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# Modelling and simulation techniques for membrane biology

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## Abstract

One of the most important aspects of Computational Cell Biology is the understanding of the complicated dynamical processes that take place on plasma membranes. These processes are often so complicated that purely temporal models cannot always adequately capture the dynamics. On the other hand, spatial models can have large computational overheads. In this article, we review some of these issues with respect to chemistry, membrane microdomains and anomalous diffusion and discuss how to select appropriate modelling and simulation paradigms based on some or all the following aspects: discrete, continuous, stochastic, delayed and complex spatial processes.

***Keywords:*** *spatial and stochastic modelling; bio-chemical kinetics; multi-scale simulation; systems biology*

## INTRODUCTION

Crucial to the understanding of the mechanisms of cellular function as a whole is the understanding of biophysical and biochemical processes taking place on the cell membrane. Recent progress in genetic sequencing, microscopy and other experimental methods has shed a great deal of light on membrane structures and phenomena, including the discovery that the membrane may possess significant lateral structure (microdomains); with concomitant progress in the understanding of transport phenomena on the membrane, of ion channel function and of transport across the membrane [1–3]. However, the integration of this information into comprehensive and coherent models of the membrane has been slower. To some extent, this is due to the sheer complexity of the interactions taking place and experimental limitations. For example, when visualizing proteins on the membrane using Transmission Electron Microscopy (TEM), only two species at a time can (at present) typically be labelled, making the determination of interactions among tens or hundreds of species very difficult.

In order to produce a coherent picture of the cell membrane, mathematical modelling and simulation provide an indispensable tool. On the other hand, the modelling of membrane processes poses mathematical challenges of its own because of the non-classical nature of biophysical media. Cellular processes take place in highly complex environments, characterised, *inter alia*, by very high local densities of species (molecular crowding), spatial segregation and organization and by the presence of various structures whose character and functions are

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generally only partly (if at all) understood. As a testament to this, diffusion on the cell membrane is not only highly anomalous but the diffusion rate of proteins on live cell membranes is between one and two orders of magnitude slower than in reconstituted artificial membranes with the same composition. Furthermore, diffusion is dependent on the dimensions of the medium so that diffusion on the highly disordered cell membrane is not a perfectly mixing process and therefore the assumptions underlying the classical theory of chemical kinetics fail, requiring new approaches to modelling chemistry on the membrane. Finally, in many cases, key species are present in small or very small numbers, so even the concept of concentration is no longer meaningful.

In their essence, all of these challenges stem from the complex spatial and non-homogenous character of the cell membrane (and cellular media in general) and so may be said to be 'spatial challenges'. In order to address them, we need methods that must represent the membrane with a higher degree of fidelity. This may mean either that we must directly include the presence and/or function of the various structures and functions in computer simulations, or that their existence and effects must be taken into account indirectly by the models.

## SPATIAL ASPECTS OF THE CELL MEMBRANE

The classical view of the plasma membrane lipid bilayer as a two-dimensional fluid acting as a neutral solvent for membrane proteins in which all particles diffuse freely [4] has been substantially modified in recent years. The plasma membrane is a highly complex structure that is compartmentalised on multiple length and time scales. This compartmentalization is driven by a variety of lipid–lipid, lipid protein and actin cytoskeleton interactions [3, 5–7].

An example of membrane microdomains is the lateral segregation of glycosphingolipids and cholesterol into liquid-ordered domains. Phase separation of cholesterol-enriched, liquid-ordered domains or lipid rafts, has been demonstrated in both model and biological membranes, although the length and time scales on which this phase separation occurs are the matter of debate [3, 6, 8]. Multiple estimates of the diameter of lipid rafts have been provided using diverse techniques, although photonic force microscopy, homo-FRET and EM provide a convergence of estimates to 6–50 nm, with the most recent studies favouring the lower end of this range [1–3]. Similar sizes, in the range of 12–32 nm have been reported for the microdomains occupied by activated H-ras and K-ras [1].

An important role that has been ascribed to all plasma membrane microdomains is that of selectively concentrating proteins to facilitate the assembly of signalling complexes [9]. Many studies have been qualitatively interpreted in terms of this type of microdomain model. However, no quantitative analysis has been attempted to explore the basic mechanics of how microdomains might drive protein–protein interactions as demanded of their role in supporting the assembly of signalling platforms. For example, if microdomains do aggregate proteins, are there any constraints on size and dynamics that need to be imposed for them to achieve this function? If so, are these constraints realistic and how do the predictions compare with recent estimates of microdomain size and dynamics? These are difficult but important questions that are especially relevant in the context of the ongoing discussions of plasma membrane structure and function.

The Mitogen-activated protein kinase (MAPK) cascade is perhaps one of the most studied of biochemical reactions, as the pathway provides molecular targets for novel anticancer therapies. The cascade transmits signals from activated growth factor receptors on the plasma membrane into the cytosol of the cell. In its simplest form it consists of three tiers in which Ras phosphorylates and activates Raf, which phosphorylates MEK which then phosphorylates ERK. There are three mayor MAPK pathways: extracellular signal regulated kinase (ERK), Jun N-terminal kinase (JNK) and p38.

A significant article in this area is that of Huang and Ferrell [10] in which the MAPK cascade is represented by a system of 25 ordinary differential equations (ODEs). In a more recent paper, Schoeberl *et al*. [11] elucidate the MAPK pathway activated by the EGF receptor in which there are 94 species and 230 reactions on the plasma membrane and in the cytosol. Yet, even this very complicated model ignores spatial effects such as kinase translocation. An important aspect of the MAPK pathway is the nature of the signal transduction module that relates the signal to the response (ERKpp). Based on the amount if concentration of ERKpp, a cell can make a decision either to proliferate or differentiate.

Huang and Ferrell suggest from their ODE study that there is a switch-like behaviour due to Ras activation. On the other hand, Mackeigan *et al*. [12] suggest that the output is graded in response to growth factor simulation. Furthermore, recent studies suggest that signal outputs of the MAPK pathway depend strongly on spatial location [13].

In order to resolve these issues a greater focus on spatial modelling and simulation will be needed. It is already known that RasGTP form nanoclusters on the plasma membrane. It has been suggested that these nanoclusters (which have a short life time of about 0.4 s and contain only a small number of molecules) have a very significant effect on the MAPK pathway. In fact in a very recent work [14], a temporal compartment model linking the signal transmission of each nanocluster, modelled by a Markov process, with a system of ODEs for the MAPK pathway in the cytosol has predicted graded signal transduction.

Another set of related questions is concerned with the nature of the random motions of proteins on the plasma membrane. Biological media exhibit a large degree of complexity and heterogeneity and often exhibit substantial compartmentalization [15], and diffusion is observed to be orders of magnitude slower than predicted by theory [16]. As a result of the non-classical nature of these random motions, biological reactions are generally complex and non-deterministic. Moreover, they are frequently characterised by low numbers of molecules of some reacting components [17]. Anomalous diffusion can arise in many ways, such as through cytoskeletal corralling whereby proteins are corralled due to the interaction with the cytoskeleton beneath the membrane [18]. Molecular crowding has been estimated to be anywhere between 5% and 40% of the total volume. It has been postulated that anomalous diffusion may be a mechanism for cells to localise receptors and control intramembrane signalling [19]. The nature of anomalous diffusion can be captured by the anomalous diffusion parameter $\alpha$, which has the value $\alpha = 1$ for pure diffusion. At the percolation threshold, where global mixing stops, the value for $\alpha$ in two and three dimensions is 0.69, and 0.54, respectively. Various techniques have been used to study such processes, including Single Particle Tracking (SPT) [20], Fluorescence Recovery after Photobleaching (FRAP) [21] and Fluorescence Correlation Spectroscopy [22]. Despite these investigations, the quantification of the degree and nature of the anomalous diffusion has proven difficult [19, 23] due to experimental limitations. Nevertheless, some estimates of the anomalous exponent and other parameters have been reported. For example, Smith and collaborators [20] estimated $\alpha \approx 0.49 \pm 0.16$ for diffusion of proteins on HeLa cell surfaces. Shav-Tal *et al*. [24] can colour fluorescent proteins to track both mRNA and translate proteins inside the nucleus of a living cell. They observed purely diffusive behaviour ~58% of the time and corralled behaviour ~42% of the time. In different settings, Wachsmuth *et al*. [25] measured the diffusion behaviour of proteins in the nucleus and estimated a value for the anomalous parameter $\alpha = 0.87$, which is far from the percolation threshold. On the other hand, Schwille *et al*. [26] showed that diffusion on membranes is anomalous with $\alpha = 0.74$ and that value is close to the percolation threshold in two dimensions, so that anomalous effects are significant on plasma membranes.

## MODELLING OF CHEMICAL KINETICS ON MEMBRANES

In order to address such important and difficult issues, modelling and simulation tools should be used hand-in-hand with experimental techniques. The crucial point is what form these models should take: deterministic versus stochastic, discrete versus continuous, spatial versus temporal, delay versus non-delay. If we are to develop realistic, experimentally verified models and simulation techniques, how should we proceed? There are essentially three modelling approaches: temporal models, temporal models that incorporate spatial information and spatial-temporal models. We discuss these aspects each in turn.

### Temporal models

In a purely temporal setting and when there are large numbers of molecules present, chemical reactions are modelled by ODEs that are based on the laws of Mass Action and that estimate reaction rates on the basis of average values of the reactant density. Any set of $m$ chemical reactions can be characterised by two sets of quantities: the stoichiometric vectors (update rules for each reaction) $\nu_1, \ldots, \nu_m$ and the propensity functions $a_1(X(t)), \ldots, a_m(X(t))$. The propensity functions represent the relative probabilities of each of the $m$ reactions occurring. Here $X(t)$ is the vector

of concentrations at time $t$ of the $N$ species involved in the reactions. The ODE that describes this chemical system is given by

$$X'(t) = \sum_{j=1}^{m} v_j a_j(X(t)).$$

Very often the size of the ODE system can be reduced by the use of the Quasi-Steady Sate Assumption (QSSA). Under the QSSA it is assumed that the fast reactions go to equilibrium much more quickly that the slow reactions. Thus a system of algebraic equations can be solved at the 'fast equilibrium' and this solution substituted back into the original system, thus reducing the dimension and altering the propensity functions to include non-linear Hill functions.

In the case of small numbers of molecules the appropriate formulation is the Stochastic Simulation Algorithm (SSA) [27], as ODEs can only describe a mean behaviour. The SSA is essentially an exact procedure that describes the evolution of a discrete non-linear Markov process. It accounts for the inherent stochasticity (internal noise) of the $m$ reacting channels and only assigns integer numbers of molecules to the state vector. At each step, the SSA simulates two random numbers from the uniform distribution U[0,1] to evaluate an exponential waiting time, $\tau$, for the next reaction to occur and an integer $j$ between 1 and $m$ that indicates which reaction occurs. The state vector is updated at the new time point by the addition of the $j$-th stoichiometric vector to the previous value of the state vector, that is

$$X(t+\tau) = X(t) + v_j.$$

The main limiting feature of SSA is that the time step can become very small, especially if there are large numbers of molecules or widely varying rate constants. In order to overcome these limitations, a number of different approaches (so-called $\tau$-leap methods) have been suggested in which the sampling of likely reactions is taken from either Poisson [28] or Binomial [29] distributions. In these cases a much larger time step can be used at the loss of a small amount of accuracy. A different approach is to note that the discrete non-linear Markov process described by the SSA has a probability density functions that is the solution of the so-called Chemical Master Equation (CME). The CME is a discrete parabolic partial differential equation in which there is an equation for each configuration of the State space. When the State space is enumerated, the CME becomes a linear ODE and the probability density function takes the form

$$p(t) = e^{At} p(0)$$

where $A$ is the state-space matrix. Even for relatively small systems, the dimension of $A$ can be in the millions, so it would appear that this is not a computationally feasible approach. But in fact a proposed finite state projection algorithm [30] reduces the size of the matrix $A$ and this together with the fact that not all the states are reachable, then the use of Krylov subspace techniques [31, 32] to efficiently compute the exponential of a matrix times a vector means that the computation of the probability density function directly is a very feasible technique.

Finally, it is important to note that there is a regime intermediate to the discrete stochastic regime and the continuous deterministic ODE regime in which the internal noise effects are still significant but continuity arguments can apply. This leads to the so-called Chemical Langevin Equation (CLE) that is an Itô stochastic ordinary differential equation (SDE), driven by a set of Wiener processes that describes the fluctuation in the concentrations of the molecular species. The CLE preserves the correct dynamics for the first two moments of the SSA and takes the form

$$\mathrm{d}X = \sum_{j=1}^{m} v_j a_j(X(t)) + B(X(t))\mathrm{d}W(t).$$

Here $W(t) = (W_1(t), \ldots, W_N(t))$ is a vector of $N$ independent Wiener processes whose increments $\Delta W_j = W_j(t+h) - W_j(t)$ are $N(0,h)$ and where

$$B(x) = \sqrt{C}, \quad C = (v_1, \ldots, v_m)\,\mathrm{Diag}(a_1(X), \ldots, a_m(X))(v_1, \ldots, v_m)^T.$$

Here $h$ is the time discretization step. Effective numerical methods designed for the numerical solution of SDEs [33–35] can be used to simulate the chemical kinetics in this intermediate regime. Furthermore, adaptive multiscale methods have been developed which attempt to move back and forth between these three regimes as the numbers of molecules change [36].

## Temporal models that use spatial information

In terms of understanding chemical dynamics in a crowded environment, there may be some hope that

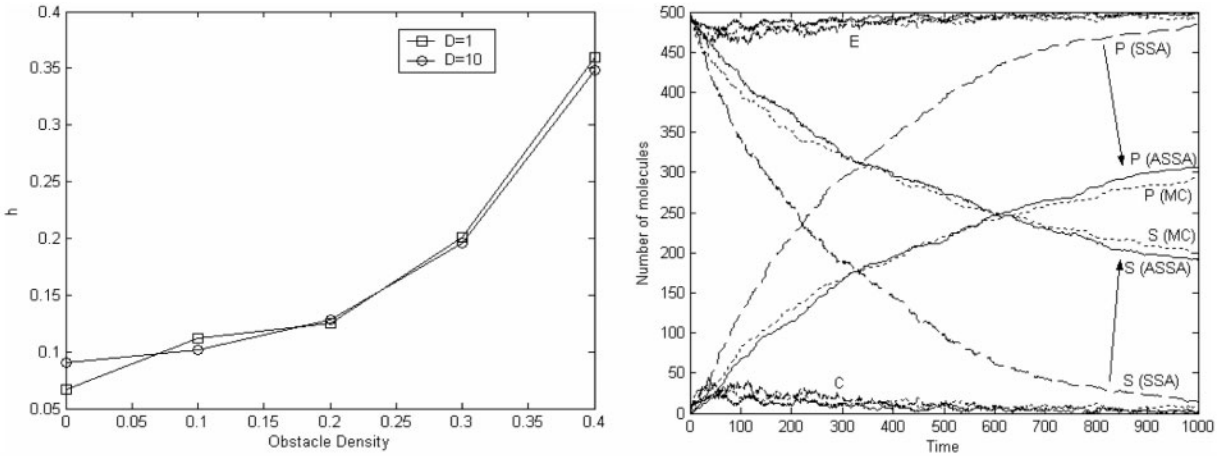


**Figure 1:** Left: the dependence of h on obstacle density and step size. Note that even at $\theta = 0$, $h \neq 0$ as diffusion is not completely classical due to competition for voxels. Right: comparison of pure SSA (dashed line), ASSA (with appropriate *h*—solid line) and Monte Carlo simulation (dotted line) for the Michaelis–Menten system in which four molecular species react according to the equation:

$$E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} C \overset{k_2}{\rightarrow} E + P$$

where *E* is the enzyme, *S* is the substrate, *C* is a complex and *P* is the product. The initial conditions are $[E(0)] = 500$, $[S(0)] = 500$, $[C(0)] = 0$, $[P(0)] = 0$ and $\theta = 0.4$. While SSA is inaccurate for this set-up, the ASSA predictions are very close to the Michaelis–Menten Monte Carlo results.

temporal models can be used that captures some of the spatial effects. For instance, Kopelman [37, 38] observed that for crystalline alloys, macromolecular crowding can affect the nature of chemical reactions and postulated a time-dependent behaviour for $k$ in reactions of the type A + A S, of the form

$$k(t) = k_0 t^{\alpha - 1}, \quad \alpha \in [0,1]. \tag{1}$$

Here the value 1-$\alpha$ is a measure of the dimensionality of the system and is sometimes called the fractal parameter. For the Michaelis-Menten reactions with different obstacle densities, Monte Carlo simulations can be used to estimate the rate constants [15, 17, 39], and it was shown that $k$ behaved as in (1) for the bimolecular reactions, but was always constant for unimolecular reactions, even in crowded environments. The simulations also show pronounced substrate-product segregation at obstacle densities comparable with *in vivo* conditions. This suggests that complicated spatial kinetic effects can be captured by simple temporal models by relating $\alpha$ to the obstacle density in a crowded environment [40]. This is done by using Monte Carlo simulations to capture a relationship between $h$ and $\theta$, the density of obstacles on the membrane—see Figure 1 (left panel). The general strategy is to replace all constant rates by (1) in the case of bimolecular reactions while for unimolecular reactions, no change is needed since spatial structure has no impact. The ensuing temporal algorithm ASSA (Anomalous Stochastic Simulation Algorithm) captures the heterogeneous dynamics of, for example, the Michaelis-Menten reactions in a crowded environment well (Figure 1, right panel).

Another way in which temporal models can use spatial information is through compartmental models. In cellular models these compartments are usually the plasma membrane, the cytosol and the nucleus. It is very rare for all three compartments to be represented and most compartment models either use the first two or the last two compartments. For example, in [41] a model is developed for the IKK-IκB-NF-κB signalling module by distinguishing between NF-κB, IκB-{$\alpha/\beta/\epsilon$} and IκB-{$\alpha/\beta/\epsilon$}-NF-κB proteins in the cytosol and in the nucleus and by taking translocations of corresponding proteins between these two compartments into account. In a more complex setting a discrete Markov model (based on the SSA) for Ras nanoclusters on the plasma membrane is coupled with an ODE model

for the MAPK pathway in the cytosol [14]. This multiscaled compartment model, along with some experimental investigations, suggest that Ras microdomains function act as sensitive switches.

Delays are an essential feature in the modelling of cellular pathways and regulatory networks. They are intrinsic to slow biochemical processes that do not occur instantaneously and are often affected by spatial inhomogeneities. For instance, delays are associated with transcription and translation, two processes that imply other spatiotemporal processes often not explicitly modelled, such as (in eukaryotes) diffusion and translocation into and out of the nucleus, RNA polymerase activation, splicing, protein synthesis and protein folding. By incorporating delays into the temporal model we can expect more accurate and reliable predictions of cellular dynamics [42]. Delay Differential Equation (DDE) models with delayed feedback have been studied since the late 1960s [43–46]. Recently, transcriptional and translational delays have been studied and modelled in the continuous deterministic regime described by DDEs for various genetic regulatory systems [47–49]. In order to take proper account of both time delays and intrinsic randomness, Barrio *et al*. [50] developed a delay stochastic simulation algorithm (DSSA) that generalises the SSA in the delayed setting. Bratsun *et al*. [51] also developed a delay SSA but they do not consider waiting times for delayed reactions and only non-consuming reactions can be delayed. The simulation proceeds by drawing reactions and their waiting times (for delayed and non-delayed reactions)—see Figure 2. Recent research [52] suggests that spatial inhomogeneities can be well captured and modelled by means of time delayed processes with specific delay distributions and, in some cases, can provide insights into complicated cellular processes more reliably and in a more reasonable time frame than many spatial models.

It is not always easy to give a recipe as to which simulation approach should be used in a given setting. However, in the case of, for example, genetic regulation when there are certain key regulatory proteins that occur in small numbers then the discrete, stochastic approach that takes into account intrinsic noise is probably the most appropriate approach. Similarly, if the accurate representation of delayed processes, such as transcription and translation, is important then the incorporation of delay into the simulation will also lead to reliable results. Otherwise, hybrid approaches that capture the diverse elements of discrete, stochastic, continuous and delayed aspects of a cellular environment may well be necessary.

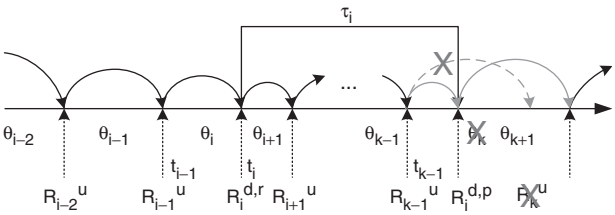


**Figure 2:** Schematic representation of the DSSA implementation. Here, $\theta_i$ refers to the waiting time until the next reaction $R_i$ is scheduled and $\tau_i$ is the delay time of reaction $R_i$. The dotted arrows point to the time line indicating when a reaction is updated. The reaction is specified below the arrow. $R^u$ denotes a non-delayed, $R^d$ a delayed reaction. Non-delayed reactions are updated when triggered. In case of a delayed consuming reaction, its reactants and products are separately updated. This is marked by $R^{d,r}$ and $R^{d,P}$, respectively. If time steps and reactions are drawn but then ignored they are crossed out. Those steps are marked as grey dashed lines and the steps replacing an ignored step are marked as grey solid lines.

## Spatial models

Many biological systems are characterised by complex spatial structure, low diffusion rates and low numbers of molecules. For example, biomacromolecular diffusion coefficients in the cytoplasm are usually 5–20 times lower than their values in saline [53] while the diffusion of lipids on cell membranes are estimated to be between 1 and 2 orders of magnitude lower than predicted by theory [54]. If we are to model accurately these processes then spatial models are mandatory. We are then faced with the problem of how to represent these complex processes. A very traditional technique is the Reaction-Diffusion Partial Differential Equation (RDPDE). However, this approach is only valid if we are dealing with large numbers of molecules. On the other hand RDPDEs can deal effectively with irregularly shaped regions through computational techniques based on finite elements and finite volumes. However, when the ultrastructure is changing dynamically or if we are dealing with complex processes such as transport by lipid rafts then this framework can become very complicated indeed.

In a subdiffusive setting the counterpart of the RDPDE is the fractional equation

$$D\rho(X,t) = D_t^{1-\alpha}\nabla^2\rho(X,t) + f(X,t)$$

where $D_t^{1-\alpha}\gamma(t)$ is the fractional Riemann-Louiville derivative operator that reduces to the identity operator when $\alpha = 1$ (pure diffusion) [55]. A number of authors have studied this framework in, for example, reaction fronts for bimolecular reactions [56]. However, currently there are few practical numerical methods for the solution of fractional differential equations [57].

In the discrete stochastic spatial setting the SSA and the CME have been generalised to describe stochastic reaction-diffusion reactions for *in vivo* biochemistry [58]. In the case of the reaction-diffusion Master Equation the volume is divided into subvolumes that are small enough to be considered homogeneous by diffusion over the time scale of the reaction. The state of the system is updated by allowing the molecules to jump at random to neighbouring subvolumes with diffusion being modelled by a unimolecular reaction. There may be many subvolumes and so this can be potentially computationally expensive, thus a next subvolume method that generalise the temporal SSA has been developed [59, 60]. This method scales logarithmically with the number of subvolumes. A similar spatial implementation is given in [61] based on the direct implementation of the SSA, with similar efficiencies. More sophisticated spatial Kinetic Monte Carlo techniques attempt to adaptively coarse grain the lattice in both time and space using ideas from Multigrid techniques for the spatial graining [62] and $\tau$ leap methods for the temporal graining [29, 63].

These remarks lead us to the very powerful spatial modelling technique known as Monte Carlo simulation, in which a two or three- dimensional computational lattice is used to represent a membrane or the interior of some part of a cell [15, 17, 64, 65]. This lattice can then be seeded with different molecular species of differing numbers. These molecules can move (diffuse) on the lattice and if they interact, then the appropriate chemical reaction takes place with a certain probability. A crowded environment can be simulated by placing inert molecules on a lattice with which the seeded molecules cannot react. Further extensions of this idea have led to simulation environments that attempt to model the behaviour of lipid rafts in which both proteins and rafts can diffuse on the lattice [65].

The advantage of this approach is that very detailed, complex systems can be simulated; the disadvantages are the large amounts of computational time and restrictions on the actual size of the domain that can be represented and practically simulated. This comes about because if we assume that a voxel can be occupied by at most one molecule at any given time and assuming an average molecular diameter of 4 nm, then even a lattice of size $500 \times 750$ voxels corresponds to a physical size of $2\,\mu\text{m} \times 3\,\mu\text{m}$ [65]. Furthermore, since the Monte Carlo technique is a stochastic method, a number of independent simulations may need to be performed in order to be able to compute statistics about mean and variance behaviour, depending on the variables of interest—in many cases the system self-averages if many molecules are present. One way to circumvent this computational bottleneck is to exploit the power of grid computing [66] and divide the computational membranes into subdomains and perform simulations independently in each subdomain with asynchronous communication (in terms of particle movement) between the regions. This can reduce the computational overheads significantly but comes at considerable human intervention via programming in parallel.

## A CASE STUDY

In this case study we discuss how Monte-Carlo simulations can be used to address very complicated membranal processes involving chemistry, microdomains, cytoskeletal partitioning and FRAP (Fluoresence Recovery after Photobleaching). This work is based on [65] and [67]. In this work, a two-dimensional lattice is used to represent a cell membrane. Each element of this lattice is a voxel that can be either occupied or unoccupied by a molecule at each time step; in the former case, a record is made of what molecule occupies the voxel. Since a voxel can only be occupied by one molecule at one time (volume exclusion), if we assume an average molecular diameter of around 4 nm (an average globular protein) then a lattice of dimensions $250 \times 378$ voxels, corresponds to an area of $1\,\mu\text{m} \times 1.5\,\mu\text{m}$. This computational membrane is large enough to obtain meaningful results of membranal dynamics—but the simulations can be very slow, especially when run over long time

intervals or if there are large numbers of molecules. At each such step, a molecule $M_1$ is chosen at random from the general population. Let the coordinates of this molecule be $(x, y)$. One of the voxels with coordinates $(x + d_i, y)$, $(x - d_i, y)$, $(x, y + d_i)$ or $(x, y - d_i)$ is also chosen at random, where $d_i$ is the step size of species $i$. This new voxel represents the location to which the molecule is moved during the current time step by Brownian motion alone. By using non-unitary and non-integral step sizes, the behaviour of systems with various degrees of stirring and/or stochasticity can be investigated

Obstacles can be represented as a separate chemical species that is inert with respect to all other species and has step size identically 0. We will denote the density of random obstacles on the membrane as $\theta$, and note that on lattices with immobile obstacle densities below the percolation threshold ($\theta_T \approx 0.4073$) accessible sites form a percolation cluster [68, 69]. Another source of anomalous diffusion is the interaction of mobile proteins and lipids with a cytoskeletal 'fence' system spanning the membrane [16]. This can be simply modelled by assuming fence lines are at right angles to each other and distributed evenly across both dimensions, with spacing between lines ('pitch'). Each fence line is made up of immobile fence posts (obstacles) and each voxel of the fence is either occupied or unoccupied by a fence post [67]. In this way, square domains are delimited by fence lines on the membrane. The only qualitative difference between fixed obstacles and fence posts is that the former are uniformly distributed on the membrane while the latter are randomly distributed only along fence lines. Proteins attempting to cross from one domain to another may be rejected (and thus retained in their current domain) by collisions with the fixed fence posts—'hop diffusion' [16].

The interaction of proteins with lipid micro-domains (lipid rafts) can be investigated in this framework [65, 67]. It is believed that proteins diffuse more slowly inside rafts than outside [70] and this has been postulated to be a possible source of anomalous diffusion [71]. A raft can be modelled as a two-dimensional, circular patch of radius $\gamma_\rho$ and area $A_\rho = \pi(\gamma_\rho)^2$. Rafts can be either fixed or diffuse in an analogous manner to proteins, with diffusion rate relative to proteins given by the Saffman-Delbruck equation [65]. A key method for measuring protein dynamics is (FRAP). The method 'bleaches' fluorescent molecules by exposure to high intensity laser radiation. As unbleached molecules move into the bleached area, the fluorescence recovers over time to its pre-bleaching state. The recovery curve can be used to infer information about the mobility of the macromolecule under investigation [72]. Simulating FRAP experiments is relatively straightforward, by giving each protein a 'tag' property, that has value 1 if they are fluorescent and 0 otherwise, and changing the tags appropriately.

Simulation results show that the most powerful constraining factor for small-scale molecular mobility is the presence of many randomly distributed, fixed (or almost fixed) obstacles. The presence of lipid rafts with biophysically realistic characteristics has a moderate effect on the anomalous exponent if proteins partition into rafts, but has a large effect if proteins are excluded from rafts. In contrast, collisions with a rectangularly organised cytoskeletal picket cannot, in this simulation framework, account for a large degree of anomalous diffusion in the absence of other interactions even if the fence lines are completely impenetrable and close together (as low as 10 protein diameters). Thus, obstacle concentration, the distribution of obstacles and the precise diffusion model (triangular versus rectangular lattice) are important parameters in characterizing the long-range diffusion of proteins. Further experimental elucidation of the likely geometries of impeding structures on cell membranes would help to focus modelling efforts in this area. Finally it is also important to note that there are other membrane/protein interactions that can be explored. For example, physical association (rather than simple collision) with the cytoskeleton could also contribute significantly to non-classical diffusion. Moreover, recent work [65] shows that the mobility of rafts as well as the ability of rafts to selectively capture and exclude different proteins can change the characteristics of the random walks executed by proteins on a cell membrane.

It is clear that Monte Carlo techniques can be used to investigate sources of anomalous diffusion in two-dimensional biological membranes such as randomly distributed fixed obstacles, lipid rafts (with proteins either partitioning into or being excluded from rafts) and a rectangularly organised cytoskeletal fence system. Simulations suggest that of these, fixed obstacles and exclusion from rafts are the mechanisms most likely to cause anomalous diffusion, in the absence of other interactions. The combination

of the three mechanisms, at biologically relevant levels, can account for experimentally reported anomalous diffusion levels. This suggests that the presence of impediments to motion in complex biological media has important effects on biochemical interactions and that in order to accurately take into account the effects of complex spatial features on dynamic (including chemical reacting) systems, spatially explicit methods such as Monte Carlo simulation are vital tools.

## KEY POINTS

In order to provide insight into cellular processes, modelling approaches must strike a balance. On the one hand, it is clear that the spatial properties and biological 'objects' populating the membrane are crucial to its various functions, so taking these spatial aspects into account is indispensable. On the other hand, direct simulation approaches can be prohibitively expensive, especially as biological relevance can happen over scales of seconds or minutes rather than micro-seconds. Thus we may attempt to capture some of the features by applying 'semi-empirical' modifications to the constitutive equations of classical non-spatial models ('smart' temporal models). This will be worthwhile if the new models retain the computational tractability of the classical ones but are considerably more accurate than these. The new methods can then be used either on their own, in conjunction with spatial methods to improve computation times, or in conjunction with non-spatial methods to improve accuracy of prediction in cases where the former are found wanting.

Nevertheless as more and more data becomes available and our knowledge base increases, the more complex the simulations become in order to gain further insights, thus compounding the problem. This relationship, confirming the adage that 'there is no free lunch', is illustrated in Figure 3. At the same time biological intuition becomes more significant, not less significant. For this reason, it will be increasingly important not only to use mathematical modelling to make sense of the flood

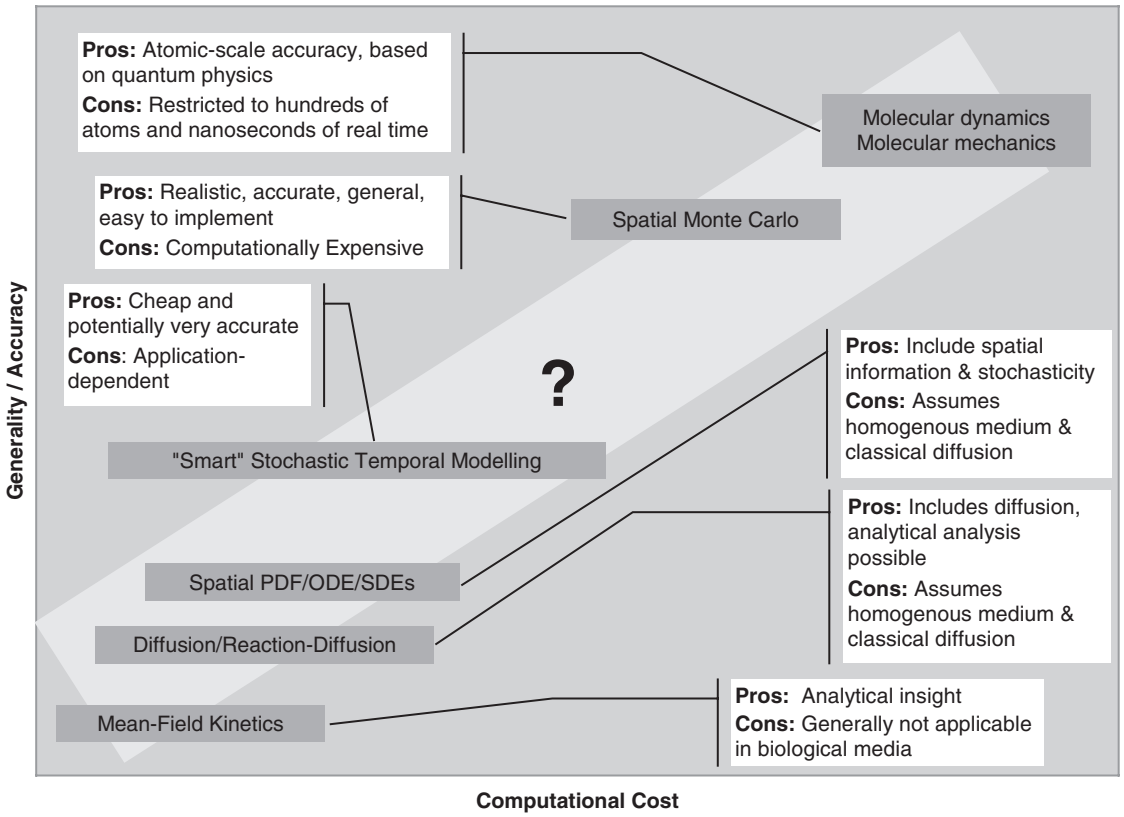


**Figure 3:** To provide insight into cellular processes, computational modelling approaches must balance the need for spatial detail and physical realism with the available computational resources. As the latter improve, it becomes possible to move further up on the scale.

of experimental findings but also for applied mathematicians and mathematical biologists to work more closely with, rather than in isolation from, experimentalists.

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