High Throughput Single Particle Characterisation of Lipid Nanoparticles (LNPs) using the Integrated Nanoparticle Size and Payload Analyser (INSPA)

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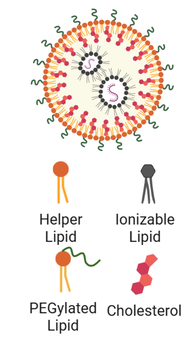
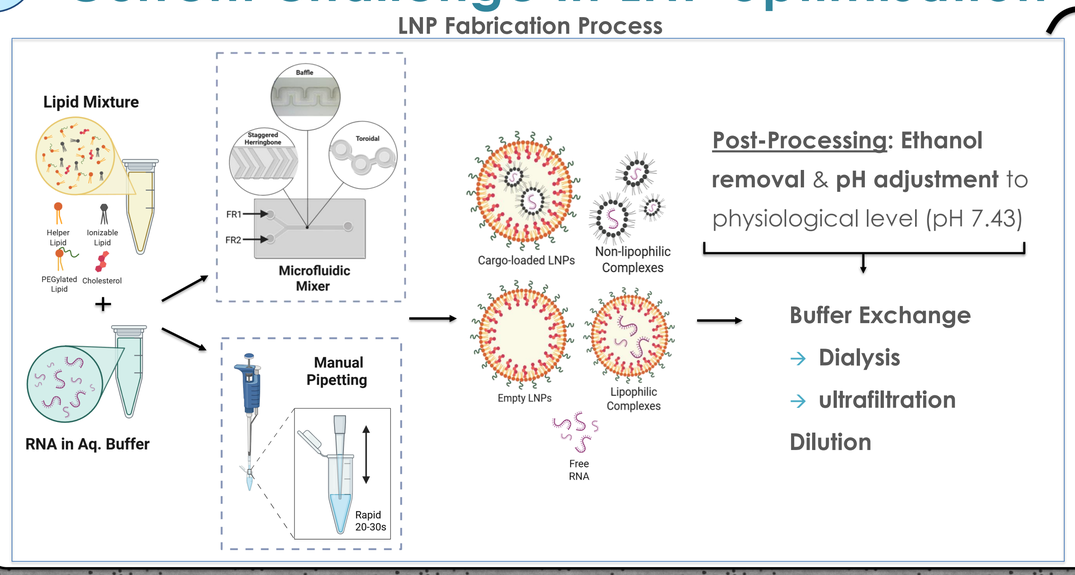
Introduction

- Development of **Lipid Nanoparticle (LNP)** formulations for nucleic acid delivery requires laborious optimisation of physical and chemical process parameters to ensure a **high loading efficiency** as well as **high reproducibility of cargo delivery to recipient cells** - an ongoing challenge the LNP field is still actively trying to tackle.[1]
- The standard LNP characterisation methods are **DLS** which **lacks single-particle resolution** and is susceptible to bias, and **Cryo-TEM**, which offers single particle resolution **but lacks throughput.**
- Other methods are limited**, i.e. NTA** lacks sensitivity with gradual fall-off for LNPs < 80 nm, while **MRPS** lacks throughput and reliability.
- We introduce the **Integrated Nanoparticle Size and Payload Analyser (INSPA)** that combines **scattering microscopy (iSCAT) for sizing** and **fluorescent staining for RNA loading efficiency quantification.**
- INSPA runs on a conventional fluorescence microscope with a simple beam splitter and uses a microfluidic flow cell to immobilize LNPs on a coverslip for imaging.



1 Current challenge in LNP optimisation



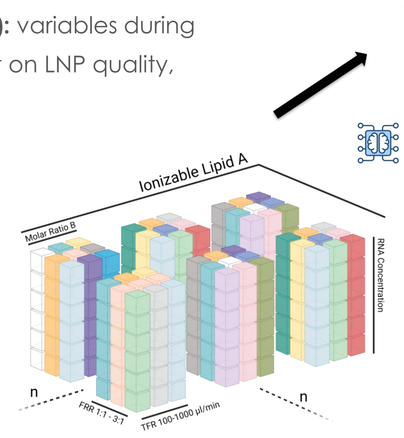
Post-Processing: Ethanol removal & pH adjustment to physiological level (pH 7.43)

Buffer Exchange
- → **Dialysis**
- → **ultrafiltration**

Dilution

Critical Process Parameters (CPPs): variables during fabrication with significant impact on LNP quality, namely:

- Component molar ratio
- Flowrate Ratio (FRR)
- Total Flowrate (TFR)
- N:P Ratio
- Dialysis/Dilution
- Ionizable lipid
- RNA Concentration
- Aqueous buffer



LNP Quality Assessment

(Transfection and Expression Efficiency)

Large parameter space → optimisation and screening become laborious and can benefit from having a **high throughput characterisation method.**

2 Integrated Nanoparticle Size and Payload Analyzer

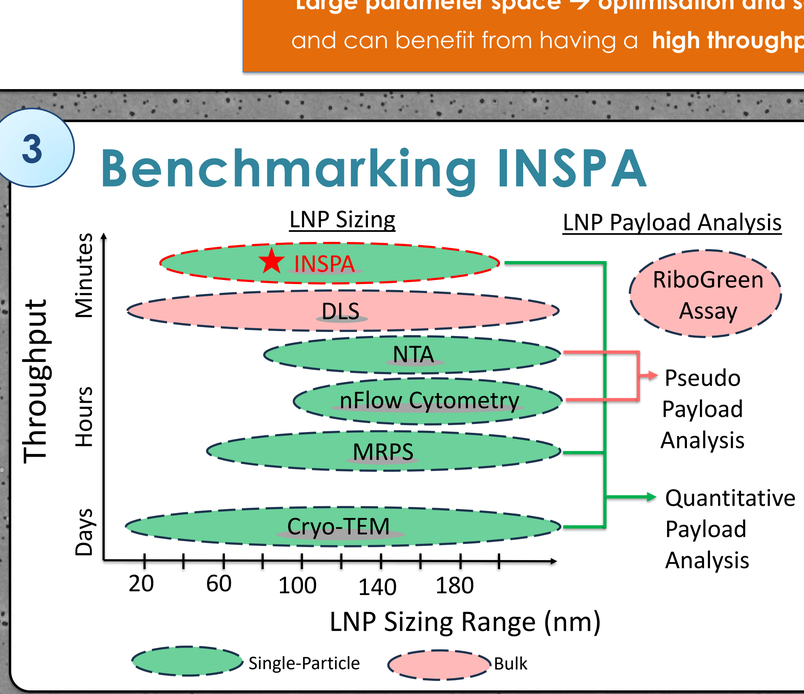
INSPA: Workflow

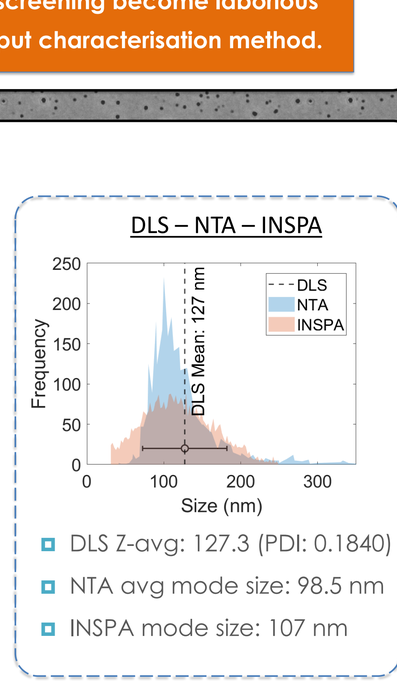
An iSCAT-based imaging platform that provides both single particle resolution sizing of LNPs and RNA quantification per LNP.

$$LE\% = \frac{\#LNP\ with\ RNA}{Total\ \#\ LNP} \times 100$$

$$E_{[R,N,A]} = T_{[RNA]} - U_{[R,N,A]},\quad EE\% = \frac{E_{[RNA]}}{T_{[RNA]}} \times 100$$

3 Benchmarking INSPA





- DLS Z-avg: 127.3 (PDI: 0.1840)
- NTA avg mode size: 98.5 nm
- INSPA mode size: 107 nm

4 Introducing a new CQA metric: Per Particle Payload (PPP)

Please visit my poster to see the results!

Conclusion & Future work

- INSPA measured both size and loading efficiency of single LNPs down to ~ 30 nm diameter in a few minutes using 15x fold less sample volume than DLS.
- INSPA shortens the design-build-characterize-learn loop, accelerate screening and upon cell transfection help uncover new structure-function relationships thanks to its integrated multiparametric size and payload analysis.
- Future work: Incorporate INPSA into a screening platform for LNP optimisation with a custom fabrication setup, low volume post processing, and low volume cell transfection.

Acknowledgements

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CANO
CENTRE FOR APPLIED NANOMEDICINE

References

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3. Ly, H. H., Daniel, S., Soriano, S. K. V., Kis, Z. & Blakney, A. K. Optimization of Lipid Nanoparticles for saRNA Expression and Cellular Activation Using a Design-of-Experiment Approach. *Molecular Pharmaceutics* **19**, 1892-1905 (2022)