MULTIMODAL CHARACTERIZATION OF TARGETED LIPID NANOPARTICLES USING CHARGE DETECTION MS AND ORTHOGONAL SEPARATION TECHNIQUES

*Rebecca D'Esposito[1]; **Esther Lewis[1]**; Matthew Lauber[1]; Mateusz Imiolek[3]; Stephan M. Koza[1]; Balasubrahmanyam Addepalli[1]; Martin Kurnik[2]; Sophia Kenrick[2]; Michelle Chen[2]; Ying Qing Yu[1]*

[1]Waters Corporation, Milford, MA; [2]Waters Corporation, Goleta, CA; [3]Waters AG, Dattwil, Switzerland

Introduction

Targeted lipid nanoparticles (tLNPs) are an emerging platform for nucleic acid delivery, enabling applications such as in vivo CAR-T therapies and programmable immunology [1,2]. These systems combine ionizable lipids, nucleic acid payloads, and surface-conjugated targeting ligands (e.g., monoclonal antibodies), resulting in increasing molecular and structural complexity. Despite this complexity, characterization is often dominated by bulk assays such as encapsulation efficiency (e.g., RiboGreen fluorescence) and ensemble sizing methods like dynamic light scattering (DLS). While useful for rapid screening, these approaches provide limited insight into particle heterogeneity and structure–function relationships [3].

Orthogonal techniques, including DLS/ELS, FFF-MALS, and chromatographic methods, expand characterization by probing size, charge, and component-level composition. However, these approaches rely on indirect or ensemble-averaged measurements, making it difficult to directly assess intact nanoparticle populations [3,4]. Charge detection mass spectrometry (CDMS) has emerged as a powerful tool for analyzing large macromolecular assemblies, enabling direct measurement of individual particle masses in the megadalton range without deconvolution [5]. This capability is particularly well-suited for LNPs, where intrinsic heterogeneity is expected but not readily resolved using conventional techniques.

Here, we apply a multi-technique analytical strategy, including CDMS, to characterize tLNPs with varying levels of antibody conjugation. CDMS directly measures whole-particle mass distributions, revealing population-level heterogeneity not captured by traditional sizing methods. Together with orthogonal techniques, this work highlights the importance of integrated physicochemical characterization for optimizing LNP-based therapeutics.

Methods

tLNP samples were sourced from Phosphorex. tLNPs were generated by conjugating azide-modified mAbs to DBCO-lipids (0, 1, 2.5 and 5%) via copper-free click chemistry. All LNPs and tLNPs were buffer exchanged into 20 mM ammonium acetate, pH 7.4, using Slide A Lyzer™ MINI Dialysis Devices (Thermo Scientific™) for 20 minutes at 4 ˚ C prior to CDMS analysis. Samples were introduced by nano electrospray ionization (nESI) into a benchtop Xevo™ CDMS Instrument (Waters) operated under native conditions. CDMS data were processed using the CDMS Toolkit within waters_connect™ Software to obtain mass distributions and assess particle heterogeneity. Complementary intact particle methods, including dynamic and electrophoretic light scattering (DLS, ELS), and FFF, provided supporting measurements of size and mobility but were secondary to CDMS.

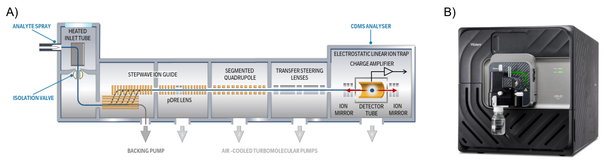


Figure 1: A) Instrument diagram, B) Picture of Xevo CDMS

Methods

Field Flow Fractionation (FFF)- Multi-Angle Light Scattering (MALS)

FFF separation was performed using neat samples with a separation method optimized for tLNP-mRNAs on an Eclipse™ FFF Instrument with a 350 µm fixed-height short channel connected to an HPLC pump and autosampler. PBS was used as the mobile phase. A DAWN™ MALS Instrument, an Optilab™ differential refractometer, and a UV detector set to 260 nm wavelength were used for online detection. The FFF system was controlled by VISION™ Software. Reported uncertainties are the standard deviations from triplicate measurements. Data acquisition and analysis were performed using the ASTRA™ Software.

Figure 2:Arc HPLC, Eclipse™FFF system, DAWN MALS detector, Optilab dRI detector

Results: FFF-MALS

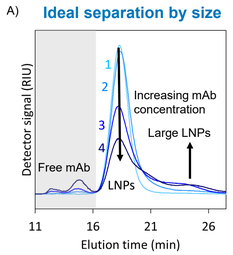
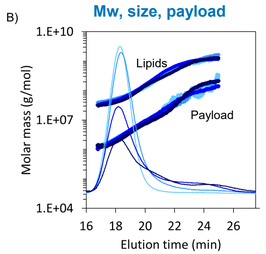
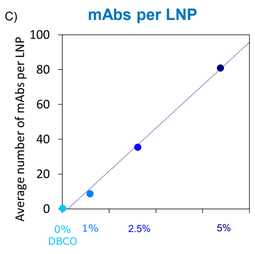


Figure 3: A) Elution chromatogram, B) Molar mass by elution time, and C) estimated mAb per tLNP by FFF-MALS (DBCO = Dibenzocyclooctyne)

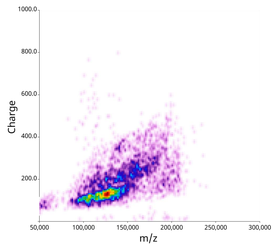
Conjugation Levels	
1	LNP **no** mAb (0% DBCO)
2	tLNP **low** mAb (1% DBCO)
3	tLNP **mid** mAb (2.5% DBCO)
4	tLNP **high** mAb (5% DBCO)





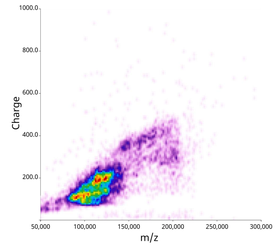
Results: CDMS

Figure 4: m/z vs charge 2D density plot of the unconjugated LNPs (0% DBCO)



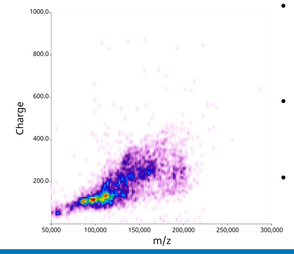
- CDMS reveals that even unconjugated LNPs exhibit substantial intrinsic heterogeneity in particle mass.
- This baseline distribution highlights the limitations of ensemble-only measurements.
- It provides a critical reference for assessing the impact of mAb conjugation.

Figure 5: m/z vs charge 2D density plot of the conjugated tLNPs (1% DBCO)



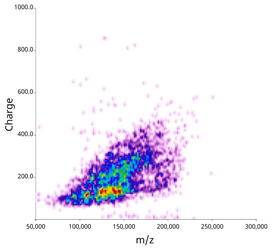
- Low-level mAb conjugation increases distribution width, indicating greater particle-to-particle variability.
- The absence of a simple mass shift suggests nonuniform antibody incorporation.
- These results demonstrate the sensitivity of CDMS to early-stage structural changes.

Figure 6: m/z vs charge 2D density plot of the conjugated tLNPs (2.5% DBCO)



- At mid-level conjugation, the emergence of broader and more complex distributions suggests multiple particle subpopulations.
- This indicates increasingly heterogeneous mAb incorporation across LNPs.
- CDMS uniquely resolves these features that are obscured in averaged measurements.

Figure 7: m/z vs charge 2D density plot of the conjugated tLNPs (5% DBCO)



- High mAb conjugation produces the broadest and most heterogeneous mass distribution.
- This suggests limits to uniform surface modification and potential structural variability at higher incorporation.
- The data reinforce the need for single-particle analysis to fully characterize tLNP populations.

Conclusion

This work demonstrates that targeted LNPs exhibit substantial particle-level heterogeneity that cannot be fully captured by conventional ensemble techniques alone. Charge detection mass spectrometry (CDMS) enables direct measurement of intact nanoparticle masses, revealing distribution breadth, subpopulations, and nonuniform antibody incorporation that are otherwise obscured.

Across increasing levels of mAb conjugation, CDMS shows progressively broader and more complex mass distributions, indicating that higher ligand incorporation amplifies variability rather than producing uniform modification. When combined with orthogonal methods such as FFF-MALS, these data provide a more complete understanding of size, composition, and functionalization.

Overall, this multimodal analytical strategy highlights the importance of integrating single-particle and ensemble measurements to characterize complex nanomedicines. Such approaches are critical for establishing robust structure–function relationships and informing the design and optimization of next-generation LNP-based therapeutics.

References

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