing intrinsic noise. Notable examples include genetic toggle switch (Shea and Acker, 1985; Ptashne and Switch, 1992; Gardner, Cantor, and Collins, 2000), genetic oscillator (Ishiura et al., 1998; Elowitz and Leibler, 2000) and frequency-modulation regulation (Cai, Dalal, and Elowitz, 2008). Intrinsic noise also enables cells to probabilistically switch between different phenotypes and allows homogeneous cell population to differentiate into sub-populations with distinct characteristics (Karen, et al., 2005; Suel, et al., 2007; Maamar, Raj, and Dubnau, 2007; Lidstrom and Konopka, 2010; Balazsi, van Oudenaarden and Collins, 2011). For instance, intrinsic noise might play key role in the stem cell differentiation (Hanna, et al., 2009, Yamanaka, 2009; Garg and Sharp 2016) and cancer development (Ao et al., 2008; Wang et al., 2014). It is also suggested that, the intrinsic noise, through allowing the cell to retain the flexibility of switching between different states, increases the adaptivity of cell population and thus provide evolutionary advantage (Thattai and van Oudenaarden, 2004; Acar, Mettetal, and van Oudenaarden, 2011).

As the intrinsic noises are heavily regulated by the regulatory system, observed data, in the form of empirical distribution of gene expression at single cell level, would offer us a glimpse into the underlying mechanism. It has been suggested that, in addition to the mean expression profile, the characteristics of intrinsic noises should also be considered for the purpose of defining phenotypes (Wills, et al., 2013). Comparing with the study utilizing only the ensemble-based data, the analysis of intrinsic noise will not only reduce the parameter uncertainties, but may also be able to reveal the information masked at ensemble level (Munsky, Fox and Neuert, 2015). Such revelation can even be gained through very simple inference approach. For instance, correlation coefficients between the expressions of different genes can be used as indication on the existence of direct or indirect regulatory relationship (Stewart-Ornstein, Weissman, and El-Samad, 2012). And if the single cell gene expression can be monitored through time, the autocorrelation function along can be used to determine the upstream or downstream relationships between genes (Dunlop et al, 2008). Moreover, under the ergodicity assumption, the snapshot of gene expression at single cell level can be used to infer the temporal property of cellular system, such as the decision of cell cell fate (Trapnell,
et al., 2014) and the oscillation of gene regulation system (Leng l, et al., 2015). Nonetheless, in order to fully utilize information contained in the single-cell level observations and to make physical meaningful quantitative conclusion, suitable stochastic models capturing key traits of biological system as well as appropriated inference method coupling with the models are necessary.

There are many existing models that can be used to study gene regulatory system, notable examples include logical model (Kauffman, et al., 2003), linear or nonlinear deterministic model (Klipp et al., 2008) and Bayesian network (Friedman, et al., 2000). In this paper, we will solely focus on the models and inference methods that are based on or closely related to the Chemical Master Equation (CME). Under this framework, a gene regulatory system consists of a number of different molecular species, and the state of system is described with discrete random vector that represents copy numbers of each molecular species at a given time. The molecular species would serve as reactants and products in a number of chemical reactions that form the core mechanism of the regulatory system. The evolution of the system are then driving by the continuous firing of various chemical reactions that modify the system state. Under appropriate assumptions, such system can be viewed as a Markov point process, described by a set of differential equation known as CME, a discrete formulation of Kolmogorov forward equation.

A CME based approach boasts quite a few advantages for modeling system at single cell level. Firstly, it captures the discrete, stochastic and dynamical nature of cellular system, and can offer a casual and physical explanation of the observed data; Secondly, this approach is quite flexible and can be used to establish models with different level of details. For instance, a simple model can be expanded by incorporating intermediate species and chemical reactions not presented in original model. And it can be used as a starting point for constructing more sophisticated model if certain key assumption, such as Markov property, has to be dropped. Thirdly, many existing models, including the stochastic differential equation, deterministic differential equation and even the logical model, can be viewed as approximations to CME. In this way, the CME based approach could allow us to unify different models under a single framework.

In this paper, we will firstly introduce the concept of CME, as well as how the CME can be used to model gene regulatory system. We will then discuss several popular alternative approximations of CME. Afterward we will discuss how to study the dynamics of a given CME system, through the means of simulation and through the means of analytically or numerically solving CME. Finally, we will discuss the related inference approaches available in current literatures to conclude our article.

2 Modeling with Master Equation

The Chemical Master Equation (CME) was firstly used to study the statistical distribution of the number of molecules in autocatalytic reaction $A + B \rightarrow 2B$ (The product molecular specie B also serve as the catalyst in its own production. In this reaction, a single B molecule convert a single
A molecule into a B molecule, Delbruck, 1940). Later, similar methods are used to study bimolecular reactions such as \( A + B \rightarrow C \) (One molecule A combines with another molecule B to form a molecule C, Renyi 1954), \( 2A \rightarrow B \) (Two copies of molecules A forms a single molecule B, Ishida, 1960). In these relatively simple cases, distribution of system states (in term of the copy number) can be analytically solved. It is also worthy to note that the major analytically tool used is the generating function rather than distribution function (Singer 1953). Thoroughly review on these early works can be found in Bharucha-Reid (1960) and McQuarrie (1967).

Within the cell, the production of mRNA and protein molecules can also be modeled based a number of chemical reactions. For instance, a system with a single gene and no regulatory mechanism can be summarized as four basic reactions: 1) Transcription process, in which a mRNA molecule is produced from the gene (usually only one copy per cell); 2) Translation process, in which a protein molecule is produced from any mRNA molecule; 3) and 4) Degradation of mRNA and protein, in which a mRNA or protein molecule is broken down into smaller molecules that might be used for other biological processes. Now if we use “\( R \)” and “\( P \)” to denote mRNA and protein species, and ignore other molecular species we are not interested in, the whole system can be represented using the following reactions:

\[
\begin{align*}
\text{Transcription:} & \quad \text{Gene} \xrightarrow{\tau_R} R + \text{Gene} \\
\text{Translation:} & \quad R \xrightarrow{\tau_P} R + P \\
\text{mRNA Degradation:} & \quad R \xrightarrow{\lambda_R} \emptyset \\
\text{Protein Degradation:} & \quad P \xrightarrow{\lambda_P} \emptyset
\end{align*}
\]

(1)

where \( \emptyset \) is used to denote the products of degradation processes (Thattai and van Oudenaarden, 2001).

Model (1) contains the following key informations: species, the composition of all chemical reactions and the rate constants for each reaction. In the following paragraph, we will show how these information can be used to construct a CME. We will start with the general form of a chemical reaction that involves \( I \) different species \( S_1, S_2, \ldots, S_I \):

\[
b_1 S_1 + b_2 S_2 + \cdots + b_I S_I \xrightarrow{\tau} c_1 S_1 + c_2 S_2 + \cdots + c_I S_I
\]

(2)

where \( b_i \) represents the number of molecule \( S_i \) required as reactant, and \( c_i \) represents number of molecule \( S_i \) in the product. When this reaction fires, \( (b_1, b_2, \ldots, b_I) \) copies of species \( S_1, \ldots, S_I \) will be transformed into \( (c_1, c_2, \ldots, c_I) \) copies of species \( S_1, \ldots, S_I \). Let us use vector \( \mathbf{X} = (X_1, \ldots, X_I)^T \) to denote the state of the system before firing, where \( X_i \) represents the the total copy number of species \( S_i \). Then the firing of the above reaction would result in a net change \( \mathbf{\xi} = \mathbf{c} - \mathbf{b} \), and update system state from \( \mathbf{X} \) to \( \mathbf{X} + \mathbf{\xi} \).

The frequency of the firing depends on the rate constant \( \tau \), current system state \( \mathbf{X} \) as well as the volume of the system. As we have discussed in
the introduction, all reactions are driven by the random collisions between molecules. Consequently, the more the number of molecules, the smaller the volume, the more frequently reaction would fire. To specify the relationship, following assumptions are usually needed: 1) the system evolves within a fixed volume $\Omega$; 2) molecules in the systems are always well-mixed; 3) the collisions between molecules are sufficiently elastic so that only a small percentage of collisions would actually result in a firing. It can be then argued that the time between successive reactions should follow exponential distribution with rate proportional to the number of different ways of selecting $(b_1, b_2, \cdots, b_I)^T$ molecules out from $(X_1, \cdots, X_I)^T$, and also inversely proportional to $\Omega^{\sum b_i - 1}$. This is equivalent to randomly assigning balls with different colors to $\Omega$ different boxes, and counting the number of ways of finding a particular color combination in one of the boxes. Thus, the rate of exponential distribution would roughly be proportional to \( \prod_{i=1}^{I} X_i^{b_i} / \Omega^{\sum b_i - 1} \) (Gillespie, 1971). If we use $\tau$ to denote the proportional rate constant and set $\Omega = 1$ for simplicity, the rate of the firing of chemical reaction (2) is

$$a(X) = \tau \prod_{i=1}^{I} X_i^{b_i}$$

(3)

which is also known as the propensity function. When multiple reactions are present, the waiting time for each reaction to fire is independently distributed with its own propensity function. The reaction that fires first may change the system state and consequently modify the propensity function of other reactions, though.

Return to the basic model (1), we will assume that the number of gene within a cell is a fixed constant (thus avoid the complexity due to cell division), and only focus on mRNA and protein species. Denote the copy numbers as $(X_R, X_P)^T$, then the propensity functions and the induced net changes of the four reactions are:

| Propensity Function | Net Change |
|---------------------|------------|
| Transcription:      | $\tau_R$ $(1,0)^T$ |
| Translation:        | $\tau_R X_R$ $(0,1)^T$ |
| mRNA Degradation:   | $\lambda_R X_R$ $(-1,0)^T$ |
| Protein Degradation:| $\lambda_P X_P$ $(0,-1)^T$ |

The independently and exponentially distributed waiting times between successive reactions allow us to model such system using point Markov Process. More generally, let us consider a system with $M$ different species and $K$ different reactions, and use discrete non-negative vector $X = (X_1, X_2, \cdots, X_M)^T$ to represent current system state. If possible, we will omit the time indicator for simplicity but will apply notation $X(t)$ whenever we need to emphasize the dynamical nature of the system.

The propensity function of the $k$th reaction is denoted as $a_k(X)$, which should only depend on time $t$ through $X$, as otherwise the system will no longer be memoryless. The net change induced by the firing of the $k$th
reaction is denoted as $\xi_k$. Then the probability of finding the system in a particular state $X$ at time $t$ obeys the CME:

$$\frac{dP_t(X)}{dt} = -\left[\sum_{k=1}^{K} a_k(X)\right]P_t(X) + \sum_{k=1}^{K} a_k(X - \xi_k)P_t(X - \xi_k), \quad (4)$$

a differential equation of $P_t(X)$ (Van Kampen, 1992). Here the time derivative of the distribution of the system is decomposed into the loss of probability mass as the system moves out $X$ and the gain of probability mass as the system moves into $X$. These two components are further decomposed according to the contribution of individual reaction.

As long as the initial distribution $P_0(X)$ is specified, the distribution $P_t(X)$ at any later time $t$ will be determined by CME. Moreover, through setting the time derivative as 0 and solve (4), we may obtain the equilibrium (steady state) distribution $P^s(X)$. This solution is unique and stable in the sense that, as $t \to \infty$, $P_t(X)$ will always converge to $P^s(X)$ starting from any proper probability vector $P_0(X)$ (Schnakenberg, 1976).

Equivalently, the stochastic process $X(t)$ can also be represented as the solution to the following stochastic equation (Ball, et al., 2006; Anderson 2007):

$$X(t) = X(0) + \sum_{k=1}^{K} Y_k \int_0^t a_k(X(s))ds \xi_k, \quad (5)$$

in which $Y_k(t)$ are independent, unit rate Poisson Processes, and the term $Y_k \int_0^t a_k(X(s))ds$ represents the total number of firing of the $k$th reaction during period $[0, t]$. For instance, if there is only one species in the system and the only reaction is to produce an additional copy of this species with constant rate $\tau$ (a pure birth process), then the solution to (5) at time $t$ will be Poisson with rate $\tau t$. One key advantage of representation is that it can be extended relatively easily to non-Markov system, such as system with time delay or with time dependent propensity functions (Anderson 2007);

It is often impossible to find analytical solution to a CME with dimension higher than one, and we may need to focus on the evolution of the moment of $X$ as an alternative. In particular, for any function $H(X)$, its expectation $\mathbb{E}_t[H(X)]$ with respect to $P_t(X)$ obeys the following equation (Van Kampen, 1992):

$$\frac{d\mathbb{E}_t[H(X)]}{dt} = \mathbb{E}_t[(H(X + \xi_k) - H(X))a_k(X)], \quad (6)$$

which can sometimes be used to solve the moment $\mathbb{E}_t[H(X)]$ or the moment at equilibrium.

Apply the above discussion to the basic model (1), we have the follow-
\[ \frac{d\mathbb{P}_t(X_R, X_P)}{dt} = -(\tau_R + X_R \lambda_R + X_R \tau_P + X_P \lambda_P)\mathbb{P}_t(X_R, X_P) \]
\[ + \tau_R \mathbb{P}_t(X_R - 1, X_P) + (X_R + 1)\lambda_R \mathbb{P}_t(X_R + 1, X_P) \]
\[ + X_R \tau_P \mathbb{P}_t(X_R, X_P - 1) + (X_P + 1)\lambda_P \mathbb{P}_t(X_R, X_P + 1). \]

Unfortunately, unless approximation is used, there is no analytical solution for either \( \mathbb{P}_t(X_R, X_P) \) at time \( t \) or \( \mathbb{P}^*(X_R, X_P) \) (Shahrezaei and Swain, 2008). Nonetheless, as the production and degradation of mRNA do not depend on the protein molecules, mRNA species alone constitutes a pure birth-death process and can be modeled with an one-dimensional CME with analytical solution. For instance, the equilibrium distribution of mRNA molecule is Poisson with rate \( \tau_R / \lambda_R \). Moreover, it is also possible to derive differential equation of certain moments, including means, variances and covariance of mRNA and protein (Thattai and van Oudenaarden, 2001; Munsch, B. Trinh, and M. Khammash, 2008). Such calculation suggests that, at equilibrium state, the expectation of the number of protein equals \( (\tau_R \tau_P) / (\lambda_R \lambda_P) \), and the corresponding variance equals the expectation multiplied by \( 1 + \tau_P / (\lambda_P + \lambda_R) \). That is, the fluctuation in the number of protein is relatively greater due to the variation in the number of mRNA. One way to utilize this simple result is through the so-called Fano factor, defined as the ratio between variance and mean. If the observed Fano factor mRNA molecules is significantly larger than 1, the underlying system must be more sophisticated than model (1) (Raser and O’Shea, 2004).

Another implication of model (1) is that if the transcription rate \( \tau_R \) is low while the transcription rate \( \tau_P \) is high, protein molecules will appear as translational “burst” following the transcription of every new mRNA molecule. Such phenomena have been observed and studied extensively in single cell gene expression experiments (Cai, Friedman, and Xie, 2006; Yu, et al., 2006).

As we have mentioned in the introduction, one key advantage of a CME based system is it can be readily expanded to incorporate more complicated mechanisms. To initiate the transcription process, an enzyme named RNA polymerase (RNAP) must attach to a specific site (promoter) near the gene. The structure of chromatin that enclosed the corresponding gene must also go through a process known as chromatin remodeling to allow the access of RNAP. All these facts suggest the gene itself should also be viewed as a dynamical system. In particular, we may assume that a given gene can stochastically switch between an inactive state and an active state (we will use notation “on” and “off” to distinguish these two states in the following representation). And the transcription can only occur when the gene is active. Assuming that all the factors (such as the number of RNAP molecules) related to the switching behavior remain consistent, the transcription process in model (1) can be expanded into the following two-states model (Kepler and Elston, 2001):
\[
\begin{align*}
\text{Gene}_{\text{off}} & \xrightleftharpoons[k_{\text{off}}]{k_{\text{on}}} \text{Gene}_{\text{on}} \\
\text{Gene}_{\text{on}} & \xrightarrow{\tau_R} R + \text{Gene}_{\text{off}}
\end{align*}
\]

in which the first line represented two coupled reactions with opposite directions. The state vector corresponding to this new model would then also include the current state of gene in addition to the numbers of RNA and protein molecules. For instance, the inactive and active states of the gene may be coded as “0” and “1” respectively. Under this model, the steady state distribution of mRNA molecule is no longer Poisson but can still be obtained analytically (Raj, et al., (2006)).

When the switch rates between “active” and “inactive” states are high, the qualitative behavior of this two-states model can be quite similar to the behavior of model (1). However, slow switch rates will generally result in transcriptional “burst” of mRNA molecules, and increase the cell-to-cell variability of mRNA numbers. Consequently, the tail of the mRNA copy number distribution will be longer than Poisson, and bi-modality might appear if the probability of gene being inactive is significant (Kepler and Elston, 2001; Shahrezaei and Swain, 2008). For the experimental evidences, readers can refer to the work of Golding, et al., (2005) and Raj, et al., (2006).

Due to the complexity of transcriptional process in different cells, scientists often expand the aforementioned two states model to incorporate sophisticated control mechanism. For instance, to describe certain features specific to eukaryotic transcription, a five-states stochastic model is used to study eukaryotic gene expression (Blake et al., 2003). The core system that control the life cycle of virus \(\text{Lambda phage}\) contains 5 different promoter regions which implies that the core system may take 32 different states (Lei et al., 2015). And as demonstrated in the work of Neuert et al., (2013), in order to fit the observed mRNA distribution of STL1 gene in \(\text{Saccharomyces cerevisiae}\), the minimal number requires for modeling the potential states of gene is four. It is safe to predict that more sophisticated models will be needed to explain the data emerging from the experiments that employ more advance technology. Still, a CME based approach, in principle, can be easily modified to reflect the newly observed phenomena.

The discussion above focuses on a single gene, but it is quite straightforward to expand both models (1) and (7) to accommodate multiple genes that act independently, each with its own mRNA and protein species. In the subsequent discussion, numerical numerical subscript 1, 2, \(\cdots\) will be used to label different species of genes, mRNA and proteins. The difficult part, however, is to incorporate the regulatory interactions between genes. And we will first focus on how to expand model (7) to account one of the most common regulatory mechanism: the transcriptional regulation.

Within cell, proteins of the same or different species often form complexes known as transcriptional factors (TF) which serve as regulators in transcriptional process. For instance, a transcriptional factor may bind with DNA near a promoter region, and would effectively block the binding of RNAP molecule, thus prevent the initiation of transcription. An-
other transcription factor might increase the affiliation between RNAP molecule and promoter, and results in an increasing of transcriptional activity. Through transcriptional factors, complex regulatory network can be formed between genes. It is often possible to identify sub-systems with distinctive pattern in the big network, and such sub-systems are called motif or module. Here we will use a common motif containing cooperative repressor that was firstly used to study the control system of bacterial known as Enterobacteria phage λ (Shea and Acker, 1985; Arkin, Ross and McAdams, 1998) as example to illustrate how to model transcription regulation within the framework of CME. This motif contains two genes, and two copies of protein of second gene (P2) can forms a dimer (P2P2) that blocks the activation of the first gene. Correspondingly, the regulated transcription of the first gene can be summarized using the following biochemical reactions:

\[
\begin{align*}
2P_2 & \xrightarrow{\frac{k_1}{k_{-1}}} P_2P_2 \\
P_2P_2 + \text{Gene}_{1,\text{off}} & \xrightarrow{k_2} \text{Gene}_{1,P_2-P_2} \\
\text{Gene}_{1,\text{off}} & \xrightarrow{k_{on}} \text{Gene}_{1,\text{on}} \\
\text{Gene}_{1,\text{on}} & \xrightarrow{T\tau}\text{R}_1
\end{align*}
\]

(8)

This mechanism suggests that, an increasing in the copy number of P2 will tend to increase the number of dimer P2-P2. Consequently, the first gene in inactive state would be more likely to bind with the dimer rather than switching into active state and initiating transcription. In this way, the increasing in the abundance molecules P2 will eventually result in an overall decreasing of the transcriptional efficiency of the first gene. That is, the second gene represses the expression of the first gene through its protein products. More specifically, as the first gene can stochastically switch between three three mutually exclusive states (inactive state Gene_{1,off}, binding state Gene_{1,P_2-P_2}, and active state Gene_{1,\text{on}}), and the abundance of P2 would increase the relative weight of the binding state, the repression mechanism discussed here would be nonlinear rather than linear. (Shea and Acker, 1985).

If the first gene also represses the expression second gene in similar fashion, then the equilibrium distribution of the system described above may contain two distinctive modes with the choice of appropriate parameters (Cherry and Adler, 2000; Kepler and Elston, 2001; Tian and Burrage 2008; Smadbeck and Kaznessis, 2012). In each mode, the expression of one of the gene will be high and the expression of the other gene will be suppressed. Switching between modes might occur if the intrinsic fluctuation is sufficiently strong to drive the system from one mode to another. Depending on the depth of the barrier between these two modes, the waiting time between switch might be too long that the system would effectively settle in one of the mode unless external stimulus is applied. This system, commonly known as genetic switch in biological literature, is the example of one of the most commonly motifs found in real biological regulatory system and had been artificially engineered in the lab (Ptashne, 1992; Gardner, Cantor, and Collins, 2000).
This example illustrates how complicated nonlinear mechanism can be modeled in a CME framework. Nonetheless, as more detailed mechanisms are incorporated into the model, the corresponded mathematical formulation can become extremely complicated. And in order to specify the CME, we need to keep track of the states of many intermediate species which are usually unobservable in practice. For instance, in model (3), the state vector of the CME should include the following variables: number of second protein molecules, number of dimers formed by the second protein, number of the first mRNA molecules, and the current state of the first gene. It is very unrealistic to imagine that we could obtain all these information from experiment, and we would need to treat the unobservable variables as missing data. Still, in a complex model with many missing information, it is very hard to assert whether the incomplete observation would contain sufficient information for us to reasonably identify and infer the parameters of interests (Azeloglu and Iyengar, 2015). It is thus of great importance to discuss approach that might be used to simplify a detailed physical model, with the goal of eliminating intermediate species/states while retaining the non-linear nature of the interaction.

In their seminar work on the kinetic of enzymatic reaction, Michaelis and Menten (1913) removed the intermediate species in the deterministic differential equation model based on the phenomenon “time scale separation”. In many chemical and biological system, the intermediate species might evolve at a much faster rates comparing with the other main species under investigation. Consequently, it is then reasonable to propose that, such species would always reaches a “quasi” stable state almost instantly relatively to the main species. Then the approximated abundance intermediate species can be determined based the current abundance of main species. This approach, commonly known as Quasi-Steady-State Approximation (QSSA) or Pseudo-Steady-State assumption, had been since widely used to simplify deterministic biochemical models involving multiple species can be justified using perturbation theory (Ackers, Johnson, and Shea, 1982; Segel and M. Slemrod, 1989; Gunawardena, 2014).

Rao and Arkin (2003) firstly apply QSSA to reduce the complexity of a CME based model. In their work, the system state vector is partitioned as $X = (Y, Z)^T$, representing the main species and intermediate species respectively. Then we have:

$$
\frac{dP_t(X)}{dt} = \frac{dP_t(Z|Y)}{dt}P_t(Y) + \frac{dP_t(Y)}{dt}P_t(Z|Y)
$$

Under the framework of stochastic model, QSSA can be expressed as following: conditioning on the main species $Y$, intermediate species $Z$ still evolve as a Markov process and reach the equilibrium distribution infinitely fast. Effectively speaking, the time-dependent conditional distribution of $Z$ given $Y$ can be replaced by time independent “conditional equilibrium distribution” where $Y$ is held as constant. That is, $P_t(Z|Y) \approx P^*(Z|Y)$ and $dP_t(Z|Y)/dt \approx 0$. Then the above equation is simplified as:
\[ \frac{dP_t(X)}{dt} \approx \frac{dP_t(Y)}{dt} \mathbb{P}^o(Z|Y). \]

A new reduced CME can then be used to model the distribution of main species \( P_t(Y) \). This new reduced CME will only take into consideration of the reactions that result in a net change of \( Y \). In the following discussion, we will use notation \( k^* \) as index for such reaction and use \( \xi_k^* \) to represent the net change of \( Y \) due to the firing of the \( k \)th reaction. Still, the propensity function of such reaction under consideration \( a_k(Y, Z) \), may still depend on intermediate species \( Z \). This issue can be resolved by replacing it with expected propensity function with respect to \( \mathbb{P}^o(Z|Y) \) or simply substituting \( Z \) with \( \mathbb{E}^o(Z|Y) \) (Such approximation will be discussed in detail in the later section). Then if we denote \( \hat{a}_k(Y) = \mathbb{E}^o[a_k(Y, Z)|Y] \), and \( \bar{a}_k(Y) = a_k(Y, \mathbb{E}^o(Z|Y)) \approx \hat{a}_k(Y) \), the reduced CME that approximates the evolution of \( Y \) can be written as:

\[ \frac{dP_t(Y)}{dt} \approx -\sum_{k^*} \bar{a}_k^*(Y)P_t(Y) + \sum_{k^*} \hat{a}_k^*(Y - \xi_k^*\bar{a}_k^*(Y)P_t(Y - \xi_k^* \bar{a}_k^*(Y)). \]

Under QSSA, given the copy number of main species \( Y \), it is usually straightforward to determine \( \mathbb{E}^o(Z|Y) \) using a deterministic approximation. More rigorous theoretical treatment of QSSA can be found in the work of Ball, et al., (2006) and Kang and Kurtz (2013). For instance, in model (8), let us say that we can treat the dimer, as well as the three different states of first gene as intermediate species. Applying the QSSA, then the propensity functions in the first three reversible reactions must be balanced on average, which would allows us to write down following equations regarding the quasi-steady-state expectation of the number of dimmer, as well as the probability of the first gene being in one of the three states:

\[ \begin{align*}
  k_1X_P^2 \mathbb{P}^o_{\text{Gene}_1, \text{off}} &= k_{-1}\mathbb{E}^o(X_{P_2-P_2})P^o_{\text{Gene}_1, \text{off}} = k_{-2}\mathbb{P}^o_{\text{Gene}_1-P_2-P_2} \\
  k_2\mathbb{E}(X_{P_2-P_2})P^o_{\text{Gene}_1, \text{off}} &= k_{-2}\mathbb{P}^o_{\text{Gene}_1-P_2-P_2} \\
  k_{\text{on}}P^o_{\text{Gene}_1, \text{off}} &= k_{\text{off}}P^o_{\text{Gene}_1, \text{on}}.
\end{align*} \]

which would allow us to derive the probability that the first gene is active as a function of \( X_{P_2} \):

\[ \mathbb{P}^o_{\text{Gene}_1, \text{on}} = F_1(X_{P_2}) = \frac{1}{1 + \frac{k_{\text{off}}}{k_{\text{on}}} + \frac{k_{\text{on}}}{k_{\text{off}}} \frac{k_1}{k_{-1}} X_{P_2}^2} = \frac{b_1}{1 + c_1 X_{P_2}^2}. \]

The average transcription rate of the first gene can be then modeled as the transcription rate at active state times the probability that the gene is active. This approximation allow us to simplify model (8) as a single reaction with a rational propensity function:
Similar nonlinear rational functions have been widely used in literatures to describe complex chemical reaction kinetics, with famous examples such as Michaelis-Menten kinetic (Michaelis and Menten, 1913), and Hill functions (Hill, 1913). In recent years, such approach had been used to study gene regulatory system as well: negative feedback loops that reduce the intrinsic noise (Paulsson and Ehrenberg, 2000; Becskei and Serrano, 2000; Thattai and van Oudenaarden, 2002); positive feedback loop that may serve as noise amplifier or as switch (Hasty et al., 2000; Isaacs et al., 2003; Maamar, Raj, and Dubnau, 2007;), enzymatic reaction (Qian 2008), the Lac operon in E.coli, the induction Enterobacteria phage λ as well as the dynamic of stem cell switch (Chickarmane, et al., 2006; Chickarmane and Peterson 2008).

This formulation can also be justified based on thermodynamic theory, in which the probability of activating the gene equals the ratio between the number of conformations of active state and the total number of all possible conformations. Generally speaking, under proper assumption on the equilibrium status of the system, the transcriptional rate in a regulatory system will be a rational functions of the abundance of transcriptional factors, which can be determined based on the abundance of protein molecules that form the corresponding TFs.(Keller, 1995; Bintu, et al., 2005(1)(2)). The algebraic terms contains in the rational functions will reflect the detailed transcriptional mechanisms and the coefficients serve to determined the strength and directions of regulation mechanisms. The following example serves as an illustration of such approach:

\[
\tau_{R_1} F_1(X_{P_1}, X_{P_2}, X_{P_3}) = \tau_{R_1} \frac{0.5 + X_{P_1} + 0.1X_{P_2}X_{P_3}}{1 + X_{P_1} + X_{P_2} + 0.4X_{P_2}X_{P_3}}.
\]

Specifically, in this equation, the ratio of constants in numerator and denominator represent a base probability of initiating transcriptional process when all the regulators are absent. The other algebraic terms contained in the equation suggest that there are three different TFs that serve as the regulator of the first gene: \(X_{P_1}\) represents \(P_1\), the protein molecule of the first gene; \(X_{P_2}^2\) represents \(P_2-P_2\), a dimer formed by two copies of protein molecules of the second gene; and \(X_{P_2}X_{P_3}\) represents \(P_2-P_3\), a complex formed by each copy of protein of the second and third genes. When a term (such as \(X_{P_2}^2\)) only appears in the denominator, it suggests that the corresponding TF, if binds with the gene, will completely block the transcriptional process. Thus, this TF will tend to slow down the overall transcriptional rate and serve the role of repressor. If a term appears in both numerator and denominator (such as \(X_{P_1}\) and \(X_{P_2}X_{P_3}\)), the role of the corresponding TF will be determined based on the comparison of the ratio of its coefficients in numerator and denominator with the base probability of initiating transcription process. For instance, in this example, \(P_1\) will serve as an activator that increases the transcriptional rate, but \(P_2-P_3\) serves as a repressor. Finally, due to the restriction posed by the thermodynamic theory, all the coefficients should be non-negative, and
the coefficient of a given algebraic term in numerator should be no larger than the corresponding coefficient in denominator.

This approach of simplifying the regulatory model has two advantages: 1) it does not require the intermediate species and would solely focus on mRNAs and proteins; 2) it retains the key characteristic of the gene regulatory systems, and would allow us to study the nonlinear regulatory relationship between genes.

Finally, in the experiment where only the mRNA species or protein species are observable at single cell level, we may need to simplify the aforementioned model further so it would only include the observable species. As the translation process is treated as a simple birth process with constant rate, we might apply QSSA and state that the copy number of mRNA molecule is proportional to the number of its own protein product. In this way, we could propose the simplified model consists of only mRNA or protein species. For a system with $K$ different genes, we may then use $S_i$ to represent the $i$th species (which could be mRNA or protein) with copy number $X_i$. Then we may represent the regulation system with the following reactions:

\[
\begin{align*}
\text{Gene}_i & \xrightarrow{\tau_i F_i(X)} S_i + \text{Gene}_i \\
S_i & \xrightarrow{\lambda_i} \emptyset
\end{align*}
\]

in which $F_i(X)$ should be a nonlinear rational function of $X = (X_1, X_2, \cdots, X_M)^T$, and would represent the detailed regulatory relationship between genes in the system under investigation following the same principle we discussed earlier.

Correspondingly, the CME should be:

\[
\frac{dP_t(X)}{dt} = - \sum_{i=1}^{M} (\tau_i F_i(X) + \lambda_i)P_t(X) \\
+ \sum_{i=1}^{M} \lambda_i (X_i + 1)P_t(X + \epsilon_i) \\
+ \sum_{i=1}^{M} \tau_i F_i(X - \epsilon_i)P_t(X - \epsilon_i)
\]

in which $\epsilon_i$ represents the $K$ dimensional unit vector whose $i$th component equals 1.

In the above discussion, we outline the general idea of applying CME to model the gene regulatory system. This approach would allow us to capture the discrete, stochastic and nonlinear traits of biological system at single cell or single molecular level, and is also flexible enough for expansion. In the following sections, I will outline the general approach that is available for studying CME based system, with a focus on the
application in gene regulatory system. In particular, I will discuss how to approximate a CME based system, how to simulate and solve a CME based system, as well as how to perform statistical inference within the framework of a CME based model.

3 Approximation of CME System

Under appropriate conditions, it is possible to approximate CME with continuous stochastic process, or even deterministic process. In this section, we will discuss several approximations schemes of CME, and demonstrates their connection and discrepancy with CME. Such discussion would not only provide us different tools to simplify the model, but also serves to illustrate the necessity of applying CME based model to study cellular system.

We will view various approximations discussed here as the asymptotic limit of the CME system. In this setting, “asymptotic limit” means the limit behavior of the system as its size expands to infinity. In our previous discussion, we assume that CME system evolves within a fixed unit volume (such as the gene regulatory system within a single cell). To expand the system, we can pool Ω identical copies of the original system together to form a larger system with volume Ω. Here the “identical copies” means that all the copies evolve according to the same CME, but the state of individual system state may still vary due to the stochastic nature of CME. We will also assume that, after pooling these identical systems together, all the particles can travel freely in the expanded system and mix instantly. We will denote the state of the expanded system as $X^{(Ω)}$, and also define the concentration of each species as the average number of molecules in unit volume:

$$x = \frac{X^{(Ω)}}{Ω}.$$ 

Then within an arbitrary unit volume, the rate for firing the $k$th reaction should roughly equal $a_k(x)$. If we do not count the interactions between different unit volume, we may deduce that the rate of firing the $k$th reaction in the expanded system is about $Ωa_k(x)$. With this in mind, we will focus on the system whose propensity function is related to $Ω$ in the following way:

$$a_k^{(Ω)}(X^{(Ω)}) = Ωf_k(x) + o(Ω), \quad (16)$$

Under this assumption, it can be shown that (Oppenheim, Shuler, and Weiss, 1969; Kurtz 1970, 1972), the trajectory of the expanded system normalized by $Ω$, would converge to the trajectory defined by the following deterministic differential equation as $Ω$ goes to $∞$:

$$\frac{dx}{dt} = \sum_{k=1}^{K} f_k(x)ξ_k, \quad (17)$$

which is essentially the macroscopic rate equation of the conventional chemical kinetic. In this equation, the unit of concentration should be...
$V^{-1}$, the unit of propensity function is $s^{-1}$ and $\xi_k$ is an unit-less vector. Then the unit on the left hand side of this equation should be $s^{-1}V^{-1}$, and consequently, the unit of function $f_k$ should be $s^{-1}V^{-1}$.

In the examples we discussed so far (including equation (3) as well as the rational nonlinear propensity function (13) under QSSA), the reminder terms $o(\Omega)$ all equal 0, and function $f_k(x)$ are identical to propensity function $a_k(X)$ in system with unit volume. It is worthy noting that, equation (3) is derived with the system size $\Omega$ in mind. And when we apply QSSA to simplify a complicated system, we essentially replace part of the system with its quasi-steady-state deterministic limit. For this reason, strictly speaking, the $X$ terms in the nonlinear rational function we derived earlier actually represent $x = X/\Omega$.

For instance, model (1) can be approximated using the following equations (using $x_R, x_P$ to denote the concentration of RNA and protein respectively):

$$\frac{dx_R}{dt} = \tau_R - \lambda_R x_R, \quad \frac{dx_P}{dt} = \tau_P x_R - \lambda_P x_P.$$

Similarly, if we use $x_{R_1}$ to represent the mRNA concentration of the first gene in the repressor model as laid out in model (14), the linear approximation will yield:

$$\frac{dx_{R_1}}{dt} = \tau_R \frac{b_0}{1 + c_1 x_P^2} - \lambda_{R_1} x_{R_1}.$$

This result connects the CME and deterministic differential equation, and also validates the use of deterministic system to model an inherently stochastic cellular system. Nonetheless, there are quite a few issues regarding the application of deterministic approximation. First of all, in order for the deterministic limit to apply, the system size must be sufficiently large. And the copy numbers of each molecular species should also be large enough so that the fluctuations are relatively insignificant. However, due to the low copy number effects within single cell, even small variations would lead to large consequence. Secondly, may key characteristics of the underlying stochastic are not invariant to the change of system size (Qian, Shi, and Xing, 2009; Vellela and Qian, 2009; Bishop and Qian, 2010.). For instance, many gene regulatory systems exhibit bimodal or even multi-modal behavior, and often randomly evolve through different trajectories that may lead to different phenotypes (Ozbudak, et al., 2004; Dubnau and Losick, 2006; Mettetal and van Oudenaarden, 2007; Kuwahara and Soyer 2012). If we increase the size of the system, technically speaking, the dynamic of stochastic system would converge towards the “most likely” path, and the chance of taking other less likely trajectories would approach zero. Such phenomenon has been observed in the bacterium Bacillus subtilis. This bacterium is often remain in dormant state but can stochastically transit into a “competent” state and gain the ability to capture DNA from surrounding environment (Suel et al., 2006). Through introducing a defect in the cell division mechanisms, “super” cells that consists of multiple individual cells sharing cytoplasm can be formed.
It was observed that the probability of transiting into "competent" state would decrease as the size of "super" cell increases (Suel et al., 2007).

In this light, cautious should be taken when comparing the inference results based on deterministic model using data at ensemble level with the inference results based on stochastic model using data at single cell level. In particular, the ensemble-level data represent the average system state across a large number of cells, while the deterministic limit of a stochastic model that resembles single-cell system would represent the most likely state of the system. And with the presence of multi-modality, the average system state can be quite different from the most likely system state. Thus, the modeling of intrinsic noise, not only can allow us to fully utilize information information contained in single-cell level data, but also offer a potential "correct" perspective for understanding the underlying system.

It is clear that the information on the intrinsic noise stored in CME will be lost when the macroscopic deterministic approximation is used. A compromise between these two extremes is to preserve the stochasticity of the system but apply continuous approximation. Still, depending on how to model "stochasticity", different approaches such as linear noise approximation (LNA) and Stochastic Differential Equation (SDE, or Langevin Equation) can be used.

We will firstly discuss LNA on the basic of the famous system size expansion approach (Van Kampan, 1976, 2007). This approach starts with the following ansatz: the probability function $P_t(X^{(Ω)})$ has a sharp peak at position of the order $Ω$, with the width of the order $Ω^{1/2}$. This ansatz will then allow us to represent $X^{(Ω)}$ as:

$$X^{(Ω)} = Ωx(t) + Ω^{1/2}y(t).$$

The first term $x(t)$ is the solution of the deterministic equation (17) and the second term $y(t)$ represents stochastic fluctuation which usually depends on $x(t)$. As also discussed in Kubo, Matsuo and Kitahara (1973), such ansatz essentially assumes that the distribution of system state would retain the uni-modal and bell-shaped characteristics throughout its time course. Under this representation, the trajectory of the system can be decomposed as the main deterministic trajectory determined by (17) plus a minor stochastic component. The evolution of stochastic component $y(t)$ can be solved by noting that, under this decomposition, $P_t(X^{(Ω)})$ can be represented as $Π_t(y)$, whose time derivative can be written as:

$$\frac{∂}{∂t}Π_t(y) = \frac{∂}{∂t}P_t(X^{(Ω)}) + Ω^{1/2} \sum_{i=1}^{M} \frac{dx_i}{dt} \frac{∂Π_t}{∂y_i}.$$ 

By the ansatz discussed above, the first term on the right hand side of the equality can be expanded based on $Ω$. The largest terms in the resulting expansion are of the order $Ω^{1/2}$, which will cancel the existing $Ω^{1/2}$ order terms given that the macroscopic deterministic equation (17) holds. Then if we only collect the terms of the order $Ω^0$ and ignore other terms that would vanish as $Ω$ goes to infinity, we have the following equality:
\[
\frac{\partial}{\partial t} \Pi_t(y) = - \sum_{i,j=1}^{M} A_{ij} \frac{\partial}{\partial y_i} [y_i \Pi_t] + \frac{1}{2} \sum_{i,j=1}^{M} B_{ij} \frac{\partial^2}{\partial y_i y_j} \Pi_t,
\]
where
\[
A_{ij} = \sum_{k=1}^{K} \xi_{ik} \frac{\partial}{\partial x_j} f_k(x), \quad B_{ij}(x) = \sum_{k=1}^{K} \xi_{ik} \xi_{kj} f_k(x),
\]
and \(\xi_k\) represents the \(i\)th entry of vector \(\xi_k\).

Given \(x\), this formula is a linear Fokker-Planck equation whose solution is Gaussian. Thus, under LAN, we can firstly solve the macroscopic equation to obtain the deterministic trajectory. Then we can construct differential equations on the first and second moments of \(y\) to solve the mean vector and covariance matrix (Van Kampan, 2007). Such strategy would then allow us to determine the distribution of system at any time \(t\) as well as the equilibrium distribution.

Using the Ito interpretation, the above Fokker-Planck equation is also equivalent to the following Stochastic differential equation (Komorowski, et al., 2009):
\[
dy_t = Ay_t dt + \Sigma dW_t,
\]
where \(A\) is a \(M \times M\) square matrix whose entry at \(i\)th row and \(j\)th column is \(A_{ij}\), \(W_t\) is a \(K\) dimensional Wiener process, and \(\Sigma\) is a \(M \times K\) dimensional matrix whose entry at \(i\)th row and \(k\)th column is \(\xi_{ik} \sqrt{f_k(x)}\).

Both drift and diffusion coefficients only depend on \(t\) through \(x\).

Take model \(\Pi\) as example, the LAN approximation of the noise term \(y = (y_R, y_P)^T\) shows that:
\[
\frac{\partial}{\partial t} \Pi_t(y) = \frac{\partial}{\partial y_R} [\lambda_{RyR} \Pi_t] - \frac{\partial}{\partial y_P} [(\tau_{PyR} - \lambda_{PyP}) \Pi_t] + \frac{1}{2} (\tau_{RxR} + \lambda_{RxR}) \frac{\partial^2}{\partial y_R^2} \Pi_t + \frac{1}{2} (\tau_{PxP} + \lambda_{PxP}) \frac{\partial^2}{\partial y_P^2} \Pi_t,
\]
and the equivalent stochastic differential equation (SDEs) are:
\[
dy_R = -\lambda_{RyR} dt + \sqrt{\tau_{RxR}} dW_1 - \sqrt{\lambda_{PxP}} dW_3,
\quad
dy_P = (\tau_{PyR} - \lambda_{PyP}) dt + \sqrt{\tau_{PxP}} dW_2 - \sqrt{\lambda_{PxP}} dW_4.
\]

It is worthy noting that, although the fluctuation in LNA appears as an additive term to the deterministic component, the strength of the fluctuation depends on the deterministic trajectory and thus reflects the underlying mechanisms of CME system. In this regard, the fluctuation in LNA should be treated as multiplicity noise rather than the additive noise whose magnitude is often independent from the deterministic trajectory. In fact, as demonstrated in the work of Frigola, et al., (2012), additive noise is often insufficient to model the underlying complex system appropriately. Nonetheless, as the center of the approximated distribution
must follow the deterministic trajectory, the effectiveness of LNA depends on how well the deterministic approximation works. In particular, LNA would fail to capture the key characteristics of the system if the system contains multiple steady states or moves past a critical point (Baras, Mansour, and Pearson, 1996).

Another alternative is to approximate the CME using the following SDE (Kurtz, 1978; Hanggi, 1980; Gillespie, 2002 (1)(2)):

$$dX_t = \sum_{k=1}^{K} \xi_k a_k(X_t) dt + \left( \xi_1 \sqrt{a_1(X_t)}, \xi_2 \sqrt{a_2(X_t)}, \cdots, \xi_K \sqrt{a_K(X_t)} \right) dW_t$$

where $W_t$ is a $K$ dimensional Wiener process. For instance, the SDEs approximation of model (1) are:

$$dX_R = (\tau_R - \lambda_R X_R) dt + \sqrt{\tau_R} dW_1 - \sqrt{\lambda_R X_R} dW_3,$$

$$dX_P = (\tau_P X_R - \lambda_P X_P) dt + \sqrt{\tau_P X_R} dW_2 - \sqrt{\lambda_P X_P} dW_4.$$ 

An intuitive understanding of this approximation can be obtained from equation (5), the Poisson process presentation of CME. Between $(t, t + \Delta t)$, the number of firing of the $k$th reactions is $Y_k[\int_{t}^{t+\Delta t} a_k(X(s)) ds]$, which can be approximated with Poisson distributed random variable with rate $\Delta t a_k(X(t))$. If we assume that the system size is sufficiently large, $\Delta t a_k(X(t))$ would also be large enough to guarantee a Normal approximation. A more rigorous treatment of this subject would show that the difference between the SDE approximation and CME will be on the order of $\log(\Omega)$ (Kurtz, 1978).

One key advantage of SDE over LNA is that it does not assume that the center of distribution must evolve along the deterministic trajectory, and thus may give a better description when the distribution of underlying system is multimodal. For instance, in certain system such as the toggle switch (Gardner, Cantor, and Collins, 2000), even if the initial distribution of the system is of single mode, it may split into a multimodal distribution in the presence of a critical point as the system evolves. However, a coupled deterministic system would only evolve toward one of the modes as determined by the initial condition. Consequently, LNA will not be able to handle such scenario property as the mode of distribution in LNA must follow the deterministic trajectory. In contrary, the diffusive nature of SDE may still allow the probability mass to be distributed towards different modes as the system evolves past the critical point and thus would offer a much better approximation of the underlying system. Still, the effectiveness of SDE approximation may still be limited if the probability mass is mainly concentrated near the boundary of state space where the discreteness of the CME can not be easily ignored. The potential discrepancy between CME and SDE approximation had been explored in simulation study (Baras, Mansour and Perason, 1996). In an experimental study of genetic bistable toggle switch (Ma, et al., 2012), it
has also been observed that the system could enter a third stable state where both gene express at very low level. Such phenomenon can not be predicted by SDE model but can be explained with CME model.

4 Simulation of Master Equation System

Simulation is often an indispensable tool in studying the property of CME system as the analytical solution of CME is usually impossible to obtain. In particular, the distribution of system state at any given time or at equilibrium can be reconstructed with samples collected from many independent trajectories. Utilizing the fact that CME system is driven by the successive firings of different reactions and the waiting times follow exponential distributions, Gillespie (1976,1977) proposed the following exact algorithm for simulating realizations of trajectories of CME system:

- Step 1: Set the starting system state as $X(0)$, and set time $t = 0$;
- Step 2: Calculate the propensity functions $a_k(X(t))$ for $k = 1, 2, \ldots, K$;
- Step 3: For each $k$, generate an exponentially distributed random variable $\tau_k$ with rate $a_k(X(t))$, and set $\tau = \min(\tau_1, \ldots, \tau_K)$.
- Step 4: Set $I = \arg\min_k \tau_k$.
- Step 5: Advance the system by updating $X \rightarrow X + \xi_I$, and $t \rightarrow t + \tau$.
- Step 5: Stop if pre-set conditions are met. Otherwise, return to step 2.

This algorithm is called Direct Method. In particular, steps 3 and 4 are used to determine the when and which of the $K$ reactions will fire next. In this algorithm, $K$ uniform distributed random variables are needed to be generated in each iteration. This cost can be reduced significantly by utilizing the memoryless property of exponential distribution. That is, the waiting time for the next reaction to fire follows exponential distribution with rate $\sum_k a_k(X(t))$, and the chance that the $k$th reaction is the next reaction is proportional to $a_k(X(t))$. Then steps 3-4 in Direct Method can be improved by following procedure (Gillespie, 1976,1977):

- Step 3*: Generate an exponentially distributed random variable $\tau$ with rate $\sum_k a_k(X(t))$ and a uniform random variable $U$.
- Step 4*: Let $I = k$ so that

$$\frac{\sum_{j=1}^{k-1} a_j(X(t))}{\sum_{k=1}^{K} a_k(X(t))} \leq U < \frac{\sum_{j=1}^{k} a_j(X(t))}{\sum_{k=1}^{K} a_k(X(t))},$$

where $\sum_{j=1}^{k-1} a_j(X(t)) = 0$ when $k = 1$.

The revised algorithm is firstly named as First Reaction Method, but is commonly known as the Stochastic Simulation algorithm (SSA) or Gillespie’s algorithm. In each iteration of this algorithm, we only need to generate two uniform random variables. Still, the computational cost related to the non-random elements, including updating all propensity functions
at Step 2 and selecting the first reaction at Step 4*, is still of the order $K$ for each iteration.

Further improvement can be achieved using the Next Reaction Method (Gibson and Bruck, 2000) through the application of two major innovations. Firstly, by exploiting the memoryless property, we may focus on the absolute time frame starting from the beginning rather than the relative waiting times between successive reactions. Such exploitation can reduce the number of uniform random variables needed for each iteration to 1, from 2 (with the exception of the first iteration). Secondly, by storing all relevant information in a tree structure, we may minimize the computing cost in determining the next reaction and avoid unnecessary reevaluation of the propensity functions. This strategy can reduce the computational complexity related the non-random elements to the order of $\log(K)$. Anderson (2007) reformulated the Next Reaction Method was reformulated using the Poisson process presentation of CME, and suggested that such approach can be use to simulate non-Markov system, such as the system with delayed reaction time or the system whose propensity functions change over time. Finally, through developing data structure that reflects the common reactants shared by different reactions, the LOLCAT method can be used to reduce the computational cost even further (Indurkhya and Beal, 2010).

While the efficiency of exact methods can be dramatically improved using the aforementioned algorithms, it may still take considerable time to simulate even a single trajectory as every reactions that fires in succession must be taken into account. Consequently, a faithful strategy can be extremely computational demanding when we need to estimate the distribution of system based on a large number of samples. And it is often necessary to adopt approximation schemes to reduce the computational burden.

One common strategy is to assume that the propensity functions remain constants during a certain period $(t, t + \tau)$ (for instance, when $X(t)$ is large comparing with all $\xi_k$, the change due to the firings would be insignificant), then the number of firing the $k$th reaction during this period would follow Poisson distribution (See equation (5)). Then we may “jump” from $t$ to $t + \tau$ using the following approximation:

$$X(t + \tau) \approx X(t) + \sum_{k=1}^{N} \xi_k M_k,$$

where $M_k$ is independent Poisson distributed random variables with rate $\tau a_k(X(t))$.

This method is called $\tau$-leaping algorithm (Gillespie 2001). Generally speaking, a large $\tau$ means fast computation time but low precision. Consequently, the performance of this algorithm would depend on the appropriate choice of step size $\tau$, which should be updated dynamically during the course of simulation (Gillespie 2001, Gillespie and Petzold 2003, Cao, Gillespie and Petzold 2006). Furthermore, the effectiveness of this algorithm can also be improved by using $a_k(X(t^*))$, where $t^*$ is chosen as a “mid-point” between $t$ and $t + \tau$, rather than $a_k(X(t))$ to approximate
the propensity function during \((t, t + \tau)\) (Gillespie 2001). This \(\tau\)-leaping algorithm can also be combined with the Next Reaction method to improve the efficiency (Puchalka and Kiezek 2004). Another similar method is known as the R-leaping method (Auger, Chatelain and Koumoutsakos 2006), in which the algorithm is set to leap forward for a fixed number of firings rather than a fixed time. Under the same constant propensity function assumption, the elapsed time for making R-leaping follows Gamma distribution.

Another strategy utilizes the QSSA. In many applications, the propensity functions often have vastly different time scales so that certain reactions fire much more frequently than others. Not surprisingly, in an exact simulation, most of the computing efforts will be invested on the “fast” reactions, while the evolution of the system is often determined by the “slow” reactions. In this regard, computational efficiency can be improved by using exact algorithm on the “slow” reactions but applying approximated scheme to handle the “fast” reactions.

In the work of Haseltine and Rawling (2002), the evolution of the system under “fast” reactions are approximated using Langevin equation, as long as the distribution of species related to “fast” reactions does not change over one iteration of SSA of “slow” reactions. In the Maximum Time Step method (Puchalka and Kiezek 2004), \(\tau\)-leaping algorithm is used to handle “fast” reactions, which are dynamically determined through the course of simulation. The criteria of allocating a reaction as “fast” are: first, the copy numbers of species involved must be greater than a threshold; second, the chance for this reaction to fire first must also be big enough. In the slow-scale stochastic simulation algorithm (Cao, Gillespie, Petzold 2004, 2005), not only the reactions are allocated into two categories, the species are also classified: “fast” species are the species that are impacted by one or several “fast” reactions, and the rest are “slow” species. By QSSA, the distribution of “fast” species are complete determined by the current states of “slow” species. And we can simply simulate the trajectory of the “slow” species based on a reduced CME in which the propensity functions are established as the expectation conditional on the “slow” species.

Our discussion has focused on simulating independent trajectories of CME so far. When the goal is to estimate the probability of particular event (such as the probability that the number of proteins in the system would reach a given threshold within given time) in a CME system, the technique of important sampling is often useful for improving the estimation. The weighted SSA is the first algorithm that incorporates important sampling into SSA (Kuwaharaa and Mura, 2008). In this algorithm, the propensity functions in the step 4* of SSA are multiplied by pre-determined scale constants, which changes the relative priorities of reactions that might fire. If appropriate scale constants are used, the chance of occurrence of the event of interests can be increased. The bias introduced in this process is adjusted by re-weighting the simulated trajectory accordingly. Within such framework, the confidence intervals of the estimator can be estimated as well (Gillespie, Roh, and Petzold, 2009). A few modifications of weighted SSA exist with the aim of locating better
scale constants. In the state-dependent SSA (Roh, Gillespie, and Petzold 2010), the scale constants can be dynamically updated during the course of simulation. In the double-weighted SSA (Daigle, et al., 2011), the scale constants are also applied in step 3* of SSA which scale the distributions of waiting times between reactions. The purpose of this scheme is to open the possibility of applying cross-entropy method to find the optimal choice of scale constants. The two strategies discussed above can be combined together to achieve a better result (state-dependent double weighted SSA, Roh, et al., 2011).

For more discussion on the issue of simulating CME system, readers can also refer to the following more in-depth review work: Gillespie (2007), Gillespie, Hollander and Petzold (2013).

5 Approximating CME by Analytical and Numerical Methods

In order to attain a more in-depth understanding of the CME system, to estimate the unknown parameters in CME model based on observed data or to compare competing models, it is often necessary to establish relatively precise relationship between the model parameters and the behavior of the system reflecting by the distribution function. For such a purpose, in addition to our discussion on how to simulate a CME system, in this section we will explore how to solve CME by analytical and numerical methods. Due to the often intractable nature of CME, most of the methods discussed in this section are approximated methods in nature.

Although the solution to the full distribution function of a CME is often impossible to obtain, the evolution functions of moments are often easier to work with. In particular, equation (6) can be used to establish differential equation of the expectation of any function $H(X)$. As an illustration, let us apply equation (6) to model (1), then for function $H(X) = X^n_{R}X^m_{P}$ where $n, m$ are non-negative integers, we have:

$$\frac{d}{dt}E(X^n_{R}X^m_{P}) = E[((X_R + 1)^n - X^n_{R})X^m_{P}\tau_{R}] + E[((X_R - 1)^n - X^n_{R})X^m_{P}\lambda_{R}X_{R}] + E[X^n_{R}((X_P + 1)^m - X^m_{P})\tau_{P}X_{R}] + E[X^n_{R}((X_P - 1)^m - X^m_{P})\lambda_{P}X_{P}].$$

In order to find $E(X^n_{R}X^m_{P})$, we have to solve the lower moments first. For instance, if we wish to find $E(X^n_{R})$, we may set $m = 0$ in the above equation and the right-hand side of it will be a linear combination of the moments of $X_R$ up to the $n$th order. Thus, we would need to set $n = 1$ and solve $E(X_R)$ first, then find $E(X^2_{R})$ and so forth. Similarly, the solution of $E(X_RX_P)$ would require the solutions of $E(X_P), E(X_R)$ and $E(X^2_R)$. Such solutions will be analytical functions of the rate parameters and can be used to infer parameters in the CME based on the observed moments.

The key reason that we can solve $E(X^n_{R}X^m_{P})$ for any integer numbers $n$ and $m$ is that all the involved propensity functions are simple: either constant or linear function. And the question of solving moments would
be much more complicated if nonlinear propensity functions are involved.

First of all, let us consider the so-called stoichiometric network, a CME based system if all the propensity functions are of the form (3). Equation (6) can still be applied for establishing differential equations, in which the derivatives of moment are represented as the linear functions of other moments. Nonetheless, as long as some propensity functions are nonlinear, such equalities will involve moments with orders higher than the order of moments in the derivatives. In short, let us use \( \mu_n \) to represent the vector of moments of \( X \) up to order \( n \), then based on equation (6) and (3), the ordinary differential equation for \( \mu_n \) can be expressed in the following form:

\[
\frac{d\mu_n}{dt} = C_n \mu_n + C_n^* \mu^*_n,
\]

where \( \mu^*_n \) is a vector which consists moments with orders higher than \( n \), \( C_n \) and \( C_n^* \) are constant matrices that only depend on the parameters and model structure used to define the propensity functions. More details regarding such method can be found in the work of Engblom (2006), Gillespie (2008), as well as Sotiropoulos and Kaznessis (2011). Due to the presence of higher-order moment terms, this equation cannot be solved analytically nor numerically. Approximation approaches collectively known as moment closure method are used in literature to resolve this issue. In a typical moment closure scheme, higher-order moments \( \mu^*_n \) are approximated as functions of lower-order moments \( \mu_n \) so that equation (18) can be effectively “closed” to include lower-order moments \( \mu_n \) only.

Numerous methods have been developed to define appropriate closure scheme. The earliest method utilized the relationship between the cumulant and moments of probability distribution. If we “truncating” all the higher-order cumulants of the solution of CME by setting them as 0, then the corresponded higher-order moments can be expressed as known functions of lower-order moments. This method was first introduced in Whittle (1957), in which all the third and higher order cumulants were set to 0. This approach was also discussed in the work of Matis and Kiffe (1996) and Nasell (2003b). Alternatively, we may also expand the propensity function around the mean, and establish closure scheme through truncating the higher-order terms in the Taylor expansion (Lee, Kim and Kim, 2009; Ale, Kirk, and Stumpf, 2013; Lee, 2013). In these approaches, the focus of truncation is on the central moments rather than cumulants. The accuracy of the truncation based approximation methods were discussed in Lee, Kim and Kim (2009) and Grima (2012).

Since truncating all the third and higher order cumulants is equivalent to a Normal distributional assumption, we may also define moment closure scheme by imposing distributional assumptions over the solution of CME. For single-variable model, popular choice of distributions includes Poisson, Log-Normal and Beta-Binomial (Nasell, 2003a; Krishnarajah et al., 2005). The latter two distributions are more suitable for modeling system with skewed distribution. For multivariable model, mixture distribution (Krishnarajah et al., 2007), as well as the multivariate version of Normal, Log-Normal and Gamma distribution (Lakators, et al., 2015) can be
used. However, such moment closure schemes all suffer a common drawback: the degree of approximation is totally determined by the choice of distribution.

Other more flexible moment closure schemes can be found in the literature as well. In the Separable Derivative-Matching moment closure approach (Singh and Hespanha, 2006; Singh and Hespanha 2011), higher-order moments are assumed to be the products of the powers of lower-order moments. The constant exponents used in this representation can be determined by matching the time derivatives of the true moment functions with the derivative of approximated moments. If we approximate all the moments with order higher than the second, this procedure is equivalent to moment closure method based on log-normal distributional assumption. In the Zero-Information moment closure scheme (Smadbeck and Kaznessis, 2013), the distribution of CME is approximated by the maximum entropy probability distribution conditional on the lower moments.

In addition to the analytical moment closure schemes, stochastic simulation can also be used for closing the dynamic of the system. In the work of Ruess et al., the higher-order moments are approximated based on samples from stochastic simulation, and an extended Kalman filter was used to provide moment estimations up to certain order. This approach has the potential of overcome the limitation of many moment closure schemes, especially when the behavior of the system can not be adequately described by moment equations.

To handle the CME system where the propensity functions can be rational, we often need to firstly multiple both sides of CME (4) by the products of denominators of the propensity functions to obtain moment equalities such as equation (18). It had been shown that, we can then proceed to construct moment equation similar to (18), and apply appropriate distributional assumption to close the moment equation (Milner, Gillespie and Wilkinson, 2011).

In the setting of gene regulation system with rational transcription rate functions, Achimescu and Lipan (2006) and Raffard et al. (2008) studied a single gene system with mRNA and protein species similar to (14). After multiplying the CME with the denominator term of the transcription rate, z-transformation can be applied to transform the probability density function $P_t(X)$ into $F(z, t) = \sum_X z^X \cdot P_t(X)$, whose taylor expansion coefficients are the factorial cumulants. Then equalities of factorial cumulants can be constructed and moment closure can be achieved through truncating the higher-order cumulants. Chao and Wong (draft paper) studied gene regulatory network consists of multiple genes as shown in (15). Based on their work, a rigorous moment equality can be constructed for a system with only one gene. For system of multiple genes, the moment equality developed on single gene system can be used to form appropriate approximated moment equality. This approximation scheme put more emphasize on the truncation of the higher-order cross moments between different variables but could relatively preserve the moments of individual variable.

Although the moment functions would grant us valuable information
regarding the CME system, complete information can only be gained through investigating the distribution function directly. Many statistical inference procedures also require likelihood functions that are analytically or numerically trackable. In the following discussion, we will review the available methods of calculating the probability distribution of CME system, including the distribution at arbitrary time $t$ and the equilibrium distribution.

As CME represents a special case of continuous time Markov Chain, if we could numerate all states in the state space as vector $S$ and denote the corresponding transition rate matrix as $Q$, under initial distribution $P_0(S)$, the distribution of system at time $t$ is

$$P_t(S) = e^{Qt}P_0(S).$$

The steady-state distribution can also be calculated based on $Q$: it is the normalized left eigenvector corresponding to the eigenvalue 0.

Nonetheless, due to the discrete nature of CME, a direct evaluation of equation (19) is a tremendous, if not impossible task even for a simple CME system. In an open system where the state variables can take any non-negative integer values, the state space consists of infinite number of states and the transition rate matrix is of infinite dimension. Even if we impose an upper bound to the value of state variable, the number of different states can still be astronomical. For instance, in a one gene system with mRNA and protein species, if we assume that the copy numbers of mRNA or protein never exceed 99, the state space would still consist of 10000 different states. As a result, suitable approximated methods will be a necessity for making the computational problem feasible.

Practically and theoretically speaking, it is highly unlikely for a biochemical system to visit the whole state space during a limited time. Similarly, the distribution of system at any finite time $t$ or the equilibrium distribution is also likely concentrated on a small subset of the whole state space. This principle forms the foundation of the Finite State Projection method (FSP) (Munsky and Khammash, 2006), in which the complete state space $S$ is decomposed into a reduced and finite subspace $J$, termed as "finite projection space" that consists of the core states frequently visited by the system, and its complement. The transition matrix $Q$ can then be partitioned accordingly. If we denote the resulting block matrix correspond to $J$ as $Q_J$, and assume that $J$ covers the non-zero support of initial distribution, then the distribution of the system over the projection space at time $t$ can be approximated as:

$$P_t^*(J) = e^{Q_J}P_0(J).$$

where $P_0(J)$ the partitioned vector of initial distribution $P_0(S)$ based on $J$.

The accuracy of approximation will increase monotonically as additional states are added into $J$. And the quality of FSP approximation can be evaluated using the following result. Let $P_t(J)$ be the partitioned vector of the exact distribution $P_t(S)$ at time $t$, then for any $\varepsilon > 0$,

$$1^T P_t^*(J) \geq 1 - \varepsilon, \text{ then } 0 \leq P_t(J) - P_t^*(J) \leq \varepsilon 1.$$
Munsky and Khammashb (2006) thus suggested an iterative procedure: starting from a relatively small subset, the finite projection space is iteratively expanded through adding new states based on the reachability from the current subspace. In each iteration, the approximation error will be estimated based on equation (21) and the algorithm will be stopped till the error falls below a pre-given threshold. This algorithm is guaranteed to terminate within finite steps if the system is bounded but can usually be applied to infinite system as well. Counter-example in which FSP algorithm fails does exist (MacNamara, Sidje and Burrage, 2007), but such system does not usually exist in physical world. Applications of FSP algorithm to study stochastic noise in gene network can be found in Munsky and Khammash (2008) as well as Neuert et al. (2013).

FSP algorithm can be further augmented in a number of ways, which usually focus on reducing the cost of computing matrix exponential and optimizing the finite projection space. In the Krylov-FSP algorithm (Burrage, et al., 2006), inexact matrix-vector routines derived from Krylov subspace method are added to the original FSP algorithm. The importance of the efficient enumerations of state space in applying FSP method was discussed in MacNamara, Sidje and Burrage (2007). Munsky and Khammashb (2007) studied the application of FSP when the initial distribution is sparse, and also propose to split $[0, t]$ into multiple small intervals in order to capture the support of distribution more efficiently. Sunkara and Hegland (2010) discussed the optimality of FSP approach focusing on the size of projection space. Hjartarson, Ruess and Lygeros (2013) proposed a method that combines stochastic simulation algorithm with FSP method which allows a reduction of the size of finite projection space without sacrificing accuracy. The FSP method can also be implemented to utilize the time scale separation commonly found in genetic system (Peles, Munsky and Khammash, 2006) and can also be applied to spatially inhomogeneous stochastic biochemical system as well (Drawert, et al., 2010).

Similar to the FSP algorithm, Wolf et al., (2010) presented a state reduction approach named sliding window method for estimating the distribution of CME. The most distinctive feature of this approach lies in its way of constructing state space. In particular, given the initial condition this algorithm divides the time course $[0, t]$ into multiple segments, and calculates the distributions of system states at the right ends of each segments sequentially. For each segment, a new reduced state space named sliding window is constructed to facilitate the calculation. The principles of constructing sliding window are: first, it must contain the major support of the distribution of system states at the left-end of current segment; second, the probability of leaving the sliding window during current segment must be sufficiently small. In this way, the series of sliding windows will likely to capture the states that will be visited by the system during $[0, t]$. Comparing with FSP algorithm, the sliding window approach requires a smaller reduced state space and can thus save computing resources.

In FSP method, the complement of the finite projection space $J$ is effectively aggregated into a single absorbing state to reduce the state space to a manageable level. Similar schemes that focus on reducing the state
space through aggregating similar states are firstly developed to study
general stochastic dynamic system (Simon and Ando, 1961; Haviv, 1987;
Meyer, 1989). In these early approaches, the state space are classified
into groups so that an approximate solution could focus on the interac-
tions between groups rather than the interactions between states within
the same group. Such approaches often work well if the transaction rates
matrix exhibits a certain block structure. For instance, in the gene regula-
tory model we considered, due to the fact that immediate transitions can
only occur between adjacent states, the transition matrix is usually very
sparse and could be organized into an almost block diagonal matrix un-
der proper enumeration of states. More generally, an aggregation method
would utilize two operators: an aggregation operator that projects the
original state space to a reduced aggregated space on which a new CME
is defined and solved, and an disaggregation operator that can be applied
to the new CME to obtain an approximated solution of the original CME.

The separation of time scale, as well as the QSSA, can serve as useful
guides for setting up these two operators. The aggregation operator can
be set up to aggregate different states of the fast species together and
disaggregation operator defined based on the distribution of fast species
conditional on the slow species can be use to approximate the solution.
For instance, in a system consisting of a mRNA specie with copy number
$X$ and corresponding protein specie with copy number $Y$, the state space
consists of all non-negative integer pairs $(x, y)$. The aggregation operator
can be defined as $(x, y) \rightarrow y$ so that all the states with the same protein
copy number will be aggregated into a single new state. Then the new
aggregated state space would only contain mRNA species as variable,
and the solution of corresponding CME, $P^*(y)(y = 0, 1, 2, \ldots)$, can be
determined analytically. Original CME can then be approximated using
disaggregation operator $P(x, y) \approx P^*(y)P_s(x|y)$ where $P_s(x|y)$ represents
the conditional steady-state distribution. Applications of such approach
can be found in the works of Peles, Munsky, and Khammash (2006)
and MacNamara. et.al (2008).

Hegland, et.al (2007) defined an aggregation operator that aggregates
every two adjacent states together. If such operator is applied to the
same system multiple times, the size of size of state space would shrink
exponentially. The disaggregation operator was defined to redistribute the
probability mass over any state after aggregation equally over the original
states. This approach essentially approximated the solution of CME with
a stepwise function and can by applied to high-dimensional system based
on sparse grid.

Waldherr, Wu, and Allgwer (2010) applied aggregation method to
study a bistable genetic switch with two activator. In this system, the
"on" state is defined as the states when both gene express highly. And
the aggregation operator would aggregate all the states in which the sum
of copy numbers of both genes is greater than a certain threshold into
a single "on" state. The the transition probability in to the aggregated
"on" state can be computed and allows an explanation on how certain
long-term characteristics (the very slow transition into a "sinking" state)
can be generated by reactions that take place on much shorter time scale.
Both FSP and many aggregation methods can reduce the complexity of state space and simplify the computation. Still, when the dimension is high, the discrete nature of CME can still pose serious challenge. One way to resolve this issue is to apply hybrid stochastic-deterministic method in which part of the system is modeled as continuous variables. Similar to the QSSA, in a hybrid approach, the system vector $X$ is partitioned as $(Y, Z)$ and the solution to CME is decomposed as $P(X) = P(Y)P(Z|Y)$. Here $Y$ represent the species with low copy numbers, and are treated as discrete variables. $Z$ represent species with high copy numbers, and are treated as continuous random variables. $Y$ can then be modeled using a reduced CME equation in which variables $Z$ are replaced by their conditional expectations. Correspondingly, the evolution functions of $Z$, are usually represented using coupled deterministic equations. In the first hybrid method for solving CME (Hellander and Lotstedt, 2007), $Z$ is assumed to be independent random variables following Normal distributions with small variance. And the expectations of $Z$ are modeled with deterministic differential equations determined by the distribution of $Y$. This hybrid system was solved numerically by approximating the distribution of discrete variables with samples SSA and calculating the expectations of continuous variables though a deterministic time stepping method. Similar approach was also explored in Henzinger et al., (2010). In Menz, et al., (2012), the explicit evolution functions of the distributions of discrete variables and the expectations of continuous variables are derived using the Laplace’s method of integral approximation. And the neglecting of fluctuations of continuous random variables was also justified based on the Wentzel-Kramers-Brillouin approximation.

In the aforementioned hybrid methods, the role of continuous species on the evolution of the system are assumed to be limited to the first moments. Such assumption may not always be appropriate, especially when the copy numbers of corresponding species are moderate or the distributions of system have multiple modes. This limitation can be addressed by the method of conditional moments (Hasenauer, et al., 2014) in which the evolution functions of the higher moments of continuous variable $Z$ conditional on the discrete variables are taken into consideration. This approach essentially combines the key principles behind both hybrid model and the moment-based approach, and thus can potentially offer a better approximation of the underlying system.

Another approximation approach worthy mentioning is the mean field approximation (Kim, Lepzelter and Wang 2007; Kim and Wang 2007). This approach assumes that the distribution of the system state equals the product of marginal distribution of individual species, and thus avoids the difficulty of handling the interacting term in CME. Despite of the simplification made in this assumption, certain key traits of the system (such as the probability of the activation of gene and the fluctuation of the molecules) may still be preserved.
6 Inference Approach

We have discussed how to construct a CME based model, as well as the available methods for studying the properties of CME. In practice, as direct observations or measurements on inner mechanism of cellular system and the values of parameters are rarely possible, we often have to rely inference approach to understand such complex systems based on observable information. In the case of gene regulatory model, the modern experimental technologies may allow scientists to obtain the following information: a) single-cell level mRNAs or proteins expressions measured at (presumably) steady state; b) snapshots of single-cell level mRNAs or proteins expressions collected at various time stages, using the samples from the same populations. c) the temporal tracking of mRNAs or proteins molecules of individual cells. From the perspective of CME model, such observations yield direct information on the equilibrium distribution, the temporal distribution at different times or realizations of the trajectories of CME. In this section, we will discuss the existing inference approaches that can utilize aforementioned information to draw meaningful conclusion of the underlying model.

To what extend the given model can be solved analytically often serves as an important factor in determining the inference approaches. If we could obtain the analytical solution of the given CME, likelihood based approaches will be an obvious choice for inferring the unknown parameters. For instance, under the two-state model (7) where gene can switch between active and inactive states, the steady state distribution of mRNA copy number can be solved analytically (Raj, et al., 2006). Then the key parameters (such as the rates of activation and deactivation of mRNA) can be inferred directly using the maximum likelihood approach (Raj, et al., 2006; Tan and van Oudenaarden, 2010). Still, in the inference of complex dynamical model, it is often hard to know in advance that whether the model is adequate for explaining the data or whether the unknown parameters are actually identifiable based on the observations (Tan and van Oudenaarden, 2010). Thus, additional measures might be needed to further validate the inference results. For instance, the adequacy of the model can be assessed by comparing the experimental data and the simulated samples from the model specified by the estimated parameters (Raj, et al., 2006). The identifiability of the parameters can be investigated by a thoroughly searching of the parameters space to see if the model can also be fitted with other values (Zenklusen, Larson and Singer, 2008).

Unfortunately, a CME is rarely analytically solvable. Still, in certain scenarios, this issue can be circumvented by introducing additional model assumptions. A notable example is the distribution of copy number of protein molecules in basic model (1), which has no analytically solution. Nonetheless, under model (1), the lifetime of a given mRNA molecule follows exponential distribution. Moreover, any given mRNA molecule will translate protein molecules as a constant rate Poisson process. Consequently, the number of proteins produced during the lifetime of a mRNA molecule follows geometric distribution. Since the lifetime of mRNA molecule is often much shorter comparing to the lifetime of pro-
tein, we may then model the translation process as “burst”. That is, a new mRNA molecule will instantly translate a geometrically distributed number of protein molecules. Based on this assumption, we may construct a new CME which predicts that, conditional on the mRNA copy number, the stationery distribution of protein copy number follows negative binomial distribution (Paulsson and Ehrenberg, 2000). Furthermore, by applying continuous approximation as well as ignoring the fluctuation in mRNA copy number, it can be shown that the distribution of protein copy numbers should follow Gamma distribution specified by two parameter \(a\) and \(b\) (Cai, Friedman and Xie, 2006):

\[
p(x) \propto x^{a-1} e^{-x/b}.
\]

Comparing to the notations we used in model (1), parameter \(a\) is roughly equivalent to \(\tau_R/\lambda_P\), and can be interpreted as the frequency of burst. \(b\) is equivalent to \(\tau_P/\lambda_R\) and represents the average number of protein molecules produced per burst. This scheme models can be easily applied to fit single-cell level protein expression data, and was used to study the expressions of \(\beta\)-galactosidase in living *Escherichia coli* cell which exhibit clear burst pattern (Cai, Friedman and Xie, 2006). In particular, this model can explain the two distinctive patterns of the distribution of protein expression observed in experiments: a pattern resembles an exponential decay with peak at zero and a bell-shaped pattern with non-zero peak. A further system-wide examination on the expressions of different protein species in *Escherichia coli* cells (Taniguchi, et al., 2011) also demonstrated that the empirical distributions of all the protein species can be well fitted by Gamma distribution with two parameters.

For general problems, it is often necessary to explore alternative formulations to counter the intractability of distribution function. One common approach is focusing on developing equality or differential equation of the moments of variables of interests. Such moment-based methods may not be able to fully utilize the observed information as we might expect for a likelihood-based approach, but can still provide valuable insight that can not be obtained by approaches that ignore the intrinsic noise. Munsky, Trinh and Khammash (2009) studied the number of measurements required to identify all parameters in model (1) as well as the initial conditions. Under CME model, it is only necessary to measure the first two moments of protein and mRNA copy numbers at two separate times. On contrary, number of measurements needed for identifying parameters under an equivalent deterministic system is far greater.

So, et al., (2011) inferred parameters in the two-states model (7) by fitting the analytical formulas of Fano factor and the square coefficient of variation (variance divided by mean square) to the observed values. Similar method was used by Gandhi, et al., (2010) regarding the coordination of genes during cell divisions. The dependence between genes introduced by cell divisions were explored by numerically fitting the analytical expression of the covariance between gene expressions. The moment-based approach can also be used to distinguish different model assumptions. In the work of Singh et al., (2012), the analytical formula of Fano fac-
tor is used as the basis for inferring whether the noise in protein level is mainly contributed by the Poisson fluctuation in RNA numbers or by the stochastic transitions between different states of gene.

If we cannot derive the exact moment equality, moment closure methods are usually applied to establish approximated expressions of key moments for inference purpose. Milner, Gillespie, Wilkinson (2013) studied the inference of model parameters based on time series observations of CME system, and modeled the observed data as Gaussian distributed random variables whose means and variance are determined by moment closure scheme. Kugler (2012) also considered the similar approach but focused on fitting the parameter by minimizing the distance between observed moments and the moments predicted by model. For biological system with rational propensity functions, Pedraza and van Oudenaarden (2005) investigated a three genes system whose interactions are modeled by Hill functions and established the moment closure schemes by applying linear expansion around the steady state. Achimescu and Lipan (2006), Raffard et al. (2008) explored the inference problem in a single gene system with mRNA and protein species in which the propensity function is rational functions. Their work also took the presence of external signals. Chao and Wong (in preparation) developed moment-closure inference approaches for analyzing multiple-gene systems and demonstrated that such approach can not only be applied to estimate the unknown parameters but also be used to infer the unknown regulatory relationship.

To quantify the uncertainty of the inferred parameters through moment-based approach, Zechner, et al., (2012), noted that, due to the large number of cells measured simultaneously in cytometry experiments, it was reasonably to expect that the sample size is large enough so that the empirical moments would roughly follow normal distribution whose means and variances can be expressed as functions of moments. Consequently, as long as we could apply suitable moment closure scheme to determine how particular moments depend on unknown parameters, the uncertainty of estimations can be quantified using the frequentist property of maximum likelihood estimator or the posterior distribution if we assign prior distribution to the unknown parameters. Similar ideas can also be found in the work of Ruess, Milias-Argeitis and Lygeros (2013), Ruess and Lygeros (2015) and Schilling, et al., (2016).

Another major source of uncertainty lies in the fact that the chosen closure scheme serves only as an approximation of the system under investigation. It is reasonable to expect that, to attain a reasonable level of approximation, we might need to apply different moment closure schemes on different systems, or even the same system for different value of parameters. However, it is often hard to evaluate the error introduced by moment closure scheme in practice. Schilling, et al., (2016) proposed a adaptive algorithm to handle this issue. In their approach, as the algorithm searches parameter spaces, stochastic simulation algorithm will be performed to generate samples using the current parameter values. The discrepancy between the simulated samples and observations can then be used to evaluate the fitness of the employed moment closure schemes and make adjustment if necessary. Consequently, this adaptive algorithm not only could select the most appropriate moment closure schemes, but can
also adopt different schemes in different parts of parameter space.

In the inference methods we discussed so far, the unknown parameters are estimated based on analytical formulas that link the observed data and unknown parameters. While such approaches are often relatively easy to implement and require minimal computation resources, there are several potential drawbacks. As have discussed, under most CME models, we cannot establish exact analytical formulas and are forced to adopt approximation formulations. However, as it is often difficult to evaluate the discrepancy between the approximation formulations and the true model, it is also hard to quantify the bias and uncertainty introduced by the approximation schemes during the inference procedure. Furthermore, the approximation formulations often focus on certain summary statistics (such as the moments) of the observed data and thus may not be able to fully utilize the information. In the following paragraphs, we will investigate the inference approaches that utilize numerical methods or simulation algorithms to bridge the gap between the data and model parameters.

The FSP approach we discussed in the previous section can be used to calculate the numerical solution of CME system within desired precision. Such numerical solution can then allow us to infer the unknown parameters by searching the parameter space and locating the values that minimize the distance between numerical solution and the empirical distribution. In Munsky, Trinh and Khammash (2009), the CME model on the lac operon of *Escherichia coli* was fitted by numerically searching the values of parameters minimizing the $L_1$ distance between the FSP solution and observed distribution. In calculating this distance metric, measurements obtained under various conditions were assigned different weights for specifying the relative importance. As for the detailed optimization procedure, a random initial guess is firstly made, then the value will be updated using gradient-based and simulated annealing searches. Similar method can also be found in Neuert, et al., (2013), Shepherd, et al., (2013) and Senecal, et al., (2014).

In the aforementioned FSP based inference approach, we have to re-calculate the numerical solution of CME throughout the optimization algorithm. Even though the FSP method can reduce the computational burden of finding the numerical solution of CME significantly, such task can still be very computational demanding, especially when the dimension of parameter space is large. In this regard, it can be worthy to consider likelihood-free inference approach to avoid the difficulty of finding solution of CME. In particular, the Bayesian method known as Approximate Bayesian Computation (ABC) (Tavaré et al., 1997; Pritchard et al., 1999; Beaumont et al., 2002) can be used for such a purpose. In a standard ABC rejection algorithm, a particle $\theta^*$ is firstly sampled from the prior distribution of unknown parameter, and is used to generate a simulated data set $X_{\theta^*}$. The proximity between $X_{\theta^*}$ and the observed data set $X$ can then be evaluated based on a chosen distance metric. The decision on whether to reject or accept the particle $\theta^*$ will then be made on whether the distance is greater or smaller than a predefined threshold $\epsilon$. This procedure essentially allow us to obtain independent sample of $\theta$ from density $p(\theta | d(X, \hat{X}) < \epsilon)$, which can be regarded as a reasonable approximation
to the posterior distribution \( p(\theta | X) \) for small \( \epsilon \). Thus, as long as we can simulate samples from the given model with specified parameters, we can obtain posterior samples of parameters without evaluating the likelihood function. Considering the numerous stochastic simulation algorithms we discussed in previous section, ABC can be an suitable choice for inferring parameters in CME.

The efficiency of a ABC sampling algorithm largely depends on the acceptance rate of particles. The acceptance rate can be improved by adopting Markov Chain Monte Carlo method (Marjoram et al., 2003) and sequential sampling technique (ABC SMC, see Toni, et al., 2009, Liepe, et al., 2014). The ABC SMC algorithm utilizes a a gradient of thresholds \( (\epsilon_1, \epsilon_2, \ldots, \epsilon_T) \) in strictly decreasing order with \( \epsilon_T \) as the desired threshold. Sampled particles will be propagated through a sequence of intermediate distributions corresponding to the intermediate thresholds until the final set of samples represents the target posterior distribution.

Another important issue in applying ABC algorithm is the control of false rejection error, the error that the proposed particle is reject even though the distribution of experimental sample is consistent with the distribution of simulated data. As such error is often due to the fact that we can only use a finite size of samples to approximate the true distribution as defined by the particle, it can be reduced by increasing the size of simulated data set at the price of increasing computational cost. In the ABC algorithm named INSIGHT for analyzing flow cytometry data (Lillacci and Khammash, 2013), it is shown that, if the Kolmogorov distance is used as the distance metric, the false rejection error will depends on the specified threshold \( \epsilon \), the size of experimental sample and the size of simulated data. Moreover, the size of experimental sample in a typical flow cytometry experiment is often large enough so that the required size of sample for attaining a reasonable false rejection error can be surprisingly small. This discovery means that the ABC algorithm can be implemented in a very efficient manner as long as the size of observed data is large enough. In addition, the use of Kolmogorov distance also allow the bounds of a mismatch index to be estimated, which is defined as the distance between the distribution of experimental data and the distribution of best fitted model. This index grants us valuable insight on the fundamental discrepancy between experimental data and the stochastic model, and can be used to determine whether alternative models should be investigated or not.

Finally, as all the Bayesian methods, ABC opens the possibility of using Bayes factor or posterior probability to compare competing models (Toni, et al., 2009, Liepe, et al., 2014). For instance, in Toni et al. (2012), the ABC SMC algorithm is used to study the MEK/ERK phosphorylation dynamics using time course data obtained from \textit{in vivo} cells. The estimated posterior probabilities are then used to rank candidate models that represent different hypothesis on the underlying systems. Moreover, by comparing the simulated samples and the observed data, ABC method also make a direct diagnostic of the discrepancy between the model and the data possible (Ratmann, et al., 2009).

If we can track the expressions of mRNA or protein within individual
cells continuously (Golding, et al., 2005, Yu, et al., 2006), then the inference problem can be handled in a quite different fashion. In particular, as laid out in Gillespie algorithm (Gillespie, 1977), as long as we have the complete information on a particular trajectory of CME over time \([t_0, t_n]\) (including the initial copy number(s) \(x(t_0)\) as well as the firing times of each reactions up to time \(t_n\)), we can easily express the likelihood function as the product of exponential and multinomial densities. The corresponding inference problem can then be easily solved. For instance, in a stoichiometric system where the propensity functions are linear functions of the unknown parameters, the maximum likelihood estimator can be analytical solved given the full trajectory (Daigle et al., 2012).

Nonetheless, the complete information is extremely hard if not impossible to obtain. In practice, we can only observe the system state at a few discrete time points. Let us represent the observed data as \(x(t_0), x(t_1), \ldots, x(t_n)\), the likelihood function is then the products of transition likelihood \(p(x(t_i) | x(t_{i-1}), \theta)\) whose expression is usually not analytical. For example, considering a single molecular specie that evolves according to a simple birth and death process, if the copy numbers are 10 and 20 at time 0 and \(t\) respectively, then any full trajectory that satisfies the following condition is consistent with the observation: 1) the total number of births minuses the total number of deaths during \((0, t]\) equals 10; 2) The birth events and the death events can occur in any order and at any time as long as the total copy number never drops below 0. Consequently, transition probability from 0 and \(t\) will be the sum of probabilities of all the consistent full trajectories, which can be hard to compute if the system is complex.

Many authors had thus explored approximated approaches to estimate the transition probability so that the unknown parameters can be inferred with conventional methods. In Reinker, Altman and Timmer (2006), under the assumption that the number of firings is limited or the propensity functions remain constant during period \((t_{i-1}, t_i]\), it was shown that the transition probability can be approximated with relatively simple analytical formulas. This approach is roughly equivalent to approximate the exact transition probability as the sum of probabilities of the most probable paths from \(t_{i-1}\) to \(t_i\). In the work Tian et al. (2007), the transition likelihood from \(t_{i-1}\) to \(t_i\) is estimated using non-parametric kernel density function based on the simulated realizations of system at \(t_i\) given initial condition \((t_{i-1}, x(t_{i-1})).\)

By treating the full trajectory as complete data, the maximum likelihood estimator of parameters can also be found using Expectation-Maximization (EM) algorithm. In the E-step, the expectation of the likelihood function of the full trajectory conditional on the observations and current value of parameters is evaluated. Then in the M-step, the value of parameters can be updated by maximizing the conditional expectation. As it is usually impossible to calculate the exact conditional expectation in the context of CME, the Monte Carlo extension of EM algorithm (MCEM) is often used in practice. In MCEM, the conditional expectation will be estimated based on the sampled full trajectories. The major difficulty in applying MCEM in CME system lies in the fact that the simulated trajectories must be consistent with the observed data, which
can be hard to achieve if we use an unmodified stochastic simulation algorithm. Horvath and Manini (2008) suggested that the full path should be simulated piece-wisely for each interval \((t_{i-1}, t_i]\). Daigle et al., (2012) argued that in order to implement MCEM efficiently, the initial choice of parameters should be the values that are likely to generate consistent trajectories. An iterative algorithm based on the cross-entropy method (Rubinstein, 1997) was used to find such initial values. In each iteration, trajectories are simulated using previous parameter values but only the trajectories that are closed to the observed path are used for updating the parameters.

Wang et al. (2010) discussed an approach in which likelihood function is maximized using stochastic gradient descent. It was shown that the gradient of likelihood function can be determined based on the expectation of the durations that the system stay on different states and the numbers of transitions between states, conditional on the observed path. A reversible jump Markov chain Monte Carlo algorithm is implemented for simulating paths that are consistent with the observations, in which new paths are proposed by adding/deleting certain set of reactions from initially proposed path. This method can be apply to the data set with only part of the species observed.

In addition to the frequentist approaches, Bayesian methods that utilize MCMC sampling algorithms can also be used to solve such problems. Boys, Wilkinson and Kirkwood (2008) use MCMC algorithm to sample the full trajectories conditional on the observations. The efficiency of MCMC sampling is improved by using reversible jumping and blocking update methods. Generally speaking, the Bayesian approach can usually be applied to system with unobserved species directly, as Bayesian approach can simply impute such missing information in the same way as imputing the full trajectories.

The discreteness of CME is often the major obstacle in obtaining its solution. As we have discussed in the previous section, the CME can be approximated by other continuous stochastic process, including the linear noise approximation (LNA) and the stochastic differential equation. In the following paragraphs, we will discuss existing inference approaches with respect to the continuous stochastic models, especially with respect to the time series data on the evolution of individual systems.

LNA approximated the CME as the sum of deterministic term and stochastic fluctuation. As noted in Komorowski et al. (2009), the stochastic fluctuation can be modeled by SDE whose drifting and diffusion terms depend on the deterministic part of LNA. Consequently, the solution of LNA is always multivariate Gaussian distribution whose mean vector and covariance matrices can be determined by the propensity functions. Thus, with suitable choice of prior over the unknown parameters, the posterior distribution can be sampled straightforwardly using standard Metropolis-Hastings algorithm. This framework can readily accompany the presence of unobserved species, as well as the measurement errors (assuming to be an additive Gaussian noise). This method was applied to estimate the GFP protein degradation rate from cycloheximide experiment. Fearnhead, Giagos, Sherlock (2014) also considered the inference problem using...
LNA and shown that such approach can be statistically and computationally more efficient than approaches based on deterministic differential equation or SDE.

Unlike LNA, the transition probability between two successive observations in a full SDE approximation is often analytically intractable. However, such transition probability can often be estimated by discretizing the trajectory of SDE system, a scheme commonly known as Euler-Maruyama approximation. This approximation discretizes the sample path between two successive observations into multiple segments, and the increment in each segment is modeled as independent Gaussian random variables whose means and values are determined by SDE. This approximation forms the basis of the Bayesian inference framework proposed by Golightly and Wilkinson (2005) for general stoichiometric model. A MCMC scheme is then used to obtain posterior samples of unknown kinetic parameters. Due to the need of imputing values to discretize the SDE, as well as handling the unobserved species, the sampling procedure is alternative between the sampling of parameters conditional on the augmented data and the sampling of missing data given observations and the current set of parameters.

This scheme can be further enhanced with advanced sampling methods. To overcome the dependence between the parameters and missing data, sequential MCMC methods can be used to sample the model parameters (Golightly and Wilkinson, 2006). The accuracy of Euler-Maruyama approximation increases as the number of imputed value increases. However, if we increase the number of imputed values, we will also increase the computational cost which could break down an ordinary Bayesian imputation algorithm. Golightly and Wilkinson (2008) proposed a global MCMC strategy with an improved Gibbs sampler, in which a Brownian motion process is used to impute values between successive observations so that the computational cost will not scaled up as the number of segments increases. The speed of such computation scheme is still limited by the complexity of the model, and particle Markov Chain Monte Carlo method can be implemented to lessen the computation burden (Golightly and Wilkinson, 2011).

As we have discussed in the second section, it is possible to propose model with different level of details for explaining the mechanics of gene regulatory system. Nonetheless, it is often hard to observe the inner mechanism of regulatory system directly and we have to rely on the available information to choose between different models. For instance, how shall we choose between the simple model (1) and the two-state model (7) by analyzing the single-cell level distribution of the copy number of protein molecules? And how can we know if our choice of model is sophisticated enough to explain what we have observed or whether the observed information is sufficient for us to infer the detail of the model we propose? In the following paragraphs, we will review the relevant literatures that deal with the problem of model selection in the context of studying CME system. Still, many of the approaches discussed here would also shed light into the inference problem of complex dynamical system in general.

To evaluate the fitness of the model, we need to propose suitable met-
rics that can be used to measure the distance between model and data. In literatures, the distances between the observed and predicted values or time derivatives of state variables or between the predictive and observed moments are often used (Kugler, 2012; Babtie, et al., 2014; Liepe, et al., 2014). If the predictive distribution can be obtained, $\chi^2$ test can be used to determine whether the prediction of model is consistent with the data (Zenkluen, Larson and Singer, 2008), and Euclidean distance or Hellinger distance can be used as metric of discrepancy the difference (Munsky, Trinh, and Khammash, 2009; Sunnaker, et al., 2013; Silk, et al., 2014). To compare model with different level of details, AIC as well as Bayes factor can be used to penalize additional model parameters or structure (Toni, et al., 2009; Sunnaker, et al., 2013; Babtie, et al., 2014; Liepe, et al., 2014; Silk, et al., 2014). The fitness and complexity of the model can also be balanced by measuring the uncertainty introduced by over-complex model. For instance, in the work of Neuert, et al., (2013), the log-likelihoods are used as measurement of fitness while the uncertainty is evaluated by cross-validation. Specifically, the uncertainty is defined as the average log-likelihoods of complete data set calculated using parameters obtained through fitting the model with sampled partial data set. Then the best model is chosen based on the balance between the fitness and uncertainty.

As many authors had pointed out, due to the complexity of dynamical system, even if we can achieve good fit using a particular model or particular set of parameters, there might be other alternative models or sets of parameters that could fit the data equally well. Consequently, it is often useful to search the space of candidate models or the parameter space thoroughly before making a final conclusion. For a fixed model, Villaverde, et al., (2015) explore the predictive accuracy of fitted model using a consensus approach. This approach will search the parameter spaces of the given model and collect sets of parameter values that all fit the data well. Then the accuracy of prediction can be analyzed based on whether these collected sets of parameter values could reach consensus or not. The burden of searching the parameter spaces can be reduced by grouping the parameters into modules of meta-parameters. The uncertainty in the structure of model is explored in the so called Topological Sensitive Analysis (Babtie, et al., 2014). In this approach, alternative structure is proposed by modifying the relationship between nodes in the given model. Restrictions are imposed to limit the search space. The fitness of the proposed structure is then evaluated using Gaussian process regression. Topological Filtering method (Sunnaker, et al., 2013) explore alternative models by constructing a tree of models. The root of this tree is the base model consists of many detailed interactions. New nodes are then created by removing interactions and the associated parameters step by step. Analysis on the fitness of the model will be carried along the way. The process of creating new nodes will only be stopped if any further simplification will make the model unfitted for the data. This approach may create multiple branches and the candidate models at the end of each branch will be collected for further study. Finally, the exploration of alternative models may guide the scientists to design further experiments to further discriminate different models. Maximizing Fisher information
has been proposed as a principle for designing new experiments to understand biochemical reaction network (Ruess and Lygeros, 2013). And the interaction between inference, model selection and design of experiment also serves as the central theme in the 2014 DREAM contest (Meyer et al., 2014).

7 Discussion

In this review article, we explored the CME based approach in modeling and analyzing the intrinsic noise in gene regulation system. We demonstrated that the CME based model offers a flexible way of incorporating physical mechanism and is capable of capturing the discrete, stochastic and dynamical nature of cellular system. We also discussed several alternative modeling approaches and viewed them as approximations of the equivalent CME model. We then provided an overview of available tools that can be used to study CME based systems. In particular, we discussed simulation methods, analytical and numerical solvers, and statistical methods that can be used to infer the unknown parameters or structures in CME model using data collected at single-cell levels.

Our discussion in this article has been focused on the intrinsic noise. It must be admitted, though, the systems we considered are also under the impact of different types of extrinsic noises. First of all, all the single-cell level experiments are subjected to measurement errors. In smFISH approach, as individual molecules can be visualized and tracked, it is possible to obtain the discrete counts of the molecular species under investigation. However, in technologies such as flow cytometry and mass cytometry, the single-cell level observations are collected through measuring the intensity signals emitted by reported tags attached to target molecules. Consequently, we would need to infer the discrete counts of molecules from the observed continuous intensity signals in order to apply the CME model. In practice, it is often assumed that the observed intensities follow Gaussian distributions whose means and variances are proportional to the counts (Munsky, Trinh and Khammash 2009; Komorowski et al., 2009; Lillacci and Khammash, 2013). Other models exist as well, such as Gaussian random variables with constant variances (Golightly and Wilkinson, 2011). In scRNA-seq experiment, how to properly normalize the observed read counts and correct bias introduced by the unobserved and dropout measurements also pose serious challenge for data analysts (Stegle, Teichmann, and Marioni, 2015; Bacher and Kendziorski, 2016).

In addition to measurement noises, system at single-cell levels are subjected to various fluctuations induced by external factors. With carefully designed experiment, it is possible to distinguish the intrinsic and extrinsic noise. For instance, in the study by Elowitz, et al., (2002), the single-cell levels expressions of two different types of proteins driving by identical promoters were observed. The extrinsic noise is defined as the fluctuations that impact the expressions of both proteins simultaneously. Still, for general cases, we need to explicitly model the external factors to
distinguish between different sources of noises. For instance, the fluctuation introduced by cell division can be modeled based on the assumption that molecules in parent cell are randomly distributed to offspring cells upon division (Rigney, 1979; Rosenfeld, et al., 2005). Cell growth, on the other hand, increases the cell volumes and thus decreases the concentrations of molecules. Then we may model the fluctuations introduced by cell growth similarly to the noises introduced by the degradation of molecular species (Lei, et al., 2015). Moreover, cells in the same population are often originated from common ancestor and share information through extracellular communications. And a full understanding of the regulatory interactions within single cell may have to be achieved in the population context (Snijder and Pelkmans, 2011).

Suffice to say, the story told by intrinsic noise is not complete. Nonetheless, information extract from intrinsic can still allow us to understand the functions of the fundamental building blocks of cellular systems. In this way, the work on the intrinsic noise would provide a foundation stone for constructing constructing more sophisticated models and serve as an integral part in the quest of understanding life.

This review are restricted to CME based approaches for analyzing intrinsic noise in gene regulation system. While such approach does have the advantage of providing a detailed, physical interpretable model, it also presents greater challenge for both modeling and inference. On the one hand, many of the approaches discussed in this article have been applied to study relatively small and compact systems with various degree of success. On the other hands, how such approaches can be applied to understand larger systems involving hundreds and thousands of species and reactions is still an interesting question without a definite answer. Still, we would like to point out that, the knowledge gained analyzing small systems via CME-based approaches can often provide valuable insight in understanding more complex systems.

For instance, under the simplest CME model (1), we can show that the Fano factor of mRNA copy number should equals 1 and the Fano factor of protein number is greater than 1. This simple conclusion would suggest that the Fano factor can be used to measure the strength of intrinsic noise and any deviation from baseline would indicate more complicated mechanisms (Thattai and van Oudenaarden, 2001; Tao, 2004). The study on Fano factor of molecule species in different cells has reveal many fundamental characteristics of cellular system. For instance, it has been observed that, in prokaryotic cell, the fluctuation of protein is often determined by and positively correlated with the translational efficiency (Ozbudak, et al. 2002). And in eukaryotic cells, strong correlation between noise and transcriptional efficiency can be found (Blake et al., 2003). In addition, the regulatory pathway would also leave impact on the strength of intrinsic fluctuations and it is commonly known that the negative feedback loop can reduce the noise and positive feedback would increase the noise level (Kepler and Elston, 200; Becskei and Serrano, 2000; Isaacs et al., 2003).

Take this discussion one step further, the study on two-gene systems suggests that the correlation between the expressions of different genes measured at the single cell level can be used as indicator of underlying
regulatory relationship. Simple analysis would show that, correlation between genes tend to be negative if one gene represses the expressions of another gene, and positive in case of activation. In the traditional ensemble based experiments, similar information can only be obtained through the introduction of perturbation. In the work of of Stewart-Ornstein, Weissman, and El-Samad (2012), such principle is used to categorize 182 studied proteins in yeast cell based on the correlation matrix measured at steady-state. It was observed that the genes within the same block often have similar functions and respond to the same upstream regulators. This study also found evidence on the correlation between intrinsic noises and external stimulus which suggests that the observed intrinsic fluctuation can be used to study the regulation pathway. In addition, if the observations can be made at different time points, then the dynamical cross-correlation function can be used to determine the direction of regulatory relationship, as the change in the expression of the upstream gene will only impact the expression of the downstream genes after delay (Dunlop et al, 2008).

To conclude, CME based approach provides a unique and indispensable perspective in understanding the role of intrinsic noises in cellular system. More importantly, the discrete and dynamical natures of CME also present fresh challenges for statisticians. First, as we have discussed in this article, the intrinsic noises contains valuable information that can only be extracted via a physical interpretable model such as CME. Nonetheless, the introduction of such model also blurs the relationship between the parameters to be inferred and the observed data. Consequently, it is often necessary to find suitable inference approaches to avoid the evaluation of distribution functions. Then what principle shall we follow so that the proposed method can fully utilize information contained in the data? Second, even for a relatively simple genetic toggle switch model, the stationary distribution of the corresponding CME can be unimodal or bimodal based on the values of parameters. And for a CME with bimodal stationary distribution, its evolution through time would exhibit phase transitions between unimodal and bimodal distributions. The low-copy-number effect of cellular system also forces us to consider discrete distributions that can not simply be approximated as continuous distribution. How well the traditional inference approaches fare with such unconventional distributions? Third, in the context of complex dynamical models such as CME, there are no satisfactory answers on how to determine whether the unknown parameters can be identified solely based on the available information, or how to compare competing models with different levels of details. Can we adjust conventional model selection approaches or do we need to develop fresh new methods to solve such problems? In this regard, we hope that our review article would not only introduce the exiting works of applying CME based model to analyze cellular system to statistical community, but can also ignite new research interests toward this direction.
8 References

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