

Engineering endosomal escape: design rules for ionisable lipid nanoparticles

Why most internalised cargo never reaches the cytosol, and what formulation scientists can change

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Summary

Lipid nanoparticles are taken up efficiently by cells, yet only a small fraction of their nucleic acid cargo — often estimated at a few per cent — reaches the cytosol. The rest is trapped in endosomes and degraded in lysosomes. Escape efficiency is therefore one of the most powerful levers on potency, dose and tolerability. This paper sets out the mechanism and the design rules we apply.

1. The pathway

- Uptake: apolipoprotein E adsorbed in blood, or a surface ligand, engages cell receptors and the particle enters by endocytosis.
- Acidification: proton pumps lower endosomal pH from about 6.5 (early) to 5.5 (late) and below in lysosomes.
- Protonation: ionisable lipids with an apparent pKa near 6.2–6.6 become cationic as pH falls.
- Disruption: cationic lipids pair with anionic endosomal lipids; cone-shaped ion pairs favour inverted hexagonal phases that breach the membrane.
- Release: cargo that crosses before lysosomal degradation reaches the cytosol, where mRNA is translated or siRNA loads into RISC.

2. Design rules

Lever	Direction	Rationale
Apparent pKa	Tune to 6.2–6.6 for IV hepatic delivery	Neutral in blood, protonated in early-to-late endosomes.
Tail geometry	Branched or unsaturated tails	Larger cone angle favours non-bilayer phases.
Helper lipid	DOPE over DSPC where escape limits potency	DOPE itself favours hexagonal phases.
PEG-lipid	Short anchors (C14) for fast shedding	A persistent PEG layer hinders membrane contact.
Cholesterol analogues	Consider β -sitosterol or other phytosterols	Reported to change particle morphology and endosomal trafficking.

3. How we measure escape

- Galectin-8/9 recruitment imaging to detect damaged endosomes.
- Split-luciferase or fluorescent reporter assays for cytosolic delivery.
- Co-localisation with endosomal and lysosomal markers over time.
- Potency per internalised dose, comparing uptake (flow cytometry) with protein output.

Key point

Uptake and escape must be measured separately. A formulation with high uptake but poor escape can look identical to one with low uptake in a single expression readout.

References available on request. This paper summarises published literature and our internal design practice.