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Computational modeling of membrane trafficking processes: From large molecular assemblies to chemical specificity

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Abstract

In the last decade, molecular dynamics (MD) simulations have become an essential tool to investigate the molecular properties of membrane trafficking processes, often in conjunction with experimental approaches. The combination of MD simulations with recent developments in structural biology, such as cryo-electron microscopy and artificial intelligence-based structure determination, opens new, exciting possibilities for future investigations. However, the full potential of MD simulations to provide a molecular view of the complex and dynamic processes involving membrane trafficking can only be realized if certain limitations are addressed, and especially those concerning the quality of coarse-grain models, which, despite recent successes in describing large-scale systems, still suffer from far-from-ideal chemical accuracy. In this review, we will highlight recent success stories of MD simulations in the investigation of membrane trafficking processes, their implications for future research, and the challenges that lie ahead in this specific research domain.

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Introduction

Membrane trafficking is critical for the proper functioning of cells, as it plays a vital role in cell homeostasis. Despite their importance, however, many of the molecular mechanisms underlying these processes are not fully understood and a better comprehension would be crucial to advance our basic understanding of cell biology.

In the last decade, thanks to the recent advancements in microscopy techniques, and especially cryo-electron microscopy (cryo-EM), the field is steadily making progresses in this direction [1–5]. However, cellular membranes are highly dynamic, owing to the fluid nature of lipid assemblies, and structural biology approaches often struggle to grasp the molecular details of such processes.

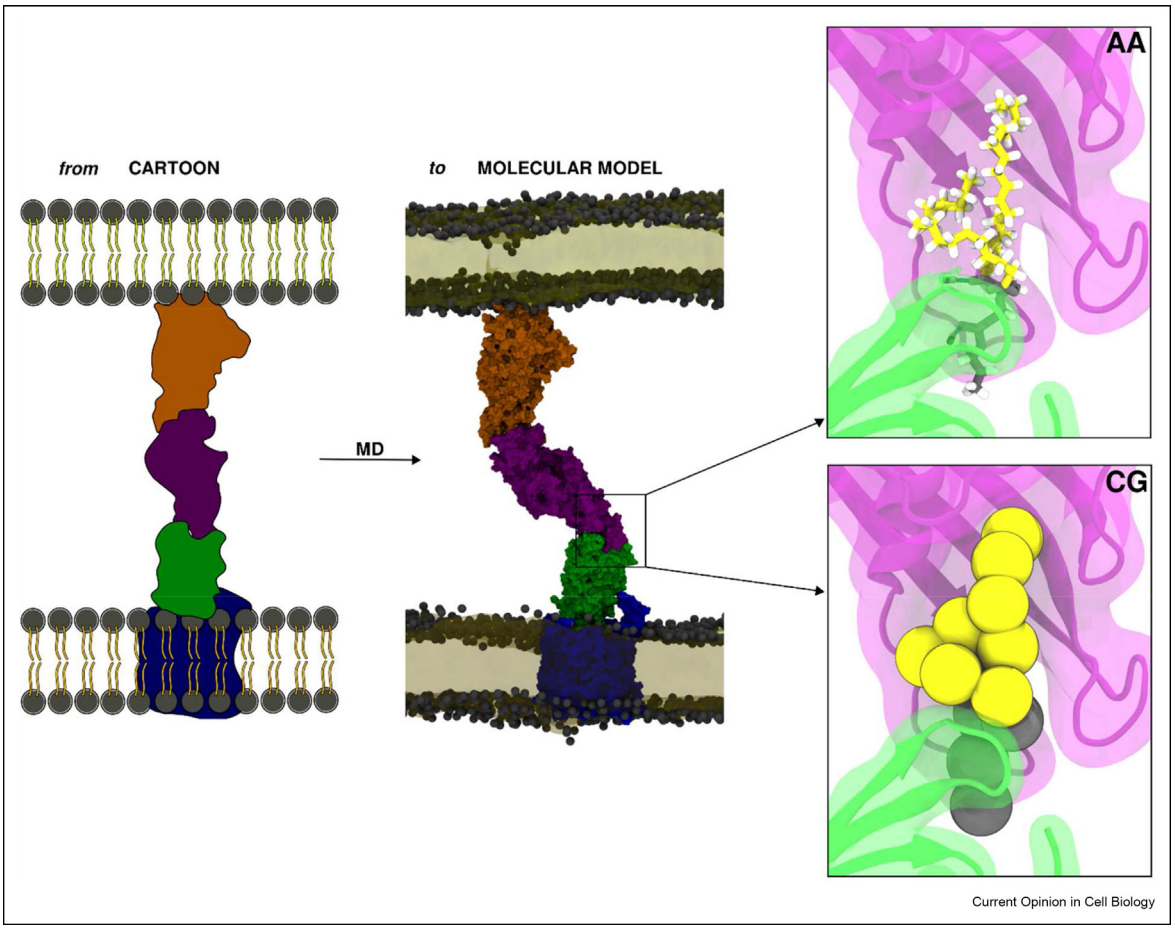
To overcome this limitation, computational methods, such as molecular dynamics (MD) simulations, have emerged as a powerful tool for studying the dynamics and mechanisms of multiple aspects of membrane trafficking, from large-scale membrane remodeling processes, such as those involved in vesicular trafficking, to local mechanisms that require extreme chemical detail and specificity, such as selective lipid binding to proteins. To this end, a careful integration of computer simulations with experimental approaches is becoming the state-of-the-art toward a better characterization of membrane trafficking processes at the molecular level.

In this review, we will provide our point of view on the current state-of-the-art of MD simulations in the study of these processes. We will highlight recent discoveries and advances in the field in a non-comprehensive way, and we will discuss the challenges and future directions for research in this area. We will argue that while the field has made tremendous progress toward a better understanding of large-scale membrane remodeling processes, it is the combination of large-scale simulations with models with improved chemical accuracy that will allow for the next step in our mechanistic understanding of membrane trafficking processes.

Method development leading the way

Biological molecules, such as proteins and lipids, are inherently multiatomic systems, and the three-dimensional organization of these atoms in space often determines both their dynamics and function (Figure 1, left panel). MD simulations are a physics-based

Figure 1



Computational models of protein-lipid systems at different resolutions. Left: MD simulations allow to describe the behavior of biological systems with atomistic or quasi-atomistic accuracy, allowing to generate experimentally testable hypotheses with finer granularity than simplistic cartoon-like models. Right: two different resolutions (AA: top; CG: bottom) for a model lipid inside a protein cavity are shown. MD, molecular dynamics.

computational approach that models the movement of atoms and molecules in a system. As such, it can be used to study the behavior of nanoscopic systems at the molecular level under well-controlled conditions (temperature, pressure, ionic strength) and can provide insights into the properties and dynamics of complex chemical and biological systems, including their structure, thermodynamic and kinetic behavior. In a sense, MD simulations can be considered as "*in silico* experiments", akin to *in vitro* reconstitutions, where the response of an artificial system to well-defined variations in environmental conditions can be investigated.

MD simulations in the biological realm have primarily focused on the investigation of protein dynamics [6]. To simulate membrane proteins, which constitute the largest class of drug targets [7], it soon became evident that a proper description of the surrounding lipidic environment was required. Once accurate models for lipids became available, and given the importance of lipid membranes in many biological contexts, this naturally led to the investigation of membrane properties *per se*.

However, two major challenges emerged, together with this shift in focus. First, the chemistry of lipid membranes is exceptionally rich and diverse, with thousands of lipid species, differing in their backbone, acyl chains, polar groups, and glycosylation, composing our cell membranes [8]. Second, the size scales at which membrane processes take place can be much larger than those of individual proteins, and the use of small-sized models for lipid bilayers might suppress important long-range properties. Hence, owing to these challenges and the frontier nature of this new research field, major advances in computational modeling of membrane trafficking processes have often been associated to key methodological developments, most notably pertaining the use of coarse-grain (CG) models for MD simulations of lipid membranes [9,10].

In CG simulations, the atomistic resolution is lost by grouping individual atoms into larger beads (Figure 1, right panel). This process results in a loss of accuracy for what pertains to chemical specificity, but the computational gain resulting from the transition to a CG representation allows simulating much larger systems than with fully atomistic simulations. Historically, continuous and sustained development of improved CG models has been largely driven by the goal of describing membrane-related processes [9–14]. Notably, while simplified CG models have been quite successful in the study of membrane-only systems [11], the study of membrane trafficking processes requires models that can also describe the interaction between lipid membranes and proteins. Development along these lines includes both models that are system-specific (also called minimal CG models) [15–17], and those that by basing their parameterization on the behavior of small molecules, aim at chemical transferability [13,18–21]. In the latter case, due to the loss of some intramolecular interactions, CG models for proteins need to include an elastic network to keep the protein secondary structure, leading to the inability of these models to appropriately describe extensive protein conformational changes.

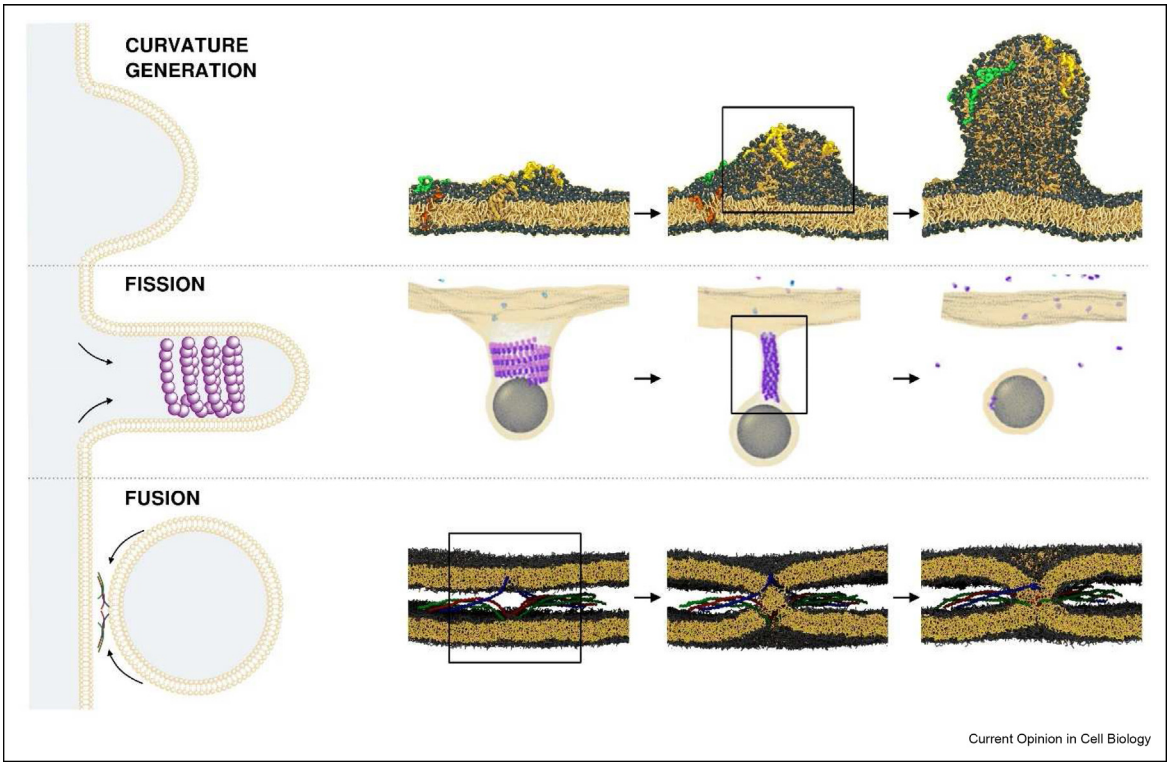
Hence, a key aspect of the ongoing developments in CG force-field modeling is to maintain their computational advantages while concomitantly increasing their chemical accuracy, that is the ability to correctly describe the underlying physicochemical behavior of different molecules.

Toward large scales: membrane remodeling processes

Far from being a passive actor, membranes rather play a major role in membrane trafficking processes. On the one hand, their chemical composition coordinates signaling via the specific and well-regulated assembly of protein complexes. On the other hand, their topological and mechanical properties control their remodeling by proteins, namely bending, fission, and fusion.

In the last decade, MD simulations have become an indispensable tool to unravel the variety of mechanisms in which proteins can generate or sense membrane

Figure 2



Membrane remodeling processes mediated by proteins using MD simulations. Top: Curvature generation, as the one induced by the RHD of RETR1/FAM134B [27,28], has been extensively investigated by MD simulations. Middle: Membrane fission, as the one promoted by the staged assembly and disassembly of copolymers in the ESCRT-III filaments [32,33]. Bottom: Membrane fusion, here catalyzed by the transmembrane domains of SNARE proteins that lower the energy barrier for stalk formation during the fusion process [34,35]. From top to bottom: Adapted from the study by Siggel et al. [27]. Adapted from the study by Jiang et al. [32]. Source data for the images provided by the authors [34]. MD, molecular dynamics; RHD, reticulon-homology domain.

curvature [22–30]. While extensive work has focused on the ability of proteins to generate curvature by scaffolding them [26], more recently, thanks to the use of multiresolution MD simulations, efforts have focused on the ability of transmembrane proteins, such as the reticulon-homology domain [27,28], to curve membranes (Figure 2, top panel). Other exciting areas are the use of multiscale schemes able to bridge different resolutions to simulate systems across different spatiotemporal scales, for example, to investigate budding by peripheral proteins [29], or the investigation of membrane bending by liquid droplets [30], an interesting model for biomolecular condensates [31].

The deformation of membranes into highly curved structures can lead to membrane fission, and the mechanism of this process has been the focus of many studies [32,33,36–41]. This activity is carried out by specific proteins, including Dynamin-like proteins, a family of GTPases with multiple domains that is responsible for the catalysis of membrane fission, or by the ESCRT-III machinery. Recently, studies using minimal CG models have unraveled the mechanism through which these proteins promote membrane fission. In detail, they have shown how the motion of a dynamin filament constricts and rotates the membrane acting as a torque, later disassembling to help achieve the complete fission [37], or how the polymeric motifs of ESCRT-III are assembled and disassembled in a sequential mechanism that is responsible of their membrane remodeling process (Figure 2, middle panel) [32,33].

The final step of vesicular trafficking, membrane fusion, has also been a subject of extensive research [34,35,42–45]. On this topic, the combination of CG simulations with methods to compute the free energy pathway for stalk formation has contributed to unravel the role of SNARE proteins in this process (Figure 2, bottom panel) [34,35], as well as the importance of the lipid composition [42]. Multiresolution MD simulations have also shed light on the activity of the influenza protein hemagglutinin during stalk formation and fusion pore opening in the context of viral infections [43].

Back to the molecular level: chemical specificity of protein-lipid interactions

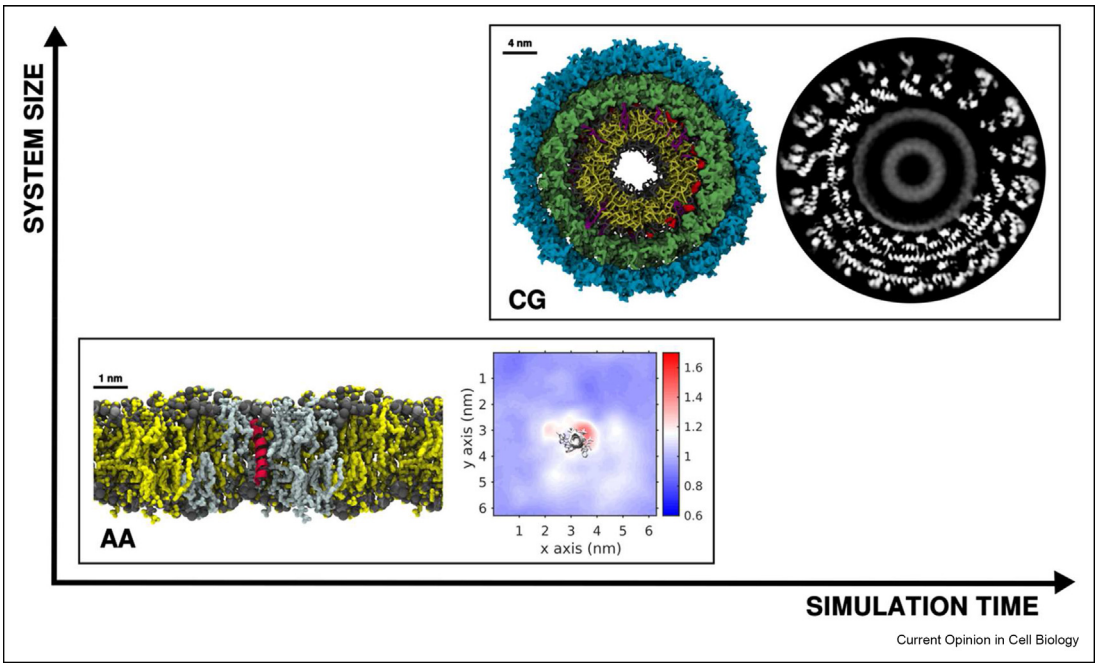
While many computational works have greatly helped improving our understanding of the mechanisms behind membrane remodeling, several other aspects of membrane trafficking processes remain poorly characterized. Most notably, many fine-tuned signaling aspects of membrane trafficking, from cargo selection to lipid recognition, have been largely overseen, possibly because of the lack of technical appeal in comparison with the large-scale simulations discussed above or because of the requirements of computationally expensive full atomistic approaches to describe the minutiae of the processes under investigation.

In particular, the accurate description and prediction of protein–protein interactions leading to cargo recognition and selection are probably still beyond the capabilities of MD simulations. However, recent artificial intelligence (AI) tools building on recent advances in protein modeling [46–50] hold major promise toward providing a starting structural (albeit not dynamical) view of the interactions between various proteins involved in membrane trafficking complexes. On the other hand, MD simulations, often in combination with progresses in mass spectrometry and microscopy techniques, have successfully investigated several aspects of protein–lipid interactions that play a major role in membrane trafficking [51].

One major example in the context of membrane traffic is the key role of lipid–protein interactions around p24/TMED2, a cargo adaptor that specifically recruits Glycosylphosphatidylinositol (GPI)-anchored proteins for their export and that cycles between the ER and the Golgi together with COPII and COPI components. Seminal work [52] combining experiments and atomistic MD simulations showed a direct and highly specific interaction of exclusively one sphingomyelin (SM) species, SM18, with the transmembrane (TM) domain of p24. More recently, using CG MD simulations in combination with super-resolution microscopy, Rodriguez-Gallardo et al. observed that in yeast both C18 and C26 ceramides accumulate around the cytosolic leaflet of the TM helix of p24. This observation suggests that the TM domain of p24 can cause an asymmetric distribution of lipids in the membrane, leading to lateral segregation of GPI-anchored proteins into discrete zones next to specific ER exit sites [53]. In human cells, a similar enrichment of ether lipids around p24 was observed using atomistic simulations (Figure 3). This observation provides an intriguing molecular explanation of the coordinate regulation of ether lipids with sphingolipids, suggesting that adaptation and functional compensation between the two lipid species, as shown by genome-wide Clustered Regularly Interspaced Short Palindromic Repeats interference (CRISPRi) screen, could be modulated by their preferential interaction with p24 proteins [54].

Parallel to membrane protein–lipid interactions, many modeling studies have also investigated the specific recognition of lipids by peripheral proteins in the context of membrane trafficking, an area that is strongly benefitting from recent progresses in CG force fields [55]. These studies mostly focused on phosphoinositides as these lipids function as signaling molecules in multiple trafficking pathways. A few recent examples

Figure 3



Insights into specific protein-lipid interactions from MD simulations. Left: Atomistic MD simulations predict an enrichment of ether lipids around the TM of p24/TMED2 (Transmembrane emp24 domain-containing protein 2) [54]. Right: CG simulations of a membrane tubule in the presence of a CHMP1B-IST1 copolymer predict an enrichment of anionic lipids (in purple) at contact sites as well as an accumulation of the highly unsaturated lipid tail of 1-stearoyl-2-docosahexaenoylphosphatidylcholine (SDPC) (in red), in good agreement with cryo-EM experiments using brominated lipid probes [60]. From left to right: Adapted from the study by Jimenez-Rojo et al. [54]. Adapted from ref. the study by Moss et al. [60]. cryo-EM, cryo-electron microscopy; CG, coarse-grain; MD, molecular dynamics.

include the interaction between phosphatidylinositol 4-phosphate 5-kinase (PIP5K) and model membranes [56], the molecular characterization of membrane binding by PH domains [57], the stabilization of the fusion stalk by Synaptotagmin C1 domains binding to PIP2 [58], or the identification of a novel PIP binding site for the tubby domain [59].

Notably, the emergence of molecular simulations as a reliable tool to describe complex membranes can also inform the development of novel experimental tools. In a recent example, the combination of a novel approach, based on cryo-EM with halogenated lipids and MD simulations, has allowed to describe with great accuracy the composition and structure of membranes (Figure 3) [60]. Therefore, using brominated lipids as contrast-enhancing probes for cryo-EM images of protein-coated membrane tubules, the authors could observe leaflet-level and protein-localized structural lipid patterns, with marked differences in comparison with protein-free, flat bilayers. Notably, they showed how protein—lipid interactions might lead to preferential localization of specific lipid classes at the sites of protein recruitment. These developments open exciting opportunities to investigate membranes at the structural level, including the dynamic molecular—scale interactions that govern their functions.

Modeling large scale processes with appropriate chemical accuracy: the challenging cases of lipid droplet (LD) biogenesis and lipid transport at membrane contact sites (MCSs)

While most molecular simulations studies on membrane trafficking have either focused on large membrane remodeling processes or specific chemical details, such as protein—lipid interactions, we suspect that the combination of the two approaches will propel the field even further, and in particular for systems that lack the geometrical periodicity that is required for the averaging process of structural methods (e.g. by cryo-EM). This is the case for two exciting and challenging areas of membrane trafficking: lipid storage by LDs and lipid trafficking at MCSs between intracellular organelles.

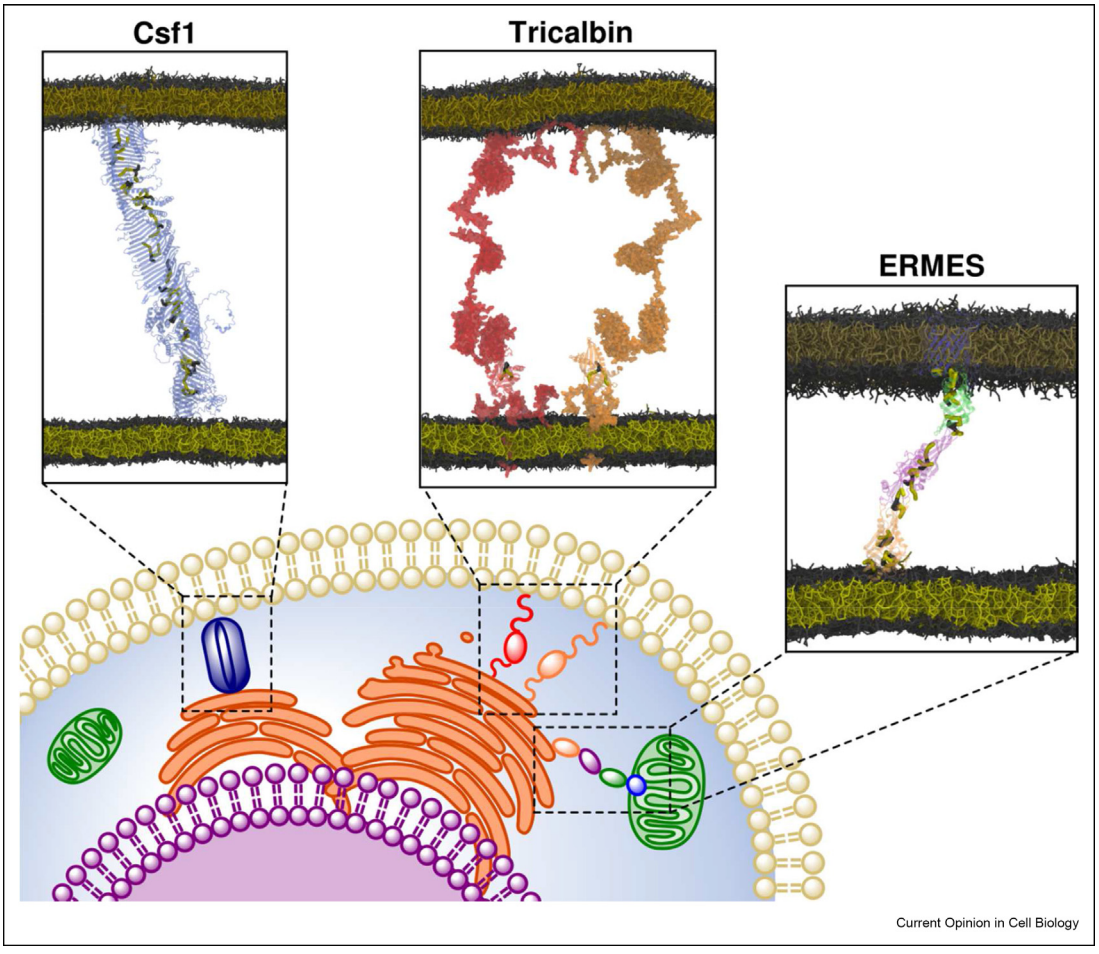
LDs are intracellular organelles consisting of a core of neutral lipids surrounded by a phospholipid monolayer. They emerge from the ER, thanks to the coordinated action of a complex protein machinery, in which the

protein seipin plays a key regulatory role [61]. Recently, MD simulations in combination with experimental approaches have significantly contributed to our current understanding of the process of LD biogenesis. In detail, MD simulations using chemical-specific CG models have identified seipin as the protein responsible for the initial stages of LD formation thanks to its ability to cluster and accumulate neutral lipids via specific interactions between polar moieties of these lipids and membrane embedded polar residues [62–64]. In addition, both chemical-specific CG models and minimal CG models have also highlighted the role of seipin and membrane properties in LD budding, a large-scale membrane remodeling process that takes place after initial LD biogenesis [65–67]. Overall, these works highlight how MD simulations have the potential to investigate multiple steps of complex biological processes, ranging from the identification of specific chemical interactions up to large-scale processes.

MCS are regions of the cell where the membranes of two distinct organelles come into close and stable contact (typically in the 10–40 nm range), thanks to the activity of tethering proteins. This organization allows for the spatial compartmentalization of specific processes, mainly lipid transport, and for the coupling of both lipid and protein content between subdomains of distinct organelles.

So far, a few studies combining simulations and experiments have contributed to our understanding of the mechanism of lipid transport by small lipid transport domains at MCS [69,70]. All of them, however, have focused exclusively on individual steps of the lipid

Figure 4



Lipid transport at membrane contact sites. Schematic representation, along with the corresponding computational model, of several LTPs at MCSs. Csf1 Bridge-Like Lipid Transfer Protein Family Member 1 (BLTP1 in humans) is a chorein motif LTP that contains a long hydrophobic channel connecting the Endoplasmic Reticulum (ER) to the Plasma Membrane (PM). Tricalbins (E-Syts in humans) possess multiple C2 domains as well as a synaptotagmin-like mitochondrial-lipid-binding protein (SMP) domain. They tether, and likely transport lipids between, the ER and the PM. The ERMES complex subunits assemble in a tunnel-like fashion between the ER and the mitochondria, as shown by a recent molecular model [68]. ERMES, ER-mitochondria encounter complex; MCS, membrane contact site.

transport mechanisms and we still lack a complete structural and dynamical modeling of entire MCS.

Because of their sizes, the investigation of entire MCS is best suited to CG approaches (Figure 4). However, to understand the fine regulation of exchange processes taking place at MCS, an adequate description of chemical interactions is paramount. In particular, the presence of intrinsically disordered domains in MCS tethering proteins [71] requires improvement in the parameterization of current CG models for proteins to correctly reproduce basic properties of disordered proteins [72–74]. In parallel, while low-resolution models of tethering complexes that are also involved in lipid transport start to emerge [4,68,71,75,76], the ability of CG models to correctly describe the lipid transport mechanism and specificity of such machineries will be subject to test.

A notable example in this direction is the recently proposed *in situ* model of the ER-mitochondria encounter complex (ERMES) [68]. There, using a combination of quantitative live-imaging, cryo-correlative microscopy, sub-tomogram averaging, and molecular modeling, the authors provide a molecular model of ERMES that is consistent with a continuous pathway for lipid transport between the two organelles. Together with the characterization of the structures of tunnel-like transport proteins of the Vps13 family [4], we foresee that continuous improvement in the accuracy of CG models will provide unprecedented insights into our mechanistic understanding of lipid fluxes at MCS and membrane trafficking processes.

Author contributions

Alvarez Lorenzo Daniel: Conceptualization, Writing – Original draft, Writing – Review & Editing, Visualization. **Sapia Jennifer:** Writing – Review & Editing, Visualization. **Vanni Stefano:** Conceptualization, Resources, Writing – Original draft, Writing – Review & Editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stefano Vanni reports financial support was provided by Swiss National Science Foundation. Stefano Vanni reports financial support was provided by European Research Council.

Data availability

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

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** of outstanding interest

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