A Definition of Cellular Interface Problems

Markus Kirkilionis, Mirela Domijan, Martin Eigel,
Erwin George, Mike Li, and Luca Sbano

Mathematics Institute, University of Warwick
Coventry CV4 7AL, UK
`mak@maths.warwick.ac.uk`

Abstract. We define a class of cellular interface problems (short: CIP) that mathematically model the exchange of molecules in a compartmentalised living cell. Defining and eventually solving such compartmental problems is important for several reasons. They are needed to understand the organisation of life itself, for example by exploring different 'origin of life' hypothesis based on simple metabolic pathways and their necessary division into one or more compartments. In more complex forms investigating cellular interface problems is a way to understand cellular homeostasis of different types, for example ionic fluxes and their composition between all different cellular compartments. Understanding homeostasis and its collapse is important for many physiological medical applications. This class of models is also necessary to formulate efficiently and in detail complex signalling processes taking place in different cell types, with eukaryotic cells the most complex ones in terms of sophisticated compartmentalisation. We will compare such mathematical models of signalling pathways with rule-based models as formulated in membrane computing in a final discussion. The latter is a theory that investigates computer programmes with the help of biological concepts, like a subroutine exchanging data with the environment, in this case a programme with its global variables.

1   Setting the Problem

Most theories about the origin of life depend on the relative closedness of a reaction volume allowing for either the protected replication of a 'replicator' like primitive RNA, or the persistence of a simple metabolic pathway where without a protective and also selective membrane the reaction system would dilute or be perturbed and cease to exist (for some speculation see for example [16]). The membranes of biology are formed by lipids which have astonishing chemical properties allowing them to build so-called vesicles in a self-organised fashion and in many different environments. By either allowing the vesicles to split and preserving the metabolic pathway, or by positioning the replicator into both daughter vesicles, it is argued that this new entity is able to allow Darwinian evolution. In this case replication may not be perfect, allowing for mutations, or the metabolic pathway becomes perturbed, or both. After the subsequent unavoidable selection process the complexity in terms of 'division of labour' and

therefore the possibilities of primitive life to adapt to changing environments, but also the precision of the replication process itself might have gradually increased.

Such speculations about different possible paths to produce stable life, i.e cells that are able to reproduce, that have a metabolism (use energy to decrease their own entropy) and are able to adapt, moreover the ability to evolve on larger time scales, enable us to better understand the organisatorial prerequisites for the working of even the simplest present cellular signalling pathway. Compartmental organisation in other words is defining the necessary system boundaries or form without which the signalling system and other cellular function would never have come into existence.

It is fruitful to approach problems in computer science with similar biological ideas and concepts. Such cross-over is called natural computing, with membrane computing being an important sub-discipline. We will introduce another type of computational device, a density approach to molecular concentrations inside the cell. In other words we will make use of continuous distributions of molecules, so go from a discrete to a continuous perspective. It is tempting to compare the latter with ideas from membrane computing.

2   A Modular Approach

The problem which we will call 'Cellular Interface Problem' (CIP) is constructed with the help of partial differential equations (PDE) defined on the cell volumes, and interface or boundary conditions defined on the membranes, depending whether a membrane is a system boundary, or whether the membrane is enclosing a compartment internal to the system. The state of the system is characterised by concentrations of molecules, again either defined on compartmental volumes or as concentrations on the interfaces. Clearly what we have in mind is a direct correspondence between a density of molecules that can be directly measured in a cell, for example with the help of a confocal microscope. We will come back to this point in an own section. In this view the system state is very closely chosen to represent measurable quantities, i.e. can be called 'empirical'. We note that 'empirical' necessarily can only be defined according to a given spatial scale, which here is the one of the microscope. We are aiming at simulating the time course of concentration changes in the cell which if predicted correctly, i.e. if there is a correspondence between measured data and simulated data, can tell us something about the working of the 'real' cell. The system state is a function of time t and space x, so is spatially explicit. We are making this choice because we are interested in transport processes not only across the membrane, but also inside each cellular compartment. Many more examples and simulations related to this definition can be found in [14].

2.1   Events

Any model describing changes in molecular distribution and reaction between species is necessarily event driven. Here the basic events are either molecular displacements, numbers of reactions between species of molecules, or conformational changes of molecules (like proteins), all relative to a given time scale

(interval) $\delta > 0$ assumed to be small but sufficiently large such that at least one displacement or reaction/conformational change is expected to happen. There is a universal clock assumed for all these events, and all possible events can take place in parallel. At the given typical physical scales related to the microscope this event structure is assumed to be close to reality.

2.2 Membrane System

The membrane system of a biological cell consists of a lipid bilayer. Its chemical properties determine which molecules can pass this membrane spontaneously, or need help in passing it. This might require energy and is then called active transport. There are other forms of transport in biological cells, for example vesicles budding from the membrane and fusing to a membrane at a different location (secretory pathway). But this will not be considered in the following as this would mean we could not work with a fixed geometry of the membrane system. Here we consider a fixed general setting, for simplicity in two spatial dimensions only. The outer membrane (cell wall) is represented by a piecewise smooth boundary denoted by Γ_c, see Fig.1. The volume encircled by Γ_c and representing the cytoplasm is given by Ω_c. There are possibly nested compartments lying entirely inside Ω_c. The largest is typically the cell nucleus, and the mathematical counterpart of the nuclear envelope is denoted by Γ_n, the volume encircled by Ω_n. There are possibly different substructures inside Ω_n, not necessarily modelling a lipid bilayer, but definitely an area with different properties of the medium where a molecule might have to pass in a different way. Such areas are modelled again by subdomains Ω_i, $i = 1, \ldots, s$, with corresponding boundaries Γ_i, $i = 1, \ldots, s$.
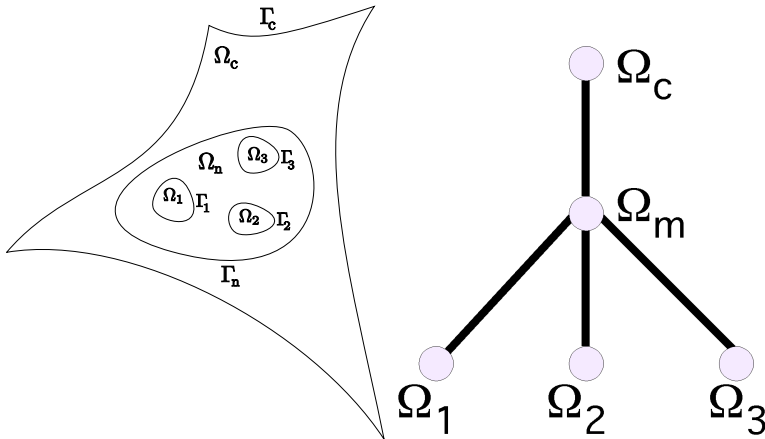

Fig. 1. A compact smooth domain $\overline{\Omega_c}$ with interior Ω_c and sub-subdomains $\overline{\Omega_i}$, $i = 1, \ldots, s$ inside a subdomain $\overline{\Omega_n}$ of Ω_c. The right graph shows the hierarchy of the domain data-structure of the example.

2.3   Ion Channels and Transporters

We are interested to consider membranes which are permeable and which allow molecules to pass from one compartment to another. Biologically one way of transport is through channels which are here considered as *molecular machines* constituted by macromolecules which can be in different conformations. Such macromolecules allow to transfer smaller molecules from one membrane side to the other, often through a regulated mechanism. Experiments show that the movement of a molecule through the channel can be considered sequentially and discretely, often corresponding to conformational changes. The nature of the steps is dictated by the molecular interactions, this implies that from the microscopic point of view the transitions form a stochastic process. In the following we consider some fundamental structure of such a process. We spend some time on this example because it will explain very easily why we need to introduce temporal and spatial scales into the derivation of a CIP. A channel can be described as a cell compartment with a certain number of internal sub-compartments. Let this number be m. If we want to include the two exits we can say that the total number is $m + 2$. A picture of this can be see in Fig.2. A molecule crossing the channel has to move from compartment 0 to compartment $m+1$. The transitions are in general reversible.
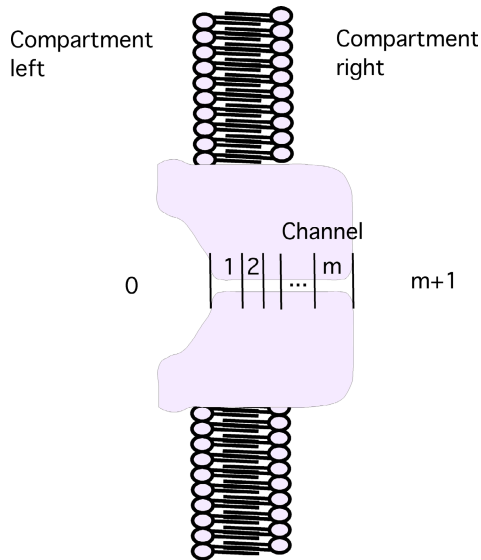

Fig. 2. A schematic view of a channel in a membrane. Compartments *left* and *right* are external to the channel.

Now let us observe that the state of the channel can be described by considering the occupation of one of the $m + 2$ sub-compartments. We now make the crucial simplifying assumption that only one single molecule can cross the channel at one time. This allows us to define the state space of the channel as

$$S \doteq \{i \in \{0, ..., m+2\}\}. \tag{1}$$

Now any motion in the channel of a molecule in the direction from A to B (see in Fig.2) will correspond to a sequence of transitions in S of the form

$$i \rightarrow i + 1.$$

Similarly any motion of a molecule from B to A (see in Fig.2) will correspond to a sequence of transitions of the type

$$i \rightarrow i - 1.$$

Since the transitions are stochastic such a structure is naturally described through the notion of a Markov Chain (MC). On S the Markov chain is constructed by determining the transition probabilities

$$P(s = i | s = j) = p_{ij} \text{ with } i, j \in S.$$

The transition probabilities satisfy the Markov property:

$$p_{i_1 i_3} = \sum_{i_2 \in S} p_{i_1 i_2} p_{i_2 i_3}. \tag{2}$$

One can easily note that since transition are possible only through adjacent sub-compartments we have that

$$p_{ij} = 0 \text{ for } j > i+1 \text{ and } j < i-1.$$

The natural way to construct the transition probability matrix $P = (p_{ij})$ is to consider its infinitesimal generator defined as

$$K = \lim_{t \to 0} \frac{P(t) - \mathbf{I}}{t}. \tag{3}$$

Let $p_i(t)$ be the probability that a time t the channel is in state i, then the time evolution of the Markov chain is governed by

$$\frac{dp_i(t)}{dt} = \sum_{j \in S} p_i(t) K_{ji}. \tag{4}$$

Looking at the scheme in Fig.2 we can encode the Markov chain in a graph, the so-called *Interaction Graph*.

Each vertex in the graph represents a sub-compartment and the arrows are drawn according to the following rule:

$$\text{There is an arrow from } i \text{ to } j \text{ if and only if } K_{ij} \neq 0. \tag{5}$$

Recall that in any Markov chain $K_{ii} = -\sum_{j \neq i} K_{ij}$ and $K_{ij} \geq 0$ for any $i \neq j$. We should notice that in the example in Fig.3 the channel is one way: a molecule that enters the channel in 0 eventually will reach the other end $m+1$.
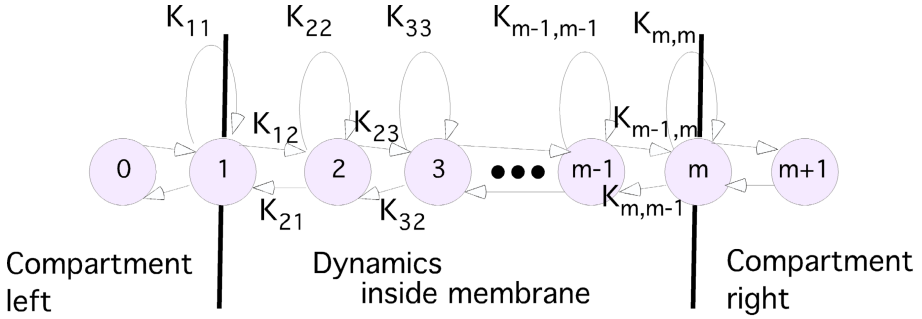
Fig. 3. The channel interaction graph

From the modelling point of view the matrix K is formed by the rates at which in unit time transitions occur. Such rates can be derived by statistical mechanics arguments. Finally recall a crucial property of Markov chains: ergodicity. For our purpose we are interested in observing that for large times $(t \rightarrow \infty)$ equation (4) a steady state solution given by solving:

$$p\,K = 0 \text{ or } K^T p = 0. \tag{6}$$

The solution of (6) can be normalised and it is called *invariant measure*. Other transporters establishing for example a so-called *symport* or *antiport* can be modelled in a similar way. The concept of assigning a graph to molecular transitions, with the interaction graph being the most basic one, can be found in [26], and for mass-action reaction systems in [10].

2.4   Fluxes across Membranes and Movement Inside Compartments

In the following we restrict our attention to derive flux conditions across just a single membrane. Let therefore Ω now just be a two dimensional domain divided into two sub-domains Ω_1, Ω_2 such that

$$\Omega = \Omega_1 \cup \Omega_2.$$

The membrane is geometrically represented by the common boundary of the two sub-domains

$$\Gamma = \Omega_1 \cap \Omega_2.$$

Fluxes across Membranes Derived from Microscopic Channel Dynamics. In the membrane (interface) Γ we assume there are channels which can be open for molecules to cross the membrane, as introduced in the previous section. For simplicity let us assume there is a simple diffusion process inside each compartment described by the standard diffusion equation in both Ω_1 and Ω_2. Each of the channels in Γ is described independently by an m-state Markov chain whose generator is $K(y)$, where $y \in \Gamma$. Then the diffusion scaling $\delta^2/\tau = D$ allows two possible boundary conditions at Γ. Let ρ_i be the molecular density in Ω_i and the membrane be given according to the convention

$$\Gamma = \{(x, y) \in \Omega_1 \cup \Omega_2 : x = 0\}.$$

As we will motivate in the following there will be two different interface conditions that are induced by the channel dynamics, and which are related to different relative time-scales of channel transitions with respect to transport inside a compartment of a given molecule. By using the interface Γ coordinates, and by denoting with $\pi(y) = (\pi_1(y), \ldots, \pi_m(y))$ the transition probabilities of all channels located at $y \in \Gamma$ (we used the notation p before for a single channel), these different interface conditions are:

1. If the Markov chain evolves on a time scale equal to the diffusion then the boundary condition is

$$\rho_1(t, 0, y) = \pi_1(t, y), \quad \rho_2(t, 0, y) = \pi_m(t, y),$$

and

$$\begin{aligned}
\frac{\partial \rho_1(t, 0, y)}{\partial x} &= \frac{1}{D} \left(k_{12}(y)\rho_1(t, 0, y) - k_{21}(y)\pi_2(t, y) \right), \\
\frac{\partial \rho_2(t, 0, y)}{\partial x} &= \frac{1}{D} \left(-k_{m-1,m}(y)\rho_2(t, 0, y) + k_{m,m-1}(y)\pi_{m-1}(t, y) \right).
\end{aligned} \tag{7}$$

As we consider our previous channel example embedded into a spatial context it should be noted that the entries of K are nonzero only in the diagonal and the two off-diagonals. Furthermore it was assumed that at the 'left' gate we have $K_{11} = -\frac{k_{12}}{\delta}$, $K_{12} = \frac{k_{12}}{\delta}$, $K_{21} = \frac{k_{21}}{\delta}$ and $K_{22} = -\frac{k_{21}}{\delta} - k_{23}$, with the k_{ij} being given positive transition rates characterising the channel (see Fig.3), and δ a spatial scale. Symmetric assumptions have been made for transition rates at the 'right' channel gate. The spatial scale δ will be the grid size of a grid introduced to perform the continuum limit of a transport process inside the volume of the compartments. This setting of the transition rates might be of course different for different kinds of transport through the membrane that do not follow the discrete diffusion logic. Here $\pi_1(t, y)$, and $\pi_m(t, y)$ are the probability distributions on the two exits of the channel at $(0, y)$. Their dynamics is given by the ODE

$$\frac{d\pi_\alpha(t, y)}{dt} = \sum_{\beta=1}^{m} K_{\alpha\beta}(y)\pi_\beta(t, y), \quad \alpha = 2, \ldots, m-1.$$

These conditions as the ones below should hold for all $y \in \Gamma$, and $t \in [t_0, T]$, with t_0 the start of the experiment, and T the time where the experiments or at least its observation stops.

2. If the Markov chain evolves on a time scale faster than the diffusion then the boundary condition is

$$\rho_1(t, 0, y) = \mu_1(y), \quad \rho_2(t, 0, y) = \mu_m(y),$$

where $\mu(y) = (\mu_1(y), \ldots, \mu_m(y))$ is the invariant measure of the Markov chain modelling the channel.

We now motivate the derivation of the interface conditions given above. Let us partition Ω into a a two dimensional lattice $\Lambda_\delta = (\delta\mathbb{Z})^2$, with $\delta > 0$ being the fundamental length of the lattice Λ_δ. Clearly as $\delta \to 0$ the lattice tends to $\mathbb{R}^2$. Each point in Λ_δ is identified by a couple of coordinates (n_x, n_y). We denote by $P_i(t, n_x, n_y)$ the probability that a particle is in the site (n_x, n_y) at time t. Let $\tau > 0$ be a given time scale. We consider the following discrete diffusion process in Ω_i:

$$P_i(t + \tau, n_x, n_y) = \frac{1}{2}\mathbf{D}_x(P_i(t, n_x, n_y)) + \frac{1}{2}\mathbf{D}_y(P_i(t, n_x, n_y),) \tag{8}$$

where $\mathbf{D}_x$ and $\mathbf{D}_y$ are operators defined by

$$\mathbf{D}_x f(n_x, n_y) \doteq f(n_x + 1, n_y) + f(n_x - 1, n_y), \tag{9}$$

$$\mathbf{D}_y f(n_x, n_y) \doteq f(n_x, n_y + 1) + f(n_x, n_y - 1). \tag{10}$$

Without loss of generality the membrane Γ can be parameterised by

$$\Gamma = \{(n_x, n_y) \in \Lambda_\delta : n_x = 0\}.$$

On the membrane the diffusion process implies that

$$P_i(t + \tau, 0, n_y) = P_i(t, -1, n_y) + \frac{1}{2}\mathbf{D}_y(P_i(t, 0, n_y)). \tag{11}$$

Now we introduce the channels into Γ. At each point $(0, n_y) \in \Gamma$ we assume that there exists a channel who has a certain number of internal m states as before (see Figure 2). Let C be the (discrete) state space of a channel. At the moment we do not enter into the details of the mechanism but simply assume that each channel is described by a Markov chain of the form

$$\Pi_\alpha(t + \tau, 0, n_y) = \Pi_\alpha(t, 0, n_y) + \tau \sum_{\beta=1}^{m} K_{\alpha\beta}(\delta, n_y)\Pi_\beta(t, n_y). \tag{12}$$

The function $\Pi_\alpha(t, 0, n_y)$ is the probability that a single molecule is in $(0, n_y) \in \Lambda_\delta$ at time t, and in the state α of the channel. By continuity we need to require the following boundary conditions:

$$P_1(t, 0, n_y) = \Pi_1(t, 0, n_y), \quad P_2(t, 0, n_y) = \Pi_m(t, 0, n_y). \tag{13}$$

Using (13) and substituting (12) into (11) we obtain:

$$P_i(t, 0, n_y) + \tau \sum_{\beta=1}^{m} K_{i\beta}(\delta, n_y)\Pi_\beta(t, n_y) = P_i(t, -1, n_y)) + \frac{1}{2}\mathbf{D}_y(P_i(t, 0, n_y)). \tag{14}$$

Next we take the continuum limit to obtain densities, denoted by ρ:

$$P_i(t, x/\delta, y/\delta) = \rho_{i,\delta}(t, x, y), \tag{15}$$

$$\Pi_1(t, y/\delta) = \rho_{1,\delta}(t, 0, y), \tag{16}$$

$$\Pi_m(t, y/\delta) = \rho_{2,\delta}(t, 0, y), \tag{17}$$

$$\Pi_\alpha(t, y/\delta) = \pi_{\alpha,\delta}(t, y) \text{ for } \alpha \neq 1, m. \tag{18}$$

Note that $\pi_{\alpha,\delta}(t, y)$ can be interpreted as the number of channels in state α present in a membrane segment of length δ in Γ. Making the substitution in (8) and taking the limit $\delta, \tau \to 0$ with $\frac{\delta^2}{\tau} = D > 0$ we obtain by a standard calculation that

$$\frac{\partial \rho_i(t, x, y)}{\partial t} = D\nabla^2 \rho_i(t, x, y), \quad i = 1, 2. \tag{19}$$

For the boundary condition, retaining δ, τ greater than zero, we obtain

$$\rho_{i,\delta}(t + \tau, 0, y) - \rho_{1,\delta}(t, 0, y) = \tau\, K_{11}(\delta, y)\rho_{1,\delta}(t, 0, y) + \tau\, K_{1m}(\delta, y)\rho_{2,\delta}(t, 0, y) +$$
$$+\tau \sum_{\beta \neq 1, m} K_{1\beta}(\delta, y)\pi_{\beta,\delta}(t, y),$$

$$\rho_{m,\delta}(t + \tau, 0, y) - \rho_{m,\delta}(t, 0, y) = \tau\, K_{m1}(\delta, y)\rho_{1,\delta}(t, 0, y) +$$
$$+\tau\, K_{mm}(\delta, y)\rho_{2,\delta}(t, 0, y) + \tau \sum_{\beta \neq 1, m} K_{1\beta}(\delta, y)\pi_{\beta,\delta}(t, y),$$

$$\pi_\alpha(t + \tau, y) - \pi_\alpha(t, y) = \tau\, K_{\alpha 1}(\delta, y)\rho_{1,\delta}(t, 0, y) + \tau\, K_{\alpha m}(\delta, y)\rho_{2,\delta}(t, 0, y) +$$
$$+\tau \sum_{\beta=1}^{m} K_{\alpha\beta}(\delta, y)\pi_{\beta,\delta}(t, y) \text{ for } \alpha \neq 1, m,$$

and

$$\rho_{1,\delta}(t, 0, y) - \rho_{1,\delta}(t, -\delta, y) = -\tau\, K_{11}(\delta, y)\rho_{1,\delta}(t, 0, y) - \tau\, K_{1m}(\delta, y)\rho_{2,\delta}(t, 0, y) +$$
$$-\tau \sum_{\beta \neq 1, m} K_{1\beta}(\delta, y)\pi_{\beta,\delta}(t, y) + \frac{1}{2}(\rho_{1,\delta}(t, 0, y + \delta) + \rho_{1,\delta}(t, 0, y - \delta)),$$

$$\rho_{2,\delta}(t, 0, y) - \rho_{2,\delta}(t, -\delta, y) = -\tau\, K_{m1}(\delta, y)\rho_{1,\delta}(t, 0, y) - \tau\, K_{mm}(\delta, y)\rho_{2,\delta}(t, 0, y) +$$
$$-\tau \sum_{\beta \neq 1, m} K_{m\beta}(\delta, y)\pi_{\beta,\delta}(t, y) + \frac{1}{2}(\rho_{2,\delta}(t, 0, y + \delta) + \rho_{2,\delta}(t, 0, y - \delta)).$$

These expression can be further simplified by considering that $K_{1m}(\delta, y) = K_{m1}(\delta, y) = 0$, because the two ends on the channel do not communicate directly. The new conditions read

$$\rho_{1,\delta}(t + \tau, 0, y) - \rho_{1,\delta}(t, 0, y) = \tau\, K_{11}(\delta, y)\rho_{1,\delta}(t, 0, y) + \tau \sum_{\beta \neq 1, m} K_{1\beta}(\delta, y)\pi_{\beta,\delta}(t, y),$$

$$\rho_{m,\delta}(t + \tau, 0, y) - \rho_{m,\delta}(t, 0, y) = \tau\, K_{mm}(\delta, y)\rho_{2,\delta}(t, 0, y) +$$
$$+\tau \sum_{\beta \neq 1, m} K_{1\beta}(\delta, y)\pi_{\beta,\delta}(t, y),$$

$$\pi_{\alpha,\delta}(t+\tau,y) - \pi_{\alpha,\delta}(t,y) = \tau\, K_{\alpha 1}(\delta,y)\rho_{1,\delta}(t,0,y) + \tau\, K_{\alpha m}(\delta,y)\rho_{2,\delta}(t,0,y) +$$

$$+\tau \sum_{\beta=1}^{m} K_{\alpha\beta}(\delta,y)\pi_{\beta,\delta}(t,y) \text{ for } \alpha \neq 1,m,$$

and

$$\rho_{1,\delta}(t,0,y) - \rho_{1,\delta}(t,-\delta,y) = -\tau\, K_{11}(\delta,y)\rho_{1,\delta}(t,0,y) +$$

$$-\tau \sum_{\beta \neq 1,m} K_{1\beta}(\delta,y)\pi_{\beta,\delta}(t,y) + \frac{1}{2}(\rho_{1,\delta}(t,0,y+\delta) + \rho_{1,\delta}(t,0,y-\delta)),$$

$$\rho_{2,\delta}(t,0,y) - \rho_{2,\delta}(t,-\delta,y) = -\tau\, K_{mm}(\delta,y)\rho_{2,\delta}(t,0,y) +$$

$$-\tau \sum_{\beta \neq 1,m} K_{m\beta}(\delta,y)\pi_{\beta,\delta}(t,y) + \frac{1}{2}(\rho_{2,\delta}(t,0,y+\delta) + \rho_{2,\delta}(t,0,y-\delta)).$$

To simplify these conditions let us consider

$$\pi_{\alpha,\delta}(t+\tau,y) - \pi_{\alpha,\delta}(t,y) = \tau\frac{\partial \pi_{\alpha,\delta}(t,y)}{\partial t} + o(\tau),$$

$$\rho_{i,\delta}(t+\tau,0,y) - \rho_{i,\delta}(t,0,y) = \tau\frac{\partial \rho_{i,\delta}(t,0,y)}{\partial t} + o(\tau),$$

$$\rho_{i,\delta}(t,0,y) - \rho_{i,\delta}(t,-\delta,y) = \delta\frac{\partial \rho_{i,\delta}(t,0,y)}{\partial x} + o(\delta),$$

and

$$\rho_{i,\delta}(t,0,y+\delta) + \rho_{i,\delta}(t,0,y-\delta) = o(\delta).$$

Using the previous approximation the boundary condition can finally be rewritten as

$$\frac{\partial \rho_{1,\delta}(t,0,y)}{\partial t} = K_{11}(\delta,y)\rho_{1,\delta}(t,0,y) + \sum_{\beta \neq 1,m} K_{1\beta}(\delta,y)\pi_{\beta,\delta}(t,y),$$

$$\frac{\partial \rho_{2,\delta}(t,0,y)}{\partial t} = K_{mm}(\delta,y)\rho_{2,\delta}(t,0,y) + \sum_{\beta \neq 1,m} K_{1\beta}(\delta,y)\pi_{\beta,\delta}(t,y),$$

$$\frac{\partial \pi_{\alpha,\delta}(t,y)}{\partial t} = K_{\alpha 1}(\delta,y)\rho_{1,\delta}(t,0,y) + K_{\alpha m}(\delta,y)\rho_{2,\delta}(t,0,y)$$

$$+\sum_{\beta=1}^{m} K_{\alpha\beta}(\delta,y)\pi_{\beta,\delta}(t,y) \text{ for } \alpha \neq 1,m, \tag{20}$$

$$\frac{\partial \rho_{1,\delta}(t,0,y)}{\partial x} = -\frac{\tau}{\delta}\, K_{11}(\delta,y)\rho_{1,\delta}(t,0,y) - \frac{\tau}{\delta} \sum_{\beta \neq 1,m} K_{1\beta}(\delta,y)\pi_{\beta,\delta}(t,y),$$

$$\frac{\partial \rho_{2,\delta}(t,0,y)}{\partial x} = -\frac{\tau}{\delta}\, K_{mm}(\delta,y)\rho_{2,\delta}(t,0,y) - \frac{\tau}{\delta} \sum_{\beta \neq 1,m} K_{m\beta}(\delta,y)\pi_{\beta,\delta}(t,y).$$

In the following we want to take the limit $\delta, \tau \to 0$ while preserving the diffusive scale $\frac{\delta^2}{\tau} = D$. By inspection of equations (20) one can note that the limits that need to be studied are

$$\lim_{\delta \to 0} K_{\alpha\beta}(\delta, y), \tag{21}$$

and

$$\lim_{\delta \to 0} \frac{\tau}{\delta} K_{\alpha\beta}(\delta, y) = \lim_{\delta \to 0} \frac{\delta}{D} K_{\alpha\beta}(\delta, y). \tag{22}$$

The limits (21) and (22) cannot be both finite and different from zero at the same time. Therefore we have two cases, as introduced in the beginning of this subsection:

(Case A): Suppose that

$$\lim_{\delta \to 0} K_{\alpha\beta}(\delta, y) = K_{\alpha\beta}(y). \tag{23}$$

Clearly we have

$$\lim_{\delta \to 0} \pi_\delta(t, y) = \pi(t, y) = (\pi_1(t, y), \dots, \pi_m(t, y))$$

and in the two compartments the two densities

$$\rho_1(t, x, y), \quad \rho_2(t, x, y)$$

are defined. They satisfy

$$\rho_1(t, 0, y) = \pi_1(t, y), \quad \rho_2(t, x, y) = \pi_4(t, y)$$

by continuity at the boundary. We just consider next the Neumann part of the interface conditions. When we apply the general equations (20) to our specific channel setting, moreover using the diffusion scaling $D = \frac{\delta^2}{\tau}$, we get

$$\begin{aligned}
\frac{\partial \rho_1(t, 0, y)}{\partial x} &= \frac{1}{D} \left(k_{12}(y)\rho_1(t, 0, y) - k_{21}(y)\pi_2(t, y) \right), \\
\frac{\partial \rho_2(t, 0, y)}{\partial x} &= \frac{1}{D} \left(-k_{m-1,m}(y)\rho_2(t, 0, y) + k_{m,m-1}(y)\pi_3(t, y) \right).
\end{aligned} \tag{24}$$

(Case B): Suppose that

$$\lim_{\delta \to 0} \frac{\delta}{D} K_{\alpha\beta}(\delta, y) = \frac{1}{D} \tilde{K}_{\alpha\beta}(y). \tag{25}$$

This implies that

$$K_{\alpha\beta}(\delta, y) \simeq \frac{1}{\delta} \tilde{K}_{\alpha\beta}(y).$$

So the boundary conditions are of the form

$$
\begin{aligned}
\frac{\partial \rho_{1,\delta}(t,0,y)}{\partial t} &= \frac{1}{\delta}\tilde{K}_{11}(y)\rho_{1,\delta}(t,0,y) + \frac{1}{\delta}\sum_{\beta \neq 1,m}\tilde{K}_{1\beta}(y)\pi_{\beta,\delta}(t,y),\\
\frac{\partial \rho_{2,\delta}(t,0,y)}{\partial t} &= \frac{1}{\delta}\tilde{K}_{mm}(y)\rho_{2,\delta}(t,0,y) + \frac{1}{\delta}\sum_{\beta \neq 1,m}\tilde{K}_{1\beta}(y)\pi_{\beta,\delta}(t,y),\\
\frac{\partial \pi_{\alpha,\delta}(t,y)}{\partial t} &= \frac{1}{\delta}\tilde{K}_{\alpha 1}(y)\rho_{1,\delta}(t,0,y) + \frac{1}{\delta}\tilde{K}_{\alpha m}(y)\rho_{2,\delta}(t,0,y)+\\
&\quad +\tfrac{1}{\delta}\sum_{\beta=1}^{m}\tilde{K}_{\alpha\beta}(y)\pi_{\beta,\delta}(t,y) \text{ for } \alpha \neq 1,m,\\
\frac{\partial \rho_{1,\delta}(t,0,y)}{\partial x} &= -\frac{1}{D}\tilde{K}_{11}(y)\rho_{1,\delta}(t,0,y) - \frac{1}{D}\sum_{\beta \neq 1,m}\tilde{K}_{1\beta}(y)\pi_{\beta,\delta}(t,y),\\
\frac{\partial \rho_{2,\delta}(t,0,y)}{\partial x} &= -\frac{1}{D}\tilde{K}_{mm}(y)\rho_{2,\delta}(t,0,y) - \frac{1}{D}\sum_{\beta \neq 1,m}\tilde{K}_{m\beta}(y)\pi_{\beta,\delta}(t,y).
\end{aligned}
\tag{26}
$$

For $\delta \to 0$ the first three equations can be solved by singular perturbation theory. The leading order solution is $\tilde{K}(y)\mu(y) = 0$ where $\mu(y)$ is the invariant measure of the Markov chain describing the channel. Therefore the interface conditions we get are

$$
\begin{aligned}
\rho_1(t,0,y) &= \mu_1(y),\\
\pi_\alpha(t,0,y) &= \mu_\alpha(y) \text{ for } \alpha = 2...m-1,\\
\rho_2(t,0,y) &= \mu_m(y).
\end{aligned}
\tag{27}
$$

Note that the choice (27) solves also the last two equations in (26).

Other Classical Boundary Conditions across Membranes. In case same relatively small molecules can just cross a lipid bilayer this can be modelled in a PDE setting by so-called Robin boundary conditions without the need to introduce an up-scaling step as we have done before in case of a simple channel dynamics. Consider again some piece of membrane denoted by Γ. Then this condition is given by

$$
a(t,y)\frac{\partial}{\partial \nu}\rho + b(t,y)\rho = c(t,y) \text{ on } [0,T] \times \Gamma,
\tag{28}
$$

where ν is the outward pointing normal vector (we have now assumed that the piece of membrane can be curved, whereas before Γ without loss of generality was considered to be a piece of straight vertical line), and a, b and c are continuous and sufficiently smooth functions characteristic for the membrane and the molecules we are considering. The concentration ρ is considered to describe the molecular distributions inside a compartment, whereas the outer compartment concentration is assumed to be constant and therefore not considered as a state variable.

Transport Inside Compartments. Let Ω now denote the volume (here area, as we are only considering the two-dimensional case) of some cellular compartment. The molecules will move inside the compartment according to some

stochastic process. Without doing the up-scaling here explicitly, but indeed following very much the considerations about the channel dynamics in our detailed previous section on channel dynamics, the resulting equation on a macroscopic scale (here assumed to be the scale of the microscope) can lead to the following equations:

$$\frac{\partial}{\partial t}\rho(t,x) - D\triangle\rho(t,x) = 0 \ \ \text{on} \ \ [0,T] \times \Omega. \tag{29}$$

This is the simplest case, i.e. here we would consider isotropic standard diffusion, with a diffusion coefficient $D > 0$. The operator denoted by $\triangle = \nabla^2$ is the so-called Laplace operator already used before in equation (19). Such an equation would be the limit equation resulting from Brownian motion. There are more sophisticated processes possible. One assumption could be that the molecules get stuck from time to time anywhere in Ω which can be modelled by introducing a mobile and an immobile sub-population of molecules:

$$\partial_t\rho_{mobile}(t,x) = D\Delta\rho_{mobile}(t,x)$$
$$+ k_b \, \rho_{immobile}(t,x) - k_d \, \rho_{mobile}(t,x) \ \ \text{on} \ [0,T] \times \Omega \tag{30}$$
$$\partial_t\rho_{immobile}(t,x) = -k_b \, \rho_{immobile}(t,x) + k_d \, \rho_{mobile}(t,x) \ \ \text{on} \ [0,T] \times \Omega \tag{31}$$

The positive constants k_b and k_d determine the rated at which 'bound' molecules are released and enter the mobile fraction, or get caught and are not able to diffuse any more. Clearly the observed molecular concentration as observed by the microscope would be given as the sum of the mobil and immobile fraction, i.e. $\rho = \rho_{mobile} + \rho_{immobile}$. There are of course other mathematical ways to model such 'sticky' behaviour, Again one can first introduce certain stochastic processes (different from Brownian motion) and derive an effective equation by up-scaling. This can lead to equations with so called fractional diffusion operators, giving rise to sub-diffusive behaviour. There can be directed movement of molecules as well, for example given by the fact that transporter molecules can actively transport other molecules along the cytoskeleton of the cell.

3   Combining the Modules

In order to obtain a complete meaningful specific molecular distribution model, i.e. one that eventually can be compared with data, we need to ascribe boundary and interface conditions to each of the boundaries/interfaces Γ_c, Γ_n and Γ_i, $i = 1, 2, 3$, see Fig. 1, and transport operators for each of the domains Ω_c, Ω_n and Ω_i, $i = 1, 2, 3$. Of course the same holds in case of a more general membrane system given by a tree describing the hierarchy of any system of nested subdomains. In the following we give just one simple possible example of such a complete model, but emphasise the modular character we have introduced. In reality biological knowledge and insight will lead to different hypothesis about movement and translocation of specific molecules across membranes, leading to different transport operators defined inside the compartments, and boundary and interface conditions between the compartments. Our example model is:

We assume that at Γ_c we can impose from outside the cell a fixed molecular concentration for some time:

$$\rho_c(t,y) = c(t,y) \text{ on } [0,t^*] \times \Gamma_c, \tag{32}$$

$$\frac{\partial}{\partial\nu}\rho_c(t,y) = 0 \text{ on } [t^*,T] \times \Gamma_c, \tag{33}$$

$$\partial_t\rho_c(t,x) = D\Delta\rho_c(t,x) \text{ in } [0,T] \times \Omega_c, \tag{34}$$

At Γ_n we assume channel dynamics:

$$\rho_c(t,y) = \pi_1(t,y), \quad \rho_{n,mobile}(t,y) = \pi_m(t,y), \text{ on } [0,T] \times \Gamma_n, \tag{35}$$

$$\frac{\partial\rho_c(t,y)}{\partial\nu} = \frac{1}{D}\left(k_{12}(y)\rho_c(t,y) - k_{21}(y)\pi_2(t,y)\right), \text{ on } [0,T] \times \Gamma_n, \tag{36}$$

$$\frac{\partial\rho_{n,mobile}(t,y)}{\partial\nu} = \frac{1}{D}\left(-k_{m-1,m}(y)\rho_n(t,y) + k_{m,m-1}(y)\pi_{m-1}(t,y)\right), \tag{37}$$
on $[0,T] \times \Gamma_n$,

$$\frac{d\pi_i(t,y)}{dt} =$$
$$k_{i-1,i}(y)\pi_{i-1}(t,y) - (k_{i,i-1}(y) + k_{i,i+1}(y))\pi_i(t,y) + k_{i+1,i}(y)\pi_{i+1}(t,y), \tag{38}$$
$$i = 2, ..., m-1, \text{ on } [0,T] \times \Gamma_n,$$

Inside Ω_n we assume molecules can possibly bind to some structure:

$$\partial_t\rho_{n,mobile}(t,x) = D\Delta\rho_{n,mobile}(t,x)$$
$$+k_b(x)\,\rho_{n,immobile}(t,x) - k_d(x)\,\rho_{n,mobile}(t,x) \text{ in } [0,T] \times \Omega_n, \tag{39}$$
$$\partial_t\rho_{n,immobile}(t,x) = -k_b(x)\,\rho_{n,immobile}(t,x) + k_d(x)\,\rho_{n,mobile}(t,x)$$
$$\text{on } [0,T] \times \Omega_n, \tag{40}$$

Inside Ω_n we assume there are some subdomains where diffusing molecules will not be able to penetrate:

$$\frac{\partial}{\partial\nu}\rho_{n,mobile}(t,y) = 0 \text{ on } [0,T] \times \Gamma_i, i = 1,2,3. \tag{41}$$

The model still needs to be complemented with initial conditions. The state of the system is given by the molecular concentrations $\rho_c(t,x)$, $\rho_n(t,x)$, and the states $\pi(t,y) = (\pi_1(t,y), \ldots, \pi_m(t,y))$, where by continuity $\pi_1(t,y) = \rho_c(t,y)$ and $\pi_m(t,y) = \rho_{n,mobile}(t,y)$ for $y \in \Gamma_n$ and al $t > 0$. The initial conditions can then be written as:

(i) Initial conditions for concentrations in compartments:

$$\rho_c(t,x) = \rho_{c,0}(x) \text{ on } \overline{\Omega}_c, \tag{42}$$

$$\rho_{n,mobile}(t,x) = \rho_{n,mobile,0}(x) \text{ on } \overline{\Omega}_n, \tag{43}$$

$$\rho_{n,immobile}(t,x) = \rho_{n,mobile,0}(x) \text{ on } \Omega_n, \tag{44}$$

(ii) For the channel variable we only need to assign initial values for the states not directly connected to the compartments:

$$\pi_2(0) = \pi_{2,0}, \ldots, \pi_{m-1}(0) = \pi_{m-1,0}. \tag{45}$$

All initial conditions which are either functions or constant values are assumed to be non-negative. We would like to put this well-posed[1] mathematical model into a cell biology context. The boundary Γ_c is modelling the outer cell wall. The cell is embedded in a solution where the concentration of a ligand activating some receptor molecules can be kept controlled in a time interval $[0, t^*]$, with $0 > t^* > T$. After time t^* this ligand is washed away. The activated receptor activates a transcription factor (TF), and it is this activated transcription factor which is assumed to be our molecular species. The activation is close to the cell wall and described by the continuous function $c(t, y) > 0$. The TF cannot leave the cell after the activation of the receptors has stopped, equation (33). Now the TF is freely diffusing in the cytoplasm (equation (34)). Some TF molecules will hit the nuclear envelope modelled by Γ_n. In order to reach any gene inside the nucleus the TF has to cross a nuclear porous complex (NPC). In the current model the NPC is simply modelled by a linear channel, modelled by a linear Markov chain. It also has to be noted that the pores are not modelled as discrete entities. We assume there is a concentration of such channels across the membrane system, of course this is a mathematical abstraction perhaps not justified at a resolution of a confocal microscope. Inside the nucleus modelled by Ω_n the TF can possibly bind to some structure, for example the chromatin. The TF can also be released again. The density of the structure and therefore the probability of a TF molecule to bind or unbind is encoded in the functions $k_b(x) > 0$ and $k_d(x) > 0$. Finally there are regions in the nucleus where the TF is not able to enter, for example a nucleosome which is assumed to be too dense. Such regions are modelled by the domains Ω_1, Ω_2 and Ω_3. At time T we stop to look at this 'in-silico'[2] experiment.

## 3.1   Reactions among Several Species of Molecules

Our model proposed in the previous section, and composed of modules described earlier, is already having some complexity. Nevertheless we have to remind ourselves that in reality every such molecule will have to take part in different molecular reactions, mostly binding to form so-called complexes. A transcription factor for example will need to bind to another molecule[3] in order to be really able to cross the NPCs. To include reactions we will need to increase the number of molecular species. In case these additional species will be present in the same domains as the TF modelled before, each of these additional species

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[1] This would not be too hard to prove by standard techniques. Nevertheless this is a non-standard PDE problem, mostly due to the interface dynamics defined on one of the boundaries.

[2] After implementation in a computer, which still needs a not straightforward discretisation step.

[3] For example to the RAN-GDP complex. See the RAN pathway.

will add the same number of state variables as was needed to model the TF. Reactions are transitions between species, and as those we can also formally define the mobile and immobile phase of the same species as two different populations of molecules. In this case equations (39) and (40) already show how reaction terms enter the model. We give a short overview to mass-action kinetics in a non-spatial setting. The resulting reaction terms would be needed to be incorporated to the spatially explicit model at each location x in a domain where such a reaction can take place. For simplicity we only consider and discuss here the well known deterministic mass-action reaction systems and fix the notation. It should be noted that the local dynamical system (i.e. for each location x in a compartment) derived from the reaction scheme in this interpretation is only valid with different implicit assumptions made for the mechanisms of the reactions and the properties and abundance of the different molecules involved. In particular there has been a law of large numbers being applied to the particle system, and the continuum limit is only represented by the so-called 'average dynamics', i.e. any stochastic fluctuations are assumed to be negligible. Also we assume each particle of every species remains unchanged in its properties during reactions and only 'varies' by forming molecules with other species or molecules of its own kind. Such a chemical mass-action reaction system with r reactions and m reacting species is then described by a time-continuous dynamical system defined for each $x \in \Omega$ which is directly associated to the reaction scheme. Each such reaction can be written in the form

$$\alpha_{1j}S_1 + \cdots + \alpha_{nj}S_n \xrightarrow{k_j} \beta_{1j}S_1 + \cdots + \beta_{nj}S_n, \quad j = 1, ..., r, \tag{46}$$
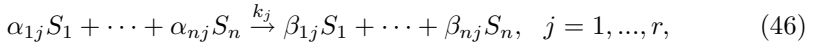
where the S_i, $1 \leq i \leq n$, are the chemical species and each $k_j > 0$ is the kinetic constant of the j-th reaction. The kinetic coefficients take into account all effects on the reaction rate apart from reactant concentrations, for example, temperature, light conditions, or ionic strength in the reaction. The coefficients α_{ij} and β_{ij} represent the number of S_i molecules participating in j-th reaction at reactant and product stages, respectively. The net amount of species S_i produced or consumed by the reaction is named the stoichiometric coefficient and defined by $n_{ij} := \beta_{ij} - \alpha_{ij}$. These coefficients are arranged in a *stoichiometric* matrix, denoted by N. The rate at which the j-th reaction takes place in mass-action kinetics takes the form of a monomial,

$$v_j(x, k_j) = k_j \prod_{i=1}^{m}(\rho^i)^{\kappa_{ij}},$$

where κ_{ij} is the molecularity of the species S_i in the j-th reaction, and ρ^i is the concentration of the ith species. Also in our mass-action kinetic interpretation the kinetic exponent κ_{ij} reduces to being simply α_{ij}. Kinetic exponents are arranged in a *kinetic* matrix, denoted by κ. The time evolution of the species concentrations is described by the following initial value problem:

$$\dot{\rho} = Nv(\rho, k), \tag{47}$$
$$\rho(0) > 0, \tag{48}$$

where $\rho(0)$ are initial molecular concentrations. The vector

$$\rho(t, x) = (\rho^1(t, x), \ldots, \rho^n(t, x))^T$$

is describing the concentrations of the n different molecular species $S_1, \ldots, S_n$ at *any* location x in one of the compartments of our membrane system. Equation (47) is delivering the so called reaction terms, it does neglect all transport or transportation over membranes of any of the species. We need to enlarge the state space associated to our complete model for a single molecular species as described in section 3. The state of the extended system is now given by the molecular concentrations

$$\rho_c(t, x) = (\rho_c^1(t, x), \ldots, \rho_c^n(t, x))^T, \rho_n(t, x) = (\rho_n^1(t, x), \ldots, \rho_n^n(t, x))^T,$$

and the states $\pi(t, y) = (\pi_1^1(t, y), \ldots, \pi_m^n(t, y))$ which can now be interpreted as a $m \times n$-matrix. Of course we make a number of assumption implicitly, like the one that molecules of different species do not interact in the channel etc. All such issues would need to be fine-tuned and checked in a realistic modelling attempt. We should also note that each single equation of the model in the beginning of section 3 is now becoming a system of n equations corresponding to the n different species we have introduced. Finally interesting qualitative behaviours such as bistability and oscillations have been observed in such reaction systems of mass-action type, see [1,6,9,17,11]. They can be interpreted to result from a bifurcation, i.e. a qualitative change in the behaviour of the system's solutions when one or more of the parameters are varied. Hence, a common approach in identifying such behaviour has been to derive conditions under which the system is able to undergo an associated bifurcation, see [29,11]. Such considerations hold for each location in space independently. In combination with transport of molecules we can expect that from local complex dynamics (such as oscillations) very complex spatial *pattern formation* can arise. See for example different chapters in [24], especially chapter 1 by Fiedler and Scheel, and chapter 3 by Paul Fife. Very often elementary chemical reactions of mass-action type are too complex for modelling biological systems. After time scaling the reactions schemes can be often simplified and then are called 'enzyme kinetics', see among others [40,23].

3.2   The Necessary Discretisation

There a various way to discretise the PDE models we have finally derived. It should be noted that the PDE did result from up-scaling of discrete objects, for example particles jumping on a fine grid which grid size was scaled to zero. In other words, and for later discussion, we make a step from discrete stochastic processes to continuum models, and finally a step back to discrete models which are discretisations of continuum models, as those are the only ones that can be currently interpreted by present standard computer architecture. We choose a form of a so-called mesh-free discretisation based on a partition of unity (PUM), a class of generalised finite element methods. We explain the discretisation step for a single given diffusion equation in one of our compartments. Let H be an

appropriate Hilbert space ($H = H^1$ for 2nd order problems like the diffusion equation). We can obtain the variational form of the PDE using a continuous bilinear form $a : H \times H \to R$ and a linear form $l \in H'$ along with appropriate boundary or interface conditions. The final problem we seek to solve may be summarised as

$$\text{Find} \quad u \in H \quad \text{s.t.} \quad a(u, v) = l(v) \qquad \forall v \in H. \tag{49}$$

The basic method of discretisation in the PUM framework is then given by the following steps:

– Given a domain Ω on which a linear scalar PDE is defined, open sets called *patches* are used to form a cover of the domain. ($\Omega_N := \{\omega_i\}_{i=1}^N$, with $\overline{\Omega} \subset \bigcup_i \omega_i$).
– A partition of unity $\{\varphi_i\}_{i=1}^N$ subordinate to the cover is constructed.
– The local function space on patch ω_i, $1 \le i \le N$, is given by $\mathcal{V}_i := \text{span}\{\psi_i^k\}_{k=1}^{p_i}$, with $\{\psi_i^k\}_{k=1}^{p_i}$ being a set of base functions defining the approximation space for each patch. The global approximation space, also called the trial or the *PUM space*, is defined by $V_{\text{PU}} := \text{span}\{\varphi_i \psi_i^k\}_{i,k}$. Replacing H by the finite dimensional subspace[4] V_{PU}, a global approximation u_h to the unknown solution u of the PDE is defined as a (weighted) sum of local approximation functions on the patches:

$$u_h(x) = \sum_{i=1}^N \varphi_i(x) \left(\sum_k \xi_i^k \psi_i^k(x) \right).$$

– The unknown coefficients ξ_i^k are determined by substituting the above approximation into the PDE and using the method of weighted residuals to derive an algebraic system of equations

$$A\xi = b. \tag{50}$$

More detail on the PUM, including a description of its approximation properties and how to construct the PUM space, may be found in, for example, [2,3,4,39]. We have implemented the PUM in a C++ code called the *Generic Discretisation Framework (GDF)*. See also [12,13,14].

4   What Can Be Measured?

Once the model is implemented on a computer we can compare the simulated solution with a measured concentration of molecules in a given cell. Typically this would currently be best done in an in-vivo situation with the help of fluorescent markers with which the molecules under investigation have been tagged. As

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[4] Note that V_{PU} is conforming for the Neumann problems we are concerned with in this article.
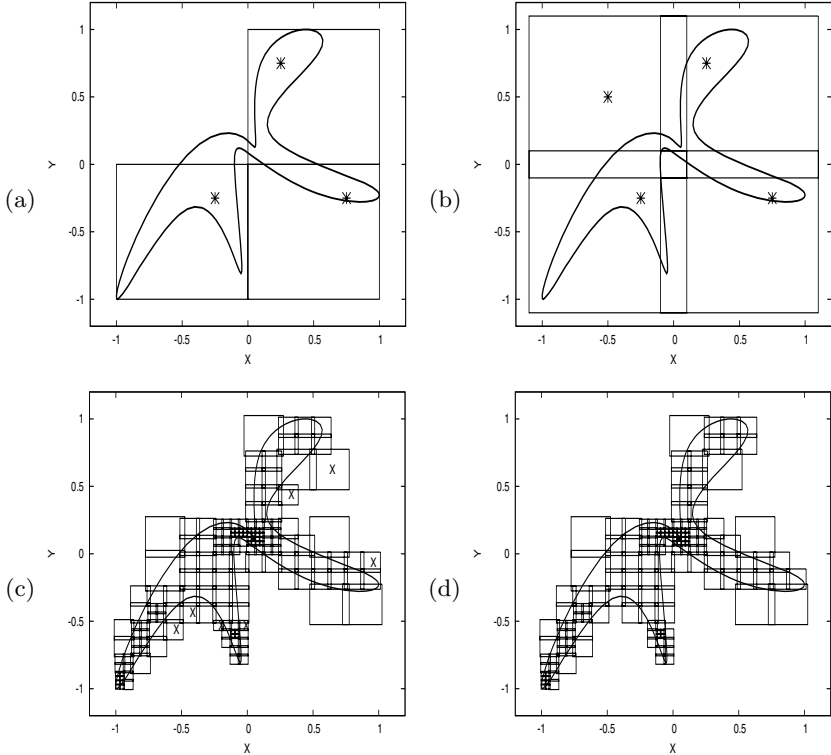
Fig. 4. Key stages of cover construction for a complex shaped compartment. **(a)** Three points distributed randomly in a complex domain and the initial cover. **(b)** An increase in the number of patches so that the whole domain is covered. Patches have also been extended by $\alpha = 1.2$. **(c)** Optional refinements of the cover. For clarity, the points are omitted and only the associated patch pictured. Seven patches whose intersection with the domain are subsets of another patch are labelled 'X' and will be removed in the final stage of basic cover construction. **(d)** The final cover of 159 patches with the seven patches from frame (c) removed.

there are only very few colours available[5] the number of different species that can be observed at the same time is very limited, and most of the time is just a single species. We observe the distribution of the molecules with the help of a microscope, in this context this will be a confocal microscope most of the time. This can be done at different magnifications which introduces the spatial scale at which we have to consider the problem. Interestingly enough the pictures we will retrieve from the microscope are again discrete, i.e. pixel based. This means we are comparing a discrete solution of a continuum model with the discretised image of a (on the typical scales given by the resolution chosen) continuous

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[5] The first one introduced and the one best known is GFP, for Green Fluorescent Protein.

distribution of fluorescence in the sample cell. The normal situation we will encounter is that the fluorescence distribution of the tagged molecules when starting the observation will be static, i.e. in equilibrium. This is because most of the experimental conditions start from an equilibrium. If there is for example a diffusion process, the molecules will have had enough time to evenly distribute in all compartments which they can enter. In order to make processes visible we will have to perturb the system. This can be done with the help of a laser with which we can bleach the fluorescent molecules. In other words we can implement a sink term for fluorescence[6] and the result is a dynamic situation where all the mechanisms responsible for protein distribution are now acting together to change the measured fluorescence distribution over time. The most frequently used approach is FRAP (Fluorescence Recovery After Photobleaching) where a certain part of the cell is bleached, i.e. all fluorescent molecules in a given area are bleached in a very short period of time. Subsequently it can be measured how the now bleached area is recovering its fluorescence. This can only happen if the tagged molecules outside the bleach area can move, for example again by a simple diffusion process. We frequently use also longer bleaching periods. This can be helpful, for example in understanding how many fluorescent molecules are estimated to be in a closed compartment, or by equilibrating a flux into a compartment with the sink created by the laser beam. The literature on FRAP and the other bleaching techniques is huge and we do not try here to give a complete overview. A paper describing and applying techniques very close to techniques we frequently use in the laboratory is [31].

The are many more ways of measuring specific parameters and mechanisms of the model proposed, most of them related to in-vitro measurements, for example measuring kinetic constants etc. We cannot go into any detail here, but emphasise that information from different sources (and on different scales) will be needed in a successful modelling attempt. Geometrical information is discussed in the next subsection.

4.1   Cell Compartmental Geometry

The images of Fig. 5 did not allow to retrieve the geometry of the chloroplast. The membrane system inside a chloropast is very complex, consisting of various so called thylakoid staples which host the light harvesting complexes. In this case it would be better get the problem geometry from other sources, for example from electron microscopy (EM) with a much higher resolution. Sometimes however the confocal microscopy resolution is sufficient. Figure 6 shows a fibroplast cell which is almost flat[7]. Here the essential geometry of the membrane system (plasma membrane, nuclear envelope) could be recovered.

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[6] There are also activatable fluorescent molecules which can be activated by a laser beam. In this case the sink becomes a source.

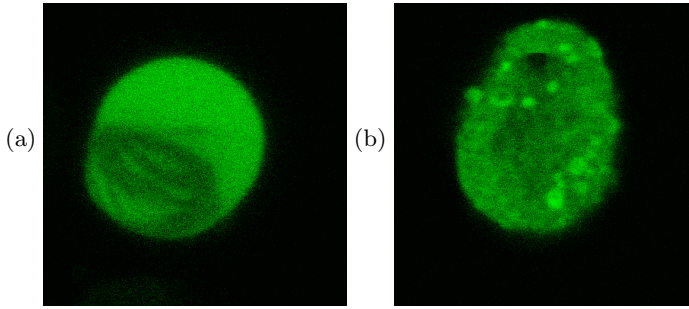[7] This justifies a 2D approach in the modelling.

Fig. 5. The start of two different continuous bleaching experiments with the help of a confocal microscope. Courtesy of Colin Robinson's laboratory (University of Warwick). Each picture shows a single chloroplast inside a single pea protoplast (the outer rigid cell wall has been removed) plant cell. **(a)** A bleaching example where pure GFP was translocated into the chloroplast. This molecule cannot bind to any membranes inside the chloroplast. As this molecule is rapidly diffusing the bleaching area is only visible indirectly as a larger area of fluorescence depletion in the lower part of the chloroplast. **(b)** A membrane protein has been translocated into the chloroplast. The bleaching area in the top of the picture can be clearly seen as the protein is largely immobile, most likely bound to the membrane system. Illustration taken from [13].
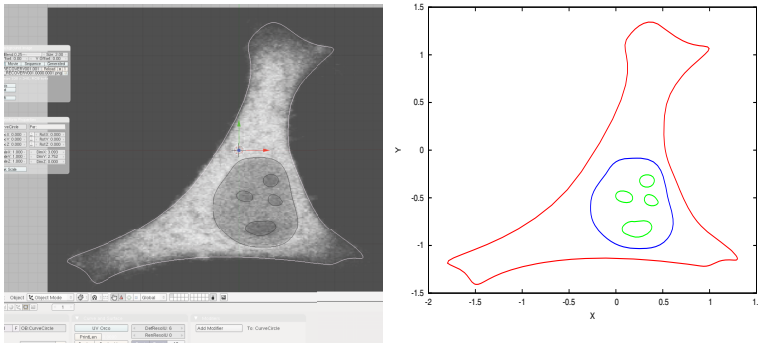

Fig. 6. Domain with nested subdomains. Left the original fibroplast microscopy image inside a modelling programme to retrieve the (sub-)domain shapes, courtesy of Gimmi Ratto, see also [33]. Right: The main domain represents the cell body, the main subdomain represents the nucleus, and the four inner sub-subdomains (green) represent nucleoli. Illustration taken from [13].

4.2   Simulation Results

In order to compare measurements with simulations we best visualise the computed solutions on the geometry we have retrieved from the experimental situation. We illustrate this in Figure 7 with the help of the fibroblast example.
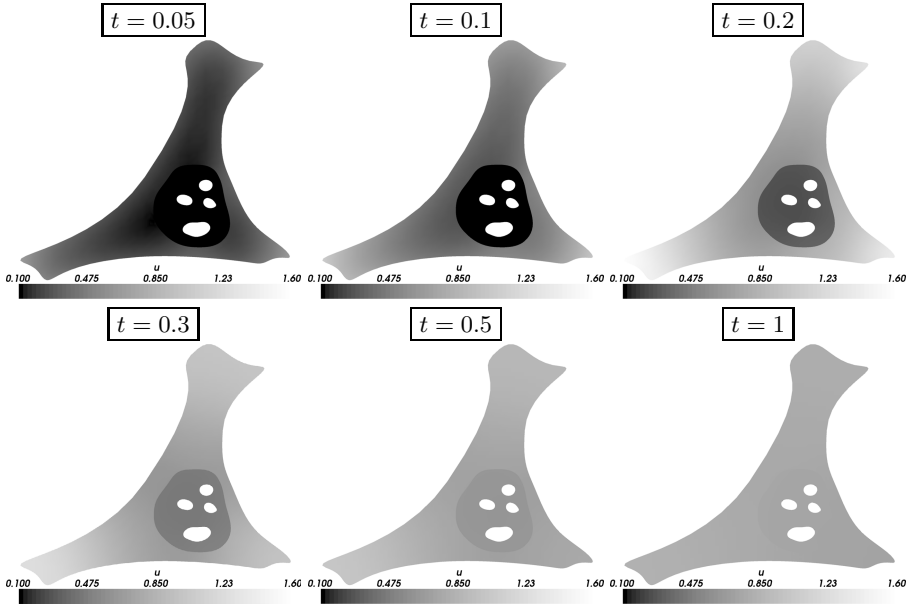
Fig. 7. Concentration function $\rho(t, x)$ at selected times for a simulation on the entire two-dimensional fibroblast shape. In this simplified signalling problem a molecule is released (activated) uniformly at the plasma membrane, diffuses inside the cytoplasm, can enter the nucleus (a sub-domain, i.e. a nested domain of level 1) following a simple linear relationship based on concentration differences (not modelling the NPCs in detail), and finally distributes itself inside the nucleus where it can get bound to the nucleoli modelled as sub-sub-domains (nested domains of level 2). Illustration taken from [13].

5 The Cell as an Information Processing Device

Membrane computing and modelling and simulation of cellular molecular distributions have been defined and introduced for completely different purposes. Membrane computing is very generally speaking taking up successfully ideas from biology to theoretically analyse algorithms. The concept is to structure algorithms that eventually can solve certain computational tasks in finite time. This is a step away from the Turing machine. This theoretical device is as unstructured as possible, i.e., it was designed to investigate a very general class of algorithms and checks whether a given programme encoded in bits stops after performing only finitely many discrete steps (stopping problem). Algorithms solving problems in a modern world, like regulating traffic in a city, are surely necessarily much more structured. They are expressed in languages that are object oriented, which means there are surely 'membranes' around the objects that protect local variables to be overwritten etc. Nevertheless information has to enter and leave the objects. This is indeed very close to the biological situation of a cell. As mentioned in the introduction it is very likely that the complex membrane systems we see in eukaryotic cell are linked to the fact that cells in a

multi-cellular organisation have to solve much more different tasks. Such tasks
are always better and more robustly solved if they can be distributed to differ-
ent specialised sub-units, the organelles. The cell developed different mechanisms
how molecules can pass the membranes, in cell biology called 'translocation'. It
was on purpose we have chosen a transcription factor (TF) to explain how mole-
cular processes do solve the task of reacting to signals stemming from outside the
cell. Such processes are usually called 'signalling pathways'. The transcription
factor is for example released or better activated close to the plasma membrane
where the receptors measuring the outside signal[8] are located. They finally have
to reach the respective gene which needs to be activated in order to respond to
the changes of the environment, here signalled by a changing signalling extra-
cellular molecular concentration. Nevertheless the task might not be so straight-
forward. The internal state of the cell may act as a filter of the incoming signal.
A gene might only be switched on if a transcription factor reaches the gene
in the right way. It may be required that the signal is repeated just with the
right amplitude and frequency[9]. To work as a filtering information processing
device the cell needs to be able to adapt its internal state, including the regu-
lation of membrane proteins enabling other molecules to cross membranes. To
understand these processes it was always good practise to try a forward simu-
lation, i.e. to model the different sub-processes that might lead to the observed
molecular distributions, assemble them in a system, and compare prediction and
measurement for a given period of time. In this paper we have defined a possible
framework with which such computations can be performed. It is striking how
close the concepts are in relation to ideas from membrane computing. This is
no coincidence, as both approaches need to directly abstract cellular biological
performance.

6   Discussion

We can easily see the similarities and differences between membrane computing
and cellular interface problems (CIP) when we look at the definitions of both
'processes'[10]. Following the definition of a transition P system in [32] (of degree
$m \geq 1$) such a system is a construct of the form

$$\Pi = (O, C, \mu, w_1, w_2, \ldots, w_m, R_1, R_2, \ldots, R_m, i_o),$$

with

1. O is the (finite and nonempty) alphabet of objects,
2. $C \subset O$ is the set of catalysts,

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[8] For example a hormone.
[9] For example such processes are important for synaptic plasticity where only a re-
peated signal should be interpreted as 'learning', i.e. increasing synaptic transmission
strength.
[10] Both membrane computing and cellular interface problems can be called processes
as they necessarily change both generically their state in time.

3. μ is a membrane structure, consisting of m membranes, labeled $1, 2, \ldots, m$; we say that the membrane structure, and hence the system, is of degree m,

4. $w_1, w_2, \ldots, w_m$ are strings over O representing the multisets of objects present in regions $1, 2, \ldots, m$ of the membrane structure,

5. $R_1, R_2, \ldots, R_m$ are nite sets of evolution rules associated with regions $1, 2, \ldots, m$ of the membrane structure,

6. i_o is either one of the labels $1, 2, \ldots, m$, and the respective region is the output region of the system, or it is 0, and the result of a computation is collected in the environment of the system.

The rules related to this system are of the form $u \rightarrow v$ or $u \rightarrow v\delta$, with $u \in O^+$ and $v \in (O \times Tar)^*$, where $Tar = \{here, in, out\}$. The rules are applied to be maximally parallel. We first focus on similarities. The membrane structure μ can be interpreted as identical in the cellular interface problem, but there will be in addition the geometrical information needed for the simulation. The 'molecules' in the P systems are also represented by a finite number of different species, labelled $w_1, w_2, \ldots$. There is no direct relationship between the evolution rules $R_1, R_2, \ldots, R_m$ and the transitions in the CIP. Of course the mass-action kinetic reactions we have briefly discussed do deliver such rules, but there are other transition rates related to the transport process (not incorporated to the transition P system) and the translocation processes[11] over membranes. The labelling of the species in order to follow their membership to certain compartments is done in the same way as in the transition P system.

Many differences between P system and CIP result from the fact that the CIP is formulated completely discrete, whereas the CIP on its macroscopic level is completely formulated in a continuous framework. This can be seen by comparing the alphabet O of the P system and the vector of concentrations ρ formulated for the CIP. This can be explained by introducing the concept of scale, both in space and time. For a P system temporal scale is largely irrelevant as it is a powerful computational concept. Like for a Turing machine it is important to perform the steps defined by the rules of the P system and the definition of a universal clock where discrete steps (events) are performed in a maximally parallel way as long as the system has not reached its final configuration. For the CIP scale is crucial, both for time and space. Processes can take place at different temporal and spatial scales, and during the so called multi-scale analysis (see [38,34]) these processes will look different in the final outcome of the model. To make this point was the reason to include a long discussion of the channel dynamics where we motivated how to derive the corresponding interface conditions.

It could be argued that this distinction between discrete and continuous frameworks is artificial. In fact we could just define all transitions of the CIP on a microscopic scale, where discrete particles would follow stochastic processes. This is in fact a true statement. Nevertheless a scale problem would arise also in this framework, the events of different molecular processes would happen at different time scales. This would mean we would need to go to the smallest time scale present in the system, and define all other processes in terms of this basic scale.

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[11] Just as an example we have discussed in detail a given channel dynamics.

The result would be an infinitely complex system which we presently could not handle even on large computers. The reason for this is that we would need to give up using all different averaging procedures which have been developped to derive simpler, macroscopic, often called 'effective' equations on relatively large temporal and spatial scales. It is in fact 'averaging' in a wider sense that motivates the use of continuum models if direct modelling and simulation of (biological) temporal and spatial processes is the given task. It would be interesting to investigate if such concepts also could apply for the further development of P systems.

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