
VALIDITY OF THE TOTAL QUASI-STEADY-STATE APPROXIMATION IN STOCHASTIC BIOCHEMICAL REACTION NETWORKS

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ABSTRACT

Stochastic models for biochemical reaction networks are widely used to explore their complex dynamics but face significant challenges, including difficulties in determining rate constants and high computational costs. To address these issues, model reduction approaches based on deterministic quasi-steady-state approximations (QSSA) have been employed, resulting in propensity functions in the form of deterministic non-elementary reaction functions, such as the Michaelis-Menten equation. In particular, the total QSSA (tQSSA), known for its accuracy in deterministic frameworks, has been perceived as universally valid for stochastic model reduction. However, recent studies have challenged this perception. In this review, we demonstrate that applying tQSSA in stochastic model reduction can distort dynamics, even in cases where the deterministic tQSSA is rigorously valid. This highlights the need for caution when using deterministic QSSA in stochastic model reduction to avoid erroneous conclusions from model simulations.

1 Introduction

Understanding the complex dynamics of biochemical reaction networks, which are fundamental to cellular processes, relies heavily on mathematical modeling [1]. For systems with homogeneous spatial distributions of molecules, models based on ordinary differential equations (ODEs) are widely used [2]. These models represent molecular concentrations as variables and define reaction rates through mass-action kinetics. In contrast, for systems exhibiting spatial heterogeneity, partial differential equations (PDEs) are employed to incorporate spatial variability [2].

When molecular copy numbers are too low to support a continuous concentration-based description, stochastic effects become significant [3]. In such cases, stochastic models based on continuous-time Markov chains (CTMCs) provide a

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more appropriate framework [4]. These models, like their deterministic counterparts, generally use mass-action kinetics to define propensity functions that govern reaction probabilities. However, analytical solutions for the probability distributions of stochastic models are rarely feasible [5], necessitating simulation approaches such as the Gillespie algorithm [6]. For systems with pronounced spatial heterogeneity, compartment-based spatial stochastic simulation algorithms (spatial SSAs) are commonly used to enhance these simulations [7].

Stochastic simulations of mass-action kinetics-based models are powerful tools for studying biochemical systems but face significant challenges. Accurately determining reaction rate constants remains difficult [8], and systems with disparate reaction timescales often require repeated simulations of fast reactions to capture the dynamics of slower processes [9]. To mitigate these challenges, simplification techniques such as the quasi-steady-state approximation (QSSA) are employed [10, 11, 12, 13]. The QSSA reduces computational complexity by neglecting fast reactions and approximating fast-scale variables in propensity functions with their stochastic QSSAs, expressed as their moments conditioned on slow variables. However, stochastic QSSAs are often analytically intractable [5]. Consequently, they are frequently replaced with deterministic QSSAs derived from deterministic models [14]. This substitution results in propensity functions that resemble concentration-dependent non-elementary reaction functions, such as Michaelis-Menten or Hill functions, transformed into count-based forms. This approach has been widely adopted in numerous studies to explore the stochastic dynamics of biochemical reaction networks. [15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36].

For stochastic model reduction using deterministic QSSA, various forms of deterministic QSSA, including the standard QSSA (sQSSA) and total QSSA (tQSSA), are available [37, 38, 14]. Among these, the tQSSA has demonstrated superior performance in accurately capturing the original deterministic dynamics [14]. For example, while the sQSSA, which leads to the Michaelis-Menten (MM) equations, often distorts the dynamics of the original deterministic system, the tQSSA has proven to be more accurate across a broader range of conditions in deterministic ODE models [14] and has improved the fidelity of PDE model simplifications [39]. This enhanced accuracy in deterministic models likely explains the superior performance of stochastic simulations employing tQSSA-based equations (stochastic tQSSA) compared to those using other deterministic QSSAs [37, 38, 14, 34]. As a result, the stochastic tQSSA has been widely regarded as reliable [37, 38, 14, 34, 40, 41, 42, 12, 35, 36, 43], particularly in scenarios where the deterministic tQSSA is rigorously valid [44], such as in rapid reversible binding processes.

However, recent studies challenge this assumption, revealing that the stochastic tQSSA is not universally valid [45, 46, 47]. This review critically examines these limitations, showing that stochastic tQSSA can introduce significant distortions in the dynamics of simple gene regulatory network models, even in cases where the deterministic tQSSA accurately captures the original dynamics. These distortions are evident in both homogeneous and heterogeneous spatial contexts, emphasizing the need for caution when applying tQSSA in stochastic simulations.

2 Results

2.1 Deterministic and stochastic tQSSA for gene regulatory network dynamics under spatial homogeneity

To evaluate the validity of the stochastic tQSSA, we first derive the deterministic tQSSA and demonstrate its application in simplifying the stochastic model for a gene regulation system. In this model, mRNA (M) is transcribed from DNA (D) at a rate α_m and degraded at a rate d_m . D reversibly binds to a repressor (P) to form a complex ($D:P$), which inhibits mRNA transcription. The binding and unbinding rates are denoted by k_f and k_b , respectively. Assuming homogeneous spatial conditions, the deterministic dynamics of the species' concentrations can be expressed using mass-action-based ordinary differential equations (ODEs):

$$\begin{aligned}
 \frac{dD}{dt} &= -k_f D \cdot P + k_b D:P, \\
 \frac{dP}{dt} &= -k_f D \cdot P + k_b D:P, \\
 \frac{dD:P}{dt} &= k_f D \cdot P - k_b D:P, \\
 \frac{dM}{dt} &= \alpha_m D - d_m M.
 \end{aligned} \tag{1}$$

By introducing the variables representing the total DNA ($D_T = D + D:P$) and repressor ($P_T = P + D:P$) concentrations instead of P and $D:P$, we can rewrite the ODE system as follows:

$$\begin{aligned}\frac{dD}{dt} &= -k_f D \cdot (P_T - D_T + D) + k_b (D_T - D), \\ \frac{dM}{dt} &= \alpha_m D - d_m M, \\ \frac{dD_T}{dt} &= 0, \\ \frac{dP_T}{dt} &= 0.\end{aligned}\tag{2}$$

Here the time derivatives of D_T and P_T are zero, indicating that these quantities remain constant over time.

Generally, the reactions involving the binding and unbinding between DNA and repressor occur in much faster time scales than mRNA production (i.e., $k_f, k_b \gg \alpha_m, d_m$). Then D , regulated by only the fast reversible binding reactions, evolves in a much faster time scale than the other slow (or fixed) variables (M , D_T , and P_T), not regulated by the fast reactions. Then, in the fast time scale, D quickly converges to its quasi-steady-state (QSS) while the other slow variables (M , D_T , and P_T) remain constant. The approximation of the QSS (QSSA; D_{tq}) can be obtained by solving $dD/dt = 0$:

$$D_{tq}(D_T, P_T) = \frac{1}{2} \left\{ (D_T - P_T - K_d) + \sqrt{(D_T - P_T - K_d)^2 + 4D_T K_d} \right\},\tag{3}$$

where $K_d = k_b/k_f$ is the dissociation constant. The derived QSSA is called the total QSSA (tQSSA) as it is a function in terms of the total variables (i.e., D_T , and P_T). Then we can derive the reduced model by replacing D by the tQSSA (D_{tq})

$$\begin{aligned}\frac{dM}{dt} &= \alpha_m D_{tq}(D_T, P_T) - d_m M, \\ \frac{dD_T}{dt} &= 0, \\ \frac{dP_T}{dt} &= 0.\end{aligned}\tag{4}$$

This reduction is mathematically justified by Tikhonov's theorem [44].

When the molecule copy numbers of the species are low, the stochastic dynamics of their interactions, which cannot be described by deterministic models, can no longer be ignored. To model these stochastic dynamics, the system can be described by a CTMC of the molecule copy numbers $n_X = X \cdot \Omega$, where X represents the species D , P , $D:P$, or M , and Ω is the system volume. The propensity functions for the reactions that alter the copy numbers follow a mass-action form (Table 1).

Table 1: Propensity functions of the full gene regulation model under spatial homogeneity.

Reactions	Propensities
$D + P \longrightarrow D:P$	$\frac{k_f}{\Omega} n_D \cdot n_P$
$D:P \longrightarrow D + P$	$k_b n_{D:P}$
$D \longrightarrow D + M$	$\alpha_m n_D$
$M \longrightarrow \emptyset$	$d_m n_M$

Simulating this model using the Gillespie algorithm allows analysis of stochastic dynamics. However, the computational cost of such stochastic simulations is high, hampering the analysis of dynamics and parameter estimation that require numerous simulations. Thus, simplifying the stochastic model is highly favorable. To simplify the stochastic model, one can consider the stochastic counterpart of the deterministic reduced model (Eq. (4)), where all fast reactions are eliminated and the fast variable n_D in the propensity functions is substituted by the tQSSA equation (Table 2). In this process, all concentration-dimension variables are scaled by Ω to convert them into count-dimension variables:

Here,

$$\begin{aligned}n_{D_{tq}}(n_{D_T}, n_{P_T}) &= D_{tq} \cdot \Omega \\ &= \frac{1}{2} \left\{ (n_{D_T} - n_{P_T} - K_d \cdot \Omega) + \sqrt{(n_{D_T} - n_{P_T} - K_d \cdot \Omega)^2 + 4n_{D_T} K_d \Omega} \right\},\end{aligned}\tag{5}$$

Table 2: Propensity functions of the reduced gene regulation model under spatial homogeneity.

Reactions	Propensities
$\emptyset \longrightarrow M$	$\alpha_m n_{D_{tq}}(n_{D_T}, n_{P_T})$
$M \longrightarrow \emptyset$	$d_m n_M$

where $n_{D_T} = n_D + n_{D:P}$ and $n_{P_T} = n_P + n_{D:P}$. The derived equation approximating the stochastic QSS (Eq. (5)) is commonly referred to as the stochastic tQSSA. By eliminating the fast reactions from the full stochastic model (Table 1; the first two reactions), the reduced model (Table 2) requires significantly fewer reactions for simulation over the same time frame. As a result, the reduced model achieves substantially lower computational costs compared to the full model.

2.2 Stochastic tQSSA can distort gene regulation network dynamics under spatial homogeneity

Under the assumption of rapid reversible binding (i.e., $k_f, k_b \gg \alpha_m, d_m$), the deterministic tQSSA (Eq. (3)) accurately captures the deterministic QSS of D [44, 48, 49, 50]. Consequently, when simulating the deterministic full (Eq. (1)) and reduced (Eq. (4)) models under conditions of binding and unbinding rates much higher than the other reaction rates, the reduced model accurately replicates the slow dynamics of the full model (Fig. 1a). Such valid reduction by using the deterministic tQSSA has led to expectations in previous studies that the reduced model by using the stochastic tQSSA (Table 2) would similarly replicate the slow dynamics of the stochastic full model (Table 1) [37, 38, 14, 34, 40, 41, 42, 12, 35, 36, 43]. Indeed, for most cases where the deterministic tQSSA is valid, the stochastic reduced model effectively captures the slow dynamics of the stochastic full model (Fig. 1b).

However, a recent study revealed that the stochastic tQSSA equation (Eq. (5)) substantially overestimates the stochastic QSS under specific conditions: when $n_{D_T} K_d \Omega < 10$ and $n_{D_T} \approx n_{P_T}$, even with rapid reversible binding [46]. The first condition arises in scenarios of tight molecular binding (i.e., small K_d) combined with low molecular copy numbers (n_{D_T}) and small system volumes (Ω), where stochastic effects dominate and the system deviates from deterministic behavior. The second condition occurs when the binding species have comparable copy numbers. Notably, this invalid condition for the stochastic tQSSA is independent of the deterministic tQSSA's validity. Thus, under the condition, the deterministic tQSSA remains valid (Fig. 1c). In contrast, the stochastic reduced model exhibits significant discrepancies compared to the stochastic full model (Fig. 1d). Specifically, the stochastic tQSSA equation (Eq. (5)) overestimates the stochastic QSS, resulting in an inflated production rate and a distorted stationary distribution of M .

These results highlight that while tQSSA-based reductions are effective in many scenarios, they are not universally applicable. Furthermore, the validity conditions for applying the tQSSA in stochastic systems are stricter than those for deterministic systems, requiring careful consideration in stochastic modeling. In such cases, an alternative QSSA, introduced in a previous study [46] and referred to as the stochastic low-state QSSA (lQSSA), offers a valid approach for stochastic model reduction.

2.3 Deterministic and stochastic tQSSA for gene regulatory network dynamics under spatial heterogeneity

Next, we derive the deterministic tQSSA and demonstrate its application in simplifying the stochastic model in a spatially heterogeneous context. Building on the gene regulation model from the previous section, we introduce diffusion for P and M in a bounded one-dimensional domain. D and $D:P$ are assumed not to diffuse as the DNA remains localized within the nucleus. This system can be described by partial differential equations (PDEs) based on mass-action kinetics:

$$\begin{aligned}
 \frac{\partial D}{\partial t} &= -k_f D \cdot P + k_b D:P, \\
 \frac{\partial P}{\partial t} &= \delta_P \Delta P - k_f D \cdot P + k_b D:P, \\
 \frac{\partial D:P}{\partial t} &= k_f D \cdot P - k_b D:P, \\
 \frac{\partial M}{\partial t} &= \delta_M \Delta M + \alpha_m D - d_m M,
 \end{aligned} \tag{6}$$

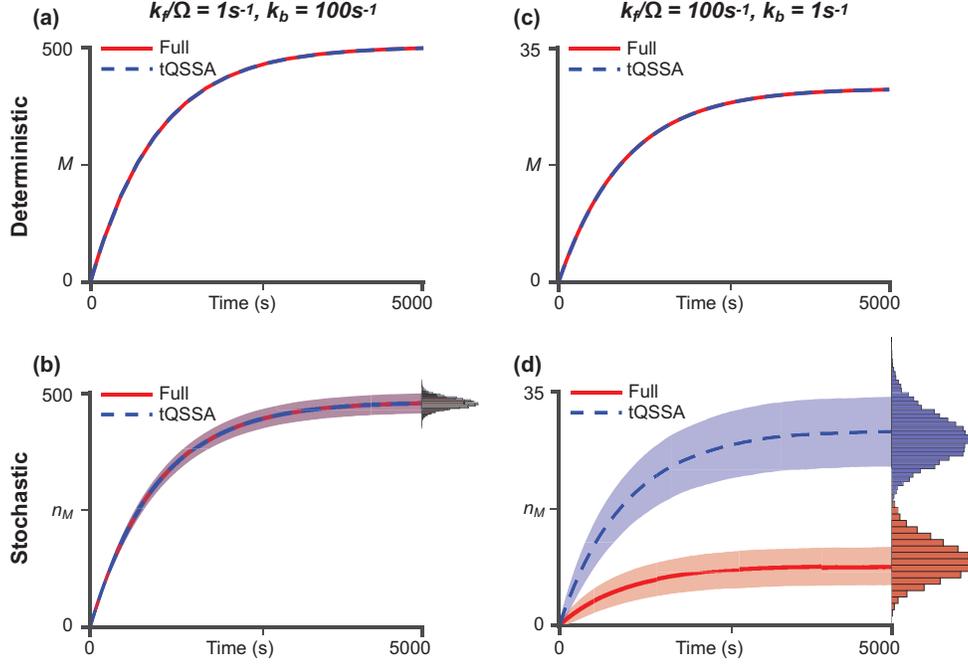


Figure 1: Stochastic tQSSA can distort dynamics even when the deterministic tQSSA is valid under spatial homogeneity. **(a)** When the binding ($k_f/\Omega = 1 \text{ s}^{-1}$) and unbinding ($k_b = 100 \text{ s}^{-1}$) rates are much faster than the other reactions ($\alpha_m = 0.1 \text{ s}^{-1}$ and $d_m = 0.001 \text{ s}^{-1}$), M simulated with the deterministic full model (Eq. 1, red solid line) and the reduced model (Eq. 4, blue dashed line) precisely match. **(b)** Under the same conditions, n_M simulated with the stochastic full model (Table 1, red solid line) and the reduced model (Table 2, blue dashed line) also match closely, as various prior studies expected. Here, the lines with shaded regions represent the mean \pm standard deviation, and the histograms depict the stationary distribution of 10^4 trajectories. **(c-d)** However, when the amounts of the rapidly binding species are similar ($n_{D_T} = n_{P_T} = 10$) and the binding becomes tight ($k_f/\Omega = 100 \text{ s}^{-1}$, $k_b = 1 \text{ s}^{-1}$), the deterministic full and reduced models still precisely match (c), but the stochastic reduced model fails to replicate the dynamics of the full model (d). Here, $\Omega = 1$ (arbitrary unit), and the initial condition is $[D, P, D:P, M] = [10, 10, 0, 0]$.

where δ_X denotes the diffusion coefficient of the corresponding species ($X = P, M$). Similar to the corresponding ODE model (Eq. 1), the full model can be rewritten by introducing $D_T = D + D:P$ and $P_T = P + D:P$:

$$\begin{aligned}
 \frac{\partial D}{\partial t} &= -k_f D \cdot (P_T - D_T + D) + k_b (D_T - D), \\
 \frac{\partial M}{\partial t} &= \delta_M \Delta M + \alpha_m D - d_m M, \\
 \frac{\partial P_T}{\partial t} &= \delta_P \Delta (P_T - D_T + D), \\
 \frac{\partial D_T}{\partial t} &= 0.
 \end{aligned} \tag{7}$$

Notably, unlike the corresponding ODE model (Eq. 1), the P_T is time-variant.

If the diffusion coefficients are large or comparable to the reaction rate constants of the fast reversible binding reactions (i.e., k_f and k_b), the spatial heterogeneity is quickly resolved, so that the PDE model (Eq. 6) becomes nearly equivalent to the corresponding ODE model (Eq. 1). Thus, we assume that diffusion coefficients are comparable or much smaller than the slow reaction rate constants (i.e., α_m and d_m). Under this condition, we can assume that D quickly converges to the QSS while the other variables remain constants, where the QSSA of D is equivalent to that obtained from the

ODE model (i.e., D_{tq} (Eq. 3)). Thus, the full PDE model can be simplified using the tQSSA [51, 52, 47]:

$$\begin{aligned}\frac{\partial M}{\partial t} &= \delta_M \Delta M + \alpha_m D_{tq} - d_m M, \\ \frac{\partial P_T}{\partial t} &= \delta_P \Delta (P_T - D_T + D_{tq}), \\ \frac{\partial D_T}{\partial t} &= 0.\end{aligned}\tag{8}$$

For simulations involving low molecular copy numbers, a compartment-based Gillespie algorithm can be employed [7, 47]. In this approach, the one-dimensional spatial domain is discretized into n compartments, with reactions occurring independently within each compartment (Table 3). The reaction propensities in the i -th compartment are determined by the copy numbers of the reactants within that compartment ($n_{X,i}$; $X = D, P, D:P, M$). Assuming the length of the bounded domain is L , the length of each compartment is $h = L/n$. Additionally, species diffusion between adjacent compartments is modeled as inter-compartmental conversion reactions. For instance, the diffusion of P from the i -th compartment to the $(i + 1)$ -th compartment is represented by the conversion reaction $P_i \longrightarrow P_{i+1}$. At the boundary compartments (the first and n -th compartments), diffusion is restricted to the adjacent compartments (the second and $(n - 1)$ -th compartments, respectively). Since the length of each compartment is h , the conversion reaction rate constant across compartments is $\tilde{\delta}_X = \delta_X/h^2$, where $X = P, M$ [7, 47].

Table 3: Propensity functions of the full gene regulation model under spatial heterogeneity.

Reactions	Propensities
$D_i + P_i \longrightarrow D:P_i, i = 1, \dots, n$	$\frac{k_f}{\Omega_i} n_{D,i} \cdot n_{P,i}$
$D:P_i \longrightarrow D_i + P_i, i = 1, \dots, n$	$k_b n_{D:P,i}$
$D_i \longrightarrow D_i + M_i, i = 1, \dots, n$	$\alpha_m n_{D,i}$
$M_i \longrightarrow \emptyset, i = 1, \dots, n$	$d_m n_{M,i}$
$P_i \longrightarrow P_{i+1}, i = 1, \dots, (n - 1)$	$\tilde{\delta}_P n_{P,i}$
$P_i \longrightarrow P_{i-1}, i = 2, \dots, n$	$\tilde{\delta}_P n_{P,i}$
$M_i \longrightarrow M_{i+1}, i = 1, \dots, (n - 1)$	$\tilde{\delta}_M n_{M,i}$
$M_i \longrightarrow M_{i-1}, i = 2, \dots, n$	$\tilde{\delta}_M n_{M,i}$

Similar to the spatially homogeneous case, we can consider the stochastic counterpart of the reduced PDE model as a reduced model of the full spatial stochastic system [47] (Table 4). In this reduced model, all fast reactions are removed, and the fast variable $n_{D,i}$ in the propensity functions is replaced by the stochastic tQSSA for each compartment:

$$\begin{aligned}n_{D_{tq},i}(n_{D_T,i}, n_{P_T,i}) \\ = \frac{1}{2} \left\{ (n_{D_T,i} - n_{P_T,i} - K_d \cdot \Omega_i) + \sqrt{(n_{D_T,i} - n_{P_T,i} - K_d \cdot \Omega_i)^2 + 4n_{D_T} K_d \Omega_i} \right\},\end{aligned}\tag{9}$$

where $n_{D_T,i} = n_{D,i} + n_{D:P,i}$, $n_{P_T,i} = n_{P,i} + n_{D:P,i}$, and Ω_i is the compartment volume. Simulating this reduced model is significantly more efficient than the full model due to the elimination of all fast reactions, as in the spatially homogeneous case.

Table 4: Propensity functions of the reduced gene regulation model under spatial heterogeneity.

Reactions	Propensities
$\emptyset \longrightarrow M_i$	$\alpha_M n_{D_{tq}}(n_{D_T,i}, n_{P_T,i})$
$M_i \longrightarrow \emptyset$	$d_m n_{M,i}$
$P_{Ti} \longrightarrow P_{T_{i+1}}, i = 1, \dots, (n - 1)$	$\tilde{\delta}_P (n_{P_T,i} - n_{D_{tq}}(n_{D_T,i}, n_{P_T,i}))$
$P_{Ti} \longrightarrow P_{T_{i-1}}, i = 2, \dots, n$	$\tilde{\delta}_P (n_{P_T,i} - n_{D_{tq}}(n_{D_T,i}, n_{P_T,i}))$
$M_i \longrightarrow M_{i+1}, i = 1, \dots, (n - 1)$	$\tilde{\delta}_M n_{M,i}$
$M_i \longrightarrow M_{i-1}, i = 2, \dots, n$	$\tilde{\delta}_M n_{M,i}$

2.4 Stochastic tQSSA can distort gene regulation network dynamics under spacial heterogeneity

When the rapid reversible binding is much faster than the other reactions and diffusion ($k_f, k_b \gg \alpha_m, d_m, \delta_M, \delta_P$), the time scale of D and the other variables are well separated in the full PDE model (Eq. (7)). Thus, the deterministic tQSSA

(Eq. (3)) is expected to accurately captures the deterministic QSS of D , even under spacial heterogeneity [51, 52, 47]. Indeed, when simulating the deterministic full (Eq. (6)) and reduced models (Eq. (8)) with binding and unbinding rates much faster than other reactions and diffusion, the reduced model accurately reproduces the dynamics of M in the full model (Fig. 2a). In this simulation, the models assumed a one-dimensional spatial domain of length $L = 10 \mu m$, with D localized in the central $10/31 \mu m$ region, and Neumann boundary conditions. The valid reductions using the deterministic tQSSA in such cases has led to the expectation that it would also hold in the stochastic context. Indeed, for various scenarios meeting the deterministic tQSSA validity conditions, the stochastic reduced model (Table 4) accurately captures the slow dynamics of the full model (Table 3) (Fig. 2b). In this simulation, the number of compartments was 31 and the D was localized only in the single center compartment.

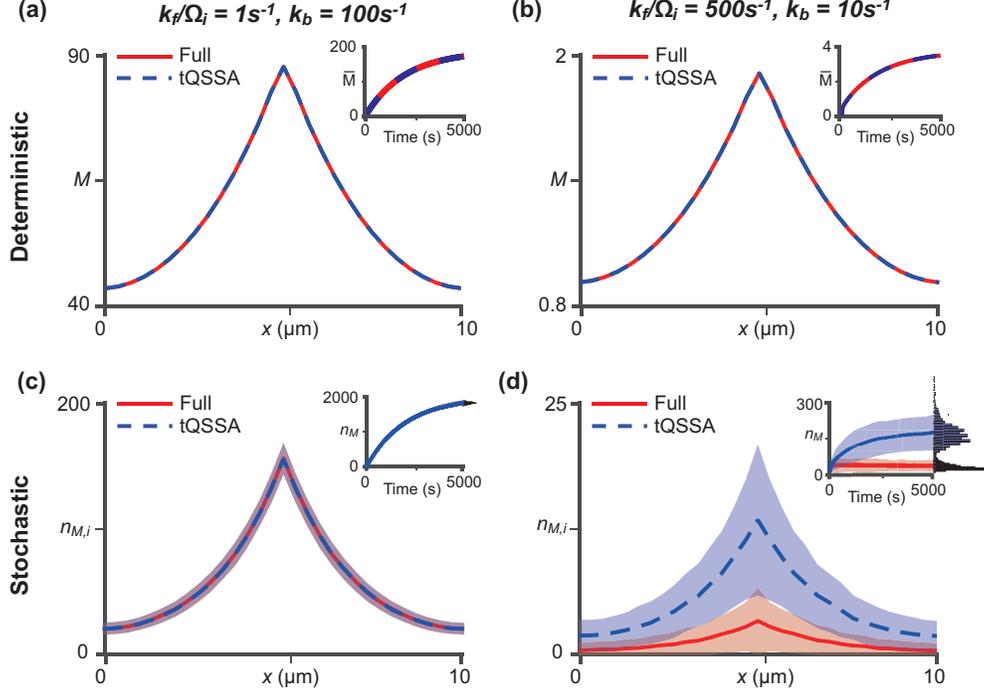


Figure 2: Stochastic tQSSA can distort dynamics even when deterministic tQSSA is valid under spatial heterogeneity. **(a)** When the binding ($k_f/\Omega_i = 1 \text{ s}^{-1}$) and unbinding ($k_b = 100 \text{ s}^{-1}$) rates are much faster than the other reactions ($\alpha_m = 0.5 \text{ s}^{-1}$, $d_m = 0.0005 \text{ s}^{-1}$) and diffusion ($\delta_M = \delta_P = 0.002 \mu\text{m}^2/\text{s}$), M at $t = 5000$ and the spatial mean M trajectory (\bar{M} ; inset) simulated with the deterministic full model (Eq. 6, red solid line) and the reduced model (Eq. 8, blue dashed line) are in precise alignment. **(b)** Under the same conditions, $n_{M,i}$ at $t = 5000$ and the spatial total $n_{M,i}$ trajectory (n_M ; inset) simulated with the stochastic full model (Table 3, red solid line) and the reduced model (Table 4, blue dashed line) also show close agreement. Here, $n_{M,i}$ were plotted at the center of the corresponding compartment on the x -axis and then interpolated. The lines with shaded regions represent the mean \pm standard deviation, and the histograms illustrate the stationary distribution from 10^3 trajectories. **(c-d)** However, when binding becomes tight ($k_f/\Omega_i = 500 \text{ s}^{-1}$, $k_b = 10 \text{ s}^{-1}$), the deterministic full and reduced models still match precisely (c), but the stochastic reduced model fails to replicate the dynamics of the full model (d). Notably, while the spatial total amounts of the reversibly binding species are not comparable ($n_{P_T} = 31$ and $n_{D_T} = 2$), their local amounts become comparable ($n_{P_T,16} \approx 1$ and $n_{D_T,16} = 2$), causing local violations of the stochastic tQSSA. Here, $L = 10 \mu\text{m}$, $n = 31$, and $\Omega_i = 1$ (arbitrary unit). The initial conditions are $D(x, 0) = 2I_{\{5-h/2 < x < 5+h/2\}}(x)$, $P(x, 0) = I_{\{0 < x < 10\}}(x)$, and $D:P(x, 0) = M(x, 0) = 0$.

However, as shown in a previous section, the stochastic tQSSA is not universally valid, even under conditions of rapid reversible binding [46]. Moreover, unlike the spatially homogeneous case, the validity of the stochastic tQSSA in spatially heterogeneous systems must be evaluated locally [47]. Specifically, while the total amounts of the two binding species across the entire spatial domain (i.e., n_{D_T} and n_{P_T} where $n_X = \sum_{i=1}^n n_{X,i}$) may differ significantly, their local amount can be comparable in certain compartments (i.e., $n_{D_T,i}$ and $n_{P_T,i}$). Furthermore, these compartments typically have smaller volumes (Ω_i) and lower copy numbers ($n_{D_T,i}$) compared to the whole system. Consequently, the invalid condition of the stochastic tQSSA may be satisfied locally (i.e., $n_{D_T,i}K_d\Omega_i < 10$ and $n_{D_T,i} \approx n_{P_T,i}$), even if

it is not met in the corresponding spatially homogeneous system. Indeed, when the rapid reversible binding becomes tight, the deterministic reduced model remains valid (Fig. 2c), but the stochastic reduced model fails to capture the dynamics of the original system (Fig. 2d). Specifically, even if the total counts of D and P across the spatial domain differ significantly in the system ($n_{D_T} = 2, n_{P_T} = 31$), one of the 31 compartments, where D is localized, experiences comparable species counts ($n_{D_T,i} = 2, n_{P_T,i} \approx 1$). This local violation of the stochastic tQSSA validity condition leads to an overestimation of the production rate of M, ultimately distorting the original system's dynamics.

These results emphasize that, similar to the spatially homogeneous case, tQSSA-based stochastic model reductions are not universally applicable, with stricter validity conditions than in deterministic models. Moreover, caution is particularly necessary under spatial heterogeneity, as it can lead to local violations of validity conditions that are otherwise satisfied in spatially homogeneous systems. For such cases, especially in compartments where the stochastic tQSSA validity condition is locally violated, the usage of the alternative QSSA (lQSSA) is required [46, 47].

3 Discussion

In this review, we examined the limitations of the stochastic tQSSA, which has been widely regarded as an efficient and accurate tool for stochastic simulations [15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36]. Specifically, we demonstrated that applying the stochastic tQSSA can significantly distort dynamics, even when the deterministic tQSSA accurately captures the original system's behavior (i.e., fast binding and unbinding reactions). This underscores that the stochastic tQSSA has stricter validity conditions than its deterministic counterpart, warranting cautious application.

The invalidity condition of the stochastic tQSSA, identified in a previous study [46] as tight binding between species with comparable counts, seems relatively narrow. This may explain why earlier studies often observed good agreement between the stochastic tQSSA and the original dynamics. However, the tight binding condition ($n_{D_T} K_d \Omega < 10$), which defines this invalidity, is plausible in real biological systems [46]. Moreover, as systems evolve, they may transiently enter invalid regimes, characterized by comparable amounts of reversibly binding species [46]. Additionally, spatial heterogeneity can locally violate the validity condition even when the corresponding spatially homogeneous system remains valid, as demonstrated in our Results section and previous work [47]. Therefore, although the invalidity condition appears narrow, it requires cautious application.

In such scenarios, the stochastic low-state QSSA (lQSSA), proposed in earlier work [46], serves as a viable alternative. The stochastic lQSSA is valid under conditions of tight binding ($n_{D_T} K_d \Omega < 10$) and can thus address scenarios where the stochastic tQSSA becomes invalid. Consequently, the adaptive application of the stochastic tQSSA and lQSSA enables a universally valid reduction of stochastic models under rapid reversible binding conditions [46]. Importantly, in spatially heterogeneous systems, the stochastic tQSSA and lQSSA should be applied on a compartmental basis, determined by local validity criteria (i.e., $n_{D_T,i} K_d \Omega_i < 10$).

If the time scales of the variables in a system are not fully separated (e.g., reversible binding is not significantly faster than other reactions), even the deterministic tQSSA may fail to accurately capture the system's dynamics [53]. To address such situations, recent studies have developed an effective time-delay scheme (ETS) [53] and its extensions [54]. This scheme rigorously estimates the time-delay effects in molecular complex formation during reversible binding, which are negligible when the QSSA approach is valid. These studies demonstrated that ETS can accurately replicate the original deterministic dynamics even in cases where tQSSA is invalid. They also applied ETS to a simple stochastic system, assuming only one DNA binding site. Future work could explore whether this scheme remains applicable under spatial heterogeneity or in stochastic models where interacting species with copy numbers greater than one undergo reversible binding. There may be scenarios where ETS fails due to fundamental differences between deterministic and stochastic models, which would warrant further investigation.

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