# Mixed-mode oscillations in a three-store calcium dynamics model 

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#### Abstract

Calcium ions are important in cell process, which control cell functions. Many models on calcium oscillation have been proposed. Most of existing literature analyzed calcium oscillations using numerical methods, and found rich dynamical behaviours. In this paper, we explore a further study on an established three-store model, which contains endoplasmic reticulum (ER), mitochondria and calcium binding proteins. We conduct bifurcation analysis to identify two Hopf bifurcations, and apply normal form theory to study their stability and show that one of them is supercritical while the other is subcritical. Further, we transform the model into a slow-fast system, and then apply the geometrical singular perturbation theory to investigate the mechanism of generating slow-fast motions. The study reveals that the mechanism of generating the slow-fast oscillating behaviour in the three-store calcium model for certain parameter values is due to the relative fast change in the free calcium in cytosol, and relative slow changes in the free calcium in mitochondria and in the bounded $\mathrm{Ca}^{2+}$ binding sites on the cytosolic proteins. A further parametric study may provide some useful information for controlling harmful effect, by adjusting the amount of calcium in a human body. Numerical simulations are present to demonstrate the correct analytical predictions.


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## 1. Introduction

Calcium ions are very important and essential substance in cells, one of the main second messengers in the intracellular signalling process [1]. Calcium ions control many cellular functions, such as neuronal differentiation [2], muscle cell contraction [3], egg activation [4] and so on. The common changes of calcium ions concentration in cells are related to oscillations [5], which has been found in experiments since 1980s [6]. After then many biologists continued experimental studies in order to explain how the oscillations occur, while applied mathematicians paid attention to theoretical studies of this phenomenon.

To understand the calcium oscillation, we first give a brief description of this phenomenon. There are several intracellular compartments involved in the general scheme of oscillation process, such as endoplasmic reticulum (ER), mitochondria, calcium binding sites, etc $[7,8]$. We call the ER, mitochondria and calcium binding sites as calcium stores in cell, among which the ER is the largest one. Suppose one intracellular signal molecule is moving to the membrane of the cell. Then,

[^0]G-protein coupled receptors (GPCR) on the cell membrane will combine with the signal molecule to activate a kind of Gproteins called phospholipase C (PLC), and the PLC decomposes phosphatidyl inositol 4,5-bisphosphate ( $\mathrm{PIP}_{2}$ ) into inositol $1,4,5$-trisphosphate $\left(\mathrm{IP}_{3}\right)$ and diacylglycerol (DAG) [9]. The $\mathrm{IP}_{3}$ is a vital substance in calcium oscillations, because the main oscillation process is the release of calcium ions from the ER into cytosol, which is sensitive to $\mathrm{IP}_{3}$ [10]. Moreover, the $I P_{3}$ can activate $I P_{3}$ receptors $\left(\mathrm{IP}_{3} R\right)$ on the membrane of ER leading to the calcium release [11]. Besides the $\mathrm{IP}_{3}$ release channel on the membrane of $E R$, another release channel is controlled by ryanodine receptor (RyR) [12], which is activated by cyclic ADP ribose. Opening of $\mathrm{IP}_{3} \mathrm{R}$ and RyR is also stimulated by calcium-induced calcium release scheme (CICR) [13], implying that the changes of calcium concentration in cytosol leads to calcium release from ER. The process described above is the release from ER. The efflux pumping from cytosol to ER is activated through the sarco-endoplasmic reticulum ATPase (SERCA) [14], which is a substance on the membrane of ER and uses ATP hydrolysis to pump calcium into ER.

The uniporter on the membrane of mitochondria is a mechanism that responds to either membrane potential, stress or ligand binding, and uptakes calcium into mitochondria [15]. The efflux channel from mitochondria is through $\mathrm{Na}^{+} / \mathrm{Ca}^{2+}$ and $\mathrm{H}^{+} / \mathrm{Ca}^{2+}$ exchangers, combined with a flux through the mitochondrial permeability transition pores (PTPs) [16].

Based on the general schemes discussed above, many mathematical models have been established, which can be classified into two types: either or not including the concentration of $\mathrm{IP}_{3}$ as a state variable in the model [17-20]. We may also categorize them according to the number of calcium stores involved, yielding two calcium stores models [20-22] and three calcium stores models [23-25]. Since different cells have different calcium oscillations, it is hard to determine which one is better. In most of existing publications, numerical simulations are used to show rich dynamical behaviours such as regular bursting, spiking, quasi-periodic bursting and chaotic bursting [26]. However, the general mechanism underlying these rich dynamical behaviours remains open. This study will focus on two typical regular bursting oscillations: point-point bursting, denoted as $1^{0}$ (one large-amplitude oscillation without small-amplitude oscillation) mixed-mode oscillation, and point-cycle bursting, denoted as $1^{s}\left(s \in \mathbb{Z}^{+}\right)$(one large-amplitude oscillation with $s$ small-amplitude oscillations between) mixed-mode oscillation. Both the two regular bursting types contain slow-fast oscillations. The motivation of this study is to use bifurcation analysis and geometric singular perturbation method to find out the mechanism of generating these two typical calcium oscillations.

In particular, we modify a three-store calcium oscillation model to exclude possibility of chaos. We apply bifurcation theory to show that the only possible bifurcation arising from the positive equilibrium of the system is Hopf bifurcation and identify two Hopf critical points. Then we apply normal form theory to study the stability of limit cycles, indicating that Hopf bifurcation is a source of the oscillation behaviour. In order to provide a further theoretical study on the oscillation induced by Hopf bifurcation, we carefully compare the order of parameters and choose an appropriate parameter as a small perturbation parameter so that the geometric singular perturbation method (GSPM) can be applied to investigate the slowfast motions, with the fast variable representing the free calcium concentration in cytosol, and the slow variable describing the free calcium concentration in mitochondria and the bounded $\mathrm{Ca}^{2+}$ binding sites concentration in cytosolic proteins. We identify folded singularities and singular orbits, which can be classified as either folded saddle or folded focus depending upon the eigenvalues at the singular points. The former can cause many small-amplitude oscillations travelling on a largeamplitude oscillation, while the latter has only large-amplitude oscillation. Two examples are given to illustrate these slowfast motions.

The rest of paper is organized as follows. In Section 2, we present an established three-store model describing calcium oscillations, and prove that the solutions of the model are well-posed and bounded. In Section 3, we analyze Hopf bifurcation by using normal form theory and show some numerical verification. In Section 4, we first derive a dimensionless model and then give a complete singular perturbation analysis to explain the mechanism of calcium oscillation. Moreover, we present two examples to demonstrate the theoretical results. Finally, conclusion is drawn in Section 5.

## 2. Mathematical model and well-posedness of solutions

### 2.1. Mathematical model

In this paper, we consider a three-store calcium oscillation model, which was developed and studied numerically in [23]. As shown in Fig. 1, this model consists of three different calcium stores, the ER, the mitochondria and the calcium binding proteins in cytosol. The ER has tree flux channels: $J_{p u m p}, J_{c h}$ and $J_{\text {leak }}$. The $J_{p u m p}$ denotes the transport of $\mathrm{Ca}^{2+}$ into the ER by SERCA, the $J_{c h}$ represents the $\mathrm{Ca}^{2+}$ release from the ER to cytosol following the CICR, and the $J_{\text {leak }}$ denotes an additional efflux channel from the ER to cytosol which is sensitive to $\mathrm{IP}_{3}$. For the mitochondria, there exist two exchange channels between mitochondria and cytosol: $J_{\text {in }}$ and $J_{o u t}$. The $J_{\text {in }}$ describes active transport of $\mathrm{Ca}^{2+}$ from cytosol to mitochondria through a specific uniporter, and the $J_{o u t}$ represents the $\mathrm{Ca}^{2+}$ release from mitochondria to cytosol through $\mathrm{Na}^{+} / \mathrm{Ca}^{2+}$ and $\mathrm{H}^{+} / \mathrm{Ca}^{2+}$ exchangers, combined with a flux through the mitochondrial PTPs.

Thus, we have the following equations for the exchange channels:

$$
\begin{aligned}
J_{p u m p} & =k_{p u m p} C a_{\text {cyt }}, \\
J_{c h} & =k_{c h} \frac{C a_{c y t}^{2}}{K_{1}^{2}+C a_{c y t}^{2}}\left(C a_{E R}-C a_{C y t}\right),
\end{aligned}
$$



Fig. 1. Schematic presentation of the model (2.2).

$$
\begin{align*}
J_{\text {leak }} & =k_{\text {leak }}\left(C a_{E R}-C a_{\text {Cyt }}\right), \\
J_{\text {in }} & =k_{\text {in }} \frac{C a_{\text {Cyt }}^{8}}{K_{2}^{8}+C a_{\text {Cyt }}^{8}}, \\
J_{\text {out }} & =k_{\text {out }} \frac{C a_{\text {Mit }}}{K_{3}+C a_{\text {Mit }}} . \tag{2.1}
\end{align*}
$$

Note that in model (2.1), except for Jout, all expressions are taken from [23], while Jout is chosen from [24], which is based on experimental results in [27]. There are five state variables involved in model (2.1): the free calcium concentration in cytosol $\left(C a_{C y t}\right)$, the free calcium concentration in the ER $\left(C a_{E R}\right)$, the free calcium concentration in mitochondria $\left(C a_{M i t}\right)$, the concentration of free $\mathrm{Ca}^{2+}$ binding sites on the cytosolic proteins (Pr), and the concentration of bounded $\mathrm{Ca}^{2+}$ binding sites on the cytosolic proteins (CaPr). Consequently, we obtain a set of ordinary differential equations (ODEs) describing the model:

$$
\begin{align*}
\frac{\mathrm{d} C a_{C y t}}{\mathrm{~d} t} & =J_{C h}+J_{\text {leak }}-J_{\text {pump }}+J_{o u t}-J_{\text {in }}+k_{-} C a P r-k_{+} C a_{C y t} P r \\
\frac{\mathrm{~d} C a_{E R}}{\mathrm{~d} t} & =\frac{\beta_{E R}}{\rho_{E R}}\left(J_{\text {pump }}-J_{c h}-J_{\text {leak }}\right) \\
\frac{\mathrm{d} C a_{\text {Mit }}}{\mathrm{d} t} & =\frac{\beta_{\text {Mit }}}{\rho_{\text {Mit }}}\left(J_{\text {in }}-J_{o u t}\right) \\
\frac{\mathrm{d} C a P r}{\mathrm{~d} t} & =k_{+} C a_{C y t} P r-k_{-} C a P r \\
\frac{\mathrm{dPr}}{\mathrm{~d} t} & =-k_{+} C a_{C y t} P r+k_{-} C a P r \tag{2.2}
\end{align*}
$$

where $\rho_{E R}$ and $\rho_{\text {Mit }}$ are the volume ratios between the ER and cytosol, and between mitochondria and cytosol, respectively. $\beta_{E R}$ and $\beta_{M i t}$ represent the ratios of the concentrations of free calcium in the $E R$ and mitochondria to the respective total concentration of calcium, $k_{+}$and $k_{-}$denote average kinetic constants of $\mathrm{Ca}^{2+}$ binding to the buffer proteins and the reverse process.

For model (2.2), in genearl we may assume the following two conditions hold.
A1. There is no calcium exchange between intracellular and extracellular, which means that the total calcium concentration $C a_{\text {tot }}$ remains a constant, i.e.,

$$
\begin{equation*}
C a_{t o t}=C a_{C y t}+\frac{\rho_{E R}}{\beta_{E R}} C a_{E R}+\frac{\rho_{M i t}}{\beta_{M i t}} C a_{M i t}+C a P r . \tag{2.3}
\end{equation*}
$$

A2. There is no protein binding site exchange between intracellular and extracellular, implying that the total concentration of bounded and unbounded proteins $P r_{t o t}$ is a constant, namely,

$$
\begin{equation*}
P r_{t o t}=P r+C a P r . \tag{2.4}
\end{equation*}
$$

These two assumptions are reasonable since the calcium and protein binding sites transport across membrane is much slower than the intracellular transport process [24]. Under (2.3) and (2.4), the five differential equations in (2.2) can be reduced to three. In this paper, we select $C a_{C y t}, C a_{\text {Mit }}$ and CaPr as state variables, and for convenience, introduce the new variables:

$$
X=C a_{C y t}, \quad Y=C a_{M i t}, \quad Z=C a P r .
$$

Table 1
Parameter values for model (2.2) [23,24].

| Parameter | Definition | Value |
| :--- | :--- | :--- |
| $C a_{\text {tot }}$ | Total concentration of calcium | $90 \mu \mathrm{M}$ |
| $P r_{\text {tot }}$ | Total concentration of binding sites | $120 \mu \mathrm{M}$ |
| $k_{\text {ch }}$ | The maximal permeability of the CICR channels | $4200 \mathrm{~s}^{-1}$ |
| $k_{\text {pump }}$ | The rate constant of the pump | $20 \mathrm{~s}^{-1}$ |
| $k_{\text {leak }}$ | The rate constant for Ca ${ }^{2+}$ leakage through The membrane of ER | $0.05 \mathrm{~s}^{-1}$ |
| $k_{\text {in }}$ | The maximal permeability of the uniporters in the mitochondrial membrane | $\mu \mathrm{Ms}^{-1}$ |
| $k_{+}$ | The average kinetic constant of Ca ${ }^{2+}$ binding to the buffer proteins | $0.1 \mu \mathrm{Ms}^{-1}$ |
| $k_{-}$ | The average kinetic constant of $\mathrm{Ca}^{2+}$ unbinding from the buffer proteins | $0.01 \mathrm{~s}^{-1}$ |
| $k_{\text {out }}$ | The maximal rate of calcium flow through $\mathrm{Na}^{+} / \mathrm{Ca}^{2+}$ and $\mathrm{H}^{+} / \mathrm{Ca}^{2+}$ exchangers | $1.9 \mu \mathrm{Ms}^{-1}$ |
| $\rho_{E R}$ | The volume ration between the ER and the cytosol | 0.01 |
| $\rho_{M i t}$ | The volume ration between the mitochondria the mitochondria and the cytosol | 0.01 |
| $\beta_{E R}$ | The constant for relating the concentrations of free calcium in the ER to the total concentrations |  |
| $\beta_{\text {Mit }}$ | The constant for relating the concentrations of free calcium in the mitochondria to the total concentrations | 0.0025 |
| $K_{1}$ | The half-activation constant | 0.0025 |
| $K_{2}$ | The half-activation constant | $5 \mu \mathrm{M}$ |
| $K_{3}$ | The half-activation constant | $0.8 \mu \mathrm{M}$ |



Fig. 2. Simulated time history for system (2.5) for (a) $k_{c h}=1200$; and (b) $k_{c h}=4200$.

Solving $C a_{E R}$ from (2.3), $\operatorname{Pr}$ from (2.4), and substituting them into the first, third and fourth equations in (2.2), we obtain the following 3-D ODE system:

$$
\begin{align*}
\frac{\mathrm{d} X}{\mathrm{~d} t}= & k_{\text {ch }} \frac{X^{2}}{K_{1}^{2}+X^{2}}\left[\frac{\beta_{E R}}{\rho_{E R}}\left(C a_{\text {tot }}-X-\frac{\rho_{\text {Mit }}}{\beta_{M i t}} Y-Z\right)-X\right] \\
& +k_{\text {leak }}\left[\frac{\beta_{E R}}{\rho_{E R}}\left(C a_{\text {tot }}-X-\frac{\rho_{\text {Mit }}}{\beta_{M i t}} Y-Z\right)-X\right]-k_{\text {pump }} X \\
& +k_{\text {out }} \frac{Y}{K_{3}+Y}-k_{\text {in }} \frac{X^{8}}{K_{2}^{8}+X^{8}}+k_{-} Z-k_{+} X\left(P r_{\text {tot }}-Z\right) \\
\equiv & f_{1}(X, Y, Z), \\
\frac{\mathrm{d} Y}{\mathrm{~d} t}= & \frac{\beta_{\text {Mit }}}{\rho_{\text {Mit }}}\left(k_{\text {in }} \frac{X^{8}}{K_{2}^{8}+X^{8}}-k_{\text {out }} \frac{Y}{K_{3}+Y}\right) \equiv f_{2}(X, Y), \\
\frac{\mathrm{d} Z}{\mathrm{~d} t}= & -k_{-} Z+k_{+} X\left(P_{\text {tot }}-Z\right) \equiv f_{3}(X, Z) . \tag{2.5}
\end{align*}
$$

All the parameter values listed in Table 1, except $k_{\text {out }}$, are taken from [23], while $k_{\text {out }}$ is chosen from [24]. These parameters vary depending on the types of cells. To study the complex intracellular $\mathrm{Ca}^{2+}$ behavior, the parameter $k_{c h}$ is usually taken as a bifurcation parameter, because the change of $k_{c h}$ corresponds to the change of inhibitor (i.e., $\mathrm{Mg}^{2+}$ ) and potentiator (i.e., ATP and caffeine) in cell, which can be changed by external stimuli [28,29].

In Fig. 2, two simulations are given to illustrate two typical oscillations for model (2.5), with $k_{c h}=1200$ for Fig. 2(a) and $k_{c h}=4200$ for Fig. 2(b). Clearly, Fig. 2(a) exhibits a relaxation oscillation, while Fig. 2(b) shows a motion involving many small-amplitude oscillations between large-amplitude oscillations.

### 2.2. Positivity and boundness of solutions

Physically meaningful solutions must be non-negative and bounded. We have the following theorem for model (2.5),
Theorem 2.1. All solutions of the calcium oscillation model (2.5) are non-negative provided the initial conditions are taken nonnegative, and bounded.

To prove the above theorem, we need the following lemma.
Lemma 2.1. (Lemma 1 in [24]) The cone $\mathbb{R}_{+}^{N}$ is invariant for the flow generated by the equation,

$$
\frac{\mathrm{d} u}{\mathrm{~d} t}=f(u)
$$

if and only if the function $f(u)$ is quasi-positive, i.e. for every $i=1, \ldots, N$ the function

$$
f_{i}\left(u_{1}, \ldots, 0, \ldots, u_{N}\right) \geq 0
$$

where 0 stands at the $i$-th position and $u_{j} \geq 0$ for $j \neq i$.
Proof. Applying Lemma 2.1 to model (2.5), we have $N=3$ and

$$
\begin{aligned}
f_{1}(0, Y, Z) & =k_{\text {leak }} \frac{\beta_{\text {ER }}}{\rho_{E R}}\left(C a_{\text {tot }}-\frac{\rho_{\text {Mit }}}{\beta_{\text {Mit }}} Y-Z\right)+k_{\text {out }} \frac{Y}{K_{3}+Y}+k_{-} Z \\
f_{2}(X, 0, Z) & =k_{\text {in }} \frac{\beta_{\text {Mit }}}{\rho_{\text {Mit }}} \frac{X^{8}}{K_{2}^{8}+X^{8}} \\
f_{3}(X, Y, 0) & =k_{+} X P r_{\text {tot }}
\end{aligned}
$$

It is seen from Table 1 that all parameter values are positive. For the first equation, since (2.3) holds, yielding $C a_{\text {tot }}-$ $\frac{\rho_{\text {Mit }}}{\beta_{\text {Mit }}} Y-Z=\frac{\rho_{E R}}{\beta_{E R}} C a_{E R}+X \geq 0$, implying that $f_{1}(0, Y, Z) \geq 0$. It is easy to see that $f_{2}(X, 0, Z) \geq 0$ and $f_{3}(X, Y, 0) \geq 0$ for nonnegative initial conditions. Thus by Lemma 2.1, the positivity of solutions of model (2.5) is proved.

It remains to prove that the non-negative solutions of (2.5) are bounded. Let $(X(t), Y(t), Z(t))$ be a non-negative solution and consider

$$
V=X(t)+\frac{\rho_{M i t}}{\beta_{M i t}} Y(t)+Z(t)
$$

Then, differentiating $V$ with repsect to $t$ and evaluating it along the trajectory of (2.5) yields

$$
\frac{\mathrm{d} V}{\mathrm{~d} t}=\frac{\left(k_{\text {leak }}+k_{c h}\right) C a_{t o t} \beta_{E R}}{\rho_{E R}}-V_{1}=\left\{\begin{array}{lll}
<0 & \text { if } & V_{1}>\frac{\left(k_{\text {leak }}+k_{c h}\right) C a_{t o t} \beta_{E R}}{\rho_{E R}} \\
>0 & \text { if } & V_{1}<\frac{\left(k_{\text {leak }}+k_{c h}\right) C a_{t o t} \beta_{E R}}{\rho_{E R}}
\end{array}\right.
$$

where $V_{1}$ is given by

$$
V_{1}=k_{p u m p} X+\left(k_{c h} \frac{X^{2}}{K_{1}^{2}+X^{2}}+k_{\text {leak }}\right)\left[\frac{\beta_{E R}}{\rho_{E R}}\left(X+\frac{\rho_{M i t}}{\beta_{M i t}} Y+Z\right)+X\right]+\frac{k_{c h} C a_{t o t} \beta_{E R}}{\rho_{E R}} \frac{K_{1}^{2}}{K_{1}^{2}+X^{2}},
$$

which defines a surface in the $R_{+}^{3}$ space. Therefore, all positive solutions are attracted into the trapping region $\Omega$, defined by

$$
\Omega=\left\{(X, Y, Z) \mid X \geq 0, Y \geq 0, Z \geq 0, V_{1} \leq 0\right\}
$$

This implies that all solutions are bounded.
The proof of Theorem 2.1 is complete.

## 3. Stability and bifurcation analysis

In this section, we choose parameter values from Table 1 except the bifurcation parameter $k_{c h}$. To facilitate symbolic computation, we transform all the values in rational numbers. For convenience, we denote $k_{\text {ch }}$ as $\alpha$. Letting $f_{1}(X, Y, Z)=$ $f_{2}(X, Y)=f_{3}(X, Z)=0$, we get the equilibrium, denoted as $E=\left(X_{0}, Y_{0}, Z_{0}\right)$. Solving $Y_{0}$ from $f_{2}\left(X_{0}, Y_{0}\right)=0$ yields $Y_{0}=Y\left(X_{0}\right)$, and $Z_{0}$ from $f_{3}\left(X_{0}, Z_{0}\right)=0$ gives $Z_{0}=Z\left(X_{0}\right)$, and then substituting these two solutions into $f_{3}\left(X_{0}, Y_{0}, Z_{0}\right)=0$ we obtain a polynomial equation in $X_{0}$ and $\alpha$ as follows:

$$
\begin{aligned}
f\left(\alpha, X_{0}\right)= & 560332800-11860377600 X_{0}-3997040640 X_{0}^{4} \\
& +3737894531250 X_{0}^{12}+232890625000 \alpha X_{0}^{12}-249036800 \alpha X_{0}^{4} \\
& +10364853515625 X_{0}^{9}+93423496875000 X_{0}^{10}-474415104 X_{0}^{3} \\
& +414594140625 X_{0}^{11}-99903602688 X_{0}^{2}+448266240 \alpha X_{0}^{2}
\end{aligned}
$$



Fig. 3. Bifurcation diagram for system (2.5) projected on the $\alpha-X$ plane, with the solid and dotted curves to denote stable and unstable equilibria, respectively.

$$
\begin{align*}
& -477328125000 \alpha X_{0}^{10}-1519124480 \alpha X_{0}^{3} \\
& +839382812500 \alpha X_{0}^{11}-596660156250 X_{0}^{8} \\
= & 0 . \tag{3.1}
\end{align*}
$$

Note that in the above calculation, we only take the numerator part of the expression, since the denominator is non-zero. The bifurcation diagram based on (3.1) is shown in Fig. 3. To find stability of the equilibrium $E$, we calculate the Jacobian of system (2.5) at $E$ to obtain

$$
\left.J\right|_{\left(X_{0}, Y_{0}, Z_{0}\right)}=\left[\begin{array}{ccc}
f_{1 x} & f_{1 y} & f_{1 z}  \tag{3.2}\\
f_{2 x} & f_{2 y} & 0 \\
f_{3 x} & 0 & f_{3 z}
\end{array}\right],
$$

which in turn generates the characteristic polynomial $P(\lambda, \alpha)=\operatorname{det}\left(\lambda I-\left.J\right|_{E}\right)=0$, where

$$
\begin{equation*}
P(\lambda, \alpha)=\lambda^{3}+a_{1}(\alpha) \lambda^{2}+a_{2}(\alpha) \lambda+a_{3}(\alpha) \tag{3.3}
\end{equation*}
$$

According to Hurwitz Criterion [30], the equilibrium $E$ is asymptotically stable if and only if all the roots of $P(\lambda, \alpha)$ have negative real part, or equivalently, if and only if all the Hurwitz arrangements $\Delta_{i}(\alpha),(i=1,2,3)$ are positive, where

$$
\Delta_{1}(\alpha)=a_{1}(\alpha), \quad \Delta_{2}(\alpha)=a_{1}(\alpha) a_{2}(\alpha)-a_{3}(\alpha), \quad \Delta_{3}(\alpha)=a_{3}(\alpha) \Delta_{2}(\alpha)
$$

Based on Hurwitz Criterion, we know that the conditions for a static bifurcation to occur from the equilibrium $E$ are $a_{3}(\alpha)=$ $0, a_{1}(\alpha)>0$ and $\Delta_{2}(\alpha)>0$. To examine if a static bifurcation may arise from equilibrium $E$, we substitute $Y_{0}=Y\left(X_{0}\right)$ and $Z_{0}=Z\left(X_{0}\right)$ into $a_{3}(\alpha)$ to get a polynomial function of $a_{3}\left(\alpha, X_{0}\right)=0$. Now combined with $f\left(\alpha, X_{0}\right)=0$, we eliminate $\alpha$ from two polynomial equations to obtain a function $\alpha\left(X_{0}\right)$ and a resultant function $S\left(X_{0}\right)=0$. Then, solving $S\left(X_{0}\right)=0$ yields a solution $X_{0}=17.14789348$, which is substituted into $\alpha\left(X_{0}\right)=0$ to obtain a solution $\alpha=-14.56668978<0$. Therefore, for the parameter values given in Table 1, there does not exist physically meaningful static bifurcation from $E$.

To identify a Hopf bifurcation in general $n$-dimensional dynamical systems, the following theorem provides sufficient and necessary conditions.

Theorem 3.1. (Theorem 2.2.1 in [31]) The necessary and sufficient conditions for a Hopf bifurcation to occur from an equilibrium of general $n$-dimensional dynamical systems are $\Delta_{n-1}=0, a_{n}>0$ and $\Delta_{i}>0(1 \leq i \leq n-2)$.

To examine if a Hopf bifurcation may arise from the equilibrium $E$, we substitute $Y_{0}=Y\left(X_{0}\right)$ and $Z_{0}=Z\left(X_{0}\right)$ into $\Delta_{2}(\alpha)=$ $a_{1}(\alpha) a_{2}(\alpha)-a_{3}(\alpha)$ to get a polynomial function $\Delta_{2}\left(\alpha, X_{0}\right)=0$. Then combined with $f\left(\alpha, X_{0}\right)=0$, we eliminate $\alpha$ from the two polynomial equations to obtain the solution $\alpha=\alpha\left(X_{0}\right)$, and a resultant function $H\left(X_{0}\right)=0$. Solving $H\left(X_{0}\right)=0$ we obtain solutions for $X_{0}$, which are substituted into $\alpha\left(X_{0}\right)$ to obtain solutions for $\alpha$. Using Theorem 3.1, we have identified two Hopf bifurcation points $\left(\alpha_{H_{1}}, X_{H_{1}}\right)=(473.83509348,0.07443800)$ and $\left(\alpha_{H_{2}}, X_{H_{2}}\right)=(4459.36206111,0.26063065)$, both of which are indicated in Fig. 3.

Moreover, we have the following result.
Theorem 3.2. The Hopf bifurcation corresponding to $\left(\alpha_{H_{1}}, X_{H_{1}}\right)$ is subcritical, while the one corresponding to ( $\alpha_{H_{2}}, X_{H_{2}}$ ) is supercritical.

Proof. We apply center manifold theory and normal form theory, and the Maple program developed by Yu [32] to system (2.5) to analyze the Hopf bifurcations which occur at the two critical points. Suppose that the Jacobian matrix of a general dynamical system, described by $\dot{x}=f(x, \alpha), x \in \mathbb{R}^{n}, \alpha \in \mathbb{R}$, evaluated on an equilibrium at a critical point $\alpha=\alpha_{c}$, contains a pair of purely imaginary eigenvalues $\pm i \omega_{c}$, and all other eigenvalues have negative real part. Then, the normal form associated with a Hopf bifurcation, describing the dynamics on a 2-dimensional center manifold near the critical point, can be written in polar coordinates as

$$
\frac{\mathrm{d} r}{\mathrm{~d} t}=r\left(v_{0} \mu+v_{1} r^{2}+\ldots\right), \quad \frac{\mathrm{d} \theta}{\mathrm{~d} t}=\omega_{c}+\tau_{0} \mu+\tau_{1} r^{2}+\ldots
$$

where $\mu=\alpha-\alpha_{c}$. The coefficient $v_{1}$ is called the first-order focus value, which determines the stability of bifurcating limit cycles: when $v_{1}<0(>0)$, the Hopf bifurcation is called supercritical (subcritical), and the bifurcating limit cycles are stable (unstable).

Before applying the Maple program [32] to system (2.5), we need to transfer the equilibria to the origin and make the Jacobian of the system in the Jordan canonical form. For the first bifurcation point ( $\alpha_{H_{1}}, X_{H_{1}}$ ), we introduce the transformations: $X_{1}=X-0.07443800, Y_{1}=Y-0.00000750, Z_{1}=Z-51.20765088, \mu=\alpha-473.83509348$. Then at $(0,0,0)$, using the Taylor expansion of the transformed system up to third order, and further introducing the following linear transformation,

$$
\left[\begin{array}{l}
X_{1} \\
Y_{1} \\
Z_{1}
\end{array}\right]=\left[\begin{array}{rrr}
0.05597993 & -0.01515741 & -0.00036637 \\
0.00000042 & -0.00000633 & 0.00100010 \\
-0.22625777 & -1.01732823 & 0.01855946
\end{array}\right]\left[\begin{array}{l}
X_{2} \\
Y_{2} \\
Z_{2}
\end{array}\right],
$$

into system (2.5) yields,

$$
\left[\begin{array}{l}
\dot{X}_{2}  \tag{3.4}\\
\dot{Y}_{2} \\
\dot{Z}_{2}
\end{array}\right]=\left[\begin{array}{l}
f_{1}\left(X_{2}, Y_{2}, Z_{2}, \mu\right) \\
f_{2}\left(X_{2}, Y_{2}, Z_{2}, \mu\right) \\
f_{3}\left(X_{2}, Y_{2}, Z_{2}, \mu\right)
\end{array}\right]
$$

where $f_{i}, i=1,2,3$ are lengthy polynomials up to third order, omitted here for brevity.
Now, the Jacobian of system (3.4) evaluated at the origin is in Jordan canonical form:

$$
\left.J\right|_{(0,0,0)}=\left[\begin{array}{ccc}
0 & \omega_{c_{1}} & 0 \\
-\omega_{c_{1}} & 0 & 0 \\
0 & 0 & -0.15324213
\end{array}\right]
$$

in which $\omega_{c_{1}}=0.38241922$. The coefficients $v_{0}$ and $\tau_{0}$ can be obtained by using the formulas given in [33]:

$$
\begin{aligned}
& v_{0}=\frac{1}{2}\left(\frac{\partial f_{1}}{\partial X_{2} \partial \mu}+\frac{\partial f_{2}}{\partial Y_{2} \partial \mu}\right)=0.02844762 \\
& \tau_{0}=\frac{1}{2}\left(\frac{\partial f_{1}}{\partial Y_{2} \partial \mu}-\frac{\partial f_{2}}{\partial X_{2} \partial \mu}\right)=-0.00079828
\end{aligned}
$$

Now applying the Maple program [32] to system (3.4) (setting $\mu=0$ ) results in $v_{1}=1.01766511$ and $\tau_{1}=-121.36968666$. Therefore, the normal form associated with the first Hopf bifurcation up to third order is given by,

$$
\begin{align*}
\frac{\mathrm{d} r}{\mathrm{~d} t} & =r\left(0.02844762 \mu+1.01766511 r^{2}+\cdots\right) \\
\frac{\mathrm{d} \theta}{\mathrm{~d} t} & =0.38241922-0.00079828 \mu-121.36968666 r^{2}+\ldots \tag{3.5}
\end{align*}
$$

which shows $v_{1}>0$, indicating that the first Hopf bifurcation associated with the critical point $H_{1}\left(\alpha_{H_{1}}, X_{H_{1}}\right)$ is subcritical.
For the second Hopf bifurcation point, similarly we apply the same procedure. Let $X_{1}=X-0.26063065, Y_{1}=Y-$ $0.00000006, Z_{1}=Z-86.72495839, \mu=\alpha-4459.36206111$. Then, introducing the following linear transformation,

$$
\left[\begin{array}{l}
X_{1} \\
Y_{1} \\
Z_{1}
\end{array}\right]=\left[\begin{array}{rrr}
1.02526315 & -0.35477053 & 0.71822809 \\
-0.02502722 & -0.82998987 & 0.74825735 \\
-0.31396442 & -0.93717881 & -0.28777169
\end{array}\right]\left[\begin{array}{l}
X_{2} \\
Y_{2} \\
Z_{2}
\end{array}\right]
$$

into the system (2.5) yields

$$
\left[\begin{array}{c}
\dot{X}_{2}  \tag{3.6}\\
\dot{Y}_{2} \\
\dot{Z}_{2}
\end{array}\right]=\left[\begin{array}{l}
F_{1}\left(X_{2}, Y_{2}, Z_{2}, \mu\right) \\
F_{2}\left(X_{2}, Y_{2}, Z_{2}, \mu\right) \\
F_{3}\left(X_{2}, Y_{2}, Z_{2}, \mu\right)
\end{array}\right],
$$

where the lengthy functions $f_{i}, i=1,2,3$ are omitted here.


Fig. 4. (a) Numerical bifurcation diagram of system (2.5); and (b) Simulated time history of system (2.5) for $k_{c h}=473$.7, converging to the equilibrium $E$.

The Jacobian of system (3.6) evaluated at the origin is in the Jordan canonical form,

$$
\left.J\right|_{(0,0,0)}=\left[\begin{array}{ccc}
0 & \omega_{c_{2}} & 0 \\
-\omega_{c_{2}} & 0 & 0 \\
0 & 0 & -0.11911178
\end{array}\right]
$$

in which $\omega_{c_{2}}=3.65233388$. Then, similarly we obtain $v_{0}, \tau_{0}, v_{1}$ and $\tau_{1}$ for the second Hopf bifurcation, yielding the following normal form up to third order,

$$
\begin{align*}
\frac{\mathrm{d} r}{\mathrm{~d} t} & =r\left(-0.00276114 \mu-137.30341233 r^{2}+\cdots\right) \\
\frac{\mathrm{d} \theta}{\mathrm{~d} t} & =3.65233388-0.00036851 \mu-32.28641861 r^{2}+\cdots \tag{3.7}
\end{align*}
$$

which shows $v_{1}<0$, indicates that the second Hopf bifurcation associated with the critical point $H_{2}\left(\alpha_{H_{2}}, X_{H_{2}}\right)$ is supercritical.

This completes the proof of Theorem 3.2.
To this end, we present some simulations to verify the analytical predictions. We use model (2.5) to perform the simulations with the parameter values taken from Table 1, except $k_{c h}(\alpha)$. To study dynamical behaviours, we show a numerical bifurcation diagram in Fig. 4(a) from which we can see that oscillation amplitude jumps up around the subcritical Hopf bifurcation point as $\alpha$ increases to cross the critical point, and disappears around the supercritcal Hopf bifurcation point. For the supercritical Hopf bifurcation (consider it from larger values of $k_{c h}$ decreased to smaller values of $k_{c h}$ ), when $k_{c h}$ passes the Hopf critical point, small oscillation happens, then with further decrease of $k_{c h}$, the amplitude of oscillation suddenly jumps up. The mechanism of this "jump" phenomenon may be related to canard explosion which we will discuss in next section. For the subcritical Hopf bifurcation, the trajectory solution converges to a stable oscillating motion due to the boundedness.

Now, we vary $k_{c h}$ from small values to large ones, taken as $k_{c h}=473.7,473,84,4459$ and 4460 , with the corresponding simulations shown in Fig. 4(b), Figs. 5, 6 and 7, respectively. When $k_{c h}=473.7<\alpha_{H_{1}}=473.83509348$, the solution trajectories converge to the equilibrium $E$, as shown in Fig. 4(b), while when $k_{c h}=473.84>\alpha_{H_{1}}=473.83509348$, it becomes unstable, and converge to a stable oscillating motion due to the boundedness of solutions (see Fig. 5). Fig. 6 shows simulation for $k_{c h}=4459<\alpha_{c_{2}}=4459.36206111$, indicating that solution trajectories converge to a stable limit cycle. The radius of the limit cycle can be estimated from (3.7) as $r \approx 0.00269833$, which agrees well with that observed from Fig. 6(b). When $k_{c h}=4460>\alpha_{H_{2}}=4459.36206111$, the solution trajectory converges to the equilibrium $E$. These simulations clearly verify the theoretical results obtained in previous sections.

## 4. Geometric singular perturbation analysis

In the previous section, simulations have shown that near the two Hopf critical points, there exist fast-slow motions. In this section, we apply the Geometric Singular Perturbation Method (GSPM) (e.g. see [34-37]) to give a more rigorous analysis on model (2.5). The GSPM is now widely used on studying fast-slow motions, which combines theory of canard cycles with a suitable global returning mechanism [38].


Fig. 5. Simulation of system (2.5) for $k_{c h}=473.84$, converging to a stable oscillating motion due to boundedness: (a) time history; and (b) phase portrait.


Fig. 6. Simulation of system (2.5) for $k_{c h}=4459$, converging to a stable limit cycle: (a) time history; and (b) phase portrait.

### 4.1. Rescaling

First, in order to apply the GSPM, we need introduce a proper scaling to model (2.5), by defining new dimensionless variables ( $x, y, z, t_{1}$ ) as follows:

$$
\begin{equation*}
X=\mu_{1} x, \quad Y=\mu_{2} y, \quad Z=\mu_{3} z, \quad t=\mu_{4} t_{1}, \tag{4.1}
\end{equation*}
$$

where $\mu_{1}$ and $\mu_{2}$ are typical calcium concentration scales in cytosol and mitochondria, $\mu_{3}$ is typical concentration scale of bounded $\mathrm{Ca}^{2+}$ binding sites, and $\mu_{4}$ is a time rescale. Substituting (4.1) into (2.5), we obtain the following dimensionless system:

$$
\begin{aligned}
\frac{\mu_{1}}{\mu_{4}} \frac{\mathrm{~d} x}{\mathrm{~d} t_{1}}= & k_{c h} \frac{\mu_{1}^{2} x^{2}}{K_{1}^{2}+\mu_{1}^{2} x^{2}}\left[\frac{\beta_{E R}}{\rho_{E R}}\left(C a_{\text {tot }}-\mu_{1} x-\frac{\rho_{\text {Mit }}}{\beta_{M i t}} \mu_{2} y-\mu_{3} z\right)-\mu_{1} x\right] \\
& +k_{\text {leak }}\left[\frac{\beta_{E R}}{\rho_{E R}}\left(C a_{\text {tot }}-\mu_{1} x-\frac{\rho_{\text {Mit }}}{\beta_{M i t}} y-\mu_{3} z\right)-\mu_{1} x\right]-k_{\text {pump }} \mu_{1} x \\
& +k_{\text {out }} \frac{\mu_{2} y}{K_{3}+\mu_{2} y}-k_{\text {in }} \frac{\mu_{1}^{8} x^{8}}{K_{2}^{8}+\mu_{1}^{8} x^{8}}+k_{-} \mu_{3} z-k_{+} \mu_{1} x\left(P r_{\text {tot }}-\mu_{3} z\right), \\
\frac{\mu_{2}}{\mu_{4}} \frac{\mathrm{~d} y}{\mathrm{~d} t_{1}}= & \frac{\beta_{\text {Mit }}}{\rho_{\text {Mit }}}\left(k_{\text {in }} \frac{\mu_{1}^{8} x^{8}}{K_{2}^{8}+\mu_{1}^{8} x^{8}}-k_{\text {out }} \frac{\mu_{2} y}{K_{3}+\mu_{2} y}\right),
\end{aligned}
$$



Fig. 7. Simulated time history of system (2.5) for $k_{c h}=4460$, converging to the equilibrium $E$.

$$
\frac{1}{\mu_{4}} \frac{\mathrm{~d} z}{\mathrm{~d} t_{1}}=-k_{-} z+k_{+} \mu_{1} x\left(\operatorname{Pr}_{t o t}-z\right)
$$

In addition, in the above equations we set $\frac{\mu_{1}^{8}}{K_{2}^{8}}=1, \frac{\mu_{2}}{K_{3}}=1, \frac{P r_{\text {tot }}}{\mu_{3}}=1$ and $\frac{\mu_{4} \beta_{\text {Mit }}}{\mu_{2} \rho_{\text {Mit }}}=1$ to get

$$
\mu_{1}=\mu_{2}=K_{3}, \quad \mu_{3}=P r_{t o t}, \quad \mu_{4}=\frac{K_{3} \rho_{M i t}}{\beta_{M i t}}
$$

and so the above differential equations become

$$
\begin{aligned}
\frac{K_{2} \beta_{M i t}}{P r_{\text {tot }} K_{3} \rho_{\text {Mit }}} \frac{\mathrm{d} x}{\mathrm{~d} t_{1}}= & \frac{k_{\text {ch }}}{P r_{\text {tot }}} \frac{K_{2}^{2} x^{2}}{K_{1}^{2}+K_{2}^{2} x^{2}}\left[\frac{\beta_{E R}}{\rho_{E R}}\left(C a_{\text {tot }}-K_{2} x-\frac{\rho_{M i t}}{\beta_{M i t}} K_{3} y-P r_{\text {tot }} z\right)-K_{2} x\right] \\
& +\frac{k_{\text {leak }}}{P r_{\text {tot }}}\left[\frac{\beta_{E R}}{\rho_{E R}}\left(C a_{\text {tot }}-K_{2} x-\frac{\rho_{\text {Mit }}}{\beta_{\text {Mit }}} K_{3} y-P_{\text {tot }} z\right)-K_{2} x\right] \\
& -\frac{k_{\text {pump }}}{P r_{\text {tot }}} K_{2} x+\frac{k_{\text {out }}}{P r_{\text {tot }}} \frac{y}{1+y}-\frac{k_{\text {in }}}{P r_{\text {tot }}} \frac{x^{8}}{1+x^{8}}+k_{-} z-k_{+} K_{2} x(1-z), \\
\frac{\mathrm{d} y}{\mathrm{~d} t_{1}}= & k_{\text {in }} \frac{x^{8}}{1+x^{8}}-k_{\text {out }} \frac{y}{1+y}, \\
\frac{\mathrm{~d} z}{\mathrm{~d} t_{1}}= & \frac{K_{3} \rho_{M i t}}{\beta_{M i t}}\left[-k_{-} z+k_{+} K_{2} x(1-z)\right] .
\end{aligned}
$$

Now, comparing the coefficients in the above system, we find that $\frac{K_{2} \beta_{\text {Mit }}}{P_{r_{\text {tot }} K_{3} \rho_{\text {Mit }}}}=5.3763 \times 10^{-4}$ and $\frac{k_{\text {leak }}}{P_{\text {tot }}}=4.1667 \times 10^{-4}$ are much smaller than all other coefficients which are in the order of $10^{-2} \sim 10^{2}$. Thus, we introduce a small perturbation parameter $\varepsilon>0$ into the above system to obtain

$$
\begin{align*}
\varepsilon \frac{\mathrm{d} x}{\mathrm{~d} t_{1}}= & \left(\frac{k_{c h}}{P r_{\text {tot }}} \frac{K_{2}^{2} x^{2}}{K_{1}^{2}+K_{2}^{2} x^{2}}+\varepsilon\right)\left[\frac{\beta_{E R}}{\rho_{E R}}\left(C a_{\text {tot }}-K_{2} x-\frac{\rho_{\text {Mit }}}{\beta_{M i t}} K_{3} y-P r_{\text {tot }} z\right)-K_{2} x\right] \\
& -\frac{k_{\text {pump }}}{P r_{\text {tot }}} K_{2} x+\frac{k_{\text {out }}}{P r_{\text {tot }}} \frac{y}{1+y}-\frac{k_{\text {in }}}{P r_{\text {tot }}} \frac{x^{8}}{1+x^{8}}+k_{-} z-k_{+} K_{2} x(1-z) \\
\equiv & f(x, y, z, \varepsilon), \\
\frac{\mathrm{d} y}{\mathrm{~d} t_{1}}= & k_{\text {in }} \frac{x^{8}}{1+x^{8}}-k_{\text {out }} \frac{y}{1+y}, \\
\frac{\mathrm{~d} z}{\mathrm{~d} t_{1}}= & \frac{K_{3} \rho_{\text {Mit }}}{\beta_{\text {Mit }}}\left[-k_{-} z+k_{+} K_{2} x(1-z)\right] . \tag{4.2}
\end{align*}
$$

System (4.2) is a singular perturbed system (a slow-fast system) with two slow variables $(y, z)$ and one fast variable $x$. The proper identification of the fast and slow variables in the three-store calcium oscillation model allows us to utilize the GSPM to investigate the dynamical behaviour of the system in geometric structure that involves mixed-mode interaction and complex oscillations.

To demonstrate the "jump" phenomenon with respect to $\varepsilon$, we vary $\varepsilon$ from $10^{-4}$ to $10^{-2}$ to obtain the numerical bifurcation diagrams, shown in Figs. 8, 9(a) and 9(b) for $k_{\text {ch }}=500,3000$ and 4200 respectively. It is seen from Figs. 8 and 9(b) that "jump" points exist, at which canard explosions happen, while the oscillation amplitude shown in Fig. 9(a) changes


Fig. 8. Numerical bifurcation diagram of system (4.2) for $k_{c h}=500$ (with other parameter value taken from Table 1).


Fig. 9. Numerical bifurcation diagram of system (2.5) for (a) $k_{c h}=3000$; and (b) $k_{c h}=4200$ (with other parameter value taken from Table 1).
smoothly. This is not surprising since $k_{c h}=500$ and $k_{c h}=4200$ are near the two "jump" points while $k_{c h}=3000$ is located at the middle smooth part, as shown in Fig. 4(a).

### 4.2. Layer problem

Further, introducing a time scale $t_{1}=\varepsilon \tau$ into (4.2) yields

$$
\begin{align*}
x^{\prime} & =f(x, y, z, \varepsilon) \\
y^{\prime} & =\varepsilon\left(k_{\text {in }} \frac{x^{8}}{1+x^{8}}-k_{\text {out }} \frac{y}{1+y}\right), \\
z^{\prime} & =\varepsilon \frac{K_{3} \rho_{\text {Mit }}}{\beta_{\text {Mit }}}\left[-k_{-} z+k_{+} K_{2} x(1-z)\right], \tag{4.3}
\end{align*}
$$

in which the prime denotes differentiation with respect to the fast time $\tau$.
Letting $\varepsilon \rightarrow 0$ in (4.3) yields the equations describing the layer problem, which approximates the dynamics for the fast variable $x$,

$$
\begin{align*}
x^{\prime} & =f(x, y, z, 0) \\
y^{\prime} & =0 \\
z^{\prime} & =0 \tag{4.4}
\end{align*}
$$

Note that the slow variables $(y, z)$ are considered as parameters in the layer problem. Then, we obtain the critical manifold, which is defined as the set of equilibria of the layer problem:

$$
\begin{equation*}
S_{0}=\left\{(x, y, z) \in \mathbb{R}^{3} \mid f(x, y, z, 0)=0\right\} \tag{4.5}
\end{equation*}
$$



Fig. 10. The critical manifold $S_{0}$ for $k_{c h}=4200$ (with other parameter values taken from Table 1 ), consisting of two attracting sheets $S_{a}^{ \pm}$, one repelling sheet $S_{r}$ and two fold curves $F^{ \pm}$(in yellow color). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The graph of the critical manifold is shown in Fig. 10, which represents a $S$-shape surface. The fold curves $L$ of $S_{0}$ are precisely the set of points where the layer problem undergoes fold bifurcations:

$$
\begin{equation*}
F=\left\{(x, y, z) \in \mathbb{R}^{3} \mid f_{x}(x, y, z, 0)=0\right\} \tag{4.6}
\end{equation*}
$$

where $f_{x}=\frac{\partial f}{\partial x}$.
It should be noted that the condition given in (4.6) is necessary but not sufficient for fold bifurcation. Fig. 10 shows two fold curves denoted as $F^{ \pm}$in yellow color for $k_{c h}=4200$. The fold curves are important because according to Fenichel theory [39] they divide the critical manifold $S_{0}$ into attracting and repelling parts. Whether the part is attracting or repelling is determined by the sign of $\left.f_{x}(x, y, z, 0)\right|_{S_{0}}$. If $\left.f_{x}(x, y, z, 0)\right|_{S_{0}} \neq 0, S_{0}$ is called normally hyperbolic, and if $\left.f_{x}(x, y, z, 0)\right|_{S_{0}}<$ 0 for $(x, y, z) \in S_{a}$, then $S_{a}$ is attracting. If $\left.f_{x}(x, y, z, 0)\right|_{S_{0}}>0$ for $(x, y, z) \in S_{r}$, then $S_{r}$ is repelling. Normally, hyperbolic disappears at $\left.f_{x}(x, y, z, 0)\right|_{S_{0}}=0$. On this critical manifold $S_{0}$, there are two attracting parts denoted by $S_{a}^{+}$and $S_{a}^{-}$, and one repelling part denoted by $S_{r}$, as shown in Fig. 10. Hence, $S_{0}=S_{a}^{+} \cup F^{+} \cup S_{r} \cup F^{-} \cup S_{a}^{-}$.

### 4.3. Reduced problem

Letting $\varepsilon \rightarrow 0$ in (4.2), we obtain the reduced problem which approximates the dynamics for the slow variables $(y, z)$,

$$
\begin{align*}
& 0=f(x, y, z, 0) \\
& \dot{y}=k_{\text {in }} \frac{x^{8}}{1+x^{8}}-k_{\text {out }} \frac{y}{1+y} \\
& \dot{z}=\frac{K_{3} \rho_{\text {Mit }}}{\beta_{\text {Mit }}}\left[-k_{-} z+k_{+} K_{2} x(1-z)\right], \tag{4.7}
\end{align*}
$$

where the dot denotes differentiation with respect to the slow time $t_{1}$.
The reduced problem is a 3-D differential-algebraic system. The first equation describes the dynamics which occur on the critical manifold $S_{0}$, while the last two equations describe the slow motions along critical manifold. Note that $y$ can be expressed explicitly as a function of $(x, z)$ through $f(x, y, z, 0)=0$, denoted as $y=\phi(x, z)$.

To analyze the flow on the critical manifold $S_{0}$, we take a total time derivative of $f(x, y, z, 0)=0$ to obtain

$$
f_{x} \dot{x}+f_{y} \dot{y}+f_{z} \dot{z}=0
$$

and then project the reduced problem (4.7) onto the $x-z$ plane. So, we have

$$
\begin{align*}
-f_{x} \dot{x} & =f_{y}\left(k_{\text {in }} \frac{x^{8}}{1+x^{8}}-k_{\text {out }} \frac{y}{1+y}\right)+f_{z} \frac{K_{3} \rho_{\text {Mit }}}{\beta_{\text {Mit }}}\left[-k_{-} z+k_{+} K_{2} x(1-z)\right], \\
\dot{z} & =\frac{K_{3} \rho_{\text {Mit }}}{\beta_{\text {Mit }}}\left[-k_{-} z+k_{+} K_{2} x(1-z)\right], \tag{4.8}
\end{align*}
$$

This system is singular along the fold curves, where $f_{x}=0$. The singularity can be removed by introducing a new rescaling time $s=-\frac{t}{f_{x}}$, which yields the desingularized reduced system:

$$
\begin{align*}
\dot{x} & =f_{y}\left(k_{\text {in }} \frac{x^{8}}{1+x^{8}}-k_{\text {out }} \frac{y}{1+y}\right)+f_{z} \frac{K_{3} \rho_{\text {Mit }}}{\beta_{\text {Mit }}}\left[-k_{-} z+k_{+} K_{2} x(1-z)\right],  \tag{4.9}\\
\dot{z} & =-f_{x} \frac{K_{3} \rho_{\text {Mit }}}{\beta_{\text {Mit }}}\left[-k_{-} z+k_{+} K_{2} x(1-z)\right],
\end{align*}
$$



Fig. 11. Phase portraits of locally linearized slow flow of the desingularized system (4.9) near a folded node. The red solid line, the blue solid line and the black dotted line denote the eigenvector, the trajectory and the folded curve, respectively. All trajectories in the shadow region, called funeral region, pass through the folded singularity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
where the dot denotes differentiation with respect to new time $s$, and $y$ is given by $y=\phi(x, z)$. The detailed lengthy expressions of $f_{x}, f_{y}, f_{z}$, and $\phi(x, z)$ are omitted here for brevity.

The flow described by desingularized system (4.9) is equivalent to the reduced system (4.8) on the attracting sets $S_{a}^{ \pm}$but has opposite orientation on the repelling set $S_{r}$ since $f_{x}<0$ on $S_{r}$.

System (4.9) has two types of singularities, one is folded singularity defined by

$$
\begin{align*}
f_{x}(x, \phi(x, z), z, 0) & =0 \\
f_{y}\left(k_{\text {in }} \frac{x^{8}}{1+x^{8}}-k_{\text {out }} \frac{\phi(x, z)}{1+\phi(x, z)}\right)+f_{z} \frac{K_{3} \rho_{\text {Mit }}}{\beta_{\text {Mit }}}\left[-k_{-} z+k_{+} K_{2} x(1-z)\right] & =0, \tag{4.10}
\end{align*}
$$

and the other is an ordinary singularity defined by

$$
\begin{gather*}
k_{\text {in }} \frac{x^{8}}{1+x^{8}}-k_{\text {out }} \frac{\phi(x, z)}{1+\phi(x, z)}=0, \\
\frac{K_{3} \rho_{\text {Mit }}}{\beta_{\text {Mit }}}\left[-k_{-} z+k_{+} K_{2} x(1-z)\right]=0 . \tag{4.11}
\end{gather*}
$$

The ordinary singularity defines the equilibria of the desingularized system (4.9), while the folded singularity does not mean any equilibria in the reduced problem, but defines a special set of points where both sides of the first equation of (4.8) become zero, which implies that $\dot{\chi}$ is finite and non-zero at the folded singularity. This allows trajectories to move across the fold curve in finite time, and such solutions are called singular canards. The following definition [34] is needed to classify the folded singularity.

Definition 4.1. Suppose the point $\left(x^{*}, z^{*}\right)$ satisfies the folded singularity condition (4.10). Denote the Jacobian matrix of the desingularized system (4.9) evaluated at $\left(x^{*}, z^{*}\right)$ as $\left.J\right|_{\left(x^{*}, z^{*}\right)}$. Let $\lambda_{1}$ and $\lambda_{2}$ (with $\left|\lambda_{1}\right|<\left|\lambda_{2}\right|$ ) be the eigenvalues of the Jacobian matrix. Then the signs of the eigenvalues $\lambda_{1}$ and $\lambda_{2}$ can be used to classify the singularities into three types: a folded node with two negative real eigenvalues, a folded focus with a complex conjugate pair of eigenvalues, and a folded saddle with two real eigenvalues having opposite signs.

In the folded node case, the trajectories of the slow flow that lie along the eigenvectors passing through the attracting sheet to the repelling sheet of the critical manifold with nonzero speed in slow time, yield singular canards. Fig. 11 depicts the geometry of a generic folded node, with associated trajectories. It is seen from Fig. 11 that the orientation of eigenvector and the direction of trajectories on the repelling part are opposite from standard node. All trajectories in the shadow region (see Fig. 11) pass through the folded singularity called funeral region, and bounded by strong eigenvector and folded curve. Mathieu [36] studied the folded node case theoretically and gave the following theorem. For the folded focus case, there does not exist singular canards (see Corollary 3.1 in [37]).

Theorem 4.1. (Theorem 2.3 in [36]) For the slow-fast system

$$
\begin{aligned}
& \dot{x}=g(x, z, \varepsilon), \\
& \dot{z}=f(x, z, \varepsilon),
\end{aligned}
$$

where $x \in \mathbb{R}^{n}, z \in \mathbb{R}^{m}$, with $\varepsilon>0$ sufficiently small, and for a folded node case, letting $\mu=\lambda_{2} / \lambda_{1}$, in which $\lambda_{1}$ corresponds to the weak eigenvector and $\lambda_{2}$ to the strong eigenvector.
(A) If $\mu<1$, the singular canard corresponding to the strong eigenvector can always be perturbed to a maximal canard. If $\mu^{-1} \notin \mathbb{N}$, then the singular canard corresponding to the weak eigenvector can also be perturbed to a maximal canard. These two canards are called primary canards.


Fig. 12. The critical manifold $S_{0}$ and a singular orbit $\Gamma$ for model (4.2) with $k_{c h}=4200$ (and other parameter values are taken from Table 1), projected onto the $z-x$ plane. The critical manifold $S_{0}$ consists of two attracting sheets $S_{a}^{ \pm}$(blue solid line), one repelling sheet $S_{r}$ (red dashed line) and two fold curves $F^{ \pm}$(black dashed line). The singular orbit contains four segments, two fast segments $\Gamma_{f 1,2}$ and two slow segments $\Gamma_{s 1,2}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
(B) Suppose $k>0$ is an integer satisfying $2 k+1<\mu^{-1}<2 k+3$ and $\mu^{-1} \neq 2(k+1)$. Then, in addition to the two primary canards, there exist at most other $k$ canards, which are called secondary canards.

### 4.4. Construction of singular orbits

Based on Theorem 4.1, we know that the folded node can produce small oscillations. Moreover, with a suitable global return mechanism, folded node can also generate mixed-mode oscillations (MMOs).

In this section, in order to well understand the "jump" phenomenon in model (2.5), we construct singular orbits for system (4.2) to show a global return mechanism, yielding slow-fast motions. The singular orbit $\Gamma$ consists of four segments, as shown in Fig. 12. The first segment $\Gamma_{f 1}$ starts from the lower fold curve $F^{-}$, where a rapid evolution happens along the flow of layer problem (4.4) to the upper attracting critical manifold $S_{a}^{+}$. Once arriving on $S_{a}^{+}$, the second segment $\Gamma_{s 1}$ begins. The trajectory moves along the flow of the reduced problem (4.7) until it reaches the upper fold curve $F^{+}$. On the fold curve $F^{+}$, the reduced problem is singular. If the singularity is a folded node, the trajectory crosses the fold curve in slow time from attracting sheet $S_{a}^{+}$to the repelling sheet $S_{r}$, inducing small oscillations. Then, after finite time in a rotation, the trajectory jumps down to the lower attracting manifold $S_{a}^{-}$. If the singularity is a folded focus, the trajectory jumps down directly to the lower attracting manifold $S_{a}^{-}$without rotation [35,36]. The jumping down is the third segment $\Gamma_{f 2}$ which is described by the reduced problem. Then the flow of layer problem takes over until reaching the lower fold curve $F^{-}$, which consists of the fourth segment $\Gamma_{s 2}$. This formed singular orbit $\Gamma$ characterizes a large-amplitude oscillation.

Having constructed the singular orbits, we now turn to the study of the oscillatory behavior of model (4.2). As mentioned in Section 2, there are two typical slow-fast oscillations which may occur in our model, one is $1^{0}$ MMOs (i.e., one large-amplitude oscillation without small-amplitude oscillations), the other is $1^{s}$ MMOs (i.e., one large-amplitude oscillation carrying $s$ small-amplitude oscillations). In next section, we will present two examples to illustrate the mechanism of slow-fast oscillations.

### 4.5. Examples

Example 1. $1^{s}$ MMOs. It follows from Theorem 4.1 that a folded node can induce small oscillations when the trajectories move across the folded singularity. As an example, we take $k_{c h}=4200$ to show how the mechanism works for $1^{s}$ MMOs. Fig. 13(a) shows a singular orbit which contains two folded singularities. To identify them, we use (4.5) and (4.10) to calculate the folded singularity points to get $P_{1}:(x, z)=(0.33532165,0.71869963)$ on the upper folded curve, and $P_{2}:(x, z)=(0.10832623,0.71242882)$ on the lower folded curve. The eigenvalues evaluated at these two folded singularity points are:

$$
\begin{array}{ll}
\text { For } & P_{1}: \\
\text { For } & P_{2}: \\
\lambda_{1}=-0.01205194, \quad \lambda_{2}=-1.15842838 \\
\end{array}
$$

According to Definition 4.1, $P_{1}$ is a folded node while $P_{2}$ is a folded focus. Folded focus can not induce singular canards. Consider the singular orbit as shown in Fig. 13(a), and suppose that the orbit starts from $P_{2}$, since $P_{2}$ is an unstable focus, and jumps up to the upper attracting sheet $S_{a}^{+}$labeled by $A$. Here we define $\delta$ to measure the distance from $A$ to the strong eigendirection (the blue line in Fig. 13(a)) [36]. $\delta>0$ means that the point $A$ is in the funeral region, otherwise it is outside


Fig. 13. (a) The critical manifold $S_{0}$ and a singular orbit $\Gamma$ for model (4.2) with $k_{c h}=4200$ (and other parameter values are taken from Table 1 ), and (b) the simulated time history.


Fig. 14. (a) The critical manifold $S_{0}$ and a singular orbit $\Gamma$ for model (4.2) with $k_{c h}=1200$ (and other parameter values are taken from Table 1), and (b) the simulated time history.
the region. If $\delta<0$, the trajectory will never cross the fold curve to reach the repelling sheet, but directly jumps down to the lower attracting sheet. In our case, the trajectory along the reduced flow goes to the folded node $P_{1}$ due to $\delta>0$, yielding singular canards in which the trajectory crosses the folded singularity from $S_{a}^{+}$to $S_{r}$ in finite speed and in finite slow time till attracted by $S_{a}^{-}$. Then, the trajectory moves fast to jump down to the attracting sheet $S_{a}^{-}$and continues along the reduced flow to reach $P_{2}$, forming a singular orbit.

The number of singular canards or the rotation near $P_{1}$ can be estimated by using Theorem 4.1. For $P_{1}, \mu^{-1}=$ 96.11967670, we have $k=47$, implying that at $P_{1}$ there exist at most 47 secondary singular canards and two primary singular canards [37], and thus the maximal number of small oscillations is 49. Fig. 13(b) shows the simulated time history, from which we can see that MMOs appear, showing large-amplitude oscillations and many small oscillations between them. A more clear picture can be viewed in a zoomed box (see Fig. 13(b)).

Example 2. $1^{0}$ MMOs. To see $1^{0}$ MMOs, we choose $k_{c h}=1200$ as an example to depict a critical manifold of system (4.2), as shown in Fig. 14(a) in which a singular orbit is formed with two folded singularities appearing on the two fold curves. In this example, the two folded singularities are obtained from (4.10) and (4.5) as $P_{1}:(x, z)=(0.45150663,0.64267178)$ on the upper folded curve, and $P_{2}:(x, z)=(0.14314289,0.61820441)$ on the lower folded curve. The eigenvalues of the system evaluated at these two folded singularity points are:

$$
\begin{array}{lll}
\text { For } & P_{1}: & \lambda_{1}=-0.03034533, \quad \lambda_{2}=-2.39949156 ; \\
\text { For } & P_{2}: & \lambda_{1,2}=0.08274715 \pm 0.03240994 i
\end{array}
$$

It is easy to see that $P_{1}$ is a folded node while $P_{2}$ is a folded focus. Similarly, we consider a trajectory starting from the unstable folded focus $P_{2}$. Then it rapidly jumps up to the landing point $A$ on the attracting sheet $S_{a}^{+}$, but for this case, the point $A$ is out of the funeral region, meaning that the trajectory does not pass $P_{1}$, and so no singular canards appear. Hence,
after arriving at the point $A$, the trajectory follows the reduced flow to reach the folded curve, and then jumps down to the lower attracting sheet $S_{a}^{-}$. Further, it follows the reduced flow to the point $P_{2}$ to form a closed orbit. The simulated time history is shown in Fig. 14(b), indeed indicating a relaxation oscillation.

## 5. Conclusions

In this paper, after a brief introduction on the general scheme of the cell process involved in calcium oscillations, we select a three-store calcium oscillation model and modify a term to exclude possibility of chaos. Then we prove that this system is well-posed with non-negative, bounded solutions. We show that this model has typical relaxtion oscillation as well as mixed oscillations, and explain how they happen, based on bifurcation theory and geometric singular perturbation method.

In order to reveal the mechanism of oscillating behaviours of the model, we apply bifurcation theory and choose the maximal permeability of the CICR channels, $k_{c h}$, as a bifurcation parameter. We have shown that the only possible bifurcation arising from the positive equilibrium of the system is Hopf bifurcation. We use linear analysis to identify two Hopf critical points, and then employ nonlinear analysis with normal form theory to study the stability of limit cycles. This study indicates that Hopf bifurcation is a source of the oscillation behaviours. To give a further theoretical investigation on the oscillation induced by Hopf bifurcation, we compared the order of parameters and introduced a small perturbation parameter $\varepsilon$ so that the geometric singular perturbation method (GSPM) can be applied. The resulting dimensionless slow-fast system contains one fast and two slow variables. The fast variable is associated with the free calcium concentration in cytosol, and the slow system describes the changes of the free calcium concentration in mitochondria and the bounded calcium concentration on the cytosolic proteins. We identified the folded singularities and singular orbits, and presented two examples to illustrate the slow-fast motion in oscillations.

With the help of GSPM, the study given in this paper reveals that the mechanism of generating the slow-fast oscillating behaviour in the three-store calcium model for certain parameter values is due to the relative fast change in the free calcium in cytosol, and relative slow changes in the free calcium in mitochondria and in the bounded $\mathrm{Ca}^{2+}$ binding sites in cytosolic proteins. A further parametric study can be performed to identify the parameter regions where such slow-fast oscillations may happen and thus may provide some useful information for controlling harmful effect, by adjusting the amount of calcium in a human body. Moreover, for those parameter values which are not close to the Hopf critical points (see Fig. 9(a)), we may apply the method developed by Zhang, et al. [40,41] and Yu, et al. [42] to identify a different type of slow-fast oscillations, and thus will give a more accurate global picture on this slow-fast oscillation phenomenon.

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