DIRECT MEASUREMENT OF LIPOPROTEIN PARTICLE HETEROGENEITY USING CDMS

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Introduction

Lipoprotein particles, including high-density lipoprotein (HDL) and low-density lipoprotein (LDL), play critical roles in cholesterol transport and lipid homeostasis (1). Beyond their classical function in cardiovascular health, these particles are increasingly implicated in a wide range of biological processes, including cardiometabolic disorders, neurodegenerative disease, inflammation, and host response to infection (1,2). Despite their importance, lipoproteins remain challenging to characterize due to their intrinsic heterogeneity in size, molecular composition, and structural organization (3,4).

Traditional analytical approaches—such as bulk biochemical assays, electrophoresis, and ensemble mass spectrometry—typically report population-averaged measurements, thereby obscuring the diversity of lipoprotein subclasses (3,5). As a result, these methods fail to capture particle-to-particle variability that may be biologically relevant, hindering efforts to fully understand how lipoprotein structure and physicochemical properties relate to function and disease (4,6).

Charge Detection Mass Spectrometry (CDMS) offers a powerful alternative by enabling direct measurement of individual particles (7,8). By simultaneously determining the mass and charge of single lipoprotein particles, CDMS provides a high-resolution, label-free approach to resolving heterogeneous populations (8,9). This capability allows for the identification of distinct lipoprotein subclasses and the characterization of their structural and compositional diversity (10).

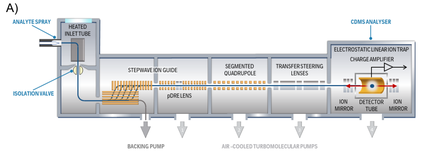
In this study, we apply CDMS to human plasma-derived lipoproteins to directly probe particle-level heterogeneity across HDL (small (S), medium (M), large (L)), LDL, lipoprotein(a) (Lp(a)) and related species (3, 6). This work establishes CDMS as a transformative analytical platform for lipoprotein research, with potential to advance mechanistic understanding and support translational applications in disease diagnostics and therapeutic development (7,10).

Methods

Lipoprotein particles were isolated from 500 µL of plasma using density-based sequential flotation ultracentrifugation in a fixed-angle rotor (TLA-110). Samples were centrifuged at 100,000rpm and subsequently at 58,000rpm in a Optima MAX-TL ultracentrifuge (Beckman Coulter) at 15 ° C to obtain fractions with a final density of 1.21 g/mL. Potassium bromide density solutions were removed using Amicon® Ultra-4 centrifugal filter units (Millipore) at 4,500rpm for 8 min in a Sorvall™ Legend™ XF centrifuge (Thermo Fisher Scientific™).

Lipoprotein fractions were buffer-exchanged into 20 mM ammonium acetate, pH 7.4 using Slide-A-Lyzer™ MINI Dialysis Devices (Thermo Scientific) for 15 minutes at 4 ° C. Following buffer exchange, samples were introduced by nano-electrospray ionization into a benchtop Xevo™ CDMS Instrument (Waters). CDMS data were processed and reviewed using the CDMS Toolkit within the waters_connect™ Software. Statistical analysis was scripted using Python. CDMS mass histograms were generated using identical logarithmic bins spanning 1 × 10^7 to 3 × 10^8 Da for all samples.

A)



B)

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

Results

Fraction 1: HDL-S

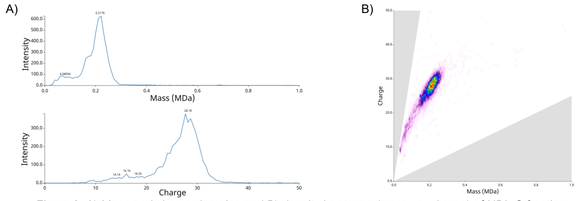


Figure 2: A) Mass and charge domains and B) density heat map (mass vs. charge) of HDL-S fraction

HDL-S particles exhibit a relatively narrow mass distribution and limited charge dispersion, consistent with a more homogeneous population of small HDL particles. These data demonstrate that even the smallest HDL subclass retains measurable particle-to-particle variability that is not captured by bulk measurements.

Fraction 2: HDL-M

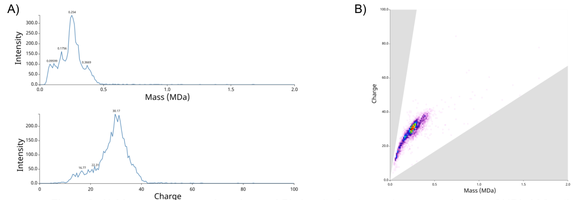


Figure 3: A) Mass and charge domains and B) density heat map (mass vs. charge) of HDL-M fraction

HDL-M displays increased mass spread and broader charge distribution relative to HDL-S, indicating the emergence of greater structural and compositional diversity. This transition highlights a continuum of particle heterogeneity across HDL subclasses rather than discrete, uniform populations.

Fraction 3: HDL-L

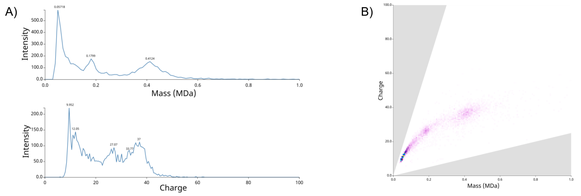


Figure 4: A) Mass and charge domains and B) density heat map (mass vs. charge) of HDL-L fraction

HDL-L exhibits substantial mass dispersion and pronounced charge heterogeneity, consistent with increased structural complexity and compositional variability in larger HDL particles. The broadened distributions suggest the presence of multiple subpopulations within this fraction.

Fraction 4: Lp(a)

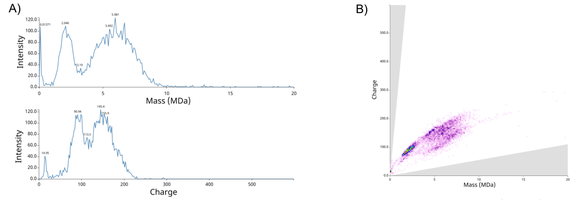


Figure 5: A) Mass and charge domains and B) density heat map (mass vs. charge) of Lp(a) fraction

Lp(a) particles show extreme mass and charge heterogeneity, spanning a wide size range consistent with known variability in apolipoprotein(a) structure. These data highlight the limitations of conventional assays and demonstrate the unique capability of CDMS to resolve highly heterogeneous lipoprotein species.

Mass and Charge Overlays

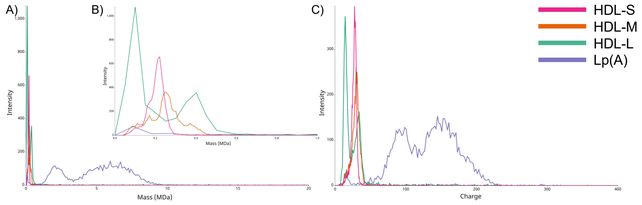


Figure 6: A) Mass overlay of all fractions, B) Zoomed inset of the lower mass range, and C) charge overlay of all fractions

Mass and charge overlays reveal a progressive shift toward higher mass and broader distributions across HDL and Lp(A) subclasses. This trend reflects increasing particle heterogeneity and structural complexity from HDL-S to HDL-L. The substantial overlap between distributions demonstrates that lipoprotein subclasses are not discrete entities but exist along a continuum. These results highlight the ability of CDMS to resolve particle-level variability that is obscured by conventional bulk measurements.

Discussion

This study demonstrates that CDMS provides a direct and quantitative view of lipoprotein particle heterogeneity that is not accessible using traditional analytical methods. By measuring the mass and charge of individual particles, CDMS resolves subclass-specific distributions and reveals substantial variability within nominally uniform fractions.

Across HDL subclasses, we observe a clear progression in both mass and heterogeneity, with HDL-L exhibiting significantly broader distributions than HDL-S. These findings support the concept that HDL particles exist along a continuum of structural and compositional diversity rather than as discrete, homogeneous classes.

The ability to resolve charge in addition to mass provides an additional dimension of particle characterization, likely reflecting differences in lipid composition, protein content, and post-translational modifications. This is particularly evident in Lp(a), where extreme heterogeneity is consistent with known variability in apolipoprotein(a) structure and glycosylation.

Importantly, these results highlight a key limitation of conventional bulk measurements, which obscure particle-level variability by reporting averaged values. CDMS overcomes this limitation and enables direct interrogation of particle distributions, which may be more closely linked to biological function and disease risk.

Together, these results establish CDMS as a powerful platform for lipoprotein analysis, with potential applications in mechanistic studies, biomarker discovery, and therapeutic development.

Conclusion

- CDMS enables direct, single-particle measurement of lipoprotein mass and charge, providing unprecedented insight into particle heterogeneity.
- HDL subclasses exhibit progressively increasing mass and distribution breadth, revealing substantial particle-level variability within each fraction.
- Lp(a) displays extreme heterogeneity, underscoring the need for analytical methods capable of resolving complex lipoprotein populations.
- These findings demonstrate that particle-resolved measurements provide fundamentally different information than bulk assays and may be critical for understanding lipoprotein biology and disease.
- CDMS represents a transformative tool for advancing lipoprotein research and enabling next-generation diagnostics.

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