APPLICATION NOTE

ACD/LABS [ADVANCED CHEMISTRY DEVELOPMENT, INC.]

Advanced Data Analysis of Peptide LC-MS Spectra through *In Silico* Fragmentation

Anne Marie Smith
Artyom Petrovskiy
Vitaly Lashin

Peptides play essential roles in biological systems and medicine, acting as hormones, neurotransmitters, and signaling molecules. While mass spectrometry (MS) is a key tool for peptide analysis, manually assigning fragment ions can be time-consuming and challenging, especially for complex structures. This study presents an *in-silico* fragmentation approach using *vendor neutral* software that enhances MS spectral interpretation by applying established fragmentation rules and automating spectral annotation—dramatically reducing manual effort and increasing analytical efficiency.

Methods

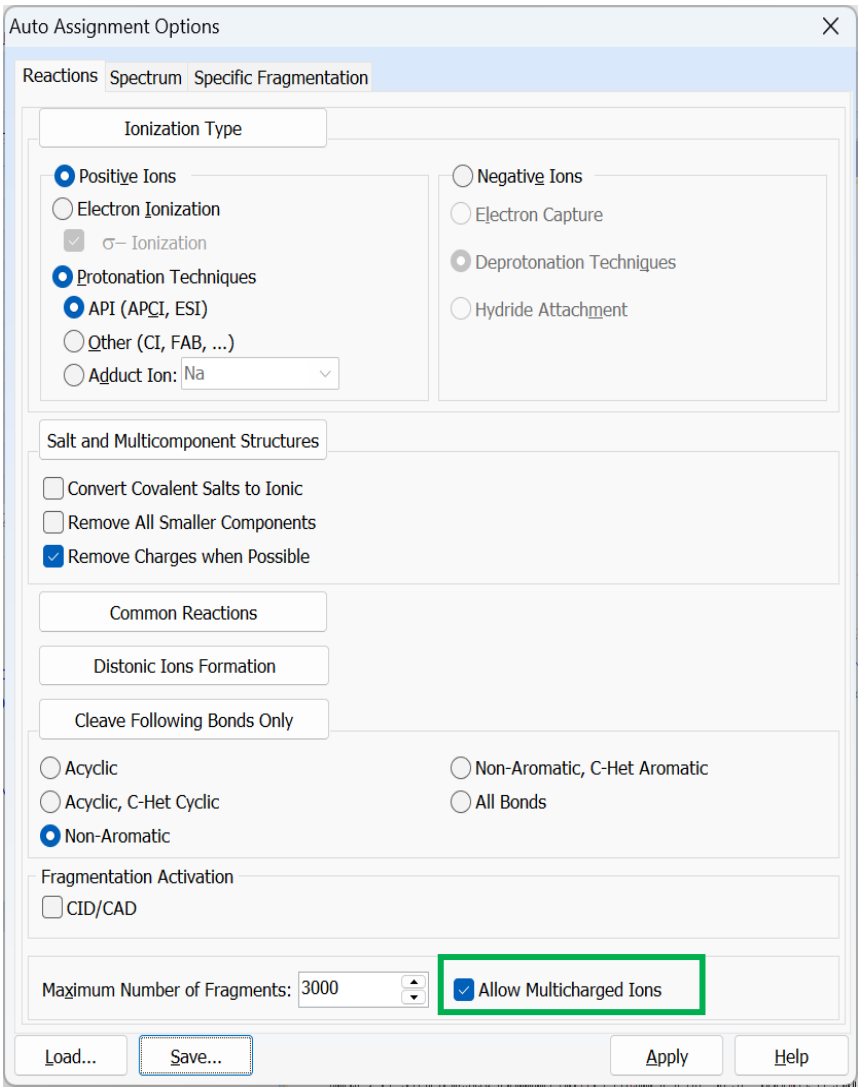
High resolution MS data was obtained from open source data[1] using a linear ion trap coupled to either an ion cyclotron resonance (ICR) or orbitrap analyzer. For LC/MS peptide analysis, ACD/Labs' vendor neutral software, MS Workbook Suite was used to streamline data interpretation by leveraging peptide-specific fragmentation rules—automatically assigning a, b, c, x, y and z ions.

Features within the software include:

- **In Silico Fragmentation**—Enhanced rules for multiply charged species and amide/disulfide bond fragmentation

- **Spectral Annotation**—Automatically label a, b, c, x, y, and z ions, linked to structural visualization

- **Assignment Scoring**—Match theoretical fragments with experimental peaks to generate a spectrum assignment score


Auto Assignment Options
Reactions
Spectrum
Specific Fragmentation
Ionization Type
Positive Ions
Electron Ionization
σ– Ionization
Protonation Techniques
API (APCI, ESI)
Other (CI, FAB, ...)
Adduct Ion:
Na
Negative Ions
Electron Capture
Deprotonation Techniques
Hydride Attachment
Salt and Multicomponent Structures
Convert Covalent Salts to Ionic
Remove All Smaller Components
Remove Charges when Possible
Common Reactions
Distonic Ions Formation
Cleave Following Bonds Only
Acyclic
Acyclic, C-Het Cyclic
Non-Aromatic
Non-Aromatic, C-Het Aromatic
All Bonds
Fragmentation Activation
CID/CAD
Maximum Number of Fragments:
3000
Allow Multicharged Ions
Load...
Save...
Apply
Help

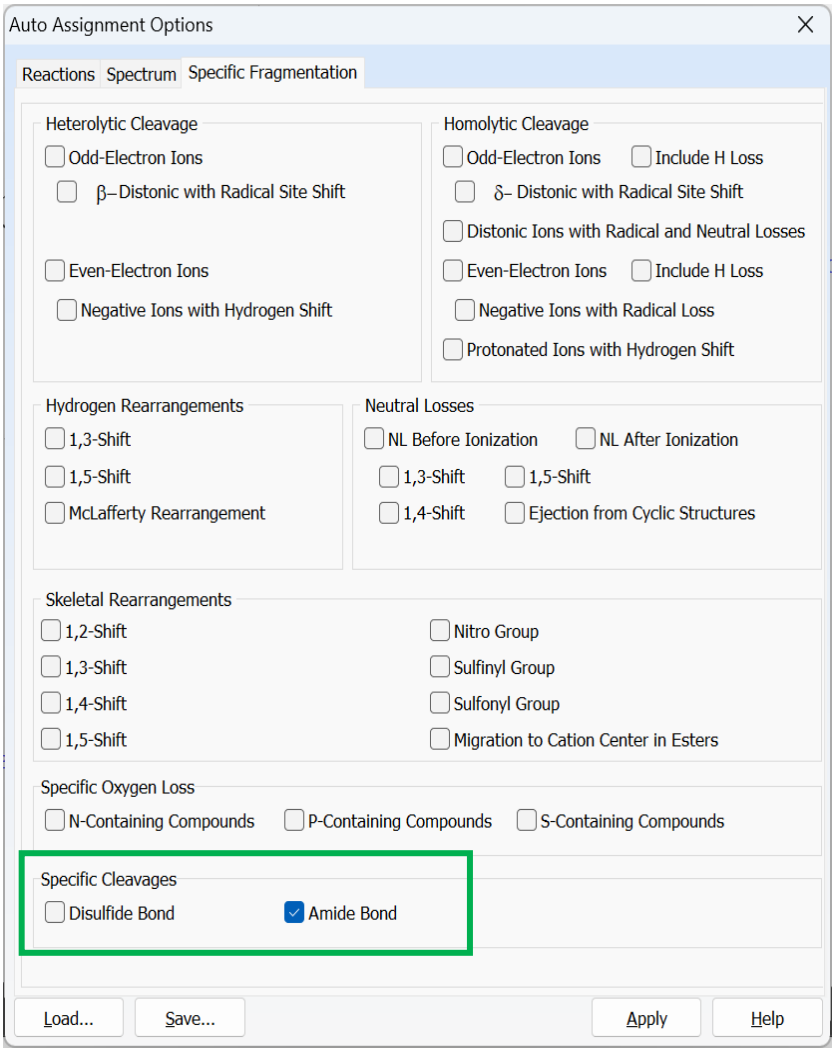


Figure 1: In Silico Fragmentation Settings – Overview of applied fragmentation rules, including the newly added Multicharged Ions, and Disulfide Bond and Amide Bond Specific Cleavages.

Key Findings

Application of the software to LC-MS data demonstrated:

Improved Fragment Assignment—Higher accuracy and score assigning MS/MS spectra to peptide structures—Assignment score rose from 28.7% to 84.5% with the inclusion of multicharged ions and new peptide fragmentation rules

Enhanced Visualization—Spectra annotated with labeled ion types and linked to structure to facilitate rapid interpretation. Formulae indicates if a multicharged ion is present.

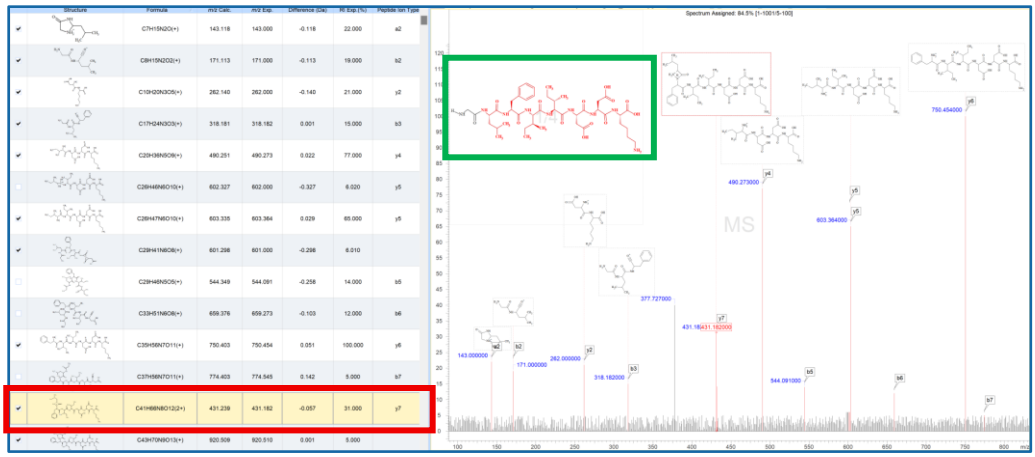


Figure 2 Annotated Spectrum with Linked Structure and Fragment Table: Selecting a row in the interactive fragment table highlights the corresponding ion on both the spectral structure (green box) and the m/z spectrum. In this example, a doubly charged (2+) ion—automatically assigned using newly implemented fragmentation rules—is emphasized (red box), demonstrating the enhanced capabilities for detecting higher charge states

Isomer Differentiation—Analyze multiple structures simultaneously. Scoring helps distinguish isomeric peptides and identify the correct structure.

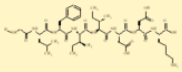
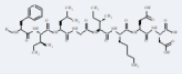
No.	Name	Structure	Formula	FW	Monoisotopic Ma...	[M+H]+	Spectrum Assigned (%)
1	GLFIIDDK		C43H69N9O13	920.075	919.501	920.509	84.5% [1-1001/5-100]
2	FILGIKDD		C43H69N9O13	920.075	919.501	920.509	41.2% [1-1001/5-100]

Figure 3 Example of structural isomers with different peptide backbones. Different assignment scores illustrate the utility of the software in identifying the correct isomer.

Automated Reporting—Configurable reporting engine streamlines data analysis, reducing manual effort. Build reports that include Table of Fragments with peptide ion type, Table of Structures with spectrum assignment score, assigned spectrum with fragment assignment, fragmentation scheme, and more.

ACD/Labs

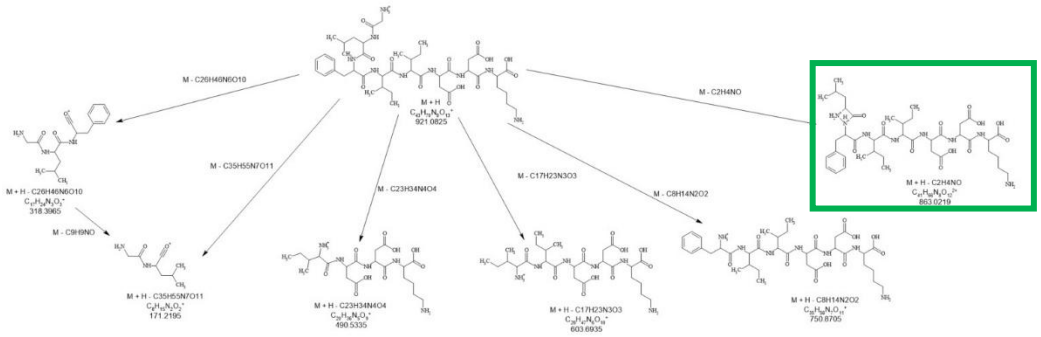


Figure 4 Customize fragmentation reporting. Multiply-charged ions (e.g., 863.0219 m/z) are now included and highlighted (green box).

Every Vendor in One Platform—Why it Matters

MS Workbook Suite supports data formats from most major instrument vendors, offering flexibility in choosing the optimal instrument for your analysis. All xC/UV/MS data can be seamlessly processed within a single interface, ensuring:

- Seamless integration between raw data and numerical results
- Centralized storage and visualization of data alongside its chemical context and analytical outcomes
- Consistent and standardized data processing across different instrument platforms