AURORA®
SERIES



AURORA SERIES® IMPACT PORTFOLIO · TARGETED PROTEOMICS

Targeted Proteomics: the peer-reviewed evidence base for Aurora Series® columns

Comprehensive review of peer-reviewed publications establishes IonOpticks Aurora Series® columns as the chromatographic platform of choice for reproducible, sensitive and portable targeted proteomics. From high-plex PRM verification panels and regulated absolute quantification through to single-copy-per-cell immunopeptidomics.

1,305

Targeted assays built from 5,437 Orbitrap Astral precursors, in days, on the same Aurora® Rapid™ 8×150 chemistry (Wahle et al., 2025). [4]

1,070

Precursors in a single injection: a 101-protein CSF neurodegeneration panel on Aurora® Elite™ 15×75 (Plubell et al., 2026). [5]

~1 copy per cell

LOQ for drug-modified $KRAS^{G12C}$ neoantigens by SureQuant on Aurora® Ultimate™ 25×75 XT (Maso et al., 2025). [9]

Highlights from the peer-reviewed evidence

Twelve peer-reviewed studies citing Aurora Series® columns in targeted workflows have been reviewed.

01 **DISCOVERY TO TARGETED ON THE SAME COLUMN**

5,437 precursors
1,305 targeted assays, in days

Orbitrap Astral DIA discovery translated to Stellar MS PRM on the same Aurora Rapid™ 8×150 chemistry.

Wahle et al., Mol Cell Proteomics 2025

02 **SCALE WITHOUT LOSING PRECISION**

1,070 precursors in a single injection

101-protein CSF neurodegeneration panel on Aurora Elite™ 15×75. ~12 s peak widths and ~7 points per peak at 62 concurrent precursors.

Plubell et al., Mol Cell Proteomics 2026

03 **NO MISSING VALUES**

Targets found in every sample

Aurora Frontier™ PRM detected clinically actionable PRAME425 in every HLA-A*02+, PRAME+ NUT sample and reversed its ranking versus untargeted DIA.

Jensen et al., J Immunother Cancer 2026

04 **EXTREME SENSITIVITY**

~1 copy-per-cell LOQ for soto-p7

Drug-modified $KRAS^{G12C}$ neoantigens quantified by SureQuant on Aurora Ultimate™ 25×75 XT, direct injection, no trap column.

Maso et al., Nature Communications 2025

05 **ABSOLUTE QUANTIFICATION**

8,571 proteins
15 CYP AQUA panel

Deepest published liver S9 proteome: 400 ng, 60 min, Aurora Ultimate™ 25×75, DIA-PASEF, defining the surrogate peptides for LC-MRM.

Singh et al., J Proteome Res 2026

06 **TARGETED THROUGHPUT AT SINGLE-CELL INPUT**

1,080 multiplexed samples per day, zero missing values

shTMTpro-triggered SureQuant on Aurora Rapid™ 8×75 quantified all five histone H3 PTMs in every low-input sample. DDA got one.

Mun et al., J Proteome Res 2026

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Why Aurora Series® columns work for targeted proteomics

The published evidence converges on one explanation: Aurora columns deliver the chromatographic resolution and retention time stability that complex targeted assays demand at the limits of detection. Five design characteristics matter most.

01 Retention time stability is the currency of scheduled acquisition

Every scheduled targeted method trades acquisition window width against the number of concurrent precursors, and therefore against injection time and sensitivity, so narrow windows are only safe if the separation is reproducible. This is borne out in the published evidence. On Aurora Rapid™ 8×150, normalised retention-time error stayed within a tight band across twelve consecutive technical replicates, and Astral to Stellar and Stellar to Stellar alignment held within ±30 s at both 60 and 100 SPD [4]. On Aurora Elite™ 15×75, average peak widths of ~12 seconds supported approximately seven points per peak at a 1.71 s cycle time, with 1.5-minute scheduled windows across 1,070 precursors [5]. In immunopeptidomics, a single unscheduled calibration run was sufficient to schedule acquisition across an entire sample series [10], and in triggered single-cell PTM work the co-elution of the heavy trigger and the endogenous peptide is itself the identification criterion [16].

02 Zero post-column dead volume and the peak shape targeted assays depend on

The Aurora packed-emitter architecture integrates the packed bed directly into the electrospray emitter and uses the nanoZero® fitting, eliminating the pre- and post-column dead volumes that dominate peak broadening in nano- and capillary-flow LC-MS. For targeted work the consequence is direct: narrower peaks concentrate the analyte into fewer, more intense scans, which is what allows a limit of quantification near one copy per cell to be distinguished from a genuine ~14 copies-per-cell measurement [9], epitopes to be resolved above 0.01 fM from roughly 1 µg of immunopeptide [10], and a lowest peptide LOQ of ~10 amol to be reached in plasma [4].

03 One chemistry across discovery and targeted platforms

The fastest published assay translations share one design decision: change the mass spectrometer, keep the column. Aurora Rapid™ 8×150 on Evosep One carried Orbitrap Astral DIA discovery, Stellar DIA-GPF and targeted PRM/MS^3 in the same study [4]. An 8 cm IonOpticks column carried both Astral discovery at 100 SPD and Stellar PRM at 60 SPD without revalidation between platforms [6]. Aurora Elite™ 15×75 generated the gas-phase fractionated library and the scheduled PRM assay built from it [5], and ran dda-PASEF, dia-PASEF and prm-PASEF acquisition in a single forensic workflow [14]. In a three-instrument, two-site comparison, Aurora Ultimate™ 25×75 was the one constant, so differences could be attributed to acquisition method rather than to separation chemistry [15].

04 Depth in discovery defines what the targeted assay can reach

A targeted panel can only contain peptides that discovery has found and characterised. Several of the studies here are, in effect, demonstrations that Aurora columns supply enough depth to populate an assay. From 400 ng loads, 8,571 proteins were quantified across rat and hamster liver S9, and surrogate peptides were selected for a 15-CYP MRM panel [11]. From urinary extracellular vesicle proteins, 5,828 proteins were identified, nearly triple the deepest prior report, and three tumour-specific mutant peptides were taken into a FAIMS-PRM assay [8]. From under 1 µL of serum, 270 alkylation biomarkers across 232 sites were narrowed to a prm-PASEF verification assay [14]. From CSF, a median of 2,135 proteins per sample at 100 SPD was reduced by machine learning to a 22-peptide clinical panel [6].

05 Formats, geometries and source interfaces for every targeted platform

Targeted proteomics is not run on one instrument ecosystem, and the studies here reflect that. The same Aurora chemistry is available in XT (Thermo EASY-Spray / Nanospray Flex), CSI (Bruker CaptiveSpray), XS (SCIEX OptiFlow) and UNI. Published targeted work on Aurora columns spans Orbitrap Fusion Lumos with FAIMS [8], Orbitrap Ascend [9, 15], Exploris 480 [7, 16], Stellar MS [4, 5, 6, 15], timsTOF Pro and Pro 2 [11, 14] and ZenoTOF 8600 via an ACQUITY M-Class [17]. Laboratories running mixed fleets can therefore standardise on a single chemistry from discovery through validation without re-optimising the separation for each source.





The analytical column is not a consumable chosen at the end of method development but the shared reference frame that makes a targeted assay portable.

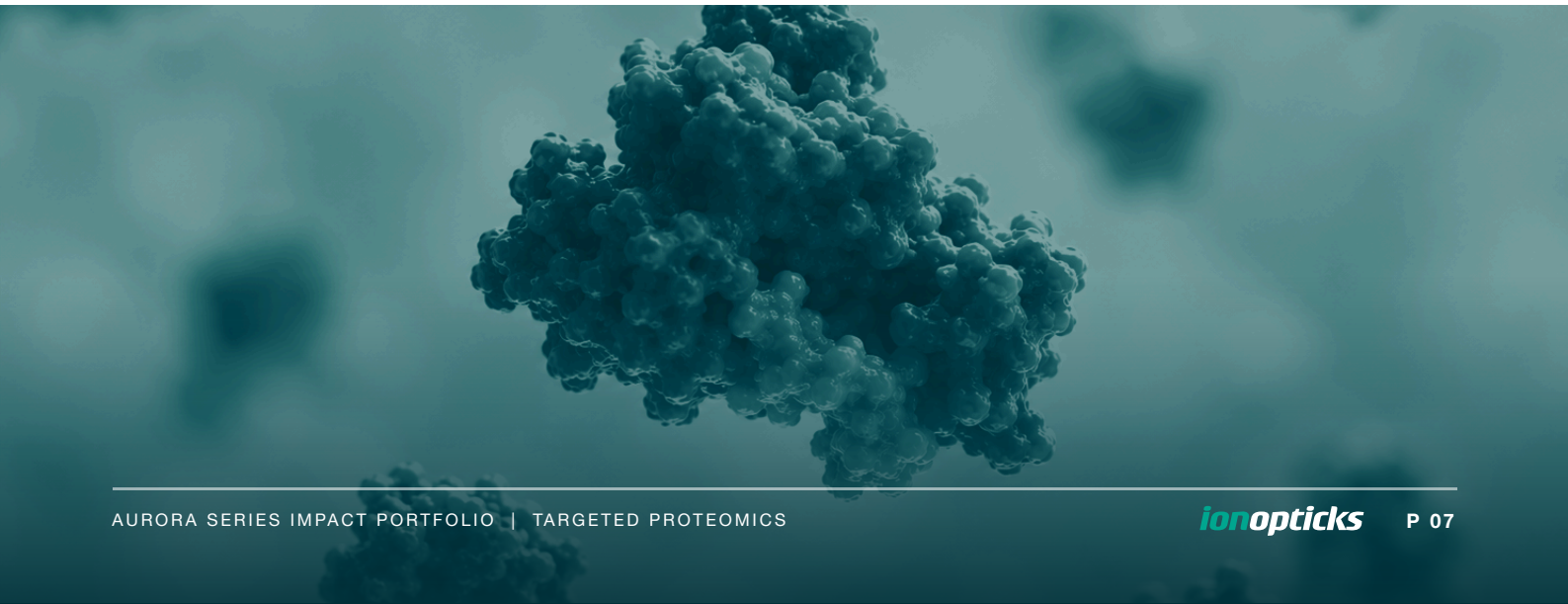
01 The targeted proteomics challenge

Discovery proteomics answers the question of what is present. Targeted proteomics answers the harder question that follows: how much of a particular analyte is present, in this sample and in every other sample in the study, measured the same way each time. Transforming qualitative assessments into robust targeted measurements is what imbues proteomics with true clinical credentials, informing regulatory toxicology, forensic casework, and therapeutic decision-making. However, this is also makes targeted acquisition unforgiving, as selected reaction monitoring (SRM/MRM), parallel reaction monitoring (PRM), prm-PASEF and triggered SureQuant methods commit in advance to a list of precursors and a schedule for measuring them, and anything that moves under the schedule is not recovered by reprocessing the data afterwards.

Over the past fifteen years, the field has defined what a trustworthy targeted measurement requires. The NCI-CPTAC multi-laboratory study showed that multiplexed MRM assays with stable-isotope dilution can be reproduced and transferred across sites and platforms when materials and protocols are standardised [1]. Parallel reaction monitoring on quadrupole-Orbitrap instruments then moved targeted work onto high-resolution, accurate-mass analysers and extended the quantifiable dynamic range in complex matrices [2]. The subsequent tiered, fit-for-purpose framework specified the analytical validation each class of targeted measurement demands, from exploratory research through to clinical use [3]. All of these consensus efforts treat reproducibility as a property of the whole measurement system, of which liquid chromatography is an inseparable part.

The last two years have raised the throughput and scale at which those principles must hold. Hybrid quadrupole/dual-cell linear ion trap instruments now acquire PRM at ~70 Hz and support MS^3, so clinical-grade targeted assays no longer require a trade-off between sensitivity and speed [4]. On the same platforms, gas-phase-fractionated DIA libraries, automated precursor filtering, and real-time retention-time alignment schedule more than a thousand precursors in a single injection [5]. Triggered acquisition has pushed the same discipline further still, to histone PTMs from single-cell input [14] and to HLA peptides present at single-digit copies per cell [9]. Assay development that was measured in months is now measured in days [4, 6].

In every one of these workflows, the chromatography sets the ceiling. Scheduled acquisition windows of 0.75–1.5 minutes only shrink that far if retention times reproduce across injections, across sample batches and, increasingly, across instruments [4, 5]. Measurements at attomole and copies-per-cell level leave little margin for poor peak shape or for dispersion between the column bed and the emitter [9, 10]. Assay transfer from a discovery instrument to a targeted instrument is only rapid if the separation is held constant while the mass spectrometry changes [4, 6, 13]. The analytical column, in other words, is not a consumable chosen at the end of method development but the shared reference frame that makes a targeted assay portable. This application note sets out the peer-reviewed evidence that IonOpticks Aurora Series® columns (Aurora Rapid™, Aurora Elite™, Aurora Ultimate™ and custom Aurora Frontier™ formats) meet that requirement across the full range of targeted proteomics applications.



02

The peer-reviewed evidence base

The studies summarised below span PRM, MRM, prm-PASEF, HRM-DIA and triggered SureQuant acquisition, on Orbitrap Astral, Exploris, Fusion Lumos, Ascend, Stellar MS, timsTOF Pro and Pro 2 and ZenoTOF 8600 platforms, interfaced through Vanquish Neo, UltiMate 3000, nanoElute, ACQUITY M-Class and Evosep One systems. Each cites IonOpticks Aurora Series® columns in its methods, and each has been published in a peer-reviewed journal.



2.1 Building targeted assays: discovery-to-PRM translation

2.2 Clinical biomarker verification in patient cohorts

2.3 Targeted immunopeptidomics and neoantigen quantification

2.4 Absolute quantification for regulated, pharmaceutical and forensic workflows

2.5 Extending the reach of targeted acquisition: multiplexing, PTMs and microproteins

2.1 Building targeted assays: discovery-to-PRM translation

The defining workflow of modern targeted proteomics is the transfer of a discovery list onto a targeted instrument without months of method redevelopment. All three studies below hold the column chemistry constant across the discovery and targeted legs, which is what makes retention-time transfer, and therefore rapid assay translation, tractable.

PAPER 01 · REFERENCE [4]

One column chemistry, two instruments, days instead of months: Aurora Rapid on Evosep One is the chromatographic constant that turns Orbitrap Astral discovery data into a validated, absolutely quantitative Stellar MS clinical assay.

From discovery to clinic in days: IonOpticks Aurora Rapid enables seamless translation of 5,400+ plasma peptides from Orbitrap Astral to Stellar MS targeted assays

Wahle, M., Remes, P. M., Albrecht, V., Baggio Lorenz, M., Mueller-Reif, J., Steigerwald, S., Heymann, T., Niu, L., Lössl, P., Horning, S., Jacob, C. C., & Mann, M. (2025). A Novel Hybrid High-Speed Mass Spectrometer Allows Rapid Translation From Biomarker Candidates to Targeted Clinical Tests Using ^{15}N-Labeled Proteins. *Molecular & Cellular Proteomics*, 24(9), 101050.

> **Study snapshot:** Wahle et al. introduce the Stellar MS, a hybrid quadrupole/dual-cell linear ion trap that performs PRM at ~70 Hz and MS3 at up to 40 Hz, and show that Orbitrap Astral DIA identifications can be moved onto Stellar PRM in a few days, facilitating the rapid development of clinical-grade targeted plasma assays. Discovery and targeted acquisition ran on the same IonOpticks Aurora Rapid 8 cm × 150 µm ID column (Evosep One, 60 SPD / 21 min), allowing retention times to transfer directly between instruments via adaptive RT realignment within ±30 s. The refined assay targeted 1,305 precursors across 410 protein groups at 10.3% median CV, and ^{15}N-labelled full-length protein standards delivered absolute quantification of seven liver disease biomarkers from 0.75 to 240 mg/L at 5.1% median CV.

INSTRUMENTATION USED



Aurora® Rapid™ 8×150
8 cm × 150 µm ID, 1.7 µm C18



Evosep One
60 SPD, 21 min



Orbitrap Astral
DIA discovery



Stellar MS
PRM and MS3 targeting

Translating biomarker panels into validated clinical assays remains the slowest step in plasma proteomics, typically taking months on triple quadrupole instruments restricted to SRM/MRM. Wahle et al. address this using the Stellar MS, which replaces the third quadrupole with a dual-cell linear ion trap so that all fragments of a targeted precursor are captured and scanned out at once. Working from a single-shot Orbitrap Astral DIA dataset of 901 protein groups and 5,437 precursors, they show that essentially all Astral-identified plasma proteins can be targeted on Stellar MS, then use PRM Conductor to filter stepwise through pre-screen and intermediate assays to a single refined method of 1,305 precursors covering 410 protein groups. A parallel Stellar DIA-GPF route yielded 739 precursors across 210 protein groups. Both instruments were coupled to an Evosep One running the same IonOpticks Aurora Rapid 8 cm × 150 µm ID column at 60 SPD, so adaptive RT realignment could reconcile discovery and targeted runs across platforms.

The authors then spiked in seven ^{15}N-labelled full-length protein standards from an alcohol-related liver disease panel, achieving >99% isotope incorporation and absolute quantification across a 0.75–240 mg/L range with a lowest peptide LOQ of 1.02×10^{-5} pmol (~10 amol). Whole-protein standards also allowed proteoform-level inference, including ITIH4 chain-specific quantification and glycosylation occupancy. This work establishes a robust pipeline for translating biomarker discovery data into targeted clinical assays with absolute quantification, providing clinical laboratories with a validated approach for protein biomarker verification.

WHY THIS PAPER MATTERS

- **Assay development compressed to days.** A single-shot Astral DIA list (901 protein groups, 5,437 precursors) became a refined 1,305-precursor targeted assay (410 proteins) after pre-screen iterations, with adaptive RT realignment reconciling retention times between Orbitrap Astral and Stellar MS to within ±30 s and allowing scheduled acquisition windows of ~1 min or less without losing targets to chromatographic drift.
- **Clinical-grade precision at scale.** The refined Astral-designed assay reached 10.3% median CV with 75% of precursors below 20%, and the Stellar-designed assay 8.8% with 85% below 20%. Astral discovery DIA achieved 15.0% median CV, and targeting the 5,347 precursor list on Stellar raised this to about 20%. MS3 targeting on the dual ion trap further improved detectability for some longer precursors, and can roughly halve their LOQs, however the overall LOD/LOQ stayed similar and the seven-protein quantification used MS2.
- **^{15}N-labelled proteins enable absolute quantification and proteoform readouts.** Seven liver disease biomarkers (SERPINC1/ANT3, QSOX1, TTR/TTHY, ALDOB, ITIH4, ATRN, SELENOP/SEPP1) were quantified from 32 heavy/light peptide pairs at 5.1% median CV, with every precursor below 20%. Because the standards are intact proteins, missed cleavages, processing, and PTM occupancy are visible: ITIH4's 30 kDa and 70 kDa chains were quantified separately, and the missing endogenous peptide IEETTMTTQTPAPIQAPSAILPLPGQSVER pointed to near-complete glycosylation at that site.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **One chromatographic constant across two instruments.** The Aurora Rapid 8×150 column on Evosep One at 60 SPD (21 min) ran Orbitrap Astral DIA discovery, Stellar DIA-GPF, and targeted PRM/MS3 assays. Matched column chemistry is what makes cross-platform retention time transfer, and therefore rapid assay translation, tractable.
- **Retention time reproducibility for adaptive realignment.** Normalised retention time error stayed within a tight band across 12 consecutive technical replicates per gradient, and Astral to Stellar and Stellar to Stellar alignment stayed within ±30 s at 60 SPD and 100 SPD which is the stability the Adaptive RT scheme depends on.
- **Sensitivity at low load and high throughput.** The 150 µm ID column format took 250–500 ng of plasma digest directly from Evotips with no offline fractionation. 1 ng-per-protein ^{15}N spike-ins on the 60 SPD method still gave 14.5 data points per peak across 112 targeted precursors at 7.9% median CV on the heavy channel. A 5 cm × 75 µm Aurora Rapid on the Evosep Whisper Zoom 80 SPD method supported the low-load DDA assessment of labelling efficiency.

PAPER 02 · REFERENCE [5]

Scaling targeted validation to over 1,000 precursors in cerebrospinal fluid demands chromatographic precision that holds across every injection. The IonOpticks Aurora Elite 15×75 column delivers the retention time stability and peak resolution needed to unlock the Stellar MS as a high-plex PRM platform for clinical biomarker verification.

1,070 precursors, one injection, real disease biology: Stellar MS PRM on an IonOpticks Aurora Elite 15×75 C18 UHPLC column resolves TAU, APOE-ε4 and 18 CSF proteins altered in Alzheimer's disease CSF

Plubell, D. L., Remes, P. M., Wu, C. C., Jacob, C. C., Merrihew, G. E., Hsu, C., Shulman, N., MacLean, B. X., Heil, L., Poston, K. L., Montine, T. J., & MacCoss, M. J. (2026). Development of Highly Multiplex Targeted Proteomics Assays in Biofluids Using a Nominal Mass Ion Trap Mass Spectrometer. *Molecular & Cellular Proteomics*, 25(2), 101506.

Study snapshot: This study demonstrates scalable targeted proteomics using scheduled PRM assays built from gas-phase fractionated DIA libraries with PRM Conductor and executed on a Stellar MS with Adaptive RT real-time alignment. A nominal-mass linear ion trap matched Orbitrap DIA quantitatively on matrix-matched calibration curves (median LOQ 9.7% versus 8.5% human dilution) with superior precision at low concentrations. Using an IonOpticks Aurora Elite 15×75 C18 UHPLC column, the MacCoss lab acquired 1,070 precursors from 101 proteins in the CSF neurodegenerative disease panel, identifying 29 peptides across 18 proteins significantly altered in Alzheimer's dementia, including TAU and the APOE-ε4 isoform-specific peptide LGADMEDVR, whose abundance tracked participant genotype. The same overall workflow (gas-phase fractionated DIA libraries, PRM Conductor, and Adaptive RT) scaled to 3,501 precursors in plasma extracellular vesicle survey assays, demonstrating multiplexing capacity across more than a threefold range.

INSTRUMENTATION USED



Aurora® Elite™ 15×75
15 cm × 75 µm ID, 1.7 µm C18



Vanquish Neo
UHPLC



Stellar MS
Scheduled PRM, Adaptive RT

Targeted assays are meant to carry discovery proteomics into clinical measurement, but they have not scaled effectively. Monitoring each target for two to five minutes when peptides elute in under 20 seconds wastes most of the duty cycle, and the number of concurrent precursors rises fast enough to starve each target of injection time. Plubell et al. loosen this constraint through three technical advances. Gas-phase fractionated DIA libraries, acquired in the sample matrix itself, identify which peptides are measurable, how their fragments behave, and when they elute. PRM Conductor filters precursors on peak width, absolute area, and interference-free transitions, then distributes them optimally across the gradient. Adaptive RT compares embedded reference DIA spectra with live acquisition five times per peak width to reschedule targets in real time, allowing acquisition windows to shrink to 0.75–1.5 minutes without losing peaks to chromatographic drift. On a Vanquish Neo and an IonOpticks Aurora 15×75 C18 column, they constructed a focussed 1,070 precursor PRM assay (maximum 62 concurrent precursors, approximately seven points across each peak) targeting 101 neurodegenerative-disease-associated proteins in CSF. The low concurrent load enabled longer ion injection times and high quantitative precision, supporting reliable measurement of APOE isoforms and other disease-linked peptides in Alzheimer's samples. The same instrument architecture also supported much larger survey-style assays of more than 2,000 precursors.

Across these experiments the authors demonstrate that reducing the number of concurrent precursors directly improves precision at low analyte concentrations, which is exactly the performance required when translating discovery findings into clinical assays. In a plasma extracellular-vesicle survey, 671 peptides from 315 protein groups distinguished Parkinson's from non-Parkinson's participants, illustrating the dual utility of hypothesis-led panels and hypothesis-free surveys on the Stellar platform.

WHY THIS PAPER MATTERS

- **Multiplex capacity on nominal-mass hardware.** GPF-DIA library generation, PRM Conductor filtering and Adaptive RT scheduling enable >2,000 precursors per injection on a nominal-mass ion trap. On a matrix-matched CSF calibration curve, Stellar LIT PRM achieved median limit of quantification of 9.7% human dilution versus 8.5% for Exploris 480 Orbitrap DIA, with better reproducibility at the dilute end, representing quantitative parity from far cheaper hardware.
- **A counter-intuitive result on where the library should be built.** The Orbitrap-derived GPF library was substantially larger, but the assay built from the Stellar's own nominal-mass GPF data reproduced better at low concentrations. Peptides selected at high resolution do not always transfer well to unit resolution. Practically, this means that the library should be built on the instrument that will run the assay, with matched chromatography where possible.
- **The multiplexing–precision trade-off, quantified.** For Mag-Net enriched plasma EVs, a 3,501-precursor assay (174 concurrent) exceeded 20% median CV by the 10% dilution, while a 1,599-precursor subset (~92-98 concurrent) maintained precision below 20% until the 1% dilution point. Fewer concurrent precursors enable longer injection times and more ions per measurement. Assay size is a design variable to be tuned to the biological question, not maximised.
- **Clinical translation for neurodegenerative disease.** Applied to CSF and Mag-Net enriched plasma EVs, the assays detect known Alzheimer's- and Parkinson's-associated proteins (TAU, APOE-ε4, TERA/VCP, CD36, RAB11, G3P/GAPDH, 1433E/YWHAE) with tight ratio distributions, enabling biomarker monitoring without prior hypotheses.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Matched library and assay on one column.** An IonOpticks Aurora 15×75 C18 UHPLC column on a Vanquish Neo UHPLC ran both the 60-minute, 2 Th window GPF DIA library (300–1100 m/z) and the scheduled PRM acquisition for the 101-protein neurodegenerative panel, ensuring matched chromatography between library generation and targeted measurement.
- **Peak shape that supports dense sampling.** Average peak widths of ~12 seconds enabled approximately seven points per peak at a 1.71 s cycle time across 1,070 precursors from 101 proteins (maximum 62 concurrent precursors, 1.5-minute scheduled windows). This retention-time stability is sufficient for Adaptive RT to keep windows narrow.
- **Delivered the measurements that carried the biology.** On this column, the assay identified peptides from 18 proteins significantly altered in Alzheimer's dementia, including TAU, ALDOA and KPYM. The APOE-ε4 isoform-specific peptide LGADMEDVR showed the largest fold change and tracked participant genotype (0,1 or 2 ε4 alleles), demonstrating single-amino-acid variant discrimination directly from crude CSF digests.

PAPER 03 · REFERENCE [6]

One column. Two platforms. Zero revalidation. The IonOpticks 8 cm bridges Astral discovery (100 SPD) and Stellar clinical deployment (60 SPD) with the retention-time stability that cuts assay transfer from months to days.

Discovery to clinical assay in a single study: 2,100 CSF proteins per sample at 100 SPD on an 8 cm IonOpticks column, translated to a 22-protein targeted panel that outperforms established Multiple Sclerosis markers

Bader, J. M., Makarov, C., Richter, S., Strauss, M. T., Held, F., Wahle, M., Lorenz, M. B., Pöschl, L., Skowronek, P., Thielert, M., Berthele, A., Zeng, W.-F., Ammar, C., Bludau, I., Schubert, B., Theis, F. J., Gasperi, C., Hemmer, B., & Mann, M. (2026). Large-scale Proteomics Across Neurological Disorders Uncovers Biomarker Panel and Targets in Multiple Sclerosis. *Cell*, 189, 2128–2147.

Study snapshot: 5,045 cerebrospinal fluid samples spanning the neurological disease spectrum were profiled by DIA, with ~2,600 re-measured on an Orbitrap Astral at 100 SPD (median 2,135 proteins per sample) using an 8 cm IonOpticks column on Evosep One. Machine learning reduced the proteome to a 30-feature panel that outperformed the established clinical CSF marker set by 0.1–0.2 ROC AUC in oligoclonal band-negative multiple sclerosis. The panel was then transferred to a 22-peptide Stellar PRM/MS3 targeted assay on the same 8 cm IonOpticks chemistry at 60 SPD, adding a further 0.02–0.1 ROC AUC and replicating in an independent 616-sample cohort.

INSTRUMENTATION USED



IonOpticks 8 cm column
8 cm, 60 and 100 SPD



Evosep One
100 SPD and 60 SPD



Orbitrap Astral
DIA discovery



Stellar MS
PRM/MS3 targeted panel

Cerebrospinal fluid is the mainstay of neurological diagnostics, yet multiple sclerosis is still misdiagnosed in roughly 1 in 7 people and underdiagnosed in about 1 in 3. Oligoclonal bands, which are central to the McDonald criteria, are absent in roughly 10% of MS cases, and this group has the highest misdiagnosis rate and experiences the longest delay in commencing clinical intervention. Bader et al. profiled 5,045 cross-sectional samples across autoimmune, neurodegenerative, infectious, neoplastic and vascular disease, alongside neurological controls. Roughly 2,600 samples were re-measured on an Orbitrap Astral coupled to an Evosep One using an 8 cm IonOpticks column, lifting coverage to approximately 2,100 proteins per sample at 100 samples per day and making protein identification largely independent of blood-CSF barrier impairment, which is the main source of CSF proteome variation. Machine learning distilled the proteome to a 30-feature panel that matched established clinical markers across all OCB statuses and outperformed them by 0.1–0.2 ROC AUC in the diagnostically challenging OCB-negative subgroup. 29 panel proteins were then transferred to a targeted PRM assay on a Stellar mass spectrometer, again using an Evosep One and an 8 cm IonOpticks column (21-minute gradient, 60 samples per day) with 20 fmol isotope-labelled standards. Filtering on workflow CV pruned the assay to 22 peptides and 22 proteins, and the improved quantification added a further 0.02–0.1 ROC AUC to the panel's lead over established clinical markers, with performance confirmed in an independent 616-sample replication cohort.

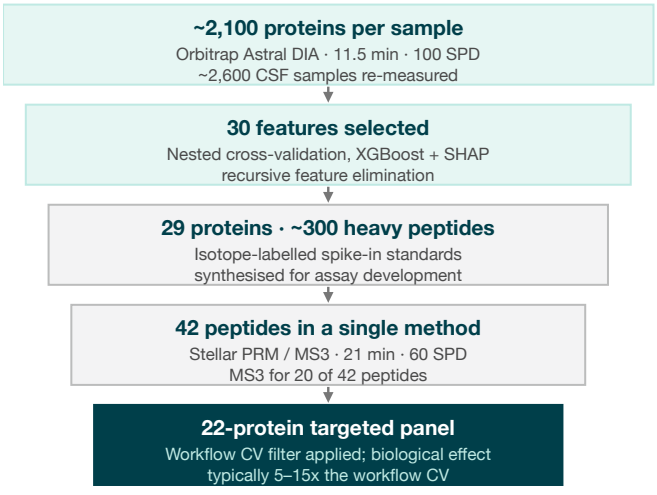
WHY THIS PAPER MATTERS

- **Scale that resolves the confounders.** An order of magnitude beyond typical CSF studies, analysis of the 5,045 sample cohort revealed blood-CSF barrier impairment as the dominant proteome confounder, explaining roughly three times more variance than clinical diagnosis, with proteome-derived substitutes validated for studies lacking albumin ratios.
- **A usable test for OCB-negative Multiple Sclerosis.** The 22-protein panel achieved superior diagnostic performance (AUC ~0.85) compared to established clinical markers in OCB-negative MS cases, addressing the 10% of MS patients who experience double the overall misdiagnosis rate.
- **Complete discovery-to-routine translation in one study.** The diagnostic panel was successfully converted to a targeted PRM assay on the Stellar mass spectrometer with heavy isotope-labeled spike-in standards, signalling readiness for bridging the discovery-to-clinic gap for routine laboratory implementation.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Clinical-depth proteomics at speed.** On Orbitrap Astral + Evosep One, the 8 cm IonOpticks column delivered ~2,100 proteins per CSF sample (median 2,135) at 100 samples per day. With ~1,000 proteins at workflow CV <20%, this is roughly twice the quantitative depth of neat plasma workflows, and provides sufficient coverage for machine learning biomarker discovery.
- **Depth that holds up in a difficult matrix.** On the timsTOF Pro 2, protein identifications fell sharply with increasing BCB impairment (QAlb). The Orbitrap Astral workflow using the 8 cm IonOpticks column flattened this dependence by ~2.3-fold (slope –127.1 to –55.6), making coverage largely independent of barrier status and removing the single largest confounder in CSF proteomics.
- **One column chemistry, two platforms.** The same 8 cm IonOpticks format carried both discovery (Astral, 100 SPD, 11.5 minute gradients) and targeted assays (Stellar, 60 SPD, 21-minute gradients). Stable retention times enabled 1.5–3 minute targeting windows, fitting all 42 peptides into a single method without revalidation between platforms.
- **Assay robustness when sample is scarce.** Biological variation across donors was 2–15× the workflow CV, enabling panel pruning to 22 high-confidence peptides even at 30% or 60% reduced peptide input (7 µL and 4 µL vs. 10 µL standard). This provides an inherent robustness advantage over single-analyte markers especially when CSF sample volume is limited.

A Discovery-to-targeted funnel on one 8 cm IonOpticks column



B ROC AUC gain over established clinical CSF markers

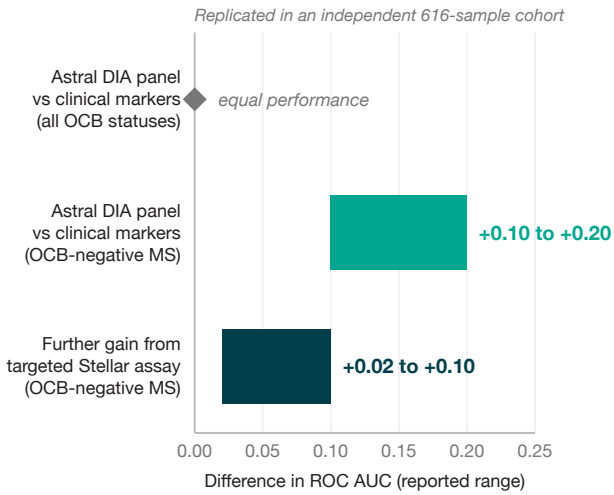


Figure 1. Discovery to targeted assay on one 8 cm IonOpticks column. Panel A - ~2,100 proteins per sample on the Orbitrap Astral at 100 SPD, narrowed to a 22-protein Stellar PRM panel at 60 SPD. Panel B - Targeting adds 0.02–0.1 ROC AUC to a panel that already outperforms established clinical CSF markers by 0.1–0.2 ROC AUC in OCB-negative MS.

Constructed from values reported in Bader et al., Cell, 2026; 189:2128–2147, https://doi.org/10.1016/j.cell.2026.01.017



Discovery on one mass spectrometer, targeted assays on another, and the same Aurora column throughout. Keeping the column constant is what let published assays move from Orbitrap Astral to Stellar MS without revalidating the separation.

2.2 Clinical biomarker verification in patient cohorts

Verification is where candidate lists meet real cohorts, orthogonal platforms and longitudinal sampling. These studies take targeted panels into independent validation cohorts and into patient follow-up, and benchmark them against established clinical assays.

PAPER 04 · REFERENCE [7]

A targeted panel is only as good as the chromatography it is built upon. The IonOpticks Aurora Elite 15×75 column powered every validation-phase acquisition in this study, turning 102 discovery candidates into a seven-protein signature that replicated in an independent cohort and matched ELISA on every overlapping marker.

Targeted HRM-DIA on Aurora Elite column validates seven-protein plasma signature for sarcopenia progression across two independent cohorts

Kong, S. H., Jeon, O. H., Kim, J. Y., Kim, M., Kim, J., Park, S. S., Chang, H. C., Won, C. W., & Han, D. (2026). Distinct Proteomic Signatures Driving Progression of Sarcopenia: A Longitudinal Multicohort Study. *Journal of Cachexia, Sarcopenia and Muscle*, 17(2), e70240.

> **Study snapshot:** Longitudinal multicohort study tracking sarcopenia progression in 171 community-dwelling older adults (discovery) and 93 participants (validation). Untargeted DIA profiling identified candidate signatures, followed by targeted HRM-DIA quantification of 31 proteins against spiked-in heavy stable-isotope-labelled reference peptides using an IonOpticks Aurora Elite 15×75 column on an UltiMate 3000 RSLC coupled to an Orbitrap Exploris 480. Seven signatures (LRG1, CST3, TIMP1, C2, ITIH1, AMBP and LYZ) replicated with consistent directionality, showing orthogonal ELISA agreement of r = 0.74–0.86 across six overlapping proteins and establishing a robust, reproducible panel for early muscle decline detection.

INSTRUMENTATION USED



Aurora® Elite™ 15×75
15 cm × 75 µm ID, 780 nL/min



UltiMate 3000 RSLC
Thermo Scientific



Orbitrap Exploris 480
HRM-DIA

Sarcopenia biomarker discovery has been held back by cross-sectional designs and single-cohort analyses that rarely survive independent replication. Kong et al. address both limitations with a two-phase longitudinal study. In the discovery phase, plasma from 171 participants in the Korean Frailty and Aging Cohort Study was profiled by data-independent acquisition and stratified by sarcopenia trajectory over two years, comprising stable nonsarcopenic (n = 65), newly incident (n = 63) and persistently sarcopenic (n = 43) cohorts. This yielded 102 differentially expressed proteins, with APOA1 falling sharply in participants who developed sarcopenia (fold change −1.42, $p < 0.001$) and B2M remaining elevated in those who stayed sarcopenic (+1.58, $p < 0.001$). Validation moved to an independent 93-participant OsteoSarc cohort. Hyper Reaction Monitoring DIA on an IonOpticks Aurora Elite 15×75 C18 UHPLC column coupled to an Orbitrap Exploris 480 via an UltiMate 3000 RSLC system delivered the retention time stability needed for reproducible quantification of 31 proteins against spiked-in heavy stable-isotope-labelled reference peptides. A further 21 markers were measured by immunoassay, and the six proteins common to both platforms agreed at r = 0.74–0.86. Seven proteins (LRG1, CST3, TIMP1, C2, ITIH1, AMBP and LYZ) held up with consistent direction and significance in both cohorts, and pathway enrichment converged on LXR/RXR signalling, acute-phase response and complement cascade activation. This work established a compact, orthogonally corroborated panel with a clear route into targeted clinical assay development.

WHY THIS PAPER MATTERS

- **Longitudinal design with genuine external validation.** Two-year follow-up in the KFACS discovery cohort (n = 171) was replicated in the clinically distinct OsteoSarc cohort (n = 93), directly addressing the reproducibility gap that the authors identified in earlier sarcopenia biomarker work. Follow-up duration was closely matched between cohorts (24.0 vs 23.8 months, p = 0.481).
- **A discovery-to-targeted funnel that narrows cleanly.** 102 differentially expressed proteins ($p < 0.05$) were reduced to a 31-protein SIL-peptide panel for HRM-DIA, of which seven kept the same direction and significance in both cohorts. LRG1 and TIMP1 correlated negatively with muscle strength, CST3 positively with physical performance, and C2 negatively with muscle mass.
- **Mechanistic convergence rather than a protein list.** LXR/RXR activation, acute-phase response signalling, and complement cascade activation were enriched in both cohorts, pointing to a shared lipid–immune axis in muscle decline and offering tractable targets for follow-up assays.
- **Orthogonal platform agreement.** Targeted MS and immunoassay measurements of six overlapping proteins (APOA1, APOA2, APOB, APOE, C3 and C4) correlated at r = 0.74–0.86, establishing that MS-based targeted panels can stand alongside established ELISA readouts in clinical cohort work.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Sole LC platform for the targeted validation leg.** IonOpticks Aurora Elite 15×75 column on an UltiMate 3000 RSLC coupled to an Orbitrap Exploris 480 carried the entire HRM-DIA phase in the 93-participant validation cohort. Both the 31-protein targeted quantification (using spiked-in heavy SIL standards) and the untargeted proteome profiles were extracted from the same acquisitions, demonstrating the column's suitability for multiplexed, multi-modal analyses.
- **Throughput suited to a clinical validation set.** The Aurora Elite 15×75 column, operated with 780 nL/min flow rate supported rapid 30-minute gradients (8–30% B), enabling efficient analysis of the validation cohort while maintaining reproducibility for longitudinal biomarker verification.
- **Retention-time stability enabled library-free targeted extraction.** Spectronaut v18 generated the direct-DIA library against the UniProt human database using iRT standard peptides, with quantification normalised via cross-run normalisation. The success of this workflow is dependent upon the consistent retention-time behaviour delivered by the Aurora Elite column.

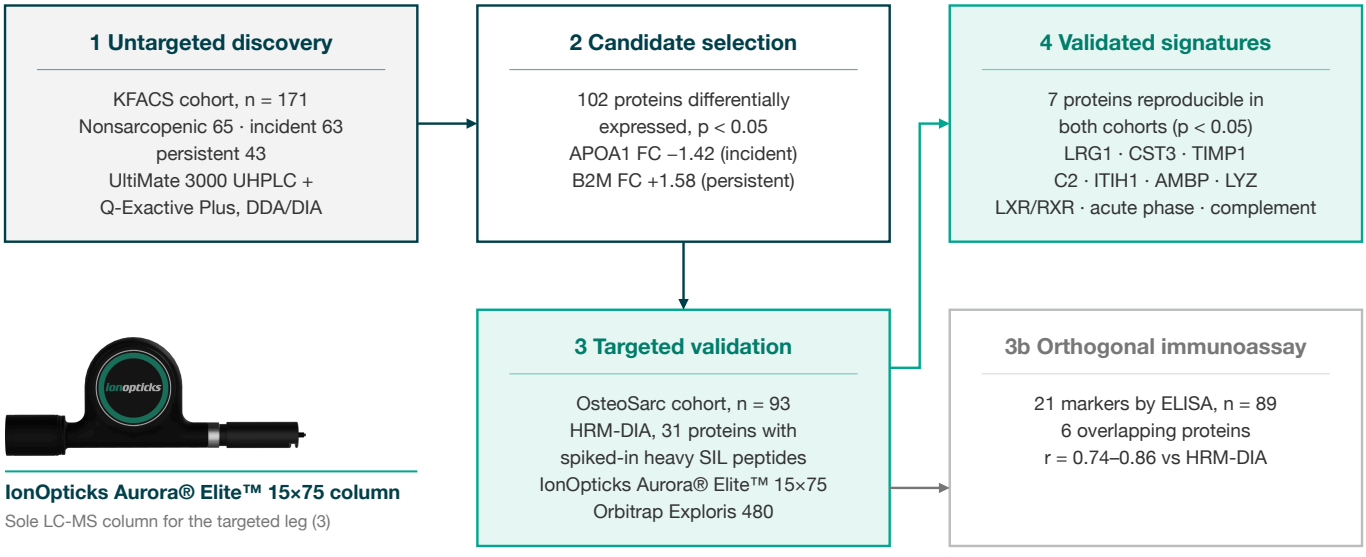


Figure 2. From untargeted discovery to validated biomarkers for sarcopenia progression. Panel 1 - Untargeted DIA-MS profiling of 171 participants (KFACS cohort) identified 102 differentially expressed proteins across three trajectory groups. Panel 2 - Candidate selection narrowed these to key markers including APOA1 (downregulated in incident cases) and B2M (upregulated in persistent cases). Panel 3 - Targeted HRM-DIA validation on IonOpticks Aurora Elite column (15 cm × 75 µm) with heavy SIL peptide standards quantified 31 proteins in an independent 93-participant cohort (OsteoSarc). Panel 3b - Orthogonal ELISA validation (21 markers, n=89) confirmed quantitative consistency (Pearson r=0.74–0.86). Panel 4 - Seven reproducible protein signatures (LRG1, CST3, TIMP1, C2, ITIH1, AMBP, LYZ) mapped to LXR/RXR signalling, acute-phase response, and complement cascade pathways.

Figure prepared from data reported in Kong et al., Journal of Cachexia, Sarcopenia and Muscle, 2026; 17:e70240, https://doi.org/10.1002/jcsm.70240

PAPER 05 · REFERENCE [8]

Proteogenomic profiling of urinary EVs achieved 5,828 protein identifications with absolute quantification of tumor-specific mutant proteins by Aurora-enabled PRM, delivering a $50, 5-hour liquid biopsy that tracks cystoscopic tumor burden with 100% cancer specificity.

Deep enough to find them, targeted enough to track them: Aurora Ultimate 25×75 maps 5,828 urinary EV proteins, nearly triple the prior depth, and monitors three tumour-specific mutant peptides in one PRM assay

Hakozaki, Y., Sugimoto, K., Yamada, Y., Danno, T., Hashimoto, K., Shinchi, H., Kume, H., & Ueda, K. (2026). Proteogenomic detection of tumor-specific somatic mutant proteins in urinary extracellular vesicles for non-invasive monitoring of bladder cancer. *Frontiers in Oncology*, 16, 1808671.

Study snapshot: Non-invasive bladder cancer monitoring is enabled by the first absolute quantification of tumor-specific mutant proteins in urinary extracellular vesicles. Using whole-exome sequencing in five patients, deep LC-MS on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column with FAIMS on an Orbitrap Fusion Lumos identified 11,207 proteins in tumor tissue and 5,828 in urinary EVs, roughly 3× deeper than previous reports. Of 47 protein-level variants, four in urinary EVs including three (LCP1_D321H, TKT_K102N, PLCD1_R639H) were quantified by FAIMS-PRM with heavy-labelled standards, decreasing post-resection and stable across 24 months recurrence-free, with an Aurora Elite 15×75 C18 UHPLC column supporting DIA profiling.

INSTRUMENTATION USED



Aurora® Ultimate™ 25×75
25 cm × 75 µm ID, 200 nL/min



Aurora® Elite™ 15×75
15 cm × 75 µm ID, 350 nL/min



Vanquish Neo
Nanoflow LC



Orbitrap Fusion Lumos
FAIMS, discovery and PRM



Orbitrap Exploris 240
Label-free DIA time course

Bladder cancer has the highest recurrence rate of any solid tumour, and surveillance currently means repeat cystoscopy every few months, which is invasive, costly, and part of a roughly $4 billion annual care burden, while urine cytology is non-invasive but only about 48% sensitive. Hakozaki et al. show that tumour-specific mutant proteins can be measured in urinary extracellular vesicles, giving a cost-effective, antibody-free approach that complements ctDNA with direct protein-level validation. This approach targets mutant proteins encoded by a patient's own somatic mutations, which are in principle absolutely cancer-specific because they come from a patient's own somatic mutations, but in practice they have been almost invisible as antibodies rarely distinguish a single amino-acid change, and public sequence databases often do not contain that patient's variants. This challenge is addressed by whole-exome sequencing each patient's tumour and buffy coat, translating the calls with Neoantimon and appending the resulting mutant peptide entries to the human reference proteome, one personalised database per patient. These customised databases enable detection of the mutant proteins in extracellular vesicles, which package even non-secreted intracellular proteins and carry them into urine. Deep LC-MS profiling of tumour tissue, cultured tissue-derived EVs and urinary EVs from five patients used an Orbitrap Fusion Lumos with FAIMS and Vanquish Neo nanoflow LC, with separations on IonOpticks Aurora Ultimate 25×75 C18 UHPLC columns at 200 nL/min for discovery and PRM, and Aurora Elite 15×75 C18 UHPLC columns at 350 nL/min for complementary DIA on an Orbitrap Exploris 240. This work confirmed 47 variant sequences at the protein level, 39 in tissue, 32 in tissue-derived EVs and 4 in urinary EVs. Three urinary EV mutant peptides (LCP1_D321H, TKT_K102N, PLCD1_R639H) were then quantified in a targeted PRM assay with stable isotope-labelled standards at tens to low hundreds of attomoles per 0.1 mL of urine. All three dropped immediately after transurethral resection and stayed low during two years of follow-up, matching negative cystoscopic findings whereas complement factor H, the antigen in the current approved BTA assay, was present in the same EVs but did not track tumour status.

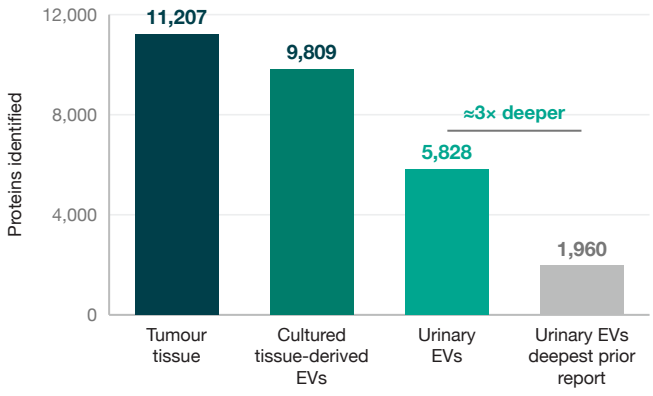
WHY THIS PAPER MATTERS

- **First direct evidence of somatic mutant proteins in urinary EVs, enabled by a personalised, per patient database.** An earlier proteogenomic study that searched a database pooled from 86 colorectal cases detected mutant proteins in only 12% of non-hypermutated tumours. In this work, mutant proteins were identified in four of five bladder cancer tissues, attributed to the lower false-positive burden of an individualised search space, with every urinary EV mutant peptide separately confirmed against a synthetic standard spectrum.
- **No reliance on public mutation catalogs.** Whole-exome sequencing (WES) of each tumour and matched buffy coat was translated with Neoantimon and appended to Swiss-Prot, so only variants that a patient actually carries enter the search rather than relying on public databases that may contain irrelevant variants.
- **The targeted assay tracked tumour burden where an approved marker did not.** Absolute quantification by PRM showed all three mutant peptides fell sharply after resection and remained flat across 24 months of cystoscopy- and cytology-confirmed, recurrence-free follow-up (patient BC4). In the same time-series samples, complement factor H, the principal target of the BTA assay, was detectable but showed no consistent pre- to post-operative change.
- **A cost and throughput argument against ctDNA panels.** At an estimated $50 for this assay ($40 for EV extraction plus $10 for protein analysis), the workflow takes roughly five hours end to end, versus $481–2,630 and about 8 hours for a 74-mutation ctDNA panel. Crucially, extra PRM targets do not raise the per-sample cost, unlike antibody-based single-EV methods that need a specific antibody for every substitution.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Depth is what made the mutant proteins visible.** An Aurora Ultimate 25×75 column at 200 nL/min over a 115-minute gradient, with FAIMS on an Orbitrap Fusion Lumos, identified 5,828 urinary EV proteins against 1,960 in the deepest prior urinary EV proteome, and 11,207 proteins from five tumour tissues against 7,526 from 86 tissues in the earlier proteogenomic study. Single mutant peptides sit far down the abundance range, as without that depth there is nothing to hand to a targeted assay.
- **Two column lengths for two jobs.** The Aurora Ultimate 25×75 column carried the discovery work, while an Aurora Elite 15×75 column at 350 nL/min over a 60-minute gradient on an Orbitrap Exploris 240 handled the DIA label-free time-course profiling across eight timepoints from 1 mL of urine, trading maximum depth for throughput with a shorter gradient and higher flow.
- **The same LC-MS stack ran the targeted assay.** The PRM work ran on the same Orbitrap Fusion Lumos, FAIMS Pro, and Vanquish Neo configuration as the discovery experiments. The resulting assay is compact and fully specified: six precursors, three endogenous mutant peptides plus their heavy counterparts, at a normalised collision energy of 30 V, each with optimised FAIMS compensation voltages (approximately spanning -42 to -64 V), delivering attomole-level sensitivity from a single injection.

A Proteome depth on the Aurora 25×75 with FAIMS



B From personalised exome to a single targeted assay

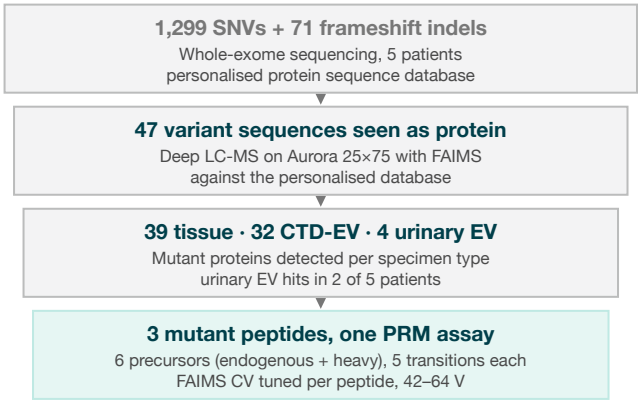


Figure 3. From deep proteome profiling to a single targeted assay. Panel A - Proteome depth achieved on the Aurora Ultimate 25×75 with FAIMS: 5,828 proteins identified in urinary EVs, nearly triple the deepest prior report (1,960). Panel B - The filtering workflow for candidate biomarkers: 1,370 exome variants (1,299 SNVs + 71 frameshift indels) from five patients were narrowed by deep LC-MS to 47 variant proteins, then to four mutant proteins detected in urinary EVs (from two of five patients), and ultimately to a three-peptide PRM assay for longitudinal monitoring.
Constructed from values reported in Hakozaki et al., Frontiers in Oncology, 2026; 16:1808671, https://doi.org/10.3389/fonc.2026.1808671

2.3 Targeted immunopeptidomics and neoantigen quantification

HLA-bound peptides are non-tryptic, recovered in vanishingly small amounts and often present at single-digit copies per cell. In both studies below it was the absolute copy numbers from targeted acquisition, rather than the discovery screen, that determined which epitope entered the therapeutic programme.

PAPER 06 · REFERENCE [9]

When a therapeutic programme turns on whether a neoantigen sits at one copy per cell or fourteen, the chromatography has to be good enough that the answer is the biology and not the separation. The Aurora® Ultimate™ XT delivered every one of those measurements.

Counting a cancer target at one copy per cell: Aurora Ultimate XT enables immunopeptidomics-guided development of a drug-modified KRAS^{G12C} T cell engager

Maso, L., Mosure, S. A., Rodriguez-Aponte, S. A., Pizzo, A., Mensah, D. N., Southard, M., Sze, S., Ahmed, T., Vash, B., Hattori, T., Rajak, E., Koide, A., Neel, B. G., Koide, S., Liu, W., Toenjes, S. T., Da Silva Jardine, P., Chopra, R., Rader, C., & Stopfer, L. E. (2025). Engineered Antibodies That Stabilize Drug-Modified KRAS^{G12C} Neoantigens Enable Selective and Potent Cross-HLA Immunotherapy. *Nature Communications*, 16, 11264.

Study snapshot: Covalent KRAS^{G12C} inhibitors leave a drug adduct on the oncoprotein that is processed and presented on HLA as a synthetic neoantigen. Maso et al. use targeted immunopeptidomics to detect and absolutely quantify these hapten-peptides for the first time, establishing a limit of quantification of about one copy per cell and showing they sit at roughly 10 copies per cell with short complex half-lives, far below what conventional pMHC targeting can reach. An IonOpticks Aurora Ultimate 25×75 XT C18 UHPLC column delivered the chromatographic sensitivity for these single-copy measurements, driving the engineering of affinity-matured T cell engagers. AETX-R114 binds sotorasib-modified peptide at 7 pM on HLA-A*03:01, extends p*MHC half-life beyond 24 h, and raises surface density in NCI-H2122 cells from below 1 to around 14 copies per cell, which is enough to drive selective killing in vitro and tumour regression in vivo. AETX-R302 extends this approach to divarasib-modified peptides across A2 and A3 supertypes for broad patient coverage.

INSTRUMENTATION USED



Aurora® Ultimate™ XT 25×75
25 cm × 75 µm ID, 55 °C



Vanquish Neo
Direct injection



Orbitrap Ascend Tribrid
DDA and SureQuant

The hard part of neoantigen immunotherapy is not finding a mutation, it is proving that the resulting peptide reaches the cell surface in enough copies to be worth targeting. KRAS^{G12C} has been especially intractable because the reactive cysteine picks up heterogeneous thiol adducts, and conventional MS workflows cleave those adducts to enable detection, which destroys the modification state that defines the hapten. Maso et al. built a targeted immunopeptidomics assay around that problem on a Vanquish Neo and an IonOpticks Aurora Ultimate 25×75 XT C18 UHPLC column, employing paired stable isotope-labelled standards to trigger acquisition of the endogenous target and calibrate copies-per-cell quantification. This gave direct evidence that sotorasib-, adagrasib- and divarasib-modified KRAS^{G12C} peptides are genuinely presented, detectable from two hours of drug treatment and peaking at 24–48 h. Haptenated p7 appeared at 10 copies per cell against 89 for the unmodified peptide, and all three drug-peptide MHC complexes had shorter half-lives than wild-type p7. Rather than abandon the target, the team affinity-matured an antibody whose slow off-rate stabilises the complex and raises its own antigen density. The resulting T cell engager, AETX-R114, killed sotorasib-resistant lines with EC50 values of 310 pM to 2.1 nM and significantly inhibited tumour growth in a co-mixed xenograft model. AETX-R302 extends recognition across the HLA-A2 and A3 supertypes, covering an estimated 66% of the U.S. population.

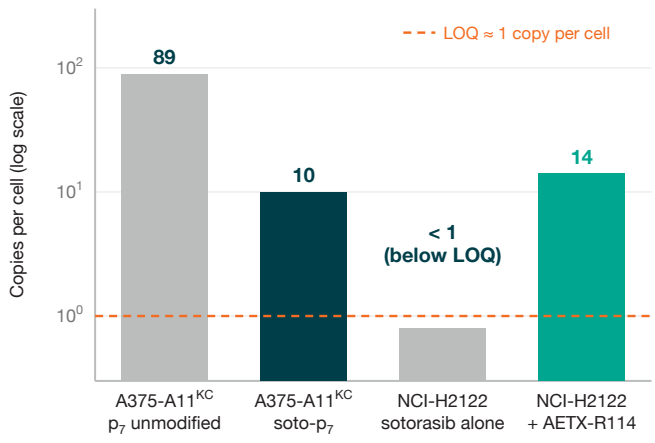
WHY THIS PAPER MATTERS

- **First direct quantification and stabilisation of drug-haptenated KRAS p*MHCs.** SureQuant immunopeptidomics detected and quantified sotorasib- and divarasib-modified KRAS peptides at single-digit copies-per-cell. Engineered antibodies that hold these unstable neoantigens on the surface were developed, extending complex half-life on A3-supertype alleles (measured out to 24 h) with no effect on the wild-type peptide complex.
- **Measurement that changed the therapeutic design.** MS and stability data prioritised HLA-A*03:01 over A*11:01 during affinity maturation because A*11 already had higher intrinsic stability and surface density. The engager reached single-digit picomolar affinity on A*03 and triple-digit picomolar affinity on A*11 and A*68 (160 pM), so potency could be matched across three high-frequency alleles.
- **Cross-HLA supertype recognition for broad population coverage.** AETX-R114 recognises sotorasib-modified p7 on A3-supertype alleles (A*03, A*11, A*68), while AETX-R302 exhibits unprecedented cross-supertype recognition of divarasib-modified peptides on both A2 and A3 supertypes, potentially covering 66% of the U.S. population.
- **Antibody binding as a way to create antigen density.** A slow off-rate binder lifted measured soto-p7 presentation in NCI-H2122 cells from below the limit of quantification to about 14 copies per cell, and the increase was itself confirmed by MS. This demonstrates that low-abundance, unstable epitopes are engineerable rather than undruggable, with implications well beyond KRAS.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Sole chromatographic platform for the MS programme.** An IonOpticks Aurora Ultimate 25×75 XT C18 UHPLC column at 55 °C on a Vanquish Neo UHPLC, coupled to an Orbitrap Ascend Tribrid, ran both the DDA discovery acquisitions and every targeted SureQuant run, and so every copies-per-cell figure in the paper is based on measurements generated on this column.
- **Direct injection, no trap column.** Peptides were loaded straight onto the analytical column via direct injection because for frontier immunopeptidomics requires preservation of maximal HLA immunoprecipitate from finite cells with targets at single-digit copies per cell, and removing the trapping step eliminates a potential source of sample loss.
- **Sensitivity at the level the biology demands.** The DDA workflow resolved endogenous hapten-peptides at a limit of quantification near one copy per cell over a 120-minute gradient at 200 nL/min, and distinguished a genuine below-LOQ signal from a real ~14 copies-per-cell measurement after antibody treatment. The claim that the binder creates antigen density depends entirely upon on those two abundances being distinguishable, a trace-level separation problem solved by the Aurora Ultimate XT 25×75 column.

A Absolute quantification of drug-modified KRAS^{G12C} neoantigens on the cell surface



B MS-guided affinity maturation gave a 14–16× tighter, slower off-rate T cell engager

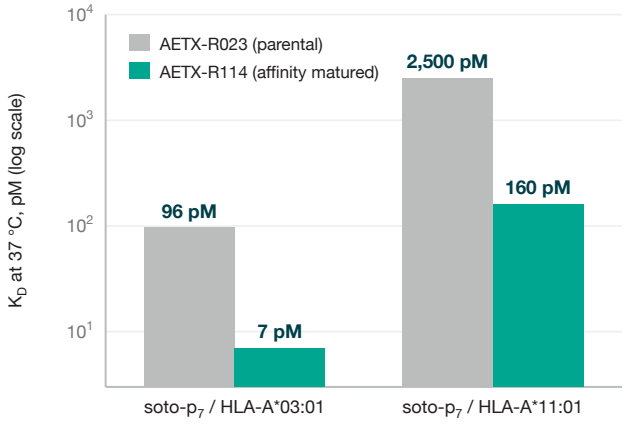


Figure 4. Aurora Ultimate XT quantified drug-modified KRAS neoantigens at single-digit copies per cell, enabling development of affinity-matured TCEs that stabilise presentation and bind with picomolar affinity across HLA supertypes. Panel A - Absolute quantification of soto-p7 showed 10 copies/cell (A375) and <1 copy/cell (NCI-H2122), increasing to 14 copies/cell upon AETX-R114 stabilisation. Panel B - MS-guided affinity maturation improved binding 14-fold (HLA-A03:01) and 16-fold (HLA-A11:01), achieving 7 pM and 160 pM affinities respectively.
Data recreated from values reported in Maso et al., Nature Communications, 2025; 16:11264, https://doi.org/10.1038/s41467-025-66132-w

PAPER 07 · REFERENCE [10]

When targeting changed the answer: a custom-length Aurora Frontier column found the clinically actionable PRAME epitope in 4 of 4 samples where shotgun immunopeptidomics found it in only 2, reversing its abundance ranking

Jensen, J. L., Peterson, S. K., Sambade, M. J., Alley, J. R., Yu, S., Kinjo, T., Bennett, S. N., Vensko, S. P., Shabrang, M., Debetta, J. D., Geyer, J. K., Price, B. A., Nickel, K. P., Kimple, R. J., Kotecha, R. S., Herring, L. E., Davis, I. J., Wang, J. R., French, C. A., Kuhlman, B., Weiss, J. M., Rubinsteyn, A., & Vincent, B. G. (2026). Shared PRAME epitopes are T-cell targets in NUT carcinoma. *Journal for ImmunoTherapy of Cancer*, 14(1), e013539.

Study snapshot: NUT carcinoma is a rare, lethal cancer with a median survival of 6.7 months and no effective standard of care. Across five cell lines and a patient-derived xenograft, DIA immunopeptidomics returned 33,644 unique peptides and 14 cancer/testis HLA class I ligands, nine from PRAME. But HLA-A*02:01/SLLQHLIGL (PRAME425), the epitope targeted by brenetafusp and anzutresgene autoleucel, appeared in only 2 of 4 PRAME+, HLA-A*02+ samples by DIA and scored below PRAME462. A targeted PRM assay on a custom-length IonOpticks Aurora Frontier 75 µm ID C18 UHPLC column, using both single-labelled and double-labelled isotopic standards, detected it in 4 of 4 above 0.01 fM and above both other PRAME epitopes. That result sent PRAME425 forward into a brenetafusp-modelled bispecific and anzutresgene-modelled TCR T-cells, both of which gave near-complete tumour cell killing at a 1:1 effector-to-target ratio, establishing PRAME as a clinically actionable therapeutic target.

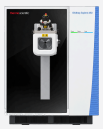
INSTRUMENTATION USED



Aurora® Frontier™ 60×75
Custom length, 75 µm ID C18



Vanquish Neo
UHPLC



Orbitrap Exploris 480
FAIMS, targeted PRM

NUT carcinoma is driven by BRD4::NUTM1 and related NUTM1 fusion oncogenes. It is PD-L1-low, mutation-poor and immunologically "cold", and it has resisted chemotherapy, checkpoint blockade and bromodomain inhibitors, with these inhibitors increasing median survival by only one to two months. Jensen et al. reasoned that if the fusion oncoprotein rewires transcription through reliable epigenetic changes, it might also switch on genes encoding targetable cancer antigens. RNA sequencing and antigen prediction pointed to the cancer/testis gene PRAME, as genetic gain- and loss-of-function work showed that BRD4::NUTM1 itself sustains PRAME levels. The critical clinical question was whether the specific HLA-A*02:01-bound PRAME peptide SLLQHLIGL (PRAME425) targeted by brenetafusp and anzutresgene autoleucel is actually presented on the tumour cell surface. Shotgun immunopeptidomics found more PRAME-derived HLA ligands (9) overall than all other cancer/testis antigens combined (5), but the actionable PRAME425 epitope in only 2 of 4 relevant samples and at lower abundance than a different PRAME epitope. Because untargeted intensities are inherently only semi-quantitative, the authors built a targeted PRM assay on a custom-length IonOpticks Aurora Frontier 75 µm ID C18 UHPLC column (Exploris 480, FAIMS, Vanquish NEO), spiking 5 fmol of stable isotope-labelled standards and achieving 0.01 fM sensitivity. Targeting found PRAME425 in 4/4 PRAME+, HLA-A*02+ sample and as the most abundant epitope, and on that basis they built an αPRAME425 TCR × CD3 bispecific, modelled on brenetafusp, and αPRAME425 TCR T-cells based on anzutregene autoleucel. Both products killed PRAME+ NUT carcinoma cells in vitro in a T-cell-dependent manner, giving near-complete tumour cell killing at 1:1 effector-to-target ratio and significant tumour growth inhibition in patient-derived in vitro models, establishing PRAME as a potentially clinically actionable therapeutic target for this lethal orphan disease.

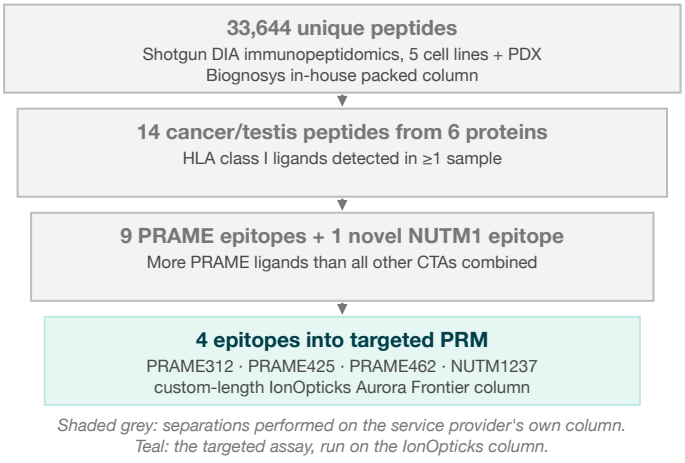
WHY THIS PAPER MATTERS

- **A first actionable immunotherapy target in a cancer that has none.** NUT carcinoma affects children and young adults, has a median survival of less than one year, and current US trials focus on bromodomain inhibitors. PRAME is not an incidental marker here, as the initiating fusion oncoprotein drives its expression, so the target and the disease mechanism are linked. Accordingly, this may make PRAME therapies work better for NUT carcinoma therapy than in genetically heterogeneous cancers.
- **The targeted assay changed the conclusion rather than confirming it.** Shotgun immunopeptidomics alone would have led to deprioritisation of PRAME425, as it had been detected in only 2/4 of the relevant samples, and ranked below another PRAME epitope on semi-quantitative intensity. Targeted quantification against labelled standards found PRAME425 in all four and ranked it first among the PRAME peptides quantified, demonstrating that the key decision on which candidate to progress in the therapeutic programme rests on the targeted numbers, not the discovery screen.
- **A short path from epitope to clinical-stage modalities.** An αPRAME425 TCR × αCD3 bispecific built with the variable regions of brenetafusp, and TCR T-cells carrying the anzutresgene autoleucel TCR, both produced complete or near-complete ablation of all three PRAME+, HLA-A*02+ lines at a 1:1 effector-to-target ratio, with no effect on PRAME-negative or haplo-incompatible controls. Peptide pulsing rescued killing in the PRAME-negative line, confirming antigen-directed activity.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Sensitivity against one of the least forgiving sample types in proteomics.** HLA class I immunopeptides are non-tryptic 7–12mers with no reliable C-terminal charge, recovered in tiny amounts from cell pellets. Each injection used half the sample's immunopeptide yield (~1 µg of total peptide anticipated) against 5 fmol spikes of heavy standard, and resolved epitopes at absolute abundances above 0.01 fM.
- **Retention-time stability for scheduled acquisition.** One unscheduled PRM run set retention-time calibration before data acquisition, then scheduled the acquisition across all six samples on a 63.3-minute gradient. A calibrate-once-then-schedule approach only holds together if retention times reliably reproduce across the run series.
- **Reproducibility across two independent standard sets.** The panel was deliberately run twice, once with single-labelled and once with double-labelled isotopic standards, as two independent experiments to build confidence in the quantitative result. Similar results and trends were reported from both. Recovering the same rankings across two separate standard chemistries and acquisition series depends on the separation behaving identically each time.

A Immunopeptidome narrowed to a four-epitope targeted assay



Shaded grey: separations performed on the service provider's own column. Teal: the targeted assay, run on the IonOpticks column.

B Detection of the actionable PRAME425 epitope

Targeting also reversed the ranking: PRAME425 scored below PRAME462 by shotgun signal, but above both other PRAME epitopes when quantified against labelled standards.

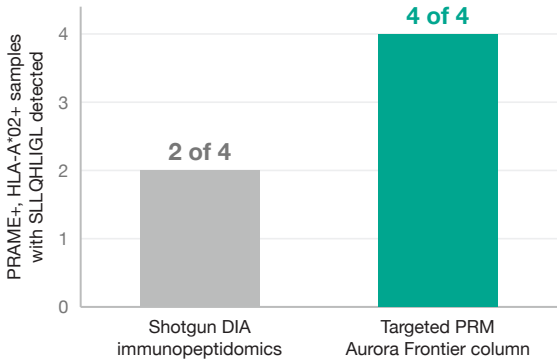


Figure 5. Targeted PRM on the custom-length Aurora Frontier column achieved complete detection of clinically actionable PRAME425 epitope, where shotgun methods achieved only 50% coverage. Panel A - Immunopeptide funnel from 33,644 unique peptides (shotgun DIA, grey) to four epitopes selected for targeted assay (Aurora, teal). Panel B - PRAME425-HLA-A*02+ detection: 2 of 4 samples by shotgun DIA versus 4 of 4 by targeted PRM on Aurora, with targeted analysis reversing epitope ranking through absolute quantification against labelled standards.
Constructed from values reported in Jensen et al., Journal for ImmunoTherapy of Cancer, 2026; 14:e013539, https://doi.org/10.1136/jitc-2025-013539.



Narrower peaks concentrate the analyte into fewer, more intense scans, enabling a limit of quantification near one copy per cell.

2.4 Absolute quantification for regulated, pharmaceutical and forensic workflows

Where a measurement has to withstand regulatory or evidential scrutiny, targeted assays with isotope-labelled standards supply traceable concentrations. The studies below apply that standard to cytochrome P450s in liver S9, the metabolic system used in DMPK and genotoxicity testing, and to retrospective verification of sulfur mustard exposure under the Chemical Weapons Convention.

PAPER 08 · REFERENCE [11]

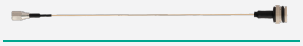
Nobody looked this deep into S9 before. The Aurora Ultimate 25×75 column delivers the depth to quantify 8,500+ proteins and absolutely quantify 15 CYP enzymes in rat and hamster S9, providing the quantitative proteomic foundation for selecting species and inducers that maximise oxidative capacity in DMPK and toxicology studies.

The deepest liver S9 proteome to date: 8,571 proteins on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column across rat and hamster enable absolute quantification of 15 CYP enzymes

Singh, J., Moniot, S., Dieckhoff, J., Bringezu, F., Simon, S., & Fischer, F. (2026). The Liver S9 Proteome of Rat and Hamster: Global Profiling and Targeted Cytochrome P450 Quantification Reveal Induction-Responsive Remodeling. *Journal of Proteome Research*, 25(7), 3415–3428.

> **Study snapshot:** A Merck KGaA study paired deep DIA-PASEF discovery with targeted LC–MRM to characterise rat and hamster liver S9 fractions under basal conditions and after Aroclor or phenobarbital/β-naphthoflavone induction. Discovery on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column with a timsTOF Pro quantified 8,571 proteins across both species, roughly three times the depth of previous S9 datasets, from 400 ng loads on a 60-minute gradient. These data selected surrogate peptides for a 15-CYP AQUA panel that absolutely quantified enzymes including CYP3A1 at 21.2 pmol/mg in controls versus 820 under Aroclor and 324 under PBNF, revealing species-specific and inducer-specific metabolic remodelling that guides species and inducer selection for DMPK and toxicology applications.

INSTRUMENTATION USED



Aurora® Ultimate™ 25×75
25 cm × 75 µm ID, 300 nL/min



UltiMate 3000 RSLCnano
Thermo Scientific



timsTOF Pro
DIA-PASEF

Liver S9 is the mandated metabolic activation system for genotoxicity testing under OECD guidelines, yet its proteomic composition has been characterised far less thoroughly than microsomes or hepatocytes. Earlier LC-MS datasets reported roughly 1,500 to 3,000 proteins, generally without replicate-resolved quantification across species and inducers. Singh et al. close that gap with a two-stage study design using an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column. Label-free DIA-PASEF discovery on a timsTOF Pro quantified 8,571 gene-level proteins across three independent lots per species and condition, with per-run identifications held at 6,100 to 6,800 and median log2 intensities varying by no more than 0.3 across all eighteen runs. That depth resolved a conserved core S9 proteome of 5,004 proteins shared between species alongside substantial species-biased subsets, and showed that induction modulates abundance rather than presence. Functional curation returned 452 xenobiotic-relevant proteins, including 142 drug-metabolising enzymes and 161 transporters. Induction remodelled the proteome extensively, such that rat CYP2B1 rose 100 to 130-fold under Aroclor, several CYP3A enzymes by 20 to 60-fold, alongside coordinated suppression of mitochondrial electron transport and activation of heat-shock chaperone networks. Surrogate peptides selected from these discovery data underpinned a targeted LC–MRM panel with AQUA stable-isotope-labelled standards, giving traceable picomole-per-milligram concentrations for 15 CYPs spanning the families most relevant to drug and nitrosamine metabolism.

This work establishes a quantitative proteomic foundation for selecting species and inducers in metabolic activation assays.

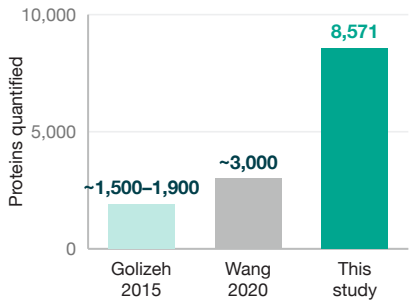
WHY THIS PAPER MATTERS

- **A reference dataset for a regulated assay system.** S9 is mandated for metabolic activation in the bacterial reverse mutation test (Ames), and this is the most comprehensive S9 characterisation published, at roughly three times the depth of prior datasets. Anyone selecting or qualifying an S9 lot now has a protein-level checklist, not just an Ames pass/fail.
- **Discovery and targeted analysis reinforce each other.** The DIA-PASEF data selected surrogate peptides, and the MRM assay supplied absolute concentrations for enzymes that discovery alone struggles to separate because of shared peptides and relaxed protein inference. Rat CYP3A1 went from 21.2 pmol/mg in controls to 820 under Aroclor (39×) and 324 under PBNF (15×).
- **Actionable study-design guidance.** Aroclor-induced rat S9 maximises CYP1A/2B/3A oxidative capacity, hamster S9 under Aroclor raises CYP2F/2G activities that matter for certain promutagens and nitrosamines, and PBNF gives the same directional profile at lower amplitude, which is useful where saturation is a concern. This bears directly on the enhanced Ames test approach recommended by the EMA for nitrosamine impurity assessment.
- **Counter-intuitive findings that only this depth reveals.** CYP2E1 fell rather than rose under both inducers in both species, with hamster levels about five times rat (64.1 vs 13.2 pmol/mg in controls). Given CYP2E1's role in bioactivating small xenobiotics, this matters when predicting metabolic capacity of induced S9. Aroclor also drove coordinated down-regulation of the electron transport chain and TCA cycle, a suppressive footprint invisible in shallower datasets.

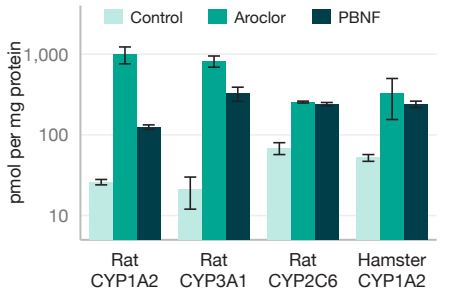
HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Deep proteome coverage for S9 characterisation.** An Aurora Ultimate 25×75 C18 UHPLC column carried all 18 DIA-PASEF runs on an UltiMate 3000 RSLCnano coupled to a timsTOF Pro, delivering 8,571-proteins from 400 ng peptide load on a 60-minute gradient at 300 nL/min. This depth captured low-abundance CYPs and complete ADME machinery (Phase I/II/III enzymes, transporters) essential for cross-species comparison.
- **Depth from a modest load without fractionation.** 6,100–6,800 protein IDs per run from 400 ng of unfractionated S9 digest, requiring no depletion, no fractionation, and only one injection per sample. Comparison datasets relied on multidimensional LC-MS/MS to reach a fraction of that depth.
- **Reproducible cross-species quantification.** Global log2 intensity medians spanned only 12.5–12.7 (hamster) and 12.4–12.7 (rat) with comparable interquartile ranges, supporting reliable differential abundance analysis and hierarchical clustering by species and treatment. Within-species overlap of 88.7% (hamster) and 81.4% (rat) depends on this stability, since library-free DIA-NN with match-between-runs requires consistent retention-time behaviour.
- **It defined the targeted panel.** Surrogate peptides for the 15-CYP MRM panel were selected from the untargeted global data. The discovery depth achieved made peptide selection possible across six CYP families and two species, including enzymes that are hard to resolve by discovery alone due to shared peptides.

A Depth of the S9 proteome

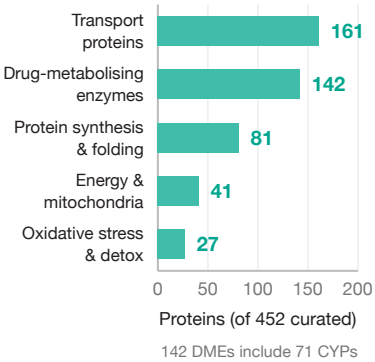


B Absolute CYP levels by LC–MRM



Rat CYP3A1: 39× under Aroclor, 15× under PBNF

C Xenobiotic machinery covered



142 DMEs include 71 CYPs

Figure 6. Deep discovery enables targeted quantification: Panel A - 8,571 proteins quantified in this study versus ~1,500–1,900 (Golizeh 2015) [12] and ~3,000 (Wang 2020) [13] in prior S9 datasets. Panel B - Absolute CYP concentrations by LC–MRM with AQUA SIL standards, mean ± SD of three biological replicates, showing inducer-responsive CYP1A2 and CYP3A1 in rat and CYP1A2 in hamster. Panel C - Coverage of 452 curated xenobiotic machinery proteins across functional categories, including 142 drug-metabolising enzymes (71 CYPs) and 161 transport proteins.
Figure recreated by IonOpticks from values reported in Singh et al., Journal of Proteome Research, 2026; 25(7):3415–3428, https://doi.org/10.1021/acs.jproteome.5c01180. Not a reproduction of a published figure.

PAPER 09 · REFERENCE [14]

Retrospective verification of sulfur mustard exposure demands sensitivity and speed. The Aurora Elite 15×75 C18 UHPLC column delivers the chromatographic performance needed to identify 270 biomarkers and verify exposure at 5 µM in blood serum, enabling high-throughput forensic toxicology workflows for chemical warfare agent detection.

One Aurora Elite column, three acquisition modes: 270 sulfur mustard biomarkers discovered and verified by prm-PASEF for forensic verification down to 5 µM

Thomann, G., Brackmann, M., Bochet, C. G., & Curty, C. (2026). Application of Proteomic Workflows for the Identification of Biomarkers for the Retrospective Verification of Sulfur Mustard Intoxication. *Analytical Chemistry*, 98(14), 10550–10561.

> **Study snapshot:** Switzerland's Spiez Laboratory built a high-throughput proteomic workflow for retrospective verification of sulfur mustard exposure, running dda-PASEF library building, dia-PASEF discovery and prm-PASEF targeted validation on a single IonOpticks Aurora Elite C18 UHPLC column at 50°C with a nanoElute and timsTOF Pro 2. Full-proteome analysis of serum exposed to three sulfur mustards revealed 270 candidate biomarkers spanning 232 modification sites, 213 of them novel, from under 1 µL of serum per sample. Human serum albumin enrichment on Cibacron Blue magnetic beads then allowed unequivocal verification at 5 µM agent on both 30-minute and 15-minute gradients, with sensitivity proposed for CWC/OPCW-relevant forensic toxicology applications at approximately 10-fold higher concentration than typical OPCW proficiency-test spike levels.

INSTRUMENTATION USED



Aurora® Elite™ 15×75
15 cm × 75 µm ID, 50 °C



nanoElute
Bruker



timsTOF Pro 2
dda-, dia- and prm-PASEF

Retrospective verification of chemical weapons use is a treaty-enforcement problem with an analytical bottleneck. The current gold standard for sulfur mustard exposure, the Proteinase K-derived Cys^{34} tripeptide adduct on human serum albumin, is specific but narrow, requires large sample volumes, and yields almost no information about the surrounding matrix. Thomann et al. took a different route and developed a comprehensive proteomic workflow using an IonOpticks Aurora Elite 15×75 C18 UHPLC column at 50°C with a nanoElute and timsTOF Pro 2. Serum from seven healthy donors was exposed in vitro to three sulfur mustard agents and processed by SP3 within the rapid-robotic R2-P1 protocol on a KingFisher APEX using under 1 µL of serum per sample. Data-dependent PASEF built the spectral library, dia-PASEF profiled the full proteome, and resulting data were mined by principal component analysis and an orthogonal random forest classifier. This yielded 270 candidate biomarkers covering 232 modification sites, of which 213 were previously undescribed. 74 sites sit in albumin alone, mostly on glutamic acid and predominantly on the protein surface. A low-mass dda-PASEF variant tuned to alkyl side-chain fragment ions at m/z 105, 137 and 165 confirmed 25 peptides unambiguously, and four alkylated amino acid building blocks were synthesised and incorporated as reference standards. Albumin enrichment on Cibacron Blue-modified magnetic beads cut protein counts by roughly 58% and enabled prm-PASEF verification of exposure at 5 µM on both 15-minute and 30-minute gradients. This work establishes a comprehensive biomarker panel and analytical workflow proposed for CWC/OPCW-relevant retrospective verification, with verified sensitivity at 5 µM representing approximately 10-fold higher concentration than typical OPCW proficiency-test spike levels. Although this work is in vitro, it supplies a much broader adduct panel than the Cys^{34} tripeptide alone and a workflow that can be run on microlitre-scale serum.

WHY THIS PAPER MATTERS

- **A much wider adduct map than the current gold standard.** This is a proteome-wide look at serum exposed in vitro to three sulfur mustard agents (mustard gas HD, sesquimustard Q, O-mustard T), identifying 270 potential biomarkers with 213 novel alkylation sites across three agent representatives and significantly expanding beyond the single Cys-34 HSA adduct currently used as gold standard. Because the workflow is non-specific and proteome-wide, the same sample preparation can in principle be directed at other alkylating agents without redesigning a new method for each suspected chemical.
- **Throughput and sample economy suited to real casework.** Less than 1 µL of serum per sample, automated SP3 on a robotic platform, tryptic digest rather than the non-specific proteases previous sensitivity work has needed, and a 30-minute gradient with a 15-minute option. Enabled detection of exposure down to 5 µM final concentration using Cibacron Blue-modified magnetic bead enrichment and prm-PASEF.
- **Discovery, statistics and synthesis close the loop.** Candidate peptides from dia-PASEF were confirmed against synthetically prepared reference material (Glu, Asp, Cys, His building blocks), the standard by which an identification becomes admissible rather than merely plausible. The authors note this combination remains underdeveloped in adductomics, yet it establishes the foundation for forensic validation standards.
- **Chemical provenancing is within reach.** Hierarchical clustering separated serum exposed to mustard gas, sesquimustard and O-mustard even though all three alkylate predominantly the same sites, because intensity distribution across peptides differs by agent. Which sulfur mustard was used can therefore be inferred from an untargeted measurement. A critical caveat, however, is that 44 of 270 sesquimustard biomarkers appeared as HETE adducts, the signature normally expected only from mustard gas, via a second hydrolysis pathway. Any attempt to read adduct type as agent identity must account for this.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **One column across the entire discovery-to-verification pipeline.** The IonOpticks Aurora Elite 15×75 C18 UHPLC column on a nanoElute coupled to a timsTOF Pro 2 carried dda-PASEF library building, dia-PASEF discovery, and prm-PASEF targeted verification, serving all three acquisition modes without changing chromatography. This delivered 270 alkylated peptides and 213 novel modification sites from under 1 µL of serum per sample.
- **Flexibility for throughput and trace analysis.** The 30-minute gradient at 50°C resolved 232 alkylation sites with sample economy that contrasts favourably with existing verification methods. When shortened to 15 minutes for trace analysis, the column still supported prm-PASEF verification at 5 µM, surfacing three modified peptides at trace level that the longer gradient did not.
- **Retention-time and mobility fidelity for evidential weight.** Biomarker confirmation rested on matching synthetically prepared reference material to the serum analyte at a stated retention time and ion mobility, exemplified by 24 minutes and 1.12 V s cm^{-2} for the HETETE-modified peptide 2b. Comparison against synthetic standards only carries evidential weight if the separation is reproducible enough to make the match meaningful.

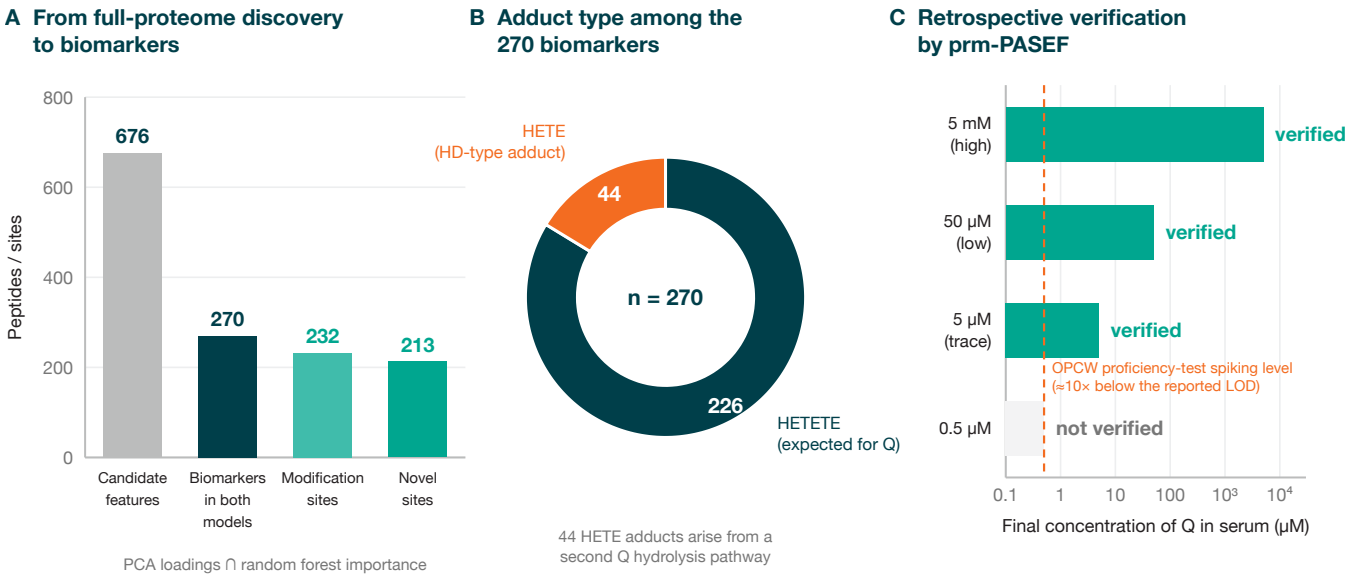


Figure 7. From discovery to forensic verification: proteomic analysis of sulfur mustard-exposed serum yields 270 biomarkers and detection down to 5 µM. Panel A - Candidate selection shows 676 features from PCA loadings reduced to 270 biomarkers significant in both PCA and random forest models, spanning 232 modification sites of which 213 are novel. Panel B - Adduct characterisation shows 44 of 270 sesquimustard (Q) biomarkers carried the HETE adduct normally associated with mustard gas (HD), arising from a secondary hydrolysis pathway. Panel C - Retrospective verification shows prm-PASEF with Cibacron Blue-based albumin enrichment verified Q exposure at 5 mM, 50 µM, and 5 µM (trace) levels, with 0.5 µM remaining below the detection limit, approximately 10× above the OPCW proficiency-test spiking level.

Figure recreated by IonOpticks from values reported in Thomann et al., Analytical Chemistry, 2026; 98(14):10550–10561, https://doi.org/10.1021/acs.analchem.5c07620

2.5 Extending the reach of targeted acquisition: multiplexing, PTMs and microproteins

The final group pushes targeted acquisition into territory it has not previously occupied: multiplexed discovery-scale quantification on targeted hardware, post-translational modifications at single-cell input, and orthogonal verification of translation products that discovery proteomics cannot see.

PAPER 10 · REFERENCE [15]

Discovery proteomics on a Stellar with no compromises. Using the IonOpticks Aurora Ultimate 25×75 column, iTMTproC matches MultiNotch MS^3 accuracy while delivering 26% more proteins, bringing multiplexed shotgun capability to quadrupole-ion traps.

Discovery proteomics on targeted hardware: IonOpticks Aurora enables 9-plex iTMTproC quantification on Stellar with accuracy matching MultiNotch MS^3 and 26% more protein coverage.

Cruz, E. R., Johnson, A. N. T., Pujari, V., Zhang, Q., Nguyen, T., Stadlmeier, M., Wang, J., Jacob, C. C., McAlister, G. C., Remes, P. M., & Wühr, M. (2025). Expanding Targeted Instrumentation for Discovery Applications: Complement Reporter Ion Quantification with a Quadrupole-Ion Trap Instrument. *Journal of Proteome Research*, 24(9), 4611–4622.

> **Study snapshot:** iTMTproC adapts complement reporter ion quantification to a quadrupole-ion trap MS, enabling 9-plex shotgun proteomics on targeted instrumentation. Against MultiNotch MS^3 on an Orbitrap Fusion Lumos, Stellar iTMTproC matched interference suppression on HeLa-yeast standard while quantifying 26–30% more proteins in single-shot analysis. On an Orbitrap Ascend analysing a 9-point Xenopus developmental time course, iTMTproC quantified 8,971 proteins versus 8,531 for MultiNotch MS^3. Every separation, on all three instruments across two different laboratories, used an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column with 90-minute gradients at 350 nL/min, ensuring chromatography was not a confounding variable.

INSTRUMENTATION USED



Aurora® Ultimate™ 25×75
25 cm × 75 µm ID, 60 °C



Vanquish Neo
UHPLC, 350 nL/min



EASY-nLC 1200
Second-site nanoflow LC



Stellar MS
iTMTproC



Orbitrap Fusion Lumos, Ascend
MultiNotch MS3

Proteomic analyses have long been split across two main instrument classes, with high-resolution analysers used for discovery and triple quadrupole or quadrupole-ion trap systems used for targeted work. Cruz et al. close that gap from the targeted side. Conducting multiplexed shotgun proteomic analyses with isobaric tags normally requires a tribrid instrument, because reporter ion ratio distortion from co-isolated peptides has to be suppressed by an extra MS^3 fragmentation step. Complement reporter ion quantification (TMTproC) offers an alternative at the MS^2 level by reading balancer-group fragment ions whose masses are precursor-specific, and until now this had only ever been implemented on high-resolution analysers. In this study, despite the low mass accuracy of ion trap MS1 scans on quadrupole-ion trap analysers, iTMTproC has been adapted for this hardware through the implementation of a ±0.2 Th filter on the theoretical M0 and M+1 peptide masses, a restriction to doubly charged precursors, and the application of empirically derived AGC and injection time settings. Benchmarked on a HeLa-yeast interference standard mixed 10:1 to mimic low-abundance dynamic proteins, Stellar iTMTproC produced 5:1 and 10:1 ratio distributions closely tracking MultiNotch MS^3 on an Orbitrap Lumos, with both substantially outperforming TMTpro-MS^2 reporter ion quantification, and quantified 26–30% more proteins in a single shot. Applied to Xenopus embryogenesis on an Orbitrap Ascend, iTMTproC and MultiNotch MS^3 produced highly similar protein quantification trajectories, indicating high overall similarity between temporal protein profiles obtained from the two approaches. All separations throughout used an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column with 90-minute gradients at 350 nL/min and 2% DMSO at 60°C, run on either a Vanquish Neo or EASY-nLC 1200 system.

By demonstrating that quadrupole-ion trap platforms can match tribrid performance when coupled with high-resolution chromatography, this work bridges the gap between targeted instrumentation and discovery-scale multiplexed proteomics.

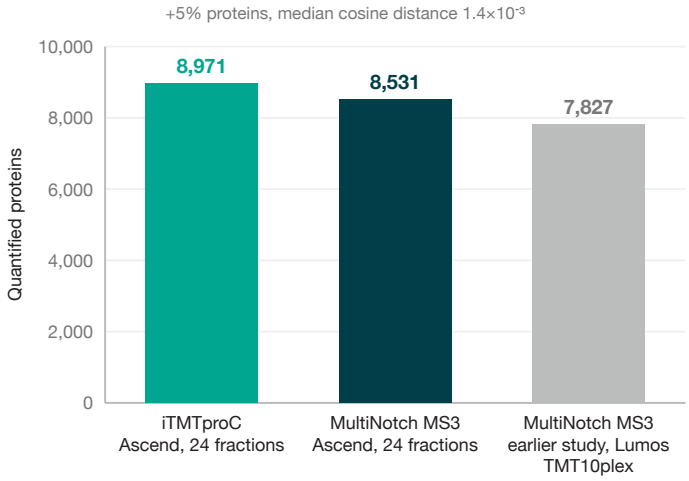
WHY THIS PAPER MATTERS

- **Democratises multiplexed proteomics.** Brings accurate TMTpro quantification to cost-effective quadrupole-ion trap instruments, eliminating the need for high-end tribrid Orbitrap systems for routine multiplexed discovery workflows.
- **Accuracy verified two ways.** On the interference standard, iTMTproC ratio distributions closely track MultiNotch MS^3 (both beating TMTpro-MS^2). Replicated on a single Ascend instrument at another site, iTMTproC, shows slightly more ratio distortion than MS^3 but quantifies more proteins, a trade-off that favours coverage for discovery.
- **Concordance with real biology, not just standards.** Across nine developmental stages, iTMTproC and MultiNotch MS^3 showed median cosine distance of 1.4×10^{-3}, indistinguishable dynamics up to the 75th percentile, matched abundance distributions, and near-identical k-means clusters.

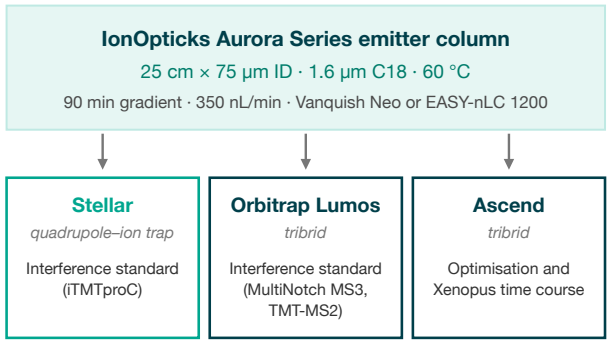
HOW IONOPTICKS COLUMNS CONTRIBUTED

- **The one constant in a three-instrument, two-site comparison.** An IonOpticks Aurora Ultimate 25×75 C18 UHPLC column was used for every separation, with Stellar and Lumos both interfaced with Vanquish Neo UHPLCs at one location, and Ascend interfaced with a EASY-nLC 1200 at a second location. Holding the column format constant is ultimately what makes the cross-platform comparison interpretable, as differences can be attributed to acquisition method rather than to separation chemistry.
- **Resolution that supports very narrow isolation.** iTMTproC accuracy depends on a 0.4 Da quadrupole isolation window and tight centring on M0/M+1 peaks, with co-eluting precursors as the primary interference source. The 90-minute gradient at 350 nL/min delivered the separation quality against which scan-speed, AGC, and injection-time were optimised.
- **Depth despite conservative filtering.** iTMTproC applies stringent thresholds, discarding triply charged precursors and requiring 450 pseudo-counts, which costs 19% of quantifiable peptides upfront (7,359 to 5,995). Even so, the 25 cm Aurora Ultimate 25×75 column delivered 8,971 quantified proteins across 24 offline fractions (interleaved with TFEA washes) on a single column, exceeding MultiNotch MS^3 on the same instrument (8,531) and a prior Lumos result (7,827) in the same lab.

A Proteins quantified in the 9-plex Xenopus time course



B One separation platform across every instrument in the study



Lumos and Stellar measurements used separate UHPLC systems in two different laboratories; the column format was the one component held constant throughout.

Figure 8. Holding Aurora chromatography constant, iTMTproC quantifies 5% more proteins than MultiNotch MS^3 on Ascend (8,971 vs. 8,531) with equivalent accuracy. Panel A - Protein coverage in 9-plex Xenopus developmental time course (24 fractions), comparing iTMTproC versus MultiNotch MS^3 on Orbitrap Ascend (cosine distance 1.4×10^{-3}), with earlier MultiNotch MS^3 data from Lumos shown for cross-platform context. Panel B - The IonOpticks Aurora Ultimate 25×75 C18 UHPLC column was the constant component across Stellar, Orbitrap Lumos, and Ascend platforms, with Ascend data collected using a different UHPLC system.

Constructed from values reported in Cruz et al., Journal of Proteome Research, 2025; 24(9):4611–4622, https://doi.org/10.1021/acs.jproteome.5c00356.

PAPER 11 · REFERENCE [16]

Twenty single cells a day becomes 1,080. An IonOpticks Aurora Rapid 8×75 column enables 20-minute cycles, delivering 72 runs per day while maintaining consistent chromatography across DDA, PRM and SureQuant, enabling zero-missing-value PTM quantification and ensuring the method comparison reflects real sensitivity gains rather than chromatographic variability.

1,080 single cells per day on an 8 cm Aurora column: shTMTpro-triggered SureQuant quantifies histone PTMs with zero missing values

Mun, D.-G., Nishida, H., Bhat, F. A., Pasupuleti, R. R., Delanghe, B., & Pandey, A. (2026). Multiplexed Quantitation of Post-Translationally Modified Peptides in Single Cells Using Triggered MS/MS Combined With Super Heavy Tandem Mass Tags. *Journal of Proteome Research*, 25(2), 1184–1190.

> **Study snapshot:** A targeted single-cell strategy pairs 15-plex TMTpro multiplexing with SureQuant triggered MS/MS, using super heavy TMTpro trigger peptides 9 Da above the sample tags. Five histone H3 PTM peptides were quantified in every sample at single-cell-equivalent input where DDA managed only one of five, and four PTMs were measured across 150 sorted Jurkat cells with median CVs of 22.6–36%. All acquisition ran on an IonOpticks Aurora Rapid 8×75 C18 UHPLC column at 400 nL/min and 50°C on an UltiMate 3000 coupled to an Orbitrap Exploris 480 with FAIMS Pro. A roughly 20-minute cycle enabled 72 runs per day and 1,080 multiplexed samples.

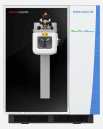
INSTRUMENTATION USED



Aurora® Rapid™ 8×75
8 cm × 75 µm ID, 400 nL/min, 50 °C



UltiMate 3000 RSLC
Thermo Scientific



Orbitrap Exploris 480
FAIMS Pro, SureQuant

Single-cell proteomics can now report thousands of proteins per cell, but untargeted acquisition leaves missing values, and post-translational modifications are the worst case because they sit at lower stoichiometry than their unmodified counterparts. Mun et al. tackle both the coverage and the throughput problem at once. Synthetic histone H3 PTM peptides are labelled with super heavy TMTpro, which is 9 Da heavier than the TMTpro tags used on the samples, and 20 fmol is spiked into a 15-plex TMTpro-labelled sample. In watch mode the Exploris 480 monitors the trigger at low resolution and a 10 ms injection time; when two of five predefined fragment ions match within 20 ppm, it switches to quantitative mode, acquires the endogenous TMTpro-labelled peptide at 60,000 resolution with a 116 ms injection, reading relative abundance from the reporter ions of each channel. Because the trigger sits at a defined mass offset rather than in a carrier channel, this approach sidesteps the quantitative accuracy penalty that carrier-proteome designs incur through co-isolation. Benchmarked against DDA and standard PRM on a serial dilution of TMTpro-labelled HeLa digest, both targeted methods held all five histone H3 PTMs at 100 pg per channel while DDA quantified only one across 45 samples, and detection persisted at 10 pg and 1 pg where DDA returned nothing. Applied to 150 Jurkat cells sorted on a cellenONE platform, this method returned four PTMs across ten TMT sets with median CVs of 22.6% (K14ac), 22.7% (K23ac), 36% (K27me3) and 24.3% (K79me). Peptide separation used an UltiMate 3000 RSLC with an IonOpticks Aurora Rapid 8×75 C18 UHPLC column (3-45% B in 10-minute analytical gradient), supporting 72 runs per day and 1,080 multiplexed channels per day.

This workflow establishes a robust platform for comprehensive single-cell PTM analysis without the missing values that have limited previous approaches, enabling high-throughput epigenetic profiling at single-cell resolution.

WHY THIS PAPER MATTERS

- **It removes the missing-value problem rather than working around it.** At single-cell-equivalent input, (100 pg per channel), DDA quantified one of five histone PTMs across 45 samples, while SureQuant and PRM quantified all five histone H3 PTMs in every sample. For single-cell work, the ambiguity between a true absence and a failure to sample is the central interpretive obstacle, and targeted acquisition removes it.
- **A neat solution to the carrier-channel problem.** Rather than boosting signal with a carrier proteome and accepting reporter ion distortion, the ~9 Da shTMTpro offset triggers tandem MS acquisition selectively without co-isolating the trigger into the single-cell measurement. The authors conclude that this represents the first demonstration of an offset-mass trigger for targeted PTM measurement at single-cell resolution.
- **Demonstrated practical throughput for single-cell PTM panels.** Achieved 72 LC-MS/MS runs per day with 15-plex TMT (1,080 samples/day), with targeted methods detecting PTMs at 10 pg and 1 pg per channel (single-cell and single-nucleus levels), which is scalable to TMT 35-plex without method modification.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Sole analytical column for every acquisition mode.** The Aurora Rapid 8×75 C18 UHPLC column served as the sole analytical platform for DDA, PRM, and SureQuant comparisons under identical chromatography conditions, ensuring that reported differences between acquisition strategies reflect genuine method performance rather than separation variability.
- **Short-column format enables high-throughput single-cell analysis.** The ~20-minute cycle delivers 72 runs per day and 1,080 multiplexed samples, with retention time stability across ten TMT sets demonstrating reproducible chromatography.
- **Co-elution of light and heavy pairs is the identification criterion.** Retention-time fidelity between the shTMTpro trigger and TMTpro-labelled endogenous peptide, confirmed by matching elution times and consistent fragmentation, places correct assignment squarely on chromatographic separation.
- **Precursor ion purity as a chromatographic read-out.** Precursor ion purity above 90% in 24 of 37 identified MS/MS scans demonstrates that reporter ions were largely unaffected by co-isolation.

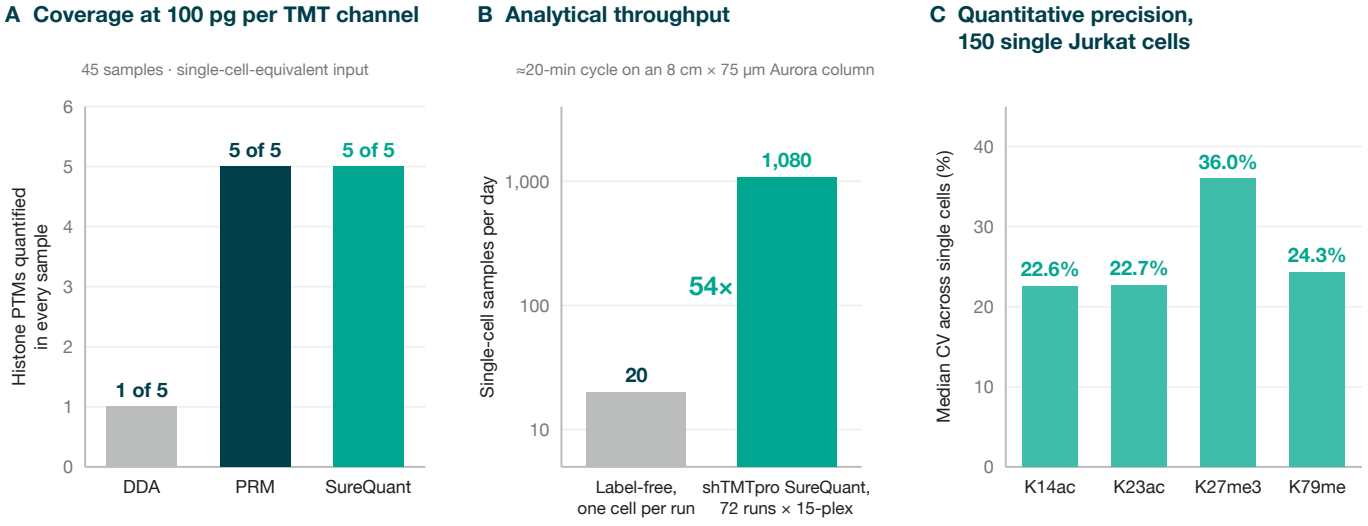


Figure 9. shTMTpro-triggered SureQuant on Aurora 8 cm columns enables complete single-cell PTM quantification at scale. Panel A - At 100 pg/channel (single-cell-equivalent), SureQuant and PRM achieved 100% coverage (5/5 histone PTMs) versus DDA (20%, 1/5). Panel B - 15-plex TMT with ~20-minute cycles delivers 1,080 samples/day, 54× faster than label-free single-cell analysis. Panel C - Robust quantification across 150 cells with median CVs of 22-36%.

Figure recreated by IonOpticks from values reported in Mun et al., Journal of Proteome Research, 2026; 25(2):1184–1190, https://doi.org/10.1021/acs.jproteome.5c00972. Not a reproduction of a published figure.

PAPER 12 · REFERENCE [17]

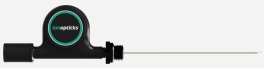
When an entry in the human reference proteome depends on proving that a 25-amino-acid microprotein is really there, Aurora Elite columns deliver the low-load sensitivity that turns a candidate ncORF peptide into an annotated protein-coding gene.

From 7,264 dark ORFs to 1,785 microproteins: TransCODE defines 'peptideins' with Aurora Elite PRM confirming endogenous expression

Deutsch, E. W., Kok, L. W., Mudge, J. M., Valls, C. F., Jungreis, I., Ruiz-Orera, J., Sun, Z., Kusebauch, U., Fierro-Monti, I., Abelin, J. G., Alba, M. M., Aspden, J. L., Bandyopadhyay, S., Banerjee, K., Baranov, P. V., Bazzini, A. A., Bourassa, F., Bruford, E. A., Calviello, L., Carr, S. A., Carvunis, A.-R., Chothani, S., Clauwaert, J., Dean, K., Faridi, P., Frankish, A., Goodale, A., Green, T., Hubner, N., Ingolia, N. T., Kellis, M., Magrane, M., Martin, M. J., Martinez, T. F., Menschaert, G., Ohler, U., Orchard, S., Potter, A., Rackham, O. J. L., Rees, M. G., Root, D. E., Roth, J. A., Roucou, X., Sialana, F. J., Slavoff, S. A., Świrski, M. I., Tierney, J. A. S., Trifiro, F.-A., Valen, E., Vasylieva, V., Wacholder, A., Wang, S., Wang, L., Weissman, J. S., Wu, W., Xie, Z., Choudhary, J. S., Bassani-Sternberg, M., Vizcaíno, J. A., Ternette, N., Brunet, M. A., Moritz, R. L., Prensner, J. R., van Heesch, S. (2026). Expanding the Human Proteome With Microproteins and Peptideins. *Nature*, 654(8021), 813–825.

Study snapshot: Thousands of ribosome-predicted non-canonical ORFs remain unvalidated as proteins because standard tryptic proteomics detects fewer than 3%. The TransCODE Consortium found HLA immunopeptidomics achieves tenfold higher detection rates (24.6% versus 2.5%) by reanalyzing 240 million immunopeptidomic spectra against 7,264 GENCODE ncORFs, enabling five annotation bodies to establish 'peptideins' as a new protein class and promoting four ncORFs to protein-coding genes. Multiplexed PRM with heavy synthetic standards on an IonOpticks Aurora Elite XS 15×75 C18 UHPLC column coupled to a Sciex ZenoTOF 8600 verified tier 1A microproteins across three cell lines, establishing a workflow from Ribo-seq to validated annotation.

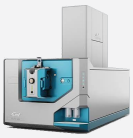
INSTRUMENTATION USED



Aurora® Elite™ XS 15×75
15 cm × 75 µm ID, 1.7 µm C18



ACQUITY UPLC M-Class
Waters



ZenoTOF 8600
SCIEX, multiplexed PRM

How many protein-coding genes does the human genome contain? Ribo-seq has nominated thousands of non-canonical ORFs (ncORFs), but annotation bodies will not add a gene until a polypeptide is shown to exist. Deutsch et al. give the TransCODE Consortium's consensus answer, built by querying 7,264 GENCODE ncORFs against 3.5 billion tryptic and 240 million HLA immunopeptidomic MS/MS spectra (95,520 experiments). Whereas conventional tryptic proteomics detected only 183 ncORFs (2.5%), immunopeptidomics found 1,785 ncORFs (24.6%), a 10-fold improvement. This is significant because many of these microproteins never produce two unique tryptic peptides of ≥9 residues, the HUPO-HPP requirement for verifying that at protein exists. In fact, of the full ncORF set, 28.3% encode products shorter than 25 amino acids and therefore sit below this threshold by design. Integration of MS evidence, Ribo-seq, the ORBL evolutionary score and CRISPR screens across eight cell lines established the 'peptidein' annotation class now adopted by GENCODE and UniProt. Targeted PRM with isotopically labelled synthetic standards, run on an IonOpticks Aurora Elite 15×75 C18 UHPLC column coupled to a Sciex ZenoTOF 8600, provided orthogonal confirmation of endogenous expression, and established a workflow that supports profiling through to validated protein annotation.

WHY THIS PAPER MATTERS

- **A new annotation class agreed across major reference projects.** GENCODE, UniProtKB, HGNC, RefSeq and HUPO-HPP will now classify confidently detected but functionally uncharacterised translation products as 'peptideins' rather than leaving them unannotated. In this study, 121 initial peptidein annotations were issued, and four ncORFs cleared the bar for full protein-coding gene status, including c2riboseqorf47 (a GMCL1 uORF, now ENSG00000310604) nominated on HLA, CRISPR and ORBL evidence without tryptic MS support.
- **It measures what tryptic discovery misses.** Across 3.5 billion spectra, validated peptides were obtained for only 0.9% of the 7,264 ncORFs, and single-peptide identifications failed manual review three times out of four (25.5% passed, versus 71.4% for two-peptide candidates). Small size, amino acid composition and possible BAG6-mediated proteasomal degradation explain this failure rate, making DIA with targeted PRM validation the necessary route forward.
- **Functional validation at scale.** Identified c10riboseqorf92 as a pan-essential peptidein (required for viability in 85.6% of 485 cancer cell lines) from the OLMALINC lncRNA, demonstrating that peptidein-class molecules can have critical cellular functions despite lacking canonical protein-coding gene status.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Targeted verification of candidate microproteins.** Multiplexed PRM assays confirming endogenous ncORF peptides against heavy synthetic standards were run on an Aurora Elite XS 15×75 C18 UHPLC column on an ACQUITY UPLC M-Class coupled to ZenoTOF 8600, and provide the orthogonal evidence underpinning tier 1A protein-coding annotations.
- **Validated a protein-coding gene from scratch.** On the ZenoTOF arm, the Aurora Elite XS column enabled detection of c11riboseqorf4 peptide (ATPGHTGCLSPGCPDQPAR) in HeLa S3, HEK293 and K562 lysates across two preparation protocols, co-eluting with the heavy standard. This PIDD1 uoORF is now GENCODE-annotated as protein coding based partly on this PRM evidence.
- **Analytical sensitivity for low-abundance ncORFs.** Injections of 250 ng digest (6 fmol heavy peptide, 5 fmol iRT) delivered the peak shape and signal-to-noise to confidently detect endogenous ncORF peptides above background, achieving the assay sensitivity required for reference-grade microprotein annotation.

PLATFORMS REPRESENTED ACROSS THIS EVIDENCE BASE

MASS SPECTROMETERS


Orbitrap Astral

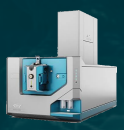

Orbitrap Exploris 480


Orbitrap Exploris 240

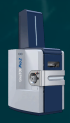

Stellar MS


Orbitrap Fusion Lumos


Orbitrap Ascend


ZenoTOF 8600


timsTOF Pro


timsTOF Pro 2

UHPLC SYSTEMS


nanoElute


Evosep One


Vanquish Neo


UltiMate 3000


ACQUITY M-Class


EASY-nLC 1200

IONOPTICKS PRODUCTS FEATURED IN THIS NOTE



Aurora® Rapid™ 5×75

5 cm × 75 µm ID. Low-load targeted and DDA characterisation of labelled standards on Evosep Whisper Zoom methods. Ref [4]



Aurora® Rapid™ 8×75

8 cm × 75 µm ID. Triggered acquisition at single-cell input, at around 72 runs per day. Ref [16]



Aurora® Rapid™ 8×150

8 cm × 150 µm ID. The column chemistry in published Orbitrap Astral to Stellar MS assay translations at 60–100 SPD. Refs [4, 6]



Aurora® Elite™ 15×75

15 cm × 75 µm ID. High-plex scheduled PRM, HRM-DIA validation panels, prm-PASEF verification and multiplexed PRM of microproteins on ZenoTOF 8600. Refs [5, 7, 8, 14, 17]



Aurora® Ultimate™ 25×75

25 cm × 75 µm ID. Deep discovery that defines targeted panels, trace-level FAIMS-PRM, and copies-per-cell SureQuant immunopeptidomics by direct injection. Refs [8, 9, 11, 15]



Aurora® Frontier™ 60×75

60 cm × 75 µm ID. Extended-length separation for applications where standard formats constrain sensitivity, such as HLA class I immunopeptidomics. Ref [10]

DECISION GUIDE

03 Choosing the right Aurora column for your targeted workflow

The Aurora Series includes several formats optimised for different targeted workflows. The table below summarises the main options and the published studies that used them.

AURORA COLUMN	FORMAT	TYPICAL TARGETED WORKFLOW	THROUGHPUT WINDOW
Aurora Rapid™ 5×75	5 cm × 75 µm ID, 1.7 µm	Low-load targeted and DDA characterisation of labelled standards; Evosep Whisper Zoom methods. [4]	**80 SPD**
Aurora Rapid™ 8×75	8 cm × 75 µm ID, 1.7 µm	High-throughput triggered acquisition at single-cell and single-nucleus input; multiplexed PTM panels. [16]	**~72 runs/day (10 min gradient)**
Aurora Rapid™ 8×150	8 cm × 150 µm ID, 1.7 µm	Discovery-to-targeted translation on Evosep One; scheduled PRM and MS^3 clinical assays in plasma and CSF. [4, 6]	**60–100 SPD (11.5–21 min)**
Aurora Elite™ 15×75	15 cm × 75 µm ID, 1.7 µm	High-plex scheduled PRM, HRM-DIA validation panels, prm-PASEF verification, matched library-plus-assay acquisition. [5, 7, 8, 14, 17]	**15–60 min gradients**
Aurora Ultimate™ 25×75	25 cm × 75 µm ID, 1.7 µm	Deep discovery feeding targeted panel design; trace-level SureQuant and FAIMS-PRM where sensitivity governs. [8, 9, 11, 15]	**60–120 min gradients**
Aurora Frontier™ (custom)	Custom bed length, 75 µm ID, 1.7 µm	Application-specific geometries where standard formats constrain sensitivity, such as HLA class I immunopeptidomics. [10]	**Method-dependent**

Practical guidance. Laboratories building a discovery-to-verification pipeline should standardise on a single Aurora chemistry across both phases rather than optimising each in isolation. Where the discovery leg needs maximum depth to populate the panel, Aurora Ultimate™ 25×75 or Aurora Elite™ 15×75 is the starting point; where the verification leg needs cohort-scale throughput, Aurora Rapid™ 8×150 on Evosep One is the format used in the published Astral to Stellar translations [4, 6]. Keeping the chemistry constant is what allows retention-time alignment to do the work of method transfer, and it removes the most common source of drift between study phases.

04

Summary

Targeted proteomics succeeds or fails on reproducibility because a scheduled assay is a set of commitments made before the samples are run, specifying which precursors, in which window, and with how much injection time. The separation is what binds these commitments together. The peer-reviewed studies in this note converge on the same finding from different directions: the Aurora Series® architecture supplies the retention-time stability, peak shape and sensitivity that high-plex, trace-level and cross-platform targeted assays require.

Across all studies the same design principle recurs: change the mass spectrometer but keep the column. This principle enables targeted assays to be portable between discovery and validation, between instruments, and between laboratories.

For laboratories translating discovery findings into clinical measurement, Aurora Rapid™ 8×150 serves as the column chemistry in published Orbitrap Astral to Stellar MS workflows, where retention times transfer within ±30 s and assay development is compressed from months to days [4, 6]. For high-plex verification panels and validation cohorts, Aurora Elite™ 15×75 carries assays of more than a thousand precursors [5], entire HRM-DIA validation legs [7], and three acquisition modes in a single forensic workflow [14]. When sensitivity matters most, Aurora Ultimate™ 25×75 and Aurora Frontier™ geometries deliver the critical measurements for detecting attomole-level mutant peptides in urinary extracellular vesicles, quantifying HLA neoantigens at copy-number depth, and performing high-plex reporter quantification [8, 9, 10, 15].

IonOpticks works directly with targeted proteomics groups worldwide at academic medical centres, pharmaceutical discovery and DMPK laboratories, CROs, government and defence laboratories, and clinical diagnostics programmes to configure the right column, cassette, heating and source interface for each instrument ecosystem, including custom geometries when applications demand them.

Contact your IonOpticks representative to discuss assay development, method transfer, or evaluation columns for your targeted proteomics workflow.

05 References

References 1–3 establish foundational context for the targeted proteomics workflows described. References 4–11 and 14-17 are peer-reviewed publications that explicitly cite IonOpticks Aurora Series® columns in their methods sections. References 12 and 13 support reference 11.

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We work directly with targeted proteomics groups at academic medical centres, pharmaceutical discovery and DMPK laboratories, CROs, government and defence laboratories, and clinical diagnostics programmes to configure the right column, cassette, heating and source interface for each instrument ecosystem.

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