



Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Endosomal Escape Is a Systems Bottleneck Rather Than a Single Material Property

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ABSTRACT

Endosomal escape is often treated as though it were a scalar characteristic of a delivery material: a carrier is described as having high or low escape efficiency, and formulation optimization is subsequently organized around maximizing that property. This interpretation compresses several biologically distinct events into one label. Productive cytosolic delivery requires cellular internalization, entry into a permissive intracellular trajectory, appropriate carrier restructuring, timely cargo-carrier dissociation, interaction with an endosomal membrane, sufficient membrane destabilization without disproportionate injury, and survival of competing recycling and degradative pathways. Failure at any one of these transitions can produce the appearance of poor “escape,” even when the material itself retains membrane-active capacity. Conversely, strong membrane perturbation can occur without efficient liberation of usable cargo. This Perspective therefore reframes endosomal escape as a systems bottleneck generated by the alignment of carrier state, cargo state, compartment identity, cell type, dose, and time. Evidence from quantitative localization, split-luciferase assays, galectin reporters, nanoscale imaging, lipid-nanoparticle structural studies, and triggered endosomal-release systems is used to separate measurable states that are frequently conflated. A proposed systems model is developed in which productive escape is an emergent transition rather than a universal material constant. The resulting framework changes how escape should be measured, compared, optimized, and validated in nucleic-acid delivery.

Keywords: Endosomal escape, Lipid nanoparticles, Cytosolic delivery, Intracellular trafficking, Cargo release, RNA delivery

Received: 27 March 2025; **Revised:** 20 June 2025; **Accepted:** 24 June 2025

INTRODUCTION

The clinical and preclinical success of nucleic-acid therapeutics has intensified interest in lipid nanoparticles (LNPs), polymeric carriers, peptide-enabled systems, and related nonviral delivery technologies. Yet delivery performance is controlled by a sequence of extracellular, tissue, cellular, and intracellular barriers rather than by formulation chemistry alone [1-3]. For RNA systems in particular, accumulation at the tissue of interest, cellular uptake, intracellular routing, release from endosomal compartments, persistence of intact cargo, and downstream translation or silencing all occupy different biological scales. A carrier can therefore perform well at one stage and poorly at another without the discrepancy being visible in a single aggregate efficacy readout.

Endosomal escape is especially vulnerable to this compression. It is frequently described as a property of an ionizable lipid, polymer, peptide, lipid phase, or membrane-active motif, implying that material ranking should

remain relatively stable across cells and cargos. Quantitative mRNA-LNP studies challenge that assumption. In tested formulations, only a small fraction of internalized material reached the cytosol, while cytosolic mRNA abundance did not translate into protein expression in a simple proportional manner [4]. This observation does not show that escape is unimportant. It shows that “escape” itself sits within a larger chain of conditional transitions.

The central argument of this Perspective is therefore that endosomal escape is more productively treated as a systems bottleneck than as a single material property. The term is used here to describe an emergent constraint produced by the alignment—or misalignment—of carrier state, cargo state, compartment identity, membrane state, cell biology, dose, and time. This proposed interpretation distinguishes at least four questions that are commonly merged: whether a carrier entered the cell; whether it reached an intracellular compartment from which release was possible; whether the carrier and cargo separated appropriately; and whether free, functional cargo actually appeared in the cytosol.

This distinction also changes the evidential standard for escape claims. A membrane-damage reporter, a colocalization image, an uptake measurement, a functional-expression assay, and a direct cytosolic-cargo assay do not measure the same event. Their disagreement is therefore not necessarily experimental noise. In many cases it is information about where the delivery system is failing.

Cellular entry does not equal cytosolic delivery

Cellular uptake is a prerequisite for intracellular delivery, but it is not evidence that a therapeutic cargo has reached the cytosol. Endocytic entry itself is heterogeneous, and route assignments can depend strongly on experimental method, nanoparticle properties, cell state, and the temporal window of observation [5]. A formulation associated with high cellular fluorescence may therefore represent surface-bound material, internalized carrier distributed across several compartments, partially degraded cargo, or productive cytosolic delivery. Collapsing these states into “uptake” obscures the very transition that endosomal-escape studies are intended to resolve.

Quantitative analysis of cell-penetrating peptides illustrates the problem directly. Increased cell association can increase absolute cytosolic delivery while leaving the fraction of internalized material that escapes comparatively unchanged [6]. In such a case, a formulation can appear superior because it delivers more material to endosomes rather than because its intrinsic probability of cytosolic release is higher. This distinction is important for dose scaling. A system that depends on overwhelming an inefficient intracellular transition with greater uptake may behave differently from one that improves productive release per internalized cargo molecule.

Compartment-specific sensors provide another layer of resolution. SNAPSwitch-type approaches were developed to quantify whether proteins, DNA, or nanoparticle-associated cargo reach specific intracellular locations rather than merely appearing inside the cell [7]. Such measurements narrow the inferential gap between entry and productive localization, although localization by itself still does not establish biological function.

High-content galectin assays extend this decomposition by measuring uptake, endosomal membrane perturbation, and downstream RNA delivery as related but nonidentical outputs [8]. Their value lies partly in the divergences they reveal. A carrier that enters efficiently need not produce extensive detectable endosomal damage; a carrier producing membrane perturbation need not generate proportionate functional RNA delivery; and the same formulation can behave differently across cell lines.

The first systems principle is therefore straightforward: cellular entry defines exposure to the intracellular trafficking network, not successful escape from it. A meaningful escape metric must be normalized to what actually entered the relevant intracellular compartments and must distinguish absolute cytosolic delivery from the fractional efficiency of crossing the endosomal boundary.

Endosomal maturation

Once internalized, a delivery carrier encounters an evolving compartment rather than a static vesicle. Endosomal identity changes through acidification, membrane remodeling, acquisition and loss of trafficking proteins, recycling, fusion, and progression toward lysosomal degradation. The carrier is simultaneously changing. Ionizable lipids alter protonation state, polymeric materials may reorganize or swell, lipid phases can rearrange, cargo can become more or less tightly associated with the carrier, and membrane contacts change as the compartment matures.

The physicochemical environment of these compartments can be measured directly. Fluorescence-lifetime approaches have resolved subcellular pH at the level of individual vesicles and have shown why assumed mechanisms require experimental testing rather than inference from formulation chemistry [9]. In one such

analysis, polyethyleneimine did not produce the vesicular-pH change expected from a simple proton-sponge explanation under the tested conditions. The appropriate conclusion is not that all proton-sponge mechanisms are impossible, but that endosomal pH should be treated as an experimentally observable state variable rather than an assumed consequence of a material label.

Maturation also creates distinct release opportunities. Nanoscale imaging has shown that delivered mRNA can escape from recycling-associated endosomal tubules [10]. This observation is important because it weakens a simplified model in which productive escape occurs only after progressive maturation toward a late endosomal state. It instead suggests that intracellular routing can generate transient spatial states in which carrier restructuring, membrane geometry, and cargo availability become favorable for release.

Recycling can therefore act as more than a neutral return pathway. Pharmacological inhibition of endocytic recycling with endosidine 5 increased delivered mRNA expression in experimental cell systems and in mice through an Annexin-A6-associated mechanism [11]. The result does not establish recycling inhibition as a general delivery strategy, but it demonstrates that a trafficking pathway can compete with productive delivery and that altering the route can change functional output without changing the underlying cargo sequence.

Endosomal state can also be pharmacologically manipulated within the formulation itself. Incorporation of esomeprazole into an mRNA-LNP cancer-vaccine system modified endolysosomal conditions and improved performance in the reported preclinical context [12]. Because such interventions can simultaneously affect pH, membrane properties, trafficking, stress responses, and tumor biology, their benefit should not be reduced automatically to an isolated increase in “escape efficiency.”

These findings suggest a second systems principle: an internalized carrier does not encounter one endosome but a trajectory of compartment states, some of which may favor recycling, degradation, carrier disassembly, membrane interaction, or productive release. Escape probability is consequently conditioned by when and where the carrier reaches a permissive state.

Cargo-carrier dissociation

Membrane crossing cannot be interpreted independently of cargo-carrier dissociation. For a nucleic-acid payload to become pharmacologically available, the carrier must do more than destabilize an endosomal membrane. It must also reorganize sufficiently for the cargo to become separable from the lipid, polymer, peptide, or supramolecular structure that protected it during earlier stages of delivery.

LNP internal structure is not fixed after formulation. Biophysical studies of siRNA-LNPs have shown that inverted lipid-phase organization can be associated with enhanced transfection [13]. Such findings demonstrate that internal carrier architecture is functionally relevant, but they do not establish that a particular structural phase is itself equivalent to cytosolic escape. Structural reorganization may instead alter several coupled processes, including membrane interaction, lipid mixing, particle disassembly, and exposure of the nucleic acid to the surrounding aqueous phase.

Direct intracellular imaging provides stronger evidence for separating these events. Live-cell and super-resolution analyses of RNA-LNP delivery have visualized disintegration of the carrier, redistribution of ionizable lipids, segregation of RNA from lipid components, membrane damage, and cytosolic RNA release as distinguishable processes [14]. Importantly, detectable endosomal damage did not map one-to-one onto detectable release of encapsulated RNA. Some damaged compartments contained little or no observable liberated cargo, demonstrating that membrane perturbation alone does not guarantee productive cargo release.

The relevant mechanistic unit is therefore not simply “carrier escapes” or “endosome ruptures.” It is the alignment of at least three evolving states: the biological compartment must remain permissive; the carrier must reorganize sufficiently; and the cargo must become available for liberation. This Perspective terms the overlap of these conditions the proposed productive-dissociation window. The term is not an experimentally validated universal state. It is a conceptual device for distinguishing productive restructuring from premature disassembly, incomplete separation, or membrane perturbation that occurs while the cargo remains effectively sequestered.

The state map below is needed because these processes run partly in parallel rather than as a single linear sequence.

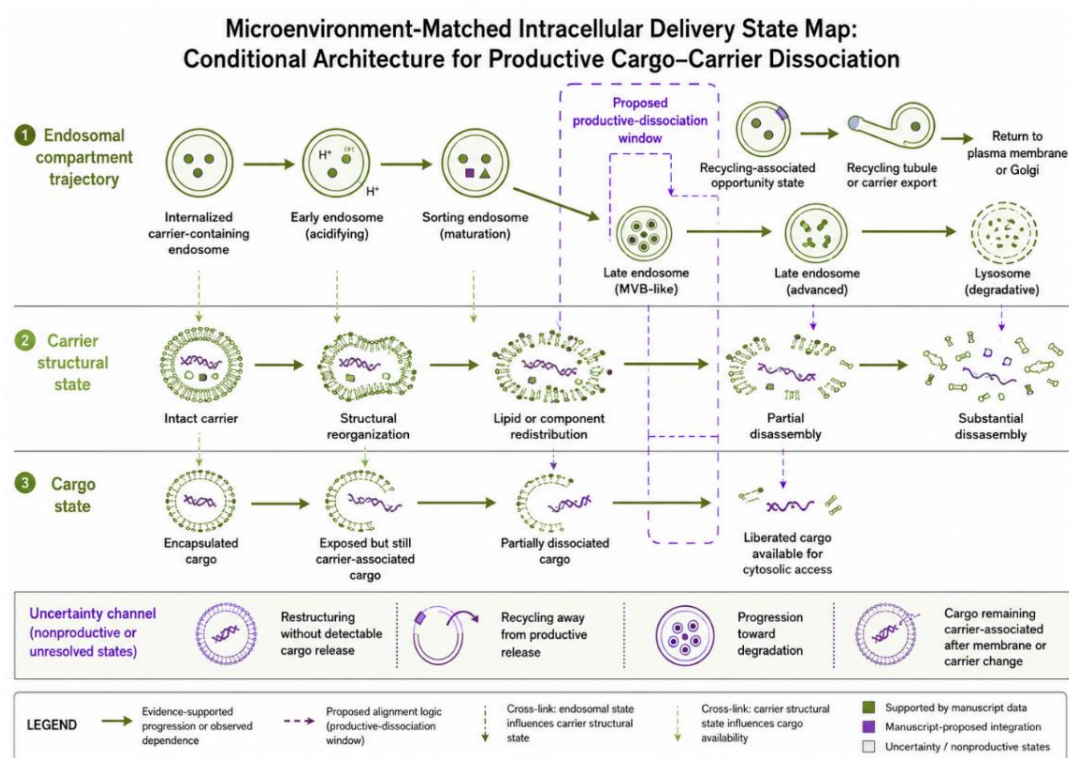


Figure 1. Cargo liberation depends on alignment between compartment trajectory, carrier restructuring, and cargo state

Membrane interaction and destabilization

The next transition occurs at the interface between the evolving carrier and the endosomal membrane. Ionizable lipids, sterols, peptides, polymers, and associated cargo can change the physical organization of that interface, but the membrane itself is biologically heterogeneous. Lipid domains, curvature, protein composition, charge, compartment identity, and maturation state vary over time. A formulation therefore does not possess a membrane-destabilizing effect independently of the membrane it encounters.

Recent analyses of mRNA-LNP delivery have emphasized the possible role of lipid-raft organization and related membrane-domain behavior in controlling intracellular release [15]. The evidence remains heterogeneous, so lipid rafts should not be elevated to a universal escape mechanism. Their broader importance is conceptual: membrane interaction is created jointly by carrier composition and biological membrane state.

Engineered triggered systems reinforce this point by showing that cytosolic release can be increased through mechanistically distinct interventions. De novo pH-responsive peptides can become more membrane-active under endosomal conditions; porphyrin-containing LNPs can use light-generated reactive processes to initiate release; and photoresponsive nanomaterials can mechanically perturb endolysosomal membranes [16–18]. These approaches are not interchangeable and should not be interpreted as evidence for one general rupture mechanism. Instead, they demonstrate experimentally that membrane destabilization can be manipulated independently and that its effectiveness depends on the trigger, material, cargo, and cellular environment.

The membrane event also has to be synchronized with carrier disassembly. Split-luciferase quantification of pH-responsive nanoparticles has shown that changing how cargo is linked to the carrier can change measured cytosolic delivery, with a cleavable disulfide-linked configuration producing greater escape in the tested system than a more stable linkage [19]. Thus, even when a carrier reaches a membrane-active state, productive delivery can still be limited by cargo retention.

For design purposes, membrane interaction should therefore be decomposed into a cross-scale sequence: the carrier reaches a specific biological compartment; its structure and charge state evolve; the carrier engages the local membrane; a degree of destabilization occurs; and cargo must be available for release. None of these states should be substituted for the next one. The relevant question is not whether a material can disrupt a membrane under some condition, but whether it can do so in the correct compartment, at the correct time, at a usable dose, while the cargo is in a releasable state.

The distinctions are easier to evaluate as cross-scale transitions than as a list of membrane-active material classes.

Table 1. Cross-scale tests that separate membrane destabilization from productive cytosolic release

Biological-compartment transition	Context	Boundary	Interpretation	Validation
Scientific anchor	Carrier state at the transition	Dominant barrier Transferability boundary	Dose dependence that must remain explicit	Discriminating experiment Article-level systems test
Internalized carrier enters an acidifying sorting endosome	Largely intact carrier beginning physicochemical reorganization	Dominant barrier or competing sink Continued sorting without productive restructuring Transferability boundary Behavior in one cell type or formulation does not establish the same endosomal state elsewhere	Carrier amount can change the number of exposed compartments without changing fractional productive release	Discriminating experiment Pair compartment-resolved localization with a direct cytosolic-cargo measurement Article-level systems test Does greater intracellular exposure increase productive release per internalized cargo, or only the size of the endosomal pool?
Maturing compartment encounters a structurally reorganizing carrier	Lipid, polymer, or peptide components become more membrane-interactive	Dominant barrier or competing sink Carrier remains intact enough to retain cargo or progresses toward degradation Transferability boundary Artificial membrane activity cannot be transferred automatically to intact cells	Trigger-responsive behavior may depend on local concentration and maturation state	Discriminating experiment Measure carrier restructuring and membrane state in the same temporal window Article-level systems test Is the putative membrane-active state reached before cargo is degraded or irreversibly retained?
Recycling-associated compartment creates a transient release opportunity	Carrier partially reorganized while trafficking remains dynamic	Dominant barrier or competing sink Recycling removes the carrier from a productive release trajectory Transferability boundary A recycling-associated route in one mRNA-LNP system is not a universal escape pathway	Increasing dose may increase recycling flux as well as potential release events	Discriminating experiment Resolve compartment identity while measuring appearance of free cytosolic cargo Article-level systems test Does rerouting change fractional cytosolic delivery independently of total uptake?
Carrier establishes direct contact with an endosomal membrane	Membrane-active components are exposed or protonated	Dominant barrier or competing sink Contact occurs without sufficient destabilization Transferability boundary Model membranes do not reproduce endosomal protein, lipid, geometry, or repair context	Membrane interaction may rise with carrier abundance without crossing a productive threshold	Discriminating experiment Combine membrane-interaction readout with compartment-resolved cargo-release assay Article-level systems test Does membrane contact predict cargo liberation in intact cells rather than only biophysical membrane activity?
Endosomal membrane becomes detectably perturbed	Carrier may be partially disassembled, reorganized, or still cargo-bearing	Dominant barrier or competing sink Perturbation occurs while cargo remains sequestered Transferability boundary Damage-reporter intensity is not equivalent to escaped-cargo fraction	More extensive perturbation need not produce proportional release	Discriminating experiment Acquire membrane-perturbation and direct cytosolic-cargo measurements independently Article-level systems test Does a stronger perturbation signal increase usable cytosolic cargo, or only membrane injury?

Carrier disassembly exposes cargo at the membrane interface	Cargo becomes partly dissociated but may remain lipid-, polymer-, or peptide-associated	Dominant barrier or competing sink Incomplete cargo–carrier separation Transferability boundary Linker or carrier chemistry effects are construct-specific	Dose changes both carrier–membrane encounters and the quantity of retained cargo	Discriminating experiment Manipulate cargo linkage while holding upstream uptake as constant as practicable Article-level systems test Is the limiting step membrane crossing, cargo dissociation, or both?
Cargo becomes free at the cytosolic boundary	Carrier no longer fully determines cargo motion	Dominant barrier or competing sink Downstream sequestration, degradation, aggregation, or inefficient utilization Transferability boundary Cytosolic appearance does not guarantee identical downstream function across cargos	Absolute cytosolic amount and fractional release should be distinguished	Discriminating experiment Measure free cytosolic cargo separately from expression or silencing Article-level systems test Does the membrane event generate pharmacologically available cargo rather than merely detectable redistribution?

Note. The final column expresses the article’s proposed systems-level discriminating tests. The table deliberately avoids numerical thresholds or rankings because none is transferable across the carrier, cargo, cell, dose, and assay contexts considered here.

Escape efficiency versus cellular damage

Membrane destabilization is useful only if it produces enough cytosolic delivery to justify its biological cost. This requirement complicates the common strategy of optimizing formulations toward stronger endosomal perturbation. Endosomal membranes are functional cellular structures, and their disruption recruits repair machinery, alters trafficking, can expose luminal contents to the cytosol, and may trigger stress or death pathways. Kinetic studies of LNP-induced endosomal disruption demonstrate that the timing and extent of membrane injury can vary substantially with formulation and can track differences in cellular toxicity [20]. Importantly, increased perturbation does not guarantee proportionate functional expression. This creates a design problem that cannot be solved by ranking materials solely according to damage-reporter intensity.

Peptide- and protein-based escape strategies illustrate the same tension. Their membrane activity can improve cytosolic access, but efficacy must be interpreted alongside membrane selectivity, concentration dependence, cargo compatibility, and toxicity [21]. The relevant optimization target is therefore not maximum membrane activity. It is sufficient and appropriately localized activity within a broader safety window.

Temporal behavior also matters. In a shielded siRNA-delivery system, substantial cellular internalization could coexist with delayed or limited release, while modulation of shielding altered both toxicity and delivery behavior [22]. This emphasizes that escape efficiency is not a static percentage detached from time. Two carriers with similar eventual cytosolic delivery could expose cells to very different durations or intensities of endosomal stress. Galectin recruitment is particularly informative here because it reports loss of membrane integrity. Phytosterol-containing LNPs have produced markedly more Galectin-8-positive endosomal events than cholesterol-containing comparators [23]. Those data demonstrate formulation-dependent differences in detectable membrane perturbation. They do not justify translating a fold-change in galectin events into an equivalent fold-change in escaped cargo. Direct cargo-fate measurements show why that conversion is unsafe: membrane damage and cargo liberation can diverge.

A useful escape metric must therefore contain at least two axes. One is productive cytosolic delivery: how much intact, usable cargo reaches the cytosol relative to what entered the relevant intracellular pathway. The other is biological cost: what membrane injury, stress, trafficking disruption, or loss of viability accompanies that delivery. The optimal point may differ by cargo, target cell, therapeutic window, and required duration of expression. This is one reason a universal “high-escape material” category is unlikely to be sufficient.

Intracellular trafficking competition

Even after a carrier displays favorable membrane interaction, intracellular trafficking remains competitive. Endosomes can recycle, mature, fuse, fragment, form tubules, deliver material toward lysosomes, or interact with other organelles. Carrier components can separate from cargo and follow different routes. Some cargo may reach

the cytosol while another fraction of the same administered dose remains trapped or is degraded. Productive delivery is therefore a probabilistic outcome of competing intracellular states rather than a deterministic consequence of entry.

Carrier nanostructure can alter those trajectories. Replacing cholesterol with naturally occurring sterol analogues has produced polymorphic LNP morphologies together with enhanced intracellular mRNA delivery [24]. These structure–function relationships show that carrier architecture influences what happens after uptake, but the precise intermediate mechanism cannot be inferred solely from morphology. A differently shaped or internally reorganized particle may alter endosomal sorting, membrane contact, disassembly, cargo retention, or several processes simultaneously.

The broader nonviral-delivery literature reinforces this mechanistic plurality. Reviews of cellular trafficking have identified numerous proposed routes of endosomal release and repeatedly highlighted the weakness of assays that infer escape from downstream function or bulk localization [25]. This creates an important interpretive asymmetry: a functional outcome can establish that at least some cargo successfully traversed the intracellular pathway, but it cannot by itself identify which endosomal transition was responsible.

Polymeric nanoparticles provide a particularly useful historical example because multiple escape mechanisms have been invoked for them, including osmotic effects, membrane interactions, polymer rearrangement, and other context-dependent processes [26]. The resulting “nanoescapology” literature is valuable not because one mechanism emerged as universally correct, but because it demonstrates how easily a complex intracellular outcome can be attributed to a favored material property without direct state-resolved evidence.

Recent interpretation of LNP escape goes even further by proposing that membrane crossing may not be the final intracellular bottleneck. One 2025 Perspective has argued for vesicle-budding-and-collapse-related release and for subsequent dissolution of lipid–nucleic-acid aggregates as an additional step required before cargo becomes fully available [27]. These ideas should remain explicitly hypothesis-level where direct validation is incomplete. Nevertheless, they expose an important weakness in the conventional endpoint: detecting material on the cytosolic side of an endosomal membrane does not necessarily establish that the cargo is chemically and physically available for downstream action.

The systems interpretation that follows is therefore broader than membrane escape alone. Intracellular delivery contains competing sinks—recycling, degradation, persistent carrier association, nonproductive membrane perturbation, and potentially post-escape sequestration—as well as productive routes. Which pathway dominates depends on carrier composition, cargo chemistry, compartment state, cell biology, dose, and time. A material can therefore change from productive to nonproductive without any alteration in its nominal chemical identity. Endosomal escape, under this interpretation, is not a fixed property carried into the cell by the formulation; it is the outcome of a conditional intracellular state trajectory.

Cell-type and cargo dependence

Escape measurements become difficult to generalize when the cell itself changes the delivery problem. Single-cell quantification of siRNA has shown substantial heterogeneity in cytosolic dose even among cells exposed to the same extracellular formulation, with cytosolic abundance relating to downstream knockdown at the individual-cell level [28]. Nominal dose therefore cannot be assumed to represent a uniform intracellular dose. Differences in uptake, trafficking capacity, endosomal maturation, membrane repair, metabolic state, and intracellular degradation can change the fraction of cells that reach a productive state.

Material comparisons are similarly context dependent. Screening of polymer libraries has demonstrated that endosomal escape can vary independently of uptake and other delivery characteristics [29]. A polymer that performs well because it enters cells efficiently is not necessarily one that converts internalized cargo efficiently into a cytosolic pool. Direct escape assays therefore provide information that conventional transfection rankings can conceal.

Quantitative split-luciferase approaches reinforce this point for pH-responsive nanoparticles. SLEEQ can distinguish cytosolic access from bulk internalization and show that material response under endosomal conditions changes fractional escape [30]. Yet an escape fraction obtained in one reporter cell line is not a universal constant of the material. Changing membrane composition, endosomal physiology, cargo size, carrier–cargo binding, or exposure time can move the system into another delivery regime.

Purpose-built architectures can overcome specific intracellular constraints. A Trojan-horse strategy has, for example, increased cytosolic nucleic-acid delivery in the engineered system in which it was tested [31]. Such

results establish that endosomal escape is designable, but not that a given escape mechanism will remain superior across cargos, tissues, or disease contexts.

This Perspective therefore proposes a cell–cargo decision boundary. On one side, carrier state, compartment state, cargo dissociation, membrane interaction, and cellular physiology align sufficiently to generate productive cytosolic delivery. On the other, one or more variables redirect the dose toward recycling, degradation, persistent carrier association, membrane injury without cargo release, or other nonproductive states. The boundary is not a universal threshold. It represents a context-dependent transition that can shift with cell type, cargo identity, carrier composition, dose, and time.

The resulting architecture is better represented as nested exposure states than as a single escape percentage.

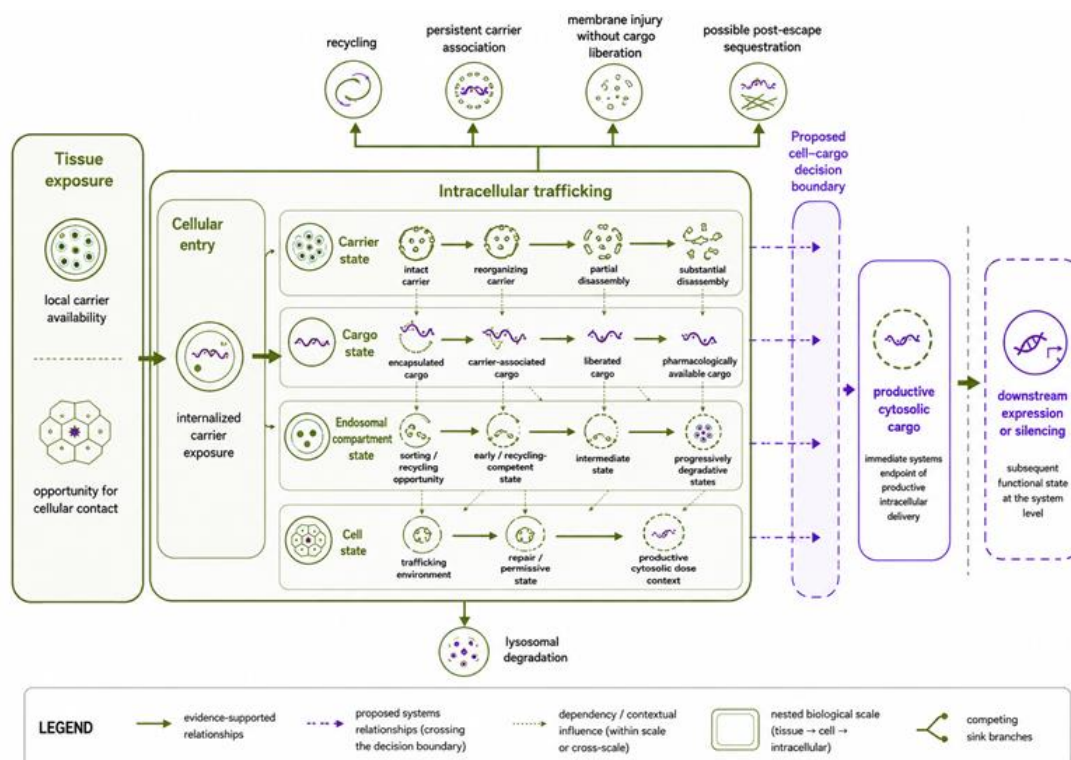


Figure 2. Productive escape emerges only after tissue exposure, cellular entry, and intracellular state become mutually permissive

The endosomal-escape systems model

The preceding evidence argues against treating escape as a carrier-specific constant. The same formulation can encounter different tissue concentrations, cell populations, uptake routes, compartment states, membrane compositions, cargo-release kinetics, and competing intracellular sinks. Tissue-scale exposure itself must be separated from downstream function. Organ-targeted reformulation of mRNA-LNPs has shown that accumulation and translation can be altered in nonidentical ways [32]. Greater material deposition therefore cannot automatically be interpreted as greater endosomal escape.

The proposed Endosomal-Escape Systems Model describes productive cytosolic delivery as the intersection of eight coupled variables: carrier state, cargo state, biological compartment, membrane interaction, cellular damage and repair, competing trafficking routes, cell type, and dose–time history. None is proposed as universally dominant. Instead, the limiting variable may migrate as the system changes. Improving uptake can expose a downstream dissociation bottleneck; increasing membrane activity can reveal toxicity; accelerating carrier disassembly can generate premature cargo loss; and improving tissue accumulation can leave cellular or intracellular barriers unchanged.

The model also separates opportunity from conversion. Tissue exposure creates an opportunity for cellular contact. Cellular uptake creates an opportunity for intracellular trafficking. A permissive endosomal state creates an opportunity for carrier restructuring. Membrane destabilization creates an opportunity for cargo crossing. None guarantees conversion to the next state.

This distinction explains why formulation rankings can change between cell types or assays. A material does not carry one fixed escape efficiency from an artificial membrane system into a cell, from one cell line into another, or from cultured cells into a tissue. What is transported across those contexts is the material architecture; what emerges is a new interaction between that architecture and a different biological system.

The systems model therefore replaces the question “Which material escapes best?” with a more discriminating question: Under which carrier, cargo, compartment, cell, dose, and temporal conditions does the delivery system convert intracellular exposure into usable cytosolic cargo without unacceptable biological cost?

The cross-scale conditions that follow from this model are summarized below.

Table 2. Proposed cross-scale conditions for deciding whether endosomal escape is the operative bottleneck

Governing condition	Carrier state	Biological compartment Dominant barrier Dose dependence Translation boundary Discriminating experiment Article-specific endpoint
Scientific anchor	Carrier state	Biological compartment Dominant barrier Dose dependence Translation boundary Discriminating experiment Article-specific endpoint
Tissue exposure is low	Intact circulating or tissue-distributed carrier	Biological compartment Extracellular/tissue space Dominant barrier or sink Insufficient delivery opportunity Dose dependence Increasing dose may increase exposure but also off-target burden Translation boundary Low tissue exposure prevents inference about intracellular escape Discriminating experiment Quantify tissue exposure before interpreting cellular assays Article-specific endpoint Is intracellular escape even reachable as the dominant limitation?
Tissue exposure is adequate but cellular uptake is poor	Intact or protein-associated carrier	Biological compartment Cell surface/extracellular interface Dominant barrier or sink Entry limitation Dose dependence More dose may raise contact without solving uptake mechanism Translation boundary Tissue accumulation cannot substitute for intracellular exposure Discriminating experiment Pair tissue distribution with cell-specific carrier uptake Article-specific endpoint Is the bottleneck cellular entry rather than endosomal escape?
Uptake is high but cytosolic cargo remains low	Internalized/restructuring carrier	Biological compartment Endosomal network Dominant barrier or sink Trafficking, dissociation, or membrane-crossing limitation Dose dependence Absolute uptake and fractional productive release must be separated Translation boundary Uptake metrics do not identify the failed intracellular step Discriminating experiment Measure uptake, carrier state, cargo release, and cytosolic cargo independently Article-specific endpoint Which intracellular transition prevents productive delivery?

Membrane perturbation is high but cargo release is low	Membrane-active carrier with incompletely liberated cargo	Biological compartment Perturbed endosome Dominant barrier or sink Cargo retention or nonproductive injury Dose dependence Increasing membrane-active dose may amplify toxicity Translation boundary Damage reporters cannot be converted into escape percentage Discriminating experiment Pair membrane-injury reporter with direct cargo-release assay Article-specific endpoint Is membrane damage productive or merely injurious?
Cytosolic cargo is detectable but function remains weak	Cargo has crossed the endosomal boundary	Biological compartment Cytosol Dominant barrier or sink Post-escape sequestration, degradation, or inefficient utilization Dose dependence Additional escape may not improve downstream effect Translation boundary Cytosolic appearance and pharmacological function remain distinct Discriminating experiment Measure free cytosolic cargo together with expression or silencing Article-specific endpoint Has the bottleneck moved downstream of endosomal escape?
Strong function occurs with modest detectable perturbation	Carrier achieves productive release without extensive reporter-positive damage	Biological compartment Endosome to cytosol Dominant barrier or sink No evidence that large-scale disruption is required Dose dependence Efficient conversion may reduce the required intracellular dose Translation boundary Low damage signal does not imply absent escape Discriminating experiment Use orthogonal direct-release and functional assays Article-specific endpoint Can productive delivery occur through limited or transient membrane disruption?
Performance changes between cell types or cargos	Same nominal carrier architecture enters a different biological system	Biological compartment Cell-specific intracellular network Dominant barrier or sink Context-dependent dominant sink Dose dependence Same nominal dose can generate different cytosolic exposure Translation boundary One cell/cargo ranking cannot establish general escape capability Discriminating experiment Repeat matched measurements across relevant cell and cargo contexts Article-specific endpoint Is “escape efficiency” stable enough to be treated as a material property?

Note. This table represents the proposed systems decision logic of the Perspective. It is not a validated scoring system, regulatory framework, or predictive classifier.

Measurement problems

Measurement is the point at which the systems interpretation becomes experimentally testable. Dynamic FRET-FLIM methodology has recently been used to distinguish mRNA release from endosomal membrane damage and to reveal formulation-dependent differences between uptake-dominated and release-favorable behavior [33]. Such approaches are important because they measure states that conventional colocalization or downstream protein-expression assays often merge.

An escape claim should therefore specify what escaped, from where, relative to what denominator, over what time interval, and by which measurement principle. Total cellular fluorescence answers a different question from free cytosolic RNA. Galectin recruitment reports membrane injury, not escaped-molecule fraction. Translation or silencing establishes downstream functionality but integrates all preceding and subsequent bottlenecks.

No single assay is likely to constitute a universal gold standard. Instead, this Perspective proposes orthogonal triangulation: measure intracellular exposure; establish compartment localization where possible; measure carrier or cargo dissociation; quantify cytosolic cargo directly; characterize membrane injury independently; and assess downstream function separately. Agreement strengthens interpretation. Disagreement identifies which transition requires mechanistic investigation.

Design and validation rules

Delivery systems should consequently be optimized against multiple endpoints rather than maximum escape alone. Degradable silicon-ether ionizable lipids illustrate how delivery potency can be considered together with carrier degradation, tissue clearance, and preclinical tolerability [34]. These data do not establish clinical superiority, but they demonstrate the broader design principle that intracellular performance cannot be isolated from disposition and safety.

Five validation rules follow from the proposed systems model. First, uptake must never substitute for cytosolic delivery. Second, membrane damage must not substitute for cargo escape. Third, escaped cargo must be distinguished from downstream expression or silencing. Fourth, rankings should be tested across relevant cell, cargo, dose, and temporal contexts before being described as material properties. Fifth, tissue-scale accumulation, cellular delivery, intracellular escape, and functional outcome should remain separate translational levels.

Experimental design should also identify the current dominant bottleneck rather than assuming endosomal escape is always the limiting stage. Improving a nonlimiting step may increase toxicity or formulation complexity without materially improving functional delivery. Conversely, a modest change in one stage may expose a previously hidden downstream limitation.

The most informative development programs will therefore use perturbation experiments that discriminate competing explanations. If uptake rises without cytosolic cargo, investigate intracellular conversion. If galectin recruitment rises without cargo release, examine dissociation and membrane-productivity coupling. If cytosolic cargo rises without function, investigate post-escape availability. If performance changes across cell types, determine whether trafficking, damage repair, cargo processing, or another contextual state shifted the decision boundary.

CONCLUSION

Endosomal escape is not adequately described by asking whether a material is intrinsically “good” or “bad” at escaping endosomes. Productive cytosolic delivery emerges from the interaction of carrier structure, cargo state, biological compartment, membrane state, trafficking competition, cell context, dose, and time.

Treating escape as a systems bottleneck explains why cellular uptake, endosomal perturbation, cytosolic cargo, and downstream function can diverge; why a carrier may change rank across models; and why stronger membrane activity can increase injury without proportionate benefit. The proposed Endosomal-Escape Systems Model therefore shifts optimization from maximizing a single apparent escape property toward identifying the transition that currently limits productive delivery.

The practical consequence is a stricter evidential standard. Escape should be measured directly where possible, triangulated using orthogonal assays, normalized to relevant intracellular exposure, and interpreted within its carrier, cargo, cell, dose, temporal, and tissue boundaries. The objective is not maximum membrane disruption. It is reliable conversion of biological exposure into usable cytosolic cargo with acceptable biological cost.

ACKNOWLEDGMENTS: None

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

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