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# Biological recognition and cellular trafficking of targeted RNA-lipid nanoparticles

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Lipid nanoparticles (LNPs) have unlocked the potential of ribonucleic acid (RNA) therapeutics and vaccines. Production and large-scale manufacturing methods for RNA-LNPs have been established and rapidly accelerate. Despite this, basic research on LNPs is still required, due to their high assembly complexity and fairly new development, including research on lipid organization, transfection optimization, and *in vivo* behavior. Understanding fundamental aspects of LNPs that is, how lipid composition and physicochemical properties affect their biodistribution, cell recognition, and transfection, could propel their clinical development and facilitate overcoming current challenges. Herein, we review recent developments in the field of LNP technology and summarize the main findings focusing on nano-bio interactions.

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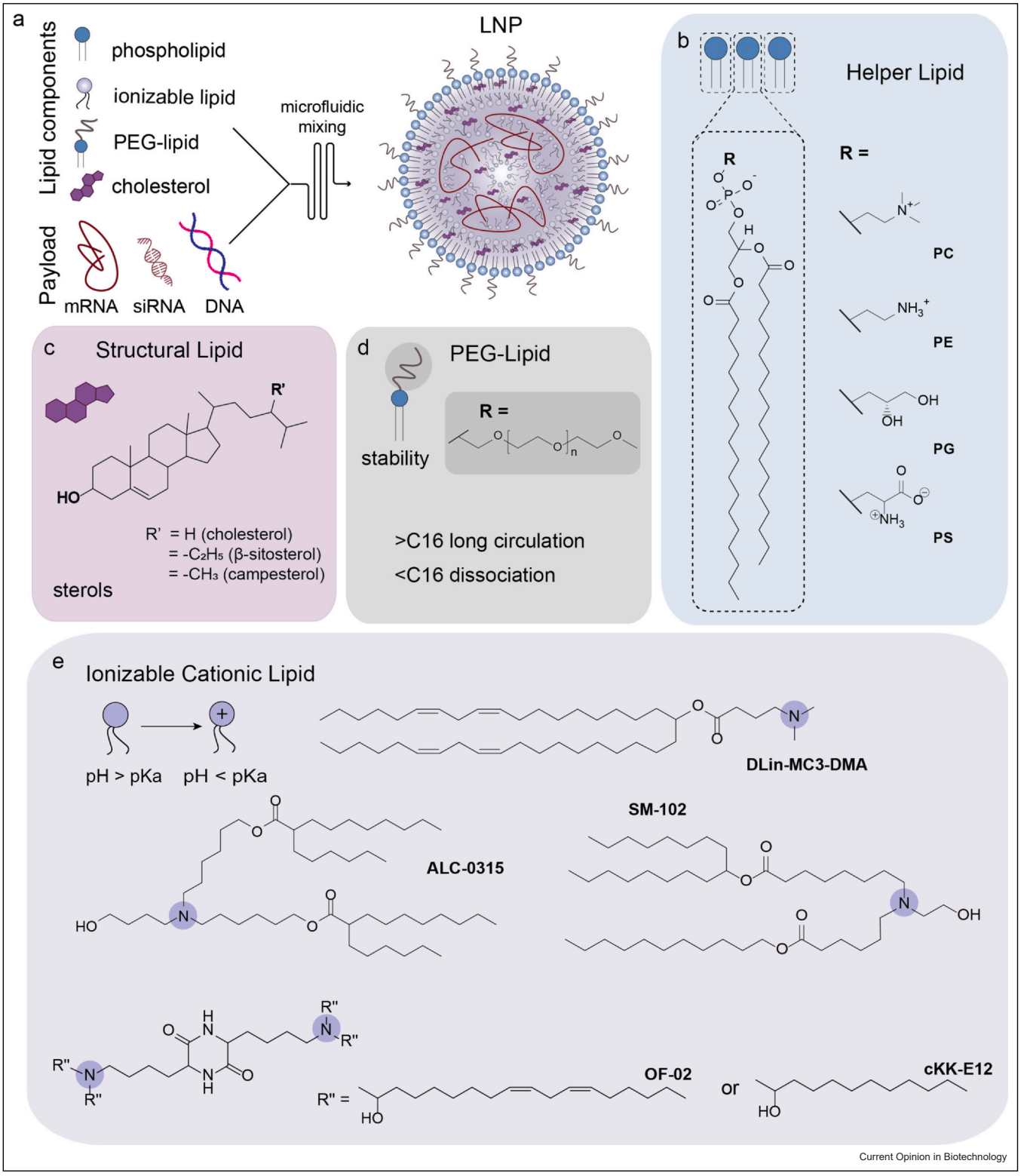
## Introduction

Lipid nanoparticles (LNPs) are the most successful nanocarriers for the intracellular delivery of exogenous ribonucleic acids (RNAs) used for gene silencing (siRNA) [1•,2] expression (mRNA) [3,4] and editing (CRISPR/Cas9) [5,6]. Three RNA-LNP products have already been approved for clinical use, including Onpattro® — the first siRNA-LNP drug for the treatment of polyneuropathies resulting from the hereditary transthyretin amyloidosis — and the two mRNA-based vaccines against severe acute respiratory syndrome coronavirus 2 [1•,7,8]. LNPs are kinetically stable spherical particles exhibiting a diameter of ~100 nm and near-neutral surface charge at physiological pH [7–9]. The 'optimal' combination of the LNP components allows for efficient RNA entrapment and cytosolic delivery. Moreover, lipid components can individually dictate circulation lifetimes, biodistribution, cell selectivity, or transfection potency and therefore rational design of LNPs is of great importance for the outcome of each individual therapeutic. LNPs have a sophisticated nanostructure based on the synergistic effect of individual lipid components comprising the assembly (Figure 1) [10]. The components include 'helper' lipids that provide structural integrity (i.e. phospholipids and polyethylene glycol [PEG]-lipids), hydrophobic 'structural' lipids that reside in the LNP core (i.e. cholesterol), and ionizable cationic lipids (ICLs), that complex RNA and facilitate transfection. ICLs with an apparent pKa between 6 and 7, allow electrostatic interaction-driven complexation with nucleic acid molecules in acidic pH, followed by LNP assembly [11].

LNPs — such as other nanocarriers — encounter several biological barriers, which could potentially reduce their performance. Upon intravenous administration, LNPs must avoid immune detection, prevent nonspecific interactions with proteins and nontarget tissues, reach and internalize into the target cells, and finally facilitate endosomal escape for payload release in the cytosol [12–14]. Notably, it has been estimated that only a small percentage of the injected LNP dose reaches its target cells, and that less than 2% of the LNP-siRNA reaches the cytosol after endocytosis [15]. On this journey, LNPs interact with several biomolecules (serum proteins) and biological structures (cell membranes), which influence the ability of LNPs to deliver their payload into the target site. Such nano-bio interactions, which in turn determine biodistribution and LNP fate, are mainly determined by the physicochemical properties of LNPs, including lipid composition, particle size, surface charge, apparent $pK_a$, and morphology. While a large number of papers reviewing the use of RNA-LNPs as therapeutics or vaccines has extensively been published [12–14], less attention has been paid to the understanding of their nano-bio interactions.

**Figure 1**


a
Lipid components
phospholipid
ionizable lipid
PEG-lipid
cholesterol
Payload
mRNA
siRNA
DNA
microfluidic mixing
LNP
b
Helper Lipid
R
R =
PC
PE
PG
PS
c
Structural Lipid
R'
HO
R' = H (cholesterol)
= -C2H5 (β-sitosterol)
= -CH3 (campesterol)
sterols
d
PEG-Lipid
R =
stability
>C16 long circulation
<C16 dissociation
e
Ionizable Cationic Lipid
pH > pKa
pH < pKa
DLin-MC3-DMA
ALC-0315
SM-102
OF-02
R" =
or
cKK-E12
Current Opinion in Biotechnology

Molecular properties of nucleic acid-based LNPs. **(a)** Individual lipid components and nucleic acid payloads used in LNPs and formation of the nanoparticle after microfluidic mixing. **(b)** Molecular structures of commonly used helper lipids. Phospholipids can vary on head group or chain length and saturation. **c)** Structural lipids are usually sterols. Clinically approved LNPs incorporate cholesterol [1,7,8], however other sterol analogs (i.e. sito-, campe-, etc.) have been observed to influence LNP morphology and mRNA transfection [54,55]. **(d)** PEG-lipids providing structural and long-term stability on the LNP are also used to prolong circulation lifetimes or can be designed to dissociate for LNP interaction when in contact with biological fluids. **(d)** ICLs contain tertiary amine-based head groups that, when the pH is lower than their $pK_a$, they become protonated and thus positively charged. Abbreviations: PC = phosphatidylcholine, PE = phosphatidylethanolamine, PG = phosphatidylglycerol.

Herein, we briefly summarize key findings that connect LNP properties and *in vivo* behavior, and critically discuss the remaining challenges and necessary steps for the advancement of the field toward clinical success.

## Lipid nanoparticle–serum protein interactions

After administration, LNPs instantly interact with biological components, particularly, plasma proteins and mononuclear phagocytic cells (monocytes, macrophages, and dendritic cells) responsible for clearance. PEGylation has shown to prolong the blood circulation half-life of LNPs by offering a 'stealth effect' that reduces interactions with immune system-triggering proteins, for example, opsonins, which recognize and bind to macrophage and phagocyte receptors [16,17]. However, PEGylation, especially in high mol% content, also decreases particle–cell membrane interactions and hence reducing transfection potency [16,18]. Considering this, PEG-lipids can be designed to dissociate from the LNP surface upon contact with biological fluids, and exchange with plasma lipoproteins, a process termed PEG-lipid shedding [16,19]. The PEG-lipid dissociation rate from LNPs in blood circulation is highly determined by the PEG-lipid anchor length (Figure 1). C14 anchor PEG-lipids dissociate faster (> 45%/h) than C18 anchor PEG-lipids (0.2%/h) [16,20]. After PEG-lipid shedding, serum proteins are rapidly adsorbed on the LNP surface, leading to the formation of a protein layer called protein corona, which modifies the physicochemical features of RNA-LNPs and determines their biodistribution and cellular uptake [21,22]. The protein corona of several LNP formulations has been recently found to be rich in apolipoproteins (Apo), which are known to facilitate cellular uptake via receptor-mediated endocytosis [23,24]. For instance, the selective recognition and hepatic uptake of Onpattro® relies on the adsorption of ApoE, which binds to low-density lipoprotein receptor (LDLR), abundantly expressed on hepatocytes [1•]. The strong interaction between the LNPs and N-terminal lipid-binding region of ApoE via tryptophan residues causes conformational changes in ApoE, which results in a high affinity to LDLRs [21].

## Interactions of lipid nanoparticles with tissues and cells

Considering that nanoparticle physicochemical properties will profoundly influence (desired and undesired) nano-bio interactions, LNPs can be engineered for cell and tissue-selective targeting and payload delivery (Table 1).

Systemic administration of Onpattro®, that exploits the ApoE-LDLR uptake pathway, resulted in liver accumulation and preferential uptake by hepatocytes [1]. In turn, by replacing the ICL Dlin-MC3-DMA for ALC-0315, a higher LNP accumulation in hepatic stellate cells (HSCs) was observed, likely due to differences in protein corona composition [25]. Moreover, hepatocyte targeting could be achieved by producing LNPs with sizes smaller than the fenestration diameter in the liver [26]. We also recently demonstrated that switching the LNP surface charge from neutral to anionic, leads to the preferential accumulation of Dlin-MC3-DMA-based LNPs in the hepatic reticuloendothelial system (RES), mediated by the scavenger receptor Stabilin-2 [27•]. In a similar fashion, Kim et al. explored the ability of LNPs to target liver sinusoidal endothelial cells (LSECs) by functionalizing their surface with mannose-based targeting ligands, resulting in selective mRNA delivery to LSECs [28].

LNPs can be also engineered to target non-liver tissues by modulating the lipid composition, and therefore modifying properties such as surface charge and apparent $pK_a$ of LNPs. For instance, the replacement of neutral phospholipids (DSPC or DOPE) with anionic (1,2-distearoyl-sn-glycero-3-phospho-(1'-rac-glycerol) or 18:PA) or cationic (DOTAP or 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine) alternatives, leads to redirection of mRNA delivery from liver to spleen and lungs, respectively [29••,30]. These differences in organ targeting can be only achieved when the phospholipid concentration in LNPs is 40 mol% [29••]. On the other hand, the Siegwart group recently developed a new strategy called selective-organ targeting (SORT) wherein a fifth component (SORT molecules) is added to LNP formulations to selectively target extrahepatic tissues through passive targeting (Figure 2) [31,32]. In this context, systemically administered LNPs containing SORT molecules such as cationic 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), anionic (18PA), and ionizable 1,2-dioleoyl-3-dimethylammonium-propane lipids enabled selective mRNA delivery and CRISPR–Cas gene editing to lung, spleen, and liver, respectively [33••]. Particularly, 50% DOTAP-SORT-LNPs preferentially accumulated in lung endothelial

**Table 1**

**A selection of reviewed LNPs that demonstrated enhanced tissue/cell targeting.**

| Lipid composition | Lipid ratio | Target tissue | Target cell | Payload | Function | Ref |
|---|---|---|---|---|---|---|
| DSPC, CHO, DLin-MC3-DMA, DMG-PEG2k | 10/38.5/50/1.5 | Liver | Hepatocytes | siRNA | Transthyretin silencing (treatment for amyloidosis) | [1] |
| DSPC, CHO, ALC-0315, DMG-PEG2k | 10/38.5/50/1.5 | Liver | HSCs | siRNA | ADAMTS13 silencing (biodistribution studies) | [26] |
| DSPG, CHO, DLin-MC3-DMA, DMG-PEG2k | 10/38.5/50/1.5 | Liver | LSECs | mRNA | eGFP/mCherry expression (biodistribution studies) | [27] |
| DOPE, CHO, 246C10, mannose-PEG2k | 20/50.5/26.5/3 | Liver | LSECs | mRNA | mFLuc expression (biodistribution studies) | [28] |
| DOPE, CHO, 306$O_{10}$, DMPE-PEG2k | 35/40/22.5/2.5 | Liver | - | mRNA | mFLuc expression (biodistribution studies) | [29] |
| DOPS, CHO, 306$O_{10}$, DMPE-PEG2k | 35/40/22.5/2.5 | Spleen | - | mRNA | mFLuc expression (biodistribution studies) | [29] |
| DOTAP, CHO, 306$O_{10}$, DMPE-PEG2k | 35/40/22.5/2.5 | Lung | - | mRNA | mFLuc expression (biodistribution studies) | [29] |
| 5A2-SC8, DOPE, CHO, DMG-PEG2k,18PA (SORT) | 16.7/16.7/33.3/3.3/30 | Spleen | Macrophages | mRNA | mFLuc expression (biodistribution studies) | [31,33] |
| 5A2-SC8, DOPE, CHO, DMG-PEG2K, DODAP (SORT) | 19.05/19.05/38.1/3.8/20 | Liver | Hepatocytes | mRNA | mFLuc expression (biodistribution studies) | [31,33] |
| 5A2-SC8, DOPE, CHO, DMG-PEG2k, DOTAP (SORT) | 11.9/23.8/11.9/2.4/50<br>11.9/11.9/23.8/2.4/50 | Lung | Endothelial cells | mRNA | mFLuc expression (biodistribution studies) | [31,33] |
| PBA-Q76-O16B, NT1-O14B (NT-lipidoid) | 5/5 and 3/7[a] | Brain | Neuronal cells | ASO | Tau silencing (biodistribution studies) | [35] |
| DOPE, CHO, BP-lipid, DMPE-PEG2k | 16:46.5:35:2.5 | Bone surface and marrow | - | mRNA | mFLuc expression (biodistribution studies) | [36] |

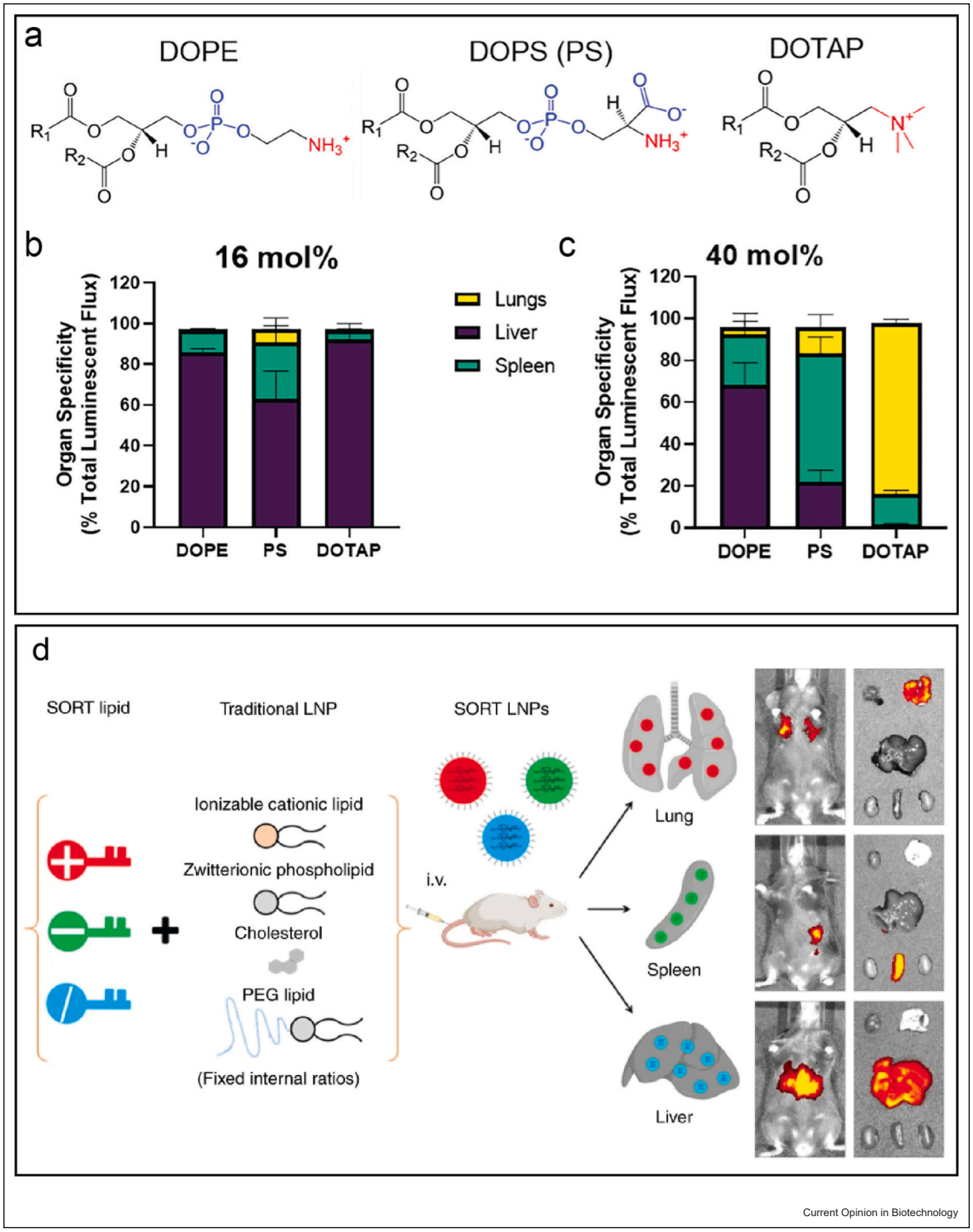
Abbreviations in the table that cannot be found in main text: ASO = antisense oligonucleotide.
1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE); 1,2-distearoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DSPG); 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP); 1,2-dioleoyl-3-dimethylammonium-propane (DODAP).
[a] w/w ratio.

cells, while 30%-18PA-SORT-LNPs did in splenic macrophages. Mechanistically, tissue-specific targeting by SORT LNPs occurs via PEG-lipid shedding, protein corona formation — which composition is determined by the SORT molecule employed — and subsequent interactions with receptors abundantly expressed in the target tissue [34••]. The addition of SORT molecules modifies the apparent $pK_a$ of LNPs and further affects the LNP–serum protein interactions. Liver-targeting SORT LNPs exhibited an apparent $pK_a$ in the range of 6–7 and acquired an ApoE-rich protein corona. SORT LNPs with higher apparent $pK_a$ values (> 9) had a protein corona rich in vitronectin, which mediated lung targeting due to the abundant expression of vitronectin receptors by lung endothelial cells. On the other hand, the protein corona displayed on spleen-targeting SORT LNPs (apparent $pK_a$ between 2 and 6) was found to be rich in β2-glycoprotein I, which bound to phosphatidylserine (PS)-exposing blood cells, promoting LNP filtration from blood circulation to the spleen [35•]. Instead of incorporating additional components to LNP formulations, synthesis of a new generation of ICLs containing targeting head groups has been also exploited in order to target different organs via active targeting. For instance, Xu and coworkers synthesized a series of neurotransmitter-based lipoids that have the ability to cross the blood–brain barrier, leading to a higher accumulation of LNPs and subsequent payload release in various different regions of the mouse brain, including cerebral cortex, hippocampus, and cerebellum [35•]. A similar approach has been employed by the Mitchell group to deliver mRNA into the bone marrow microenvironment [36]. They designed ICLs containing bisphosphonate (BP) head groups that displayed a high affinity for hydroxyapatite and bone surfaces, which improved LNP accumulation and mRNA transfection in the bone microenvironment.

## Cellular uptake

Receptor-mediated endocytosis is the main process by which RNA-LNPs are taken up by cells. Early studies showed that fluorescently labeled siRNA-LNPs enter cells via clathrin-mediated endocytosis [15], which was further corroborated for mRNA-LNPs [21]. This endocytic pathway is responsible for the cellular uptake of lipid- and cholesterol-enriched LDLs by binding to ApoE that targets them to LDLRs [37]. As mentioned above, the LNP–protein corona complex is enriched with ApoE after PEG-lipid shedding, to endogenously target LDLRs [15,38]. It has recently been shown that ApoE adsorption causes rearrangement of LNP components, affecting the LNP nanostructure, which is expected to affect the intracellular RNA delivery mediated by LNPs [21]. LNPs can be also functionalized with antibodies for cytosolic mRNA delivery via caveolae-mediated endocytosis in lung endothelial cells

**Figure 2**


a
DOPE
DOPS (PS)
DOTAP
b
16 mol%
Organ Specificity (% Total Luminescent Flux)
120
100
80
60
40
20
0
DOPE
PS
DOTAP
Lungs
Liver
Spleen
c
40 mol%
Organ Specificity (% Total Luminescent Flux)
120
100
80
60
40
20
0
DOPE
PS
DOTAP
d
SORT lipid
Traditional LNP
SORT LNPs
Ionizable cationic lipid
Zwitterionic phospholipid
Cholesterol
PEG lipid
(Fixed internal ratios)
i.v.
Lung
Spleen
Liver
Current Opinion in Biotechnology

Lipid components used for extrahepatic LNP targeting. **(a)** Phospholipids, such as DOPE, DOPS, and DODAP, were used as helper lipids in LNP formulations in **(b)** moderate (16% mol) or **(c)** high (40% mol) content. In moderate helper, lipid content LNPs targeted mostly the liver. In high content, DOPS and DOTAP formulations shifted the biodistribution from liver to spleen and lungs, respectively. **(d)** SORT strategy. SORT lipids, particularly a cationic (DOTAP), an anionic (18PA), or an ionizable (DODAP) lipid, are added as a fifth lipid component to LNP formulations for selective targeting of lung, spleen, and liver, respectively. This image is used with permission by reference [33].
Image was adapted from reference [29].

expressing caveolae-associated proteins [39]. Importantly, studies using small molecules to block endocytic pathways revealed that macropinocytosis and phagocytosis also contribute to the cell internalization of some LNP formulations [15,40]. In fact, immunes cells commonly use phagocytosis to engulf and destroy exogenous particles, including LNPs [41••,42]. Note that positively charged LNPs may be taken up by clathrin- or caveolae-mediated endocytosis after electrostatic interactions with anionic macromolecules, such as proteoglycans and glycosaminoglycans of cell membranes [41••]. Finally, membrane fusion has been recently employed to deliver siRNA and mRNA in mouse and human cells via LNPs containing membrane-fusogenic cationic lipids or fusogenic coiled-coil peptides [42,43]. Direct fusion with the cell membrane allows for payloads to be directly delivered to the cytosol, avoiding therefore endocytic routes from where LNPs need to escape [43].

## Intracellular trafficking

Following endocytosis, LNPs are entrapped in endosomes that, eventually, fuse with lysosomes [11,38]. In early endosomes, physiological pH (~7.4) decreases to acidic values (pH~6.5), and after further endosome maturation (late endosome), pH decreases even further (pH~6.0) before final fusion with lysosomes (pH~5.0) [44]. For cytosolic RNA delivery, LNPs must escape the endosomes before lysosome formation that eventually leads to LNP and RNA degradation (Figure 3). The pH-responsive property of ICLs is pivotal for endosomal escape. In the acidic endosomes, ICLs are positively charged and electrostatically interact with negatively charged phospholipids of the endosomal membrane, creating nonbilayer liquid-crystalline phases (i.e. inversed hexagonal phase [$H_{II}$]), which facilitate endosomal disruption and escape of RNA into the cytosol (Figure 3) [9,11,45]. The optimal $pK_a$ of ICLs has been intensively studied and LNPs with excellent transfection potency display apparent $pK_a$ values between 6.0 and 6.7 [3,4,46]. However, not only the $pK_a$, but also other structural properties affect the ability of ICLs to induce transfection. ICLs with an accentuated cone-shape molecular structure, such as the clinically approved Dlin-MC3-DMA, ALC-0315, and SM-102, have shown to destabilize the lipid bilayer of cell membrane [46–48]. Moreover, a recent study showed that LNPs with cubic and hexagonal nanostructures fuse more easily with endosomal membranes than lamellar LNPs, resulting in higher endosomal escape rates [45]. In a similar fashion, induction of phase-separated blebs in the LNP nanostructure led to enhanced transfection efficiency [49••].

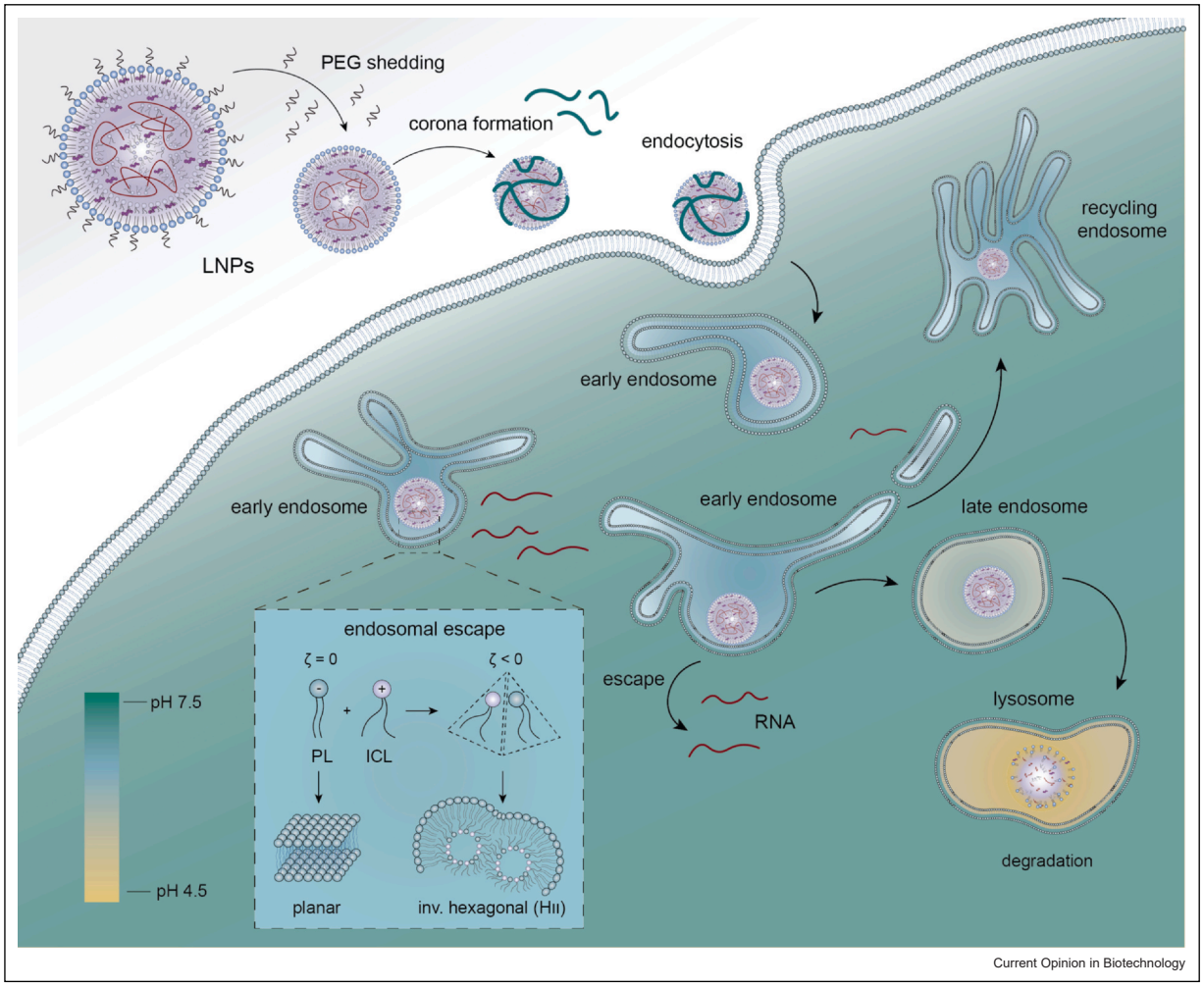
Although the process of endosomal escape by LNP has not been completely resolved yet, pioneering studies reported that LNP-RNA escape occurs in the early and late endosomes [15,50,51]. Recruitment of cytosolic galectins by damaged endosomes in response to LNPs as well as colocalization of LNPs with endosomal trafficking proteins, such as the EEA1, the APPL1, and Rab11, corroborate endosomal escape [15,41,52]. More recently, Zerial and coworkers showed that mRNA-LNPs displaying high transfection efficiency accumulated in early- and recycling endosomes, where escape mainly occurred, probably because of their positive and negative membrane curvatures that favor interactions with ICLs, leading to membrane leakages [41]. In this respect, it is interesting that Patel et al. showed that by substituting cholesterol to 25% and 50% 7a-hydroxycholesterol in mRNA-LNPs reduced the presence of recycling endosomes and observed enhanced mRNA delivery to primary human T cells *ex vivo* by 1.8-fold and 2.0-fold, respectively [53]. Moreover, other sterol analogs have been also observed to induce morphological changes in LNPs and increase mRNA potency, likely due to different endosomal trafficking and enhanced endosomal escape [54,55].

## Discussion and conclusion

Multicomponent systems, such as LNPs, exert intricate morphologies and properties and, due to the large variety of all individual lipid components and their combinations, LNPs appear to be endlessly tunable.

Despite the exciting advancements made in the field of lipid-based nanomedicine, cell/tissue selectivity for LNPs, accompanied with efficient RNA delivery and expression, remains a challenge. The 'liposome boom' in the 1990s that — up to today — has failed to deliver a product with extrahepatic targeting to the clinic, is a lesson to be learned for LNPs [56]. The preclinical development pipeline of nanomedicines usually follows that of traditional drug discovery, however, there are two main aspects that require consideration. First, LNP-based therapeutics still presents somewhat uncharted regulatory territories, which could lead to several implications in drug development. For example, it is not

**Figure 3**




Internalization of RNA-LNPs and endosomal escape. After RNA-LNPs reach the bloodstream, PEG-shedding can take place, which facilitates adsorption of serum proteins onto the LNP surface, which in turn could influence nanoparticle clearance and phagocytosis, but also enable receptor-mediated endocytosis in target cells. Once endocytosed, RNA-LNPs are entrapped in early, recycling, and late endosomes where pH drops to acidic values (pH ~6.0–6.5). At this stage, the ICL will be positively charged in the acidic lumen of the endosomal compartments, and therefore electrostatically interacts with anionic endosomal phospholipids. Abundant membrane phospholipids prefer planar membrane geometries. However, electrostatic interaction with the ICLs leads to the formation of conical-shaped lipid pairs inducing liquid-crystalline phases in the endosomal membrane (i.e. inverse hexagonal phase $H_{II}$). Such nonbilayer phases disrupt the endosome, resulting in subsequent cytosolic escape of the RNA payload. If would not escape the endosomes, RNA-LNPs will either recycle back to the bloodstream via the recycling endosomes or will be degraded when endosomes fuse with lysosomes. Abbreviations: PL = phospholipid, ζ = membrane curvature, inv. hexagonal = inverse hexagonal.

yet clear whether LNP components should be considered as active ingredients or drug excipients, and as a result regulation can be complex [57]. Second, a myriad of preclinical nanoparticle studies achieves very poor translational prediction [58]. A top-down approach is commonly used for targeted LNP discovery, where empirical screenings narrow down formulations with optimal *in vivo* behavior. However, exhaustive large-scale experiments, high costs, and ethical considerations involved in animal testing, will force us to narrow down nanoparticle candidates for evaluation in rodents. Consequently, many 'poor *in vitro* performers' are disregarded and the potential of a plethora of LNP formulations is not assessed further.

In contrast, a better translation of nanomedicines can be achieved by understanding key molecular interactions that drive biological mechanisms underpinning nanoparticle fate, biodistribution, and payload delivery. In this regard, we have recently shown that anionic LNPs preferentially accumulate in the sinusoidal endothelial cells (SECs) of zebrafish embryos — functionally equivalent to LSECs in mammalian animals — through an interaction with two

transmembrane receptors, Stablin-1 and Stabilin-2, depending on nanoparticle size [59,60]. Instead of endless nanoparticle screenings, by simply understanding the effect of nanoparticle charge in cell selectivity, and by generating LNPs with anionic surface charge, it was possible to redirect nanoparticle accumulation toward SECs over hepatocytes [27]. This event was found to be conserved in mouse models. Moreover, as with Onpattro's ApoE- mediated mechanism, endogenous lipid transport mechanisms can be explored as a strategy to guide LNPs to specific tissues or cells, that is, employing triglyceride lipase (TGL)-mediated pathways for nanoparticle cell-specific targeting and transport. Spatiotemporal regulation of TGLs and their involvement in lipid transport and metabolism, make them interesting targets for nanoparticle cell specificity. We recently described the discovery of a phase-separated lipid-based formulation consisting of just two lipids, which was capable of accumulating within brain endothelial cells of zebrafish embryos by hijacking a TGL-mediated lipid transport pathway [61]. This selective *in vivo* behavior was a result of a specific nanoparticle–TGL interaction, which was taking place due to lipid composition and the phase-separated morphology [62]. Building upon the liposome design, we similarly developed novel phase-separated mRNA-LNPs that were able to selectively target brain endothelial cellls of zebrafish, with concomitant selective mRNA delivery and protein expression [62]. By acquiring a more comprehensive picture on the biological identity of LNPs influenced by their physicochemical properties, nanoparticle *in vivo* behavior can be more accurately predicted.

Overall, this review is an effort to summarize key findings in the field of LNP development that will help understand LNP *in vivo* behavior. Lipid composition, morphology, size, colloidal stability, and charge will all affect *in vivo* performance, corona formation, selective nanoparticle-protein communications, and promote different interactions with endogenous mechanisms. Applying a biocentric approach on LNP development — considering how physicochemical properties and properties of each individual lipid component can affect the nano-bio interface — is vital for rational design strategies that could help overcome current challenges and lead to more precise technologies.

## CRediT authorship contribution statement

**O. Escalona-Rayo:** Conceptualization, Investigation, Writing – original draft. **P. Papadopoulou:** Conceptualization, Investigation, Writing – original draft. **B. Slütter:** Conceptualization, Writing – review & editing, Supervision. **A. Kros:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## Data Availability

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

## Declaration of Competing Interest

I hereby confirm that there is no conflict of interest regarding the paper.

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- • of special interest
- •• of outstanding interest

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