Acta Pharmaceutica Sinica B 2026;16(9):5674−5708

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

Lipid nanoparticles as active biointerfaces: From membrane interaction to systemic dysregulation

Falko Seger[a,†], **L. Maria Gutschi**[b,†], **Stephanie Seneff**[c,*,†]

[a]*Independent Researcher, Berlin 10117, Germany*
[b]*Pharmacy Consultant, Retired, Ottawa K1P 1C1, Canada*
[c]*Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, MA 02139, USA*

Received 19 December 2025; received in revised form 21 February 2026; accepted 25 March 2026

KEY WORDS

Lipid nanoparticles;
Ionizable lipids;
mRNA therapeutics;
Oxidative stress;
Phosphatidylinositol cycle;
Membrane disruption;
Signaling cascades;
Cell homeostasis

Abstract Lipid nanoparticles (LNPs) are central to modern mRNA therapeutics, including COVID-19 vaccines. Far from passive carriers, their ionizable lipids actively interact with cellular membranes. Evidence from cellular, transcriptomic, and proteomic studies indicates that LNPs, with or without nucleic acid, alter gene and protein expression, thereby initiating inflammatory, detoxification, and stress responses at the membrane. Key pathways affected include lipid metabolism and detoxification, with roles for Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) and cytochrome P450 enzymes. We hypothesize that the phosphatidylinositol (PI) cycle is the primary site of LNP-induced perturbations, regulating membrane restructuring and organelle trafficking during endocytosis. Disruption of this cycle triggers downstream signaling cascades, including nuclear factor kappa B (NF-κB), mitogen-activated protein kinases (MAPKs), Janus kinase/signal transducers and activators of transcription (JAK/STAT), and mechanistic target of rapamycin (mTOR). We term this systemic effect lipid-nanoparticle-driven membrane dysfunction (L-DMD), characterized by dysregulated cellular communication, stress responses, and energy balance. This review provides a mechanistic framework for understanding the persistent biological effects of modified modRNA-LNP exposure and emphasizes a systems-level intracellular perspective.

© 2026 The Authors. Published by Elsevier B.V. on behalf of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

*Corresponding author.
E-mail address: seneff@csail.mit.edu (Stephanie Seneff).
[†]These authors made equal contributions to this work.
Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2026.07.001
2211-3835 © 2026 The Authors. Published by Elsevier B.V. on behalf of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Lipid nanoparticles (LNPs) used in mRNA vaccine technology are engineered to resemble low-density lipoprotein (LDL) particles, thereby improving cellular penetration and facilitating endosomal escape[1]. Four distinct lipid types comprise LNPs: cationic ionizable lipids, cholesterol, phospholipids, and PEGylated lipids. Cationic ionizable lipids aid endosomal escape, cholesterol stabilizes the membrane and supports fusion, and phospholipids stabilize the LNP[2]. PEGylation, the addition of polyethylene glycol (PEG), masks the lipid particle's cationic surface charge and provides a hydrophilic stealth coating. Ionizable lipids interact with negatively charged endosomal phospholipids by forming cone-shaped ion pairs[3]. PEG coatings shield the surface from aggregation, opsonization, and phagocytosis[4].

While LNPs are recognized as biointeractive entities that integrate into cellular membranes and influence membrane structure, leaflet asymmetry, and local electrostatics, current pharmacodynamic and toxicological frameworks treat lipid-mediated effects as secondary or transient to the resulting protein expression (Fig. 1). These frameworks focus primarily on nucleic acid payload, generalized innate immune responses, and protein expression, without linking membrane-level perturbations to downstream signaling dysregulation. We propose lipid-nanoparticle-driven membrane dysfunction (L-DMD), a hypothesis centered on phosphatidylinositol (PI) cycle perturbations, as a unifying mechanistic framework connecting structural membrane changes to possible persistent alterations in intracellular signaling pathways, and consequently at the transcriptional level, including nuclear factor kappa B (NF-κB), mitogen-activated protein kinases (MAPKs), Janus kinase/signal transducers and activators of transcription (JAK/STAT), and mechanistic target of rapamycin complex 1/2 (mTORC1/2).

1.1. *L-DMD—a rational hypothesis*

LNPs are often seen as delivery vehicles for nucleic acids, but their biological activity goes beyond payloaded transport. Their physicochemical properties, particularly those of ionizable lipids and PEGylated lipids, render them inherently biointeractive (Fig. 1). The ionizable lipids are engineered to undergo charge transitions in response to their local environment[5], enabling membrane interaction, bilayer penetration, and endosomal escape[6,7]. We propose LNPs act as active modifiers of membrane structure and function rather than passive carriers; cryogenic transmission electron microscopy (Cryo-TEM) indicates that LNPs lack a hollow aqueous core or stable internal membrane bilayers[2,8]. Instead, the encapsulated ribonucleic acid is intimately associated with ionizable lipids through electrostatic interactions[9]. The negatively charged phosphate backbone of the ribonucleic acid interacts directly with positively charged or protonatable lipid headgroups, forming a compact, disordered lipid-nucleic acid core[10] that reflects a metastable, non-crystalline lipid-nucleic acid assembly capable of structural reorganization, including bleb formation[11].

LNPs are stabilized predominantly by weak, non-covalent interactions, including electrostatic attraction, van der Waals forces, and hydrophobic effects[8,10]. No single interaction maintains structural integrity; stability arises from the collective interactions and flexibility of lipids and nucleic acids. The core is best seen as a dynamic network stabilized by the high configurational freedom of its components rather than from fixed architectural elements[12].

Consequently, the outer lipid shell forms through spontaneous self-organization of lipids at the interface, not *via* an internal scaffold or layered membrane system[2]. This structure is sufficiently stable to permit formulation, storage, and systemic transport[13], yet it remains deliberately metastable[14]. Its metastability enables structural rearrangements in response to environmental cues, such as pH shifts or membrane contact, thereby facilitating cellular uptake and release of nucleic acid payload.

Given these properties, LNPs are best described as supramolecular assemblies rather than fixed molecules[15]. Their behaviour reflects their multiparticulate, dynamic, and colloidal nature, with structure arising from collective interactions[16]. This distinction is not merely semantic; it has implications for how LNPs are perceived, their interactions with biological systems, their responses to environmental stimuli, and their structural transitions *in vivo.*

Having outlined key structural and organizational features of LNPs, it is necessary to briefly discuss how these structures function *in vivo*. Such a discussion is essential to bridge the gap between formulation design concepts derived from physicochemical considerations and the actual biological behavior of LNPs after administration.

In biological environments, LNPs are exposed to complex and heterogeneous conditions, including variable pH, ionic strength, protein coronas, and membrane interfaces. Under these conditions, their colloidal organization enables adaptive responses that may not be readily predictable from static structural models alone. As a result, LNPs may exhibit emergent nonlinear biological effects stemming from the interplay among particle composition, membrane interactions, and cellular context. Understanding these behaviors requires explicit consideration of both their physicochemical design principles and their dynamic reorganization *in vivo*. This structural characterization describes LNPs in isolation. To fully evaluate their biological impact, it should be considered how these metastable assemblies may behave in the complex environment of living systems.

1.2. *The special properties of ionizable lipids*

Central to this *in vivo* reorganization may be the ionizable lipids themselves, whose unique physicochemical properties could drive membrane interaction and integration. At membrane interfaces, the metastable behaviour of LNPs decomposes into the actions of individual lipids, which mediate interactions with cell membranes. Recent studies show that ionizable lipids can directly integrate into phospholipid bilayers[17,18]. Molecular dynamics simulations reveal that ionizable lipid nanodroplets merge spontaneously with model membranes, with insertion of lipid components into the bilayer rather than transient surface adsorption[17]. These simplified models isolate key physicochemical interactions that are otherwise masked in fully biological systems. Free energy profiles indicate several clinically used ionizable lipids favor partitioning into phospholipid membranes, with insertion depth and stability depending on lipid chemistry, bilayer composition, and phase state[18].

These findings are in keeping with the structure-function relationships described by Atmuri et al.[19], who demonstrated experimentally that molecular geometry (*i.e.*, branched *vs.* linear tails, ionizable head-group, p*Ka*[1], and linker biodegradability) can influence bilayer disruption and retention. Notably, membranes with higher phase-transition temperatures and greater lipid order (such as liquid-ordered cholesterol-enriched domains) are more

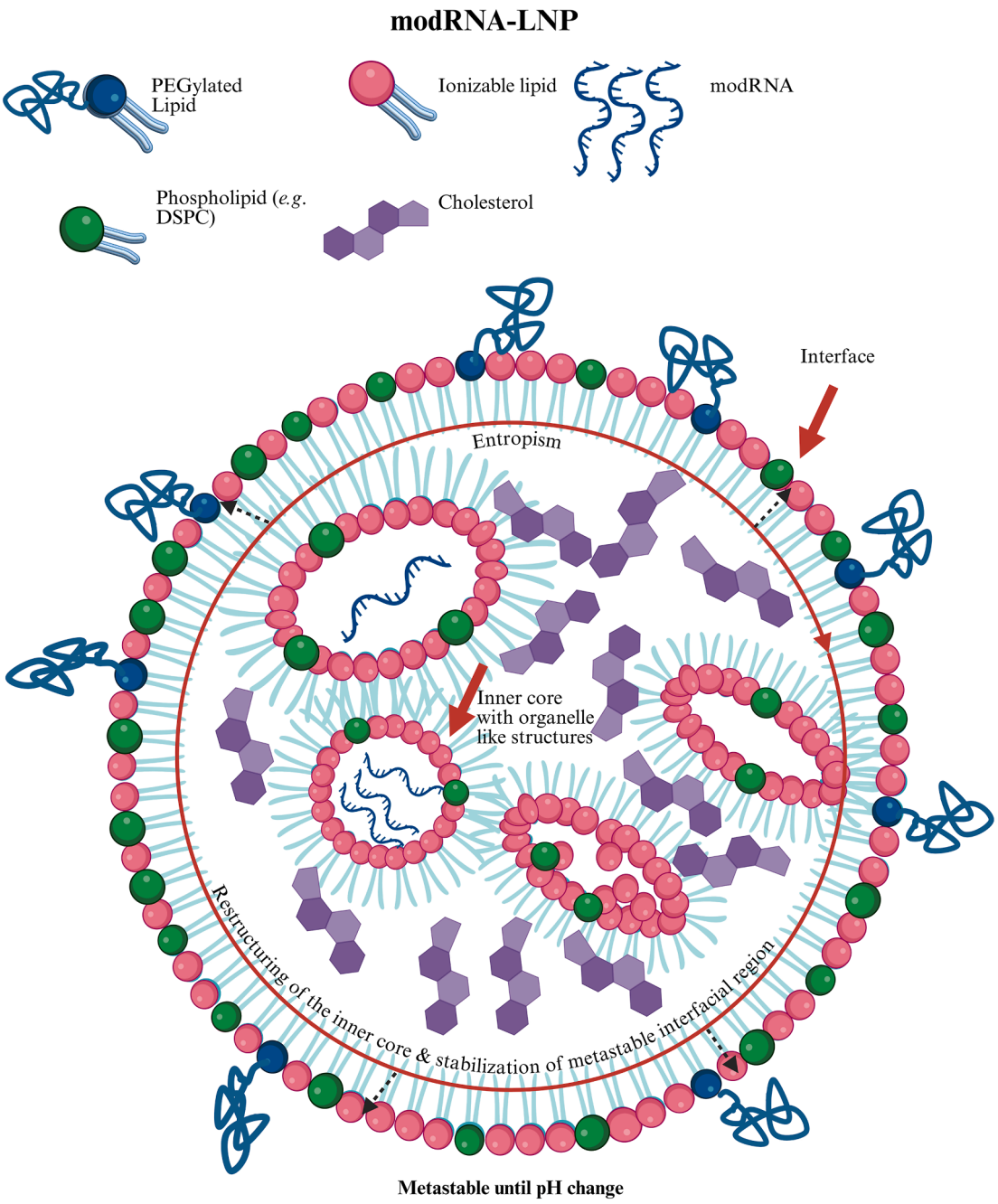


Figure 1 Schematic structural representation of an LNP (*via* Cryo EM). Diverse weak-loaded forces keep the LNP metastable. Created in BioRender. Seger, F. (2026). https://BioRender.com/ysmgilk.

susceptible to penetration by ionizable lipids, indicating that the membrane's physical state critically determines lipid integration. Importantly, such insertion represents the expected behavior of a metastable supramolecular assembly undergoing dynamic interactions with the lipid bilayer.

Ionizable lipids may cause lasting changes in membrane organization beyond insertion, affecting leaflet asymmetry, cholesterol distribution, lipid packing density, and membrane fluidity, often locally, at signaling microdomains[17]. These perturbations may alter the electrostatic and dielectric properties of the inner leaflet[20], crucial for membrane signaling. Since phosphoinositide (PIP)-dependent signaling relies on membrane-protein interactions and exhibits[21] modest but long-lived perturbations in lipid packing, local charge density can be sufficient to destabilize them[22].

Building on this conceptual gap, we present our L-DMD hypothesis, introducing how lipid-induced membrane perturbations may drive system-wide signaling dysregulation.

1.3. Powerful signaling effects of phosphoinositides (PIPs)

Within this context, phosphoinositides (PIPs; also referred to as PtdInsP) play a central mechanistic role. Phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) and Phosphatidylinositol-3,4,5-trisphosphate ($PI(3,4,5)P_3$) are low-abundance lipids that nonetheless control a disproportionate share of cellular membrane

proximal−proximal signaling processes[21−24]. They serve as spatial organizers for second messengers, regulate ion channels and vesicular trafficking, organize actin-membrane interactions, and act as obligatory cofactors for enzymes such as phospholipase C and phosphoinositide 3-kinase (PI3K)[21]. Importantly, their signaling function is governed not by bulk concentration but by electrostatics, dielectric interactions, spatial availability, and orientation within the inner plasma membrane.

$PI(4,5)P_2$ organization is dictated by electrostatic interactions between its highly negative headgroup and clusters of basic residues in intrinsically disordered protein domains. Proteins such as myristoylated alanine-rich C kinase substrate (MARCKS), epithelial sodium channel (ENaC) and growth-associated protein 43 (GAP-43) reversibly bind multiple $PI(4,5P)_2$ molecules through multivalent electrostatic binding, forming separate lipid pools[21,25,26]. This dynamic system depends on input: increased calcium promotes calcium-calmodulin binding, displaces proteins, and releases sequestered lipids, while phosphorylation neutralizes positive charges and releases $PI(4,5)P_2$. Consequently, membrane surface charge and lipid packing regulate the formation of signaling complexes productively[27].

Perturbations of membrane electrostatics therefore have immediate and predictable consequences for PIP-dependent signaling. Integration of ionizable lipids into the plasma membrane is expected to alter local charge density, modify lipid headgroup spacing, and change the effective dielectric environment experienced by PIPs and their binding partners. Even modest shifts in these parameters can disrupt the balance between sequestration and release of $PI(4,5)P_2$ and $PI(3,4,5)P_3$, thereby reshaping the spatial logic of membrane signaling[28]. Given that ionizable lipids demonstrably integrate into membranes and alter local charge density (as discussed in Section 1.2), such perturbations represent a mechanistically plausible and testable scenario warranting investigation.

From this electrostatic perspective, signal-regulatory disorder represents a direct physicochemical consequence rather than a secondary biological effect. Signaling pathways depend on precise PIPs positioning, including PI3K and serine/threonine-specific protein kinases (PKB, also known and referred to as AKT). Phospholipase C-dependent calcium signaling, small GTPase activation, and actin-regulating cascades are inherently susceptible to disruptions in membrane lipid organization because these pathways are organized at the membrane through lipid−protein co-assembly rather than isolated protein−protein interactions. Therefore, alterations in lipid availability propagate across multiple signaling axes simultaneously[29].

Despite advances in understanding LNP physicochemical behaviour and membrane interactions, current pharmacodynamic and toxicological frameworks largely treat LNPs as inert delivery vehicles, attributing adverse effects primarily to the encoded antigen (*e.g.*, the spike protein) or non-specific innate immune activation (*e.g.*, Toll-like receptor (TLR) activation[30]). This view may underestimate the direct bioactive potential of lipid components, both individually and in assemblies, particularly their ability to induce sustained alterations in membrane electrostatics and PIP organization: the integration of exogenous ionizable lipids into cell membranes may lead to durable changes in local charge density and lipid packing, disrupting spatially limited PI/$PI(4,5)P_2$/$PI(3,4,5)P_3$ pools exceeding thresholds, subsequently dysregulating multiple membrane−proximal signaling pathways (*e.g.*, PI3K/Akt/mTORC1) axis[31], phospholipase C-calcium signaling[32,33], small guanosine triphosphatases (GTPases)[34], and actin regulatory cascades[35] in a cell-type- and context-dependent manner.

L-DMD describes physicochemical disruption of membrane-organized signaling rather than nonspecific toxicity or antigen-driven immune activation. Our hypothesis predicts comparable pathway effects from empty and payloaded LNPs, dependence on ionizable lipid partitioning, mitigation *via* restoration of $PI(4,5)P_2$/$PI(3,4,5)P_3$ homeostasis, and cumulative effects after repeated exposure. The following sections review supporting evidence for membrane integration, PIP dysregulation, downstream signaling alterations, and the pharmacokinetic and biodistribution properties underlying LNP *in vivo* behavior.

2. Lipid nanoparticle for RNA delivery: biological properties and effects on cellular systems

Having outlined the conceptual framework of L-DMD, we now examine the physicochemical, pharmacological and biological properties of LNPs that provide the mechanistic basis for this hypothesis.

2.1. *Factors influencing nanoparticle bioactivity*

Nanoparticle uptake and immune activation arise from their high surface area-to-mass ratio and reflect their integrated physical features, including size, charge, shape, and lipid composition, rather than any individual lipid[36]. Ionizable-lipid chemistry (unsaturation, branching, p*K*a), formulation conditions and manufacturing variables together influence membrane fusion, biodegradability, persistence, safety, and effectiveness[36,37], producing emergent, process-dependent behaviors that cannot be inferred from individual components.

modRNA-LNP architectures are heterogeneous due to self-assembly, ranging from multilamellar vesicles with blebs to core-shell-like morphologies[8,38]. Across published platforms, estimates suggest approximately 12%[39] to 80%[40] of particles are empty or minimally loaded with modRNA, depending on formulation or assay used (*e.g.*, dye-binding, confocal methods) as well as batch- or manufacturing-related variability[41]. Importantly, these minimally loaded or empty LNPs (eLNPs) exhibit distinct physicochemical and functional properties[3,4,7,11,] may be structurally more fluid[3,8], and can exhibit higher per-particle fusogenicity and membrane disruption[8]. Payload content alters zeta potential, corona composition[11,12,] and cellular uptake kinetics[40,42], creating a biologically mixed exposure profile[43] that requires human-relevant modeling to define functional response which may differ between eLNPs and loaded particles (see Section 3). Fig. 2 schematizes the physicochemical and biological characteristics of a modRNA-loaded (LNP).

2.2. *The biocorona and biodistribution of lipid nanoparticles*

Upon entry into biological fluids, LNPs rapidly acquire a dynamic coating, a biocorona, comprised of serum proteins, primarily of lipoproteins, immunoglobulins, albumin, complement, and coagulation factors that confer a new biological identity. It varies by species[45], and is determined by both LNP characteristics (size, charge, PEG density, rigidity) and host or environmental factors[46]. In humans, it remains incompletely defined, with ApoE, vitronectin, C-reactive protein (CRP), and alpha-2-macroglobulin

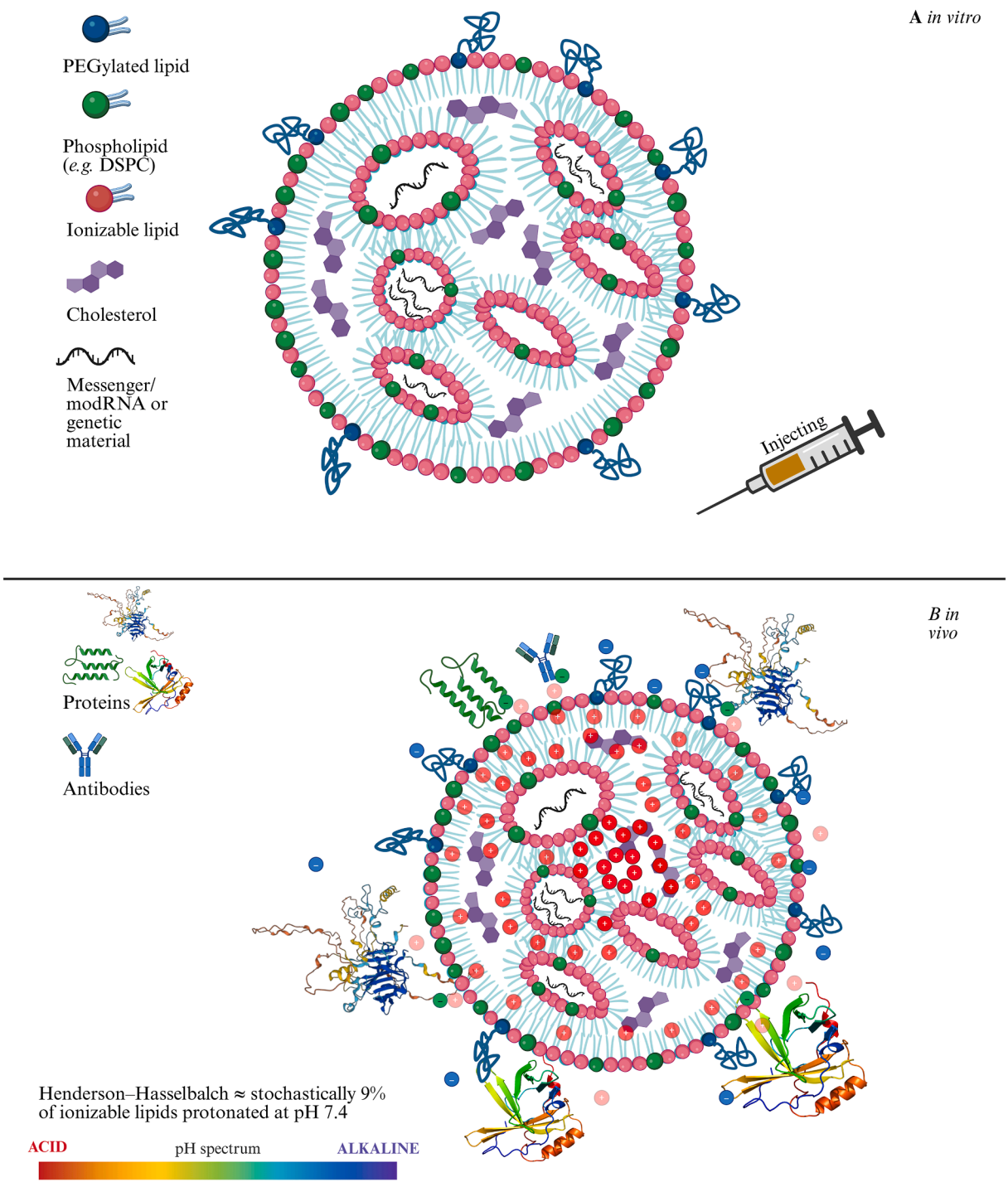


Figure 2 Schematic representation of the physicochemical and biological characteristics of a modRNA-loaded lipid nanoparticle (LNP). (A) Physicochemical structure of the modRNA−LNP. The lipid components self-assemble into a quasi-bilayer, forming ionizable lipid-rich domains that bind with the mRNA payload (black). The inset box shows the apparent p*K*a (∼6.2) of the ionizable lipid, resulting in about 10% protonation at neutral-to-slightly-basic pH, whether in the formulation buffer (pH 7.4 ± 0.5) or under physiological conditions (pH 7.4), thereby providing a near-neutral surface charge during circulation. A syringe icon indicates typical routes of administration (intravenous [IV] or intramuscular [IM]). (B) Protein corona formation and surface charge neutralization after plasma exposure. The same LNP after incubation with blood plasma or serum. A dense protein corona quickly forms on the LNP surface, consisting of diverse serum proteins (alpha-helices, beta-sheets, and globular structures in blue, orange, gold, and green) and immunoglobulins (Y-shaped antibodies in blue). Counterions (blue (−) symbols) on the corona interface electrostatically shield the internal positive potential, producing an apparently neutral surface (near-zero zeta potential). Red plus (+) signs inside represent residual positive electrostatic potential within the ionizable lipid-rich core that diminishes in density towards the corona, indicating charge reduction through the lipid shell and adsorbed proteins[44]. This internal-external charge asymmetry affects serum stability, biodistribution, and cellular uptake, and influences endosomal escape behavior. Created in BioRender. Seger, F. (2026). https://biorender.com/do3y6xk.

consistently present[47], and it can alter LNP structure, biodistribution, and modRNA stability[48].

LNPs remodel in plasma and lymph. Indeed, recent cryo-EM work demonstrates that LNPs do not simply adsorb proteins but rather exchange lipids and apolipoproteins with endogenous lipoproteins, producing hybrid LNP-lipoprotein assemblies resembling physiological LDL[44]. These fusions modify surface potential and fluidity, redirect uptake *via* the LDL-receptor (LDL-R) pathway and by apolipoprotein-dependent routes, and influence stability, tissue tropism, and immune recognition[49,50].

Considering these distinctions, it is crucial to evaluate LNP distribution within the body and across cell types, as these factors affect uptake, antigen presentation, and downstream cellular processes. Biodistribution indicates where LNPs or drugs are in the body but does not itself imply cell entry or gene expression. Transfection requires the uptake and endosomal escape of nucleic acids into cells. Gene expression depends on the persistence of intact modRNA translating into protein. These processes are often conflated in studies and regulatory submissions. Recognizing this distinction is crucial for understanding toxicity, durability and efficacy, as biodistribution alone cannot predict which specific cells are transfected or the levels of protein expression.

Among the factors affecting LNP distribution, the route of administration exerts the greatest influence, more so than in small-molecule pharmacology. Factors such as syringe pressure, perfusion rate, proximity to blood and lymphatic vessels, local pH, and temperature can influence particle dispersion during intramuscular (IM) administration[51]. Following intramuscular administration, LNPs remain initially at the injection site before traversing lymphatics to draining nodes, later entering circulation primarily as lipoprotein-hybrids.

After a single intravenous infusion in rats (patisiran-like), 90% of radioactivity was found in the liver within 4 h, indicating fenestrated endothelium and ApoE/LDL-R uptake. Conversely, a single intramuscular dose of modRNA-LNP (Moderna-like) remained at the injection site for 24−48 h and only appeared in the liver after 8−48 h, indicating that the route of administration affects timing and distribution patterns, even under controlled conditions[45].

LNPs primarily accumulate in the liver, spleen, and draining lymph nodes, which are rich in phagocytic cells of the reticuloendothelial system (RES), such as monocytes, Kupffer cells, and macrophages[52]. Such tropism toward the hepatic and lymphoid RES compartments indicates that immune activation and detoxification pathways are primary determinants of systemic LNP fate.

Recruited antigen-presenting cells (APCs) can internalize Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) LNPs, translate the encoded spike protein, and then subsequently migrate to nearby lymph nodes, where T cell priming occurs[53]. Lesser amounts reach the heart, lungs, adrenal glands, ovaries, eyes, and other tissues *via* transcytosis or direct penetration[3,7,54−58].

In humans, the ionizable lipids and the vaccine modRNA (Moderna®), quantified by mass spectrometry and quantitative polymerase chain reaction (qPCR), respectively, appeared in plasma, with T max (time to maximum observed concentration) between 4 h and 2 days, with wide inter-individual variability in timing and magnitude[59]. Buckley et al.[60] used PET-CT in non-human primates to track rapid, stochastic LNP trafficking to lymph nodes after IM injection of a Moderna-like formulation. Quadriceps injections consistently drained to the iliac nodes with clear muscle distribution at 4 h. Deltoid injections varied, draining to axillary, apical, or pectoral nodes, with little spleen signal and no uptake in heart or other tissues (plasma kinetics not measured). These findings show that intramuscular LNP administration triggers early lymphatic trafficking and distribution prior to immune activation and clearance. Variability in T_{max} likely results from administration factors, tissue biology, and particle interactions with cell membranes affecting sequestration and redistribution.

Conventional radiotracing and fluorescence-based labelling approaches blur intact *vs* degraded components and lack the subcellular resolution within tissues. New peptide-tag imaging confirms the presence of intact hybrid particles in tissue[61], highlighting the extent to which existing approaches may underestimate the complexity and heterogeneity of LNP distribution.

Uptake, or endocytosis, occurs *via* both receptor-mediated and receptor-independent mechanisms, often simultaneously, with the protein corona modulating these interactions[62,63]. Receptor-independent uptake relies on nonspecific hydrophobic and/or electrostatic forces, whereas receptor-mediated pathways, including clathrin-mediated endocytosis, depend on the local microenvironment and lipid−membrane interactions. Specific lipid components, such as the helper lipid 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), can alter membrane conformation and signaling to facilitate G-protein coupled receptor (GPCR) engagement without direct binding, and lipoprotein-enriched coronas further enhance internalization[64]. Overall, the biocorona determines biodistribution, uptake and persistence, linking formulation chemistry to L-DMD risk.

2.3. *Endosomal escape and membrane destabilization due to ionizable lipids*

Endosomal escape is the rate-limiting step for RNA translation. After endocytosis, progressive endosomal acidification protonates ionizable lipids, thereby altering charge balance and triggering lipid rearrangement and membrane destabilization. Several, not mutually exclusive, mechanisms have been proposed to explain this process, including proton-driven osmotic swelling (*i.e.*, proton-sponge effect)[65,66], promotion of non-bilayer hexagonal phases, and directed lipid mixing or pore formation facilitated by inverted-cone lipid geometries[18]. Endosomal damage, evidenced by galectin recruitment, can occur solely from ionizable lipids without cytosolic RNA delivery[67]. Smaller membrane perturbations induced by LNPs are detected and rapidly sealed by the Endosomal Sorting Complexes Required for Transport-III (ESCRT-III) machinery. Computational and experimental studies suggest that LNPs can transiently tear membranes, disrupt lipid raft organization, and tether to the endosomal membrane, enhancing escape[66,68,69].

Escape is inefficient and remains a central bottleneck for RNA therapeutics[65,70]. Here, 'endosomal release/escape efficiency' refers to assay-specific estimates of the fraction of internalized LNP payload that reaches the cytosol in a functionally available form. Values are not directly comparable across assays and formulations[42]. Across representative studies, estimated endosomal release efficiencies are generally low and span roughly ∼1%−15%, as summarized in Müller et al.[42], based on data drawn from Sabnis et al.[71], Maugeri et al.[72], and Gilleron et al.[73]. These efficiencies depend on the ionizable lipid structure, p*K*a, particle topology, cell type activation state, and the timing of the transient "burst" fusion window during early endosome acidification (pH < p*K*a)[42,65,74−76]. Escape occurs within minutes to a few hours after uptake, depending on formulation, cargo and assay

methodology. If modRNA does not release into the cytoplasm, then maturing endosomes fuse with lysosomes, degrading modRNA and lipids. Accumulation of LNP components in lysosomes can impair function, block receptor recycling, create a cellular "traffic jam", and prolong lipid retention, which may reduce therapy effectiveness and cause toxicity[65,76–79].

Indeed, LNP uptake follows a bell-shaped, nonlinear response influenced by particles and cell-specific factors. Immune cells like monocyte-derived macrophages and THP-1 cells show limited cytotoxicity, likely because their phagocytic pathways better handle LNP uptake and membrane stress[80]. Uptake and endosomal escape are further influenced by interactions between the LNP surface and surrounding biomolecules[81,82], leading to a stochastic distribution of particle identities and protonation thresholds[76,82]. The relationship between dose and functional delivery is probabilistic, rather than linear, leading to variability in cellular responses and signaling, which are highly dose dependent[83].

2.4. *Spread to distant sites via exosomes*

Once the genetic material (RNA/modRNA/DNA) escapes the endosome, it can be packaged into naturally secreted membrane-bound vesicles along with ionizable lipids and intact mRNA. Maugeri et al.[72] showed that, in a murine model, LNPs in recycling endosomes are either expelled intact or partially degraded, thereby affecting transfection efficiency. This process involves the trafficking of a fraction of LNP-delivered mRNA together with LNP-derived lipids into intraluminal vesicles of multivesicular endosomes (MVBs), which are subsequently released *via* extracellular vesicles, thereby enabling intercellular transfer. In such models, these exosomes circulate systemically and have been shown to mediate functional RNA transfer to distant cells and enable protein translation[72]. The evidence for modRNA-LNPs transfer in humans remains preliminary and needs further investigation[84,85].

Exocytosis serves as both a clearance route and a secondary distribution mechanism, extending modRNA or lipid fragments to the surrounding microenvironment in a paracrine manner[86], where functional translation is possible. Importantly, recycling of endosomes, as well as eLNPs or those with blebs, may cause cellular stress, oxidative damage, and chronic inflammation[87]. These factors are not considered in biodistribution studies and may contribute to cumulative toxicity, especially with repeated doses[88].

Spike-carrying exosomes prior to seroconversion have been detected in humans[89], supporting the plausibility of protein relay *via* vesicular pathways, potentially extending antigen exposure and immune activation beyond initially transfected cells. However, definitive evidence for exosome-mediated transfer of vaccine-modRNA in humans remains limited and warrants further study.

2.5. *LNP metabolism and oxidative stress mechanism*

The process by which the LNPs disassemble and are metabolized is typically described as hydrolysis and clearance[90,91]. Fatty acid metabolites derived from these lipids can activate PPARs, initiating lipid-sensing and stress response pathways[78]. The long-term persistence[91] and biodistribution[92] have not been systematically characterized *in vivo*, particularly with respect to tissue-specific retention and potential for bioaccumulation following repeated dosing.

In silico studies suggest these lipids may localize within the bilayer[18,66], further complicating predictions of LNP stability and *in vivo* behavior. Intracellularly, LNP components intercalate directly with cell membranes, triggering both repair and oxidative-stress pathways, leading to endosomal damage (detectable by galectins), immune signaling, and the production of reactive oxygen species (ROS)[93]. Oxidative stress can arise from incomplete lipid degradation, membrane perturbation, and lysosomal processing, and it is modulated by lipid structure, intracellular localization, and cellular context[67].

Tertiary amine-based ionizable lipids can generate *N*-oxides and reactive aldehydes during manufacturing and storage, which may covalently modify modRNA, rendering it untranslatable. Packer et al.[94] first identified such adducts in 2021. They can be mitigated by buffering or novel lipid designs[95]. These adducts may generate aberrant molecules that activate interferon-driven innate immune pathways[96,97] or potentially modify cellular proteins through electrophilic mechanisms. Ionizable lipids commonly incorporate unsaturated acyl chains susceptible to peroxidation, producing electrophilic aldehydes such as 4-hydroxynonenal (4-HNE), a well-established oxidative-stress product known to disrupt protein folding and lysosomal function[78,98]. However, direct evidence that modRNA-LNP administration generates 4-HNE at biologically significant levels in humans is currently lacking, and any clinical impact remains speculative.

Nonetheless, LNP metabolism may be viewed not only as producing degradative metabolites but also as a potential source of reactive, bioactive lipid intermediates that contribute to sustained inflammatory or stress signaling[90,91].

2.6. *Activation of the immune system*

LNPs can activate the immune system independently of spike protein expression. Within the adaptive immune system, two mechanisms have been identified. First, IgE-mediated anaphylaxis arises from antibodies induced against PEGylated particles in the biocorona[3,99,100]. Such reactions are rare but potentially life-threatening. Second, exposure to PEGylated LNPs can induce anti-PEG IgM antibodies, leading to the accelerated blood clearance (ABC) phenomenon, in which subsequent doses are rapidly cleared, reducing efficacy and altering exposure profiles[101].

Third, LNPs also activate innate immunity through complement- and receptor-mediated pathways. Complement activation-related pseudo-allergy (CARPA) represents a blood-facing innate immune reaction to nanoparticles, including PEGylated drugs[102,103]. CARPA is mediated by complement activation and anaphylatoxins (Complement (C)3a, C5a), often triggered by anti-PEG IgM antibodies, which can activate mast cells and release histamine, leading to acute infusion reactions[103].

Evaluation of CARPA typically relies on porcine and non-human primate models that recapitulate human cardiopulmonary responses; to date, to our knowledge, only one published study[104] has directly examined modRNA-LNP administration in a porcine CARPA model using intravenous administration, confirming complement activation and CARPA-like hemodynamics. Allergic reactions observed after modRNA COVID-19 vaccination may be mediated by this phenomenon[105] and have prompted calls for alternative PEG excipients to mitigate CARPA[106]. Clinically, patisiran (an siRNA-LNP therapeutic for the treatment of hereditary transthyretin-mediated amyloidosis, Onpattro®) infusions

can also trigger CARPA, sometimes severely, through complement activation[107,108].

LNPs can activate the innate immune system by transfecting immune cells, providing an intrinsic adjuvant-like activity[79] that aids in antibody production; LNPs can activate TLR3, 7, 8, and 9 in the endosome[109]. Additionally, LNPs bind to diverse cell membrane receptors such as CRP, TLR4, and TLR2, and can activate the NOD-Like Receptor Protein 3 (NLRP3) inflammasome[110]. These immune effects highlight the need to distinguish among biodistribution, transfection, and gene expression, as they are context-dependent and may cause pathogen-like effects beyond just cytotoxicity.

2.7. Delivery architecture as a determinant of membrane stress and possible systemic risk

The biological effects of nucleic acid therapeutics are determined not solely by RNA modality, but also by delivery architecture and membrane interactions. Comparative analysis of approved platforms reveals a continuum of membrane engagement and persistence not captured by conventional pharmacokinetic or biodistribution frameworks.

This principle is evident when examining clinically approved RNA therapeutics. The siRNA therapeutic inclisiran (Leqvio®) achieves durable LDL-cholesterol lowering through triantennary *N*-acetylgalactosamine (GalNAc)-mediated receptor-targeted delivery to hepatocytes, and has been evaluated in large, repeated-dose clinical trials with extended follow-up[111,112]. Its favorable safety profile, supported by precise cellular targeting, low administered doses, and infrequent dosing, mitigates systemic exposure and inflammatory risk, suggesting that the delivery strategy, not the RNA modality itself, may be a key determinant of long-term tolerability. Patisiran (Onpattro®) has a safety profile that includes transient elevations in transaminase levels (often resolved with dose adjustment) and infusion reactions, but little evidence of progressive hepatotoxicity over 18 months of treatment[107,113]. This contrasts with the acute reactogenicity observed with modRNA-LNP vaccines, although direct comparisons are confounded by route (IV *vs.* IM) and dosing frequency.

In contrast, the European Medicines Agency (EMA)'s assessment of the self-amplifying-RNA COVID-19 vaccine zapomeran (Kostaive®), which employs LUNAR (ATX-126) ionizable lipids designed for improved degradability[114], reported very slow clearance of ATX-126 with tissue half-life not determinable for most organs, and an estimated half-life in muscle of 31-64 days[115]. Collectively, these products define a continuum of RNA delivery: from targeted, non-particle delivery (Leqvio®) to hepatotrophic siRNA-LNP delivery (Onpattro®), and to lipid-based vaccine platforms employing ionizable lipids tuned for increased endosomal fusion and cytosolic release (Kostaive®) (Table 1[19,53,107,111–114,116,117]). This is consistent with experimental structure−activity analyses showing that lipid architecture and ionization state influence bilayer disruption and toxicity[19].

These data indicate that ionizable LNPs cannot be regarded as biologically inert carriers whose effects end once RNA is released. They function as supramolecular, membrane-active assemblies that persist within endosomal and lysosomal membranes, engaging repair circuits, oxidative stress pathways, and innate immune signaling. These processes are highly context-dependent, vary across tissues and individual cells[118], and are not fully captured by conventional biodistribution or transfection metrics. Together, the continuum of observations supports the lipid-driven membrane dysfunction (L-DMD) hypothesis as a coherent explanatory framework for the durable, tissue-specific and heterogeneous outcomes associated with modRNA-LNP exposure. These mechanistic insights give rise to the core L-DMD hypothesis: membrane- and vesicle-level perturbations may represent the missing link that couples delivery architecture to cellular dysregulation.

Building on these considerations, the following section investigates how the biological disposition, pharmacodynamic, and physicochemical properties of modRNA-LNP systems may produce detectable molecular signatures *in vivo*. If membrane-associated uptake, intracellular trafficking, endosomal escape, or LNP restructuring perturb local signaling environments, such effects are expected to produce coordinated, system-level transcriptomic and proteomic shifts rather than isolated pathway activation. Omics-based profiling therefore provides a suitable framework to evaluate whether modRNA-LNP exposure is associated with broad molecular changes consistent with membrane-centered dysregulation.

3. Omics data indicating membrane dysfunction secondary to LNP transfection

Omics approaches, including transcriptomics, metabolomics, lipidomics, and proteomics, are exploratory tools for analyzing cellular and organismal responses in experimental systems. They generate high-dimensional datasets that reveal molecular patterns and pathway alterations, providing reproducible, falsifiable signatures that support hypothesis generation rather than causal proof. In the context of modRNA-LNP COVID-19 vaccines, these datasets enable systematic assessment of intracellular responses to both eLNPs and payloaded formulations following uptake, endosomal processing, and translation of modRNA. Although primarily descriptive, omics data provide a foundation for mechanistic interpretation, including evaluation of membrane-associated dysfunction. A key limitation is that transcriptomic changes alone cannot establish functional pathway activation without proteomic validation, while proteomic data alone cannot fully reconstruct upstream regulatory or membrane-level processes.

To date, omics analyses of LNPs remain limited, particularly for longitudinal human comparisons between vaccinated and unvaccinated cohorts. Transcriptomic, lipidomic, and proteomic datasets are therefore of primary interest, as they provide the most direct insights into altered signaling and protein activity states following LNP exposure. Established signaling pathway reconstructions, especially from cancer and immunologic research, provide a validated framework for interpreting pathway crosstalk and network dynamics and guide the hypothesis-driven part of this analysis. Accordingly, we organized this section into three parts: (1) omics data from eLNP-treated mouse models, (2) available human omics datasets, and (3) evaluation of whether convergent transcriptomic and proteomic signatures support the L-DMD hypothesis, while clearly distinguishing exploratory associations from mechanistic inference. Later sections integrate these findings across models and interpret them within the L-DMD framework.

3.1. Mouse data

In 2021, Ndeupen et al.[119] published a pioneering *in vivo* study, in which they analyzed how a biological system responds to eLNPs.

Table 1 Comparative pharmacology and safety profile of approved RNA nanoparticle therapeutics.

Platform (example)	Route/vehicle	Primary delivery mechanism	Approximate endosomal escape[a]	Membrane activity[b]	Potential membrane risk[c]	Common safety signals	Ref.
GalNAc−siRNA (inclisiran)	SC, Ligand−siRNA conjugate, No LNP.	Hepatocyte-targeted receptor uptake (ASGPR).	Sufficient at low doses (No LNP).	Minimal: Negligible disruptive capacity.	√ (No ionizable lipids).	Primarily injection site only.	Di Fusco et al.[111] Wright et al.[112]
siRNA−LNP (patisiran)	i.v., Ionizable lipid, (DLin-MC3-DMA).	Hepatic fenestration + ApoE mediated LDL-R.	∼1% (model-dependent).	Modest; Transient endosomal lesions repaired by ESCRT.	√√ (Hepatotropic, low pK_a 6.4).	↑Transaminases; ↓ Vitamin A levels; Infusion reactions (CARPA).	EMA Onpattro EPAR 2018.[107] Urits et al.[113]
modRNA−LNP (SARS-CoV-2 vaccines)	i.m., Ionizable lipid, (ALC-0315/SM-102).	Immune activation + lymphatic drainage + systemic distribution.	≤∼15% (context-dependent).	High; Sustained endosomal/lysosomal engagement.	√√√ (systemic distribution, pK_a 6.1−6.7).	Systemic reactogenicity (fever myalgias), Rare myocarditis, Rare anaphylaxis.	EMA EPAR Comirnaty 2021.[53] EMA EPAR Moderna 2021[116]. Wong et al. [117]
saRNA−LNP (zapomeran/ SARS-CoV-2 vaccine)	i.m., Ionizable lipid, (ATX-126/ LUNAR lipids).	Prolonged RNA replication + front-loaded lipid-mediated delivery.	Variable; amplification design.	High; ionizable lipid with saRNA prolonging innate activation.	√√√ (very slow clearance, pK_a undisclosed/proprietary).	Systemic reactogenicity ≈ modRNA-LNPs.	Atmuri et al.[19] EMA EPAR Kostaive[114]

√, minimal, √√√, highest relative risk category. This framework requires prospective validation.

ApoE, apolipoprotein E; ASGPR, asialoglycoprotein receptor; CARPA, complement activation-related pseudoallergy; ESCRT, endosomal sorting complexes required for transport; GalNAc =*N*-acetylgalactosamine; i.m., intramuscular; i.v., intravenous; LDL-R, low-density lipoprotein receptor; L-DMD, lipid-driven membrane dysfunction; LNP, lipid nanoparticle; LUNAR, Lipid-enabled and Unlocked Nucleomonomer Agent modified RNA; modRNA, nucleotide-modified RNA; p*K*a, acid dissociation constant; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SC, subcutaneous; saRNA, self-amplifying RNA; siRNA, small interfering RNA.

[a] Endosomal escape varies by cell type, ionizable lipid p*K*a, and assay method (*e.g.*, fluorescence dequenching, galectin recruitment). Values represent estimates from *in vitro* and animal models; human data are limited.

[b] Membrane activity represents qualitative assessment from regulatory filings and published mechanistic studies and does not imply difference in lipid persistence or systemic half-life.

[c] Potential L-DMD risk: based on ionizable lipid p*K*a, membrane fusogenicity, and biodistribution.

Their pharmacokinetic investigation, in which wild-type (WT) C57BL/6 (B6) mice were injected intramuscularly with 10 μg of eLNPs, revealed thousands of changes in gene expression. The chosen dosage corresponds to the typical design for pharmacodynamic evaluation[120,121]. Furthermore, these changes were also evident in the Luminex panel at the protein level.

Regarding the number of up- or down-regulated genes, the study analysis revealed that "*with $P < 0.05$ and FDR [False Discovery Rate] < 0.05, 9508 genes and 8883 genes, respectively, were differentially expressed*." A marked upregulation of genes involved in monocyte and granulocyte development, recruitment, and function (*e.g.*, *Ccl2*, *Ccl3*, *Ccl4*, *Ccl7*) was observed. The Kyoto encyclopedia of genes and genomes (KEGG) transcriptomic gene set enrichment analysis (GSEA) revealed a pronounced induction of inflammatory cytokines, including Interleukin-1 (IL-1) beta (β), granulocyte−macrophage colony-stimulating factor, and IL-6 signaling pathways, which are hallmark markers of acute innate and adaptive immune activation[122−126].

NF-κB was upregulated by an estimated 2.5−2.6 $\log_2$-fold. In contrast, the TCA cycle and PPAR signaling pathways were markedly downregulated, with an approximately 2.0-$\log_2$ fold reduction in expression. The upregulation of genes associated with TLRs, NOD-like receptors, and RIG I-like receptors suggests a robust system-wide immunological response[127]. Hematopoietic cell lines, in particular, showed a more than 2 $\log_2$-fold increase in gene expression, indicating pronounced activation within stem and progenitor compartments that may be driven by the combined signaling of TLRs[128], NOD receptors[129], and RIG I[130] pathways and/or the internalization pathways of the LNPs. These findings are consistent with the single-cell-based flow cytometric experiment by Parhiz et al.[131].

Furthermore, the observations of Ndeupen et al.[119] are consistent with the *in vitro* experiment made by Zelkoski et al.[132]. in human monocytes, showing that eLNPs trigger TLR4-dependent signaling that bifurcates into a strong myeloid differentiation factor 88 (Myd88)/NF-κB response and a parallel, though weaker, TIR-domain-containing adapter-inducing interferon-β (TRIF)-mediated interferon regulatory factor (IRF) activation. Their enzyme-linked immunosorbent assay and transcription analysis revealed that Jun, a component of the AP-1 transcription factor complex, activated *via* the c-Jun N-terminal kinase (JNK)-MAPK cascade downstream of TLR4/MyD88 signaling, exhibited a 1.93-fold $\log_2$ expression change, whereas the JAK−STAT axis, typically associated with interferon signaling[133], showed a more modest 1.13-fold $\log_2$ change.

Korzun et al.[134] investigated the reactogenicity of eLNPs compared with Luciferase (Luc) mRNA-LNPs *in vivo,* both as a single dose and after multiple doses, in C57BL/6 (wild-type) mice and in MyD88, TLR4, and TRIF-knockout lines. The LNPs were produced microfluidically (Onpattro®-like, Pfizer®-like, Moderna®-like) and injected intraperitoneally. The ionizable lipids were the only variable changed. Reactogenicity was quantified by sickness behavior (reduced food intake and weight loss). TLR4 dependence was tested by pharmacological inhibition with resatorvid (TAK-242) (3 mg/kg) given 2 h prior to each dose in the eLNPs groups. Gene expression was analyzed using the NanoString Counter Mouse Inflammation Panel (liver and hypothalamus tissue) and qRT-PCR, supplemented by Enzyme-Linked Immunosorbent Assay (IL-6, IL-1β, TNF-α), Lipocalin 2, and Fluorescence-Activated Cell Sorting analysis of peritoneally obtained immune cells.

The authors showed that TLR4 and MyD88, in particular, are necessary for the initiation of these reactions and that pharmacological inhibition of TLR4 (*e.g.*, with TAK-242) can attenuate the reactogenicity of eLNPs, and thus also the disease-related behavioral changes. These findings underscore the importance of the TLR4/MyD88 axis not only for proinflammatory gene expression, but also for physiological behavioral responses associated with sickness behavior. This finding is consistent with *in vitro* experiments performed by Zelkoski et al.[132].

Similarly, Korzun et al.[134] described an inflammatory signature for ionizable lipids, including induction of the proinflammatory cytokine genes *Il1b* and *Il6*, as well as the chemokine genes *Ccl2*, *Ccl4*, *Cxcl2*, and *Cxcl10*, findings consistent with Ndeupen et al.[119] and indicative of enhanced inflammatory gene expression and downstream pathway activation.

Further examination of the raw transcriptional data provides additional support for the observations of Ndeupen et al.[119]. Specifically, analyses of whole-tissue expression reveal substantial regulation of key signaling nodes, including Ras homolog gene family member A (*Rhoa*), phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 gamma (*Pik3c2g*), Rho-associated coiled-coil containing protein kinase 2 *(Rock2*), *Mapk3* (also known as extracellular signal-regulated kinase 1 (*Erk1*), and *Stat3*. These changes, observed in whole tissue, indicate a systemic activation of RAS/PI3K/ROCK/ERK signaling pathways that likely involves multiple cell types, including immune and epithelial cells. Analyses restricted to isolated immune cell subsets may not fully capture these effects[133−136]. Canonical genes such as Kirsten Rat Sarkoma (*Kras*) were not significantly altered in immune-cell-enriched subsets, whereas downstream and parallel pathways show robust modulation, highlighting the importance of considering global transcriptional responses to eLNPs.

Another study supporting these observations published by Luo et al.[137] investigated proteomic alterations induced by eLNPs lacking a modRNA payload. Depending on the formulation, the various nanocarriers were administered intranasally, intramuscularly, intravenously, orally, or intradermally, with doses ranging from 0.0005 mg/kg to 0.5 mg/kg (LNPs, liposomes, polyethylenimine complexes) or 50.725 mg/kg (DNA origami) and 10^{13} GC/mL adeno-associated viruses.

The authors compared payload-containing and no-payload LNPs with phosphate-buffered saline [PBS] controls. They detected 375 differentially expressed proteins, of which 240 were upregulated and 135 downregulated in the no-payload LNP group *in vivo*. As determined by Reactome pathway analysis, these changes were linked to metabolic processes, particularly ribosome function, protein translation, and RNA metabolism. Specific markers, including Ribosomal Protein 2, 6, 11, 15, Eukaryotic Initiation Factor 4B, 2B3 were found to be differentially regulated.

These data, derived from *in vivo* Omics analyses, provide early insights into the broad immunological and signaling effects elicited by LNPs.

3.2. *Human data*

To the best of our knowledge, there are only two existing *ex vivo* human studies providing direct molecular readouts following COVID-19 modRNA-LNP vaccination: Knabl et al.[138] (concerning *ex vivo* buffy coat transcriptomics) and Hickey et al.[139] (concerning longitudinal serum proteomics). Other studies are cited solely to provide context.

Knabl et al.[138] conducted an *ex vivo* transcriptomic study to assess the systemic effects of modRNA packaged in LNPs in both elderly individuals with comorbidities and healthy younger vaccinated individuals.

The first study group consisted of nine hospitalized Beta-variant COVID-19 patients, of whom four had received the first dose of BNT162b2 8-11 days before symptom onset, while five remained unvaccinated; all vaccinated patients and three of the five unvaccinated received dexamethasone. Immune transcriptomes (MSigDB-GSEA) were analyzed at days 7−13, 20−32, and 42−60 after symptom onset. In the raw data, thousands of genes in the transcriptomics were statistically significantly changed (up- or downregulated) after the BNT162b2-application. As reported by the authors, COVID-19 symptoms (Beta variant) developed in all patients, including those infected shortly after the first BNT162b2 dose[138]. MSigDB-GSEA analysis of the four vaccinated patients revealed acute changes in gene expression across thousands of genes. One elderly comorbid patient died before the end of the study. Signaling pathways affected included JAK−STAT3, mTORC1, and KRAS, among others, with many transcriptomic changes reaching statistical significance.

The canonical ERK−MAPK signaling pathway involves several key proteins: rat sarcoma (RAS), rapidly accelerated fibrosarcoma (RAF), mitogen-activated protein kinase (MEK), and extracellular signal-regulated kinase (ERK), and it has extensive crosstalk with the mTORC1/2-pathways through feed-forward and feed-back mechanisms[140].

Notably, *TNFA*, *via* NF-κB signaling, was upregulated in both study groups (see next paragraphs), similar to the activation observed by Zelkoski et al.[132] and Ndeupen et al.[119]. It remains unclear whether this reflects canonical (*RELA*/p65−*IKB*-dependent) or non-canonical (*NIK*−*RELB*-mediated) NF-κB activation[141].

Moreover, Knabl et al.[138] detected significant mTORC1 signaling across doses 1 to 3, with 10 overlapping genes reaching statistical significance, underscoring that both mTORC1 and p53 are co-activated under physiological and genotoxic stress. KRAS signaling can engage NF-κB through multiple downstream routes (including RAF/MEK/ERK and PI3K/AKT branches)[142], thereby promoting transcriptional programs that support cell survival and stress adaptation. Distinguishing canonical from non-canonical NF-κB activation in these samples requires targeted inspection of pathway components (*e.g.*, *RELA*/p65 phosphorylation and *IKB* degradation for the canonical arm; *NIK*/*RELB* dynamics for the non-canonical arm) at the transcript and protein level[142]. Moreover, the four elderly patients showed significant upregulation of the JAK-STAT5 pathway with *IL-2* and the JAK−STAT3 pathway with *IL6*.

The authors later included eight healthy, naive individuals receiving their first vaccine dose and analyzed transcriptional changes 7−10 days post-vaccination. In these naive individuals, selective upregulation of the transcription factor *E2F8* and specific interferon-stimulated genes was observed *via* p53-regulated pathways, while *E2F1* and cyclin A1 (*CCNA1*) remained largely unchanged. Similarly, only three canonical mTORC1 target genes (cell division cycle 25A (*CDC25A*), ribonucleotide reductase family member 2 (*RRM2*), and BUB1 mitotic checkpoint kinase (*BUB1*)) were modulated, indicating that global mTORC1 activation was minimal yet statistically significant at this early time point. *CDC25A*, *RRM2*, and *BUB1* mark the transitions G1/S (*CDC25A*), DNA synthesis (*RRM2*), and mitosis (*BUB1*)[143].

Taken together, these findings indicate that the observed mTORC1-related transcriptional patterns are unlikely to be primarily driven by dexamethasone treatment, particularly given its half-life of 36−54 h[144], which is unlikely to account for weeks-long pathway activation. If corticosteroid exposure were the dominant driver, suppression rather than persistence of mTORC1-associated transcriptional programs would be expected.

Hickey et al.[139] conducted one of the first proteomic analyses examining molecular alterations following BNT162b2 and mRNA-1273 vaccination. Serum samples from adults vaccinated with BNT162b2 or mRNA-1273 were analyzed longitudinally for proteomic changes. Statistical analyses, including predictive modeling, identified key markers and signaling pathways. One month post-third dose, both modRNA vaccines modulated pathways linked to RAS/MAPK and ubiquitin-mediated protein regulation, with consistent upregulation of UB2D1/PolyUbiquitin K48, indicating shared activation of protein degradation. Phosphoinositide-dependent signaling, including PI3K−PI(3,4,5)P_3−AKT, PIP_2-dependent calcium mobilization, and PIP-regulated endocytic trafficking, was modulated, reflecting early membrane−proximal post-translational signaling. Downstream effects varied by vaccine platform and gender.

ChaC glutathione-specific gamma-glutamylcyclotransferase 1 and insulin signaling upregulation diverged between BNT162b2 and mRNA-1273, suggesting platform-specific LNP/ionizable lipid effects on stress and metabolic signaling. A transient induction of cytochrome P450 phase I metabolic pathways occurred exclusively in male BNT162b2 recipients, without activation of the constitutive androstane receptor (CAR)/pregnane X receptor (PXR), arguing against xenobiotic induction and pointing to sex-specific or context-dependent hepatic modulation. In the long term, six-month assessments show persistent activation of translational and ribosomal pathways, with male mRNA-1273 recipients exhibiting additional cap-dependent translation initiation and AKT signaling.

3.3. *Convergent findings across studies and platforms*

Despite differences in model systems (mouse *versus* human), omics platforms (transcriptomics *versus* proteomics), LNP formulations (MC3, ALC-0315, SM-102), and experimental designs, several molecular signatures appear consistently across studies.

(1) Multiple lines of evidence indicate modulation of phosphoinositide-related pathways. These include downregulation of *PIK3C2G* (class II PI3K) across independent mouse datasets, phosphoinositide-dependent signaling alterations observed in human serum proteomics, and dysregulation of ESCRT-associated pathways involved in membrane repair and endocytic trafficking.
(2) Inflammatory and stress-associated signaling pathways are reproducibly engaged, including NF-κB activation, upregulation of TNF-α, IL-6, and IL-1β, chemokine induction, and evidence of NLRP3 inflammasome involvement (for proteomics and transcriptomics across species). TLR4/MyD88-dependent signaling is consistently implicated, with indications of pathway bias, depending on cellular and experimental context.
(3) Metabolic and detoxification pathways are affected in a context-dependent manner. These include downregulation of cytochrome P450-associated xenobiotic metabolism,

suppression of PPAR and AMPK signaling pathways, and attenuation of TCA cycle activity, consistent with altered lipid and energy homeostasis.

(4) Multiple signaling cascades downstream of membrane−proximal events are activated, including RAS/MAPK, PI3K/AKT/mTOR, and JAK−STAT pathways, as observed in both mouse and human datasets.

Importantly, several of these molecular perturbations are observed following exposure to empty LNPs lacking RNA payload, indicating that components alone are sufficient to induce broad transcriptomic and proteomic responses. The convergence of phosphoinositide-dependent signaling changes, PI3K modulation, engagement of the membrane repair pathway, and downstream signaling activation provides empirical support for the L-DMD hypothesis, which posits membrane-level dysregulation as a central initiating event.

However, the biggest hurdle is distinguishing between payloaded LNPs and eLNPs due to their altered *in vivo* behavior, as outlined in Section 2. In addition, antagonistic and/or synergistic effects are expected during endosomal escape, subsequent payload release, and transcription. This was demonstrated by Luo et al.[137] in their proteomics analysis comparing empty *versus* payloaded LNPs.

Taken together, the observed disparities in LNP behavior suggest a shift from empirical description toward a systems-level mechanistic interpretation.

4. Proposed mechanistic hypothesis derived from the omics data: L-DMD as a central node

In this section, we present and discuss several pathways at the level of mechanistic signaling nodes, with direct experimental support (derived from transcriptomic and proteomic data). This serves as a reference framework for network dynamics, rather than as a complete causal chain or disease model. The previous omics signatures will be interpreted through three types of evidence: 1) Direct observations (*e.g.*, PIK3C2G downregulation), 2) Mechanistic nodes with cross-study validation (*e.g.*, TLR4/MyD88), and 3) System-level implications requiring prospective studies (*e.g.*, cell-cycle dysregulation). Phosphoinositides (PIPs) are emphasized as central organizers of these pathways, providing membrane−proximal coordination of signaling nodes and network integration.

4.1. Disruption of the ESCRT circuit and phosphatidylinositol signaling

The most striking observation from the proteomics study by Hickey et al.[139] concerns a reduction in endocytic activity and a downregulation of the ESCRT-circuit, both of which could indicate a disruption of the PI cycle (which refers to the conversion and recycling of PIPs)[145-147]. It has been shown that the modRNA-LNPs induce endosomal membrane ruptures[88], which are detected by the ESCRT machinery. Small disruptions are quickly sealed by the ESCRT-circuit[148] in crosstalk with PIPs before disruption accelerates, and larger perturbations trigger NLRP3 and binding by galectins to signal repair. Interestingly, Förster III et al.[149] demonstrated that mRNA-carrying LNPs can also induce lysosomal rupture, which likewise activates the NLRP3 inflammasome and reduces mRNA translation efficiency.

This is consistent with the review by Hurley et al.[150], which summarizes ESCRTs as central to cell membrane repair mechanisms and, beyond that, to the organelle transport system. ESCRT complexes are recruited to membrane domains in part through interactions with phosphoinositide pools that define membrane identity, and their function in sorting ubiquitinated payload integrates ubiquitin signaling with membrane remodeling[148]. Distinct ESCRT subunits contain ubiquitin-binding motifs that recognize ubiquitylated membrane proteins and phosphoinositides, allowing coordinated recruitment and assembly of ESCRTs at phosphoinositide-rich membranes, thereby linking ubiquitin-driven payload recognition to membrane deformation and trafficking. In summary, this pathway identified by proteomics provides supporting evidence for our hypothesis.

4.2. Downregulated xenobiotic metabolism by cytochrome P450 enzymes

A notably downregulated KEGG pathway identified by Ndeupen et al.[119] and sex-specific for BNT162b2 in Hickey et al.[139] was the metabolism of xenobiotics by cytochrome P450 (CYP) enzymes across species. Available data also imply perturbations in hepatic xenobiotic metabolism, warranting closer examination of CYP enzymes as membrane-embedded pharmacologic sensors. Biochemical and structural studies demonstrate that these enzymes are functionally embedded in biological membranes[151−153].

Rises in pro-inflammatory cytokines, including IL-6, TNF-α, IFN-γ, IL-2, IL-1α, and IL-1β further suppress hepatic CYP1A2, CYP2C9, CYP2C19, and CYP3A4[154,155]. Such reductions usually only occur after marked systemic inflammation (*e.g.*, sepsis, CRP $>$100 mg/L)[156]. Clinically, case reports and cohort analyses document relevant changes in clozapine pharmacokinetics post-vaccination, in some cases leading to neutropenia and hospitalization[157−159].

The mechanism is consistent with inflammation-mediated suppression of CYP enzymes, particularly CYP1A2 and CYP3A4, which are central to clozapine metabolism[160]. Comparable effects have been reported for other CYP-metabolized drugs, including statins, benzodiazepines, antiepileptics, and immunosuppressants, which are predominantly CYP3A4[161] or CYP2C9 substrates[162], likely reinforced by altered membrane conditions. Together, these findings identify context-dependent modulation of CYP-associated pathways following modRNA-LNP vaccination, prompting mechanistic exploration of membrane-related processes.

CYP enzymes are essential for xenobiotic detoxification and endogenous lipid metabolism. They perform crucial biological functions, including cholesterol and fatty acid metabolism, vitamin D activation, and the synthesis of prostacyclins and thromboxanes from arachidonic acid[163].

ModRNA-LNPs can transiently remodel local cellular and vesicular membranes by altering phospholipid organization and membrane curvature through fusogenic and/or receptor-binding interactions[164]. Ionizable lipids with specific amine headgroups have been reported to directly interact with membrane immune receptors such as TLR4 and Cluster of Differentiation 1d, supporting the possibility that LNP components can perturb native lipid microdomains that normally stabilize receptor complexes and membrane-bound enzymes[110].

The exact mechanisms are still under intense investigation. However, such microdomain disruption provides a plausible mechanistic basis for the ‘decoupled’ TLR4 signaling patterns[165]

observed in murine studies by Korzun et al.[134] and Ndeupen et al.[119], as well as in luminescence assays by Zelkoski et al.[132], and could additionally contribute to CYP activity at the hepatocellular membrane interface[166]. In addition, independent biophysical studies have further demonstrated rapid pH-responsive structural transformations of ionizable LNPs that induce curvature stress and lipid packing rearrangements consistent with membrane remodeling behavior[74,167].

Furthermore, CYP enzymes are functionally embedded in biological membranes, with their binding, orientation activity depending crucially on the lipid composition: Anionic phospholipids such as phosphatidylserine or phosphatidylglycerol mediate stable docking of positively charged CYP domains *via* electrostatic interactions[168], while phosphatidylethanolamine, due to its small head group, induces membrane curvature and loosening of the packing, thus enabling partial insertion and correct orientation of the enzyme; this lipid-dependent embedding is dynamic, influences the opening of membrane-side substrate channels and coupling to redox partners[169], and thus directly determines catalytic efficiency, whereby this principle is not limited to ER-localised CYPs, but also applies to mitochondrial steroidogenic CYP enzymes[170]. Therefore, perturbations to membrane composition would be expected to directly alter CYP and catalytic performance.

In summary, CYP downregulation likely reflects both cytokine-mediated transcriptional suppression and direct perturbation of membrane microdomains by ionizable lipids, consistent with the L-DMD prediction that membrane-embedded enzyme function is sensitive to lipid reorganization. Corroborating these molecular events, clinical evidence of post-vaccination hypercholesteremia[171] supports the concept that impaired hepatic detoxification and membrane-associated enzyme modulation translate into measurable serum lipid changes at the population level.

4.3. Are the TLR4 reactions biased? What the mouse data reveal

In line with the CYP observations, the results by Ndeupen et al.[119], Korzun et al.[134], and Zelkoski et al.[132] indicate a biased TLR4 dual-pathway activation pattern, with evidence pointing toward a stronger MyD88-dependent response relative to TRIF-dependent signaling in certain contexts. These observations raise the possibility of divergent or potentially decoupled TLR4 signaling. Such a bias may originate upstream at the cell membrane level and could be linked to early membrane dysfunction, consistent with patterns described by Zelkoski et al.[132] and related literature on ligand-specific TLR4 modulation[172–174].

Building on considerations of membrane structure and lipid microdomain organization, it is mechanistically plausible that early plasma membrane alterations could perturb TLR4 receptor localization, orientation, and/or associated phosphoinositide dynamics (*e.g.*, $PI(4,5)P_2$). This could potentially contribute to biased or divergent dual-pathway activation. As a working hypothesis, this concept may connect our proposed membrane-level dysregulations to the observed TLR4 signaling patterns, given the established interconnection of TLR4 with $PI(4,5)P_2$[172–174].

modRNA N1-methylpseudouridine (m1ψ) modifications suppress endosomal TLR recognition[175], but ionizable lipidamine head groups are capable of simultaneously activating membrane-associated TLR4 and Cluster of Differentiation 1d[110], which could lead to a paradoxical state of simultaneous TLR suppression and activation. This should also be considered in omics data. This dual, potentially opposing regulation may contribute to complex or seemingly contradictory immune activation signatures and should therefore be carefully considered when interpreting omics datasets.

Within the context of the L-DMD hypothesis, such a mechanism provides a plausible explanation for divergent TLR4 signaling patterns and supports the concept that supramolecular LNP properties can modulate receptor signaling independently of the RNA payload. While direct experimental evidence linking LNP-induced membrane perturbation to TLR4 pathway bias remains limited, extensive literature demonstrates that TLR4 signaling is highly sensitive to lipid raft integrity and local $PI(4,5)P_2$ availability, thereby making this a testable mechanistic link within the L-DMD framework[176,177].

4.4. Upregulation of multiple inflammatory markers

Ndeupen et al.[119] showed a remarkable cytokine and chemokine profile. The simultaneous upregulation of TNF-α IL-1β, and IL-17 signaling pathways is notable, since these cytokines form a pro-inflammatory axis frequently implicated in the pathogenesis of autoimmune and chronic inflammatory conditions[178].

While IL-6 can modulate autophagy in the context of oxidative stress[179], and IL-1β levels may be influenced by autophagic clearance of pro-IL-1β[180], their elevation in this setting more likely reflects a broader integration of NLRP3-inflammasome activation[179], NF-κB signaling, and MAPK pathway dysregulation[181–183]. Similarly, β-chemokines such as CCL2, CCL3, CCL4, and CCL7 contribute not only to immune cell recruitment[184,185], but may also intersect with autophagy-related transcriptional regulation (*e.g.*, *via* FOXK1)[186,187]. Korzun et al.[134] observed comparable patterns.

Taken together, the coordinated upregulation of IL-6, IL-1β, TNF-α, IL-17, and β-chemokines (CCL2, CCL3, CCL4, CCL7) across species at the transcriptional and protein levels indicates activation of converging inflammatory pathways, including NLRP3 inflammasome signaling, NF-κB activation, and MAPK pathway dysregulation. Additionally, marked increases were observed in CXC chemokines (CXCL1, CXCL2, CXCL5, CXCL10) and colony-stimulating factor 2 and 3, which displayed the highest fold changes. Interestingly, CXCL5 has been shown to orchestrate the recruitment and spatial organization of innate and adaptive leukocytes in the lung during influenza infection, highlighting its role in regulating local inflammatory responses[188].

Notably, this cytokine and chemokine signature is reproducible across independent studies and experimental models[119,134], arguing against experimental artifacts and supporting the presence of an LNP-associated inflammatory program.

4.5. Complement activation

Korzun et al.[134] observed in the raw data, that all models used C3 activation with a 1.5-log fold change. This suggests that Complement 3 activation was not primarily driven by TLR4. G-protein coupled receptors, such as the C3a receptor, are highly dependent on cell membrane integrity and cholesterol ratios[189–194].

Interestingly, the study by Luo et al.[137] investigated interactions between different ionizable LNP lipids (MC3, Lung SORT, SM-102, and ALC-0315) and cell surface proteins to identify potential competitors for LNP attachment forming a proteinaceous corona. In this context, vitronectin and ficolin-1

were found to particularly facilitate attachment of LNPs to heart tissue cells. Interactions with vitronectin may promote cell proliferation and enhance tumor growth and metastatic potential[195], while ficolin-1, through binding to transforming growth factor (TGF-β1), may modulate the lectin pathway and the complement component of innate immunity[196]. This suggests that the protein corona itself may already have direct effects on membrane organization.

Notably, analysis of the raw data reported by Korzun et al.[134] revealed that PIK3C2G, which encodes a class II PI3K, was consistently downregulated across all datasets, providing additional evidence in support of our L-DMD hypothesis. The combination of TLR4-independent C3 activation and evidence that protein corona components directly engage membrane-associated receptors supports the L-DMD model, in which LNPs can initiate immune signaling through biophysical membrane reorganization prior to, or in parallel with, classical receptor-mediated pathways.

4.6. *Downregulation of PPAR and AMPK signaling*

Given that peroxisome proliferator-activated receptor gamma (PPARγ) is a central regulator of lipid metabolism, mitochondrial function, and anti-inflammatory signaling[119], and is modulated through AMPK-dependent phosphorylation[197,198], its strong downregulation at the transcriptional level suggests disruption of homeostatic lipid and energy control. Such suppression, as reported under inflammatory or stress conditions[199–201], may exacerbate NF-κB activation (as shown by the data) and contribute to chronic inflammation and/or unpredictable outcomes. The expression pattern is indicative of MAPK pathway dysregulation affecting PPARγ phosphorylation and signaling[200,201]. Notably, in macrophages, PPARγ regulates inflammatory gene expression[200]. Ballav et al.[202] highlight that partial PPARγ dysregulation can broadly disrupt cellular metabolism and signaling, influencing disease fate decisions across multiple tissues.

If such PPAR suppression were sustained across tissues, as documented in Section 2.2 (liver, spleen, lymph nodes), this would be consistent with the systemic, multi-organ distribution pattern of the LNPs and the L-DMD framework. However, direct evidence of persistent PPAR dysregulation in human tissues following modRNA-LNP administration is lacking, and extrapolation to chronic disease outcomes requires longitudinal validation.

4.7. *RAS (Rat sarcoma) and MAPKs*

In summary, the insights of Knabl et al.[138] and Korzun et al.[134] support the assumption that the inflammatory properties of LNPs observed in mice by Ndeupen et al.[119] and the findings of Luo et al.[137] are also present following BNT162b2 transfection. Moreover, not all immune cells internalize LNPs equally, as uptake efficiency is influenced by factors such as the protein corona and the modRNA payload (Section 2.2).

Such induction is consistent with stress-responsive transcriptional reprogramming. In this context, KRAS activation represents a central signaling hub, as RAS-driven pathways can engage, for example, both RAF/MEK/ERK, p38-MAPK pathways and PI3K/AKT cascades (which was also observed in the proteomics by Hickey et al.[139]), thereby integrating inflammatory, metabolic, and genotoxic stress signals and promoting short-term cellular adaptation. The concurrent upregulation of p53, as also reported by Knabl et al.[138], suggests the activation of compensatory checkpoint mechanisms that counterbalance proliferative or stress-related cues[203,204].

Growing evidence indicates that GTPases, particularly RAS isoforms and RHOA, are spatially localized at the plasma membrane, where they play crucial roles in vesicle trafficking and membrane organization. Additionally, these GTPases can interact with lipids of the inner membrane leaflet, including phosphatidylserine and PIPs, forming lipid-protein interfaces that may influence local signaling nanodomains[34,205–210].

The partial heterogeneity of transcriptional changes among different age groups highlights limitations in generalizing the transcriptomic effects observed in Knabl et al.[138] and sets the stage for the subsequent discussion of more controlled and consistent experimental models.

However, these observations position RAS[211,212] and other related GTPases, like RHO[213], as membrane−proximal integrators of phosphoinositide- and voltage-dependent signaling perturbations, providing a direct mechanistic bridge between membrane dysregulation and downstream transcriptional reprogramming, including the translation of altered electrical signaling into sustained cellular state changes[211,212]. Interestingly, Guillot-Ferriols et al.[214] and Chen et al.[215] demonstrated that electric fields affect intracellular signaling pathways.

4.8. *Transcription factors (E2F1, E2F8) and mechanistic target of Rapamycin complex (mTORC)*

Knabl et al.[138] note: "*Interestingly E2F8, but not CCNA1 or E2F1, was modestly upregulated 7 days post-vaccination in the naïve vaccinated individuals (1.5-fold, padj = 0.04).*" However, growing evidence suggests that *E2F1/2/4* expression correlates with immune cell infiltration and may modulate immune responses in proliferative or stressed cells[216–218]. It is known that E2F1 is in tight crosstalk with the 7 other members of the E2F transcription factor family[219–221] during cell cycle phases. Furthermore, it has been shown that E2F1 plays a crucial role in immune cell differentiation and cloning[222,223].

These findings support the notion that modRNA-LNP exposure can induce selective, systemic transcriptomic changes even in the absence of active viral infection, with partial activation of cell cycle genes, interferon responses, and partial mTORC1 engagement without coherent downstream cell-cycle coordination, consistent with a fragmented or dysregulated cell cycle program rather than a synchronized proliferative response[224,225].

Furthermore, the consistent active p53 upregulation in both age groups, together with a decoupled *E2F1* from *E2F8* transcription factor transcriptome, indicates that cell cycle dysfunctionality was observed in the immune cells, which depended on the mTORC1 signaling regulating *CDC25A*, *RRM2* and *BUB1*[226,227]. Another indication that the cell cycle is affected at the transcriptomic level is the coordinated enrichment of gene sets associated with mitosis, checkpoint control, and Rho-GTPase signaling. All analyses were performed using KEGG and showed that the signals were active.

Emerging evidence suggests that PI(3,5)P_2 may act as an upstream lipid regulator by forming a triad of lipid−lipid, lipid−protein, and protein−protein interactions, thereby modulating PI3K class I signaling while being generated by PI3K class III and phosphoinositide kinase FYVE-type zinc finger-containing activity. These pathways are functionally linked to mTORC1 and mTORC2 regulation[228–230].

This decoupled activation pattern indicates a state of cellular stress adaptation rather than controlled expansion, with potential

long-term consequences for immune cell homeostasis and functional changes.

4.9. *Conceptual consolidation of sections 3 and 4*

Of note, Luo et al.[137] also observed with empty LNPs the activation of eucaryotic initiation factors (eIF) family. Driven by these data, we suggest that signaling transduction pathways such as mTORC and the MAPKs regulating eIFs may also be involved in endosomal escape processes[231,232].

However, *in vitro*, *ex vivo*, and *in vivo* mouse data demonstrate that lipid-formulated constructs, such as liposomes and LNPs, alter cellular transcriptomics and proteomics by modulating signal transduction pathways, receptor activation, and phosphorylation cascades[119,132,233–235]. This finding is of considerable significance, as the omics data we have discussed here, along with shifts in signaling pathways, cannot be attributed solely to the presence of modRNA and/or spike proteins.

This is reasonable, given the sequence of events: the LNPs are first taken up *via* membrane physics and chemistry (time point 0), after which modRNA is released into the cytosol with a delay. In fact, release rates, estimated to be at 10%−15% or less, as suggested by Müller et al.[42], appear far more likely to indicate that most of the modRNA remains within the LNP and is subsequently exocytosed. Importantly, this delayed release and translation may occur at anatomical sites distant from the injection site (Section 2.3).

Our L-DMD hypothesis implies that LNPs may also drive the immune system's initial response through membrane-driven effects on transfected cells, thereby affecting systems beyond immune cells. This is further supported by the study by Connors et al.[236], which showed that eLNPs induce activation and maturation of monocyte-derived dendritic cells (MDDCs) and upregulate CD40 expression, leading to the recruitment of pro-T follicular helper cell cytokines, IL-6, IL-12, and IL-21. These findings are also consistent with Amor et al.[237]. Furthermore, Qin et al.[233] showed in mice that these effects have transgenerational immunological consequences.

Taken together, these findings indicate that LNP-driven immune activation is not restricted to acute innate immune responses or transient antigen-presentation-linked effects, but can also engage epigenetically mediated regulatory programs, consistent with recent evidence demonstrating short-term and persistent epigenetic memory in innate immune cells following BNT162b2 mRNA vaccination[238,239]. In this context, recent work by Chyła et al.[240], for example, has highlighted that PI(4,5)P_2 signaling is not confined to the plasma membrane but also operates at the nuclear level, where it contributes to transcriptional regulation and chromatin organisation[241].

We next summarize the given evidence supporting our working hypothesis. Table 2[119,132,134,137–139,159,171,242] organizes the omics-derived pathway alterations (Sections 3 and 4) into functional categories that reflect the proposed L-DMD mechanistic cascade. Primary evidence comes from the previously discussed omics studies, supported by human observations, to show the functional relevance of molecular findings in human populations. Data serves as supporting evidence of pathway engagement rather than as validated clinical evidence outcomes.

Table 3[119,134,137–139] presents primary omics and functional studies that inform the pathway classifications in Table 2. Each record details the model, material type, route of exposure, data modality, and timeline used to resolve membrane-associated signaling alterations described within the L-DMD framework.

Taken together (Tables 2 and 3), these findings suggest that the earliest perturbations following LNP exposure likely originate at the plasma membrane, with the PI cycle as a central regulatory hub. Even subtle disturbances can cascade through lipid raft organization, receptor localization, and downstream signaling, producing transcriptomic and proteomic alterations, as shown by Hickey et al.[139] and Luo et al.[137].

Establishing precise *in vivo* mechanistic patterns remains challenging, especially without large cohorts stratified by age, vaccine type, dose, batch, comorbidities, prior illnesses, genetic predisposition, and LNP formulations. Extrapolation to chronic outcomes (*e.g.*, autoimmunity[243], myocarditis[117]) requires longitudinal validation. The pathway enrichments presented here are hypothesis-generating rather than definitive disease mechanisms.

Omics data from Sections 3 and 4 demonstrate multi-pathway perturbations following LNP exposure that cannot be attributed to the payload alone. Convergent alterations—including phosphoinositide signaling[139], *Pi3kc2g* downregulation[134], mTOR and MAPK engagement, ESCRT dysregulation[139,149], and metabolic pathway suppression[119] across studies and platforms support the L-DMD hypothesis.

These observations indicate that early plasma membrane perturbations propagate through lipid rafts, receptor localization, and downstream signaling cascades, resulting in the observed transcriptomic and proteomic changes. Such early membrane-centered dysregulation, though not reflected in conventional lab values, may initiate broader signaling alterations, including paracrine and endocrine effects[244].

In the next section, we examine membrane organization, focusing on lipid rafts, endosomal-lysosomal, and autophagosomal aspects that may drive changes in gene expression in the PI3K/AKT/mTORC1[245,246], JAK−STAT[247,248], and MAPK[249] cascades observed in the omics data.

5. Breaching the plasma membrane: important roles for phosphoinositides (PIPs)

In Section 2, we outlined that LNP/modRNA complexes enter cells *via* receptor-dependent and receptor-independent endocytosis-like mechanisms, including membrane breaching. LNPs transfect diverse cell types, including epithelial cells, B cells, T cells, macrophages, and dendritic cells, depending on formulation and *in vivo* transformations, though the contribution of individual cell types to COVID-19 modRNA-LNP transfection remains under investigation. Computational simulations show that various ionizable lipids can integrate into the inner plasma membrane leaflet[17–19], making early membrane-level alterations of receptor localization or conformation mechanistically plausible. LNP uptake occurs *via* endocytosis and/or macropinocytosis, as demonstrated by Panariti et al.[250], Voigt et al.[251], and reviews by Wang et al.[252] and Gerelli[253].

Building on the phosphoinositide-centered signaling framework, we expand the hypothesis to include membrane biophysical and biochemical constraints that may couple LNP exposure to intracellular trafficking dynamics, integrating previously discussed multi-omics observations with membrane-scale processes. Membrane penetration and direct interactions of ionizable lipids with the bilayer and membrane-bound receptors may drive membrane reorganization events, including alterations in lipid raft

Table 2 Membrane-associated signaling pathways perturbed by LNP exposure.

Section	Pathway	Membrane connection	Representative evidence	Ref.
Membrane structure and maintenance				
4.1	ESCRT/Endocytosis	• Membrane repair following endosomal stress. • Recruitment depends on phosphoinositides.	H-Px, ↓ESCRT proteins and ↓endocytic activity after modRNA-LNP injection.	Hickey et al.[139]
4.2	CYP	• ER/mitochondrial embedded membranes. • Catalytic geometry depends on local phospholipids and charge.	↕ M-Tx (eLNP), H-Px, H-Obs, ↓CYP metabolism post i.m. injection in mice; ↑Phase I enzymes (♂ only, BNT162b2 specific, hepatocellular leakage[a]), clozapine toxicity post-vaccination (functional corroboration[b]).	Ndeupen et al.[119] Hickey et al.[139] Thompson et al.[159]
Innate immune signaling				
4.3	TLR4 axis (MyD88/TRIF bias)	• Plasma lipid membrane organization. • Intact phosphoinositides needed for balanced signaling.	M-Tx (eLNP), M-Fx, MyD88-dominant NF-κB activation, reversible with TLR-4 blockade (TAK-242) in human THP-1 cells *in vitro via* NF-κB.	Ndeupen et al.[119] Korzun et al.[134] Zelkoski[c] et al.[132]
4.4	Pro-inflammatory cytokines	• Membrane-associated PRR signaling (NF-κB/MAPK). • Functional organization of opes within inner-leaflet lipid raft.	↕ (M-Tx, M-Tx (eLNP)), H-Obs, ↑NF-κB targets and canonical cytokines in murine eLNP, Concordant upregulation in human datasets, cytokine-mediated drug interactions documented.[d]	Ndeupen et al.[119] Korzun et al.[134] McColl et al.[242]
4.4	B-Cytokines, CXC- chemokines, IL-6, IFNs (across species)	• Membrane proximal NF-κB activation.	↕ (MTx, MFx (eLNP)), H-Tx, Concordant CCL family upregulation in murine eLNP and human buffy-coat transcriptomes (CXCL 9,10).	Ndeupen et al.[119] Korzun et al.[134] Knabl et al.[138]
4.5	Complement (C3, lectin)	• Membrane-bound GPCR receptors (C3aR). • Protein corona effects.	M-Tx (eLNP), ↑C3. M-Px, LNP-vitronectin/ficolin-1 binding facilitates C3 activation events.	Korzun et al.[134] Luo et al.[137]
Growth and metabolic signaling				
4.7	RAS−RAF−MEK−ERK (MAPK)	• RAS GTPases anchor lipids at plasma membrane nanodomains. • Translate local charge shift to signal amplification.	↕ M-Tx (eLNP), H-Tx, H-Px, MAPK pathway upregulation across species omes.	Korzun et al.[134] Knabl et al.[138] Hickey et al.[139]
4.7	PI3K/AKT/mTOR	• Membrane-localized PI3K. • Partial mTORC1 engagement indicates upstream dysregulated lipid signaling *via* RAS.	↕ M-Tx (eLNP), H-Tx, H-Px, Partial MAPK pathway + PI3K modulation post LNP exposure.	Korzun et al.[134] Knabl et al.[138] Hickey et al.[139]
4.4	PI3K class II	• Often activated downstream of RTKs + GPCRs. • Act independently of regulatory subunits characteristic of PI3Ks.	M-Tx (eLNP), Consistent ↓*Pik3c2g* across datasets.	Korzun et al.[134]
4.6	PPARγ	• Integrates lipid-derived signals. • Suppression favors NF-κB activation.	↕ M-Tx (eLNP), H-Obs, ↓PPARγ pathway → loss of anti-inflammatory tone + lipid metabolism imbalance, post-vaccination hypercholesterolemia (OR, 1.54, 95% CI: 1.36 −1.74; population level corroboration)[e].	Ndeupen et al.[119] Huang et al.[171]
4.6	AMPK	• Senses membrane-linked stress. • Suppression favors NF-κB activation.	M-Tx (eLNP), ↓AMPK → reduced oxidative metabolism and ↑NF-κB.	Ndeupen et al.[119]
Cell cycle and stress response				
4.8	p53 + *E2F* Network	• Downstream of RAS−MAPK and PI3K−mTOR signaling. • Cell-cycle checkpoint.	H-Tx: p53 + *E2F8,* partial mTORC1 activation.	Knabl et al.[139]

Section numbers correspond to their respective sections in the main text.

H, human, M, mouse; Tx, transcriptomic; Px, proteomic; Obs, observational; Fx, functional; ↕, cross-species concordance (human + mouse); AKT, protein kinase B (also PKB); AMPK, AMP-activated protein kinase; C3, complement component 3; C3aR, complement C3a receptor; CAR, constitutive androstane receptor; CCL, C−C motif chemokine ligand; CI, confidence interval; CXCL, C−X−C motif

chemokine ligand; CYP, cytochrome P450 enzyme; E2F, E2 transcription factor family; eLNP, empty lipid nanoparticle (no mRNA payload); ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ESCRT, endosomal sorting complex required for transport; GPCR, G protein-coupled receptor; GTPase, guanosine triphosphatase; i.m., intramuscular administration; KRAS, Kirsten rat sarcoma viral oncogene homolog; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase (also MAPK/ERK kinase); modRNA−LNP, nucleoside-modified mRNA formulation encapsulated in a lipid nanoparticle; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa B; OR, odds ratio; p53, tumor protein p53; PI3K, phosphatidylinositol 3-kinase; PIK3C2G, phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 gamma; PIP, phosphatidylinositol phosphate; PM, plasma membrane; PPARγ, peroxisome proliferator-activated receptor gamma; PRR, pattern recognition receptor; PXR, pregnane X receptor; RAF, rapidly accelerated fibrosarcoma (serine/threonine kinase); RAS, rat sarcoma; RTK, receptor tyrosine kinase; TLR4, toll-like receptor 4, TRIF, toll/interleukin-1 receptor/resistance protein (TIR)-domain-containing adapter-inducing interferon-β.

[a] Sex- and vaccine-specific enzyme elevation without CAR/PXR indicates leakage, rather than de novo synthesis[139].

[b] Clinical corroboration: Clozapine (CYP1A2/3A4 substrate) toxicity post vaccination supports enzyme suppression observed in omics datasets. Observational data included as supportive evidence of pathway engagement.

[c] Not Omics data but included as supportive evidence for the mechanistic inference.

[d] Cytokine-mediated mechanism reviewed by McColl et al.[242]; observational reports provide complementary validation.

[e] Population-based association for lipid metabolic imbalance; causality requires further investigation.

stability, which serve as organizational platforms for receptors such as G protein-coupled receptors (GPCRs) and receptor tyrosine kinases.

This aligns with observations by Wang et al.[252] and Aliabarinodehi et al.[74]. Lavagna et al.[254] suggest that size-dependent aggregation of hydrophobic nanoparticles within lipid membranes induces local curvature, folding, and transient phase transitions, acting as an initial mechanical trigger perturbing phosphoinositide organization and receptor localization, and setting the stage for altered signaling and recycling dynamics.

5.1. *Brief overview of the phosphatidylinositol (PI)-cycle*

Given that membrane interactions are evident, the membrane structuring and recycling process must be examined by investigating the PI-cycle (Fig. 3 and Supporting Information Table S1) and the potential effects LNPs may have on it.

The eukaryotic plasma membrane is a phospholipid bilayer forming a stable barrier between aqueous compartments[255]. Phospholipids are amphipathic, with hydrophobic tails oriented inward and hydrophilic heads facing the aqueous environment, thereby enabling selective transport. Membrane fluidity depends on fatty acid composition and cholesterol content. Embedded proteins, such as receptors (*e.g.*, GPCRs), ion channels, and transmembrane enzymes (*e.g.*, ACE2), mediate transport, signaling, and cell−cell recognition *via* extracellular signals[256]. Fatty acid composition often follows the 18:0/18:1 or 18:1/18:1 rule, where unsaturated chains (*e.g.*, 18:1) reduce packing density and increase lipid mobility[257]. The bilayer should be regarded as a fluid mosaic[258].

Lipid rafts are specialized microdomains enriched in sphingolipids and cholesterol, with specific phospholipid composition, including phosphoinositides, influencing membrane order and permeability[259,260]. They create local spatial and electrical potential differences, promoting receptor clustering and anchoring of signaling molecules. Phosphoinositides act as first intracellular messengers, initiating downstream cascades at plasma and endosomal membranes after receptor internalization[261]. These cascades regulate the cycle *via* feedback loops [*e.g.*, *via* RHO-GTPases (Ras homologous) and cell division cycle 42 (Cdc42—part of the RHO family)] [262,263].

Fallahi-Sichani & Linderman[264] showed that lipid rafts as membrane microdomains influence the localization of receptors like GPCRs. With rapid dimerization and monomerization, frequent partner changes lead to the formation of oligomeric receptor clusters. In addition, the effect of ligands varies broadly: "*while some GPCRs are stimulated to dimerize by ligands, others are inhibited-indicating a differential, ligand-dependent receptor organization.*"[264].

It has been suggested that these membrane microdomains also play a critical role in mediating interactions with exogenous particles. They can facilitate interactions between LNPs and the cellular membrane, thereby enhancing intracellular uptake through endocytosis[265]. Furthermore, the specific organization of these domains improves recognition and internalization processes, coordinates receptor localization and membrane trafficking and influences downstream signaling events, thus highlighting their central role in understanding how LNPs influence cellular homeostasis.

Although not yet demonstrated *in vivo*, it is conceivable that lipid nanoparticles may interfere with autophagic lysosome reformation (ALR)[266,267], a process essential for sustaining lysosomal homeostasis and autophagic flux[268,269]. Since ALR depends

Table 3 Summary of omic studies informing Table 2 (model, methods, route, and data type).

Study and reference	Model/species	Material tested	Route/dose	Data type/readout	Timeline/endpoints
Ndeupen et al.[119]	Mouse (C57BL/6, WT).	eLNP (no mRNA).	i.m. 10 μg in PBS.	M-Tx, (Whole tissue RNAseq + GSEA) -seq + GSEA.	~24 h post injection.
Korzun et al.[134]	Mouse (WT + KO lines).	Luc mRNA LNP + eLNP.	Luc mRNA + eLNP groups received 5 μg in 100 μl PBS *via* i.p. as a single dose (acute) or i.p. once every 24 h for 3 doses (chronic). $n = 4$ groups total. eLNP groups received TAK-242 inhibitor 2 h prior to each dose.	M-Tx, M-FX, (behavior + ELISA + Transcriptome).	6 h post last dose.
Luo et al.[137]	Mouse/cell models.	eLNP (no payload) + comparators.	Multiple routes (i.n., i.m., i.v., PO, ID), 0.0005−0.5 mg/kg.	M-Px, (quantitative proteomics).	≤24 h post dose.
Hickey et al.[139]	Human (adults post BNT162b2/mRNA1273 vaccination)[a].	modRNA-LNP vaccination.	i.m., clinical dose.	H-Px, (SomaScan v4.1 proteomics).	1 month and 6 months post-dose dose 3.
Knabl et al.[138]	Human (patients and healthy volunteers)[a] Elderly patients (3 doses, postvaccination or infection, treatment with dexamethasone) Younger healthy group (naive, 1st dose).	modRNA-LNP vaccination/ infection cohort.	i.m., clinical dose.	H-Tx, (whole blood buffy coat transcriptomics)	Elderly, Days 7−60. Younger, Days 7−10.

C57BL/6, inbred mouse strain C57 black/6; eLNP, empty lipid nanoparticle (no mRNA payload); Fx, functional assays; GSEA, gene set enrichment analysis; H, human; i.m., intramuscular administration; i.p., intraperitoneal administration; i.v., intravenous administration; KO, knockout; Luc, luciferase; SQ, subcutaneous administration; i.n., intranasal administration; M, mouse; PO, oral administration; ID, intradermal administration; Px, proteomic profiling; seq, sequencing (or sequence data); Tx, transcriptomic profiling; WT, wild type.

[a] Post-vaccination samples; Knabl cohort included patients with concurrent SARS-CoV-2 Beta-variant infection, potentially confounding attribution to LNP-only effects. Hickey cohort represents healthy adults post 3rd dose.

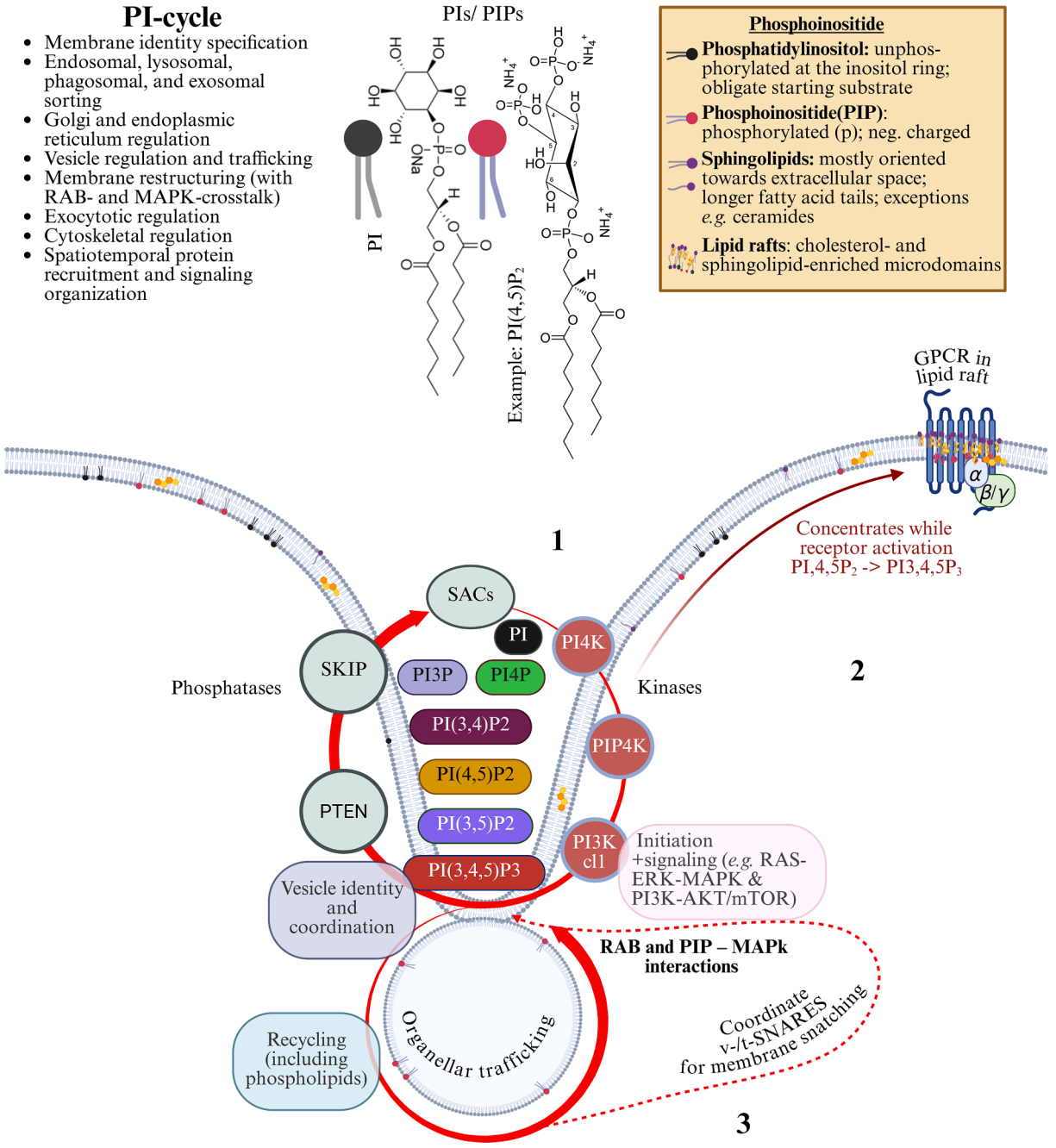


Figure 3 Cell membrane organization and the phosphatidylinositol (PI)-cycle. This schematic summarizes established PI-cycle biology and its role in defining membrane identity, vesicle trafficking, and endosomal maturation through spatially restricted phosphoinositide-species. Distinct phosphorylated PI pools coordinate receptor-proximal signaling and trafficking decisions, thereby linking membrane dynamics to downstream pathways such as PI3K−AKT−mTOR and RAS−ERK/MAPK signaling. (1) Phosphoinositide phosphatases remove phosphate groups, ensuring spatial and temporal signal termination within the PI cycle. (2) Phosphoinositide kinases generate specific phosphoinositide-species through site-specific phosphorylation of phosphatidylinositol. (3) Phosphoinositides cooperate with SNARE-proteins and small GTPases (*e.g.*, RAB) to regulate membrane curvature, fusion, and biophysical properties that underlie vesicular transport and signal compartmentalization. Abbreviations: AKT, protein kinase B; ERK, extracellular signal-regulated kinase, GPCR, G-protein coupled receptor; MAPK, mitogen-activated protein kinase; mTOR, mechanistic target of rapamycin; mTORC, mechanistic target of rapamycin complex; PI, phosphatidylinositol; PIP, Phosphoinositide; PI4P, phosphatidylinositol 4-phosphate; PI(3,4)P_2, phosphatidylinositol 3,4-bisphosphate; PI(3,5)P_2, phosphatidylinositol 3,5-bisphosphate; PI(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PI(3,4,5)P_3, phosphatidylinositol 3,4,5-triphosphate; PI3K, phosphoinositide 3-kinase; PI3Kcl1, class 1 phosphoinositide 3-kinase; PI4K, phosphatidylinositol 4-kinase; PTEN, phosphatase and tensin homolog; SAC, sac domain-containing phosphoinositide phosphatase; RAB, ras-related in brain (GTPase family); RAS, rat sarcoma (GTPase family); SKIP, sphingosine kinase-interacting protein; SNARE, soluble NSF attachment protein receptors. Created in BioRender by Seger, F. (2026). https://BioRender.com/unux4hx.

on phosphoinositide transitions[270] and clathrin-mediated membrane remodeling[271], the insertion of ionizable lipids into these organelles could compromise the delicate balance of lysosomal recycling. Such perturbations may not only disturb autophagic flux but could also contribute to organ-specific toxicities reported in the nanomedicine safety literature[108].

5.2. *Signaling through phosphorylation states of phosphatidylinositol*

Phosphatidylinositols (PIs) are amphiphilic glycerophospholipids that insert into the plasma membrane with their inositol headgroups facing the cytosol. At physiological pH, PI is monoanionic (net charge ∼ −1), constitutes ∼5%−10% of membrane phospholipids[272,273], and serves as a structural membrane component. Phosphorylated derivatives, phosphoinositides (PIPs), are low-abundance signaling lipids (∼1%−5%) that remain membrane-bound, define organelle identity, and regulate trafficking, including endocytosis, phagocytosis, and receptor-mediated internalization.

The PI cycle, described by Posor et al.[274] (Table S1 for detailed PIPs-transitions with enzymes and kinases involved), is a dynamic network of phosphorylation transitions, *e.g.*, $PI(4,5)P_2 \leftrightarrow PI(3,4,5)P_3$, controlled by kinases (*e.g.*, PI3K), phosphatases (*e.g.*, phosphatase and tensin homolog, inositol polyphosphate phosphatases), and downstream effectors like AKT. These transitions govern organelle maturation, endosomal−lysosomal trafficking, phospholipid recycling, and membrane integrity[274−277], integrating with Ras-related in brain (Rab) GTPases and signaling cascades such as AKT/mTOR and ERK. Exposure to LNPs is hypothesized to perturb membrane dielectrics, electrostatics and biophysics, phosphoinositide organization, and endocytic trafficking, with secondary effects on PI-dependent signaling and organelle identity[278,279].

5.3. *The LNP components and their effects*

5.3.1. *Ionizable lipids*

Given that PIPs mark endosomal membranes and tightly regulate organelle maturation and trafficking, disruption of their dynamic phosphorylation-dephosphorylation cycles can compromise membrane integrity (Fig. 4). These lipids interact with negatively charged PIPs and the surrounding phospholipid matrix, potentially destabilizing the tightly regulated membrane environment described above. Consequently, this can induce transient endosomal and lysosomal membrane rupture or permeabilization, as demonstrated in recent studies, thereby facilitating the cytosolic release of encapsulated payloads, such as modRNA, while bypassing the canonical degradation pathways normally orchestrated by the PI cycle[88,149]. This is especially notable because only a proportion of payload-containing LNPs (depending on the formulation and cell type) reach the phase of endosomal/lysosomal escape[72], and it is not yet known how many remain in endosomal/lysosomal/autophagosome transition[76] (discussed in Section 2).

A recent spatiotemporal analysis[87] using sensitive LNP labeling platforms further supports this notion, showing that continuous endocytosis and endolysosomal trafficking are required to maintain pools of releasable compartments, thereby mechanistically explaining why only a fraction of LNPs achieve cytosolic release while the remainder remain in lysosomes. This partial efficiency highlights the unresolved question of how the remaining LNPs, which do not induce endosomal rupture and are trafficked to lysosomes, are subsequently degraded or recycled within the cell, and whether their presence might disrupt these processes.

LNPs, upon endocytic uptake, introduce high local concentrations of cationic or ionizable lipids into the endosomal membrane, a phenomenon highlighted as a major determinant of both efficacy and toxicity of LNP formulations. Several studies, including Čechová et al.[17], Ermilova and Swanson[18], and Atmuri et al.[19], have proposed that such lipids can trigger integrative membrane events. Jørgensen et al.[90] further report that ionizable lipid−membrane interactions may lead to endosomal disruption, and that even biodegradation products of protonated tertiary amines-containing ionizable lipids, such as ALC-0315 and SM-102, remain bioactive. The authors note: "*However, they are neither synthesized from endogenous or natural structures nor are they degraded into typical biocompatible building blocks.*" How the integrative events[17−19] relate to the direct disruption observed by Jørgensen et al.[90] remains to be clarified.

5.3.2. *DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine)*

In the context of the recycling processes and membrane organization, another aspect that has received little attention is the phospholipid, DSPC[280,281], which is used as a helper lipid in LNPs. DSPC (also known as 18:0/18:0) has two saturated stearic acid chains. While structurally similar to the phospholipids naturally found in plasma membranes, DSPCs are more tightly packed, more rigid, and more thermally stable[282] than the typical 18:0/18:1 and 18:1/18:1 phospholipids found in eukaryotic membranes[257,283]. It has a high gel-liquid transition temperature (T_m approx. 55 °C), which gives it unusual rigidity[284]. It is a natural phospholipid, primarily found in human pulmonary surfactant[285].

This raises the question of how DSPC affects the PI-cycle, given that phosphatidylcholines are recycled into various phospholipids, including PI. Phospholipase D, which converts phosphatidylcholines into phosphatidic acid, plays an essential role in this process[286−288]. Phosphatidic acid is converted into CDP-diacylglycerol by CDP-diacylglycerol synthase, a key step in channeling phosphatidic acid into the biosynthesis of PI and other phospholipids[273,289]. CDP-diacylglycerol subsequently serves as the immediate precursor for PI, synthesized by PI synthase at the endoplasmic reticulum, forming the core PI scaffold[273,290].

Data on how DSPC is degraded and whether it can interfere with membrane physiology beyond the transfection process *via* recycling is sparse. After 24 h, most DSPC primarily accumulates in the liver, spleen, heart, kidney, and lung, indicating that these organs' plasma membranes take up the lipid[291]. This confirms DSPC's tissue delivery and retention. However, liposomes composed of phospholipids such as DSPC can markedly alter membrane structure, as previously discussed in the review by Lonez et al.[234]. Studies indicate that DSPC-containing liposomes can undergo hydrolytic and oxidative degradation, producing phospholipid-derived products that may alter membrane structure and stability in unpredictable ways[292,293]. However, the exact cellular mechanisms *in vivo* remain under investigation.

5.3.3. *Oxysterol-binding proteins*

Oxysterol-binding protein-related proteins coordinate PI metabolism with sterol transport[294,295], adding regulatory complexity to the PI cycle. Whether LNP-delivered cholesterol engages this pathway, particularly under inflammatory conditions[296] that alter sterol trafficking and phosphoinositide turnover, warrants systematic investigation to determine if oxysterol-binding protein-related proteins mediate lipid exchange or signaling events.

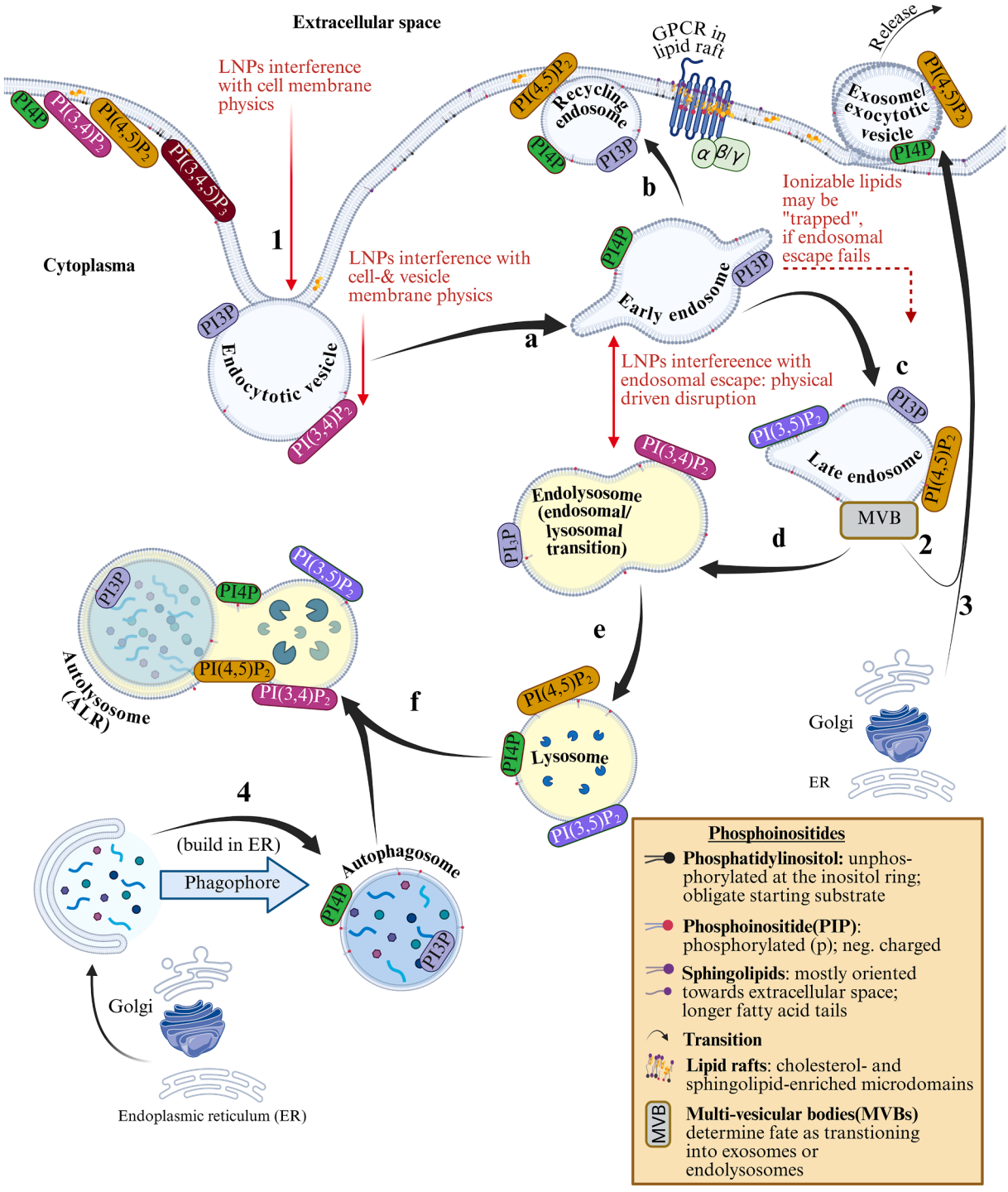


Figure 4 Organelle trafficking, organelle membrane identity, and lipid nanoparticle influence. (1) Internalization. Lipid nanoparticles are taken up by endocytosis and enter early endocytic vesicles. LNPs may interfere with physics of plasma and vesicle membranes during uptake and early endosomal trafficking. (a) Early endosome. Defined predominantly by PI3P, with contributions from PI4P. PI3P is generated by class III PI3K, which establishes endosomal identity by recruiting effector proteins, such as ESCRT-0 and SNX, for cargo sorting. (b) Recycling endosome. Enriched in $PI(4,5)P_2$, PI4P, and PI3P, supporting membrane recycling, cargo resorting, and rebalancing of phosphatidylinositol species. (c) Late endosome. Characterized by PI3P with increasing levels of $PI(3,5)P_2$, generated by PIKfyve. Regulates cargo sorting and MVB formation, and trafficking toward exosome release or lysosomal fusion. Incomplete endosomal escape may trap ionizable lipids within these compartments. (d) Endolysosomal transition. Characterized by elevated $PI(3,5)P_2$ with residual PI3P supporting lysosomal maturation processes, including ion channel activity and V-ATPase function. (e) Lysosome. Dominated by $PI(3,5)P_2$ and variable phosphoinositide species depending on functional state. Coordinates degradation of intracellular components and autophagic lysosomal reformation (ALR). (f) Autolysosome. Contains PI3P derived from autophagosomes and $PI(3,5)P_2$ derived from lysosomes. During ALR, $PI(4,5)P_2$ and PI4P are involved, with $PI(4,5)P_2$ recruiting clathrin and the AP-2 complex to support membrane tubulation and lysosome reformation. (2) and (3) Exocytosis pathways. Cargo release occurs

5.3.4. *A role for lipid impurities*

As we suggested in Section 2, LNPs act as bioactive particles with unpredictable biodistribution and transfection pathways. This makes it difficult to determine the membrane conditions under which their proposed mechanisms of action will occur. Furthermore, the process by which lipid impurities or their by-products may influence endosomal-lysosomal transition, autophagosome formation, and the subsequent autophagosome-lysosome fusion that leads to autolysosome formation requires further study.

Covalent RNA-lipid adducts formed during manufacturing[94,95], and possible oxidative degradation products (*e.g.*, 4-HNE), may compound membrane perturbations (see Section 2.6). In particular, 4-HNE, a highly reactive α,β-unsaturated aldehyde generated from peroxidation of omega-6 polyunsaturated fatty acids, readily forms Michael adducts with nucleophilic amino acid residues (cysteine, histidine, lysine) in proteins[297,298] If sufficiently generated *in vivo*, 4-HNE and related electrophilic lipid species merit systematic investigation as they are established modifiers of MAPK components and other signaling pathways[297,298]. Such modification could disrupt phosphoinositide homeostasis, whether by altering PI(4)P pools at the Golgi, impairing PI(4,5)P_2-mediated membrane dynamics, or modulating PI(3,4,5)P_3 signaling cascades that control vesicle trafficking, autophagy, and immune sensing, or directly modifying phosphoinositide-metabolizing enzymes that may contribute to sustained pathway dysregulation beyond direct membrane perturbations.

5.4. *Small perturbations can lead to major shifts in PIP signaling*

Recent work by Fung et al.[22] demonstrates that phosphoinositide (PIP) interconversion is governed by feedback and feedforward circuits, excitable and oscillatory regimes, and kinetic thresholds, such that minor perturbations in membrane composition, enzyme activity, or lipid transport can trigger disproportionately large and sometimes paradoxical shifts in PIP balance. This nonlinearity implies that local LNP-associated perturbations (*e.g.*, ionizable lipids, DSPC, or pH changes) may push membranes across critical dynamical thresholds in downstream signaling[299], altering endosomal identity, trafficking, and payload release. Consequently, simple formulation optimization may not ensure predictable delivery across diverse cell types or physiological states.

This perspective highlights that LNP-induced effects are not purely additive but may exceed critical system thresholds that conventional toxicological frameworks[299], which often inadequately model complex, self-assembling bioactive nanosystems, may fail to capture. Models of lipid nanoparticle activity that neglect intracellular phosphoinositide processing and downstream signaling cascades, including MAPK pathways, therefore remain incomplete. Fung et al.'s[22] findings furthermore align with broader evidence that PIPs function as central hubs integrating signaling and mechanical regulation through feedforward and feedback interactions with cytoskeletal dynamics and membrane remodeling[300−302].

6. Discussion

In this hypothesis-driven review, we proposed lipid nanoparticle-driven membrane dysfunction (L-DMD) as a mechanistic framework that may explain the persistent biological effects reported after exposure to LNPs. The model focuses on the bioactive properties of the ionizable lipid components and their interactions with cell membranes independent of any nucleic acid payload. In contrast to models which are limited to innate immune activation or modRNA translation kinetics, L-DMD emphasizes how direct physicochemical interactions with cellular membranes can induce sustained dysregulation in cellular signaling, metabolism, and homeostasis.

The core premise of the L-DMD hypothesis is that the metastable, supramolecular nature of LNPs, together with the electrostatic properties of ionizable lipids, can allow them to integrate into cellular membranes, including disruption of endosomal vesicles. This could lead to local changes in charge density, lipid packing, and the dielectric environment. Even minor alterations in these parameters may perturb localized pools of key phosphoinositides (*e.g.*, PI(4,5)P_2, PI(3,4,5)P_3, PI4P, PI(3,4)P_2), which are central to membrane−proximal signaling and organelle identity. Therefore, their dysregulation can propagate to downstream pathways including NF-κB, MAPK, JAK/STAT, and mTORC1/2, and through bidirectional feedback affecting upstream signaling nodes. This leads to the broad inflammatory stress responses and metabolic patterns observed in cellular, transcriptomic, and proteomic analyses.

Experimental lipidomic analyses of nanoparticle−plasma interactions support lipid-mediated membrane remodeling at the nano−bio interface. To the best of our knowledge, only Pepafilippou et al.[303] have examined *ex vivo* lipidomic profiles of liposome-corona assemblies. Using HSPC/cholesterol liposomes incubated with human plasma, they identified selective enrichment of sphingomyelins, ceramides, and acylglycerols in the corona by mass spectrometry. Although not directly transferable to ionizable LNPs due to their pH-dependent biophysics, the study demonstrates selective lipid−lipid interactions at the nano-bio interface and supports the conceptual basis of our hypothesis.

Signal transduction through PI3K/AKT/mTOR and RAS/ERK provides an example of this principle. These cascades are nonlinear, threshold-dependent systems with feedforward and feedback loops, where small shifts in lipid composition or charge distribution can destabilize network homeostasis[304−306]. One

via MVB fusion with the plasma membrane or *via* the trans-Golgi network, involving PI4P and PI(4,5)P_2, which regulate vesicle trafficking and exocytosis. (4) Autophagosome formation. Generated at the ER, PI3P produced by class III PI3K recruits WIPI2, promotes phagophore expansion and LC3 lipidation. Abbreviations: ALR, autophagic lysosomal reformation; AP-2, adaptor protein complex-2; ER, endoplasmic reticulum; ESCRT, endosomal sorting complexes required for transport; LC3, microtubule-associated protein 1 light chain 3; MVB, multivesicular body; PI3K, phosphoinositide 3-kinase; PI3K class III, class III phosphoinositide 3-kinase; PI3P, phosphatidylinositol-3-phosphate; PI3,4P_2, phosphatidylinositol-3,4-bisphosphate; PI3,4,5P_3, phosphatidylinositol-3,4,5-trisphosphate; PI3,5P_2, phosphatidylinositol-3,5-bisphosphate; PI4P, phosphatidylinositol-4-phosphate; PI4,5P_2, phosphatidylinositol-4,5-bisphosphate; PI(3,4,5)P_3, phosphatidylinositol-3,4,5-triphosphate; PIKfyve, phosphoinositide kinase FYVE-type zinc finger-containing; SNX, sorting nexin; V-ATPase, vacuolar-type H^+-ATPase; WIPI2, WD repeat domain phosphoinositide-interacting protein 2. Created with BioRender. Seger, F. (2026). https://BioRender.com/s8tc5vs.

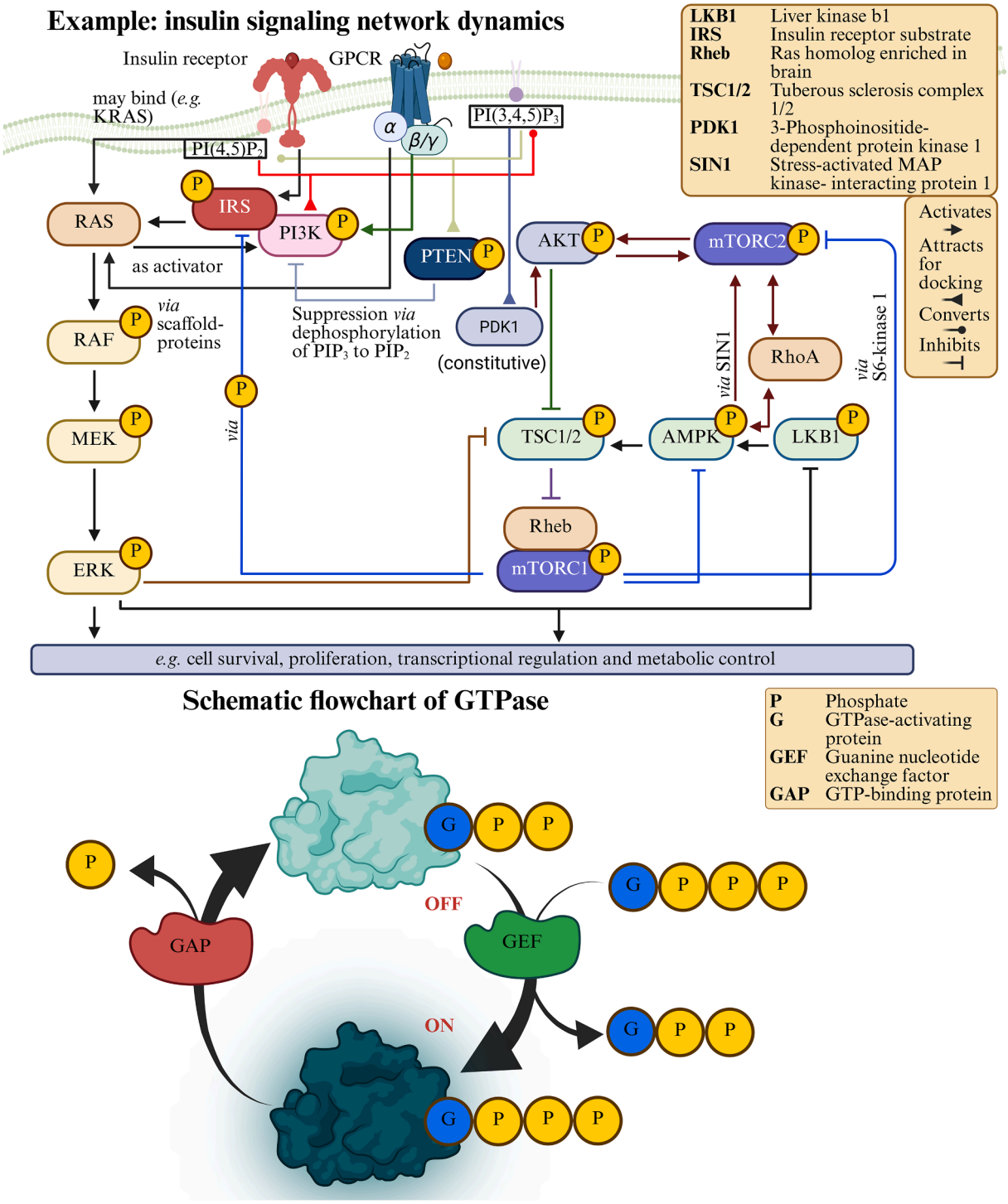


Figure 5 Membrane-organized phosphoinositide regulation: insulin pathway as an example. Dynamic routes of inhibition and activation are integrated through multi-layered feedback loops: mTORC1 acts as a central homeostatic regulator, providing inhibitory feedback to both the upstream IRS scaffold and the autocrine mTORC2−RHO−AMPK signaling axis to ensure signal termination and metabolic stability. The molecular logic of these transitions is governed by the GTPase cycle (GEF/GAP) illustrated below, which functions as the fundamental binary switch for network activation. GPCR activation at the plasma membrane, involving heterotrimeric G-protein subunits (Gα, Gβ, and Gγ), triggers PI3K-dependent conversion of PIP_2 to PIP_3, thereby linking receptor signaling to downstream AKT/mTOR and RAS/ERK cascades that govern cell growth, survival, and metabolic control. Insulin receptor activation triggers phosphorylation of the IRS scaffold, which recruits PI3K and Grb2/SOS to initiate the AKT and RAS/ERK cascades. This axis is linked to the GPCR system *via* RGS proteins that modulate G-protein activity, ensuring the synchronized control of metabolic and proliferative cellular responses. Note: This diagram is a simplified representation of the insulin signalling network, highlighting key regulatory axes rather than providing an exhaustive map of all participating molecules. Abbreviations: AKT, protein kinase B; AMPK, AMP-activated protein kinase; ERK, extracellular signal-regulated kinase; GBP, GTP-binding protein; GAP, GTPase

example, in which LNP-driven alterations at the plasma membrane or endosome could theoretically affect both the amplitude and duration of signaling outputs is shown schematically in Fig. 5 (*e.g.*, insulin signaling pathways).

This framework addresses an existing explanatory gap in current pharmacodynamic and toxicological models, which often treat LNPs primarily as delivery vehicles and attribute observed effects primarily to the persistence of the modRNA-encoded protein[85,307] or to transient innate immunity[308,309].

The phenomenon of persistent detection of spike protein and modRNA in certain individuals is consistent with this framework and is of particular interest to us. L-DMD suggests that such persistence may arise from signaling dysregulation rather than intrinsic molecular stability. If LNP-induced perturbations create conditions under which MAPK signaling platforms activation becomes spatially and temporally dysregulated[19], such dysregulation could, in principle, influence RNA turnover, translation shutdown and protein degradation mechanisms, producing functional persistence of modRNA and prolongation of protein half-life, even in the absence of chemical "indestructibility"[310−314]. We assume that the decisive factor is not the intrinsic chemical stability of modRNA, but a systems-level failure of cellular homeostatic shutdown mechanisms. As detailed in Section 3, the data point to alterations in phosphoinositide-regulated signaling nodes and do not provide evidence for a single dominant upstream trigger, leaving open the possibility of effects on higher-order signal termination processes.

Experimental human *ex vivo* data support this interpretation[315]. Sequence-optimized, but chemically unmodified PpLuc mRNA formulated in LNPs demonstrated significantly higher luciferase expression at 24 and 48 h compared with unencapsulated, uridine-unmodified mRNA, suggesting that the supramolecular colloidal structure of the mRNA−LNP complex enhances intracellular persistence.

Furthermore, activation of innate immune sensors such as Toll-like receptor 4 (TLR4) may be associated with modulation of intracellular RNA processing and proteostatic pathways. Experimental studies demonstrate that RNA sensing by endosomal Toll-like receptors requires coordinated RNase-dependent processing, including RNase T2, RNase 2, and OAS-RNase L systems, linking innate immune activation to RNA turnover regulation[316−318]. In parallel, TLR4 signaling has been shown to interact with proteolytic and ubiquitin-dependent protein regulatory networks[319−321]. Comparable innate immune activation has also been described for cationic lipid transfection systems such as Lipofectamine, supporting the concept that lipid-mediated immune sensing may influence intracellular RNA and protein processing dynamics[322].

Together, these findings may indicate a shift in cellular homeostatic regulation rather than effects solely attributable to nucleoside modification. They support our argument that the functional behavior of modRNA LNP complexes can be influenced by supramolecular and biophysical properties, including entropic aspects of lipid organization, lipid−RNA interactions, phase behavior, and interactions with membrane-bound receptors and with plasma and vesicle membranes, which may in turn affect intracellular processing, RNA stability, and protein persistence.

The L-DMD hypothesis is testable with current tools. Endosomal function and LNP escape can be quantified using galectin-8-GFP or SNAPSwitch reporter assays and confocal microscopy; proteasome and RNase activities can be measured using Proteasome Glo kits, RNase L substrates, or pulse-chase experiments with actinomycin D, each under the control of MAPK inhibitors (*e.g.*, SB203580, U0126) or mTORC blockers (rapamycin). KEGG pathway enrichment analyses of RNA-Sequence data (6−48 h post-LNP transfection) can reveal dysregulation of MAPK, mTORC, RNA degradation, and ribosome biogenesis pathways, complemented by phospho-Western blots for key proteins (p-ERK, p-p38, p-S6K).

In *in vivo* mouse models, bioluminescence imaging combined with tissue Quantitative Polymerase Chain Reaction/Enzyme-Linked Immunosorbent Assay enable the correlation of prolonged luciferase or spike persistence with individual signaling variability. By comparing modified *vs*. unmodified mRNA, empty LNPs, and genetic/pharmacological interventions, it is possible to distinguish effects driven by the supramolecular properties of the LNPs from those linked to the modRNA payload.

Variability in spike/modRNA persistence across individuals may reflect genetic or physiological differences in membrane signaling tolerance, feedback-loop robustness, or stress adaptation thresholds. These factors determine the rate at which a cell restores homeostasis after LNP-induced perturbations. Individuals with reduced ability or tolerance for membrane asymmetry or weaker feedback control could experience prolonged signaling activity and delayed molecular turnover.

Current post-marketing pharmacovigilance frameworks[323] are optimized for detecting and attributing immunologically mediated adverse events with acute temporal profiles, typically within hours to weeks post-vaccination. The L-DMD hypothesis, however, predicts a distinct class of lipid-mediated responses characterized by low-grade activation of signaling pathways, phosphoinositide dysregulation, autophagy impairment, and metabolic imbalance, manifesting as delayed (weeks to months), cumulative, multisystemic effects of mild-to-moderate severity. These attributes fall outside standard case definitions, temporal windows, and biological plausibility assessments linked to known immune mechanisms and are associated with high confounding in causality assessment in multifactorial pathology[323,324]. Empirical testing of L-DMD requires mechanism-informed biomarker development and prospective cohorts designed to detect non-immunological, delayed manifestations that current surveillance systems are not designed to capture.

Recognizing LNPs as active biointerfaces rather than inert delivery vehicles may enable the rational design of next-

activating protein; GEF, guanine nucleotide exchange factor; GPCR, G protein-coupled receptor; GRB protein 2, growth factor receptor-bound protein 2; IRS, insulin receptor substrate; LKB1, liver kinase B1; MEK, MAPK/ERK kinase; mTORC1/2, mechanistic target of rapamycin complex 1/2; P, phosphate; PDK1, 3-Phosphoinositide-dependent protein kinase 1; PI3K, phosphoinositide 3-kinase; PIP_2, phosphatidylinositol-4,5-bisphosphate; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; RAF, rapidly accelerated fibrosarcoma kinase; RAS, rat sarcoma (small GTPase); RGS, regulators of G-protein signaling; Rheb, ras homolog enriched in brain; RhoA, Ras homolog family member A (small GTPase); SIN1, stress-activated map kinase-interacting protein 1; SOS, son of sevenless homolog; TSC1/2, tuberous sclerosis complex 1/2. Created in BioRender. Seger, F. (2026). https://BioRender.com/m6o2bva.

generation LNPs with improved safety profiles, enhanced controllability, and tunable persistence characteristics. Insights from the L-DMD hypothesis can guide three strategic directions.

1. Rational LNP design

Adjusting ionizable lipid composition, charge density, and membrane affinity could allow decoupling of delivery efficiency from prolonged signaling perturbation, enabling safer and more predictable nanomedicine platforms.

2. Spatiotemporal control of signaling

Determining how LNPs influence membrane signaling domains may facilitate deliberate tuning of MAPK, mTOR, or innate immune pathways for therapeutic benefit, including cancer immunotherapy or transient immune modulation.

3. Predictive biomarker development

Establishing readouts for phosphoinositide dynamics, MAPK deactivation kinetics, and ubiquitin−protease activity as candidate biomarkers to assess long-term cellular responses to LNP exposure.

L-DMD is important beyond modRNA vaccines. Similar lipid-based nanocarriers are increasingly used in gene therapy, oncology, and protein replacement medicine. Understanding how supramolecular lipid organization governs intracellular network behavior will likely have broad implications for nanomedicine, integrating biophysics, physicochemistry, pharmacology, and systems biology. Since open systems like LNPs operate *in vivo* alongside open systems like cells, this perspective may be useful for developing a consistent model[325−328] for future advancements.

Future research should prioritize analytical frameworks that characterize the supramolecular properties of LNPs, which are not fully addressed by current or emerging compendial standards[329,330]. While recent regulatory guidelines establish quality control parameters, including particle size, encapsulation efficiency, and chemical modifications such as adducts, they do not systematically assess biophysical determinants, such as ionizable lipid protonation state[9], lipid domain organization[48], membrane fusion kinetics, or structural heterogeneity[331], that may directly influence the membrane perturbations of the L-DMD hypothesis. Advances in structural and functional assays, including cryo-EM, small-angle X-ray and neutron scattering, and single-particle fluorescence methods, provide nanoscale resolution of lipid packing and particle morphology, informing next-generation quality control strategies[48,332].

In vivo, emerging tracking technologies, such as SPARKLE[61], alongside mass spectrometry imaging, enable spatiotemporal resolution of LNP particle biodistribution that current fluorescent labeling cannot achieve without altering particle biophysics. Standardizing these methodologies will be essential for testing L-DMD predictions, identifying biomarkers of membrane dysregulation, and rationally engineering next-generation LNPs with improved safety and controllability. We see this as one of the largest gaps and one of the greatest opportunities for optimizing future research.

While the L-DMD framework raises important questions about long-term cellular responses to LNP exposure, it remains a hypothesis-generating model awaiting rigorous experimental corroboration. Until such validation is obtained, any extrapolation to clinical risk or public health implications should be approached with caution.

In summary, the evidence compiled here, from structural studies, molecular dynamics simulations, biophysical experiments, and omics datasets, indicates that the lipid components of LNPs possess intrinsic bioactivity that can contribute to longer-term cellular alterations, including effects observed with empty LNPs.

While comprehensive, this hypothesis-driven review remains primarily conceptual. Several limitations must be acknowledged:

1. Evidence hierarchy and model dependence: Most data integrated here derive from *in vitro* and murine studies employing high LNP doses and simplified cellular systems. Their pharmacodynamic and metabolic contexts differ from human administration, particularly with respect to intramuscular injection, depot kinetics, and systemic clearance. Translation to human physiology, therefore, requires caution.
2. Absence of long-term human datasets: There are currently no controlled longitudinal omics or lipidomic studies comparing vaccinated versus unvaccinated cohorts, nor studies isolating the effects of *empty* LNPs in humans. As a result, the persistence and mechanistic implications of the proposed L-DMD phenomena remain inferential.
3. Confounding from infection, comorbidities, and drug exposure: The majority of available transcriptomic and proteomic datasets were collected under non-standardized clinical conditions or concurrent illness, complicating attribution of observed molecular patterns specifically to LNP exposure.
4. Methodological gaps in membrane biology and lipid analytics: Quantitative measurement of membrane phosphoinositides, ionizable-lipid partitioning, or phospholipid-domain dynamics *in vivo* is technically challenging. Present biomarker assays (*e.g.*, lipidomics or phospho-proteomics) lack temporal resolution and standardization across laboratories.
5. Unresolved dose, composition, and batch variability: Manufacturing differences in ionizable lipid identity, particle size distribution, and PEG density can alter biological activity. Without harmonized formulations, cross-study comparison and extrapolation remain uncertain.
6. Speculative extensions to clinical outcomes: Although pathways such as NF-κB, MAPK, JAK/STAT, and mTORC1/2 are well established in stress and immune regulation, linking their modulation by LNPs to specific human disease states or chronic conditions remains speculative. No causal relationships have been established. Similarly, any sex-specific vaccine effects require validation.
7. Omics data require confirmation: Transcriptomics cannot provide information about protein activity, nor can proteomics identify the upstream signals that led to altered protein expression. Furthermore, protein functionality, including correct folding and binding regulation, must also be experimentally determined. Lipidomics should therefore be applied as a complementary tool to trace upstream signaling inputs and the resulting suspected perturbations of the plasma membrane and vesicular transport pathways (see experimental design proposal in the Discussion).
8. modRNA is based on the concept of suppressing intracellular pattern recognition: receptors: A crucial aspect of modRNA's success is that it is chemically modified (*e.g.*, m1ψ) to suppress pattern recognition receptors such as TLRs, thereby preventing

premature mRNA degradation and increasing translation efficiency through nucleoside modifications[333−336]. At the same time, however, TLRs are functionally cross-talking with LDL receptors involved in LNP uptake, as well as with the complement system[337−340]. In addition, TLRs interact closely with membrane lipids[341,342]. This represents a potential limitation. TLR suppression (*via* modRNA modifications) combined with simultaneous TLR4 activation (*via* ionizable lipids) could produce contradictory immunological effects with additive, antagonistic, and/or synergistic consequences. This complicates attribution of observed cellular responses to the LNP structure, the modRNA payload, or their interaction.

9. Future confirmation of the L-DMD framework will require coordinated systems toxicology studies: combining standardized LNP formulations, dose-dependent multi-omic profiling, and mechanistic perturbation experiments to distinguish membrane-driven signaling effects from payload-mediated responses.

7. Conclusions

Collectively, structural, biophysical, and omics data indicate that LNPs possess intrinsic bioactivity that can modulate intracellular signaling independent of mRNA payload. The L-DMD framework hypothesis provides a conceptual bridge between colloidal physics and the regulation of cell systems, explaining persistence phenomena following SARS-CoV-2 mRNA vaccination, and offering practical routes for design improvement.

Lipid nanoparticles exemplify a notable intersection of colloidal chemistry and cell biology. Their interaction with biological membranes is not merely passive diffusion but rather a transient, tightly coordinated *in vivo* process governed by electrostatic charge, membrane curvature, and lipid composition. This process is further shaped by the Vroman effect and the pH-dependent protonation dynamics of ionizable lipids, leading to stochastic protein corona formation and the potential modulation of canonical and/or non-canonical signaling pathways at the membrane level. These membrane−proximal events can initiate cellular stress and metabolic signaling networks, including NF-κB, MAPK, JAK/STAT, and mTOR, thereby constituting one of the earliest biochemical interfaces between a synthetic delivery vector and the living cell.

The concept of lipid-nanoparticle-driven membrane dynamics (L-DMD) describes how electrostatic and compositional disturbances may contribute to system-level transcriptional reprogramming. Future quantitative studies should aim to determine the resolution kinetics of these responses, helping to clarify where beneficial adaptive signaling concludes and where sustained or unintended persistence may start.

By viewing lipid nanoparticles as active participants in cellular decision-making rather than passive delivery vehicles, researchers may gain greater control over efficacy, safety, and temporal behavior, paving the way for a new generation of rationally engineered nanomedicines.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

The authors used AI-assisted tools (ChatGPT, Claude, and AlterAI) for language refinement and rewording of author-drafted text, and Grammarly for grammar and spelling checking. No AI tool was used to generate original scientific content, data, interpretations, synthesis or conclusions. All authors reviewed and approved the final manuscript and take full responsibility for its content.

Acknowledgements

S. Seneff received funding from Quanta Computer, Inc. under contract number 6950759. The other authors received no funding for this work. We thank our reviewers for their comments, and Dr. Myra Forster-van Hijfte for reading a draft and offering valuable feedback, which has led to a significantly improved, more focused version of the manuscript. We also thank David Cowley (independent researcher, Nottingham, United Kingdom) for constructive feedback and a thoughtful review of the final manuscript. All figures were created by F. Seger using BioRender in accordance with BioRender's terms of use and academic license. The graphical abstract figure was created in BioRender. Seger, F. (2026) https://BioRender.com/a75beyg.

Author contributions

Falko Seger: Conceptualization, Writing−original draft, Writing−review and editing, Visualization.

L. Maria Gutschi: Conceptualization, Writing−review and editing. Stephanie Seneff: Conceptualization, Writing−review and editing, Funding acquisition, Project administration.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2026.07.001.

References

1. Khurana A, Allawadhi P, Khurana I, Allwadhi S, Weiskirchen R, Banothu AK, et al. Role of nanotechnology behind the success of mRNA vaccines for COVID-19. *Nano Today* 2021;**38**:101142.
2. Hald Albertsen C, Kulkarni JA, Witzigmann D, Lind M, Petersson K, Simonsen JB. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. *Adv Drug Deliv Rev* 2022;**188**:114416.
3. Swetha K, Kotla NG, Tunki L, Jayaraj A, Bhargava SK, Hu H, et al. Recent advances in the lipid nanoparticle-mediated delivery of mRNA vaccines. *Vaccines (Basel)* 2023;**11**:658.
4. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Adv Drug Deliv Rev* 2016;**99**:28−51.
5. Kloczewiak M, Banks JM, Jin L, Brader ML. Perspective on higher-order structure and thermal stability of mRNA vaccines. *Mol Pharm* 2022;**19**:2022−31.
6. Trollmann MFW, Böckmann RA. Decoding pH-driven phase transition of lipid nanoparticles. *Small* 2026;**14**:e11381.
7. Haghighi E, Abolmaali SS, Dehshahri A, Mousavi Shaegh SA, Azarpira N, Tamaddon AM. Navigating the intricate *in vivo* journey of lipid nanoparticles tailored for the targeted delivery of RNA therapeutics: a quality-by-design approach. *J Nanobiotechnol* 2024;**22**:710.
8. Brader ML, Williams SJ, Banks JM, Hui WH, Zhou ZH, Jin L. Encapsulation state of messenger RNA inside lipid nanoparticles. *Biophys J* 2021;**120**:2766−70.
9. Eygeris Y, Gupta M, Kim J, Sahay G. Chemistry of lipid nanoparticles for RNA delivery. *Acc Chem Res* 2022;**55**:2−12.

10. Kulkarni JA, Cullis PR, van der Meel R. Lipid nanoparticles enabling gene therapies: from concepts to clinical utility. *Nucleic Acid Therapeut* 2018;**28**:146−57.
11. Simonsen JB. A perspective on bleb and empty LNP structures. *J Control Release* 2024;**373**:952−61.
12. Trollmann MFW, Böckmann RA. mRNA lipid nanoparticle phase transition. *Biophys J* 2022;**121**:3927−39.
13. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. *Nat Rev Mater* 2021;**6**:1078−94.
14. Cárdenas M, Campbell RA, Arteta MY, Lawrence MJ, Sebastiani F. Review of structural design guiding the development of lipid nanoparticles for nucleic acid delivery. *Curr Opin Colloid Interface Sci* 2023;**66**:101705.
15. Nele V, Campani V, Alia Moosavian S, De Rosa G. Lipid nanoparticles for RNA delivery: self-assembling *vs* driven-assembling strategies. *Adv Drug Deliv Rev* 2024;**208**:115291.
16. Manning AM, Tilstra G, Khan AB, Couture-Senécal J, Lau YMA, Pang J, et al. Ionizable lipid with supramolecular chemistry features for RNA delivery *in vivo*. *Small* 2023;**19**:e2302917.
17. Čechová P, Paloncýová M, Šrejber M, Otyepka M. Mechanistic insights into interactions between ionizable lipid nanodroplets and biomembranes. *J Biomol Struct Dyn* 2025;**43**:9983−93.
18. Ermilova I, Swenson J. Ionizable lipids penetrate phospholipid bilayers with high phase transition temperatures: perspectives from free energy calculations. *Chem Phys Lipids* 2023;**253**:105294.
19. Atmuri NDP, Saadati F, Kulkarni J, Witzigmann D, Cullis PR, Ciufolini MA. Design of cationic ionizable lipids for the delivery of therapeutic nucleic acids. *Mol Ther Methods Clin Dev* 2025;**33**:101585.
20. Gurtovenko AA, Lyulina AS. Electroporation of asymmetric phospholipid membranes. *J Phys Chem B* 2014;**118**:9909−18.
21. McLaughlin S, Murray D. Plasma membrane phosphoinositide organization by protein electrostatics. *Nature* 2005;**438**:605−11.
22. Fung SYS, Xǔ XJ, Wu M. Nonlinear dynamics in phosphoinositide metabolism. *Curr Opin Cell Biol* 2024;**88**:102373.
23. Ellenbroek WG, Wang YH, Christian DA, Discher DE, Janmey PA, Liu AJ. Divalent cation-dependent formation of electrostatic PIP_2 clusters in lipid monolayers. *Biophys J* 2011;**101**:2178−84.
24. Mandala VS, MacKinnon R. The membrane electric field regulates the PIP_2-binding site to gate the KCNQ1 channel. *Proc Natl Acad Sci U S A* 2023;**120**:e2301985120.
25. Yue Q, Al-Khalili O, Moseley A, Yoshigi M, Wynne BM, Ma H, et al. PIP_2 interacts electrostatically with MARCKS-like Protein-1 and ENaC in renal epithelial cells. *Biology* 2022;**11**:1694.
26. Yamaga M, Martin TFJ. $PI(4,5)P_2$ is a master regulator for Ca^{2+}-triggered vesicle exocytosis. *Biochim Biophys Acta Mol Cell Biol Lipids* 2025;**1870**:159651.
27. Gada KD, Logothetis DE. PKC regulation of ion channels: the involvement of PIP_2. *J Biol Chem* 2022;**298**:102035.
28. Tariq K, Luikart BW. Striking a balance: PIP_2 and PIP_3 signaling in neuronal health and disease. *Explor Neuroprotective Ther* 2021;**1**:86−100.
29. Clarke RJ. Electrostatic switch mechanisms of membrane protein trafficking and regulation. *Biophys Rev* 2023;**15**:1967−85.
30. Tanaka T, Legat A, Adam E, Steuve J, Gatot JS, Vandenbranden M, et al. DiC14-amidine cationic liposomes stimulate myeloid dendritic cells through toll-like receptor 4. *Eur J Immunol* 2008;**38**:1351−7.
31. Glaviano A, Foo ASC, Lam HY, Yap KCH, Jacot W, Jones RH, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Mol Cancer* 2023;**22**:138.
32. Ivanova A, Atakpa-Adaji P, Rao S, Marti-Solano M, Taylor CW. Dual regulation of IP_3 receptors by IP_3 and PIP_2 controls the transition from local to global Ca^{2+} signals. *Mol Cell* 2024;**84**:3997−4015.e7.
33. Putney JW, Tomita T. Phospholipase C signaling and calcium influx. *Adv Bio Regul* 2012;**52**:152−64.
34. Cao S, Chung S, Kim S, Li Z, Manor D, Buck M. K-Ras G-domain binding with signaling lipid phosphatidylinositol (4,5)-phosphate (PIP_2): membrane association, protein orientation, and function. *J Biol Chem* 2019;**294**:7068−84.
35. Janmey PA, Bucki R, Radhakrishnan R. Regulation of actin assembly by $PI(4,5)P_2$ and other inositol phospholipids: an update on possible mechanisms. *Biochem Biophys Res Commun* 2018;**506**:307−14.
36. Abbasi R, Shineh G, Mobaraki M, Doughty S, Tayebi L. Structural parameters of nanoparticles affecting their toxicity for biomedical applications: a review. *J Nano Res* 2023;**25**:43.
37. Yuan Z, Yan R, Fu Z, Wu T, Ren C. Impact of physicochemical properties on biological effects of lipid nanoparticles: are they completely safe. *Sci Total Environ* 2024;**927**:172240.
38. Szebeni J, Kiss B, Bozó T, Turjeman K, Levi-Kalisman Y, Barenholz Y, et al. Insights into the structure of Comirnaty COVID-19 vaccine: a theory on soft, partially bilayer-covered nanoparticles with hydrogen bond-stabilized mRNA-lipid complexes. *ACS Nano* 2023;**17**:13147−57.
39. Münter R, Larsen JB, Andresen TL. The vast majority of nucleic acid-loaded lipid nanoparticles contain cargo. *J Colloid Interface Sci* 2024;**674**:139−44.
40. Li S, Hu Y, Li A, Lin J, Hsieh K, Schneiderman Z, et al. Payload distribution and capacity of mRNA lipid nanoparticles. *Nat Commun* 2022;**13**:5561.
41. Chen X, Ye Y, Li M, Zuo T, Xie Z, Ke Y, et al. Structural characterization of mRNA lipid nanoparticles (LNPs) in the presence of mRNA-free LNPs. *J Control Release* 2025;**386**:114082.
42. Müller JA, Schäffler N, Kellerer T, Schwake G, Ligon TS, Rädler JO. Kinetics of RNA-LNP delivery and protein expression. *Eur J Pharm Biopharm* 2024;**197**:114222.
43. Liao S, Wang S, Wadhwa A, Birkenshaw A, Fox K, Cheng MHY, et al. Transfection potency of lipid nanoparticles containing mRNA depends on relative loading levels. *ACS Appl Mater Interfaces* 2025;**17**:3097−105.
44. Grumelot S, Mohammed N, Colonrosado J, Sadeghi SA, Fang F, Hilsen K, et al. Lipid nanoparticle protein coronas form *via* lipoprotein fusion rather than shell-like adsorption. *bioRxiv* 2025. https://doi.org/10.64898/2025.12.21.695162.
45. Rampado R, Crotti S, Caliceti P, Pucciarelli S, Agostini M. Recent advances in understanding the protein corona of nanoparticles and in the formulation of "stealthy" nanomaterials. *Front Bioeng Biotechnol* 2020;**8**:166.
46. Sun Y, Zhou Y, Rehman M, Wang YF, Guo S. Protein corona of nanoparticles: isolation and analysis. *Chem Bioeng* 2024;**1**:757−72.
47. Voke E, Arral ML, Squire HJ, Lin TJ, Zheng L, Coreas R, et al. Protein corona formed on lipid nanoparticles compromises delivery efficiency of mRNA cargo. *Nat Commun* 2025;**16**:8699.
48. Sebastiani F, Yanez Arteta M, Lerche M, Porcar L, Lang C, Bragg RA, et al. Apolipoprotein E binding drives structural and compositional rearrangement of mRNA-containing lipid nanoparticles. *ACS Nano* 2021;**15**:6709−22.
49. Kim W, Ly NK, He Y, Li Y, Yuan Z, Yeo Y. Protein corona: friend or foe? Co-opting serum proteins for nanoparticle delivery. *Adv Drug Deliv Rev* 2023;**192**:114635.
50. Paunovska K, Da Silva Sanchez AJ, Lokugamage MP, Loughrey D, Echeverri ES, Cristian A, et al. The extent to which lipid nanoparticles require apolipoprotein E and low-density lipoprotein receptor for delivery changes with ionizable lipid structure. *Nano Lett* 2022;**22**:10025−33.
51. Naasani I. Establishing the pharmacokinetics of genetic vaccines is essential for maximising their safety and efficacy. *Clin Pharmacokinet* 2022;**61**:921−7.
52. Hosseini-Kharat M, Bremmell KE, Prestidge CA. Why do lipid nanoparticles target the liver? Understanding of biodistribution and liver-specific tropism. *Mol Ther Methods Clin Dev* 2025;**33**:101436.
53. European Medical Assessment. *Comirnaty: european public assessment report. EMEA/H/C/005735/0000*. Amsterdam: European Medicines Agency; 2020. Corr.1. [cited 2026 Feb 25]. Available from:

https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf.
54. Therapeutic Goods Administration. *Nonclinical evaluation report BNT162b2 [mRNA] COVID-19 vaccine (Comirnaty). Freedom of Information Document FOI 2389-06*. Canberra: Australian Government Department of Health and Aged Care; 2021 [cited 2026 Mar 31]. Available from: https://www.tga.gov.au/sites/default/files/foi-2389-06.pdf.
55. Neves AR, Queiroz JF, Costa Lima SA, Figueiredo F, Fernandes R, Reis S. Cellular uptake and transcytosis of lipid-based nanoparticles across the intestinal barrier: relevance for oral drug delivery. *J Colloid Interface Sci* 2016;**463**:258−65.
56. Khare P, Edgecomb SX, Hamadani CM, Tanner EEL, S Manickam D. Lipid nanoparticle-mediated drug delivery to the brain. *Adv Drug Deliv Rev* 2023;**197**:114861.
57. Chen J, Xu Y, Zhou M, Xu S, Varley AJ, Golubovic A, et al. Combinatorial design of ionizable lipid nanoparticles for muscle-selective mRNA delivery with minimized off-target effects. *Proc Natl Acad Sci U S A* 2023;**120**:e2309472120.
58. Younis MA, Sato Y, Elewa YHA, Kon Y, Harashima H. Self-homing nanocarriers for mRNA delivery to the activated hepatic stellate cells in liver fibrosis. *J Control Release* 2023;**353**:685−98.
59. Kent SJ, Li S, Amarasena TH, Reynaldi A, Lee WS, Leeming MG, et al. Blood distribution of SARS-CoV-2 lipid nanoparticle mRNA vaccine in humans. *ACS Nano* 2024;**18**:27077−89.
60. Buckley M, Araínga M, Maiorino L, Pires IS, Kim BJ, Michaels KK, et al. Visualizing lipid nanoparticle trafficking for mRNA vaccine delivery in non-human primates. *Mol Ther* 2025;**33**:1105−17.
61. Balcorta HV, Mata Corral MY, Gallegos A, Chavez J, Perez J, Balivada S, et al. Development of chemical tags for universal lipid nanoparticle visualization and tracking in 2D and 3D imaging. *Nano Lett* 2025;**25**:7682−9.
62. Akhter MH, Khalilullah H, Gupta M, Alfaleh MA, Alhakamy NA, Riadi Y, et al. Impact of protein corona on the biological identity of nanomedicine: understanding the fate of nanomaterials in the biological milieu. *Biomedicines* 2021;**9**:1496.
63. Behzadi S, Serpooshan V, Tao W, Hamaly MA, Alkawareek MY, Dreaden EC, et al. Cellular uptake of nanoparticles: journey inside the cell. *Chem Soc Rev* 2017;**46**:4218−44.
64. Lavington S, Watts A. Lipid nanoparticle technologies for the study of G protein-coupled receptors in lipid environments. *Biophys Rev* 2020;**12**:1287−302.
65. Chatterjee S, Kon E, Sharma P, Peer D. Endosomal escape: a bottleneck for LNP-mediated therapeutics. *Proc Natl Acad Sci U S A* 2024;**121**:e2307800120.
66. Vermeulen LMP, Brans T, Samal SK, Dubruel P, Demeester J, De Smedt SC, et al. Endosomal size and membrane leakiness influence proton sponge-based rupture of endosomal vesicles. *ACS Nano* 2018;**12**:2332−45.
67. Johansson JM, Du Rietz H, Hedlund H, Eriksson HC, Oude Blenke E, Pote A, et al. Cellular and biophysical barriers to lipid nanoparticle-mediated delivery of RNA to the cytosol. *Nat Commun* 2025;**16**:5354.
68. Er-Rafik M, Ferji K, Combet J, Sandre O, Lecommandoux S, Schmutz M, et al. Tear of lipid membranes by nanoparticles. *Soft Matter* 2022;**18**:3318−22.
69. Pilkington EH, Suys EJA, Trevaskis NL, Wheatley AK, Zukancic D, Algarni A, et al. From influenza to COVID-19: lipid nanoparticle mRNA vaccines at the frontiers of infectious diseases. *Acta Biomater* 2021;**131**:16−40.
70. Dowdy SF. Endosomal escape of RNA therapeutics: how do we solve this rate-limiting problem?. *RNA* 2023;**29**:396−401.
71. Sabnis S, Kumarasinghe ES, Salerno T, Mihai C, Ketova T, Senn JJ, et al. A novel amino lipid series for mRNA delivery: improved endosomal escape and sustained pharmacology and safety in non-human primates. *Mol Ther* 2018;**26**:1509−19.
72. Maugeri M, Nawaz M, Papadimitriou A, Angerfors A, Camponeschi A, Na M, et al. Linkage between endosomal escape of LNP-mRNA and loading into EVs for transport to other cells. *Nat Commun* 2019;**10**:4333.
73. Gilleron J, Querbes W, Zeigerer A, Borodovsky A, Marsico G, Schubert U, et al. Image-based analysis of lipid nanoparticle-mediated siRNA delivery, intracellular trafficking and endosomal escape. *Nat Biotechnol* 2013;**31**:638−46.
74. Aliakbarinodehi N, Niederkofler S, Emilsson G, Parkkila P, Olsén E, Jing Y, et al. Time-resolved inspection of ionizable lipid-facilitated lipid nanoparticle disintegration and cargo release at an early endosomal membrane mimic. *ACS Nano* 2024;**18**:22989−3000.
75. Schlich M, Palomba R, Costabile G, Mizrahy S, Pannuzzo M, Peer D, et al. Cytosolic delivery of nucleic acids: the case of ionizable lipid nanoparticles. *Bioeng Transl Med* 2021;**6**:e10213.
76. Paramasivam P, Franke C, Stöter M, Höijer A, Bartesaghi S, Sabirsh A, et al. Endosomal escape of delivered mRNA from endosomal recycling tubules visualized at the nanoscale. *J Cell Biol* 2022;**221**:e202110137.
77. Sahay G, Querbes W, Alabi C, Eltoukhy A, Sarkar S, Zurenko C, et al. Efficiency of siRNA delivery by lipid nanoparticles is limited by endocytic recycling. *Nat Biotechnol* 2013;**31**:653−8.
78. Bitounis D, Jacquinet E, Rogers MA, Amiji MM. Strategies to reduce the risks of mRNA drug and vaccine toxicity. *Nat Rev Drug Discov* 2024;**23**:281−300.
79. Moghimi SM, Simberg D. Pro-inflammatory concerns with lipid nanoparticles. *Mol Ther* 2022;**30**:2109−10.
80. Bates SM, Munson MJ, Trovisco V, Pereira S, Miller SR, Sabirsh A, et al. The kinetics of endosomal disruption reveal differences in lipid nanoparticle induced cellular toxicity. *J Control Release* 2025;**386**:114047.
81. Zheng L, Bandara SR, Tan Z, Leal C. Lipid nanoparticle topology regulates endosomal escape and delivery of RNA to the cytoplasm. *Proc Natl Acad Sci U S A* 2023;**120**:e2301067120.
82. Lu ZR, Sun D. Mechanism of pH-sensitive amphiphilic endosomal escape of ionizable lipid nanoparticles for cytosolic nucleic acid delivery. *Pharm Res* 2025;**42**:1065−77.
83. Su J, Song Y, Zhu Z, Huang X, Fan J, Qiao J, et al. Cell−cell communication: new insights and clinical implications. *Signal Transduct Targeted Ther* 2024;**9**:196.
84. Pateev I, Seregina K, Ivanov R, Reshetnikov V. Biodistribution of RNA vaccines and of their products: evidence from human and animal studies. *Biomedicines* 2023;**12**:59.
85. Fertig TE, Chitoiu L, Marta DS, Ionescu VS, Cismasiu VB, Radu E, et al. Vaccine mRNA can be detected in blood at 15 days post-vaccination. *Biomedicines* 2022;**10**:1538.
86. Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics−developing a new class of drugs. *Nat Rev Drug Discov* 2014;**13**:759−80.
87. Cheng Y, Zhao E, Yang X, Luo C, Zi G, Wang R, et al. Entrapment of lipid nanoparticles in peripheral endosomes but not lysosomes impairs intracellular trafficking and endosomal escape. *Int J Pharm* 2025;**669**:125024.
88. Omo-Lamai S, Wang Y, Patel MN, Milosavljevic A, Zuschlag D, Poddar S, et al. Limiting endosomal damage sensing reduces inflammation triggered by lipid nanoparticle endosomal escape. *Nat Nanotechnol* 2025;**20**:1285−97.
89. Bansal S, Perincheri S, Fleming T, Poulson C, Tiffany B, Bremner RM, et al. Cutting edge: circulating exosomes with COVID spike protein are induced by BNT162b2 (Pfizer-BioNTech) vaccination prior to development of antibodies: a novel mechanism for immune activation by mRNA vaccines. *J Immunol* 2021;**207**:2405−10.
90. Jörgensen AM, Wibel R, Bernkop-Schnürch A. Biodegradable cationic and ionizable cationic lipids: a roadmap for safer pharmaceutical excipients. *Small* 2023;**19**:e2206968.
91. Knaggs KLM, Sun Y, Walz BA, Pang J, Khan OF. The role of excipients in lipid nanoparticle metabolism: implications for enhanced therapeutic effect. *Ther Deliv* 2025;**16**:687−700.
92. Wang W, Deng S, Lin J, Ouyang D. Modeling on *in vivo* disposition and cellular transportation of RNA lipid nanoparticles *via* quantum

mechanics/physiologically-based pharmacokinetic approaches. *Acta Pharm Sin B* 2024;**14**:4591−607.
93. Yu Z, Li Q, Wang J, Yu Y, Wang Y, Zhou Q, et al. Reactive oxygen species-related nanoparticle toxicity in the biomedical field. *Nanoscale Res Lett* 2020;**15**:115.
94. Packer M, Gyawali D, Yerabolu R, Schariter J, White P. A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. *Nat Commun* 2021;**12**:6777.
95. Hashiba K, Taguchi M, Sakamoto S, Otsu A, Maeda Y, Ebe H, et al. Overcoming thermostability challenges in mRNA−lipid nanoparticle systems with piperidine-based ionizable lipids. *Commun Biol* 2024;**7**:556.
96. Cordes J, Zhao S, Engel CM, Stingele J. Cellular responses to RNA damage. *Cell* 2025;**188**:885−900.
97. Maelfait J, Liverpool L, Rehwinkel J. Nucleic acid sensors and programmed cell death. *J Mol Biol* 2020;**432**:552−68.
98. Li Y, Zhao T, Li J, Xia M, Li Y, Wang X, et al. Oxidative stress and 4-hydroxy-2-nonenal (4-HNE): implications in the pathogenesis and treatment of aging-related diseases. *J Immunol Res* 2022;**2022**:2233906.
99. Zhou ZH, Stone Jr CA, Jakubovic B, Phillips EJ, Sussman G, Park J, et al. Anti-PEG IgE in anaphylaxis associated with polyethylene glycol. *J Allergy Clin Immunol Pract* 2021;**9**. 1731−3.e3.
100. Kozma GT, Shimizu T, Ishida T, Szebeni J. Anti-PEG antibodies: properties, formation, testing and role in adverse immune reactions to PEGylated nano-biopharmaceuticals. *Adv Drug Deliv Rev* 2020;**154**−**155**:163−75.
101. Fu S, Zhu X, Huang F, Chen X. Anti-PEG antibodies and their biological impact on PEGylated drugs: challenges and strategies for optimization. *Pharmaceutics* 2025;**17**:1074.
102. Szebeni J. Complement activation-related pseudoallergy: a new class of drug-induced acute immune toxicity. *Toxicology* 2005;**216**:106−21.
103. Szebeni J, Simberg D, González-Fernández Á, Barenholz Y, Dobrovolskaia MA. Roadmap and strategy for overcoming infusion reactions to nanomedicines. *Nat Nanotechnol* 2018;**13**:1100−8.
104. Dézsi L, Mészáros T, Kozma G, H-Velkei M, Oláh CZ, Szabó M, et al. A naturally hypersensitive porcine model may help understand the mechanism of COVID-19 mRNA vaccine-induced rare (pseudo) allergic reactions: complement activation as a possible contributing factor. *GeroScience* 2022;**44**:597−618.
105. Bakos T, Mészáros T, Kozma GT, Berényi P, Facskó R, Farkas H, et al. mRNA-LNP COVID-19 vaccine lipids induce complement activation and production of proinflammatory cytokines: mechanisms, effects of complement inhibitors, and relevance to adverse reactions. *Int J Mol Sci* 2024;**25**:3595.
106. Song J, Su D, Wu H, Guo J. Implications of anaphylaxis following mRNA-LNP vaccines: It is urgent to eliminate PEG and find alternatives. *Pharmaceutics* 2025;**17**:798.
107. European Medicines Agency. *Onpattro: European Public Assessment Report. EMA/554262/2018*. Amsterdam: European Medicines Agency; 2018 [cited 2026 Mar 31]. Available from: https://www.ema.europa.eu/en/documents/assessment-report/onpattro-epar-public-assessment-report_en.pdf.
108. Wang J, Ding Y, Chong K, Cui M, Cao Z, Tang C, et al. Recent advances in lipid nanoparticles and their safety concerns for mRNA delivery. *Vaccines (Basel)* 2024;**12**:1148.
109. Dai W, Xing M, Sun L, Lv L, Wang X, Wang Y, et al. Lipid nanoparticles as adjuvant of norovirus VLP vaccine augment cellular and humoral immune responses in a TLR9- and type I IFN-dependent pathway. *J Virol* 2024;**98**:e0169924.
110. Chaudhary N, Kasiewicz LN, Newby AN, Arral ML, Yerneni SS, Melamed JR, et al. Amine headgroups in ionizable lipids drive immune responses to lipid nanoparticles by binding to the receptors TLR4 and CD1d. *Nat Biomed Eng* 2024;**8**:1483−98.
111. Di Fusco SA, Maggioni AP, Bernelli C, Perone F, De Marzo V, Conte E, et al. Inclisiran: a new pharmacological approach for hypercholesterolemia. *Rev Cardiovasc Med* 2022;**23**:375.
112. Wright RS, Koenig W, Landmesser U, Leiter LA, Raal FJ, Schwartz GG, et al. Safety and tolerability of inclisiran for treatment of hypercholesterolemia in 7 clinical trials. *J Am Coll Cardiol* 2023;**82**:2251−61.
113. Urits I, Swanson D, Swett MC, Patel A, Berardino K, Amgalan A, et al. A review of patisiran (ONPATTRO®) for the treatment of polyneuropathy in people with hereditary transthyretin amyloidosis. *Neurol Ther* 2020;**9**:301−15.
114. Thi TTH, Suys EJA, Lee JS, Nguyen DH, Park KD, Truong NP. Lipid-based nanoparticles in the clinic and clinical trials: from cancer nanomedicine to COVID-19 vaccines. *Vaccines (Basel)* 2021;**9**:359.
115. European Medicines Agency. *Kostaive (zapomeran): european public assessment report. Sections 2.4.3 (pharmacokinetics) and 2.4.6 (discussion on non-clinical aspects)*. Amsterdam: European Medicines Agency; 2024. Available from: https://www.ema.europa.eu/en/documents/assessment-report/kostaive-epar-public-assessment-report_en.pdf.
116. European Medicines Agency. *COVID-19 Vaccine Moderna: european public assessment report. EMEA/H/C/005791/0000*. Amsterdam: European Medicines Agency; 2021 [cited 2026 Mar 31]. Available from: https://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf.
117. Wong HL, Hu M, Zhou CK, Lloyd PC, Amend KL, Beachler DC, et al. Risk of myocarditis and pericarditis after the COVID-19 mRNA vaccination in the USA: a cohort study in claims databases. *Lancet* 2022;**399**:2191−9.
118. Avraham R, Haseley N, Brown D, Penaranda C, Jijon HB, Trombetta JJ, et al. Pathogen cell-to-cell variability drives heterogeneity in host immune responses. *Cell* 2015;**162**:1309−21. Erratum in: *Cell* 2015; **163**: 523.
119. Ndeupen S, Qin Z, Jacobsen S, Bouteau A, Estanbouli H, Igyártó BZ. The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory. *iScience* 2021;**24**:103479.
120. Finney DJ. The median lethal dose and its estimation. *Arch Toxicol* 1985;**56**:215−8.
121. Avila AM, Bebenek I, Bonzo JA, Bourcier T, Davis Bruno KL, Carlson DB, et al. An FDA/CDER perspective on nonclinical testing strategies: classical toxicology approaches and new approach methodologies (NAMs). *Regul Toxicol Pharmacol* 2020;**114**:104662.
122. Luster AD. The role of chemokines in linking innate and adaptive immunity. *Curr Opin Immunol* 2002;**14**:129−35.
123. Esche C, Stellato C, Beck LA. Chemokines: key players in innate and adaptive immunity. *J Invest Dermatol* 2005;**125**:615−28.
124. Tao T, Jiang G, Su Y, He D, Zhu L, Jiang Q, et al. The multiple roles of GM-CSF in autoimmune and autoinflammatory uveitis. *Biochem Pharmacol* 2025;**240**:117090.
125. Rincón M, Anguita J, Nakamura T, Fikrig E, Flavell RA. Interleukin (IL)-6 directs the differentiation of IL-4-producing $CD4^+$ T cells. *J Exp Med* 1997;**185**:461−9.
126. de Jong AJ, Pollastro S, Kwekkeboom JC, Andersen SN, Dorjée AL, Bakker AM, et al. Functional and phenotypical analysis of IL-6-secreting $CD4^+$ T cells in human adipose tissue. *Eur J Immunol* 2018;**48**:471−81.
127. Jeannin P, Jaillon S, Delneste Y. Pattern recognition receptors in the immune response against dying cells. *Curr Opin Immunol* 2008;**20**:530−7.
128. Nagai Y, Garrett KP, Ohta S, Bahrun U, Kouro T, Akira S, et al. Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment. *Immunity* 2006;**24**:801−12.
129. Fritz JH. Nod-like receptors have a grip on stem cells. *Cell Host Microbe* 2014;**15**:659−61.
130. Ji Y, Kumar R, Gokhale A, Chao HP, Rycaj K, Chen X, et al. LRIG1, a regulator of stem cell quiescence and a pleiotropic feedback tumor suppressor. *Semin Cancer Biol* 2022;**82**:120−33.
131. Parhiz H, Brenner JS, Patel PN, Papp TE, Shahnawaz H, Li Q, et al. Added to pre-existing inflammation, mRNA-lipid nanoparticles

induce inflammation exacerbation (IE). *J Control Release* 2022;**344**: 50−61.
132. Zelkoski AE, Lu Z, Sukumar G, Dalgard C, Said H, Alameh MG, et al. Ionizable lipid nanoparticles of mRNA vaccines elicit NF-κB and IRF responses through toll-like receptor 4. *NPJ Vaccines* 2025; **10**:73.
133. Raftery N, Stevenson NJ. Advances in anti-viral immune defense: revealing the importance of the IFN JAK/STAT pathway. *Cell Mol Life Sci* 2017;**74**:2525−35.
134. Korzun T, Moses AS, Jozic A, Grigoriev V, Newton S, Kim J, et al. Lipid nanoparticles elicit reactogenicity and sickness behavior in mice *via* toll-like receptor 4 and myeloid differentiation protein 88 axis. *ACS Nano* 2024;**18**:24842−59.
135. Mendoza MC, Er EE, Blenis J. The Ras−ERK and PI3K−mTOR pathways: cross-talk and compensation. *Trends Biochem Sci* 2011; **36**:320−8.
136. Soriano O, Alcón-Pérez M, Vicente-Manzanares M, Castellano E. The crossroads between RAS and RHO signaling pathways in cellular transformation, motility and contraction. *Genes* 2021;**12**:819.
137. Luo J, Molbay M, Chen Y, Horvath I, Kadletz K, Kick B, et al. Nanocarrier imaging at single-cell resolution across entire mouse bodies with deep learning. *Nat Biotechnol* 2025;**43**:2009−22.
138. Knabl L, Lee HK, Wieser M, Mur A, Zabernigg A, Knabl L Sr, et al. BNT162b2 vaccination enhances interferon−JAK−STAT-regulated antiviral programs in COVID-19 patients infected with the SARS-CoV-2 Beta variant. *Commun Med* 2022;**2**:17.
139. Hickey TE, Mudunuri U, Hempel HA, Kemp TJ, Roche NV, Talsania K, et al. Proteomic and serologic assessments of responses to mRNA-1273 and BNT162b2 vaccines in human recipient sera. *Front Immunol* 2025;**15**:1502458.
140. Panwar V, Singh A, Bhatt M, Tonk RK, Azizov S, Raza AS, et al. Multifaceted role of mTOR (mammalian target of rapamycin) signaling pathway in human health and disease. *Signal Transduct Targeted Ther* 2023;**8**:375.
141. Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduct Targeted Ther* 2020;**5**:209.
142. Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, et al. NF-κB in biology and targeted therapy: new insights and translational implications. *Signal Transduct Targeted Ther* 2024;**9**:53.
143. Bassermann F, Eichner R, Pagano M. The ubiquitin proteasome system—implications for cell cycle control and the targeted treatment of cancer. *Biochim Biophys Acta* 2014;**1843**:150−62.
144. Yasir M, Goyal A, Sonthalia S. Corticosteroid Adverse effects. [Updated 2023 Jul 3]. In: *StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing; 2026: Jan [cited 2026 Mar 31]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK531462/.
145. Gulluni F, Martini M, Hirsch E. Cytokinetic abscission: phosphoinositides and ESCRTs direct the final cut. *J Cell Biochem* 2017;**118**: 3561−8.
146. Tan X, Thapa N, Choi S, Anderson RA. Emerging roles of PtdIns(4,5)P_2—beyond the plasma membrane. *J Cell Sci* 2015;**128**: 4047−56.
147. Ajazi A, Bruhn C, Shubassi G, Lucca C, Ferrari E, Cattaneo A, et al. Endosomal trafficking and DNA damage checkpoint kinases dictate survival to replication stress by regulating amino acid uptake and protein synthesis. *Dev Cell* 2021;**56**: 2607−22.e6.
148. Giannini C, Ponzone L, Barroero N, Hirsch E. The interplay between phosphoinositides and ESCRT proteins. *Adv Bio Regul* 2025;**9**: 101126.
149. Forster IIIJ, Nandi D, Kulkarni A. mRNA-carrying lipid nanoparticles that induce lysosomal rupture activate NLRP3 inflammasome and reduce mRNA transfection efficiency. *Biomater Sci* 2022; **10**:5566−82.
150. Hurley JH, Coyne AN, Miączyńska M, Stenmark H. The expanding repertoire of ESCRT functions in cell biology and disease. *Nature* 2025;**642**:877−88.
151. Baylon JL, Lenov IL, Sligar SG, Tajkhorshid E. Characterizing the membrane-bound state of cytochrome P450 3A4: structure, depth of insertion, and orientation. *J Am Chem Soc* 2013;**135**:8542−51.
152. Hackett JC. Membrane-embedded substrate recognition by cytochrome P450 3A4. *J Biol Chem* 2018;**293**:403704046.
153. Mukherjee G, Nandekar PP, Wade RC. An electron transfer competent structural ensemble of membrane-bound cytochrome P450 1A1 and cytochrome P450 oxidoreductase. *Commun Biol* 2021; **4**:55.
154. Liu F, Aulin LBS, Manson ML, Krekels EHJ, van Hasselt JGC. Unraveling the effects of acute inflammation on pharmacokinetics: a model-based analysis focusing on renal glomerular filtration rate and cytochrome P450 3A4-mediated metabolism. *Eur J Drug Metab Pharmacokinet* 2023;**48**:623−31.
155. Stanke-Labesque F, Gautier-Veyret E, Chhun S, Guilhaumou R; French Society of Pharmacology and Therapeutics. Inflammation is a major regulator of drug metabolizing enzymes and transporters: consequences for the personalization of drug treatment. *Pharmacol Ther* 2020;**215**:107627.
156. Koozi H, Lengquist M, Frigyesi A. C-reactive protein as a prognostic factor in intensive care admissions for sepsis: a Swedish multicenter study. *J Crit Care* 2020;**56**:73−9.
157. Bayraktar İ, Yalçın N, Demirkan K. The potential interaction between COVID-19 vaccines and clozapine: a novel approach for clinical trials. *Int J Clin Pract* 2021;**75**:e14441.
158. Imai T, Ochiai S, Ishimaru T, Daitoku H, Miyagawa Y, Fukuhara R, et al. A case report: clozapine-induced leukopenia and neutropenia after mRNA COVID-19 vaccination. *Neuropsychopharmacol Rep* 2022;**42**:238−40.
159. Thompson D, Delorme CM, White RF, Honer WG. Elevated clozapine levels and toxic effects after SARS-CoV-2 vaccination. *J Psychiatry Neurosci* 2021;**46**:E210−1.
160. Eiermann B, Engel G, Johansson I, Zanger UM, Bertilsson L. The involvement of CYP1A2 and CYP3A4 in the metabolism of clozapine. *Br J Clin Pharmacol* 1997;**44**:439−46.
161. Villemure S, Trenaman SC, Goralski KB. The impact of COVID-19 infection on cytochrome P450 3A4-mediated drug metabolism and drug interactions. *Expert Opin Drug Metab Toxicol* 2023;**19**:329−32.
162. Lim SYM, Al Bishtawi B, Lim W. Role of cytochrome P450 2C9 in COVID-19 treatment: current status and future directions. *Eur J Drug Metab Pharmacokinet* 2023;**48**:221−40.
163. Hossam Abdelmonem B, Abdelaal NM, Anwer EKE, Rashwan AA, Hussein MA, Ahmed YF, et al. Decoding the role of CYP450 enzymes in metabolism and disease: a comprehensive review. *Biomedicines* 2024;**12**:1467.
164. Akanchise T, Luo F, Angelov B, Deng Y, Manna G, Angelova A. Rapid structural transformation of ionizable lipid nanoparticles involving omega-3 polyunsaturated fatty acids enhances antioxidant defense and mitochondrial proteins activity in pH-responsive drug delivery. *J Colloid Interface Sci* 2026;**704**:139420.
165. Ruysschaert JM, Lonez C. Role of lipid microdomains in TLR-Mediated signalling. *Biochim Biophys Acta* 2015;**1848**:1860−7.
166. Chaluvadi MR, Nyagode BA, Kinloch RD, Morgan ET. TLR4-dependent and -independent regulation of hepatic cytochrome P450 in mice with chemically induced inflammatory bowel disease. *Biochem Pharmacol* 2009;**77**:464−71.
167. Li Z, Carter J, Santos L, Webster C, van der Walle CF, Li P, et al. Acidification-induced structure evolution of lipid nanoparticles correlates with their *in vitro* gene transfections. *ACS Nano* 2023;**17**: 979−90.
168. Kim KH, Ahn T, Yun CH. Membrane properties induced by anionic phospholipids and phosphatidylethanolamine are critical for the membrane binding and catalytic activity of human cytochrome P450 3A4. *Biochemistry* 2003;**42**:15377−87.
169. Mustafa G, Nandekar PP, Mukherjee G, Bruce NJ, Wade RC. The effect of force-field parameters on cytochrome P450-membrane interactions: structure and dynamics. *Sci Rep* 2020;**10**:7284.

170. Asady B, Sampels V, Romano JD, Levitskaya J, Lige B, Khare P, et al. Function and regulation of a steroidogenic CYP450 enzyme in the mitochondrion of *Toxoplasma gondii*. *PLoS Pathog* 2023;**19**: e1011566.
171. Huang Z, Xu X, Zhu G. Association between coronavirus disease 2019 vaccination and hypercholesterolemia: a cross-sectional study from the 2023 National Health Interview Survey. *Medicine (Baltim)* 2025;**104**:e46221.
172. Luo L, Wall AA, Yeo JC, Condon ND, Norwood SJ, Schoenwaelder S, et al. Rab8a interacts directly with PI3Kγ to modulate TLR4-driven PI3K and mTOR signalling. *Nat Commun* 2014;**5**:4407.
173. Płóciennikowska A, Hromada-Judycka A, Dembińska J, Roszczenko P, Ciesielska A, Kwiatkowska K. Contribution of CD14 and TLR4 to changes of the $PI(4,5)P_2$ level in LPS-stimulated cells. *J Leukoc Biol* 2016;**100**:1363−73.
174. López-Haber C, Levin-Konigsberg R, Zhu Y, Bi-Karchin J, Balla T, Grinstein S, et al. Phosphatidylinositol-4-kinase IIα licenses phagosomes for TLR4 signaling and MHC-II presentation in dendritic cells. *Proc Natl Acad Sci U S A* 2020;**117**:28251−62.
175. Quan Y, Yang H, Li W, Li L. mRNA vaccines: immunogenicity and quality characteristics. *J Nanobiotechnol* 2025;**24**:6.
176. Shi Y, Ruan H, Xu Y, Zou C. Cholesterol, eukaryotic lipid domains, and an evolutionary perspective of transmembrane signaling. *Cold Spring Harbor Perspect Biol* 2023;**15**:a041418.
177. Barnett KC, Kagan JC. Lipids that directly regulate innate immune signal transduction. *Innate Immun* 2019;**26**:4−14.
178. Zheng Y, Sun L, Jiang T, Zhang D, He D, Nie H. TNFα promotes Th17 cell differentiation through IL-6 and IL-1β produced by monocytes in rheumatoid arthritis. *J Immunol Res* 2014;**2014**: 385352.
179. Marasco MR, Conteh AM, Reissaus CA, Cupit 5th JE, Appleman EM, Mirmira RG, et al. Interleukin-6 reduces β-cell oxidative stress by linking autophagy with the antioxidant response. *Diabetes* 2018;**67**:1576−88.
180. Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. *Nat Rev Immunol* 2013;**13**:722−37.
181. Chi DS, Fitzgerald SM, Pitts S, Cantor K, King E, Lee SA, et al. MAPK-dependent regulation of IL-1- and beta-adrenoreceptor-induced inflammatory cytokine production from mast cells: implications for the stress response. *BMC Immunol* 2004;**5**:22.
182. Yang HT, Cohen P, Rousseau S. IL-1beta-stimulated activation of ERK1/2 and p38alpha MAPK mediates the transcriptional up-regulation of IL-6, IL-8 and GRO-alpha in HeLa cells. *Cell Signal* 2008;**20**:375−80.
183. Tengesdal IW, Dinarello A, Powers NE, Burchill MA, Joosten LAB, Marchetti C, et al. Tumor NLRP3-derived IL-1β drives the IL-6/STAT3 axis resulting in sustained MDSC-mediated immunosuppression. *Front Immunol* 2021;**12**:661323.
184. Li H, Wu M, Zhao X. Role of chemokine systems in cancer and inflammatory diseases. *MedComm* 2022;**3**:e147.
185. Unver N. Macrophage chemoattractants secreted by cancer cells: sculptors of the tumor microenvironment and another crucial piece of the cancer secretome as a therapeutic target. *Cytokine Growth Factor Rev* 2019;**50**:13−8.
186. Nakatsumi H, Matsumoto M, Nakayama KI. Noncanonical pathway for regulation of CCL2 expression by an mTORC1-FOXK1 axis promotes recruitment of tumor-associated macrophages. *Cell Rep* 2017;**21**:2471−86.
187. Huda N, Khambu B, Liu G, Nakatsumi H, Yan S, Chen X, et al. Senescence connects autophagy deficiency to inflammation and tumor progression in the liver. *Cell Mol Gastroenterol Hepatol* 2022; **14**:333−55.
188. Guo L, Li N, Yang Z, Li H, Zheng H, Yang J, et al. Role of CXCL5 in regulating chemotaxis of innate and adaptive leukocytes in infected lungs upon pulmonary influenza infection. *Front Immunol* 2021;**12**:785457.
189. Jastrzebska B, Debinski A, Filipek S, Palczewski K. Role of membrane integrity on G protein-coupled receptors: rhodopsin stability and function. *Prog Lipid Res* 2011;**50**:267−77.
190. Gimpl G. Interaction of G protein-coupled receptors and cholesterol. *Chem Phys Lipids* 2016;**199**:61−73.
191. Prasanna X, Sengupta D, Chattopadhyay A. Cholesterol-dependent conformational plasticity in GPCR dimers. *Sci Rep* 2016;**6**:31858.
192. Prasanna X, Mohole M, Chattopadhyay A, Sengupta D. Role of cholesterol-mediated effects in GPCR heterodimers. *Chem Phys Lipids* 2020;**227**:104852.
193. Kumar GA, Chattopadhyay A. Cholesterol-dependent endocytosis of GPCRs: implications in pathophysiology and therapeutics. *Biophys Rev* 2021;**13**:1007−17.
194. Ray AP, Thakur N, Pour NG, Eddy MT. Dual mechanisms of cholesterol-GPCR interactions that depend on membrane phospholipid composition. *Structure* 2023;**31**: 836−47.e6.
195. Lin Y, Bian L, Zhu G, Zhang B. Vitronectin promotes proliferation and metastasis of cervical cancer cells *via* the epithelial-mesenchymal transition. *Front Oncol* 2024;**14**:1466264.
196. Gao P, Lu Y, Tang K, Wang W, Wang T, Zhu Y, et al. Ficolin-1 ameliorates pulmonary fibrosis *via* directly binding to TGF-β1. *J Transl Med* 2024;**22**:1051.
197. Sozio MS, Lu C, Zeng Y, Liangpunsakul S, Crabb DW. Activated AMPK inhibits PPAR-α and PPAR-γ transcriptional activity in hepatoma cells. *Am J Physiol Gastrointest Liver Physiol* 2011;**301**: G739−47.
198. Sun H, Zhu X, Lin W, Zhou Y, Cai W, Qiu L. Interactions of TLR4 and PPARγ, dependent on AMPK signalling pathway contribute to anti-inflammatory effects of vaccariae hypaphorine in endothelial cells. *Cell Physiol Biochem* 2017;**42**:1227−39.
199. Harada K, Isse K, Kamihira T, Shimoda S, Nakanuma Y. Th1 cytokine-induced downregulation of PPARγ in human biliary cells relates to cholangitis in primary biliary cirrhosis. *Hepatology* 2005; **41**:1329−38.
200. Yin R, Dong YG, Li HL. PPARγ phosphorylation mediated by JNK MAPK: a potential role in macrophage-derived foam cell formation. *Acta Pharmacol Sin* 2006;**27**:1146−52.
201. Su AC, Zhang LY, Zhang JG, Hu YY, Liu XY, Li SC, et al. The regulation of autophagy by p38 MAPK-PPARγ signaling during the brain ischemic tolerance induced by cerebral ischemic preconditioning. *DNA Cell Biol* 2022;**41**:838−49.
202. Ballav S, Biswas B, Sahu VK, Ranjan A, Basu S. PPARγ partial agonists in disease-fate decision with special reference to cancer. *Cells* 2022;**11**:3215.
203. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Montalto G, Cervello M, et al. Mutations and deregulation of Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascades which alter therapy response. *Oncotarget* 2012;**3**:954−87.
204. Nigam M, Punia B, Dimri DB, Mishra AP, Radu AF, Bungau G. Reactive oxygen species: a double-edged sword in the modulation of cancer signaling pathway dynamics. *Cells* 2025;**14**:1207.
205. Worthylake RA, Burridge K. RhoA and ROCK promote migration by limiting membrane protrusions. *J Biol Chem* 2003;**278**: 13578−84.
206. Yoshizaki H, Ohba Y, Parrini MC, Dulyaninova NG, Bresnick AR, Mochizuki N, et al. Cell type-specific regulation of RhoA activity during cytokinesis. *J Biol Chem* 2004;**279**:44756−62.
207. Omerovic J, Prior IA. Compartmentalized signalling: ras proteins and signalling nanoclusters. *FEBS J* 2009;**276**:1817−25.
208. Schmick M, Vartak N, Papke B, Kovacevic M, Truxius DC, Rossmannek L, et al. KRas localizes to the plasma membrane by spatial cycles of solubilization, trapping and vesicular transport. *Cell* 2014;**157**:459−71.
209. Zhou Y, Prakash PS, Liang H, Gorfe AA, Hancock JF. The KRAS and other prenylated polybasic domain membrane anchors recognize phosphatidylserine acyl chain structure. *Proc Natl Acad Sci USA* 2021;**118**:e2014605118.

210. Munro E. Anillin puts RhoA in touch with PIP_2. *Dev Cell* 2019;**49**:819−20.
211. Hancock JF, Parton RG. Ras plasma membrane signalling platforms. *Biochem J* 2005;**389**:1−11.
212. Jang H, Abraham SJ, Chavan TS, Hitchinson B, Khavrutskii L, Tarasova NI, et al. Mechanisms of membrane binding of small GTPase K-Ras4B farnesylated hypervariable region. *J Biol Chem* 2015;**290**:9465−77.
213. Mosaddeghzadeh N, Kazemein Jasemi NS, Majolée J, Zhang SC, Hordijk PL, Dvorsky R, et al. Electrostatic forces mediate the specificity of RHO GTPase-GDI interactions. *Int J Mol Sci* 2021;**22**:12493.
214. Guillot-Ferriols M, Lanceros-Méndez S, Gómez Ribelles JL, Gallego Ferrer G. Electrical stimulation: effective cue to direct osteogenic differentiation of mesenchymal stem cells?. *Biomater Adv* 2022;**138**:212918.
215. Chen Z, Chen Y, Zhe M, Jiang J, Liu H, Qin L, et al. Engineered smart piezoelectric materials facilitate bone defect regeneration. *Mater Des* 2026;**262**:115501.
216. Chen Y, Gong W, Dai W, Jiang H, Xu X. E2F1/2/4 mRNA is associated with immune infiltration and are potential biomarkers for the prognosis of human gastric carcinoma. *Transl Cancer Res* 2021;**10**:2801−11.
217. Daigh LH, Saha D, Rosenthal DL, Ferrick KR, Meyer T. Uncoupling of mTORC1 from E2F activity maintains DNA damage and senescence. *Nat Commun* 2024;**15**:9181.
218. Zhang Y, Hao X, Han G, Lu Y, Chen Z, Zhang L, et al. E2F1-mediated GINS2 transcriptional activation promotes tumor progression through PI3K/AKT/mTOR pathway in hepatocellular carcinoma. *Am J Cancer Res* 2022;**12**:1707−26.
219. Calzone L, Gelay A, Zinovyev A, Radvanyi F, Barillot E. A comprehensive modular map of molecular interactions in RB/E2F pathway. *Mol Syst Biol* 2008;**4**:173.
220. Bertonnier-Brouty L, Andersson J, Kaprio T, Hagström J, Bsharat S, Asplund O, et al. E2F transcription factors promote tumorigenicity in pancreatic ductal adenocarcinoma. *Cancer Med* 2024;**13**:e7187.
221. Wasserman D, Nachum S, Cohen M, Enrico TP, Noach-Hirsh M, Parasol J, et al. Cell cycle oscillators underlying orderly proteolysis of E2F8. *Mol Biol Cell* 2020;**31**:725−40.
222. Timmers C, Sharma N, Opavsky R, Maiti B, Wu L, Wu J, et al. E2f1, E2f2, and E2f3 control E2F target expression and cellular proliferation *via* a p53-dependent negative feedback loop. *Mol Cell Biol* 2007;**27**:65−78. Erratum in: Mol Cell Biol 2012;**32**:1758.
223. Spitschak A, Dhar P, Singh KP, Casalegno Garduño R, Gupta SK, Vera J, et al. E2F1-induced autocrine IL-6 inflammatory loop mediates cancer-immune crosstalk that predicts T cell phenotype switching and therapeutic responsiveness. *Front Immunol* 2024;**15**:1470368.
224. Li J, Ran C, Li E, Gordon F, Comstock G, Siddiqui H, et al. Synergistic function of E2F7 and E2F8 is essential for cell survival and embryonic development. *Dev Cell* 2008;**14**:62−75.
225. Zheng J, Huang J, Xia J, Zhou W, Dai L, Lin S, et al. Transcription factor E2F8 is a therapeutic target in the basal-like subtype of breast cancer. *Front Oncol* 2023;**13**:1038787.
226. Vigo E, Müller H, Prosperini E, Hateboer G, Cartwright P, Moroni MC, et al. CDC25A phosphatase is a target of E2F and is required for efficient E2F-induced S phase. *Mol Cell Biol* 1999;**19**:6379−95.
227. Delgado M, Washam CL, Urbaniak A, Heflin B, Storey AJ, Lan RS, et al. Phosphoproteomics provides novel insights into the response of primary acute lymphoblastic leukemia cells to microtubule depolymerization in G1 phase of the cell cycle. *ACS Omega* 2021;**6**:24949−59.
228. Wang X, Hills LB, Huang YH. Lipid and protein co-regulation of PI3K effectors Akt and Itk in lymphocytes. *Front Immunol* 2015;**6**:117.
229. Sun F, Yang Y, Tu H, Cai H. Gradients of $PI(4,5)P_2$ and $PI(3,5)P_2$ jointly participate in shaping the back state of dictyostelium cells. *Front Cell Dev Biol* 2022;**10**:835185.
230. Sun J, Zalejski J, Song S, Sharma A, Wang W, Hu Y, et al. $PI(3,5)P_2$ controls the signaling activity of class I PI3K. *bioRxiv [Preprint]* 2025. https://doi.org/10.1101/2023.01.25.525550. Originally published 2023 Jan 25. [Version 2].
231. Yang M, Lu Y, Piao W, Jin H. The translational regulation in mTOR Pathway. *Biomolecules* 2022;**12**:802.
232. Shveygert M, Kaiser C, Bradrick SS, Gromeier M. Regulation of eukaryotic initiation factor 4E (eIF4E) phosphorylation by mitogen-activated protein kinase occurs through modulation of Mnk1−eIF4G interaction. *Mol Cell Biol* 2010;**30**:5160−7.
233. Qin Z, Bouteau A, Herbst C, Igyártó BZ. Pre-exposure to mRNA-LNP inhibits adaptive immune responses and alters innate immune fitness in an inheritable fashion. *PLoS Pathog* 2022;**18**:e1010830.
234. Lonez C, Vandenbranden M, Ruysschaert JM. Cationic liposomal lipids: from gene carriers to cell signaling. *Prog Lipid Res* 2008;**47**:340−7.
235. Lonez C, Vandenbranden M, Ruysschaert JM. Cationic lipids activate intracellular signaling pathways. *Adv Drug Deliv Rev* 2012;**64**:1749−58.
236. Connors J, Joyner D, Mege NJ, Cusimano GM, Bell MR, Marcy J, et al. Lipid nanoparticles (LNP) induce activation and maturation of antigen presenting cells in young and aged individuals. *Commun Biol* 2023;**6**:188. Erratum in: Commun Biol 2025;**8**:285.
237. Amor NP, Guo K, Zhang S, Xia J, Yang Y, Lin A. Lipid nanoparticle: beyond delivery vehicle-unveiling its immunological adjuvant potential. *FASEB J* 2025;**39**:e70641.
238. Yamaguchi Y, Kato Y, Edahiro R, Søndergaard JN, Murakami T, Amiya S, et al. Consecutive BNT162b2 mRNA vaccination induces short-term epigenetic memory in innate immune cells. *JCI Insight* 2022;**7**:e163347.
239. Simonis A, Theobald SJ, Koch AE, Mummadavarapu R, Mudler JM, Pouikli A, et al. Persistent epigenetic memory of SARS-CoV-2 mRNA vaccination in monocyte-derived macrophages. *Mol Syst Biol* 2025;**21**:341−60.
240. Chytła A, Rattay S, Akgül B, Sztacho M. Plasma membrane and nuclear phosphatidylinositol 4,5-bisphosphate signalling in cancer. *Lipids Health Dis* 2025;**24**:39.
241. Hifdi N, Vaucourt M, Hnia K, Panasyuk G, Vandromme M. Phosphoinositide signaling in the nucleus: impacts on chromatin and transcription regulation. *Biol Cell* 2025;**117**:e2400096.
242. McColl ER, Croyle MA, Zamboni WC, Honer WG, Heise M, Piquette-Miller M, et al. COVID-19 Vaccines and the virus: impact on drug metabolism and pharmacokinetics. *Drug Metab Dispos* 2023;**51**:130−41.
243. Jung SW, Jeon JJ, Kim YH, Choe SJ, Lee S. Long-term risk of autoimmune diseases after mRNA-based SARS-CoV2 vaccination in a Korean, nationwide, population-based cohort study. *Nat Commun* 2024;**15**:6181.
244. Sellegounder D, Ferrucci L, Anbazhagan R, Basisty N. Editorial: molecular crosstalk between endocrine factors, paracrine signals, and the immune system during aging. *Front Endocrinol* 2023;**14**:1203755.
245. Chen M, Tan JX, Sun Y, Thapa N, Cryns VL, Anderson RA. Agonist- and stress-driven compartmentalized phosphoinositide signaling in cells. *Biochim Biophys Acta Mol Cell Biol Lipids* 2025;**1870**:159662.
246. Ebner M, Sinkovics B, Szczygieł M, Ribeiro DW, Yudushkin I. Localization of mTORC2 activity inside cells. *J Cell Biol* 2017;**216**:343−53.
247. Rädler PD, Wehde BL, Wagner KU. Crosstalk between STAT5 activation and PI3K/AKT functions in normal and transformed mammary epithelial cells. *Mol Cell Endocrinol* 2017;**451**:31−9.
248. Flori E, Cavallo A, Mosca S, Kovacs D, Cota C, Zaccarini M, et al. JAK/STAT inhibition normalizes lipid composition in 3D human

epidermal equivalents challenged with Th2 cytokines. *Cells* 2024;**13**:760.
249. Koester AM, Tao K, Szczepaniak M, Rames MJ, Nan X. Nanoscopic spatial association between Ras and phosphatidylserine on the cell membrane studied with multicolor super resolution microscopy. *Biomolecules* 2022;**12**:1033.
250. Panariti A, Miserocchi G, Rivolta I. The effect of nanoparticle uptake on cellular behavior: disrupting or enabling functions?. *Nanotechnol Sci Appl* 2012;**5**:87−100.
251. Voigt J, Christensen J, Shastri VP. Differential uptake of nanoparticles by endothelial cells through polyelectrolytes with affinity for caveolae. *Proc Natl Acad Sci U S A* 2014;**111**:2942−7.
252. Wang T, Bai J, Jiang X, Nienhaus GU. Cellular uptake of nanoparticles by membrane penetration: a study combining confocal microscopy with FTIR spectroelectrochemistry. *ACS Nano* 2012;**6**:1251−9.
253. Gerelli Y. Chapter three: exploring interactions between lipid membranes and nanoparticles through neutron and X-ray reflectometry techniques. In: Iglič A, Rappolt M, Losada-Pérez P, editors. *Advances in biomembranes and lipid self-assembly*, vol. 38. Academic Press; 2023. p. 37−61.
254. Lavagna E, Barnoud J, Rossi G, Monticelli L. Size-dependent aggregation of hydrophobic nanoparticles in lipid membranes. *Nanoscale* 2020;**12**:9452−61.
255. Cooper GM. *The cell: a molecular approach*. 2nd ed. Sunderland (MA): Sinauer Associates; 2000 [cited 2026 Mar 31]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK9839/.
256. Hammond GRV, Burke JE. Novel roles of phosphoinositides in signaling, lipid transport, and disease. *Curr Opin Cell Biol* 2020;**63**:57−67.
257. Skotland T, Sandvig K. The role of PS 18: 0/18: 1 in membrane function. *Nat Commun* 2019;**10**:2752.
258. Heimburg T. The excitable fluid mosaic. *Biochim Biophys Acta Biomembr* 2023;**1865**:184104.
259. Lupyan D, Mezei M, Logothetis DE, Osman R. A molecular dynamics investigation of lipid bilayer perturbation by PIP_2. *Biophys J* 2010;**98**:240−7.
260. Pike LJ. Lipid rafts: bringing order to chaos. *J Lipid Res* 2003;**44**:655−67.
261. Levin R, Grinstein S, Schlam D. Phosphoinositides in phagocytosis and macropinocytosis. *Biochim Biophys Acta* 2015;**1851**:805−23.
262. Weiner OD, Neilsen PO, Prestwich GD, Kirschner MW, Cantley LC, Bourne HR. A PtdInsP(3)- and Rho GTPase-mediated positive feedback loop regulates neutrophil polarity. *Nat Cell Biol* 2002;**4**:509−13.
263. Guan K, Curtis ER, Lew DJ, Elston TC. Particle-based simulations reveal two positive feedback loops allow relocation and stabilization of the polarity site during yeast mating. *PLoS Comput Biol* 2023;**19**:e1011523.
264. Fallahi-Sichani M, Linderman JJ. Lipid raft-mediated regulation of G-protein coupled receptor signaling by ligands which influence receptor dimerization: a computational study. *PLoS One* 2009;**4**:e6604.
265. Wang X, Shi X, Wang R. Regulating mRNA endosomal escape through lipid rafts: a review. *Int J Pharm* 2025;**675**:125571.
266. Sarkar S, Carroll B, Buganim Y, Maetzel D, Ng AH, Cassady JP, et al. Impaired autophagy in the lipid-storage disorder Niemann-Pick type C1 disease. *Cell Rep* 2013;**5**:1302−15.
267. Dall'Armi C, Devereaux KA, Di Paolo G. The role of lipids in the control of autophagy. *Curr Biol* 2013;**23**:R33−45.
268. Zhang S, Peng X, Yang S, Li X, Huang M, Wei S, et al. The regulation, function, and role of lipophagy, a form of selective autophagy, in metabolic disorders. *Cell Death Dis* 2022;**13**:132.
269. Jarocki M, Turek K, Saczko J, Tarek M, Kulbacka J. Lipids associated with autophagy: mechanisms and therapeutic targets. *Cell Death Discov* 2024;**10**:460.
270. Karim M, Mishra M, Lo CW, Saul S, Cagirici HB, Gourdelier M, et al. PIP4K2C inhibition reverses autophagic flux impairment induced by SARS-CoV-2. *Nat Commun* 2025;**16**:6397.
271. Haucke V, Kozlov MM. Membrane remodeling in clathrin-mediated endocytosis. *J Cell Sci* 2018;**131**:jcs216812.
272. Vaithianathan T, Bukiya A, Liu J, Liu P, Asuncion-Chin M, Fan Z, et al. Direct regulation of BK channels by phosphatidylinositol 4,5-bisphosphate as a novel signaling pathway. *J Gen Physiol* 2008;**132**:13−28.
273. Blunsom NJ, Cockcroft S. Phosphatidylinositol synthesis at the endoplasmic reticulum. *Biochim Biophys Acta Mol Cell Biol Lipids* 2020;**1865**:158471.
274. Posor Y, Jang W, Haucke V. Phosphoinositides as membrane organizers. *Nat Rev Mol Cell Biol* 2022;**23**:797−816.
275. Lolicato F, Nickel W, Haucke V, Ebner M. Phosphoinositide switches in cell physiology — from molecular mechanisms to disease. *J Biol Chem* 2024;**300**:105757.
276. Balla T. Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol Rev* 2013;**93**:1019−137.
277. Eramo MJ, Mitchell CA. Regulation of PtdIns(3,4,5)P_3/Akt signalling by inositol polyphosphate 5-phosphatases. *Biochem Soc Trans* 2016;**44**:240−52.
278. Xu S, Cao B, Xuan G, Xu S, An Z, Zhu C, et al. Function and regulation of Rab GTPases in cancers. *Cell Biol Toxicol* 2024;**40**:28.
279. Koike S, Jahn R. Rab GTPases and phosphoinositides fine-tune SNAREs dependent targeting specificity of intracellular vesicle traffic. *Nat Commun* 2024;**15**:2508.
280. Puranik A, Lenehan PJ, Silvert E, Niesen MJM, Corchado-Garcia J, O'Horo JC, et al. Comparative effectiveness of mRNA-1273 and BNT162b2 against symptomatic SARS-CoV-2 infection. *Med* 2022;**3**:28−41.e8.
281. Granados-Riveron JT, Aquino-Jarquin G. Engineering of the current nucleoside-modified mRNA-LNP vaccines against SARS-CoV-2. *Biomed Pharmacother* 2021;**142**:111953.
282. Harvey RD, Ara N, Heenan RK, Barlow DJ, Quinn PJ, Lawrence MJ. Stabilization of distearoylphosphatidylcholine lamellar phases in propylene glycol using cholesterol. *Mol Pharm* 2013;**10**:4408−17.
283. Skotland T, Kavaliauskiene S, Sandvig K. The role of lipid species in membranes and cancer-related changes. *Cancer Metastasis Rev* 2020;**39**:343−60.
284. Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, et al. A review on phospholipids and their main applications in drug delivery systems. *Asian J Pharm Sci* 2015;**10**:81−98.
285. McMaster CR. From yeast to humans—roles of the Kennedy pathway for phosphatidylcholine synthesis. *FEBS Lett* 2018;**592**:1256−72.
286. Cummings R, Parinandi N, Wang L, Usatyuk P, Natarajan V. Phospholipase D/phosphatidic acid signal transduction: role and physiological significance in lung. *Mol Cell Biochem* 2002;**234−5**:99−109.
287. Wagner K, Brezesinski G. Phospholipase D activity is regulated by product segregation and the structure formation of phosphatidic acid within model membranes. *Biophys J* 2007;**93**:2373−83.
288. Bruntz RC, Lindsley CW, Brown HA. Phospholipase D signaling pathways and phosphatidic acid as therapeutic targets in cancer. *Pharmacol Rev* 2014;**66**:1033−79.
289. Semenkovich CF, Goldberg AC, Goldberg IJ. Chapter 37: disorders of lipid metabolism. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM, editors. *Williams textbook of endocrinology*. 13th ed. Elsevier; 2016. p. 1660−700.
290. Pearce B, Jakobson K, Morrow C, Murphy S. Phosphatidic acid promotes phosphoinositide metabolism and DNA synthesis in cultured cortical astrocytes. *Neurochem Int* 1994;**24**:165−71.
291. Quick J, Santos ND, Cheng MHY, Chander N, Brimacombe CA, Kulkarni J, et al. Lipid nanoparticles to silence androgen receptor variants for prostate cancer therapy. *J Control Release* 2022;**349**:174−83.

292. Jeschek D, Lhota G, Wallner J, Vorauer-Uhl K. A versatile, quantitative analytical method for pharmaceutical relevant lipids in drug delivery systems. *J Pharm Biomed Anal* 2016;**119**:37−44.
293. Rezaei S, Blick EE, Mineart KP, Kelley EG. Chapter Three - linking chemical degradation and physical instability of lipid vesicles. In: Iglič A, Rappolt M, Losada-Pérez P, editors. *Advances in biomembranes and lipid self-assembly*, vol. 41; 2025. p. 47−64.
294. Nishimura T, Gecht M, Covino R, Hummer G, Surma MA, Klose C, et al. Osh proteins control nanoscale lipid organization necessary for PI(4,5)P_2 synthesis. *Mol Cell* 2019;**75**:1043−57.e8.
295. Heckle LA, Kozminski KG. Osh-dependent and -independent regulation of PI4P levels during polarized growth of *Saccharomyces cerevisiae*. *Mol Biol Cell* 2023;**34**:ar104.
296. Raychaudhuri S, Prinz WA. The diverse functions of oxysterol-binding proteins. *Annu Rev Cell Dev Biol* 2010;**26**:157−77.
297. Leonarduzzi G, Robbesyn F, Poli G. Signaling kinases modulated by 4-hydroxynonenal. *Free Radic Biol Med* 2004;**37**:1694−702.
298. Forman HJ, Fukuto JM, Miller T, Zhang H, Rinna A, Levy S. The chemistry of cell signaling by reactive oxygen and nitrogen species and 4-hydroxynonenal. *Arch Biochem Biophys* 2008;**477**:183−95.
299. Thiemicke A, Neuert G. Rate thresholds in cell signaling have functional and phenotypic consequences in non-linear time-dependent environments. *Front Cell Dev Biol* 2023;**11**:1124874.
300. Hou X, Chen Y, Carrillo ND, Cryns VL, Anderson RA, Sun J, et al. Phosphoinositide signaling at the cytoskeleton in the regulation of cell dynamics. *Cell Death Dis* 2025;**16**:296.
301. Adhikari H, Cullen PJ. Role of phosphatidylinositol phosphate signaling in the regulation of the filamentous-growth mitogen-activated protein kinase pathway. *Eukaryot Cell* 2015;**14**:427−40.
302. Houthaeve G, De Smedt SC, Braeckmans K, De Vos WH. The cellular response to plasma membrane disruption for nanomaterial delivery. *Nano Converg* 2022;**9**:6.
303. Papafilippou L, Nicolaou A, Kendall AC, Camacho-Muñoz D, Hadjidemetriou M. The lipidomic profile of the nanoparticle-biomolecule corona reflects the diversity of plasma lipids. *Nanoscale* 2023;**15**:11038−51.
304. Gual P, Grémeaux T, Gonzalez T, Le Marchand-Brustel Y, Tanti JF. MAP kinases and mTOR mediate insulin-induced phosphorylation of insulin receptor substrate-1 on serine residues 307, 612 and 632. *Diabetologia* 2003;**46**:1532−42.
305. Minard AY, Tan SX, Yang P, Fazakerley DJ, Domanova W, Parker BL, et al. mTORC1 is a major regulatory node in the FGF21 signaling network in adipocytes. *Cell Rep* 2016;**17**:29−36.
306. Le TKC, Dao XD, Nguyen DV, Luu DH, Bui TMH, Le TH, et al. Insulin signaling and its application. *Front Endocrinol* 2023;**14**:1226655.
307. Krauson AJ, Casimero FVC, Siddiquee Z, Stone JR. Duration of SARS-CoV-2 mRNA vaccine persistence and factors associated with cardiac involvement in recently vaccinated patients. *NPJ Vaccines* 2023;**8**:141.
308. Rosati M, Terpos E, Homan P, Bergamaschi C, Karaliota S, Ntanasis-Stathopoulos I, et al. Rapid transient and longer-lasting innate cytokine changes associated with adaptive immunity after repeated SARS-CoV-2 BNT162b2 mRNA vaccinations. *Front Immunol* 2023;**14**:1292568.
309. Lee Y, Jeong M, Park J, Jung H, Lee H. Immunogenicity of lipid nanoparticles and its impact on the efficacy of mRNA vaccines and therapeutics. *Exp Mol Med* 2023;**55**:2085−96.
310. Iordanov MS, Paranjape JM, Zhou A, Wong J, Williams BR, Meurs EF, et al. Activation of p38 mitogen-activated protein kinase and c-Jun NH_2-terminal kinase by double-stranded RNA and encephalomyocarditis virus: involvement of RNase L, protein kinase R, and alternative pathways. *Mol Cell Biol* 2000;**20**:617−27.
311. Jurkiewicz A, Graczyk D. MAP kinases are involved in RNA polymerase III regulation upon LPS treatment in macrophages. *Gene* 2022;**831**:146548.
312. Zhang G, He LS, Him Wong Y, Xu Y, Zhang Y, Qian PY. p38 MAPK regulates PKAα and CUB-serine protease in *Amphibalanus amphitrite* cyprids. *Sci Rep* 2015;**5**:14767.
313. Tang T, Zhu Q, Li X, Zhu G, Deng S, Wang Y, et al. Protease Nexin I is a feedback regulator of EGF/PKC/MAPK/EGR1 signaling in breast cancer cells metastasis and stemness. *Cell Death Dis* 2019;**10**:649. Erratum in: Cell Death Dis 2020;**11**:13.
314. Sala-Gaston J, Costa-Sastre L, Pedrazza L, Martinez-Martinez A, Ventura F, Rosa JL. Regulation of MAPK signaling pathways by the large HERC ubiquitin ligases. *Int J Mol Sci* 2023;**24**:4906.
315. Lutz J, Lazzaro S, Habbeddine M, Schmidt KE, Baumhof P, Mui BL, et al. Unmodified mRNA in LNPs constitutes a competitive technology for prophylactic vaccines. *NPJ Vaccines* 2017;**2**:29.
316. Pang SJ, Jiang TY, Wang NG, Cui XW, Wang H, Pan YF, et al. RNA polymerase II subunit 5-mediating protein limits TLR4-induced innate immune activation in macrophages by inhibiting IKKβ/NF-κB signaling during sepsis. *Cell Commun Signal* 2025;**23**:274.
317. Ostendorf T, Zillinger T, Andryka K, Schlee-Guimaraes TM, Schmitz S, Marx S, et al. Immune sensing of synthetic, bacterial, and protozoan RNA by toll-like receptor 8 requires coordinated processing by RNase T2 and RNase 2. *Immunity* 2020;**52**:591−605.e6.
318. Lee D, Le Pen J, Yatim A, Dong B, Aquino Y, Ogishi M, et al. Inborn errors of OAS-RNase L in SARS-CoV-2-related multisystem inflammatory syndrome in children. *Science* 2023;**379**:eabo3627.
319. Bucci M, Vellecco V, Harrington L, Brancaleone V, Roviezzo F, Mattace Raso G, et al. Cross-talk between toll-like receptor 4 (TLR4) and proteinase-activated receptor 2 (PAR_2) is involved in vascular function. *Br J Pharmacol* 2013;**168**:411−20.
320. Zhong B, Liu X, Wang X, Liu X, Li H, Darnay BG, et al. Ubiquitin-specific protease 25 regulates TLR4-dependent innate immune responses through deubiquitination of the adaptor protein TRAF3. *Sci Signal* 2013;**6**:ra35.
321. Papendorf JJ, Krüger E, Ebstein F. Proteostasis perturbations and their roles in causing sterile inflammation and autoinflammatory diseases. *Cells* 2022;**11**:1422.
322. Guo X, Wang H, Li Y, Leng X, Huang W, Ma Y, et al. Transfection reagent Lipofectamine triggers type I interferon signaling activation in macrophages. *Immunol Cell Biol* 2019;**97**:92−6. Erratum in: *Immunol Cell Biol* 2020;**98**:88.
323. World Health Organization. *Causality assessment of an adverse event following immunization (AEFI): user manual for the revised WHO classification*. 2nd ed. Geneva: World Health Organization; 2019 [cited 2026 Mar 31]. Available from: https://www.who.int/publications/i/item/9789241516990.
324. Bellavite P. Causality assessment of adverse events following immunization: the problem of multifactorial pathology. *F1000Research* 2020;**9**:170.
325. Islam S, Bhattacharya S. Dynamical systems theory as an organizing principle for single-cell biology. *NPJ Syst Biol Appl* 2025;**11**:85.
326. Philipps M, Schmid N, Hasenauer J. Current state and open problems in universal differential equations for systems biology. *NPJ Syst Biol Appl* 2025;**11**:101.
327. Simons BD, Karin O. Cell cycle criticality as a mechanism for robust cell population control. *Mol Syst Biol* 2025;**22**:241−58.
328. Fischer DS, Villanueva MA, Winter PS, Shalek AK. Adapting systems biology to address the complexity of human disease in the single-cell era. *Nat Rev Genet* 2025;**26**:514−31.
329. United States Pharmacopeia. *Analytical procedures for quality of mRNA vaccines and therapeutics: draft guidelines*. 3rd ed. Rockville (MD): United States Pharmacopeia; 2024 [cited 2026 Feb 13]. Available from: https://go.usp.org/mRNAVaccineQuality.
330. European Medicines Agency. *Guideline on the quality aspects of mRNA vaccines: draft. EMA/CHMP/BWP/82416/2025*. Amsterdam: European Medicines Agency; 2025 [cited 2026 Mar 31]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-quality-aspects-mrna-vaccines_en.pdf.

331. Hassett KJ, Higgins J, Woods A, Levy B, Xia Y, Hsiao CJ, et al. Impact of lipid nanoparticle size on mRNA vaccine immunogenicity. *J Control Release* 2021;**335**:237−46.
332. Arral ML, Whitehead KA. Design principles of lipid nanoparticles for RNA delivery. *Nat Rev Bioeng 2026* 2026;**4**:417−34.
333. Karikó K, Ni H, Capodici J, Lamphier M, Weissman D. mRNA is an endogenous ligand for toll-like receptor 3. *J Biol Chem* 2004;**279**: 12542−50.
334. Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* 2005;**23**:165−75.
335. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Mol Ther* 2008;**16**:1833−40.
336. Monroe J, Eyler DE, Mitchell L, Deb I, Bojanowski A, Srinivas P, et al. N1-Methylpseudouridine and pseudouridine modifications modulate mRNA decoding during translation. *Nat Commun* 2024;**15**:8119.
337. Ghosh TK, Mickelson DJ, Solberg JC, Lipson KE, Inglefield JR, Alkan SS. TLR-TLR cross talk in human PBMC resulting in synergistic and antagonistic regulation of type-1 and 2 interferons, IL-12 and TNF-alpha. *Int Immunopharmacol* 2007;**7**:1111−21.
338. Xu XH, Shah PK, Faure E, Equils O, Thomas L, Fishbein MC, et al. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. *Circulation* 2001;**104**:3103−8.
339. Hovland A, Jonasson L, Garred P, Yndestad A, Aukrust P, Lappegård KT, et al. The complement system and toll-like receptors as integrated players in the pathophysiology of atherosclerosis. *Atherosclerosis* 2015;**241**:480−94.
340. Luo L, Wall AA, Tong SJ, Hung Y, Xiao Z, Tarique AA, et al. TLR crosstalk activates LRP1 to recruit Rab8a and PI3Kγ for suppression of inflammatory responses. *Cell Rep* 2018;**24**:3033−44.
341. Köberlin MS, Heinz LX, Superti-Furga G. Functional crosstalk between membrane lipids and TLR biology. *Curr Opin Cell Biol* 2016; **39**:28−36.
342. Kornilov FD, Shabalkina AV, Lin C, Volynsky PE, Kot EF, Kayushin AL, et al. The architecture of transmembrane and cytoplasmic juxtamembrane regions of toll-like receptors. *Nat Commun* 2023;**14**:1503.