Designing Next-Gen LNPs: Tools, Challenges, and Opportunities

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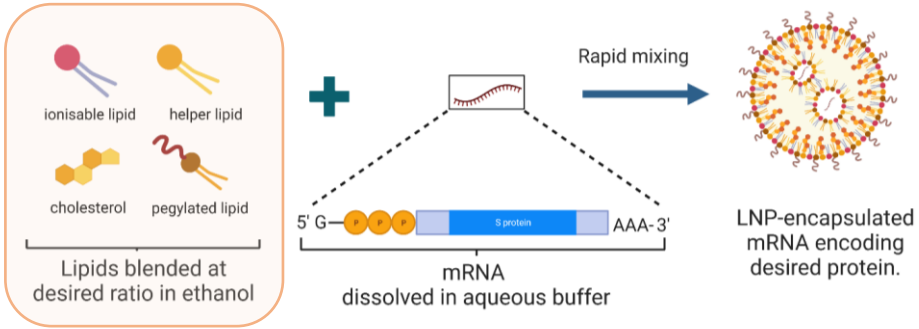
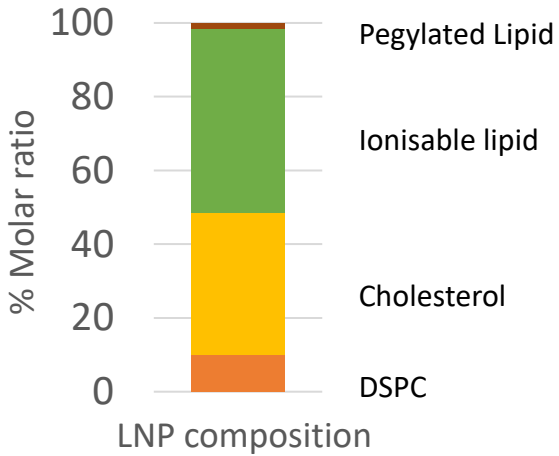
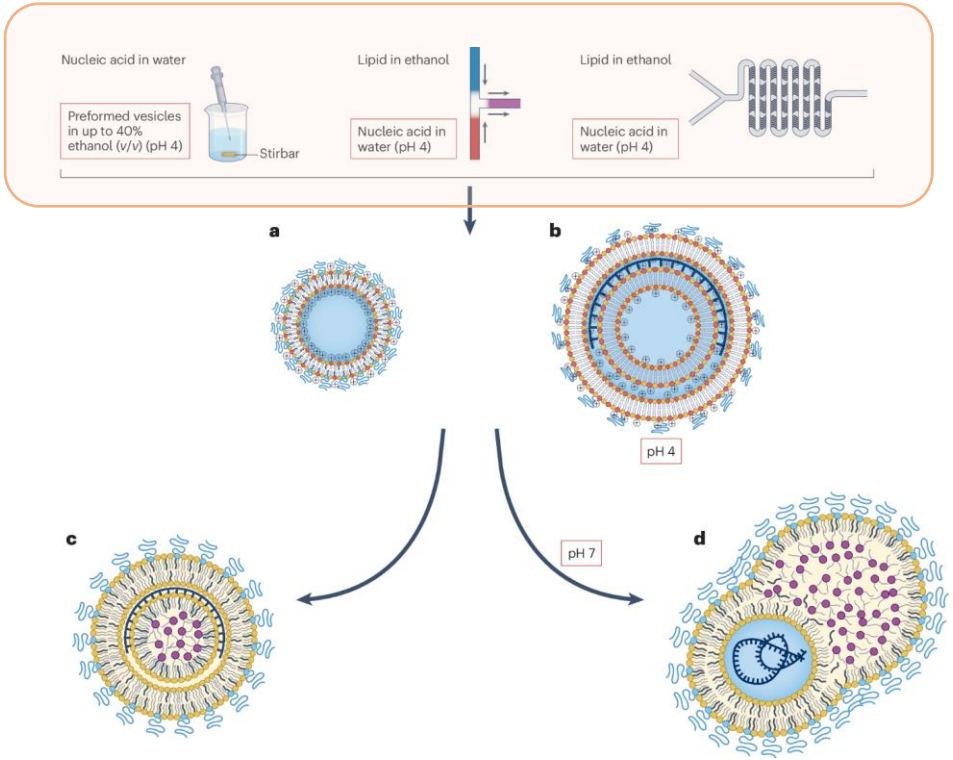
CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Design considerations for LNPs

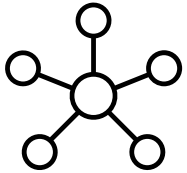




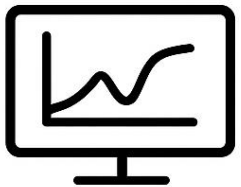


Formation of lipid nanoparticles.
From: [The 60-year evolution of lipid nanoparticles for nucleic acid delivery]

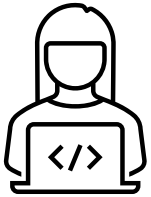
Our research question?



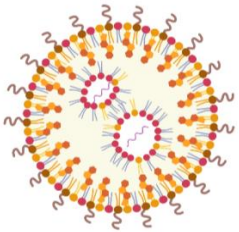
Formulation?



Manufacture?

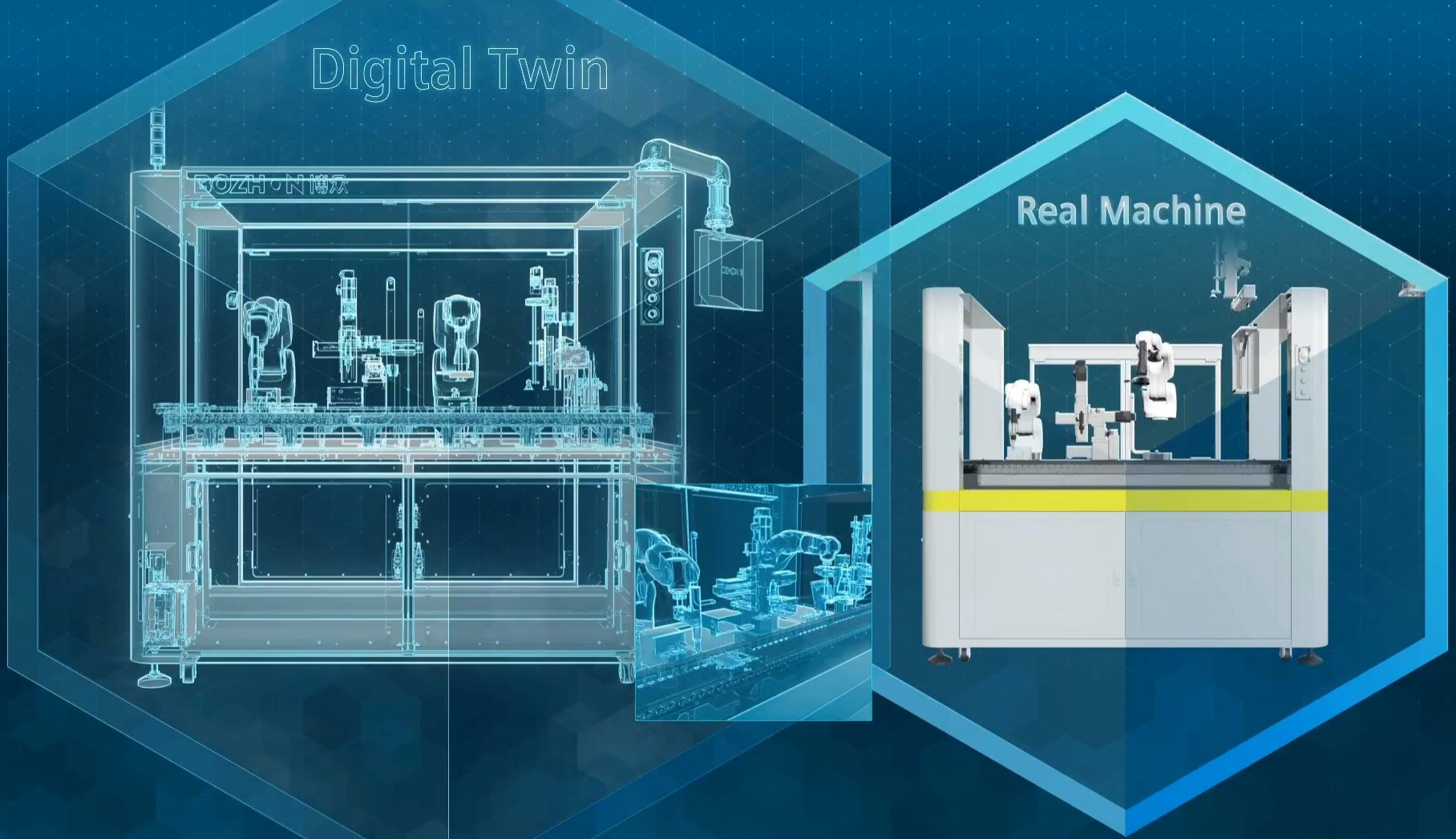


Develop next-generation LNPs?



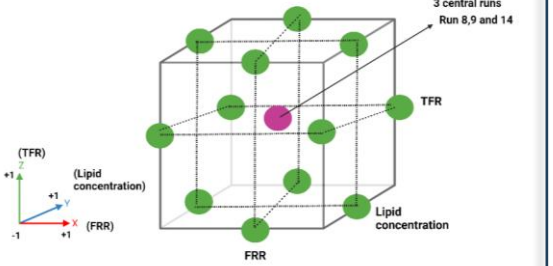
Tools and technologies?

Predictive models?

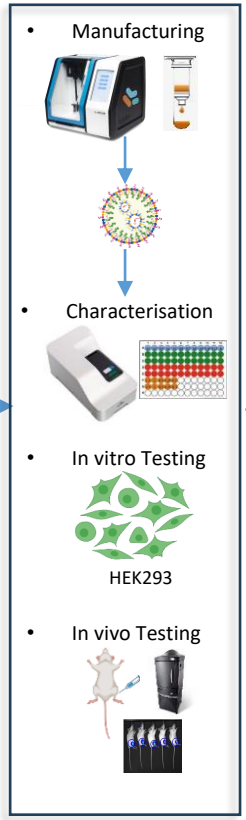

Digital Twin
Real Machine

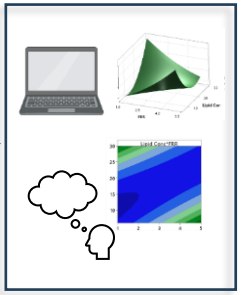
Applying QbD Principles:
Use DoE to Define a Design Space for LNP Manufacture ?

DoE Design: Box Behnken



Runs	FRR	TFR (mL/min)	Lipid Concentration (mM)
1	1 (-1)	4 (-1)	18 (0)
2	1 (-1)	12 (0)	30 (1)
3	5 (1)	4 (-1)	18 (0)
4	5 (1)	12 (0)	30 (1)
5	3 (0)	4 (-1)	30 (1)
6	3 (0)	20 (1)	30 (1)
7	5 (0)	20 (1)	18 (0)
8	3 (0)	12 (0)	18 (0)
9	3 (0)	12 (0)	18 (0)
10	3 (0)	20 (1)	6 (-1)
11	5 (1)	12 (0)	6 (-1)
12	1 (-1)	12 (0)	6 (-1)
13	3 (0)	4 (-1)	6 (-1)
14	3 (0)	12 (0)	18 (0)
15	1 (-1)	20 (1)	18 (0)

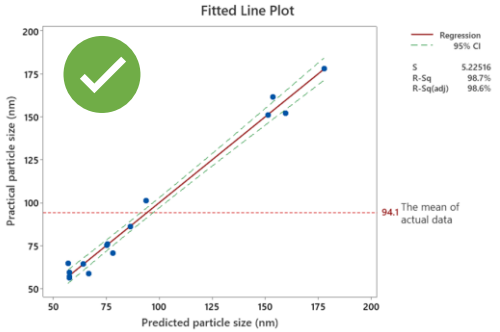


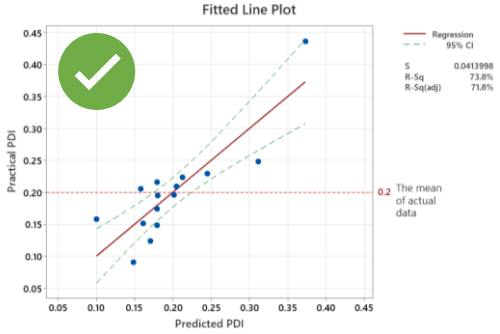


Design → Experiment → Analysis

Modelling

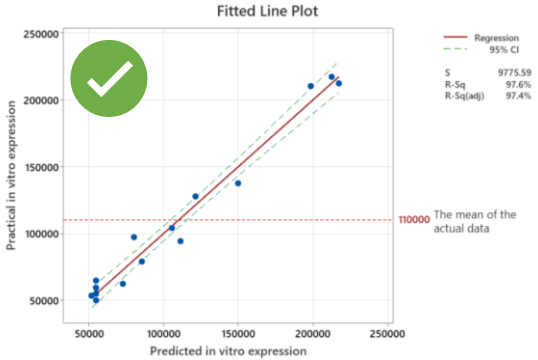
Production Process

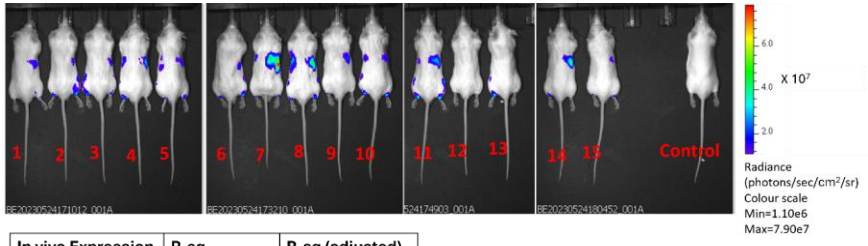




✓ We can model the manufacturing process

Potency

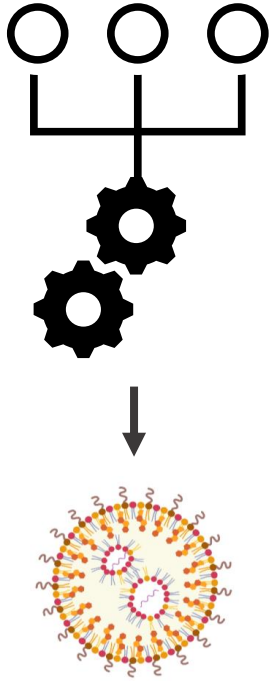




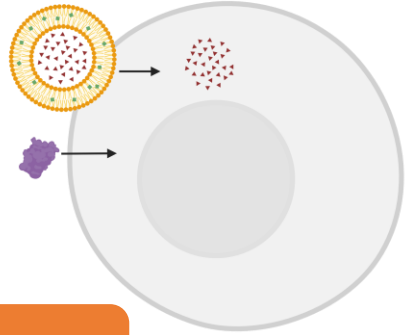
In vivo Expression	R-sq	R-sq (adjusted)
Injection site	81.2%	47.4%
liver	84.6%	57.0%

Manufacture dominates LNP CQAs

Cellular interactions dominate potency

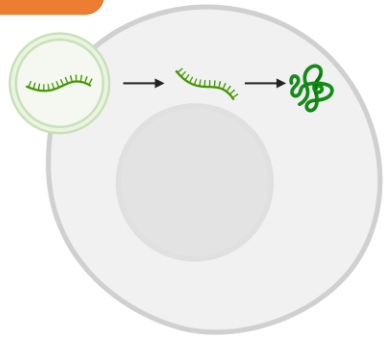


Drugs/proteins:
direct action



Successful down-selection?

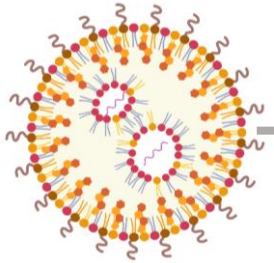
mRNA uses the cell
to make the protein



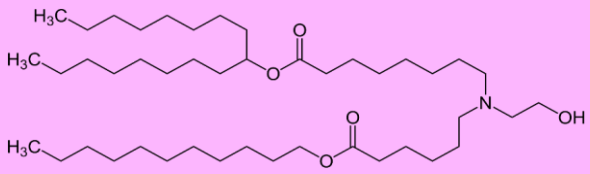
Easy to model

Challenge

Key Drivers in Formulation?

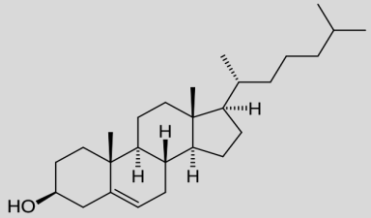


Ionisable lipid



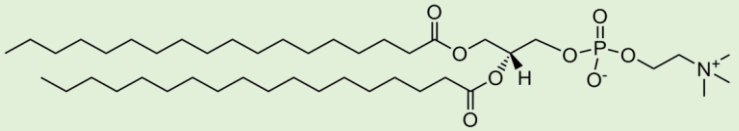
SM-102 50 %

Cholesterol



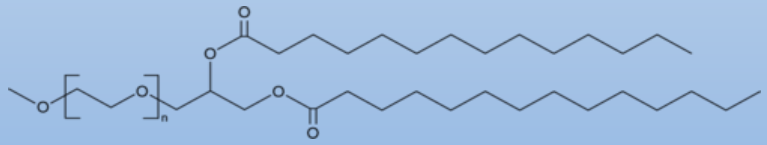
38.5 %

Helper lipid



DSPC 10 %

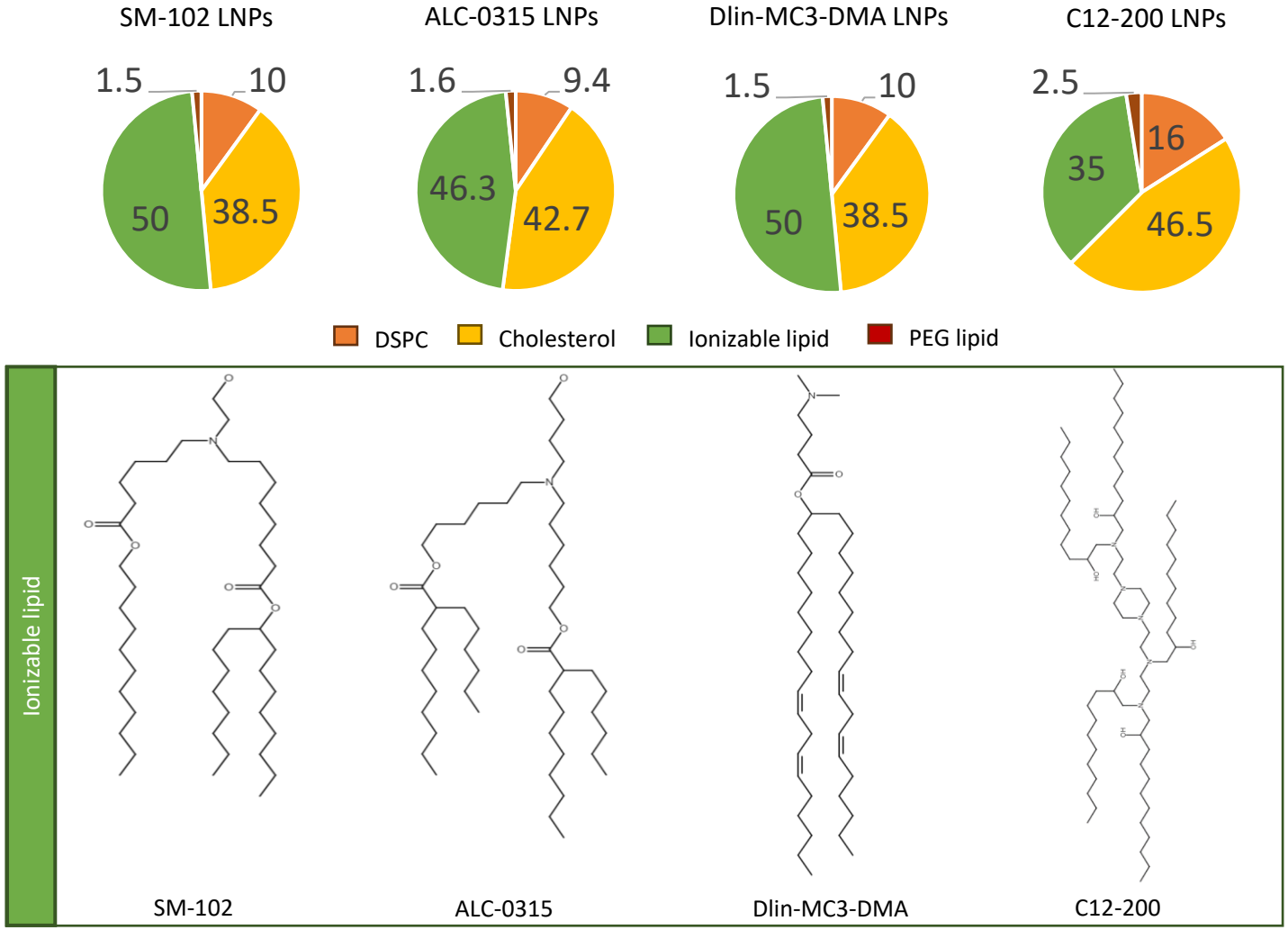
PEGylated lipid



DMG-PEG(2000) 1.5 %

?

Formulation and Function



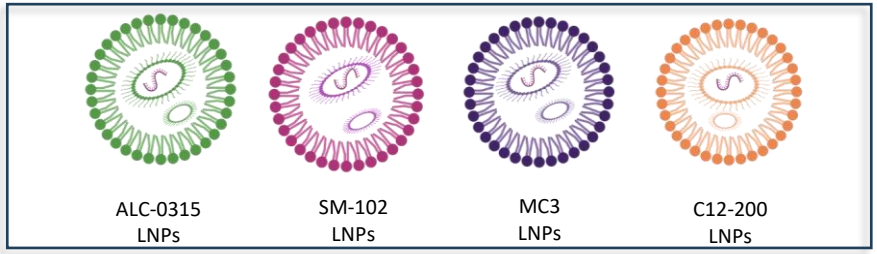
LNP physico-chemical attributes:

	Size (nm)	PDI	Zeta (mV)	%EE
mRNA	**Fluc**	**Fluc**	**Fluc**	**Fluc**
SM102	97 ± 7	0.07 ± 0.02	9 ± 4	94 ± 2
ALC-0135	85 ± 6	0.09 ± 0.02	-2 ± 3	85 ± 5
MC3	93 ± 3	0.05 ± 0.02	7 ± 2	92 ± 1
C12-200	73 ± 2	0.08 ± 0.03	-4 ± 4	98 ± 2

1. Formulation makes no notable difference
2. Type of mRNA makes no notable difference

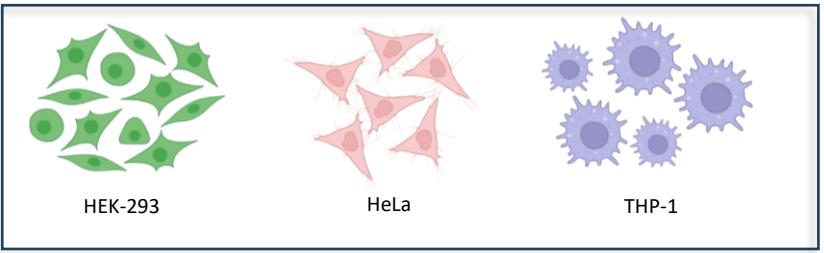
Manufacturing Process is driving the Particle characteristics

In Vitro & In Vivo

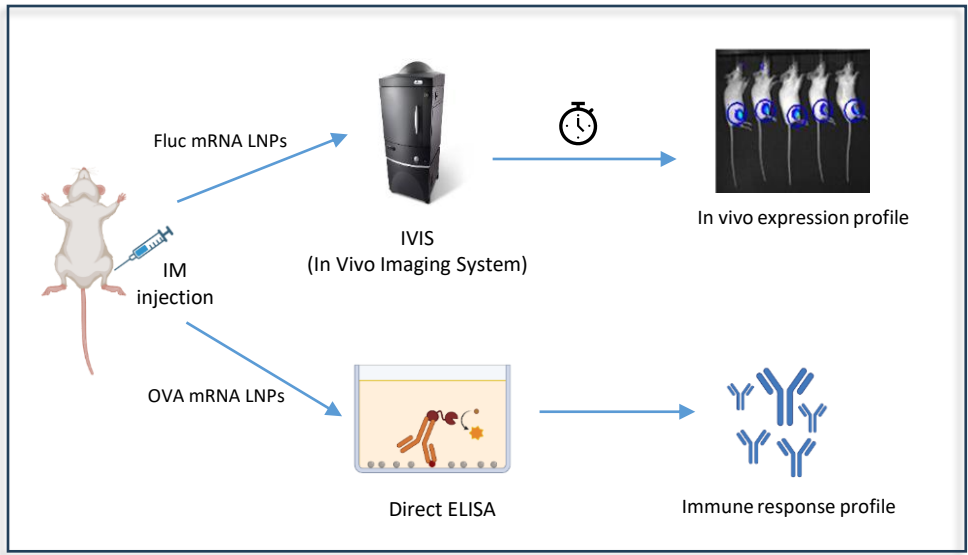


In vitro testing

In vivo testing



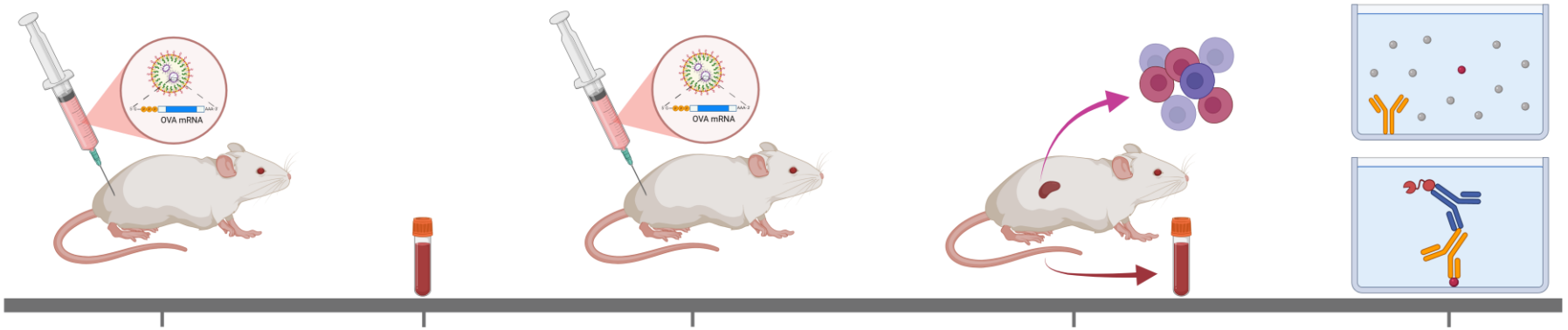
In vitro Cell Uptake
in vitro expression Profile



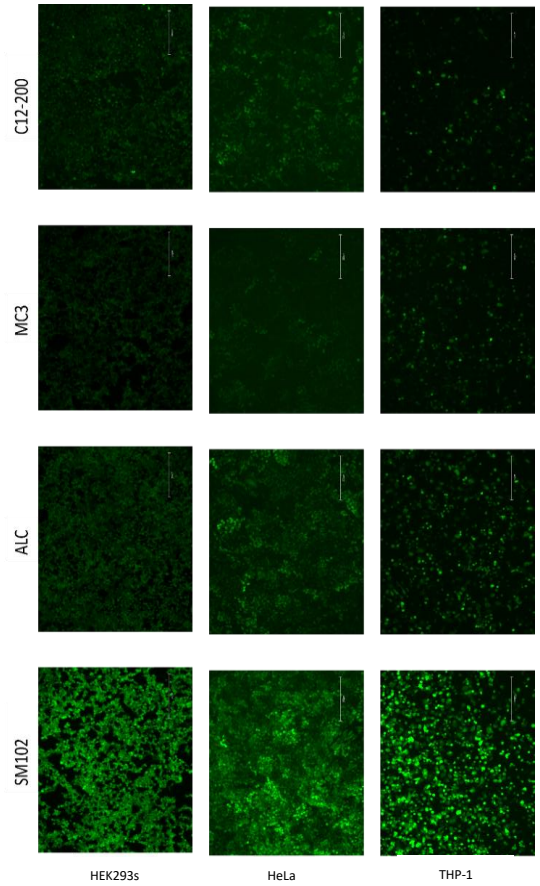
S Linday, B Binici, M Hussain et al., 2025;

Created by Biorender

Vaccine Protocol

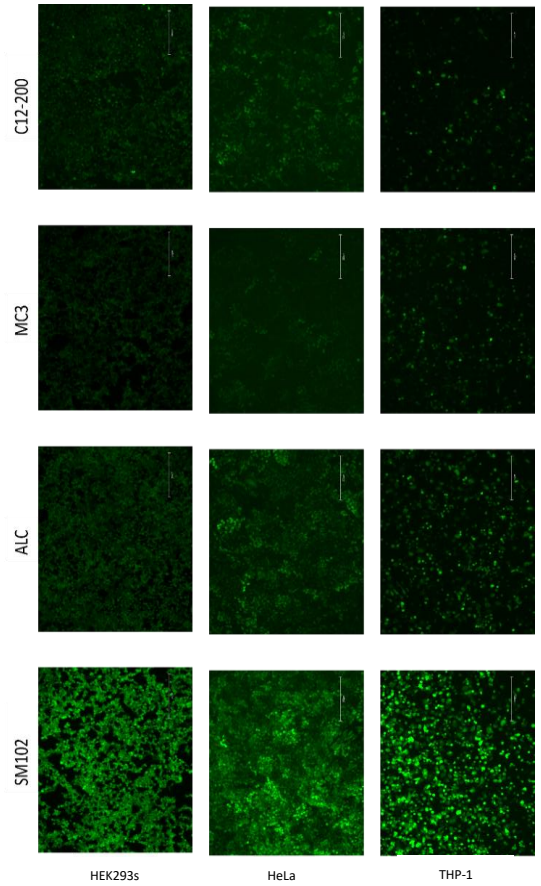


Cell expression | Protein expression | Antibody responses



Cell images of HEK293, HeLa and THP-1 cells treated with 2 µg/mL GFP-mRNA.

Cell expression | Protein expression | Antibody responses



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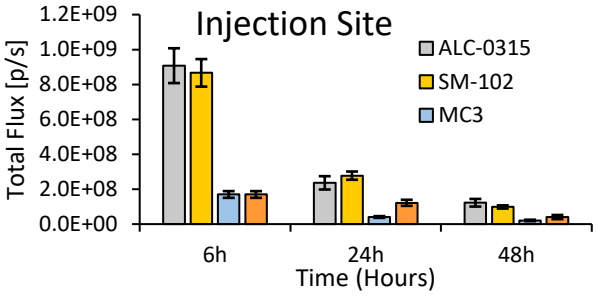
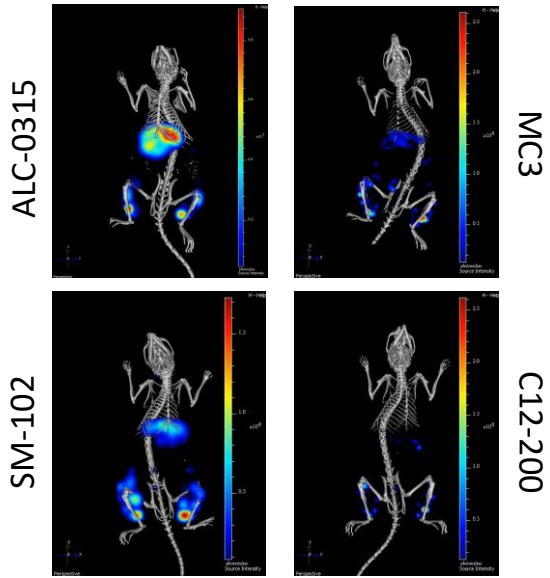
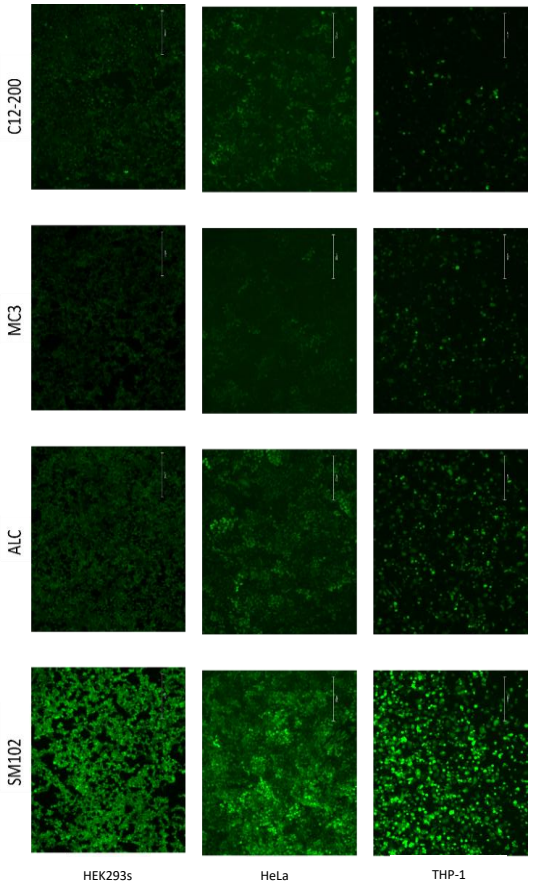




Cell expression



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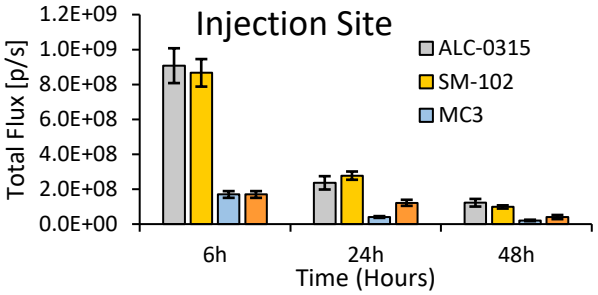
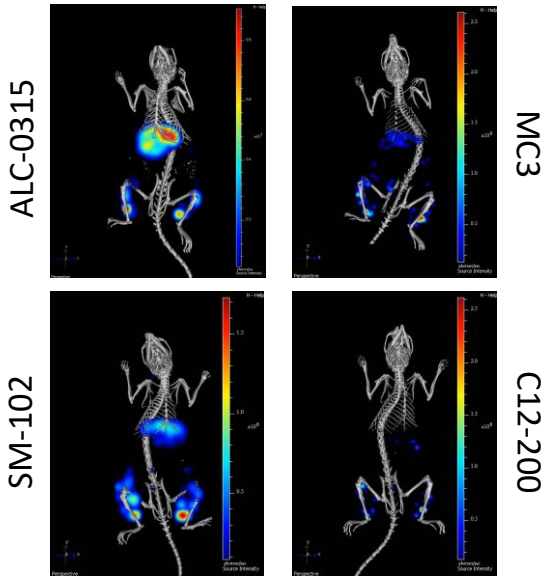
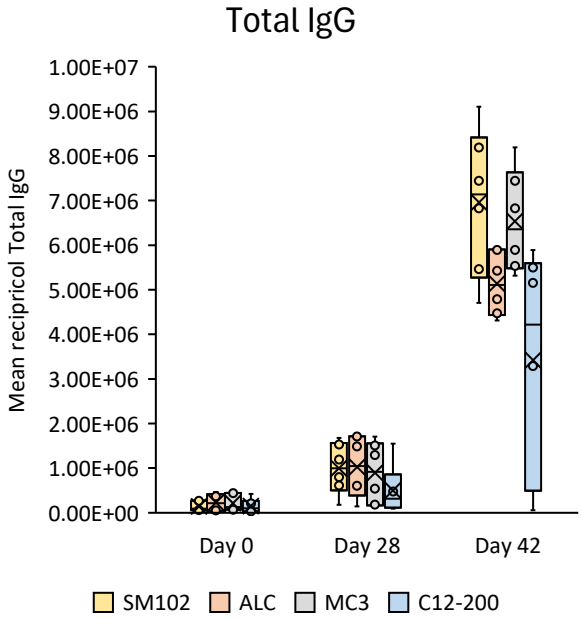
Protein expression



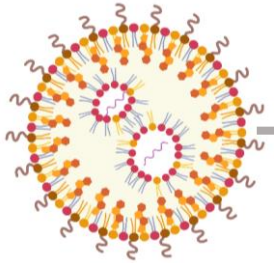


Antibody responses

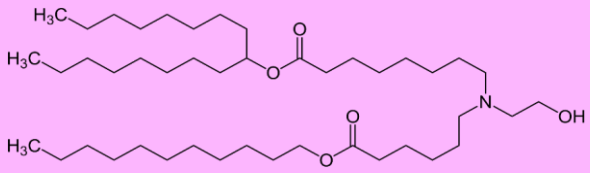


Key Drivers in Formulation

Sterols?

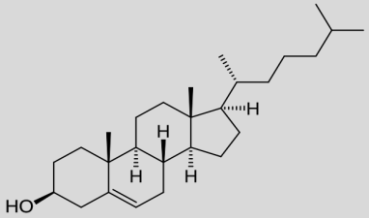


Ionisable lipid



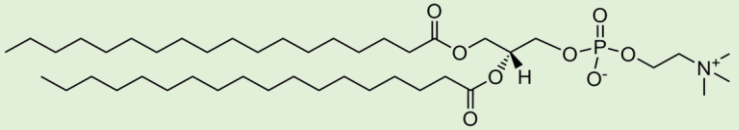
SM-102 50 %

Cholesterol



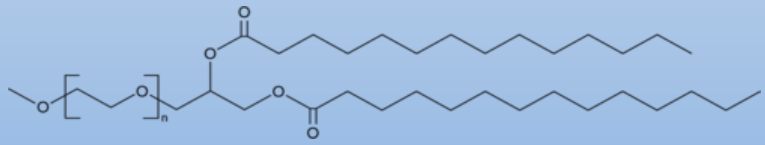
38.5 %

Helper lipid



DSPC 10 %

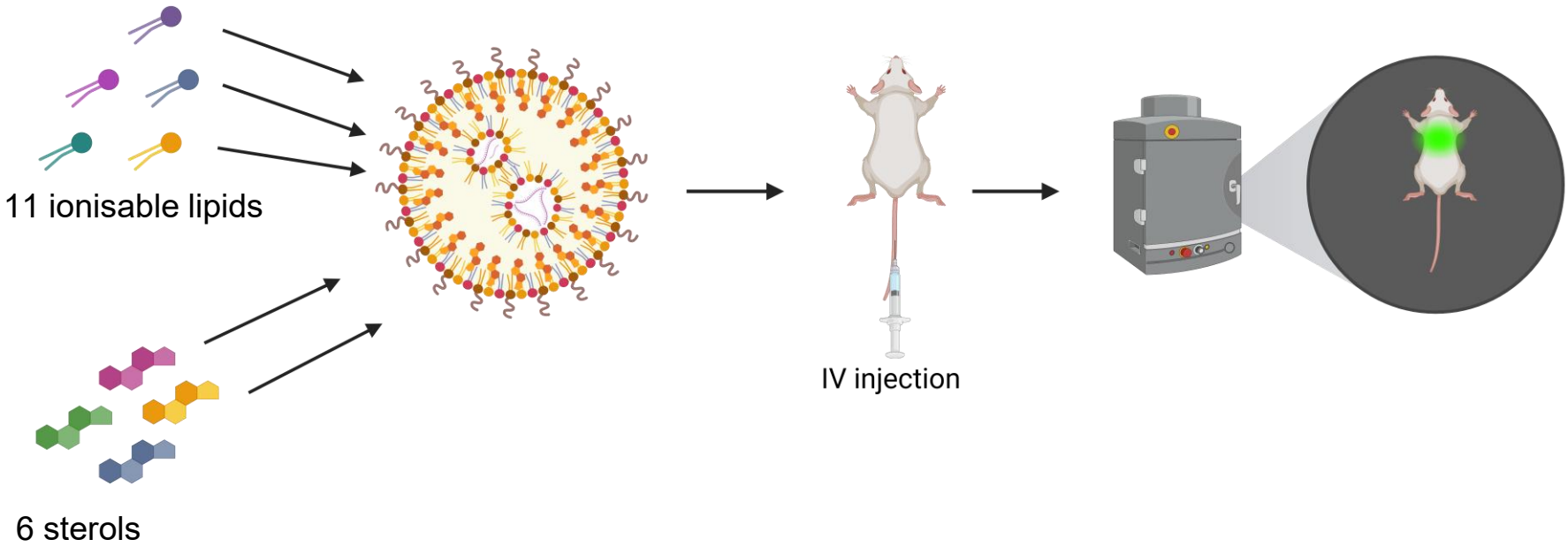
PEGylated lipid



DMG-PEG(2000) 1.5 %

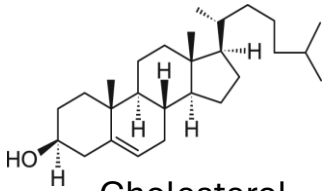
?

Which is dominant - ionisible or helper lipid?

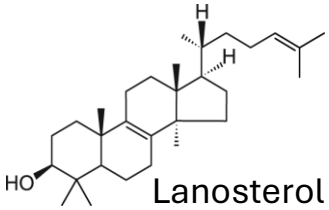


Caitlin McMillan et al., 2025 *Influence of Ionisable Lipid and Sterol Variations on Lipid Nanoparticle Properties and Performance* (under review)

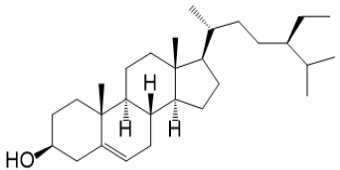
Sterols can influence responses, but ionisable lipids are the key driver



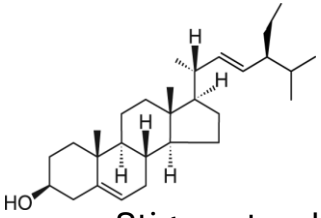
Cholesterol



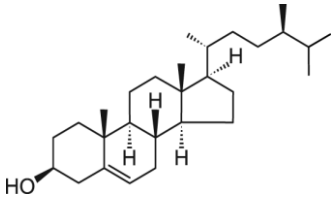
Lanosterol



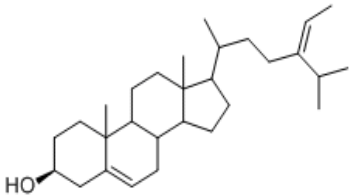
β-sitosterol



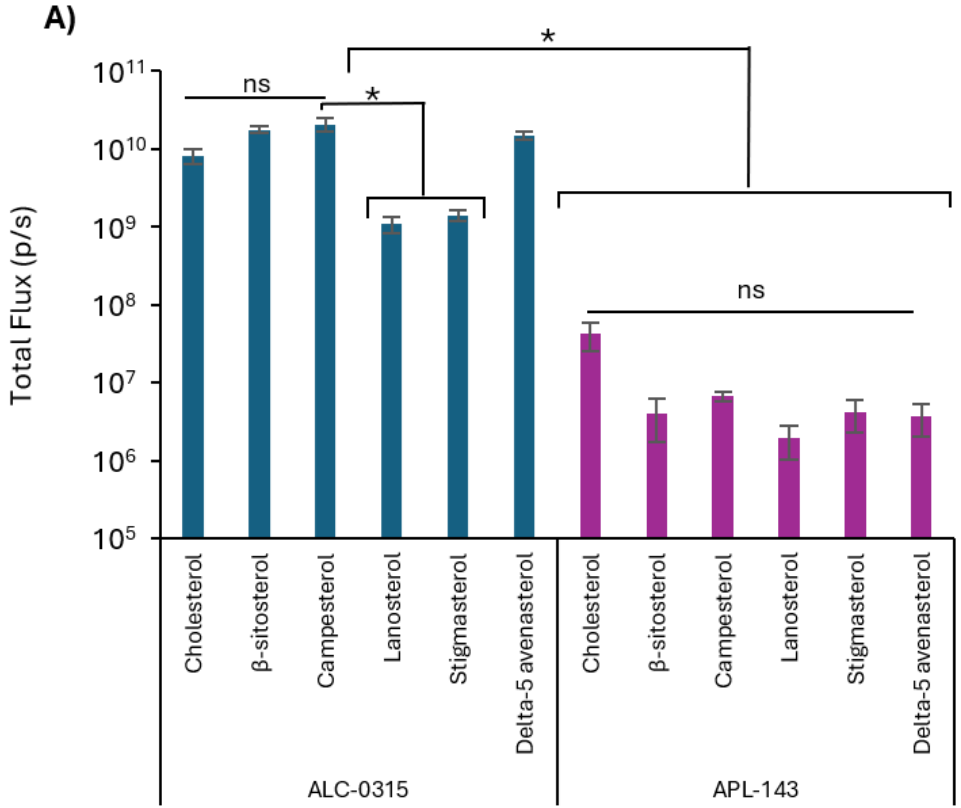
Stigmasterol



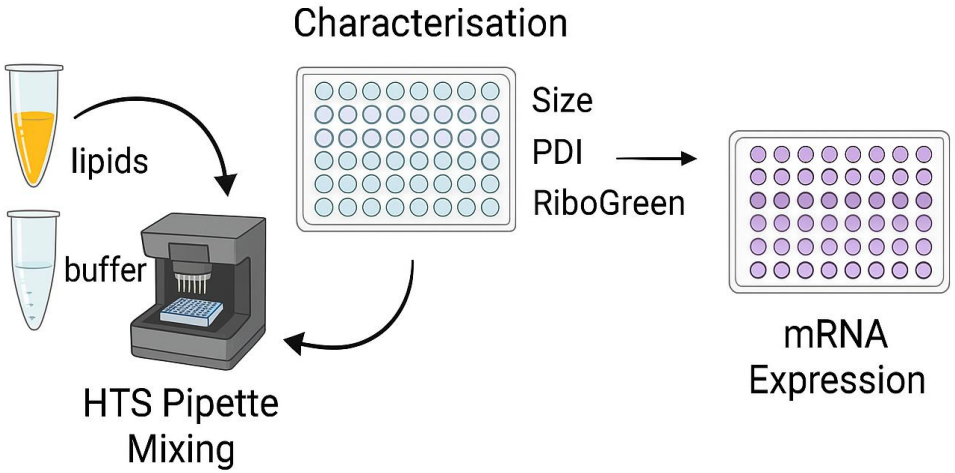
Campesterol



Delta 5-avenasterol



HTS Automated Screening – bigger dataset



Automated pipette mixing to produce the lipid blends and LNPs

Automated characterisation

(DLS size, PDI, encapsulation efficacy)

Semi-automated cell culture

(viability and expression)

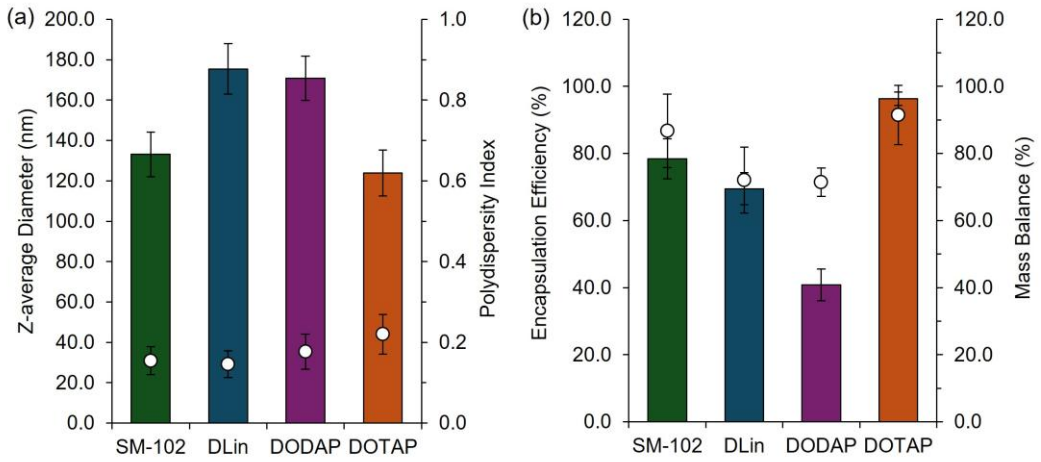
Data capture and visualisation

Developed and run by CPI as part of IDDC Programme

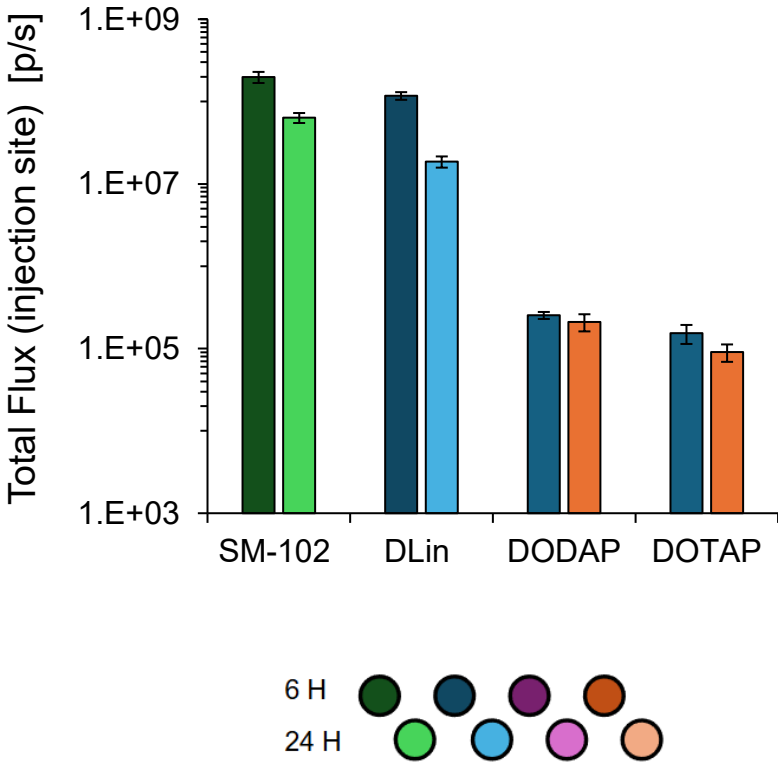
HTS: Predictive in vitro

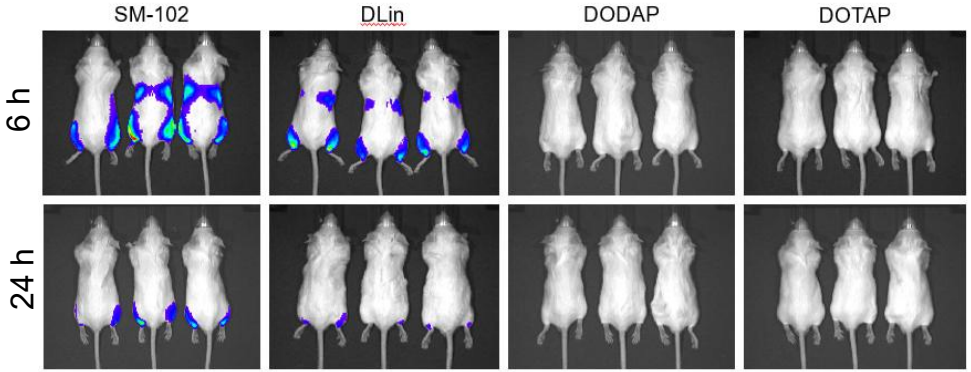
LNPs produced in 96 well plate format:

CQAs less controlled



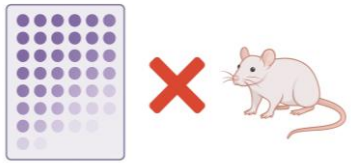
HTS formulations are predictive in vivo





In vivo is not HTS

In Vitro vs. In Vivo



Summary

What we know

- **Manufacture** tends to drive LNP CQAs
- **Lipid selection** dictates biological function.
- **Ionisable lipids** dominate
- **In vitro assays alone are not predictive.**

What we need

- **HTS Strategies** provides power discovery engine but how to we correctly down select?
- **Predictive, translatable models** with biologically relevant in vitro systems

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