Particle-Based Stochastic Simulation in Systems Biology

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Abstract: Computational modeling and simulation have become invaluable tools for the biological sciences. Both aid in the formulation of new hypothesis and supplement traditional experimental research. Many different types of models using various mathematical formalisms can be created to represent any given biological system. Here we review a class of modeling techniques based on particle-based stochastic approaches. In these models, every reacting molecule is represented individually. Reactions between molecules occur in a probabilistic manner. Modeling problems caused by spatial heterogeneity and combinatorial complexity, features common to biochemical and cellular systems, are best addressed using Monte-Carlo single-particle methods. Several software tools implementing single-particle based modeling techniques are introduced and their various advantages and pitfalls discussed.

Keywords: Spatial modeling, discrete modeling, mesoscopic.

1. INTRODUCTION

Advances in Molecular Biology and the advent of High-Throughput Biology and Bioinformatics have in recent years renewed interest in the branch of biology termed Systems Biology [1]. Systems Biology focuses on the interactions of biological entities within a biological system. Alongside the structural and functional interactions between the diverse elements, such as enzymes and metabolites in a biochemical pathway, or transcription factors and genes in a transcription regulatory network, a systems approach attempts to decipher the control-logic of the system which in turn determines the systems dynamics. The dynamics and interactions of individual components give rise to emergent properties which only make sense in the context of the system as a whole [2]. These systems can consist of a large number of interacting entities [3, 4] and, as a consequence, mathematical modeling and computational simulation are invaluable tools for the biological sciences and Systems Biology in particular.

Computational simulations are a useful tool as it is frequently the case that laboratory experiments only provide a snapshot of a system at any given time and do not fully capture the temporal aspect of the system. If changes over time are measured, these often only follow the change in concentration of one entity. Yet even systems of few interacting components can give rise to complex behavior [5] which can be missed if not all entities are monitored. Apart from aiding in the comprehension of the complexity arising from large numbers of interactions, there are many other reasons why computational tools have become useful, if not necessary, in biological research. The complete control a researcher has over the model allows for manipulation which is difficult or impossible *in vivo* or *in vitro* experimental set-ups, such as alterations of diffusion speed of molecules [6]. In addition, models can generate new hypotheses which can anticipate biochemical characterization, as was the case, for example, with the non-cooperative ultrasensitivity predicted a decade before the experimental discovery of the MAP kinase cascades [7, 8]. Finally, models are also time- and cost-effective in comparison to laboratory experiments. Once a detailed model has been created, it can be re-used with only minor modifications in order to test new hypotheses and does not incur the costs of consumables [9, 10].

Most kinetic models of biological processes treat the reacting components as population pools. These are either continuous concentrations or discrete populations. In either case, the components are an indistinguishable mass of identical elements. This inability to accurately depict single-particles gives rise to two main problems: (i) inability to represent spatially heterogeneous populations, and (ii) combinatorial explosion caused by molecular entities that can assume multiple distinct states, such as the phosphorylation states for a protein. Modeling problems caused by spatial heterogeneity and combinatorial complexity, features common to biochemical and cellular systems are best addressed using these Monte-Carlo single-particle methods. We shall first describe the two most common approaches to population based modeling. We will then describe in more detail the main problems associated with population based models and how particle-based models resolve these issues. In these models, every reacting molecule is represented individually. Reactions between molecules occur in a probabilistic manner. Software tools implementing particle-based modeling techniques are introduced along the way.

2. POPULATION BASED MODELING

2.1. Mass Action Kinetics Models

Many different types of kinetic models using various methodologies can be created to simulate any given biological system. By far the most common methodology used to create models is based on Mass Action kinetics. The empirical law of Mass Action tells us that the rate at which a chemical reaction proceeds is proportional to the amount of reacting species [11]. The state of the model at any time is defined by the population of its molecular components at that time. The main assumptions of the model are that the reactants are well-mixed, that is spatially uniformly distrib-

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uted within a finite reaction volume, and that the chemical species are present in large numbers, allowing us to treat the populations of molecular species (pools) as concentrations varying on a continuous scale. The dynamical behaviour of the system can then be expressed using Ordinary Differential Equations (ODEs). Although both these assumptions often do not hold for living systems [12], ODEs do provide a useful and powerful methodology for the analysis of some biological problems, such as the cell cycle control mechanism in budding yeast [13]. Using mostly ODEs, a model was created which was able to explain to a large degree the phenotype of over 100 yeast cell cycle mutants, and allowed for prediction of new mutant combinations and estimation of biochemical rate constants. Additionally, many models of gene regulatory networks [14], metabolic networks [15] and signalling pathways [2] use ODEs. Although the resulting ODEs can be numerous and coupling between equations can make solving them analytically difficult, methods for solving ODEs numerically are well established and standard mathematical software if often enough to run simulations based on Mass Action kinetics.

2.2. Stochastic Kinetics Models

One distinguishing feature and frequent criticism of the use of Mass Action kinetics in biological modeling is the fact that these models are continuous and deterministic. The assumption of Mass Action kinetics that molecules are present in large numbers allows us to treat them as continuous concentrations. The deterministic nature of ODEs used to calculate the dynamics of the system imply that once the starting conditions for a system are known, it is possible to predict the systems state at any time. The same starting conditions will always yield the same result. Nature, however, is far from continuous or deterministic. Molecules are discrete entities and reactions are discrete events. Many important molecular entities exist only in small copy numbers, and these numbers change in discrete steps. The smaller the number of molecules involved the more important the fluctuations about the mean value become. If copy numbers are small, ensemble averages result in a poor approximation of the process and fluctuations can have a discernible impact on the system. This is often the case in biological systems. Indeed, many processes are influenced or governed by fluctuations (see [16] and [17] for more detailed examples). Apart from the deviation from mean values exacerbated by small copy numbers, molecular events themselves are not deterministic, as not every molecular collision leads to a molecular reaction between two potential reacting partners. For all intent and purposes these events are probabilistic. If the molecular population pools are treated as discrete populations and Mass Action reaction rates are mapped onto probabilities, one arrives at a stochastic formulation of chemical kinetics in the form of the chemical master equation (CME). Although analytically extremely difficult to solve, models based on the CME can be computationally simulated by using the algorithm presented by Gillespie [18], based on a kinetic Monte Carlo scheme originally developed by Bortz *et al*. [19]. The Gillespie algorithm, also known as the Stochastic Simulation Algorithm (SSA), uses probabilities called reaction constants, derived from the chemical kinetics rate constants, that determine whether a reaction occurs. Briefly, the algorithm commences with initial conditions that specify the molecular population numbers and the reaction constants for each reaction these molecules can undergo. Random numbers then determine what length of time elapsed and what reaction occurred within that timespan. The molecular population numbers are then adjusted, alongside with the dependent probabilities, and the cycle re-commences. As such, the SSA is an event-driven algorithm. The SSA has been used extensively, most notably with gene regulatory networks [20]. It should be noted that, although models based on the CME take account of the discrete and random nature of chemical reactions, the reacting species are still described as populations with no distinction made between individual entities. The state of a model is generally described by population number of all the species involved in the reaction network. Furthermore, the reacting entities are still considered spatially uniformly distributed.

2.3. Limitations of Population-Based Approaches

2.3.1. Spatial Resolution

Population-based modeling techniques assume that all the reactive chemical species are contained in a well mixed reactor. While this is generally true in a test tube, biological systems exhibit much more complex spatial heterogeneity. For example, the cytoplasm of even the simplest cell contains many distinct compartments, each with its own specific protein complement. Even within a single compartment, localization of molecules can be influenced in many different ways, such as by anchoring to structures like the plasma membrane or the cytoskeleton. The relative positioning of biological entities with respect to one another is fundamental for the proper working of many molecular biological systems and can give rise to quite complex behaviour. For example, enzymes acting in the same pathway are often found co-localised, such as the enzymes of fatty acid biosynthesis or glycolysis [21, 22]. As the product of one reaction is the substrate for the next reaction along the pathway, this co-localisation increases substrate availability, and concomitantly enhances catalytic activity, by giving rise to increased local concentration of substrates. Additionally, substrates are less likely to be captured by competing enzymes. This phenomenon is often refered to as Substrate Channelling. [23]. Equally, signaling molecules are often confined to specific regions or found in close proximity to the proteins responsible for their activation, such as calcium/calmodulin-dependent protein kinase II (CaMKII) and calmodulin association with the N-methyl-D-aspartate receptor at the mammalian glutamatergic synapse [24]. As calcium enters the cell through the receptor, there will be an elevated local concentration of calcium available to calmodulin, which in turn will be in close proximity to CaMKII. At times this co-localisation creates supramolecular assemblies composed of many interacting partners; the postsynaptic density found just below the postsynaptic membrane of the glutamatergic synapse, which contains a large host of signaling proteins and their potential targets, is an example of such a structure [25, 26]. Co-localisation of signaling molecules is thought to prevent crosstalk and may aid protein interaction by concentrating potential partners in the same area [27]. Indeed local microdomains of signalling molecules have been shown to be important in some biological systems [28]. Equally diffusion plays an important role in cellular processes. Molecules

move by diffusion and it is through the diffusion of signaling molecules that information gets relayed from one cellular location to another such as, for example, from plasma membrane receptors to the nucleus. Indeed, using concepts borrowed from Metabolic Control Analysis, Kholodenko *et al*. have shown that diffusion can exert a significant amount of control in a signaling pathway if the relative positions of signaling molecules are taken into account [29]. Co-localisation of molecules and effects dealing within the microdomain range cause problems for population based methods as these do not operate at a sufficent high spatial resolution. When spatial representation is introduced into population based methods, the well-stirred approximation is nonetheless applied locally, and the differential localisation of reactants still cannot be addressed, as molecular resolution is not available. Algorithms have been developed, derived from the SSA, in order to address the problem of spatial heterogeneity in reaction-diffusion systems [30]. Those methods, when employed by simulation software such as MesoRD [31] and SmartCell [32], allow us to tackle some issues of spatial heterogeneity, but cannot be used if molecular resolution is required. Spatial dimensions can be added to Mass Action based approaches, turning the ODEs into corresponding partial differential equations (PDEs), though these are hard to solve in comparison to the ODEs. Finally, as reactive species are treated as populations rather than as individual molecules, there is no way of addressing the geometry of components and the effects arising from this geometry. Yet geometry of molecular entities is often important when considering their function. Microtubules are an excellent example. Not only do microtubules have a specific geometry, but their polarity arises from the geometry of their tubulin components. Recent reviews describe in detail the numerous methods employed by modeling software to deal with spatial representation and associated diffusion [33, 34], including solutions for incorporating space into population-based methods.

2.3.2. Combinatorial Explosion

A population-based model generally includes as many populations as there are reacting molecular species. Yet many molecular species can assume different states [36]. These states could correspond to, for example, post-translational modifications, ligand occupation or conformation states. Additionally, molecules can aggregate to form molecular complexes. Each state and complex is treated as a distinct molecular species and demands its own population pool. This can lead to the problem of combinatorial complexity in the form of an explosion of distinct chemical species, and a concomitant explosion of chemical reactions. An example will illustrate this point: Let us say a biological system consists of two chemical species, A and B. Species A reacts with species B in a bimolecular reaction. Further let us assume there are 500 molecules of species A and 100 molecules of species B. If species A possesses 10 phosphorylation sites, a modest number compared to some biological molecules [37], the total number of different molecular states it can assume is $2^{10} = 1024$. Therefore, population-based models would need to create 1024 population pools for species A, alone. However, we mentioned that there are only 500 molecules of species A. Clearly, using computational resources to handle 1024 distinct populations, when the total number of actual molecules cannot exceed 500 presents a waste of computational power. The number of individual reactions that need to be modelled also increases rapidly. If there are only species A and B which interact, and neither possesses a phosphorylation site, only one reaction needs to be considered:

$A + B$

If species A however possesses one phosphorylation site, the number of reactions climbs to 3:

$A + B$

$AP + B$

$A \rightarrow AP$

Table **1** illustrates the growth of states (each of which capable of reacting with B) and state conversion reactions for this simple system. The number of states grows exponentially. The situation becomes even more wasteful if species B can also assume multiple states. If, for example, A can assumes n_A states and B can assume n_B states, the number of potential bimolecular reactions is $2^{nA-1} \times 2^{nB-1}$. This can also lead to a waste in computational resources if not every state of B affects its reaction with A. A number of attempts have been made to tackle this problem for population-based methods [38, 39].

Table 1. Explosion of States and Increase in Number of Reactions with Respect to States

Number of Features	Number of States	Number of State Converson Reactions
0	1	0
1	2	1
2	4	4
3	8	12
4	16	32
5	32	80
7	128	448
8	254	1025
n	2^n	$n2^{n-1}$

3. PARTICLE-BASED STOCHASTIC APPROACHES

We can broadly classify molecular biological models into three categories: macroscopic, mesoscopic and microscopic. Microscopic models encompass Molecular Dynamics (MD) simulations. MD simulations use Newtonian laws to determine the dynamics of groups of atoms. In general, these are computationally expensive and, as a consequence, MD can only capture the dynamics of a few molecules at best. At the other end of the scale, macroscopic models focus on the dynamics of populations. As mentioned above, they are often based on chemical kinetics and utilised mostly ODEs. Particle-based modelling falls under the classification of mesoscopic modelling. Unlike macroscopic models, mesoscopic models treat biological entities, such as proteins, as individual objects. They do not attempt to model the dynamics of every atom within a molecule, but instead centre on the molecule as a whole. Most quantum-physical details are either assumed to take on average values or are ignored for the

sake of simplicity. As every particle of interest is considered in particle-based models, the state of the model is given by the collective states of all the particles in the model. The focus on individual entities allows for the observation of the behaviour of individual particles within the ensemble and how individual particles contribute to the behaviour of the system. For reaction diffusion systems, such as a signal transduction pathway or a metabolic pathway, many particle-based models are derivatives of the Smoluchowski model for reaction-diffusion systems. In effect, the Smoluchowski model describes a solution of interacting chemical particles as spheres which move by Brownian motion until two spheres come within a certain distance of each other causing them to react. The general applicability of this model led to the development of a number of general particle-based simulation software. Most programs use Brownian dynamics algorithms to simulate the Brownian motion of the particles of interest, and implement their own algorithms to handle the simulation of reactions. Most of the algorithms used to simulate reaction events are Monte Carlo algorithms, i.e. they use random numbers when computing an outcome, which take into account experimentally measured reaction rates when calculating reaction probabilities. Some of the general biochemical network simulators that implement the above framework are MCell [40], Smoldyn [41] and ChemCell [42].

The MCell program [40], which was originally developed to model the neuromuscular junction [43], is a general simulator of biochemical reaction networks that takes account of positional information. Biological entities within MCell fall into two categories: freely diffusing ligand molecules and stationary effector molecules, such as receptors. Only the effector molecules are capable of undergoing reactions and as such simulated reactions occur at surfaces within the MCell software. The ligand molecules diffuse using a Brownian Dynamics algorithm. MCell keeps track of any interaction between the ligand molecules and the effector tiles. If a ligand molecule and an effector site do come into contact, the program uses probabilities to determine the chance of a reaction occurring.

Similar to MCell in the free diffusion of molecules in a 3D simulation volume is the simulation software Smoldyn [41]. Although molecules do not take up volume, each molecule within a Smoldyn simulation has an identity and a location in space. The state of the system is entirely determined by the 3D locations of all the molecules. The molecules diffuse through the simulation volume using a 3D random walk algorithm. Smoldyn, unlike MCell, is capable of simulating reactions in solution.

Within the above mentioned programs, simulated molecules are propagated in the simulation volume over a series of fixed time-steps. At the end of every time-step the programs check whether a reaction event has occurred. In order to capture every collision event, and hence possible reaction, small time-steps are required. A recently published method, Green's Function Reaction Dynamics (GFRD) [44], offers an attractive alternative to the Brownian Dynamics approach. In analogy to the SSA, GFRD is an event-driven algorithm, in which the next reaction is determined stochastically alongside the time which is required to pass to yield the reaction, and the entire system is advanced by this time interval. This can significantly improve on the Brownian Dynamics approach with respect to speed [44].

3.1. Spatial Resolution

Since the particle state can include positional information, for example in a coordinate system, it is possible to determine all particles positions, or even orientations, with respect to each other. This enables the simulation of subtle spatial interactions, such as local concentration and diffusion effects. As an example Shimizu and Bray used the single-particle simulation software StochSim, to investigate one of the most studied cellular signalling systems, the chemotactic response of *Escherichia coli*. This system relies on the existence of a lattice of membrane receptors [45]. Their results fit better with experimentally observed results than a similar simulation which did not take account of local effect [46], The previously mentioned general reaction network simulators MCell and Smoldyn have also been used to great effect when investigating spatially heterogeneous models. Problems that were addressed using MCell include modeling of glutamate spill-over leading to activation of extra-synaptic receptors and buffered diffusion effects due to trapping of neurotransmitter by receptors at the glutamatergic synapse [9], or variability in the size of postsynaptic potentials caused by release of glutamate from different sites in the active zone and effects of glutamate receptor positioning in the postsynaptic density on receptor opening times [10]. These simulations required knowledge of the relative positions of diffusing particles (glutamate) and effector sites (receptors) and would have been difficult to do using non-single particle methods. Smoldyn has been used to model the diffusion of phosphorylated CheY (CheYp) through the cytoplasm in bacterial chemotaxis and its interaction with the phosphatase CheZ [47]. Realistic 3D modeling allowed for a detailed diffusion trace of individual CheY protein molecules moving through the cytoplasm and of the examination of the change in lifetime and cytosolic distribution of CheYp for different cytological distributions of CheZ. Further, the effect of molecular crowding on the CheYp gradient was investigated. Again, none of these simulations would have been possible without taking into account 3D space.

The geometry of reacting components can also be incorporated using particle-based models. Nédélec [48] used a particle-based model to investigate the interations generated by motor proteins between antiparallel microtubules. The model includes the polarity inherent in microtubules and their motor proteins and the simulation was capable of reproducing structures similar to those seen in mitotic spindles.

Meredys, a mesoscopic simulator currently being developed in our laboratory, incorporates a particles geometric information. Based on the the Abstracted Protein Simulator (APS) software [49] written by Dan Mossop and Fred Howell, it represents biological entities as single particles, spheres or cylinders, or as compound objects formed from the two. Every basic particle can have a number of binding sites associated with it. Particles and compound objects diffuse through the simulation volume using a 3D random walk algorithm. Bonds between particles are broken and created as determined by the user-defined rules. A collision detection algorithm establishes whether particles come sufficiently close to allow bond formation. Although conceptually sim-

ple, the use of rules to determine binding allows for creation of quite complex models. Currently Meredys is used in our laboratory to model the movement of α-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid (AMPA) subtype of glutamate receptors in the post-synaptic membrane, as was observed in glutamatergic neurons [50]. Evidently, positional information is key to these investigations and single-particle modeling will allow modeling of local concentration effects produced by mobile receptor, glutamate and cytoplasmic signaling molecules.

3.2. Combinatorial Explosion

With single particle modeling every molecule has an identity, so the number of distinct populations is limited by the number of molecules. Superfluous species pools are avoided. In a recent paper by Pettigrew and Resat [51], the computational efficiency of two Stochastic kinetic simulation algorithms where compared: a variant of the population-based SSA and the StochSim algorithm [52, 53]. The authors conclude that the SSA outperforms the StochSim algorithm as the models species population increases, however the StochSim algorithm performs much better when the species population remains small, but the network connectivity (in terms of simulated phosphorylation sites) increases. The single-particle based biological simulator StochSim represents each biological entity by one software object. The simulated molecules in Stochsim are capable of being in more than one state. These states are maintained by a vector of binary flags. Before any reaction is attempted, the algorithm inspects the current state of the reacting molecules, as determined by the binary flags, and modifies any flags representing fast reactions, based on a probability. These include, for example ligand-binding or conformational transition flags and other operations which occur at a speed greater than uni-/bimolecular reactions. The state of the molecule effects the reaction probability of the that molecules possible reactions. For example, a flag representing a phosphorylation state, can make possible, or completely disallow, a bimolecular reaction. Although StochSim can be used as a general biological simulator, it has been developed specifically to model features of chemotaxis in E. coli. It lends itself particularly well to this problem set due to its ability of representing many distinct states of individual molecules. For example, the aspartate receptor in E. coli possesses four methylation states, as well as binding sites for ligands and several protein interaction partners. Using StochSim for the simulations of the aspartate response pathway in E. coli, Shimizu *et al*. examined the effect of receptor coupling on the range of signal response and gain and compared results to experimental observations [46].

4. CONCLUSION

Computational modeling of biological systems is becoming an ever more important part of biological research. Kinetic models of biological reaction networks can be created using a variety of methodologies. The majority of models created to date use ODE to model populations of chemical species. In this review we attempted to show that when models are created to address the effect of spatial heterogeneity on biological function, or if the network is composed of a small number of molecules with high connectivity, mesoscale models of biological systems, where individual biological entities are represented, can overcome modeling pitfalls that other, population-based modeling techniques are prone to. Although the paucity of quantitative data is a clear bottleneck and often frustrates attempts of building accurate molecular models, it should not detract from well thought-through efforts. Basic models can be extended as more biological data is aquired. Indeed, often these models are essential in guiding traditional experiments to yield new insight. Many software tools already exist that function as general biological simulators and permit the modeling of many biological phenomena using single-particle methods. It is likely that modeling software will become a routine part of the biologists toolbox within the foreseeable future. Single particle based modeling techniques offer users the ability to create some of the most realistic models of biological systems and as such it is imperative that development and improvement of particel-based simulation software continues.

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