Quantifying the Roles of Space and Stochasticity in Computer Simulations for Cell Biology and Cellular Biochemistry

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E20-08-0530
TITLE: Quantifying the Roles of Space and Stochasticity in Computer Simulations for Cell Biology and Cellular Biochemistry

Dear Authors, two reviewers have looked at your manuscript, and in general they are positive, as am I. There is one caveat: one of the reviewers, and myself, think that MBoC is not the best outlet for the paper. Cell biologists would not be too interested in minute details and issues of modeling. They normally just want to know very general modeling outline, and then focus on biological topics. Your focus is much more fit for something like PLoS Comp Biol or BMC Bioinformatics. This said, I will leave the door open. As far as you understand that only a small fraction of readership will be interested and OK with it, here are two main revisions you'll have to do:
1) the first section requires revision to present a fair and comprehensive overview of spatial stochastic modeling/simulation approaches - see specifics in the report of reviewer II.
2) please discuss model reduction and parameter estimation issues.
3) address constructively numerous specific comments of both reviewers.

If you choose to do that, I will send your revised manuscript to reviewer II for the second look.

Sincerely,

Alexander Mogilner
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Johnson,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the

Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you haveopted out of publishing the review history.

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In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

As the title states this paper looks at quantifying the Roles of Space and Stochasticity in Computer Simulations for Cell Biology and Cellular Biochemistry. The authors have their own particular view on this in terms of very detailed spatial simulations and that is fine.
But I am disappointed as a review that there is very little on model reduction and parameter estimation, for example. The review could be much stronger if the authors had taken a more wide ranging view point. But that is their decision.

I have marked up in the attached document a number of issues. They are not extensive and if they are done the paper is acceptable.

Reviewer #2 (Remarks to the Author):

Summary:

The authors provide an overview of methods for resolving spatial dynamics and stochasticity in modeling biological systems. The paper has three main components, beginning with a review of ODE models, well-mixed stochastic models, PDE models, and concluding with a more detailed overview of spatial, stochastic modeling approaches and associated simulation packages. After this survey, the authors present a series of basic chemical and biological models, illustrating differences that may arise between different scales of models (deterministic vs stochastic, well-mixed vs spatial,...) and between different simulation packages. Finally, the authors conclude with some speculation on where they feel the field should go.

Overall the paper was well-written, and I very much appreciated the effort to build a library of core examples through which different modeling approaches and simulators can be assessed. As I discuss below, I feel the first section requires some revision to present a fair and comprehensive overview of spatial stochastic modeling/simulation approaches. Should the authors address those comments, I would recommend publication, but with one caveat. As it is primarily focused on modeling regimes and simulation software, and not a specific driving biological application, I am unsure if the manuscript is appropriate for publication in Molecular Biology of the Cell. I leave it to the editor to make this assessment.

Main Comments:
1. pg 14 - Discussion of "single-particle Kinetic Monte Carlo schemes". ReaDDy seems to me to be just as microscopic and physically realistic in its underlying modeling approach as eGFRD and other "Green's Function" approaches. It certainly approximates a well-defined underlying physical model:

- ReaDDy supports volume exclusion through calibrated soft-core repulsive potentials. Is there any experimental evidence, or comparisons to more detailed MD-type simulations of proteins, that demonstrate soft-core repulsive potentials are less accurate in modeling volume exclusion between proteins than approximating them as spheres and using a hard-core potential?

- The bimolecular reaction model in ReaDDy is based on the Doi / volume-reactivity / lambda-rho model, where two particles react with a fixed rate when sufficiently close. Is there any experimental evidence, or comparisons to more microscopic models of protein interactions such as MD, that demonstrate this reaction model is less accurate for approximating biological reactions than the Colins-Kimball / Smoluchowski model (particularly if one is able to model volume exclusion through the use of a repulsive potential)?

- ReaDDy is timestep based, and error control should be just as feasible as for typical BD simulations since there is an underlying continuous-time model that it approximates (the Doi model, with particles moving by drift-diffusion and experiencing interaction potentials).

- Figure 2 caption; I would not say that Doi model simulators like ReaDDy use a "macroscopic rate". They use an intrinsic microscopic rate that, like a Collins-Kimball rate, is often calibrated to macroscopic rates, but could in theory be calibrated from more microscopic simulations or experimental data.

I would suggest a rewrite of this section to more appropriately frame the physical accuracy of, at least, ReaDDy.

2. While volume exclusion is a nice feature in many of the Green's function and Smoluchowski simulators, my impression, further reinforced by the limitations discussed in the manuscript for Fig. 4,

is that it is generally not feasible to resolve in anything but very small systems. As such, many spatial, stochastic modeling studies ignore general volume exclusion. In this context, it is unclear to me whether retaining volume exclusion for just bimolecular reactions offers an appreciable benefit in most biological models (where there are many types of species, and "collisions" between non-reactive pairs may dominate). Can the authors point to some literature on the benefit of keeping volume exclusion just for bimolecular reactions compared to using a pure point-particle model without volume exclusion?

3. pg 9 and 10, discussion on spatial lattice methods such as the RDME:

- pg 9 - The STEPS RDME simulator should also be referenced, along with the associated publications, see
http://steps.sourceforge.net/STEPS/research.php

- Box 3, second paragraph - The small voxel size issue mentioned by the authors was shown/proven in Isaacson SIAP 2009 and Hellander, Hellander and Petzold, PRE 2012.

- It should be mentioned that a variety of lattice methods have been designed to overcome the small voxel size issue, including SpatioCyte, renormalized RDME methods that match statistics of the Collins-Kimball model (Hellander, Hellander and Petzold, PRE (2015), Hellander and Petzold, J. Chem. Phys (2017)), and the CRDME (convergent RDME), which overcomes the issue by converging to the Doi / volume reactivity / lambda-rho model (Isaacson J. Chem. Phys (2013), Isaacson and Zhang J. Comp. Phys. (2018)).

4. The excellent recent review by Smith and Grima, "Spatial Stochastic Intracellular Kinetics: A Review of Modelling Approaches" Bull. Math. Bio (2019), should be cited. It provides a detailed discussion and comparison of spatial, stochastic modeling approaches, with significant attention to biophysical properties of different models.

Other Comments:
5. Though I realize this might not be possible, I think it would be very helpful to readers and the broader modeling community if one or more of the test examples could be compared to a "ground truth" given by experimental data, or compared to a more microscopic model/simulation (like MD). This would be helpful in understanding which of the considered models really gives the "right" behavior, as opposed to the current comparison, which focuses more on the differences between models.

6. Box 2 - Last sentence of the first paragraph, "A intuitively simple...": I'm not sure what the authors mean here by a fixed time step. Do the authors mean approximating the master equation as a discrete time Markov chain? If so, one would expect that as dt -> 0 the approximation converges, so the error is still controlled in dt and can be made arbitrarily small.

7. pg 13, box 5, first paragraph - It should perhaps be mentioned that methods like BD or eGFRD may still require significantly reduced time steps as particle densities increase to avoid missing reactions (for the former) or due to the decreased size of pair-protective domains (for the latter). My understanding is that as densities increase, ultimately Green's function methods may become less efficient than brute force BD with a small timestep. Can the authors comment on this?

8. pg 18 - Fig 3 - Do the ODE models used here have two compartments (i.e. a 2D compartment

and a 3D compartment)?

9. pg 23 - Middle paragraph - Can the authors say more about what approximation within Smoldyn leads to the discrepancy for 2D problems?

10. pg 31 - I didn't understand the sentence "This is in part..." Why does lack of volume exclusion lead to accumulation within a small space rather than spreading out across the whole membrane? Doesn't the PDE model also lack volume exclusion; if so, why is it not affected like Smoldyn?

Typos:
pg 3 - last sentence: Are these Gillespie references misplaced?

pg 4 - First full paragraph: Similar comment after "neuronal axon".

Box 2 - Should the sum be over little "r"?

Response to referees. Author responses are in red. In the main text, our edits are all highlighted in yellow.

Reviewer #1 (Remarks to the Author):

As the title states this paper looks at quantifying the Roles of Space and Stochasticity in Computer Simulations for Cell Biology and Cellular Biochemistry. The authors have their own particular view on this in terms of very detailed spatial simulations and that is fine.
But I am disappointed as a review that there is very little on model reduction and parameter estimation, for example. The review could be much stronger if the authors had taken a more wide ranging view point. But that is their decision.
We agree with the reviewer that both model reduction and parameter estimation play central roles for computational models of cellular behavior. For our study, which focuses on the influence of spatial resolution and stochasticity, these aspects become important as one switches between different levels of complexity (spatial vs. non-spatial, deterministic vs. stochastic). However, a systematic treatment at the level of detail we try to provide for the core issues (space, stochasticity) would require a major extension of the manuscript and of the computational studies presented in it. We also felt that these aspects are more technical and less fundamental than the decision as to whether a model should, for instance, describe a cellular process with or without spatial resolution. In our revised text we therefore tried to point out when considerations should be made regarding model reduction and parameter estimation but would prefer to avoid extending the (already rather hefty) manuscript by additional studies. Please see additional paragraphs regarding these issues added on page 5 and again on page 6, and a few mentions in the results and discussion.

I have marked up in the attached document a number of issues. They are not extensive and if they are done the paper is acceptable.

We thank the reviewer for their careful reading of the manuscript and numerous comments, queries and suggestions. We have tabulated all but the most minor of these and respond below.

- pp. 3-4. [The reviewer notes several spurious references to Gillespie papers.]

These were included by mistake. These may have added to the reviewer's impression of our manuscript being biased in favor of Gillespie's contributions to the field, for which we apologize. The references have now been corrected and updated to include the original references that we intended to cite here. We have attempted to credit the wide range of contributions to each of the areas highlighted. We have also changed all occurrences of the term "Gillespie Algorithm" in the paper to "Stochastic Simulation Algorithm" or SSA. We further address this issue for specific comments below.

- pp. 4. An important area missing here is modeling of plasma membrane.

We agree that the plasma membrane and how it is modeled is an important topic. In the methods (Fig 2b) we illustrate aspects of the membrane that impact model selection and include these features in Table S2. In the discussion, we return to the topic particularly with regard to the significance and challenge of integrating membrane mechanics with biochemical interactions that couple to it (page 37).
We have now also added a comment on moving boundaries, and specifically note that models allowing moving boundaries (frequently representing membrane) do not necessarily capture the biophysics of membrane dynamics. They can be decoupled methodologically, although fundamentally, they are not (see page 10).

- p. 6, Fig. 1. “PDEs are numerically solved on a mesh as shown.” This is not correct. There are mesh free approaches!

We agree with the reviewer’s point, but to keep the presentation simple here we have chosen not to explicitly mention mesh-free methods, but rather to rephrase the statement as, “*For example*, PDEs *can be* numerically solved on a mesh as shown.” (emphasis added). We explicitly note mesh free approaches are an option in Box 3.

- p. 7. “In most practical modeling applications the CME cannot be solved analytically…” Absolutely not true. The solution of the CME is

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

The issue is in computing this.

By “analytical solution” we mean a closed-form expression for the *exact* solution of the CME, in accordance with the standard definition of this term. The above equation is most decidedly *not* an analytical solution: it is an equation that describes the *form* of the solution, which actually must be computed numerically (and hence *approximately*) for problems with more than a few degrees of freedom because of combinatorial explosion (bounded systems) or infinite state space (unbounded systems). To clarify what me mean by our statement that the CME cannot be solved analytically, we have added the parenthetical remark “(i.e., with a closed-form expression).” We feel that to go into further details about this would go beyond the interest of our target audience.

- p. 8, Box 2. “However, the fixed time step would always introduce the potential for errors….” The time steps can vary!

This is true, but variable time step size does not eliminate the errors due to a finite difference approximation, as we now explain more clearly in the revised text, “However, the fixed time step in this integration scheme has finite error that is only eliminated in the limit $\Delta t \to 0$, since reactions may occur even during shorter time steps than the one chosen to propagate the system in time.” The point we are trying to get to is that it’s useful to have an exact algorithm to get around this problem, and such an algorithm happens to exist for stochastic systems.

- p. 8, Box 2. “One popular and precise method used to generate trajectories through the state space sampled by the CME without the need to choose a discrete time step is the Gillespie algorithm, also known as the stochastic simulation algorithm (SSA) (Bortz et al., 1975) (Gillespie, 1976a; Lopez et al., 2013). in a Gillespie simulation” You are missing key references, e.g., Tom Kurtz. It is not a “Gillespie simulation”. Be impartial. [Many more instance of this complaint throughout the manuscript.]

Although usage of the term “Gillespie algorithm” to refer to the described method for stochastic simulation of chemical reaction kinetics is widely used in the literature (as a quick Google search will reveal), we understand that the reviewer and others can see our usage of the term as biased or neglecting the contributions of others, which is unfair and draws attention away from the basic concepts we are trying to illustrate. We have therefore replaced the term “Gillespie algorithm” with “Stochastic Simulation Algorithm” or “SSA” throughout the manuscript. Although Gillespie was not the first person to perform stochastic simulations of chemical kinetics, he was the first to describe the most commonly used algorithms for their exact simulation (e.g., the exact treatment of time obtained by exponential sampling). So, as references to the SSA we now cite Gillespie’s original papers on the SSA and a 2013 review he co-authored with Petzold and Hellander, which provides a broad overview of stochastic algorithms for chemical kinetics and provides a detailed review of the literature placing the SSA in its proper context (he cites and discusses the contributions of Kurtz, for example). We don’t feel that reviewing this history is within the scope of the current work.

- p. 9. Paragraph beginning, “The most important difference between spatial and non-spatial simulations…” Note that often the boundaries are not fixed.

Good point. Figure 2b includes an example of moving boundaries, and we have now added in more text to discuss moving boundaries on page 10.

- p. 9. “Similar to the non-spatial case, it is usually not possible to solve the RDME analytically…” This is not true. The issue is computation.

Here again we are using the standard definition of “analytical” as admitting a closed form solution, which is generally not possible for the RDME.

- p. 10. Box 3. Again, there are mesh-free approaches.

We now note this in Box 3.

- p. 12. Box 4. Should not it be b(\sigma)? There is no \rho in RHS.

Thanks, the density rho was not explicitly defined, as it now is. The length scale b depends more sensitively on the maximum particle density, which is defined as the number of A or B particles (max) divided by surface area.

- p. 13. Box 5. “The time evolution of the molecules’ positions is described by a stochastic differential equation…” Usually driven by Wiener processes.

Noted, but we would like to avoid additional jargon and have left out this comment.

- p. 18. “For reversible bimolecular association of well-mixed reactants in a closed system, the *equilibrium is theoretically well-defined* and the kinetics for non-spatial rate equations can be derived analytically.” What does this mean?

We meant that the equilibrium state can be solved for analytically, and for non-spatial rate equations, so can the kinetics. We have edited this sentence to make this clearer.

- p. 18. “Because Smoldyn approximates the dynamics of the Smoluchowski model, *the kinetics can be off for specific parameter regimes*, with deviations being typically very small in 3D but significant in 2D.” What do you mean?

We clarified our explanation of why Smoldyn sometimes gives inaccurate results in two places. On page 5:

“Smoldyn is also derived to use large time-steps (albeit without excluded volume), and it is simpler to implement than GF approaches (Andrews, 2017; Andrews et al., 2010; Andrews and Bray, 2004b). However, the reaction parameters are coupled to the time-step size, rather than representing independent model features (e.g. binding radii and microscopic rates), meaning that the time-dependence (and the equilibrium in 2D) are not as rigorously correct.”

On page 18:

“The Smoldyn method uses the steady-state solution to the Smoluchowski model to derive reaction parameters (Andrews and Bray, 2004a), but in 2D there is no steady-state, and thus the reaction parameters are approximate. Because of this, Smoldyn can generate inaccurate kinetics in certain parameter regimes, with deviations being typically small in 3D but significant in 2D.”

- p. 21. “As crowders become larger and more immobile (e.g. vesicles)….” Vesicles are usually not immobile.

We agree that the wording here was confusing as we did not mean to imply that vesicles are immobile. We changed to “As crowders become larger and less mobile (e.g., vesicles)…”.

- p. 21. Double citation of Andrews (2020).

Thanks, we reworded this sentence to clarify the meaning and avoid the double citation: “In contrast, simulations that immobilized the crowders caused them to act as a rigid barrier, leading to a reduction in reaction rates despite using the same reactant concentrations studied here (Andrews, 2020).”

- p. 30. “We show that by increasing the error tolerance on the numerical integration or by increasing the PDE mesh size one can delay the onset of this transition,…” Be more specific on values.

We added in the magnitude of the changes on pg 31 now. The specific values are reported in the supplemental figure S6 (as linked in this sentence).

Reviewer #2 (Remarks to the Author):

Summary:
The authors provide an overview of methods for resolving spatial dynamics and stochasticity in modeling biological systems. The paper has three main components, beginning with a review of ODE models, well-mixed stochastic models, PDE models, and concluding with a more detailed overview of spatial, stochastic modeling approaches and associated simulation packages. After this survey, the authors present a series of basic chemical and biological models, illustrating differences that may arise between different scales of models (deterministic vs stochastic, well-mixed vs spatial,...) and between different simulation packages. Finally, the authors conclude with some speculation on where they feel the field should go.

Overall the paper was well-written, and I very much appreciated the effort to build a library of core examples through which different modeling approaches and simulators can be assessed. As I discuss below, I feel the first section requires some revision to present a fair and comprehensive overview of spatial stochastic modeling/simulation approaches. Should the authors address those comments, I would recommend publication, but with one caveat. As it is primarily focused on modeling regimes and simulation software, and not a specific driving biological application, I am unsure if the manuscript is appropriate for publication in Molecular Biology of the Cell. I leave it to the editor to make this assessment.
We are grateful to the reviewer for their positive and constructive comments. Please see our detailed responses below, along with highlighted changes in the text, which we think have clearly strengthened our paper. We acknowledge the concern about the journal choice, but as we state above, we are excited about the opportunity to share this work with a broader cell biology audience, as we believe interest in quantitative modeling is only increasing along with a corresponding increase in quantitative experimental measurements.

Main Comments:
1. pg 14 - Discussion of "single-particle Kinetic Monte Carlo schemes". ReaDDy seems to me to be just as microscopic and physically realistic in its underlying modeling approach as eGFRD and other "Green's Function" approaches. It certainly approximates a well-defined underlying physical model:
We thank the reviewer for this detailed description of READDY. We revisited our classification scheme and we agree that the description of the methods like READDY and SpringSalad as purely based on a macroscopic rate was not accurate. Our initial classification was motivated by the extent of approximations used in different methods,

which led to some muddiness in our characterization. We have thus modified this section on Single-particle methods substantially and updated our classification scheme to collision-based reactions and volume-based reactions (with corresponding update to Table S2). We then discuss how the different algorithms for each model are based on distinct approximations that introduce sources of error as a function of time-step, and due to the introduction of potentials.

- ReaDDy supports volume exclusion through calibrated soft-core repulsive potentials. Is there any experimental evidence, or comparisons to more detailed MD-type simulations of proteins, that demonstrate soft-core repulsive potentials are less accurate in modeling volume exclusion between proteins than approximating them as spheres and using a hard-core potential?
Introducing potentials to capture volume exclusion is not inherently problematic; it is indeed necessary in the Molecular Modeling fields (MD-type simulations), as hard boundaries are not continuous. The challenge for Reaction-Diffusion, however, is to calculate reaction probabilities in the presence of these potentials. READDY1 did not do this, but quite recently, they have considered the effect of the potential on reaction probabilities, and the connection to the macroscopic rate. We indicate this challenge more explicitly in the text.

- The bimolecular reaction model in ReaDDy is based on the Doi / volume-reactivity / lambda-rho model, where two particles react with a fixed rate when sufficiently close. Is there any experimental evidence, or comparisons to more microscopic models of protein interactions such as MD, that demonstrate this reaction model is less accurate for approximating biological reactions than the Colins-Kimball / Smoluchowski model (particularly if one is able to model volume exclusion through the use of a repulsive potential)?
No, we did not intend to say that the Doi model of RD is less accurate. The algorithm implemented in READDY, however, is less accurate, due to their use of a short-time approximation and their addition of potentials. If a GF approach was applied to the Doi model, the algorithm would be accurate, although as you note, it would not naturally capture excluded volume. We have updated the text to make this more clear.

- ReaDDy is timestep based, and error control should be just as feasible as for typical BD simulations since there is an underlying continuous-time model that it approximates (the Doi model, with particles moving by drift-diffusion and experiencing interaction potentials).
Yes, we agree there should be error control, as it is based on a small time-step approximation. We note that there can be other sources of error--the READDY 2 software uses two integrators, only one of which is theoretically derived to recover detailed balance, which is an error that may not be controlled by the time-step. The other algorithm only recovers the proper kinetics in dilute limits. We have removed this general criticism, and focus more on explicit comparisons of the methods/approximations.

- Figure 2 caption; I would not say that Doi model simulators like ReaDDy use a "macroscopic rate". They use an intrinsic microscopic rate that, like a Collins-Kimball rate, is often calibrated to macroscopic rates, but could in theory be calibrated from more microscopic simulations or experimental data.
Agreed, we have edited this.

I would suggest a rewrite of this section to more appropriately frame the physical accuracy of, at least, ReaDDy.

2. While volume exclusion is a nice feature in many of the Green's function and Smoluchowski simulators, my impression, further reinforced by the limitations discussed in the manuscript for Fig. 4, is that it is generally not feasible to resolve in anything but very small systems. As such, many spatial, stochastic modeling studies ignore general volume exclusion. In this context, it is unclear to me whether retaining volume exclusion for just bimolecular reactions offers an appreciable benefit in most biological models (where there are many types of species, and "collisions" between non-reactive pairs may dominate). Can the authors point to some literature on the benefit of keeping volume exclusion just for bimolecular reactions compared to using a pure point-particle model without volume exclusion?

Agreed that for many biological systems it has limited impact on the observed kinetics or spatial distribution of species. For densely crowded systems, it is important, however, and although it is true that the system size is limited, as the reviewer notes, we nonetheless think it is beneficial to have many-body reaction-diffusion software that are capable of performing this quantitative assessment of crowding on reaction rates. We were also able to perform simulations with 1000s of particles as well using FPR/NERDSS, but not with eGFRD. Theoretically predicting the influence of crowding is not often possible, and as we discuss in the results, crowding has qualitatively different effects on rates depending on if they are large or small, mobile or immobile.

A broader class of biological models that require excluded volume, however, are systems that exhibit clustering or self-assembly, which occurs with multi-valent species (e.g. globular proteins or polymers like DNA). These do not have to be dense systems. If particles can pass through each other in these cases, one cannot retain the steric exclusion or localization that can be a critical factor controlling the speed of assembly and the structure of assembled species. Recent work using SpringSaLad has studied interactions among multi-site polymers (see Ref (Chattaraj et al., 2019)). The NERDSS software has recently been used to study assembly in rigid molecules with orientational constraints, forming flat and spherical lattices as occurs in clathrin coated cages and virus shells. Without excluded volume, the multi-valent assemblies pass through one another, which is highly unphysical and prevents comparison to experiment (see Ref (Varga et al., 2020)). We add a comment in the discussion (page 36) and in the methods (page 11) to highlight this important application of excluded volume models.

3. pg 9 and 10, discussion on spatial lattice methods such as the RDME:

- pg 9 - The STEPS RDME simulator should also be referenced, along with the associated publications, see
http://steps.sourceforge.net/STEPS/research.php
Thanks, we have included references to STEPS.

- Box 3, second paragraph - The small voxel size issue mentioned by the authors was shown/proven in Isaacson SIAP 2009 and Hellander, Hellander and Petzold, PRE 2012.
Thanks, we have added in these references.

- It should be mentioned that a variety of lattice methods have been designed to overcome the small voxel size issue, including SpatioCyte, renormalized RDME methods that match statistics of the Collins-Kimball model (Hellander, Hellander and Petzold, PRE (2015), Hellander and Petzold, J. Chem. Phys (2017)), and the CRDME (convergent RDME), which overcomes the issue by converging to the Doi / volume reactivity / lambda-rho model (Isaacson J. Chem. Phys (2013), Isaacson and Zhang J. Comp. Phys. (2018)).
Thanks, we have added this comment and references into Box 3.

4. The excellent recent review by Smith and Grima, "Spatial Stochastic Intracellular Kinetics: A Review of Modelling Approaches" Bull. Math. Bio (2019), should be cited. It provides a detailed discussion and comparison of spatial, stochastic modeling approaches, with significant attention to biophysical properties of different models.
Thanks for pointing this out, we cite it now on page 9.

Other Comments:
5. Though I realize this might not be possible, I think it would be very helpful to readers and the broader modeling community if one or more of the test examples could be compared to a "ground truth" given by experimental data, or compared to a more microscopic model/simulation (like MD). This would be helpful in understanding which of the considered models really gives the "right" behavior, as opposed to the current comparison, which focuses more on the differences between models.
We agree that comparison to experiment would certainly be valuable, but we think it is beyond the scope of our study. It is challenging to construct a model that quantitatively describes a biological process, and although we would be able to then use it to distinguish which features were captured by which simulation approach, the analysis would proceed similar to what we have done here with pre-defined models. We instead choose to use theoretical results as the ground truth or right answer when they are available, as for (some of) the kinetics, and the equilibrium of bimolecular reactions, and for the equilibrium or steady-states of the membrane model and the phosphorylation model. We thought this was particularly useful since even these right answers are not reproduced by all methods, despite providing the foundation for more complex processes.

Comparison to MD is even more challenging for multiple reasons: we are restricted by the capacity to simulate any long-time scales with MD, by the limitations of MD force-fields, and by the inability to break bonds (do chemistry) in MD. Even in the MD field, it is not typically represented as the “ground truth”, due to its own approximations (e.g the force-fields are empirical). We do agree that, for example, the crowding model could be usefully compared to an MD-type simulation, which captures inertial motion, as we note more explicitly now on page 22. However, chemical reactions are generally not accessible in any MD-type simulation, so the reaction we simulate (A+B->C+B) has not been performed and is not possible in current software.

6. Box 2 - Last sentence of the first paragraph, "A intuitively simple...": I'm not sure what the authors mean here by a fixed time step. Do the authors mean approximating the master equation as a discrete time Markov chain? If so, one would expect that as dt -> 0 the approximation converges, so the error is still controlled in dt and can be made arbitrarily small.
True, it would converge for small dt--we have edited this statement accordingly.

7. pg 13, box 5, first paragraph - It should perhaps be mentioned that methods like BD or eGFRD may still require significantly reduced time steps as particle densities increase to avoid missing reactions (for the former) or due to the decreased size of pair-protective domains (for the latter). My understanding is that as densities increase, ultimately Green's function methods may become less efficient than brute force BD with a small timestep. Can the authors comment on this?
The GFRD method does indeed become less efficient in dense systems, due to the overhead costs of selecting the next event and of sampling positions from the 3D GF, and automatically converts to a BD solver. The FPR method, which combines the GF approach with Brownian updates and a reweighting factor, does not have the overhead of GFRD. Thus, the FPR method is still efficient even for small time-steps. We decided to note this more explicitly in the section on crowding (page 21) rather than in Box 5, since it is not systematically true of all GF-based methods.

8. pg 18 - Fig 3 - Do the ODE models used here have two compartments (i.e. a 2D compartment and a 3D compartment)?
There is a Volume and an Area that are specified depending on whether the species are restricted to the solution or the surface, and these are used to define the relative concentrations of those species. While there is no spatial ‘compartments’, the rates reflect the relative dimensionality of the reactions. One can think of it as tracking copy numbers, where the solution rates (units V/s) of bimolecular reactions are divided by V, and the rates of bimolecular reactions on the membrane (units of A/s) are divided by A.

9. pg 23 - Middle paragraph - Can the authors say more about what approximation within Smoldyn leads to the discrepancy for 2D problems?
Smoldyn derives a binding radius and unbinding radius based on the macroscopic rate, diffusion constant, and time-step for a process. This requires the steady-state solution to the Green’s function, and in 2D, there is no steady-state rate. They have not yet derived what this definition of the unbinding and binding radii should be in 2D (but are

working on it—personal communication with S. Andrews). They are currently approximating the radii just using the 3D GF, for lack of another option. We added a brief comment to make this more explicit in the text on page 19.

10. pg 31 - I didn't understand the sentence "This is in part..." Why does lack of volume exclusion lead to accumulation within a small space rather than spreading out across the whole membrane? Doesn't the PDE model also lack volume exclusion; if so, why is it not affected like Smoldyn?
This was not stated properly. For Smoldyn, we are not exactly sure why the oscillations disappear, but we speculated that when particles were collecting on top of each other (due to a lack of excluded volume), they were not properly accounting for the increasing density of molecules on the surface. Hence, the number of reactive collisions was undercounted. The PDE would correctly produce an increasing density with additional recruitment events, so the problem is not really due to excluded volume but possible errors in accounting for all reaction pairs. However, since we did not prove that was the source of the discrepancy for Smoldyn, we have removed this speculation entirely.

Typos:
pg 3 - last sentence: Are these Gillespie references misplaced?
pg 4 - First full paragraph: Similar comment after "neuronal axon".
Yes, this happened in a few places accidentally, always Gillespie references—some kind of user-error with EndNote. They have been deleted.

Box 2 - Should the sum be over little "r"?
Yes, thank you for catching that.

RE: Manuscript #E20-08-0530R
TITLE: "Quantifying the Roles of Space and Stochasticity in Computer Simulations for Cell Biology and Cellular Biochemistry"

Dear Prof. Johnson:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell. Congratulations on the job well done. Just please make sure to fix a couple of typos pointed out by one of the reviewers.

Sincerely,
Alexander Mogilner
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Johnson:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The response to reviewer's comments are detailed and appropriate. The paper can go to publication.

Reviewer #2 (Remarks to the Author):

The authors have addressed my comments and I now recommend publication. Below are a few typos, and what seem like a few misplaced references, I noticed.

Minor Comments:
pg 6 - "hill-type" -> "Hill-type"

pg 10 -> "Currently, there are only few simulation"

pg 10 -> "(frequently representing membrane) do"

Box 3, last sentence -> I was not aware of the Boutillier or Tiger references, which look very interesting. Are these meant to be included somewhere else though? Looking at these manuscripts they do not seem to be addressing spatial lattice models and their convergence, but more focused on frameworks for studying large non-spatial network models?