FlashNote

# Multimodal Fragmentation of Cyclic Peptides with timsOmni

The timsOmni™ platform enables efficient ring opening and sequencing of cyclic peptides through combined collision and electron based fragmentation, while OmniScape™ provides dedicated processing tools for confident structural elucidation of complex macrocycles.

## Challenge

Cyclic peptides present significant analytical hurdles due to their closed ring architecture and frequent incorporation of non canonical amino acids. Traditional CID based MS/MS struggles to generate clear b/y ion ladders, as ring structure prevents canonical N and C terminal fragmentation pathways. Multiple possible ring opening sites generate numerous linearized isomers, each capable of producing distinct fragment sets. As a result, fragmentation spectra are often complex, discontinuous, and difficult to map unambiguously. Moreover, backbone heterogeneity, hydrophobicity, and unusual side chain chemistries further complicate confident structural elucidation. Comprehensive characterization requires tools that can reproducibly linearize the sequence to generate interpretable $MS^n$ fragment and map them against all possible permutations.

## Solution

The timsOmni platform integrates resonant CID (rCID), collision cell CID (cCID), and electron based dissociation (ECD/EID) to overcome these challenges.

- **Efficient ring opening:** rCID and cCID reliably generate families of linearized precursors, enabling comprehensive backbone fragmentation.
- **Deep sequencing capability:** Multi modal $MS^n$ experiments yield rich, orthogonal fragment ladders that reveal ring opening pathways and support identification of non canonical residues.
- **Enhanced structural confidence:** ECD is particularly effective for doubly charged cyclic peptides, while EID enables high information fragmentation on singly charged species—critical for hydrophobic macrocycles.
- **OmniScape processing:** A dedicated workflow evaluates each spectrum against all linearized sequence permutations, assigns modifications, and maps c/z, a/x, and b/y fragments including highly modified residue and termini (Figure 1).

Together, timsOmni and OmniScape deliver near complete backbone coverage and confident structure assignment for complex cyclic peptides

## Comprehensive Cyclic Peptide Sequencing Demonstrated on Cyclosporin A

Using rCID on the timsOmni platform, Cyclosporin A was efficiently linearized into multiple sequence permutations. Subsequent $MS^2/MS^3$ experiments generated robust fragment ladders that enabled insight in residue level mapping across all candidate sequences.

### Electron Based Dissociation

ECD provided rich fragmentation for doubly charged species, while EID produced extensive cleavages for singly charged ions—an essential feature for hydrophobic cyclic peptides. Combined datasets increased structural depth and enabled confident discrimination among isomeric linearized forms.

### OmniScape Analysis

OmniScape evaluated all linearized permutations and termini modification scenarios, taking into account multiple side chain modifications (set as fixed). The best scoring match was selected identifying CSA6 and CSA11 as the most abundant linearized sequences. CSA5 was also observed and could effectively be disambiguated from CSA11 with an $MS^3$ experiment.

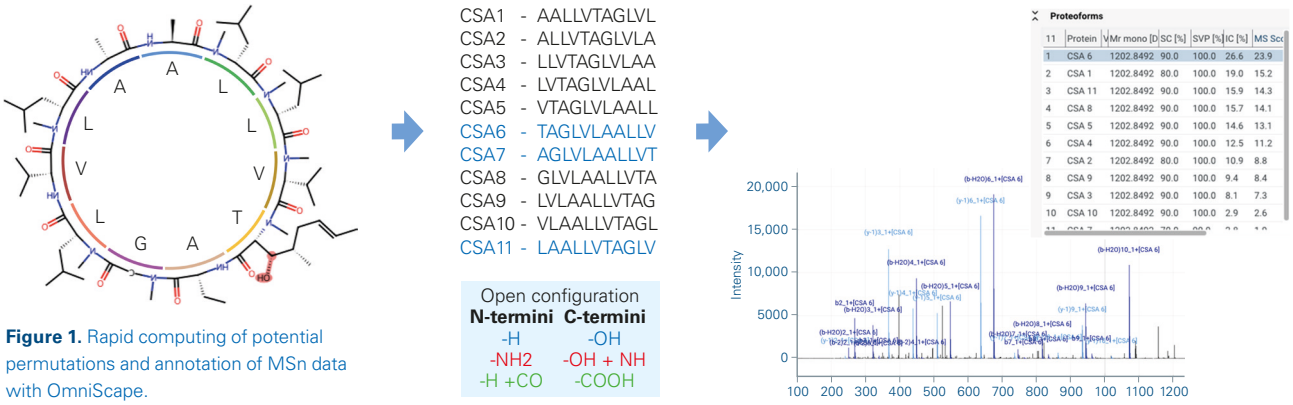


**Figure 1.** Rapid computing of potential permutations and annotation of MSn data with OmniScape.

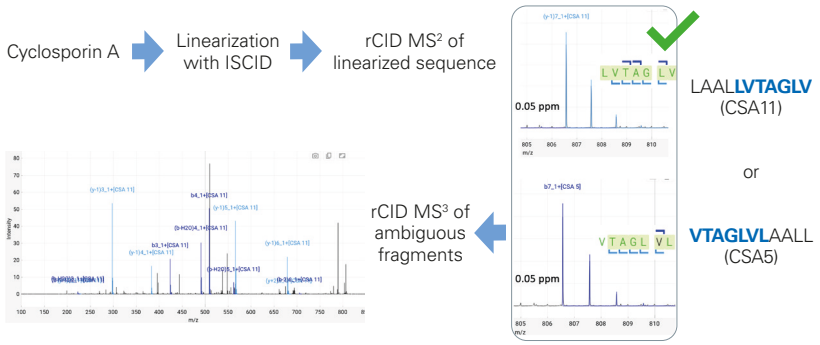


**Figure 2.** Use of MS3 for disambiguation of isobaric fragments. Here selected fragment is assigned to CSA11 sequence due to higher y-ion coverage than for CSA5, differing only by N or C-term position of Leucine

## Key Benefits

- Reliable cyclic peptide linearization
- Deep multi modal $MS^n$ fragmentation
- Accurate mapping of non canonical amino acids
- Automated sequence variant evaluation
- High confidence structural elucidation for discovery and QC workflows

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