Orthogonal Pipelines for Lipid Nanoparticle Evaluation

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University of Strathclyde Science

UKRI Engineering and Physical Sciences Research Council

Background

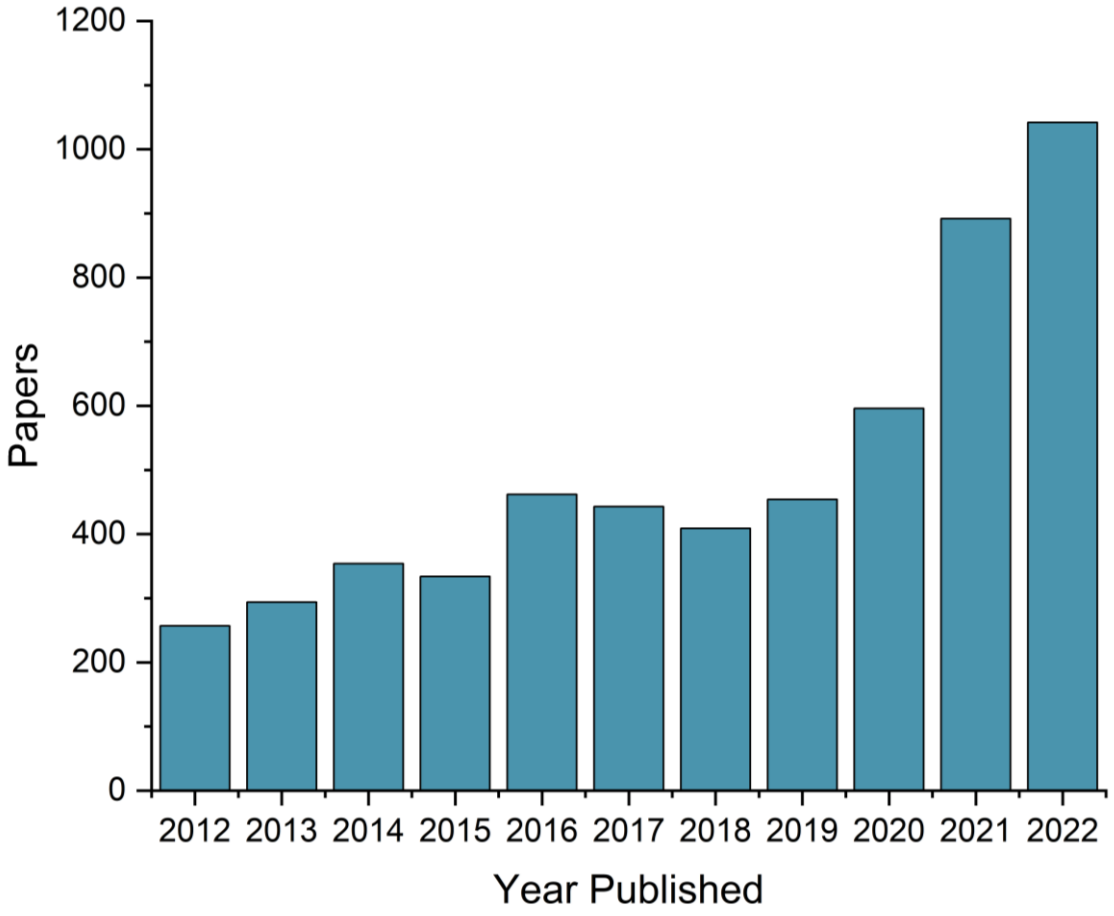

Fig.1 *– "Lipid nanoparticle" search into PubMed.*

- Ribonucleic acid (RNA) drugs encapsulated in lipid nanoparticles pose promising candidates for gene therapy in treatment resistant conditions and rare diseases.
- Clinical trials have shown gaps within our understanding of lipid nanoparticle design, associated critical quality attributes and their *in-vitro/in-vivo* biological performance.
- High resolution analytical techniques are required to correlate design, characteristic and performance to overcome nanomedicine translational barriers.

Background

Fig.2 *– Nanomed characterisation bodies.*

- Challenges faced by LNP candidates include, stability, supply chain, storage conditions variation in raw materials, case-by-case basis.
- To overcome this FDA-NCL and EUNCL has published guidance and recommendations on the characterisation of nanomedicines. Material reference standards remain in the development pipeline.
- More than one analytical technique required to measure associated physiochemical attributes of LNPs and nanomeds with one measurement using orthogonal high-resolution technique.

Background

Nanoparticle Tracking Analysis

- Size
- Size distribution
- Estimated concentration

Future?

Zetasizer

- Size
- Size distribution
- Surface charge

Field Flow Fractionation

- Size
- Size distribution
- Surface charge
- Molecular weight
- Shape factor

Fig.3 *– Flow diagram made with BioRender.*

Increasing Technique Resolution ↑

Decreased Adoption in Routine Analysis ↓

Aims & Objectives

- **Aim**
  - To develop high resolution orthogonal analytical pipelines for the evaluation of prototype lipid nanoparticle formulations.

- **Objectives**
  - Design, manufacture, and screen prototype LNP critical quality attributes.
  - Develop high resolution analytical methods to evaluate LNPs beyond the scope of routine techniques.
  - Cross-validate results obtained with orthogonal high resolution analytical techniques.

Methodology

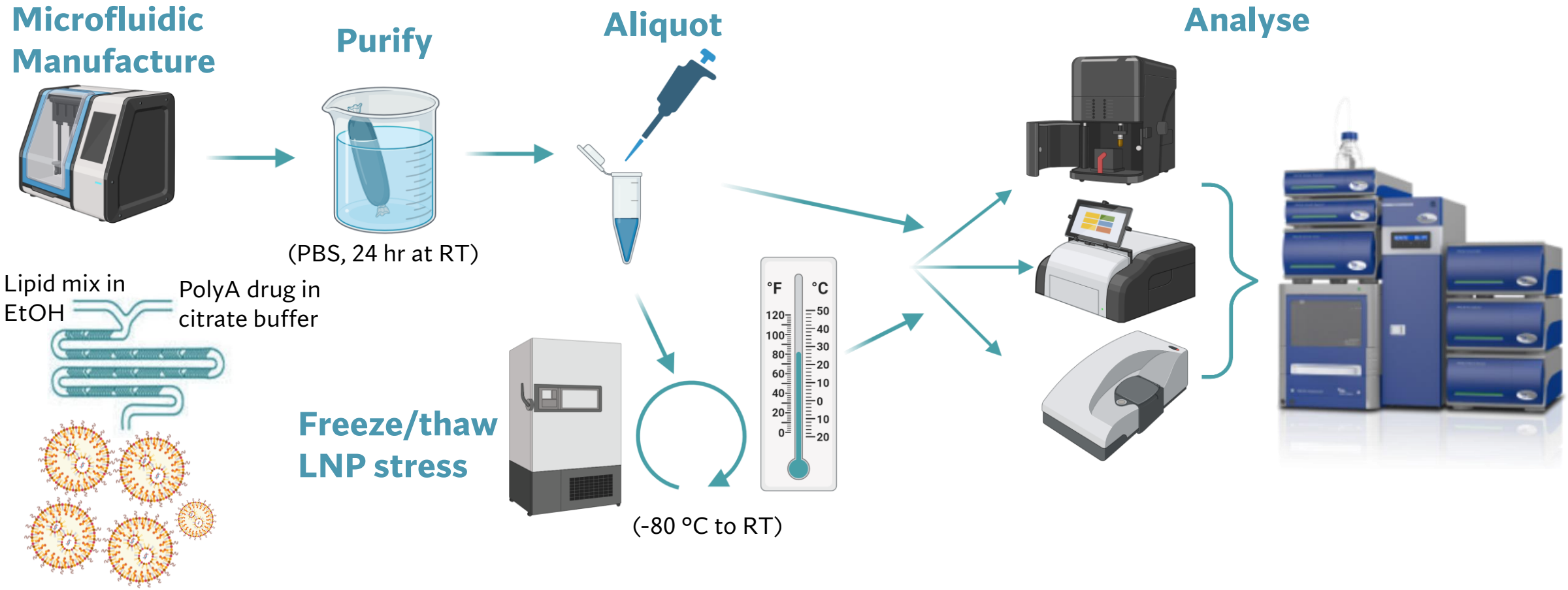

Fig.4 *– Flow diagram of RNA-LNP manufacture, dialysis and analysis. Made with BioRender.*

Results – LNP Screening (DLS)

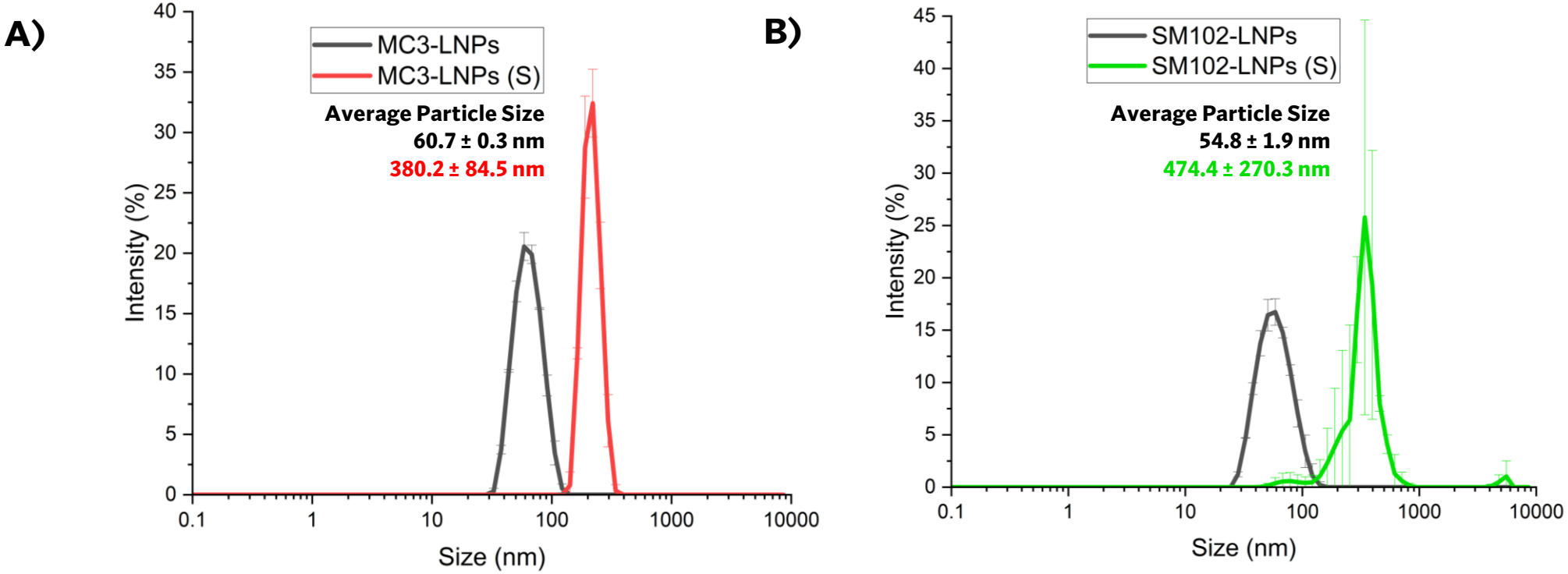

Fig.5 *– DLS results of* ***A)*** *MC3-LNPs post-formulation and stressing.* ***B)*** *SM102-LNPs post formulation and stressing, (mean ± SD, n=2).*

Results – LNP Screening (DLS)

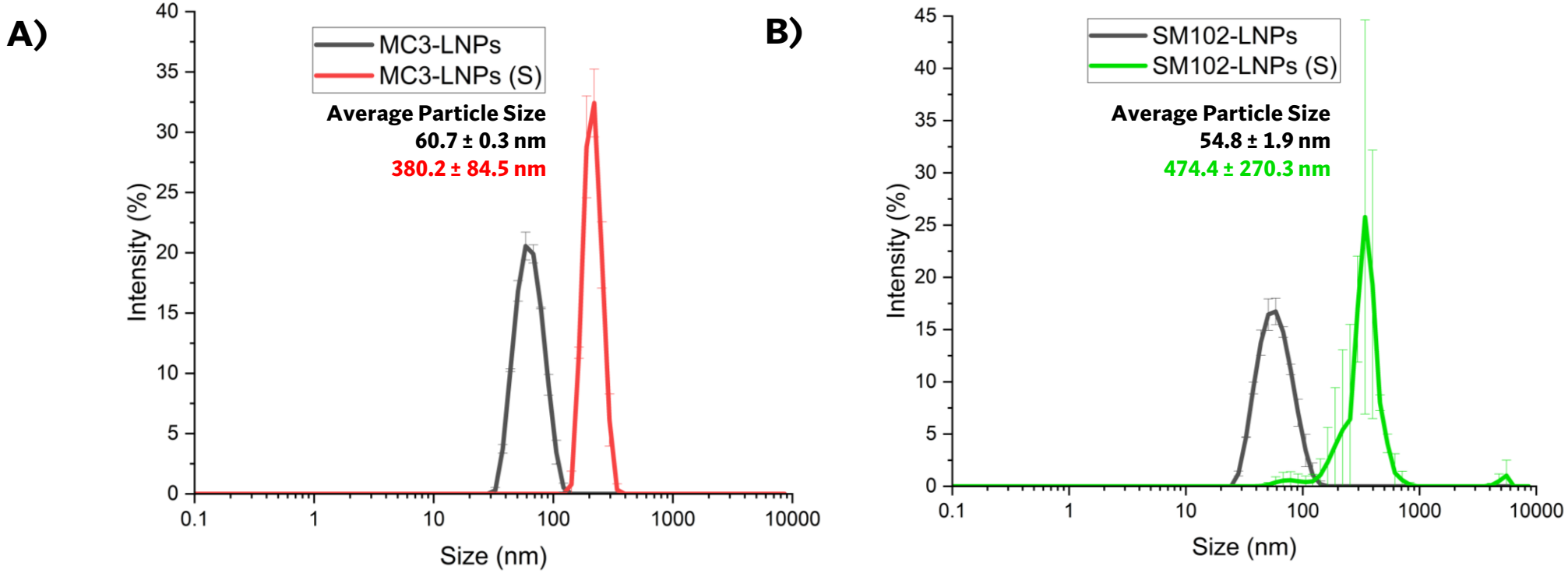

Fig.5 *– DLS results of* ***A)*** *MC3-LNPs post-formulation and stressing.* ***B)*** *SM102-LNPs post formulation and stressing, (mean ± SD, n=2).*

Table 1 *– LNP formulation zetasizer and RiboGreen™ assay results, (mean ± SD, n=2).*

	Zetasizer		RiboGreen™	
LNP CQA	**PDI**	**ZP (mV)**	**Loading (%)**	**Recovery (%)**
MC3-LNP	0.059 ± 0.02	-3.34 ± 0.86	96.4 ± 1.29	81.7 ± 4.5
MC3-LNP (S)	0.444 ± 0.07	-8.28 ± 1.82	62.9 ± 0.04	79.6 ± 1.5
SM102-LNP	0.109 ± 0.03	-4.53 ± 0.87	98.4 ± 0.37	95.2 ± 2.1
SM102-LNP (S)	0.443 ± 0.14	-2.89 ± 0.56	77.0 ± 12.2	88.3 ± 1.2

DLS indicates stability but insufficient detail to distinguish aggregation induced sub-population formation.

Results – LNP Screening (NTA)

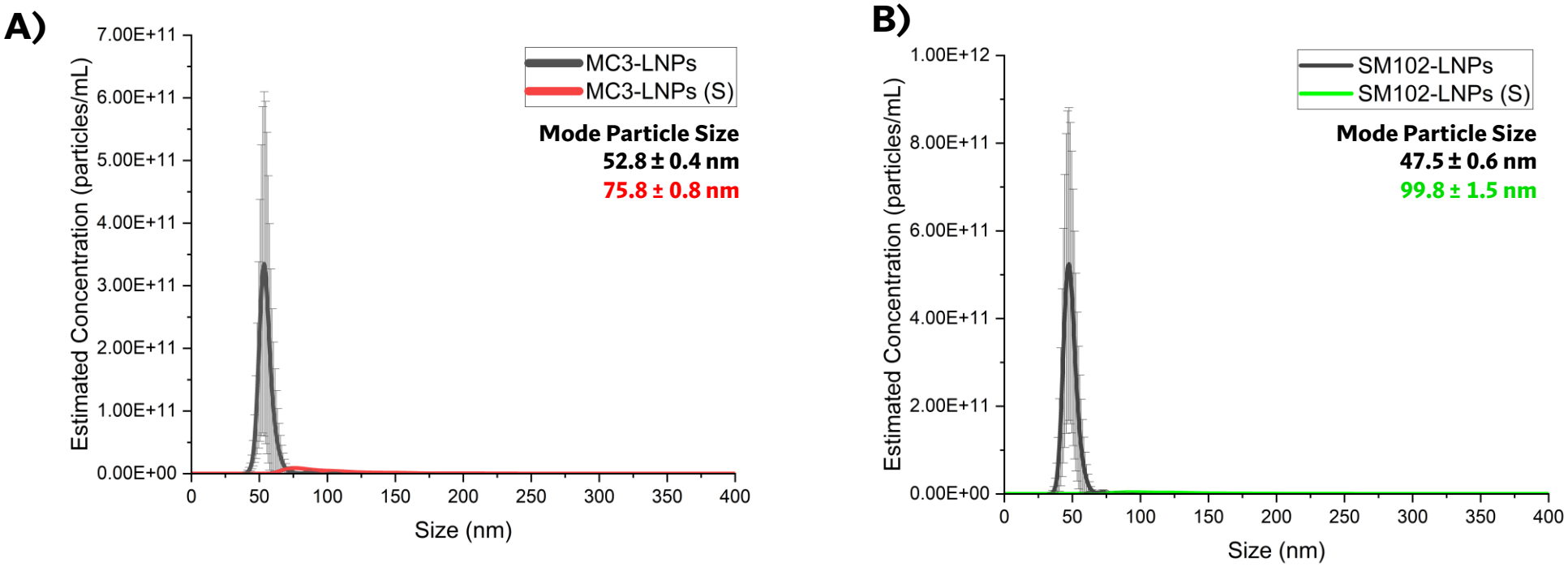

Fig.6 *– NTA results of* ***A)*** *MC3-LNPs post-formulation and stressing.* ***B)*** *SM102-LNPs post formulation and stressing, (mean* ± *SD, n=2).*

Results – LNP Screening (NTA)

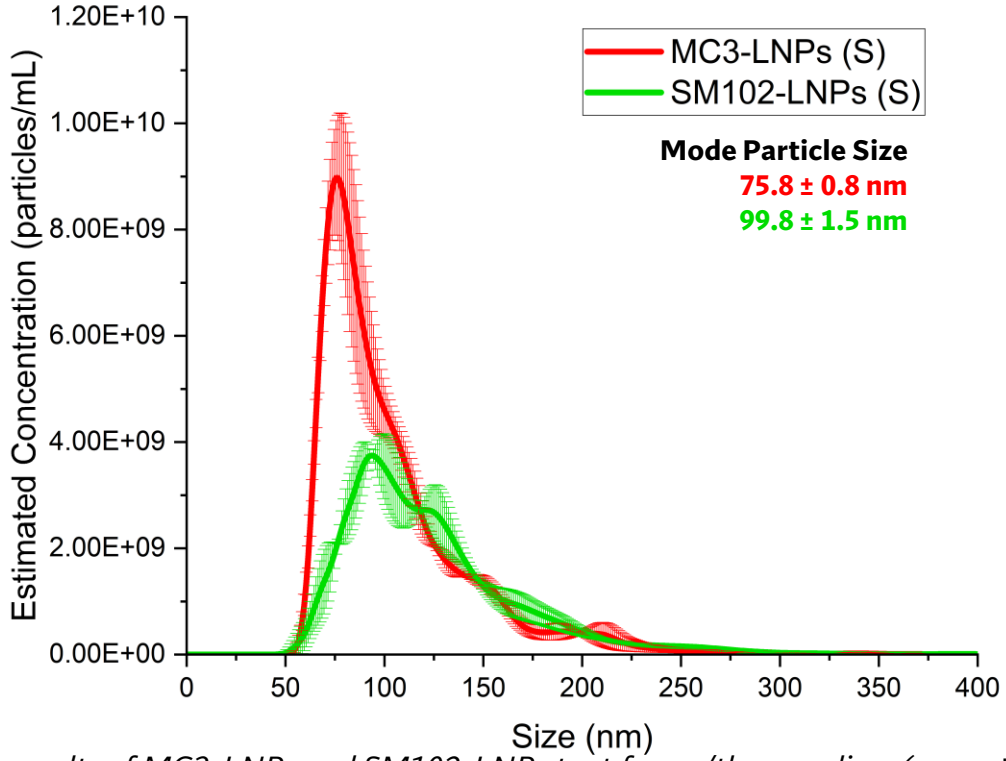

Fig.6 *– Focussed NTA results of MC3-LNPs and SM102-LNPs post freeze/thaw cycling, (mean ± SD, n=2).*

Table 2 *– LNP formulation NTA and RiboGreen™ assay results, (mean ± SD, n=2).*

	NTA		RiboGreen™	
LNP CQA	**Mean Size (nm)**	**Span**	**Loading (%)**	**Recovery (%)**
MC3-LNP	55.1 ± 0.8	0.22 ± 0.01	96.4 ± 1.29	81.7 ± 4.5
MC3-LNP (S)	104.2 ± 2.9	0.92 ± 0.03	62.9 ± 0.04	79.6 ± 1.5
SM102-LNP	49.6 ± 0.7	0.25 ± 0.02	98.4 ± 0.37	95.2 ± 2.1
SM102-LNP (S)	123.2 ± 5.0	0.90 ± 0.02	77.0 ± 12.2	88.3 ± 1.2

NTA indicates stability and distinguishes aggregation induced sub-population formation.

Results – FI-AF4-MALS Method

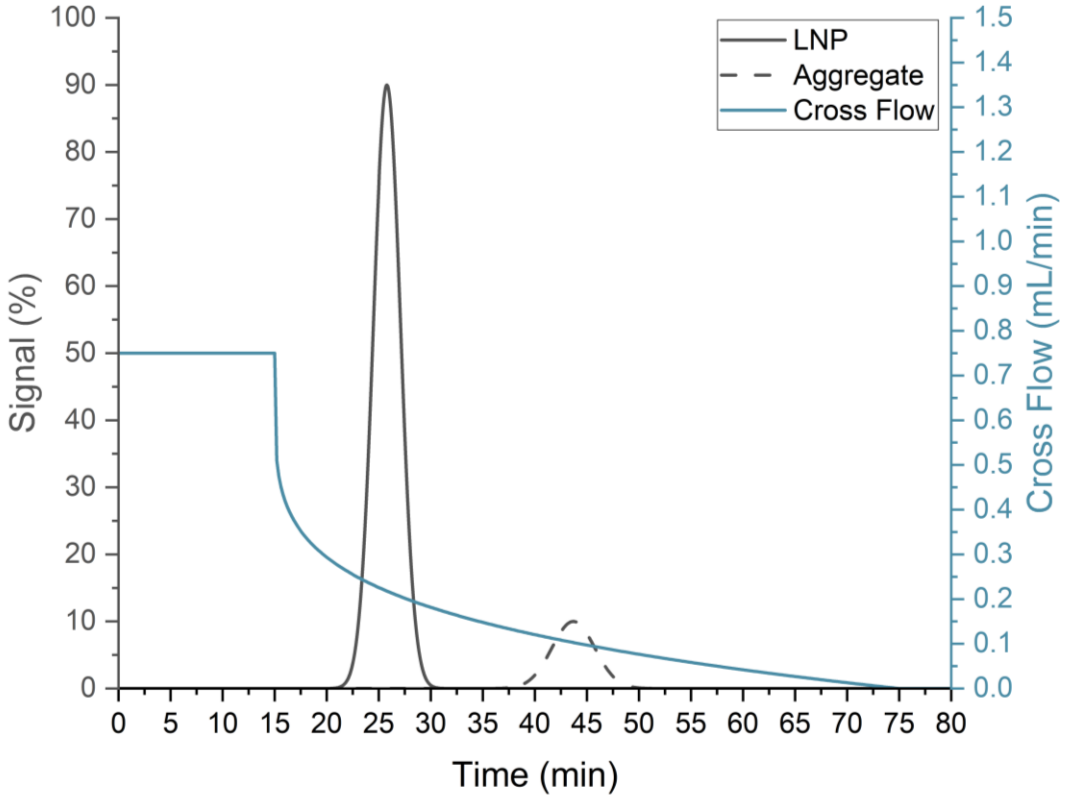

Fig.7 *– Simulated method of Frit Inlet AF4-MALS separation and detection using 60 nm LNPS and 150 nm aggregates.*

Table.3 *– Method parameters of Frit Inlet AF4-MALS.*

Frit Inlet AF4-MALS Method Parameters						
LNP Conc (mg/mL)	Membrane	Spacer (µm)	Eluent	Detector Flow (mL/min)	Injection Flow (mL/min)	Delay Time (min)
1.25	Amph RC (10 kDa MWCO)	350	PBS pH 7.4	0.3	0.2	2

Modelling method can simulate and predict separation profiles of LNP colloidal systems.
Frit Inlet AF4 method used with hyphenated inline MALS detector.

Results – FI-AF4-MALS MC3-LNPs

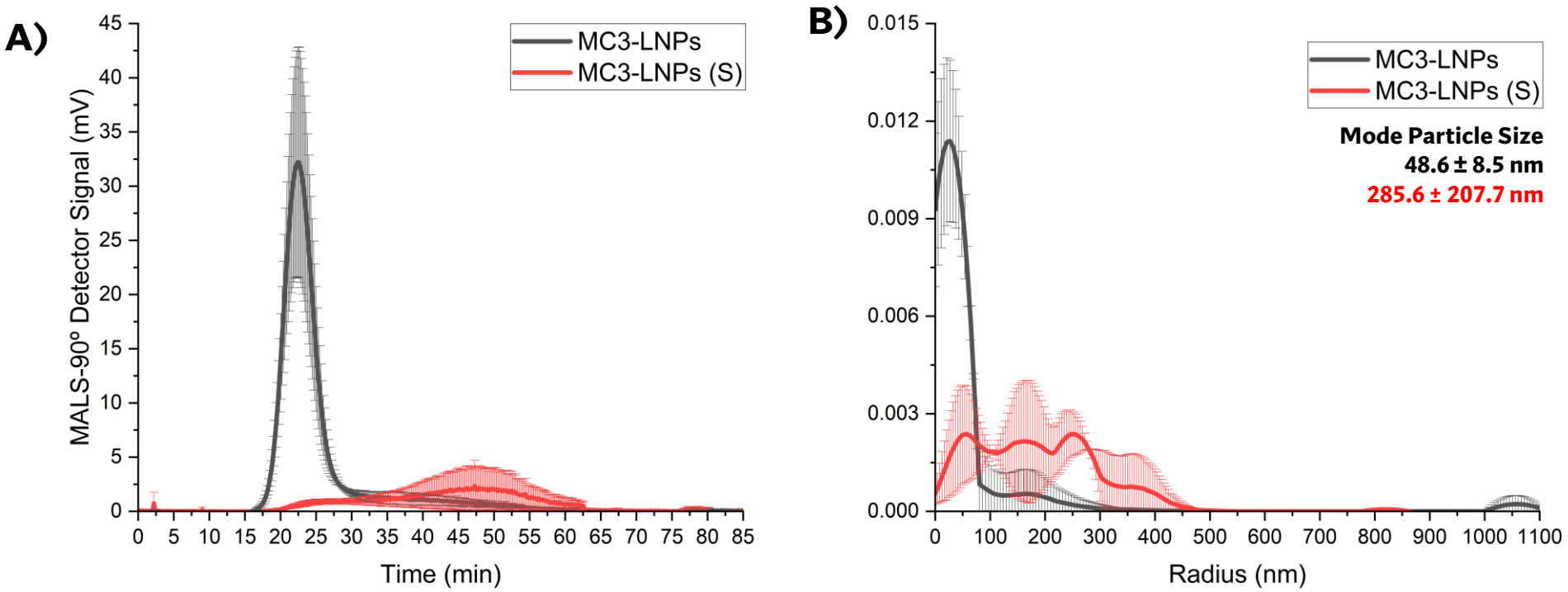

Fig.8 *- MC3-LNP FFF analysis.* ***A)*** *Elution profiles of LNPs, and* ***B)*** *Cumulative size distributions of prototype LNP formulations, (mean ± SD, n=2).*

Post-formulation sample elution profile indicates monodisperse sample population with sharp narrow peak, whereas stressed MC3-LNP profile signifies aggregation. Membrane retention time increases by ~ 20 mins on MC3-LNP aggregation.

Results – FI-AF4-MALS SM102-LNPs

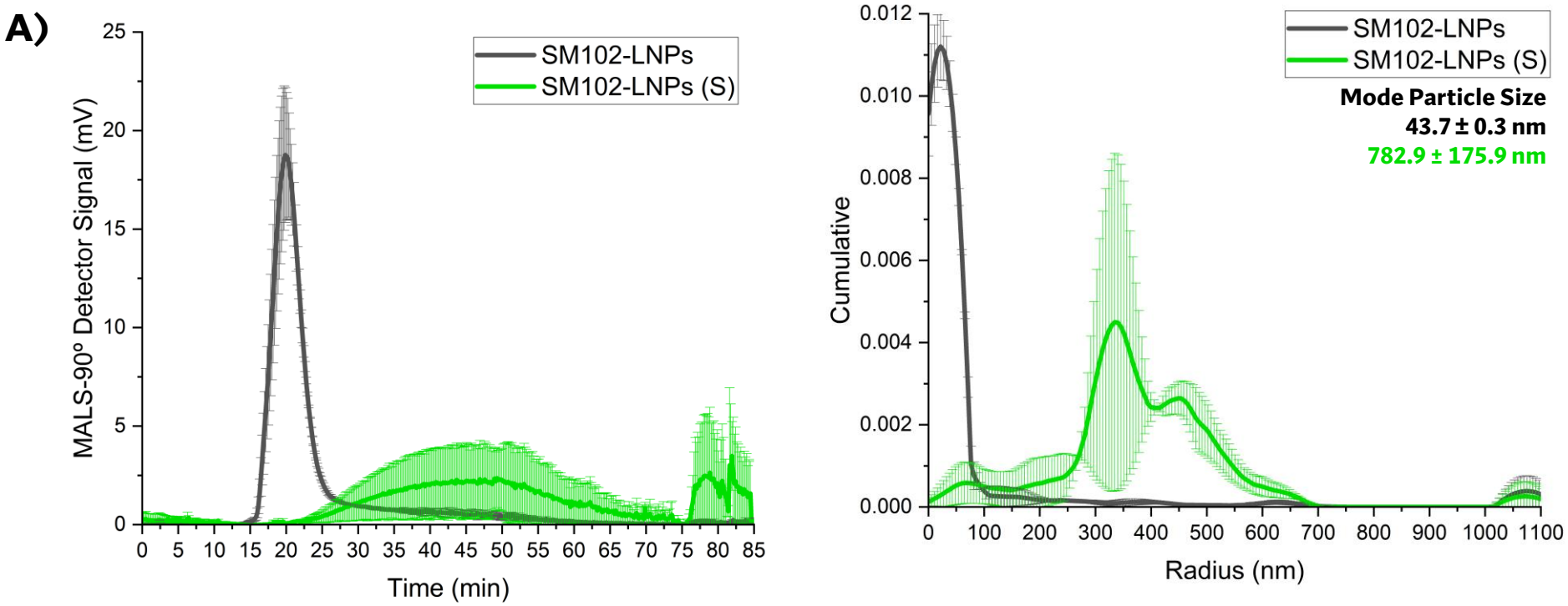

Fig.9 *– SM102-LNP FFF analysis.* ***A)*** *Elution profiles of LNPs, and* ***B)*** *Cumulative size distributions of prototype LNP formulations, (mean ± SD, n=2).*

Post-formulation sample elution profile indicates monodisperse sample population with sharp narrow peak, whereas stressed SM102-LNP profile signifies aggregation. Membrane retention time increases by ~ 25 mins on SM102-LNP aggregation.

Results – Method Validation

NTA and FI-AF4-MALS

Table.4 *– Comparison of NTA and FFF results.*

	NTA	FI-AF4-MALS
LNP CQA	**Mode Size (nm)**	
MC3-LNPs	52.8 ± 0.4	48.6 ± 8.5
MC3-LNPs (S)	75.8 ± 0.8	285.6 ± 207.7
SM102-LNPs	47.5 ± 0.6	43.7 ± 0.3
SM102-LNPs (S)	99.8 ± 1.5	782.9 ± 175.9

Post formulation prototype LNPs produce similar mode size values between techniques.
Stressed samples show high variation between techniques.
More optimisation required for cross-validation of orthogonal high-res techniques.

Conclusions

Manufacture	Method Development	Validation
Manufactured biorelevant LNP formulations for stressing and analysis.	Analytical Pipelines have been developed for low- and high-resolution characterization of LNPs.	FFF and NTA data produce similar LNP sizes values. More method optimization needed for cross-validation.

Fig.10 *– Made with BioRender.*

Conclusions

- Future looks bright for the LNP nanomedicine field!
- Correlations needed for future harmonization of LNP development.. Begins with statistical modelling
- New models of developed technologies
- Hyphenated technologies
- New technologies

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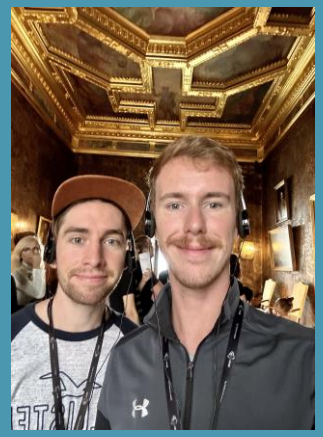

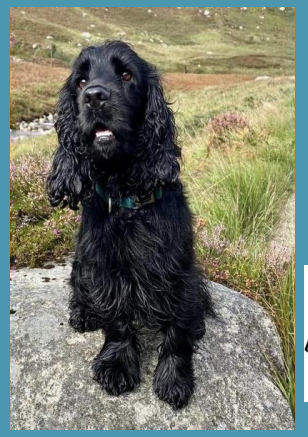

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