A BIDOMAIN MODEL FOR THE CALCIUM DYNAMICS IN LIVING CELLS

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The aim of this paper is to analyze, via periodic homogenization techniques, the effective behavior of a non-linear system of coupled reaction-diffusion equations appearing in the modeling of calcium dynamics in living cells under the action of buffering proteins. We obtain, at the macroscale, a calcium bidomain model governing the evolution of the concentration of the calcium ions and of the buffers in the cytoplasm.

Key words: asymptotic analysis, bidomain model, imperfect interface.

1. INTRODUCTION

Our goal in this paper is to analyze the macroscopic behavior of a non-linear system of coupled reaction-diffusion equations describing the calcium dynamics in living cells. Calcium is an important intracellular messenger in any biological cell, taking part in a wide range of cellular processes, such as muscle contraction, gene expression, cell cycle, protein synthesis, or metabolism (see, for example, [1–3] and the references therein). The study of the calcium dynamics in living cells is a challenging problem, both from a theoretical and, also, from a computational point of view. Intracellular calcium concentrations need to be controlled and several buffer proteins, pumps or calcium carriers take part at this extremely complicated process. We analyze here only the evolution of the calcium ions in the presence of buffering proteins that can bind calcium ions and of the Serca pumps acting on the membrane between the cytosol and the endoplasmic reticulum, but we can include in our analysis other mechanisms governing the evolution of calcium in living cells (see, for instance, [4]). Moreover, to simplify the presentation, we consider the case in which only one buffering protein acts in the cytosol and only one in the endoplasmic reticulum. The case in which we have several buffers in each phase can be addressed in a similar way. A large amount of \( Ca^{2+} \) is buffered by specific proteins, called \( Ca^{2+} \) buffers. Such proteins differ as structure or affinity and capacity for \( Ca^{2+} \). All of them have other roles within a living cell; for instance, they can catalyze the correct folding of other cellular proteins, they regulate \( Ca^{2+} \) release and retention, or they can exchange information about calcium levels within various organelles to other proteins. The buffer binding and unbinding of calcium can be modeled by mass-action kinetic terms. However, various types of kinetics can be can be consi-
dered, depending on the type of buffering proteins or on the type of the cells. For more details and examples of such buffering proteins, see [5].

We consider a microscopic model given by a system of reaction-diffusion equations, with nonlinear reaction terms and suitable boundary conditions, which describes the dynamics of the concentrations of the calcium ions in living cells in the presence of buffering proteins. More precisely, at the microscale, we consider reaction-diffusion equations for the concentration of the calcium ions in the cytosol and, respectively, in the endoplasmic reticulum, coupled through an interfacial exchange term. The evolution of the buffering proteins, in the cytosol and in the endoplasmic reticulum, is taken into account in this model, as well.

From a mathematical point of view, the microscopic domain is considered to be a complex $\varepsilon$-periodic heterogeneous structure, formed by two connected components, both reaching the external fixed boundary of the domain and separated by an imperfect heterogeneous interface. Here, $\varepsilon$ is a real positive small parameter, related to the characteristic dimension of the two constituents. The first component is given by the cytosol, whereas the other component is occupied by the endoplasmic reticulum. Indeed, since the endoplasmic reticulum can be considered to form a periodic interconnected network through the cytoplasm and the period of this network is much smaller than the characteristic dimension of a typical cell, one can use homogenization techniques to obtain a macroscopic model, the so-called calcium bidomain model. Thus, at a macroscopic scale, we are led to a strongly coupled nonlinear system of partial differential equations governing the evolution of the concentrations of the involved species. The derived effective model is consistent with several experimental data or numerical simulations performed in order to analyze the dynamics of the calcium ions inside biological cells (see [2], [3], [6], [7], and [8]).

We point out that some mathematical models for calcium dynamics with buffering proteins are derived in the literature by using systems of ordinary differential equations, describing the time-evolution of the involved proteins. However, the complexity of the mechanisms governing the calcium dynamics is better captured by mathematical models involving systems of partial differential equations.

There is an extensive literature concerning such problems. Various theoretical and computational methods and models were considered; they are complementary and they are all useful for a reasonable understanding of the complex mechanisms involved in calcium dynamics in living cells.

The problem of deriving the calcium bidomain equations using periodic homogenization techniques was addressed, for instance, by a formal approach in [3] and by using the two-scale convergence method in [9] or the periodic unfolding method in [10]. We include in our model the action of buffering proteins and we use here a rigorous homogenization method based on suitable unfolding operators (see [11] and [12]) to derive a macroscopic problem, which solution represents a
good approximation of the solution of the microscopic one.

We mention here that bidomain models obtained via homogenization tools arise also in other contexts, such as the modeling of diffusion processes in porous media (see [13–19]) or the modeling of the electrical activity of the heart (see [20–23]). Related homogenization problems for reaction-diffusion problems in various porous media were addressed, for instance, in [24–30].

The structure of the rest of this paper is as follows: in Section 2, we set the microscopic problem and we state our main convergence result, which is proven in Section 3. We end our paper with some concluding remarks.

2. SETTING OF THE MICROSCOPIC PROBLEM AND THE MAIN RESULT

Let \( \Omega = (0,1)^n \subset \mathbb{R}^n \) \((n \geq 3)\), with Lipschitz continuous boundary \( \partial \Omega \). The cellular domain \( \Omega \) is assumed to be a periodic structure made up of two connected components, denoted by \( \Omega_1^\varepsilon \) and \( \Omega_2^\varepsilon \), separated by an imperfect interface \( \Gamma^\varepsilon \). Both phases are assumed to reach the boundary \( \partial \Omega \) of \( \Omega \). Let \( Y = (0,1)^n \) be the reference cell in \( \mathbb{R}^n \). We suppose that \( Y_1 \) and \( Y_2 \) are disjoint Lipschitz connected open subsets of \( Y \), with a common boundary \( \Gamma \) and such that both reach the boundary \( \partial Y \) of \( Y \). We set \( \partial Y_1 = \Gamma \cup \Gamma_1 \) and \( \partial Y_2 = \Gamma \cup \Gamma_2 \), where \( \Gamma_i \), for \( i \in \{1,2\} \), are the intersections of \( \partial Y_i \) with \( \partial Y \). We suppose that \( \Gamma_i \) are identically reproduced on the opposite faces of \( Y \). Also, for each \( k \in \mathbb{Z}^n \), we denote \( Y_k^{i} = k + Y_i \), for \( i \in \{1,2\} \).

Let \( \varepsilon \in (0,1) \) be a small parameter related to the characteristic dimension of the periodic structure, taking values in a positive real sequence tending to zero, such that the stretched domain \( \varepsilon^{-1} \Omega \) can be represented as a finite union of axis-parallel cuboids having corner coordinates in \( \mathbb{Z}^n \) (see [31]). For each \( \varepsilon \), we set \( Z_\varepsilon = \{ k \in \mathbb{Z}^n | \varepsilon Y_k \cap \Omega \neq \emptyset, i \in \{1,2\} \} \) and we define \( \Omega_2^\varepsilon = \Omega \setminus \bigcup_{k \in Z_\varepsilon} \left( \varepsilon Y_k \right) \), \( \Omega_1^\varepsilon = \Omega \setminus \overline{\Omega_2^\varepsilon} \), and the Lipschitz surface \( \Gamma^\varepsilon = \partial \Omega_1^\varepsilon \cap \Omega = \partial \Omega_2^\varepsilon \cap \Omega \). We denote by \( \nu \) the unit outward normal to \( \Omega_1^\varepsilon \). We point out that the results given in this paper hold true for more general domains \( \Omega \) (see [32]).

We denote by \( (\cdot,\cdot)_H \) the inner product on a Hilbert space \( H \). Finally, throughout the paper, we denote by \( C \) a generic fixed positive constant, whose value can change from line to line.

In this periodic microstructure, we shall consider a coupled system of non-linear reaction-diffusion equations, with suitable boundary and initial conditions. More precisely, if \((0,T)\), with \( T < \infty \), is the time interval of interest, we shall analyze the macroscopic behavior, as the small parameter \( \varepsilon \to 0 \), of the solution
((\(u_1^\varepsilon, v_1^\varepsilon\)), (\(u_2^\varepsilon, v_2^\varepsilon\))) of the following system:

\[
\begin{align*}
\partial_t u_1^\varepsilon - \text{div}(A_1^\varepsilon \nabla u_1^\varepsilon) &= f_1^\varepsilon(x, u_1^\varepsilon, v_1^\varepsilon) \quad \text{in } (0, T) \times \Omega_1^\varepsilon, \\
\partial_t u_2^\varepsilon - \text{div}(A_2^\varepsilon \nabla u_2^\varepsilon) &= f_2^\varepsilon(x, u_2^\varepsilon, v_2^\varepsilon) \quad \text{in } (0, T) \times \Omega_2^\varepsilon, \\
\partial_t v_1^\varepsilon - \text{div}(B_1^\varepsilon \nabla v_1^\varepsilon) &= g_1^\varepsilon(x, u_1^\varepsilon, v_1^\varepsilon) \quad \text{in } (0, T) \times \Omega_1^\varepsilon, \\
\partial_t v_2^\varepsilon - \text{div}(B_2^\varepsilon \nabla v_2^\varepsilon) &= g_2^\varepsilon(x, u_2^\varepsilon, v_2^\varepsilon) \quad \text{in } (0, T) \times \Omega_2^\varepsilon,
\end{align*}
\]

(2.1)

with the boundary and the initial conditions

\[
\begin{align*}
A_1^\varepsilon \nabla u_1^\varepsilon \cdot \nu &= A_2^\varepsilon \nabla u_2^\varepsilon \cdot \nu \quad \text{on } (0, T) \times \Gamma^\varepsilon, \\
A_1^\varepsilon \nabla u_1^\varepsilon \cdot \nu &= \varepsilon h^\varepsilon(x, u_1^\varepsilon, u_2^\varepsilon) \quad \text{on } (0, T) \times \Gamma^\varepsilon, \\
B_1^\varepsilon \nabla v_1^\varepsilon \cdot \nu &= 0, \quad B_2^\varepsilon \nabla v_2^\varepsilon \cdot \nu = 0 \quad \text{on } (0, T) \times \Gamma^\varepsilon, \\
u_1^\varepsilon(0, x) &= u_1^0(x), \quad v_2^\varepsilon(0, x) = v_2^0(x) \quad \text{in } \Omega_1^\varepsilon, \quad i \in \{1, 2\}.
\end{align*}
\]

(2.2)

As already mentioned, the model problem (2.1)-(2.2) arises, for instance, in the modeling of calcium dynamics in the presence of buffering proteins in biological cells. In such a case, the domain \(\Omega_1^\varepsilon\) represents the cytosol, whereas the domain \(\Omega_2^\varepsilon\) represents the endoplasmic reticulum. We suppose that calcium reacts with buffering proteins that are present in the cytosol and, also, in the endoplasmic reticulum. The cytosolic calcium concentration is denoted by \(u_1^\varepsilon\), while the calcium concentration in the endoplasmic reticulum is denoted by \(u_2^\varepsilon\). Intracellular calcium is buffered by proteins and we denote the concentrations of the buffer with calcium bound by \(v_1^\varepsilon\) and \(v_2^\varepsilon\), in the cytosol and in the endoplasmic reticulum, respectively. The matrices \(A_i^\varepsilon\) and \(B_i^\varepsilon\), for \(i \in \{1, 2\}\), are the diffusion matrices of the calcium and of the buffers, respectively. The membrane \(\Gamma^\varepsilon\), which separates the cytosol and the endoplasmic reticulum, is supposed to be imperfect and heterogeneous (physically and chemically). For the kind of problem we analyze here, the normal fluxes of the calcium ions through the interface are usually taken to be continuous; the function \(h^\varepsilon\) arising in the transmission condition on \(\Gamma^\varepsilon\) couples the bulk-concentrations of the calcium ions, modeling the kinetics of the transport through the membrane between the cytosol and the endoplasmic reticulum. The appearance of the small parameter \(\varepsilon\) in (2.2) in front of \(h^\varepsilon\) is related to the physical properties of the Serca pumps. We assume that we have a nonhomogeneous spatial distribution of the pump proteins on the boundary. As in [3], more general forms for the Serca flux \(h^\varepsilon\) can be considered. We suppose that the buffering proteins are confined to their respective domains and, so, we impose homogeneous Neumann boundary conditions on \(\Gamma^\varepsilon\) for them.

Examples of buffers acting in the cytosol or in the endoplasmic reticulum are calmodulin, calbindin, calreticulin, troponin, or calsequestrin (see [5]).

We list below the assumptions we impose for the data involved in our microscopic problem.
(A₁) For $\alpha, \beta \in \mathbb{R}$, with $0 < \alpha < \beta$, we denote by $\mathcal{M}(\alpha, \beta, Y)$ the set of all the matrices $A \in (L^\infty(Y))^{n \times n}$ having the property that, for any $\xi \in \mathbb{R}^n$, $(A(y)\xi, \xi) \geq \alpha|\xi|^2$, $|A(y)\xi| \leq \beta|\xi|$, almost everywhere in $Y$. We consider the matrices $A^\varepsilon(x) = A(x/\varepsilon)$ defined a.e. in $\Omega$, where $A \in \mathcal{M}(\alpha, \beta, Y)$ is a $Y$-periodic symmetric smooth matrix, and we denote the matrix $A$ by $A_1$ in $Y_1$ and by $A_2$ in $Y_2$, respectively. In a similar way, we define the matrices $B^\varepsilon$.

(A₂) For $i \in \{1, 2\}$, the reaction rate is given by $f_i^\varepsilon(x, w) = f_i(x/\varepsilon, w)$, where $f_i : \mathbb{R}^n \times \mathbb{R}^2 \rightarrow \mathbb{R}$ satisfies the following hypotheses: $f_i$ is continuous; $f_i(\cdot, w)$ is a $Y$-periodic function for all $w \in \mathbb{R}^2$; $f_i(y, \cdot)$ is Lipschitz continuous for all $y \in \mathbb{R}^n$ with constant independent of $y$; $f_i(y, 0) = 0$ for all $y \in \mathbb{R}^n$. Similar hypotheses for the functions $g_i^\varepsilon$ are imposed.

(A₃) For the nonlinear function $h^\varepsilon$, we suppose that

$$h^\varepsilon(x, u_1^\varepsilon, u_2^\varepsilon) = h_0^\varepsilon(x)(u_2^\varepsilon - u_1^\varepsilon),$$

(2.3)

where $h_0^\varepsilon(x) = h_0(x/\varepsilon)$ and $h_0 = h_0(y)$ is a real $Y$-periodic function in $L^\infty(\Gamma)$, with $h_0(y) \geq \delta > 0$. Further, we consider that

$$H = \int_{\Gamma} h_0(y) \, d\sigma_y \neq 0.$$  

(A₄) Let $(\cdot)_- = \min \{\cdot, 0\}$ and $i \in \{1, 2\}$. We assume that for a.e. $(x, w) \in \mathbb{R}^n \times \mathbb{R}^2$ it holds that

$$f_i^\varepsilon(x, w)(w_1)_- + g_i^\varepsilon(x, w)(w_2)_- \leq C \left( (w_1)_-^2 + (w_2)_-^2 \right),$$

(2.4)

where $w = (w_1, w_2)$ and $C$ is a positive constant independent of $\varepsilon$.

(A₅) There exist $\Lambda > 0$ and $M > 0$ such that (see [28]), for $i \in \{1, 2\},$

$$\begin{align*}
&f_i(\cdot, w) \leq Mw & \text{for all } w \in \mathbb{R}^2 \text{ with } w_i \geq \Lambda, \\
g_i(\cdot, w) \leq Mw & \text{for all } w \in \mathbb{R}^2 \text{ with } w_i \geq \Lambda. 
\end{align*}$$

(2.5)

(A₆) The initial concentrations $u_1^0, u_2^0, v_1^0, v_2^0 \in L^2(\Omega)$ are non-negative and bounded independently with respect to $\varepsilon$ by a constant $\Lambda$ (the same constant appearing in assumption (A₅)).

Using the Lipschitz continuity, it follows that for all $(x, w) \in \mathbb{R}^n \times \mathbb{R}^2$ one has:

$$|f_i^\varepsilon(x, w)| \leq C(1 + |w_1| + |w_2|), \quad i \in \{1, 2\}$$

(2.6)

and

$$|g_i^\varepsilon(x, w)| \leq C(1 + |w_1| + |w_2|), \quad i \in \{1, 2\},$$

(2.7)

where $C$ is a positive constant independent of $\varepsilon$.

**Remark 2.1** As in [9], we can deal in a similar way with the case in which the function $h^\varepsilon(x, w_1, w_2) = h(w_1, w_2)$, where $h$ is Lipschitz-continuous in both arguments.
and
\[ h(w_1, w_2) = \bar{h}(w_1, w_2)(w_2 - w_1), \tag{2.8} \]
with \( 0 < h_{\text{min}} \leq \bar{h}(w_1, w_2) \leq h_{\text{max}} < \infty \). Also, more general functions \( h^\varepsilon \) can be considered (see [34] and [35]).

We point out that we imposed here, for our geometry, a Dirichlet condition on the external boundary of the domain \( \Omega \), which simplifies the mathematical treatment of the problem under study, since it ensures the existence of suitable extensions of our solutions (preserving the non-negativity, the essential boundedness, and the a priori estimates for our solution). However, for suitable geometries, it is possible to deal with more realistic boundary conditions, such as homogeneous Neumann conditions, provided that we can take care of the existence of appropriate extension operators.

We can treat also the more general case of an heterogeneous medium represented by matrices of the form \( A^\varepsilon = A(x, x/\varepsilon) \) or \( A^\varepsilon = A(t, x/\varepsilon) \), under suitable assumptions on the matrices \( A \) (see [9]). Moreover, we can address the case in which the diffusion matrices \( A^\varepsilon_i \) and \( B^\varepsilon_i \), with \( i \in \{1, 2\} \), depend on the concentrations \( u^\varepsilon_i \) and \( v^\varepsilon_i \), respectively (see [33]).

**Remark 2.2** As in [9] and [26], one can prove that the concentration fields \( u^\varepsilon_1 \), \( v^\varepsilon_1 \), \( u^\varepsilon_2 \), and \( v^\varepsilon_2 \) are positive and essentially bounded, which is a natural requirement for such a biological model. So, as in [24], [26], and [27], as relevant particular examples of nonlinearities \( f_i \) and \( g_i \) which satisfy our assumptions we can consider functions of the type
\[ f_i(y, w_1, w_2) = \lambda_i(y) \frac{P_i(w_1, w_2)}{Q_i(w_1, w_2)}. \]
Here, \( \lambda_i \) is a bounded measurable \( Y \)-periodic function and \( P \) and \( Q \) are suitable polynomials which satisfy structural conditions enabling us to use our theoretical results. Since we can prove that our solutions are positive and essentially bounded, as concrete examples we can take, for \( i \in \{1, 2\} \),
\[ f_i(y, w_1, w_2) = a^0_i(y) \frac{w_1 w_2}{1 + w_1 + w_2 + w_1 w_2} + a^1_i(y) w_1 + a^2_i(y) w_2, \]
where \( a^0_i, a^1_i \) and \( a^2_i \) are bounded measurable \( Y \)-periodic functions. Similar examples can be considered for the functions \( g_i \).

Typically, for modeling calcium dynamics in the presence of buffering proteins, one assumes that \( g_i = -f_i \), for \( i \in \{1, 2\} \) (see, for example, [3] and [33]). However, we consider here general functions satisfying hypotheses (A_1) – (A_6).

One can impose other conditions on the data involved in our problem in order to ensure that our concentration fields are non-negative and bounded (see, for example, [33]). Such assumptions cover also various buffering proteins dynamics. For instance, one can deal, in accordance with the law of mass action, with nonlinear...
functions $f_1$ or $f_2$ of the following form (see [3] and [33]):

$$f_i(y,w_1,w_2) = k^1_i(y)w_2 - k^2_i(y)w_1(K_i - w_2),$$

with positive rates coefficients. Usually, for simplicity, the linear mass conservation law is used. Still, this is valid only when the concentrations are not limited. If this is not the case, we can use the Michaelis-Menten law or even Hill’s law, if we don’t want to keep in our model the linear conservation law for small concentrations of the involved fields.

Since it is not easy to obtain an explicit solution of the microscopic problem (2.1)–(2.2) (numerical simulations are difficult to be performed, as well), we apply an homogenization procedure for getting a suitable model that describes the averaged features of the complex microstructure. By using the periodic unfolding method (see [11] and [12]), we can obtain the asymptotic behavior of the solution of our problem. This behavior is described by a continuous model, a so-called bidomain model, similar to the ones arising in the context of the modeling of diffusion processes in porous media or in the case of the study of the electrical activity of the heart. The homogenized solution fits well with experimental data (see, among others, [2], [3], [6], [7], and [8]).

Let us state now our main convergence result.

**Theorem 2.3** The unique solution $((u^ε_1, v^ε_1),(u^ε_2, v^ε_2))$ of system (2.1)–(2.2) converges, in the sense of (3.8)–(3.9), as $ε → 0$, to the unique solution $(u_1, v_1), (u_2, v_2)$ of the following macroscopic problem, set in $(0,T) × Ω$:

$$\begin{align*}
|Y_1| \partial_t u_1 - \text{div}(\overline{A}^1 \nabla u_1) - H(u_2 - u_1) &= \int_{Y_1} f_1(y,u_1,v_1) \, dy, \\
|Y_2| \partial_t u_2 - \text{div}(\overline{A}^2 \nabla u_2) + H(u_2 - u_1) &= \int_{Y_2} f_2(y,u_2,v_2) \, dy, \\
|Y_1| \partial_t v_1 - \text{div}(\overline{B}^1 \nabla v_1) &= \int_{Y_1} g_1(y,u_1,v_1) \, dy, \\
|Y_2| \partial_t v_2 - \text{div}(\overline{B}^2 \nabla v_2) &= \int_{Y_2} g_2(y,u_2,v_2) \, dy,
\end{align*}$$

(2.9)

together with the initial conditions

$$\begin{align*}
u_1(0,x) &= u^0_1(x), \quad u_2(0,x) = u^0_2(x) \quad \text{in } Ω, \\
v_1(0,x) &= v^0_1(x), \quad v_2(0,x) = v^0_2(x) \quad \text{in } Ω.
\end{align*}$$

(2.10)

Here, $\overline{A}^1$ and $\overline{A}^2$ are the homogenized matrices, given by:

$$\overline{A}^1_{ij} = \int_{Y_1} \left( a^1_{ij} + \sum_{k=1}^n a^1_{ik} \frac{\partial \chi_{1j}}{\partial y_k} \right) \, dy,$$

$$\overline{A}^2_{ij} = \int_{Y_2} \left( a^2_{ij} + \sum_{k=1}^n a^2_{ik} \frac{\partial \chi_{2j}}{\partial y_k} \right) \, dy,$$

where $a^1_{ij} = (A_1)_{ij}$, $a^2_{ij} = (A_2)_{ij}$, and $\chi_{1k}, \chi_{2k} ∈ H^1_{\text{per}}(Y_1)$, $\chi_{2k} ∈ H^1_{\text{per}}(Y_2), k = 1, \ldots, n$. 

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are the weak solutions of the cell problems

\[
\begin{align*}
\begin{cases}
-\text{div}_y (A_1(y)(\nabla_y \chi_k + e_k)) &= 0, \quad y \in Y_1, \\
A_1(y)(\nabla_y \chi_k + e_k) \cdot \nu &= 0, \quad y \in \Gamma, \quad \int_\Gamma \chi_k \, d\sigma_y = 0, \\
A_2(y)(\nabla_y \chi_k + e_k) \cdot \nu &= 0, \quad y \in \Gamma, \quad \int_\Gamma \chi_k \, d\sigma_y = 0.
\end{cases}
\tag{2.11}
\end{align*}
\]

\[
\begin{align*}
\begin{cases}
-\text{div}_y (A_2(y)(\nabla_y \chi_{2k} + e_k)) &= 0, \quad y \in Y_2, \\
A_2(y)(\nabla_y \chi_{2k} + e_k) \cdot \nu &= 0, \quad y \in \Gamma, \quad \int_\Gamma \chi_{2k} \, d\sigma_y = 0.
\end{cases}
\tag{2.12}
\end{align*}
\]

The effective diffusion matrices \( \overline{B}^1 \) and \( \overline{B}^2 \) for the buffers are defined similarly.

3. PROOF OF THE MAIN RESULT

Let \( V \) be an arbitrary Banach space. We denote by \( V' \) its dual and we consider the space \( \mathcal{W}(0, T; V, V') := \{ w \in L^2(0, T; V) : \partial_t w \in L^2(0, T; V') \} \), where the time derivative \( \partial_t w \) is understood in the distributional sense. \( \mathcal{W}(0, T; V, V') \) is a Banach space if we endow it with the norm of the graph \( \|w\|_{\mathcal{W}} := \|w\|_{L^2(0, T; V)} + \|\partial_t w\|_{L^2(0, T; V')} \). Let \( H^1_{\partial \Omega}(\Omega^i_1) = \{ v \in H^1(\Omega^i_1) \mid v = 0 \text{ on } \partial \Omega \cap \partial \Omega^i_1 \}, \) for \( i \in \{1, 2\} \).

**Definition 3.1** We say that \( (u^1_1, v^1_1), (u^2_1, v^2_1) \in \mathcal{W}(0, T; H^1_{\partial \Omega}(\Omega^1_1), (H^1_{\partial \Omega}(\Omega^2_1))' \times \mathcal{W}(0, T; H^1_{\partial \Omega}(\Omega^2_1), (H^1_{\partial \Omega}(\Omega^1_1))' \) is a weak solution of problem (2.1)–(2.2) if for any \( w_i \in H^1_{\partial \Omega}(\Omega^i_1) \), with \( i \in \{1, 2\} \), and for a.e. \( t \in (0, T) \) we have

\[
\begin{align*}
&\langle \partial_t u^1_1, w_1 \rangle_{\Omega^1_1} + \langle \partial_t u^2_1, w_2 \rangle_{\Omega^2_1} + \int_{\Omega^1_1} A^1_1 \nabla u^1_1 \cdot \nabla w_1 \, dx + \int_{\Omega^2_1} A^2_1 \nabla u^2_1 \cdot \nabla w_2 \, dx = \\
&\quad -\int_{\Omega^1_1} f^1(x, u^1_1, v^1_1) w_1 \, dx + \int_{\Omega^2_1} f^2_1(x, u^2_1, v^2_1) w_2 \, dx + \\
&\quad \varepsilon \int_{\Gamma_x} h^\varepsilon(x, u^1_1, u^2_1)(w_1 - w_2) \, d\sigma_x, \tag{3.1}
\end{align*}
\]

\[
\begin{align*}
&\langle \partial_t v^1_1, w_1 \rangle_{\Omega^1_1} + \langle \partial_t v^2_1, w_2 \rangle_{\Omega^2_1} + \int_{\Omega^1_1} B^1_1 \nabla v^1_1 \cdot \nabla w_1 \, dx + \int_{\Omega^2_1} B^2_1 \nabla v^2_1 \cdot \nabla w_2 \, dx = \\
&\quad \int_{\Omega^1_1} g^1(x, u^1_1, v^1_1) w_1 \, dx + \int_{\Omega^2_1} g^2_1(x, u^2_1, v^2_1) w_2 \, dx, \tag{3.2}
\end{align*}
\]

together with the initial conditions

\[
u^1_1(0) = u^0_1, \quad v^1_1(0) = v^0_1 \quad \text{in } \Omega^i_1, \quad i \in \{1, 2\}. \tag{3.3}
\]

Here, \( \langle \cdot, \cdot \rangle_{H^1_{\partial \Omega}(\Omega^i_1), (H^1_{\partial \Omega}(\Omega^i_1))'} \) denotes the duality pairing \( \langle \cdot, \cdot \rangle_{H^1_{\partial \Omega}(\Omega^i_1), (H^1_{\partial \Omega}(\Omega^i_1))'} \) of \( H^1_{\partial \Omega}(\Omega^i_1) \) with its dual space \( (H^1_{\partial \Omega}(\Omega^i_1))' \).

Following the technique used in [9] and [26], one can prove the following result.

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Proposition 3.2 There exists a unique weak solution \((u_1^\varepsilon, v_1^\varepsilon), (u_2^\varepsilon, v_2^\varepsilon)\), in the sense of Definition 3.1, of problem (2.1)–(2.2). Moreover, the concentration fields \(u_1^\varepsilon, v_1^\varepsilon, u_2^\varepsilon, v_2^\varepsilon\) are non-negative and bounded almost everywhere.

Let \(i \in \{1, 2\}\). Taking \(u_i^\varepsilon\) and \(v_i^\varepsilon\) as test functions in (3.1)–(3.2), integrating with respect to time and taking into account that \(u_i^\varepsilon\) and \(v_i^\varepsilon\) are bounded and non-negative, it follows that there exists a constant \(C > 0\), independent of \(\varepsilon\), such that

\[
\|u_i^\varepsilon\|_{L^2(0,T;H^1_{\text{div}}(\Omega_i^\varepsilon))} + \|v_i^\varepsilon\|_{L^2(0,T;H^1_{\text{div}}(\Omega_i^\varepsilon))} \leq C, \tag{3.4}
\]

\[
\|u_i^\varepsilon\|_{L^\infty(0,T;L^2(\Omega_i^\varepsilon))} + \|v_i^\varepsilon\|_{L^\infty(0,T;L^2(\Omega_i^\varepsilon))} \leq C, \tag{3.5}
\]

\[
\varepsilon(h^\varepsilon(x, u_1^\varepsilon, u_2^\varepsilon), u_2^\varepsilon - u_1^\varepsilon)_{L^2(0,T;L^2(\Gamma^\varepsilon))} \leq C. \tag{3.6}
\]

Also, as in [26], we can see that there exists a positive constant \(C > 0\), independent of \(\varepsilon\), such that

\[
\|\partial_t u_i^\varepsilon\|_{L^2(0,T;H^1_{\text{div}}(\Omega_i^\varepsilon))'} + \|\partial_t v_i^\varepsilon\|_{L^2(0,T;H^1_{\text{div}}(\Omega_i^\varepsilon))'} \leq C. \tag{3.7}
\]

The above a priori estimates will be used for obtaining, via the periodic unfolding method, the limit problem (2.9)–(2.10). To this aim, we use the unfolding operators \(\mathcal{T}_i^\varepsilon\), \(\mathcal{T}_i^\varepsilon\), and \(\mathcal{T}_i^\varepsilon\), which transform functions defined on oscillating domains into functions given on fixed domains (for the definitions and the main properties of these operators, see [11] and [12]).

For the connected-connected geometry we consider here, there exist linear and bounded extension operators \(P_\varepsilon : L^2(0,T;H^1_{\text{div}}(\Omega_i^\varepsilon)) \rightarrow L^2(0,T;H^1_0(\Omega))\), which preserve the non-negativity and the a priori estimates (3.4)–(3.6) (see [26] and [32] and the references therein). For simplicity, we denote the extensions of the solutions \(u_i^\varepsilon\) and \(v_i^\varepsilon\), for \(i \in \{1, 2\}\), by the same symbol. Further, for our solutions \(u_i^\varepsilon\) and \(v_i^\varepsilon\), we consider the time derivatives \(\partial_t \pi_i^\varepsilon\) and \(\partial_t \varphi_i^\varepsilon\) of the extensions by zero of \(u_i^\varepsilon\) and \(v_i^\varepsilon\), respectively, to the whole of \(\Omega\); then, the weak convergence of the extensions of time derivatives of our microscopic solutions can be proven and this will be enough to pass to the limit in the equation terms containing time derivatives (see [26]).

Proof of Theorem 2.3. Let \((u_1^\varepsilon, v_1^\varepsilon), (u_2^\varepsilon, v_2^\varepsilon)\) be the unique solution of problem (2.1)–(2.2). Using our a priori estimates and the properties of the above mentioned unfolding operators, it follows that there exist \(u_1, u_2 \in L^2((0,T) \times \Omega; H^1_{\text{per}}(Y_1)/\mathbb{R})\), \(\tilde{u}_1 \in L^2((0,T) \times \Omega; H^2_{\text{per}}(Y_1)/\mathbb{R})\), \(\nu_2 \in L^2((0,T) \times \Omega; H^1_{\text{per}}(Y_2)/\mathbb{R})\), such that, up to a subsequence, for \(\varepsilon \to 0\), one has:

\[
\begin{align*}
\mathcal{T}_1^\varepsilon(u_1^\varepsilon) &\to u_1 \quad \text{weakly in } L^2((0,T) \times \Omega; H^1_0(Y_1)), \\
\mathcal{T}_1^\varepsilon(\nabla u_1^\varepsilon) &\to \nabla u_1 + \nabla_y \tilde{u}_1 \quad \text{weakly in } L^2((0,T) \times \Omega \times Y_1), \\
\mathcal{T}_2^\varepsilon(u_2^\varepsilon) &\to u_2 \quad \text{weakly in } L^2((0,T) \times \Omega; H^1_0(Y_2)), \\
\mathcal{T}_2^\varepsilon(\nabla u_2^\varepsilon) &\to \nabla u_2 + \nabla_y \tilde{u}_2 \quad \text{weakly in } L^2((0,T) \times \Omega \times Y_2). 
\end{align*}
\]
exist \( v_1, v_2 \in L^2(0, T; H^1_0(\Omega)) \), \( \hat{v}_1 \in L^2((0, T) \times \Omega, H^1_{\text{per}}(Y_1)/\mathbb{R}) \), \( \hat{v}_2 \in L^2((0, T) \times \Omega; H^1_{\text{per}}(Y_2)/\mathbb{R}) \) such that, up to a subsequence, for \( \varepsilon \to 0 \), we have:

\[
\begin{align*}
&\mathcal{T}^\varepsilon_i(v^\varepsilon_i) \rightharpoonup v_1 \quad \text{weakly in } L^2((0, T) \times \Omega; H^1(Y_1)), \\
&\mathcal{T}^\varepsilon_i(\nabla v^\varepsilon_i) \rightharpoonup \nabla v_1 + \nabla_y \hat{v}_1 \quad \text{weakly in } L^2((0, T) \times \Omega \times Y_1), \\
&\mathcal{T}^\varepsilon_2(v^\varepsilon_2) \rightharpoonup v_2 \quad \text{weakly in } L^2((0, T) \times \Omega; H^1(Y_2)), \\
&\mathcal{T}^\varepsilon_2(\nabla v^\varepsilon_2) \rightharpoonup \nabla v_2 + \nabla_y \hat{v}_2 \quad \text{weakly in } L^2((0, T) \times \Omega \times Y_2).
\end{align*}
\]

(3.9)

Here, \( H^1_{\text{per}}(Y_i)/\mathbb{R} \) is the quotient space of functions defined in \( H^1_{\text{per}}(Y_i) \) up to an additive real constant. Moreover, for \( i \in \{1, 2\} \), we get (see [26]):

\[
\begin{align*}
&\mathcal{T}^\varepsilon_i(u^\varepsilon_i) \to u_i \quad \text{strongly in } L^2((0, T) \times \Omega \times Y_i), \\
&\mathcal{T}^\varepsilon_i(v^\varepsilon_i) \to v_i \quad \text{strongly in } L^2((0, T) \times \Omega \times Y_i), \\
&\mathcal{T}^\varepsilon_i(\nabla v^\varepsilon_i) \to \nabla v_i \quad \text{strongly in } L^2((0, T) \times \Omega \times \Gamma).
\end{align*}
\]

(3.10)

Using the hypotheses we imposed for our data, it follows that \( \mathcal{T}^\varepsilon_i(f^\varepsilon_i(x, u^\varepsilon_i, v^\varepsilon_i)) \) converges weakly to \( f_i(y, u_i, v_i) \) in \( L^2((0, T) \times \Omega \times Y_i) \) and \( \mathcal{T}^\varepsilon_i(g^\varepsilon_i(x, u^\varepsilon_i, v^\varepsilon_i)) \) converges weakly to \( g_i(y, u_i, v_i) \) in \( L^2((0, T) \times \Omega \times Y_i) \), for \( i \in \{1, 2\} \).

For getting the limit problem (2.9)–(2.10), we take in (3.1)–(3.2) suitable admissible test functions. More precisely, let us take in (3.1)

\[
\begin{align*}
w_1(t, x) &= \left( \varphi_1(x) + \varepsilon \varphi_2 \left( x, \frac{x}{\varepsilon} \right) \right) \Phi(t), \\
w_2(t, x) &= \left( \psi_1(x) + \varepsilon \psi_2 \left( x, \frac{x}{\varepsilon} \right) \right) \Phi(t),
\end{align*}
\]

(3.11)

where \( \varphi_1, \psi_1 \in \mathcal{D}(\Omega) \), \( \varphi_2 \in C^\infty_0(\Omega; H^1_{\text{per}}(Y_1)) \), \( \psi_2 \in C^\infty_0(\Omega; H^1_{\text{per}}(Y_2)) \), and \( \Phi \in \mathcal{D}((0, T)) \). Integrating in time from \( 0 \) to \( T \), applying in each term the corresponding unfolding operators, and passing to the limit, by using the above convergence results and Lebesgue’s convergence theorem (for more details, see, for instance, [10], [11], [12], and [26]), we get:

\[
\begin{align*}
&- \int_0^T \int_{\Omega \times Y_1} u_1 \varphi_1 \Phi' \, dx \, dy \, dt - \int_0^T \int_{\Omega \times Y_2} u_2 \psi_1 \Phi' \, dx \, dy \, dt + \\
&\int_0^T \int_{\Omega \times Y_1} A_1(y)(\nabla u_1 + \nabla_y \hat{u}_1) \cdot (\nabla \varphi_1 + \nabla_y \varphi_2) \Phi \, dx \, dy \, dt + \\
&\int_0^T \int_{\Omega \times Y_2} A_2(y)(\nabla u_2 + \nabla_y \hat{u}_2) \cdot (\nabla \psi_1 + \nabla_y \psi_2) \Phi \, dx \, dy \, dt + \\
&\int_0^T \int_{\Omega \times \Gamma} h_0(y)(u_1 - u_2)(\varphi_1 - \psi_1) \Phi \, dx \, ds_y \, dt = \\
&\int_0^T \int_{\Omega \times Y_1} f_1(y, u_1, v_1) \varphi_1 \Phi \, dx \, dy \, dt + \\
&\int_0^T \int_{\Omega \times Y_2} f_2(y, u_2, v_2) \psi_1 \Phi \, dx \, dy \, dt.
\end{align*}
\]

(3.12)
From (3.12), by using standard density arguments, we are led to the limit problem (2.9)–(2.10) for the calcium concentrations. Indeed, taking \( \varphi_1 = 0 \) and \( \psi_1 = 0 \), and, then, suitable functions \( \varphi_2 \) and \( \psi_2 \), we obtain the cell problems (2.11)–(2.12) and

\[
\hat{u}_1 = \sum_{k=1}^{n} \frac{\partial u_1}{\partial x_k} \chi_{1k}, \quad \hat{u}_2 = \sum_{k=1}^{n} \frac{\partial u_2}{\partial x_k} \chi_{2k}.
\]

(3.13)

Next, choosing \( \varphi_2 = 0 \) and \( \psi_2 = 0 \), using (3.13), and, then, taking suitable functions \( \varphi_1 \) and \( \psi_1 \), we get the limit equations for the calcium concentrations in (2.9). We can proceed in the same manner for deriving the effective equations in (2.9) for the buffers \( v_1 \) and \( v_2 \), with similar matrices \( \overline{B}_1 \) and \( \overline{B}_2 \) and similar cell functions \( \overline{\chi}_{1k} \in H^1_{\text{per}}(Y_1) \), \( \overline{\chi}_{2k} \in H^1_{\text{per}}(Y_2) \), \( k = 1, \ldots, n \), defined with the aid of the matrix \( B \).

We remark that passing to the limit, with \( \varepsilon \to 0 \), in the initial conditions, we obtain \( u_i(0, x) = u_{0i}(x) \) and \( v_i(0, x) = v_{0i}(x) \) in \( \Omega \), for \( i \in \{1, 2\} \).

The uniqueness for the solutions to the effective model (2.9)–(2.10) can be proved by usual methods. Since \( u_1, v_1, u_2, \) and \( v_2 \) are uniquely determined, the above convergences for the microscopic solutions are valid for the whole sequence. This ends the proof of Theorem 2.3.

4. CONCLUSIONS

By using the periodic unfolding method, the effective behavior of the unique solution of a system of coupled partial differential equations arising in the modeling of calcium dynamics in living cells under the action of buffering proteins was analyzed. At the macroscale, we get a calcium bidomain model, consisting of coupled reaction-diffusion equations for the concentration of the calcium ions and of the buffers in the cytoplasm.

REFERENCES