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Modeling membranes *in situ*

Chelsea M. Brown and Siewert J. Marrink

Abstract

Molecular dynamics simulations of cellular membranes have come a long way—from simple model lipid bilayers to multi-component systems capturing the crowded and complex nature of real cell membranes. In this opinionated minireview, we discuss the current challenge to simulate the dynamics of membranes in their native environment, *in situ*, with the prospect of reaching the level of whole cells and cell organelles using an integrative modeling framework.

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Introduction

What I cannot simulate, I cannot understand. Paraphrasing Feynman, this statement illustrates the importance of computational modeling in modern science. The field of molecular dynamics (MD) simulations, in particular, has made an enormous impact on our understanding of a wide variety of processes, including those taking place inside cells. This certainly holds for membrane-related processes, which is the topic of this minireview. Thanks to the steady increase in computational power over the past decades, we have witnessed a transition from pioneering simulations of model lipid membranes and isolated membrane proteins to extensive studies capturing protein–lipid interplay in ever-more realistic membrane environments [1–5].

Capturing the complexity of realistic cell membranes is far from trivial. Typical cell membranes comprise 10s or 100s of different lipid types, and are packed with a huge diversity of membrane proteins. Moreover, the composition strongly depends on the type of organelle or cell, changes during the cell's life cycle, and is distinctly non-homogeneous. To add to this challenge, cell membranes are not isolated entities: they are in continuous interaction with their surroundings. Signaling pathways connect the inside of cells or cell organelles to the outside, and may trigger local structural changes, such as the formation of nanodomains, all the way to large-scale membrane-remodeling events during fusion or fission. In turn, dynamic processes happening at the membrane trigger numerous downstream processes in other parts of the cell.

The increasing recognition that the cytoplasm also constitutes a very crowded and heterogeneous environment makes it clear that we are only at the beginning of our quest for modeling membranes in their full complexity, i.e. residing in a native (*in situ*) context. In the end, whole-cell models at molecular resolution are key. For this to happen, integrative modeling approaches are needed, combining state-of-the art experimental techniques with multiscale simulation workflows [6]. Fortunately, we are witnessing immense progress in elucidating the architecture and stoichiometry of cellular components at unprecedented resolutions, setting the stage for spatially detailed simulations of membranes of whole cells or cell organelles.

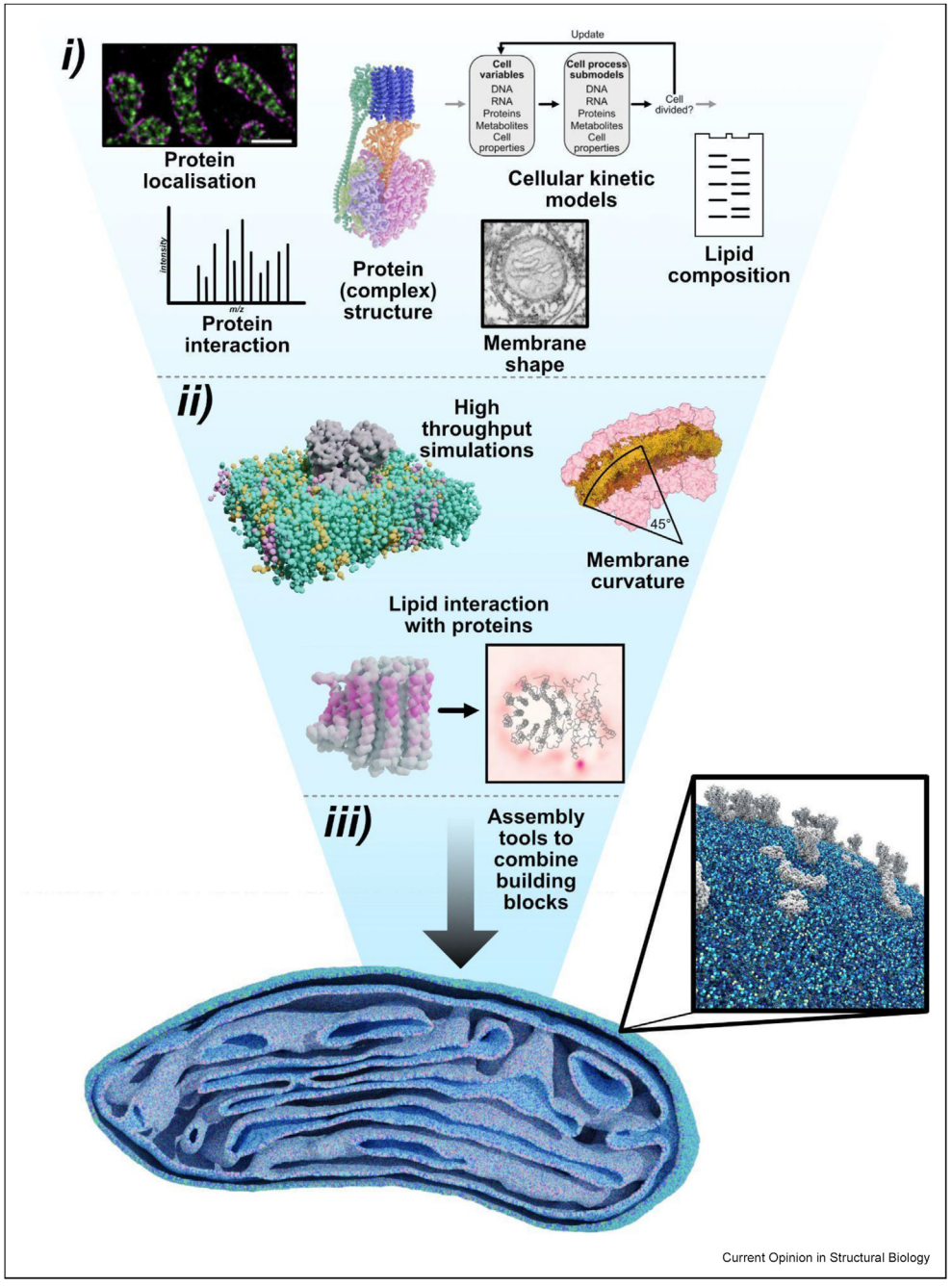
The scope of this opinionated minireview is as follows. We first provide some examples of current integrative multiscale modeling pipelines that aim to bring the field to the next level. This is followed by a section with selected recent examples of the major progress along the lines sketched earlier, i.e. to simulate membranes in a realistic environment, culminating with current attempts to bridge to the whole cell or organelle level. We finish with a short section with our ideas on current issues and possible future roads ahead.

Integrative multiscale membrane modeling

From the early days on, computational membrane models have depended upon a large variety of experimental data. For instance, Nuclear Magnetic Resonance (NMR) and diffraction data have been extensively used to calibrate and validate the lipid force fields. In turn, simulations of lipid membranes have aided in the interpretation of experimental data and provided testable hypotheses [7].

To model membranes as part of a realistic environment, an integrative modeling framework is required combining experimental data, high-throughput modeling pipelines, and multiscale workflows. Recent examples of such an integrative approach can be found in the modeling of membrane-enveloped viruses [8–13] and the construction of organelle and whole-cell models [14,15]. The underlying workflow typically consists of three parts (Figure 1): (i) obtaining the relevant data on composition, geometry, and structure;

Figure 1



Integrative multi-scale modeling framework. Step (i) obtaining target data from experimental assays or kinetic network models, step (ii) validating and pre-equilibrating individual components with high-throughput pipelines, and step (iii) building the final system using multiscale assembly tools. The protein localization is taken from Ref. [18], membrane shape from Ref. [37], and the kinetic workflow is adapted from Ref. [38]. The protein structure was downloaded from the protein data bank (PDB ID: 6N2Y). Membrane curvature is taken from Ref. [39]. The lipid interaction visualization was modified from Ref. [25]. The complex assembly example, the membranes of an entire mitochondrion, is adapted from Ref. [34].

(ii) validating individual components at target resolution; and (iii) multiscale building of the final system.

The first step requires the availability of state-of-the-art experimental data. Foremost, data on the composition of the membrane and its environment are needed, ideally with information on the stoichiometries of the constituents. Impressive progress is being made in fluorescent-labeling techniques [14,16–18] and mass spectrometry [19] to provide this information for membranes in their natural context. An alternative source of information is offered by kinetic network models, which can predict the composition of the cell along the cell's life cycle [20]. Next, geometrical information on the overall membrane shape, and the position of large complexes, helps to construct relevant starting states of the simulation, whereas for lipid bilayers with a few components, one could start from a randomized initial structure of all the components; such an approach becomes infeasible for more complex systems. The required information can be extracted, at ever increasing precision, from an array of biophysical approaches, most directly from cryogenic electron tomography (cryo-ET) [21]. Finally, structural data for all complexes that either reside in the membrane or make up the environment are required. With the vast amount of membrane protein complexes nowadays available from cryogenic electron microscopy (cryo-EM) [22], together with ongoing increase in accuracy of artificial intelligence–assisted predictions (e.g. AlphaFold2 [23]), reliable starting structures for most biomolecular complexes can be generated.

The second step involves optimization of the key components at the target resolution. For realistic membrane models, this is either at the all-atom (AA) level, providing the highest level of accuracy, or at the coarse-grain (CG) level, providing optimal speed. For both AA and CG MD simulations, a number of established force fields are available that have been validated and optimized over the years and can accurately capture the behavior of multicomponent native membranes representative of a large variety of cells including mammalian, yeast, neuronal, mitochondrial, thylakoid, and bacterial [4,24]. In conjunction with high-throughput pipelines [25–27], thousands of small-scale systems can be simulated to pre-equilibrate, and if necessary, further optimize the building blocks. The latter part is essential when resorting to CG models, which often benefit from optimization against reference simulations at the AA resolution. Pre-equilibration is particularly useful to ensure that the local environment of a biomolecular complex is realistic and therefore is stable when integrated into the bigger system. As an example, pre-equilibration of protein–lipid interactions has been performed to smoothen the integration of proteins including their lipid annular shell into the membrane envelope of the SARS-CoV-2 virus [10] and into the membrane of a protocell [28].

The third step concerns building the initial configuration of the full-complexity model. This is typically achieved using a multiscale procedure. To construct membrane envelopes from scratch, a number of popular tools exist, e.g. Charmm-GUI [29], Insane [30], and others, which can put arbitrary numbers of lipids and proteins into planar or spherical membrane geometries. For more complex membrane shapes, mesoscale techniques such as dynamic triangulated surface simulations [31] can be used to obtain the required membrane shape, followed by a backmapping step to the target resolution [32,33]. Alternatively, membrane volume maps from cryo-ET can serve as a starting point [34]. More fancy multiscale workflows that couple resolutions on the fly are also promising, as exemplified by the Multiscale Machine-Learned Modeling Infrastructure (MuMMI) [35,36]. In MuMMI, three resolution scales are dynamically coupled using machine learning: a continuum model able to simulate milliseconds of time for a macroscopic (1 μm^2) membrane patch, a middle scale at the CG level to explore protein–lipid interactions, and an AA model to capture specific details of these interactions.

Simulating membranes in their native environment

The steady progress in our ability to model a variety of multicomponent membranes, as well as reaching the required spatiotemporal scales to capture protein–lipid and protein–protein interplay, has opened the way to simulate collective behavior of large assemblies of proteins and lipids. State-of-the-art membrane simulations showing such emerging behavior include simulations of membrane-lytic proteins cutting membranes to pieces (so called 'cookie-cutters') [40,41], alignment or oligomerization of proteins due to membrane-mediated interactions [42,43], lipid-based regulation of membrane channel activity [44,45], nascence of lipid droplets through the combined action of lipids and proteins [46–49], changes in membrane viscosity as a function of protein crowding [50], protein-mediated lipid transport [51,52], simulations of large-membrane protein assemblies such as the nuclear pore complex [53] and centriole [54], studies addressing membrane fusion in complex settings [55–58], formation of lipid aggregates in aerosols [59], and protein-induced membrane curvature generation [39,60–63].

Pushing this further, an increasing number of studies aim to capture the interaction of the membrane with its environment, i.e. the cytosolic or periplasmic regions, thus exploring membrane dynamics *in situ*. A first step is looking at membrane–cytoskeleton interactions, as exemplified by simulations of actin filaments with lipid bilayers showing preferential adsorption/desorption of the filaments depending on lipid composition as well as ion types present in the solution [64], or specific

deformation of vesicles dependent on length and stiffness of the filament [65]. More and more realistic descriptions of bacterial membranes are also appearing, including the peptidoglycan layers and other parts of the periplasm [66–68] (Figure 2a).

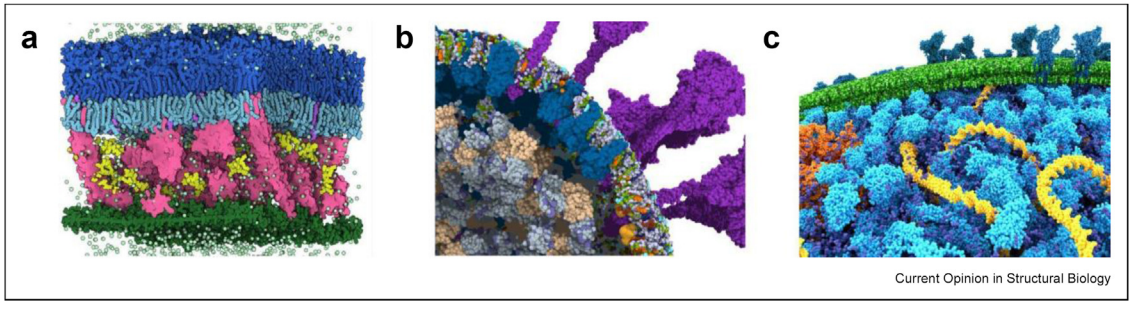
To arrive at simulations that include the full complexity of the membrane environment, more realistic descriptions of the cytoplasm itself are needed. Ongoing efforts are aiming at constructing such models, which come with their own challenges [69] and are therefore still limited with regard to applications addressing cytosol–membrane interactions. Recent work in this direction addresses the effect of crowding of cytosolic proteins near a membrane, showing a distinct effect on membrane-bending rigidity [70]. Along these lines, we also see pioneering simulations of biomolecular condensates interacting with membranes. For instance, different groups showed that, depending on lipid composition or pH, condensates can either adopt a more spherical shape, or wet the membrane thereby inducing negative membrane curvature [71,72]. Taking this further with lower-resolution models, coacervation-induced vesicle remodeling was captured, explaining endocytosis and exocytosis pathways observed experimentally [73,74].

Given the complex coupling between the cell membrane and its environment, arguably, to fully understand membrane behavior, simulations of entire organelles and whole cells are required. The community is currently taking the first steps in this direction. For instance, simulations of near-complete models of membrane-enveloped viruses have emerged. Examples are the recent models of the SARS-CoV-2 viral particle, including all known protein complexes, some with realistic glycosylation patterns, embedded in a spherical or ellipsoidal multicomponent lipid envelope [8–11]. Modeling the RNA content inside the virus is still a challenge as the structural organization is not known in detail. However, in one of these studies [11], the RNA fragments bound to the proteins have been included, providing a realistic view of the complex environment of the viral membrane itself (Figure 2b). Other enveloped viruses that have been simulated in great detail include HIV [12], the influenza virus [75], and the hepatitis B virus [76].

Beyond viruses, membrane-bound organelles are interesting targets. A key pioneering example is the modeling, at AA level, of a light-harvesting organelle [14]. This system shows how membrane curvature can separate groups of proteins into discrete domains. The disorder in the lipid environments found in these domains was found to change the efficiency of excitation transfer. Another breakthrough was achieved in simulating the convoluted membranes of an entire mitochondrion, making use of an implicit solvent CG model [34]. Although this work is a proof of principle study (in the absence of proteins one would not expect the mitochondrial membranes to be able to keep their folds), it opens the way to simulate more realistic models of mitochondria and other membrane-rich organelles such as chloroplasts and the endoplasmic reticulum/Golgi membranes.

The ultimate goal is, arguably, simulations of whole cells. On the way to this aim, a cell-scale membrane envelope was constructed and simulated [28]. In this study, they successfully simulated a protocell with a diameter of 200 nm for 500 ns, which contained 1397 proteins. The protocell stayed assembled for the entire simulation length, with water only passing through dedicated proteins and not the lipid bilayer. The most complex system generated to date is the model of the JCVI-Syn3A minimal cell, measuring 400 nm in diameter [15]. While the membrane itself contained five lipid types and 2200 proteins, the cytosolic components demonstrate how complicated it is to capture all of the relevant biological details. Within the cytosol, there were 60,000 proteins, about 500 ribosomes, 1.7 million metabolites, and a 543-kbp length of DNA. These

Figure 2



Membranes simulated *in situ*. Selected examples of membranes interacting with the environment. **(a)** Model of bacterial periplasm connected to the outer membrane [66], **(b)** close-up of a viral membrane interacting with bound RNA [11], and **(c)** membrane–cytosol interaction of a minimal cell [15].

components undoubtedly interact with the membrane and membrane proteins, influencing their behavior and dynamics. All of the included components are needed to accurately simulate a membrane in its biological state (Figure 2c).

Challenges ahead

When modeling membranes *in situ*, system sizes inevitably increase, which involves a number of challenges: (i) equilibration, i.e. lack of convergence and sensitivity to starting structures; (ii) force field imbalances, mounting when systems contain different classes of biomolecules and are simulated over longer time scales; (iii) biological relevance, in particular capturing the nonequilibrium nature of real cells; and (iv) analysis, the extraction of important features of complex systems. Many of these (and related) challenges plaguing large-scale MD simulations in general are discussed in a recent paper [77]. In the following, we briefly point out particular challenges and possible roads ahead for membrane-based systems.

Challenges in achieving equilibration

Traditionally, membrane patches are simulated long enough (either brute force, or with enhanced sampling) to assure the properties of interest are converged. With increasing complexity, this becomes more challenging—even the timescales for converging the distributions of lipids around membrane proteins are tens of microseconds or more. Cumulative sampling, when multiple copies of the same protein are present, might help to alleviate this problem to some extent. In general, however, care has to be taken to assess which properties are converged and which ones are affected by an initial-model bias. There is a clear need for enhanced sampling schemes that can increase sampling of the slow collective modes, such as the oligomerization of membrane proteins, formation of large-scale domains, or coupling between the membrane and cytosolic organization. Some steps in this direction are being taken [78,79]. More extensive sampling can be performed on subcompartments, i.e. smaller-scale systems extracted from the full one, with the additional challenge of making these compatible with the periodic boundary conditions that are typically used in MD simulations.

A particular challenge of simulating asymmetric membranes is in dealing with stresses due to imbalances in lipid placement. For a flat bilayer, this can be managed with careful consideration of the average area per lipid for each leaflet, which is automatically considered in Charmm-GUI [29] or can be manually controlled with tools such as Insane [30]. These implementations have been used in recently published complex asymmetric membranes [25]. Lipid preference for membrane curvature and hence placement in the system should be considered if the membrane is not flat, for example, the preference of cardiolipin at highly curved regions of the bilayer [80–83]. Domains of this nature can be added when building the membrane [34]. In systems featuring closed compartments (e.g. liposomes), this is further compounded by the sensitivity to the initial water and ionic content. A misjudgment in the number of molecules added inside can give rise to hyper or hypo-osmotic shocks causing membrane crumpling or rupture. In the SARS-CoV-2 envelope and protocell models discussed earlier, the authors needed to make many iterations of their model to get rid of the initial imbalance.

Challenges in dealing with force field imbalances

An accurate force field forms the basis for any successful simulation, but any type of force field relies on underlying assumptions and therefore faces limitations. Some shortcomings only become noticeable when time and length scales increase as small systematic errors accumulate and impact the results. A key example is the over estimation of protein–protein interactions with AA models, resulting from the historical bias to stabilize the folded state [69]. The same problem might hold for membrane proteins, only showing up with large-scale assemblies when deviations at the level of protein–lipid interactions get compounded in a crowded environment. Such problems have recently been notified for the CG Martini model, warranting a significant reparameterization effort [84]. Similarly, traditional force fields face problems capturing the configurational ensembles of intrinsically disordered proteins (IDPs) and polysaccharides [85,86]. Considering the complexity of interactions involving different classes of biomolecules, such as between sugars, nucleotides, proteins, and lipids, the level of accuracy remains uncertain; yet these are central for our ability to simulate membranes *in situ*. Force fields therefore need to be continuously being refined, with a key challenge to improving the overall balance.

Challenges in capturing biological relevance

The aim to model membranes *in situ* to capture biological relevance raises the question how to define our system. Knowing that there is a huge variability between individual cells for which, despite progress in experimental measurements as discussed earlier, mostly data averaged over many cells are available, representing any particular cell with full realism remains a challenge. Moreover, the composition of the cell strongly depends on the life cycle, changing across timescales that are inaccessible to current MD simulations. Living cell membranes are essentially nonequilibrium structures, subject to and driven by active processes, constant exchange of membrane-bound materials and the action of active proteins that shuttle between different states, driving changes in the local structure and properties of the membrane upon energy consumption [87].

Additionally, membranes are in contact with the surrounding milieu that is also the source of many out of equilibrium processes, e.g. causing the fluidization of the cytosol upon increasing metabolic activity [70] or the formation and dissolution of membrane-adhering condensates [88,89].

Therefore, to capture biological relevance, we have to resort to simulating prototypical systems, or 'instances, reflecting a possible temporary condition of the membrane and its surroundings. By systematic variation of the conditions, we can expect to learn what matters and what does not. To mimic cell conditions along the life cycle, the prospect of coupling to kinetic network models looks powerful, providing the ability to generate different starting states in a serial workflow. With further progress on the experimental side on revealing the spatial intricacies of the cell, and ongoing efforts to optimize the molecular mechanics force fields, we foresee the concept of 'living' simulations, i.e. complex systems that are continuously being simulated, regularly updated to incorporate the latest improvements.

Challenges in performing analysis

While in the 'old' days it took months to produce enough data that could be analyzed in a day or two, currently the workflow pertaining to complex systems is turned around. Terabytes of data are generated overnight, requiring months of dedicated analysis. The community is active in developing novel platforms streamlining the analysis, also specifically for membranes [90]. Expanding our efforts into simulations of membranes *in situ*, these issues will only become bigger, questions one would like to address going far beyond those solvable by most standard analysis tools. What interacts with what in such a complex membrane environment? Which processes are diffusion controlled, and which require activation? Which cytoplasmic elements interact directly with membranes, and to what extent do the cytoplasm and membrane change each other? Are there depletion zones, or do certain molecules accumulate near (or inside) the membrane? To what extent are strongly curved membranes enriched with certain lipids and membrane proteins? Do correlations exist between the different membrane protein complexes in adjacent membranes, as observed in thylakoid stacks? Answering these and related questions, and obtaining correlations that are not easily predictable on the forehand, requires the use of machine learning, which is a rapidly evolving field, already tackling membrane-specific problems [91–93].

Another challenge is the storage and handling of large amounts of data, with important community-driven efforts underway to make simulation-based data available and searchable (e.g., https://mddbr.eu/, [94–97]). It is important for the membrane community to be involved and benefit from these initiatives. Finally, visualization of such complex data is a challenge per se. Fortunately, methods for visualizing large systems are in continuous development [98], with one example being the recently introduced Molecular Nodes plug-in for Blender [99].

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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