Minireview

Space in systems biology of signaling pathways – towards intracellular molecular crowding in silico

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Abstract How cells utilize intracellular spatial features to optimize their signaling characteristics is still not clearly understood. The physical distance between the cell-surface receptor and the gene expression machinery, fast reactions, and slow protein diffusion coefficients are some of the properties that contribute to their intricacy. This article reviews computational frameworks that can help biologists to elucidate the implications of space in signaling pathways. We argue that intracellular macromolecular crowding is an important modeling issue, and describe how recent simulation methods can reproduce this phenomenon in either implicit, semi-explicit or fully explicit representation.
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Keywords: Systems biology; Signaling pathway; Simulation; Molecular crowding; Excluded volume effect; Intracellular space

1. Introduction

Systems biology brings engineering disciplines such as control systems and signal processing into molecular biology at the level of biomolecular interaction networks, or pathways. Dynamical system characteristics and signal response functions of cellular signaling pathways are some of the main topics in systems biology.

Extracellular signals captured by receptor proteins on the cell surface are transduced inward to control target proteins or gene expression. Two interconnected underpinnings of this cellular response are molecular mobility (e.g., diffusion and active transport) and the signal transduction reactions. Despite its equal importance, little attention has been paid to the former biophysical properties of the cellular environment, which can contribute to overall signaling characteristics of the system by introducing non-linear signal delays. The Stokes–Einstein relation implies slow liquid phase diffusion speed of protein macromolecules, which are key players in the signaling. The significance of diffusion in reaction–diffusion systems becomes marked when reactions are comparatively faster than diffusion rates. The phosphorylation state of target molecules with spatially separated membrane-localized protein kinases and cytosolic phosphatases depends heavily on diffusion [1]. Subcompartments diffusively formed by localized proteins can significantly alter the effect of noise on signaling outcomes [2], implying the crucial coupling of noise and diffusion.

Extremely high protein density in the intracellular space, commonly called molecular crowding, can magnify the spatial effect. In a typical cell, the total macromolecular density is 50–400 mg/ml [3], far higher than typical in vitro conditions (1–10 mg/ml). If a solution contains 30% by volume of identical globular molecules, less than 1% of the remaining space is available to an additional molecule of the same size due to the excluded volume effect caused by steric repulsion, resulting in a mutual impenetrability of macromolecular solutes [4]. In such an environment, slow (5–20 times lower than saline solutions) apparent translational diffusion speed is observed [5], which in turn is caused by anomalous diffusion. Anomalous diffusion is defined as sub-linear scaling of mean-squared displacement of the molecule over time, and is used as a measure for cytoplasmic crowding [6]. Molecular crowding can also alter protein activities and break down classical reaction kinetics [7]. Minton has given excellent reviews about recent works on the influence of molecular crowding on thermodynamics and volume exclusion, including experimental findings, non-steric (weak) interactions, and biochemical reactions in physiological media [8,9].

In the remainder of this article, we review the computational frameworks that can be used to model and simulate the consequences of spatial features. Although we will mainly consider cytosolic signaling pathways, most discussions in this paper should also be applicable to other cellular phenomena that involve diffusion-limited reactions and localized proteins. This paper is written to attract the community's attention to the importance of considering space when modeling biochemical signaling cascades and other cellular phenomena. Due to the length limitation, however, this article is not intended to be a complete review of all aspects of the spatial effects and modeling issues. Interested readers are referred to other review and research papers.

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Abbreviations: 3D, three-dimensional; BD, Brownian dynamics; CA, cellular automata; DPD, dissipative particle dynamics; FCS, fluorescence correlation spectroscopy; FRAP, fluorescence recovery after photobleaching; GFRD, Green's function reaction dynamics; HP, hydrophobic-polar; LB, lattice Boltzmann; MD, molecular dynamics; ODE, ordinary differential equation; PDE, partial differential equation

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2. Spatial simulation methods

With the recent realization of the importance of noise in cellular information processing [10], preference for stochastic discrete-events [11] to conventional ordinary differential equations (ODEs) has become the norm in biochemical simulations. However, as we have seen above, the coupling of space *with* noise should be addressed when the effect of protein localization is to be investigated [2]. Therefore, the preference for stochastic methods should be retained in spatial simulations.

Ideally, to reproduce the crowding effects and protein localizations in silico, spatial simulation methods should be able to depict coarse-grained shapes and sizes of molecules and their positions in three-dimensional (3D) space. Proteins stay localized at certain parts of the cell as a result of cell compartmentalization and non-covalent weak interactions such as ionic, van der Waals, hydrogen bonds and hydrophobic-polar (HP) interactions [12]. Weak interactions, which can also influence the reaction and diffusion rates of molecules should be considered during simulation [8,13]. More importantly, simulation approaches should be computationally scalable to support simulation of large intracellular systems. A summary of methods exhibiting these simulation requirements is presented in Table 1. We discuss these methods in the following sections.

2.1. Molecular dynamics

Motions regulating all molecules constituting the cell arise from fundamental physical rules. By computing the forces affecting every molecule from some many-body potential in a particle space (Fig. 1(a)) and numerically integrating Newton's laws over a small discrete time-step, the molecular dynamics (MD) approach could potentially be used to compute the macroscopic behavior of molecules in a system [14]. The computational cost of MD simulation increases linearly with the number of interacting atoms [15]. Despite being the most accurate and fundamental approach [16], MD cannot be used to simulate whole cell systems, which consist of very large number of atoms arising from macromolecules. It has only been used in problems involving time-scales of nanoseconds and space-scales of tens of nanometers. For example, it was employed to illustrate the effects of cellular crowding on a small number of molecules [17,18].

The dissipative particle dynamics (DPD) simulation approach [19,20] is a coarse-grained approximation of MD. It was applied in a hydrophobicity study of a protein aggregation system which was at least three orders of magnitude larger ($\sim$20 000 nm^3) than previous investigations [21]. In spite of its reduced computational costs and support for weak interactions, DPD currently cannot be used in cell simulations because it does not permit biochemical reactions.

2.2. Partial differential equations

While the MD simulation approach deals with reaction and diffusion at the molecular level (i.e., micro-scale), the spatial partial differential equations (PDEs) approach, on the other hand, computes the intracellular kinetics at the macroscopic level. The Virtual Cell [22] employs PDEs with the finite volume method to correspond to reaction and diffusion rates of mobile molecules in its spatial simulation framework. Compartments in the framework can be adopted to depict the cell's spatial structures. These compartments are further divided into finite subvolumes through a mesh-generator (Fig. 1(e)). Numerical methods are used to solve the differential equations. Finer time-step and subvolume sizes produce more accurate solutions but with higher computational overhead. Despite being one of the most computationally scalable spatial simulation algorithms, PDEs cannot accurately represent intracellular noise because it is a deterministic approach. Noise has profound implications, especially when the number of molecules is small (e.g., transcription factors) [10]. Moreover, noise is further amplified in finite subvolumes such as the one used by the Virtual Cell because molecule numbers in each subvolume will be smaller than when they are taken as a whole [2]. Stochastic-based simulation approaches should be considered in such conditions. Next, we look at other methods which are more sophisticated than spatial PDEs, but unlike MD, are still computationally tractable.

2.3. Brownian dynamics

Brownian dynamics (BD) is a stochastic simulation approach with continuum space and time. In this particle-based

Table 1
Spatial simulation methods

Method	Space	Scale	Time	Stochastic	Excluded	Weak	References
MD	Particle	Micro	DES	–	+	+	[17,18]
BD	Particle	Micro	DES	+	+	+	[23,39]
GFRD	Particle	Micro	DEV	+	+	–	[24]
Smoldyn	Particle	Micro	DT	+	–	–	[25]
Lattice Gas CA	Discrete	Micro	DT	+	+	–	[33]
Weimar CA	Discrete	Meso	DT	+	+	–	[36]
Spatial Gillespie	Discrete	Meso	DEV	+	–	–	[45,46]
PDE	Mesh	Macro	DES	–	–	–	[22]
Gillespie	–	Meso	DEV	+	–	–	[11]
ODE	–	Macro	DES	–	–	–	

Some methods that can be used in simulation of biochemical pathways with space are listed. Non-spatial Gillespie and ODE are included for comparison. MCell, DPD, and some variations of CA introduced in the text are not shown due to their inability to represent cytosolic biochemical reactions effectively. Space: see Fig. 1. Scale: In 'Micro'-scopic methods, each instance of molecule is distinguished from others, and modeled as an object with a position either in a continuum space or a discrete lattice. 'Macro'-scopic schemes represent the system state as a mean-field concentration gradient. There are many possible 'Meso'-scopic schemes between macro and micro realms. Mesoscopic methods in this table treat molecules discretely, but do not track positions in a compartment or within a subvolume. Time: time-stepping scheme. 'DEV', 'DT', and 'DES' mean discrete-event, discrete-time, and numeric solution of a continuous differential equation system, respectively. Stochastic: if the method is stochastic. Excluded: if the method can represent the excluded volume effect [8]. See the text for the explanation of why Smoldyn and spatial Gillespie cannot fully represent excluded volume effect. Weak: the weak molecular interactions.

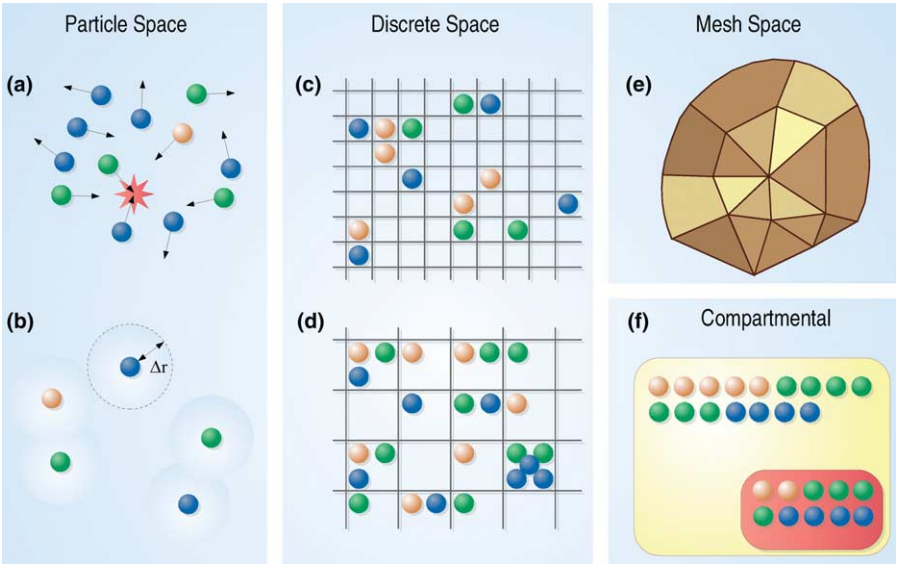

Fig. 1. *Representations of space*. In 'Particle' space, molecules are represented as individual particles with positions in a continuum space. (a) Particles are usually given motions according to some kind of force equations that are numerically integrated to advance time. Reactions are represented as collisions between particles. (b) Some methods including GFRD employ an optimization technique that allows particles to 'jump' in time and space by calculating the maximum distance (Δr) that the particle can travel in the time slot. 'Discrete' space representation discretizes the space either by subvolumes (voxels) of an identical shape (typically cubic) or a regular lattice. (c) In this 'microscopic' lattice, at most one particle is allowed to occupy a lattice site. (d) Some methods allow multiple particles to reside in a single lattice site. This class of discrete space representation is often called mesoscopic. (e) 'Mesh' space in this paper means conventional structured or unstructured meshing schemes of a concentration field. (f) Non-spatial biochemical simulators usually make use of 'Compartmental' space, which assumes a chemical equilibrium in each compartment, and molecular transfers between compartments are not modeled as implicit built-in rules in the simulation method (such as diffusion), but in an explicit way such as membrane transporters.

approach (Fig. 1(a)), the molecules exhibit noise as they are propagated according to the Langevin equation. The equation contains random forces that are intended to represent the interaction between the diffusing and the implicitly represented solvent molecules. BD has been applied successfully to investigate electrostatic competition effects between substrates binding to an enzyme [13] and to observe how crowder molecules influence the GroEL–GroES chaperonin machinery at the atomic scale [23]. Hence, this approach can effectively simulate crowded environment, given that the crowder molecules are explicitly represented in the simulation space. Such representation, however, will incur very high computational costs, owing to the increased frequency of collision events and the smaller time-steps required to resolve them.

BD can be viewed as a numerical procedure to solve the Smoluchowski equation, which describes the diffusive encounter of molecules in solution. On the other hand, for two-body problems, it is possible to analytically solve the equation by using the Green's function. This approach was adopted by van Zon and ten Wolde when they developed an event-driven simulation algorithm called Green's function reaction dynamics (GFRD) [24] (Fig. 1(b)). The basic idea is to reduce the many-body problem that constitutes the biochemical system into a set of two-body problems by determining the length of the timestep to be sufficiently small. Although, GFRD permits larger time-steps when the particles are too far apart to react, this advantage is lost when simulating crowded environments. This is because GFRD retains the drawbacks of BD, which is the dependency of step sizing scheme to the frequency of collision events. Additionally, it also does not consider weak interactions between molecules. Nevertheless, this method can represent the excluded volume effect and active transportation, and can give different sizes and shapes to molecules.

Smoldyn (Smoluchowski dynamics) [25] is another approach to numerically realize the Smoluchowski model of diffusion-limited reactions. The molecules are represented as point particles (Fig. 1(a)) with binding and unbinding radii, which are computed from each species' macroscopic reaction rates. A disadvantage of discrete-time approaches in continuum space such as Smoldyn is that it is possible to miss collisions when the length of time-steps are set not sufficiently small. Smoldyn can represent reduced diffusion speed in crowded environment by placing impenetrable blocks in space [26]. One of the major consequences of the excluded volume effect is the dependency of the diffusive movements on physical sizes (and shapes) of the diffusing molecules. Unlike GFRD, dimensionless particles used in the current version of Smoldyn does not permit accurate representation of the effect.

MCell [27] is a unique BD simulation approach that is specialized to simulate reactions between free-diffusing ligand molecules and stationary surface receptors. The surfaces are constructed using convex polygon meshes as illustrated in Fig. 1(e). Its current version, however, does not support bimolecular reactions in 3D space. MCell has recently been extended to run on distributed computing environment to permit large scale simulations [28].

2.4. Lattice-based methods

Cellular automata (CA) is a lattice of uniform sites with a finite number of states that evolves in discrete-time [29,30]. The transition of each automaton (i.e., molecule) at the sites is fully specified in terms of its local interaction. The molecule can propagate either along its velocity vector or according to

its diffusion rate to arrive at another lattice site, and then collide or react with other molecules. CA can be used to simulate reaction and diffusion at both microscopic [7,31–34] (Fig. 1(c)) and mesoscopic (Fig. 1(d)) scales, having single and multiple molecules at a site, respectively. Lattice size and geometry (e.g., square, hexagonal or trigonal) can also influence the outcome of simulation, as reported by Shimizu et al. [35] when they analyzed the *Escherichia coli* chemotaxis signaling pathway using a CA-based Ising model.

Large differences in molecule sizes and numbers in biological cells motivated Weimar [36] to use CA to simulate enzymatic reaction networks at both meso-scale (metabolites) and micro-scale (enzymes) simultaneously on a two-dimensional lattice. This approach reduces a sizable amount of memory requirement, especially when considering larger systems such as the whole cell. The size of the lattice sites can be larger to accommodate large molecules, and as a result, fewer sites will need to be created and stored in the memory. The reduced resolution of the lattice would, however, translate to lower precision of the molecular diffusions at each time-step.

Tremmel et al. [37] took a step further by simulating diffusion of plastoquinol molecules in a thylakoid membrane with the integral thylakoid proteins having different shapes and sizes. The simulation was carried on the same lattice at micro-scale. Some of the large integral thylakoid proteins were stationary and could span more than a single site. Nevertheless, their CA implementation does not support biochemical reactions.

Chan and Dill [38] introduced the HP lattice model which takes into account the charges of molecules on the lattice. Ping et al. [39] later extended this approach to include BD to investigate the effects of crowder molecules on protein folding and stability.

For more accurate representation of the cell, 3D CA would be required. The local interaction nature of CA makes it suitable for implementation on parallel architectures and hence, supports reduction in the computational time required for 3D simulations. Examples of parallel 3D implementations include a life-like cell membrane simulation undertaken by the CyberCell group [40] and an amphiphilic hydrodynamic simulation work by Love et al. [41]. In the CyberCell approach, biochemical reactions were not implemented, instead, the cell membrane was simulated based on three variants of local interactions between particles: (1) attraction, (2) dispersion and (3) alignment.

At the completion of multiple CA simulation runs using the same parameters and model, one can obtain each molecule's distribution function based on the average number of molecules at a specific lattice site with a given velocity. Following this, the lattice Boltzmann (LB) method [42] uses lattice sites to hold each molecule's distribution function instead of the molecules themselves. In addition to parallel 3D simulations of amphiphilic fluids [43], LB has been applied successfully in simulations of chemical dissolution in porous media with molecular diffusion, surface reaction and forced convection [44].

2.5. Spatial Gillespie

Stundzia and Lumsden [45] extended the Gillepie's stochastic approach [11] to be used in subvolumes for spatial simulation. Their method was employed to simulate the propagation of a calcium wave by reaction–diffusion across a cell. Elf et al. [46], on the other hand, extended the fast version of the Gillepie's algorithm, the Next Reaction [47] method, to be used in subvolumes. The SmartCell simulator [48] also implements a similar scheme. The subvolume sizes, as shown in Fig. 1(d), are determined such that all reactive molecular species, represented as point particles, are almost uniformly distributed in each subvolume's space. This is done by ensuring that the diffusion of reactants in a subvolume takes place more frequently (e.g., more than 100 times) than their respective reactions. At each time-step, each molecule can either react in its current subvolume or diffuse to an adjacent one. The diffusional probability at each time-step is obtained by mapping the bulk diffusion constant in Fick's law using the Green's function. Similar to the original Next Reaction method, the computation time increases only logarithmically with the number of subvolumes in the system. Nonetheless, it is not possible to reproduce crowded conditions because volume exclusion from both reactive and non-reactive crowder molecules cannot be represented explicitly when they are depicted as point particles.

3. Data availability

Consideration for the balance between demand and availability of input data is extremely important for successful modeling and simulation of real world systems. Here, we consider simulation of a partial signaling pathway in a whole cell-scale space.

In addition to conventionally used quantities in non-spatial biochemical models, such as reaction rate constants and initial concentrations, spatial methods may require knowledge about (1) proteins' mobilities, and (2) their abundance and localization in the cell. To model the mobility adequately, depending on the modeling scheme being used, (a) translational diffusion constants, and (b) existence and quantitative properties of active transportations should be examined for all protein species involved in the pathway. Additionally, if the crowded environment is taken into account, sizes (which could be to some extent estimated from molecular weights assuming a globular shape) and localization of all macromolecular species present in the target cell must be measured or estimated to give a 'crowding map' in the simulation.

Despite the seemingly exploding demand for numbers, recent advancements in measurement technologies and bioinformatics are making the picture not entirely pessimistic. An optical technique called fluorescence correlation spectroscopy (FCS) [49] can be used to access information about (1) local concentrations, (2) apparent translational diffusion constants, (3) non-Brownian movements such as active transport and anomalous sub-diffusions [50] of fluorescent proteins. Although highly sensitive and versatile, a drawback of FCS is its inability to examine cells smaller than the detection volume of about 1 femto-liter, which is about the size of an *E. coli* cell. Fluorescence recovery after photobleaching (FRAP) is another measurement technique for apparent diffusion constants that makes use of fluorescent proteins [51], even though it cannot quantify anomalous diffusion, immobility and active transport of proteins. FRAP has been successfully used for *E. coli* cells [5], which are generally too small for FCS.

It would be possible to computationally construct the crowding map from the archives of protein localization GFP

images available through the Yeast GFP Fusion Localization Database [1] for yeast cells and the GenoBase [2] for *E. coli* cells. Molecular weights and other properties of the proteins in various organisms can be obtained from UniProt [3] and Ensembl. [4]

4. Discussion

The significance of molecular crowding with regard to biochemical simulation is twofold [33]. First, the apparent diffusion constant D is no longer a constant, but is a function that depends on interactions of the size, shape, and chemical properties of the molecule and the crowder agents. This makes it difficult to quantify diffusion speed without conducting in vivo measurements or detailed microscopic simulations. Second, high macromolecular densities increase effective protein concentrations (activity coefficients), and make reaction kinetics fractal. This suggests a preference for explicit modeling of crowding environments over an implicit representation which simply lowers diffusion coefficients and increases reaction rate constants k in normal reaction–diffusion simulations. The explicit representation may be either in a semi-explicit way using a field of total protein density ('crowding map') and making D and k density-dependent, or fully explicitly using crowding particles.

Among the computational approaches presented in this paper, the class of methods based on CA is promising, in terms of its versatility, simplicity and scalability. Considering that CA is currently one of the most actively studied methods for (bio)physical simulations, it would not be surprising to see a variation of it that works as a standard way of spatial biochemical simulations in near future. By consolidating the approaches, described by Weimar [36], Tremmel et al. [37] and Chan and Dill [38], it would be possible to arrive at an ideal CA-based approach that meets all of the simulation requirements with fully explicit representation of crowding. One probable remaining drawback is the very large computational time arising from simulation of whole cell systems. However, CA is one of the computational frameworks that are most efficiently parallelizable, as exemplified by, among many others, the CyberCell [40] or the Love group [41].

Ideally MD or BD should give the most precise computational reproduction of the intracellular dynamics with crowding and weak interactions, but digital computers may not become fast enough to simulate on physiological timescales for years to come. Replacing the Green's function in GFRD by some kind of non-Gaussian function that models anomalous diffusion could potentially produce an approximate method with the semi-explicit treatment for the crowding that can overcome the degraded speed in highly crowded simulations.

Although it is based on gas-phase kinetics and can treat crowding consequences only implicitly, spatial Gillespie class of methods has a good chance to find many useful applications in areas where spatial resolution of protein localization can be treated crudely and the effect of crowding can be ignored, due to its extremely high efficiency. The standard Gillespie's Next Reaction method has been implemented on parallel architectures and favorable speedups have been reported [52,53] with increasing number of processors. Similarly, the spatial Gillespie class of methods is also a good candidate for parallelization because of its local interaction nature between the subvolumes.

No single simulation method is likely to work effectively and efficiently for highly heterogeneous and multi-scale system like the cell [54]. This becomes apparent for simulation of signaling pathways when the model includes small molecules and proteins that have different scales of diffusion speed. Investigation of coupling effect of the signaling system with other cellular phenomena such as metabolic reactions and gene expression is another interesting application of simulation that introduces multi-scaleness in time, space and concentration. The multi-algorithm framework that combines modular submodels driven by different algorithms to make a composite simulator is a feasible solution for this problem [55]. An integrative cellular model constructed on this framework, for example, may have a modular architecture that has slow-diffusing protein molecules on CA, small molecules that have little effect from crowding on spatial Gillespie, gene expression on Gillespie and slow reactions in metabolism on ODE.

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