

Multi-Domain Programmable Lipid Nanoparticles for Targeted Delivery

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Abstract

Programmable lipid nanoparticles, or LNPs, represent a breakthrough in the realm of targeted drug delivery, offering precise spatiotemporal control essential for the treatment of complex diseases such as cancer and genetic disorders. In order to provide a more modular perspective and a more balanced analysis of the mechanism, this review presents a novel Four-Domain Model that consists of Architecture, Interface, Payload, and Dispersal Domain. We explored the dynamical equilibrium between LNPs components and the surroundings throughout their destiny, from formulation to release. On the basis of this, we delve deep into manufacturing challenges, scalability issues, and regulatory hurdles, associated with the clinical translation of LNP technology. Within the framework focusing on the programmability in each domain, we prioritized patient-centric factors like dosing regimens, administration techniques, and potential consequences. Notably, this review expands to innovative anatomical routes, such as intranasal and intraocular administration, offering a thorough examination of the advantages and disadvantages of each route. We also offered a comprehensive comparison between artificial LNPs and natural exosomes in terms of functionality, biocompatibility, and therapeutic potential. Ultimately, this review highlights the potential of programmable LNPs to evolve into more intelligent, naturally integrated systems, achieving optimal biocompatibility and functionality.

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1 Introduction

Complex diseases like cancer and genetic disorders often exhibit heterogeneous and dynamic characteristics within the body, making uniform drug distribution challenging^{1,2}. The treatment of these diseases necessitates precise and effective drug-delivery systems with high spatiotemporal control for maximized therapeutic impact^{3,4}. Up to date, delivery loaders such as hydrogels⁵, polymeric nanoparticles⁶, molecular conjugates⁷, viral vectors⁸, and lipid-based nanoparticles⁹ (LBNPs) are getting widely used. Particularly, LBNPs are increasingly recognized for their ability to protect and deliver sensitive agents like mRNA to target sites, locally or systematically, while maintaining biocompatibility^{9,10}. LBNPs allow targeted administration to specified diseased cells or tissues, enhancing therapeutic efficacy via controlled release mechanisms and minimizing systemic side effects. Among the diverse LBNP formulations, lipid nanoparticles (LNPs) are distinguished by their inclusion of ionizable lipids. They incorporate helper lipids, cholesterol, PEGylated lipids, and other functional components into their self-assembled nanoscale structure, just like other LBNPs^{11,12}. Their impact has been evidenced by numerous clinical trials^{13,14} and emergency use authorizations for SARS-CoV2 mRNA vaccines by Pfizer and Moderna^{15–17}.

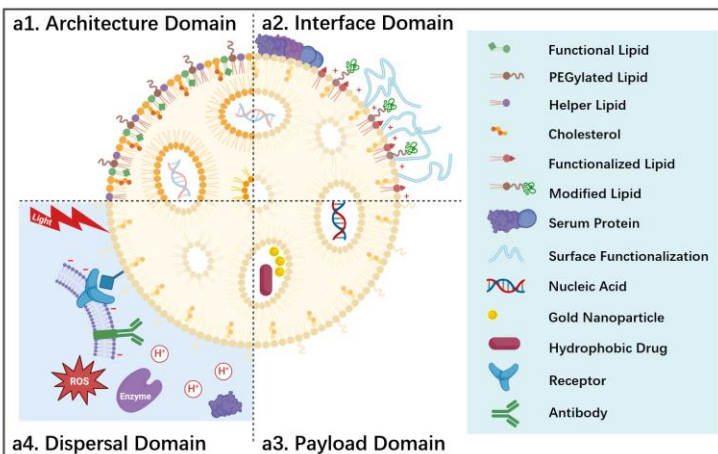
Conceptually, LNPs simulate a “thermodynamic system” where the mass is enclosed within confined spaces under equilibrium circumstances and released when the system is non-equilibrium. In this review, we examine the equilibrium and non-equilibrium states of LNPs as semi-closed systems at different stages of their life cycle. While existing reviews comprehensively covered aspects such as development⁹, component¹⁸, application¹⁹, and clinical aspects of LNPs¹⁹, our perspective further integrates structural, environmental, and functional aspects within a thermodynamically informed framework. On this basis, we introduce the Four-Domain Model, comprising the Architecture, Interface, Payload, and Dispersal Domain (**Fig. 1**). This modular framework fills in the gaps in the present studies by dissecting the advancement of LNPs with particular attention on each domain's programmability. We apply the term “programmability” to LNPs drug delivery systems here, even though it was originally used to describe microrobots in computer systems or any intelligent system that reacts to external stimuli or acts on its initiative in a predictable and regulated manner. The term “programmability” in this review refers to nanoparticles' capacity to be engineered to respond to certain environmental cues for focused and regulated drug release. The programmability of LNPs, including response to pH^{20,21}, temperature¹¹, or enzymatic activity^{22,23}, has been hugely advanced by developments in materials science and nanotechnology. This advancement has increased therapeutic efficacy and reduced off-target consequences.

In the Four-Domain Model, the Architecture Domain subjects the structural components of LNPs, analogous to the container in a thermodynamic system that separates the payload from the surroundings. This domain's programmability was mostly concentrated on the mesophase structure, which reacts to pH variations and fusion mechanisms in endosome escape. The Interface Domain investigates the interactions between LNPs and their surroundings, focusing on how different functional components respond to environmental factors such as pH, redox potential, and enzymes. The Payload Domain concentrates on the encapsulated mass and its

applications, encompassing therapeutic agents and functional payloads with their therapeutic benefits. The Dispersal Domain provides a thorough picture of the lifecycle of LNPs at different stages of delivery by addressing the different administration routes and biodistribution patterns of LNPs. As a result, this architecture provides a modular foundation for the purposeful and clear design of programmable LNPs. "Modularity" refers to the flexibility to add, remove, or modify the components for specific functions or responses, such as targeting ligands or responsive triggers^{24–26}.

In biomedical contexts, LNPs monitor the environment and maintain equilibrium or close-to-equilibrium before receiving specific cues. The on-demand responsive release, including multi-step delivery^{27,28}, is conceptualized as a non-equilibrium transition triggered by external stimuli such as pH changes or enzyme presence. By applying these equilibrium descriptions, we gain an intuitive understanding of the dynamic interplay within LNPs. We presented the newest discovery regarding the structure of LNPs by examining manufacturing and regulatory barriers, offering practical recommendations for overcoming these obstacles, and advancing toward clinical adoption. We also suggested potential anatomical delivery routes, such as intracerebral and intraocular methods, that are associated with their challenges and opportunities. Furthermore, we examined and prospected for future research directions to maximize LNPs' potential in precision medicine for the ultimate clinical application. Despite progress on programmable LNPs, natural cells or biological vesicles are non-equilibrium compartments that are far more sophisticated than those in artificial compartments, maintaining intricately interlinked mass and information fluxes. This review further demonstrated a detailed comparison between artificial LNPs and natural exosomes using our Four Domain Model to demonstrate our model's advantage. This comparative analysis provides a roadmap for developing more intelligent, naturally integrated systems. Although LNPs are the review's main focus, we believe these ideas can be expanded to different delivery systems and medical applications to address unmet medical requirements and provide creative answers to challenging health problems.

a. Four-Domain model of LNPs



b. Thermodynamic perspective

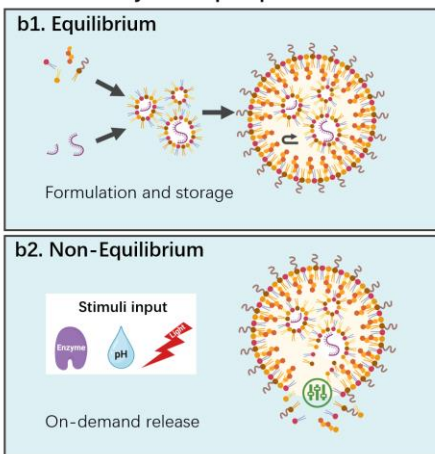


Fig. 1 Illustration of the Four-Domain Model and thermodynamic perspective of programmable LNPs. **a.** Schematic overview of the Four-Domain Model of LNPs. **a1.** The Architecture Domain focuses on the structural integrity and composition of LNPs, including ionizable lipids, PEGylated lipids, helper lipids, and cholesterol. **a2.** The Interface Domain addresses interactions with the biological environment, enhancing stability, reducing immunogenicity, and improving targeting efficacy. This domain is the focus of the programmability of LNPs. **a3.** The payload Domain highlights encapsulated therapeutic agents and functional components, focusing on therapeutic effect and programmability. **a4.** The Dispersal Domain explores administration route, biodistribution, and trafficking analysis, which are critical for optimizing delivery strategies and clinical outcomes. **b.** Schematic illustrating LNPs to a semi-closed thermodynamic system in equilibrium (**b1**) and non-equilibrium (**b2**) conditions. **b1.** LNP formulation to achieve equilibrium during storage and delivery. **b2.** LNPs transition to a non-equilibrium state programmed to respond to external stimuli, enabling controlled and on-demand release of payloads.

2 Architecture Domain: Structure and Formulation of LNPs

The Architecture Domain is analogous to the container in a thermodynamic system, encapsulating the payload to separate it from the environment. It ensures that the LNPs maintain stability throughout the delivery process, protecting the payload from degradation until it reaches the target site. Architecture Domain comprises the majority of LNPs by molar fraction and primarily consists of cationic ionizable lipids (CILs), PEGylated lipids, helper lipids, and cholesterol, each serving distinct functional roles. These components are self-assembled to form a core-shell architecture that encapsulates and delivers therapeutic payloads while influencing the stability of LNPs and interaction with biological environments^{9,19}.

2.1 Structural Dynamics and Programmability of Architecture Domain: pH-Responsive Behavior and Fusion Mechanisms in the Architecture Domain

The structure of LNPs contains two distinct parts: the shell and the core^{29,30}. The shell is a single lipid layer enveloping the LNPs, with its hydrophilic phase facing the exterior to separate the inner and outer environments. The shell component mainly comprises helper lipids, PEGylated lipids, and cholesterol, with only a small portion of CILs³¹. Helper lipids comprise the largest proportion of the LNPs composition and are the primary structural elements. PEGylated lipids are essential for preventing the aggregation of lipid crystalline structures, thereby facilitating the formation of small, discrete nanoparticles. This property is particularly important for maintaining the colloidal stability of LNPs in physiological environments¹⁹. Cholesterol, another vital component, can modulate the fluidity of lipid membranes, improve their resistance to serum-induced degradation³², and facilitate endosome escape²⁹. The core composition of lipid nanoparticles primarily includes CILs¹¹, which have largely replaced traditional permanent cationic lipids in liposomal formulations. The inclusion of CIL has significantly reduced biotoxicity and inflammation¹⁹. Additionally, small amounts of helper lipids and cholesterol are still present in the core³¹.

Recent studies suggest that the core is characterized by lipid mesophases that encapsulate aqueous content, forming complex structures such as inverse micellar, inverse hexagonal, cubic, or lamellar phases³³. The various mesophases that LNPs core can adopt, while not fully understood, are largely governed by the constituent lipids' critical packing parameter (CPP), particularly the CILs and helper lipids³⁴. The CPP, defined as the ratio of the amphiphile's hydrophobic molecular volume to the product of its hydrophobic tail length and effective headgroup area (**Fig. 3a**), serves as a predictive tool for lipid self-assembly behavior. A CPP value close to unity typically results in lamellar phase formation. In contrast, values less than one tend to produce inverse hexagonal phase or cubic phase, where the payload is encapsulated within the aqueous center of these inverse structures^{35–37} (**Fig. 3b**). This relationship between molecular geometry and supramolecular organization underpins the engineering of programmability into the Architecture Domain of LNPs, which allows us to tune the structure by tuning CPP.

The nature of containing CIL leads to the pH responsiveness of the core. Protonation or deprotonation of these CILs can affect the electrostatic forces between their heads, indirectly affecting the CPP, which results in a change in their structure and mesophase³³. For instance, at endosomal pH (4), LNPs demonstrate increased volume and core water content compared to physiological pH (7.4), accompanied by disruption of distinct mult-layer shell structure³⁸. These pH-induced structural transitions are further exemplified by the mesophase shift from micellar to cubic structures with increasing protonation of CILs in follow-up experiments³⁹. Such transitions underpin the inherent programmability without the need to incorporate extra functional components, which are utilized in small molecule release through diffusion and promoting endosomal escape (**Fig. 3c**).

Site-specific delivery of small molecules, such as fluconazole for antifungal therapy⁴⁰, can take advantage of this characteristic. Fluconazole can be released by diffusion through water channels in the cubic phase after reaching the acidic environment⁴⁰. Endosomal escape is a limiting step in LNPs delivery. After cellular intake, the LNPs are encapsulated in the endosome and will be degraded when the endosome develops into a lysosome. Escape is essential as therapeutics such as nucleic acids need to reach the cytosol to perform the effect⁴¹. One primary mechanism of endosome escape is through membrane fusion, where the pH-responsive structural dynamics of LNPs are particularly important⁴². The fusion process is profoundly influenced by the LNP's nanostructure, which can be explained by calculating the energy difference between before and after fusion. The elastic energy can be determined by the Canham-Helfrich formula, $E/A = \frac{\kappa}{2}(J - C_0)^2 + \bar{\kappa}K$, where E is the elastic energy of a membrane and A is the area, κ is the bending rigidity, J is the mean curvature and C_0 is the intrinsic curvature, $\bar{\kappa}$ is the Gaussian curvature modulus, and K is the Gaussian curvature, which is the product of the two principal curvatures. If we calculate the energy before and after two vesicle fusion, we can see $\Delta E_{fusion} = -4\pi\bar{\kappa}$, which depends on the gaussian modulus. Therefore, a positive Gaussian curvature modulus would promote membrane fusion. While most biology membranes have negative $\bar{\kappa}$, cubic mesophase has a positive $\bar{\kappa}$, which aligns with it being stable with a saddle structure with negative Gaussian curvature K . Therefore, compared to laminar or micellar phases, the cubic phase achieves higher fusogenic potential compared to

other phases^{43,44}(**Fig. 3d**). An alternative experiment posits that the insertion of ionizable lipids into the endosome membrane can lead to a hexagonal transformation of the endosome membrane itself⁴⁵, facilitating the escape of the payload from the endosome, which can be possibly explained by altering of $\bar{\kappa}$ of the endosome membrane. This intrinsic dynamic of LNPs reveals the principle behind it and can potentially inspire researchers to leverage this characteristic for other uses and invent more novel endosome escape techniques.

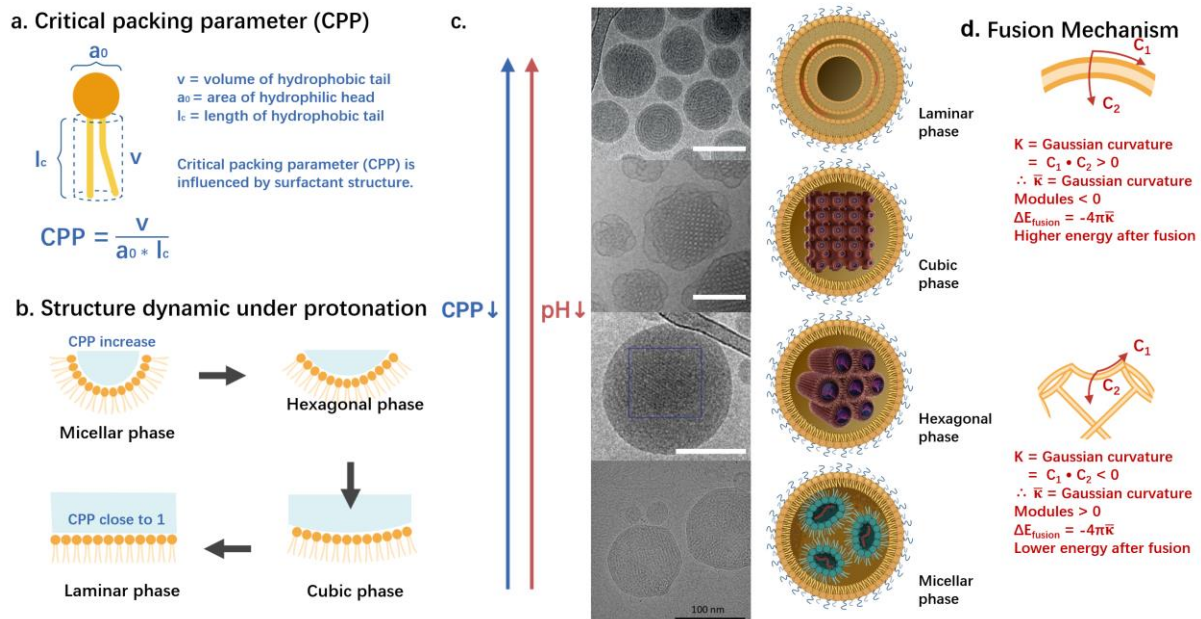


Fig. 2 Structural of LNPs and mechanisms of endosomal escape. **a.** The CPP is a key determinant of the self-assembly structure of surfactants. **b.** A demonstration of how CPP affects the membrane curvature, with blue the hydrophilic phase. **c.** This panel depicts four distinct internal phases of LNPs with Cryo-TEM image: cubic, hexagonal, micellar, and lamellar structures, which CPP and environmental pH levels influence. Changes in CPP and pH drive transitions between these phases, as indicated by the arrows. All scale bars are 100 nm. Cubic and hexagonal phases adapted from ref. ²⁹ with permission. © 2023 The Authors. Published by Elsevier Ltd. **d.** This panel explains how different phases affect the energy during membrane fusion.

2.2 Formulation, Scalability, Stability, and Characterization

The core-shell architecture of LNPs is formulated through advanced formulation techniques that have significantly evolved in recent years^{46,47}. Traditional methods⁴⁸, like thin-film hydration, microemulsion, and solvent injection, have been largely supplanted by more simple and efficient approaches (**Table 1**). Notably, microfluidic technologies have revolutionized the field, offering improved control over particle size, polydispersity, and encapsulation efficiency^{49–51} by leveraging laminar flow for precise mixing at the microscale, which is fundamentally governed by thermodynamic and fluid dynamic principles^{46,52}.

Moreover, the rapid mixing kinetics in microfluidic systems, occurring on millisecond timescales, enable the encapsulation of labile molecules under mild conditions, preserving their integrity. This process involves the rapid mixing of lipids dissolved in an organic phase with an aqueous phase containing nucleic acids, inducing supersaturation and leading to spontaneous nucleation and growth of LNPs encapsulating the payload.

The scalability of LNP production remains a critical concern as these nanoparticles transition from laboratory-scale synthesis to industrial-scale manufacturing for clinical applications. Microfluidic technologies have progressed in addressing this challenge through the principle of parallelization, wherein multiple microfluidic units operate simultaneously to increase production volume while maintaining precise control over particle characteristics achieved at smaller scales⁵³.

During storage, LNPs face potential degradation pathways, including aggregation^{54,55}, lipid oxidation⁵⁶, payload leakage⁵⁷, and payload degradation⁵⁸. The composition of the core is carefully engineered to mitigate these issues¹⁸. PEG chains, typically incorporated as PEG-lipid conjugates, serve as primary stabilizing agents by creating a steric barrier around the LNP⁵⁹. This PEG-mediated stabilization involves both enthalpic and entropic contributions, with hydrated PEG chains forming a localized aqueous phase and configurational entropy resisting compression when particles approach each other. Other components of the core also contribute to storage stability. Cholesterol enhances structural stability, which is particularly important for maintaining LNPs effectiveness³². Lyophilization (freeze-drying) is often employed to enhance the long-term stability of LNPs during storage. However, this process can induce stress on the nanoparticles, potentially leading to aggregation or structural changes. To mitigate the effects of lyophilization, cryoprotectants such as trehalose or sucrose are often added to the formulation to prevent fusion and maintain the structural integrity of LNPs during the freeze-drying process⁶⁰.

The structure of LNPs can be characterized with microscopic and scattering techniques. Cryogenic transmission electron microscopy (cryo-TEM) provides a direct way to visualize, offering high-resolution imaging of LNPs, revealing intricate details of their internal mesophase, organization, and morphology⁶¹. Complementing direct visualization, small-angle X-ray scattering (SAXS)⁶¹ and small-angle neutron scattering (SANS)⁶² provide periodic arrangements of lipid molecules in various mesophases, helping us characterize the LNPs' mesophase. Synchrotron-based SAXS, in particular, offers superior resolution and the capability for time-resolved studies, capturing dynamic structural changes during LNP formation and payload release⁶². Complementing experimental approaches, computational simulation techniques, as summarized in **Table 2**, offer advantages by passing wet lab, enabling atom-level kinetic studying, and potential to be scaled with more computation units (CPUs and GPUs). Generally, developing computational simulation techniques for LNPs aims to optimize their design to predict behavior in physiological conditions while reducing the time and cost of drug development. However, computation power can also be a limiting factor, as complex computation models for simulation, especially when utilizing neural networks⁶³, can require high-end computational units while only simulating hundreds of atoms, which is not directly applicable to LNPs with many more molecules.

3 Interface Domain: Key to Programmability

The Interface Domain represents the boundary between the LNPs and their surrounding environment, which is analogous to the interface in a thermodynamic system. It encompasses the surface properties, such as charge, affinity to plasma proteins, and ligand presentation, which govern the interaction of the LNPs with biological membranes and other cellular components. Comprising the outermost layer of the LNP, this domain includes polyethylene glycol (PEG) coatings, surface functionalization, and various modifications that collectively determine the particle's *in vivo* fate¹⁹.

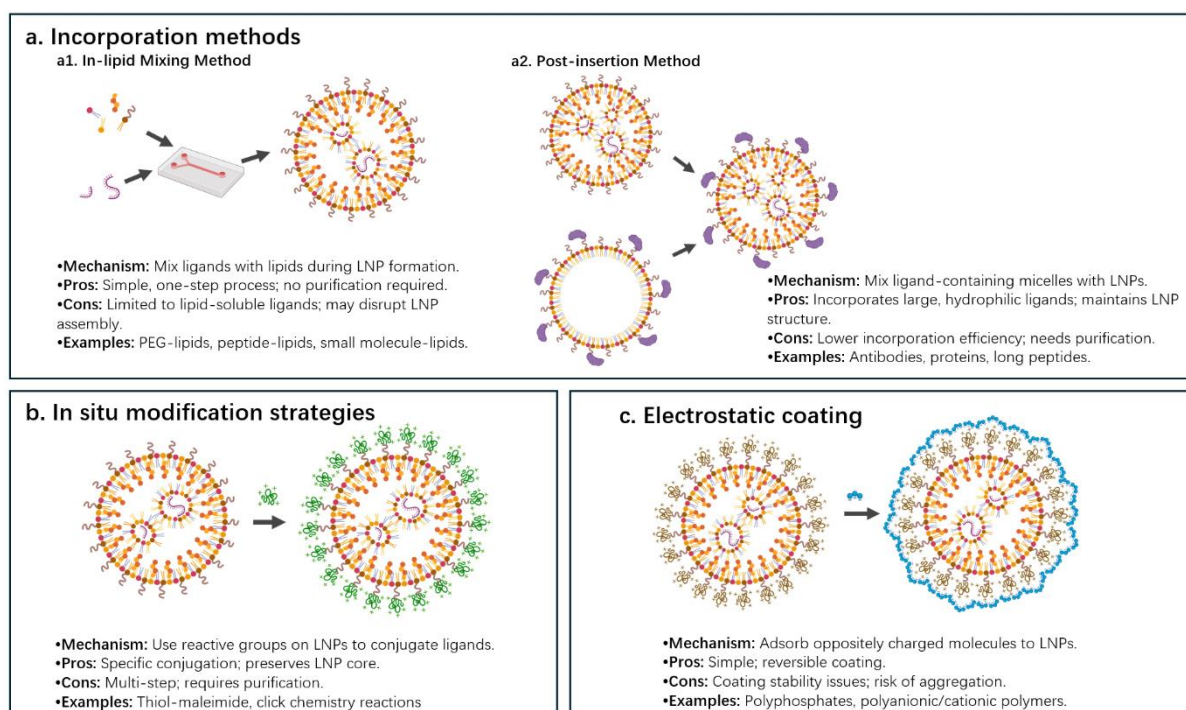


Fig. 3 Surface engineering of the interface domain. **a.** Incorporation methods. Techniques for integrating functional molecules directly into nanoparticles during lipid molecule synthesis. This approach involves pre-synthesizing the nanoparticles with desired molecules to achieve surface engineering. **b.** *in situ* modification strategies. Approaches for modifying nanoparticle surfaces post-synthesis include conjugating targeting ligands or other functional groups via covalent bonds. **c.** Electrostatic coating. Utilizes electrostatic interactions to coat nanoparticles with charged molecules, enhancing stability and providing additional functionalization options. Adapted from ref.⁶⁴ with permission. © 2023 The Author(s). Published by Elsevier B.V.

3.1 Component of Interface Domain and Surface Engineering Technique

The Interface Domain of LNPs can achieve programmability by integrating various kinds of function components on the surface, including small molecules, targeting proteins, nucleic

acid aptamers, functional lipids, and other stimuli-responsive elements. This requires sophisticated engineering of the LNPs interface, necessitated by the complexity of biological systems and the need to control particle-environment interactions precisely. Researchers have developed three primary techniques for integrating functional elements into the Interface Domain to achieve surface engineering. Incorporation of pre-synthesized ligands⁶⁵, in which lipid molecules with modification are synthesized before incorporating on LNPs. The modified lipid molecules can be integrated into LNPs by introducing them during formulation (**Fig. 3a1**) or by mixing micellar with functional lipids with LNPs to incorporate these lipids (**Fig. 3a2**). *in situ* modification strategies⁶⁵ involve modification of the surface of LNPs by forming a covalent bond of the modifier and the surface of LNPs (**Fig. 3b**). Electrostatic coating⁶⁴ utilizes electrostatic interactions to coat nanoparticles with charged molecules, enhancing stability and providing additional functionalization options (**Fig. 3c**). Different approaches can be applied for incorporation and *in situ* methods, which has been extensively covered by previous review⁶⁵.

3.2 Programmability of Interface Domain

Programmability in the Interface Domain of LNPs is achieved through three primary strategies: active targeting, the incorporation of environment-responsive elements, and endogenous targeting (**Fig. 4**).

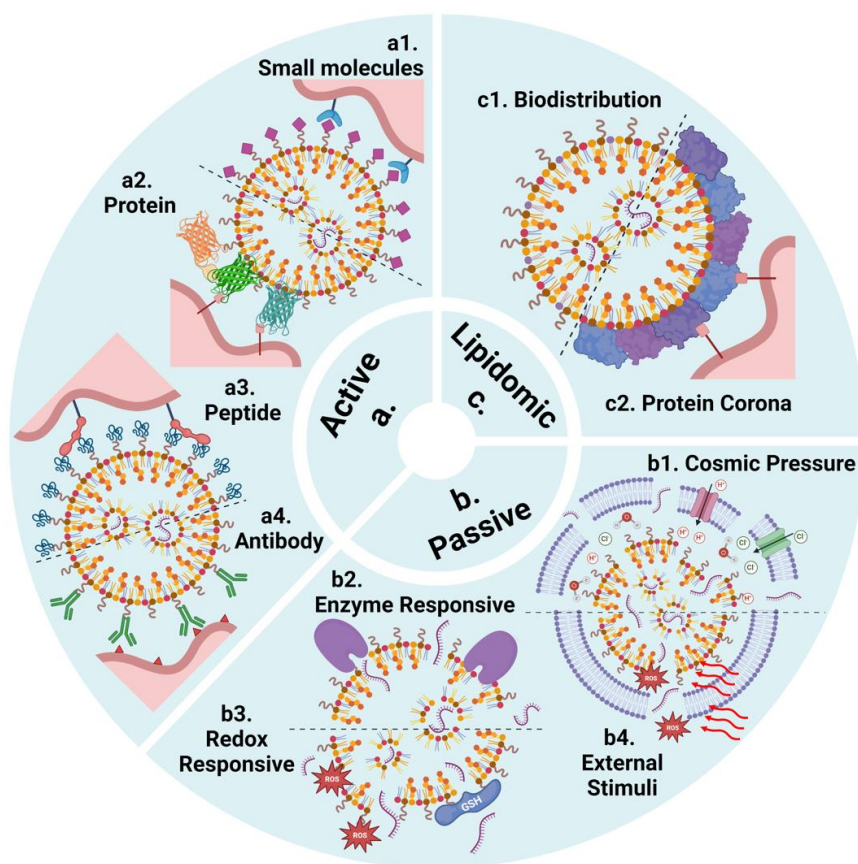


Fig. 4 Strategies for enhancing programmability in the Interface Domain of LNPs. Programmability in the Interface Domain of LNPs is achieved through three primary strategies:

a. Active targeting, which involves integrating targeting molecules such as small molecules, peptides, proteins, and nucleic acid aptamers. b. Passible targeting by incorporating environment-responsive elements to respond to various stimuli such as pH changes, redox potential, and enzymatic activity. c. Lipidomic engineering, which can affect the properties of LNPs and the protein corona formation, can affect the targeting ability.

3.2.1 Active Targeting: Small Molecules, Peptides, Proteins, and Nucleic Acid Aptamers

As the most popular and researched area, the integration of targeting molecules on the LNPs surface capitalizes on the selectivity of biological interactions to guide nanoparticles to specific cell types or tissues. This strategy employs three main categories of targeting ligands: small molecules, peptides and proteins, and nucleic acid aptamers.

Small molecules can be sugar monomers, nucleoside bases, or even vitamins. They target cancer cells or other specific cells with corresponding receptors on the surface. Folic acid, which is a B vitamin, has been integrated into LNPs as many cancer cells contain folate receptors⁶⁶. Similarly, mannose⁶⁷, a sugar monomer, is used to target liver sinusoidal cells with mannose receptors, while adenosine-functionalized LNPs are employed to target astrocyte cells⁶⁸.

Peptide ligands can selectively guide LNPs to cells with higher affinity to the peptide. These cells may naturally interact with certain peptides, enabling us to target healthy cells, such as neurons in the neural retina with MH42 peptides-modified LNPs⁶⁹. Sometimes, the cell is only accessible by the peptide under pathological conditions, therefore allowing targeting malfunctioning cells, such as targeting PD-L1-expressing PTEN-deficient triple-negative breast cancer cells with PD-L1 binding peptides decorated LNPs⁷⁰. Antibody modification provides another approach to directly targeting immune cells, with notable progress of Anti-Ly6c antibody modification for targeted delivery to inflammatory leukocytes⁷¹ and Anti-CD5 antibody modification to T cells⁷².

Nucleic acid aptamers represent a versatile option, combining the specificity of antibodies with easier production. The effectiveness of aptamers was demonstrated by targeting osteoblasts to achieve gene silencing⁷³, although the LBNP involved does not perfectly align with our definition. While limited work utilizes this, recent FDA approval of the second aptamers demonstrates an emerging interest and opportunity in this field⁷⁴. In conclusion, these three approaches highlight the versatility and potential of targeted LNPs systems in advancing precision medicine and targeted therapies.

3.2.2 Functional Components for Stimuli Responsive: pH, Redox, Enzymatic, and Light-Triggered Components

Environment-responsive elements incorporated into the Interface Domain enable LNPs to respond dynamically to their surroundings, facilitating controlled payload release. Various

stimuli, including pH changes, redox potential, enzymatic activity, and light irradiation, can trigger the responsiveness of these elements.

pH-sensitive element functionalized LNPs focusing on resolving problems related to endosome escape, on top of the programmability in the Architectural Domain introduced earlier. Three primary mechanisms exist: enhancing interaction with the negatively charged membrane after gaining a positive charge in acidic pH, enhancing endosomal escape and cytosolic delivery via the proton sponge effect, and, in a recent study, applying mechanical force to the endosome membrane.

The first approach involves utilizing the pH-activated lipid-like material ssPalm, incorporating vitamin E scaffolds, which destabilizes membranes at low pH through the protonation of tertiary amines²⁰. PGlu(DET-Car)30 LNPs, having similar principles, present an active charged surface that interacts with negatively charged membranes in response to pH changes⁷⁵. The proton sponge effect can be achieved by integrating pH buffers, such as using imidazole-based lipids such as O12-D3-I3, which exhibit a buffering effect that induces swelling and enhances endosomal escape⁷⁶. It is also possible to leverage both mechanisms to promote endosome escape, demonstrated by a recently developed acid-degradable anionic lipid LNPs that will undergo hydrolysis of azido-acetal linker under acidic conditions in the endosome and increase the osmolarity while shifting to a positive charge to promote fusion with endosome membrane⁷⁷. The third approach to enhancing endosome escape involves incorporating “nanomachine” lipid components capable of rotation and mechanical transition under a low pH environment, which disrupts the endosome membrane⁷⁸.

Redox-responsive elements, including disulfide bonds and thioketal linkages, usually target tumor tissues by exploiting the reducing microenvironment to initiate payload release. Disulfide bonds, commonly used in biologics and subject to specific use case approvals, are cleaved by glutathione (GSH) within the hydrophobic tails of the lipids, leading to truncation and disintegration of the LNPs structure⁷⁹. More advanced designs, such as the BAmP-TK-12 ionizable lipid with a thioketal linkage, respond to elevated reactive oxygen species (ROS) levels in tumor cells⁸⁰, where the oxidation and cleavage of the thioketal linkage destabilize the LNPs, triggering payload release. DDA-SS-DMA ionizable lipid contains disulfide bonds that are cleaved in the high GSH environment of tumors, causing nanoparticle disassembly and controlled release of the therapeutic cargo⁸¹.

Enzymatically cleavable linkers are incorporated to treat specific enzymes present locally as input to control the cellular intake or payload release. Existing systems generally go through alterations in the surface charge as enzyme cleavage will remove old groups and induce new groups with different charges. For instance, decationization can be achieved by the corporation of esterase-labile quaternium lipidoid (AMP-POC18)⁸², which remain permanently charged until exposed to esterase. Polyphosphate (TPP) incorporated LNPs, which undergo charge reversal triggered by alkaline phosphatase (ALP), can enhance cellular uptake and transfection efficiency at the target site⁶⁴. This change of ionization can also lead to mesophase changes. Phospholipid D (PLD) LNPs with initially cubic structure changed to hexagonal phase after being cleaved by PLD enzymes²³.

Light-responsive elements, such as second near-infrared (NIR-II) dye-conjugated lipids and porphyrin-lipids, offer externally controlled, spatiotemporally precise activation of therapeutic functions through photothermal and ROS-generating mechanisms. NIR-II dye conjugated lipids (Cy-lipids) exhibit increased NIR-II absorption upon protonation in acidic conditions, facilitating photothermal endosomal escape⁸³. Similarly, porphyrin-lipids generate ROS upon near-infrared (NIR) light irradiation, disrupting endosomal membranes and promoting payload release⁸⁴. With all these different modalities that take different input stimuli and actuate precise release, we can utilize external stimuli to expand the scope of programmability and potentially achieve intelligence beyond the natural-system level.

3.2.3 Lipidomic Engineering of LNPs: Tailoring Physicochemical Properties and Biodistribution for Enhanced Precision in mRNA Delivery.

By adjusting the ratios and types of lipids in the formulation, researchers can modulate the LNP's surface solubility, surface charge, and surface affinity, allowing for fine-tuning the LNP's pharmacokinetics and therapeutic efficacy¹⁸. The proportion of saturated, unsaturated, and cholesterol lipids can contribute to surface fluidity, affecting the stability of LNPs, and affecting processes such as endosomal escape. Recent insights into lipid saturation reveal that unsaturated lipids enhance mRNA transfection efficiency due to increased fluidity, decreased phase transition temperature, and enhanced fusogenic properties⁸⁵. The surface charge at the physiological condition is determined by the proportion of permanent cationic lipids and ionizable lipids and their pK value. Tuning of surface charge can lead to organ-targeted delivery by affecting their interaction with the immune system in different organs. For instance, negative-charged LNPs are usually used to target the spleen, while positive-charged LNPs can target the lung.

Notable advancements in the tuning of surface affinity include the development of Selective Organ Targeting (SORT) technology, which leverages specific SORT molecules to dramatically alter biodistribution for precise organ targeting⁸⁶. These SORT molecules, which can be permanently cationic (e.g., DOTAP), anionic (e.g., 18PA), or ionizable cationic lipids (e.g., DODAP), dramatically alter the biodistribution of LNPs, enabling precise targeting to organs such as the lungs, spleen, or liver. The type and percentage of SORT molecule used can be finely tuned to achieve the desired tissue tropism, representing a powerful new tool in the lipid-based nanoparticle arsenal. While efficient incorporation of SORT molecules has been a limiting factor, a novel synthesis method of amidine-incorporated degradable (AID) lipids via a streamlined one-pot tandem multi-component reaction (T-MCR) addresses synthesis challenges⁸⁷.

Moreover, reassessing traditional LNPs components has shown that helper lipids and cholesterol, once deemed essential, may not be mandatory for LNPs functionality. This paradigm shift is exemplified by the finding that cholesterol removal can address the persistent challenge of preventing nanoparticle accumulation in hepatic tissues, which can be explained by tuning the affinity to reduced lipoprotein adsorption⁸⁸. By modulating and simplifying intrinsic LNPs components, researchers have achieved concurrent mRNA accumulation and translation in targeted organs like the lung and liver. This innovative targeting strategy shows

promise for enhancing the precision of mRNA therapies across diverse diseases and may apply to existing LBNPs systems. However, it is important to note that the optimal LNPs formulation likely depends on the specific therapeutic target and delivery requirements, and traditional components may still play crucial roles in various applications. These advancements collectively represent a significant step forward in the development of more precisely targeted and efficient mRNA delivery systems, potentially expanding the therapeutic potential of mRNA-based treatments.

3.2.4 Multiple Input Programmability: Leveraging Modularity for LNPs Design

The forthcoming discussion illuminates the transformative potential of LNPs that incorporate multiple responsive elements. LNPs can process complex environmental cues and execute sophisticated, preprogrammed behaviors by incorporating pH-sensitive, redox-responsive, enzymatically cleavable, and light-triggered elements. Works already exist that process more than one input, such as incorporating pH-sensitive NIR-II dye, whose absorption at 1055 nm increased 7.8-fold in acidic conditions (pH 5.0) compared to pH 7.4⁸³, to achieve photo-thermal effect only at endosome, where low pH exists. It is also to integrate redox-responsive disulfide bonds with pH-activate ssPalm that enhance endosome escape, maximizing therapeutic effects only at tumor sites²⁰.

In conclusion, the Interface Domain of LNPs represents a dynamic and highly tunable platform for achieving programmable drug delivery. By leveraging advanced engineering techniques and materials, researchers are creating increasingly sophisticated nanocarriers capable of navigating complex biological barriers and responding intelligently to their environment. As our understanding of the interplay between LNPs interfaces and biological systems deepens, we can anticipate developing next-generation programmable LNPs with higher intelligence and close to natural biology systems.

4 Payload Domain: Extending Beyond Therapeutic Effects

The Payload Domain corresponds to the therapeutic agent encapsulated within the core of Architecture Domain, akin to the mass in a thermodynamic system. This domain focuses on encapsulated payloads, their clinical application, and their programmable aspects. The encapsulated payloads accommodate diverse therapeutic agents, encompassing both hydrophilic and hydrophobic molecules. Hydrophilic components, typically encapsulated within the aqueous phase, interact electrostatically with the cationic headgroups of the lipids²⁹. In contrast, hydrophobic payloads are incorporated within the lipid structure itself, partitioning into the hydrophobic regions of the LNP⁸⁹. This dual capacity for payload incorporation allows LNPs to serve as versatile carriers for various therapeutic agents, from small-molecule drugs to macromolecules such as nucleic acids and proteins (**Table 3**).

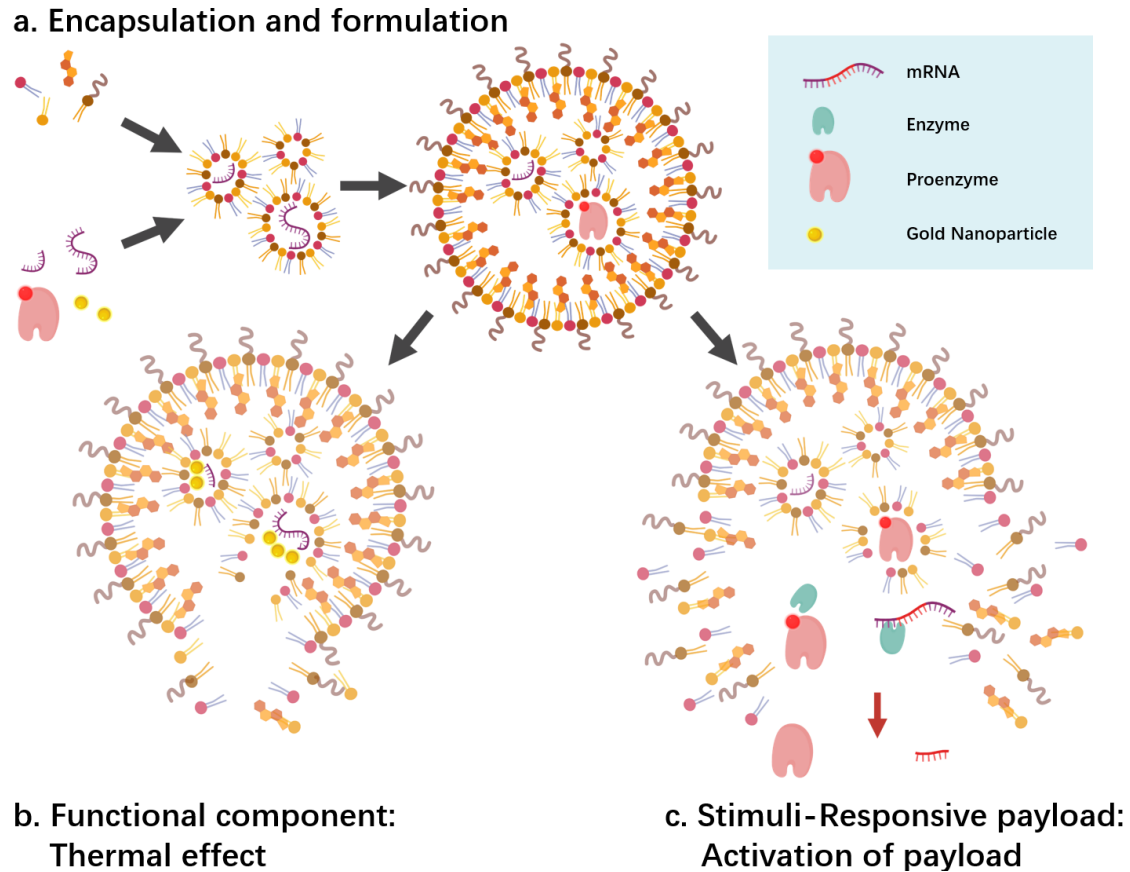


Fig. 5 Programmability of LNPs utilizing the Payload Domain. **a.** The programmability of LNPs can be achieved by encapsulating responsive payloads that react to specific cellular or environmental stimuli. Two primary approaches are highlighted: **b.** Incorporating stimuli-responsive components like gold nanoparticles or photosensitive molecules that trigger payload release upon external stimuli. **c.** Encapsulating enzyme-responsive therapeutic agents that activate selectively in specific intracellular environments. These strategies enhance the precision and efficacy of LNP-based drug delivery systems.

4.1 Therapeutic Agent

Contemporary LNP payloads encompass a range of therapeutic agents, each with specific functions and applications. mRNA^{13,90} directly delivers genetic instructions for antigen or protein production to cells, finding applications in vaccines, protein replacement therapy, and cancer immunotherapy. saRNA^{91,92} allows for intracellular replication and prolonged antigen expression, potentially reducing vaccine doses while enhancing immune response duration. DNA enables sustained antigen production through nuclear transcription and cytoplasmic translation, improving the immunogenicity of DNA vaccines. siRNA^{93–95} facilitates gene silencing by downregulating specific genes, with applications in cancer therapy and treating genetic disorders such as hereditary transthyretin-mediated amyloidosis. miRNA^{96–98} modulates gene expression by simultaneously targeting multiple genes, offering potential in cancer therapy and treating complex genetic disorders. CRISPR-Cas9^{99,100} components enable

direct correction of genetic mutations, opening avenues for gene therapy across various genetic conditions. Beyond nucleic acids, LNPs can encapsulate traditional small-molecule drugs with enhanced delivery properties. Chemotherapeutic agents like doxorubicin and paclitaxel benefit from improved solubility, circulation time, and tumor accumulation when delivered via LNPs^{101,102}. Antifungal drugs like fluconazole exhibit enhanced delivery and efficacy when formulated in LNPs, improving the treatment of systemic fungal infections⁴⁰.

4.2 “Debugging” of Programmable LNPs

In programming, achieving accuracy on the first attempt is often challenging. In computer programming, we can always use the ‘print()’ function to track the change of a particular variable in the terminal. In the context of programmable LNPs, the payload is the ‘print()’ function, and various imaging techniques will be the terminal. The payload can be engineered to help access the pharmacokinetics (PK) and the pharmacodynamics (PD) of the LNPs, particularly in the context of absorption and distribution.

PK is the study of how the body affects LNPs after administration, and the Payload Domain can be used to access the absorption and distribution of LNPs. Absorption is how the LNPs reach from the administration site into the bloodstream, which can be accessed by analyzing the content in the blood. This can be achieved by detecting the payload mRNA concentration. This can also be performed by introducing radioactive isotopes such as ³H to track the presence in blood¹⁰³. Distribution is how LNPs spread throughout the body and how they behave during cellular intake, and this intricate process can be tracked by integrating fluorophores. Imaging techniques such as fluorescence imaging and bioluminescence imaging can be used to estimate this process, even in a real-time manner¹⁰⁴. Formulation of protein corona is also an important process during distribution. However, accessing protein corona is challenging due to the difficulty of separating LNPs from unbounded plasma proteins. The payload can be tailored to solve this problem by integrating superparamagnetic iron oxide nanoparticles (IONPs) in their core and separating them with the magnetic separation method¹⁰⁵.

PD is the study of the effects of the payload of LNPs after delivery, including the subsequent protein expression and biological effects. This can be studied by using a payload that leads to the expression of bioluminescent proteins, such as luciferase, to measure the expression level of proteins in different conditions to model the link between LNPs design and their PD¹⁰³.

4.3 Programmability of the Payload Domain: Tailoring Therapeutic Activation through External and Intracellular Stimuli

The versatility of payload encapsulation in LNPs has unveiled new horizons for programmability by integrating inherently responsive payloads designed to react to specific cellular or environmental stimuli (**Fig. 5a**). Programmability in the Payload Domain of LNPs can be achieved through two primary approaches. The first approach involves encapsulating stimuli-responsive functional components, such as gold nanoparticles or photosensitive molecules, within the LNPs structure (**Fig. 5b**). These components act as nanoscale transducers, converting external stimuli like light or heat into localized physical or chemical changes, triggering payload release or activation through energy transduction mechanisms¹⁰⁶. However,

the encapsulated interactions between functional components and therapeutic agents should be carefully examined. The second approach focuses on encapsulating enzyme-responsive therapeutic agents, which are activated selectively in specific intracellular environments (**Fig. 5c**). For instance, NQO1-responsive proenzymes represent an innovative strategy wherein the therapeutic payload is activated exclusively by enzymes present in the tumor cell cytosol, thereby achieving a high degree of specificity in anti-cancer treatments²². Similarly, another work designed IL-2F, a construct comprising interleukin-2 (IL-2), a cleavable linker, and CD25, as the payload¹⁰⁷. This payload is engineered to be cleaved by matrix metalloproteinases (MMPs) prevalent in the tumor microenvironment, resulting in the selective release of active IL-2 within cancer tissues. These approaches achieve programming of LNPs with certain intracellular environments as input stimuli while being completed solely by incorporating stimuli responsive payloads. This marks the potential for complex programmable LNPs.

5 Dispersal Domain: Administration, Extracellular, and Intracellular Trafficking

The Dispersal Domain is the external milieu that interacts with the system and influences its behavior through cross-border exchanges of matter and energy, similar to the surroundings of a thermodynamic system. Similar to the dispersal of mass in a thermodynamic system, the Dispersal Domain is concerned with the lifetime of LNPs *in vivo*. This domain involves the administration route of LNPs as well as the post-injection dynamics of LNPs.

5.1 Administration Route and Extracellular Trafficking of LNPs

The administration route of LNPs determines their site of action, biodistribution, and subsequent biological response. This route influences not only the physiological milieu in which the LNPs are introduced but also the therapeutic efficacy and safety profile of the delivered payload. By selecting and understanding the administration routes, researchers can precisely identify the requisite stimuli for programmable LNPs, thereby optimizing their function to achieve targeted therapeutic delivery. This strategic approach ensures that the LNPs interact effectively with the intended biological targets, minimizing off-target effects and enhancing overall therapeutic outcomes (**Fig. 6**).

5.1.1 Parenteral Route

The parenteral route involves the direct injection of LNPs into the body, bypassing the gastrointestinal tract. This approach, the most widely used method, can be classified into several subcategories based on the site of administration, each with distinct characteristics and implications for LNPs' biodistribution, therapeutic efficacy, and patient experience. The intramuscular (IM) route is particularly favored within this category, especially for SARS-CoV-2 mRNA vaccines. This method introduces local immune responses by delivering the vaccine directly into muscle tissue. In this case, prolonged retention at the injection site will exist¹⁰⁸, and the immune response will be triggered at the lymphatic nodes, as LNPs travel through the lymphatic system without being affected by anti-PEG Antibodies¹⁰⁹. A recent study with a mouse model investigating the dynamic process between the IM route and the intravenous (IV) route observed that after the IM administration, partial LNP was taken up by muscle cells right after administration and movement of LNPs into the lymphatic system, and

bloodstream afterward. The LNPs are transported to the liver within 3 hours of injection, and the protein expression was observed within 1 hour and 3 hours at the injection site and the liver, respectively¹¹⁰.

The IV route is achieved by directly injecting LNPs into the circulation system, achieving the highest bioactivity¹¹¹. It is widely studied and used by Doxil and Onpattro, with different pharmacokinetics compared to the IM route. After injection, LNPs rapidly accumulate in the liver, as found in the same comparison study¹¹⁰. The protein expression was detectable after 1 hour from the liver. It peaked at 3 hours while substantially reducing after 3 hours, indicating a more rapid therapeutic effect, which might result from the direct administration without retention as in the IM route. This characteristic makes the IV route suitable for applications that need rapid and short response, with a higher need for drug concentration.

Subcutaneous (SC) injections are injecting drugs between the skin and muscle, which do not require special personnel and can be completed by patients. Furthermore, SC injections are less painful, have a lower infection rate, and, in rare cases, lead to systemic infection¹¹². Similar to IM injection, SC also requires an absorption process after administration. A comparison between SC injection and IV injection demonstrates that while 97.6% of the protein was expressed in the liver hepatocytes in IV injection, around 99% of expression was in the local injection site in the case of SC injection, with predominate expression in adipocytes, supplemented by fibroblasts and macrophages. The plasma concentration for the express protein is 20-fold lower for SC injection than IV injection, while it has a more prolonged effect when applied at the same dose¹¹³. Therefore, it is critical to consider the pharmacokinetic profile when designing LNPs that potentially require self-administration.

Intradermal (ID) injections are defined as the administration of a drug into the dermis, which is the layer just below the outmost layer, with a slight needle angle. While not commonly used in LNPs administration, it is getting attention as the skin is easily accessible while immune competent¹¹⁴. After ID injection, the expression can be observed at the injection site and near the lymph node in a mouse experiment, which can stem from the traveling of either transfected antigen-presenting cells (APCs) or LNPs¹¹⁵.

Specialized forms of parenteral administration have also been investigated in LNPs delivery. The intracerebral injection has been developed for direct delivery to the brain¹¹⁶, achieving local transfection and bypassing the blood-brain barrier (BBB) while requiring a highly invasive procedure with associated risks. Local transfection in the eye has also been demonstrated via subretinal injection in mice¹¹⁷. The intracerebroventricular injection is another type of parenteral administration where LNPs are delivered into the cerebrospinal fluid (CSF) within the brain's ventricles. After injection into the lateral ventricles of a mouse model, LNPs or injected substances can spread out with CSF flow, which will then later penetrate into brain tissue. However, LNPs are blocked away from the brain tissue. They are primarily expressed in lining cells of the ventricles and blood vessels, resulting in a lower in vivo transfection rate compared to free oligonucleotides¹¹⁸. While this is a promising route, it would require future optimization on the LNPs. A novel approach, the needle-free (NF) injection, is also explored. This method has several advantages, including addressing needle phobia,

reducing reliance on trained professionals, improved LNPs distribution, and the versatility to select between IM or SC injection¹¹⁹. The injected LNPs remain integrated after NF injection while covering a larger area with a higher antibody response than needle-injected IM injection in a rabbit model¹¹⁹. However, a real-time trafficking analysis is necessary to further understand the kinetic after NF injection.

5.1.2 Topical Route

The topical route refers to applying substances directly onto the skin or mucous membranes to achieve a therapeutic effect, which can include eye drops, ear drops, and lotions. This method is preferred if we aim to deliver to a target site non-invasively, especially at the open wound where LNPs can pass through the skin barrier. For instance, applying LNPs encapsulating locked nucleic acids within hydrogels on the burnt wound enhances the expression of proteins essential for skin barrier function. It accelerates wound healing, with repeated administration possible¹²⁰. Eye drops are also a promising administration route due to their non-invasive nature and ease of use. However, the limited efficacy of the tear fluid and ocular barriers prevents this approach for LNPs application¹²¹. Previous investigations of eye drop with liposomes show that smaller liposomes are easier to travel to the retinal pigment epithelium 5 minutes after eye drop administration, potentially through choroidal vessels¹²². Despite the process, direct trafficking of liposomes may be needed to understand the pharmacokinetics of LBNP administration with eyedrops.

Pulmonary delivery can also be seen as a form of the topical route, which involves delivering LNPs to the surface of pulmonary mucosa via inhalation. This approach achieves non-invasive targeted delivery to the lung and reduces off-target effects in other organs. While challenges such as stabilization of LNPs during nebulization and penetration exist, recent advances have explored the role of PEGylated lipids in addressing these issues^{123,124}. Increased stability, enhanced mucus penetration, and elevated alveoli and respiratory bronchioles expression levels have been observed. Moreover, sustained protein production is achieved through repeated administration¹²³. Similar to the pulmonary route, the intranasal route involves administration into the nasal cavity and aims to target the nasal mucosa and the upper respiratory track. Similar to the pulmonary route, LNPs need to travel through nasal mucosal for effective delivery, and this process is demonstrated to be facilitated by the incorporation of extra cationic lipids in LNPs¹²⁵. After intranasal administration, LNPs are taken up by the nasal epithelium and the dendritic cells and macrophages in the nasal mucosa¹²⁵. In an example using SARS-CoV-2 spike protein mRNA LNPs, the spike protein can be produced locally and drained to lymph nodes for presentation and induce immune response¹²⁵.

5.1.3 Enteral Route

The enteral route is medication delivery through oral ingestion into the gastrointestinal (GI) tract. Oral drug delivery is a common and convenient method, especially when delivering therapeutic agents targeting the GI tract. However, harsh environments in the GI tract, including steep pH change, pepsin, bile salts, mucin, mucosa, and the enterocytes barrier at the intestine, present significant challenges to delivering LNPs effectively¹²⁶. Screening through different formulations can aid in the design of LNPs that are capable of oral delivery. However,

existing work primarily focuses on LBPs without ionizable lipid compounds, as the pH responsiveness can be a disadvantage when encountering complex pH conditions in the GI tract. One siRNA-LBPs formed using 306O₁₃ lipidoid¹²⁷, which has three fatty acid chains, was successfully taken up at the intestine and colon cells. Despite the successful intake, no significant gene silencing was observed¹²⁶. Natural plant-based lipid is also explored in the context of oral delivery, providing higher stability in GI tract while potentially offering higher biocompatibility due to the nature of the lipid, with which several medicines treating ulcerative colitis (UC) are developed. LBPs can be formulated with lipids derived from ginger nanoparticles, which maintain stability in the GI tract and effectively target the colon. They can deliver mRNA to the target site and promote healing during UC¹²⁸. Natural lipids extracted from mulberry leaves are also used in LBPs formulation, and significant gene silencing after delivery of LBPs with the CRISPER Cas9 system¹²⁹ was observed for UC. An enzyme-responsive system is also developed by utilizing esterase presence in the UC area, and drug release is triggered by the cleavage of chitosan by esterase at the target site¹³⁰.

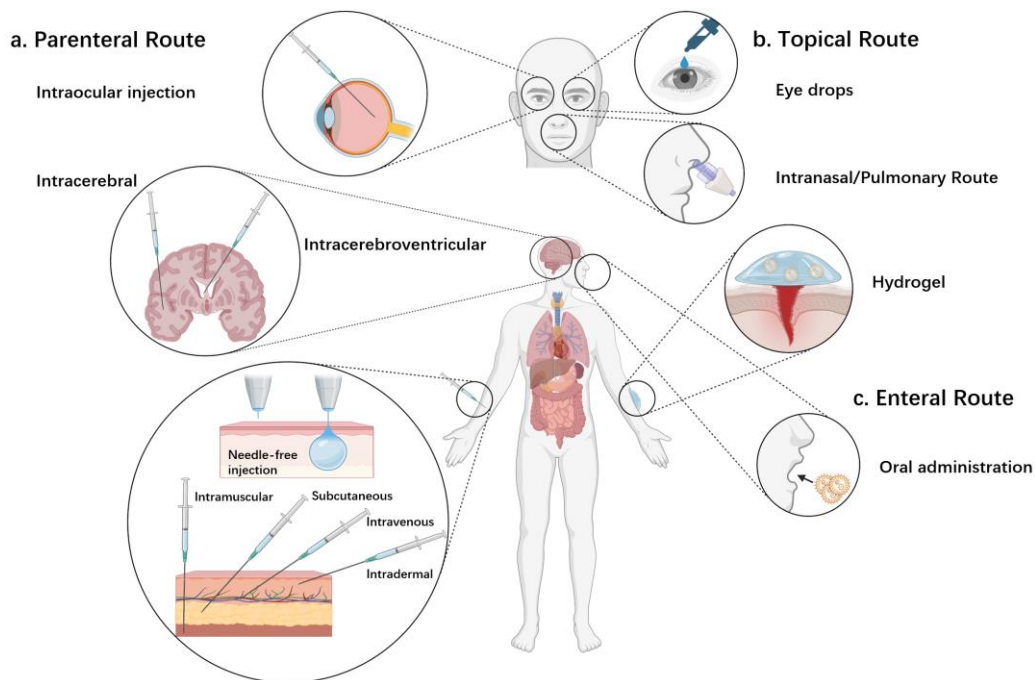


Fig. 6 Dispersal Domain: Administration Route for LBPs. **a.** Parenteral Route involves injection with a needle. **b.** Topical Route refers to applying drugs through the surface of the skin or mucosa. **c.** Enteral Route refers to oral intake in the context of LBPs.

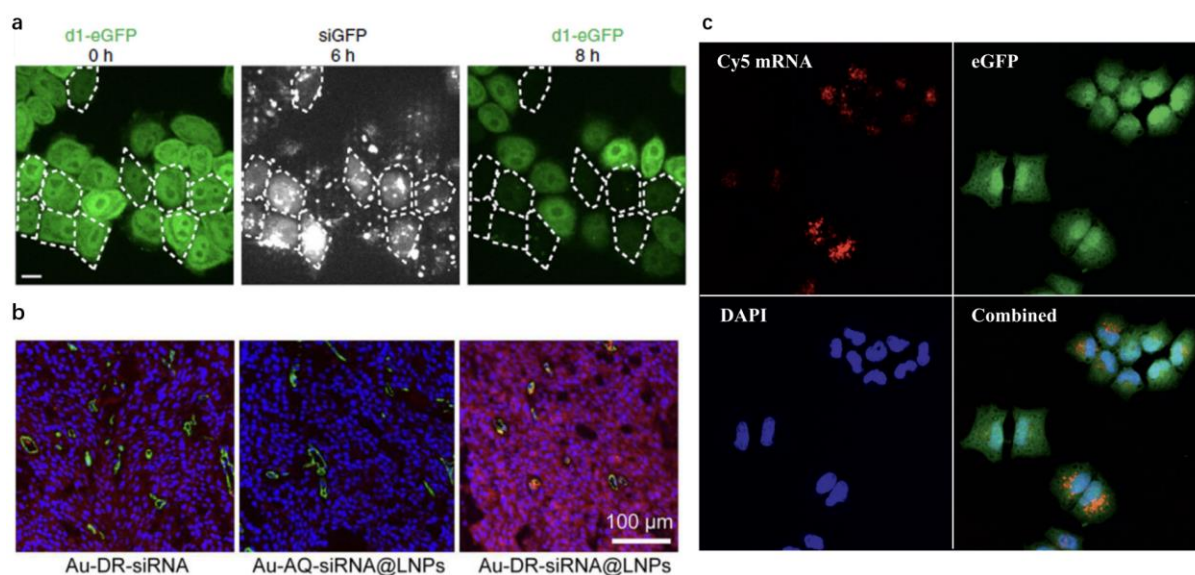


Fig. 7 *In vivo* dynamics of LNPs. **a.** Time-lapse fluorescence microscopy of HeLa-d1-eGFP cells treated with siGFP-Cy3 lipoplexes. Images show eGFP fluorescence at the start ($t = 0$ hours, left) and end ($t = 8$ hours, right) of the experiment. The middle panel outlines cells exhibiting cytosolic release events of siGFP-Cy3 within the first 6 hours, demonstrating the kinetics of siRNA delivery and target gene silencing. Scale bar: 10 μm . Adapted from ref¹³¹ with permission. © 2024 Springer Nature Limited. **b.** Confocal laser scanning microscopy (CLSM) images of tumor tissue sections after treatment with LNP-encapsulated Cy5-labeled siRNA. Red fluorescence indicates the presence and localization of released siRNA within tumor cells. This visualization demonstrates the tumor-targeting capability of LNPs and the intracellular release of functional siRNA, which transitions from a quenched to an active state upon Dicer-mediated cleavage. Adapted from ref¹⁰⁴ with permission. © 2022 Shenyang Pharmaceutical University. Published by Elsevier B.V. **c.** Schematic representation of intercellular LNP-mRNA delivery via exosomes. Cells treated with LNPs containing Cy5-eGFP mRNA secrete exosomes containing the delivered mRNA, potentially facilitating its spread to neighboring cells. This mechanism suggests a novel mode of LNP-mediated nucleic acid dissemination beyond direct cellular uptake. Adapted from ref¹³² with permission. © 1999-2024 John Wiley & Sons, Inc or related companies.

5.2 Intracellular Trajectory of LNPs: Navigating Endosomal Pathways and Cytosolic Delivery for Enhanced Therapeutic Efficacy

Once LNPs reach their target cells, their intracellular journey begins, marking a critical phase in the delivery of therapeutic payloads. The process of cellular uptake, typically via endocytosis, initiates a complex series of intracellular events that ultimately determine the fate and efficacy of the encapsulated drug. Recent studies have provided detailed insights into the kinetics of this uptake process. For instance, in HTB-177 cells, approximately 80% of radiolabeled LNPs disappeared from cell culture supernatants within 5 hours, indicating rapid cellular internalization¹³². Moreover, flow cytometry analysis revealed that over 90% of cells

were positive for Cy5-mRNA within 2 hours of LNPs administration, demonstrating the efficiency of LNP-mediated mRNA delivery.

Upon internalization, LNPs are initially contained within early endosomes (EE). These vesicles represent the first sorting station in the endocytic pathway. While the mildly acidic environment (pH ~6.5) of early endosomes does trigger some degree of change in LNPs, the main stage for endosome escape occurs in the subsequent phase. As trafficking progresses, early endosomes mature into late endosomes (LE), characterized by a further drop in pH (to ~5.5) and the accumulation of specific protein markers. This increasingly acidic environment poses challenges for payload stability but also offers opportunities for triggered release mechanisms. The “proton sponge” effect, combined with the phase transition of LNPs mediated by ionizable lipids, becomes increasingly significant within late endosomes, enhancing endosomal escape, which has been visualized via fluorescence microscopy¹³¹ (**Fig. 7a**).

The cytosol represents the ultimate destination for many LNPs payloads, particularly for nucleic acid therapeutics that exert their effects in this compartment. The dynamics of intracellular drug release from LNPs are complex and can vary depending on the specific payload and LNPs design. For nucleic acid cargoes, dissociating the payload from its lipid carrier in the cytosol is crucial for therapeutic activity. This process is influenced by factors such as the strength of electrostatic interactions between the payload and cationic lipids, the presence of cytosolic proteins that may compete for binding, and the stability of the payload itself. Recent research has provided quantitative insights into the efficiency of this process. In HTB-177 cells, it was observed that from 6.5×10^{12} VEGF-A mRNA copies delivered via LNPs, approximately 1×10^{12} VEGF-A protein copies were detected after 24 hours¹³². This translates to about 0.15 copies of VEGF-A protein produced per mRNA copy, highlighting both the potential and current limitations of LNP-mediated mRNA delivery and translation. The real-time dissociation of siRNA from LNPs in the cytosol was visualized by FRET techniques, providing valuable insights into the kinetics of this process and informing the design of more efficient delivery systems¹⁰⁴ (**Fig. 7b**). Interestingly, a fraction of internalized LNP-delivered mRNA can be packaged into exosomes and secreted, potentially extending the functional reach of the therapeutic mRNA beyond the initially targeted cells^{41,132} (**Fig. 7c**). The secreted exosomes containing the therapeutic mRNA can then be taken up by other cells, as demonstrated by the delivery of Cy5-eGFP mRNA via exosomes to HTB-177 cells, resulting in eGFP protein production¹³².

In conclusion, the dispersal domain of LNPs encompasses a complex journey from initial administration to intracellular payload delivery. Recent advances in LNPs design, imaging techniques, and mechanistic studies have significantly enhanced our understanding and control over LNPs behavior *in vivo*. The detailed kinetics of LNPs uptake, intracellular processing, and the newly discovered exosomes-mediated redistribution of mRNA add layers of complexity to this process. As we continue to unravel the intricacies of LNPs dispersal dynamics, we move closer to realizing the full potential of these versatile nanocarriers to achieve biology-level intelligence.

6 How can Artificial LNPs inspired by (Active, bioinspired) Natural Systems

As we move toward more sophisticated drug delivery systems, it is crucial to recognize the parallels between artificial LNPs and natural exosomes. Here, we demonstrated the potential of our Four Domain Model—Architecture, Interface, Payload, and Dispersal—to provide a structured framework that not only elucidates the thermodynamic behavior of LNPs but also serves as a roadmap for future innovations.

Regarding the Architecture Domain, exosomes hold a lipid layer membrane, which is more similar to liposomes compared to LNPs, with size variation from 40 – 160 nm in diameter, comparable to LNPs¹³³. Lipid composition differs from that of LNPs, emphasizing the inclusion of Phosphatidylserine (PtdSer)¹³⁴, which can be used for detection and diagnosis. For instance, PtdSer might be helpful for early cancer detection in mice¹³³.

As for the Interface Domain, unlike LNPs, surface proteins are usually observed in exosomes, and their roles include activating antitumor response¹³⁵, cell targeting¹³⁶, adhesion¹³⁷, and signaling¹³⁸, antigen presentation¹³⁹, protection from phagocytosis¹⁴⁰, and enzymes that modulate the biology process¹⁴¹. In contrast, the surface of LNPs, while potentially able to incorporate a broader category of substances, has much fewer kinds of bioreactivity compared to exosomes and mainly focuses on targeted release. While potentially safer, this also indicates that there is still much room for the programmable LNP intelligence to grow.

Another huge gap between LNPs and exosomes is in the Payload Domain. While current payloads in artificial LNPs demonstrate various therapeutic effects and exhibit programmable features (**Table 3**), extending the payload domain by examining natural exosome systems offers valuable insights for enhancing LNP versatility and efficacy. Protein is an important payload encapsulated in exosomes, while less is the case in LNPs. These proteins can be transcription factors¹⁴² or histones¹⁴³ that regulate the gene expression and DNA packaging in the cell, which can be a promising approach adapted by LNPs. However, encapsulating proteins in LNPs is not as straightforward as encapsulating negatively charged nucleic acids, and advances in this field are required. Metabolites small molecules such as amino acids, nucleotides, and citric cycle intermediates also exist in exosomes, potentially affecting metabolism in the recipient cell¹³³. Encapsulation of small molecules in LNPs, while it can lead to promising applications, can be challenging as LNPs can act as semi-closed systems and have difficulty keeping small molecules from diffusing without additional molecular-level forces.

The Dispersal Domains for the two systems are also different due to their different origin and whether they are required to be administered. However, as they mainly function in biological environments, comparing these two systems is reasonable and will give important insights into intelligent systems in the body. In conclusion, LNPs are different from exosomes in all four domains, demonstrating the effectiveness of understanding LNPs and similar systems. These insightful comparisons are expected to significantly advance the field of programmable drug delivery systems.

7 Ethical Considerations

The development of LNPs has prompted ethical considerations that must be addressed to ensure responsible innovation and clinical application. A primary concern is the safety of these systems, which can be affected by the complex and potentially hidden side effects of lipid and nucleic acid components, as highlighted by the SARS-CoV-2 mRNA vaccines. Additionally, the evolving understanding of in vivo effects of nucleic acids and other therapeutic agents, especially in the context of sophisticated programmable LNPs, requires a balance between development speed and safety.

Predictability is another major concern as programmable LNPs integrate diverse functional components and complex therapeutic mechanisms. This increases the complexity of interaction with biological environments, necessitating a thorough understanding of physiology to predict pharmacodynamics and pharmacokinetics, thereby minimizing risks of unexpected side effects and long-term health issues.

As programmable LNPs approach the complexity of living systems, ethical considerations relating to artificial life emerge. If LNPs can navigate the body and provide therapeutic effects, they may be perceived as artificial exosomes or viruses. Their potential environmental impact also arises, as these complex systems could interact with natural cells, bacteria, and viruses, requiring rigorous assessment criteria beyond clinical considerations.

Ultimately, ethical questions arise regarding the accessibility of sophisticated programmable LNPs. As their complexity increases, so does the likelihood of higher costs and the need for specialized expertise and equipment, potentially exacerbating healthcare inequality. Ensuring equitable access to these technologies is a significant challenge.

8 Conclusion and Outlook

In conclusion, this research presents a new conceptual framework that breaks down the key components of programmable LNPs into four distinct domains: Architecture, Interface, Payload, and Dispersal Domain. This modular model, akin to Lego blocks, enables the rational design of programmable LNPs for precise payload control and reduced off-target effects. Unlike traditional models such as liposome models¹⁴⁴ and polymeric nanoparticle models¹⁴⁵, which often treat LNPs as an indivisible entity, the Four-Domain Model identifies LNPs as dynamic, semi-closed systems in equilibrium, acknowledging potential matter exchange due to their porous surfaces and diffusion process⁴⁰. **Table 4** provides a comprehensive overview of various stimuli-responsive mechanisms employed in programmable LNPs across these domains. We hope this novel model can accelerate advancements in precision medicine and programmable drug delivery systems.

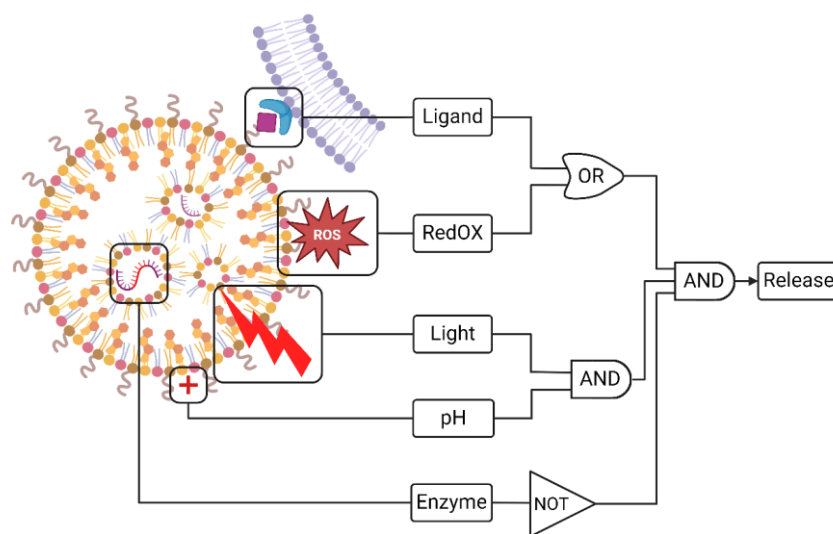


Fig. 8 Outlook of programmable LNPs. The future of programmable LNPs involves integrating multiple stimuli-responsive elements to achieve precise and controlled therapeutic cargo release under specific conditions, as illustrated in this schematic. AND gate indicates release or therapeutic activity only when both stimuli are present. OR gate indicates release when only one of the stimuli is present. The NOT gate indicates the failure of the release or loss of therapeutic effect when a certain input stimulus is present.

Despite notable progress in the development of programmable LNPs, liposomes remain dominant in the therapeutic environment as seen by their established track record (**Table 5**). Programmable LNPs must overcome development and clinical translation obstacles in order to go from theoretical promise to real-world use. This involves refining their design, ensuring regulatory compliance, optimizing clinical trials, engaging with stakeholders, and establishing scalable manufacturing processes. Post-approval collection of real-world evidence further substantiates their long-term safety and efficacy, facilitating broader clinical adoption.

The inherent complexity of programmable LNPs, due to their multi-component nature, poses challenges in design and formulation. The vast number of variables in LNP design makes exhaustive testing of all potential formulations difficult and inefficient, risking the omission of optimal combinations. Recent developments in machine learning have introduced innovative methods that enable the prediction of therapeutic outcomes by analyzing the properties of individual components. Machine learning advancements, such as the AGILE platform using deep learning with graph neural networks and molecular descriptors¹⁴⁶, and the TransLNPs model based on transformers¹⁴⁷, have shown promise in predicting mRNA transfection potency and efficiency. These tools streamline LNP design, enabling more efficient exploration of the high-dimensional design space and potentially uncovering novel delivery mechanisms. On the other hand, the current microfluidic formulation techniques might not be universally applicable due to the complex physiochemical properties of novel functional components. Engineering

techniques for proteins to achieve orthogonal modification are developed to face this challenge. However, to truly unleash the potential of programmable LNPs, a novel universal engineering technique is required.

In addition to the complexity of refining programmable LNP designs, clinical adaptation presents another challenge. Novel materials contain a variety of functional aspects, thus, it's important to assess their metabolism, bioactivity, biocompatibility, and potential side effects. The intricacy of these combined impacts might be more than current clinical trials can handle. Therefore, Clinical trial protocols must be updated to consider the complexity of these cutting-edge technologies.

This review compared artificial LNPs and natural exosomes with our Four Domain Model, highlighting their functionality, biocompatibility, and therapeutic potential. Programmable LNPs provide precise control over therapeutic payloads and can be engineered for targeted delivery, while natural exosomes offer a biocompatible, endogenous platform with the potential for reduced immunogenicity. LNPs benefit from scalable manufacturing and real-time monitoring capabilities but face design complexity and regulatory challenges. With their natural cargo and targeting properties, exosomes struggle with production scalability and consistency. The choice between these delivery systems depends on the therapeutic application, balancing the need for customization and control against biocompatibility and natural delivery mechanisms. This comparative analysis also provides a roadmap for developing more intelligent, naturally integrated systems.

Nevertheless, the existing artificial interface remains simply a “boundary” of a thermodynamic system instead of an intelligent gatekeeper for complex molecular fluxes, as provisioned in **Fig. 8**. As programmable LNPs evolve to incorporate multiple functions and stages, the outlined framework serves as a solid basis for overcoming challenges and maximizing their potential in various therapeutic contexts. Furthermore, we discussed novel anatomical delivery routes, such as intranasal and intraocular methods, that are associated with their challenges and opportunities. We prospect for future research directions to maximize LNP potential in precision medicine for the ultimate clinical application. When producing a well-controlled and adaptative artificial interface becomes a reality, so will artificial functional vesicles.

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Z. Liu and Y. Wei conceived and designed the review theme; Z. Liu gathered and analyzed the literature; Z. Liu and Y. Wei drafted the manuscript; Z. Liu created the tables; J. Chen created the figures; Y. Wei, H. Ho, K. Yong, and M. Xu critically revised the manuscript for important intellectual content; H. Ho oversaw the project and acquired funding. All authors discussed the results and implications and commented on the manuscript at all stages.

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12 Ethics declarations

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Additional information

Table 1. Comparative analysis of formulation methods for programmable LNPs.

Method	Description	Drug degradation	Scalability	Size & PDI Control	Advantages	Encapsulation Efficiency	Disadvantages	Complexity	Accessible Volume	Applicability to Different Payloads	Reference
Microfluidics Approaches	Introduction of turbulence in a microfluidic channel facilitates the formation of LNPs.	Low	High	Excellent	High reproducibility	Excellent	Cost of initial investment and possibility of clogging in microchannels	Moderate	uL to L	Broad (small molecules, proteins, nucleic acids)	Ref. ^{50,148}
Ethanol Injection	Dissolution of the lipid matrix in a water-miscible solvent followed by rapid injection into a mixed aqueous phase under constant stirring.	Low	Medium	Average	Versatile and of simple implementation	Good	Residual solvent, heterogenous	Low	uL to mL	Limited (small molecules, some proteins)	Ref. ^{148,149}
Thin-film Hydration	Dissolution of lipids in organic solvents followed by evaporation forms a thin film that, upon hydration in aqueous media, produces LNPs.	Medium	Medium	Poor	Cost effective and simple	Low	Extra steps needed for homogeneity (i.e., extrusion), time consuming	Low	uL to mL	Broad (small molecules, nucleic acids)	Ref. ^{148,150}
T-junction Mixing	T-Junction mixing involves using high-speed impingement jet mixers for flash nanoprecipitation, where solvent and anti-solvent phases are mixed nearly instantly, facilitating the efficient production of nanoparticles.	Medium	High	Excellent	Efficient and high throughput	Good	Requires large volumes of liquids, making it less suitable for small-scale or early-stage development.	Moderate	mL to L	Limited (small molecules, some proteins)	Ref. ¹⁴⁸
Microemulsion	Microemulsions create LNPs by forming nanometer-sized droplets where lipids self-assemble at the interface, stabilized by surfactants, allowing controlled nucleation and growth.	Medium	Low	Poor	Suitable for small volume vs. surfactants	Average	High proportion of surfactants (20-40%)	Low	uL to mL	Limited (small molecules, some proteins)	Ref. ^{148,151}
Microfluidic Vortex Mixing	Utilization of a vortex flow field to achieve high throughput synthesis of size-tunable lipid vesicles by focusing the lipid stream within a rotating aqueous buffer.	Low	High	Good	Efficient and high throughput	Good	May need downstream processing for concentration and purification.	Moderate	mL	Broad (small molecules, nucleic acids)	Ref. ¹⁵²
Spray Drying	Atomization of a liquid feed containing dissolved or suspended LNPs into a hot drying gas stream to produce a dry powder.	Medium (7.5-14% siRNA loss)	High	Good	Produces dry powder suitable for inhalation and Single-step process	Good	Heat exposure can damage thermolabile components	Moderate	mL to L	Broad (small molecules, nucleic acids)	Ref. ^{153,154}

Table 2. Overview of computational models for LNPs design and analysis.

Computational Model	Model Principle	Application	Strength	Weakness	Typical Software/Tools	Time Scale	Data Input Requirements	Reference
All Atom Molecular Dynamics	Simulates atomic interactions over time using classical mechanics.	Study LNPs formation, structure, and RNA encapsulation; Examine effects of pH, lipid composition, and RNA on LNPs properties; Investigate lipid phase behavior and RNA-lipid interactions.	Provides atomistic insights into LNPs structure and dynamics. Can simulate self-assembly process. Allows systematic variation of LNPs composition and conditions.	Limited to small system sizes and short timescales compared to real LNPs. Force field accuracy can impact results. Difficult to directly compare to experimental LNPs characterization.	GROMACS, NAMD, OpenMM, AMBER	Nanoseconds to microseconds	Lipid and RNA structures and parameters; System composition and initial configuration; Simulation parameters (temperature, pressure, etc.).	Ref. ^{155–157}
Coarse-Grained Molecular Dynamics Simulations	Reduces atomic detail by grouping atoms into larger “beads”.	Study LNPs self-assembly and structure. Investigate LNP-membrane fusion and RNA release. Examine effects of LNPs size and composition.	Enables simulation of larger systems over longer time scales; computationally less demanding than full MD. Allows study of collective lipid behavior and phase transitions.	Loses atomic level detail. Force field development and validation is challenging. Mapping between CG and atomistic representations not always straightforward.	MARTINI, SIRAH, ELBA, SDK	Microseconds to milliseconds	CG mapping scheme for lipids and RNA; CG force field parameters; System composition and initial configuration.	Ref. ^{158–160}
Monte Carlo Simulations	Uses probabilistic methods to explore configurations; can handle a vast parameter space. For LNPs, they often use coarse-grained models and can access larger time and length scales than all-atom MD.	Study pH-dependent behavior of LNPs. Investigate lipid ionization states. Examine lipid packing and phase behavior. Explore RNA-lipid interactions in LNPs core.	Effective for sampling large configuration spaces; can incorporate complex energy landscapes; can access larger system sizes and longer timescales.	May require extensive sampling to achieve convergence; lacks explicit dynamics information; may not capture kinetic effects.	LAMMPS, HOOMD-blue	Varies widely	Coarse-grained models for lipids and RNA; system composition; Interaction potentials; Simulation parameters (temperature, chemical potentials, etc.).	Ref. ^{160,161}
Poisson-Boltzmann Calculations	Solves the Poisson-Boltzmann equation to model electrostatic interactions in solution.	Predict electrostatic potential distributions in LNPs; Estimate lipid ionization states; Study pH-dependent behavior of LNPs core; Investigate ionic strength effects on LNPs structure.	Computationally efficient compared to molecular simulations; Can handle large systems and long-range electrostatic effects; Provides continuum description of ionic atmospheres; Allows systematic variation of solution conditions (pH, ionic strength).	Neglects ion-ion correlations and ion-specific effects; May not capture molecular-level details or lipid packing effect; Assumes thermal equilibrium; Simplified geometry may not fully represent complex LNPs structures.	APBS (Adaptive Poisson-Boltzmann Solver), DelPhi, MEAD	Not applicable - provides equilibrium electrostatic properties	System geometry (e.g., cylindrical model of LNPs core); Charge distributions or fixed charges; Dielectric constants for different regions; Ionic strengths and species; pH and pKa values for ionizable groups.	Ref. ^{160,162}
PBPK/PD Model for LNP-mediated mRNA Delivery	Physiologically based pharmacokinetic/ pharmacodynamic modeling that accounts for LNPs distribution, degradation, and mRNA expression.	Predict biodistribution and transgene expression of mRNA-LNPs in mice; Evaluate effects of targeting on LNPs behavior.	Bridges gap between LNPs delivery kinetics and delayed transgene expression; Accounts for physiological factors and active targeting; Can be expanded to various conditions and species.	Requires experimental data for parameter estimation and validation; May not capture all molecular-level interactions.	ADAPT 5 (BMSR)	Hours to days	Blood and tissue PK data; Luciferase expression kinetics; Physiological parameters (organ volumes, blood flows); LNPs properties.	Ref. ¹⁰³
Machine Learning Based	Utilizes conventional machine learning models such as support vector machines (SVM), random forests, and other regression techniques to predict key LNPs characteristics.	Predicts LNPs properties like encapsulation efficiency, transfection efficiency, and stability by leveraging structural and physicochemical features of LNPs components.	Offers rapid and scalable predictions across diverse datasets; Efficiently captures nonlinear relationships in data; Easily integrates with high-throughput screening workflows; Suitable for exploratory data analysis and feature selection.	Performance may be limited when handling complex systems with intricate interactions; Model accuracy heavily depends on the quality and diversity of training data; Generalization to novel lipid structures can be challenging.	Scipy, MATLAB	Not applicable – provides predictions of static properties rather than dynamic behavior.	LNPs characteristics, including molecular descriptors, structural features (e.g., SMILES), and experimental measurements (e.g., encapsulation efficiency, transfection efficiency).	Ref. ^{103,146,147}
AGILE	The AGILE platform uses a deep learning approach combining graph neural networks (GNNs) and molecular descriptor encoding to predict the mRNA transfection potency of ionizable lipids.	Accelerating the discovery and optimization of LNPs for mRNA delivery to specific cell types.	Rapid screening of large virtual lipid libraries Ability to identify novel lipid structures. Cell-type specific optimization Integration of structural and molecular property data. Interpretable results providing insights into structure-activity relationships	Reliance on experimental data for fine-tuning, which can be time-consuming to generate. Potential limitations in predicting performance of lipid structures significantly different from the training set.	PyTorch, RDKit	Not applicable – provides design but rather kinetics or dynamics	SMILES representations of lipid structures; Molecular descriptors calculated using Mordred.; Experimental mRNA transfection potency data for model fine-tuning.	Ref. ¹⁴⁶

TransLNP	TransLNPs is a transformer-based deep learning model that combines pretraining on large molecular datasets with fine-tuning on limited LNPs data to predict the transfection efficiency of ionizable LNPs for mRNA delivery.	Accelerating the screening and optimization of LNPs for mRNA delivery by predicting their transfection efficiency.	Combines both coarse-grained atomic sequence and fine-grained 3D structural information. Utilizes pretraining on large molecular datasets to improve performance on limited LNPs data. Incorporates data balancing techniques (BalMol block) to manage imbalanced datasets Outperforms AGILE in predicting LNPs transfection efficiency.	Still requires experimental data for fine-tuning, which can be time-consuming to generate. Performance may be limited by the “transfection cliff” phenomenon, where small structural changes lead to significant differences in transfection efficiency.	PyTorch, RDKit, Custom BalMol block for data balancing	Not applicable – provides design but rather kinetics or dynamics	SMILES representations of lipid structures; 3D conformational information for molecules; Experimental transfection efficiency data for fine-tuning.	Ref. ¹⁴⁷
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Table 3. Characteristics and applications of payloads encapsulated in programmable LNPs¹.

Payload Type	Example	Clinical Applications	Solubility	Charge	Encapsulation Location	Encapsulation Efficiency	Stability Consideration		Stability Concerns & Disadvantages of Direct Delivery	Packaging and Delivery Enhancements	Half-life in Circulation	Mechanism of Action	Reference
							Storage Conditions	Formulation Additives					
mRNA	mRNA vaccines, eGFP mRNA, Luciferase mRNA, IL-2 mRNA	Gene therapy, vaccines, reporter gene expression, immunotherapy	Hydrophilic	Negative	Aqueous core	82-95%	-80°C to 20°C	Lipids, PEGylated lipids, cholesterol	Rapid degradation by RNases, poor cellular uptake, immune stimulation	Lipid formulation enhances delivery	Minutes to hours	Induces protein expression	Ref. 13,90
siRNA	PKN3 shRNA	Gene silencing	Hydrophilic	Negative	Aqueous core	>93%	-80°C to 20°C	Lipids, PEGylated lipids, cholesterol	Rapid degradation by RNases, poor cellular uptake, off-target effects	Targeted delivery to specific tissues	Minutes to hours	RNA interference (RNAi)	Ref. 93,94
microRNA	miR-193b-3p mimic, miR-21, miR-18a, let-7c	Gene therapy, Cancer therapy	Hydrophilic	Negative	Aqueous core	>90%	-80°C to 20°C	Lipids, PEGylated lipids, cholesterol	Rapid degradation by RNases, Poor cellular uptake	Enhanced delivery with LNPs	Minutes to hours	Regulation of gene expression, Modulation of translation	Ref. 96-98
Self-amplifying RNA	SARS-CoV-2 spike protein RBD, influenza hemagglutinin	Vaccines	Hydrophilic	Negative	Aqueous core	>90%	-80°C to 20°C	Lipids, PEGylated lipids, cholesterol	Rapid degradation by RNases, poor cellular uptake, potential for immunogenicity	Lipid formulation enhances delivery	Hours to days (longer than conventional mRNA)	Self-amplification in cytoplasm leads to elevated levels of antigen expression, inducing strong immune responses	Ref. 91,92
Translation-activating RNA	PTV-IIIab-based taRNA, mini taRNA (94 nt)	Potential for treating haploinsufficiency diseases	Hydrophilic	Negative	Aqueous core	-	-	Formulation Additives: Stabilizing hairpins added to 5' and 3' ends	Transient effect (12-24 hours) when delivered as unmodified RNA	AAV delivery also demonstrated	-	Recruiting translation initiation factors to target mRNA to enhance translation	Ref. 163
CRISPR Components	Cas9 mRNA, sgRNA	Gene editing	Hydrophilic	Negative	Aqueous core	>90% for most formulations	-80°C to 20°C	Stabilizers, enzymes	Rapid degradation, potential off-target effects, immune responses	Enhanced delivery with LNPs	Minutes to hours	Genome editing	Ref. 99,100
DNA	Plasmid DNA (pDNA)	Gene therapy	Hydrophilic	Negative	Aqueous core	~60-80%	-80°C to 20°C	Lipids, polyethyleneimine	Poor cellular uptake, nuclear entry challenges, immune responses	Enhanced cellular uptake	Hours to days	Gene expression	Ref. 20,93
Antifungals	Fluconazole (Fluc)	Antifungal therapy	Hydrophilic	Neutral, subject to ionization	Aqueous core, lipid bilayer	~59%	-	-	Poor aqueous solubility, limited bioavailability	Enhanced solubility and stability	-	Inhibition of fungal growth	Ref. 40

¹ Notes:

- 1) The encapsulation efficiency percentages are approximations and may vary based on specific formulations and conditions.
- 2) Storage conditions can vary significantly based on the specific formulation and stability of the payload type.
- 3) The bioavailability section is generalized; specific formulations may have different characteristics.

Chemotherapeutics	Dox, Paclitaxel	Cancer therapy	Amphiphilic (Dox), Hydrophobic (Paclitaxel)	Neutral (Paclitaxel), Positive (Dox)	Aqueous core & lipid bilayer (Dox), Lipid bilayer (Paclitaxel)	~100% (Dox), ~95% (Paclitaxel)	-	-	Cardiotoxicity, myelosuppression (Dox), Poor aqueous solubility, systemic toxicity (Paclitaxel)	Targeted delivery reduces side effects	-	Cytotoxicity	Ref. ^{101,102}
Enzymes	Proenzymes, Coenzyme Q10	Enzyme replacement therapy, metabolic disorders	Hydrophilic/hydrophobic (CoQ10)	Variable	Aqueous core/lipid layer (CoQ10)	~70%	-20°C to 80°C	- Stabilizers, cryoprotectants	Rapid clearance, immunogenicity, denaturation	Improves stability and activity	-	Enzyme replacement	Ref. ^{22,164}
Metal Nanoparticles	Gold nanoparticles	Photothermal therapy	Generally hydrophobic	Neutral to negative	Aqueous core, surface modified	92 ± 5%	-	-	Potential toxicity, aggregation, stability under physiological conditions	-	-	Photothermal effect for targeted drug release	Ref. ⁸⁴

Table 4. Overview of stimuli-responsive mechanisms across programmable LNPs domains.

Domain	Input	Functional Component	FDA Approval Status	Mechanism	Characterization Techniques	Application	Functional Component Synthesis	LNPs Formation	Encapsulation Efficiency	Payload	Reference
Architecture	pH	DLin-MC3-DMA (MC3)	Approved as part of mRNA-based vaccines (COVID-19)	transition from inverse spherical micellar to inversehexagonal lipid structures in lipid excess phases.	SAXS, Cryo-TEM, DLS, Fluorescence spectroscopy, Gradient UPLC, bDNA analysis, ELISA, Fluorescence microscopy, Fluorescence anisotropy	mRNA Delivery	Purchased from AstraZeneca	ethanol injection method	Not specified	mRNA	Ref. ³⁹
		Phytantriol-based Ionizable Lipid Nanoparticles (ALC-0315/PT, SM-102/PT)	Components like ALC-0315 and SM-102 approved in COVID-19 mRNA vaccines	Lyotropic liquid crystalline mesophase transitions from cubic to hexagonal structures with pH changes to enhance endosomal escape.	DLS, SAXS, 2-(p-toluidino) naphthalene-6-sulfonic acid assay, Zeta Potential Measurement	Drug Delivery	Purchased from Advanced Molecular Technologies (Scoresby, Victoria, Australia)	thin-film hydration method followed by probe sonication	Not specified	Various	Ref. ³³
		2-morpholinoethyl oleate (O2ME)	No specific approval; research stage	Transition from hexagonal (H2) to cubic (cubosomes) phase with pH changes, which interacts with Fungal cells.	NMR, DLS, SAXS, HPLC, UV-vis, CLSM, SEM	Antifungal Therapy	Esterification reaction between oleic acid and 2-(2-hydroxyethyl) morpholine using EDC and DMAP as catalysts, followed by purification using flash chromatography	thin-film hydration method followed by probe sonication	59	Fluconazole (Fluc)	Ref. ⁴⁰
	Light	NIR-II dyes conjugated lipid (Cy-lipid)	No specific approval; research stage	Protonation of Cy-lipid in acidic conditions increases NIR-II absorption, facilitating photothermal endosomal escape.	NMR, HRMS, TEM, DLS, UV-vis-NIR, CLSM, Fluorescence spectroscopy, Thermal imaging, Fluorescence microscopy, Bioluminescence imaging	mRNA Delivery	Nucleophilic substitution between thiol lipid and polymethine dye	Mixing of lipids and mRNA in citrate buffer, followed by dialysis	~90%	eGFP and luciferase mRNA	Ref. ⁸³
		Porphyrin-lipid	No specific approval; research stage	Generates ROS upon NIR light irradiation, disrupting endosomal membranes.	DLS, TEM, cryo-EM, UV-vis, STED, RT-qPCR, Fluorescence spectroscopy, Confocal microscopy, Bioluminescence imaging	Gene	Acylation reaction between lysophosphatidylcholine and pyropheophorbide (a chlorophyll-derived porphyrin analogue)	Microfluidic rapid mixing	>95%	siRNA	Ref. ⁸⁴
	Payload	Enzyme	IL-2F (IL-2, cleavable linker, CD25)	Components (like IL-2) approved, but not specific construct	Cleavage of linker by MMP-14 in tumor microenvironment, releasing active IL-2.	DLS, TEM, ELISA, CLSM, Immunohistochemistry, Western blot, Flow cytometry, Immunofluorescence, Hematoxylin and eosin (H&E) staining, TUNEL staining	Cancer	Cleavable linker (SGRSEN↓IRTA) inserted between IL-2 and CD25 sequences using PCR and Gibson assembly	Microfluidics with ethanol and aqueous phases	Not specified	IL-2 mRNA
NQO1-responsive proenzyme			No specific approval; research stage	Activation in the presence of overexpressed NQO1 in tumor cells.	MALDI-TOF MS, SDS-PAGE, TEM, DLS, T7EI assay	Cancer	The QPN ligand was attached to lysine residues at or near the active sites of the target proteins	Rapid mixing	Not specified	Proenzymes	Ref. ²²
Light		5 nm gold nanoparticles	Approved for some medical uses, not specifically in lipids	Localized heating of AuNPs causes thermal decomposition of adjacent lipid lamellae, releasing payload.	DLS, UV-vis, Cryo-TEM, FM, fluorescence spectroscopy	Cancer	AuNPs: Commercially obtained (Ted Pella Inc, 5 nm PELCO NanoXact Gold Nanoparticles)	T-tube protocol mixing lipids (DODAP, DSPC, cholesterol, PEG-DSPE) with AuNPs, followed by dialysis	~100%	Doxorubicin	Ref. ¹⁰⁶

Interface	pH	imidazole based lipid (O12-D3-I3)	Not specifically approved; similar lipids used in approved formulations	Buffering induced Swelling enhance Endosomal Escape.	TEM, TNS fluorescence titration, DLS, colorimetric method, HPLC-MS	Cancer	Multi-step synthesis involving tert-butyl carbamate protection, amine alkylation, and imidazole derivatization	preformed vesicle method	93	siRNA	Ref. ⁷⁶
		PGlu(DET-Car)30 LNPs	No specific approval; research stage	Active charged surface to interact with negative charged membrane.	DLS, TEM, Fluorescence Spectroscopy, FCM, CLSM, Bioluminescence Imaging, NMR Spectroscopy, HPLC	Cancer	Multi-step reaction involving biphasic solvent system and purification by acetonitrile precipitation and centrifugation	t-BuOH dilution procedure	95 ± 3%	siRNA	Ref. ²¹
		double pH-responsive lipo-amino fatty acid (LAF) – Stp oligoaminoamide (OAA) carriers	No specific approval; research stage	Increased solubility and amphiphilic if carriers disrupt the membrane.	DLS, ELS, Agarose Gel Shift Assay, RiboGreen Assay, Flow Cytometry, MTT Assay, CLSM, RT-PCR, Gel Electrophoresis, In Vitro Cleavage Assay, PCR, Sanger Sequencing, TIDE Analysis, Ex Vivo Luciferase Assay	Gene Editing	use of Fmoc solid-phase assisted peptide synthesis (SPPS) to covalently connect Stp and LAF units via branching lysine, forming various topologies (combs, bundles, T-shapes, and U-shapes) with different Stp/LAF ratios.	microfluidic mixing method	>90% for most formulations	Cas9 mRNA/sgRNA	Ref. ¹⁶⁵
		pH-activated lipid-like material (ssPalm) with vitamin E scaffolds	No specific approval; research stage	Destabilizing membranes at low pH by Tertiary amines.	DLS, PicoGreen, IVIS, FC, CLSM, ELISA	Vaccine	Performed mesylation and amination followed by acylation, stirred in chloroform with catalysts at room temperature. Purified by silica gel column chromatography using a chloroform	ethanol injection method	94±4	DNA	Ref. ²⁰
	Enzyme	Esterase-labile Quaternium Lipidoid (AMP-POC18)	No specific approval; research stage	Stable lipidoid underwent decationization to interact with endosome membrane.	DLS, Cryo-EM, HPLC, DSC, FACS, CLSM, IVIS, ELISA, ELISpot	mRNA Delivery	Combinatorial synthesis of quaternium lipidoids involving the reaction of amines with acrylate ester linkers followed by alkylation with bromides	Ethanol injection method, dialysis	82	mRNA	Ref. ⁸²
		Phospholipid D (PLD)	Not specifically approved; general class used in various formulations	PLD enzymes cleave choline headgroups from phospholipids, forming membrane-bound anionic phosphatidic acid.	SPARTA, SAXS, SANS, Cryo-TEM, DLS, Fluorescence assays	neurodegeneration, vascular disease, and cancer	Purchased from Sigma Aldrich, used directly.	Thin-film hydration method followed by probe sonication	Not Provided	Not Provided	Ref. ²³
		Polyphosphate (TPP)	Not specifically approved; used in research	Charge reversal triggered by ALP, enhancing cellular uptake and transfection efficiency.	DLS, ELS, FT-IR, UV-vis, Fluorescence spectroscopy, Agarose gel electrophoresis	Cancer	Polyphosphate coated on LNPs with Prot-Palm	Solvent injection method, followed by TPP coating	Not specified	pDNA	Ref. ⁶⁴
	Redox potential	Disulfide bonds	Common in biologics; specific use case approvals	GSH cleaves disulfide bonds within the hydrophobic tails of the lipids, leading to truncation and disintegration.	MALDI-TOF MS, SDS-PAGE, TEM, DLS, T7EI assay	Cancer	Michael Addition Reaction	Rapid mixing	Not specified	Proenzymes	Ref. ²²
		BAmP-TK-12 (ionizable lipid with thioetal linkage)	No specific approval; research stage	Oxidation and cleavage of thioetal linkage by elevated ROS in tumor cells, destabilizing LNPs.	MALDI-TOF MS, SDS-PAGE, TEM, DLS, T7EI assay	Cancer	Not mentioned	Rapid mixing	Not specified	Proenzymes	Ref. ²²

		Disulfide bonds in DDA-SS-DMA ionizable lipid	No specific approval; research stage	High GSH levels in tumor environment cleave disulfide bonds, triggering nanoparticle disassembly and payload release.	NMR, TOF-MS, DLS, TEM, CLSM, qRT-PCR, Agarose Gel Electrophoresis, Western Blot, Hematoxylin and Eosin (H&E) Staining	Cancer	1) Oxidation of thioglycerol, 2) Esterification with dodecanoic acid, 3) Reaction with 2-(dimethylamino) ethane-1-thiol	Microfluidic rapid mixing	Not specified	PKN3 shRNA	Ref. ⁷⁹
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Table 5. Overview of clinical advancements in programmable lipid-based nanoparticles.

Formulation/Brand Name	Targeting Mechanism	Product Overview	Target Disease/Clinical Trial	Mechanism of Action	Formulation Type	Administration Route	Clinical Trial Phase	Notable Results	Side Effects/Adverse Reactions	Market Availability	Clinical Trial Identifier
Onivyde (Irinotecan Liposome)	Enhanced permeability and retention (EPR) effect	Liposomal formulation of irinotecan	Metastatic pancreatic cancer	Inhibition of topoisomerase I	Liposome	Intravenous	Approved	Improved overall survival	Neutropenia, diarrhea	Available	-
Doxil (Liposomal Doxorubicin)	EPR effect	PEGylated liposomal doxorubicin	Multiple cancer types (Ovarian cancer, multiple myeloma, Kaposi's sarcoma)	Intercalates DNA, inhibits topoisomerase II	Liposome	Intravenous	Approved	Reduced cardiotoxicity compared to free doxorubicin	Hand-foot syndrome, mucositis	Available	-
Myocet (Liposomal Doxorubicin)	EPR effect	Non-PEGylated liposomal doxorubicin	Breast cancer	Intercalates DNA, inhibits topoisomerase II	Liposome	Intravenous	Approved (EU)	Reduced cardiotoxicity	Neutropenia, alopecia	Available in EU	-
Vyxeos (CPX-351, Liposomal Daunorubicin and Cytarabine)	EPR effect	Liposomal combination of daunorubicin and cytarabine	Acute myeloid leukemia (AML)	DNA intercalation and inhibition of DNA synthesis	Liposome	Intravenous	Approved	Improved overall survival	Febrile neutropenia, thrombocytopenia	Available	-
Visudyne (Verteporfin)	Targeted photodynamic therapy	Liposomal verteporfin	Age-related macular degeneration	Generates reactive oxygen species upon light activation	Liposome	Intravenous	Approved	Stabilization of vision loss	Visual disturbances, injection site reactions	Available	-
ThermoDox (Celsion Corporation)	Heat-triggered release	Thermosensitive liposomal doxorubicin	Liver cancer	Heat-triggered release of doxorubicin	Thermosensitive liposome	Intravenous	Phase III	Enhanced drug accumulation at tumor site	Fever, nausea	Not available	NCT00617981
Balstilimab	Ultrasound-triggered BBB opening	Liposomal Doxorubicin	Glioblastoma	Ultrasound enhances drug penetration and activation	Liposome	Intravenous	Phase IIa	Enhanced drug delivery to brain tumors	Photosensitivity, nausea	Not available	NCT05864534
Comirnaty (Pfizer-BioNTech) and Spikevax (Moderna)	Lipid nanoparticle encapsulation	mRNA vaccines encoding SARS-CoV-2 spike protein	COVID-19	Induces immune response against SARS-CoV-2	LNPs	Intramuscular	Approved	High efficacy in preventing COVID-19	Injection site reactions, fever	Available	-
Onpattro (Patisiran)	Lipid nanoparticle delivery	siRNA targeting transthyretin (TTR) mRNA	Hereditary transthyretin-mediated amyloidosis	RNA interference reduces TTR protein production	LNPs	Intravenous	Approved	Reduction in TTR protein levels	Infusion-related reactions	Available	-
Marqibo (Vincristine Liposome Injection)	EPR effect	Liposomal vincristine	Acute lymphoblastic leukemia (ALL)	Inhibits microtubule formation	Liposome	Intravenous	Approved	Improved drug delivery to tumor cells	Peripheral neuropathy, constipation	Available	-
Abraxane (Paclitaxel Nanoparticle Albumin-Bound)	EPR effect	Albumin-bound paclitaxel nanoparticles	Breast, lung, and pancreatic cancer	Inhibits microtubule disassembly	Nanoparticle	Intravenous	Approved	Enhanced delivery and reduced toxicity	Neutropenia, neuropathy	Available	-
Eteplirsen	Targeted delivery	Phosphorodiamidate morpholino oligomer (PMO)	Duchenne muscular dystrophy	Skips exon 51 in dystrophin pre-mRNA	Lipid nanoparticle	Intravenous	Approved	Increased dystrophin production	Infusion-related reactions	Available	-