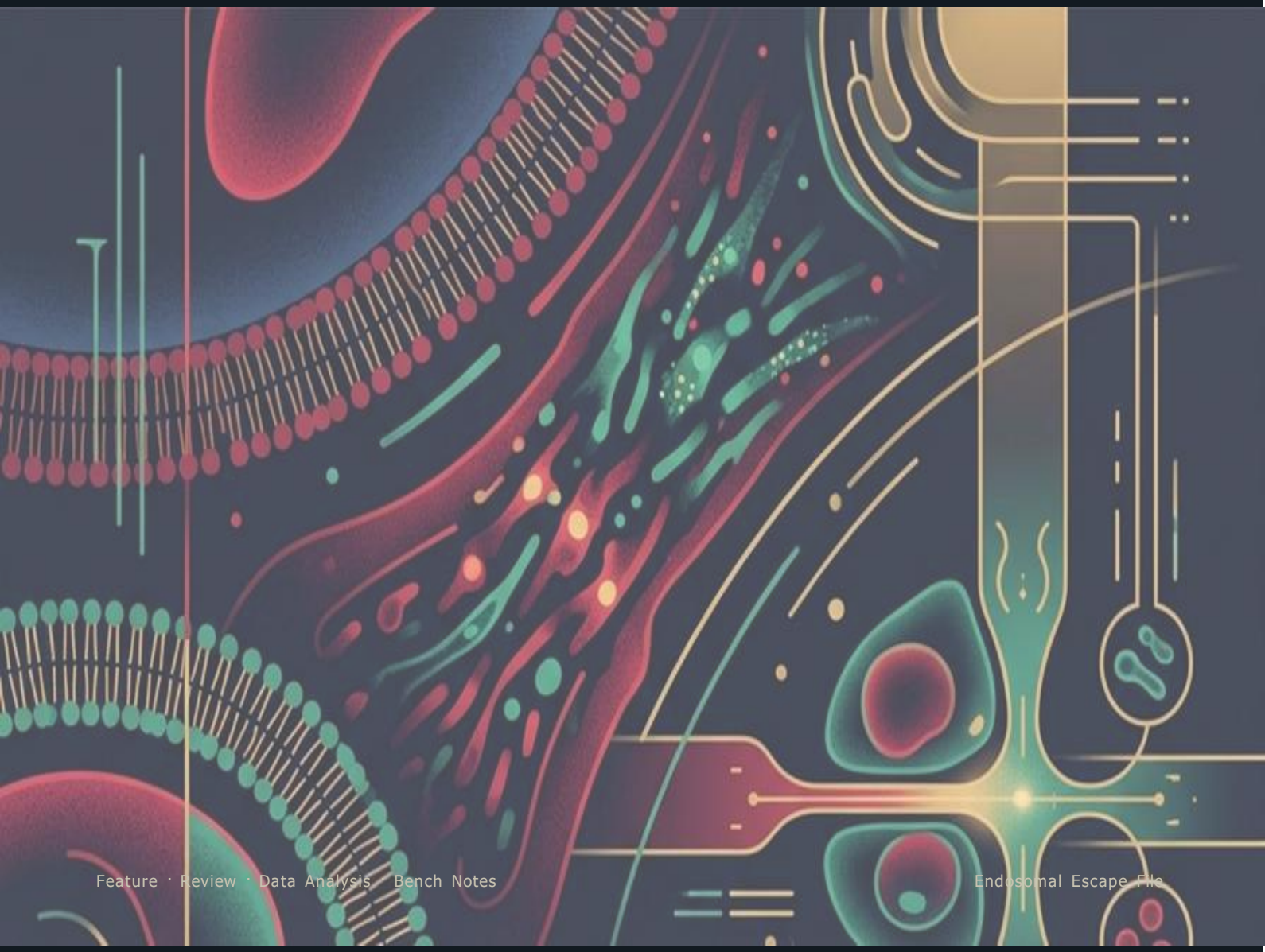
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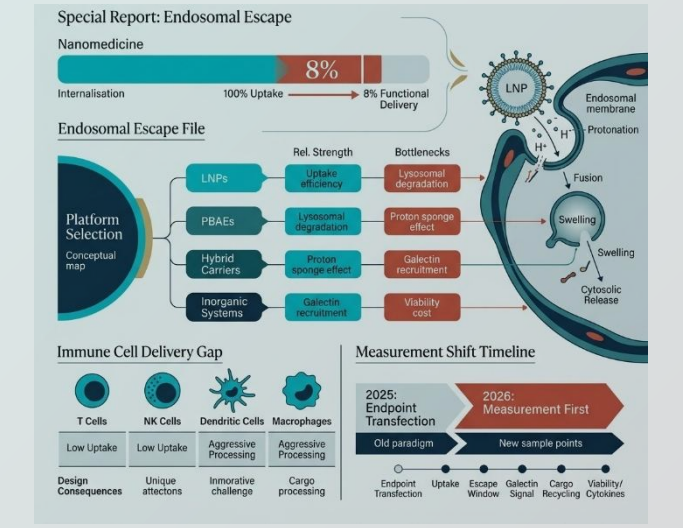
# The 8% Problem

What 2026 changed about endosomal escape — a research report on the narrow interval between uptake and functional cytosolic delivery.



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## About This Issue

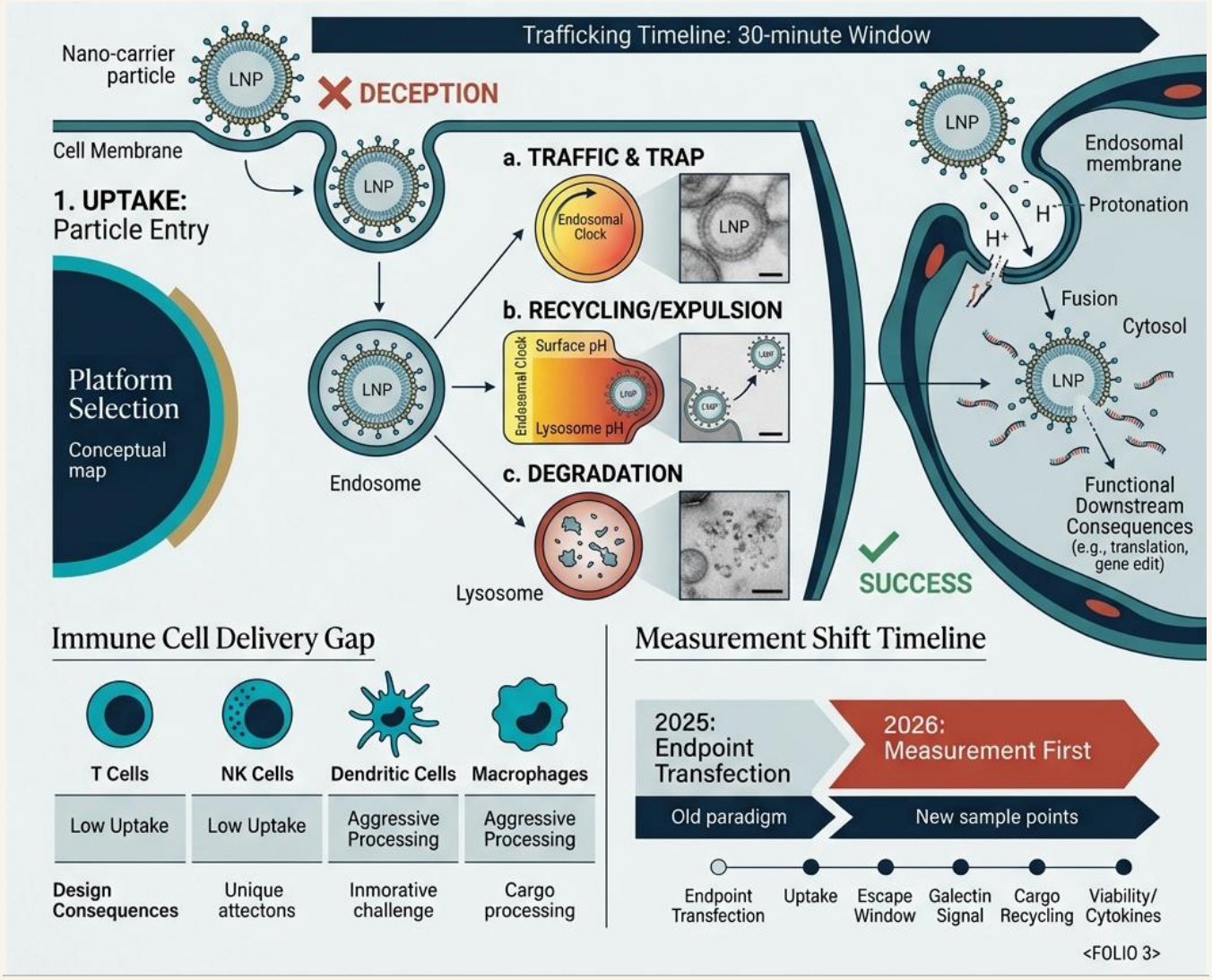
### About This Issue

A focused report for researchers working on non-viral delivery, gene engineering and endosomal escape.

# Uptake is not delivery.

The field spent a decade asking whether cargo entered the cell. The useful question begins after that.

| 08 | 7 | 1 |
|---|---|---|
| sections | data tables | endosomal clock |



# The experiment begins after internalisation

A particle inside a cell is not yet a delivered payload. It may be in an early endosome, a late endosome, a recycling compartment or a lysosome — and those are different outcomes.

For years, the working estimate was that only 1–2% of internalised cargo reached the cytosol. The figure became a standard sentence in introductions, even as methods differed in what they could resolve. The more important change in 2026 is not simply a larger percentage; it is a sharper distinction between what has entered a cell and what has become functionally available.

An in-vivo study using intact lysosomes isolated from mouse liver, together with barcoded cargo counted in each fraction, measured trapped material against cytosolic material directly. The reported fraction was roughly **8%**, with the escape event largely complete within about **30 minutes** after dosing. That timing makes the first hour an experimental window, not a footnote.

**SCOPE MATTERS**

The 8% and 30-minute values belong to the cited mouse-liver experiment. They should be treated as evidence that older estimates were method-limited — not as universal specifications for every carrier, tissue or cell type.

> “The reporting question: When did cargo become cytosolic, in which cells, measured by which assay, and with what viability cost?”

The implication is procedural. A bright internalisation image is not evidence of delivery, and a transfection percentage is not the same measurement as an escape fraction. The two must be reported separately, with the cargo, cell model and time point stated.

# Acidification is a clock

Endosomes mature continuously. A carrier does not get an unlimited opportunity to cross the membrane; it encounters a changing chemical and structural environment.




Endosomal maturation is not a passive backdrop. The pH drops progressively from the cell surface to the lysosome, and the membrane's lipid composition and curvature change with it. A carrier that buffers at one pH but not another may find its window narrow and early.

### TRAFFICKING IS PART OF THE FORMULATION

Knocking down Rab7 increased escape in the cited study. Slowing maturation may hold a productive window open, shifting attention from carrier-only design toward host trafficking.

### WHY RESULTS MOVE

Cell type, confluence, passage number and serum conditions alter trafficking kinetics. A reagent that works in HEK293 may fail in a primary immune cell for reasons that are not captured by uptake.

# Proton sponge is not the whole story

The classic account says that amine-rich polymers buffer incoming protons; chloride and water follow; the endosome swells and ruptures. It is a tidy model, but a membrane is not an abstract osmometer. The physical question is what the carrier does to a bilayer under acidification, crowding and curvature stress.

Molecular-dynamics work comparing branched PEI, amphiphilic PBAEs and a PEI-polycaprolactone block copolymer points to a combined mechanism. The strongest PBAE in that comparison carried more oleylamine: hydrophobic segments inserted into the bilayer and promoted fusion alongside buffering. The design implication is not "add hydrophobe" indiscriminately; it is to tune membrane interaction without converting delivery into uncontrolled damage.

### A BETTER WORKING MODEL

Buffering changes charge. Molecular shape and hydrophobicity change how that charge interacts with a stressed membrane. Both can matter, and neither alone is a complete mechanism.

| MECHANISM | WHAT IT CAN EXPLAIN | WHAT IT CANNOT PROVE |
|---|---|---|
| **Buffering** | pH-responsive charge and electrostatic interaction | That osmotic rupture is the dominant event |
| **Fusion** | Hydrophobic insertion, lipid mixing and local defects | That every defect produces useful cytosolic release |
| **Shape** | Cone geometry and non-lamellar phases | A universal optimum across cell types |

# What the attached review argues

The review by Gharatape and colleagues is not a catalogue of nanoparticles. It is a decision framework built around a simple premise: a carrier is only useful if its cargo reaches the cytosol in the right cell, at the right time, without unacceptable activation or toxicity.

The authors begin by separating the visible success of uptake from the less visible failure of intracellular release. Macrophages and dendritic cells can internalise particles efficiently because they are professional eaters; that does not mean their cargo survives the route to the cytosol. T cells and NK cells present the opposite challenge: uptake itself can be restrictive. A universal carrier is therefore unlikely because the upstream and downstream barriers are cell-type specific.

Their review follows the cargo through multiple competing fates: endosomal escape, lysosomal degradation, recycling-mediated exocytosis, active expulsion and, in some settings, transfer between immune cells. This wider map matters because a formulation that improves one route can worsen another. Increasing retention may help escape, while excessive retention may increase degradation or inflammatory signalling.

> “The question is not ‘How much nanoparticle entered?’ It is ‘Which fraction reached a productive compartment, by which route, and with what functional consequence?’”

### WHY IT BELONGS IN THIS ISSUE

The review supplies the broader immune-cell map; the 2026 endosomal-escape article supplies the sharper time window. Together they point toward measurement-first design.

# Entry is a route, not a result

The attached review emphasises that endocytosis is plural. The same material can use different entry machinery in different cells, and the route can change the intracellular fate.

| CELL CONTEXT | LIKELY DELIVERY CHALLENGE | DESIGN CONSEQUENCE |
|---|---|---|
| **T cells** | Low baseline uptake; productive release must be achieved without broad membrane disruption | Prioritise ionizable systems, selective ligands and low-charge exposure |
| **NK cells** | Uptake and cytosolic access are both constrained; transient expression may be useful | Use clinically mature LNP logic, but tune composition for NK membranes |
| **Dendritic cells** | Phagocytosis and macropinocytosis bring cargo in, followed by aggressive processing | Pair uptake with fusogenic or pH-responsive release |
| **Macrophages** | High uptake but rapid degradation, recycling and inflammatory sensing | Use hybrid carriers, active escape and immune-compatible surfaces |

### ONE OF THE REVIEW'S USEFUL CAUTIONS

Pathway assignment by inhibitor experiments is fragile. Pharmacological inhibitors have off-target effects and cell-line-dependent activity. A conclusion about "the" uptake route should therefore be treated as provisional unless imaging, genetics and functional delivery point in the same direction.

# No carrier wins every barrier

Platform choice is a trade between cargo, cell type, escape mechanism, manufacturing maturity and immune compatibility. The attached review ranks platforms by the gap they close, not by novelty alone.

| PLATFORM | WHERE IT IS STRONG | WHERE IT BREAKS |
|---|---|---|
| **Ionizable LNP** | mRNA/siRNA delivery; pH-triggered membrane interaction; clinical manufacturing precedent | Composition-sensitive; inflammatory risk; tissue tropism can dominate |
| **Polymer / PBAE** | Tunable chemistry, sustained release, hydrophobic and pH-responsive design | Cytotoxicity at high charge; release and reproducibility remain difficult |
| **Lipid-polymer hybrid** | Combines loading and structural roles; useful for difficult immune cells | More variables, more complex scale-up and characterisation |
| **Inorganic carrier** | Imaging, magnetic or photothermal functions; stable surfaces | Often weak standalone escape; oxidative or heavy-metal concerns |
| **Protein / EV-mimetic** | Biological compatibility and receptor interactions | Cargo loading, batch consistency and scale are limiting |

### DESIGNER'S SUMMARY

For T-cell mRNA, ionizable LNPs are a practical starting point. For macrophage editing, hybrid systems or escape-assisted polymers may be more appropriate. The right answer is cell-specific, not platform-loyal.

# Size and charge are upstream levers

The review's design discussion places size, shape, charge, PEGylation and targeting ligands in the same multidimensional space. These variables govern circulation, tissue access and cell capture. They are powerful — but they mostly govern delivery to the cell, not release from the endosome.

Size can change which endocytic pathway dominates. Surface charge can bias which cells capture a carrier and how strongly it associates with membranes. Internal architecture can alter both loading and uptake, meaning that two particles with similar external dimensions may not behave as equivalent objects. The practical consequence is to treat particle characterisation as a mechanistic record, not as a cosmetic specification.

**SIZE**

Size is not a single "better" direction. Small particles may drain or circulate differently; larger constructs may favour phagocytic uptake. The optimum is coupled to cell type and tissue route.

**CHARGE**

Charge improves interaction only until it becomes a liability. Transient or pH-dependent charge can separate circulation behaviour from endosomal membrane activity.

*The design rule: Use size and charge to reach the right cell. Use membrane chemistry and trafficking control to reach the cytosol.*

# The strongest signal may be a warning

Galectin recruitment is a sensitive readout of endosomal membrane damage. That makes it useful — and conceptually uncomfortable. The same event that permits release can trigger innate sensing.

When an endosomal membrane is torn, cytosolic galectins rush to damaged glycans. The signal is visual, live and increasingly common in escape studies. But damage is not neutral. In immune cells, the membrane event can intersect with inflammatory signalling, viability loss and activation states that alter the therapeutic outcome.

This is why "high potency, high toxicity" is not always a formulation accident. The two outputs can share a physical cause. A gentler reagent that never reaches the cytosol is not necessarily safer in a useful sense; it may simply be inactive. The better question is whether the carrier creates a productive, repairable and cell-compatible escape event.

> **PAIR THE READOUTS**
>
> Functional expression, cytosolic release, galectin recruitment, viability and inflammatory markers should be interpreted as a panel. No single assay can carry the mechanism alone.

| | LOW DAMAGE | INTERMEDIATE WINDOW | HIGH DAMAGE |
|---|---|---|---|
| **Low escape** | Low expression | Useful signal | Strong signal + stress |
| **Productive release** | — | Design target | — |
| **Broad membrane disruption** | — | — | Toxicity-limited |

# What counts as evidence of escape?

A bright uptake image can be true and still be irrelevant to cytosolic delivery. Evidence becomes stronger when orthogonal measurements converge on the same event.

| READOUT | WHAT IT TELLS YOU | WHAT IT DOES NOT TELL YOU |
|---|---|---|
| **Fluorescent uptake** | Association and internalisation of labelled material | Whether cargo is free in cytosol |
| **Colocalisation** | Compartment proximity at a time point | Functional release or productive translation |
| **Galectin recruitment** | Endosomal membrane damage | Whether the damage is tolerable or useful |
| **Reporter expression** | A functional downstream consequence | How much cargo escaped or by which route |
| **Direct cytosolic fraction** | Physical distribution of cargo | Whether the released cargo remains active |
| **Viability / cytokines** | Cell cost of the intervention | Whether low toxicity reflects no delivery |

### THE MISSING STANDARD

The attached review repeatedly returns to the absence of a standardised, validated and cross-comparable escape assay. That absence is not a minor caveat. It makes platform comparisons fragile because reported percentages can refer to different cargos, cell models, time points and definitions of "escape."

# A measurement-first workflow

The goal is not to turn one paper into a universal protocol. It is to make the hidden variable visible in the system you actually care about.

## 01
### Map uptake

Measure association and internalisation with a labelled cargo, but do not call it delivery.

## 02
### Resolve the first hour

Use time points that can see early trafficking and transient release, not only 24–48 hour expression.

## 03
### Pair mechanism and function

Combine a release-sensitive readout with a functional reporter and a viability / inflammatory panel.

## 04
### Repeat in the target cell

Compare the same carrier in the actual primary or immune-cell model; cell-line transfer is not guaranteed.

## 05
### Record the carrier state

Report size, PDI, charge, pKa or ionisation behaviour, loading, serum condition and batch.

## 06
### Define the failure route

Ask whether the cargo was degraded, recycled, exocytosed, expelled, trapped or released but inactive.

# The immune cell decides what “good delivery” means

The review’s most important translational point is that immune cells are not interchangeable recipients. Macrophages and dendritic cells internalise readily but process aggressively. T cells and NK cells resist uptake, so increasing endosomal escape cannot compensate for a missing entry event. The same physicochemical choice can therefore help one cell and hurt another.

For T cells, the design problem is often a narrow balance: enough membrane interaction to enter, enough pH response to release, and little persistent cationic exposure to preserve viability and function. For macrophages, the challenge is more often to redirect or accelerate a carrier away from degradative compartments while avoiding inflammatory activation. “Transfection efficiency” without a cell-state readout is incomplete.

> **TRANSLATIONAL TEST**
>
> A platform comparison should be repeated across the intended cell types, with the same cargo and matched functional endpoint. A winner in an easy line is a hypothesis, not a product decision.

| CELL | PRIMARY BOTTLENECK | USEFUL DESIGN EMPHASIS |
|---|---|---|
| **T cell** | Entry + escape | Ionisable, low-charge, selective |
| **NK cell** | Entry + transient function | Mature LNP logic, composition tuning |
| **DC** | Processing after uptake | Fusogenic / pH-responsive release |
| **Macrophage** | Degradation + activation | Hybrid architecture, controlled damage |

# The 8% result is a measurement story

**8%**
Cytosolic fraction reported in the cited mouse-liver experiment

**~30**
Minutes — approximate time for the escape event to be largely complete

**1–2%**
Older working estimate for internalised cargo reaching the cytosol

The supplied feature article places the new figure in context: the field did not suddenly make every carrier eight times better. It measured a previously hidden fraction more directly.

The cited in-vivo work used lysosome isolation and barcoded cargo to distinguish material still trapped in degradative compartments from material available in the cytosol. That design addresses a weakness of endpoint microscopy: a particle can look intracellular while its cargo remains functionally inaccessible. The result, roughly 8% in mouse liver, is therefore valuable as a recalibration of the measurement problem.

The timing is equally important. If the escape event is largely complete within about 30 minutes, then downstream assays at 24 or 48 hours compress many separate processes into one number: translation, degradation, cell state, toxicity and perhaps cargo recycling. A better experiment samples the early window and uses the late readout only as the functional consequence.

> **DO NOT OVERGENERALISE**
>
> The value is not a universal benchmark for LNPs, PBAEs, hepatocytes or immune cells. It is a strong argument for measuring cytosolic availability directly and reporting the biological context precisely.

The most useful shift is from “transfection percentage” to **“escape fraction, time window, cell type and cost.”**

OUTLOOK

# Where delivery goes next

A measurable bottleneck becomes an engineering target. The next phase is less about declaring a winner and more about controlling the window.

01

### Report escape fraction

State the cargo, cell, tissue, time point, assay and viability context whenever cytosolic delivery is reported.

02

### Target host trafficking

If Rab7, recycling and endosomal residence shape the ceiling, host biology becomes a co-target that may improve more than one chemistry.

03

### Engineer fusion deliberately

Polymer and lipid design may move beyond osmotic rupture toward controlled membrane phase changes.

04

### Break potency–toxicity coupling

The long-term goal is delivery that does not require the same membrane damage that drives inflammation.

05

### Build for manufacture

The best mechanism will not translate without batch control, scalable upstream processes and a reproducible release assay.

09 — Closing Note

# The bottleneck is finally visible

The field is getting better at measuring a process it still cannot fully control. That is progress, but it is not a universal recipe. The 8% result should make us more demanding about methods, not more casual about transferring numbers between systems.

The attached review expands the same lesson across immune-cell engineering: uptake is a route, escape is a gate, and functional delivery is the outcome. A carrier should be judged by the barrier it closes in the intended cell — and by the biological cost of closing it.

**THE EDITORIAL TAKEAWAY**

Do not optimise the easiest measurement. Separate entry from release, sample the first hour, compare the target cell, and treat toxicity as mechanistic information.

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# Sources and reading notes

The issue combines the supplied feature article, the attached International Journal of Nanomedicine review and the primary references cited within those texts.


1. JoziC A, Le Roux C, Kim J, et al. In vivo endosomal escape assay identifies mechanisms for efficient hepatic LNP delivery. Nature Biotechnology, 11 March 2026. DOI 10.1038/s41587-026-03022-6.
2. Liu Z, Dong Y. Quantifying endosomal escape in vivo to guide lipid nanoparticle design. Nature Biotechnology News & Views, 11 March 2026. DOI 10.1038/s41587-026-03047-x.
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4. Gharatape A, Alikhani A, Verdi J, et al. The Nanoparticle Delivery Gap in Immune Cell Engineering: From Uptake to Endosomal Escape. International Journal of Nanomedicine 2026;21:650145. DOI 10.2147/JIN.S650145.
5. Proton sponge or membrane fusion? Endosomal escape of siRNA polyplexes illuminated by molecular dynamics simulations. bioRxiv preprint, March 2026.
6. Spatial conformation of ionizable lipids regulates endosomal membrane disruption. JACS, 2025. DOI 10.1021/jacs.5c10908.
7. Mechanistic insight into ionizable cationic lipid-mediated endosomal escape via transient lipid complexes. Small 22(32), e13399, 2026. PMID 42035446.
8. The endosomal escape problem solved? New insights into lipid nanoparticle delivery efficiency. Materials Today 98, 103398, September 2026.


**EDITORIAL METHOD**

Summaries distinguish reported findings from interpretation. Numerical claims retain the scope and caveats of their source.




The question after uptake

# If the cargo is inside the cell, the experiment has only begun.

A first issue on endosomal escape, immune-cell engineering and the measurements that connect carrier chemistry to functional delivery.

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