In-vitro high-throughput screening for delivering genome editing reagents

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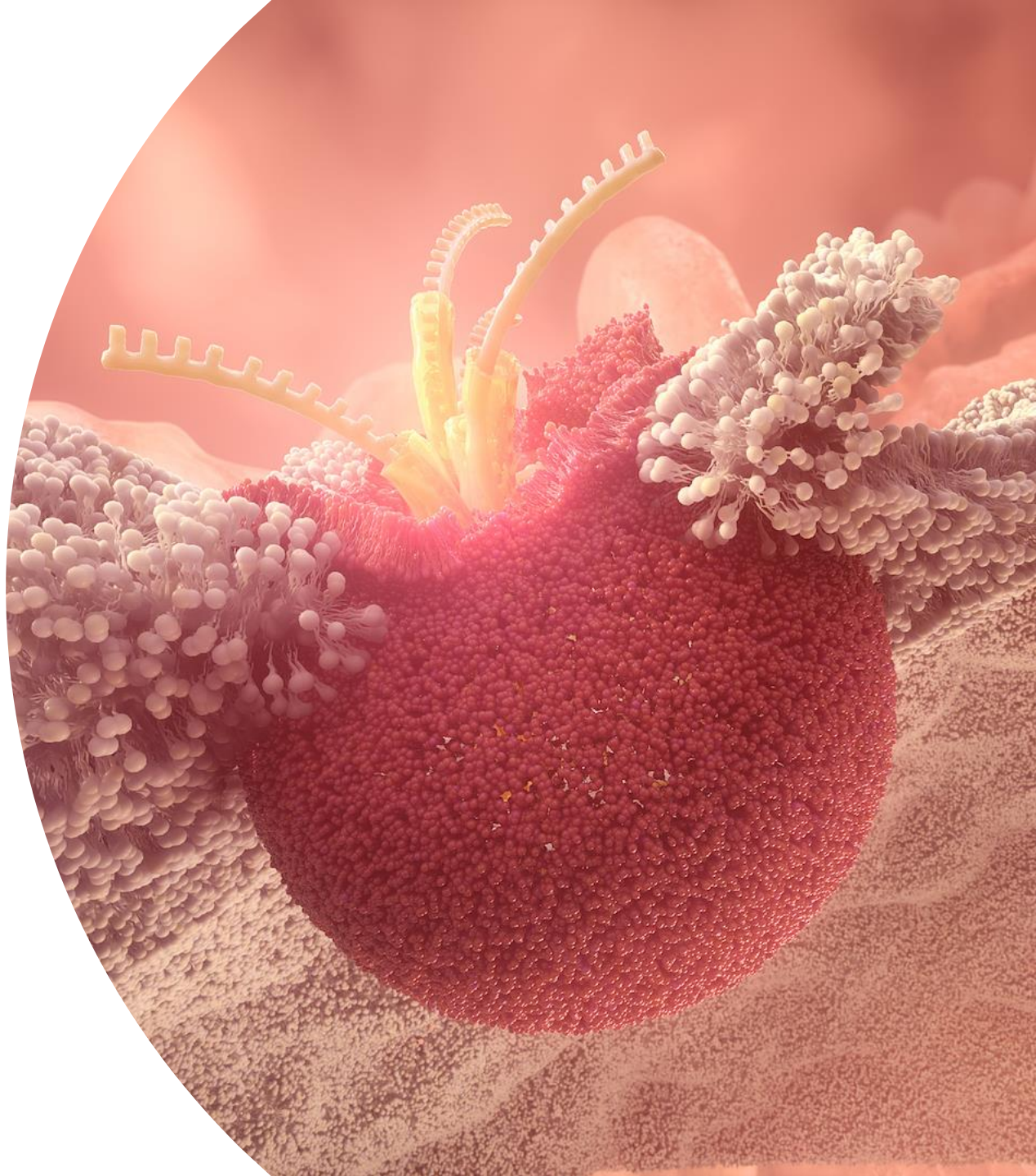


Overview

1. **Why:**
   The value of *in vitro* screening
2. **What:**
   Industrial screening workflow for CRISPR delivery
3. **How:**
   Focus on some unique screening challenges
4. **What next:**
   What is missing and what is on the way

1

In vitro screening and added value

Complexity and LNP engineering opportunities

Component Ratios

[mRNA:gRNA], [N:P],
[ionizable lipid:sterol:phospholipid:PEG-lipid]

Formulation Buffer

Type, pH, ionic strength, salts/additives, etc.

Formulation Method

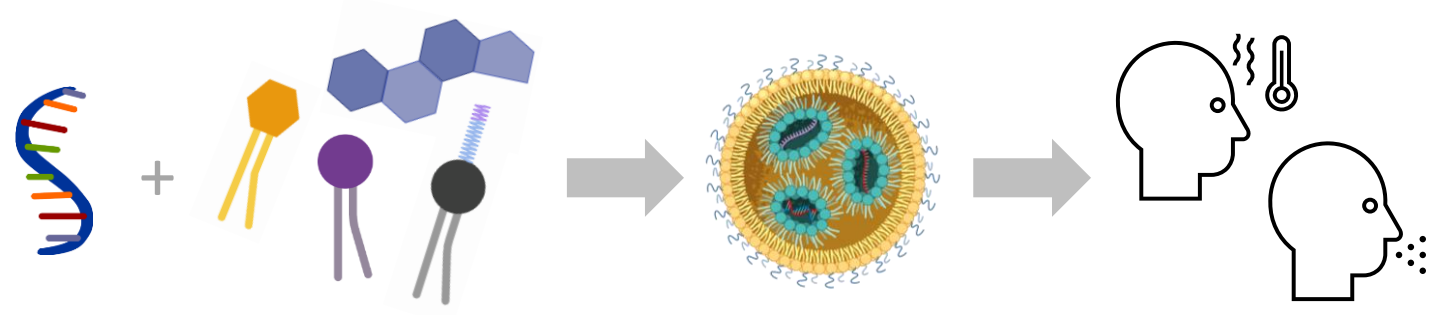
Platform, flow rate, volume, etc.

Post Processing

Method, buffer, pH, salts/additives, concentration, etc.

Components

mRNA, gRNA, mcDNA, ionizable lipid, sterol, phospholipid, PEG-lipid, etc.



Cargoes **Lipids** **LNPs** **Contexts**

Storage

State, temperature, light, time, etc.

All these parameters, individually or taken together, impact:

Efficacy, Safety, Stability, Manufacturability, IP, Cost

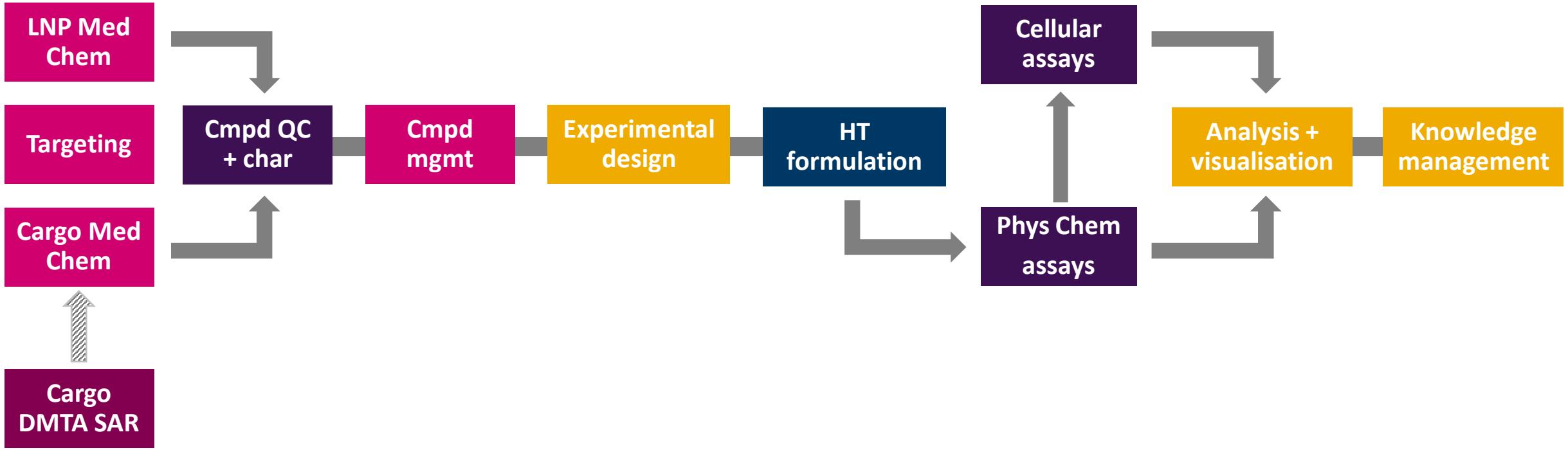
For CRISPR delivery, compare in vitro and in vivo

In-vivo (bar code)	Common	In-vitro (± bar code)
pharmacokinetics	bar coding possible	inexpensive
uptake, clearance, excretion	combinatorial	high throughput
corona formation	requires compound libraries	data rich
admin routes	dose response possible	very low reagent use
targeting	NGS possible/necessary	pharmacodynamics
safety	corona	mechanistic SAR (delivery, DDR, tox)
	efficacy SAR	no animals required
	test formulation process parameters	untested or toxic compounds
	translation unclear	reporter systems easy to build
	humanised systems	modular
	standardized workflows	easier humanised systems
	predictable costs	

2

Industrial HT screening of CRISPR delivery systems. What do you need?

Current AstraZeneca workflow



Molecular Biology
Bioinfomatics
Cell Biology
Chemistry

Compound Management
Digitisation

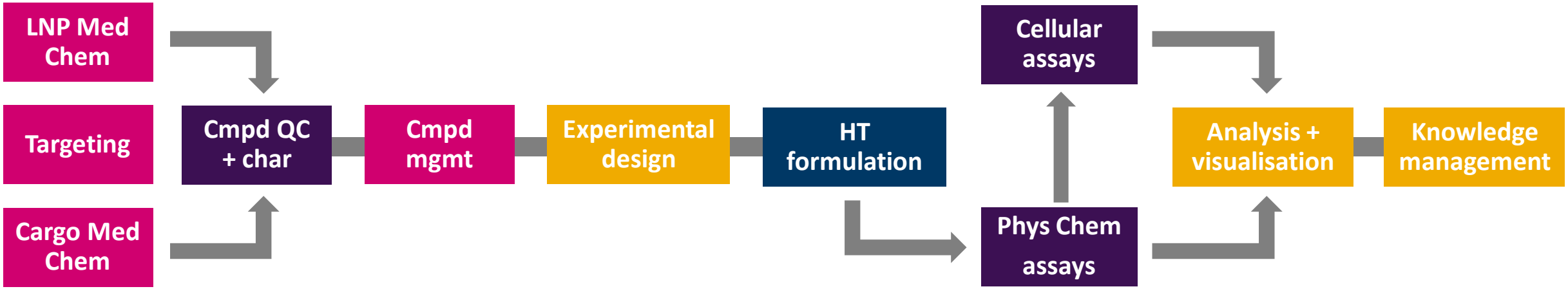
Data Science
Chemistry
Cell Biology
Formulation
Next Gen Seq

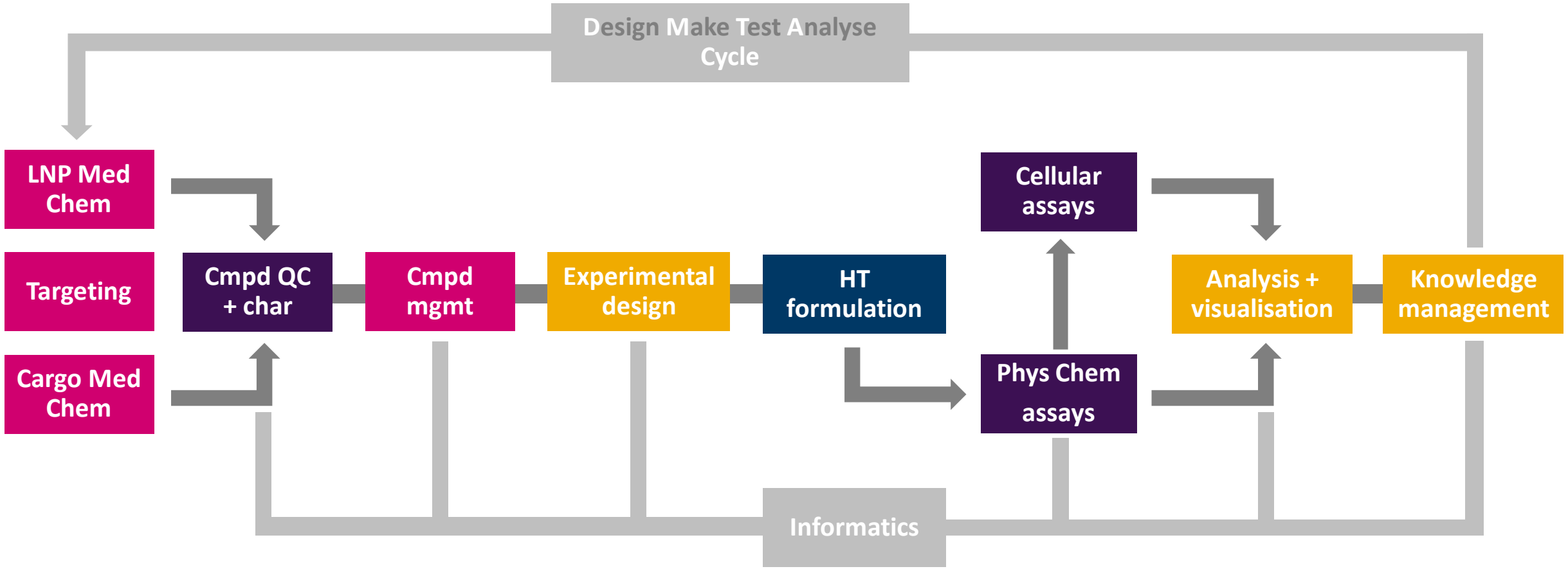
Formulation

Cell Biology
Formulation
-Omics
Next Gen Seq

Data Science

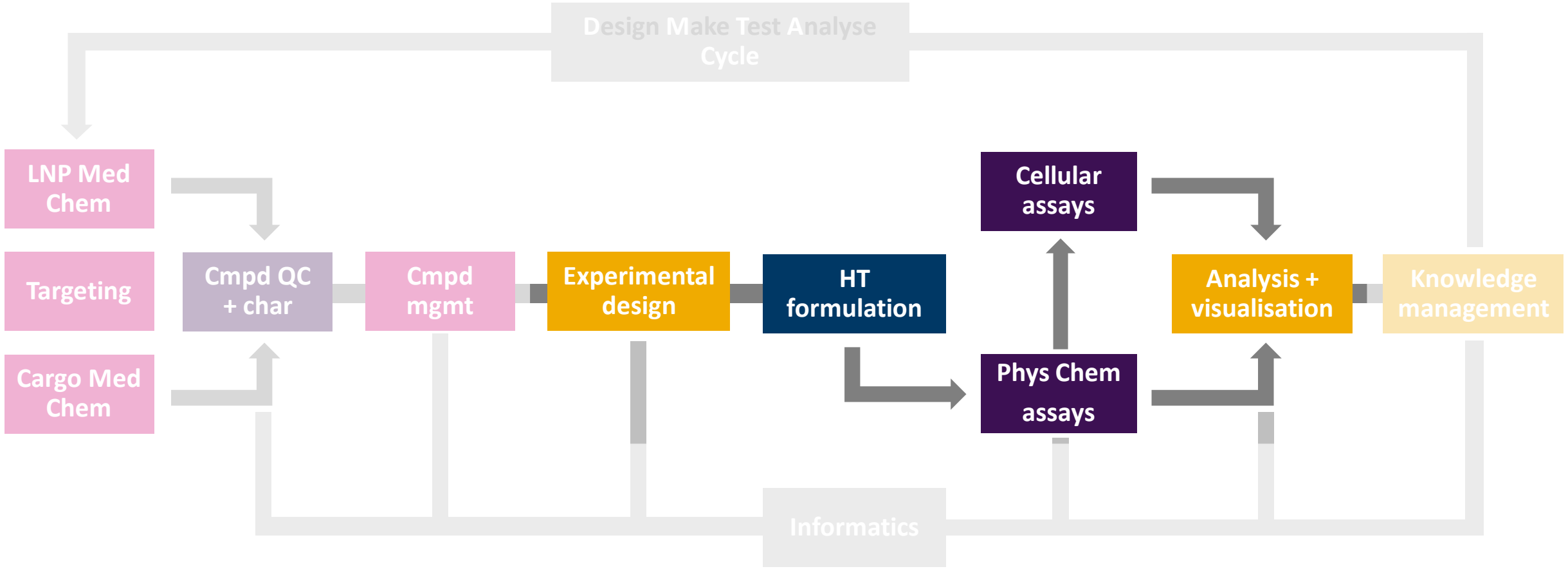
Data Science
Digitisation





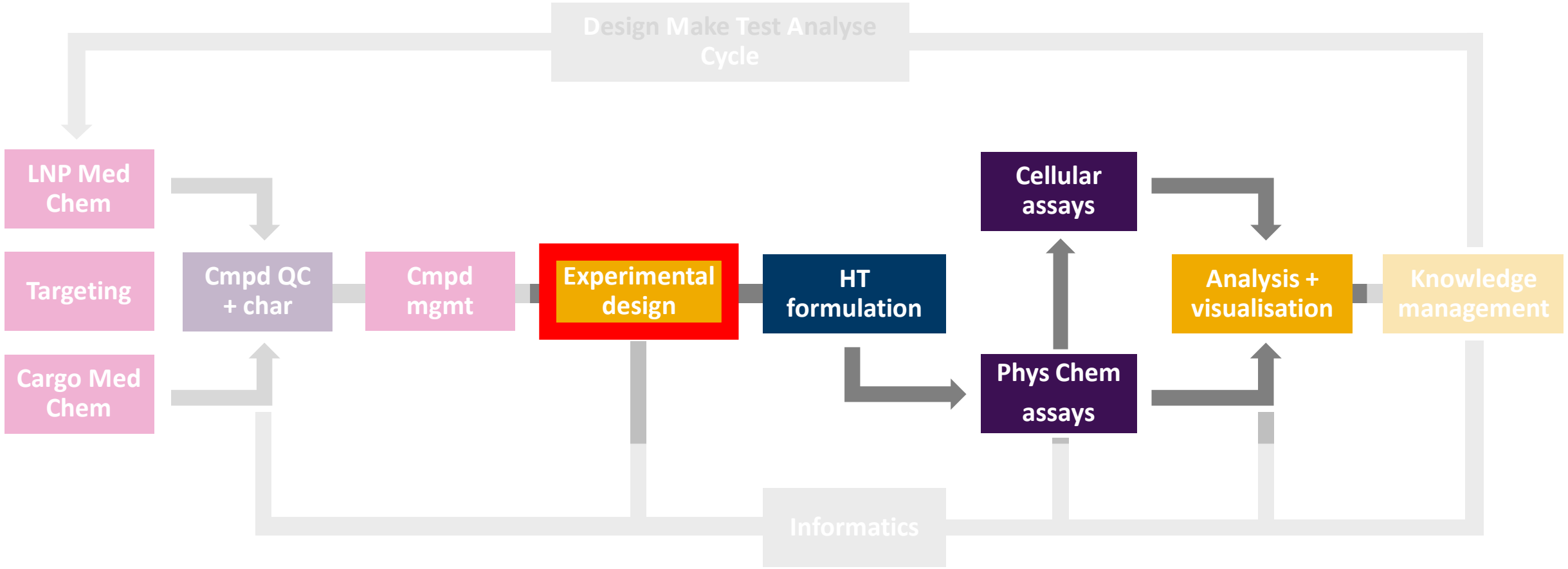
Digitisation/Informatics

IT


Design Make Test Analyse Cycle
LNP Med Chem
Targeting
Cargo Med Chem
Cmpd QC + char
Cmpd mgmt
Experimental design
HT formulation
Cellular assays
Phys Chem assays
Analysis + visualisation
Knowledge management
Informatics

3

CRISPR delivery – unique screening challenges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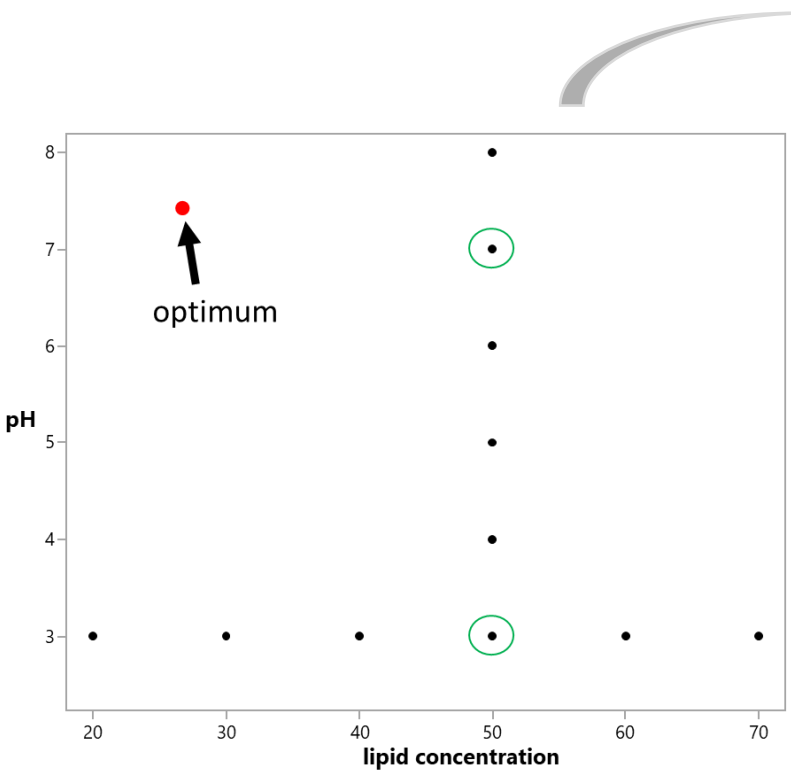
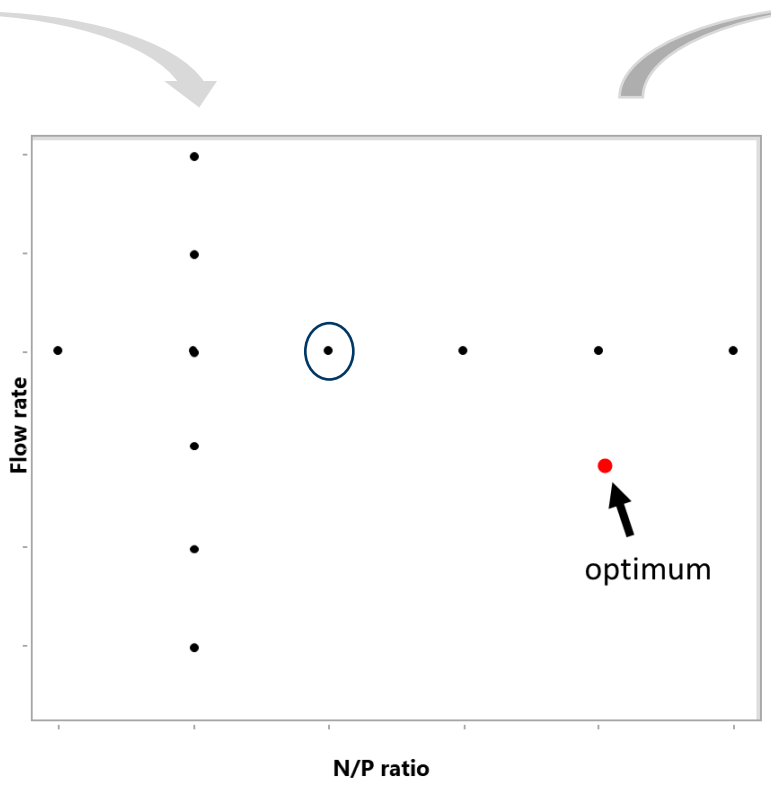
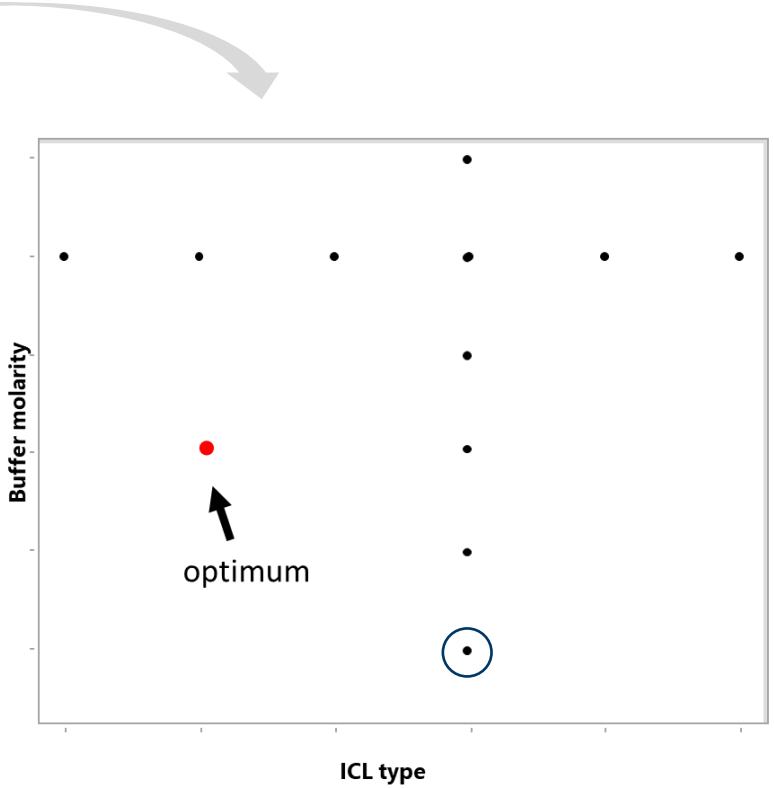
Limitations of traditional experimental approach: Multiple One-Factor-At-A-Time (OFAT)

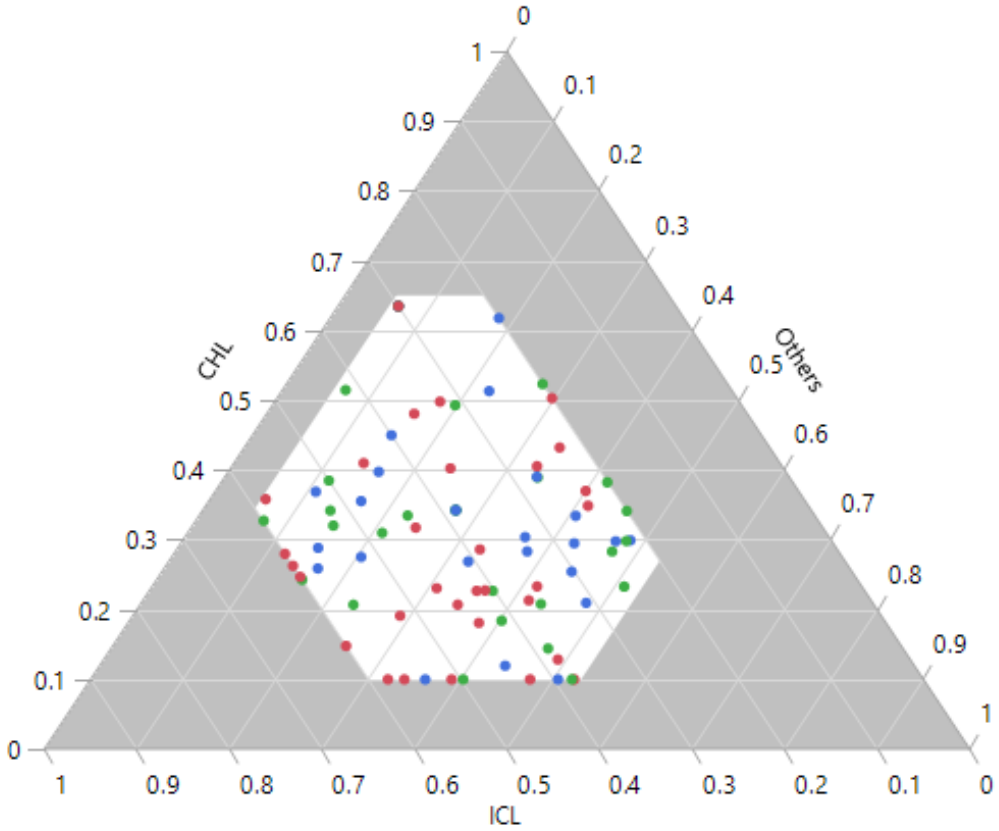




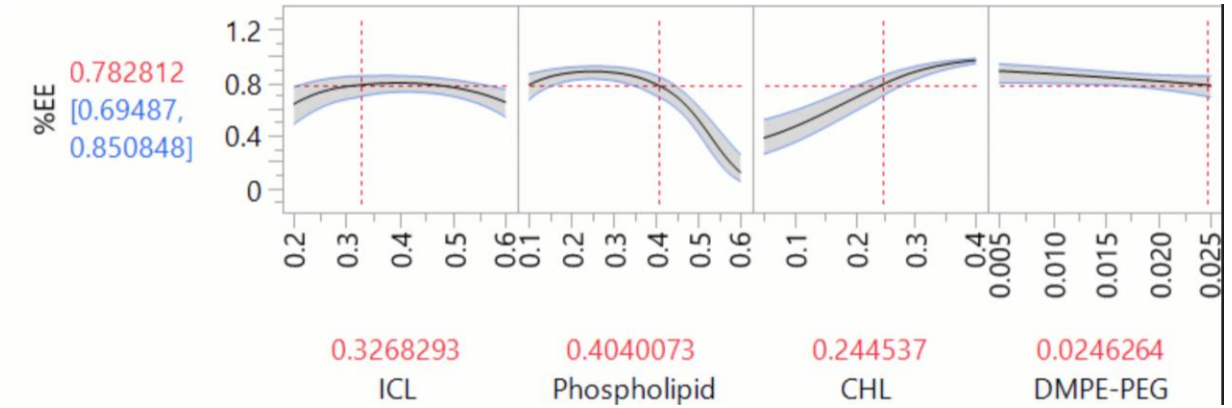


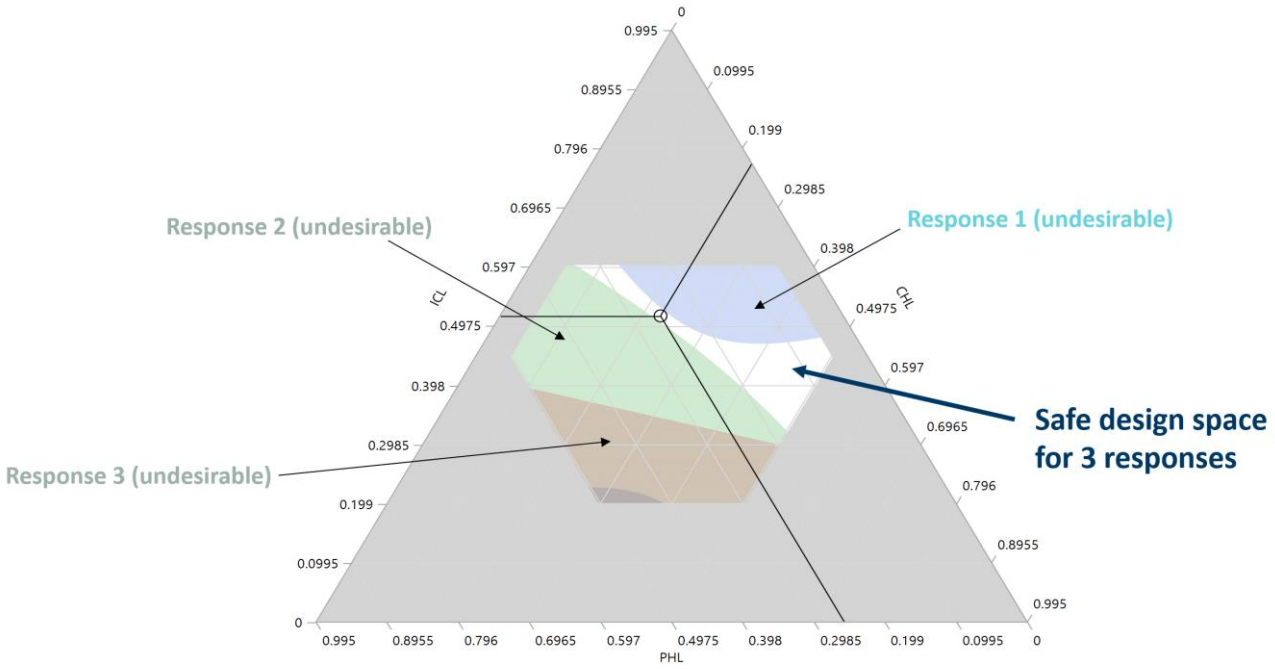
- Multiple OFAT experiments is a risky approach for complex systems
- Can lead to sequential loss of optimum conditions
- Ignores interactions between factors
- Higher costs, slower, dead-ends

Statistical modelling and optimisation of LNP composition, for cargo AND target

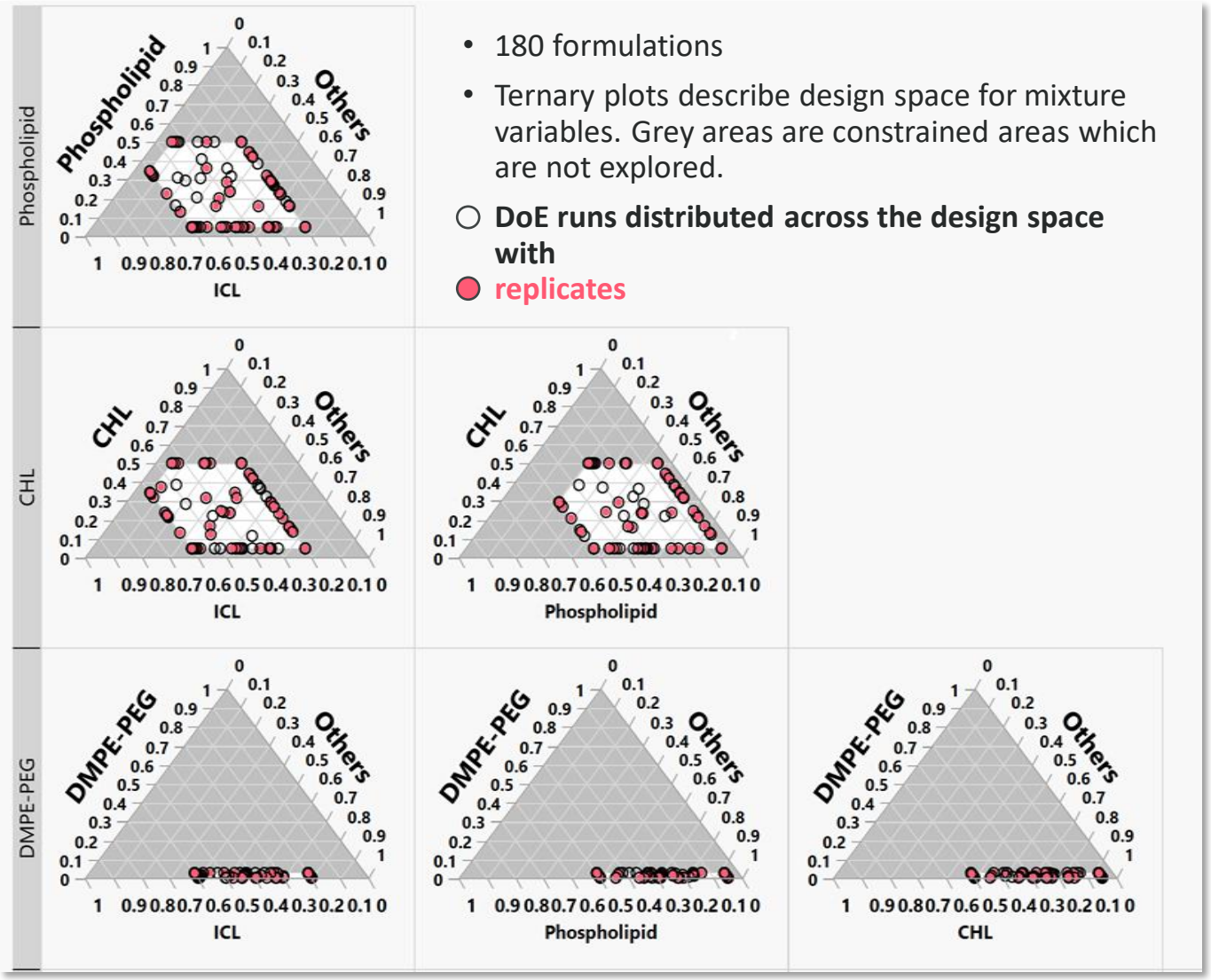


Different colours indicate different cationic lipids (ICLs)





Example: Avoid liver cells, transfect immature dendritic cells



Vary proportion of :

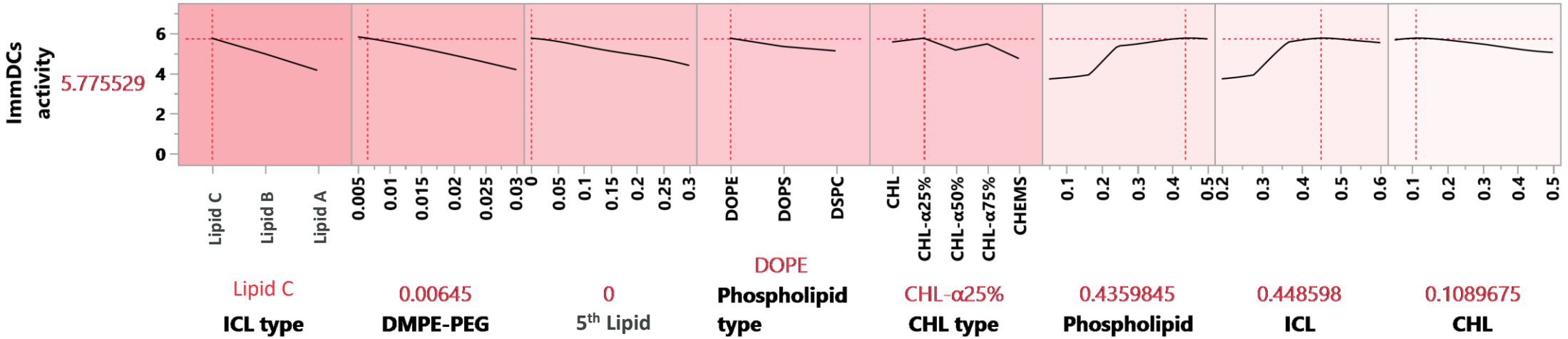
- Ionisable lipid
- Phospholipid
- Cholesterol
- DMPE-PEG
- 5th lipid

Vary identity of:

- Phospholipid (DSPC, DOPS, DOPE)
- Ionisable lipid type (3)
- Cholesterol type (CHEMS, unmodified + 7α hydroxycholesterol)

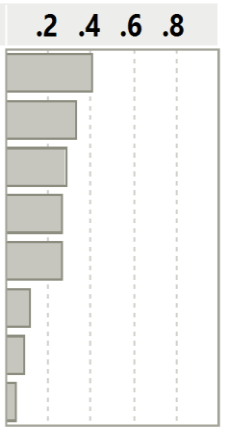
How the choice of lipids affect dendritic cell activity?

There are complex non-linear relationships between LNPs components and activity



Ranking LNPs components by importance

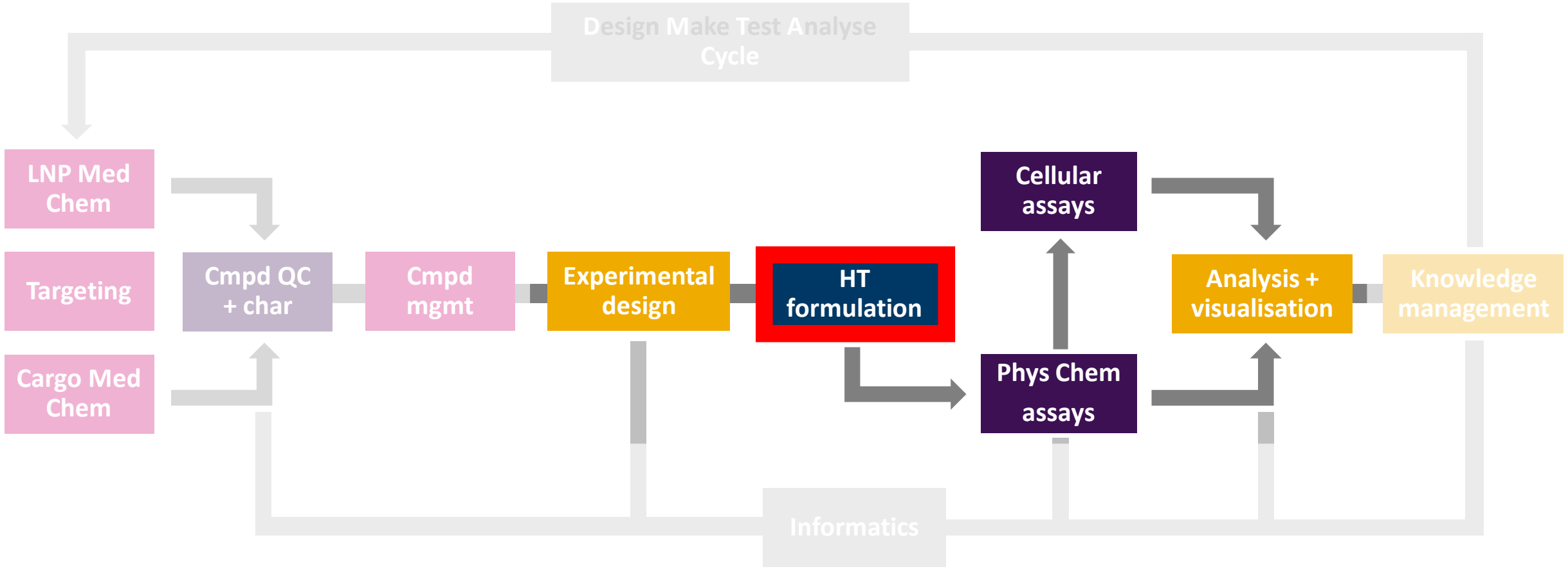
Column	Main Effect	Total Effect
ICL type	0.262	0.403
DMPE-PEG	0.097	0.333
5th Lipid	0.043	0.279
Phospholipid type	0.262	0.262
CHL type	0.262	0.262
Phospholipid	0.03	0.112
ICL	0.021	0.083
CHL	0.024	0.05



General Trends:

- ❑ **ICL type is the most important :** Lipid C > Lipid B > Lipid A
- ❑ PEG seems to have stronger impact on immDC activity
- ❑ CHEMS has negative impact on activity
- ❑ Higher 5th lipid generally reduces activity

Nice, but it’s a lot of LNPs…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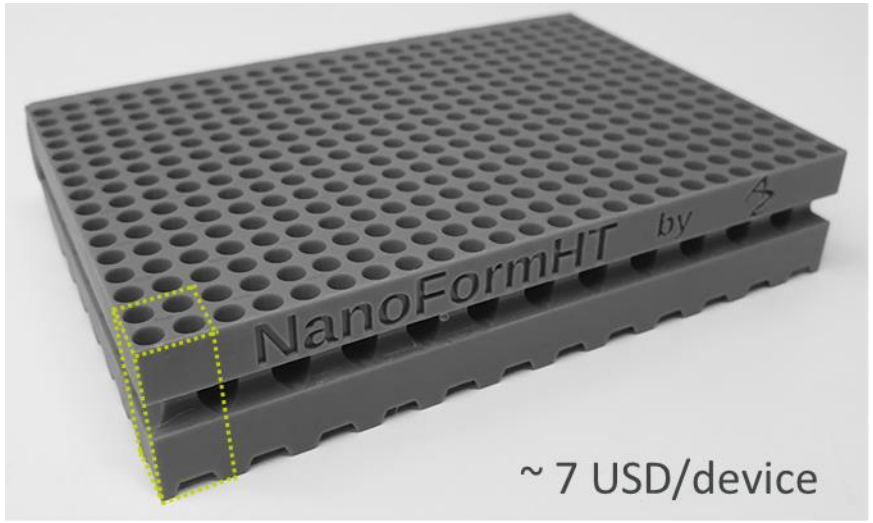
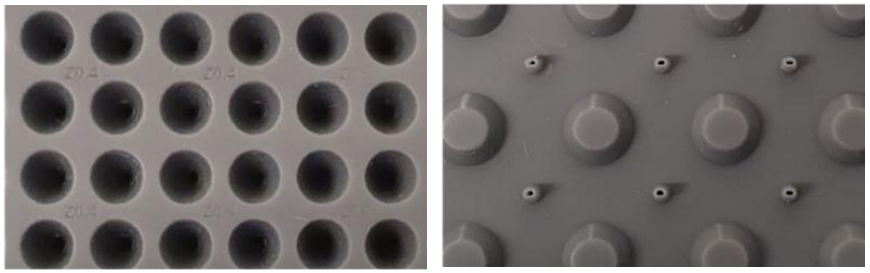
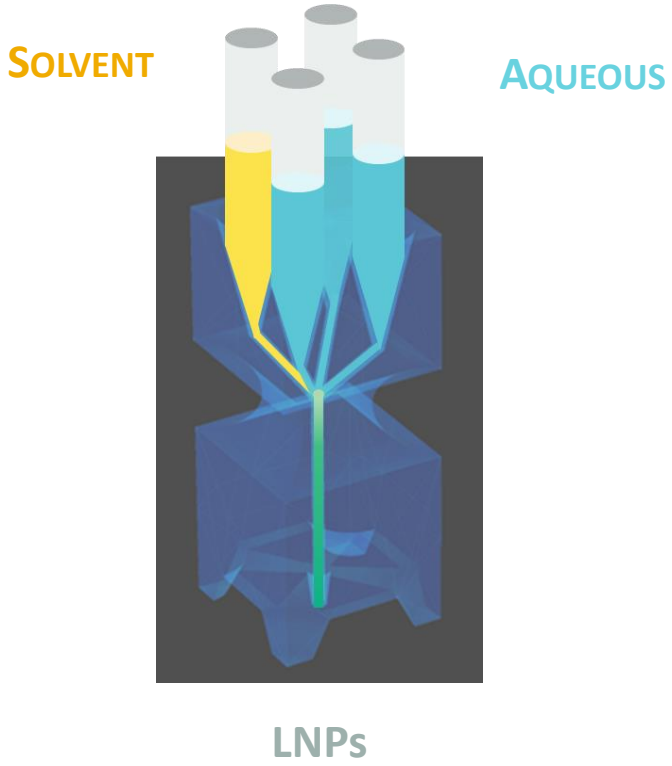
The NanoFormHT platform for parallel LNP formulation

3D printed micromixer array





Fluid routes in one mixer



Compatible with automation

Patented, Gallud A. et al. in preparation, 2024

Up to 96 LNPs takes less than 1 minute to complete. Automation compatible. Very small volumes.

How do we build the NanoFormHT source plate?

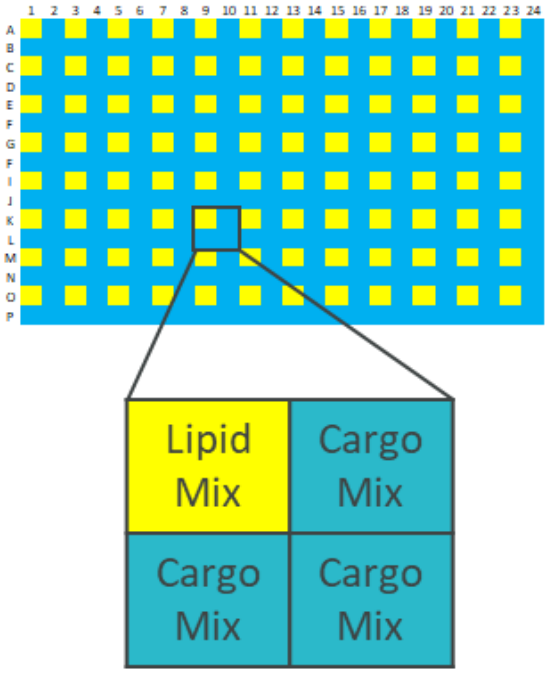
1. Input .csv files
2. Perform the dispensing on individual components using dragonfly

Aspirating components

Lipids
EtOH
Cargo
Buffer

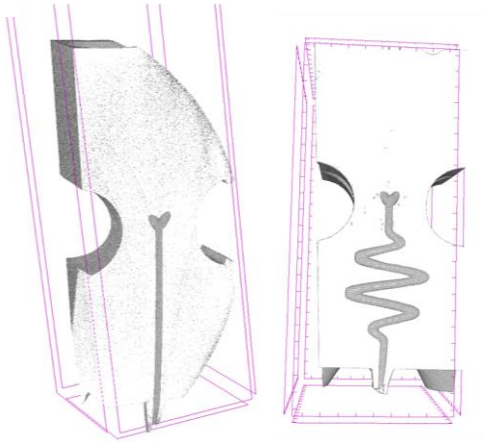
Dispensing in plate

Source plate prepared



Quality control, performance validation

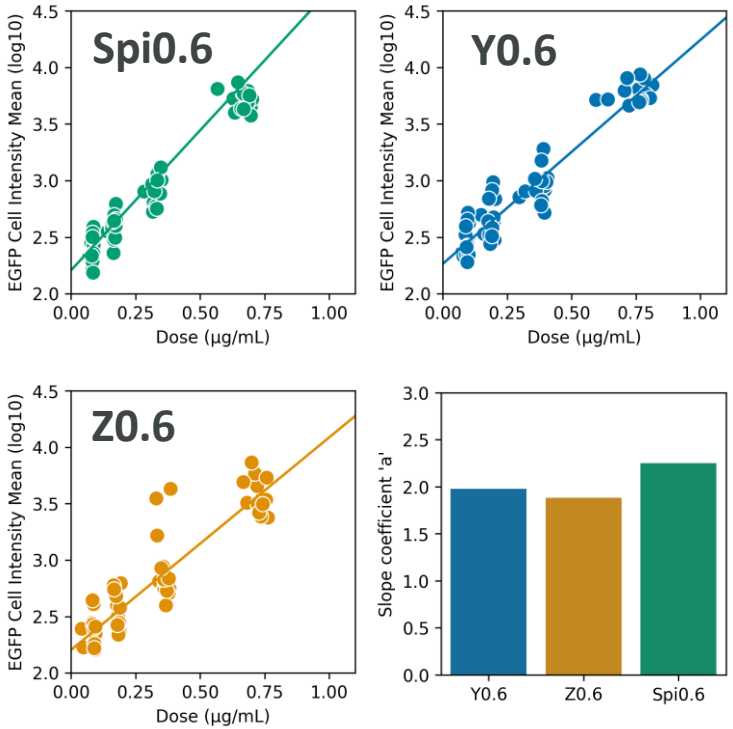
X-ray microtomography followed by alignment and reconstitution



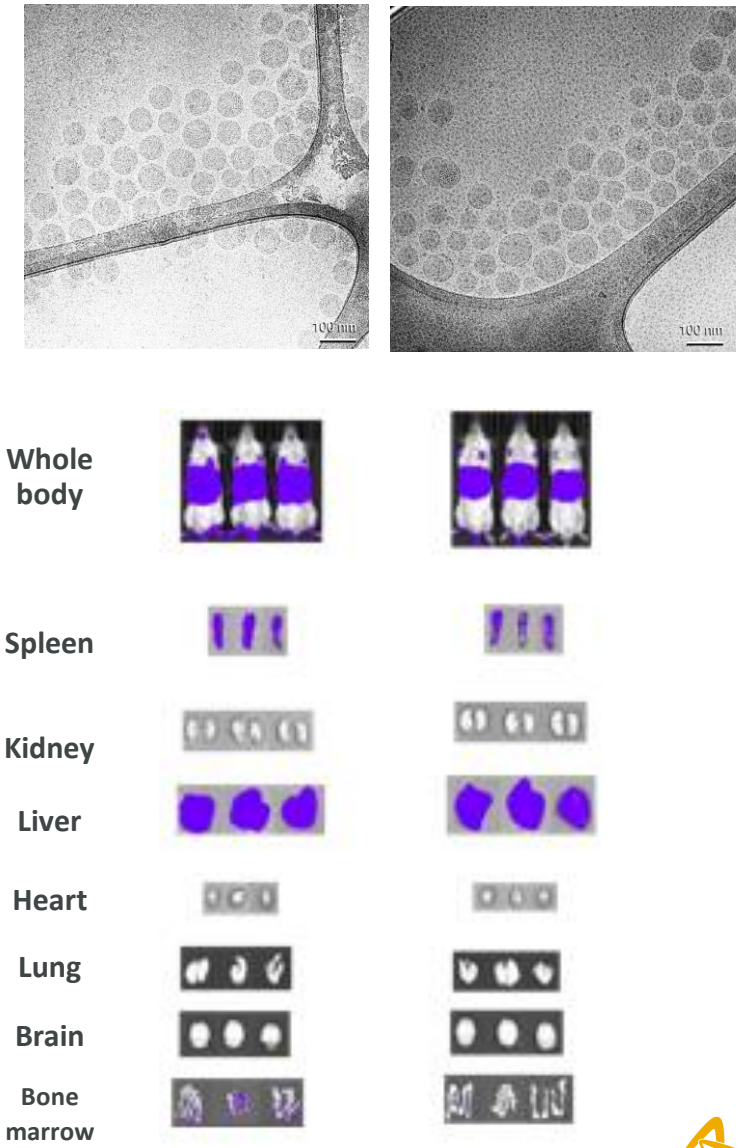
Y0.6 Z0.6

Channel diameter
Theoretical: 600 µm
Experimental: 460 ± 10 µm

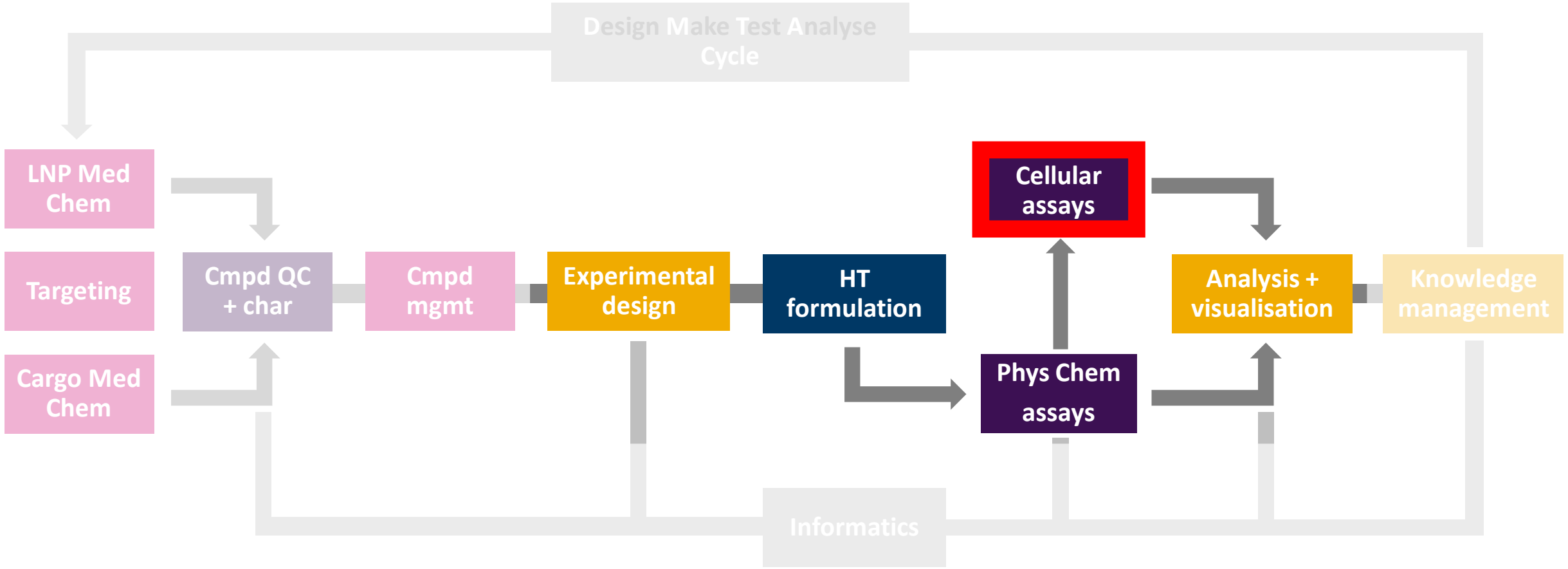
Internal geometry does not affect LNP performance *in vitro*



HEK293 cells doses for 24 h, imaged and analyzed for eGFP expression



Gallud A. et al. in preparation, 2024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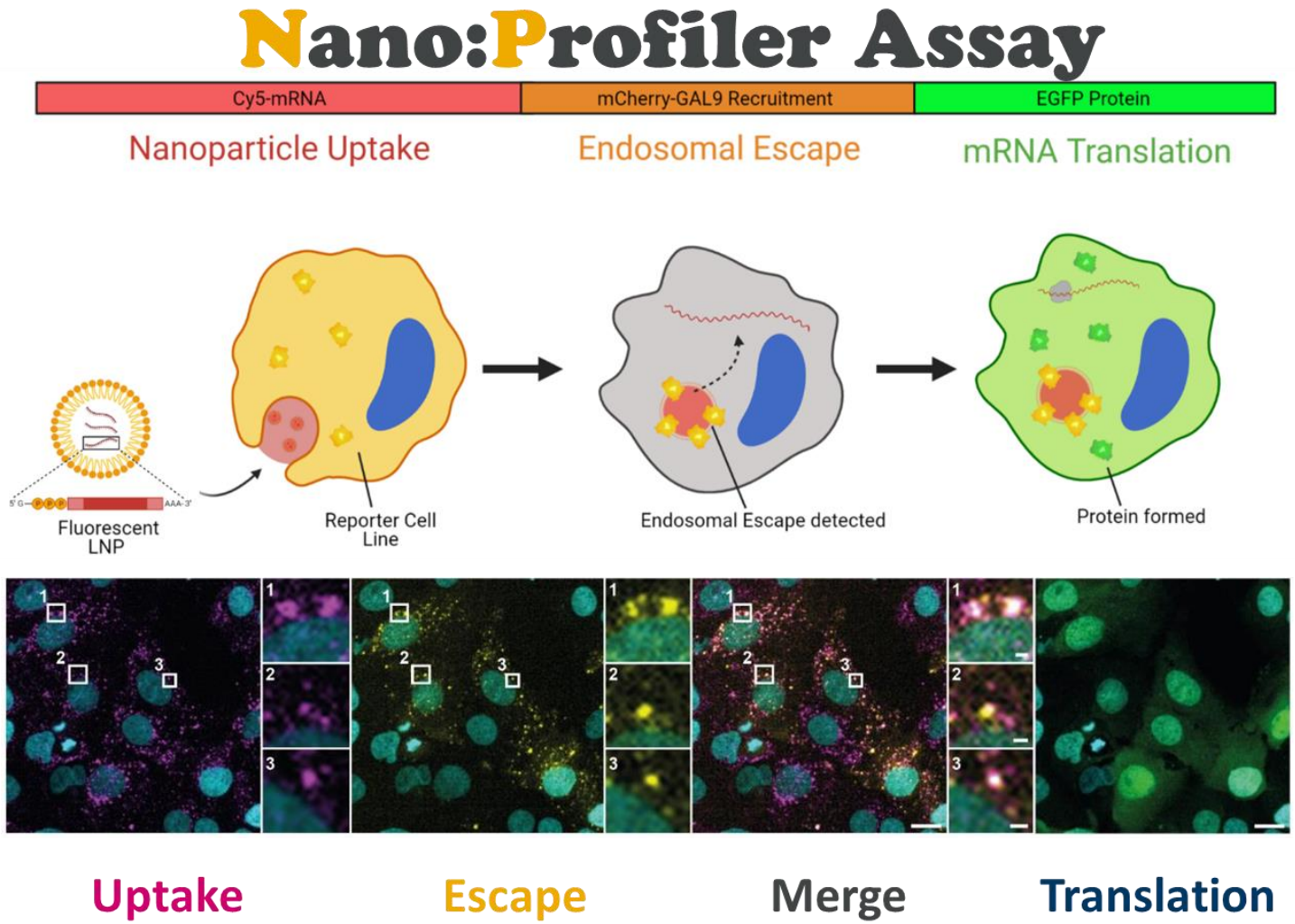
HT uptake, endosome remodelling, productive delivery and tox



Key Features:

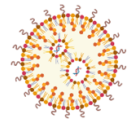
Miniaturised to 384/1536-well format

Robust assay (Z' Factor = +0.74)

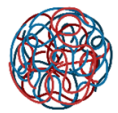
No assay processing steps required

Detects many leading delivery technologies

Stable integration in any human cell type



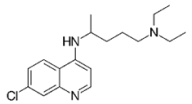
Lipid Nanoparticles



Polymer Nanoparticles

Munson et al, 2021

Ulkoski et al, 2021



Endosomal Escape Enhancers

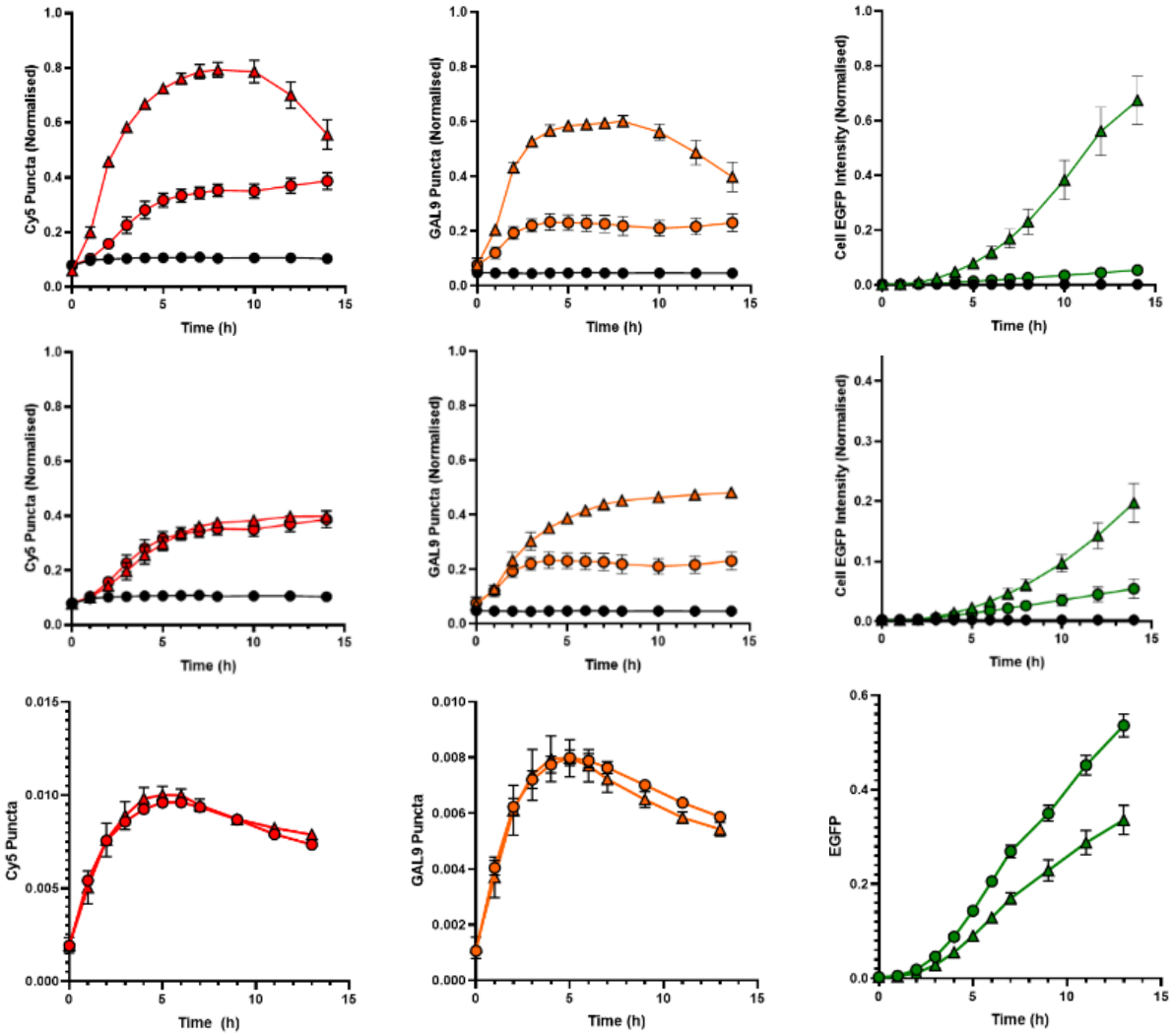
Dendrimers

Bost et al, 2022

Joubert et al, 2023

Imaging reporters for SAR deconvolution and kinetic analysis

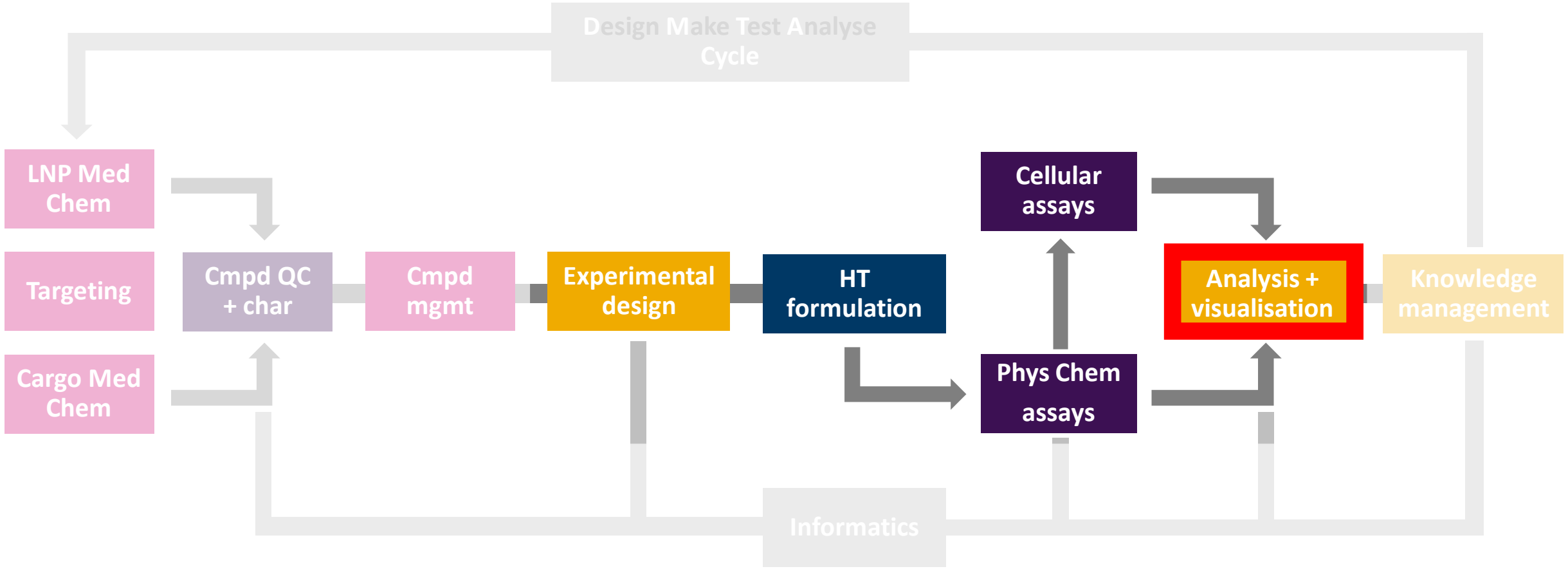
Uptake Escape Delivery



Uptake Difference

Endosomal Escape Difference

Translation Difference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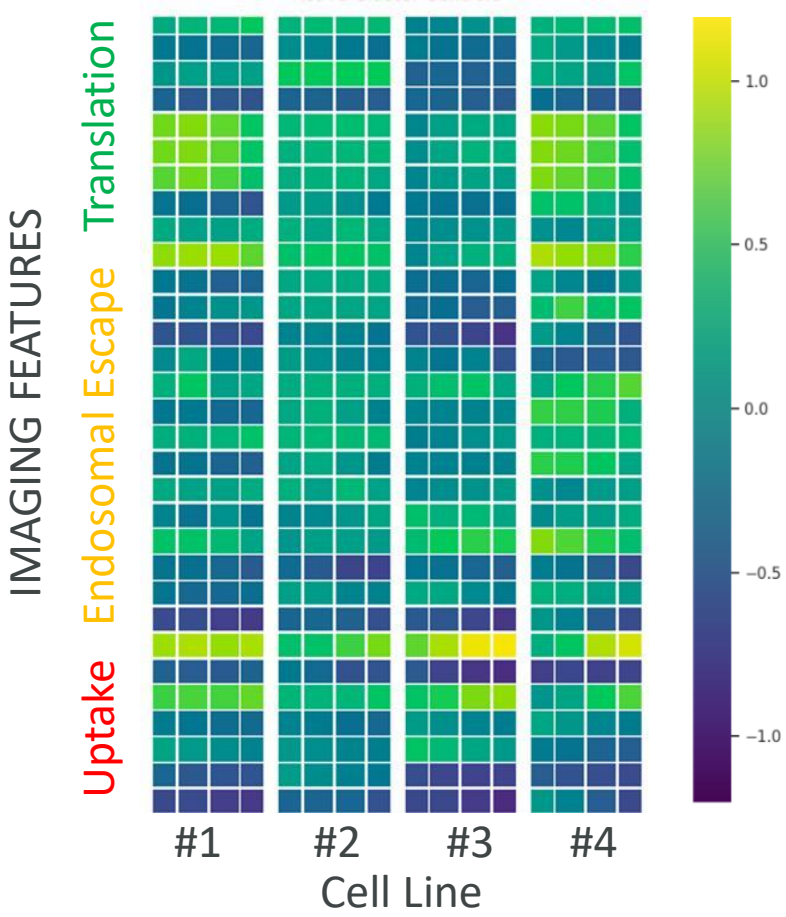
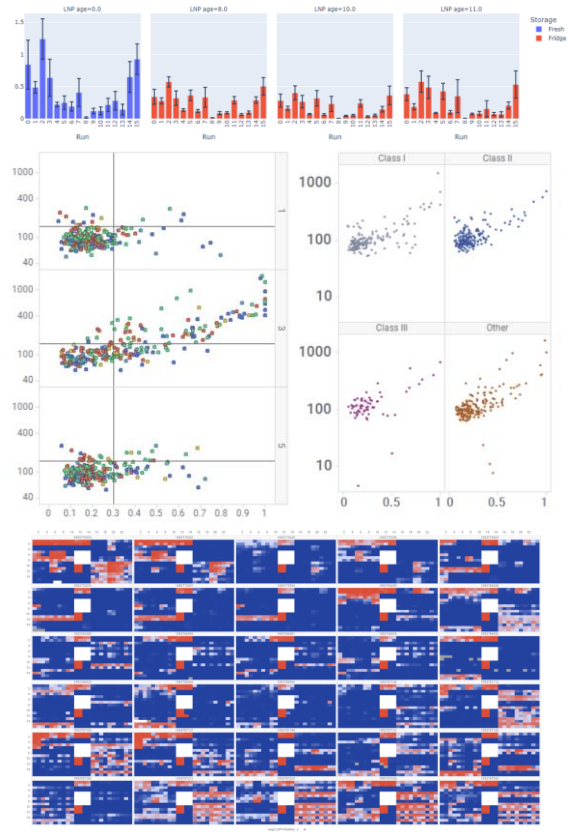
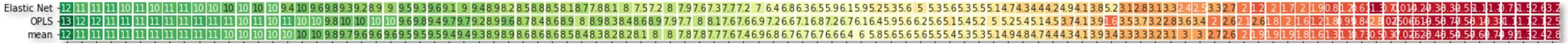
DMTA data overview

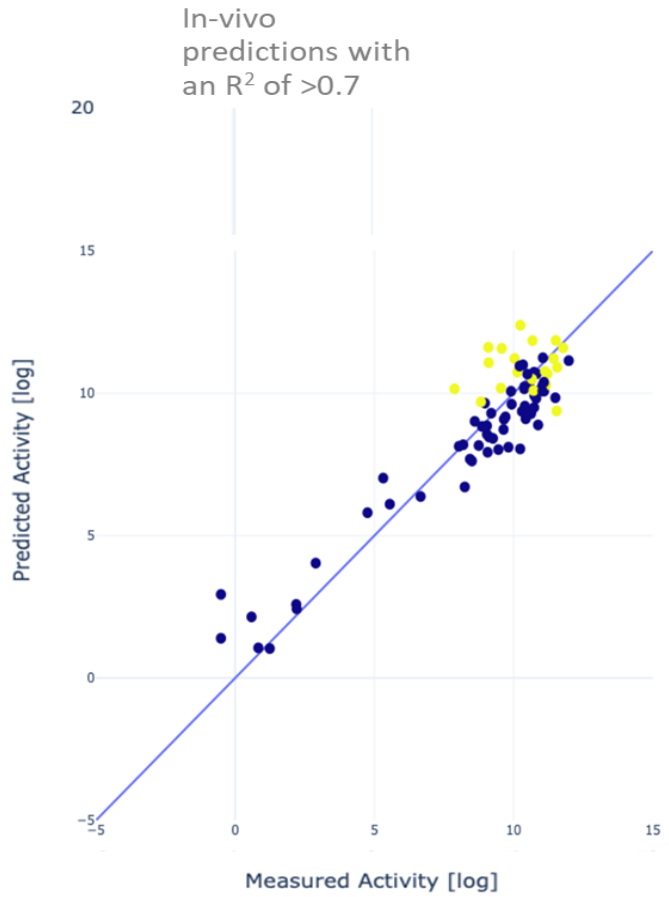
- **4** diverse cell lines with imaging reporters
- **4** different doses
- **5** different LNP formulations reference MC3 composition repeated twice (1 and 5)
- **Hundreds of measurements**/ cationic lipid
- **Thousands** of formulations





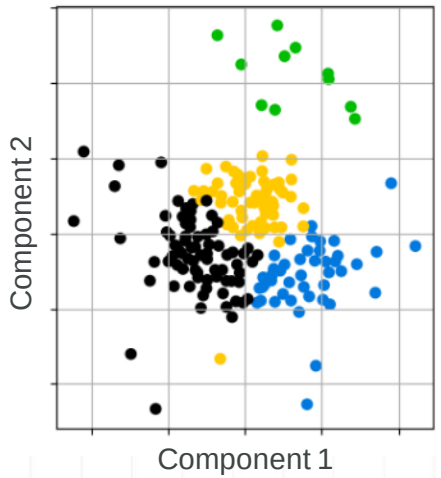
Prediction of In-Vivo Liver Activity



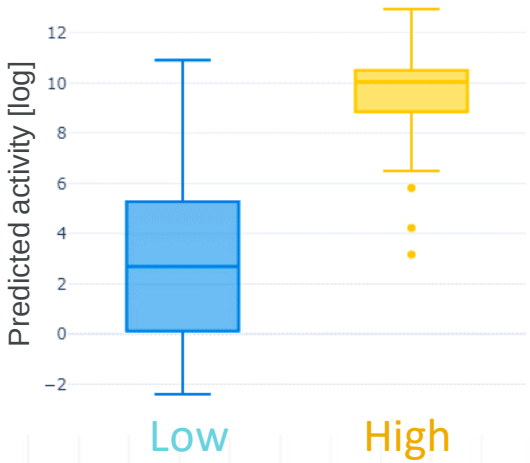


Data Insights

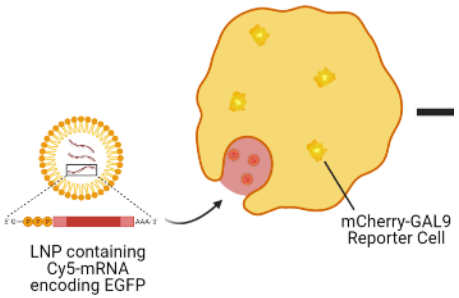
Group Clustering:



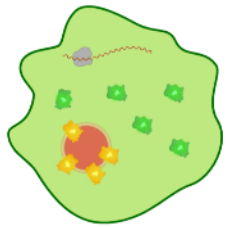
Activity:

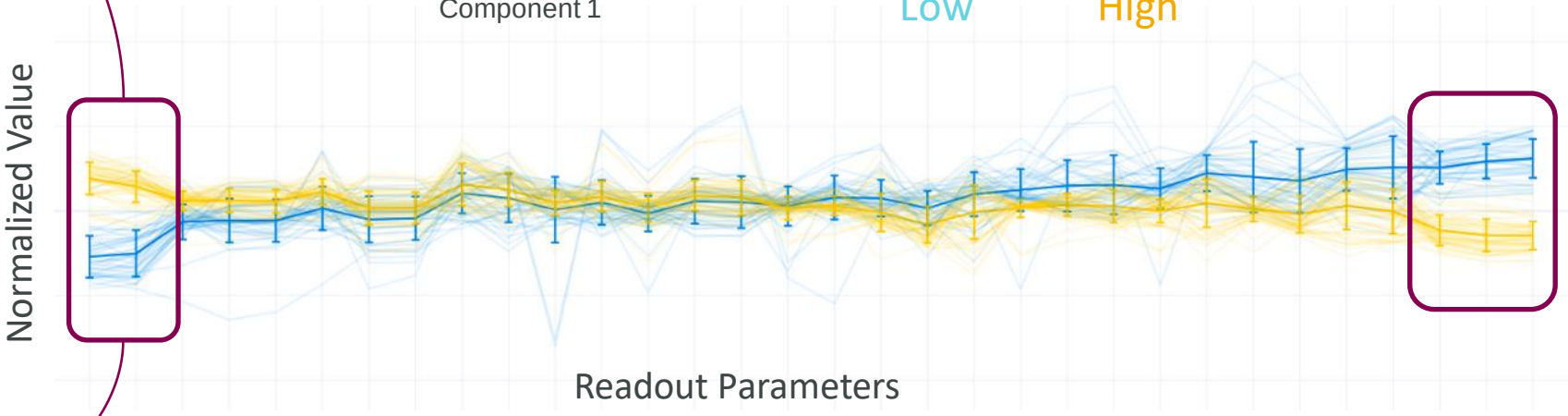


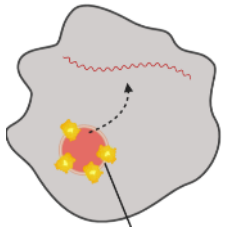
Particle uptake



EGFP production
per particle







Release events
per particle

LNPs can be clustered by features

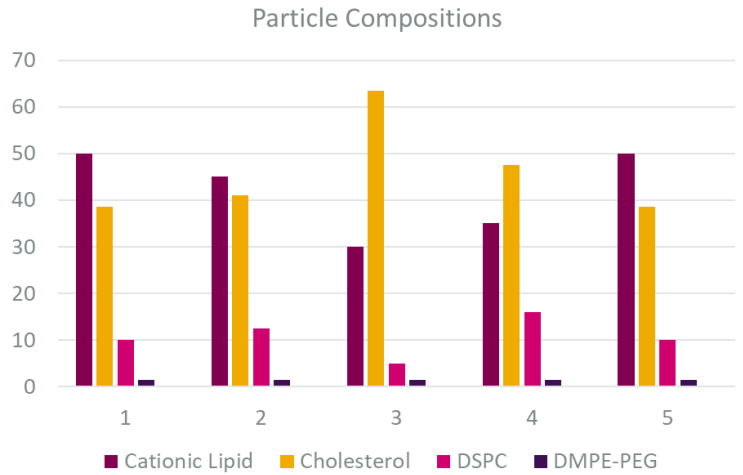
Active LNP clusters have distinct in vitro ‘fingerprints’ related to LNP processing

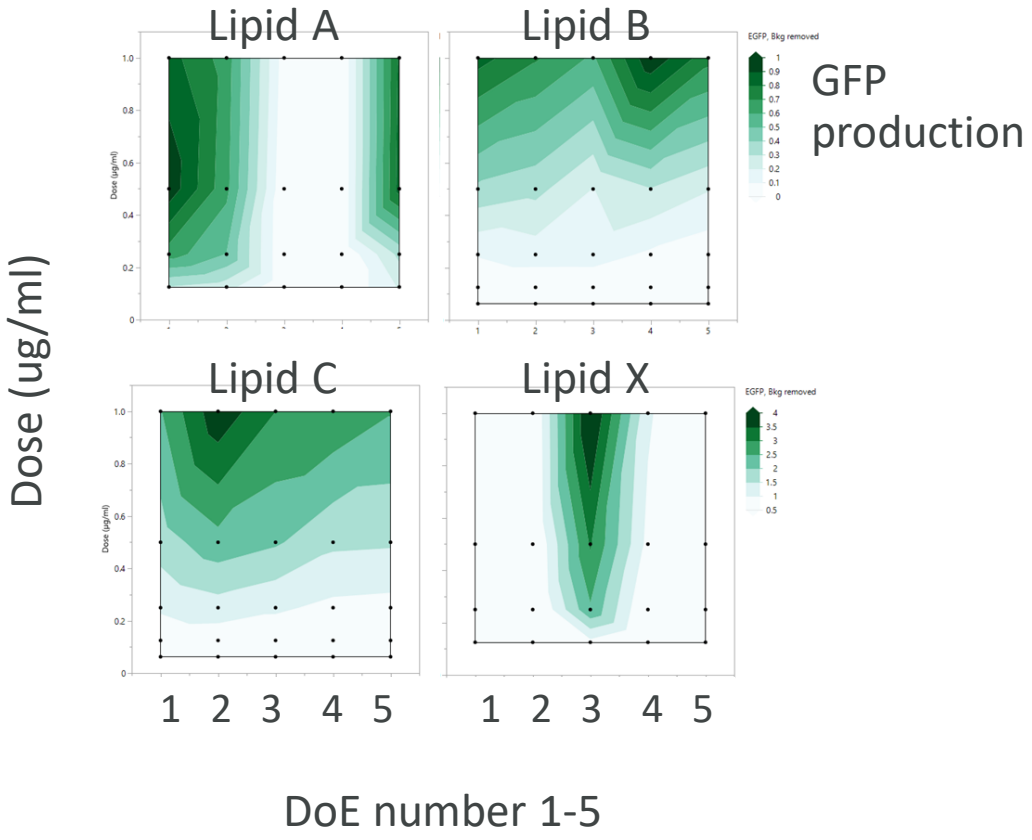
Composition reveals true activity

- Using only one formulation masks activity of some lipids
- Chemists need accurate activity measurements for building SAR models
- Case study, lipid “X”

  DoE 3 improves GFP production by 15x!

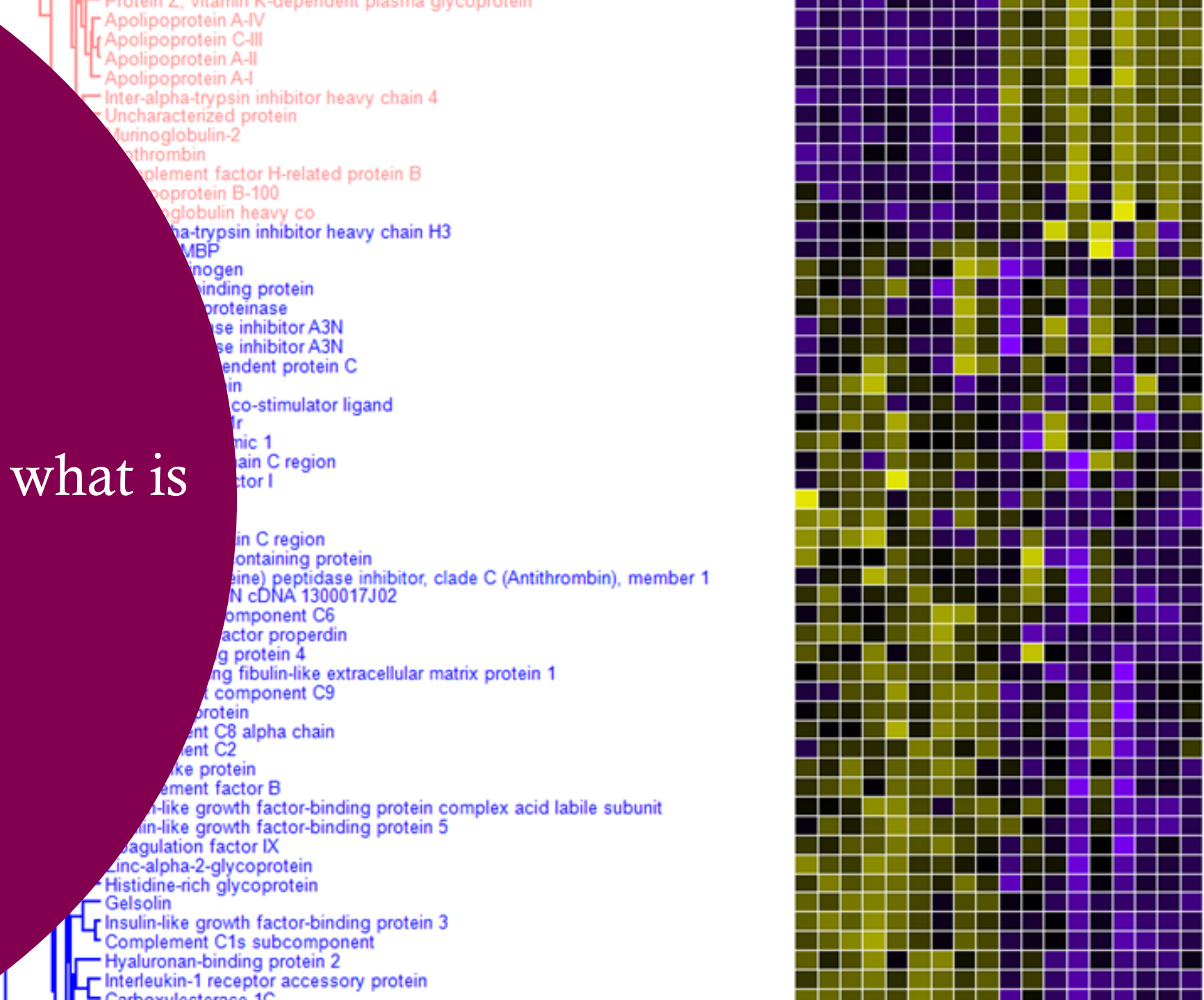
  Best lipid in standard formulation is 8x above background





4

What is missing and what is on the way



HT screening for delivering genome editing systems, To do…

Component Ratios

[mRNA:gRNA], [N:P], [ionizable lipid:sterol:phospholipid:PEG-lipid]

Formulation Buffer

Type, pH, ionic strength, salts/additives, etc.

Formulation Method

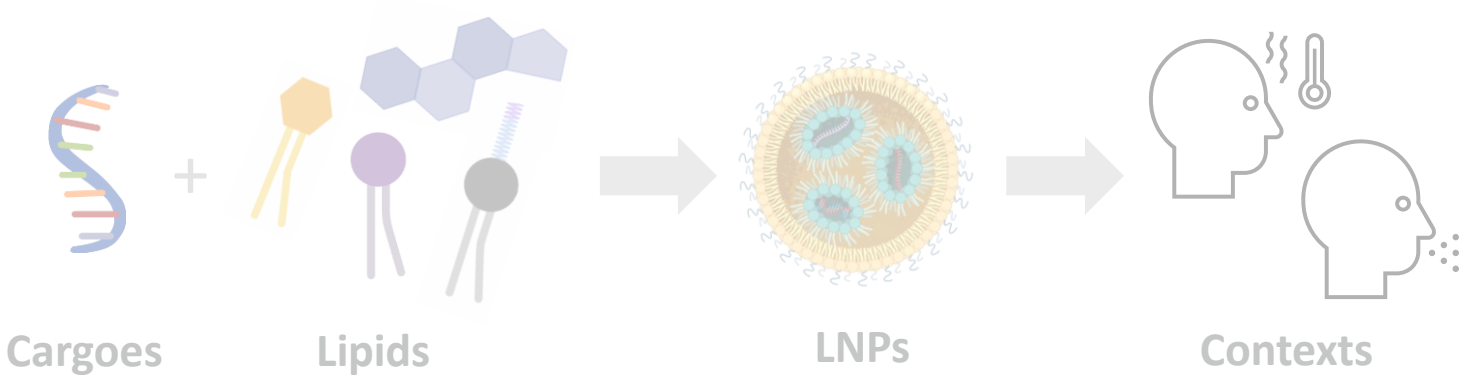
Platform, flow rate, volume, etc.

Post Processing

Method, buffer, pH, salts/additives, concentration, etc.

Components

mRNA, gRNA, mcDNA, ionizable lipid, sterol, phospholipid, PEG-lipid, etc.



Storage

State, temperature, light, time, etc.

All these parameters, individually or taken together, impact:

Efficacy, Safety, Stability, Manufacturability, IP, Cost

HT screening for delivering genome editing systems, To do…

HT OMICS

- Coronal proteomics, lipidomics and glycomics; pathological context

Particle manipulation

- HT-FANPS – high-throughput fluorescence activated nanoparticle sorting

HT structure

- Low cost, high throughput structural information; translational or mechanistic value

Data-driven translational models

- Bar-code-mediated refinement

The phenotypes of nanomedicine mechanism and function

- What characteristics should we optimise for?
- The nuances of endosomal escape and relation to toxicity and cargo expression or function

Thank you!

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