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Calcium Dynamics in Neuronal Microdomains: Modeling, Stochastic Simulations, and Data Analysis

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Definition

Calcium is a key but a ubiquitous messenger in cell physiology. Yet direct electrophysiological or light imaging measurements are limited by the intrinsic small nano- to micrometer space where chemical reactions occur and also by the small number of molecules. Thus any fluorescence dye molecule added to measure the number of calcium ions can severely perturb the endogenous chemical reactions. Over the years, an alternative approach based on modeling, mathematical analysis, and numerical simulations has demonstrated that it can be used to obtain precise quantitative results about the order of magnitude, rate constants, the role of the cell geometry, and flux regulation across scales from channels to the cell level.

The aim of this ECN is to present physical models of calcium ions from the molecular description to the concentration level and to present the mathematical tools used to analyze the model equations. From such analysis, asymptotic formulas can be obtained, which are usually valid for a certain range of parameters. However these formulas allow exploring at low cost the parameter space. The methods to analyze these equations are part of the classical analysis of partial differential equations and stochastic processes, which will not be reviewed here (see Schuss 1980, 2010a). We shall present several models related to diffusion, where formulas can be derived, and we shall specify how these formulas are used to extract parameters from experimental measurements. But in general models are far too complicated to lead to equations that can be analyzed, and most of the time, numerical simulations have to be built. Building rational simulations requires discretizing the physical equations and bridging the gap between the limits of the equations and the physical description that they account for. We will present here several stochastic simulations and their rules, limitations, and tricks that have been developed over the years. As we shall see here, any bottleneck in the equation can lead to heavy simulations running for days. In that case, coarse graining is a key step to reduce the complexity of the equation so that some analysis can be obtained and can be used to check in some limit the validity of the simulations.

All together physical modeling, mathematical analysis, numerical simulation, and their application to the statistical analysis of experimental data form an ensemble of approaches that are used today to better understand molecular interaction in nano- to microdomains. But the most striking convergence of these methods is to derive physiological laws from their first physical principles. We shall present (1) stochastic modeling of calcium ions and their trajectories, (2) modeling of local interactions with discussion of the rate constants, (3) derivation of asymptotic formulas for the residence of calcium in microdomains and dendritic spines, (4) modeling and simulation of diffusion in a crowded three-dimensional dendrite, (5) modeling of calcium in the spines, (6) modeling the CaM activation pathway, and (7) modeling of coarse-grained Markov chain to estimate the probability that the number of calcium ions bound to key molecule is reached. Finally, we shall discuss

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Detailed Description

Neuronal Microdomains

Neurons can be decomposed into several key microdomains involved in specific functions: dendrites integrate electrical signals, whereas dendritic spines are microcompartments receiving the postsynaptic terminal of excitatory synapses (Fig. 1). The presynaptic terminal is also a key compartment that controls calcium in relation to vesicular release. Dendrites further decompose into distal and apical dendrites which seem to have different electrophysiological properties that can be correlated with a difference in the structure. The soma and the axon are also separate compartments. We show in Fig. 1 different distinct compartments. Finally, it was found that aspiny neurons can confine how these simulations can be used to study physiological processes such as transient calcium ions in a dendritic spine, long-term potentiation induction, or what limits the spread of calcium following synaptic stimulations.

Fig. 1 Neuronal microdomains. (a) Electron microscopy of a synapse. The postsynaptic terminal is located on a dendritic spine (S) branched in the dendrite (D). Located around the axon (A) are the glial cells (G). (b) Three-dimensional EM reconstruction of two dendrites from the hippocampus. The PSDs of excitatory synapses are marked in red and of inhibitory synapses in blue. Filopodia (marked F) and mushroom spines (marked M) are clearly seen. (c) All possible types of the dendritic spines, for example, a 3D reconstructed hippocampal dendrite, 3 weeks old in primary culture.
calcium for a time scale comparable to a dendritic spine (Goldberg et al. 2003). Can organelles such as vesicles or endoplasmic reticulum control calcium concentration?

**Diffusion in Neuronal Microdomains: Stochastic Modeling**

We start with a Brownian description of calcium ions. Indeed motion due to thermal fluctuations is the main driving force for the motion of particles such as ions or molecules. Calcium motion has been well approximated as diffusion, which confirms that the ionic charge is screened. This is not the case for calcium influx through channels, where the nanometer space restriction is a dominant factor for the interaction between the channel charges and the ion (Benazilla 2000; Eisenberg et al. 1995).

In cells, long distances are overcome mostly by the diffusion process. For example, free calcium ions diffuse in the dendritic spines or any neuronal microdomain in the dendrite or the axons (Korkotian et al. 2004). At the molecular level, diffusion of ions is described by a random walk. When an ion meets any plasma membrane, it is reflected or translocated inside an organelle at pumps or exchangers. The transition from one region to another can be modeled by specifying absorbing conditions in specific boundary regions, such as the fictive separation between a dendrite and a spine.

**Description of Calcium Stochastic Trajectories**

A calcium ion trajectory is described by the Smoluchowski limit in the large damping approximation of the Langevin equation. The position $x$ at time $t$ satisfies the stochastic equation

$$\gamma [\dot{x} - V(x, t)] + F(x) = \sqrt{2\gamma \epsilon} w.$$  (1)

Here $\epsilon = k_B T/m$, where $T$ is the temperature and $k_B$ the Boltzmann constant; $\gamma = 6\pi a \eta$ is the dynamical viscosity, where $\eta$ is the viscosity coefficient per unit mass and $a$ is the radius of the ion. $w$ is a random white noise modeling the thermal fluctuations, and the electrostatic force is

$$F(x) = -ze \nabla_x U_0(x),$$

where $U_0$ is the potential created by the site where the proteins are located. In a first approximation, each protein creates a localized parabolic potential, where the depth can be calibrated by using the backward-binding rate and the radius by using the forward-binding rate (Korkotian and Segal 2006).

The frictional drag force, $-\gamma [\dot{x} - V(x, t)]$, is proportional to the relative velocity of the ion and the cytoplasmic fluid. The field of fluid $V(x, t)$ induced by calcium ions will be discussed below.

**The Langevin Equations**

We shall here explain the physical consideration behind the reduction to Eq. 1. For a dendritic spine containing $N$ ions of different species (e.g., Ca$^{++}$, Na$^+$, Cl$^-$, and so on), $x_i(t)$ is the displacement vector of the $i$-th ion, $m_i$ is its mass, and $z_i$ is its valence. $\vec{x} = (x_1, x_2, \cdots, x_N)$ is the coordinate of the $N$ ions in configuration space. We consider a given flow field $V(x, t)$ (see description below) and that ions interact with a fixed potential of the charges on the proteins, $U_0(x)$, and with the variable potential of all other ions. The variable potential consists of both the electrostatic ion-ion interaction potential, $U_{ii}(\vec{x})$, and the potential of Lennard-Jones-type repulsions, $U_{LJ}(\vec{x})$ (that represents the finite size of the ions). The force per unit mass on the $i$-th ion is

$$F_i(\vec{x}) = -z_i e \nabla_x [U_0(x_i) + U_{ii}(\vec{x})] - \nabla x_i U_{LJ}(\vec{x}).$$
The dynamics of the $i$-th ion is given by the Langevin equation

$$\dot{x}_i + \gamma_i [\dot{x}_i - V(x_i, t)] + F_i(\dot{x}) = \sqrt{2\epsilon_i \gamma_i} \dot{w}_i,$$

(2)

where $\epsilon_i = k_BT/m_i$, $T$ is the temperature, $\gamma_i = 6\pi \eta_i a_i$ is the dynamical viscosity, $\eta_i$ is the viscosity coefficient per unit mass, and $a_i$ is the radius of the ion. The frictional drag force, $-\gamma [\dot{x}_i - V(x_i, t)]$, is proportional to the relative velocity of the ion and the cytoplasmic fluid. The accelerations $\dot{w}_i$ represent the thermal fluctuations of the fluid. The relation between the velocity diffusion constant and the friction coefficient,

$$D_i = \frac{k_BT}{m_i \gamma_i},$$

is Einstein’s fluctuation-dissipation principle (Schuss 1980). In the Smoluchowski limit of large damping (Schuss 1980) the Langevin equation (2) reduces to

$$\gamma_i [\dot{x}_i - V(x_i, t)] + F_i(\dot{x}) = \sqrt{2\epsilon_i \gamma_i} \dot{w}_i,$$

(3)

When neglecting the ion-ion interactions, we set $U_{ij}(\dot{x}) = U_{ii}(\dot{x}) = 0$, so that Eq. 3 becomes

$$\gamma_i [\dot{x}_i - V(x_i, t)] + F(x_i) = \sqrt{2\epsilon_i \gamma_i} \dot{w}_i,$$

(4)

where

$$F(x_i) = -z_i e \nabla x_i U_0(x_i).$$

Since we are interested in tracing only one species in the spine, namely, the concentration of calcium, we assume that $\gamma_i = \gamma_{Ca^{2+}}$, $m_i = m_{Ca^{2+}}$, $z_i = z = 2$. Under these assumptions, equations in (4) are independent and identical, so that their transition probability densities are identical. We denote the transition probability density function (pdf) of each ion by $p(x, t|x_0, t_0)$ so that the calcium concentration is

$$c(x, t) = \int_{\Omega} p(x, t|x_0, t_0)c_0(x_0)dx_0,$$

where $c_0(x_0)$ is the initial calcium density.

**Specification of the Hydrodynamic Flow**

The flow of the incompressible cytoplasmic fluid in the spines is generated by the local contraction of actin-myosin complexes saturated by calcium ions. We assume that the flow field is derived from a potential $\phi(x, t)$ (see, e.g., Landau and Lifshitz 1975),

$$V(x, t) = \nabla \phi(x, t).$$

(5)

The incompressibility condition, $\nabla \cdot V(x, t) = 0$, reduces to the Laplace equation in the head $\Omega_H$ of the spine at time $t$. The surface of the head, $\Sigma(t)$, is partitioned into the surface $\Sigma_{\partial H}(t)$ of the spine.
head, that does include the surface common with the neck, and the cap $\Sigma_N(t)$ of the surface of the head inside the neck, $\Sigma(t) = \Sigma_P(t) \cup \Sigma_N(t)$. The Laplace equation in $\Omega_H(t)$ is

$$\Delta y \phi(y, t) = 0 \quad \text{for} \ y \in \Omega_H(t), \ t > 0,$$

with the boundary conditions

$$\frac{\partial \phi(y, t)}{\partial n} \bigg|_{y \in \Sigma_H(t)} = -V(t), \quad \frac{\partial \phi(y, t)}{\partial n} \bigg|_{y \in \Sigma_N(t)} = F(V(t)),$$

where $V(t)$ is the average velocity induced by the deformation of the head (see Eq. 8 below) (Holcman and Schuss 2004), due to the sum of all the local contractions, and $F(V(t))$ is the induced field velocity at the top of the neck $\Sigma_N(t)$: for a volume displaced per unit time equal to $4\pi R^2(t)V(t)$ in dimension 3 and $2\pi R(t)V(t)$, where $R(t)$ is the instantaneous radius of the head, then $\dot{R}(t) = -V(t)$.

The flux through $\Sigma_N$ is $|\Sigma_N|v(t)$; hence

$$v(t) = F(V(t)) = \begin{cases} \frac{4\pi R^2(t)V(t)}{|\Sigma_N|} & \text{in dimension 3} \\ \frac{2\pi R(t)V(t)}{|\Sigma_N|} & \text{in dimension 2} \end{cases},$$

when the field is due to the contraction of myosin after 4 calcium ions are bound. The total number of sites bound to 4 calcium is $S^{(4)}(t)$ and can be obtained by solving a system of reaction-diffusion equations (Holcman and Schuss 2004). Finally the velocity at the boundary is given by

$$V(t) = \nu_Q S^{(4)}(t),$$

where $\nu_Q$ is a constant velocity. The quantities $V(t)$ and $F(V(t))$ are stochastic processes, that are proportional to the number of saturated proteins at any given time $t$. The flow field can be expressed explicitly in terms of the functions $V(t)$ and $F(V(t))$ by Green’s function for the Neumann problem for Poisson’s equation in a sphere (or a disk) through Stokes’ formula. Green’s function $G(x, y, t)$ is the solution (defined up to a constant) of the equation

$$-\Delta_y G(x, y, t) = \delta(x - y) - \frac{1}{|\Omega|} \quad \text{for} \ x, y \in \Omega_H(t)$$

$$\frac{\partial G(x, y, t)}{\partial \nu(y)} = 0 \quad \text{for} \ x \in \Omega_H(t), y \in \Sigma(t).$$

Multiplying Eq. 6 by $G(x, y, t)$ and Eq. 9 by $\phi(y, t)$ and integrating with respect to $y$ over the domain, using Stokes’ theorem and the boundary condition (7), we get
\[ \phi(x, t) = \int_{y \in \Sigma(t)} \frac{\partial \phi(y, t)}{\partial n} G(x, y, t) dS_y - \int_{y \in \Sigma(t)} \frac{\partial G(x, y, t)}{\partial n} \phi(y, t) dS_y \]

\[ + \frac{1}{V_H} \int_{\Omega_H(t)} \phi(y, t) dy \]

\[ = \int_{y \in \Sigma(t)} \frac{\partial \phi(y, t)}{\partial n} G(x, y, t) dS_y + \frac{1}{V_H} \int_{\Omega_H(t)} \phi(y, t) dy \]

\[ = -\int_{\Sigma_H(t)} V(t) G(x, y, t) dS_y + \int_{\Sigma_H(t)} F(V(t)) G(x, y, t) dS_y \]

\[ + \frac{1}{V_H} \int_{\Omega_H(t)} \phi(y, t) dy \]

\[ = -V(t) \int_{\Sigma_H(t)} G(x, y, t) dS_y + F(V(t)) \int_{\Sigma_H(t)} G(x, y, t) dS_y \]

\[ + \frac{1}{V_H} \int_{\Omega_H(t)} \phi(y, t) dy. \]

The flow field is given by

\[ \nabla \phi(x, t) = -V(t) \int_{\Sigma_H} \nabla_x G(x, y) dS_y + F(V(t)) \int_{\Sigma_H} \nabla_x G(x, y) dS_y. \]

In the neck, due to the symmetries and the uniform initial conditions, we simplify the flow field by assuming its velocity is parallel to the axis of the neck. It is given by

\[ \nabla \phi(x, t) = V(x, t) = F(V(t)) k, \]

where \( k \) is a unit vector along the axis of the neck. We note that according to Eq. 8, as the number of saturated proteins increases, the hydrodynamic flow begins to dominate the diffusion. In Holcman and Schuss (2004), and Holcman et al. (2004), we connected the strength of flow field to the number of bound myosin molecules induced by calcium. This results in nonlinear coupled partial differential equations, that can be solved numerically by stochastic simulations.

**Rate Constants and Molecular Dynamics**

We shall now recall how to model chemical reactions, described by the backward- and the forward-binding rate, which are usually obtained in aqueous solution, where diffusion is not limited by space. In confined microdomains where the number of ions involved can be small, the binding rates have to be reinterpreted.

**Backward-Binding Rate** The mean time that two molecules react chemically is modeled as the mean time the first molecule stays imprisoned in the potential well of the second. The random time interval between the binding and the reappearance of the binding molecule into the free state is exponentially distributed with a rate constant equal to the backward-binding reaction. The exponentially distributed waiting time for the backward reaction is based on Kramers’ theory of activated barrier crossing, as described in Matkowsky et al. (1982). Thus for transient chemical reactions, each
molecule reacting with a calcium ion has two consequences: first, the molecule can become activated, and second, the time course of calcium is delayed. The unbinding events are modeled as Poissonian processes. Fixing a scale $\Delta t$, the probability to unbind is $k_b \Delta t$ (take a uniform variable and check whether it is above or below $k_b \Delta t$).

**Forward-Binding Rate** The forward-binding rate $K_{\text{for}}$ corresponds to the flux of particles to the binding sites. Contrary to the backward-binding rate, this rate does not contain local properties only, but includes the effect of the global geometry of the domain, where the chemical reaction occurs. Such a rate has been computed at equilibrium by Smoluchowski and can be converted as the effective radius $R_a$ of a ball that mimics the binding site and so that the average probability that an ion meets such ball is equal to the forward rate. The radius is calibrated according to the formula

$$K_{\text{for}} = 2\pi R_a D [\text{Ca}^{2+}], \quad (10)$$

where $[\text{Ca}^{2+}]$ is the initial calcium concentration and $D$ the diffusion constant. This calibration can also be used to estimate the effective radius of a binding molecule in a transient state, calibrated for the initial concentration condition. When the binding sites are located on the boundary, the narrow escape formula should be used (Holcman and Schuss 2013a).

**Modeling the Interaction of Calcium Ion with Surface or Receptors** We shall now specify the interaction conditions between a calcium ion and a receptor or a membrane, the model at a molecular and population level, and the physical laws that can be derived from elementary physical principles.

**Absorption** Absorption at surface $\partial \Omega$ is the process by which a particle is removed after it hits $\partial \Omega$, which can be an artificial interface such as the one between the spine and the dendrite or an effective one such as a channel. Indeed, during a simulation, when an ion hits such a surface, it disappears. In general, absorption on a surface is modeled by killing the trajectories when it encounters or passes over the surface $\partial \Omega$. Thus the probability density function to make a transition from $x \in \partial \Omega$ to any point $y$ during time $t$ is zero: $p(y; t|y) = 0$.

**Partial Absorption** Partial absorption accounts for a probability that a particle arriving at a surface is reflected with a probability $p$ or absorbed with probability $1 - p$. This condition can be calibrated to describe the interaction between the moving particle and the binding site of a receptor. This condition is formulated at a molecular level for stochastic simulations or with a probability density function to describe the macroscopic level. This condition is called also radiation or reactive or Robin boundary conditions and has been widely used to describe diffusion in a biological cell with chemical reactions on its surface (Andrews and Bray 2004; Batsilas et al. 2003; Berezhkovskii et al. 2004; Erban and Chapman 2007; Monine and Haugh 2005; Lamm and Schulten 1983; Tai et al. 2003; Zwanzig 1990).

**Schematic Description** The overdamped Langevin equation can be written as a stochastic equation

$$\dot{x} = a(x, t) + \sqrt{2\sigma(x, t)} \dot{w}. \quad (11)$$
The process \( x(t) \) defined by Eq. 11 with partially absorbing boundaries can be defined as the limit of Markovian jump processes generated by the Euler scheme

\[
x_{\Delta t}(t + \Delta t) = x_{\Delta t}(t) + a(x_{\Delta t}(t), t)\Delta t + \sqrt{2\sigma(x_{\Delta t}(t), t)\Delta t} + w(t, \Delta t)
\]

for \( t \geq s \)

\[
x_{\Delta t}(s) = x
\]

in the interval \( x > 0 \), for \( 0 \leq t - s \leq T \), with \( \Delta t = T/N \), \( t - s = iT/N \) \((i = 0, \ldots, N)\), where for each \( t \) the random variables \( \Delta w(t, \Delta t) \) are normally distributed and independent with zero mean and variance \( \Delta t \). In dimension one, with boundary at 0, the boundary behavior for the simulated trajectories that cross the boundary, identified by

\[
x_{\Delta t}(t) + a(x_{\Delta t}(t), t)\Delta t + \sqrt{2\sigma(x_{\Delta t}(t), t)\Delta t} \Delta w < 0,
\]

is described by

\[
x_{\Delta t}(t + \Delta t) = \begin{cases} 
- (x_{\Delta t}(t) + a(x_{\Delta t}(t), t)\Delta t + \sqrt{2\sigma(x_{\Delta t}(t), t)\Delta w}) & \text{w.p.} 1 - P\sqrt{\Delta t} \\
\text{terminate trajectory otherwise.} & 
\end{cases}
\]

Thus the exiting trajectory is normally reflected w.p.

\[
R = 1 - P\sqrt{\Delta t}
\]

and is otherwise terminated (absorbed). The scaling of the termination probability with \( \sqrt{\Delta t} \) reflects the fact that the discrete unidirectional diffusion current at any point, including the boundary, is \( O(1/\sqrt{\Delta t}) \) (Singer et al. 2008). This means that the number of discrete trajectories hitting or crossing the boundary in any finite time interval increases as \( 1/\sqrt{\Delta t} \).

**Partial Reflecting Condition in Dimension Larger than 2**

The scheme (14) is generalized to diffusion with drift and anisotropic constant diffusion matrix \( \sigma(t) \) in the half space, \( x_1 > 0 \), with partial oblique reflection. The Robin boundary condition is recovered if and only if trajectories are reflected in the direction of the unit vector

\[
v = \frac{\sigma n}{\|\sigma n\|},
\]

where \( n \) is the unit normal to the boundary. The radiation parameter \( k(x, t) \) in the \( d \)-dimensional Robin boundary condition and the absorption parameter \( P(x) \) are related by

\[
k(x, t) = rP(x)\sqrt{\sigma_n(t)}, \quad x_1 = 0,
\]

with \( r = 1/\sqrt{\pi} \) and \( \sigma_n(t) = n^T\sigma(t)n \). The relation (17) can also be adapted for curved boundaries and applied to the tangent plane at each point of the boundary. Indeed this is due to the fact that a smooth local mapping of the domain to a half space with an orthogonal system of coordinates preserves the constant isotropic diffusion matrix, though the drift changes according to Itô’s formula. In this case the vector \( v \) coincides with the normal \( n \).
The reflection law and the relation are new for diffusion in higher dimensions. The constant \( r \) for the Euler scheme is not the same as that for other schemes, e.g., for a discrete random walk with radiation boundaries, \( r = 1/\sqrt{2} \). The reflection can be constructed explicitly. Indeed, the \( d \)-dimensional stochastic dynamic is

\[
\dot{x} = a(x, t) + \sqrt{2} B(t) \dot{w}
\]  

in the half space

\[
\Omega = \{ x = (x_1, x_2, \ldots, x_d) \in \mathbb{R}^d : x_1 > 0 \}
\]

where \( \dot{w} \) is a vector of \( d \) independent Brownian motions and when we assume that the diffusion tensor \( \sigma(t) = B(t)B^T(t) \) is uniformly positive definite for all \( t \geq s \). We use henceforward the abbreviation \( \sigma(t) = \sigma \). The radiation condition (35) becomes

\[
-J(y, t | x, s) \cdot n = \kappa(y, t) p(y, t | x, s), \quad \text{for } y \in \partial \Omega, \quad x \in \Omega,
\]

where the components of the flux vector \( J(y, t | x, s) \) are defined by

\[
J^k(y, t | x, s) = -[a^k(y, t) p(y, t | x, s)] + \sum_{j=1}^{d} \frac{\partial}{\partial y_j} [\sigma^{k,j} p(y, t | x, s)].
\]  

The Fokker-Planck equation for the pdf of \( x(t) \) can be written as

\[
\frac{\partial p(y, t | x, s)}{\partial t} = -\nabla y \cdot J(y, t | x, s) \text{ for all } y, x \in \Omega.
\]  

If \( x \in \Omega \), but

\[
x' = x + a(x, t) \Delta t + \sqrt{2} B(t) \Delta \dot{w}(t, \Delta t) \notin \Omega,
\]

the Euler scheme for Eq. 18 with oblique reflection in \( \partial \Omega \) reflects the point \( x' \) obliquely in the constant direction of \( v \) to a point \( x'' \in \Omega \), as described below. First, we denote by \( x'_B \) the normal projection of a point \( x' \) on \( \partial \Omega \), that is, \( x'_B = x' - (x' \cdot n)n \). Then we write the Euler scheme for Eq. 18 with partially reflecting boundary as

\[
x(t + \Delta t) = \begin{cases} 
  x' & \text{for } x' \in \Omega \\
  x'' & \text{w.p. } 1 - P(x'_B) \sqrt{\Delta t}, \text{ if } x' \notin \Omega, \\
  \text{terminate trajectory w.p. } P(x'_B) \sqrt{\Delta t}, \text{ if } x' \notin \Omega.
\end{cases}
\]  

The value of the termination probability \( P(x'_B) \sqrt{\Delta t} \), that varies continuously in the boundary, is evaluated at the normal projection of the point \( x' \) on the boundary. The oblique reflection in the direction of the unit vector \( v(v_1 \neq 0) \) is defined by

\[
x'' = x' - \frac{2x'_1}{v_1} v.
\]
Note that $x_{10} = \frac{1}{C_0}$ guarantees that the reflected point of a crossing trajectory is inside the domain $\Omega$. The fact that the normal components of $x_{00}$ and $x_0$ are of equal lengths makes the high-dimensional boundary layer analysis similar to that in one dimension. Normal reflection corresponds to $v = n = (1, 0, \ldots, 0)$. We note that for a point $y \in \Omega$, we can write $P_r[x' = y] = P[x' = y']$, where

$$y = y' - \frac{2y' \cdot n}{v_1} v$$

(24)

is the oblique reflection of $y'$ (see Fig. 2). If the scheme described above is not used, a paradox can arise (Singer et al. 2008): while the pdf of the solution of Eqs. 12 and 13 converges to the solution of the FPE Eq. 32 and the initial condition Eq. 34, it does not satisfy the boundary condition Eq. 35, leading to a boundary layer, due to the diffusion approximations in the Markovian jump process.

**Generic Modeling of Calcium Ions at Pumps or Exchangers**

Partial absorbing boundary condition can be used to model the behavior of calcium ion near a channel or a pump. However cooperativity should be implemented for each case at hand. For example, after entering inside an exchanger, an ion takes a certain time to exit: this is modeled by changing a partial reflecting boundary condition to a reflecting one as long as the ion is inside the exchanger.

Some pumps can work with several ions. As the intrinsic biophysical mechanism of permeability is not necessarily understood, there is no consensus for a universal coarse-graining scheme of ion extrusion. If two ions are required, we propose that once the first ion enters the channel, it cannot move before another has hit the binding area. If the second ion enters while the first one is not returned (after an exponential waiting time), then the first one can be extruded. During that time, no other ions can enter the channel.
Modeling Calcium Influx from Channels: NMDA Receptors, AMPA Receptors, and VSCC

We shall now present examples of three classical channels such as NMDA, AMPAR (GluR2-calcium permeable), and voltage-sensitive calcium channel (VSCC) for simulating the flux of calcium inside neuronal cells. The flow of ions through open channels has been studied using Langevin equation (Eisenberg et al. 1995; Roux et al. 1995). The fluxes of ions can be implemented numerically as follows.

Calcium Influx Through NMDAR

Calcium influx through NMDA channels can be approximated by (Koch 1999, p. 99)

\[ I_N(t) = g_N \frac{e^{-t/\tau_{N,1}} - e^{-t/\tau_{N,2}}}{1 + 0.33[Mg^{2+}]e^{-0.061V_m}}(V_m - E_N), \]

(25)

where the conductance is \( g_N = 0.16 \) nS and the Nernst potential \( E_N = 0 \), \( V_m \) is the membrane potential. When the potential \( V_m \) is fixed and the fraction of current carried by calcium ions is 15\%, we can simulate such a flux by injecting particles at random times such that the instantaneous rate is the one obtained from relation Eq. 25. Indeed, the entrance is a Poissonian process with a time-dependent rate \( \lambda(t + \Delta t) = \frac{I_{Ca}(t)\Delta t}{2e} \). The number of entering ions is \( N(t)\Delta t = \frac{I_{Ca}(t)\Delta t}{2e} \), where \( I_{Ca}(t)\Delta t = \int_t^{t+\Delta t} I_{Ca}(t)dt \). An example of stochastic calcium ion entry is presented in Fig. 4 (discretized at a time step \( \Delta t = 0.1 \) ms). In some dendritic spine models (Holcman et al. 2004; Holcman and Schuss 2004), the entrance dynamics is neglected and ions are initially placed at channels, located at the top of the dendritic spine head. Typically, the total charge is \( Q_N = \int_0^\infty I(s)ds \); thus the fraction of calcium is \( Q_{Ca} = 0.15 Q_N \) Coulomb. The number of calcium ions entering is \( N_{N, Ca} = \frac{Q_{Ca}}{2e} \), where the \( e \) is the electron charge. Using parameters of Table 1, we obtain that \( Q_N = 6.38 pC \) and thus there are about \( N_{N, Ca} = 3,000 \) ions entering in average inside a single NMDA receptor (for a fixed mean voltage \( V_m = -65.1 \) mV).

Calcium Influx Through AMPAR

The stochastic arrival of ions entering through an AMPAR is computed exactly with the same method as for NMDA, except that the mean flux is given by

\[ I_A = g_A \frac{t}{\tau_A} e^{-t/\tau_A}(V_m - E_A), \]

(26)

where \( g_A = 0.3 \) nS and the Nernst potential \( E_A = 0 \) mV. The fraction of AMPAR current carried by calcium ions is 1.4\%; thus we find that the total charge is \( Q_A = 11.51 fC \), leading to approximately 500 ions.

Influx Through VSCC

Calcium influx through VSCC requires computing the changes in the membrane potential depolarization. One possibility is to use the simplified Hodgkin-Huxley model (Hille 2001). The voltage change follows the dynamics

\[
\begin{align*}
C \frac{dV}{dt} &= -I_{Na}(V, n) - I_{K}(V, n) - I_{L}(V) - I_{Ca}(V, m, h) - \gamma I_N - \eta I_A \\
\frac{dx}{dt} &= 0.1 \frac{E_{Na}(1-x) - E_N}{\tau_{Na}}, \quad \text{for } x = n, m, h,
\end{align*}
\]
where the currents are

\[ I_{Na} = g_{Na} f^3 (0.89 - 1.1 n) (V - E_{Na}) \]
\[ I_K = g_K n^4 (V - E_K) \]
\[ I_{Ca} = g_{Ca} m^3 h (V_m - E_{Ca}) \]
\[ I_L = g_L (V - E_L) \]

with parameters

\[ p = \frac{\alpha_p}{\alpha_p + \beta_p}, \quad \alpha_K = \frac{\theta_K - V_m}{\tau_K}, \quad \beta_K = \eta_k e^{\frac{V_m - 65}{zK}}, \text{ for } k = n, m, h, p. \]

where \( \gamma \) and \( \eta \) are summarized in Table 1. The total charge becomes \( Q_V = 0.6 fC \), which leads approximately to 2,000 ions entering through VSCC.

### Stochastic Simulation of Calcium in a Dendritic Spine

The major benefit of stochastic simulations is to access the total number of biochemical bonds induced by calcium ions on specific molecules and to quantify the amount of structural changes occurring at the spine level. There are many other consequences such as computing the hydrodynamic component that changes the nature of the ion trajectories or distinguishing the periods of calcium dynamics. Novel coarse-grained equations are derived in (Holcman and Schuss 2004).

### Space Exploration

The geometric characteristics of ionic trajectories with the hydrodynamic flow are distributed differently from pure diffusion (Holcman and Schuss 2004) (see Fig. 3). Not only the nature of the movement is different, but the hydrodynamic flow causes the ions to drift in the direction of the neck, and consequently the time they spend in the spine head is reduced. As a consequence, the probability of a trajectory to leave through a pump located in the head decreases. Similarly, the probability to return to the head from the spine neck is reduced if it has to diffuse upstream, against

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( g_{Na} )</td>
<td>NMDAR conductance</td>
<td>0.16 nS</td>
</tr>
<tr>
<td>( E_{Na} )</td>
<td>Equilibrium potential (NMDAR)</td>
<td>0 mV</td>
</tr>
<tr>
<td>( \tau_{n,1} )</td>
<td>NMDAR time constant</td>
<td>11.5 ms</td>
</tr>
<tr>
<td>( \tau_{n,2} )</td>
<td>NMDAR time constant</td>
<td>0.67 ms</td>
</tr>
<tr>
<td>( g_A )</td>
<td>Conductance (AMPA)</td>
<td>0.3 nS</td>
</tr>
<tr>
<td>( E_A )</td>
<td>Equilibrium potential (AMPA)</td>
<td>0 mV</td>
</tr>
<tr>
<td>( \tau_A )</td>
<td>AMPA time constant</td>
<td>0.2 ms</td>
</tr>
<tr>
<td>( I_N(V_{NMDAR}) )</td>
<td>Total charge entering ( I_N ) (NMDAR)</td>
<td>( 1.11 ) ( \times ) 10^{-15} C</td>
</tr>
<tr>
<td>( I_V(V_{NMDAR}) )</td>
<td>Total charge entering ( I_V ) (NMDAR)</td>
<td>( 1.11 ) ( \times ) 10^{-15} C</td>
</tr>
<tr>
<td>( I_{Ca}(V_{NMDAR}) )</td>
<td>Calcium ions entering through NMDAR</td>
<td>( \approx 3000 ) ions</td>
</tr>
<tr>
<td>( I_{Ca}(VSCC) )</td>
<td>Calcium ions entering through VSCC</td>
<td>( \approx 2000 ) ions</td>
</tr>
</tbody>
</table>
Fig. 3  The filling of space by five random trajectories in the spine with no drift (a) and with drift (b) Each color corresponds to a trajectory. Proteins are uniformly distributed in the spine head and are represented by circles and crossed circles, respectively. A trajectory starts at the top of the spine head where channels are located and continues until it is terminated at the dendritic shaft or at an active pump. The parameters for the simulation are $\delta_1 = 0.02 \, \mu m$, $\delta_2 = 0.01 \, \mu m$, $K_{\text{back}}^{\text{AM}} = 10^4 s^{-1}$, $K_{\text{back}}^{\text{cal}} = 2.10^3 s^{-1}$, $R = 0.5 \, \mu m$, $d/2 = 0.21794 \, \mu m$, $l = 1.5 \, \mu m$, $N_{\text{pumps}} = 10$.

Fig. 4  Calcium entry and dynamics. (a) Time course of calcium entry inside a spine through an NMDA receptor. (b) Schematic representation of the calcium and calmodulin (CaM) pathway inside a dendritic spine (Guerrier and Holcman 2014a)
the hydrodynamic drag force. Thus the ionic trajectory stays inside the spine a shorter time in the presence of the hydrodynamic flow, as compared to the time without it. As discussed in (Holcman et al. 2004), the total number of bound molecules can change as much as 30% with and without the flow.

**Two Stages of Calcium Concentration Decay**

Two very distinct decay rates of the fast extrusion periods have been reported in (Majewska et al. 2000; Sabatini et al. 2001). The second decay period is identified as purely driven by random movement, and the time constant equals the first eigenvalue of the Laplacian on the spine domain, with the adequate boundary conditions. It was shown that the first period corresponds to a fast calcium extrusion, measured in (Majewska et al. 2000) with an exponential decay rate $\lambda = 0.14 \text{ s}^{-1}$ and is due to the diffusion of saturated buffers, binding kinetics of endogenous buffers, diffusion of buffers, buffered calcium diffusion across the spine neck, and the effect of the pumps. The first period dynamics is defined by the fast binding to calcium stores (Majewska et al. 2000; Sabatini et al. 2001). On the other hand, in the simulation resulting from the model (Holcman et al. 2004), based on fast spine motility, the first time period has an exponential decay rate, constant $\lambda_t = 0.16 \text{ s}^{-1}$ derived in Holcman and Schuss (2004). The decay seems to be a consequence of the dynamics created by the push effect, since stores were neglected. Further studies that include large numbers of buffers should reveal the precise contribution of buffers to the calcium fast decay rate, as compared to the rate imposed by the spine contraction.

**Multiscale Modeling of Connecting a Continuum Bath with Single Molecular Dynamics**

There are various interesting multiscale modeling approaches where the goal is to connect a discreet description of Brownian particles with a continuum (Franz et al. 2013; Flegg et al. 2012). The conservation of fluxes at the connecting interface generates boundary layer behavior that needs to be specifically studied.

**Recipe for a Successful Stochastic Simulation**

- **Choosing a time step for a simulation.** The time step of a simulation is critical: when it is too small, the simulations take forever, while if it is too large, then binding to buffers of a certain size is missed. For a time step $\Delta t$ and a diffusion coefficient $D$, a Brownian particle moving in three dimensions jumps to a distance $\Delta x^2 = 6D\Delta t$. Thus the constraint $|\Delta x| = a/4$ provides an estimate for the time step. In general the size of the smallest target defines the condition for the time step $\Delta t$. In most cases, where target sites are fixed, it is more interesting to refine the time step when a particle enters its neighborhood.

- **Positioning a particle that has unbound.** In the Smoluchowski limit equation (where there is no dynamic for the velocity), the coarse graining of a potential well is usually done by freezing the position of the particle at the binding position during an exponential waiting time, the rate of which is the backward rate (reciprocal of the mean time to escape the well). After the particle unbinding, where should the particle be positioned? Certainly not at the absorbing boundary defining the well; otherwise the particle is immediately absorbed, unless the boundary condition is changed from absorbing to reflecting during a certain refractory time. Another possibility is to position the particle outside the boundary layer of the binding site: a small distance away from the site (3–4 radii away). This is possible if the sites are not surrounded by other absorbing sites. Inside a structure that contains a high concentration of binding sites, a different simulation approach is needed: either to derive a homogenized equation or the dynamics in the wells should not be coarse grained.
• **Partial absorption.** When there are many absorbing sites located at close proximity, nonlinear effects should be taken into account. It is possible to derive homogenized boundary conditions (see for details (Taflia and Holcman 2011)).

• **Coarse graining a simulation with the narrow escape rate.** Instead of running a full Brownian simulation where the position of each calcium ion and calmodulin molecule is computed with the Euler scheme, it is possible to coarse grain it into a rate model based on the narrow escape theory (Schuss and Holcman 2013; Holcman and Schuss 2013a). The gain of this procedure is to avoid lengthy simulation imposed by the smallest length. In a Brownian simulation, due to the small calmodulin-binding site of radius $R_{CaM} = 2 \text{ nm}$, in order to ensure that the process does not jump over the site, the MSD formula leads to a time step of $10^{-6}$ ms (with $D_{Ca} = 200 \mu \text{m}^2 \text{ms}^{-1}$), which would lead to days of simulations. To circumvent this difficulty, it is possible to compute directly the rate of arrival of calcium ions to one of the free calmodulin-binding sites. The mean first binding time $\tau_B$ of a Brownian particle diffusing with diffusion coefficient $D_1$ in a spherical domain $\Omega$, to a small spherical target (radius $r$) diffusing with coefficient $D_B$, is $\tau_B = \frac{4r^4}{(D_1 + D_B)^{5/2}}$. Due to the small size $r$, the binding times are exponentially distributed with a mean $\lambda_1 = \frac{1}{\tau_B}$. For $N$-independent calcium ions diffusing within $\Omega$, the first binding time $T_b$ to a calmodulin site is distributed with rate

$$\hat{\lambda}(N) = \lambda_1 N.$$  \hspace{1cm} (27)

Thus, the probability that a binding event occurs between time $t$ and $t + \Delta t$ is $P(t \leq T_b < t + \Delta t) = \hat{\lambda}(N) \Delta t$. An application consists in replacing the Brownian movement of calcium ions in a spine head by the binding rate $\hat{\lambda}(N) \Delta t$ at each calmodulin site. We thus need to compute at each time step the number $N$ of free calcium in the spine. Furthermore, calcium ions can escape a dendritic spine head $\Omega_{\text{head}}$ through small pumps. To model this calcium escape, we use a similar method as described above where we approximate the binding of calcium to a pump by using the mean first passage time of a diffusing ion to a small target located on the boundary. When there are $N$ calcium ions, the first binding time is

$$\mu(N) = \frac{4R_{\text{pump}} D_{Ca} N}{|\Omega|_{\text{head}}}.$$  \hspace{1cm} (28)

It is also possible to account for the competition with escaping through the spine neck using the narrow escape formula for a spine (Eq. 45). This procedure accelerates the simulations and gives excellent results (Fig. 5).

In Figs. 4, 5, we present a simulation of calcium entry in a dendritic spine binding to calmodulin (Guerrier and Holcman 2014a).

**Diffusion Laws in Microdomains with Small Openings**

Synaptic input creates calcium transients in single dendritic spines and dendrites (Fig. 6). We shall now present the modeling approach used to study calcium transients.
The stochastic nature of the calcium motion requires a probabilistic approach. Indeed, the location of an ion is not certain, and the probability $p(x,t)$ to find an ion at time $t$ at a position $x$ satisfies the standard Fokker-Planck or diffusion equation

$$\frac{\partial p}{\partial t}(x,t) = D\Delta p(x,t),$$

(29)

where $\Delta$ is the Laplacian operator and $D$ is the diffusion constant in the cytoplasm. The solution of Eq. 29 requires specifying initial and boundary conditions and allows the entire characterization of a transient regime or the steady state distribution of a single ion. For a general domain, the solution cannot be derived analytically, but it is possible to obtain long- and short-time asymptotic estimations. As we shall see, these expressions provide the dependency with respect to many geometrical parameters. When necessary, numerical simulations are used to obtain the missing information.
They are usually tedious to obtain and require careful discretization of the domain, especially when small and large scales are present. For many independent calcium ions, the concentration $c(x,t)$ is given by $c(x,t) = Np(x,t)$, where $N$ is the initial number of ions. Ignoring at this stage the effect of any chemical reaction, the microdomain geometry $\Omega$ is the main determinant of the characteristic time scale involved in diffusion. When the boundary decomposes into two parts, $\partial\Omega_a$ the absorbing part, made of key fast-binding elements, and $\partial\Omega_r$ the reflective part, then it is usually a critical aspect to quantify the mean time $\mathbb{E}(\tau)$ for an ion to reach $\partial\Omega_a$. The boundary conditions are

**Fig. 6** *Upper: Isolated calcium transients in a single dendritic spine.* Synaptic activity generates a transient calcium change in a spine and a whole dendritic segment, triggered by a back-propagating action potential. *Lower: Spontaneous calcium activity in neurons.* Synchronized and not synchronized activity as well as small (probably EPSPs), larger (probably single spike), and very large events (burst of several spikes). The decay phase of calcium is approximated by exponentials or sum of exponentials.
\[
\frac{\partial p(x, t)}{\partial n} = 0 \text{ on } \partial \Omega_r, \\
c(x, t) = 0 \text{ on } \partial \Omega_a.
\]

The general solution of the diffusion equation can be formally expanded as

\[
c(x, t) = \sum_{k=1}^{\infty} c_k u_k(x) \exp(-\lambda_k t)
\]

where \(\lambda_k > 0\) are the eigenvalues; \(u_k, k = 1\), are the eigenfunctions; and \(c_k\) are the constants. The general explicit computation of the solution of diffusion equations can be found in Carslaw and Jaeger (1959) and Crank (1975). Although expression (30) justifies fitting a sum of exponentials to experimental data, connecting the eigenvalues with the precise geometry is in general very difficult, except in a few cases where the geometry contains a narrow passage or small hole. This is the case of a dendritic spine or a narrow domain in dendrites. When \(|\partial \Omega_a| \ll |\partial \Omega_r|\), the reciprocal of the mean time to escape a domain \(E(t)\) is the first eigenvalue (Schuss et al. 2007). Indeed, \(1/\lambda_0\) is usually very large, so that there is a large gap with the rest of the eigenvalue \(\lambda_0 \ll \lambda_1\), and thus the solution 30 can be further approximated by a single exponential for a time \(t > 1/\lambda_1\),

\[
c(x, t) \approx c_0 \exp(-\lambda_0 t).
\]

We conclude that for domains with narrow neck, the arrival of diffusing particles to the small domain is almost Poissonian. This is a consequence of the geometry.

**Transition Probability Density Function with Partial Reflecting Boundary Condition**

The transition probability density function (pdf) of the limit process \(11, p(y, t|x, s) = \Pr\{x(t) = y| x(s) = x\}\), is the solution of the FPE

\[
\frac{\partial p(y, t|x, s)}{\partial t} = -\frac{\partial [a(y, t)p(y, t|x, s)]}{\partial y} + \frac{\partial^2 [\sigma(y, t)p(y, t|x, s)]}{\partial y^2},
\]

or equivalently,

\[
\frac{\partial p(y, t|x, s)}{\partial t} = -\frac{\partial J(y, t|x, s)}{\partial y} \text{ for all } y, x > 0,
\]

where

\[
J(y, t|x, s) = a(y, t)p(y, t|x, s) - \frac{\partial [\sigma(y, t)p(y, t|x, s)]}{\partial y}
\]

is the flux. The initial condition is

\[
p(y, t|x, s) \to \delta(y - x) \text{ as } t \downarrow s,
\]

and the radiation boundary condition is
\[-J(0,t|x,s) = \kappa p(0,t|x,s), \quad (35)\]

where \(\kappa\) is a constant related to the constant \(c\) and to the values of the coefficients at the boundary. The no flux and Dirichlet boundary conditions are recovered if \(c = 0\) and \(c = \infty\), respectively. The relation between the reactive “constant” \(\kappa(t)\) and the absorption parameter \(P\) for the dynamics (Eq. 11) on the positive axis with drift and with a variable diffusion coefficient is

\[\kappa(t) = rP\sqrt{\sigma(0,t)}, \quad r = \frac{1}{\sqrt{\pi}}. \quad (36)\]

The relation Eq. 36 is true for diffusion with variable coefficients. The value \(r = 1/\sqrt{\pi}\) is different than values obtained for other schemes, e.g., than the value \(r = 1/\sqrt{2}\), predicted by the discrete random walk theory of radiation boundaries (Collins and Kimball 1949). Values of \(r\) for other schemes are given in Erban and Chapman (2007).

**Exit Diffusion Rate from Dendritic Spines**

We summarize in this section the approach used to derive asymptotic formulas for the rate of diffusional exit from the spines. A dendritic spine with a narrow neck has very degenerate geometry (Andrews and Bray 2004), but the exit time of a diffusing particle, which is the reciprocal of the first eigenvalue, can be directly measured from fluorescence imaging. We shall now recall the main formula for the exit rate that was obtained in the context of the narrow escape (NET) and dire strait (DST) theory (Holcman and Schuss 2013b, c).

A free Brownian particle moves in a bounded domain \(D \subset \mathbb{R}^d(d = 2, 3)\), whose boundary \(\partial \Omega\) is sufficiently smooth (the analysis in higher dimensions is similar to that for \(d = 3\)). The Brownian trajectory \(x(t)\) is reflected at the boundary, except for a small hole \(\partial \Omega_{ur}\), where it is absorbed, as shown in Fig. 7. The reflecting part of the boundary is \(\partial \Omega_r = \partial \Omega \setminus \partial \Omega_{ur}\). The lifetime in \(\Omega\) of a Brownian trajectory that starts at a point \(x \in \Omega\) is the first passage time \(\tau\) of the trajectory to the absorbing boundary \(\partial \Omega_{ur}\). The NET

![Fig. 7 Brownian escape from a spherical window](image)
\[ v(x) = \mathbb{E}[\tau | x(0) = x] \] (37)

is finite under quite general conditions (Schuss 2010b). As the size (e.g., the diameter) of the absorbing hole decreases to zero, but that of the domain remains finite, the NET increases indefinitely. A measure of smallness can be chosen as the ratio between the surface area of the absorbing boundary and that of the entire boundary, for example,

\[ \varepsilon = \left( \frac{|\partial \Omega_a|}{|\Omega|} \right)^{1/(d-1)} \ll 1, \] (38)

provided that the isoperimetric ratio remains bounded:

\[ \frac{|\partial \Omega|^{1/(d-1)}}{|\Omega|^{1/d}} = O(1) \text{ for } \varepsilon \ll 1. \] (39)

The NET \( v(x) \) can be obtained by solving the Pontryagin-Andronov-Vitt (PAV) mixed boundary value problem for Poisson’s equation (Pontryagin et al. 1933; Pontryagin et al. 1989; Schuss 2010b)

\[ \Delta v(x) = -\frac{1}{D} \text{ for } x \in \Omega \] (40)

\[ v(x) = 0 \text{ for } x \in \partial \Omega_a \] (41)

\[ \frac{\partial v(x)}{\partial n(x)} = 0 \text{ for } x \in \partial \Omega_r, \] (42)

where \( D \) is the diffusion coefficient and \( n(x) \) is the unit outer normal vector to the boundary at \( x \in \partial \Omega \). For a circular window of radius \( a \ll |\partial \Omega|^{1/2} \) (Fig. 7),

\[ \mathbb{E}_x \tau = \frac{|\Omega|}{4aD \left[ 1 + \frac{L(0) + N(0)}{2\pi} \text{log} a + o(\text{log} a) \right]} \text{ for } a \ll |\partial \Omega|^{1/2}. \] (43)

The MFPT to the absorbing boundary at the end of the funnel of a solid of revolution obtained by rotating the symmetric planar domain \( \mathcal{Q} \) (see Holcman and Schuss (2013a) of Sect. 7) is given by

\[ \tau = \frac{1}{\sqrt{2}} \left( \frac{\ell_+}{a'} \right)^{3/2} \frac{V}{\ell_+ D (1 + o(1))} \text{ for } a' \ll \ell_+, \] (44)

where \( V = |\Omega| \) is the volume of the domain. The NET \( \tau_{x \to \partial \Omega_a} \) of a diffusing narrow circular cylinder of cross-sectional area \( \pi a^2 \) is given by
where $R_c$ is the curvature at the cusp. The asymptotic expression is derived in Holcman and Schuss (2011). The order 1 term can be computed for the sphere using the explicit expression of the Neumann-Green function (Cheviakov et al. 2010). When the spine radius is small, the leading order term for the mean exit time (which is also the rate of diffusion extrusion) was initially presented in Svoboda et al. (1996). This term accounts for the many returns of an ion between the spine neck and head (Biess et al. 2007). This term is not present when an ion cannot return to the head once it enters the neck (Korkotian et al. 2004). Because the other terms in formula 45 diverge to infinity, their contribution cannot be neglected and they affect significantly the residence time of a diffusing particle in a dendritic spine (see Fig. 8). We conclude from these analytical formulas that the spine connection determines the rate of extrusion. For short spines, all terms are significant.

Remarks The presence of a spine apparatus inside the spine might affect the extrusion of calcium or any other diffusing molecules. In addition, molecular binding affects calcium extrusion, and the rate is no longer Poissonian.

Fig. 8 Residence time of calcium in a dendritic spine. Left: mean residence time of calcium $\tau_{3D}$ as a function of the spine radius $a$. Right: $\tau_{3D}$ is plotted as a function of the spine length $L$. 
Influence of Calcium Buffers on the Residence Time of Calcium in Microdomains such as the Dendritic Spines

The residence of calcium in a spine can be influenced by other mechanisms than pure diffusion. Binding and unbinding to calcium buffers affect the time course. In that case, the entire system of partial differential equations (PDE) describing the process should be solved. Only numerical simulations are available. The generic example is

\[ \text{Ca} + B_{\text{free}} \xrightarrow{k_f} \text{Ca} - B, \]  

which leads to the PDE system of equations

\[ \frac{\partial c}{\partial t}(x, t) = D \Delta c(x, t) - k_{\text{in}} c(x, t) B(x, t) + k_b [c - B](x, t), \]  
\[ \frac{\partial [c - B]}{\partial t}(x, t) = D_B \Delta c(x, t) B(x, t) + k_{\text{in}} c(x, t) B(x, t) - k_b [c - B](x, t). \]

\( c - B \) is the bound calcium that can diffuse with diffusion coefficient \( D_B \), but stays confined in the spine head, while the calcium can be extruded at pumps or at the spine neck, which translates into the following boundary conditions:

\[ \frac{\partial c(x, t)}{\partial n} = 0 \text{ on } \partial \Omega_r, \]
\[ c(x, t) = 0 \text{ on } \partial \Omega_a, \]
\[ \frac{\partial [c - B](x, t)}{\partial n} = 0 \text{ on } \partial \Omega_r. \]

when there is cooperativity at pumps, more elaborated boundary conditions are needed (Hille 2001) and will be discussed later on. There is no general solution of such equations. If binding is fast, since \( c(x, t) + [c - B](x, t) = N_0 e^{-\lambda t} \), the decay rate depends on the unbinding time, such that for a long enough time (so that binding occurred), the asymptotic decay rate is well approximated by a sum of two exponentials

\[ c(x, t) = A e^{-\lambda t} + B e^{-k_{\text{fast}} t}, \]

where A and B are constants.

Residence Time of Calcium in the Dendritic Spines with a Hydrodynamic Flow

Dendritic spines can change shape in a few hundred milliseconds (Fischer et al. 1998; Holcman et al. 2004), after calcium ions flow in. This fast change of shape decreases the spine head volume. Spine motility was proposed by Blomberg et al. (1977), and the fast twitching movement of the spine was anticipated by F. Crick in (1982). Spine fast contraction was attributed to actin-myosin molecules or troponin C, which were observed inside the spine head. As in muscle cells, high concentrations of actin molecules indicate that rapid movement can follow the arrival of calcium ions. It has been proposed in Holcman et al. (2004) that calcium ions set the spine in motion by initiating the contraction of actin-myosin (AM) as they bind at active sites. Each molecule is
assumed to give rise to a local contraction. In a simplified model, all contractions add up to achieve a global contraction, neglecting anisotropic contraction due to a delay interval between each molecule contraction. Once calcium ions enter the spine, they arrive to the binding sites by diffusion and can bind there. When four calcium ions bind to a single troponin C protein, a local contraction of the protein occurs. Adding all local contractions at a given time produces a global contraction and induces a hydrodynamic movement of the cytoplasmic fluid. Calcium trajectories are no longer pure Brownian, but contain a drift, and thus the probability to reach the dendritic shaft through the spine neck is increased (Holcman et al. 2004). The model requires feedback of a flow field $\mathbf{v}(x,t)$ on the velocity. The flow field can be computed using Green’s function of the spine domain (Holcman et al. 2004). Adding the effect of diffusion and hydrodynamics, the transient escape rate is not necessarily a single exponential. The concentration inside the spine follows:

$$c(x,y,z,t) = C \exp\left\{-A_1 t + \frac{v_0^2}{4D} t\right\},$$

where the hydrodynamic decay time is

$$\tau = \frac{4D}{v_0^2},$$

where $v_0$ is the initial average velocity (Holcman et al. 2004). Figure 3 illustrates the effect of adding a hydrodynamic drift on pure diffusion for the exploration of the spine head. Contrary to the effect of buffer, the hydrodynamic effect shifts the extrusion rate, but does not lead to a sum of exponentials.

### Crowding Model of a Dendrite

Molecular crowding reduces diffusion, and this effect can be estimated by computing the effective diffusion coefficient of a Brownian particle moving between obstacles in dimension 2. For obstacles positioned periodically, the effective coefficient of diffusion decays nonlinearly with the density of obstacles (Holcman et al. 2011). This decay also depends on the shape of the obstacles. The effective diffusion coefficient can be computed asymptotically by conformal mapping (Holcman et al. 2011). In addition, when obstacles are positioned with additional variabilities, local narrow passages, funnels, or dead ends can be observed that lead to heterogeneity in the diffusion trajectories (Ghosh et al. 2012; Holcman and Schuss 2012, 2013a). However due to the complexity of obstacle geometry, there is no achieved theory of diffusion with obstacles in three dimensions.

We present here a local model and numerical simulations to study calcium diffusion in a dendrite. The dendrite cytoplasmic medium is highly heterogeneous and filled with many organelles. Thus the motion of a diffusing particle is affected by many interactions with its environment. The functional consequences of these interactions are difficult to access directly experimentally due to ubiquitous pathways especially for calcium dynamics. In a reduced model of diffusion in dendrites, the one-dimensional effective diffusion equation and an effective diffusion constant account for the presence of heterogeneity in the medium.

### Modeling Diffusion in a Heterogeneous Dendritic Cytoplasm

To characterize diffusion in a heterogeneous dendrite, containing various organelles such as mitochondria, spine apparatus, endoplasmic reticulum, and other structures, one method (Biess et al. 2011) consists of coarse graining a three-dimensional cylindrical dendrite into a one-dimensional effective diffusion equation in the limit where the space between organelles is small. Diffusing ions can still move inside
a dendritic domain $\Omega$, and the nature of the motion is not impaired and is well approximated by the Smoluchowski limit of the Langevin equation (Schuss 2010a): a particle at position $X(t)$ at time $t$ is described by

$$
\dot{X} + \frac{1}{\gamma} \nabla \Phi(X) = \sqrt{2D} \dot{w}(t),
$$

(51)

where $\Phi$ is a potential per unit of mass, $\gamma$ is the friction coefficient, $D$ is the aqueous diffusion constant, and $\dot{w}(t)$ is the Gaussian white noise. The potential $\Phi$ represents the effective force on the particle. When a moving molecule hits impenetrable organelles $O_i$, it is reflected.

The distribution of independent molecules is characterized by the probability density function (pdf) $p(x,t)$ which satisfies the Fokker-Planck equation

$$
\frac{\partial p}{\partial t} = D \Delta p + \nabla \left[ \frac{1}{\gamma} (\nabla \Phi(X)) p \right]
$$

(52)

in the domain $\widetilde{\Omega} = \Omega \setminus \bigcup_i O_i$ and a zero flux condition on the organelles and the dendritic membrane $\partial \Omega$:

$$
J \cdot n = -D \frac{\partial p}{\partial n} + p \frac{\partial \Phi}{\partial n} = 0,
$$

(53)

where $J$ is the flux and $n$ the outer normal of the domain $\widetilde{\Omega}$.

To account for the overall effect of crowding on diffusion, we adopt an approach based on a compartmentalization of the dendritic domain and the narrow escape theory (Holcman and Schuss 2013a), which provides the mean time for a Brownian particle to exit a domain through a small absorbing opening. The dendrite is divided into periodic compartments of length $l$ and volume $V$, separated from their neighbors by a reflecting cross section, except for a small opening of radius $a$. This compartment should be large enough so that the organelle density is the same in each of them. The small openings allow diffusing molecules to move across compartments. In contrast to previous models where crowding has been described by spherical obstacles (Biess et al. 2011) that pose barriers to diffusing molecules, crowding is modeled as a sequence of periodic compartments and small openings at the boundaries of neighboring compartments. A compartment $k$ starts at position $x_k$ and ends at position $x_{k+1}$. The number $N_k(t)$ of particles in compartment $k$ changes according to the net flux across the small windows. The flux is estimated by the small hole approximation (see Biess et al. (2011) for details).

The concentration $c(x,t) = N(x,t)/V(x,t)$ satisfies

$$
\frac{\partial c(x,t)}{\partial t} = \frac{4l^2 D}{V(x)} \frac{\partial}{\partial x} \left[ a(x) \frac{\partial}{\partial x} c(x,t) \right].
$$

(54)

Similar equations have been derived in other contexts (Zwanzig 1990; Biess et al. 2007; Berezhkovskii et al. 2004). When the parameters $a(x)$ and $V(x)$ are spatially independent, Eq. 54 simplifies to
\[
\frac{\partial c(x,t)}{\partial t} = D_{\text{eff}} \frac{\partial^2 c(x,t)}{\partial x^2},
\]
(55)

where \( D_{\text{eff}} = \frac{4a}{3} D \), the effective diffusion constant, and \( V = Sl \), with \( S \) the cross-sectional area. The effective diffusion constant depends on two parameters: the compartment length \( l \) and the size of the opening \( a \).

The model parameters are determined by (i) measuring the ratio of diffusion constants \( D_{\text{eff}}/D \) and (ii) a calibration condition of the form \( l/a = 4 \). The calibration condition expresses that the parameters \( l \) and \( a \) do not necessarily have direct physiological meanings, and we can thus set one parameter arbitrarily within the limits of the small hole approximation. Additional measurements of the diffusion constant will then fix the other parameter. A Brownian simulation of diffusing ions around an obstacle is presented in Fig. 9. Other applications of reducing equations are to compute the mean time for calcium ions or diffusing molecules to travel along crowded dendrites or axons.

**Calcium Spread Following High-Frequency Stimulation** The one-dimensional effective diffusion equation presented in the last paragraph allows analyzing calcium spread originating from localized inputs such as synapses. At dendritic synapses calcium can enter through NMDA receptors. To estimate calcium spread as a function of the synaptic input frequency, Ca\(^{2+}\) influx was simulated in the middle of a dendritic segment (Fig. 10) with buffers and pumps (see Biess et al. (2011) for the reaction-diffusion equation). The different input frequencies are \( f = 5, 10, 20, 50, 80 \) Hz. Interestingly, for input frequencies larger than 20 Hz, the calcium signal in the dendrite reaches a stationary value. For high input frequencies (\( > 20 \) Hz) calcium spread does not exceed 2.5 \( \mu m \) (\( = 0.5 \times \text{FWHM} \)) as measured from the input source. Buffers and pumps limit calcium spread to a few micrometers (Biess et al. 2011).

**Calcium Extrusion along a Cylinder: Homogenization of Hole into a Killing Rate** Computing the final distribution of calcium ions between two possible fates is a generic problem. It can be the proportion of bound calcium ions versus the number extruded or the fraction that reached the dendrite versus the fraction that got pumped. We present a general approach based on homogenization to reduce the complexity of this computation.

**Homogenization of Perforated by Partially Reflecting Boundary** We approximate the flux through a reflecting boundary, perforated by many small independent absorbing holes (Berg and Purcell 1977; Berezhkovskii et al. 2004). For diffusion, the problem can be solved using the eigenvalues of the Laplace equation in the domain \( Q \) with mixed Neumann boundary conditions on \( \partial Q \) and Dirichlet conditions on \( \partial Q_A = \bigcup_{i=1}^N A_i \). If the holes \( A_i \) are sufficiently far apart, the smallest eigenvalue \( \lambda_1^{A_i} \) is asymptotically the sum of the eigenvalues \( \lambda_1^{A_i} \) of the Laplace operator in \( Q \) with mixed Neumann-Dirichlet boundary conditions on \( \partial Q - A_i \) and \( A_i \), respectively, which can be calculated from the narrow escape theory (Holcman and Schuss 2013a).

For circular holes with fixed radius \( e \), we have the formula

\[
\lambda_1^A \approx \frac{4e D}{|\Omega|}, \quad \lambda_1^A = \frac{4e DN}{|\Omega|}.
\]
(56)

The pdf of the Brownian motion in the perforated domain relaxes to a quasi-steady state and can be described by a single exponential decay in time with rate \( \lambda_1^A \) and a uniform quasi-steady state distribution in \( \Omega \), except in boundary layers near \( A_i \). The total absorption probability flux on the
Fig. 9 Brownian simulations of uncaging experiments. (a) Model glass pipette. Shown is the initial particle distribution as taken from the experimental data and the sampling volumes (white cylindrical disks) at different locations from the uncaging spot. (b) Compartmentalized model dendrite. (c) Compartmentalized model dendrite with attached spine (dendrite geometry as in B with spine neck radius 0.3 μm, spine neck length 0.2 μm, spine head radius 0.4 μm). (d) Comparison of 3D Brownian simulations with the uncaging experiments and the results derived from the solutions of the 1D effective diffusion equation. The normalized concentration profiles are shown for the glass tube (a), the dendrite (b), and the dendrite with attached spine (c) at three locations from the uncaging spot (Adapted from Biess et al. (2011))
boundary, \( \lambda_1^A \), is partitioned among the holes with probabilities 
\[ P_i = \frac{\lambda_i^A}{\sum_{i=1}^{N} \lambda_i^A} . \]
If the holes are distributed with surface density \( n(x) \) on \( \partial \Omega \), the normal absorption flux density is
\[ J(x) \cdot \nu(x) = \frac{4\varepsilon Dn(x)}{|\Omega|} . \hspace{1cm} (57) \]

**Fig. 10 Lateral extent of calcium driven by high-frequency stimulation.** (a) Calcium diffusion in an aqueous solution contained in a pipette. (b) Calcium diffusion in a crowded dendrite. The initial concentration is equal to about 600 particles and evaluates to about 470 particles per micron for a dendrite. (c) Same settings as in (a) but with additional buffers (medium buffer concentration) and pumps. (d) Same settings as in (b) but with additional buffers (medium buffer concentration) and pumps. (e) Calcium was injected at 20 Hz for 1 s at the location of the NMDAR in the middle of the dendritic segment as shown in the upper and middle panel. The resulting spatiotemporal profile in the dendrite is shown in the lower panel. (f) Spatiotemporal profiles in the dendrite for different influx frequencies at the location of the NMDAR. (g) Corresponding calcium spread in the dendrite as measured by the full width at half maximum (FWHM) of the calcium signal (Adapted from Biess et al. (2011)). Figure 11 shows the plot of \( r_T^{\text{prev}} \) for several values of the threshold \( T \), compared to Brownian simulations in a circular disk \( \Omega = D(R) \) with reflecting boundary, except at the targets.
The homogenization procedure consists in replacing the perforated holes by a radiation condition that preserves asymptotically the same quasi-steady state distribution. We replace the leading eigenfunction and eigenvalue of the Robin problem by a Laplace equation with a small radiation function $k(x)$:

$$D\Delta p(x) = -\lambda p(x) \text{ for } x \in \Omega$$  \hspace{1cm} (58)

$$D \frac{\partial p(x)}{\partial n} = -k(x)p(x) \text{ for } x \in \partial\Omega.$$  \hspace{1cm} (59)

Matching the flux density in Eqs. 57 and 59, we obtain in dimensional coordinates

$$k(x) = 4\varepsilon D p_o(x)n(x) + O\left(\frac{\varepsilon^2}{L^2}\log\frac{\varepsilon}{L}\right).$$  \hspace{1cm} (60)

The quasi-steady state pdf is

$$p_o(x) = \frac{1 + o(k_o)}{[\Omega]},$$

except in boundary layers near $A_i$.

After the first homogenization which consists in replacing a three-dimensional diffusion with total and/or partial absorption by a reduced diffusion equation in dimension one, with a killing rate $k$, the case of a cylindrical geometry can be treated immediately. The one-dimensional case of a diffusion with uniform killing inside an interval (Holcman et al. 2005) with reflecting and absorbing endpoints has a survival pdf of a particle satisfying the equation

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig11.png}
\caption{The MFTT. Left: trajectories of diffusing molecules in a microdomain containing five binding sites on the boundary. Right: the time $t_r^{\text{MFTT}}$ is plotted as a function of the threshold $T$. We present the Brownian simulations (dotted blue line, variance in black), the theoretical formula 74 (dotted black line), and its approximation 75 (continuous blue line) for a circular disk in the irreversible case ($k_{-1} = 0$). The other parameters are $S_0 = 15$, $M_0 = 10$, $\varepsilon = 0.05$, $D = 0.1 \mu m^2 s^{-1}$, and the radius of the disk $R = 1 \mu m$ (200 runs)}
\end{figure}
\[ \frac{\partial p(x,t)}{\partial t} = -\nabla \cdot J(x,t) - kp(x,t) \]

where the probability flux density is given by

\[ J(x,t) = -D \frac{\partial}{\partial x} p(x,t) + b(x)p(x,t). \]

For long-time asymptotic, the solution of Eq. 61 is approximated by the first eigenfunction:

\[ p(x,t) = p_o(x)e^{-\lambda_o t} + O(e^{-\lambda_d t}), \]

normalized by

\[ \int_0^L p_o(x)dx = 1. \]

When the killing term \( k \) is a constant, the eigenvalue problem

\[ D \frac{\partial^2 p(x)}{\partial x^2} - k(x)p(x) = -\lambda p(x) \]

\[ p(L) = 0, \quad \frac{\partial p(0)}{\partial x} = 0. \]

In case that \( k(x) = k = \text{const} \), the first eigenfunction and eigenvalue are

\[ p_o(x) = \Omega \frac{\cos(\Omega x)}{\sin(\Omega L)}, \quad \Omega = \frac{\pi}{2DL}, \quad \lambda_o = D \frac{\pi^2}{4L^2} + k. \]

The total flux \( J \) of killed diffusing particles is given by

\[ J = \int_0^L k(x)p_o(x)dx = k. \]

**Particles Splitting: Absorbed Versus Arrived**

To quantify how ions split between pumps located on the surface of a cylinder and the ones that arrive at the end, we use the ratio

\[ R_s = \frac{\int_{\partial \Omega_a} J(x|y) \cdot v(x) dS_x}{\int_{\Omega} k(x) p(x|y) dx} = \frac{\int_{\partial \Omega} \Phi(x) dS_x - \int_{\Omega} k(x) p(x|y) dx}{\int_{\Omega} k(x) p(x|y) dx} \]

that can be computed in the limit of a very thin cylinder. The flux and the killing term are computed from solving the steady state using Fokker-Planck.
\[ 0 = D \Delta p(x|y) - k(x)p(x|y) \text{ for } x, y \in \Omega. \]  

(67)

The boundary conditions are

\[ p(x|y) = 0 \text{ for } x \in \partial \Omega, \ y \in \Omega_d \]
\[ J(x|y) \cdot \nu(x) = 0 \text{ for } x \in \partial \Omega - \partial \Omega_d - \partial \Omega_i, y \in \Omega, t > 0. \]
\[ J(x|y) \cdot \nu(x) = -\Phi(x) \text{ for } x \in \partial \Omega_i. \]

The time-independent flux is \( \Phi(x) \geq 0 \). The external steady-state flux of absorbed particles is

\[ J_a = \int_{\partial \Omega_d} J(x|y) \cdot \nu(x) dS_x. \]  

(68)

The total inward flux is

\[ J_i = \int_{\partial \Omega_i} J(x|y) \cdot \nu(x) dS_x = \int_{\partial \Omega_i} \Phi(x) dS_x. \]  

(69)

If the killing measure is uniform in the interval \([0, L]\), then \( R_s(L) = \frac{1}{\cosh(bL)} - 1 \), where \( b = \sqrt{\frac{N}{D}} \).

The case where the killing is a Dirac \( k(x) = k \delta(x - x_1) \), located at a single point \( x_1 \), and \( k \) is a constant has been treated in Holcman et al. (2005). Interestingly, changing the distribution of killing from uniform to concentrated at one point has a drastic effect on the final repartition of ions (see Fig.2,

Holcman et al. (2005)).

**Calcium Cascade Initiating Cellular Activation**

The molecular implementations that describe the transformation of a transient signal to cell activation are still unclear. We shall here present a threshold-based model: when the number of bound molecules equals a given number, we suppose that a cellular change is initiated. We present a Markov chain model that reduces the geometrical complexity based on the narrow escape rate formula (Schuss et al. 2007).

**Probability to Bind a Fixed Number of Molecules During a Transient Process**

To illustrate the need of a multiscale approach to bridge the molecular to the cellular scale, we recall that during long-term potentiation (LTP) induction, a transient calcium signal is converted into a long-term change in the synaptic properties (Bliss and Collingridge 1993; Malenka and Nicoll 1999; Lee et al. 2009; Lisman et al. 2012). This process specifically involves a class of kinases (CaMKII) that have complex local cooperative binding sites organized into a ring structure.

However, the first problem is to define the meaning of activation. For example, it can either be a single binding by a \( CaM(Ca)_3 \) or \( CaM(Ca)_4 \) molecules, several bonds, or one to six phosphorylations. Once a criterion is chosen, it becomes a computational question to estimate the probability \( P_k \) that \( k \) CaMKII molecules are activated and also compute statistical quantities such as the mean number \( <N_{act}> \) of CaMKII that are activated following a transient calcium entry.

More complicated calcium patterns are also generated where calcium ions are flowing inside a synapse. In that case, it is worthwhile to compute the number of bound molecules before an arbitrary time \( t \) or the probability \( P_{act}(t) \) and the mean number \( <N_{act}(t)> \) of activations before time \( t \). These quantities are in practice difficult to compute and depend on many parameters such as the
dendritic spine geometry, the intrinsic property rate constants, the binding site interaction forces, their localization, and so on. There are two complementary approaches:

1. **Coarse-grained Markov models**: this approach is based on the narrow escape methodology, where instead of accounting for the entire time dynamics within the complex geometry organization of the microdomain, a Poissonian rate of arrival to small targets is used to approximate the target search and finding. Various quantities of interest such as the activation probability and the statistic for the number of bound CaMKII can be estimated (Holcman and Schuss 2005; Dao Duc and Holcman 2010, 2012; Holcman et al. 2013).

2. **Brownian simulations**: Contrary to the previous approach, it is not possible to obtain the exact dependency of the probability; rather this approach is tedious and allows estimating any moment of interest for a given set of parameters.

For a microstructure such as a dendritic spine, there is no equilibrium, because the steady state is zero. Thus the relaxation time is the time for a diffusing particle to be extruded by diffusion, which is in the range of tens of ms (see Holcman and Schuss 2011). The probability for \( N \) CaMKII to be activated and the time to induction is the mean time for this to happen during a repetitive stimulation. Because the calcium to CamKII pathways requires CaM intermediates, there is no coarse-grained model yet (Guerrier and Holcman 2014a). To present the threshold method, we shall now present a simplified model where a diffusing molecule can bind to a ligand. The probability to reach a threshold has been developed in Dao Duc and Holcman (2010) and is reviewed here briefly.

**Coarse-Grained Markov Models** Traditional chemical kinetics, based on mass-action laws or reaction-diffusion equations, give an inappropriate description of the stochastic chemical reactions in microdomains, where only a small number of substrate and reactant molecules is involved. A reduced Markovian description of the stochastic dynamics of the binding and unbinding of molecules is given in Holcman and Schuss (2005) and applied in Dao Duc and Holcman (2010, 2012). Specifically, consider two finite species, the mobile reactant \( M \) that diffuses in a bounded domain \( \Omega \) and the stationary substrate \( S \) (e.g., a protein) that binds \( M \). The boundary \( \partial \Omega \) of the domain \( \Omega \) is partitioned into an absorbing part \( \partial \Omega_a \) (e.g., pumps, exchangers, another substrate that forms permanent bonds with \( M \), and so on) and a reflecting part \( \partial \Omega_r \) (e.g., a cell membrane). In this model, the volume of \( M \) is neglected. In terms of traditional chemical kinetics, the binding of \( M \) to \( S \) follows the law

\[
M + S_{\text{free}} \xrightleftharpoons[k_b]{k_f} MS,
\]

where \( k_f \) is the forward-binding rate constant, \( k_b \) is the backward-binding rate constant, and \( S_{\text{free}} \) is the unbound substrate. We assume in our model of the reaction that the \( M \) molecules diffuse in \( \Omega \) independently and, when bound, are released independently of each other at exponential waiting times with rate \( k_{-1} \).

To calculate the average number of unbound (or bound) sites in the steady state, the following reduced model is used. The number \( k(t) \) of unbound receptors at time \( t \) is a Markovian birth-death process with states \( 0, 1, 2, \ldots, \min \{M, S\} \) and transition rates \( \dot{\lambda}_{k \rightarrow k+1} = \lambda_k, \dot{\lambda}_{k \rightarrow k-1} = \mu = k_{-1} \). The boundary conditions are \( \dot{\lambda}_{S \rightarrow S+1} = 0 \) and \( \dot{\lambda}_{0 \rightarrow -1} = 0 \). Setting \( P_k(t) = \Pr\{k(t) = k\} \), the Kolmogorov equations for the transition probabilities are given by (Holcman and Schuss 2005)
\[
\dot{P}_k(t) = -[\lambda_k + k_-(S - k)]P_k(t) + \lambda_{k+1}P_{k+1}(t) + k_-(S - k + 1)P_{k-1}(t)
\]

for \( k = (S - M)^+ + 1, \ldots, S - 1 \) \hspace{1cm} (71)

with the boundary equations

\[
\dot{P}_{(S-M)^+}(t) = -k_-(S-M)^+P_{(S-M)^+}(t) + \lambda_1P_{(S-M)^++1}(t)
\]

\[
\dot{P}_S(t) = -\lambda_SP_S(t) + k_1P_{S-1}(t)
\]

and initial condition \( P_{k,q}(0) = \delta_{k,q}\delta_{q,0} \). In the limit \( t \to \infty \) to the model (71) gives the average number

\[
\langle k_\infty \rangle = \sum_{j=(S-M)^+}^{S} jP_j,
\]

where \( P_j = \lim_{t \to \infty} P_j(t) \). Similarly, the stationary variance of the number of unbound sites is

\[
\sigma^2(M, S) = \langle k_\infty^2 \rangle - \langle k_\infty \rangle^2, \quad \text{where} \quad \langle k_\infty^2 \rangle = \sum_{j=(S-M)^+}^{S} j^2P_j
\]

The rates \( \lambda_k \) are modeled as follows. For a single diffusing molecule, the time to binding is the first passage time to reach a small absorbing portion \( \partial \Omega_a \) of the boundary, which represents the active surface of the receptor, whereas the remaining part of \( \partial \Omega \) is reflecting. Due to the small target and to the deep binding potential well, the binding and unbinding of \( M \) to \( S \) are rare events on the time scale of diffusion (Schuss et al. 2007). This implies that the probability distribution of binding times is approximately exponential (Schuss 2010b) with rate \( \lambda_1 = 1/\mathbb{E}[\tau_1] \), where the NET \( \mathbb{E}[\tau_1] \) is the MFPT to \( \partial \Omega_a \).

When there are \( S \) binding sites, \( k(t) \) of which are unbound, there are \( N = [M - S + k]^+ \) free diffusing molecules in \( \Omega \), where \( x^+ = \max\{0, x\} \). The arrival time of a molecule to the next unbound site is well approximated by an exponential law with state-dependent instantaneous rate (see discussion in Holcman and Schuss (2005))

\[
\lambda_k = \frac{Nk}{\mathbb{E}[\tau_1]} = \frac{k(M - S + k)^+}{\mathbb{E}[\tau_1]}
\]

The results of the Markovian model (71) are

\[
\langle k_\infty \rangle = P_S \sum_{k=S-1}^{(S-M)^+} (S - k) + \frac{\prod_{i=S-k+1}^{S} i(M - S + i)^+}{k!(\mathbb{E}[\tau_1])^k}
\]

\[
\langle k_\infty^2 \rangle = P_S \sum_{k=S-1}^{(S-M)^+} [(S - k)^+]^2 \frac{\prod_{i=S-k+1}^{S} i(M - S + i)^+}{k!(\mathbb{E}[\tau_1])^k}
\]

\[
\sigma^2(M) = \langle k_\infty^2 \rangle - \langle k_\infty \rangle^2
\]

(see Holcman and Schuss (2005) for further details).

These formulas are used to estimate the fraction of bound receptors in photoreceptor outer segments and also to interpret the channel noise measurement variance in Holcman and Schuss.
(2005). In Holcman and Triller (2006) this analysis was used to estimate the number of bound AMPA receptors in the postsynaptic density. A similar gated Markovian model was proposed in Bressloff and Earnshaw (2009).

The reduced Markovian model is used for the calculation of the mean time of the number of bound molecules to reach a given threshold \( T \) (MFTT). In a cellular context, the MFTT can be used to characterize the stability of chemical processes, especially when they underlie a biological function. Using the above Markov chain description, the MFTT can be expressed in terms of fundamental parameters, such as the number of molecules and ligands and the forward- and backward-binding rates. It turns out that the MFTT depends nonlinearly on the threshold \( T \).

Specifically consider \( M \) Brownian molecules that can bind to immobile targets \( S \) inside a microdomain, modeled generically by Eq. 70. The first time the number \( [MS](t) \) of \( MS \) molecules at time \( t \) reaches the threshold is defined as

\[
\tau_T = \inf\{t > 0 : [MS](t) = T\},
\]

and its expected value is \( \tau_T \). Consider the case of an ensemble of the targets initially free and distributed on the surface of a closed microdomain and assume that the backward rate vanishes \( (k_{-1} = 0) \) and \( k_f > 0 \). The dynamical system for the transition probabilities of the Markov process \( MS(t) \) is similar to that above, but for the absorbing boundary condition at the threshold \( T \), which gives Eq. 71 (Dao Duc and Holcman 2010). When the binding is irreversible \( (k_{-1} = 0) \), \( \tau_T \) is the sum of the forward rates

\[
\tau_T^{\text{irrev}} = \frac{1}{\lambda} \sum_{k=0}^{T-1} \frac{1}{\lambda_{k}} + \frac{1}{\lambda_{T-1}}
= \frac{1}{\lambda} \sum_{k=0}^{T-1} \frac{1}{(M_0 - k)(S_0 - k)}. \tag{74}
\]

In particular, when \( M_0 = S_0 \) and \( M_0 \gg 1 \), Eq. 74 becomes asymptotically \( \tau_T^{\text{irrev}} \approx T/\lambda M_0(M_0 - T) \).

In addition, when the number of diffusing molecules greatly exceeds the number of targets \( (M_0 \gg S_0, T) \), (74) gives the asymptotic formulas

\[
\tau_T^{\text{irrev}} \approx \begin{cases} 
\frac{1}{\lambda M_0} \log \frac{S_0}{S_0 - T} & \text{for } M_0 \gg S_0, T \\
\frac{1}{\lambda S_0} \log \frac{M_0}{M_0 - T} & \text{for } S_0 \gg M_0, T \\
\frac{T}{\lambda M_0 S_0} & \text{for } M_0, S_0 \gg T.
\end{cases} \tag{75}
\]

**Discussion and Conclusion**

We have summarized here biophysical models at a molecular level, mathematical analysis, and coarse-graining models to study calcium dynamics in cellular microdomains. The present approach can be implemented to better understand how calcium dynamics can induce sophisticated processes such as long-term potentiation or depression, cellular process that are responsible for long-lasting changes in physiological properties. Yet what calcium is doing at synapses still remains unclear: where calcium is accumulating, what is the number of activated CaMKII (it is not exactly clear what is the meaning of being activated; it can be that a single site is phosphorylated), and what other calcium-activated molecules are important. We presented as an example of complex calcium...
feedback how spine twitching can be described at a molecular level. We also described Brownian
and simplified simulation of calcium and the CamKII pathway. The CamKII molecule can be
modeled as a simplified ring made of six balls. A calibration procedure is then use to determine
the radius $a$ of a sphere or disk in that matches the Smoluchowski formula. We have not reviewed the
presynaptic terminal, which is a critical microdomain where calcium modulates the release of
vesicles. Vesicular release is triggered by calcium entrance at specific calcium channels (Holderith
et al. 2013). The exact steps from the calcium entrance to the vesicular release are still under
investigation. The steps of calcium diffusion have been investigated both experimentally and
numerically. In particular the distance from the channel to the vesicle is a key parameter. Channel
clustering also would be interesting to investigate. The possibility that channels are not fixed and the
membrane is constantly remodeled shows that this process is quite complex. Interestingly, the
release probability can be modulated by six orders of magnitude (Kochubey et al. 2011;
Schneggenburger et al. 2012). Simulations of presynaptic calcium are based on numerically solving
partial differential equations (Matveev et al. 2004; Zucker and Regehr 2002; Zucker 1993);
however, it is hard to account for the cusp nature of the region between the vesicles and the plasma
membrane (Neher 2010). Analyzing diffusion in cusps can be studied by mapping locally the cusp
conformally to a nonsingular domain or to use analytical computation (Holcman and Schuss 2012;
Guerrier and Holcman 2014b). Another difficulty is to account for various varicosities, leading to
divergences, and a multiscale analysis should be developed. Modeling approaches are already
shedding a new light on the unsolved debate about the role of the dendritic spines in regulating
electrical versus chemical activity. It is certainly time to revisit the electrical properties of the spines
based on solving the full Poisson-Nernst-Planck equation. Chemical properties should consider
surface receptors trafficking as a chemical reservoir.

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## Author Queries

<table>
<thead>
<tr>
<th>Query Refs.</th>
<th>Details Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>Please check the edits made in Table 1 is ok.</td>
</tr>
<tr>
<td>Q2</td>
<td>Please check sentence starting “A calmodulin molecule…” for sense.</td>
</tr>
<tr>
<td>Q3</td>
<td>Please check if edit to sentence starting “The general solution…” is okay.</td>
</tr>
<tr>
<td>Q4</td>
<td>Please check if edit to sentence starting “The no flux…” is okay.</td>
</tr>
<tr>
<td>Q5</td>
<td>Please check if Fig. 7 caption is complete sentence.</td>
</tr>
<tr>
<td>Q6</td>
<td>Is this the cross reference to Eq. ??</td>
</tr>
<tr>
<td>Q7</td>
<td>Both “CaMKII” and “CamKII” have been used in text. Please check if one form should be made consistent.</td>
</tr>
<tr>
<td>Q8</td>
<td>Please check if inserted publisher location for reference Carslaw and Jaeger (1959) is okay.</td>
</tr>
<tr>
<td>Q9</td>
<td>Please provide page range for Holeman and Schuss (2013a)</td>
</tr>
<tr>
<td>Q10</td>
<td>Please provide volume id and page range for Holeman and Schuss (2013b, c), Holeman et al. (2013), Schuss and Holeman (2013).</td>
</tr>
<tr>
<td>Q11</td>
<td>Please check if inserted page range for Holcman and Triller (2006), Holcman et al. (2005) is okay.</td>
</tr>
</tbody>
</table>