Reactive clusters on a membrane

R Thul and M Falcke

Hahn-Meitner Institut, Abteilung Theorie, Glienicker Str. 100, D-14109 Berlin, Germany

Received 23 September 2004
Accepted for publication 24 February 2005
Published 17 March 2005
Online at stacks.iop.org/PhysBio/2/51

Abstract
We investigate the reaction dynamics of diffusive molecules with immobile binding partners. The fixed reactants build clusters that are comprised of just a few tens of molecules, which leads to small cluster sizes. These molecules participate in the reaction only if they are activated. The dynamics of activation is mapped to a time-dependent size of an active region within the cluster. We focus on the deterministic description of the dynamics of a single cluster. The spatial setup accounts for one of the most important determinants of the dynamics of a cluster, i.e. diffusional transport of reaction partners towards or away from the active region of the cluster. We provide numerical and analytical evidence that diffusion influences decisively the dynamic regimes of the reactions. The application of our methods to intracellular Ca\textsuperscript{2+} dynamics shows that large local concentrations saturate the Ca\textsuperscript{2+} feedback to the channel state control. It eliminates oscillations depending on this feedback.

1. Introduction

A basic task of cells is to respond to inner and outer stimuli, which involves sequences of chemical reactions that form the signaling pathway. While numerous reactions take place between dissolved binding partners in the cytosol, others occur in the plasma or organelle membranes. A third class of reaction happens between cytosolic molecules and membrane-bound reaction partners. Often these reactions involve only a small region of the membrane at a time because the cell does not only interpret the total amount of additional substances, but also the precise location of its production. A well-known example of this type of reaction is in the formation of the second messenger cyclic adenosine monophosphate (cAMP). Dissolved adenosine triphosphate (ATP) binds to adenylyl cyclase, which is fixed in the plasma membrane, to synthesize cAMP. Depending on where this reaction occurs, it may trigger a break down of glycogen to glucose or gene expression [2].

The fixed membrane-bound reaction partners are often concentrated in a small membrane area, which we call a cluster. We will refer to the molecules in the cluster as fixed elements. Generally, not all molecules in the membrane patch participate in the reaction: only activated elements join in. Although the activating mechanism varies among membrane-bound processes, this principle is ubiquitous in biology. A single cluster with several tens of fixed elements is the focus of the present work.

The small number of fixed elements in a cluster implies small cluster areas. This strong localization of the reactions causes large spatial gradients of diffusing species. The cluster diameters are much smaller than the diffusion length of the species in solution. This imposes limits on the reactions due to diffusion of dissolved reaction partners towards or away from the cluster. Here, we will model the dynamics of an active cluster accounting explicitly for the diffusion of dissolved reaction partners.

Beside cAMP, cells employ other second messengers in the abovementioned signaling tasks. Ca\textsuperscript{2+} is another prominent representative of a second messenger that participates in numerous processes [4]. It communicates the fertilization through an egg cell [20], controls apoptosis [25], and is vital for the excitation–contraction coupling in cardiac myocytes [5]. The mechanism by which a cell regulates the concentration of cytosolic Ca\textsuperscript{2+} involves receptors on the membrane of intracellular compartments. Here, the inositol-1,4,5 trisphosphate (IP\textsubscript{3}) receptor channel (IP\textsubscript{3}R) on the membrane of the endoplasmic reticulum (ER) serves as an illustrative example. Upon activation, the channel opens, which in turn results in a transient flux of Ca\textsuperscript{2+} from the ER to the cytosol. Importantly, the open probability of the channel depends sensitively on the concentration of free cytosolic Ca\textsuperscript{2+}. A moderate increase raises the tendency to release calcium, whereas a high concentration of Ca\textsuperscript{2+} causes inhibition and closes the channel. Thus, the channel releases the species that controls its state.

The feedback of Ca\textsuperscript{2+} on the channel dynamics becomes even more relevant when we take the spatial organization of IP\textsubscript{3} receptor channels into account. Generically, the receptor...
channels form clusters that are randomly distributed on the membrane of the ER. The typical inter-cluster distance is 2–7 µm [19]. The number of IP₃Rs within a cluster has not yet been established experimentally. However, it is estimated that a cluster comprises 1–40 channels. Using the size of 18 nm for a single IP₃R with all four subunits [12], we arrive at a cluster diameter of 18–100 nm. Thus, IP₃ receptor channels are tightly coupled by diffusion within a cluster because the Ca²⁺ concentration decays on length scales of about 1 µm [26].

We see that intracellular Ca²⁺ dynamics is another example of a reaction between partners fixed in a small membrane area and diffusing species. The channels are the fixed elements, Ca²⁺ is the diffusing species and the reactions are production (here as release) and binding to and dissociation from the binding sites on the channel.

To date, modeling of intracellular Ca²⁺ dynamics has proceeded along two distinct paths. In the first approach, deterministic models and spatially averaged concentrations have been used [7, 15]. Since this approach neglects any spatial information such as gradients, the Ca²⁺ concentration changes by only one order of magnitude during oscillations. However, simulations show that large gradients occur and that the Ca²⁺ concentration changes by 3–4 orders of magnitude during oscillations. Changes by only one order of magnitude during oscillations. However, simulations show that large gradients occur and that the Ca²⁺ concentration changes by 3–4 orders of magnitude during oscillations.

The procedure follows results by Swillens and Dupont [24]. The system contains m diffusive species. They are described by the concentration fields \( c(r, t) := \{c_1(r, t), \ldots, c_m(r, t)\} \). Their dynamics are of the general form

\[
\dot{c} = D \nabla_r^2 c + f_1(c) \Theta(a - r) + f_2(c) \Theta(r - a) .
\]

Here \( \nabla_r^2 \) denotes the radial part of the Laplacian in three dimensions. \( \Theta(x) \) with \( \Theta(x) = 1 \) for \( x \geq 0 \), \( \Theta(x) = 0 \) otherwise represents the Heaviside step function. The functions \( f_1 \) and \( f_2 \) subsume the details of the dynamics for \( r < a \) and \( r > a \), respectively. Most commonly, \( f_1 \) is dominated by production and \( f_2 \) by consumption.

The state of a fixed element is controlled by binding of the diffusive species to binding sites. The occupation of these binding sites determines the state. Usually, there are several binding sites per element. We denote with \( p_i, i = 1, \ldots, n \) the fraction of elements in the state \( i \) and refer to the \( p_i \) as gating variables. The dynamics of the gating variables are governed by the general equation

\[
\dot{p}_i = g_i(c, p_1, \ldots, p_n), \quad i = 1, \ldots, n.
\]

Some of the \( n \) states correspond to the activated state of the elements, so that the fraction of activated elements is determined by the sum of the corresponding gating variables. Consequently, the gating variables determine the radius \( a \) of the active area by some function \( f \) as

\[
a = f(c(a), p_1(a), \ldots, p_n(a)).
\]

We include a dependence on the diffusive species and on all \( n \) gating variables in \( f \) to account for the most general case. The values of the concentration fields and of the gating variables do not vary significantly within a cluster because the diffusion lengths are larger than \( a_0 \). Therefore, we can pick a typical value to compute \( c \) and \( p_i \). We have chosen the value at the boundary of the cluster, which turns equation (3) into an implicit expression.

The behavior of the equations (1)–(3) can be investigated by a bifurcation analysis, which determines the stationary states and their stability. We begin with the stationary states, i.e. the solutions of the equations

\[
0 = D \nabla_r^2 c + f_1(c) \Theta(a - r) + f_2(c) \Theta(r - a) ,
\]

\[
0 = g_i(c, \tilde{p}_1, \ldots, \tilde{p}_n), \quad i = 1, \ldots, n.
\]

The overbar indicates the stationary states of the Ca²⁺ concentration profile and of the gating variables, respectively. The constant \( \tilde{a} \) denotes the stationary value of the active area. Equation (4a) can be treated separately for \( r < \tilde{a} \) and \( r > \tilde{a} \) due to the Heaviside step function. Since we demand the concentration profiles to be \( C^1 \) functions with respect to \( r \), the matching conditions for the stationary solutions read as

\[
\tilde{c}_i(\tilde{a}) = \tilde{c}_i(\tilde{a}) \quad \text{and} \quad \partial \tilde{c}_i / \partial r(\tilde{a}) = \partial \tilde{c}_i / \partial r(\tilde{a}).
\]

The subscripts \( i \) and \( o \) indicate the inner and outer solution, respectively.
The computation of in proceeds in two steps. Firstly, we solve equation (4a) with a fixed, but arbitrary value of . This results in a solution for , which includes as a still undetermined parameter. Secondly, we determine self-consistently from equation (3) after inserting the solutions for and for .

Figure 2 shows a graphical method for determining . The dotted line is the bisection line, whereas the full lines represent the rhs of equation (3) for a specific model (see below). The stationary states are given by the intersections. Upon changing one parameter, the curve of is shifted. It results in a change of the values or the number of fixed points. The existence of a saddle node bifurcation is easily deduced from such a plot. It occurs when touches the bisection line. This is equivalent to the condition \( f'(\alpha) = 1 \).

Knowing the stationary points \((\bar{c}, \bar{a})\), we investigate their stability. A linearization of the reaction–diffusion dynamics in (1) and the gating dynamics in (2) results in the equations

\[
\dot{y} = D \nabla^2_y y + f_1(\bar{c}) - f_2(\bar{c}) \delta_D(\bar{a} - r) \delta a(y, z) + \left\{ \frac{\partial f_1}{\partial c}(\bar{c}) \Theta(\bar{a} - r) + \frac{\partial f_2}{\partial c}(\bar{c}) \Theta(r - \bar{a}) \right\} \cdot y \tag{5a}
\]

\[
\dot{z}_i = \sum_{j=1}^{m} \frac{\partial g_i}{\partial c_j}(\bar{c}, \bar{p}_1, \ldots, \bar{p}_n) z_j + \sum_{j=0}^{n} \frac{\partial g_i}{\partial p_j}(\bar{c}, \bar{p}_1, \ldots, \bar{p}_n) z_j. \tag{5b}
\]

We define as the perturbations of the diffusing species, and as the perturbations of the gating variables. \( \delta_D \) denotes Dirac’s delta function. Although \( \alpha \) is not a dynamical variable in our model, it still changes in time. This is a consequence of equation (3) because \( \alpha \) is computed from the evolving concentration fields and gating variables. \( \alpha \) can be written as \( \alpha = \bar{a} + \delta a \) with \( \delta a = \delta a(y, z) \). To evaluate \( \delta a \) from equation (3), we expand the expression

\[
\bar{a} + \delta a = f(\bar{c}(r) + y(r), \bar{p}(r) + z(r)) |_{r = \bar{a}} + \delta a \tag{6}
\]

to linear order:

\[
\delta a = \sum_{i=1}^{n} \frac{\partial f}{\partial c_i}(\bar{a}) \cdot \delta a \frac{\partial f}{\partial c_i}(\bar{a}) \tag{7}
\]

The denominator of \( f \) have to be taken at \((\bar{c}(\bar{a}), \bar{p}(\bar{a}))\). The denominator only arises because of the evaluation of \( f \) at \( r = \alpha \) in equation (3).

When we combine \( y \) and \( z \) to an \((n + m)\)-dimensional vector \( \mathbf{x} = (y, z)' \), the linearized equations take the matrix form \( \dot{x} = M \mathbf{x} \). If \( M \) can be diagonalized, the general solution for \( x \) is given by a linear combination of terms \( v_j \exp(\omega_j t) \), \( v_j \) represents an eigenvector of \( M \) and \( \omega_j \) the corresponding eigenvalue. Consequently, the linear stability is uniquely determined by the eigenvalues of \( M \). As shown in the appendix, \( M \) can be diagonalized. The eigenvalues \( \lambda_j \) that originate from the gating variables constitute a subset \( \{\lambda_i\} \) of all eigenvalues \( \{\omega_j\} \) and are all non-positive, i.e. \( \lambda_i \leq 0 \ \forall i \), if the gating variables dynamics are rate equations derived from a master equation. Moreover, the eigenvectors \( v_j \) of \( M \) that correspond to the eigenvalues \( \lambda_i \) possess the structure \( v_j = (0, \ldots, 0, q_j)' \) with \( \dim q_j = n \). This has two important consequences. Firstly, the eigenvalues from the gating dynamics do not contribute to any linear instability. Secondly, the solution for \( y \) does not depend on \( \exp(\lambda_j t) \). Therefore, the time dependence of \( y \) is solely governed by the eigenvalues that originate from equations (8) and (5a).

Equation (5a) can be solved separately for \( r < \bar{a} \) and \( r > \bar{a} \) because of the Heaviside step function. The matching conditions are now \( y_1(\bar{a}) = y_2(\bar{a}) \), and the first derivative jumps according to

\[
\left[ \frac{dy_a}{dr} - \frac{dy_i}{dr} \right] + \frac{f_1(\bar{c}) - f_2(\bar{c})}{D} \delta a(y, z) = 0, \tag{8}
\]

due to Dirac’s delta function in equation (5a). The continuity at \( \bar{a} \) and equation (8) fix the still undetermined coefficients of \( y \). The resulting system of equations is homogeneous. It has a non-trivial solution only if the determinant vanishes. This yields an implicit equation for the eigenvalues of the concentration fields and thus determines their linear stability.

3. Calcium dynamics

We now apply the method of section 2 to the dynamics of cytosolic calcium as a prototypic model system. The cytosolic \( \text{Ca}^{2+} \) concentration \( c \) is governed by

\[
\dot{c} = D \nabla^2 c + k_1(E - c) - k_{pc} + k_1(E - c) \Theta(a - r). \tag{9}
\]

The constants \( D \) and \( E \) denote the diffusion coefficient of \( \text{Ca}^{2+} \) in the cytosol and the \( \text{Ca}^{2+} \) concentration in the ER, respectively. The term \( k_1(E - c) \) refers to a leak current, whereas \( k_{pc} \) describes the calcium uptake by the ER through sarco-endoplasmic reticulum calcium ATPase (SERCA) pumps. Although it would be more realistic to model SERCAs using a Hill equation with coefficient 2, we approximate them by a linear expression for the sake of an analytical treatment. The last term in equation (9) describes the flux of \( \text{Ca}^{2+} \) through IP3 receptor channels. They represent the fixed elements, and the radius \( a \) of the active area is determined by the fraction of open IP3 receptor channels.

The state of a single IP3R is controlled by the state of its four subunits [6]. Each subunit expresses binding sites for \( \text{Ca}^{2+} \) and IP3. Their occupation determines the state of the subunit. De Young and Keizer introduced a model to describe the dynamics of one subunit [7]. It consists of three binding sites: an activating \( \text{Ca}^{2+}\)-binding site, an inhibitory \( \text{Ca}^{2+}\)-binding site and an IP3 binding site. Therefore, the state of a subunit can be specified by a binary triplet \( ijk \). The first index represents the IP3 binding site, the second the activating \( \text{Ca}^{2+}\)-binding site and the last the inhibiting \( \text{Ca}^{2+}\)-binding site. An index equals 1 when a site is occupied and 0 otherwise. The eight states that originate from the three binding sites are depicted in figure 1. Each state corresponds to one corner of the cube. The transition rates between the different states are indicated at the arrows. Binding of \( \text{Ca}^{2+} \) and IP3 is always proportional to the \( \text{Ca}^{2+} \) and IP3 concentration, respectively, whereas unbinding is independent of these concentrations. Since a subunit is activated when IP3 and activating \( \text{Ca}^{2+} \) are bound, \( p_{110} \) denotes the fraction of open subunits. The probability of finding a conducting IP3R is \( 4p_{110}^3 - 3p_{110}^4 \) because a channel is open when at least three out of four subunits are activated. The size of the active area is set to
investigations of the Ca$^{2+}$ dynamics start with the stationary the Ca$^{2+}$ dynamics of the De Young–Keizer (DK) model. Our representations the dissociation constant for the activating Ca$^{2+}$ site $c(r)$ and $\partial c(r)/\partial r$. We applied $\bar{c}(r)$ with $\bar{c}(a)$ reads as $\bar{c}(a) = k(\bar{k}_a)/\Theta(\bar{a} - r)$ with $\bar{A}(a) = k(\bar{k}_a)/\Theta(\bar{a} - r)$ and $\bar{B}(a) = \frac{\exp(\bar{k}_a)}{\Theta(\bar{a} - r)}$. The latter complies with the base level of the system. The stationary value of $p_{110}$ in dependence on $\bar{c}$ and the IP$_3$ concentration $I$ reads as $p_{110} = \frac{d_1 I}{(\bar{c} + d_0)(d_1 + \bar{c} I + d_2 I)}$. Here, $d_1$ and $d_2$ denote the dissociation constants for IP$_3$ when Ca$^{2+}$ is not and is bound to the inhibiting site, respectively. The parameters $d_3$ and $d_4$ refer to the dissociation constants for the inhibiting Ca$^{2+}$ processes dependent on IP$_3$ binding. $d_5$ represents the dissociation constant for the activating Ca$^{2+}$ site [7].

Inserting $p_{110}$ with $\bar{c} = \bar{c}(a)$ into equation (10) determines the stationary values of $\bar{a}$. They correspond to the intersections of the dotted bisection line and the curve of $f$ is depicted by solid lines in figure 2. When we increase the IP$_3$ concentration $I$, the curve of $f$ is shifted upward. Although $\partial f/\partial I \geq 0$ always holds, the effect on the number of stationary points depends on the parameter values. There is one fixed point at low $I$ for the parameter values chosen in figure 2. Three stationary values exist at an intermediate regime, and one stationary point is present at high IP$_3$ concentrations. Thus, two saddle node bifurcations occur upon increasing $I$. For other parameter values, we find only one fixed point for the whole range of the IP$_3$ concentration or just a single saddle node bifurcation (see below).

The DK model assumes that the IP$_3$ dynamics is much faster than calcium binding and unbinding. This entails a fast equilibration between states with IP$_3$ bound and not bound. We eliminate the IP$_3$ dynamics adiabatically in the following and use the stationary values of the states with IP$_3$ bound and not bound. As shown in the appendix, the value of $p_{110}$ is not changed by this approximation. Thus, the above analysis remains valid and we proceed to the stability of the fixed points.

The linearization of equation (9) results in $\dot{y} = D\nabla^2 y - (k_1 + k_p)y - \Theta(\bar{a} - r)k_c y + f_{\delta a}(\bar{a} - r)$. (14)

We define $f_c := k_c(E - \bar{c})\delta a$. Note that the inner concentration field $y$, is still restricted to $r \leq \bar{a}$. In linear order, the varying value of $a$ is translated into an additional flux density $f_c$ at the rim of the stationary active area. The solution of (14) is $y(r, t) = \exp(\omega t)\bar{u}(r)$ with $u(r) = A \frac{\sinh(k_1 r)}{r} \Theta(\bar{a} - r) + B \frac{\exp(-k_2 r)}{r} \Theta(r - \bar{a})$. (15) and $k_1 = \sqrt{\frac{k_1 + k_p + k_c}{D}}$, $k_2 = \sqrt{\frac{k_1 + k_p + \omega}{D}}$. (16) We used the boundary conditions $\partial u/\partial r(0) = 0$ and $u(b) = 0$. The still unknown coefficients $A$ and $B$ are fixed by the continuity of $u$ and the discontinuity of $\partial u/\partial r$ at $\bar{a}$ (see also equation (8)). The latter is a direct consequence of the last term in equation (14). The homogeneous system of equations for $A$ and $B$ possesses a non-trivial solution only if its determinant equals zero. This leads to the equation $k_2 + k_1 \coth(k_1 \bar{a}) - \frac{k_c(E - \bar{c}(\bar{a}))}{D} \eta = 0$, (17) that determines the eigenvalue $\omega$. $\eta$ is given by $\partial \delta a/\sinh(k_1 \bar{a})$, which can be cast into the form

![Figure 1](image1.png)

Figure 1. Transition scheme of the De Young–Keizer model. The dissociation constants $d_i$ are defined as $d_i := b_i/a_i$.

![Figure 2](image2.png)

Figure 2. Stationary values of $a$ given by the intersections of the bisection line (dotted) and the curve of $f$ (solid). For $f$, $I$ increases from bottom to top. Parameter values are $d_1 = 0.13 \mu M$, $d_2 = 3 \mu M$, $d_3 = 0.9434 \mu M$, $d_4 = 0.4133 \mu M$, $d_5 = 0.8234 \mu M$, $k_p = 80 s^{-1}$, $k_c = 0.002 s^{-1}$, $k_c = 34.500 s^{-1}$, $E = 750 \mu M$, $a_0 = 0.03 \mu M$ and $D = 40 \mu m^2 s^{-1}$. The DK model assumes that the IP$_3$ dynamics is much faster than calcium binding and unbinding. This entails a fast equilibration between states with IP$_3$ bound and not bound. We eliminate the IP$_3$ dynamics adiabatically in the following and use the stationary values of the states with IP$_3$ bound and not bound. As shown in the appendix, the value of $p_{110}$ is not changed by this approximation. Thus, the above analysis remains valid and we proceed to the stability of the fixed points.

The linearization of equation (9) results in $\dot{y} = D\nabla^2 y - (k_1 + k_p)y - \Theta(\bar{a} - r)k_c y + f_{\delta a}(\bar{a} - r)$. (14)

We define $f_c := k_c(E - \bar{c})\delta a$. Note that the inner concentration field $y$, is still restricted to $r \leq \bar{a}$. In linear order, the varying value of $a$ is translated into an additional flux density $f_c$ at the rim of the stationary active area. The solution of (14) is $y(r, t) = \exp(\omega t)\bar{u}(r)$ with $u(r) = A \frac{\sinh(k_1 r)}{r} \Theta(\bar{a} - r) + B \frac{\exp(-k_2 r)}{r} \Theta(r - \bar{a})$. (15) and $k_1 = \sqrt{\frac{k_1 + k_p + k_c}{D}}$, $k_2 = \sqrt{\frac{k_1 + k_p + \omega}{D}}$. (16) We used the boundary conditions $\partial u/\partial r(0) = 0$ and $u(b) = 0$. The still unknown coefficients $A$ and $B$ are fixed by the continuity of $u$ and the discontinuity of $\partial u/\partial r$ at $\bar{a}$ (see also equation (8)). The latter is a direct consequence of the last term in equation (14). The homogeneous system of equations for $A$ and $B$ possesses a non-trivial solution only if its determinant equals zero. This leads to the equation $k_2 + k_1 \coth(k_1 \bar{a}) - \frac{k_c(E - \bar{c}(\bar{a}))}{D} \eta = 0$, (17) that determines the eigenvalue $\omega$. $\eta$ is given by $\partial \delta a/\sinh(k_1 \bar{a})$, which can be cast into the form

54
Diffusion of calcium plays a central role for the selection of dynamic regimes of the Ca2+ dynamics besides the dynamics of the IP3 receptor channel. Hence, we present results for a representative cluster radius \( R \) in equation (9) to a discrete model. To this aim, we rescale equation (17). Indeed, using the identity

\[
\kappa(\omega) = \left[ a_0 \frac{4(1 - \overline{p}_{110})}{\sqrt{4 - 3(\overline{p}_{110})^2}} \frac{\overline{p}_{110}}{c} \right] \times \left[ \frac{d_5}{\overline{a}} + d_5 \overline{c} - \frac{\overline{c}}{\overline{a} + d_5 + \overline{c}} \right]_0
\]

(19)

and \( a_0 = (a_2 \mu + d_2(\omega)) / (1 + d_1) \). If the system exhibits a zero eigenvalue bifurcation for a given pair \((\overline{a}, I)\), then \( \omega = 0 \) should solve equation (17). Indeed, the eigenvalue bifurcation for a given pair \((\overline{a}, I)\) should solve equation (17). Indeed, using the identity

\[
\frac{k_c}{D} \frac{E - \overline{c}(\overline{a})}{k_2 + k_1 \coth(k_2 \overline{a})} = \frac{\overline{E}}{\overline{a}} \sinh(k_2 \overline{a}),
\]

(20)
equation (17) can be transformed into \( 1 = f' (\overline{a}) \). This is one of the conditions for a saddle node bifurcation.

### 4. Results and Discussion

Some results obtained with the model concerning Ca2+ dynamics have already been reported in [27]. Here, we consider parameter values beyond the range relevant for intracellular Ca2+ release by IP3 Rs to demonstrate model behavior which might be significant for other membrane-bounding reactions.

Diffusion of calcium plays a central role for the selection of dynamic regimes of the Ca2+ dynamics besides the dynamics of the IP3 receptor channel. Hence, we present results for \( D = 40 \mu m^2 s^{-1} \) and \( D = 220 \mu m^2 s^{-1} \). Diffusion of Ca2+ can be easily changed in experiments by application of Ca2+-binding proteins (buffers).

The original DK model is based on a continuous distribution of IP3 receptor channels. Two Hopf bifurcations bounding an oscillatory regime are the most prominent features of the Ca2+ dynamics. We test whether this property is conserved when going from spatially continuous source terms in equation (9) to a discrete model. To this aim, we rescale the flux density with a typical cluster spacing \( R \) and a representative cluster radius \( a_0 \) while keeping the total flux constant, i.e. \( k_c = k_c^{DK} R^3 / a_0 \). The resulting \( k_c \) of \( 3 \times 10^5 \) s\(^{-1}\), which agrees well with realistic values [26], leads to a loss of the oscillatory regime. We find a single stationary state for all IP3 concentrations, which is linearly stable. Decreasing the flux density by several orders of magnitude and thus approaching the Ca2+ concentration values of the DK model does not restore oscillations. This holds because gradients still prevail.

These results do not mean that a model like equations (1)–(3) does not exhibit oscillations for some parameter values. In the following, we investigate oscillations of the model. In order to obtain oscillations, we choose parameter values supported by experiments, but a value of the activating Ca2+ dissociation constant \( d_3 \) such that we obtain an oscillatory regime. We use \( d_2 = 3 \mu M \) for the inhibitory process in agreement with recent measurements [1] (Mak et al. found dissociation constants up to 45 \( \mu M \), but based on a specific model [18]). According to the experiments in [1], the coefficients for binding to the inhibiting site, \( a_2 \) and \( d_4 \), are both set to 0.2 (\( \mu M s^{-1} \)). The binding rate constant for the activating site can be evaluated from puff frequencies [28]. This implies \( a_5 \geq 1 \) (\( \mu M s^{-1} \)). Assays of the dissociation constant for Ca2+ activation yield values from 77 nM to 309 nM [16, 17, 22]. We chose \( d_3 = 0.823 \mu M \), which is motivated by the results depicted in figure 3. It shows the dynamic regimes of the model in dependence on \( d_3 \) and \( I \). Oscillations occur only for larger values of \( d_3 \).

Two saddle node bifurcations and a Hopf bifurcation terminate in a cusp. The oscillations arising at the Hopf bifurcation vanish via a bifurcation close to the lower saddle node bifurcation, which involves an increase in period. We assume it to be a homoclinic bifurcation. Typical oscillations are shown in figure 4 and in [27] for smaller values of \( a_5 \). The pattern is the same for all examples. Upon increasing the IP3 concentration, the system responds with a huge spike of Ca2+ activation and amplitude decays in space to negligible values within 1.6 ms. This implies \( a_5 \geq 1 \) (\( \mu M s^{-1} \)). Assays of the dissociation constant for Ca2+ activation yield values from 77 nM to 309 nM [16, 17, 22]. We chose \( d_3 = 0.823 \mu M \), which is motivated by the results depicted in figure 3. It shows the dynamic regimes of the model in dependence on \( d_3 \) and \( I \). Oscillations occur only for larger values of \( d_3 \).

Two saddle node bifurcations and a Hopf bifurcation terminate in a cusp. The oscillations arising at the Hopf bifurcation vanish via a bifurcation close to the lower saddle node bifurcation, which involves an increase in period. We assume it to be a homoclinic bifurcation. Typical oscillations are shown in figure 4 and in [27] for smaller values of \( a_5 \). The pattern is the same for all examples. Upon increasing the IP3 concentration, the system responds with a huge spike of Ca2+ activation and amplitude decays in space to negligible values within 1.6 ms. This implies \( a_5 \geq 1 \) (\( \mu M s^{-1} \)). Assays of the dissociation constant for Ca2+ activation yield values from 77 nM to 309 nM [16, 17, 22]. We chose \( d_3 = 0.823 \mu M \), which is motivated by the results depicted in figure 3. It shows the dynamic regimes of the model in dependence on \( d_3 \) and \( I \). Oscillations occur only for larger values of \( d_3 \).

Figure 5 shows that the structure of the bifurcation diagram does not change with a higher value of the activating dissociation constant. There is a single fixed point for almost all IP3 concentrations. Stable limit cycles exist close to the bistable area. They only extend to IP3 concentrations where the upper branch is unstable. Thus, we again find a very small band of IP3 concentrations in which the system oscillates. The oscillations behave in the same way as described above.

The existence of oscillations does not solely depend on dissociation constants. It depends on rate constants as well. Therefore, we test the influence of the binding rate constant \( a_5 \)
homoclinic bifurcation. The results in figure 7 illustrate a parameter values like those in figure 8. For oscillatory regime. The oscillations are again considerably unstable for these parameter values, too, which leads to a small occurrence. Oscillations appear only when the upper branch is the right panel depicts a period-4 example. Higher periods appear, too. Oscillations are again considerably damped at a distance of 1.6 μm.

We find a different structure of the bifurcation diagram for parameter values like those in figure 8. For \( D = 40 \, \mu m^2 \, s^{-1} \), there are two saddle node bifurcations and a Hopf bifurcation. This is similar to the results obtained above. However, increasing the diffusion coefficient changes the topology of the bifurcation diagram. A value of \( D = 220 \, \mu m^2 \, s^{-1} \) yields only one saddle node bifurcation. It leads to a bistable regime that extends infinitely towards high IP_3 concentration values.

These two examples illustrate that diffusion influences essentially the dynamical behavior. Generally, the impact of diffusion on the fixed points can be deduced from \( \partial c/\partial D \). The derivative simplifies to

\[
\frac{1}{\xi} \frac{\partial \bar{c}}{\partial D} (a) = \frac{\bar{k}_1}{\partial D} (\bar{k}_2 a + 1) \left\{ \frac{k_1 - \sinh(2\bar{k}_1 a)}{2a} \right\}
\]

The statement implies a stretching of \( f \) to the right upon increasing the diffusion coefficient. The exact effect on the stationary points depends on their properties. A continuous increment of \( D \) leads first to a higher value of \( \bar{a} \) and then to the disappearance of the fixed point, when \( \partial f (\bar{a})/\partial \bar{a} < 0 \). In the case of figure 8, \( f \) is stretched to such an extent that an increment of \( I \) does not shift \( f \) upward enough to cause a second saddle node bifurcation (see figure 2).
Figure 7. Oscillations of the Ca\textsuperscript{2+} concentration at \( r = 0 \) \( \mu \text{m} \) for different values of the IP\textsubscript{3} concentration. At \( t = 100 \) s, we decreased \( I \) from 0.22 \( \mu \text{M} \) to 0.218 \( \mu \text{M} \) (left panel), whereas \( I = 0.215 \) \( \mu \text{M} \) for all times in the right panel. Parameter values as in figure 8 and \( D = 50 \mu \text{m}^2 \text{ s}^{-1} \).

Figure 8. Stationary values of the Ca\textsuperscript{2+} concentration for \( D = 40 \mu \text{m}^2 \text{ s}^{-1} \) (left) and \( D = 220 \mu \text{m}^2 \text{ s}^{-1} \) (right). Solid lines denote linearly stable fixed points, dotted lines linearly unstable fixed points. Parameter values are \( d_1 = 0.13 \mu \text{M}, d_2 = 12.588 \mu \text{M}, d_3 = 0.9434 \mu \text{M}, d_4 = 1.7346 \mu \text{M}, d_5 = 2.4702 \mu \text{M}, k_x = 80 \text{ s}^{-1}, k_i = 0.002 \text{ s}^{-1}, k_r = 700 \text{ s}^{-1}, E = 750 \mu \text{M}, \omega_0 = 0.11 \mu \text{m}, \omega_2 = \omega_4 = 0.0167 (\mu \text{M} \text{ s})^{-1} \) and \( \omega_1 = 0.667 (\mu \text{M} \text{ s})^{-1} \).

5. Conclusion and outlook

We have presented an extended study of a new modeling concept for diffusive species that react with immobile reaction partners. The fixed reactants are confined to small clusters. Our approach to describe the cluster dynamics is always applicable when the diffusion length is much larger than the cluster size. We applied the above method to the dynamics of intracellular calcium mediated by IP\textsubscript{3} receptor channels. The spatial restriction of the Ca\textsuperscript{2+} flux to small membrane areas led to the disappearance of Ca\textsuperscript{2+} oscillations computed in spatially continuous models. The enlarged values of the Ca\textsuperscript{2+} concentration at the cluster resulted in a single linearly stable stationary state. Choosing smaller values of the channel flux constant \( k_i \) did not restore Ca\textsuperscript{2+} oscillations. Hence, the strong impact of spatial gradients on dynamic regimes will most likely also apply to localized reactions generating much smaller gradients than the gradients around a releasing Ca\textsuperscript{2+} channel.

The flux constants we have used and which entail the large concentrations occurring at releasing clusters are based on recent simulation results. These simulations of Ca\textsuperscript{2+} liberation close to experimental conditions show that Ca\textsuperscript{2+} concentrations span the range of 25–170 \( \mu \text{M} \) in the center of an open cluster [26]. This is 3–4 orders of magnitude larger than the base level. At the same time, the concentration increases only 1–2 times base level at neighboring clusters. The existence of propagating waves proofs that the activation process is sensitive to these small concentration changes. Since the channel control processes experience concentration changes of several orders of magnitude and react to small changes already, the possibility of eliminating large concentrations from the dynamics by the rescaling of binding constants must be ruled out.

Even if the Ca\textsuperscript{2+} concentration oscillated according to a deterministic model, these oscillations are not the oscillations observed in experiments [27]. Two observations led to this conclusion. Firstly, the range of IP\textsubscript{3} concentrations providing oscillations is too small. Secondly, the amplitude and the mean of the oscillations are already considerably damped in a distance of 1.6 \( \mu \text{m} \) from the cluster. Thus, they cannot represent the global Ca\textsuperscript{2+} oscillations seen in experiments. The spatial damping of the oscillations applies to each reaction that produces a diffusing species.

Our findings support earlier results that deterministic models, including only activation by IP\textsubscript{3}, activation by Ca\textsuperscript{2+} and inhibition by Ca\textsuperscript{2+}, do not capture intracellular Ca\textsuperscript{2+} oscillations [27]. Oscillations are driven by fluctuations in channel opening. The stochasticity of intracellular Ca\textsuperscript{2+} dynamics is caused by the stochastic binding and unbinding of IP\textsubscript{3} and Ca\textsuperscript{2+} to the small number of receptor molecules. Fluctuations cause spontaneous release in a single cluster. This leads to a release spike like the initial spikes in figures 4 and 7. Such a large amplitude event can lead to the opening of neighboring clusters and finally, via a nucleation process, to a wave traveling through the whole cell. If that occurs repeatedly, oscillation-like processes follow [8]. Thus, the amplitude of the initial spike is responsible for the amplitude of the oscillations. Nucleation may occur at different spots in...
the cell essentially at the same time when the IP₃ concentration is high [8], leading to almost regular periods.

Oscillations might as well be introduced by additional feedback, e.g., a Ca²⁺ feedback on IP₃ production or the filling state of the endoplasmic reticulum. Our findings suggest that the initiation of global Ca²⁺ release would still occur by wave nucleation, since the Ca²⁺ dynamics would not undergo a local instability. The additional feedbacks would just modulate the nucleation probability periodically.

The present study sheds new light on the interplay between localization and fluctuations. This feature does not directly imply that equations (A.3) lead to the same stationary values \( \bar{p}_{ij} \) as the DK model, e.g., \( \bar{p}_{110} = I/(I + d_3) \bar{p}_{10} \).

A widely used simplification of the DK model is the Li–Rinzel (LR) model [15]. Therefore, we derive the function \( \kappa(\omega) \) (equation (19)) for this model, too. The LR model uses a timescale separation between Ca²⁺ activation and Ca²⁺ inhibition. Experiments show that the inhibitory processes are much slower than activation. Thus, it is possible to eliminate Ca²⁺ activation by using the corresponding equilibrium value. It results in a single gating variable \( p_i \), which denotes the fraction of states that are not yet inhibited. Its stationary value is

\[
\bar{p}_h = \frac{d_6}{d_6 + \bar{\epsilon}}.
\]  

As for the lumped states, the stationary values \( \bar{p}_{ij} \) calculated from the LR model result from the two of De Young and Keizer. For instance, we arrive at

\[
\bar{p}_{110}^{LR} = \frac{\bar{I}}{\bar{I} + d_1 \bar{\epsilon} + d_5} \bar{p}_{110}^{DK}
\]

for the fraction of open subunits. This identity is directly reflected in the calculation of \( \kappa(\omega) \) for the LR model. It can be cast into the form

\[
\kappa(\omega) = \left[ \frac{4(1 - \bar{p}_{110})}{\sqrt{4 - 3 \bar{p}_{110}^2}} - \frac{\bar{\epsilon}}{\bar{\epsilon} + d_6 + \bar{\epsilon}} \right]_{\bar{\omega}}
\]

that is very similar to equation (19).

**Appendix C. Numerical methods**

The geometry of the IP₃R cluster imposes considerations on the discretization. As stated above, the radius of the active area measures only tens of nanometers, but the outer boundary is 5–100 μm away. A constant grid size that sufficiently resolves the dynamics in the cluster would lead to an enormous computational effort. To reduce computation time, we use a grid with non-uniform spacing. The mesh size is sufficiently small for \( r \leq a_0 \) and saturates at a larger value in the bulk. It implies that the usual discretization of the radial Laplacian

\[
\nabla_r^2 = \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial}{\partial r} \right) = \frac{\partial^2}{\partial r^2} + \frac{2}{r} \frac{\partial}{\partial r}
\]

cannot be applied. Let \( \{r_i\} \) denote the set of grid points, \( \Delta r_i := r_i - r_{i-1} \) the spacing, and \( u_i \) an approximation to the concentration profile. Then a second-order scheme for equation (C.1) reads

\[
P_L(u_i) = \frac{1}{r_i^2} \left( \frac{r_i + \Delta r_i}{2} \frac{\partial^2 u_{i+1} - u_i}{\partial r^2} \frac{u_i + u_{i-1}}{\Delta r_i} - \left( \frac{r_i - \Delta r_i}{2} \frac{u_i - u_{i-1}}{\Delta r_i} \right) \frac{2}{\Delta r_i + \Delta r_i} \right)
\]  

(C.2)
Moreover, we adopt a first-order scheme for the time integration and 50% of the stability criterion [21].

**Glossary**

**Fixed element.** Any reactive substance that is restricted to small spatial regions and that possesses two distinct states: activated and deactivated. A fixed element participates only in a reaction when it is activated.

**Bistability.** Existence of two linearly stable solutions of a nonlinear system.

**Cytoplasm.** The fluid portion of the cell in which all other internal compartments, e.g., the endoplasmic reticulum, are embedded.

**Endoplasmic reticulum.** An extensive membranous network within the cell, which serves as a major intracellular Ca²⁺ store.

**Hopf bifurcation.** Emergence or disappearance of an oscillatory solution upon variation of a parameter in a nonlinear system.

**Linear stability.** Property of a stationary solution of a nonlinear system. The system relaxes back to this stationary state upon any infinitesimal perturbation from this state, if it is linearly stable, and amplifies any infinitesimal perturbation, if it is linearly unstable.

**Receptor.** A specialized protein on a cell’s membrane that binds to substances that effect the activities of the cell.

**Saddle node bifurcation.** Emergence or disappearance of two stationary states upon variation of a parameter in a nonlinear system.

**Second messenger.** A chemical signal that relays a hormonal message from a cell’s surface to its interior.

**References**

[1] Adkins C E and Taylor C W 1999 Lateral inhibition of inositol 1,4,5-trisphosphate receptors by cytosolic Ca²⁺. *Curr. Biol.* 9 1115–8
[2] Alberts B, Bray D, Lewis J, Raff M, Roberts K and Watson J D 1994 *Molecular Biology of the Cell* (New York: Garland)
[3] Bär M, Falcke M, Tsimring L and Levine H 2000 Discrete stochastic modeling of calcium channel dynamics *Phys. Rev. Lett.* 84 5664–7
[4] Berridge M J, Lipp P and Bootmann M D 2000 The versatility and universality of calcium signaling *Nature Rev. Mol. Cell Biol.* 1 11–21
[5] Bers D 2002 Cardiac excitation-contraction coupling *Nature* 415 198–205
[6] Bezprozvanny I, Watras J and Ehrlich B E 1991 Bell-shaped calcium-response curves of Ins(1,4,5)P₃ - and calcium-gated channels form endoplasmic reticulum of cerebellum *Nature* 351 751–4
[7] De Young G W and Keizer J 1992 A single inositol 1,4,5-trisphosphate-receptor-based model for agonist-stimulated oscillations in Ca²⁺ concentration *Proc. Natl Acad. Sci. USA* 89 9895–9
[8] Falcke M 2003 On the role of stochastic channel behavior in intracellular Ca²⁺ dynamics *Biophys. J.* 84 42–56
[9] Falcke M 2004 Reading the pattern in living cells—the physics of Ca²⁺ signaling *Adv. Phys.* 53 255–440
[10] Falcke M, Tsimring L and Levine H 2000 Stochastic spreading of intracellular Ca²⁺ release *Phys. Rev. E* 62 2636–43
[11] Howard M and Rutenberg A 2003 Pattern formation inside bacteria: fluctuations due to low copy number of proteins *Phys. Rev. Lett.* 90 128102–14
[12] Jiang Q-X, Thrower E C, Chester D W, Ehrlich B E and Sigworth F J 2002 Three-dimensional structure of the type 1 inositol 1,4,5-trisphosphate receptor at 24 Å resolution *EMBO J.* 21 3575–81
[13] Jung P and Shuai J W 2001 Optimal sizes of ion channel clusters *Eurphys. Lett.* 56 29–35
[14] Keener J and Sneyd J 1998 *Mathematical Physiology* (New York: Springer)
[15] Li Y and Rinzler J 1994 Equations for InsP₃ receptor-mediated [Ca²⁺], oscillations derived from a detailed kinetic model: a Hodgkin-Huxley like formalism *J. Theor. Biol.* 166 461–73
[16] Mak D D, McBride S and Foskett J K 1998 Inositol 1,4,5-trisphosphate activation of inositol tris-phosphate receptor Ca²⁺ channel by ligand tuning of Ca²⁺ inhibition *Proc. Natl Acad. Sci. USA* 95 15821–5
[17] Mak D D, McBride S and Foskett J K 2001 Regulation by Ca²⁺ and Inositol 1,4,5-trisphosphate (InsP₃) of single recombinant type 3 InsP₃ receptor channels: activation uniquely distinguishes type 1 and type 3 InsP₃ receptors *J. Gen. Physiol.* 117 435–46
[18] Mak D D, McBride S and Foskett J K 2003 Spontaneous channel activity of the inositol 1,4,5-trisphosphate (InsP₃) receptor (InsP₃R). Application of allosteric modeling to calcium and InsP₃ regulation of the InsP₃R single-channel gating *J. Gen. Physiol.* 122 583–603
[19] Marchant J S and Parker I 2001 Role of elementary Ca²⁺ puffs in generating repetitive Ca²⁺ oscillations *EMBO J.* 20 65–76
[20] Miyazaki S, Shirakawa H, Nakada K and Honda Y 1993 Essential role of the inositol 1,4,5-trisphosphate receptor/Ca²⁺ release channel in Ca²⁺ waves and Ca²⁺ oscillations at fertilization of mammalian eggs *Dev. Biol.* 158 62–76
[21] Press W, Teukolsky S, Vetterling W and Flannery B 2002 *Numerical Recipes in C++* 2nd edn (Cambridge: Cambridge University Press)
[22] Ramos-Franco J, Fill M and Mignery G A 1998 Isoform-specific function of single inositol 1,4,5-trisphosphate receptor channels *Biophys. J.* 75 834–9
[23] Shuai J and Jung P 2002 Stochastic properties of Ca²⁺ release of inositol 1,4,5-trisphosphate receptor clusters *Biophys. J.* 83 87–97
[24] Swillens S, Dupont G, Combettes L and Champeil P 1999 From calcium blips to calcium puffs: theoretical analysis of the requirements for interchannel communication *Proc. Natl Acad. Sci. USA* 96 13750–5
[25] Szalai G, Krishnamurthy R and Hajnoczky G 1999 Apoptosis driven by IP₃–linked mitochondrial calcium signals *EMBO J.* 18 6349–61
[26] Thul R and Falcke M 2004 Release currents of IP₃ receptor channel clusters and concentration profiles *Biophys. J.* 86 2660–73
[27] Thul R and Falcke M 2004 Stability of membrane bound reactions *Phys. Rev. Lett.* 93 181103-1–4
[28] Yao Y, Choi J and Parker I 1996 Quantal puffs of intracellular Ca²⁺ evoked by inositol trisposphate in xenopus oocytes *J. Physiol.* 482 533–54