[13] M. Hou, "Estimation of sinusoidal frequencies and amplitudes using adaptive identifier and observer," *IEEE Trans. Automat. Contr.*, vol. 52, no. 3, pp. 493–499, Mar. 2007.
[14] W. Lohmiller and J. J. E. Slotine, "On contraction analysis for nonlinear systems," *Automatica*, vol. 34, no. 6, pp. 683–96, 1998.
[15] W. Lohmiller, "Contraction Analysis of Nonlinear Systems," Ph.D. Thesis, Dept. Mech. Eng., MIT, Cambridge, MA, 1999.
[16] W. Lohmiller and J. J. E. Slotine, "Nonlinear process control using contraction theory," *AIChE J.*, vol. 46, no. 3, pp. 588–96, 2000.
[17] W. Lohmiller and J. J. E. Slotine, "Control system design for mechanical systems using contraction theory," *IEEE Trans. Automat. Control*, vol. 45, no. 5, pp. 884–889, May 2000.
[18] J. J. E. Slotine and W. Li, *Applied Nonlinear Control*. Englewood Cliffs, NJ: Prentice Hall, 1991.
[19] D. Angeli, "A Lyapunov approach to incremental stability properties," *IEEE Trans. Automat. Control*, vol. 47, no. 3, pp. 410–21, Mar. 2002.
[20] A. Pavlov, A. Y. Pogromsky, N. van de Wouw, and H. Nijmeijer, "Convergent dynamics, a tribute to Boris Pavlovich Demidovich," *Syst. Control Lett.*, vol. 52, pp. 257–261, 2004.
[21] A. Pavlov, N. van de Wouw, and H. Nijmeijer, "The global output regulation problem: An incremental stability approach," in *Proc. 6th IFAC Symp. Nonlinear Control Systems (NOLCOS)*, Stuttgart, Germany, 2004, pp. 119–124.
[22] V. Fromian, G. Scorletti, and G. Ferreres, "Nonlinear performance of a PI controlled missile: An explanation," *Int. J. Robust Nonlinear Control*, vol. 9, no. 8, pp. 485–518, 1999.
[23] W. Lohmiller and J. J. E. Slotine, "On metric observers for nonlinear observers," in *Proc. IEEE Int. Conf. Control Applications*, Dearborn, MI, Sep. 1996, pp. 320–326.
[24] W. Lohmiller and J. J. E. Slotine, "Applications of metric observers for nonlinear observers," in *Proc. IEEE Int. Conf. Control Applications*, Dearborn, MI, Sep. 1996.
[25] J. Jouffroy and J. Lottin, "On the use of contraction theory for the design of nonlinear observers for ocean vehicles," in *Proc. American Control Conf.*, Anchorage, AK, May 8–10, 2002, vol. 4, pp. 2647–2652.
[26] J. Jouffroy and J. Lottin, "Integrator backstepping using contraction theory: A brief technological note," in *Proc. IFAC World Cong.*, Barcelona, Spain, 2002.
[27] J. Jouffroy, "A simple extension of contraction theory to study incremental stability properties," in *Proc. European Control Conf.*, Cambridge, U.K., 2003.
[28] J. Jouffroy, "Some ancestors of contraction analysis," in *Proc. Conf. Decision and Contr.*, Seville, Spain, 2005, pp. 5450–55.
[29] J. Jouffroy and J. J. E. Slotine, "Methodological remarks on contraction theory," in *Proc. IEEE Conf. Decision and Contr.*, Atlantis, Paradise Island, Bahamas, 2004, pp. 2537–2543.

Evaluation of Stochastic Effects on Biomolecular Networks Using the Generalized Nyquist Stability Criterion

Jongrae Kim, Declan G. Bates, and Ian Postlethwaite

***Abstract*—Stochastic differential equations are now commonly used to model biomolecular networks in systems biology, and much recent research has been devoted to the development of methods to analyse their stability properties. Stability analysis of such systems may be performed using the Laplace transform, which requires the calculation of the exponential matrix involving time symbolically. However, the calculation of the symbolic exponential matrix is not feasible for problems of even moderate size, as the required computation time increases exponentially with the matrix order. To address this issue, we present a novel method for approximating the Laplace transform which does not require the exponential matrix to be calculated explicitly. The calculation time associated with the proposed method does not increase exponentially with the size of the system, and the approximation error is shown to be of the same order as existing methods. Using this approximation method, we show how a straightforward application of the generalized Nyquist stability criterion provides necessary and sufficient conditions for the stability of stochastic biomolecular networks. The usefulness and computational efficiency of the proposed method is illustrated through its application to the problem of analysing a model for limit-cycle oscillations in cAMP during aggregation of *Dictyostelium* cells.**

***Index Terms*—Dictyostelium, cAMP oscillations, stochastic noise, Nyquist stability criterion.**

I. Introduction

In Systems Biology, analysis of biomolecular networks is now routinely carried out using computer modelling and simulation. Two types of models are generally employed for such analyses, namely deterministic rate-equation based models with continuous reactant concentrations or stochastic representations based on discrete and probabilistic changes in reactant molecule numbers [1]. For systems with low molecular concentrations, it has been argued that stochastic representations are essential in order to represent accurately the effects of the intrinsic noise, which has been shown to have the potential to qualitatively change the dynamics of such networks, [2]. Stochastic simulation, [1], however, can easily become prohibitively time-consuming for networks of even moderate size, especially if a systematic mapping of the system's parameter space is required. In addition, there is a serious lack of analytical tools for analyzing the qualitative dynamical behavior of stochastic simulation models. In [3], a novel method referred to as the Effective Stability Approximation was introduced for analyzing the stability of stochastic genetic circuits. This approach works by including an additional stochastic perturbation in the deterministic linearization of the ordinary differential equation model [4]. In this approach, however, it is necessary to approximate the dominant term in the stochastic perturbation by calculating an exponential matrix with time involved as a symbolic parameter. Since even the calculation of numerical matrix exponentials for large size matrices is computationally extremely expensive, [5], the analysis method as formulated in [3] would appear to be restricted to quite small-scale circuits, of the order of two or three states at most. To extend the applicability of the approach to larger size problems, we develop a novel approximation for the dominant term in the stochastic perturbation to the ordinary differential equation model of the biomolecular network. As shown below, this approximation does not introduce any significant additional error in the problem formulation, and facilitates the stability analysis of significantly higher order networks via the generalized Nyquist stability criterion.

The paper is organized as follows. Firstly, the current procedure for approximating the dominant stochastic perturbation in the method for genetic circuit analysis of [3] is summarized, and the associated computational problem is highlighted. Secondly, a new method for approximating the stochastic term is derived. Thirdly, it is shown how the resulting analysis problem may be formulated and solved using the generalized Nyquist stability criterion. Finally, the proposed method is applied to analyze the stability of a stochastic model for the network underlying cAMP oscillations in *Dictyostelium discoideum*. As the dimension of this network is seven, the corresponding symbolic exponential matrix calculation is computationally intractable in this case.

Manuscript received February 14, 2008; revised May 30, 2008 and July 3, 2008. This work was supported by BBSRC research Grant BB/D015340/1. First published September 12, 2008; current version published September 24, 2008. Recommended by Associate Editor Ji-Feng Zhang.

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Digital Object Identifier 10.1109/TAC.2008.929463

II. Computational Cost of Approximating the Stochastic Perturbation

We consider biomolecular interactions represented by nonlinear differential equations of the form

$$\frac{dx(t)}{dt} = f[x(t)] \tag{1}$$

where $x \in \mathbb{R}^n$, $f[x(t)]$ satisfies the standard conditions for the existence and uniqueness of the solution of the differential equation, $\mathbb{R}$ is the real number field and n is a positive integer. Linear stability analysis of such equations is performed around the equilibrium point, x_s, which satisfies $f(x_s) = 0$, as follows:

$$\frac{d\Delta x(t)}{dt} = \left.\frac{\partial f(x)}{\partial x}\right|_{x=x_s} \Delta x(t) \equiv \Gamma \Delta x(t) \tag{2}$$

where we assume that all real parts of the eigenvalues of Γ are strictly less than zero, hence, the system is Hurwitz stable. Now, introduce a small perturbation which is added to $\Delta x(t)$, to represent some stochastic noise $\Omega\alpha(t)$, where Ω in the set of positive real number, $\mathbb{R}^+$, is in general inversely proportional to the square root of the cell volume, V_{cell}, i.e., $\Omega \approx 1/\sqrt{V_{\text{cell}}}$, and $\alpha(t)$ in $\mathbb{R}^n$ is the stochastic noise whose mean value is zero. Then, the above perturbation including the stochastic fluctuation can be approximated as follows:

$$\begin{aligned}\frac{d\delta x(t)}{dt} &\approx \Gamma \delta x(t) + \Omega \frac{\partial}{\partial \Omega}\left[\frac{\partial f(x)}{\partial x}|_{x=x_s+\Omega\alpha(t)}\right]|_{\Omega=0}\delta x(t) \\ &\equiv \Gamma \delta x(t) + \Omega J[\alpha(t)]\delta x(t).\end{aligned} \tag{3}$$

We are interested in the mean trajectory of $\delta x(t)$ as follows:

$$\frac{d\mathbf{E}[\delta x(t)]}{dt} \approx \Gamma \mathbf{E}[\delta x(t)] + \Omega \mathbf{E}\{J[\alpha(t)]\delta x(t)\} \tag{4}$$

where $\mathbf{E}(\cdot)$ is the expectation. The following Bourret's approximation is derived after neglecting the terms in Ω higher than the second order and assuming that $\alpha(t)$ varies much faster than $e^{-\Gamma t}\delta x(t)$ [6]

$$\frac{d\mathbf{E}[\delta x(t)]}{dt} \approx \Gamma \mathbf{E}[\delta x(t)] + \Omega^2 \int_0^t \mathbf{E}[J_c(t-\tau)]\mathbf{E}[\delta x(\tau)]d\tau \tag{5}$$

where $J_c(t-\tau) = J[\alpha(t)]e^{\Gamma(t-\tau)}J[\alpha(\tau)]$. Note that each term of $J_c(t-\tau)$ is a linear combination of $\alpha_i(t)\alpha_j(\tau)$, $\alpha_i(t)$ is the ith element of $\alpha(t)$ and the covariance of $\alpha(t)$ is derived from the linearised Fokker-Plank equations as follows: $\mathbf{E}[\alpha(t)\alpha^T(\tau)] = e^{\Gamma(t-\tau)}\Xi$, where Ξ is given by the solution of Lyapunov equation, $\Gamma\Xi + \Xi\Gamma^T + D = 0, D = S\text{diag}[v]S^T, f(x) = Sv$, and S is the stoichiometry matrix for the network [4]. Then, $J_c(t-\tau)$ is a function of the time difference only. Since the integral in the right hand side of (5) is a convolution integral, the Laplace transform of both sides is given by [3]

$$\delta X(s) = [sI - \Gamma - \Omega^2 \hat{J}_c(s)]^{-1}\delta X(0) \tag{6}$$

where I is the identity matrix and $\hat{J}_c(s)$ is the Laplace transform of $\mathbf{E}[J_c(t)]$. The main problem with the application of the above method to higher order differential equations is the calculation of $J_c(t)$. Since the symbol t is involved in calculating the matrix exponential, $e^{\Gamma t}$, the Laplace transform cannot in practice be computed when n is large. In the next section, we present an approximation method to avoid this difficulty with a certain level of error, which can be made arbitrarily small with increasing computation cost.

III. A New Method for Approximating the Dominant Stochastic Perturbation

Recall that the original linearized differential equation is assumed to be stable, i.e., $e^{\Gamma t} \to 0$ as $t \to \infty$.

Proposition 3.1: For all Hurwitz stable Γ and any δ greater than zero, there always exists a positive number, τ_c, such that

$$\|\mathbf{E}\{J[\alpha(t)]e^{\Gamma t}J[\alpha(0)]\}\| < \delta \tag{7}$$

for all $t > \tau_c$.

Proof: Since each element of the matrix in the brackets is a linear combination of the multiplication of two elements of $e^{\Gamma t}$, the proof is straightforward and is omitted. ■

In this paper, the matrix norm could be any matrix norm.

Theorem 3.2: The Bourret's representation may be approximated as follows:

$$\frac{d\mathbf{E}[\delta x(t)]}{dt} \approx \Gamma \mathbf{E}[\delta x(t)] + \Omega^2 \int_0^t \mathbf{E}[T_c(t)]\mathbf{E}[\delta x(t-\tau)]d\tau \tag{8}$$

where

$$T_c(t) = \begin{cases} J_c(t), & \text{for } t \le \tau_c \\ 0, & \text{for } t > \tau_c \end{cases} \tag{9}$$

and the approximation error is bounded by

$$(\text{approximation error}) \le \Omega^3 \left\| \int_{\tau_c}^t \mathbf{E}[\delta x(t-\tau)]d\tau \right\|. \tag{10}$$

This approximation does not introduce any significant additional error beyond the level of approximation that is imposed in the standard Bourret's representation.

Proof: Split the integral in (8) into two subintervals, i.e., $\tau \in [0, \tau_c)$ and $\tau \in [\tau_c, t)$. From the Proposition 3.1, set δ equal to Ω, then the integral from $\tau = \tau_c$ to $\tau = t$ is bounded by

$$\begin{aligned}&\Omega^2 \left\| \int_{\tau_c}^t \mathbf{E}[J_c(t)]\mathbf{E}[\delta x(t-\tau)]d\tau \right\| \\ &= \Omega^2 \left\| \int_{\tau_c}^t \mathbf{E}\{J[\alpha(t)]e^{\Gamma t}J[\alpha(0)]\}\mathbf{E}[\delta x(t-\tau)]d\tau \right\| \\ &\le \Omega^2 \left\| \int_{\tau_c}^t \|\mathbf{E}\{J[\alpha(t)]e^{\Gamma t}J[\alpha(0)]\}\|\mathbf{E}[\delta x(t-\tau)]d\tau \right\| \\ &= \Omega^3 \left\| \int_{\tau_c}^t \mathbf{E}[\delta x(t-\tau)]d\tau \right\|.\end{aligned} \tag{11}$$

Since the standard Bourret's representation ignores all terms higher than Ω^2, no significant additional error is introduced in the approximation. Note that since the local stability around the equilibrium point is checked by inspecting the eigenvalues of the perturbed equation, the norm of the perturbed state is assumed to be sufficiently small so that the last integration of the perturbed state in (11) from time τ_c to t remains smaller than $1/\Omega$. ■

The stability of the stochastic network can be checked by analyzing the following equation:

$$\delta X(s) = [sI - \Gamma - \Omega^2 \hat{T}_c(s)]^{-1}\delta X(0) \tag{12}$$

where

$$\hat{T}_c(s) = \int_0^\infty \mathbf{E}[T_c(t)]e^{-st}dt = \int_0^{\tau_c} \mathbf{E}[J_c(t)]e^{-st}dt. \tag{13}$$

However, it is still difficult in general to obtain an exact closed form solution for this integral. The following theorem gives a way to approximate the integral numerically.

Theorem 3.3: The Laplace transform of $T_c(t)$ is given by

$$\hat{T}_c(s) = \sum_{k=1}^{N} F_k[k\Delta t, \Gamma, \Xi] \frac{e^{-s(k-1)\Delta t} - e^{-sk\Delta t}}{s} \tag{14}$$

where

$$F_k[k\Delta t, \Gamma, \Xi] = \mathbf{E}\{J[\alpha(k\Delta t)] e^{\Gamma k \Delta t} J[\alpha(0)]\} \tag{15}$$

$\Delta t = \tau_c/N$ and the error between $\hat{T}_c(s)$ and the Laplace transform of $J_c(t)$ can be made arbitrarily small for all $s = j\omega, \omega \in [0, \infty)$, by increasing N and τ_c while keeping Δt small. The matrix exponential $e^{\Gamma k \Delta t}$ is approximated by $[I + (\Delta t/r)\Gamma]^{rk}$ and $\mathbf{E}[\alpha(t)\alpha^T(0)]$ is approximated by $[I + (\Delta t/r)\Gamma]^{rk}\Xi$, where r is a positive real number greater than or equal to Δt.

Proof: To obtain an approximate integral, the interval from 0 to τ_c is divided into the sum of N subintervals, whose length equals $\Delta t = \tau_c/N$ such that

$$\mathbf{E}\{J[\alpha(t)] e^{\Gamma t} J[\alpha(0)]\} \approx \mathbf{E}\{J[\alpha(k\Delta t)] e^{\Gamma k \Delta t} J[\alpha(0)]\} \tag{16}$$

for a sufficiently large N, for all $t \in [(k-1)\Delta t, k\Delta t)$, then

$$\begin{aligned} \hat{T}_c(s) &= \int_0^{\tau_c} \mathbf{E}[J_c(t)] e^{-st} dt \\ &= \sum_{k=1}^{N} \int_{(k-1)\Delta t}^{k\Delta t} \mathbf{E}\{J[\alpha(t)] e^{\Gamma t} J[\alpha(0)]\} e^{-st} dt \\ &\approx \sum_{k=1}^{N} \mathbf{E}\{J[\alpha(k\Delta t)] e^{\Gamma k \Delta t} J[\alpha(0)]\} \\ &\quad \times \frac{e^{-s(k-1)\Delta t} - e^{-sk\Delta t}}{s} \end{aligned} \tag{17}$$

where the matrix exponential for k is approximated as mentioned in the above. The approximation error for $\hat{T}_c(s)$ is bounded by

$$\begin{aligned} &\left\| \sum_{k=1}^{N} \int_{(k-1)\Delta t}^{k\Delta t} \{\mathbf{E}[J_c(k\Delta t)] - \mathbf{E}[J_c(t)]\} e^{-st} dt \right\| \\ &\qquad \le \Delta t^2 \sum_{k=1}^{N} \Delta J_k \le \frac{\tau_c^2}{N} \Delta \bar{J} \end{aligned} \tag{18}$$

where the first inequality is satisfied because the integral of e^{-st} for the given interval is bounded by Δt, ΔJ_k is the maximum of $\|\mathbf{E}[J_c(k\Delta t)] - \mathbf{E}[J_c(t)]\|$ for $t \in [(k-1)\Delta t, k\Delta t)$, and $\Delta \bar{J}$ is the maximum of ΔJ_k for $k \in [1, N]$. As N and r grow, $\Delta \bar{J}$ converges to zero and the approximation error approaches to zero. ∎

Hence, the stability of the stochastic network may be checked via the following characteristic equation:

$$\left| sI - \Gamma - \Omega^2 \sum_{k=1}^{N} F_k[\Delta t, \Gamma, \Xi] \frac{e^{-s(k-1)\Delta t} - e^{-sk\Delta t}}{s} \right| = 0 \tag{19}$$

where $|\cdot|$ is the determinant and Δt is a fixed positive real number. Before proceeding, we note several properties of the irrational term in (19), $(e^{-s(k-1)\Delta t} - e^{-sk\Delta t})/s$. The derivations of these properties are straightforward and are omitted for brevity.

Remark 3.4:

1) The irrational term is analytic over the whole complex plane.
2) The magnitude is bounded by Δt.
3) The irrational term is BIBO (bounded input bounded output) stable.

IV. Main Results

For stability analysis, we need to check the signs of the real parts of all roots of the characteristic equation, and hence we need to obtain all roots of (19). To avoid dealing with infinite polynomials, we first write (12) as follows:

$$\delta X(s) = [I - M(s)\Delta(s)]^{-1} M(s) \delta X(0) \tag{20}$$

where $M(s) = [sI - \Gamma]^{-1}$ and $\Delta(s) = \Omega^2 \hat{T}_c(s)$. Note that since the irrational term is BIBO stable, $\Delta(s)$ also does not have any pole in the right half of the complex plane. Therefore, the following result is an immediate consequence of the application of the generalized Nyquist criterion:

Theorem 4.1: Let τ_c be generated from Proposition 3.1 and N is a sufficiently large integer and $\Delta t = \tau_c/N$. The deterministic differential equation, (2), is stable with respect to stochastic perturbation $\Delta(s) = \Omega^2 \hat{T}_c(s)$, where $\hat{T}_c(s)$ is defined in (13), if and only if the following holds:

$$\left| I - M(j\omega)\Omega^2 \sum_{k=1}^{N} F_k[\Delta t, \Gamma, \Xi] \frac{e^{-j\omega(k-1)\Delta t} - e^{-j\omega k \Delta t}}{j\omega} \right| \tag{21}$$

does not encircle the origin for $\forall \omega \in (-\infty, \infty)$.

Proof: Since the irrational term is analytic on the whole complex plane, it does not affect the number of encirclements of the origin. Hence, the result is direct by application of the generalized Nyquist stability criterion. The proof is straightforward and is omitted. ∎

Checking the above necessary and sufficient condition for stability involves counting the number of encirclement of the origin made by the Nyquist plot, which can sometimes be cumbersome, and requires a certain number of frequency evaluations. The following sufficient conditions for stability can be checked even more efficiently, at the expense of some possible conservatism.

Corollary 4.2: Let the norm of $M(j\omega)$ be bounded by a positive real number, γ, for all $\omega \in [0, \infty)$ and τ_c be generated from Proposition 3.1, and N is a sufficiently large integer and $\Delta t = \tau_c/N$. The deterministic differential equation, (2), is stable with respect to stochastic perturbation $\Delta(s) = \Omega^2 \hat{T}_c(s)$, where $\hat{T}_c(s)$ is defined in (13), if either of the following holds:

$$\begin{aligned} &\|M(j\omega)\Delta(j\omega)\| \\ &\quad = \Omega^2 \left\| M(j\omega) \sum_{k=1}^{N} F_k[\Delta t, \Gamma, \Xi] \frac{e^{-j\omega(k-1)\Delta t} - e^{-j\omega k \Delta t}}{j\omega} \right\| \\ &\quad \le 1 \end{aligned} \tag{22}$$

or

$$\Omega^2 \gamma \Delta t \sum_{k=1}^{N} \|F_k[\Delta t, \Gamma, \Xi]\| \le 1. \tag{23}$$

Proof: The sufficient conditions are direct consequence of the Nyquist stability criterion and the triangle inequality, and thus the proofs are straightforward and are omitted. ∎

V. Example: *Dictyostelium* cAMP Oscillations

The deterministic model for cAMP oscillations used in this study is taken from [7] and is given by

$$\begin{aligned} d[\mathrm{ACA}]/dt &= k_1[\mathrm{CAR1}] - k_2[\mathrm{ACA}][\mathrm{PKA}] \\ d[\mathrm{PKA}]/dt &= k_3[\mathrm{cAMPi}] - k_4[\mathrm{PKA}] \\ d[\mathrm{ERK2}]/dt &= k_5[\mathrm{CAR1}] - k_6[\mathrm{PKA}][\mathrm{ERK2}] \end{aligned}$$

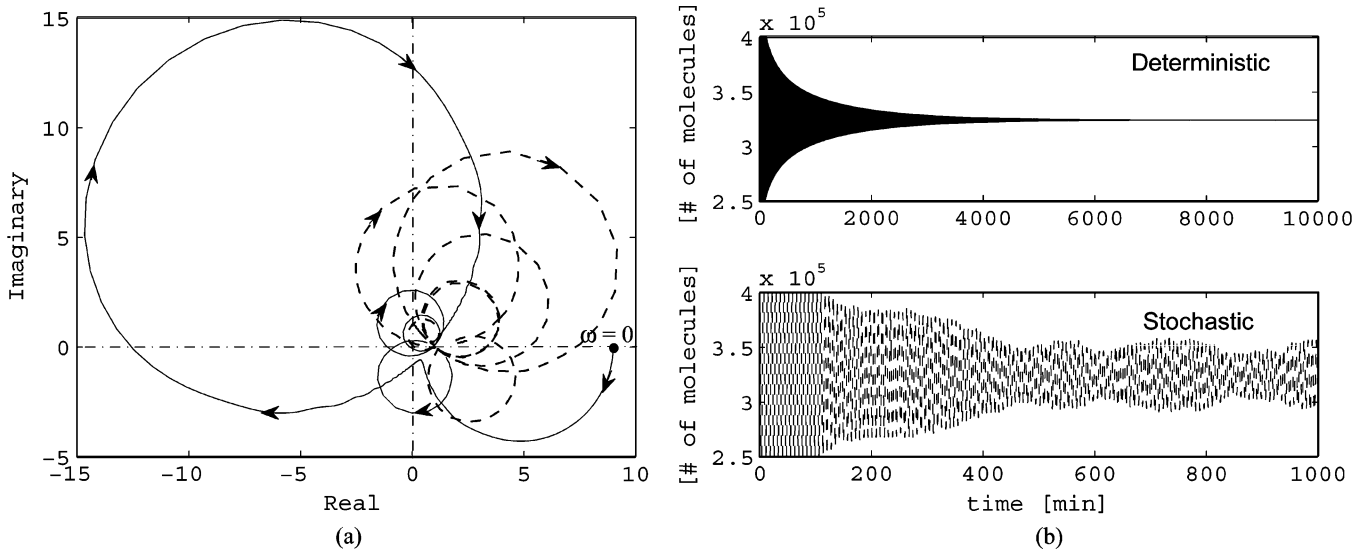

Fig. 1. Nyquist plot and the internal cAMP time histories of the deterministic and the stochastic simulations. (a) Nyquist Plot: $p_\delta = 0.6$. (b) Internal cAMP time history: $p_\delta = 0.6$.

$$\begin{aligned} d[\mathrm{RegA}]/dt &= k_7 - k_8[\mathrm{ERK2}][\mathrm{RegA}] \\ d[\mathrm{cAMPi}]/dt &= k_9[\mathrm{ACA}] - k_{10}[\mathrm{RegA}][\mathrm{cAMPi}] \\ d[\mathrm{cAMPe}]/dt &= k_{11}[\mathrm{ACA}] - k_{12}[\mathrm{cAMPe}] \\ d[\mathrm{CAR1}]/dt &= k_{13}[\mathrm{cAMPe}] - k_{14}[\mathrm{CAR1}] \end{aligned} \tag{24}$$

where ACA is adenylyl cyclase, PKA is the protein kinase, ERK2 is the mitogen activated protein kinase, RegA is the cAMP phosphodiesterase, cAMPi and cAMPe are the internal and the external cAMP concentrations, respectively, and CAR1 is the cell receptor. Each kinetic parameter in the model is represented in $k_i = \bar{k}_i(1 + p_\delta \delta_i/100)$ for $i = 1, 2, \ldots, 13, 14$. $\bar{k}_i$ is the nominal value of each k_i, which are given by [8], [9]: $\bar{k}_1 = 2.0\ \mathrm{min}^{-1}$, $\bar{k}_2 = 0.9\ \mu\mathrm{M}^{-1}\ \mathrm{min}^{-1}$, $\bar{k}_3 = 2.5\ \mathrm{min}^{-1}$, $\bar{k}_4 = 1.5\ \mathrm{min}^{-1}$, $\bar{k}_5 = 0.6\ \mathrm{min}^{-1}$, $\bar{k}_6 = 0.8\ \mu\mathrm{M}^{-1}\ \mathrm{min}^{-1}$, $\bar{k}_7 = 1.0\ \mu\mathrm{M}\ \mathrm{min}^{-1}$, $\bar{k}_8 = 1.3\ \mu\mathrm{M}^{-1}\ \mathrm{min}^{-1}$, $\bar{k}_9 = 0.3\ \mathrm{min}^{-1}$, $\bar{k}_{10} = 0.8\ \mu\mathrm{M}^{-1}\ \mathrm{min}^{-1}$, $\bar{k}_{11} = 0.7\ \mathrm{min}^{-1}$, $\bar{k}_{12} = 4.9\ \mathrm{min}^{-1}$, $\bar{k}_{13} = 23.0\ \mathrm{min}^{-1}$, and $\bar{k}_{14} = 4.5\ \mathrm{min}^{-1}$, while δ_i represents uncertainty in the kinetic parameters. In [10], the worst-case direction for perturbations in the parameter space which destroy the stable limit cycle was identified as $\delta_1 = -1, \delta_2 = -1, \delta_3 = 1, \delta_4 = 1, \delta_5 = -1, \delta_6 = 1, \delta_7 = 1, \delta_8 = -1, \delta_9 = 1, \delta_{10} = 1, \delta_{11} = -1, \delta_{12} = 1, \delta_{13} = -1$ and $\delta_{14} = 1$. p_δ represents the magnitude of the parameter-space perturbation in percent. For p_δ equal to zero, the above set of differential equations exhibits a stable limit cycle. However, for values of p_δ greater than 0.6, the equilibrium point becomes stable and the limit cycle disappears. Here, we are going to study whether this is also true for the corresponding stochastic model.

To transform the above ordinary differential equations into the corresponding stochastic model, the following 14 chemical reactions are deduced [11]:

$$\begin{aligned} \mathrm{CAR1} &\xrightarrow{k_1} \mathrm{ACA} + \mathrm{CAR1} \\ \mathrm{ACA} + \mathrm{PKA} &\xrightarrow{k_2/n_A/\mathcal{V}/10^{-6}} \mathrm{PKA} \\ \mathrm{cAMPi} &\xrightarrow{k_3} \mathrm{PKA} + \mathrm{cAMPi} \\ \mathrm{PKA} &\xrightarrow{k_4} \oslash \\ \mathrm{CAR1} &\xrightarrow{k_5} \mathrm{ERK2} + \mathrm{CAR1} \\ \mathrm{PKA} + \mathrm{ERK2} &\xrightarrow{k_6/n_A/\mathcal{V}/10^{-6}} \mathrm{PKA} \\ \oslash &\xrightarrow{k_7 \times n_A \times \mathcal{V} \times 10^{-6}} \mathrm{RegA} \\ \mathrm{ERK2} + \mathrm{RegA} &\xrightarrow{k_8/n_A/\mathcal{V}/10^{-6}} \mathrm{ERK2} \\ \mathrm{ACA} &\xrightarrow{k_9} \mathrm{cAMPi} + \mathrm{ACA} \\ \mathrm{RegA} + \mathrm{cAMPi} &\xrightarrow{k_{10}/n_A/\mathcal{V}/10^{-6}} \mathrm{RegA} \\ \mathrm{ACA} &\xrightarrow{k_{11}} \mathrm{cAMPe} + \mathrm{ACA} \\ \mathrm{cAMPe} &\xrightarrow{k_{12}} \oslash \\ \mathrm{cAMPe} &\xrightarrow{k_{13}} \mathrm{CAR1} + \mathrm{cAMPe} \\ \mathrm{CAR1} &\xrightarrow{k_{14}} \oslash \end{aligned} \tag{25}$$

where $\oslash$ represents some relatively abundant source of molecules or a non-interacting product, n_A is Avogadro's number, 6.023×10^{23}, $\mathcal{V}$ is the average volume of a *Dictyostelium* cell, $0.565 \times 10^{-12} l$ [12], and 10^{-6} is a multiplication factor due to the unit μM. The probability of each reaction occurring is defined by the rate of each reaction. For example, the probabilities during a small length of time, dt, that the first and the second reactions occur are given by $k_1 \times \mathrm{CAR1}$ and $k_2/n_A/\mathcal{V}/10^{-6} \times \mathrm{ACA} \times \mathrm{PKA}$, respectively. The probabilities for all the other reactions are defined similarly. Based on these, the chemical master equation is obtained and solved using standard numerical routines [1].

For the Bourret's approximation, the system volume, V_{cell}, has the following relation to the density and the number of molecules:

$$\begin{aligned} V_{\mathrm{cell}} &= x\frac{(\#\text{of molecules})}{\mu\mathrm{M}} = 1\ \mu\mathrm{M} \times \mathcal{V} \\ &= \frac{10^{-6} \times 6.023 \times 10^{23}}{\mathrm{liter}} \times \mathcal{V} = 3.403 \times 10^5. \end{aligned} \tag{26}$$

For this problem, since the state dimension is seven, calculating the matrix exponential symbolically is not computationally feasible. Hence, the new approximation proposed in this paper has to be used. From now on, N is fixed to 200, τ_c is chosen such that $\tau_c = \ln 0.01/\max_{i=1,2,\ldots 7} \Re[\lambda_i]$, where $\Re(\lambda_i)$ is the real part of the eigenvalues of Γ and $r = 1000$. The r is equal to the number of intervals to approximate the exponential function in Theorem 3.3. The analysis result for $p_\delta = 0.6$ is shown in Fig. 1. The deterministic model converges to a steady state and ceases to oscillate. However, since the Nyquist plot has more than one encirclement of the origin, it cannot converge to a steady state if the stochastic effect is taken into account. Stochastic simulations using Gillespie's direct method

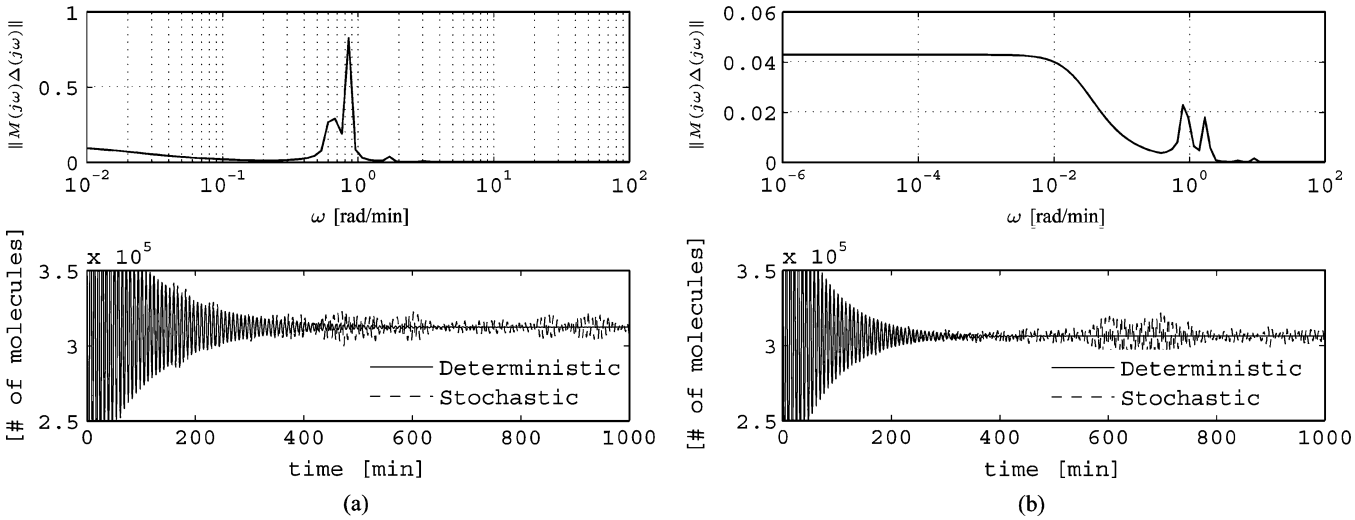

Fig. 2. Sufficient condition and the internal cAMP time histories of the deterministic and the stochastic simulations. (a) For the case of $p_\delta = 1.5$. (b) For the case of $p_\delta = 2.0$.

confirm this result, i.e., the model including stochastic noise continues to oscillate. We note that this result is of independent biological interest, since it represents an example of stochastic noise changing the qualitative behaviour of a network model even at very high molecular concentrations. Here, however, we are primarily interested in the computational complexity of the stability calculation. It takes about 54 h to perform the stochastic simulation, however, the proposed analytical method for determining the stability of the stochastic model gives the answer in less than one hour. When the magnitude of the perturbation in the model's parameters p_δ is increased to 1.5 and 2.0, the analysis results are shown in Fig. 2. In both cases, the first sufficient condition for stability, (22), is now satisfied. For $p_\delta = 2.0$ the second sufficient condition is also satisfied as the left hand side of (23) is about 0.3. We can thus conclude that the stochastic model will be stable without even checking the Nyquist plot. The stochastic time histories for both cases, of course, do not converge exactly to steady states in a deterministic sense because of the existence of noise. However, the oscillation amplitudes are almost negligible compared to the case of $p_\delta = 0.6$ and therefore we can conclude that these two cases are not oscillating. The calculation time for the stochastic simulations for both cases takes about 15 h while for the suggested algorithm it takes less than 15 min. All calculations were performed on a 3.06-GHz Pentium IV machine with 1 GB of RAM.

VI. Conclusion

We presented a novel method for approximating the Laplace transform used in evaluating the stability of stochastic differential equation models of biomolecular networks. The key advantage of the proposed method is that it does not require the matrix exponential to be calculated explicitly. Thus, the computation time associated with the proposed method does not increase exponentially with the order of the system. Moreover, we showed that the approximation error associated with the proposed approach is of the same order as existing methods. Using this approximation method, we showed how a straightforward application of the generalized Nyquist stability criterion provides necessary and sufficient conditions for the stability of stochastic biomolecular networks. The usefulness and computational efficiency of the proposed method was illustrated through its application to the analysis of a network model for cAMP oscillations in aggregating *Dictyostelium* cells. The extension of the method to nominally unstable systems is the subject of current research by the authors.

Acknowledgment

The authors are pleased to acknowledge helpful discussions with Dr. M. L. Kerr, Control and Instrumentation Research Group, University of Leicester, who is now with Deimos Space S.L. in Spain.

References

[1] D. T. Gillespie, "Exact stochastic simulation of coupled chemical reactions," *J. Phys. Chem.*, vol. 81, no. 25, pp. 2340–2361, Dec. 1977.
[2] J. M. G. Vilar, H. Y. Kueh, N. Barkai, and S. Leibler, "Mechanisms of noise-resistance in genetic oscillators," *Proc. Nat. Academy of Sciences*, vol. 99, no. 9, pp. 5988–5992, Apr. 2002.
[3] M. Scott, T. Hwa, and B. Ingalls, "Deterministic characterization of stochastic genetic circuits," *Proc. Nat. Academy of Sciences*, vol. 104, no. 18, pp. 7402–7407, May 2007.
[4] J. Elf and M. Ehrenberg, "Fast evaluation of fluctuations in biochemical networks with the linear noise approximation," *Genome Res.*, vol. 13, no. 11, pp. 2475–2484, Nov. 2003.
[5] C. Moler and C. Van Loan, "Nineteen dubious ways to compute the exponential of a matrix," *SIAM Rev.*, vol. 20, pp. 801–836, 1978.
[6] N. G. van Kampen, "Stochastic differential equations," *Phys. Rep.*, vol. 24, no. 3, pp. 171–228, 1976.
[7] M. T. Laub and W. F. Loomis, "A molecular network that produces spontaneous oscillations in excitable cells of *Dictyostelium*," *Molec. Biol. of the Cell*, vol. 9, pp. 3521–3532, 1998.
[8] L. Ma and P. A. Iglesias, "Quantifying robustness of biochemical network models," *BMC Bioinformatics*, vol. 3, no. 38, 2002.
[9] M. Maeda, S. Lu, G. Shaulsky, Y. Miyazaki, H. Kuwayama, Y. Tanaka, A. Kuspa, and W. F. Loomis, "Periodic signaling controlled by an oscillatory circuit that includes protein kinases ERK2 and PKA," *Science*, vol. 304, no. 5672, pp. 875–878, May 2004.
[10] J. Kim, D. G. Bates, I. Postlethwaite, L. Ma, and P. Iglesias, "Robustness analysis of biochemical networks models," *IEE Proc.—Syst. Biol.*, vol. 153, no. 3, pp. 96–104, May 2006.
[11] D. J. Wilkinson, *Stochastic Modelling for Systems Biology.*. Boca Raton, FL: CRC Press/ Taylor & Francis, 2006.
[12] D. R. Soll, J. Yarger, and M. Mirick, "Stationary phase and the cell cycle of *dictyostelium discoideum* in liquid nutrient medium," *J. Cell Sci.*, vol. 20, no. 3, pp. 513–523, 1976.