

# Complementary fragmentation modes enhance endogenous peptidomics analyses on the ZenoTOF 8600 system

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This technical note demonstrates a high-sensitivity workflow for deep characterization of colon cancer cell-derived peptidomes using the ZenoTOF 8600 system, with in-depth data interpretation in PEAKS Studio software. At a sample loading of 100 ng per injection, joint analysis of complementary CID, EAD and EAciD acquisitions yielded more than 38,000 peptide identifications, including >21,600 database-matched peptides and >16,460 de novo-only peptide candidates. The distinct, sequence-informative fragmentation generated by CID, EAD and EAciD extends coverage beyond CID alone, revealing low-abundance, unknown and analytically challenging peptides while strengthening sequence interpretation and identification confidence. For quantitative profiling, ZT Scan DIA continuously scans the quadrupole isolation window across the m/z range to broaden precursor sampling and deepen MS/MS coverage, enabling reproducible and reliable quantitative performance. Together, high sensitivity, complementary fragmentation and comprehensive DIA workflows on the ZenoTOF 8600 system provide information-rich solutions for rapid, deep and confident characterization of complex endogenous peptidomes.

## Key features of endogenous peptidomics on the ZenoTOF 8600 system

- **High sensitivity with substantially enhanced detection capability:** More than 38,000 peptides can be identified from approximately 100 ng of sample on the ZenoTOF 8600 system, demonstrating its powerful detection capability.
- **Versatile fragmentation modes with strong complementarity:** CID and EAD provide complementary peptide sequence and modification information. When combined as EAciD, the workflow enables more complete sequence coverage for complex peptides, including large and multiply modified peptides. The synergistic use of multiple fragmentation modes markedly improves the completeness of peptide sequence interpretation and identification confidence.
- **Powerful, intuitive data analysis ecosystem:** PEAKS Studio software supports both de novo sequencing and database searching, enhancing identifications and enabling high-accuracy matching and functional interpretation of complex peptidomics datasets.

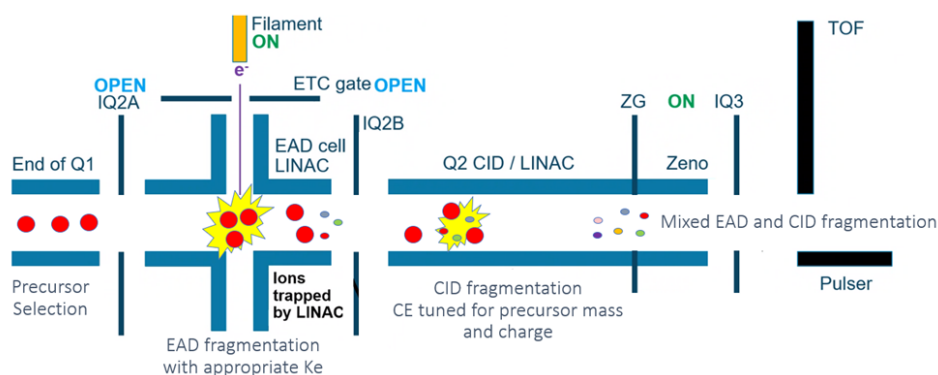


**Figure 1. Schematic representation of EAciD MS/MS fragmentation on the ZenoTOF 8600 system.** Following electron activated dissociation (EAD), unfragmented precursors can undergo collision-induced dissociation (CID) in the same scan cycle, resulting in MS/MS spectra with a broader range of fragment ion types to further increase identification confidence.

## Introduction

Peptidomics is a rapidly emerging frontier branch of omics research. Unlike conventional proteomics, which primarily focuses on the identification and quantitation of proteins, peptidomics emphasizes the systematic characterization of endogenous peptides. These peptides are derived mainly from specific protein cleavage events, degradation processes, or post-translational processing and therefore exhibit highly diverse biological functions. Thus, peptidomics is not only an important complement to proteomics but also serves as a critical bridge between the proteome and metabolome, helping to comprehensively elucidate molecular networks and regulatory mechanisms in biological systems at multiple levels. At the same time, peptidomics shows considerable potential in disease mechanism research and clinical translation. Because peptide molecules are more sensitive to physiological changes, dynamic alterations in peptide expression profiles often occur earlier than changes at the protein level, giving peptides unique advantages as biomarkers for early disease diagnosis. Within the framework of precision medicine, peptidomics-based screening of candidate molecules with specific biological activities can further provide innovative targets and support the development of peptide therapeutics [1-2].

The ZenoTOF 8600 system features significant innovations in fragmentation technology by integrating two complementary mechanisms within a QTOF system, collision-induced dissociation (CID) and electron activated dissociation (EAD), and further incorporating the hybrid fragmentation mode EAciD. CID efficiently generates b/y ions, providing stable backbone information for peptide sequencing, whereas EAD generates c/z ions through a gentler fragmentation process, helping to preserve post-translational modification (PTM) information and improve the accuracy of modification localization [3]. By integrating the fragmentation characteristics of EAD and CID within a single analysis, EAciD delivers higher sequence coverage and richer structural information, thereby substantially enhancing the ability to identify complex and modified peptides with greater confidence. These alternative fragmentation strategies, combined with Zeno trap technology to enhance fragment-ion sensitivity, enable the ZenoTOF 8600 system to improve the quality of peptide fragmentation information, making the in-depth analysis of low-abundance endogenous peptides possible.

## Methods

**Sample preparation:** Approximately  $3 \times 10^7$  HCT116 or Colo320 cell pellets were collected and lysed with 600  $\mu$ L of lysis buffer mix containing 8 M urea, 1 $\times$  protease inhibitor cocktail [Roche, 04693116001], and 1 $\times$  phosphatase inhibitor cocktail [Roche, 04906845001]. The cells were homogenized to achieve complete lysis, and the supernatant was collected for subsequent analysis. The protein lysate was separately loaded onto centrifugal ultrafiltration devices with molecular-weight cutoffs of 10 kDa and 3 kDa [Millipore], followed by centrifugation at  $13,000 \times g$  for 40 min. Fractions corresponding to molecular-weight ranges of 3–10 kDa and <3 kDa were collected for subsequent analysis. Reduction and alkylation were performed on the collected fractions using 10 mM TCEP and 40 mM IAA, respectively. A two-step enzymatic digestion was then performed with trypsin at protein-to-enzyme ratios of 1:50 (w/w, 4 h) and 1:100 (w/w, 12 h). Following digestion, the resulting peptides were desalted using SOLA $\mu$  SPE well plates according to the manufacturer's instructions and subsequently subjected to LC-MS/MS analysis.

**Chromatography:** Chromatographic separation was performed using an Evosep Eno LC system with the Whisper Zoom 20 SPD method. An IonOpticks Aurora Gen3 Elite XS C18 analytical column (15 cm  $\times$  75  $\mu$ m) was used, and the column temperature was set to 65°C. Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in acetonitrile.

**Mass spectrometry:** Data acquisition was performed on a ZenoTOF 8600 system. Table 1 shows key parameters of CID, EAD or EAciD data-dependent acquisition (DDA) experiments. ZT Scan DIA acquisition was performed using CID fragmentation with dynamic collision energy, a Q1 precursor isolation width of 5 Da, and a cycle time of 1.5 sec.

**Data processing:** DDA data [CID, EAD, and EAciD] were processed using PEAKS Studio software [v13.5]. A human protein FASTA database downloaded from UniProt was selected, and data analysis was performed using the Proteome workflow. Carbamidomethylation of cysteine was specified as a fixed modification. After de novo sequencing, database searching

Table 1: DDA acquisition parameters for CID, EAD, and EAciD modes.

| Parameter                      | CID          | EAD          | EAciD        |
|--------------------------------|--------------|--------------|--------------|
| Acquisition mode               | Positive     | Positive     | Positive     |
| Ion spray voltage              | 2250 V       | 2250 V       | 2250 V       |
| Gas 1                          | 10 psi       | 10 psi       | 10 psi       |
| Curtain gas                    | 35 psi       | 35 psi       | 35 psi       |
| CAD gas                        | 7            | 7            | 7            |
| Source temperature             | 250 °C       | 250 °C       | 250 °C       |
| Acquisition range              | 100–1,800    | 100–1,800    | 100–1,800    |
| MS1 accumulation time          | 0.1 s        | 0.1 s        | 0.1 s        |
| MS2 accumulation time          | 0.035 s      | 0.035 s      | 0.035 s      |
| Q Jet DP                       | 20 V         | 20 V         | 20 V         |
| Collision energy               | Dynamic      | 10 V         | Dynamic      |
| Electron kinetic energy        | n/a          | 7 eV         | 7 eV         |
| ETC                            | n/a          | Dynamic      | Dynamic      |
| Electron beam current          | n/a          | 5,500 nA     | 5,500 nA     |
| EAD reaction time              | n/a          | 30 ms        | 30 ms        |
| EAD RF                         | n/a          | 150 Da       | 150 Da       |
| Zeno trap                      | On           | On           | On           |
| Zeno threshold                 | 100,000      | 100,000      | 100,000      |
| Q1 resolution                  | Unit         | Unit         | Unit         |
| Maximum candidate ions         | 40           | 40           | 40           |
| Cycle time                     | 1.7 s        | 1.7 s        | 1.7 s        |
| Dynamic background subtraction | On           | On           | On           |
| Exclude former target ions     | 4 s after 2x | 4 s after 2x | 4 s after 2x |
| Charge-state selection         | 1 to 5       | 1 to 5       | 1 to 5       |

was performed to assign peptide sequences to protein entries. All other parameters were kept at their default settings. ZT Scan DIA data were processed using DIA-NN [version 1.9.1] [4]. Direct DIA searches were performed with a human protein FASTA database downloaded from UniProt. Unless otherwise specified, default settings were used. The precursor m/z range was set to 400–900 Da, and the fragment ion m/z range was set to 140–1750 Da. Triplicate ZT Scan DIA datafiles for a given sample were searched together, with match between runs (MBR) enabled. Protein groups and precursors identified and quantified [with CV<20%] were determined from the output “pg\_matrix.tsv” and “pr\_matrix.tsv” files.

## Protein and peptide identifications by IDA using multiple MS/MS fragmentation modes

For each sample, a 100 ng injection was analyzed using CID, EAD, and EAciD DDA. The three datasets were jointly processed by de novo sequencing and database searching in PEAKS Studio to maximize peptide and protein identifications. Sample preparation significantly affected identification performance [Table 2], with effects from both enzymatic digestion and ultrafiltration cutoff. The 3–10 kDa fraction provided the greatest depth, identifying approximately 2,500 proteins and more than 38,000 peptides regardless of digestion status and supporting its suitability for endogenous peptide enrichment.

Many peptide candidates remain unmatched by conventional database searching, making de novo sequencing critical for direct sequence inference from MS/MS spectra. PEAKS Studio identified numerous de novo-only peptides [Table 2]. The example peptide VKEKTHLNLVVL showed extensive fragmentation coverage under EAciD fragmentation (Figure 2), illustrating the enhanced sequence information provided by this hybrid mode. Interestingly, this peptide aligns to a segment of eukaryotic elongation factor 1 alpha 2 protein [VKEKTHINIVVI], which is an oncogenic protein known to be abnormally upregulated in colorectal cancer, promoting tumor growth, cell proliferation, and metastasis [5]. Venn diagram analysis showed that EAD and EAciD substantially expanded de novo-only peptide coverage relative to CID (Figure 3); in the digested Colo320-derived 3–10 kDa sample, they identified an additional 8,531 peptides not detected by CID. These findings highlight the value of complementary fragmentation for characterizing low-abundance and unknown peptides and more deeply exploring the hidden peptidome.

Table 2. Proteins and peptides identified from each sample.

| Sample         | Ultrafiltration fraction | Digestion  | Protein IDs<br>Database search | Peptide IDs<br>Database search | De novo-only<br>peptide IDs<br>[ALC >70%] | Total peptide IDs |
|----------------|--------------------------|------------|--------------------------------|--------------------------------|-------------------------------------------|-------------------|
| HCT116_3K_D    | <3 kDa                   | Digested   | 216                            | 549                            | 5303                                      | 5852              |
| colo320-3-10_N | 3–10 kDa                 | Undigested | 2339                           | 30363                          | 6405                                      | 36768             |
| colo320-3-10_D | 3–10 kDa                 | Digested   | 2547                           | 21646                          | 16460                                     | 38106             |

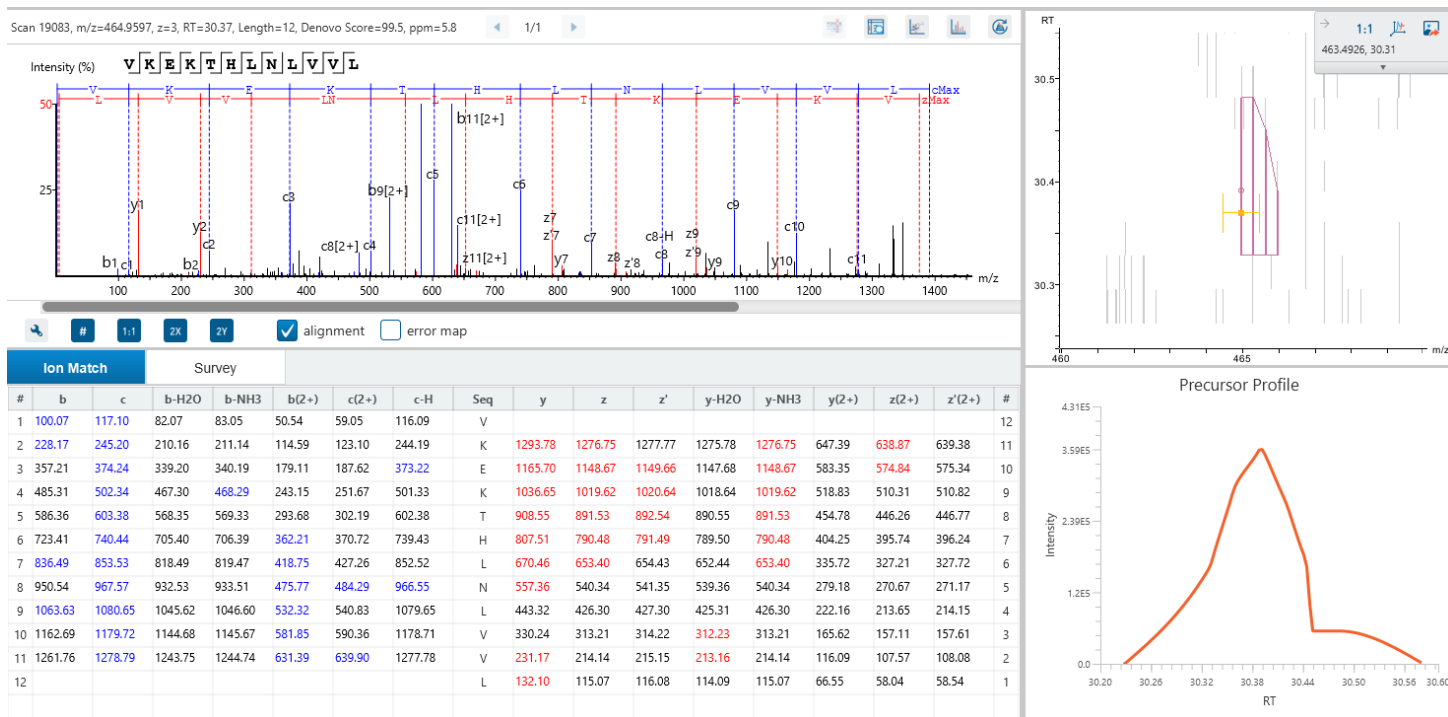


Figure 2. MS/MS spectrum of peptide VKEKTHLNLVL identified by EAcid. PEAKS Studio software shows complete sequence coverage for this peptide, with MS/MS ion matching (left). The precursor isotope distribution and extracted ion chromatogram are also shown (right).

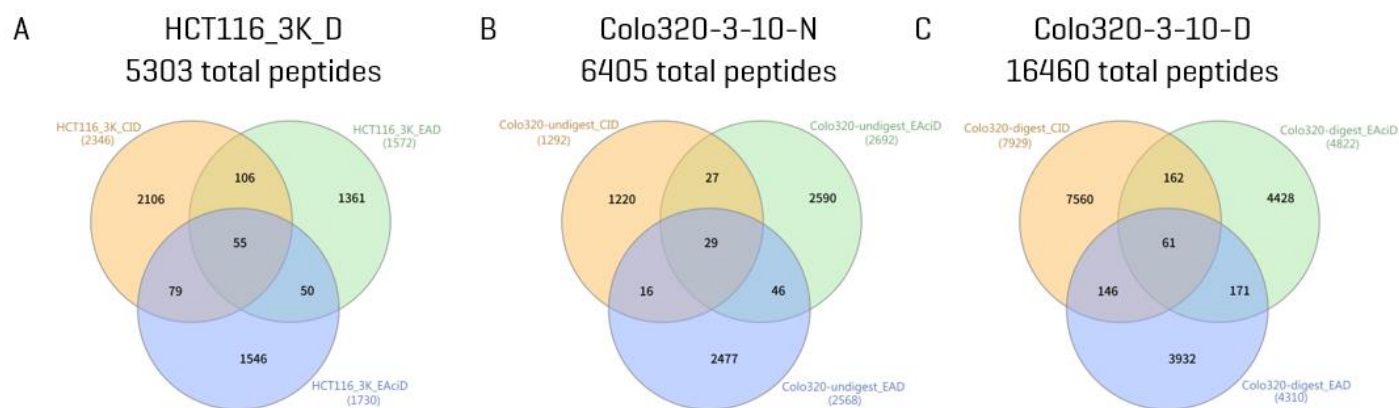


Figure 3. Venn diagrams of de novo only peptide identifications across samples, comparing CID, EAD and EAcid. [A] HCT116\_3K\_Digested; [B] Colo320\_3-10 kDa\_Digested; [C] Colo320\_3-10 kDa\_Undigested.

## Protein and peptide identifications by IDA using ZT Scan DIA

ZT Scan DIA is a method that combines the Zeno trap and a continuously scanning quadrupole for precursor isolation, improving both qualitative and quantitative performance relative to discrete-window DIA methods, i.e., Zeno SWATH DIA [6]. To enable high-throughput qualitative and quantitative analysis, the identification capability and quantitative performance of ZT Scan DIA with CID fragmentation were evaluated. Data acquired from the digested Colo320 3–10 kDa sample using ZT Scan DIA were analyzed with DIA-NN 1.9.1. As shown in Figure 4, ZT Scan DIA identified a total of 2,489 protein groups, of which 93.2% were reproducibly detected across three replicate injections. In addition, 80.4% of quantified proteins exhibited CV values below 20%, indicating excellent reproducibility and quantitative reliability. At the precursor level, reproducibility across the three replicate injections was 80.4%, and 61.8% of precursors exhibited CV values below 20%, meeting commonly accepted quality-control criteria for quantitative proteomics.

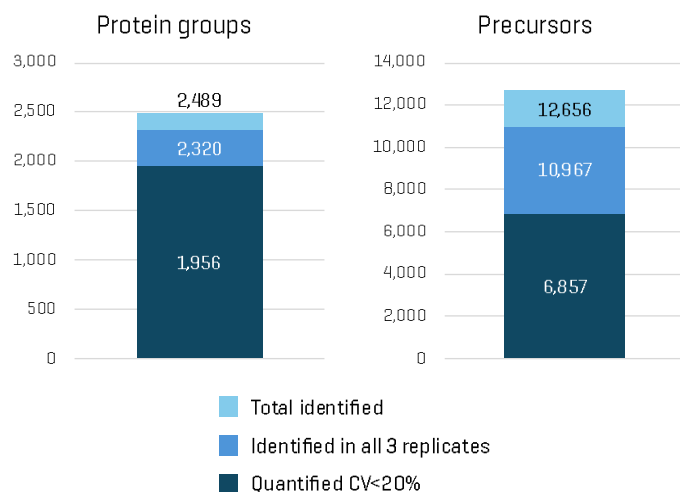


**Figure 4. Protein groups and precursors identified and quantified using ZT Scan DIA.** A total of 2,489 protein groups and 12,656 precursors were identified. More than 80.4% of protein groups and 61.8% of precursors had CV values below 20% across triplicate injections.

## Conclusions

- In-depth endogenous peptidome characterization was achieved on the SCIEX ZenoTOF 8600 platform by integrating multiple fragmentation strategies, including collision-induced dissociation [CID], electron activated dissociation [EAD], and the hybrid EAciD mode, together with an advanced data analysis workflow.
- The complementary fragmentation behavior of EAD and CID, particularly in EAciD mode, significantly improved peptide sequence coverage and enabled more reliable identification of complex and modified endogenous peptides. The hybrid fragmentation approach generated more information-rich MS/MS spectra and supported more complete sequence coverage for representative peptides.
- The high sensitivity of the platform enabled the identification of more than 38,000 peptides and approximately 2,500 proteins from only 100 ng of sample, demonstrating its strong capability for detecting low-abundance peptide species.
- ZT Scan DIA acquisition provided robust and reproducible quantitative performance, with protein group-level reproducibility exceeding 93% and most quantified protein groups and precursors showing coefficients of variation [CVs] below 20%. These results support the use of this workflow as a reliable technical foundation for comprehensive identification and accurate quantitation in complex peptidomics studies.

## References

1. Tran, N.H. et al. [2019]. Deep learning enables de novo peptide sequencing from data-independent acquisition mass spectrometry. [Nature Methods, 16:63-66.](#)
2. Shi, C., et al. [2025]. Comprehensive discovery and functional characterization of the nanocanonical proteome. [Cell Research, 35:186-204.](#)
3. Improving complex phosphopeptide characterization with hybrid EAD/CID MS/MS fragmentation. [SCIEX technical note, MKT-29611-A.](#)
4. Demichev, V., et al. [2020]. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. [Nature Methods, 17:41-44.](#)
5. <https://www.proteinatlas.org/ENSG00000101210-EEF1A2/cancer>.
6. Continuing the data independent acquisition revolution: Introducing ZT Scan DIA for quantitative proteomics. [SCIEX technical note, MKT-31819-A.](#)

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