Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

CO-DESIGNING MRNA CARGO AND LIPID NANOPARTICLES ACROSS SEQUENCE FUNCTION, INTRACELLULAR TRAFFICKING, BIODISTRIBUTION, AND MANUFACTURABILITY

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ARTICLE INFO

Received:
28 March 2026
Received in revised form:
21 July 2026
Accepted:
25 July 2026
Available online:
28 August 2026

Keywords: MRNA sequence design, Lipid nanoparticles, Endosomal escape, Innate immunity, Biodistribution, Pharmaceutical manufacturability

ABSTRACT

Messenger RNA therapeutics depend on a coupled molecular and delivery system in which sequence design, chemical composition, particle assembly, intracellular trafficking, tissue exposure, and manufacturing conditions jointly shape performance. Yet development programs commonly optimize cargo and lipid nanoparticles in partially separated workflows, often privileging a single readout such as expression, uptake, or encapsulation. This article addresses that fragmentation by proposing an original mRNA–lipid nanoparticle co-design architecture that treats the cargo, carrier, process, biological context, and therapeutic objective as interacting design states rather than independent modules. The approach is a theory-building synthesis of recent pharmaceutical, bioengineering, and computational evidence, organized around causal uncertainty, multiobjective trade-offs, and staged validation. The central contribution is a proposed architecture in which sequence–function relationships, innate-immune effects, lipid composition, particle formation, endosomal escape, biodistribution, and manufacturability are connected through explicit intermediate states and feedback loops. The architecture distinguishes predictive ranking from experimental confirmation, particle uptake from productive cytosolic delivery, organ exposure from target-cell function, and laboratory feasibility from scalable pharmaceutical control. It further argues that no universal optimum exists across vaccination, protein replacement, gene editing, immune modulation, and ex vivo engineering because each context assigns different weights to expression kinetics, inflammatory signaling, targeting, durability, safety, and process robustness. The proposal is not an empirically validated model, regulatory framework, or operational decision tool. Its value is to provide a disciplined structure for designing experiments, integrating heterogeneous evidence, reporting uncertainty, and preventing locally optimized cargo or formulation choices from being mistaken for a therapeutically and manufacturably coherent product.

To Cite This Article: Ali H, Farooq M, Shah U, Khan B, Malik S. Co-Designing mRNA Cargo and Lipid Nanoparticles across Sequence Function, Intracellular Trafficking, Biodistribution, and Manufacturability. Pharmacophore. 2026;17(4):71-82. https://doi.org/10.51847/4YlxCn8KFc

Introduction

Messenger RNA has become a programmable therapeutic modality because its encoded function can be altered without rebuilding the entire production platform. That apparent modularity, however, can obscure a deeper systems problem: the biological activity of an mRNA product does not reside in the nucleotide sequence or the carrier alone. Therapeutic performance is jointly conditioned by cargo engineering, extracellular and intracellular delivery barriers, and the multicomponent lipid nanoparticle that protects, transports, and releases the transcript [1-3]. Sequence-dependent translation, innate recognition, particle morphology, organ exposure, and process history can therefore converge on the same observed

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endpoint. A high expression value may reflect favorable cargo stability, efficient cytosolic release, preferential tissue delivery, or a combination of these factors, while a weak value may arise from failure at any one of several coupled stages. Treating the system as separable can consequently produce misleading attribution and fragile optimization.

This coupling matters across a therapeutic landscape that includes vaccination, transient protein replacement, immune modulation, ex vivo cell engineering, and in vivo genome editing. These uses impose different requirements for cell targeting, duration of expression, inflammatory signaling, repeat dosing, and acceptable risk; they cannot be reduced to a universal formulation objective [4]. A carrier that is useful for antigen presentation may be inappropriate for sustained protein replacement, while a sequence optimized for maximal translation may be undesirable when controlled immune tolerance or short editing exposure is required. The central pharmaceutical question is therefore not simply which mRNA or which lipid nanoparticle performs best. It is which cargo–particle–process combination produces the intended biological program within a defined route, tissue, dosing regimen, manufacturing environment, and evidence standard.

The unresolved gap is both scientific and computational. Experimental programs often optimize sequence elements, nucleotide chemistry, ionizable lipids, helper lipids, mixing conditions, or biological readouts in separate stages. Computational models inherit the same fragmentation when they predict one endpoint from one design space without representing the intermediate physical and biological states that connect cargo identity to therapeutic function. Vaccine development illustrates the need to integrate sequence design, formulation, innate immunobiology, and clinical context rather than treating any one layer as a sufficient determinant of translation [5]. More generally, retrospective predictive performance does not establish mechanistic explanation, prospective utility, manufacturability, or clinical relevance. A design architecture is needed that can preserve these distinctions while still enabling coordinated search across a large, heterogeneous decision space.

This article develops the proposed Cargo–LNP Co-Design Architecture as an original RNA-delivery design construct. The architecture represents cargo identity and quality, lipid composition and processing, particle state, intracellular trafficking, biodistribution, manufacturing state, and therapeutic-context utility as linked but separately observable layers. It uses feedback between layers to identify where evidence is missing, where uncertainty should constrain extrapolation, and where an apparently favorable result should trigger redesign rather than advancement. The contribution is conceptual rather than empirical: it organizes evidence-supported relationships and proposes how they can be evaluated without claiming a validated optimizer, universal causal model, regulatory decision rule, or deployment-ready system. Its intended role is to improve experimental design, computational representation, and pharmaceutical interpretation by making cross-domain dependencies explicit.

Why mRNA Cargo and Delivery Vehicle Cannot Be Optimized Separately

The first reason cargo and vehicle cannot be optimized separately is that the cargo is itself a multidimensional physical object. Coding-region secondary structure can change functional half-life and protein-expression kinetics, while 3′ untranslated-region architecture can independently augment expression [6, 7]. These effects mean that “the same mRNA dose” is not a stable design concept when transcripts differ in structure, degradation behavior, ribosome engagement, or untranslated regulatory elements. They also imply that the delivery problem presented to the nanoparticle changes with the cargo. Length, folding, charge distribution, chemical modification, and impurity state may affect encapsulation, internal organization, release, and exposure to sensors. The proposed architecture therefore treats sequence function as an input to particle design rather than as a fixed upstream specification. This relationship is evidence-supported at the level of sequence-dependent expression, but the full cargo-by-formulation interaction space remains incompletely measured and should not be assumed to be additive.

The second reason is that observed protein production is a composite system output. Functionalized lipid nanoparticles can reprogram cellular protein production by delivering mRNA to a biologically productive compartment [8]. Such an outcome cannot be attributed solely to sequence quality or particle uptake: it requires cargo protection, cell access, intracellular routing, cytosolic release, transcript competence, and translation. A formulation comparison performed with one reporter transcript may therefore identify a locally favorable carrier while failing to predict behavior with a different length, structure, encoded protein, or required expression profile. Conversely, sequence screening outside the intended delivery context may select transcripts that translate well after direct transfection but perform poorly when encapsulated, stored, administered, and trafficked in vivo. Co-design requires crossed experiments or models that can estimate interaction terms rather than parallel leaderboards for cargo and carrier.

The third reason is that lipid composition produces intermediate particle states that reshape the meaning of a cargo result. Naturally occurring cholesterol analogues can alter nanoparticle polymorphism and intracellular mRNA delivery [9]. This establishes a direct route from helper-lipid identity to morphology and biological performance, but it does not justify a universal ranking of cholesterol variants. The effect is conditional on the remaining formulation, the payload, the manufacturing method, the assay, and the biological context. Within the proposed architecture, composition is therefore not linked directly to therapeutic utility by a single arrow. It acts through particle formation, structural organization, surface and protein interactions, trafficking, and tissue exposure. Computational models that omit these intermediates may still predict within a narrow dataset, but their features can become nontransportable when process conditions, cargo properties, or biological environments change.

The fourth reason is that a formulation with high expression may still be unacceptable because escape, pharmacology, and safety are coupled. Amino-lipid structure can influence endosomal escape, persistence of pharmacological activity, and tolerability [10]. The design objective must consequently include not only the magnitude of expression but also its location,

duration, inflammatory consequences, dosing compatibility, and manufacturability. The proposed non-separability construct does not claim that every cargo variable interacts strongly with every lipid variable; rather, it requires the possibility of interaction to be tested where a shared intermediate state makes it plausible. It also requires negative and discordant results to be retained, because a candidate that succeeds in one layer and fails in another is informative about the architecture. **Table 1** organizes the evidence, constructs, and interpretation boundaries needed to develop why mrna cargo and delivery vehicle cannot be optimized separately within the article's central argument.

Table 1. Evidence domains, core questions, scientific requirements, and interpretation boundaries for Why mRNA cargo and delivery vehicle cannot be optimized separately

Construct or evidence domain	Core question	Scientific basis	Role in the article	Failure or overclaiming risk	Boundary statement
Cargo sequence architecture	How do coding-region structure and untranslated regions alter the delivery problem presented to the nanoparticle?	Sequence structure and regulatory regions affect functional stability and translation.	Establishes the cargo as a physical and functional design state rather than a fixed payload label.	Treating an expression score obtained in one assay as an intrinsic property of the sequence.	Sequence performance remains conditional on cell type, assay, formulation, route, and therapeutic objective.
Cargo–particle compatibility	Does changing the cargo alter nanoparticle formation, organization, stability, or release?	Cargo length, folding, chemistry, charge, and impurity state may interact with formulation and process conditions.	Creates an explicit interface between cargo design and particle design.	Assuming formulation performance transfers unchanged between reporter and therapeutic transcripts.	Cargo-by-formulation interactions require controlled crossed experiments and cannot be inferred from separate optimization studies alone.
Productive intracellular delivery	Does internalized mRNA reach the cytosol in a translation-competent form?	Functional delivery requires protection, uptake, trafficking, escape, cargo integrity, and translation.	Prevents uptake or total particle exposure from being treated as the final delivery outcome.	Equating cellular association, uptake, or endosomal disruption with productive mRNA release.	Productive delivery requires orthogonal trafficking, cytosolic-release, and functional-expression evidence.
Lipid-dependent particle state	Through which particle properties does lipid composition influence cargo performance?	Helper-lipid and ionizable-lipid identity can change morphology, organization, membrane interaction, and intracellular behavior.	Introduces particle state as an intermediate between nominal composition and biological function.	Drawing a direct composition-to-efficacy relationship while omitting particle structure and biological context.	A favorable lipid or ratio is not universally optimal across cargos, processes, routes, species, or indications.
Expression kinetics and localization	Is the magnitude, duration, and anatomical location of expression appropriate for the intended use?	Peak expression alone does not capture persistence, cell specificity, pharmacological timing, or off-target activity.	Converts expression from a single score into a context-dependent response profile.	Advancing a candidate because of maximal reporter expression despite unsuitable duration or localization.	Desired expression profiles must be defined prospectively for each therapeutic context.
Innate-immune and safety compatibility	Does the combined system produce an acceptable and therapeutically appropriate biological response?	Cargo chemistry, impurities, lipid structure, route, and exposure pattern may jointly shape inflammatory and safety outcomes.	Incorporates benefit–risk and immune intent into the co-design objective.	Treating low inflammation or high immune activation as universally desirable.	The desired immune profile differs across vaccination, protein replacement, gene editing, and immune tolerization.
Manufacturability and process dependence	Can the selected cargo–LNP combination be produced reproducibly	Mixing, purification, concentration, storage, and scale can affect cargo quality and	Prevents biological optimization from being separated from pharmaceutical	Assuming laboratory-scale composition defines the same product after	Manufacturability must be evaluated as a design constraint; it cannot be inferred from small-scale

	without changing its critical states?	nanoparticle attributes.	production feasibility.	scale-up or process transfer.	biological performance.

Sequence Function, Structure, Stability, and Innate Immune Effects

Sequence function begins with the recognition that untranslated and coding regions jointly determine how a transcript behaves before and during translation. Massively parallel assays have shown that human 5′ untranslated-region variants can be designed and modeled to alter translation efficiency [11]. For co-design, the relevant output is not a single intrinsic "sequence score," but a context-indexed description of initiation efficiency, structural accessibility, cell-type dependence, and compatibility with the intended expression duration. The proposed cargo state should therefore include cap configuration, 5′ and 3′ untranslated regions, coding sequence, poly(A) architecture, modified nucleosides, and encoded-protein requirements. These features should be represented alongside assay provenance because sequence–function maps learned in one cellular system may not transfer to another, and because direct-transfection assays do not reproduce nanoparticle assembly, trafficking, or in vivo exposure.

Stability and translation are related objectives rather than interchangeable measures. Combinatorial design can improve mRNA structure, stability, and translation together, but the resulting gains remain sequence- and assay-dependent and require experimental confirmation [12]. A more stable transcript may extend the window for protein production, yet excessive structural stabilization can also alter accessibility to the translational machinery or shift degradation pathways. The co-design architecture therefore represents stability as a time-dependent state that includes chemical integrity, structural persistence, and functional competence after formulation, storage, administration, and cytosolic release. The relevant question is not whether a transcript is stable in isolation, but whether the complete cargo–particle system preserves a translation-competent molecule for the duration required by the therapeutic context. This framing also prevents accelerated degradation and prolonged expression from being treated as universally unfavorable or favorable.

Innate-immune effects connect sequence chemistry directly to manufacturing and therapeutic intent. Nucleotide chemistry and process-derived features of manufactured mRNA can jointly influence innate immune activation [13]. This relationship complicates optimization because inflammatory signaling may suppress translation, contribute to reactogenicity, support vaccine adjuvanticity, or undermine immune-tolerizing applications depending on context. The architecture therefore separates three questions: whether the cargo contains structures or impurities capable of activating innate sensors; whether the delivery system changes the location and timing of sensor exposure; and whether the resulting response is aligned with the intended therapeutic program. It does not reduce immune compatibility to minimization of cytokine signals. Instead, it requires a prespecified desired immune profile and orthogonal evidence linking cargo chemistry, particle delivery, cellular sensing, protein expression, and downstream function.

Cargo quality is the manufactured realization of sequence design and must remain distinct from the intended digital sequence. Double-stranded RNA contaminants generated during in vitro transcription can be removed through dedicated purification, making impurity control an integral design variable rather than a downstream quality correction [14]. At the same time, computational and machine-learning approaches may help prioritize sequence variants, predict regulatory-element behavior, or identify promising combinations, but their usefulness is conditional on assay-matched training data, explicit applicability domains, uncertainty assessment, and prospective experimental testing [15]. The proposed sequence module therefore produces a structured evidence package rather than a ranked sequence alone: identity, integrity, structural features, impurity state, expression kinetics, innate-sensing profile, model provenance, and uncertainty. This package becomes an input to nanoparticle and process design, while discordant formulation results feed back to revise the cargo representation.

Lipid Composition, Particle Formation, Trafficking, and Endosomal Escape

Lipid composition defines more than the chemical inventory of a nanoparticle. Ionizable lipids, helper phospholipids, cholesterol or cholesterol analogues, PEG-lipids, targeting additives, and their relative proportions jointly influence assembly, stability, membrane interactions, and biological identity. Formulations optimized for intramuscular mRNA vaccination illustrate that composition must be aligned with administration route, expression profile, immune objective, and tolerated exposure rather than transferred unchanged between applications [16]. The proposed architecture therefore treats lipid composition as a conditional design state. A formulation is not "optimal" in isolation; it is preferable only within a specified cargo, process, route, biological model, and therapeutic objective.

Particle structure provides the intermediate physical layer through which nominal composition becomes biological behavior. Structural deconvolution studies show that lipid nanoparticles cannot be adequately described by mean diameter and encapsulation alone [17]. Internal organization, cargo distribution, lipid packing, surface state, and particle heterogeneity may affect storage stability, protein adsorption, cell entry, and release. These characteristics are only partially observable and may vary with the analytical method. The architecture therefore requires orthogonal characterization and records uncertainty when structural states cannot be directly resolved. It also separates compositional identity from particle identity because nominally identical lipid ratios may yield different particle populations when cargo or processing conditions change.

Particle formation is a process-dependent event rather than an automatic consequence of combining lipids and nucleic acid. Ionizable-lipid nanoparticles develop through assembly pathways shaped by molecular composition, rapid mixing, solvent conditions, pH, flow, and payload characteristics [18]. Although formation studies using other nucleic-acid cargos can inform mechanistic hypotheses, transfer to mRNA requires explicit confirmation because transcript length, structure, flexibility, and

impurity profile may alter assembly. The proposed process layer therefore records mixing geometry, flow history, solvent exchange, purification, concentration, and storage as design variables. It does not assume that a laboratory formulation remains the same product after changes in equipment, throughput, batch size, or cargo.

Intracellular trafficking is the decisive bridge between particle exposure and functional cargo availability. Nanoscale imaging indicates that productive mRNA escape can occur from specific endosomal recycling structures, while high-throughput assays demonstrate that uptake, endosomal disruption, and functional RNA delivery are distinguishable events [19, 20]. A strong uptake signal may therefore coexist with poor cytosolic delivery, and membrane damage may not correspond to intact, translation-competent cargo release. The architecture requires orthogonal measurements of particle uptake, endosomal routing, cytosolic cargo, and protein output, interpreted over time and across relevant cell types. **Table 2** organizes the evidence, constructs, and interpretation boundaries needed to develop lipid composition, particle formation, trafficking, and endosomal escape within the article's central argument.

Table 2. Components, relationships, uncertainties, and validation needs within Lipid composition, particle formation, trafficking, and endosomal escape

Component or layer	Inputs	Core function	Expected output	Uncertainty or limitation	Validation requirement
Ionizable-lipid state	Molecular structure, apparent ionization behavior, molar fraction, degradation characteristics	Supports cargo complexation during formation and membrane interaction under intracellular conditions	Stable particle formation with context-appropriate intracellular release	Structure–function relationships may be formulation-, cargo-, route-, and species-dependent	Controlled chemical-series studies with physicochemical, trafficking, expression, and safety readouts
Helper-lipid and cholesterol state	Phospholipid identity, cholesterol or analogue, relative proportions	Modulates membrane packing, particle morphology, stability, and biological interactions	Reproducible particle architecture compatible with storage and delivery	Nominal composition may not reveal internal organization or polymorphic subpopulations	Orthogonal structural characterization linked prospectively to delivery behavior
PEG-lipid and surface state	PEG-lipid chemistry, anchor length, molar fraction, desorption behavior	Controls aggregation, circulation behavior, surface accessibility, and protein interactions	Appropriate colloidal stability and exposure profile	Excess stabilization may reduce cell interaction or alter intracellular processing	Time-resolved surface, protein-adsorption, biodistribution, and functional-delivery studies
Cargo–lipid assembly interface	mRNA length, folding, chemistry, integrity, impurity state, charge environment	Couples the transcript to lipid assembly and determines encapsulated cargo organization	Protected, structurally competent, releasable mRNA	Reporter cargos may not reproduce therapeutic-cargo assembly or release	Crossed cargo-by-formulation studies rather than separate sequence and carrier screens
Particle-formation process	Mixing rate, geometry, flow ratios, solvent, pH, temperature, buffer exchange	Converts molecular inputs into a nanoparticle population	Controlled size distribution, morphology, encapsulation, and surface state	Scale or equipment changes may alter local mixing and product heterogeneity	Process-characterization studies across scale with physicochemical and functional comparability
Cellular uptake and routing	Particle surface, protein corona, receptor environment, cell state	Determines cell association, internalization, and endosomal pathway selection	Entry into relevant cells and trafficking toward a potentially productive compartment	High uptake may represent sequestration, degradation, or off-target-cell exposure	Cell-resolved uptake and colocalization measurements using relevant biological models
Endosomal escape	Ionizable-lipid behavior, endosomal maturation, membrane interaction, cargo release	Enables intact mRNA to enter the cytosol	Translation-competent cytosolic cargo	Escape is infrequent, spatially restricted, and difficult to measure directly	Orthogonal imaging, cytosolic-cargo, membrane-response, and functional-expression assays
Functional intracellular delivery	Cytosolic cargo integrity, ribosome access, innate sensing, cell viability	Converts delivered mRNA into the intended protein program	Context-appropriate magnitude, duration, and	Expression may conceal toxicity, off-target delivery, or unsuitable kinetics	Time-resolved protein-function, cell-state, viability, and immune-response assessment

	localization of expression

Joint Design Architecture across Cargo and Nanoparticle

The proposed mRNA–Lipid Nanoparticle Co-Design Crucible treats candidate development as an iterative, multi-layer reasoning process rather than a sequential handoff from sequence engineering to formulation. Orthogonal design-of-experiments strategies in mRNA engineering demonstrate that formulation variables can interact and that optimization must remain tied to the relevant cell and functional context [21]. In the proposed construct, cargo identity, cargo quality, lipid composition, manufacturing conditions, particle state, trafficking, biodistribution, and therapeutic utility remain separately represented so that failure can be localized without assuming a single cause. **Figure 1** presents the mrna–lipid nanoparticle co-design crucible, showing how the article's key components and boundaries are connected within joint design architecture across cargo and nanoparticle.

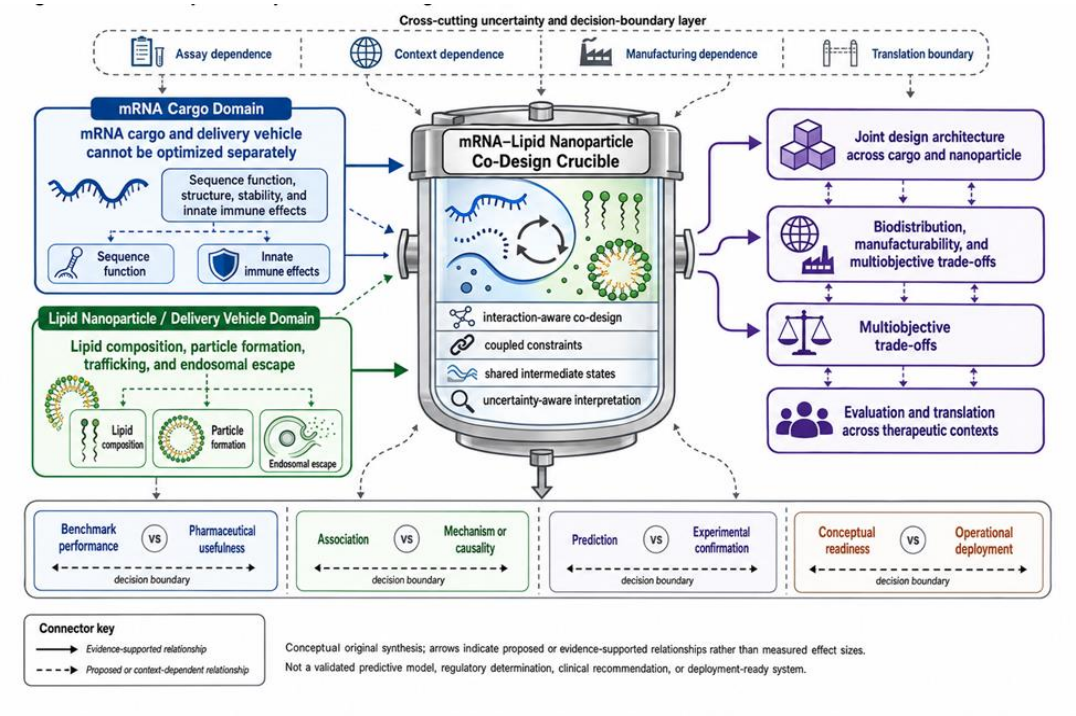

Figure 1. mRNA–Lipid Nanoparticle Co-Design Crucible

The figure is an original conceptual synthesis that organizes the article's central contribution across mRNA cargo and delivery vehicle cannot be optimized separately, sequence function, structure, stability, and innate immune effects, sequence function, innate immune effects, lipid composition, particle formation, trafficking, and endosomal escape, lipid composition. Arrows and grouping indicate proposed or evidence-supported relationships rather than measured effect sizes. The figure does not represent a validated predictive model, regulatory determination, clinical recommendation, or deployment-ready system.

The architecture operates through linked design states and redesign triggers. A cargo proposal enters with declared sequence features, predicted expression behavior, manufactured quality attributes, and uncertainty. A formulation proposal enters with lipid identities, ratios, anticipated ionization and degradation behavior, and process assumptions. These inputs are combined experimentally to determine particle state, trafficking, expression, and biological response. Multiresponse design-of-experiments studies for hepatic mRNA delivery show why the selected formulation depends on balancing several outputs rather than maximizing one measurement [22]. The architecture consequently preserves a vector of outcomes and constraints instead of collapsing them into an opaque composite score.

Computational models can support this process by prioritizing candidates, identifying interactions, and proposing informative experiments. Machine learning combined with combinatorial chemistry has accelerated ionizable-lipid discovery within experimentally defined chemical spaces [23]. Within the proposed architecture, however, model outputs remain hypotheses indexed to their training data, assay conditions, and applicability domain. Candidate ranking does not establish particle formation, productive trafficking, safety, manufacturability, or therapeutic value. Uncertainty should therefore affect experimental priority: high predicted performance with high uncertainty may justify a discriminating experiment, whereas high confidence outside the training domain should not be inferred merely from model output.

Interpretable artificial-intelligence approaches may help expose molecular features associated with delivery and guide subsequent lipid design, but experimental confirmation remains essential [24]. The architecture accordingly distinguishes four levels of computational contribution: descriptive association, predictive prioritization, mechanistic hypothesis generation, and experimentally supported explanation. Advancement between levels requires new evidence rather than stronger language. The

co-design crucible is therefore not an autonomous optimizer. It is a proposed coordination structure that connects computational search to physical characterization, biological testing, process evaluation, and human review. Its central decision product is not a universally best candidate but a traceable set of Pareto-relevant candidates whose strengths, weaknesses, uncertainties, and evidence gaps are explicit.

Biodistribution, Manufacturability, and Multiobjective Trade-Offs

Biodistribution is a design objective because composition can redirect organ-level delivery rather than merely alter total expression. Selective organ-targeting formulations demonstrate that added lipid components can shift the tissue distribution of mRNA nanoparticles [25]. Such results support the possibility of composition-guided targeting, but they do not establish universal organ-specific rules. Distribution depends on route, dose, species, host physiology, disease state, particle properties, and cargo. The architecture therefore distinguishes particle exposure, cargo exposure, target-cell entry, cytosolic release, and protein function. Whole-organ signal is insufficient when the therapeutic objective requires delivery to a specific cell population or when off-target expression carries disproportionate risk.

The synthetic particle acquires a biological identity after administration. Mechanistic work on tissue-specific delivery indicates that protein adsorption and associated biological interactions contribute to organ tropism [26]. This means that biodistribution cannot be modeled exclusively from nominal lipid composition. Host proteins, receptor environments, immune state, and disease-associated physiology may intervene between the manufactured particle and the observed tissue profile. The architecture therefore places biological identity between particle state and organ exposure, with dashed relations when evidence is formulation- or model-specific. A targeting hypothesis is treated as conditionally supported until it is reproduced across relevant doses, biological contexts, and independent measurement methods.

Manufacturability is similarly upstream of therapeutic usefulness. Microfluidic production studies show that flow, mixing, solvent conditions, purification, and payload variables can alter critical nanoparticle attributes [27]. A biologically attractive candidate may therefore be unsuitable if its particle state cannot be reproduced, analytically controlled, concentrated, stored, or transferred between scales. The architecture treats manufacturing settings as causal design variables and requires them to be recorded with the same precision as lipid ratios and sequence features. This prevents a formulation name from concealing process-dependent differences and reduces the risk that later process changes create a materially different product while retaining the same nominal composition.

Throughput scaling can preserve selected particle properties and biological potency within a defined platform, but comparability must be demonstrated rather than presumed [28]. Multiobjective co-design therefore includes expression, tissue selectivity, innate-immune compatibility, trafficking, safety, stability, scalability, batch reproducibility, and analytical controllability. These objectives may conflict. Increased surface shielding may improve colloidal stability while reducing cell interaction; stronger membrane activity may improve escape while narrowing tolerability; highly specialized targeting may increase process complexity. The architecture proposes Pareto-based comparison rather than a single weighted score, because objective weights are context dependent and can conceal unacceptable failure in a safety- or manufacturing-critical dimension.

Evaluation and Translation across Therapeutic Contexts

Protein-replacement applications require a cargo–LNP system that delivers expression to an appropriate organ and sustains functional protein production for a clinically meaningful interval. Systemic factor IX mRNA delivery illustrates the connection between liver exposure, translated protein, and replacement function in a preclinical context [29]. Evaluation should therefore extend beyond reporter expression to protein identity, activity, duration, target-cell localization, dose–response behavior, repeat-dose compatibility, and process consistency. These requirements do not establish clinical effectiveness, but they define the evidence chain needed to interpret whether a co-designed candidate is relevant to replacement therapy rather than merely capable of transient expression.

Metabolic-disease applications further require disease-linked functional evidence. Experimental mRNA treatment for acute intermittent porphyria connects hepatic delivery and enzyme production to correction of disease-relevant biology in preclinical models [30]. This type of evidence is stronger than expression alone because it tests whether the encoded product performs its intended biological role. Nevertheless, model-specific correction does not establish human efficacy, long-term safety, or generalizability to other proteins. Within the architecture, therapeutic function is therefore an additional layer after exposure and expression, not a substitute for them. Discordant findings should remain visible—for example, adequate protein expression with insufficient biochemical correction or acceptable efficacy with an unsuitable dosing burden.

In vivo gene editing imposes a different evaluation structure because delivery must coordinate multiple cargos and the downstream genomic change may be durable or irreversible. Early clinical evidence for systemic CRISPR–Cas9 delivery in transthyretin amyloidosis demonstrates the translational importance of target-cell exposure, co-delivery, editing confirmation, and clinically interpretable downstream effects [31]. For such applications, transient expression cannot be treated as the final endpoint. Evaluation must include editing specificity, distribution of editing across relevant cell populations, persistence, off-target assessment, immunogenicity, and long-term follow-up. The architecture can organize these requirements but cannot determine acceptable risk or replace clinical, ethical, and regulatory judgment.

Immune modulation illustrates why desired biological responses cannot be generalized across therapeutic contexts. A noninflammatory mRNA vaccine strategy in experimental autoimmunity shows that antigen expression may be designed to promote tolerance rather than immunogenic activation [32]. Thus, the same innate signal that supports prophylactic vaccination

could undermine a tolerizing intervention. Evaluation should be indexed to therapeutic intent and should distinguish analytical quality, cellular delivery, tissue exposure, immune phenotype, functional outcome, manufacturing robustness, and readiness for prospective study. **Table 3** organizes the evidence, constructs, and interpretation boundaries needed to develop evaluation and translation across therapeutic contexts within the article's central argument.

Table 3. Evaluation, implementation, and research priorities arising from Co-Designing mRNA Cargo and Lipid Nanoparticles across Sequence Function, Intracellular Trafficking, Biodistribution

Evaluation dimension	What must be tested	Suitable evidence or assessment	Failure signal	Interpretive limitation	Decision relevance
Cargo identity and manufactured quality	Whether the intended transcript is intact, correctly configured, and acceptably free of process-derived impurities	Orthogonal identity, integrity, cap, poly(A), residual impurity, and double-stranded RNA assessments	Identity mismatch, degradation, heterogeneous integrity, or uncontrolled impurity burden	Analytical conformity does not establish delivery, translation, or therapeutic function	Determines whether biological testing evaluates the intended cargo product
Particle state and batch reproducibility	Whether composition and process produce a consistent nanoparticle population	Size distribution, morphology, encapsulation, composition, surface, stability, and batch-variance measurements	Uncontrolled heterogeneity, instability, cargo leakage, or scale-dependent particle change	Physicochemical similarity does not alone prove functional comparability	Supports formulation selection, process control, and scale-transition decisions
Productive intracellular delivery	Whether internalized particles release intact, translation-competent mRNA into the cytosol	Orthogonal uptake, trafficking, endosomal-response, cytosolic-cargo, and expression assays	Strong uptake without cytosolic cargo or functional protein production	Escape assays may be indirect, formulation specific, or perturbative	Distinguishes a trafficking failure from a sequence or translation failure
Expression kinetics and localization	Whether protein magnitude, duration, and location match the intended application	Time-resolved protein abundance, cell-resolved localization, and functional-protein assays	Excessively brief, prolonged, off-target, or nonfunctional expression	Reporter behavior may not transfer to a therapeutic protein	Supports context-specific dose, cargo, and formulation comparison
Biodistribution and target-cell exposure	Whether particle, cargo, and expressed product reach the required organs and cell populations	Time-resolved organ and cell measurements, biological-identity characterization, and off-target assessment	Whole-organ exposure without target-cell delivery or unacceptable off-target localization	Species, dose, route, and disease state can shift distribution	Determines whether a candidate merits disease-relevant functional testing
Innate, adaptive, and repeat-dose response	Whether the immune profile matches therapeutic intent and remains acceptable across the intended regimen	Cytokine and innate-sensing panels, adaptive responses, complement, anti-carrier responses, pathology, and recovery	Unwanted inflammation, loss of activity, hypersensitivity, or cumulative tissue injury	Preclinical immune findings do not establish clinical tolerability	Informs benefit–risk assessment and suitability for repeated administration
Therapeutic-context function	Whether delivered expression produces the intended biological or disease-relevant effect	Functional protein assays, disease-relevant biomarkers, editing confirmation, immune phenotype, or cell-product potency	Adequate expression without functional correction or desired biological action	Model-specific benefit does not prove clinical utility	Separates molecular plausibility from therapeutic relevance
Manufacturability and comparability	Whether the system remains controlled across throughput, equipment, site,	Process characterization, scale studies, stability, analytical comparability, and	Scale-dependent change, unstable storage, excessive variation, or loss of potency	One platform's manufacturing solution may not transfer to another	Determines whether a candidate is suitable for continued

	storage, and batch	functional-potency testing			pharmaceutical development
Computational model validity	Whether predictions remain reliable within the intended design and assay domain	Prospective testing, external validation, calibration, uncertainty analysis, and out-of-domain challenges	Confident failure on new cargo, lipid, process, route, or biological context	Retrospective benchmark performance does not establish prospective usefulness	Governs whether model outputs can prioritize experiments or only generate hypotheses
Translation readiness	Whether the combined evidence supports the next bounded development step	Integrated evidence review with prespecified use case, uncertainties, stop/go criteria, and human oversight	Advancement based on one favorable endpoint despite unresolved critical failures	No universal threshold establishes clinical or regulatory readiness	Supports traceable research decisions without replacing expert or regulatory judgment

Figure 2 presents the evidence, validation, and translation boundary observatory for co-designing mrna cargo and lipid nanoparticles across, showing how the article's key components and boundaries are connected within evaluation and translation across therapeutic contexts.

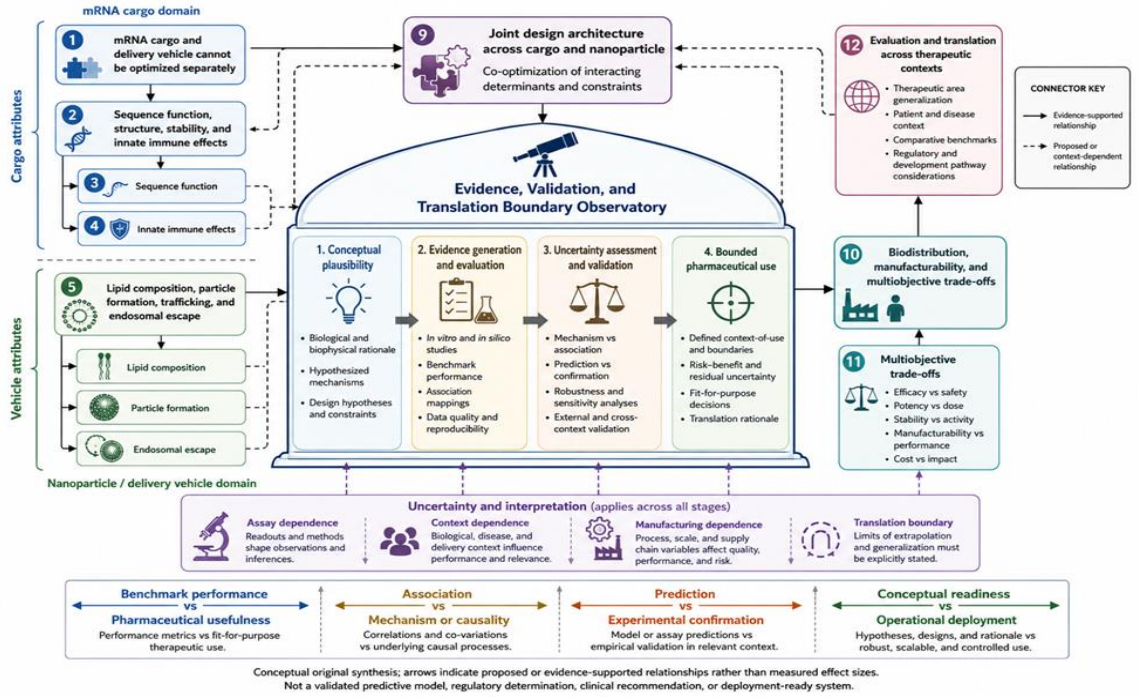

Figure 2. Evidence, Validation, and Translation Boundaries

The figure is an original conceptual synthesis that shows how claims move from conceptual plausibility through evaluation, uncertainty assessment, and bounded pharmaceutical use. Arrows and grouping indicate proposed or evidence-supported relationships rather than measured effect sizes. The figure does not represent a validated predictive model, regulatory determination, clinical recommendation, or deployment-ready system.

Limitations and Boundary Conditions

The proposed architecture is limited by the evidence from which its relationships are inferred. The selected literature contains heterogeneous cargos, lipid chemistries, manufacturing platforms, routes, biological models, assays, and therapeutic objectives. Relationships that appear consistent across several contexts may still fail when cargo length, protein identity, species, disease state, or process changes. Computational design reviews also indicate that training-domain and validation limits remain central to the interpretation of mRNA models [15]. The architecture therefore cannot supply universal feature weights, mechanistic coefficients, formulation rules, or acceptance thresholds. It organizes questions and dependencies; it does not quantify them without context-specific data.

Measurement limitations are especially important for intracellular delivery. Uptake, endosomal disruption, cytosolic cargo, translation, and cell function can generate discordant signals, and no single assay establishes the complete pathway. High-throughput escape measurements can improve experimental resolution, but their outputs remain assay dependent and require orthogonal confirmation [20]. Similar limitations apply to biodistribution and manufacturing: whole-organ measurements can obscure cell-level exposure, while apparently comparable physicochemical properties may conceal differences in biological

potency. The architecture therefore favors convergent evidence and explicit uncertainty rather than declaring one assay or attribute a universal surrogate for therapeutic performance.

The contribution is also bounded by its intended use. It is not a clinical recommendation, regulatory submission framework, autonomous laboratory controller, or validated product-selection algorithm. Manufacturing studies show that comparability and scale relationships must be demonstrated within each platform [28]. Human oversight remains necessary when objectives conflict, evidence is incomplete, or irreversible consequences are possible. Misuse would include converting the architecture into an unvalidated composite score, treating model confidence as calibrated uncertainty, assuming causal mechanisms from associations, or advancing a candidate because it performs well in one domain while unresolved safety, targeting, functional, or manufacturing failures remain.

Research Agenda and Implementation Priorities

The first research priority is to generate crossed datasets in which cargo, formulation, and process variables are changed together. Orthogonal design-of-experiments approaches provide a starting point for identifying interactions, but future studies should incorporate sequence architecture, cargo quality, lipid chemistry, mixing conditions, particle state, trafficking, expression kinetics, immune response, and manufacturing robustness within the same experimental logic [21]. Negative results and failed candidates should be retained because they define the boundaries of the design space. Prospective study plans should specify which interactions are hypothesized, which outcomes are primary, and which discordant findings trigger redesign.

The second priority is measurement standardization and evidence infrastructure. Process variables that influence microfluidic manufacturing should be recorded alongside cargo and formulation metadata rather than relegated to incomplete methods descriptions [27]. Shared datasets should include raw and processed measurements, assay conditions, material provenance, batch identifiers, negative data, uncertainty, and links between analytical, cellular, organ-level, and functional outcomes. Standardized reporting should distinguish nominal composition from measured particle state, uptake from productive release, organ exposure from target-cell function, and laboratory scale from manufacturing scale. Such infrastructure would improve both experimental reproducibility and the interpretability of computational models.

The third priority is staged external validation across therapeutic contexts. Organ-targeting hypotheses should be tested across route, dose, species, disease state, biological identity, and independent laboratories rather than inferred from one formulation–model pairing [25]. Computational tools should undergo prospective and out-of-domain evaluation before they influence candidate advancement. Implementation should proceed from retrospective evidence mapping, to prospective single-site co-design studies, to multi-site replication, and only then to bounded decision support. At each stage, stop/go criteria, uncertainty, human review, and the consequences of false advancement or false rejection should be recorded. This sequence tests whether the proposed architecture improves reasoning without presuming that it does.

Conclusion

Co-designing mRNA cargo and lipid nanoparticles requires a shift from separate sequence and formulation optimization toward a coupled pharmaceutical architecture. The proposed mRNA–Lipid Nanoparticle Co-Design Crucible links cargo identity and quality, lipid composition, particle formation, intracellular trafficking, biodistribution, manufacturing state, and therapeutic-context utility while preserving the uncertainty and evidence boundaries between them. Its central contribution is not a universal optimizer or validated decision rule, but a structured means of identifying interactions, localizing failures, planning discriminating experiments, and comparing candidates across multiple objectives without allowing one favorable metric to stand in for pharmaceutical usefulness. The architecture remains conditional on assay quality, context-specific validation, process control, and human interpretation. Its practical value will depend on prospective testing, transparent reporting, negative-data retention, and external replication across

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

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