

Light scattering solutions for multi-attribute quantification of mRNA-lipid nanoparticle therapeutics

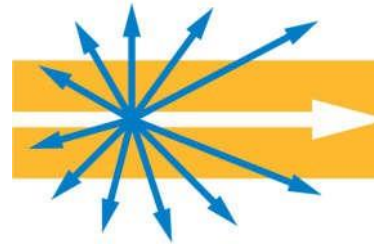
Martin Kurnik

Application Scientist II

Light scattering techniques for RNA and LNP multi-attribute quantification (MAQ)

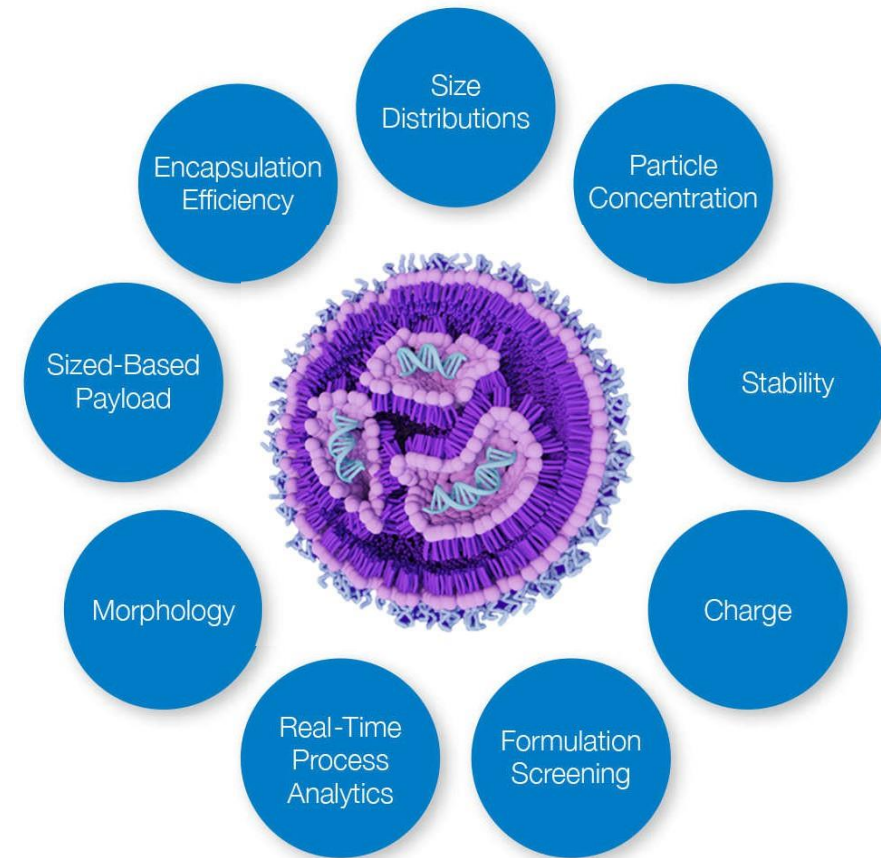
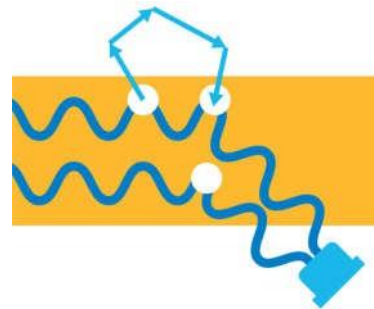
Static Light Scattering (a.k.a. multi-angle light scattering, MALS)

- ✓ Molar mass, MW
- ✓ RMS radius or radius of gyration, R_g



Dynamic Light Scattering (DLS)

- ✓ Translational diffusion coefficient, D_t
- Hydrodynamic radius, R_h





DLS Plate Reader

Size distribution and LNP concentration with built-in automation



ZetaStar™

Automated zeta potential measurement with an autosampler



ultraDAWN™

Real-time LNP size and concentration for process development and PAT



SEC – Multi-Detector (MD)

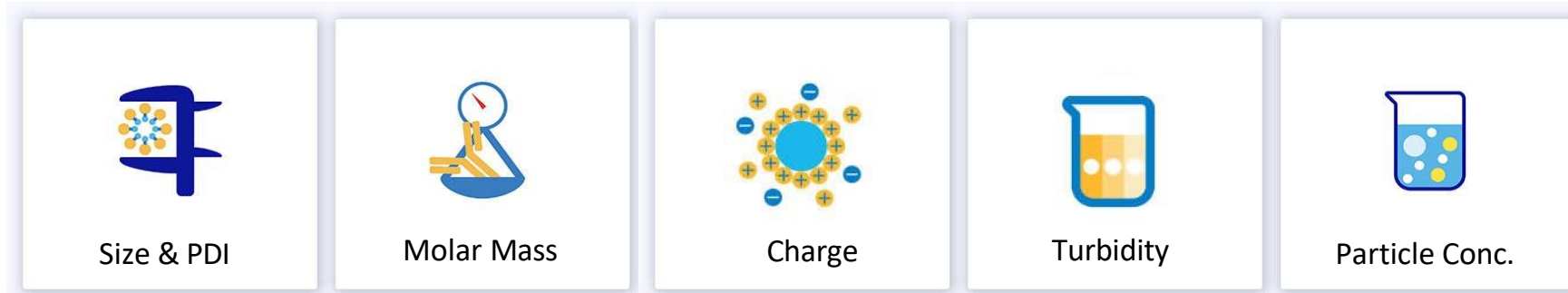
For RNA, RNP or LNPs when FFF is not available, as LNPs may be caught by or interact with the SEC columns



FFF – Multi-Detector (MD)

Ideal tool for separating and characterizing RNA, LNP, LNP-RNA, and many other bioNPs

ZetaStar: Developability and formulation



- Six parameters in a single, simple workflow
- Small sample volumes
- Fast analysis
- Automation with HPLC pump and autosampler
- GMP compliance



DLS: NanoStar and Plate Reader



2 μ L or 4 μ L



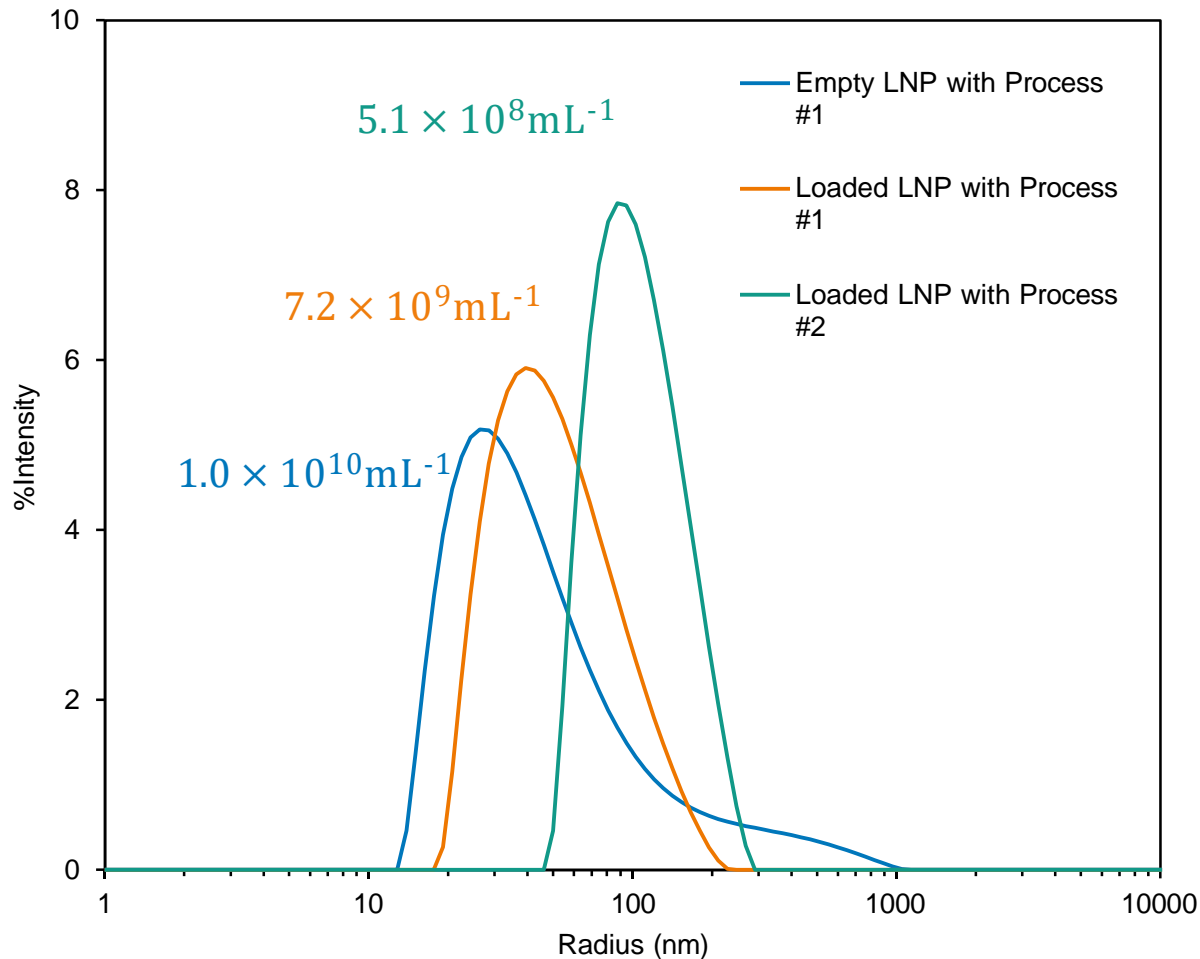
Off-shelf well plates

NanoStar™: Simultaneous SLS and DLS analysis in quartz or disposable cuvettes

- Extremely low sample volume: 2 μ L or 4 μ L
- Rapid, walk-up measurements for untrained users

Plate Reader: Measure DLS and SLS *in situ* in standard microtiter plates

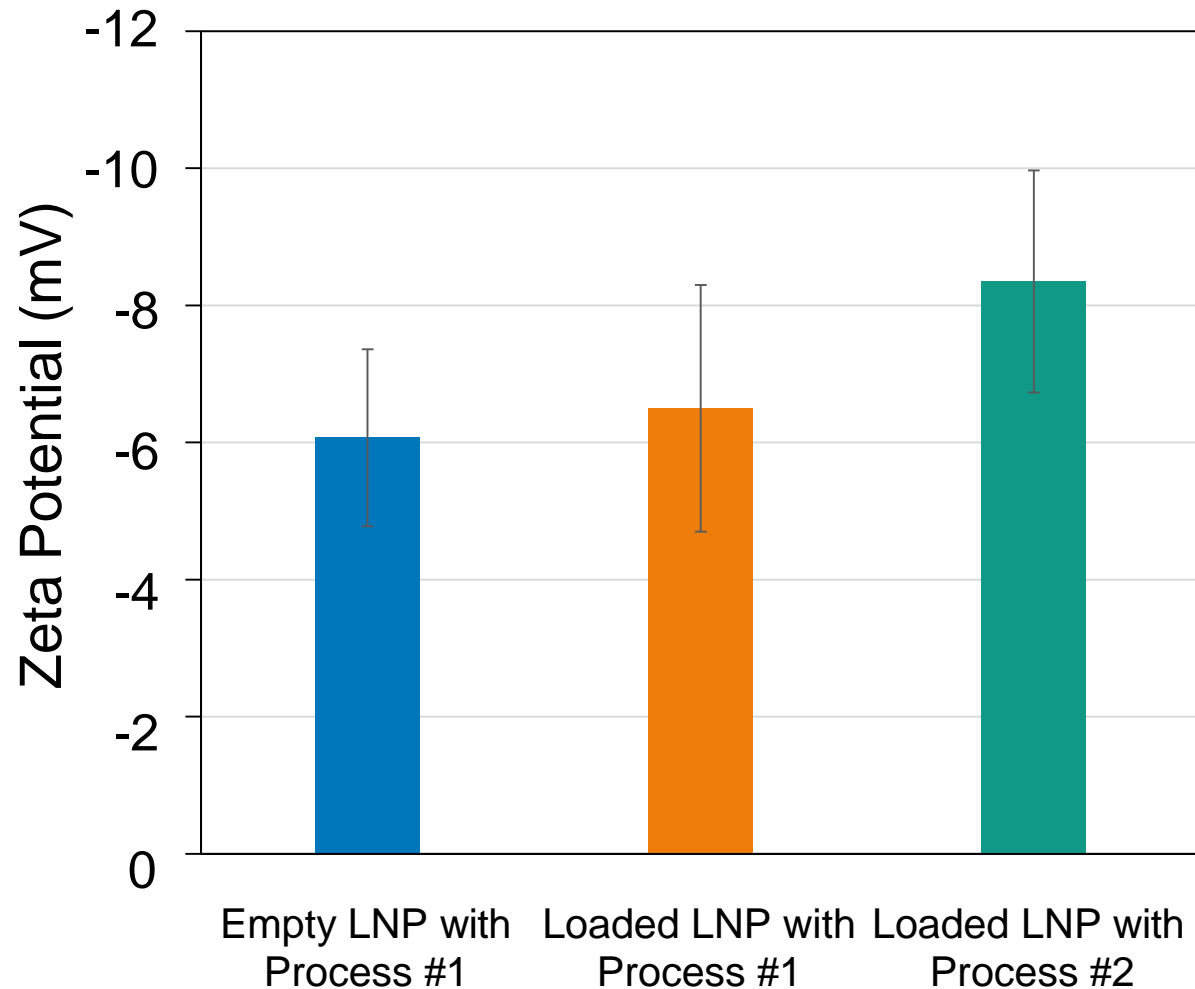
- 96, 384, and 1536 standard well plates
- No cross-contamination
- Automated analyses
- High-throughput
- Ideal to study trends and screen conditions
- Automated with liquid handling robot



Determine size, polydispersity and size distribution of empty and mRNA-loaded LNPs

- Empty LNP exhibit noticeable broad distribution
- mRNA-LNP median is clearly larger (~ 45 nm)
- Particle concentration is quickly determined
~ 1×10^{10} particles/mL

Characterizing charge / zeta potential of LNPs



ELS determines charge / zeta potential of LNPs, which is intrinsically related to the ability to enter specific organs and cells and deliver its payload effectively and where needed.

- All samples are negatively charged under test conditions
- Process conditions seem to have no or little influence on LNP surface charge
- Measurements can be performed under physiological conditions

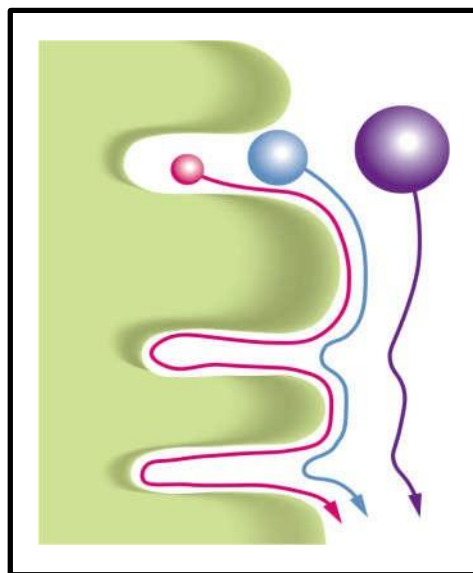
ZetaStar provides rapid answers for scientists to make faster and more informed decisions to improve LNP size consistency

mRNA-LNP Physical attributes and assays

Attribute	Assay	SEC/FFF-MALS-UV-dRI
mRNA integrity	Gel, qPCR	✓
LNP size	DLS	✓
LNP distribution	DLS	✓
Physical stability	DLS	✓
LNP number concentration	NTA	✓
LNP morphology	TEM, cryo-EM	✓ (R_g/R_h)
LNP charge	PALS	Possibly with EAF4
Encapsulation efficiency	Fluorescence	✓ new
mRNA concentration	Fluorescence	✓ new
Lipid concentration	LC-MS	✓ new

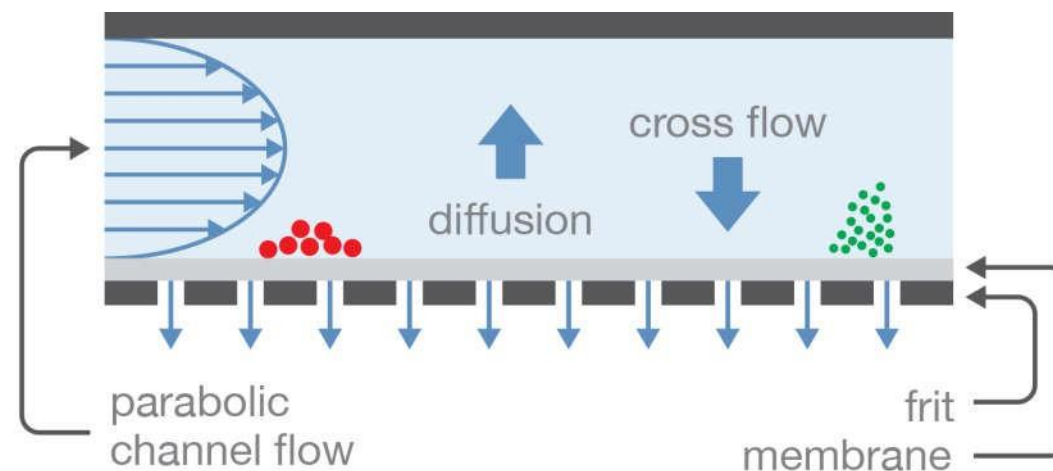
- SEC or FFF provides size-based separation

SEC



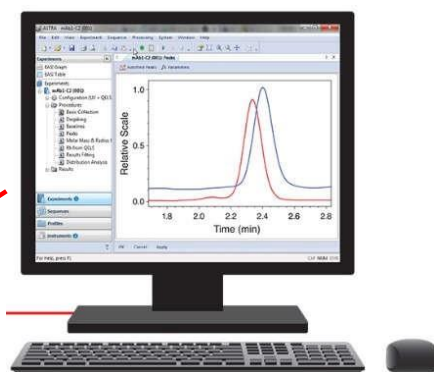
SEC: elution order by hydrodynamic volume

FFF (AF4)

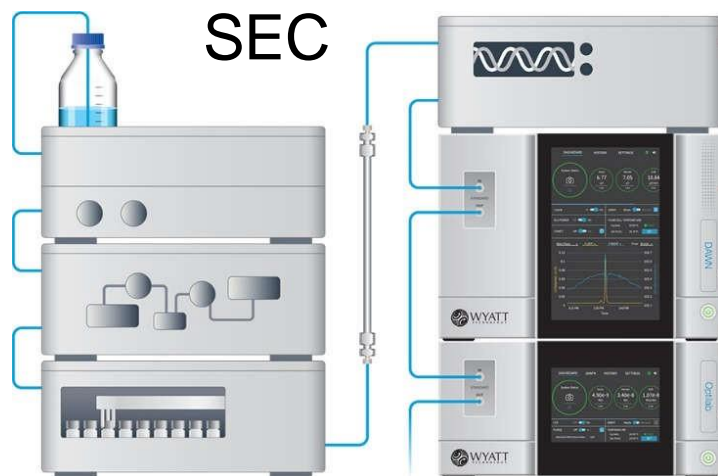


FFF: the separation tool of choice for LNPs and samples that stick to columns and are sensitive to shearing.

Optimal system configuration for LNP-MAQ



- *Online multiple-detector: DAWN™ (MALS-DLS), UV (260 nm), Optilab™ (dRI)*
- Software packages are 21 CFR Part 11 compliant



Support to ensure your success

LNP Analysis Module and Guidance Manual in ASTRA™ 8.1+.

Online Resources & Technical Notes : <https://www.wyatt.com/support>

Data review with the Wyatt Analytical Sciences team




Registration is free
for all Wyatt Users



What can we help you with?

[My Support Requests](#)



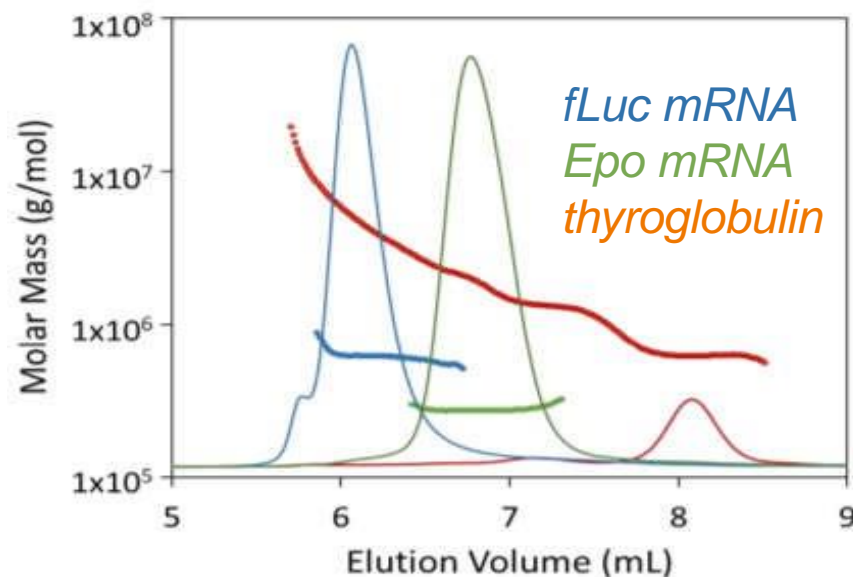
 (805) 681-9009 option 4

 support@wyatt.com

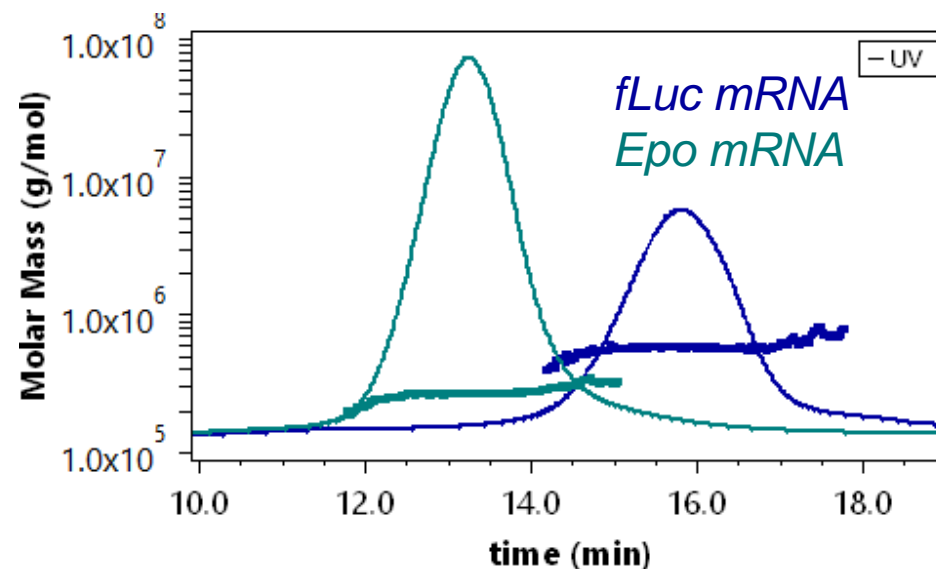
SEC or FFF to separate mRNA



SEC



FFF



WYATT TECHNOLOGY APPLICATION NOTE

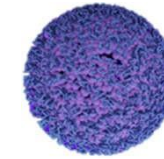
AN1616: SEC-MALS Method for Characterizing mRNA Biophysical Attributes

Pam Wang, Ph.D., Rama Akula, Moderna Therapeutics
Michelle Chen, Ph.D., Kristine Legaspi - Wyatt Technology

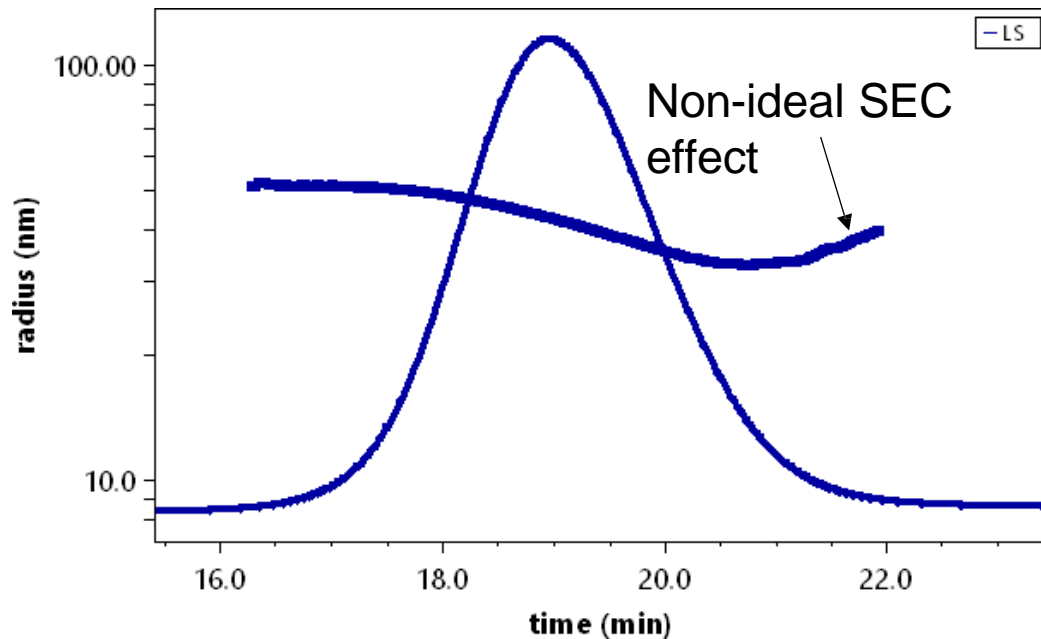
	M_w [kDa]	Agg [%]	R_g [nm]	R_h [nm]	R_g/R_h
EPO	272 ± 1	4.8	15 ± 1	12 ± 1	1.2 ± 0.1
fLuc	622 ± 1	2.6	20 ± 1	17 ± 1	1.2 ± 0.1

MALS-DLS-UV-dRI following SEC or FFF

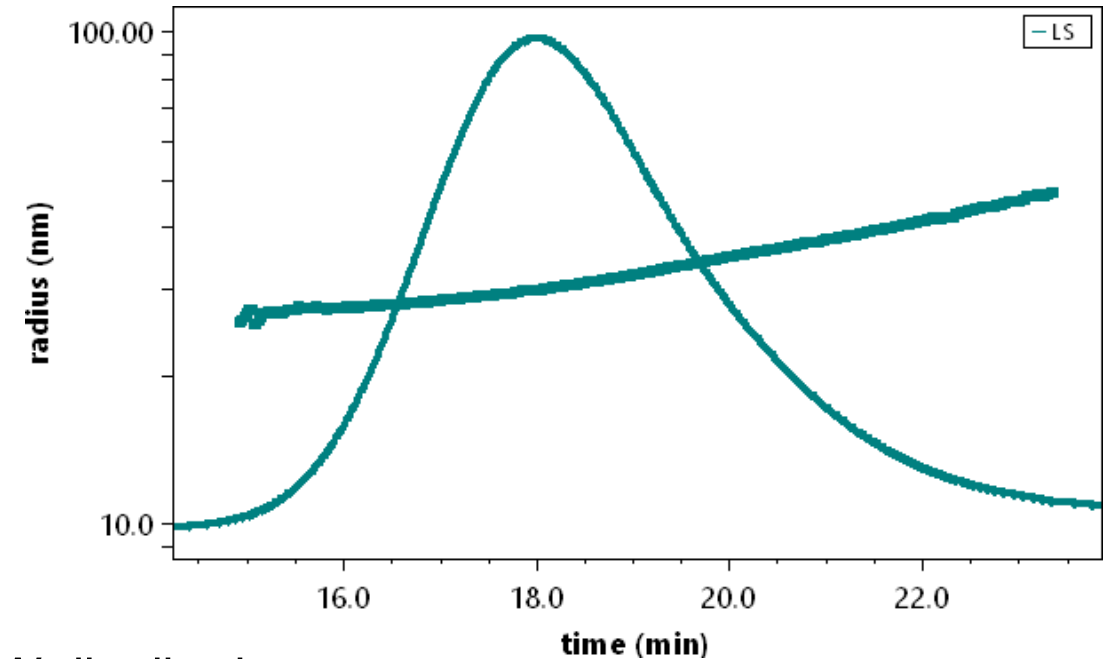
- SEC or FFF provides size-based separation



SEC



FFF

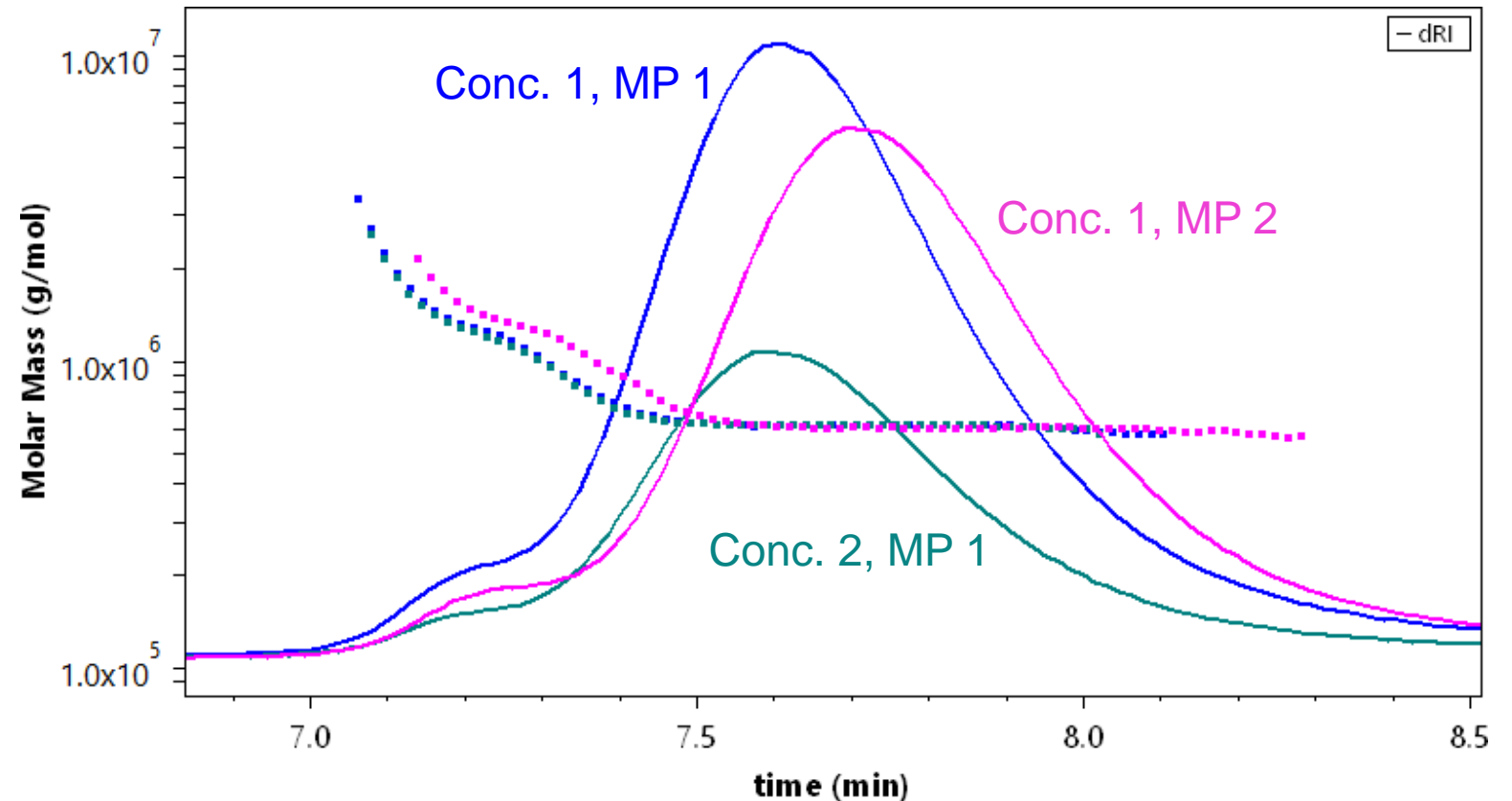


- LNP is polydisperse with continuous size/MW distribution.
- FFF is preferred over SEC: LNP stability study, separation between free RNA and LNP-RNA, and sticky LNPs.

Measuring RNA integrity by SEC/FFF-MALS

Attribute
✓ RNA integrity
LNP size
LNP distribution
LNP number
Physical stability
LNP morphology
Encapsulation efficiency
mRNA concentration
Lipid concentration

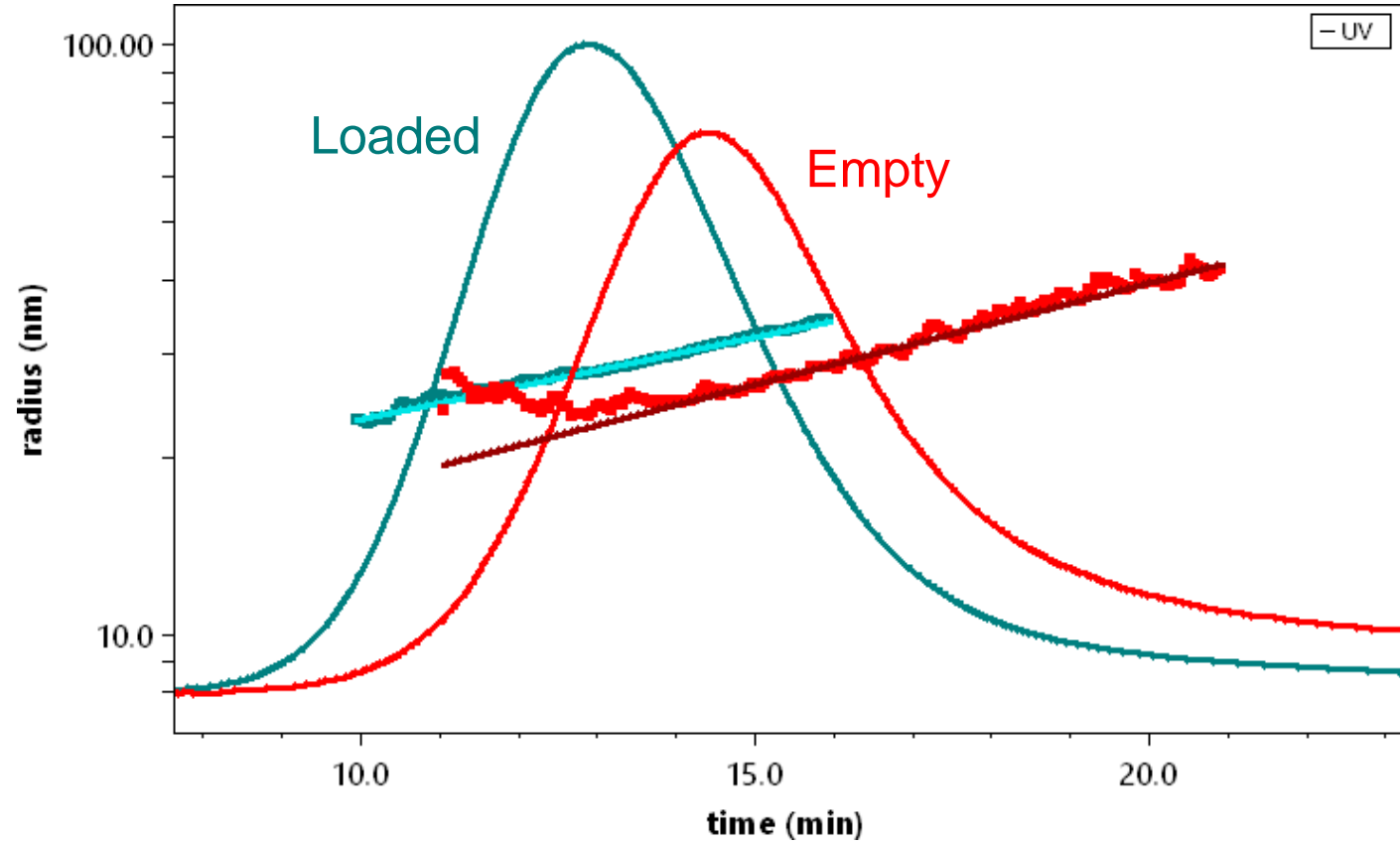
Effect of mRNA concentration and mobile phase



LNP size: average size and polydispersity



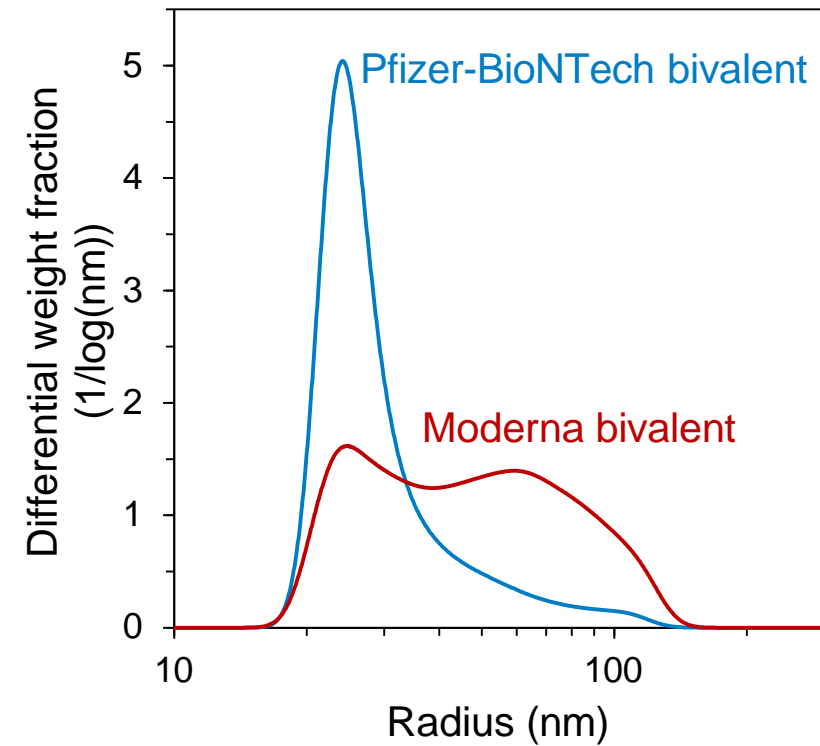
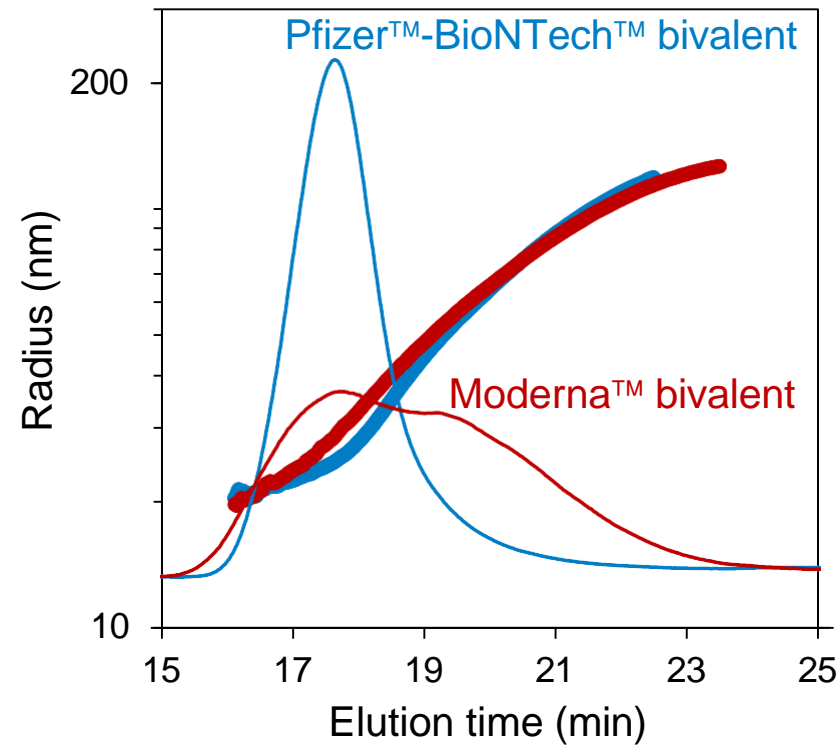
Attribute
✓ RNA integrity
✓ LNP size
LNP distribution
LNP number
Physical stability
LNP morphology
Encapsulation efficiency
mRNA concentration
Lipid concentration



LNP size distribution



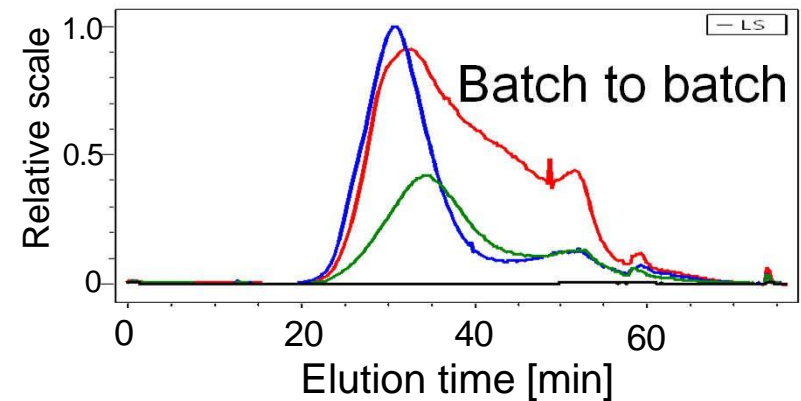
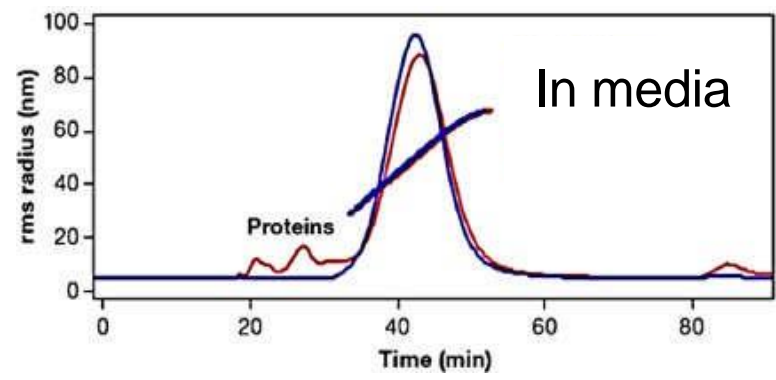
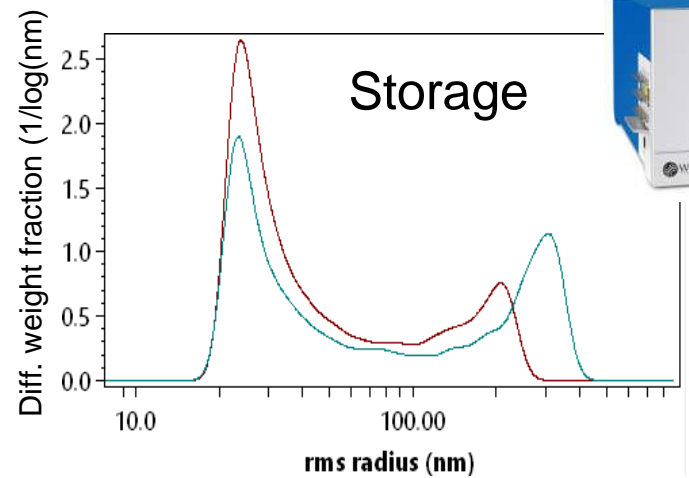
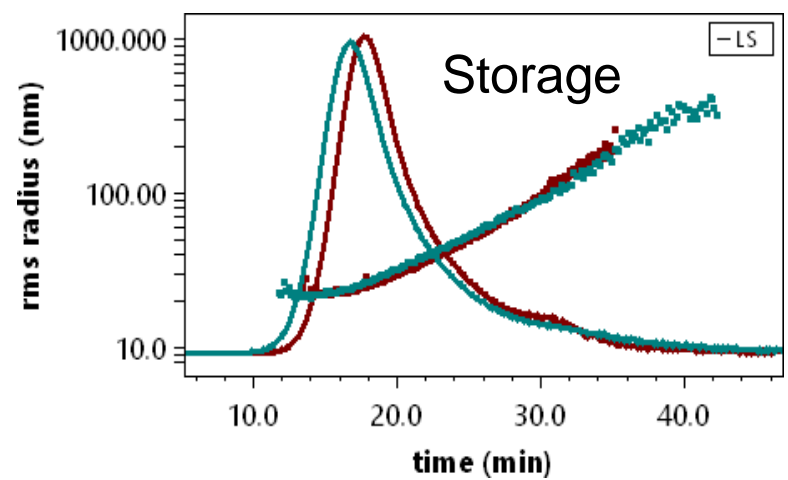
Attribute
✓ RNA integrity
✓ LNP size
✓ LNP distribution
✓ LNP number
Physical stability
LNP morphology
Encapsulation efficiency
mRNA concentration
Lipid concentration



Physical stability

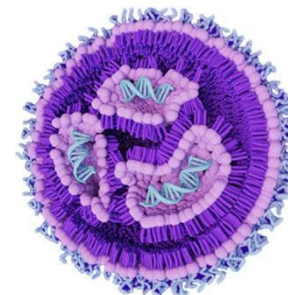


- Attribute
- ✓ RNA integrity
- ✓ LNP size
- ✓ LNP distribution
- ✓ LNP number
- ✓ Physical stability
- LNP morphology
- Encapsulation efficiency
- mRNA concentration
- Lipid concentration



Graph courtesy of Dr. Fanny Caputo, SINTEF

LNP morphology



Attribute

✓ RNA integrity

✓ LNP size

✓ LNP distribution

✓ LNP number

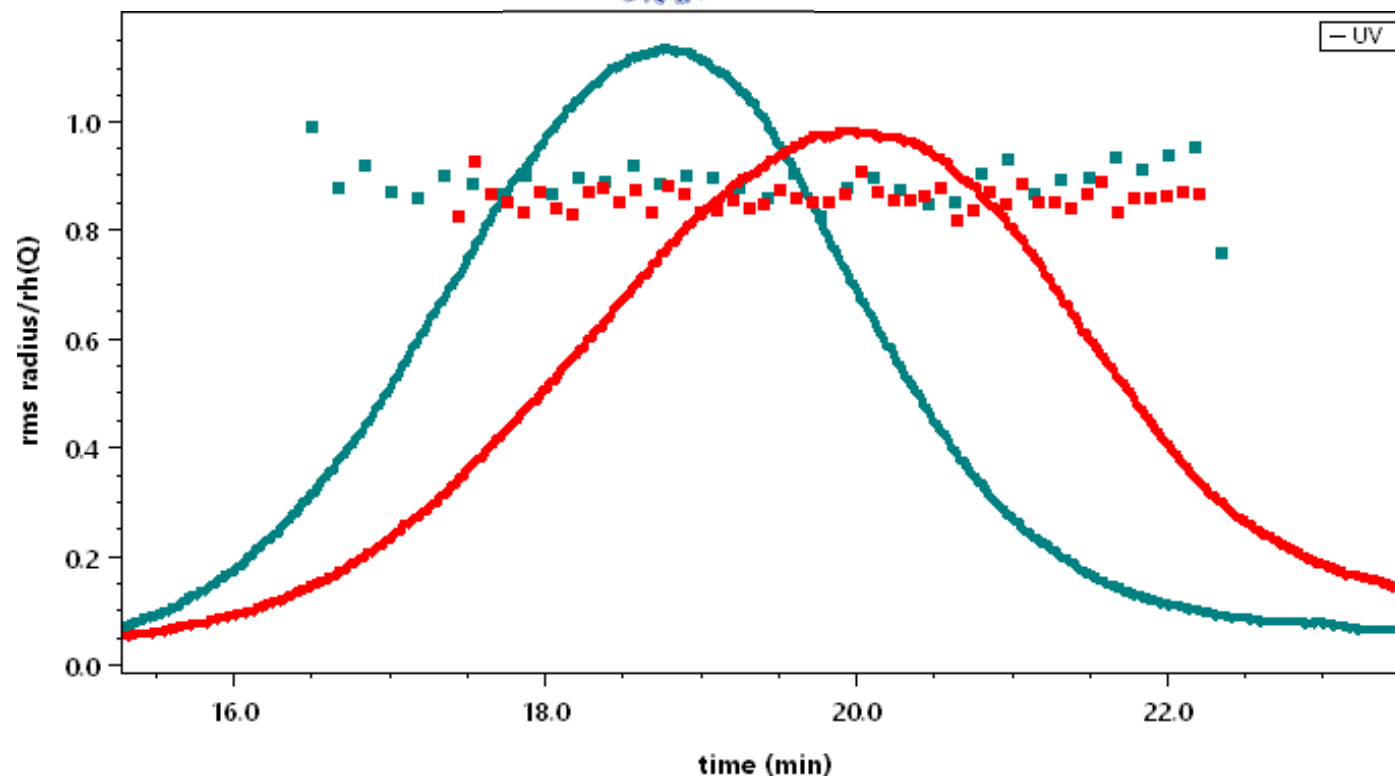
✓ Physical stability

✓ LNP morphology

Encapsulation efficiency

mRNA concentration

Lipid concentration

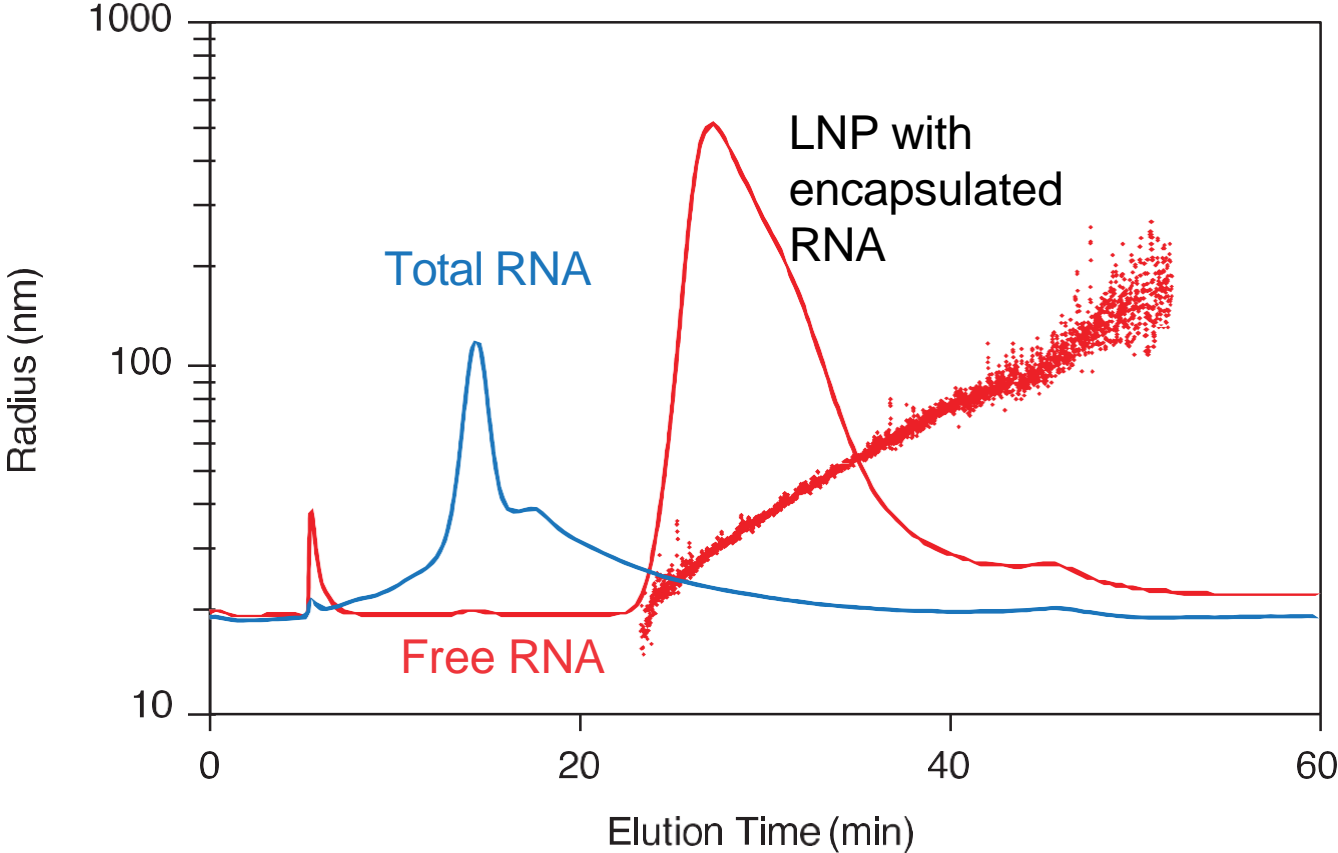


Encapsulation efficiency (EE)



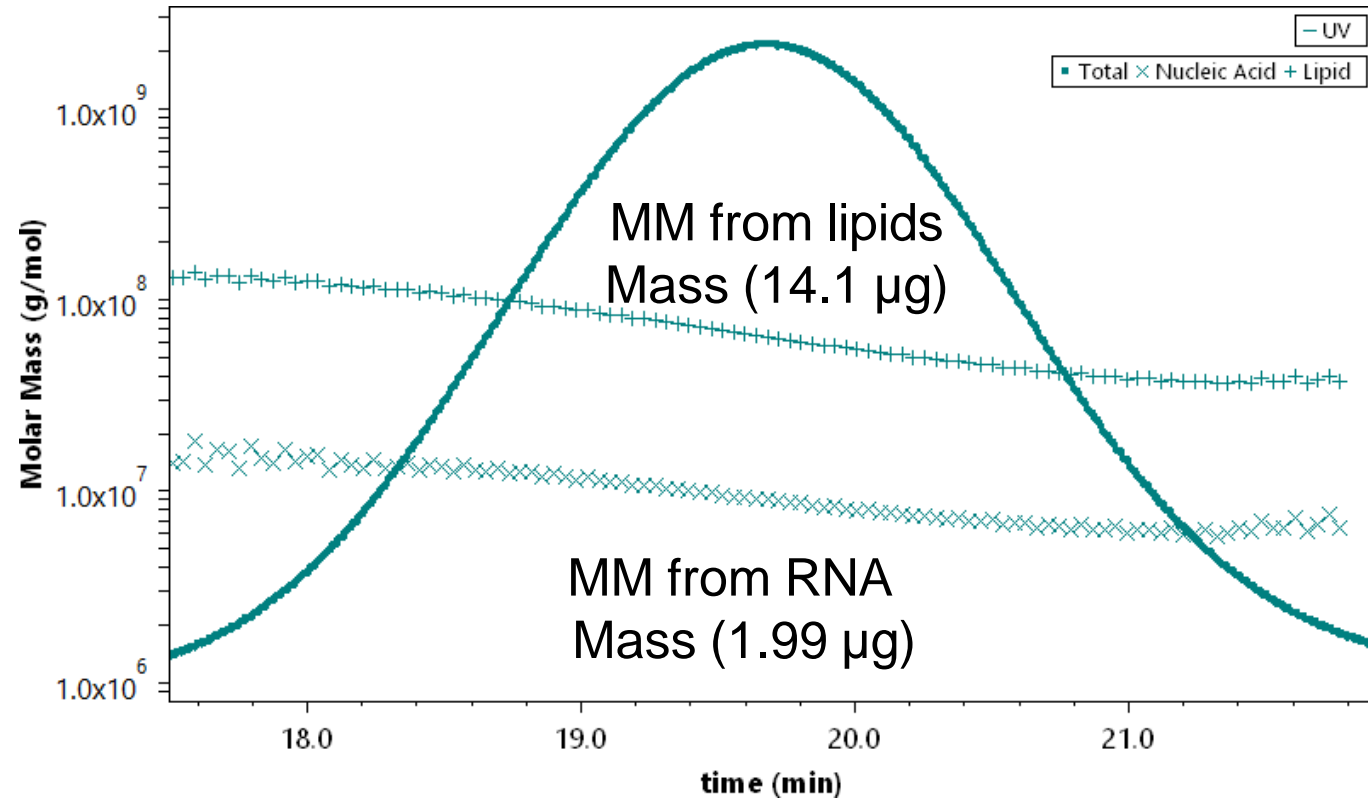
Attribute
✓ RNA integrity
✓ LNP size
✓ LNP distribution
✓ LNP number
✓ Physical stability
✓ LNP morphology
✓ Encapsulation efficiency
mRNA concentration
Lipid concentration

$$EE = (C_{\text{Total RNA}} - C_{\text{Free RNA}}) / C_{\text{Total RNA}}$$



Attribute
✓ RNA integrity
✓ LNP size
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✓ Physical stability
✓ LNP morphology
✓ Encapsulation efficiency
✓ mRNA concentration
✓ Lipid concentration

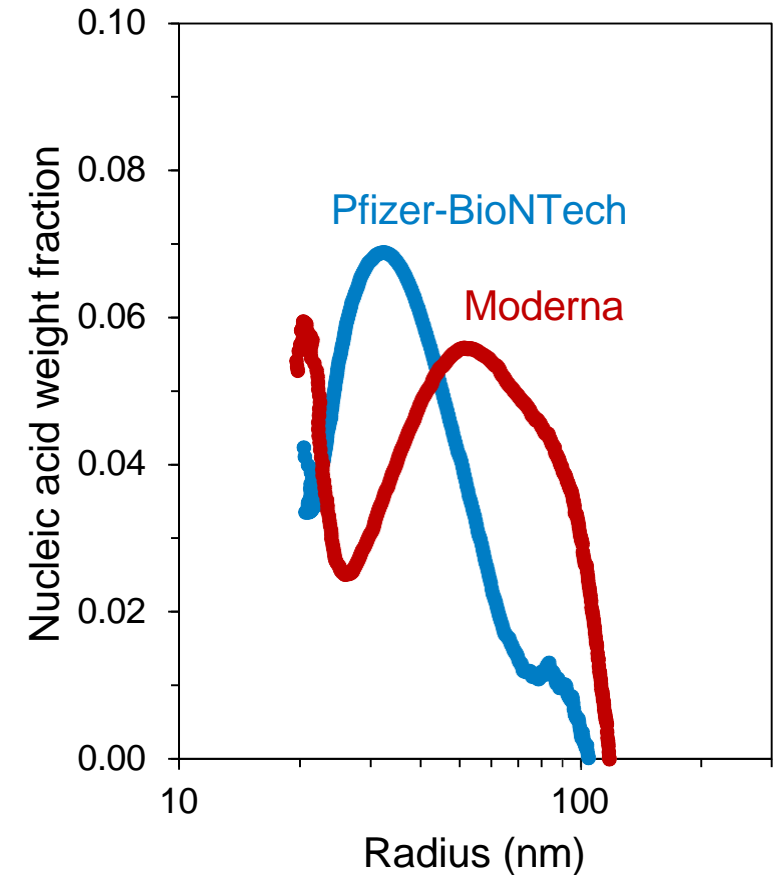
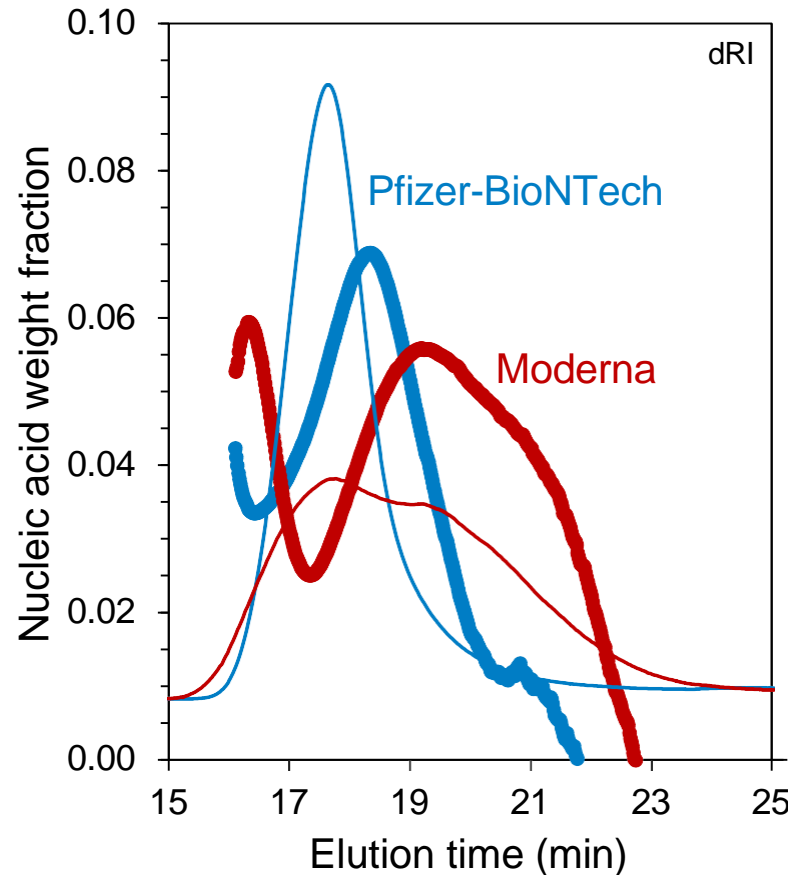
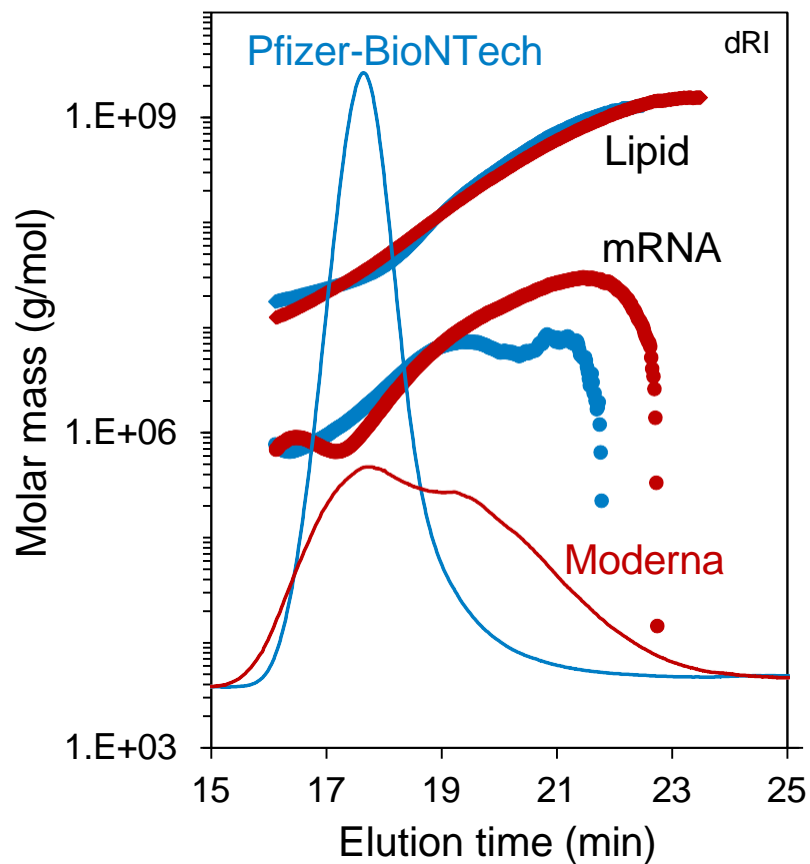
LNP Analysis for measuring payload



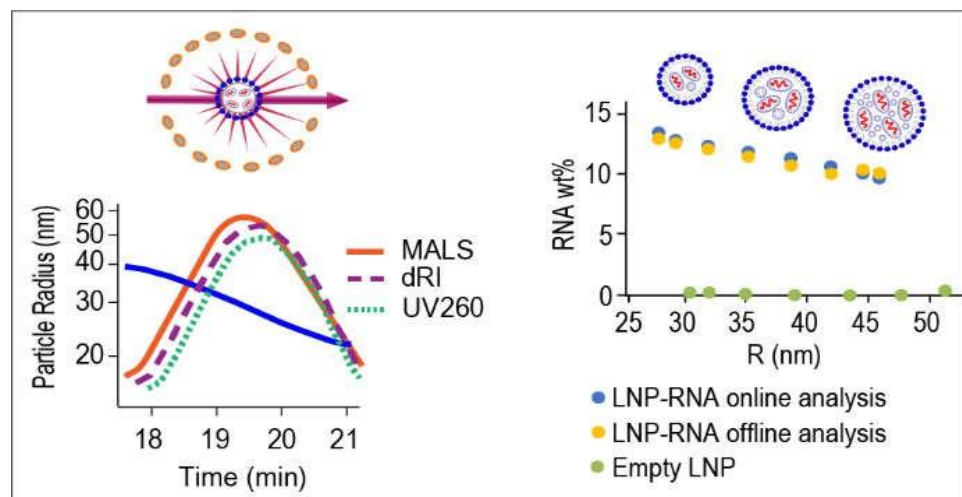
Example results from FFF-MALS LNP payload analysis



- The mRNA payload is *not* distributed equally across all particles
- Measure mRNA weight fraction or number as a function of particle size and concentration

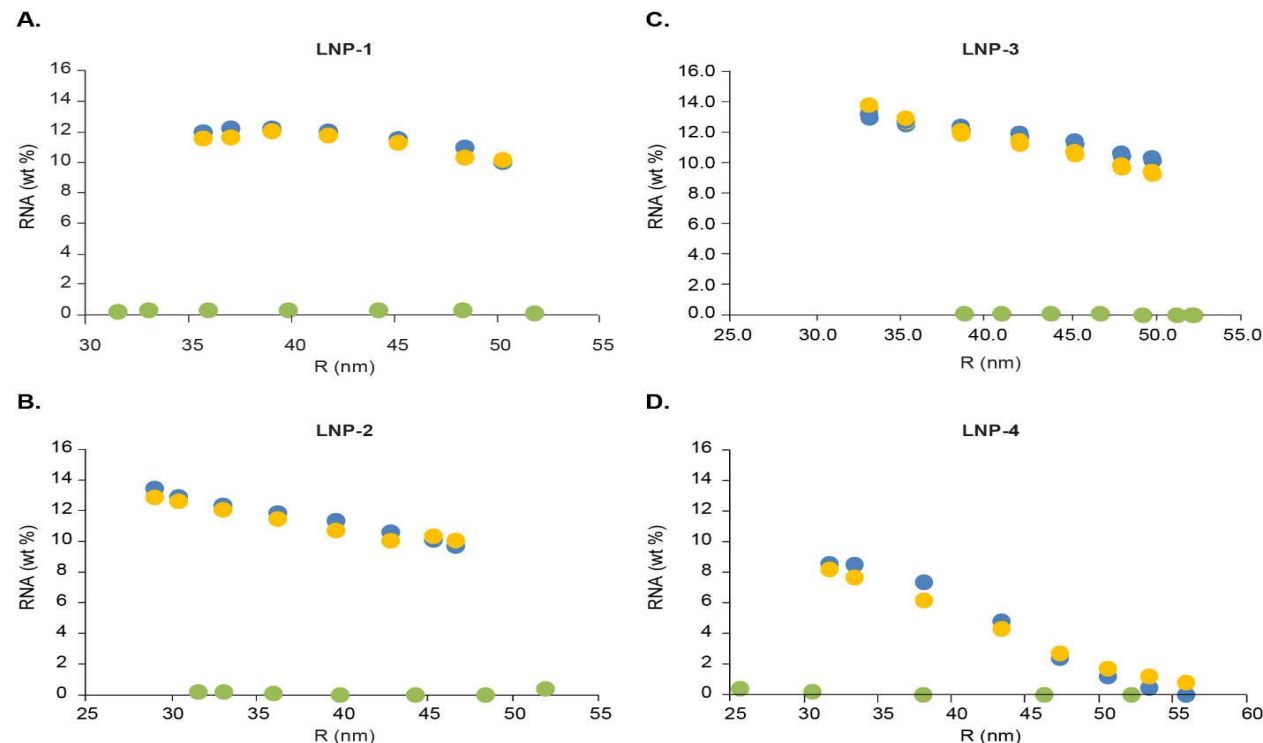


Cross-verification (Merck™): LNP-siRNA by SEC-MALS-UV-dRI



X. Jia, et al, "Enabling online determination of the size-dependent RNA content of lipid nanoparticle-based RNA formulations", X. Jia, et al., *Journal of Chromatography B* 1186 (2021): 123015.

<https://doi.org/10.1016/j.jchromb.2021.123015>

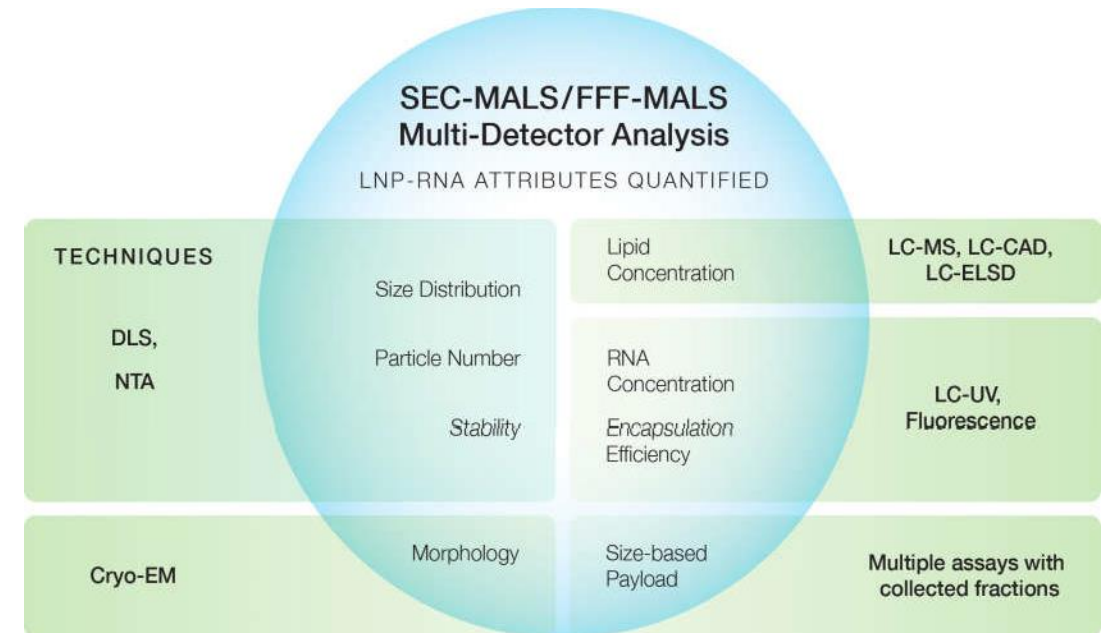


LNP-RNA, RNA wt% (online)
LNP-RNA, RNA wt% (offline)
empty LNP, RNA wt% (online)

Summary of LNP-MAQ

- SEC or FFF separates mRNA and mRNA-LNP with high resolution
- Online detectors (MALS, UV at 260 nm, and dRI) provide comprehensive characterization and multi-attribute quantitation
- The new LNP Analysis Module enables size-based nucleic acid payload
- MD-SEC and MD-FFF are essential tools for measuring mRNA and LNP size, concentration, payload, and product quality
- Software packages are 21 CFR 11 compliant
- MD-SEC and MD-FFF are automated, robust, easy to adopt, less prone to experimental errors, and require minimal hands-on time

SEC/FFF-MALS for LNP-MAQ



- www.wyatt.com/LNP



- **DLS Plate Reader:** “Polysarcosine-functionalized lipid nanoparticles for therapeutic mRNA delivery”, Nogueira, S.S et al., *ACS Appl. Nano Mat.* 3(11), 10634-10645 (2020). <https://doi.org/10.1021/acsanm.0c01834>
- **SEC-MALS:** “Polydispersity characterization of lipid nanoparticles for siRNA delivery using multiple detection size-exclusion chromatography”, Zhang, J. et al., *Anal. Chem.* 84(14), 6088-6096 (2012). <https://doi.org/10.1021/ac3007768>
- **FFF-MALS:** “Improved multidetector asymmetrical-flow field-flow fractionation method for particle sizing and concentration measurements of lipid-based nanocarriers for RNA delivery”, Mildner, R., et al., *Euro. J. Pharm. Biopharm.* 163 (2021): 252-265. <https://doi.org/10.1016/j.ejpb.2021.03.004>
- **FFF-MALS:** “Physical characterization of liposomal drug formulations using multi-detector asymmetrical flow field flow fractionation”, Parot, J. et al. *J. Cont. Rel.* 320, 495-510 (2020). <https://doi.org/10.1016/j.jconrel.2020.01.049>
- **Size-based payload distribution:** “Enabling online determination of the size-dependent RNA content of lipid nanoparticle-based RNA formulations”, X. Jia, et al., *Journal of Chromatography B* 1186 (2021): 123015. <https://doi.org/10.1016/j.jchromb.2021.123015>