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Plant Lipid Droplets and Derived Lipidic Nano-Assemblies: Structure, Biogenesis and Pharmaceutical Applications

Abdulsamie Hanano[1] | Amal Yousfan[2] | Denis J. Murphy[3]

[1]Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria (AECS), Damascus, Syria | [2]Department of Pharmaceutics and Pharmaceutical Technology, Pharmacy College, Al Andalus University for Medical Sciences, Tartus, Syria | [3]School of Applied Sciences, University of South Wales, Pontypridd, Wales, UK

Correspondence: Abdulsamie Hanano (ashanano@aec.org.sy)

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ABSTRACT

Lipid droplets (LDs) serve as the primary storage site for neutral lipids in plant cells, with growing evidence supporting many additional biological roles, such as in lipid homeostasis, signalling, trafficking, inflammatory responses and inter-organelle communication. While the biogenesis and structure of LDs in seeds and other plant tissues have been well-documented, the full range of their functions has yet to be elucidated. Plant LDs encapsulate a hydrophobic neutral lipid core, enveloped by a phospholipid monolayer embedded with specific proteins. Despite their tissue-specific diversity, a range of methods for LD isolation from plant materials has been established, facilitating lipidomic and proteomic characterisation. This knowledge has facilitated studies into the potential applications of LDs, particularly in pharmaceutical biotechnology. This review explores the multifunctional nature and biogenesis of plant LDs, highlights recent advances in LD fractioning from plant materials, explores factors affecting their stability, and discusses the potential of mimicking natural LDs using artificial lipid nano-droplets (ALNDs) and similar synthetic lipid-based formulations. It also underscores the significance of LD-based delivery systems in pharmaceutical applications, emphasising their emerging potential in enhancing drug solubility, bioavailability and targeted delivery. Finally, future research directions are highlighted, focusing on scaling up LD isolation, optimising ALND and other formulations, and investigating their pharmacokinetics and long-term stability for more widespread clinical applications.

1 | Introduction

Plant lipid droplets (LDs), formerly known variously as oleosomes, lipid bodies, or oil bodies, are dynamic organelles that are most abundant in seeds, although they are also present ubiquitously in all other plant tissues (Murphy 2001, 2012). While plant LDs were initially recognised as subcellular storage sites for neutral lipids such as triacylglycerols (TAGs) and sterol esters (SEs), recent studies have revealed their broader biological roles in plant development and wide-ranging responses to biotic and abiotic factors. Structurally, LDs originate via a complex and highly regulated mechanism within the endoplasmic reticulum (ER), where neutral lipids such as TAGs and SEs are synthesised *de novo* and accumulate within the bilayer membrane core, forming droplet-like structures. These nascent droplets expand in size, acquire a specific protein complement, and eventually bud off from the cytosolic face of the ER membrane (Chapman et al. 2012; Yu et al. 2021; Zhao et al. 2023). A mature LD consists of a lipid-rich core, primarily composed of TAGs plus other neutral lipids such as SEs or wax esters, that is enclosed by a phospholipid monolayer embedded with a mixture of proteins that play a crucial role in its biogenesis and in maintaining its structure and functionality (A. H. Huang 1996; Laibach et al. 2018; Murphy 1993;

Tzen et al. 1993; Tzen and Huang 1992). Emerging insights into the evolutionary history of canonical LD proteins, particularly SEIPINs, suggest that complex LDs were present in very early eukaryotes and subsequently played key roles in all modern clades including algae, fungi, plants and animals (Le Moigne et al. 2025).

The functionality of plant LDs is regulated by their associated proteins. Although LD proteomes may vary across cell types, a core set of high-confidence LD-associated proteins has been consistently identified (Cai and Horn 2025; Gidda et al. 2016; A. H. C. Huang 2018; Kretzschmar et al. 2020; Richardson 2020). These proteins have been broadly classified into two main classes (Olzmann and Carvalho 2019; Yang et al. 2012), with the ERTOLD protein class being tightly embedded in the LD monolayer membrane, performing diverse functions, and including oleosins, caleosins and steroleosins (Frandsen et al. 1996; Lin et al. 2002; Murphy 1993) and the CYTOLD protein class, such as the perilipins that associate with LDs directly from the cytosol (Cai and Horn 2025; Kretzschmar et al. 2020; Richardson 2020). An interesting recent development has been the first model system for an in vitro assay to study de novo LD biogenesis. In this assay, a supported lipid bilayer is deposited on a glass coverslip for the addition of neutral lipids and LD-related proteins (Deo et al. 2025). An important advance in understanding LD structure and function has been the development of highly efficient artefact-free isolation protocols from plant tissues. Despite their tissue-specific diversity, these protocols have enabled comprehensive lipidomic and proteomic characterisation (Hanano et al. 2006, 2022; A. H. C. Huang 2018; Zhi et al. 2017). This foundational knowledge greatly broadens the potential applications of LDs, particularly as related to pharmaceutical biotechnology.

The unique micro-lipidic structure and exceptional stability of LDs in general, and plant LDs in particular, have generated significant interest from industrial and pharmaceutical biotechnologists for providing safe, effective and stable delivery systems that can be adapted to transport and deliver a wide range of cargoes for a diversity of applications (Chiang et al. 2011; Oliver et al. 2020; Xu et al. 2023; Yousfan, Moursel, and Hanano 2024; Zhang et al. 2024). As a result, research into the exploitation of LDs and related nano-emulsion systems including essential oils has expanded across various fields with some of the most notable success stories relating to the food and pharmaceutical sectors (Cui et al. 2024; Kumar et al. 2025, 2024; Medeleanu et al. 2023). In this context, optimising LD extraction and purification remains a critical challenge for enhancing yield and stability, especially if deployment is required in clinical settings. A particular challenge is developing scalable and cost-effective protocols that will be essential for enabling large-scale pharmaceutical applications that fulfil the most stringent criteria that include reproducibility, sterility and hypo-allergenicity (A. H. C. Huang 2018). Additionally, advanced bioengineering strategies, including genetic modification such as genome editing and synthetic biology, offer opportunities to tailor LD composition, enabling the production of specialised LDs enriched with tailored populations of bioactive lipids and/or engineered proteins (Chen et al. 2004; Chiang et al. 2010; Greenberg et al. 1991; Hanano et al. 2016a; Yi et al. 2015; Yousfan, Moursel, and Hanano 2024; Yuan et al. 2024).

For several decades, the pharmaceutical potential of plant LDs and LD-associated proteins such as oleosins has been the subject of much research (Nykiforuk et al. 2006; Parmenter et al. 1995; Qiang et al. 2020; van Rooijen and Moloney 1995; Yu et al. 2024). The expanding pharmaceutical applications of LDs continue to evolve, driving interest in their multifunctional potential. This review explores the structure of plant LDs, their biogenesis, advances in fractionation techniques, and factors influencing their stability. It also highlights the potential of artificial lipid nanodroplets (ALNDs) as novel biomimetic alternatives. A particular emphasis is placed on the significance of LD-based delivery systems in pharmaceuticals, especially their role in improving drug solubility, bioavailability and targeted delivery. Lastly, future research directions are outlined, highlighting the need for scalable LD isolation, optimization of ALND and other formulations, and the need for comprehensive investigations into their pharmacokinetics and long-term clinical stability.

2 | Recognition of LDs as Multifunctional Cellular Organelles

LDs were first described in the 19th century, with Richard Altmann and E.B. Wilson observing fat droplets in cells and speculating on their origins (Altmann 1890; Wilson 1896). By the early 1900s, LDs were widely recognised as a new form of cellular component in biological systems and termed "liposomes." However, with the invention of artificial liposomes in the late 1960s, the term was repurposed, leading to the adoption of various alternative names, including LDs, lipid bodies, fat bodies and fat droplets (Murphy and Vance 1999). Interest in these organelles in animal systems was sparked in 1991 with the discovery of perilipin, a phosphoprotein associated with LDs in mammalian adipocytes, highlighting their functional significance (Greenberg et al. 1991). Knowledge of the roles and significance of perilipins has since expanded, and they are now recognised as abundant LD proteins present in all metazoans and also in Amoebozoa and fungi (Farese and Walther 2025; Griseti et al. 2024). A useful resource for LD researchers that mainly focuses on human-related LDs is the online 'Lipid Droplet Knowledge Portal' (Mejhert et al. 2022).

In the early 2000s, it was realised that virtually all cells, from archaea to humans, contained morphologically similar neutral LDs encapsulated by a phospholipid/protein membrane, and a consensus was adopted that all of these organelles, regardless of origin, were to be called LDs (Murphy 2012). However, in some of the earlier literature from plants, LDs were referred to by various names, including fat droplets, lipid bodies, lipid globules, oil droplets, oleosomes and spherosomes. The presence of LDs in plant cells has been recognised for over a century. As early as 1882, Sachs observed LDs in the parenchyma cells of root and shoot axils, while Walker documented their presence in seeds in 1888 (Pack 1925). LDs are present in cells and tissues throughout plant organs where they serve diverse functions. In some seeds, they accumulate in large quantities, particularly in oil-rich seeds and nuts where they can make up over half of the seed dry weight. These storage lipids are compartmentalised

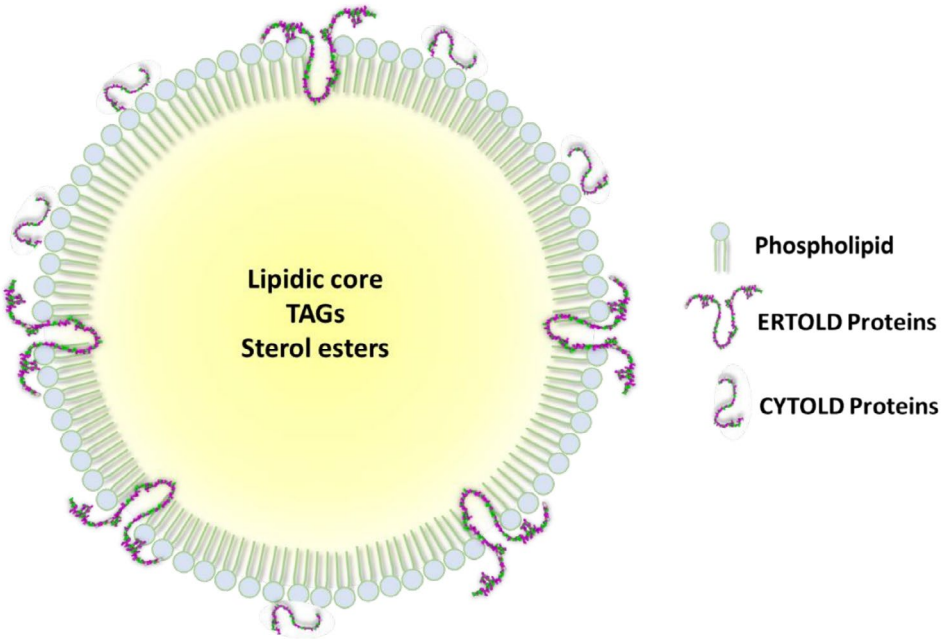

FIGURE 1 | A universal representative model of plant LD structure. All plant LDs have a similar structural organisation that sets them apart from other organelles. The LDs are surrounded by a phospholipid monolayer enclosing a core filled by neutral lipids, most commonly triacylglycerols and sterol esters. The phospholipids have their polar head groups oriented towards the cytosol whereas their acyl chains contact the hydrophobic neutral lipid core. Associated with the monolayer are a variety of proteins, mainly grouped into two major classes: ERTOLD proteins which are stably associated with membranes through their specific transmembrane domains and exhibited diverse biological functions; CYTOLD proteins are recruited to the LD surface directly from the cytosol.

into micrometre-sized cytosolic LDs, which are distributed across different seed tissues. However, the abundance and spatial distribution of LDs within seed tissues can vary significantly among plant species, with some accumulating mainly in embryos while others are preferentially found in endosperm or scutellum tissues (Bouchnak et al. 2023; Hanano et al. 2016a; Murphy 2001).

Ultrastructural studies have documented the presence of LDs within the pollen grains of some entomophilous plants (Piffanelli et al. 1997). These LDs accumulate during pollen grain maturation (Rotsch et al. 2017) and subsequently migrate into the growing pollen tube upon germination where the energy provided by catabolism of their stored lipids supports the rapid growth of the pollen tube (Rodriguez-Garcia et al. 2003). Mutations in key transcription factors regulating genes involved in TAG synthesis have been shown to reduce LD abundance in pollen grains, ultimately leading to pollen sterility (Guan et al. 2014; Zheng et al. 2018). LDs are also found in roots, with their presence notably reported in Yellow nutsedge (*Cyperus esculentus*), one of the rare plant species that accumulates oil-rich LDs in underground tissues during tuber development (Turesson et al. 2010). Additionally, LDs can transiently appear in non-dormant vegetative tissues, such as leaves and roots, depending on the plant's developmental stage and physiological state (Hanano et al. 2016b; Zienkiewicz and Zienkiewicz 2020). Tissues such as roots, leaves generally contain limited amounts of TAGs under standard conditions, and LDs were rarely documented in leaves until the last decade. Lersten et al. (2006) reviewed the existing literature on LDs in mesophyll cells and expanded on it by examining fresh leaf sections from over 300 plant species. They observed LDs in the leaves of 23% of the surveyed species, with a broad distribution across the plant kingdom.

LDs in many leaf cells are constitutively present in relatively small numbers during normal development but are strongly upregulated under stress conditions such as senescence or pathogen infection (Shimada et al. 2014). Vertebrate LDs contain a family of fat-specific proteins (FSP27) involved in the LD enlargement and proliferation that occurs during apoptosis. Expression of one of these animal proteins in transgenic *B. napus* plants led to a similar pattern of LD proliferation in the leaf tissues, although there was no cell death (Xiong et al. 2025). This implies that leaf LDs can respond dynamically to several external signals. Moreover, pathogen-induced leaf LDs can also act as sites for the synthesis and accumulation of a lipid-soluble phytoalexin that is involved in mediating the plant response to pathogen attack (Shimada et al. 2014). Gidda et al. (2016) also demonstrated that LD abundance in leaves fluctuates during diurnal cycles, with a peak in accumulation occurring at the end of the night. More recently, a detailed analysis of leaf LDs from Arabidopsis mutant accumulating high levels of sterols identified a wide range of novel proteins including several myosin-binding proteins that might facilitate LD movement along actin filaments (Omata et al. 2024). Similarly, varying amounts of LDs are present in many algal cells where they fluctuate on a diurnal basis as they track the incident sunlight in order to either reduce or increase buoyancy according to radiation levels (Ischebeck et al. 2020).

3 | Fine Structures of Plant LDs

Electron microscopy shows fractionated plant LDs as spherical organelles (Figure 1) with diameters typically ranging from 0.1 to 5 μm, depending on the plant species and tissue type (Murphy 1993). Structurally, LDs are relatively simple, consisting of a lipid-rich core surrounded by a phospholipid monolayer, which separates them from the aqueous cytosol and is stabilised by the integration of various proteins (Pyc et al. 2017). In this context, it is worth noting that lipids can also assemble into other structures, such as plastoglobules and stromules within chloroplasts. Plastoglobules are plastid-localised LDs enclosed by a monolayer membrane with a specific population of proteins, connected to the stromal leaflet of the thylakoid membrane. They are particularly abundant in floral tissues where their lipid cargoes include lipophilic pigments such as carotenoids as well as in the etioplasts and plastids of senescent leaves (Murphy 2012). Similarly to cytosolic LDs in leaves, plastoglobules are induced in response to various environmental stresses and are part of the recycling of photosynthetic membranes during leaf senescence (Nacir and Brehelin 2013). Stromules, on the other hand, are tubular extensions of the chloroplast envelope that extend into the cytosol, containing stroma but lacking thylakoids. Similar to plastoglobules, stromules increase in number under environmental stress conditions and during senescence (Hanson and Hines 2018; Mortimer et al. 2017).

3.1 | The Phospholipid Monolayer of LDs

The monolayer surrounding LDs likely reflects the membrane composition of the ER domain within which LD biogenesis occurs. In plants, as well as in non-photosynthetic organisms, the monolayer primarily consists of phospholipids (Tzen and Huang 1992). LD-bound phospholipids function as surfactants, stabilising hydrophobic neutral lipids within the aqueous cytosol by reducing LD surface tension and enhancing structural integrity by forming an emulsion system (Thiam et al. 2013). Moreover, the lipid composition of the LD monolayer actively influences LD morphology, structure and function. Protein recruitment to LDs can be regulated by a variety of factors. In the case of binding to LD monolayers, lipid packing defects play important roles (Kim and Swanson 2020), as do electrostatic interactions (e.g., binding to anionic lipids) and fluidity of the monolayer, thereby modulating LD-associated processes (Olarte et al. 2022; Thiam et al. 2013).

Phospholipids, which contain various phosphate-based headgroups, are key components of cellular membranes. Common headgroups include choline, ethanolamine, serine and inositol, giving rise to phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI), respectively. Additionally, phospholipids vary in the length and degree of unsaturation of their fatty acyl chains, which influences membrane fluidity and other biophysical properties. These variations play a crucial role in determining membrane characteristics and are unevenly distributed across different cellular membranes (Makarenko et al. 2000; Nakamura 2017; Reszczynska and Hanaka 2020). In most cases, the phospholipid monolayer of LDs primarily consists of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), lysophosphatidylcholine (lysoPC) and lyso-phosphatidylethanolamine (lysoPE), each contributing unique properties that influence LD stability, morphology, and function (Bartz et al. 2007; Leber et al. 1994). Free cholesterol (FC) is also likely present in the LD surface layer (Prattes et al. 2000), though the possibility that certain phospholipids and small amounts of FC may also reside within the lipid core cannot be excluded (Hevonoja et al. 2000).

3.2 | Lipidic Cores of LDs

The neutral lipid core of most LDs appears to consist of a simple uniform spherical liquid or semi-liquid globule or droplet and, while this might be the case in plants, there are several examples in animals where specific proteins are at least partially sequestered in this highly hydrophobic space. Such examples include histones and transcriptional regulators in the embryos of mice and some insects as well as in some somatic cells, although the various interacting components with different mechanisms occur according to the species and LD functionality (Fujimoto and Parton 2011; Stephenson et al. 2021; Welte 2015). In most cases, TAGs and SEs are the main lipid classes reported in the LD neutral core lipidome of plants (Baud 2018; Ferrer et al. 2017), although in a few cases, other neutral lipids are the dominant components, most famously in the desert shrub Jojoba (*Simmondsia chinensis*) where wax esters are the main storage lipid (Kawinski et al. 2021). Spherical wax ester-rich LDs have also been reported in some *Acinetobacter* spp., although other species accumulated rectangular or rod-shaped wax ester structures (Waltermann et al. 2005), while other bacteria are able to accumulate both TAG and wax esters thanks to a multifunctional wax ester synthetase/diacylglycerol acyltransferase (Kalscheuer and Steinbuchel 2003).

TAGs consist of three fatty acyl residues esterified to a glycerol backbone. Their physical properties are influenced by the specific fatty acids attached, as well as their positional arrangement on the glycerol molecule (Lawson and Hughes 1988; Marchin et al. 2017; Xu and Shanklin 2016). The presence of TAGs was reported in all eukaryotic organisms, including animals, plants, fungi and protists, and also in some prokaryotes (Alvarez and Steinbuchel 2002; Hanano et al. 2019, 2015, 2017). In most plants, the fatty acid composition of TAGs is relatively simple, with six fatty acids—palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3)—typically comprising over 90% of the total fatty acids, although there are exceptions, especially in seeds that accumulate unusual fatty acids such as hydroxy, epoxy or very long-chain variants (He et al. 2020; Reszczynska and Hanaka 2020).

Due to their crucial roles as a relatively inert storage component, the TAG biosynthetic pathway is conserved across diverse organisms, from bacteria to plants and humans (Athenstaedt and Daum 2006). Quantitatively, TAGs represent the primary seed storage reserve in many plant species, including oil crops such as sunflower (*Helianthus annuus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*) and maize (*Zea mays*), as

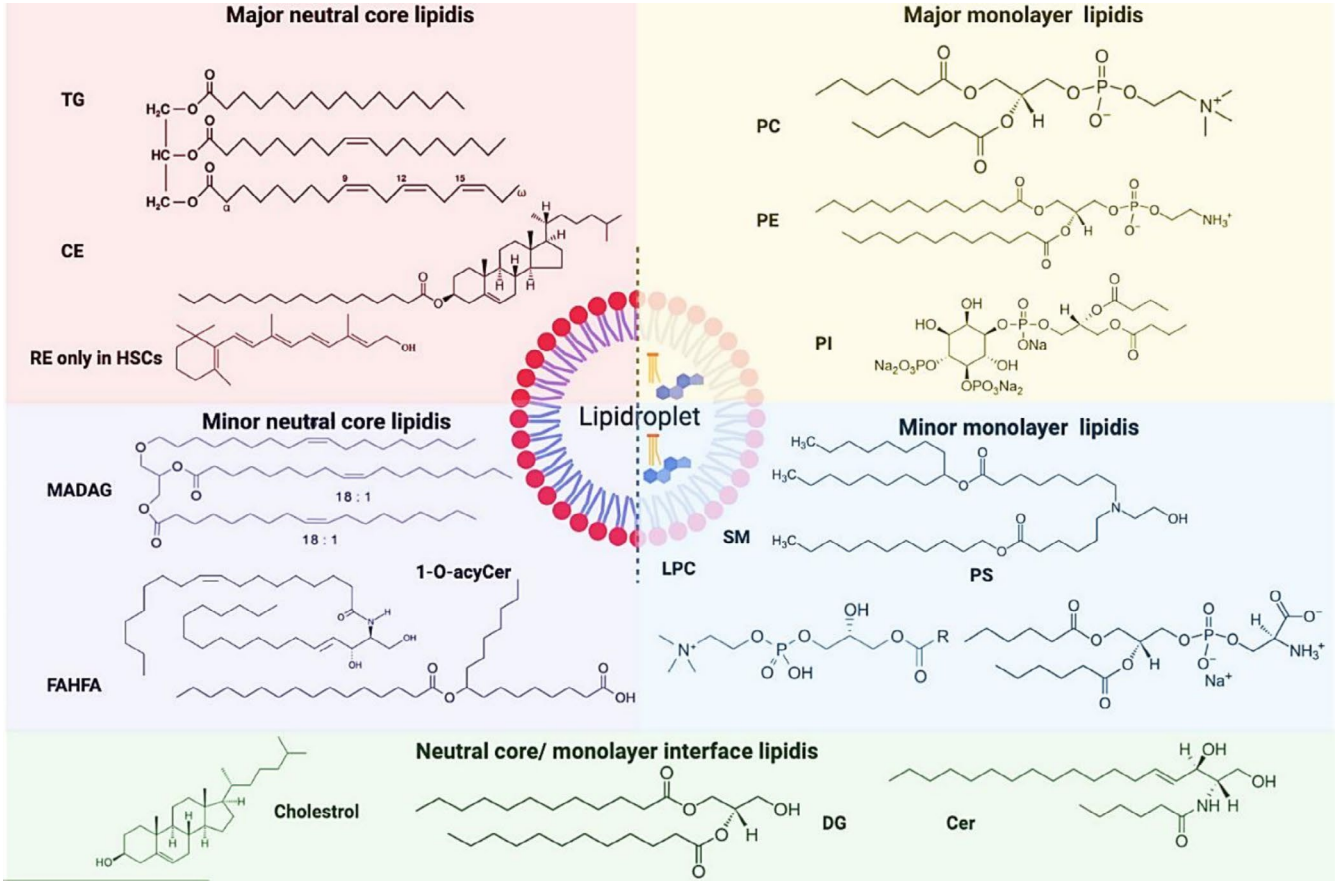

FIGURE 2 | Schematic overview of the current knowledge of the LD lipidome. The major and minor lipids of both the neutral core and phospholipid monolayer are shown. 1-0-acylCer, acylceramides; Cer, ceramides; CE, cholesteryl esters; DG, diacylglycerols; FAHFA, fatty acid esters of hydroxy fatty acids; HSC, hepatic stellate cells; lysoPC, lysophosphatidylcholine; MADAG, monoalk(en)yl diacylglycerols; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; RE, retinyl esters; SM, sphingomyelin; TG, triacylglycerols. This figure was adopted from (Wolk and Fedorova 2024).

well as in trees like olive and yew (Graham 2008; Hanano et al. 2016a, 2006, 2022). Beyond their significance for human nutrition, TAGs are gaining prominence as raw materials for various industrial applications (Durrett et al. 2008). They can also be transmethylated to generate renewable biofuels such as biodiesel, offering a sustainable alternative to fossil-derived oil (Cao et al. 2023).

Alongside TAGs, SEs contribute to the formation of the lipidic core of LDs. Free sterols are synthesised in the ER from acetyl-CoA via the mevalonate pathway, leading to the production of cycloartenol, the precursor of cholesterol—a minor sterol in plants. The conversion of cycloartenol into the predominant plant sterols, such as sitosterol, stigmasterol and campesterol, involves a series of enzymatic modifications, including methylation, demethylation, reduction, isomerization and desaturation (Benveniste 2004; Harker et al. 2003; Valitova et al. 2016). However, the relative abundance of TAGs and SEs, along with the presence of other hydrophobic molecules, can vary depending on the cellular context, cell type and environmental conditions (Wang et al. 2022; Wolk and Fedorova 2024). Figure 2 provides a schematic overview of the current understanding of the plant LD lipidome, highlighting the major and minor components of both the neutral lipid core and the surrounding phospholipid/protein monolayer.

Although the lipidic cores of plant LDs are most commonly made up of fatty acyl esters such as TAGs, SEs or wax esters, there are several examples of transgenic plants that have been bioengineered to accumulate acyl-CoA-derived biopolymers such as polyhydroxyalkanoates (PHAs) (Aghaali and Naghavi 2023; Dobrogojski et al. 2018). PHAs normally accumulate in spherical semi-solid LD-like structures in bacteria and archaea as an alternative storage product to acyl esters (Murphy 2012). Until very recently, it was thought that archaea were unable to accumulate acyl esters or TAGs, but a new report has demonstrated that halophilic archaea produce wax esters, meaning that they might accumulate similar types of acyl ester-rich LDs as are found in bacteria and eukaryotes (Grossi et al. 2025).

In connection with LD composition, it was reported that the lipid composition of animal LDs strongly influences their internal physical phase, organelle contact sites and accessibility of stored lipids to metabolic enzymes or transporters (Mahamid et al. 2019). Structural phase transitions, such as the shift from an amorphous to a smectic-crystalline state, can restrict lipid exchange with surrounding membranes and alter the binding landscape of LD-associated proteins (Hsieh et al. 2012). Under normal culture conditions, LDs are amorphous. However, during certain metabolic states or cell cycle stages, they transition into a smectic liquid-crystalline phase surrounding an amorphous

core at physiological temperature. During mitotic arrest or starvation, cholesteryl ester (CE) levels rise, likely due to TAG consumption for membrane synthesis or mitochondrial respiration. This correlates with direct visualisation of LD–mitochondrial contacts, suggesting that such transitions significantly impact lipid accessibility and turnover (Mahamid et al. 2019).

3.3 | LD-Associated Proteins

Various proteomics-based approaches have been employed to characterise the full repertoire of LD-associated proteins (Brasaemle et al. 2004). While LD proteomes may differ across cell types and analytical methods, a core set of high-confidence plant LD-associated proteins has been consistently identified (A. H. C. Huang 2018). Some aspects of potential biotechnological applications of plant LD-associated proteins and phospholipids, for example, as self-assembled nano aggregates, have been reviewed recently (Liao et al. 2025).

LD-associated proteins can be categorised into two main classes (Olzmann and Carvalho 2019; Roberts and Olzmann 2020; Yang et al. 2012). The class ERTOLD proteins are stably associated with membranes that partition between LDs and the ER, performing diverse functions. In plants, this group includes three major subclasses: oleosins (A. H. Huang 1996; Murphy 1993), caleosins (Frandsen et al. 1996) and steroleosins (Lin et al. 2002). While CYTOLD proteins are synthesised on free cytoplasmic ribosomes and subsequently recruited directly to the LD surface, either from the cytosol or from other organelles such as mitochondria, lysosomes and peroxisomes, they do not rely on the ER for targeting. Instead, they associate with LDs through direct binding, typically mediated by amphipathic helices that recognise phospholipid packing defects and exposed neutral lipids within the LD monolayer (Dhiman et al. 2020; Olzmann and Carvalho 2019; Yang et al. 2012). They are often referred to as LD-associated proteins, and their roles include participation in structural or regulatory functions at the LD surface (Horn et al. 2013; Lopez-Ribera et al. 2014).

In plants, the most common ERTOLD proteins are oleosins, which are small proteins typically ranging from 15 to 24 kDa (A. H. Huang 1996). Oleosins are characterised by short amphipathic N- and C-terminal peptides that either orient horizontally on the LD surface or extend from it. In addition, they possess a highly conserved central hydrophobic hairpin motif of about 72 uninterrupted non-charged residues, which penetrates the LD core, playing a crucial role in stabilising the structure of the organelle (Murphy 1993; Roberts et al. 1993, 1995). Oleosins are well-documented as the most abundant LD proteins in plant seeds (A. H. Huang 1996) and are anchored to the LD core via their characteristic hairpin motif (Lacey et al. 1998). These proteins are known to play a crucial role in maintaining the stability of the LD monolayer, preventing fusion of LDs, especially during desiccation, rehydration and freezing of seeds. Their presence helps maintain structural integrity and ensures proper function during critical stages of seed development (Heneen et al. 2008; Li et al. 2012; Miquel et al. 2014; Shimada et al. 2008).

Caleosins were first identified as LD-associated proteins in plant seeds (Frandsen et al. 1996; Yamaguchi-Shinozaki et al. 1992), and are now believed to be present in most, if not all, plant and algal tissues (Murphy 2012). Their name derives from the presence of a canonical calcium-binding EF-hand motif, a characteristic feature of all known caleosins (Hanano et al. 2006). This structural motif suggests a potential role in calcium-mediated signalling or regulation, further highlighting the functional diversity of LD-associated proteins in plants (Rahman, Hassan, Hanano, et al. 2018; Rahman, Hassan, Rosli, et al. 2018). Although caleosins were initially discovered as LD-associated proteins, it soon became clear that only some isoforms bind to LDs while other isoforms bind instead to various bilayer membranes including the ER and plasma membranes (Murphy 2012). Unlike oleosins, which are only found in plants and some algae, caleosins are also present in a wide variety of eukaryotes including fungi, plants and some protists where they also have LD-related functions, for example related to stress-related signalling (Rahman, Hassan, Hanano, et al. 2018; Rahman, Hassan, Rosli, et al. 2018). Caleosins are ancient proteins that may have been present in some of the earliest groups of early eukaryotes that arose over a billion years ago as part of the adaptation to increasing oxygen levels (Rahman, Hassan, Hanano, et al. 2018; Rahman, Hassan, Rosli, et al. 2018). This is consistent with a recent report that in the microalga *Auxenochlorella protothecoides*, caleosin acts as a regulator of intracellular oxidative stress via modulation of LD formation (Fu et al. 2025).

Caleosins also exhibit catalytic activity as peroxygenases (PXGs), and are now commonly referred to as CLO/PXGs. Initially, peroxygenases were identified for their role in catalysing the hydroxylation of aromatic compounds and the epoxidation of polyunsaturated fatty acids (Blee and Schuber 1990). This enzymatic function is closely linked to oxylipin metabolism and relies on an iron-coordinating heme ligand (Blee et al. 2014; Hanano et al. 2006). The biochemical structure, enzymatic properties and physiological functions of CLO/PXGs have been the subject of recent comprehensive reviews, highlighting their diverse roles in plant metabolism and stress responses (Hanano et al. 2023), including their recently described involvement in LD-mediated cadmium sequestration (Fu et al. 2025).

Like caleosins, steroleosins possess a hydrophobic segment with conserved proline residues, potentially forming a hairpin structure that penetrates the LD monolayer (Pasaribu et al. 2016). These proteins contain both a sterol-binding site and a NADPH-binding site, classifying them within the large family of hydroxysteroid dehydrogenases found in plants and various other organisms (Lin et al. 2002; Zhang et al. 2017). Functionally, steroleosins catalyse the conversion of sterol substrates into brassinosteroids, which are plant hormones that regulate several key aspects of growth and development (Lin et al. 2002).

CYTOLD proteins, as previously mentioned, are recruited to the LD surface directly from the cytosol or other non-ER organelles. Among them are LD-associated protein (LDAP) and OB-associated protein (OBAP), two recently identified LD proteins in plants. These proteins are relatively small, ranging from 25 to 27 kDa, and lack well-defined binding or functional motifs in plant LDs compared to their equivalent proteins in animal LDs. These proteins are relatively small (25–27 kDa) and, in plant LDs, lack the well-defined binding or functional motifs that are typically present in their counterparts in animal LDs.

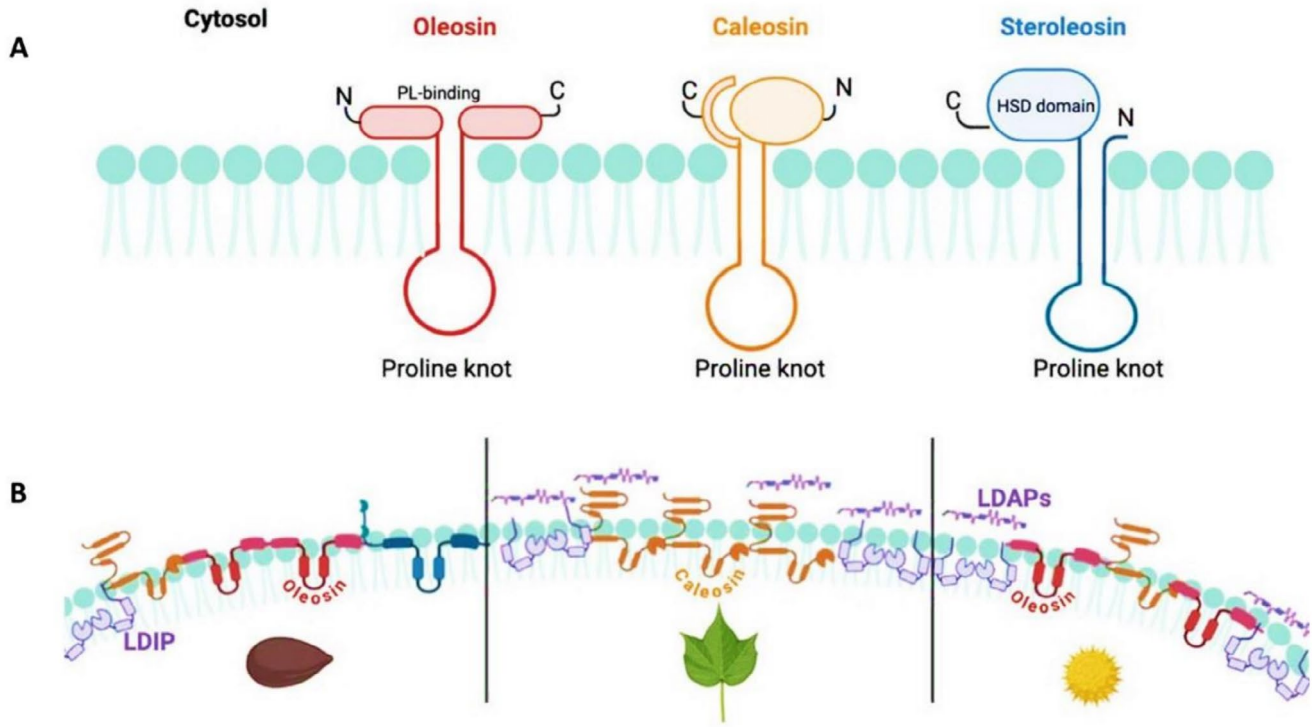

FIGURE 3 | Schematic representations of the structures of oleosin, caleosin, and steroleosin at the surface of LDs in plants. (A) Structural and positional characteristics of oleosin, caleosin and steroleosin at the LD surface. The cytosolic-facing, N- and C-terminal domains for oleosins, including two transmembrane domains, are involved in their interaction with the phospholipid monolayer. Caleosins exhibit a highly conserved Ca^{+2}-binding EF-hand motif exposed to the cytoplasm involved in its enzymatic activity. Like oleosins and caleosins, steroleosins possess a hydrophobic transmembrane domain and contain both a sterol-binding site and an NADPH-binding site. Each of these proteins is depicted as penetrating into the TAG-filled core of the LD and includes a so-called "proline knot". (B) Differential distribution of the three types of LD-associated proteins in plant tissues. Oleosins and steroleosins are mainly, but not exclusively, present in seed LDs, while caleosins are mainly found in vegetative tissues. All three proteins are also found in the pollen LDs.

Unlike ERTOLD proteins, they can recognise and bind to the LD surface where the phospholipids in the LD monolayer are not tightly packed, indicating that they likely do not play a structural role in LD stability (Gidda et al. 2016; Lopez-Ribera et al. 2014). However, in the case of the seed LD protein, which serves as an LD-targeting signal that binds to a specific oleosin isoform (OLEO1), it is involved in the control of LD size in Arabidopsis seeds and seedlings (Doner et al. 2025). These proteins tend not to be tightly bound to LDs and are often absent from fractionated seed LDs, especially if they have been washed with saline buffers (Hanano et al. 2016a). Figure 3 shows a summary overview of the current understanding of the plant LD proteome, highlighting ERTOLD and CYTOLD proteins along with their key components.

4 | LD Biogenesis

The biogenesis of LDs is a complex process that involves the coordinated stoichiometric assembly of the phospholipid monolayer, TAGs/SEs and specific populations of LD-associated proteins. Once these elements are produced, they assemble to generate nascent LDs, which may subsequently fuse to form larger structures depending on the cellular context. LDs maintain a close association with the ER, a connection observed at the electron microscopy level across various organisms, including plants (Kang et al. 2022). It is likely that specific ER subdomains are dedicated to LD assembly in response to requirements at the level of cells, tissues, or the whole organism (Gidda et al. 2011; Herman 2008). Although several studies support a uniform model of LD biogenesis in animal and yeast cells—mediated by SEIPIN proteins encoded by a single gene—the situation appears more complex in plants (Chung et al. 2019; Klug et al. 2024; Le Moigne et al. 2025; Salo 2023). In Arabidopsis, for example, LD biogenesis likely follows a similar mechanism, but important questions remain due to the presence of three distinct SEIPIN isoforms encoded by three separate genes (Klug et al. 2024). Seipin proteins from all described eukaryotes encompassing over a billion years of evolution are strongly conserved in sharing a luminal domain flanked by two transmembrane domains (Liao et al. 2025). This highlights the ancient origins of LD organelles in eukaryotes and the constraints on the seipin mechanism that have persisted intact throughout their evolution. More broadly, a universal model of LD formation could be proposed to account for variations arising from cell type, tissue, organ and organism-specific proteins, as well as differences in physiological and energetic demands. As illustrated in Figure 4, LD biogenesis can be described as a dynamic three-stage process.

Step I includes neutral lipid synthesis and intra-bilayer lens formation. The process begins with the synthesis of neutral lipids, primarily TAGs and SEs (Wilfling et al. 2014). These are formed

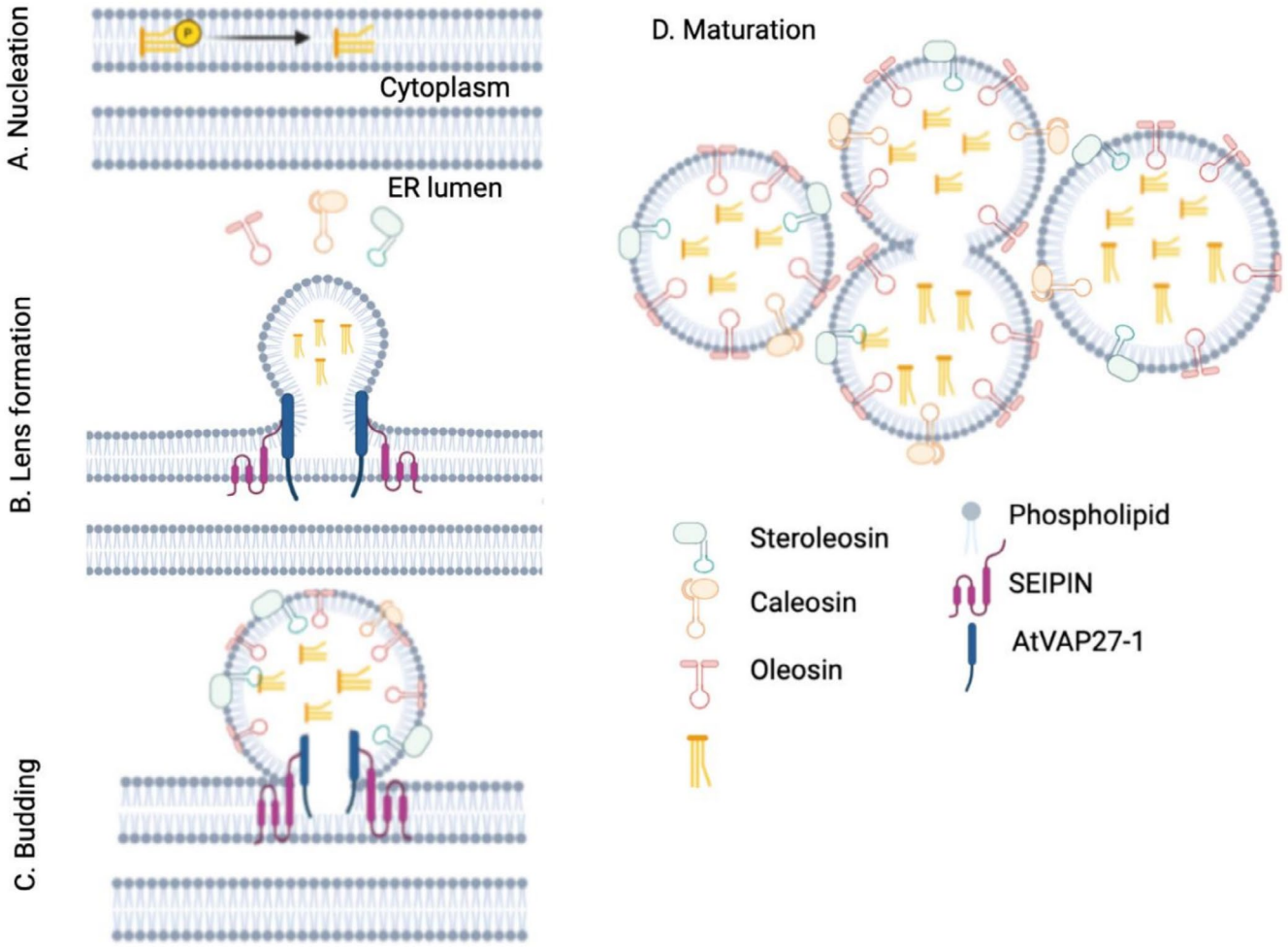

FIGURE 4 | Steps in lipid droplet biogenesis. Lipid droplets emerge from the endoplasmic reticulum (ER). The correct shape and composition of the ER membranes, which are likely affected by the fat storage-inducing transmembrane 2 (FIT2) protein and other ER-resident proteins, is an important determinant of lipid droplet biogenesis. Step 1: Triacylglycerol (TAG) synthesis (see inset) and cholesterol ester synthesis enzymes deposit neutral lipids in between the leaflets of the ER bilayer. Beyond a certain concentration, the neutral lipids demix and coalesce into a lens. Step 2: Seipin and other LD biogenesis factors define the LD nucleation site and are recruited to the lens structure to facilitate the growth of the nascent LD. For further recent views and discussions see (Chung et al. 2019; Klug et al. 2024; Le Moigne et al. 2025; Salo 2023). The emergence of the LD into the cytosol is affected by differences in surface tension of the luminal and cytosolic leaflets of the ER bilayer, likely determined by asymmetrical protein binding and phospholipid composition. Small nascent LDs can be filled with more TAGs or fused together to form bigger LDs.

through the esterification of activated fatty acids with either diacylglycerol (for TAGs) or sterols (for SEs), catalysed by specific enzymes localised to the ER. Diacylglycerol acyltransferases (DGATs) drive TAG formation, while acyl-CoA: cholesterol O-acyltransferases (ACATs) mediate SE synthesis. Initially, the newly formed neutral lipids remain dispersed within the ER membrane, but as their concentration increases, they undergo demixing and coalescence, leading to the formation of an oil lens in the interior of the ER membrane bilayer. In model membranes, this phase transition typically occurs when TAG concentration reaches 5–10 mol% (Duelund et al. 2013; Khandelia et al. 2010). At the end of this step, small lens-like structures (30–60 nm in diameter) emerge between the two leaflets of the ER membrane, causing a transient bulge or lens-like structure.

Step II leads to LD budding from the ER membrane. The composition of phospholipids and the properties of integrated proteins play crucial roles in this process. To minimise the exposure of neutral lipids to the aqueous cytosol, surface tension becomes a key factor in shaping LDs into their characteristic spherical structure (Ben M'barek et al. 2017; Thiam and Foret 2016). During LD budding, ERTOLD proteins, which possess hydrophobic hairpin domains, diffuse laterally within the ER membrane and integrate into the emerging LD monolayer. For instance, oleosins are co-translationally inserted into the ER, where they localise to specific domains enriched in accumulating TAGs. This facilitates their orientation, with the N- and C-terminal regions exposed to the cytosol, while the hydrophobic core adopts an extended hairpin configuration, embedding deeply into the TAG matrix. This integration stabilises the LD structure and contributes to its formation and function (Chapman et al. 2012).

Step III includes LD growth and maturation in the cytosol (Doner et al. 2025). After budding from the ER, LDs are released into the cytosol, where they undergo further growth and maturation. At this stage, the newly formed LDs are enveloped by a monolayer of phospholipids and functional proteins, with their core typically being made up of TAGs and SEs. These nascent

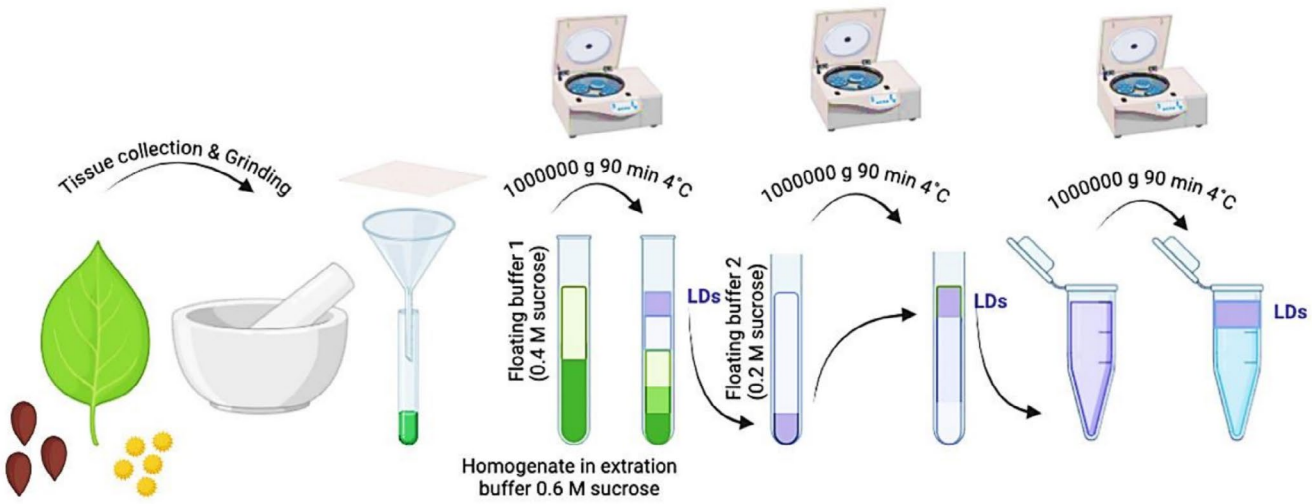

FIGURE 5 | A standard protocol outlining the key sequential steps of LDs fractioning from plant materials (PM) (seeds, leaves and pollen). Routinely, five grams of PM is enough to obtain a LD-enriched fraction. To preserve the integrity and functionality of LDs, the PM is grinding with cooling. The powder is hydrated with 10 mL of floating buffer 1. After thorough homogenisation, the extract is subjected to two successive differential centrifugations (at 10 000 × g, followed by a second centrifugation at 100 000 × g). After this stage, a floating white pad layer—consisting of LDs—is clearly appeared as upper layer. Next, the LD fraction is washed twice using a washing a floating buffer 2 (without sucrose). After a final centrifugation at 100 000 × g, the purified LD fraction is resuspended in the same buffer and stored at 4°C for further analysis.

LDs, typically around 60 nm in diameter, are referred to as "nano LDs" (Guzha et al. 2023; Olzmann and Carvalho 2019). Once in the cytosol, nano LDs can increase in size through the fusion of multiple droplets (Doner et al. 2025). This fusion process is mediated by LD-associated proteins, particularly caleosins, which have amphipathic C- and N-terminal regions exposed on the LD surface. Caleosins can form dimers with other caleosins on adjacent LDs, facilitating their merging and leading to the formation of mature LDs ranging from 0.1 to 2 μm in diameter (Hanano et al. 2023, 2006).

Another recently applied productive approach to investigating LD biogenesis involves the use of molecular dynamics simulations. These simulations extend the conventional use of conventional mimetic models that typically involve neutral lipid-enriched bilayer membranes and/or ternary trilayer systems containing a central neutral lipid bulk phase held between two phospholipid monolayers. Progress in the simulation studies is being driven by several factors, such as improved computational models for neutral lipids and their assemblies as well as more reliable structural models of LD proteins from AI-based tools such as Alpha-Fold (Dhiman et al. 2024; Mathiowetz and Olzmann 2024; Sapia and Vanni 2024). In the future, we can expect analogous advances from applying molecular dynamics simulations to nano-lipid assemblies and hybrid systems with some of their emerging biomedical applications.

5 | Fractioning of LDs From Plant Materials

For analytical purposes, lipid extraction from plant materials typically involves a mixture of polar and non-polar organic solvents, most commonly chloroform and methanol with water, to create a monophasic system that enables efficient lipid solubilisation (Bligh and Dyer 1959). However, the isolation of intact LDs from plant materials is based on their low density, allowing them to float during differential centrifugation in sorbitol gradients. This method ensures the purification of LD fractions while maintaining their native structure and preserving the catalytic activity of their associated proteins (Hanano et al. 2023). The literature provides a variety of detailed protocols for isolating LDs from plant materials. Early protocols were primarily developed for seed tissues, particularly oilseeds, where LDs are abundant. However, more recent protocols have emerged to efficiently fractionate LDs from vegetative tissues, where their abundance is comparatively low. These advances have improved the ability to study LDs across different plant organs and developmental stages (Izquierdo et al. 2020).

Regardless of whether the tissue is plant- or animal-derived, the fundamental principle of LD isolation remains the same. LDs are the least dense cellular organelles, allowing them to float during centrifugation. The process involves gently homogenising the tissue in an appropriate buffer, followed by centrifugation with a less dense overlaying buffer (floating buffer). As a result, LDs separate from the extraction buffer, rise through the floating buffer, and accumulate in a visible fat pad at the top of the centrifuge tube (Hammoudeh et al. 2020a, 2023; Hanano et al. 2006; Horn et al. 2021; Izquierdo et al. 2020). In this process, certain steps require particular attention. For instance, the composition of floating and extraction buffers presents a critical challenge for experimenters. The buffer stringency and the number of centrifugation steps must be carefully optimised to achieve a balance between obtaining clean LDs free from other cell components and preserving the full complement of LD-associated proteins (A. H. C. Huang 2018).

For the isolation of LDs from plant seeds, the first and crucial step is to effectively grind the seeds into a very fine powder. To facilitate this process, it is sometimes recommended to briefly rehydrate dry seeds in water before grinding, ensuring better homogenisation and efficient LD extraction (Hanano et al. 2016a).

Figure 5 presents a standard protocol outlining the key sequential steps involved in isolating LDs from plant material. Routinely, five grams of powdered seed is recommended for LD-enriched isolation. To preserve the integrity and functionality of LDs, all isolation steps should be performed under cold conditions. The process begins with grinding the seed tissues in the presence of liquid nitrogen to prevent enzymatic degradation. The resulting powder is then hydrated with 10 mL of floating buffer, composed of 0.1 M potassium pyrophosphate and sucrose (pH 7.4), to maintain LD stability during the isolation process (Hanano et al. 2016a, 2022). After thorough homogenisation, the preparation undergoes centrifugation at 10000×g, followed by a second centrifugation at 100000×g. At this stage, a floating white pad layer—consisting of LDs—is clearly separated from the rest of the cellular components. To ensure high purity and eliminate cellular contaminants, the LD fraction is subjected to at least two sequential washing steps using a washing buffer (floating buffer without sucrose). After a final centrifugation at 100000×g, the purified LD fraction is resuspended in the same buffer and stored at 4°C for further analysis.

The purity, encapsulation and abundance of LDs can be assessed using light microscopy, fluorescence microscopy, or flow cytometry. Staining with lipophilic dyes, such as Nile Red and BODIPY 493/503, allows visualisation and quantification of LDs in the sample (Gocze and Freeman 1994; Hammoudeh et al. 2020b; Hanano et al. 2019, 2015). In addition, immunodetection targeting LD-associated proteins can be used to examine the fractioned LDs. This involves the use of antibodies specific to proteins associated with LDs, such as anti-caleosin antibodies. Immuno-detection helps confirm the presence of specific LD proteins, allowing for further characterisation of the isolated LDs and providing insight into their functional composition (Hanano et al. 2006; Izquierdo et al. 2020).

The size and number of fractioned LDs can vary according to the plant material used. Generally speaking, seed LD diameters typically range from 0.5 to 2.5 μm, with the number of LDs per mL ranging from 10×10^3 to 65×10^3. In contrast, when LDs are isolated from vegetative tissues, this range is generally lowered to approximately 1500–2000 LDs mL^{-1} (Fabre et al. 2023; Hanano et al. 2016a; Izquierdo et al. 2020; Tzen et al. 1993). Table 1 provides a summary of the size, number per mL, and lipid-to-protein composition of fractionated LDs from various oil and non-oil seeds, as well as vegetative plant tissues. This table offers a comprehensive overview of the key characteristics of LDs isolated from different plant sources, aiding in comparisons across species and tissue types.

6 | Factors Influencing the Stability of LD Fractions

Generally, LDs in seeds carry a net negative surface charge, which helps maintain their stability as in vivo emulsion systems via electrostatic repulsion. Additionally, several studies have suggested that the proteins coating the LD surface offer physical and chemical protection, shielding them from environmental stresses such as changes in ionic strength, pH and temperature fluctuations (Chen et al. 2012; Hanano et al. 2016a; Iwanaga et al. 2007).

Enzymes, physicochemical factors and environmental conditions can degrade fractioned LDs. Understanding their performance under varying conditions is essential to improving stability for effective utilisation in different applications. Of these, pH is a critical factor influencing LD stability. As the pH of the emulsion decreases, the ζ-potential of LDs shifts from negative to positive, with the isoelectric point generally around pH 3–6 (Chen et al. 2012; Hanano et al. 2023; Iwanaga et al. 2007; Nham Tran et al. 2020). This change occurs because active protein molecules remain around the LDs after extraction. For example, the ζ-potential of LDs isolated from thraustochytrids shifted from −57 mV at pH 8 to +4 mV at pH 2.5, with the isoelectric point around pH 3 (Nham Tran et al. 2020). Additionally, when the pH of the emulsion is far from the isoelectric point,

TABLE 1 | Characteristics of fractioned LDs for various seeds and vegetative plant tissues.

Plant material	LD size (μm)	Yield	Lipid/protein/other (%)	References
Seeds				
Rapeseed	0.6–1.5	124 ± 7 per cell	75.8/14.7/9.5	Tzen et al. (1993); Yin et al. (2018)
Sunflower	2.0–2.6	NA	73.9/13.2/12.9	Gromova et al. (2015)
Flaxseed	1.5–2.0	NA	75/9.5/15.5	Boulard et al. (2015)
Soybean	0.2–0.5	NA	71.1/8.8/20.1	Iwanaga et al. (2007)
Peanut	0.6–5.4	NA	97.1/1.27/1.7	Sukhotu et al. (2016)
Coconut	1–20	NA	38.2/4.1/57.7	
Maize	0.95–2.55	NA	95.9/1.4/2.7	Sukhotu et al. (2014)
Oat	1.5–2.5	90×10^3 per mL	NA	Hanano et al. (2006)
Date palm	1.8–2.2	65×10^3 per mL	78.5/1.1/20.4	Hanano et al. (2016a)
Arabidopsis	0.5–2.0	MA	57.1/31.8/11.1	Kretzschmar et al. (2020)
Vegetative tissues				
Arabidopsis leaves	0.5–2.0	1700 per mg FW	NA	Izquierdo et al. (2020)

the mean diameter of the LDs remains small, and strong electrostatic repulsion prevents aggregation, enhancing their stability. However, when the pH approaches the isoelectric point, LD aggregation occurs, resulting in the formation of larger particles (Hanano et al. 2016a, 2022).

Regarding the effects of pH on the oxidative stability of LDs, studies have shown that lipid oxidation products, especially hydroperoxides, increase as pH decreases, due to lipoxygenase activity that particularly affects polyunsaturated residues. This indicates that oxidative stability is enhanced at higher pH values (Chen et al. 2012; Kapchie et al. 2013; Zhao et al. 2016). Moreover, salt concentration also influences the stability of LD fractions. Studies have shown that as salt concentration, particularly NaCl, increases, the ζ-potential of LDs decreases, while their mean particle size is reduced at lower NaCl concentrations but increases significantly with higher NaCl concentrations (Hou et al. 2019; Sukhotu et al. 2014). This is due to the electrostatic screening effect, where high salt concentrations reduce the ζ-potential, leading to LD aggregation. Additionally, at $CaCl_2$ concentrations of 5 mM, LDs showed a significant increase in diameter (White et al. 2008). This might be related in some part to the ability of caleosins in the presence of Ca to form dimers with other caleosins on adjacent LDs, which can lead to their merger and the formation of larger LDs (Hanano et al. 2023, 2006). Also, the presence of ferric chloride negatively affected the stability of soybean LDs at pH 7 (Kapchie et al. 2013).

Thermal treatment is another factor that significantly affects the stability of LDs. For example, heating LDs above 70°C does not cause a significant change in particle size, nor does it lead to aggregation or flocculation in the emulsions (Iwanaga et al. 2007; Sukhotu et al. 2014). However, heating does result in a change in the ζ-potential of LDs, likely due to the loss of some extrinsic proteins at the interface. The extent of the change in ζ-potential is influenced by both the heating temperature and the duration of heating (De Chirico et al. 2020; Sukhotu et al. 2014; Zhao et al. 2016). Overall, LDs show good thermal stability; for example, the composition and function of soybean LD emulsions remained stable even after prolonged storage times following heating (Zhao et al. 2016).

7 | Mimicking Natural LDs Using Artificial Lipid Nano-Droplets

The reconstruction of stable artificial LDs is technically feasible using the three fundamental components found in natural LDs across various seeds, namely, non-polar lipids such as TAGs, amphipathic phospholipids and the various LD-associated proteins (Peng et al. 2003). In recent years, ALNDs have received significant attention from the pharmaceutical industry as promising carriers for therapeutic applications (Dilliard and Siegwart 2023; Lim et al. 2025; Tenchov et al. 2021; Zhao et al. 2024). ALNDs can be synthesised through different techniques, including film hydration, reverse-phase evaporation and microfluidic hydrodynamic focusing (Maherani et al. 2012; Pattni et al. 2015). Additionally, ALNDs can undergo surface modification with specific ligands that enable targeted binding to cellular receptors, enhancing the efficiency of drug and vaccine delivery (Deshpande et al. 2013; Weissmann et al. 1975). Figure 6 shows a schematic workflow for generating ALNDs by incorporating LD-resident or structural-like proteins.

Stable ALNDs have been successfully assembled using TAGs, PLs and oleosins, either extracted from seed LDs or expressed in *Escherichia coli* (Tai et al. 2002). In this context, Chen et al. were the first to investigate whether ALNDs composed of TAGs and PLs could be stabilised by different LD-associated proteins, namely oleosin, caleosin and steroleosin. Their findings indicated that while oleosins and caleosins effectively stabilised ALNDs, steroleosins did not. The ALNDs formed with oleosins ranged in size from 0.5 to 2 μm, whereas those stabilised by caleosins were significantly smaller (50–200 nm), resembling natural LDs or being up to 10 times smaller (Chen et al. 2004). Further analysis using proteinase K digestion revealed that caleosins anchored the ALNDs via their central hydrophobic domain, approximately 4 kDa in size. The isoelectric point of caleosin-stabilised ALNDs was around pH 4.0, and ALND aggregation occurred at pH values below 4.5, suggesting that their stability and integrity were primarily maintained by surface-bound caleosins through electronegative repulsion and steric hindrance. Moreover, these caleosin-stabilised ALNDs demonstrated thermal stability up to 70°C, making them potentially valuable for various biotechnological applications (Chen et al. 2004, 2005; Chiang et al. 2010, 2011).

Recent research on ALNDs has explored their composition and the factors affecting their stability. Particular emphasis has been placed on their potential applications in pharmaceutical biotechnology and the food industry, along with strategies for enhancing their functionality (Cui et al. 2024; Yuan et al. 2024). Solid lipid nanoparticles (SLNs) are a term commonly used to describe a class of ALNDs that resembles biological LDs (Hou et al. 2021; Majsiak et al. 2021; Musielak et al. 2022). SLNs generally consist of spherical droplet-like structures of 10–1000 nm diameter with a phospholipid monolayer that surrounds a lipid core that might contain a mixture of lipidic molecules such as TAGs, DAGs, monoacylglycerols, unesterified fatty acids and waxes (Arabestani et al. 2024; Bukke et al. 2024). Various proteins can be attached to the outer monolayer of the SLN in the same way as proteins such as oleosins or perilipins are attached to biological LDs. The core lipids in the interior of SLNs surround and stabilise its principal cargo, such as anti-viral mRNA molecules, and SLNs, along with other nano-lipid assemblies, are now being used in hundreds of different biomedical applications (Zhang et al. 2023). The synthesis and therapeutic applications of SLNs based on medicinal plant formulations have recently been reviewed (Mottaqi et al. 2025). The following section provides an overview of other recent applications of LDs in pharmaceutical biotechnology.

8 | Natural and Artificial LDs as Vehicles for Pharmaceutical Applications

8.1 | LDs as Carriers of Lipophilic Therapeutics

The delivery of lipophilic drugs in therapeutic settings often poses a challenge due to their poor water solubility, which significantly reduces bioavailability and limits many of their practical clinical applications. To address this, both natural LD

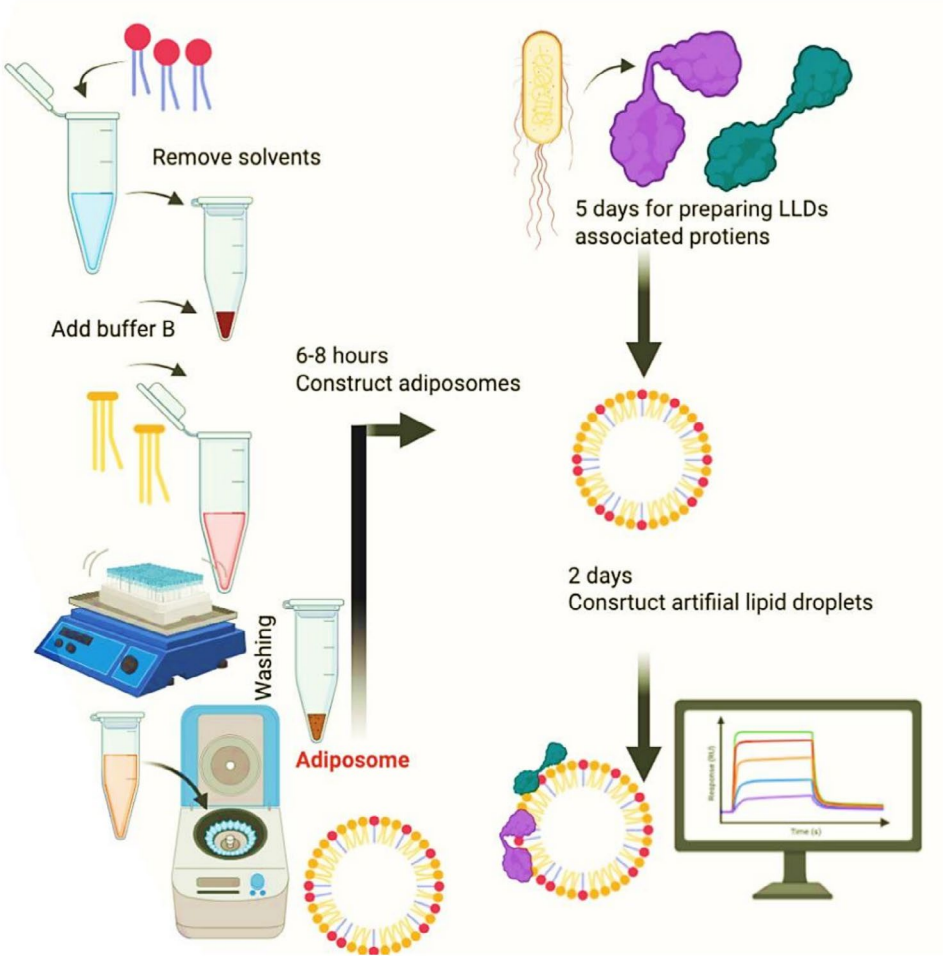

FIGURE 6 | A schematic workflow for generating artificial lipid nano-droplets (ALNDs). Adiposomes are generated by two successive steps. Step 1: formation of a phospholipid monolayer to obtain empty nanovesicles made up of a unique phospholipid layer. Step 2: The nanovesicles are filled with TAGs by simple incubation, washing, and centrifugation to finalise the formation of adiposomes, which takes about 6–8 h. Next, the adiposomes are incubated with native or recombinant LD-associated proteins (mainly oleosins or caleosins), allowing the recruitment of these proteins within the phospholipid monolayer. The ALNDs are then separated by centrifugation at 100000×g, resuspended in a floating buffer, and stored at 4°C for further analysis.

fractions and ALNDs have been proposed as carriers for lipophilic molecules. These stable oil-core carrier systems, whether derived from plants or synthetically engineered, can greatly enhance the stability and solubility of lipophilic compounds. For instance, Acevedo et al. utilised LDs fractionated from rapeseed to stabilise astaxanthin, a keto-carotenoid, demonstrating that LD-based astaxanthin exhibited much higher stability than its free form. This and similar findings highlight the potential of LDs as innovative drug delivery systems (Acevedo et al. 2014; Hama et al. 2012; Pan et al. 2018).

Another novel approach is the use of plant-derived extracellular nanovesicles which, in the case of many edible plants, have excellent biocompatibility properties that render them suitable for biomedical applications. Particularly successful examples include extracellular nanovesicles derived from ginger (Zhang et al. 2016), lemon (Yang et al. 2020) and tea flowers (Chen et al. 2022) all of which have significant anticancer properties. In a recent study, *Citrus limon*-derived nanovesicles were efficiently internalised into triple-negative breast cancer cell lines where they inhibited cell migration and proliferation, probably by disrupting several key signalling pathways (Cui et al. 2024). In all cases, these plant-derived extracellular nanovesicles were similar to the exosomes of diameter 30–150 nm that naturally play roles in the transfer of lipids, proteins and nucleic acids between many types of prokaryotic and eukaryotic cells, including plants (Kalluri and LeBleu 2020; Wang et al. 2020). Naturally occurring exosomes, with their varied cargoes that include lipids, are released from cells via exocytosis and can then be taken up by other cells via various mechanisms. Exosomes are involved in responses to many disease processes—including viral infections—where they can block viral entry function, although in some cases they can themselves be subverted for replication by certain viruses such as cytomegalovirus and herpes simplex virus 1 (Blandin et al. 2023; Kalluri and LeBleu 2020; Welch et al. 2018).

Very recent reports have uncovered intimate associations between extracellular vesicles such as exosomes and LDs, which further suggests that both plant-derived extracellular nanovesicles and LDs are worthy of further study, particularly for their potential roles in cancer treatment (Genard et al. 2024).

Indeed, in one case, the extracellular vesicles released during melanoma cell division contained a cargo that included LDs and mitochondria (Karbanova et al. 2024). Similarly, the small bilayer-enclosed exosome vesicles that are released from adipocytes can contain one or more fully intact LDs, which further reinforces the close association between these two lipidic organelles (Flaherty 3rd et al. 2019). In these cases, the exosome cargo was mainly made up of one or more micro-LDs located inside the exosome vesical, as shown in Figure 7. One can propose that such LDs could be engineered to contain lipophilic compounds of pharmaceutical interest, while their exosome carriers could also be engineered to transport them to a specific cellular target.

Another system that is already in use is artificial LDs stabilised by plant caleosins. These engineered LDs have been used to enhance the bioavailability of cyclosporine A (CsA), a hydrophobic immunosuppressive drug, when supplied to patients via oral administration (Chen et al. 2004). In this case, the CsA medication was either directly incorporated into olive oil or encapsulated in ALNDs composed of olive oil and phospholipids, with or without recombinant plant caleosin that had been expressed in and then purified from *E. coli*. Among these formulations, CsA-loaded ALNDs stabilised by recombinant caleosin demonstrated superior bioavailability compared to a commercial formulation, achieving the highest maximum whole-blood concentration, offering a promising approach for encapsulating hydrophobic drugs for oral delivery (Chen et al. 2005).

Chiang et al. (2011); Chiang et al. (2012) explored the potential of ALNDs assembled with caleosin for targeted drug delivery. Recombinant caleosin was genetically fused with an anti-HER2/neu affibody (ZH2) and expressed in *E. coli*. The Cal-ZH2-assembled ALNDs were selectively internalised by HER2/neu-positive tumour cells, achieving an internalisation efficiency of up to 90%. Additionally, the hydrophobic anticancer drug Camptothecin (CPT) was encapsulated within the Cal-ZH2-assembled ALNDs, forming ~200 nm nanoparticles that were resistant to hemolysis. CPT release from these ALNDs followed a sustained and prolonged profile under non-permissive conditions. Upon administration, Cal–ZH2-displayed ALNDs demonstrated potent antitumor activity against HER2/neu-positive cells both in vitro and in vivo, highlighting their potential as a targeted delivery system for hydrophobic drugs (Chiang et al. 2011).

Due to its poor water solubility and the presence of the blood–brain barrier (BBB), the use of paclitaxel, a highly lipophilic anticancer drug, in brain tumour treatment remains a significant challenge (Bhunia et al. 2023; Yousfan, Al Rahwanji, et al. 2024). To address this problem, a recent study was carried out using natural LDs isolated from date palm stones as carriers for paclitaxel, enhancing brain cancer therapy via intranasal delivery.

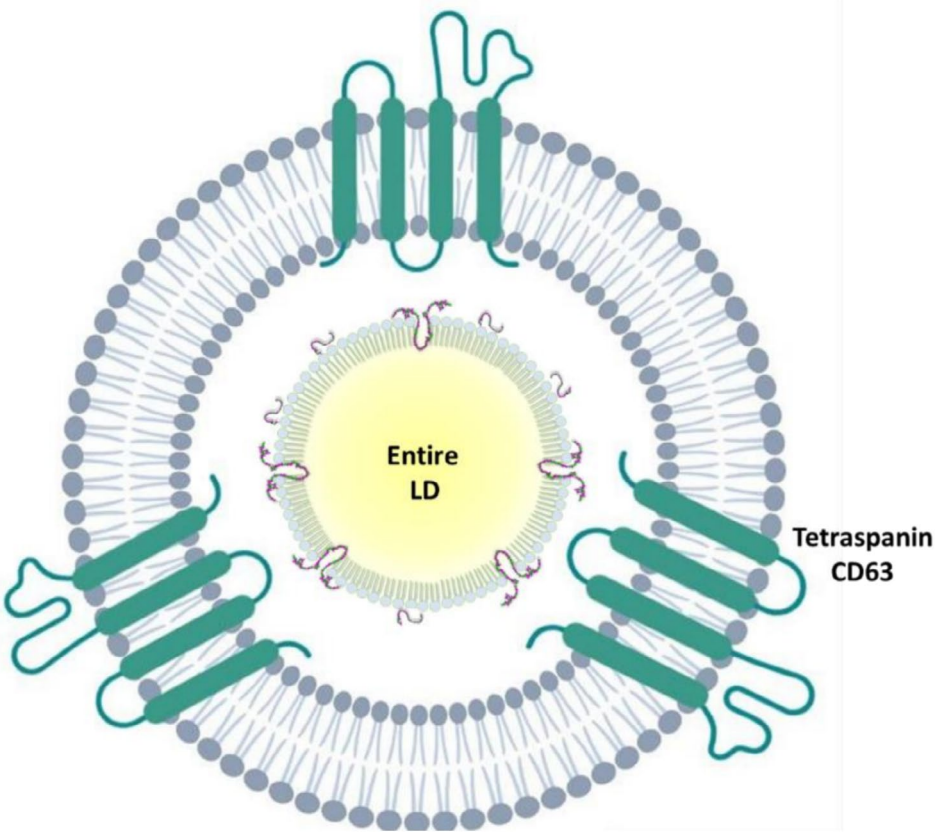

FIGURE 7 | Lipid droplet being transported as a cargo within a bilayer-bound exosome vesicle as found in adipose tissues. Exosomes are nanovesicles of 30–150 nm diameter and are ubiquitous organelles that transport lipids, proteins and nucleic acids between many cell types, but they have only rarely been found to carry intact LDs as depicted here. However, this raises the possibility of engineering exosomes to carry specific lipophilic molecules of interest within their LD cargo and delivering to a target cell of interest, such as a cancer cell. Adapted from Flaherty 3rd et al. (2019).

The results showed that LD-encapsulated paclitaxel exhibited significantly higher bio-distribution in the mouse brain compared to other organs, highlighting its potential for improved brain tumour targeting (Yousfan, Moursel, and Hanano 2024).

A further recent application of plant lipid-based nano-emulsions is based on the lipidic constituents of essential oils. Essential oils are volatile mixtures extracted from different parts of plants including seeds, flowers, fruits and leaves (Liew et al. 2020). Their major constituents include volatile monoterpenes, oxygenated monoterpenes, sesquiterpenes, esters, plus other lipophilic aromatic and aliphatic compounds (Tongnuanchan and Benjakul 2014; Yazgan et al. 2019). Uses of pure essential oils are limited due to their volatility and susceptibility to oxidative damage that limits their functionality. This means that their practical use requires some form of nano-encapsulation that enhances the chemical stability, organoleptic properties and delivery of essential oil-based products (Donsì and Ferrari 2016). Among some of the nano-encapsulation methods used are polymer-based nanocarriers (nano-capsules, nano-gels), lipid-based nanocarriers (liposomes, nano-emulsions, solid lipid nanoparticles and self-nano-emulsifying drug delivery systems) and molecular complexes. In the case of applications based on citrus oils, nano-emulsions have been particularly effective at maintaining the integrity of the oils and associated cargos over a wide range of conditions over lengthy timescales (Oprea et al. 2022). Using these technologies, it will be possible to extend the usefulness of many essential oil products by increasing their stability, extending their shelf-life and enhancing their sensory characteristics for consumers. The development of more reliable and reproducible delivery systems could also lead to additional uses of essential oil-based formulations in certain clinical settings, for example, as alternatives to antibiotics in combating microbial resistance in both human and veterinary therapy (Dupuis et al. 2022).

Plant-derived LDs are routinely used as ingredients in many cosmetic formulations where, in addition to their well-known moisturising properties, they are able to facilitate the uptake of active ingredients through the dermal barrier (Ornella et al. 2022; Pavlou et al. 2021; Pereira et al. 2016). The scope of these applications has now been extended to medical applications, such as the treatment of deep-burn injuries caused by heat or caustic substances that result in over 300 000 fatalities each year. LDs from the small oilseed plant, *Camelina sativa*, have already shown promise for biotechnology applications (Haslam et al. 2025), including as topical agents due to their high biocompatibility and capacity to carry large payloads as well as their rapid absorption into inner dermal layers (Gao et al. 2021). Freeze-dried *Camelina* LDs have now been used to deliver a formulation of the polypeptide agent, Human Basic Fibroblast Growth Factor-2 (hFGF2), expressed as a recombinant oleosin fusion that was present on the engineered LD surfaces (Gao et al. 2023). Free hFGF2 can be used to prevent inflammation and facilitate repair after serious wounds and chronic ulcers, but its absorption into damaged skin can be slow. However, when hFGF2 was present as an oleosin fusion on *Camelina* LDs, the entire ensemble was efficiently taken up through the stratum corneum cuticular barrier and delivered as an active therapeutic agent into deep layers of skin damaged by second-degree burns (Gao et al. 2023). These achievements should encourage research into the wider use of different forms of LD systems and derivatives for a broad range of biotechnological applications.

8.2 | LDs as Carriers of Polypeptides

Several studies have demonstrated that plant LDs can serve as effective expression systems for producing recombinant pharmaceutical peptides in addition to full-length proteins. Examples include growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), interleukin (IL) and nerve growth factor (NGF), all of which have been successfully expressed in plant LDs. For instance, human insulin-like growth factor 1 (hIGF-1) was expressed in *Arabidopsis thaliana* seed LDs using oleosin fusion technology, achieving an expression level of approximately 0.17% of total seed protein. In vitro cell experiments confirmed the biological activity of the plant-expressed hIGF-1 (Li et al. 2011). In the same fashion, recombinant human fibroblast growth factor 9 (rhFGF9) was successfully expressed in LDs of *A. thaliana* and *Carthamus tinctorius*. The plant-derived rhFGF9 was shown to effectively promote cell proliferation, stimulate hair growth and enhance wound healing, demonstrating its potential for therapeutic application (Cai et al. 2018; Yi et al. 2015). Moreover, Qiang et al. (2019) successfully expressed recombinant acidic FGF in the LDs of *A. thaliana* and assessed its dermal safety. In another example, LD-oleosin-fusion technology was used to efficiently express recombinant human precursor insulin (DesB30-insulin) in *A. thaliana* LDs (Nykiforuk et al. 2006). The purified recombinant insulin was then digested and matured by trypsin before clinical application (Kjeldsen et al. 2001). The biological activity tests showed that the matured plant-derived insulin could lower blood glucose as effectively as Humulin and Roche insulin, with no reported adverse side effects (Nykiforuk et al. 2006).

In addition to the expression of soluble peptides or proteins fused to oleosin on LDs, it was recently shown that hydrophobic proteins could be expressed as oleosin fusions (Fontaine 2025). As proof of concept, the E and M membrane proteins of the virus SARS-CoV-2 were expressed as recombinant oleosin fusions in *A. thaliana* and *Nicotiana benthamiana* (Gissot et al. 2023). In a wide-ranging study, several different LD protein-based carrier systems were compared with several types of cargo proteins (Fontaine 2025). One conclusion of this study was that the use of predictive methods such as ColabFold: AlphaFold2 or Membranome 3.0 simulations to predict the structures of LD-associated proteins was often unreliable, as these AI models had been trained almost exclusively on lipid bilayer-associated proteins. Therefore, one recommendation for future research is to develop new versions of AlphaFold2 or similar tools where the software has been trained using proteins located on phospholipid monolayers as part of LD or LD-mimetic systems.

The potential role of LDs as expression systems can be considered in two ways: independent and dependent. For LDs to independently translate RNA codons into peptides, their internal environment would need to recruit a full set of translational components—including tRNAs, ribosomes and mRNA—and maintain them in a compatible physicochemical state. However, given the lipidic nature of the LD core, it is predicted that lipids

would interact with these components in ways that disrupt or inhibit the process, which is naturally optimised to occur in the aqueous cytosolic environment.

In contrast, nuclear LDs may contribute to gene expression in a dependent manner. They can provide lipids for nuclear envelope expansion and serve as platforms for regulating transcription by scaffolding specific transcription factors. For example, in yeast, the transcriptional repressor Opi1, which regulates phospholipid biosynthetic genes, is tethered to nuclear LDs in close proximity to its target genes at the nuclear envelope (Olzmann and Carvalho 2019). Similarly, in hepatocytes, nuclear LDs associate with promyelocytic leukaemia nuclear bodies, which play a role in transcriptional regulation (Ohsaki et al. 2016). Moreover, in mammals, PLIN5 has been observed in the nucleus, where it forms part of a transcriptional regulatory complex that controls mitochondrial gene programs (Gallardo-Montejano et al. 2016).

8.3 | LDs as Carriers of Nucleic Acids

Messenger RNA (mRNA) has become a promising therapeutic agent for preventing and treating various diseases, particularly in the case of mammalian viruses (Pardi and Krammer 2024; Shi et al. 2024). However, for mRNA to function effectively in vivo, it requires a stable and efficient delivery system that protects it from degradation while facilitating cellular uptake and release. ALNDs have proven to be a successful platform for mRNA delivery, with ALNDs-based mRNA vaccines now in clinical use against COVID-19, marking a significant advance in mRNA therapeutics (Oliver et al. 2020; Zhang et al. 2020).

LDs/ALNDs encapsulate negatively charged nucleic acids through the formation of electrostatic complexes with ionizable lipids within lipid nanoparticles. This process relies on interactions between the positively charged ionizable lipids, phospholipids, cholesterol and PEGylated lipids with the negatively charged phosphate backbone of nucleic acid polymers, thereby promoting their incorporation into the emerging nanoparticle. To achieve efficient protonation of the ionizable lipids, ALNDs are typically prepared under acidic conditions (~pH 4), which is substantially lower than the apparent pK_a of the ionizable lipids (~6.5), and subsequently adjusted through a buffer/pH exchange step (Arte et al. 2025; Chen et al. 2024). Effective nucleic acid encapsulation is enabled by the arrangement of ionizable lipids into an 'inverted micellar' structure within the ALNDs core (Leung et al. 2012). This structure is driven by the inverted cone geometry of the lipids, where the hydrophobic tail region is larger than the polar headgroup, creating a favourable configuration for surrounding and stabilising nucleic acids. The combined action of these lipids results in the formation of stable, monodisperse nanoparticles that protect the nucleic acid cargo from enzymatic degradation and other environmental stresses, while also enhancing cellular uptake.

In addition to ALNDs, plant seed-derived LDs have also been utilised for vaccine development, as originally described in a patent by SemBioSys Genetics Inc. (https://patents.google.com/patent/WO2004111244A2/en). This patent outlines two approaches whereby the antigen can either be physically associated with the LD surface or alternatively expressed as a recombinant fusion protein with oleosin, ensuring its targeted presentation on the LDs. Following cleavage of the fusion protein from its oleosin carrier, these LD-based vaccines can be purified and subsequently administered to patients via percutaneous or mucosal routes to effectively induce an immune response against various antigens (Deckers et al. 2004).

8.4 | LDs as Carriers of Nutrient and Health Supplements

LD-based natural emulsions have diverse applications in dairy-based foods, including yogurt, imitation (non-dairy-based) milk, salad dressings and creams. For instance, LDs extracted from plants and microalgae can serve as alternatives to milk fat globules in yogurt, yielding products with comparable lipid content (~3% w/w) while enriching them with polyunsaturated fatty acids for added health benefits (Mantzouridou et al. 2019). There are increasing markets for such products among consumers who wish to avoid foods derived from animal sources. Additionally, LDs can replace egg yolk emulsifiers in salad dressings, enhancing flavour and product stability, and improving rheological properties (Hou et al. 2019; Nikiforidis 2019). Moreover, natural LDs fractionated from rapeseed have been utilised to encapsulate curcumin, a pH-dependent fluorescent therapeutic. Under acidic conditions, curcumin is converted into its hydrophobic keto form, enabling rapid diffusion into LDs within seconds. This highlights the efficient loading mechanism of lipophilic molecules into plant-derived LDs (Vardar et al. 2024).

Compared to natural LDs, ALNDs offer greater flexibility in food formulations by allowing compositional optimization to enhance metabolic properties. Current research primarily explores the impact of lipid composition on human metabolism (Yuan et al. 2024). Typically, the hydrophobic core of ALNDs consists of vegetable oils rich in TAGs. However, excessive TAG intake can lead to fat accumulation and obesity. In contrast, diacylglycerol (DAG) has been shown to more effectively inhibit fat accumulation (Yu et al. 2017). Lu et al. (2020) demonstrated that consumption of dietary DAG-enriched oils significantly reduced body weight, kidney weight and serum TAG levels in mice compared to TAG-enriched oils, thereby improving lipid metabolism. In a parallel study, Liu et al. (2022) found that supplementing a high-fat diet with fish oil led to reduced body weight and alleviated symptoms of dyslipidaemia and hepatic steatosis in mice. Overall, replacing TAG with DAG or incorporating fish oil into TAG are both effective strategies for enhancing lipid metabolism in ALNDs.

9 | Conclusions and Future Prospects

Plant LDs have garnered increasing interest as natural, biocompatible carriers with an ever-increasing list of real-life or potential applications in pharmaceuticals, food and biotechnology. Despite significant progress in understanding LD structure, biogenesis and fractionation, several challenges and opportunities remain for future research and industrial applications. One key area of interest is the optimization of LD extraction and purification techniques to enhance yield, purity and stability. Developing scalable and cost-effective fractionation methods

will be crucial for the large-scale production of plant LDs for pharmaceutical use. Advanced bioengineering approaches, such as genetic modification, genome editing and synthetic biology, can also be leveraged to tailor LD composition, enabling the production of specialised LDs enriched with bioactive lipids, engineered proteins and a range of functional cargo molecules for therapeutic applications.

The latter approach could functionalize plant LDs for targeted delivery of a wide range of drugs or other therapeutic agents. By incorporating specific ligands or fusion proteins, LDs can be designed to deliver such agents with high precision, enhancing their bioavailability and clinical efficacy. This is particularly relevant for the encapsulation of hydrophobic drugs, which suffer from poor water solubility and bioavailability. Further investigation into LD stability under physiological conditions will be essential to improve their performance in in vivo applications. Additionally, plant LDs offer potential as vaccine delivery systems, as demonstrated by recent patents and experimental studies. Future research should explore the immunogenicity of LD-based formulations and their potential for mucosal or transdermal vaccination strategies. Another requirement will be to ensure that any LD-based formulations for use in humans are thoroughly tested for allergenicity. For example, it is already known that some seed oleosins, such as in peanuts, can act as allergens in some contexts (Majsiak et al. 2021), although it might be possible to remove problematic epitopes as part of the process of engineering a recombinant version of these proteins (He et al. 2024). Finally, the sustainability and regulatory aspects of plant-derived LDs need to be addressed to facilitate their commercialization. Establishing standardised guidelines for safety, efficacy and production will be vital for their acceptance in pharmaceutical and nutraceutical industries. Overall, advancing our understanding of plant LDs and their functionalization will unlock new possibilities for drug delivery, therapeutic formulations and beyond, making them a promising frontier in biotechnology and medicine.

Author Contributions

Abdulsamie Hanano: conceptualisation, data curation and writing the original draft of the manuscript; **Amal Yousfan:** data curation and visualisation; **Denis J. Murphy:** co-writing, reviewing and editing.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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