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PERSPECTIVE

# Molecular dynamics simulations of intracellular lipid droplets: a new tool in the toolbox

Jennifer Sapia[1] and Stefano Vanni[1,2,3]

1 Department of Biology, University of Fribourg, Fribourg, Switzerland
2 Université Côte d'Azur, Inserm, CNRS, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne, France
3 Swiss National Center for Competence in Research (NCCR) Bio-inspired Materials, University of Fribourg, Fribourg, Switzerland

**Correspondence**
S. Vanni, Department of Biology, University of Fribourg, Chemin du Musée 10, Fribourg 1700, Switzerland
Tel: +41 26 300 88 96
E-mail: stefano.vanni@unifr.ch

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**Lipid droplets (LDs) are ubiquitous intracellular organelles with a central role in multiple lipid metabolic pathways. However, identifying correlations between their structural properties and their biological activity has proved challenging, owing to their unique physicochemical properties as compared with other cellular membranes. In recent years, molecular dynamics (MD) simulations, a computational methodology allowing the accurate description of molecular assemblies down to their individual components, have been demonstrated to be a useful and powerful approach for studying LD structural and dynamical properties. In this short review, we attempt to highlight, as comprehensively as possible, how MD simulations have contributed to our current understanding of multiple molecular mechanisms involved in LD biology.**

**Keywords:** computational biochemistry; fat storage; lipid droplets; membrane biology; molecular dynamics

Lipid droplets (LDs) are ubiquitous cellular structures that store fats in the form of neutral lipids (NLs). Because of this key function, they play vital roles in energy metabolism and lipid homeostasis, and they are a key hub for both physiological and pathological processes [1–3], including targeting by numerous pathogens [4,5], metabolic syndrome [6], diabetes [7], and many liver diseases [8–10]. Yet, despite their physiological importance, many mechanistic aspects of LD biology remain poorly understood.

From a physicochemical point of view, LDs are oil-in-water emulsions constituted by a core of NLs, mainly triglycerides (TG) and sterol esters (SE), encircled by a phospholipid monolayer [11]. LDs emerge from the endoplasmic reticulum (ER) membrane, where NLs originate from the enzymatic action of acyltransferases such as ACAT1/2 [12,13] and DGAT1/2 [14,15]. A diverse array of LD specific proteins decorates the lipid envelope surrounding the oil core, each performing distinct functions.

While "classical" biology and biochemistry experimental approaches remain the most frequent methodology to gain insights into LD biology, computational studies have slowly gained traction in recent years, yielding fresh perspectives. Notably, computational approaches, particularly molecular dynamics (MD) simulations, have emerged as a valuable tool for probing aspects of LDs that conventional wet-lab techniques may find challenging to address and that would have otherwise remained elusive.

MD simulations are a physics-based computational approach that models the movement of atoms and

**Abbreviations**
AA, all-atom; ACAT1/2, acyl-coenzyme A:cholesterol acyltransferases 1/2; AH, amphipathic helix; BSCL2, berardinelli-Seip congenital lipodystrophy type 2; CG, coarse-grain; CYTOLD, cytosol to lipid droplets; DG, diacylglycerol; DGAT1/2, diacylglycerol O-acyltransferases 1/2; ER, endoplasmic reticulum; ERTOLD, endoplasmic reticulum to lipid droplets; HH, hydrophobic helix; LDs, lipid droplets; LPDs, lipid packing defects; MD, molecular dynamics; NLs, neutral lipids; SE, sterol esters; TG, triglycerides; TM, transmembrane.

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, etc.) and can provide insights into the properties and dynamics of complex biological systems, including their structure, as well as 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. Hence, this technology is particularly well-suited, and complementary to experimental approaches, to describe structural and dynamical properties of LDs, including their interactions with proteins.

In this *Perspective* review, we will provide an overview of recent LD-related computational studies using MD simulations, with a specific focus on the intricate processes of LD formation and targeting by proteins. We will retrace advancements in the field, predominantly focusing on MD simulations at different scales, from all-atom (AA) to coarse-grain (CG) resolution. Starting from exploring the physicochemical properties of LD-like systems, we will provide our point of view on this emerging field, and we will highlight recent discoveries about LD proteins that have further enriched our understanding of this fascinating research area.

## MD simulations of lipid droplets: models and parameters

The conventional models employed to mimic LD-related processes using MD simulations involve two kinds of systems: (a) bilayer membranes enriched in NLs (Fig. 1), which represented the first attempt to study NL aggregation and the LD nucleation process [17–19], and (b) ternary trilayer systems, characterized by a central bulk phase of neutral lipids (mainly TG and SE) at different ratios, encased between two phospholipid monolayers (Fig. 1). The latter has emerged as the most used system to investigate the physical properties of the monolayer constituting the outer envelope of LDs [20–24], even if other models have also been used in the past [25,26].

Simulations of model LDs are not as straightforward as those of "classical" lipid bilayers, for at least three main reasons. First, while bilayers are approximately 4–5-nm thick, MD models of LD-like trilayers need to be, in principle, sufficiently thick to reach bona fide bulk properties (i.e., not affected by the phospholipid monolayer) in their core. This implies that one box dimension in trilayer simulations must be several times bigger than in lipid bilayer simulations, causing a significant increase in the associated computational cost.

Second, MD simulations of lipid bilayers are generally carried out at near-zero bilayer tension for at least two reasons: (a) to mimic the coupling of the system under study to an infinite reservoir of phospholipids and (b) based on theoretical considerations of thermodynamic equilibrium [27]. On the other hand, experimental measurements of the surface tension of the LD monolayer, both *ex vivo* [18] and *in vitro* [28], suggest that the LD monolayer could be under non-negligible tension, generally in the range 1–5 $mN{\cdot}m^{-1}$. Further, the presence of NL in lipid bilayers also influences bilayer tension [18], effectively making both bilayer and monolayer tensions two additional adjustable parameters to consider when performing MD simulations of model LDs.

Third, conflicting observations (see the next section) reported using MD simulations by various labs have led to the realization that the field lacks a well-tested set of force-field parameters capable of faithfully describing the behavior of NL molecules in all environments. To address this issue, specific parameters for NLs have been developed in the last few years. These include AA parameters for TG [29,30] and SE molecules [23] compatible with CHARMM36 [31]; CG parameters compatible with the SDK/SPICA force field [32] for TG, SE, and TG-precursor diacylglycerol (DG) [21,22,33]; an implicit-solvent CG model for TG and phospholipids [30]; a still unpublished model for TG compatible with MARTINI 3 [34] that has been recently used to investigate properties of LD growth [35]; and an ultra-coarse-grain model consisting of only four CG beads per lipid (phospholipids and TG) to study TG nucleation [36]. A list of available parameters has been recently highlighted in a technical review on the subject [37], and, while a general consensus has not been yet reached, it is our opinion that these developments are dramatically helping the field move forward by identifying potential pitfalls and by iteratively improving the quality of the computational results. Hence, we foresee that future force field developments, likely in combination with experimental efforts specifically tailored to address this issue, will continue to further improve the reliability of MD simulations to describe LD properties.

## MD simulations of protein-free LD-like systems

The development of parameters specifically tailored for NLs has increased the toolkit available for the

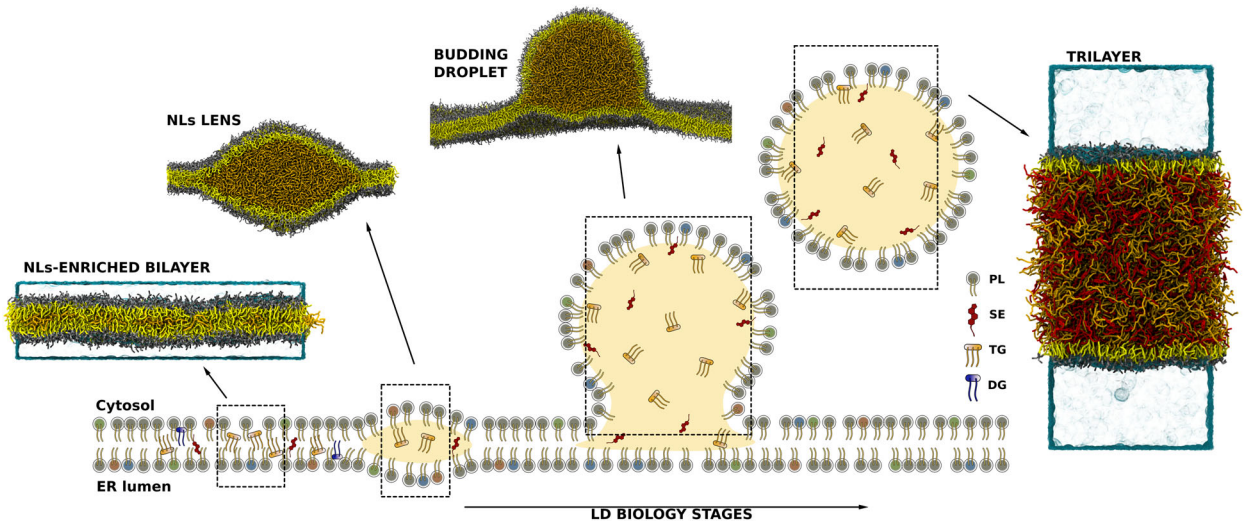


**Fig. 1.** LD-like models used in MD simulations. Representative snapshots from MD simulations highlighting different *in silico* models used to study LD biology. From left to right: NLs-enriched lipid bilayer, with NLs encased between the two bilayer leaflets; nascent NLs lens (adapted from [16]); budding NL droplet (adapted from [16]); trilayer system with bulk NLs shielded by water by two lipid monolayers. MD snapshots color code: PL (phospholipid) tails, yellow; PL head groups, gray; TG, orange; SE, red; DG, violet.

investigation of LD biology at the molecular level, both in the presence and absence of proteins. Specifically, two main processes have been extensively studied: (a) LD biogenesis and growth, and (b) protein targeting to the LD surface. Despite the important role played by proteins in these two processes, MD simulations in protein-free systems have also contributed to our understanding of several mechanistic aspects of these phenomena.

Concerning LD biogenesis, it is well established that LD formation takes place in the ER membrane (Fig. 1), and, based on the physicochemical properties of NL molecules, it had been proposed in experimental studies that diluted NL molecules in the ER membrane could undergo a spontaneous demixing within the phospholipid bilayer upon reaching a critical concentration [1,38–41]. CG-MD simulations have indeed been used to thoroughly characterize this process, and to understand its underlying molecular determinants. Specifically, they have been instrumental not only to show that NLs can form distinctive disk-shaped lenses (Fig. 1) that constitute the condensed phase in a process named nucleation [17–19,41] but also to determine how membrane properties can modulate their formation. Specifically, MD simulations have revealed how the dynamic process of LD formation, driven by the chemical potential of TG molecules, is intimately linked to the physicochemical attributes of the surrounding membrane. Those include membrane tension, which inhibits the channeling of NLs from the phospholipid bilayer to the LD nucleation site, resulting in the accumulation of TG molecules inside the lipid bilayer [18], membrane rigidity [36], and lipid composition [18,19], with DG, cholesterol, and phospholipids with conical shapes promoting the phase separation process by lowering the threshold of TG nucleation [19].

Following the nucleation phase, LDs expand by sequestering NLs from the ER. Upon reaching a certain dimension, LDs can eventually undergo budding toward the cytosol (Fig. 1). This stage is subject to various influences that drive the directionality of budding. In combination with experimental studies, MD simulations have contributed to our understanding that two important parameters in this respect are (a) surface tension and (b) membrane asymmetry. Indeed, MD simulations have proven instrumental in showing that surface tension is a key property driving the evolution from flat oil lenses during the nascent stage to spherical-shaped LDs during growth and budding [18,35,36]. Additionally, MD simulations have been used to show that leaflet imbalance, arising from an excess of phospholipids in one leaflet or the presence of embedded proteins, ensures a more extensive coverage of the oil phase on the cytoplasmic side, thereby prompting budding in that specific direction [35,36,42].

Computational approaches were not solely employed for deciphering the complexities of the LD biogenesis process. Simulations of LD-like ternary systems, initially carried out at the atomistic level using the Berger

lipid force field [43,44], have proven to be exceptionally well-suited to investigate LD structure and specifically the interactions between NLs and the phospholipid monolayer [20,22]. This highlighted how, within LDs, TG molecules can undergo extensive interdigitation with phospholipids [22]. This phenomenon minimizes the contacts between hydrophobic and hydrophilic moieties and, furthermore, can promote the formation of membrane defects, which have been proposed to be crucial for protein targeting on LDs [22].

Later studies using the CHARMM36 force field [24,45], following up on this idea, have confirmed these observations but have also, quite surprisingly, found a large enrichment (5–8%) of TG molecules on the monolayer surface and 10 times more water in the LD core than experimentally reported, even at zero surface tension [24]. Recent works have shown that these contrasting findings likely originate from the treatment of partial charges on the glycerol moiety of TG molecules [29,30]. Specifically, the original CHARMM36 parameters for TG molecules have partial charges on their glycerol moiety that are identical to those of phospholipids, leading to MD simulations of TG molecules that have an interfacial tension of 17.3 $mN{\cdot}m^{-1}$ [29], rather than 29–32 $mN{\cdot}m^{-1}$ as measured experimentally [46,47]. This observation has two interesting consequences. First, the TG molecules in the original CHARMM36 description are more hydrophilic than the experimental data suggest. Second, because their interfacial tension is compatible to that experimentally measured for diacylglycerol (DG) (17.2 $mN{\cdot}m^{-1}$) [48], their behavior in the simulations is unsurprisingly extremely similar to that of DG, which has a reported experimental solubility in lipid bilayer of around 15–20% [29,49–52]. This would correspond to a monolayer solubility of 7–10% in line with what observed in the simulations [24]. Interestingly, DG is also a biologically-active lipid intermediate in the TG synthesis pathway [53] and various reports describe DG-dependent binding of proteins on LDs [54,55].

Following these conflicting observations, efforts to improve the parameters by reducing the partial charges on the glycerol moiety according to quantum-mechanical data and physicochemical experiments [29,30] have resulted in MD simulations of trilayers that correctly reproduce the interfacial tension between TG and water using modified CHARMM36 parameters. However, these new models remain unable to reproduce the depletion of phospholipids from the LD surface observed in one specific *in vitro* reconstitution model [56]. We note, however, that this depletion is observed also in the presence of other oils (squalene, dodecane) [56], and this discrepancy is thus unlikely to originate from the description of TG–water interactions in the model. Rather, its origin has been suggested to arise from too weak TG–phospholipid interactions in the force field, also according to a continuum model description of the system [57]. This suggests that further force field developments to accurately describe LD properties might extend beyond TG molecules, and also encompass phospholipids, to further improve the agreement between MD simulations and experimental measurements in reconstituted systems.

More recently, MD simulations have also demonstrated how the presence of SE molecules influences the oil core architecture [21,23]. Specifically, MD simulations have shown that, at high concentrations, SE can spontaneously demix within the LD core [21] potentially seeding toward the formation of a SE-rich crystalline lattice just below the phospholipid monolayer. This physico-chemical phenomenon confers to the droplet a characteristic "onion-like" configuration where concentric SE layers surround an amorphous TG nucleus, that is also experimentally observed indirectly using polarization microscopy or directly using cryo-electron tomography [21,58].

## LD-specific protein targeting

In addition to the mechanical and physicochemical properties of the lipidic components, LD-resident proteins also play major roles in LD biology. The LD proteome consists of a diverse array of proteins and enzymes, mostly identified using proteomics approaches [59–62], each with distinct functions in lipid metabolism and promoting interactions of LDs with other organelles.

Interestingly, it appears that LDs lack a dedicated protein machinery responsible for the direct incorporation of proteins onto their surfaces [63]. Rather, the current paradigm posits that protein binding to LDs is essentially governed by the unique properties of the LD surface monolayer arrangement or by interactions with protein already residing on the LD surface. It is thus not surprising that physicochemical properties of the LD surface, such as interfacial tension, have been suggested as main contributors not only in LD formation and budding [18] but also in protein targeting [22,64].

In this context, LD-associated proteins have been conceptually divided into two main classes (reviewed by Olarte *et al.* [63]), depending on their localization in the absence of intracellular LDs and their likely targeting mechanism: CYTOLD proteins (also known as

Class II proteins), which stands for "Cytoplasm to LD," and ERTOLD proteins (also known as Class I proteins), or "Endoplasmic Reticulum to LD" [63] (Fig. 2A). The CYTOLD pathway refers to the mechanisms by which proteins are directed from the cytoplasm of a cell to the LD surface. These proteins often contain targeting signals or motifs, like amphipathic helices (AH) [3,63]. In the ERTOLD pathway, on the other hand, proteins target LDs via diffusion from the ER membrane and interact with the LD surface likely through helical hairpin motifs (helix-turn-helix). This transition is supposed to be mediated by the presence of ER-LD bridges that physically keep the connection between the organelles [66], and that could also modulate lipid diffusion [67].

How proteins belonging to both classes specifically partition to the LD surface has not only been examined through experimental methods, which typically aim to investigate how proteins behave in cells using fluorescence microscopy [68–71] or in reconstituted mixed bilayer/monolayer systems [65,72] but also with computational approaches. MD simulations have been pivotal in understanding how the unique surface properties of LDs can influence specific protein targeting, providing valuable insights into the molecular properties of the monolayer. Specifically, MD simulations have characterized how the presence of a lipid core affects the surface area per phospholipid and the persistence of lipid-packing defects (LPDs) [22], thus imparting a unique membrane packing to LDs compared to phospholipid bilayers (Fig. 2B). These "voids," that in lipid bilayers are generated by the exposure of the hydrocarbon atoms belonging to the lipid acyl chains to the surface [22,73], affect LD surface properties [22] and, consequently, protein targeting [45,72,74–76]. To this end, all-atom simulations have suggested that LPDs are possibly more persistent in LDs, compared with phospholipid bilayers [72,75], and, as such, can constitute specific binding sites for the preferential targeting of proteins [24,72].

Rather than being static, however, LD surface properties are likely to be dynamically modulated during different stages of lipid metabolism: LD surface expansion, possibly during LD growth, increases LD surface tension and, consequently LPDs [22], allowing new proteins to bind to the LD surface; in contrast, LD shrinkage in the late stages increases lipid packing and causes the displacement of weakly bound proteins [77]. This concerted mechanism provides a scenario where the protein composition of LDs is modulated by biophysical properties of the LD membrane.

In addition, MD simulations have provided a dynamical perspective on LD targeting. For instance, CYTOLD proteins have been proposed to bind to the monolayer following a multi-stage pathway: from their initial unfolded configuration in solution, proteins can fold upon anchoring to LPDs thanks to hydrophobic residues [72]. Indeed, specific sequence "signatures," in particular bulky hydrophobic residues, have been demonstrated by MD simulations to be crucial for specific binding to LDs [45,72,76].

Finally, the targeting of model peptides was also studied using MD, in an attempt to investigate the LD-targeting behavior of integral membrane proteins of the ERTOLD pathway [65,78]. When incorporated and randomly distributed in a phospholipid bilayer, these peptides, enriched in hydrophobic residues, showed a spontaneous tendency to diffuse toward the monolayer surface, indicating a lower free energy of the system [65,78]. All atom simulations were also used to identify specific sequence features of hydrophobic membrane motifs that mediate LD targeting,

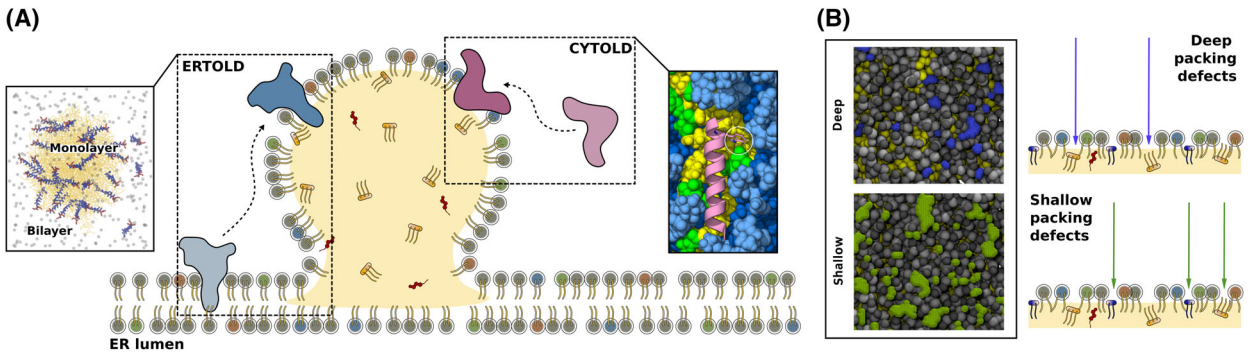


**Fig. 2.** LD-targeting by proteins. (A) Conceptual scheme of LD-targeting pathways by protein, and representative examples of MD simulations to study both ERTOLD (left; image adapted from [65]) and CYTOLD (right; snapshot adapted with permission from [45]. Copyright 2021 American Chemical Society) pathways. (B) MD snapshots and representative scheme of deep (blue, top) and shallow (green, bottom) lipid packing defects in MD simulations of model LDs.

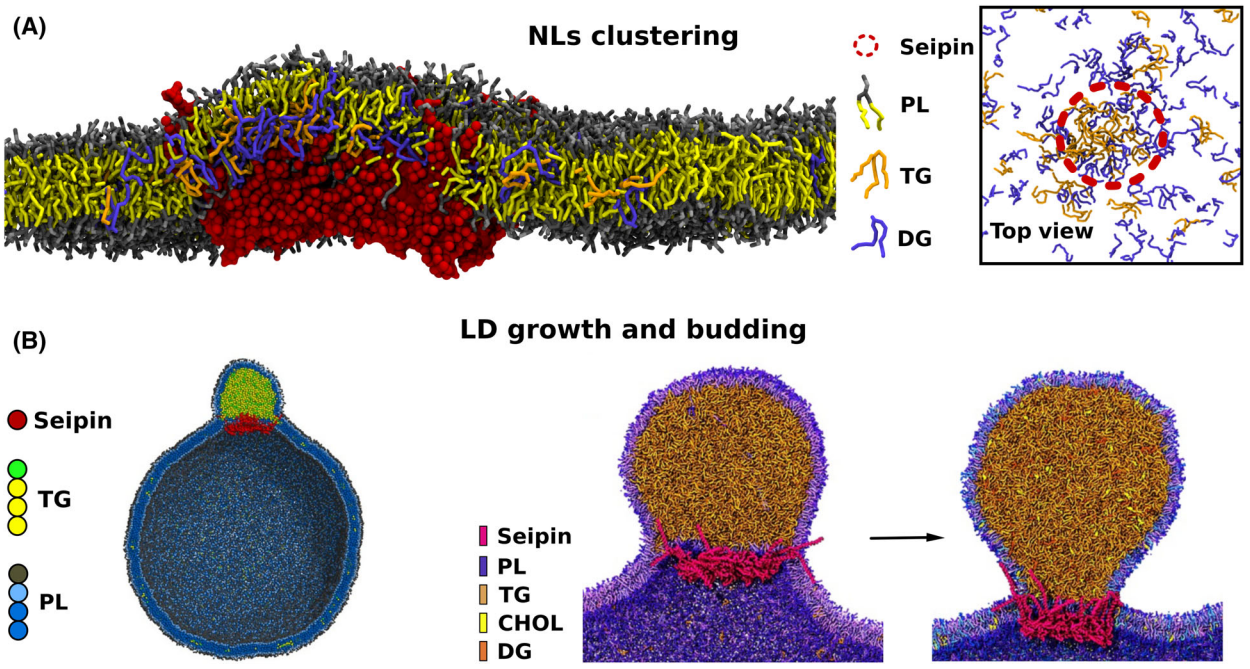


**Fig. 3.** The role of seipin in the early stages of LD biogenesis. (A) MD snapshots showing the ability of seipin (red) to cluster NLs. (B) MD simulations highlighting the role of seipin in LD growth and budding. Representative snapshots adapted from [95] (left) and [35] (right).

identifying a prominent role for deeply inserted tryptophan residues that promote subtle conformational changes of the protein structure in the bilayer versus a LD-like environment [78]. We caution, however, that all protein–LD interactions described using all-atom MD so far in this context, both for CYTOLD and ERTOLD proteins [45,72,76,78], have been carried out using the old, more hydrophilic, CHARMM parameters for TG molecules. Future studies, possibly in combination with experimental methods that probe protein–membrane interactions with high resolution, will certainly help shed light on this fascinating molecular mechanism.

## LD activity of ER integral membrane proteins

It is important to note that it is not only proteins that directly target the LD surface that contribute in a major way to the LD biogenesis process. An array of other proteins and enzymes, primarily situated within the ER, also occupy a central position in regulating the various facets of LD formation and dynamics. Among these, seipin represents a prominent example, as its localization at the ER membrane defines LD formation sites that are characterized by a single seipin oligomer associated with each emerging LD [79,80]. At the molecular level, seipin homo-oligomerizes, forming a ring-like structure constituted by 10–12 subunits, depending on species, with each monomer featuring two transmembrane (TM) segments encasing a highly conserved luminal domain folded as an 8-stranded β-sandwich [81,82] along with a hydrophobic helix (HH) [83,84].

However, seipin is not just a structural scaffold at ER-LD contact sites [85–88], as it has been suggested to play multiple functions. These include, among others, (a) the regulation of phospholipid metabolism and lipid distribution in the ER [83,89,90]; (b) control of adipogenesis, as mutations in its sequence can lead to a congenital disorder known as Berardinelli-Seip congenital lipodystrophy type 2 (BSCL2) [91,92], and (c) modulation of the early stages of LD formation, as seipin-deficient cells result in TG accumulation in the ER and delayed formation of aberrant LDs, which are smaller, supersized, and sometimes clustered [85,93,94].

Recent computational studies have made significant contributions to our understanding of seipin-mediated LD formation, offering molecular insights beyond the capabilities of current experimental methods. *In silico* work employing MD simulations at CG level of detail has demonstrated the prominent role of seipin in clustering NLs at the LD formation site (Fig. 3A). Indeed, seipin can trap and concentrate NL molecules, as well as TG precursor DG, in both luminal and TM regions, thanks to specific interactions between the polar

glycerol moieties of TG and key polar residues [41,81,96–98]. In detail, a pair of serine residues in the HH of the luminal domain acts as a binding site for membrane-embedded NLs, and their mutation into more hydrophobic residues results in delayed LD formation [97].

Ultra-CG MD simulations have further emphasized the importance of seipin in facilitating TG nucleation: despite the low resolution of this model, it has been possible to prove that key regions, such as the TM domains, contribute to the generation of a unique ER-LD neck structure, and they have provided initial molecular information concerning the early stages of LD growth and budding [95] (Fig. 3B). Along this line of research, a recent study taking advantage of a grand-canonical simulation scheme, in which TG and phospholipids are computationally "synthesized" at specific locations, was able to reproduce various stages of LD formation and maturation using the MARTINI CG force field. In the presence of seipin, nascent LDs formed within the seipin ring, which subsequently grow and expand towards the cytosolic side [35] (Fig. 3B).

## Conclusions and outlook

In the last 5–6 years, MD simulations have emerged as a complementary approach to investigate the biology of LDs. Thanks to improved computational models of neutral lipids, this methodology has contributed to advancing our understanding of the unique and fascinating molecular properties of LDs. When used appropriately, often in combination with experimental studies, the findings from MD simulations have translated into the discovery of important LD-related physiological mechanisms.

Yet, these pioneering studies only represent the tip of the iceberg, as several outstanding challenges and opportunities remain. In our opinion, three main subjects are primed for important contributions from MD simulations in the next few years. First, a detailed understanding of the exact correlation between the complex lipid composition of LDs and their molecular properties in different metabolic states or upon different genetic or environmental perturbations remains limited. In this context, the molecular properties of their neighboring ER membrane (lipid composition, membrane tension, membrane curvature) are also likely to play a major role in LD formation, budding, and growth that remains mostly unexplored. We expect that future MD studies will highlight in greater detail how all these properties affect LD biology and how this plays a role in physiological contexts.

Second, we still lack a convincing characterization of proteins at the LD surface. While MD simulations are a powerful methodology to characterize protein–membrane interactions, both AA and CG simulations have limitations, especially regarding conformational changes that might occur specifically at the LD surface. We expect that a combination of MD studies with experimental approaches (e.g., using spectroscopy approaches on chemically-labeled protein residues) will be required to further clarify the molecular details of protein binding to LDs.

Finally, the availability of recent experimental structures of enzymes in the neutral lipid synthesis pathway [12–15,99,100], as well as structural models of LD proteins from AI-based tools such as AlphaFold and Rosetta [101–105], constitutes an incredible resource to investigate LD biology in molecular terms. We foresee that the combination of this wealth of structural information with accurate lipid models mimicking mature or nascent LDs using MD simulations will further affirm the ever-increasing role of molecular simulations in this research field.

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## Author contributions

JS and SV wrote this manuscript.

## References

1 Walther TC and Farese RV (2012) Lipid droplets and cellular lipid metabolism. *Annu Rev Biochem* **81**, 687–714.
2 Walther TC, Chung J and Farese RV (2017) Lipid Droplet Biogenesis. *Annu Rev Cell Dev Biol* **33**, 491–510.
3 Olzmann JA and Carvalho P (2019) Dynamics and functions of lipid droplets. *Nat Rev Mol Cell Biol* **20**, 137–155.
4 Herker E and Ott M (2012) Emerging role of lipid droplets in host/pathogen interactions. *J Biol Chem* **287**, 2280–2287.

5 Bosch M, Sweet MJ, Parton RG and Pol A (2021) Lipid droplets and the host–pathogen dynamic: fatal attraction? *J Cell Biol* **220**, e202104005.
6 Xu S, Zhang X and Liu P (2018) Lipid droplet proteins and metabolic diseases. *Biochim Biophys Acta Mol basis Dis* **1864**, 1968–1983.
7 Tong X, Liu S, Stein R and Imai Y (2022) Lipid droplets' role in the regulation of β-cell function and β-cell demise in type 2 diabetes. *Endocrinology* **163**, bqac007.
8 Gluchowski NL, Becuwe M, Walther TC and Farese RV (2017) Lipid droplets and liver disease: from basic biology to clinical implications. *Nat Rev Gastroenterol Hepatol* **14**, 343–355.
9 Mashek DG (2021) Hepatic lipid droplets: a balancing act between energy storage and metabolic dysfunction in NAFLD. *Mol Metab* **50**, 101115.
10 Seebacher F, Zeigerer A, Kory N and Krahmer N (2020) Hepatic lipid droplet homeostasis and fatty liver disease. *Semin Cell Dev Biol* **108**, 72–81.
11 Thiam AR, Farese RV and Walther TC (2013) The biophysics and cell biology of lipid droplets. *Nat Rev Mol Cell Biol* **14**, 775–786.
12 Qian H, Zhao X, Yan R, Yao X, Gao S, Sun X, du X, Yang H, Wong CCL and Yan N (2020) Structural basis for catalysis and substrate specificity of human ACAT1. *Nature* **581**, 333–338.
13 Guan C, Niu Y, Chen SC, Kang Y, Wu JX, Nishi K, Chang CCY, Chang TY, Luo T and Chen L (2020) Structural insights into the inhibition mechanism of human sterol O-acyltransferase 1 by a competitive inhibitor. *Nat Commun* **11**, 2478.
14 Sui X, Wang K, Gluchowski NL, Elliott SD, Liao M, Walther TC and Farese RV Jr (2020) Structure and catalytic mechanism of a human triacylglycerol-synthesis enzyme. *Nature* **581**, 323–328.
15 Wang L, Qian H, Nian Y, Han Y, Ren Z, Zhang H, Hu L, Prasad BVV, Laganowsky A, Yan N *et al*. (2020) Structure and mechanism of human diacylglycerol O-acyltransferase 1. *Nature* **581**, 329–332.
16 Zoni V, Nieto V, Endter LJ, Risselada HJ, Monticelli L and Vanni S (2019) To bud or not to bud: a perspective on molecular simulations of lipid droplet budding. *Front Mol Biosci* **6**, 124.
17 Khandelia H, Duelund L, Pakkanen KI and Ipsen JH (2010) Triglyceride blisters in lipid bilayers: implications for lipid droplet biogenesis and the mobile lipid signal in cancer cell membranes. *PLoS One* **5**, 1–8.
18 Ben M'barek K, Ajjaji D, Chorlay A, Vanni S, Forêt L and Thiam AR (2017) ER membrane phospholipids and surface tension control cellular lipid droplet formation. *Dev Cell* **41**, 591–604.e7.
19 Zoni V, Khaddaj R, Campomanes P, Thiam AR, Schneiter R and Vanni S (2021) Pre-existing bilayer stresses modulate triglyceride accumulation in the er versus lipid droplets. *elife* **10**, 1–24.
20 Ollila OHS, Lamberg A, Lehtivaara M, Koivuniemi A and Vattulainen I (2012) Interfacial tension and surface pressure of high density lipoprotein, low density lipoprotein, and related lipid droplets. *Biophys J* **103**, 1236–1244.
21 Rogers S, Gui L, Kovalenko A, Zoni V, Carpentier M, Ramji K, Ben Mbarek K, Bacle A, Fuchs P, Campomanes P *et al*. (2022) Triglyceride lipolysis triggers liquid crystalline phases in lipid droplets and alters the LD proteome. *J Cell Biol* **221**, e202205053.
22 Bacle A, Gautier R, Jackson CL, Fuchs PFJ and Vanni S (2017) Interdigitation between triglycerides and lipids modulates surface properties of lipid droplets. *Biophys J* **112**, 1417–1430.
23 Braun RJ and Swanson JMJ (2022) Capturing the liquid-crystalline phase transformation: implications for protein targeting to sterol ester-rich lipid droplets. *Membranes (Basel)* **12**, 949.
24 Kim S and Swanson JMJ (2020) The surface and hydration properties of lipid droplets. *Biophys J* **119**, 1958–1969.
25 Chaban VV and Khandelia H (2014) Lipid structure in triolein lipid droplets. *J Phys Chem B* **118**, 10335–10340.
26 Chaban VV and Khandelia H (2014) Distribution of neutral lipids in the lipid droplet core. *J Phys Chem B* **118**, 11145–11151.
27 Jähnig F (1996) What is the surface tension of a lipid bilayer membrane? *Biophys J* **71**, 1348–1349.
28 Santinho A, Chorlay A, Foret L and Thiam AR (2021) Fat inclusions strongly alter membrane mechanics. *Biophys J* **120**, 607–617.
29 Campomanes P, Prabhu J, Zoni V and Vanni S (2021) Recharging your fats: CHARMM36 parameters for neutral lipids triacylglycerol and diacylglycerol. *Biophys Rep* **1**, 100034.
30 Kim S and Voth GA (2021) Physical characterization of Triolein and implications for its role in lipid droplet biogenesis. *J Phys Chem B* **125**, 6874–6888.
31 Klauda JB, Venable RM, Freites JA, O'Connor JW, Tobias DJ, Mondragon-Ramirez C, Vorobyov I, MacKerell AD Jr and Pastor RW (2010) Update of the CHARMM all-atom additive force field for lipids: validation on six lipid types. *J Phys Chem B* **114**, 7830–7843.
32 Shinoda W, Devane R and Klein ML (2007) Multi-property fitting and parameterization of a coarse grained model for aqueous surfactants. *Mol Simul* **33**, 27–36.
33 Campomanes P, Zoni V and Vanni S (2019) Local accumulation of diacylglycerol alters membrane properties nonlinearly due to its transbilayer activity. *Commun Chem* **2**, 72.

34 Souza PCT, Alessandri R, Barnoud J, Thallmair S, Faustino I, Grünewald F, Patmanidis I, Abdizadeh H, Bruininks BMH, Wassenaar TA *et al.* (2021) Martini 3: a general purpose force field for coarse-grained molecular dynamics. *Nat Methods* **18**, 382–388.
35 Nieto V, Crowley J, Santos D and Monticelli L Birth of an organelle: molecular mechanism of lipid droplet biogenesis. *bioRxiv* 10.1101/2023.07.28.550987 [PREPRINT]
36 Kim S, Li C, Farese RV, Walther TC and Voth GA (2022) Key factors governing initial stages of lipid droplet formation. *J Phys Chem B* **126**, 453–462.
37 Kim S, Swanson JMJ and Voth GA (2022) Computational studies of lipid droplets. *J Phys Chem B* **126**, 2145–2154.
38 Choudhary V, Ojha N, Golden A and Prinz WA (2015) A conserved family of proteins facilitates nascent lipid droplet budding from the ER. *J Cell Biol* **211**, 261–271.
39 Thiam AR and Forêt L (2016) The physics of lipid droplet nucleation, growth and budding. *Biochim Biophys Acta Mol Cell Biol Lipids* **1861**, 715–722.
40 Molenaar MR, Yadav KK, Toulmay A, Wassenaar TA, Mari MC, Caillon L, Chorlay A, Lukmantara IE, Haaker MW, Wubbolts RW *et al.* (2021) Retinyl esters form lipid droplets independently of triacylglycerol and seipin. *J Cell Biol* **220**, e202011071.
41 Dumesnil C, Vanharanta L, Prasanna X, Omrane M, Carpentier M, Bhapkar A, Enkavi G, Salo VT, Vattulainen I, Ikonen E *et al.* (2023) Cholesterol esters form supercooled lipid droplets whose nucleation is facilitated by triacylglycerols. *Nat Commun* **14**, 915.
42 Chorlay A, Monticelli L, Veríssimo Ferreira J, Ben M'barek K, Ajjaji D, Wang S, Johnson E, Beck R, Omrane M, Beller M *et al.* (2019) Membrane asymmetry imposes directionality on lipid droplet emergence from the ER. *Dev Cell* **50**, 25–42.e7.
43 Berger O, Edholm O and Jähnig F (1997) Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature. *Biophys J* **72**, 2002–2013.
44 Hall A, Repakova J and Vattulainen I (2008) Modeling of the triglyceride-rich core in lipoprotein particles. *J Phys Chem B* **112**, 13772–13782.
45 Kim S, Oh MI and Swanson JMJ (2021) Stressed lipid droplets: how neutral lipids relieve surface tension and membrane expansion drives protein association. *J Phys Chem B* **125**, 5572–5586.
46 Couallier E, Riaublanc A, David Briand E and Rousseau B (2018) Molecular simulation of the water-triolein-oleic acid mixture: local structure and thermodynamic properties. *J Chem Phys* **148**, 184702.
47 Mitsche MA, Wang L and Small DM (2010) Adsorption of egg phosphatidylcholine to an air/water and triolein/water bubble interface: use of the 2-dimensional phase rule to estimate the surface composition of a phospholipid/triolein/water surface as a function of surface pressure. *J Phys Chem B* **114**, 3276–3284.
48 Nakajima Y (2004) Water-retaining ability of diacylglycerol. *J Amer Oil Chem Soc* **81**, 907–912.
49 Carrasco S and Mérida I (2007) Diacylglycerol, when simplicity becomes complex. *Trends Biochem Sci* **32**, 27–36.
50 Gómez-Fernández JC and Corbalán-García S (2007) Diacylglycerols, multivalent membrane modulators. *Chem Phys Lipids* **148**, 1–25.
51 Furt F and Moreau P (2009) Importance of lipid metabolism for intracellular and mitochondrial membrane fusion/fission processes. *Int J Biochem Cell Biol* **41**, 1828–1836.
52 Alwarawrah M, Hussain F and Huang J (2016) Alteration of lipid membrane structure and dynamics by diacylglycerols with unsaturated chains. *Biochim Biophys Acta Biomembr* **1858**, 253–263.
53 Eichmann TO and Lass A (2015) DAG tales: the multiple faces of diacylglycerol – stereochemistry, metabolism, and signaling. *Cell Mol Life Sci* **72**, 3931–3952.
54 Skinner JR, Shew TM, Schwartz DM, Tzekov A, Lepus CM, Abumrad NA and Wolins NE (2009) Diacylglycerol enrichment of endoplasmic reticulum or lipid droplets recruits perilipin 3/TIP47 during lipid storage and mobilization. *J Biol Chem* **284**, 30941–30948.
55 Choi YM, Ajjaji D, Fleming KD, Borbat PP, Jenkins ML, Moeller BE, Fernando S, Bhatia SR, Freed JH, Burke JE *et al.* (2023) Structural insights into perilipin 3 membrane association in response to diacylglycerol accumulation. *Nat Commun* **14**, 3204.
56 Chorlay A and Thiam AR (2020) Neutral lipids regulate amphipathic helix affinity for model lipid droplets. *J Cell Biol* **219**, e201907099.
57 Chorlay A and Thiam AR (2018) An asymmetry in monolayer tension regulates lipid droplet budding direction. *Biophys J* **114**, 631–640.
58 Mahamid J, Tegunov D, Maiser A, Arnold J, Leonhardt H, Plitzko JM and Baumeister W (2019) Liquid-crystalline phase transitions in lipid droplets are related to cellular states and specific organelle association. *Proc Natl Acad Sci USA* **116**, 16866–16871.
59 Brasaemle DL, Dolios G, Shapiro L and Wang R (2004) Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3-L1 adipocytes. *J Biol Chem* **279**, 46835–46842.
60 Bersuker K and Olzmann JA (2017) Establishing the lipid droplet proteome: mechanisms of lipid droplet

protein targeting and degradation. *Biochim Biophys Acta Mol Cell Biol Lipids* **1862**, 1166–1177.
61 Bersuker K, Peterson CWH, To M, Sahl SJ, Savikhin V, Grossman EA, Nomura DK and Olzmann JA (2018) A proximity labeling strategy provides insights into the composition and dynamics of lipid droplet proteomes. *Dev Cell* **44**, 97–112.e7.
62 Krahmer N, Hilger M, Kory N, Wilfling F, Stoehr G, Mann M, Farese RV and Walther TC (2013) Protein correlation profiles identify lipid droplet proteins with high confidence. *Mol Cell Proteomics* **12**, 1115–1126.
63 Olarte MJ, Swanson JMJ, Walther TC and Farese RV (2022) The CYTOLD and ERTOLD pathways for lipid droplet–protein targeting. *Trends Biochem Sci* **47**, 39–51.
64 Thiam AR, Antonny B, Wang J, Delacotte J, Wilfling F, Walther TC, Beck R, Rothman JE and Pincet F (2013) COPI buds 60-nm lipid droplets from reconstituted water-phospholipid-triacylglyceride interfaces, suggesting a tension clamp function. *Proc Natl Acad Sci USA* **110**, 13244–13249.
65 Caillon L, Nieto V, Gehan P, Omrane M, Rodriguez N, Monticelli L and Thiam AR (2020) Triacylglycerols sequester monotopic membrane proteins to lipid droplets. *Nat Commun* **11**, 1–12.
66 Wilfling F, Wang H, Haas JT, Krahmer N, Gould TJ, Uchida A, Cheng JX, Graham M, Christiano R, Fröhlich F *et al*. (2013) Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocalizing from the ER to lipid droplets. *Dev Cell* **24**, 384–399.
67 Puza S, Caesar S, Poojari C, Jung M, Seemann R, Hub JS, Schrul B and Fleury JB (2022) Lipid droplets embedded in a model cell membrane create a phospholipid diffusion barrier. *Small* **18**, 2106524.
68 Khaddaj R, Mari M, Cottier S, Reggiori F and Schneiter R (2022) The surface of lipid droplets constitutes a barrier for endoplasmic reticulum-resident integral membrane proteins. *J Cell Sci* **135**, jcs256206.
69 Wang H, Sreenivasan U, Hu H, Saladino A, Polster BM, Lund LM, Gong DW, Stanley WC and Sztalryd C (2011) Perilipin 5, a lipid droplet-associated protein, provides physical and metabolic linkage to mitochondria. *J Lipid Res* **52**, 2159–2168.
70 Gross DA, Snapp EL and Silver DL (2010) Structural insights into triglyceride storage mediated by fat storage-inducing transmembrane (FIT) protein 2. *PLoS One* **5**, e10796.
71 Garcia A, Sekowski A, Subramanian V and Brasaemle DL (2003) The central domain is required to target and anchor perilipin a to lipid droplets. *J Biol Chem* **278**, 625–635.
72 Prévost C, Sharp ME, Kory N, Lin Q, Voth GA, Farese RV Jr and Walther TC (2018) Mechanism and determinants of amphipathic helix-containing protein targeting to lipid droplets. *Dev Cell* **44**, 73–86.e4.
73 Vamparys L, Gautier R, Vanni S, Bennett WFD, Tieleman DP, Antonny B, Etchebest C and Fuchs PFJ (2013) Conical lipids in flat bilayers induce packing defects similar to that induced by positive curvature. *Biophys J* **104**, 585–593.
74 Cui H, Lyman E and Voth GA (2011) Mechanism of membrane curvature sensing by amphipathic helix containing proteins. *Biophys J* **100**, 1271–1279.
75 Vanni S, Vamparys L, Gautier R, Drin G, Etchebest C, Fuchs PFJ and Antonny B (2013) Amphipathic lipid packing sensor motifs: probing bilayer defects with hydrophobic residues. *Biophys J* **104**, 575–584.
76 Speer NO, Braun RJ, Reynolds EG, Brudnicka A, Swanson JMJ and Henne WM (2024) Tld1 is a regulator of triglyceride lipolysis that demarcates a lipid droplet subpopulation. *J Cell Biol* **223**, e202303026.
77 Kory N, Thiam AR, Farese RV and Walther TC (2015) Protein crowding is a determinant of lipid droplet protein composition. *Dev Cell* **34**, 351–363.
78 Olarte MJ, Kim S, Sharp ME, Swanson JMJ, Farese RV Jr and Walther TC (2020) Determinants of endoplasmic reticulum-to-lipid droplet protein targeting. *Dev Cell* **54**, 471–487.e7.
79 Salo VT, Li S, Vihinen H, Hölttä-Vuori M, Szkalisity A, Horvath P, Belevich I, Peränen J, Thiele C, Somerharju P *et al*. (2019) Seipin facilitates triglyceride flow to lipid droplet and counteracts droplet ripening via endoplasmic reticulum contact. *Dev Cell* **50**, 478–493.e9.
80 Chung J, Wu X, Lambert TJ, Lai ZW, Walther TC and Farese RV Jr (2019) LDAF1 and Seipin form a lipid droplet assembly complex. *Dev Cell* **51**, 551–563.e7.
81 Klug YA, Deme JC, Corey RA, Renne MF, Stansfeld PJ, Lea SM and Carvalho P (2021) Mechanism of lipid droplet formation by the yeast Sei1/Ldb16 Seipin complex. *Nat Commun* **12**, 5892.
82 Arlt H, Sui X, Folger B, Adams C, Chen X, Remme R, Hamprecht FA, DiMaio F, Liao M, Goodman JM *et al*. (2022) Seipin forms a flexible cage at lipid droplet formation sites. *Nat Struct Mol Biol* **29**, 194–202.
83 Yan R, Qian H, Lukmantara I, Gao M, du X, Yan N and Yang H (2018) Human SEIPIN binds anionic phospholipids. *Dev Cell* **47**, 248–256.e4.
84 Sui X, Arlt H, Brock KP, Lai ZW, DiMaio F, Marks DS, Liao M, Farese RV Jr and Walther TC (2018) Cryo–electron microscopy structure of the lipid droplet–formation protein seipin. *J Cell Biol* **217**, 4080–4091.
85 Wang H, Becuwe M, Housden BE, Chitraju C, Porras AJ, Graham MM, Liu XN, Thiam AR, Savage DB, Agarwal AK *et al*. (2016) Seipin is required for converting nascent to mature lipid droplets. *elife* **5**, e16582.

86 Szymanski KM, Binns D, Bartz R, Grishin NV, Li W-P, Agarwal AK, Garg A, Anderson RGW and Goodman JM (2007) The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology. *Proc Natl Acad Sci USA* **104**, 20890–20895.
87 Salo VT, Belevich I, Li S, Karhinen L, Vihinen H, Vigouroux C, Magré J, Thiele C, Hölttä-Vuori M, Jokitalo E *et al.* (2016) Seipin regulates ER –lipid droplet contacts and cargo delivery. *EMBO J* **35**, 2699–2716.
88 Grippa A, Buxó L, Mora G, Funaya C, Idrissi FZ, Mancuso F, Gomez R, Muntanyà J, Sabidó E and Carvalho P (2015) The seipin complex Fld1/Ldb16 stabilizes ER-lipid droplet contact sites. *J Cell Biol* **211**, 829–844.
89 Han S, Binns DD, Chang YF and Goodman JM (2015) Dissecting seipin function: the localized accumulation of phosphatidic acid at ER/LD junctions in the absence of seipin is suppressed by Sei1pΔNterm only in combination with Ldb16p. *BMC Cell Biol* **16**, 29.
90 Wolinski H, Hofbauer HF, Hellauer K, Cristobal-Sarramian A, Kolb D, Radulovic M, Knittelfelder OL, Rechberger GN and Kohlwein SD (2015) Seipin is involved in the regulation of phosphatidic acid metabolism at a subdomain of the nuclear envelope in yeast. *Biochim Biophys Acta Mol Cell Biol Lipids* **1851**, 1450–1464.
91 Boutet E, el Mourabit H, Prot M, Nemani M, Khallouf E, Colard O, Maurice M, Durand-Schneider AM, Chrétien Y, Grès S *et al.* (2009) Seipin deficiency alters fatty acid Δ9 desaturation and lipid droplet formation in Berardinelli-Seip congenital lipodystrophy. *Biochimie* **91**, 796–803.
92 Magré J, Delépine M, Khallouf E, Gedde-Dahl T Jr, van Maldergem L, Sobel E, Papp J, Meier M, Mégarbané A, Bachy A *et al.* (2001) Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. *Nat Genet* **28**, 365–370.
93 Fei W, Shui G, Gaeta B, du X, Kuerschner L, Li P, Brown AJ, Wenk MR, Parton RG and Yang H (2008) Fld1p, a functional homologue of human seipin, regulates the size of lipid droplets in yeast. *J Cell Biol* **180**, 473–482.
94 Cartwright BR, Binns DD, Hilton CL, Han S, Gao Q and Goodman JM (2015) Seipin performs dissectible functions in promoting lipid droplet biogenesis and regulating droplet morphology. *Mol Biol Cell* **26**, 726–739.
95 Kim S, Chung J, Arlt H, Pak AJ, Farese RV Jnr, Walther TC and Voth GA (2022) Seipin transmembrane segments critically function in triglyceride nucleation and lipid droplet budding from the membrane. *elife* **11**, e75808.
96 Prasanna X, Salo VT, Li S, Ven K, Vihinen H, Jokitalo E, Vattulainen I and Ikonen E (2021) Seipin traps triacylglycerols to facilitate their nanoscale clustering in the endoplasmic reticulum membrane. *PLoS Biol* **19**, e3000998.
97 Zoni V, Khaddaj R, Lukmantara I, Shinoda W, Yang H, Schneiter R and Vanni S (2021) Seipin accumulates and traps diacylglycerols and triglycerides in its ring-like structure. *Proc Natl Acad Sci USA* **118**, 1–11.
98 Renne MF, Corey RA, Ferreira JV, Stansfeld PJ and Carvalho P (2022) Seipin concentrates distinct neutral lipids via interactions with their acyl chain carboxyl esters. *J Cell Biol* **221**, e202112068.
99 Khayyo VI, Hoffmann RM, Wang H, Bell JA, Burke JE, Reue K and Airola MV (2020) Crystal structure of a lipin/Pah phosphatidic acid phosphatase. *Nat Commun* **11**, 1309.
100 Gu W, Gao S, Wang H, Fleming KD, Hoffmann RM, Yang JW, Patel NM, Choi YM, Burke JE, Reue K *et al.* (2021) The middle lipin domain adopts a membrane-binding dimeric protein fold. *Nat Commun* **12**, 4718.
101 Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A *et al.* (2021) Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589.
102 Tunyasuvunakool K, Adler J, Wu Z, Green T, Zielinski M, Žídek A, Bridgland A, Cowie A, Meyer C, Laydon A *et al.* (2021) Highly accurate protein structure prediction for the human proteome. *Nature* **596**, 590–596.
103 Evans R, O'Neill M, Pritzel A, Antropova N, Senior A, Green T, Žídek A, Bates R, Blackwell S, Yim J *et al.* (2021) Protein complex prediction with AlphaFold-multimer. *bioRxiv* 10.1101/2021.10.04.463034 [PREPRINT]
104 Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, Lee GR, Wang J, Cong Q, Kinch LN, Schaeffer RD *et al.* (2021) Accurate prediction of protein structures and interactions using a three-track neural network. *Science* **373**, 871–876.
105 Baek M, Anishchenko I, Humphreys IR, Cong Q, Baker D and DiMaio F Efficient and accurate prediction of protein structure using RoseTTAFold2. *bioRxiv* 10.1101/2023.05.24.542179 [PREPRINT]