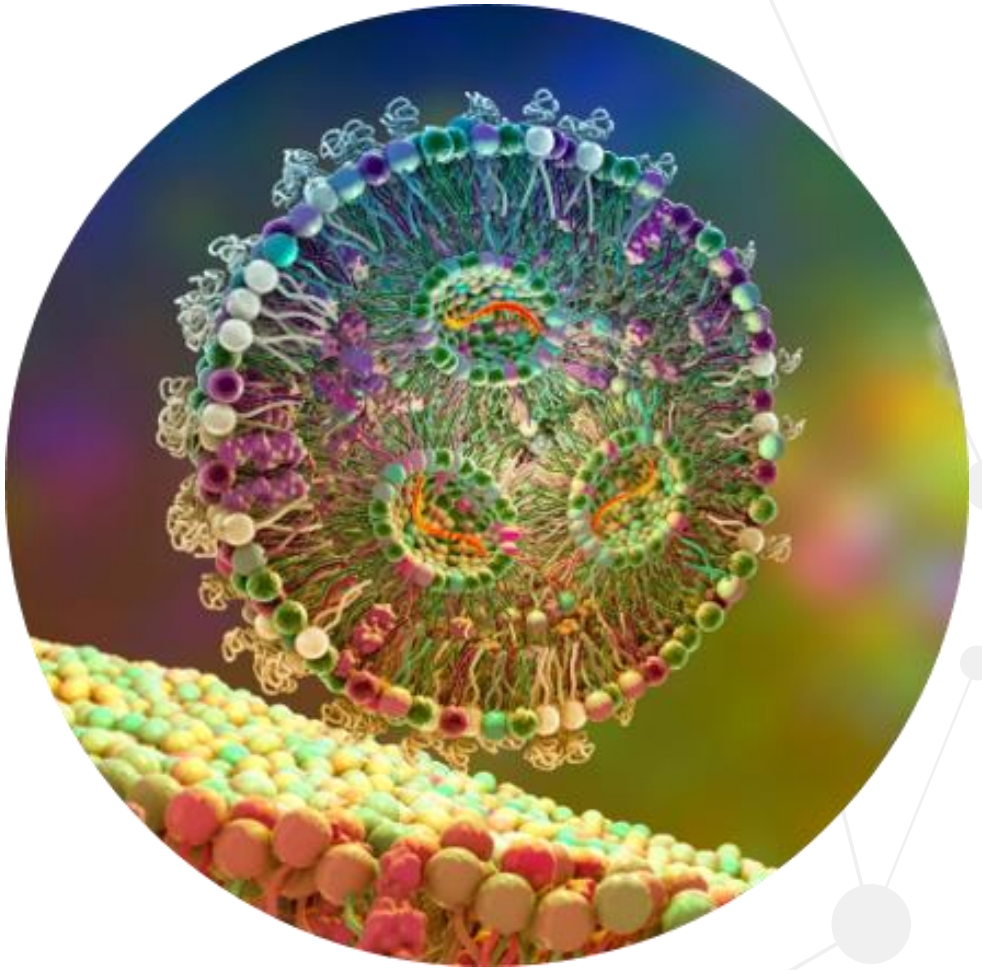
AI assisted development of lipid nanoparticles for RNA therapeutics



Nima Hojat

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Intracellular Drug Delivery Centre (IDDC)

- Centre of Excellence for companies/academics to access state of the art capabilities and expert support
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- R&D programmes to critical scientific and commercial challenges

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LNP formulation screening and characterisation

Enhanced methods to enable in vitro -in vivo correlation

Smart, next gen manufacturing

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Overarching technology focus areas

Screening & Formulation

Manufacturing

AI for lipid design, novel lipid synthesis and screening	Automated HT formulation to screen lipid & formulation against multiple payloads	Cobot integration – lab of the future	HT Characterisation (physicochemical, cell based toxicity and transfection)	In depth, advanced characterisation (in vitro, in vivo, advanced imaging)	Next gen LNP manufacture (digitally enabled, sustainable)	New integrated PAT, advanced process models, digital twins

Underpinning digital capability – upload data to cloud, apply advanced models to enable adaptive (intelligent) DoE, model development and deployment, simulation

Creating a platform for screening, characterisation and manufacture that is flexible and adaptable to answer multiple research questions (apply to different delivery tech, tune for thermostability, targeting, immunogenicity, test new PAT and models)

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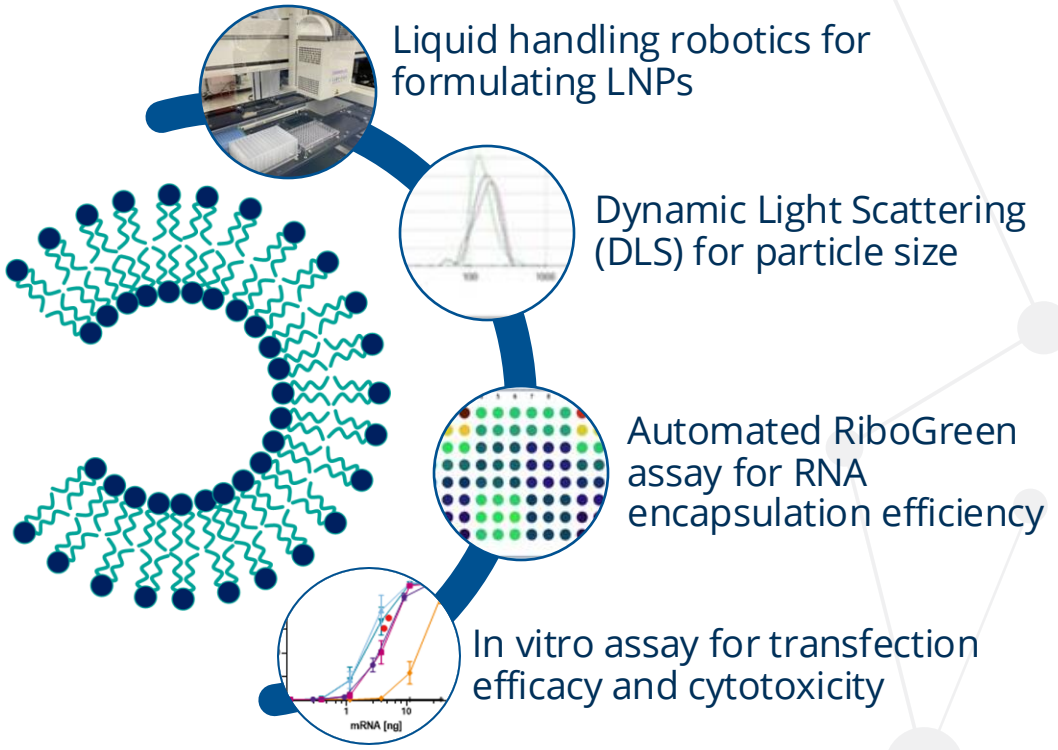
Project Objectives

Develop a **high throughput screening workflow** to **design & formulate LNPs** for optimal performance, efficacy & safety

Use the flexible automated platform for **screening** of novel and existing **lipid libraries**, in addition to different **RNA payloads**

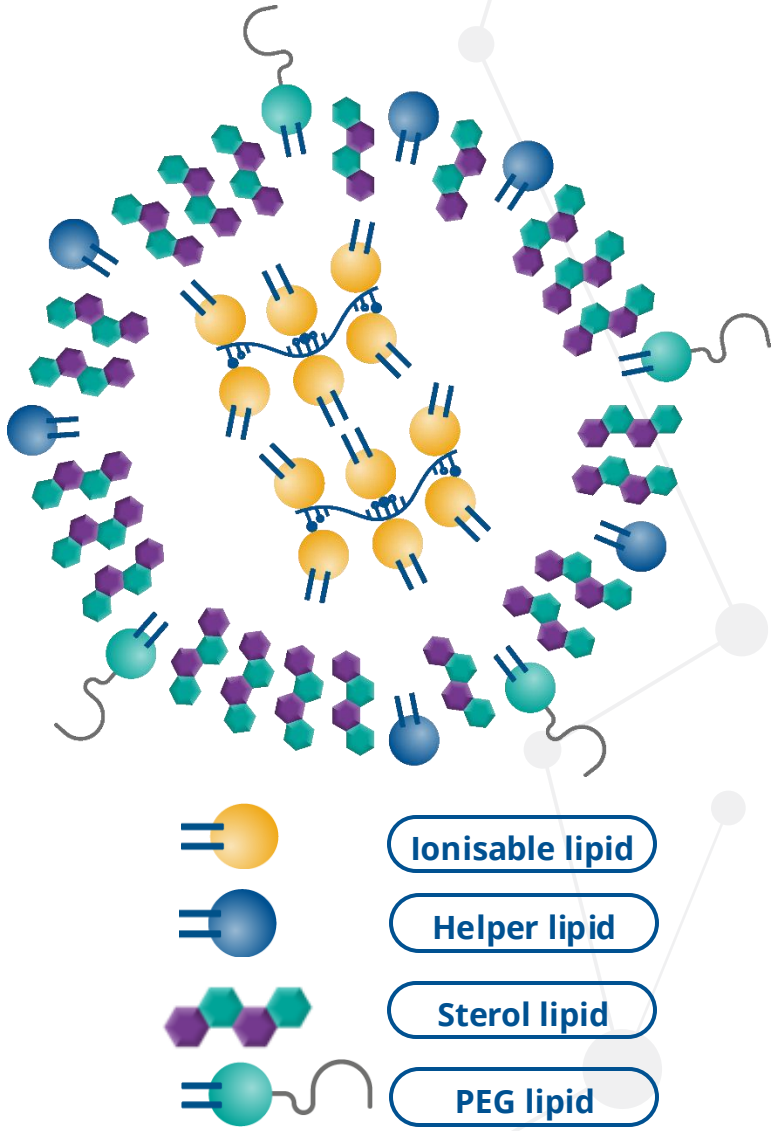
Use data collected *via* automated workflows to build a **structure-function relationship model**, allowing eventual ***in silico* selection of interesting candidates** for further experimentation



Encapsulation

- **Problem**
  - mRNA is repelled by mammalian cell surface
  - mRNA's are relatively large = natural uptake by cells extremely low
  - mRNA is highly susceptible to degradation by ribonucleases

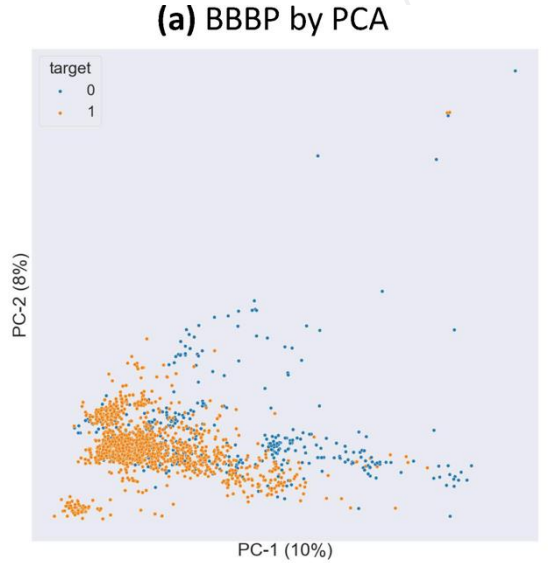
- **Solution** = Encapsulation in lipid nanoparticles (LNPs)
  - Composed of four different lipids (a cationic / ionizable lipid; a PEGylated lipid; a phospholipid; and cholesterol) that are capable of encapsulating mRNA Molecules
    - Ionisable: Complex with RNA
    - Peg: Stabilisation
    - Sterol and helper: Aid endosomal escape
  - LNPs are engulfed by cells by endocytosis
  - Release the mRNA to the cytosol, where it can be translated

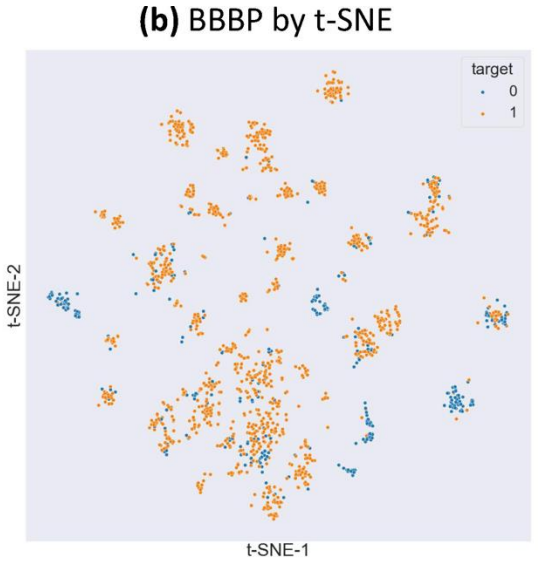


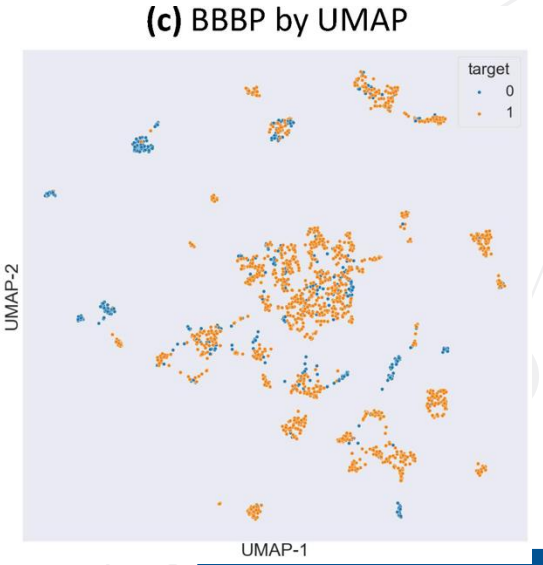
Collection and Visualisation of Lipid Data

- Similarity analysis in python for chemical space visualisation
    1. High dimension array reduced to 2d/3d by PCA, t-SNE or UMAP.
    2. Reduced chem space converted to interactive visual representation.
- CPI Modifications to
    - Improve image size
    - Show properties
    - Add filtering criteria (e.g. charge, pKa)

Source	No Lipids
A	47,000
B	1,800
C	500
D	220

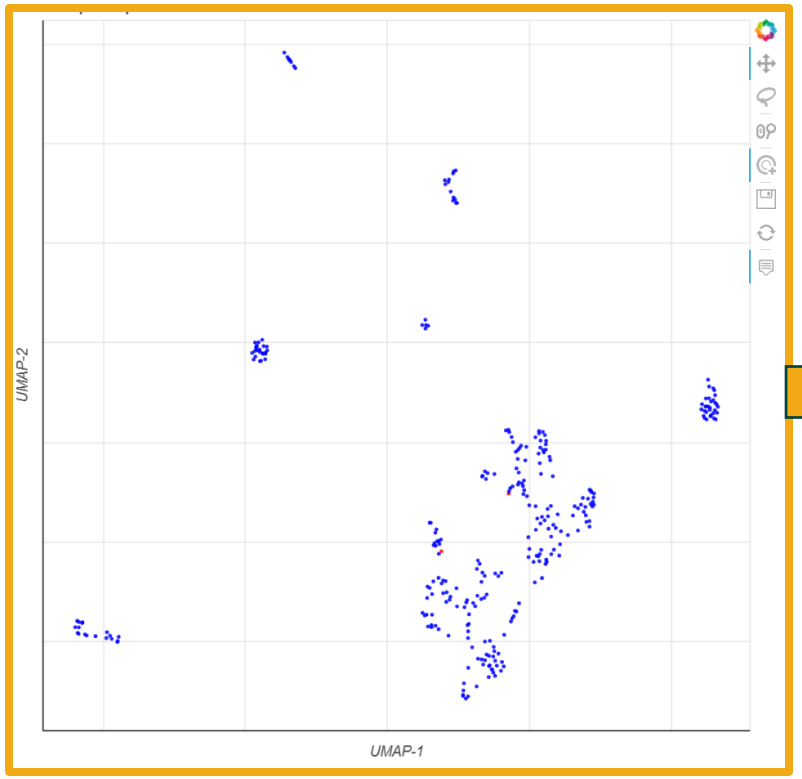


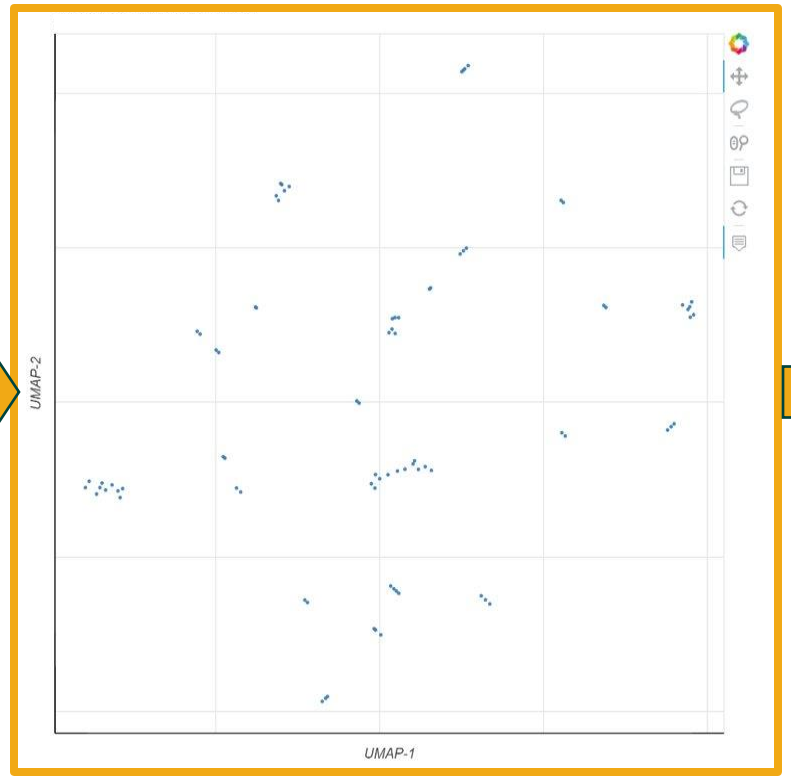




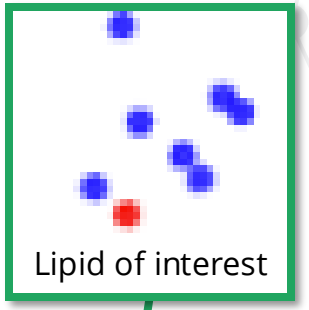
Source: https://chemplot.readthedocs.io/
Martins, Ines Filipa, et al. (2012). 52.6, 1686-1697

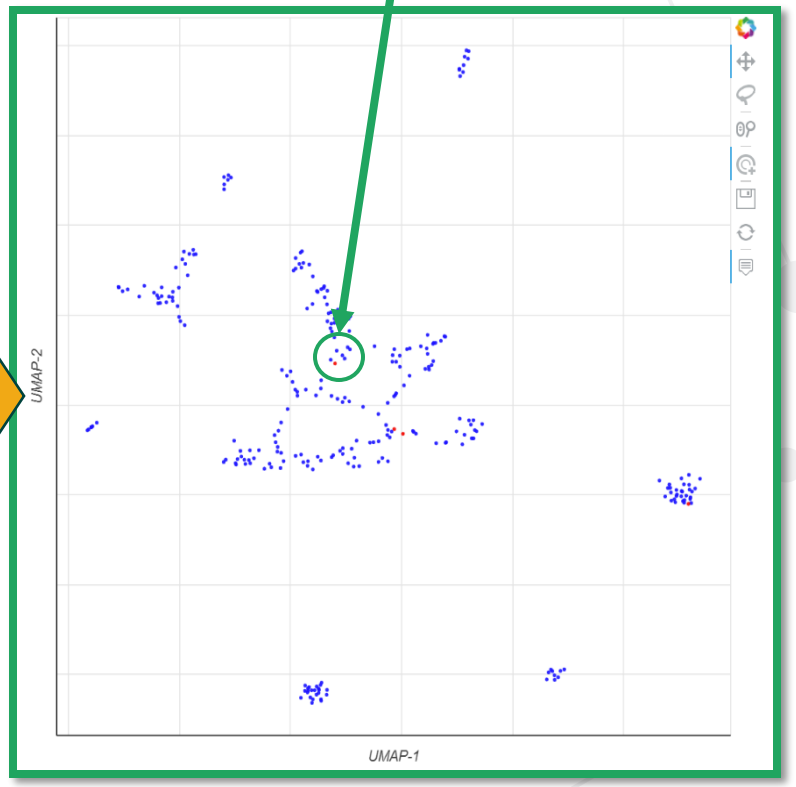
Lipid Downselection





Filtering based on critical criteria to identify new clusters of interest

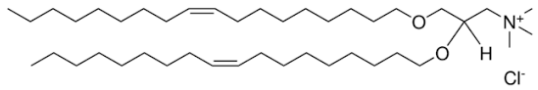




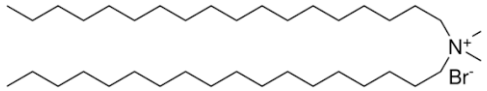
Nearest Neighbours Analysis of lipids known to perform well

Selected candidates in initial lipid screen

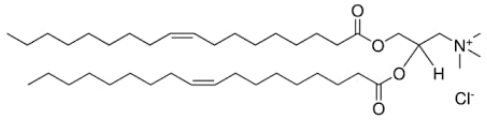
Cationic/ Ionisable



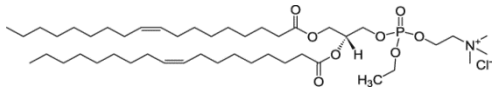
DOTMA



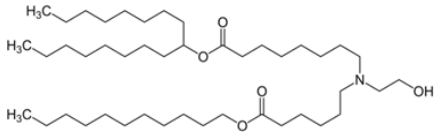
DDAB



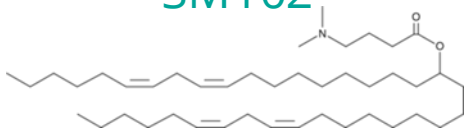
DOTAP



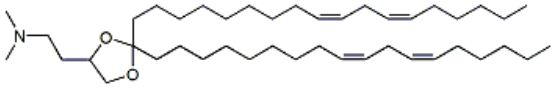
EPC



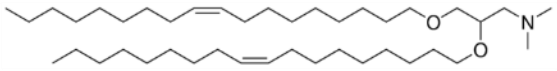
SM102



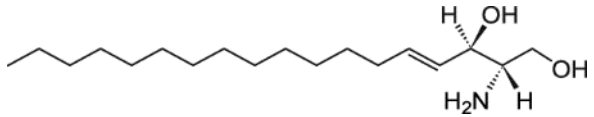
D-Lin-MC3-DMA



DLin-KC2-DMA

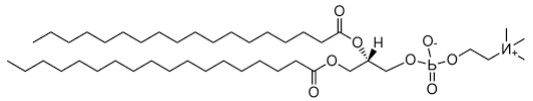


DODMA

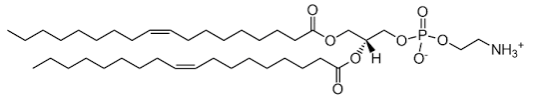


Sphingosine

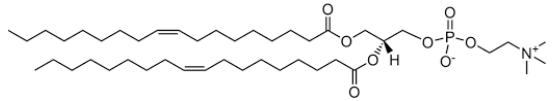
Neutral



DSPC

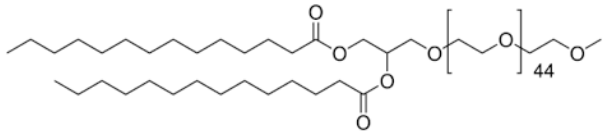


DOPE



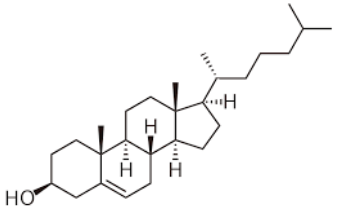
DOPC

PEG

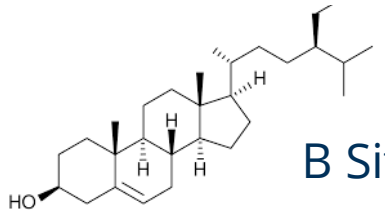


PEG 2000 DMG

Sterol



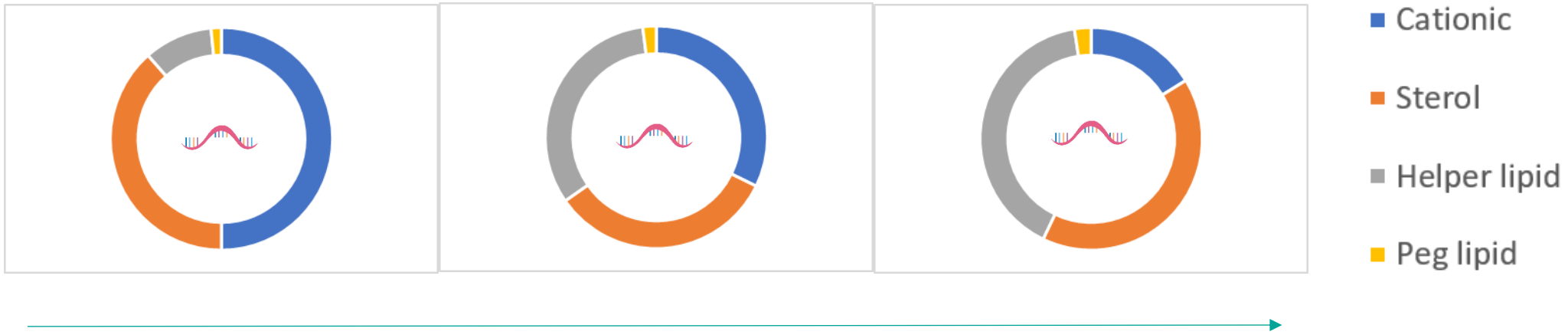
Chol



B Sitosterol

SM102 and D-Lin MC3 DMA used as controls and reference datasets

Initial lipid DoE design (First stage)

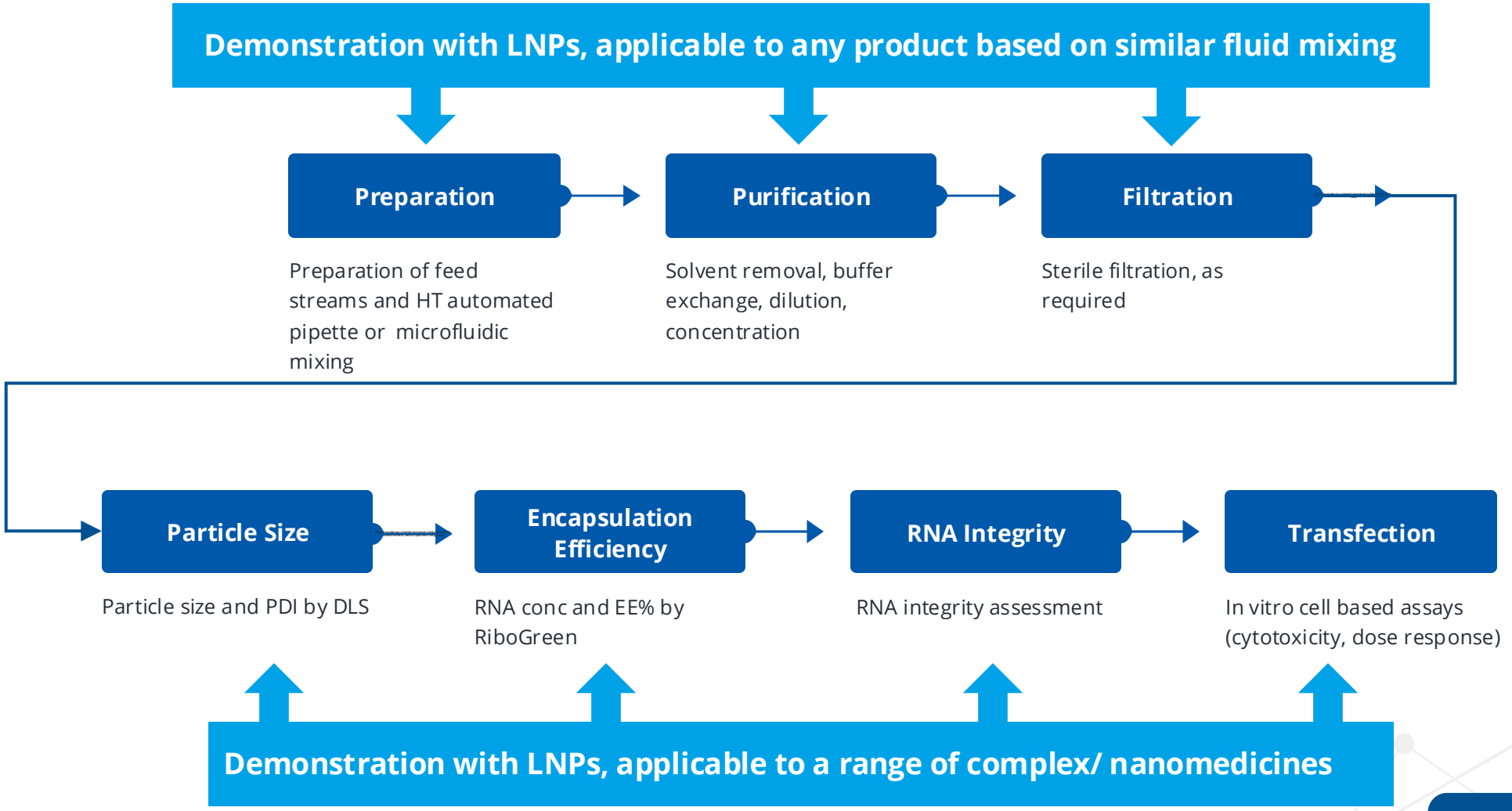


Vary lipid composition, while keeping ratio of total lipid: RNA payload the same

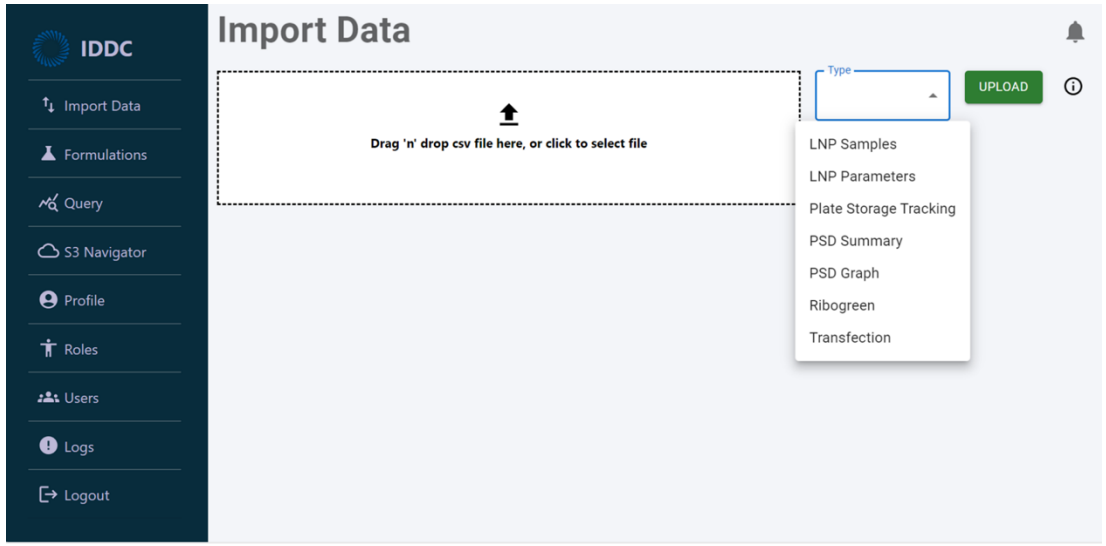
Full factorial DOE

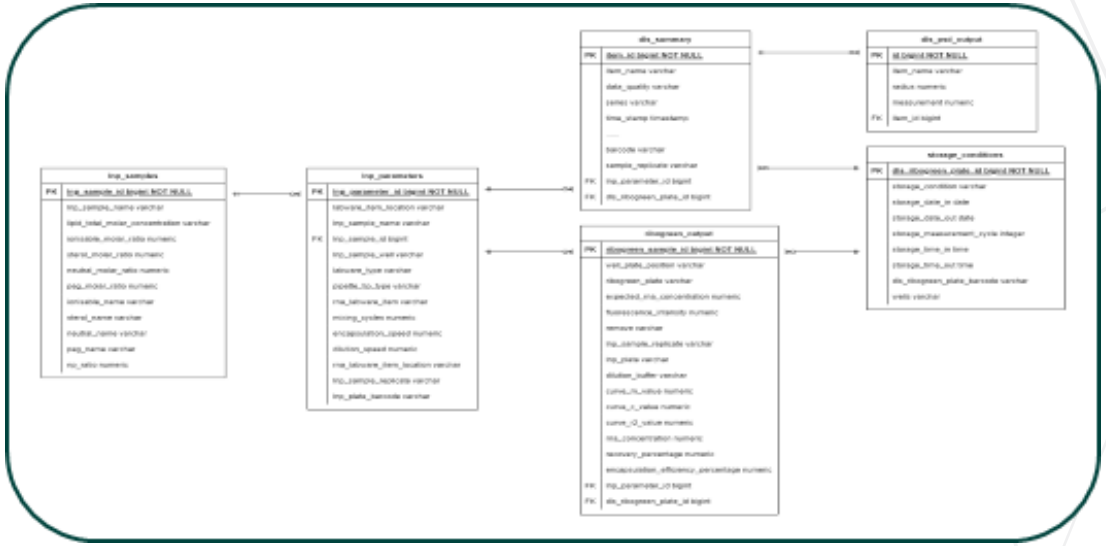
- RNA concentration fixed
- Vary cationic composition: 50, 33, 16mol% and corresponding to N/P 6, 4, 2
- Vary PEG level: 1.5 and 5 mol%
- Vary ratio of helper to sterol (4 levels): 1:1, 1:2, 1:2, 1:4
- Total sterol/helper composition fixed by cationic and PEG levels.
- Three replicates per formulation

Building a HT Screening & Characterisation Platform

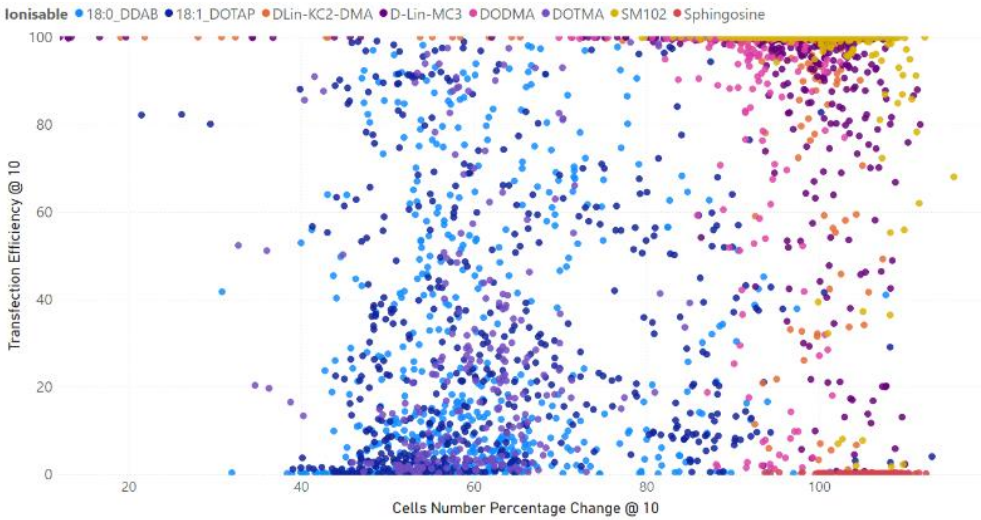


Database Interaction





Data Processing and Modelling

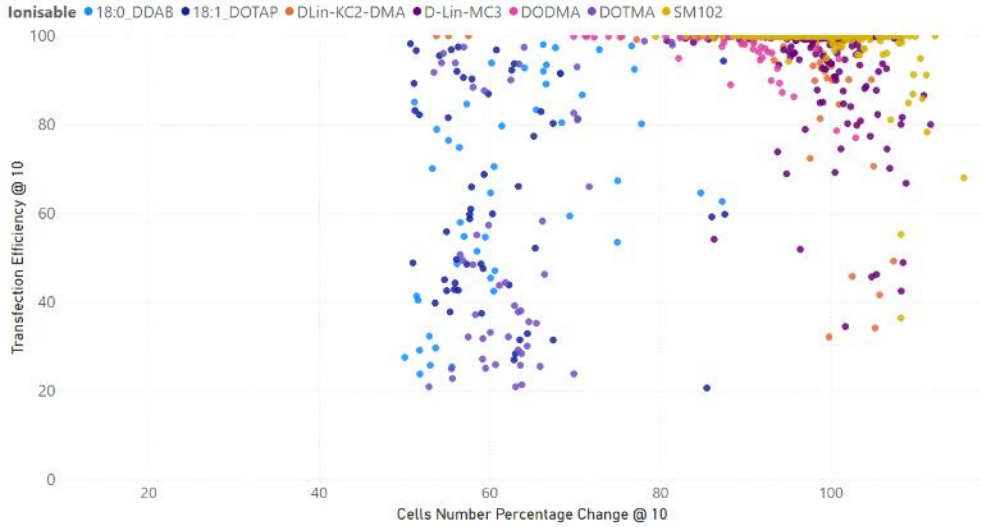


Filter to remove very poor/ unrepresentative data points

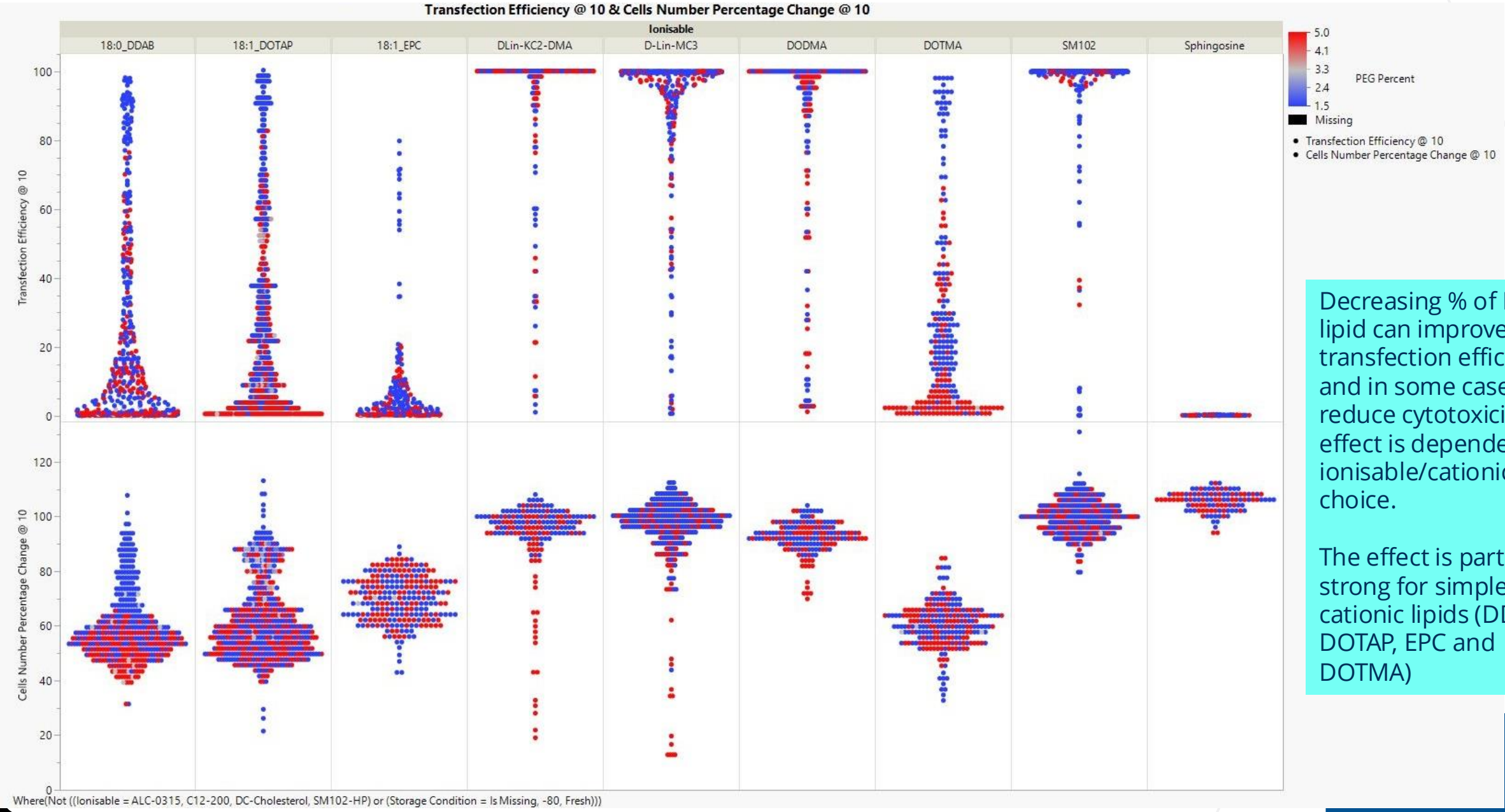
Particle diameter > 400nm
PDI > 0.4
EE % < 50%
Recovery 30% to 120%

Transfection efficiency
>20% @ 10ng dose
> 60% @ 30ng dose

Relative Cell viability
>50% @ 1ng and 10ng dose



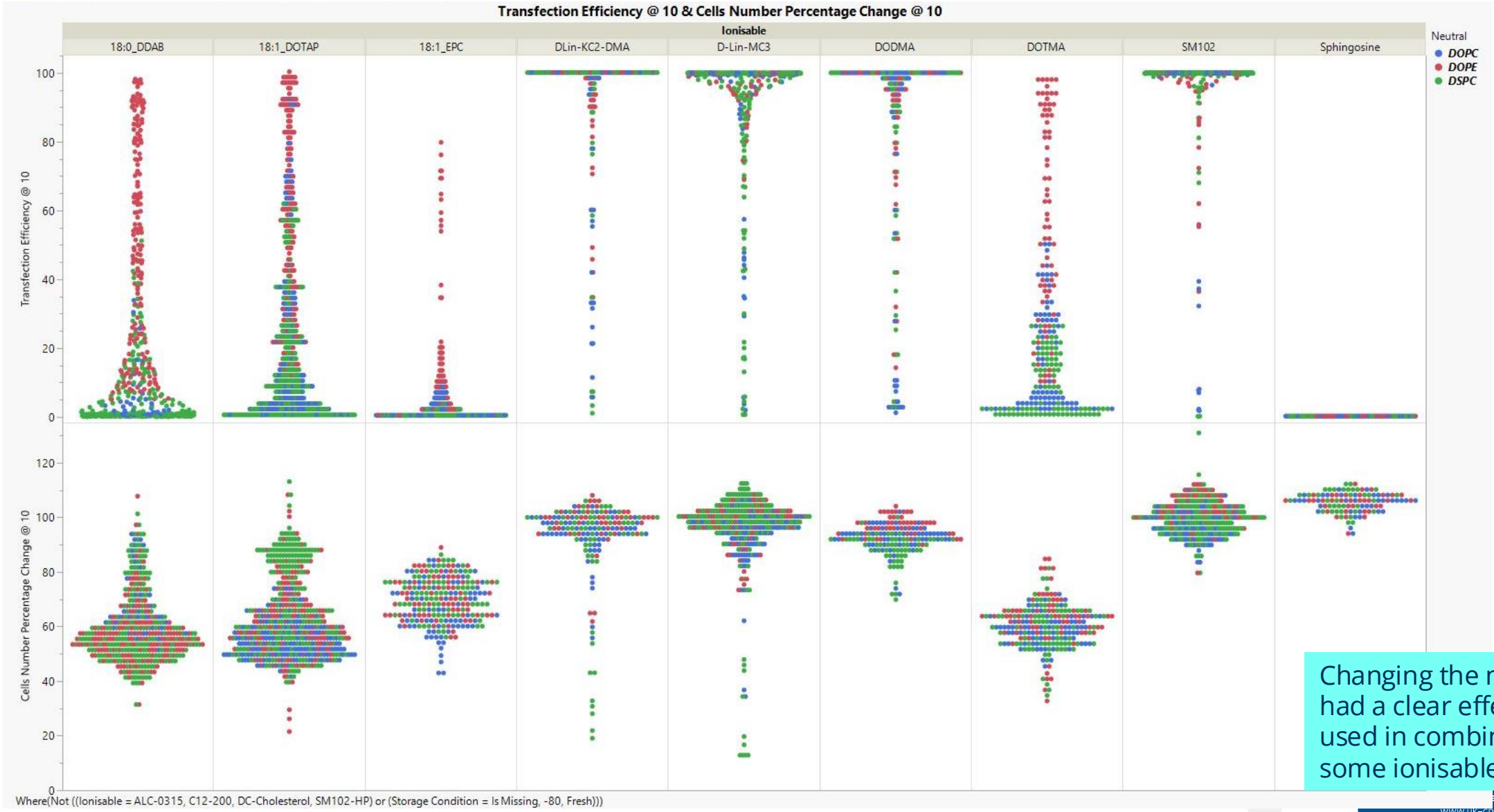
Using Models and Predictions



Decreasing % of PEG lipid can improve transfection efficiency, and in some cases reduce cytotoxicity, but effect is dependent on ionisable/cationic lipid choice.

The effect is particularly strong for simple cationic lipids (DDAB, DOTAP, EPC and DOTMA)

Using Models and Predictions



Changing the neutral lipid had a clear effect when used in combination with some ionisable lipids.

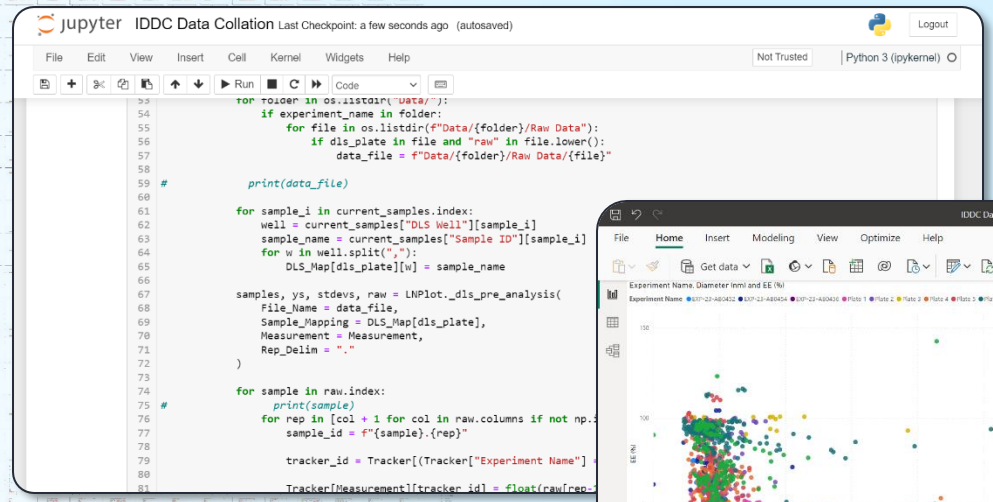
Complex Data managed by Underpinning Data Capability

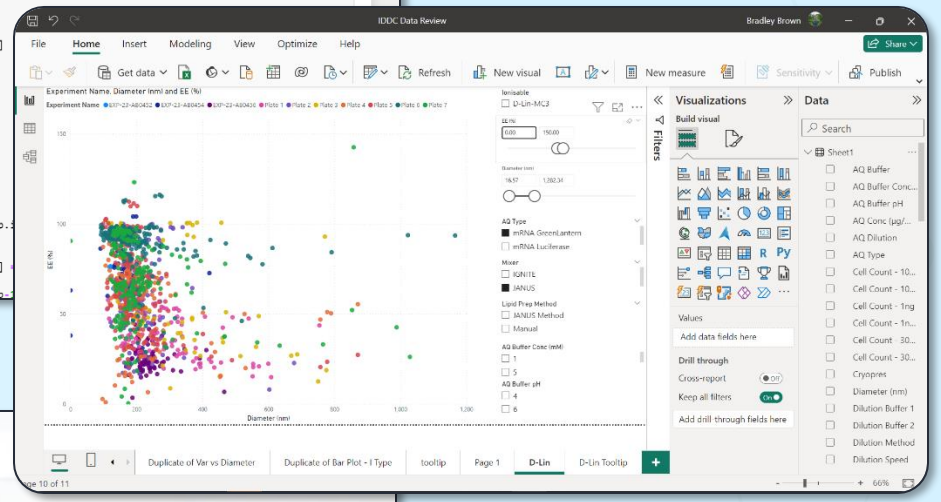
Problem

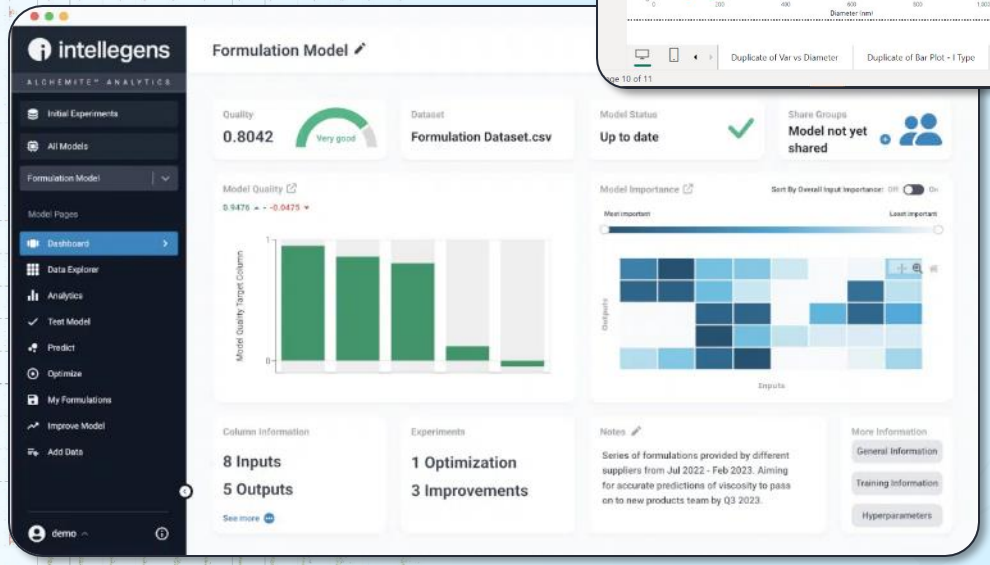
- Automation allows for large amounts of data to be collected
- Lots of variables to be investigated
- The dataset is highly multi-dimensional
- Trends can be difficult to spot

Solution

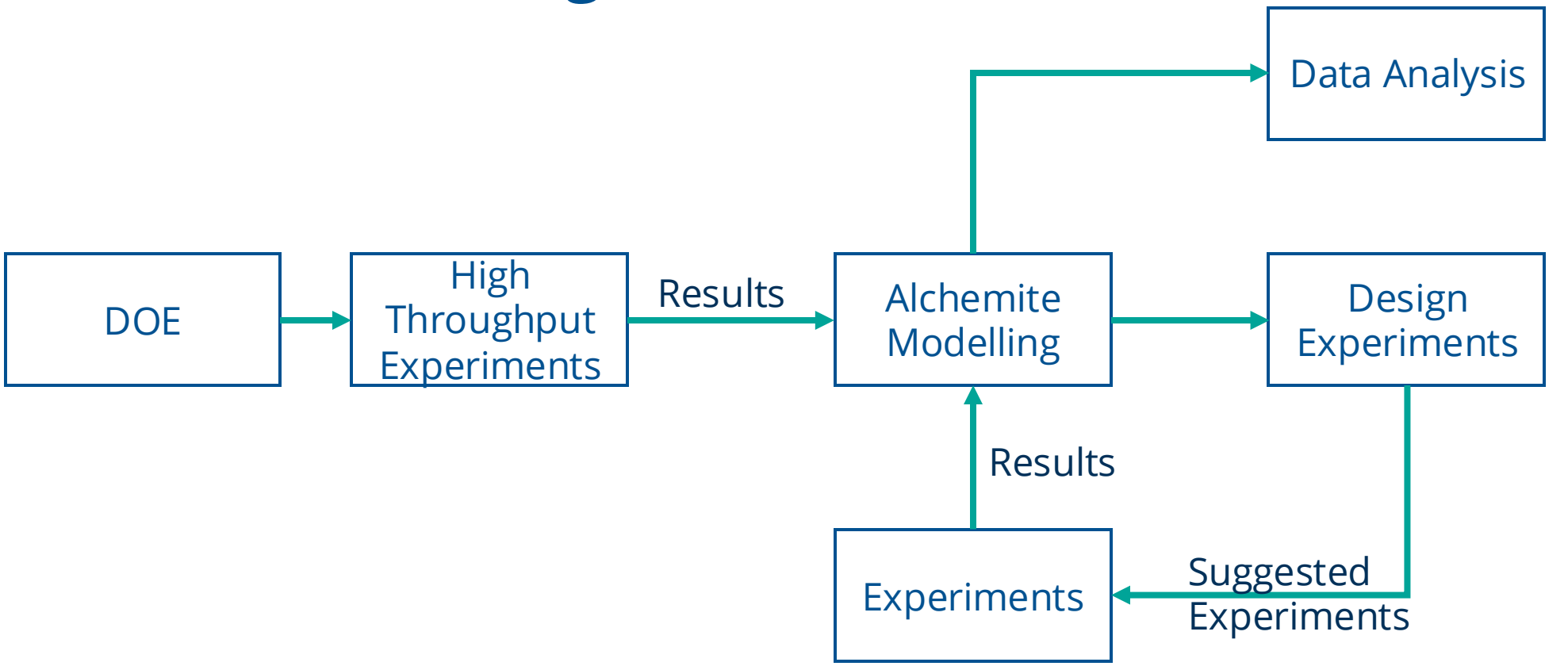
- Collaborative multi-partner database of 1000's of LNP formulations
- Machine-learning based decision-support techniques to guide lipid selection
- Use of predictive modelling and adaptive DoE techniques to design and optimise lipid formulation
- Advanced & interactive visualisation techniques







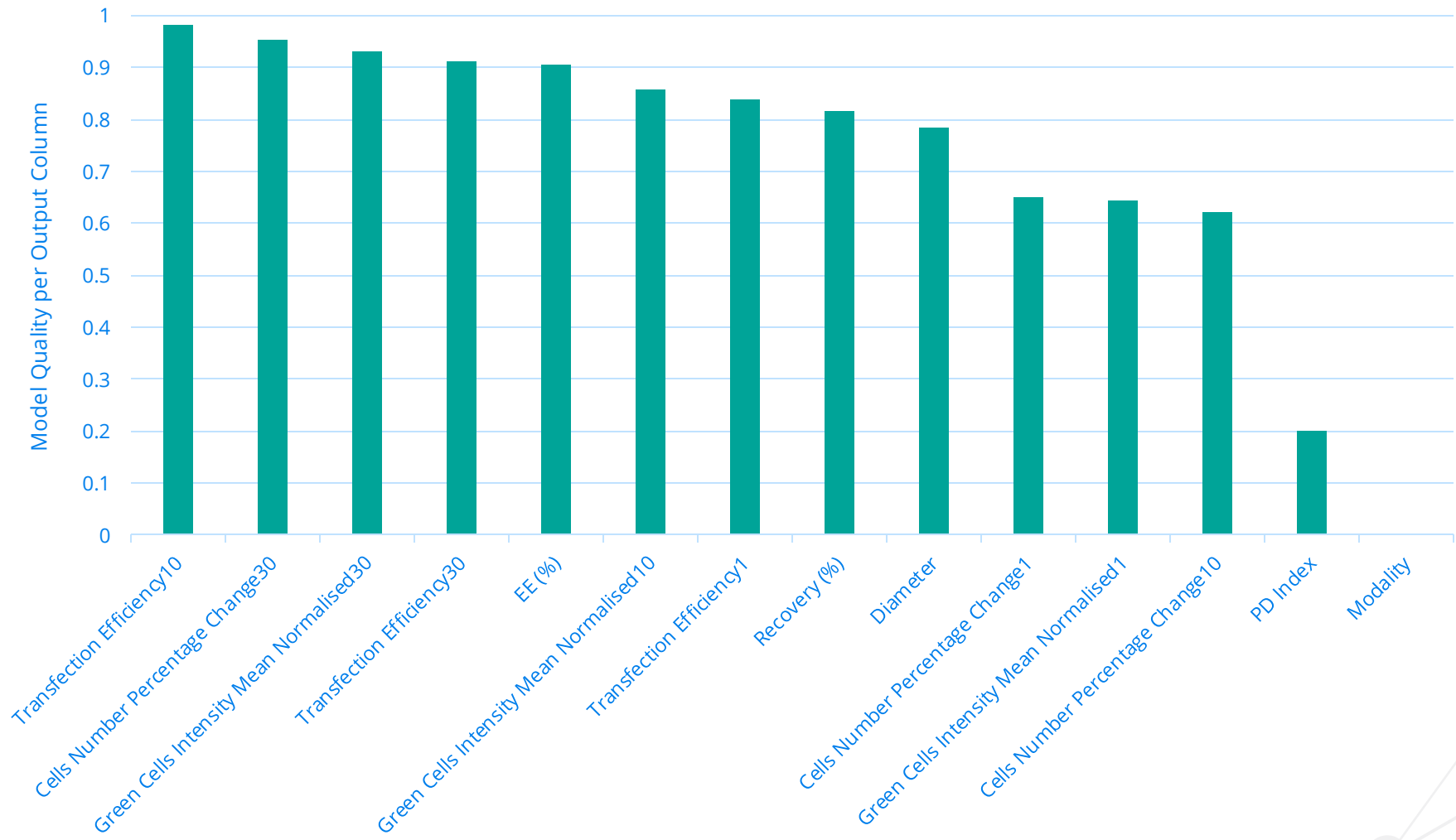
Alchemite Integration



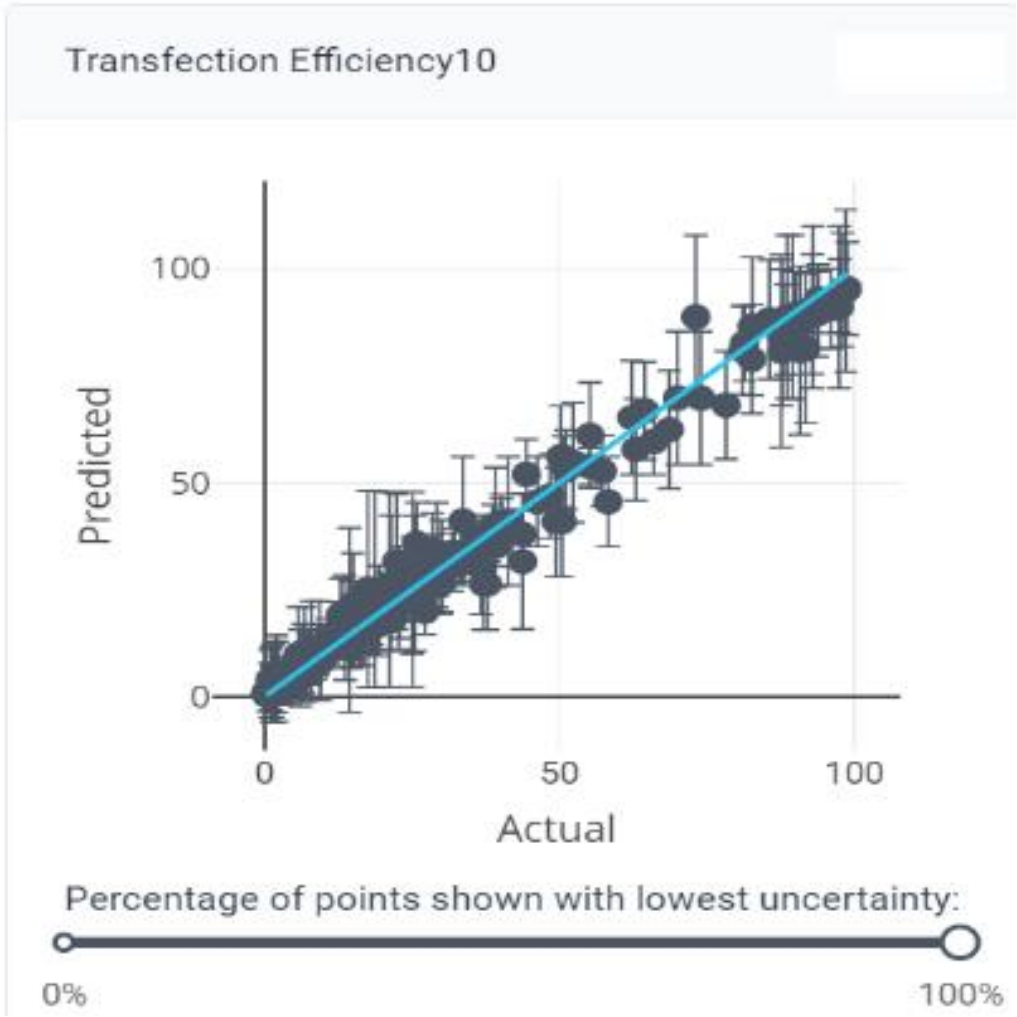
Inputs:
- Desired Experimental results e.g. EE%
- Experimental space to explore

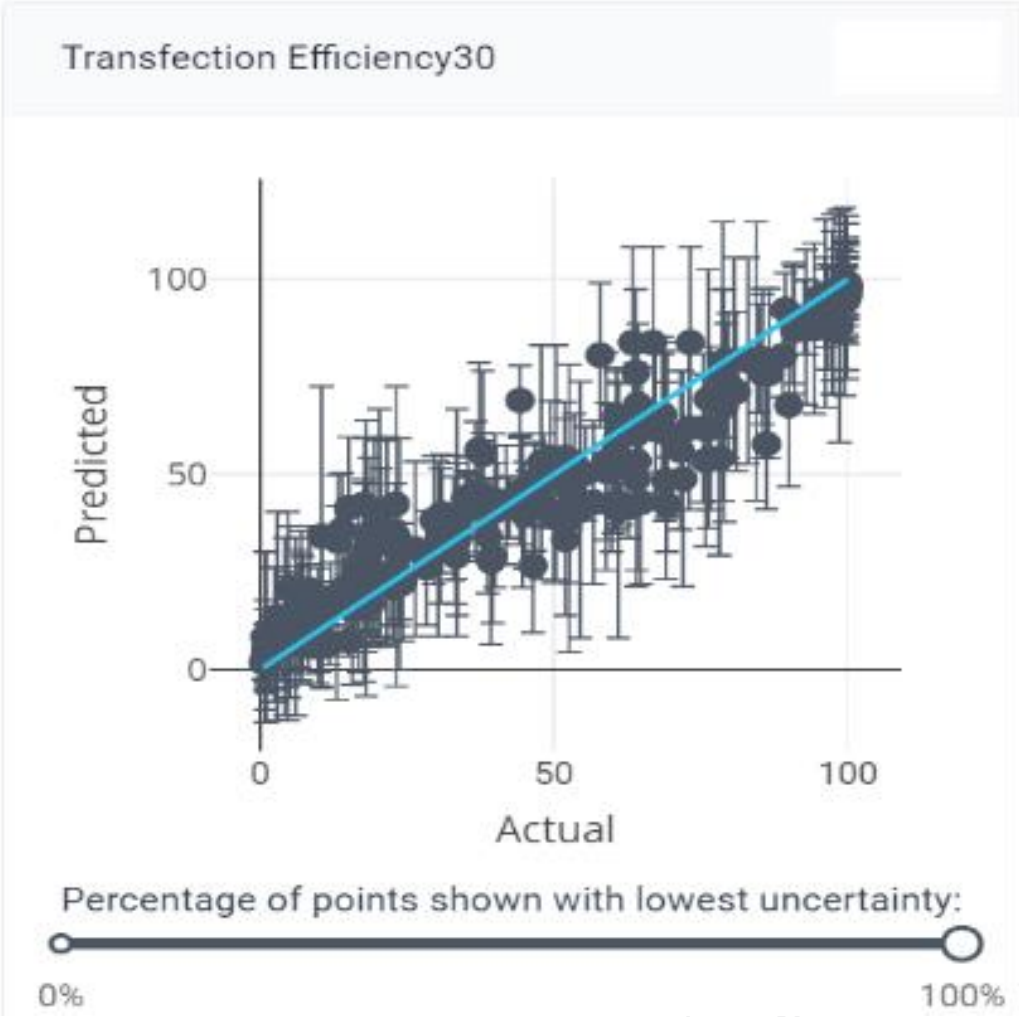
Output:
- Suggested Experiments
- Predicted experiment results

Model Quality: DOTMA

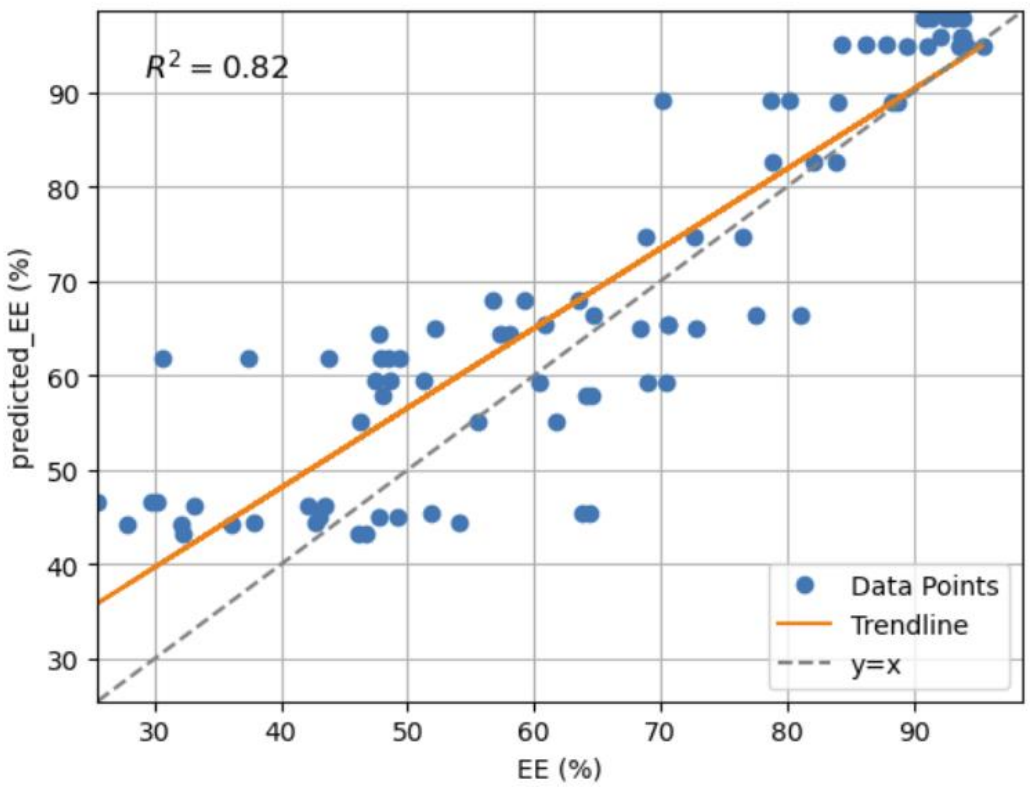


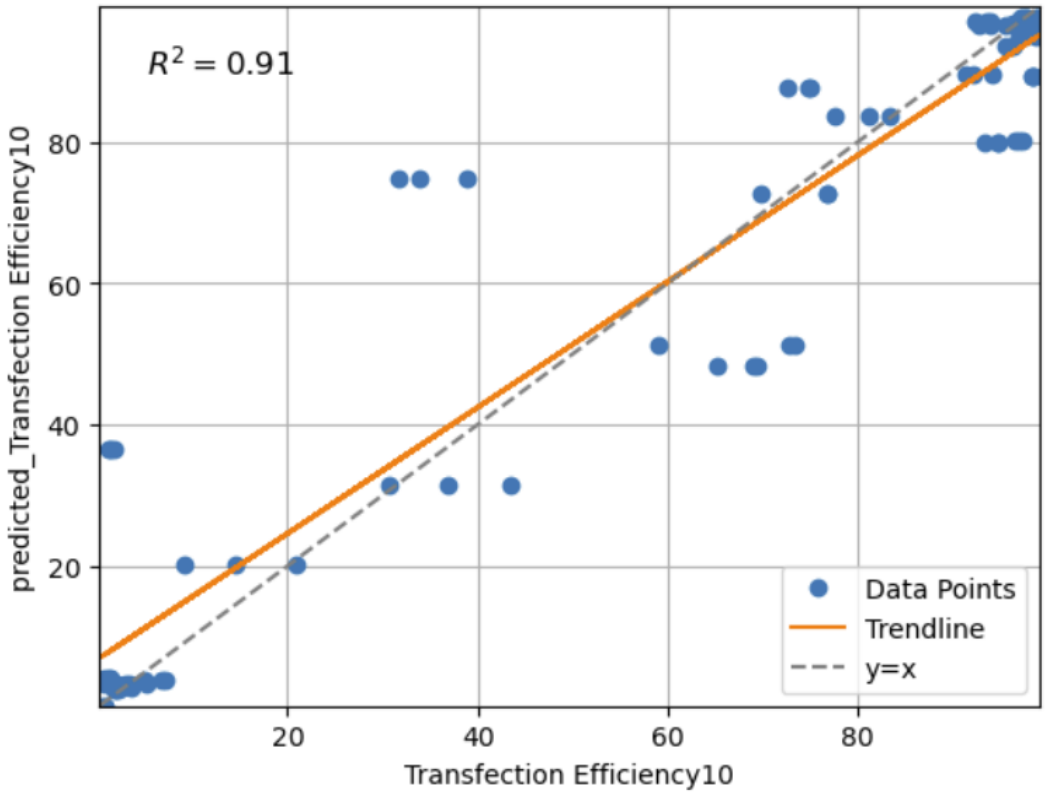
Predicted versus Experimental: DOTMA



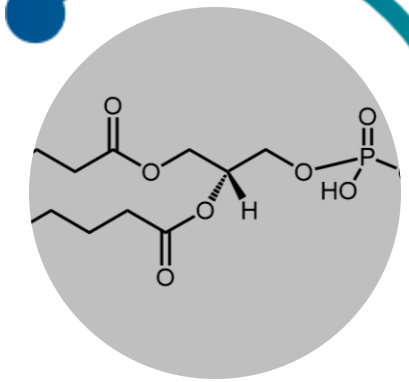


Experimental results





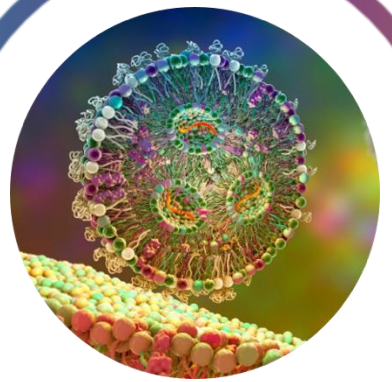
What's next



integrating dataset to help us understand what makes a good lipid

Development of data pipeline to Alchemite. Integration of Intellegens Ichnite

intellegens
Applied machine learning



Expansion of database to include partner data for in-depth screening

Producing lipid predicted by models

Recap

Objectives	Current Status	What's next
Develop high throughput screening workflow	High throughput screening workflow developed	Gain better understanding of what makes a good lipid
Screening of lipid libraries	Utilisation of AI suggested experiments	Development of data pipeline to Alchemite
Structure-function relationship model, for in silico selection of interesting LNP candidates	Analysis of results using Alchemite	Expansion of database to include partner data
	Integration of workflow with collaborative robots	Synthesis and evaluation of lipids/LNPs predicted by models

Thank you

For more information visit **www.uk-cpi.com**

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