APTILA

APPLICATION NOTE
DIA-NN ENTERPRISE

24 NOVEMBER 2026

DIA-NN 2.8: major identification gains

Support for Hybrid-DIA and PRM

Release highlights

- Major identification gains, particularly for low sample amounts and in InfinDIA mode.
- Support for Hybrid-DIA, PRM and multi-CV FAIMS.
- A reimplemented GUI engine, with several-fold speed-ups for some QC and statistical workflows.

01 / IDENTIFICATION

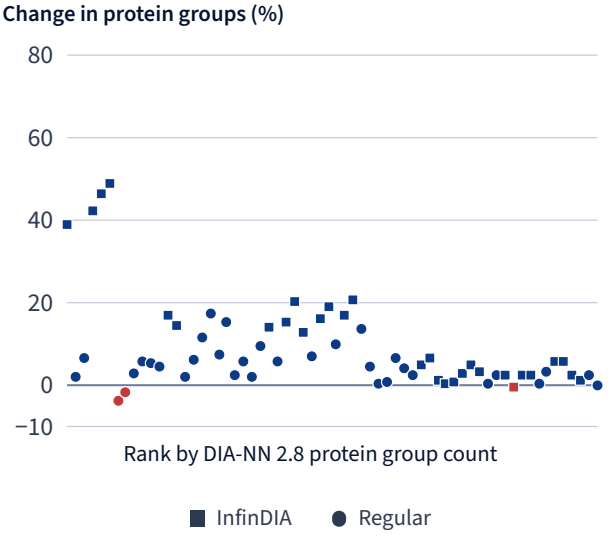
Largest gains in InfinDIA mode

DIA-NN 2.8 Enterprise demonstrates ~9% mean gain over 2.7 Enterprise and ~19% over 2.7 Academia in our internal test suite. In particular, we have introduced major improvements in the InfinDIA mode, which now achieves, in many cases, comparable identification depth to the regular mode while being capable of handling very large search spaces with ease (full-length non-specific digests, large numbers of PTMs, metaproteomics). Each point on the plots below is the number of protein groups at 1% global q-value, ordered by the DIA-NN 2.8 protein-group count.

DIA-NN 2.8 Enterprise vs 2.7 Enterprise

Figure 1. Gain between versions.

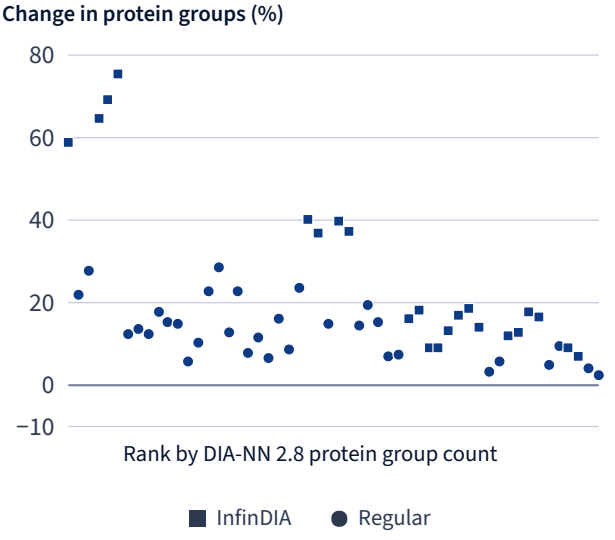
Mean gain	Median gain
+8.68%	+5.2%



DIA-NN 2.8 Enterprise vs 2.7 Academia

Figure 2. Gain including Enterprise-only features.

Mean gain	Median gain
+19.2%	+14.7%



02 / HYBRID-DIA

Scheduled targets and the proteome from the same acquisitions

Hybrid-DIA acquires scheduled PRM scans for selected targets alongside DIA scans. DIA-NN 2.8 processes both scan types together in an automated fashion, calibrating the PRM analysis based on the DIA data. Below, we reanalysed three 250 ng mouse embryonic fibroblast acquisitions from Sanner et al. [1], whose PRM scans targeted 103 lysosomal peptide precursors. The predicted library of the reviewed mouse proteome included all 103.

DIA-NN identifications required global and run-specific precursor q-values ≤1%.

Scheduled targets identified

98 / 103

precursors identified in PRM
in at least one acquisition

Identified in both DIA and PRM	**77**
Identified only in PRM	**+21**

Combined precursor coverage

83,298

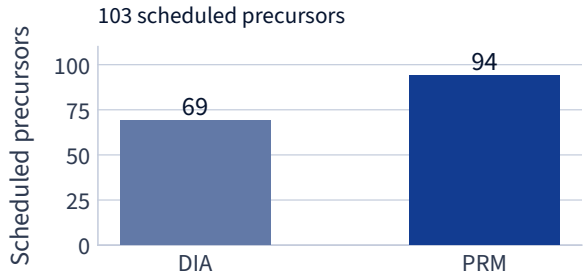
distinct precursors in DIA and PRM
across the three acquisitions

Identified in DIA	**80,586**
Identified only in PRM	**+2,712**

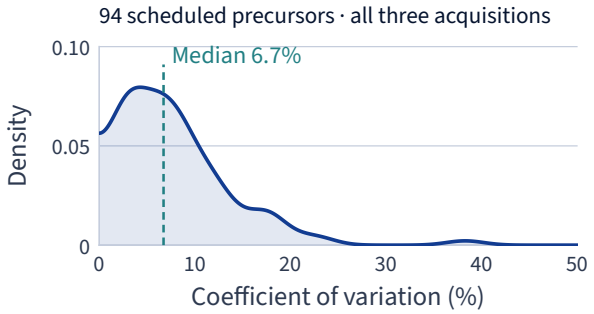
Figure 3. Every scheduled precursor identified in DIA was also identified in PRM. Of the 2,712 precursors identified only in PRM, 21 belonged to the scheduled panel; the other 2,691 were precursors co-isolated in the PRM windows, identified by searching the full library.

Figure 4. PRM identified 25 more scheduled precursors than DIA in all three acquisitions, at similar precision: median coefficient of variation 6.7% for 94 precursors in PRM and 6.1% for 69 in DIA.

Identified in all three acquisitions



Variation in PRM quantities



02 / HYBRID-DIA

Fragment evidence behind the identifications

DIA-NN's XIC export saves the fragment ion and MS1 chromatograms supporting each identification, enabling identifications and quantities to be inspected directly if needed. The exported fragment chromatograms below show examples of what the PRM scans can add: less interference for a scheduled target, and an unscheduled precursor that was not identified from the DIA data alone.

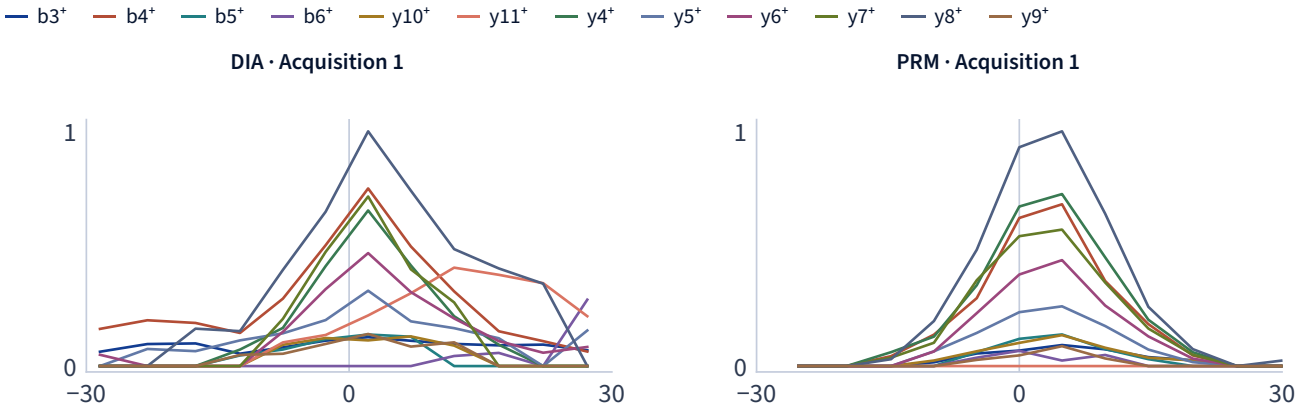
Figure 5.

Same scheduled precursor · DIA and PRM

Intensity relative to panel maximum
Time from PRM retention time (s)

Progranulin (Grn) · DSAGVWACCPYLK 2+ · Cys: propionamide

The same fragment ions from the DIA and PRM scans of the first acquisition, on a common time axis. PRM isolation windows are 1.2 m/z wide, against 24.1 m/z for DIA.

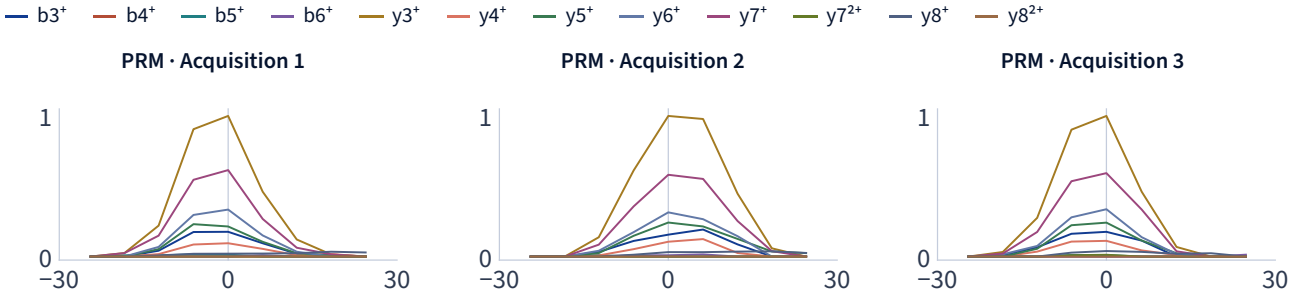


PRM · outside the scheduled panel

Intensity relative to panel maximum
Time from reported retention time (s)

MAP kinase 3 (Mapk3) · NYLQSLPSK 2+

A co-isolated precursor that was not scheduled, identified at 1% q-value from the PRM scans of all three acquisitions and from none of their DIA scans.



03 / STANDALONE PRM

In PRM, DIA-NN reports both scheduled and co-isolated precursors, in each case using target-decoy FDR control

We reanalysed internal-standard-triggered PRM data from Kennedy et al. [2] A 50 µg MCF10A human-cell digest was spiked with heavy peptide standards for a 5,176-precursor assay and separated by high-pH reversed-phase chromatography; the 96 collected fractions were pooled into six (F01–F06). In each LC–PRM injection, detection of a heavy standard triggers acquisition of the corresponding light peptide. We analysed one injection from each fraction.

DIA-NN 2.8 searched the PRM scans against a full predicted library together with the assay panel. The identifications outside the panel need no additional acquisition time: they come from the scans acquired for the assay.

Identifications required global and run-specific precursor q-values ≤1%; light assay precursors also required channel q-values ≤1%. Counts refer to light assay precursors and unlabelled precursors outside the assay; heavy standards are excluded.

Distinct precursors across six fractions

3,340 assay panel precursors + **6,714** outside the assay panel = **10,054** total light precursors

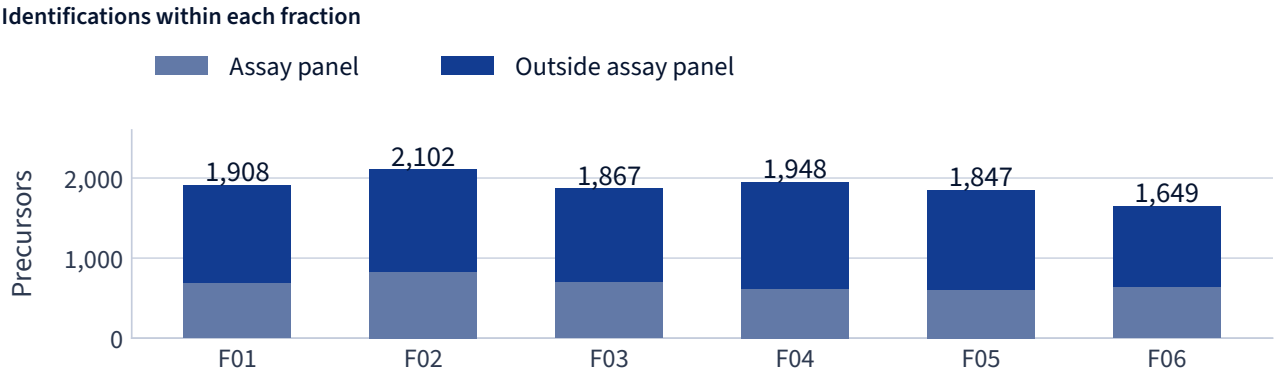


Figure 6. Of the 6,714 additional precursors, 6,636 have sequences outside the assay panel; 78 are other charge or modification states of panel sequences.

04 / METHODS

Analysis methods and sources

Hybrid-DIA acquisition and library

Sanner et al. acquired the Hybrid-DIA data on an Orbitrap Fusion Lumos with a 120 min LC gradient, 36 DIA windows of 24.1 m/z and scheduled PRM windows of 1.2 m/z. [1] The mouse library was generated from 17,283 reviewed UniProt sequences downloaded on 18 October 2026. Propionamide (C) was fixed. Five scheduled precursors from the deposited Skyline document were added, giving 3,035,972 precursors.

DIA-NN 2.8 predicted the mouse library using its deep learning retention time (RT) prediction model fine-tuned for propionamide-modified peptides. Fine-tuning used an empirical library generated with InfinDIA from the DIA scans of the first Hybrid-DIA acquisition.

Standalone PRM acquisition and libraries

Kennedy et al. used an Orbitrap Eclipse with 1.0 m/z isolation and resolution 7,500 for heavy-standard scans, and triggered light-peptide scans at 0.7 m/z and resolution 60,000. [2] DIA-NN 2.7.0 predicted libraries from 20,422 reviewed human UniProt sequences (2023_02) and 6,067 reviewed yeast sequences downloaded on 19 October 2026. Carbamidomethyl (C) was fixed.

The complete library combined 4,535,500 human and yeast precursors with the assay panel. Assay sequences, charges, cysteine modifications and isotope-label positions came from the deposited Skyline document; spectra and RTs were predicted with DIA-NN 2.7.0. We analysed the third injection of each of the six fractions from the study's 100% MCF10A sample (undiluted MCF10A human-cell digest).

Library generation and analysis

Library prediction used Trypsin/P, up to two missed cleavages, peptide lengths of 7–30 residues, charges 2–3, precursor m/z 350–1250 for mouse and human or 300–1500 for yeast, and fragment m/z 150–2000. N-term M excision was enabled.

DIA-NN 2.8.0 Enterprise analysed the three Hybrid-DIA acquisitions jointly and the six standalone PRM fractions jointly, using their respective full libraries, Knowledge base, match-between-runs and IDs, RT & IM profiling. Hybrid-DIA processing used QuantUMS. [3]

References

1. Sanner A et al. *Data-Independent Acquisition–Parallel Reaction Monitoring Acquisition Reveals Age-Dependent Alterations of the Lysosomal Proteome in a Mouse Model of Metachromatic Leukodystrophy.* Anal Chem. 2024;96:19567–19575. doi:10.1021/acs.analchem.4c04378. Data: PXD052313.
2. Kennedy JJ et al. *Internal Standard Triggered-Parallel Reaction Monitoring Mass Spectrometry Enables Multiplexed Quantification of Candidate Biomarkers in Plasma.* Anal Chem. 2022;94:9540–9547. doi:10.1021/acs.analchem.1c04382. Data and Skyline analysis: Panorama Public.
3. DIA-NN 2.8.0 Enterprise user guide. *PRM and hybrid-DIA; --hybrid; Fine tuning prediction models.*