Comprehensive sequencing of cyclic peptides by top-down MS on a timsOmni™ platform using next-generation fragmentation and OmniScape™ processing

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Introduction

Cyclic peptides are molecules abundant in nature with industrial applications ranging from drug development to food additives.

Synthetic methods have dramatically improved over the last few decades enabling pharmaceutical companies to expand the number of drug discovery projects investigating cyclic peptides. 39 cyclic peptide drugs have been approved globally since 2000.

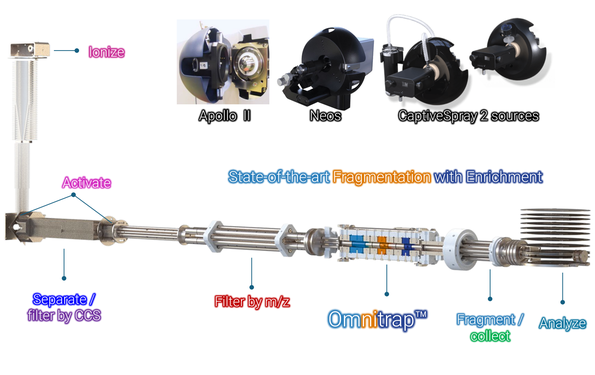
Structurally, cyclic peptides are modular polypeptide chains composed of canonical and non-canonical amino acids that are connected at distant positions to form macrocyclic structures. Large libraries can be easily created for biological screening.

Cyclic peptides' strengths include high antigen binding affinity and specificity, high proteolytic stability, and improved membrane permeation compared to non-cyclic peptides.

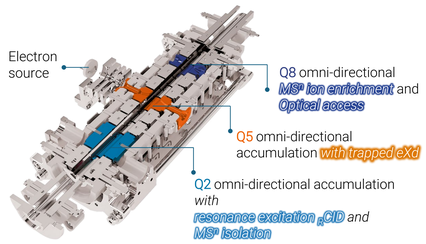
The unique timsOmni-OmniScape HW/SW combination excels in its ability to characterize cyclic peptides.

Instrumentation

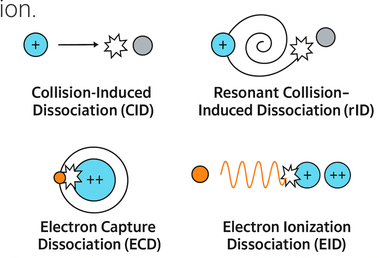
Schematic of a Bruker timsOmni™ IMS Q-Omnitrap™-ToF mass spectrometer. The Omnitrap cell with its electron source is highlighted below.



Cyclic peptides can be sprayed either in positive or negative ion mode. Following activation in the source, ion mobility separation occurs in the tims cartridge. The species of interest are then mass-selected before accumulation and fragmentation in the Omnitrap.



Fragmentation can involve various flavors of collision-induced dissociation as well as electron-based fragmentation.



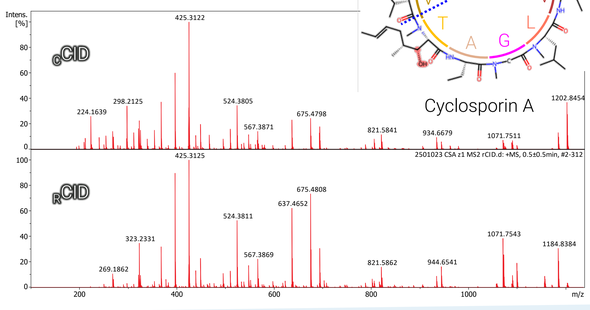
Following collection and conditioning in the collision cell with AIP, the precursor and its fragments are detected in the ToF.

Methods

The limited degree of flexibility and inherent strain in the backbone of cyclic peptides as well as the use of non-canonical amino acids precludes the use of proteases and methods commonly used for peptide sequencing. We use the timsOmni collision-based ($_C$CID and $_R$CID) fragmentation techniques to efficiently linearize and sequence cyclic peptides. Processing is performed in OmniScape.

Results

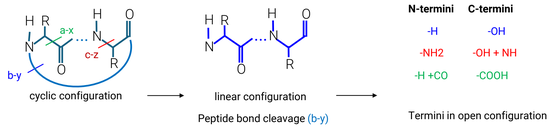
MS² xCID fragmentation spectra



Cyclosporin A

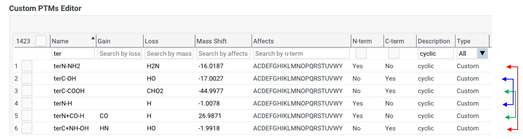
OSc processing accounting for all cleavage types

The analysis of 1 cyclic peptide of 11 amino acids potentially involves reviewing the fragmentation of 33 different linearized sequences:



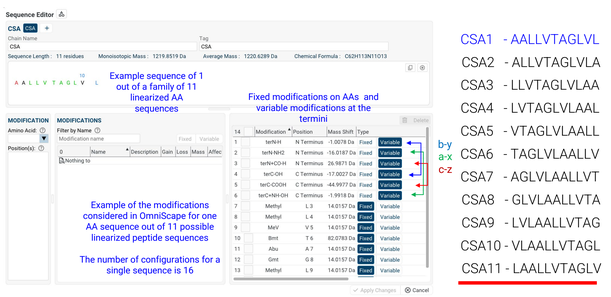
Water elimination upon cyclization can yield 3 alternative pairs of termini.

Modifications considered for the termini, as part of the linearization process



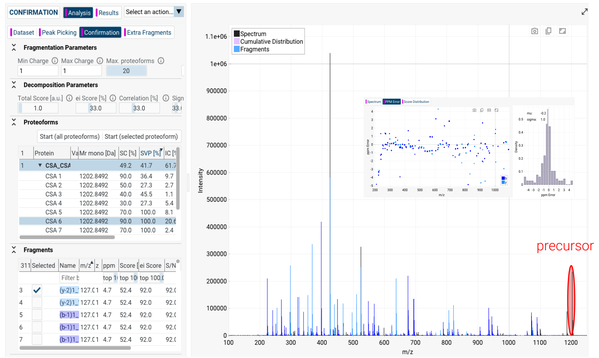
Systematic linearization of the cyclic peptides

All linearized sequences with modifications are populated enabling exhaustive comparison with the experimental data:



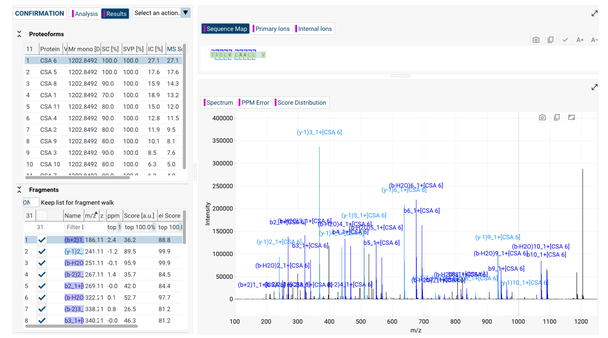
Beyond sequence coverage: full spectrum explanation

Linearized sequence screening provides an indication of which sequence best explains the data and if any major peak remains unexplained:



Confirmation and identification of main cleavage sites

Confirmation of assignments and location of preferential cleavage sites: (top next column)



Confirmation workflow involves using the linearized sequences originating from the same family of 11 sequences carrying a specific termini modification.

The best-scoring linearized sequence is CSA6, with 100% sequence coverage and only a single y cleavage missing.

TAGLV LAALL V

The second-best-scoring sequence (CSA5) yields 100% sequence coverage but does not include several b-type fragments which results in one less cleavage.

VTAGL VLAAL L

The third best-scoring sequence (CSA8) is also a good match, close to the top-scoring sequence.

GLVLA ALLVT A

Conclusion

The timsOmni–OmniScape (OSc) combination enables **a robust workflow for cyclic peptide analysis**, as shown for cyclosporin A, where the top-scoring linearized sequence is unambiguously identified.

OSc efficiently generates and evaluates all feasible linearized representations of a cyclic peptide, allowing **comprehensive exploration of the resulting sequence and fragment space** through PTM screening and confirmation workflows.

Sequence assignment is supported by multiple independent scoring metrics and by high-confidence annotation of fragment ions, providing **strong validation of the primary linearized sequence**.

Lower-abundance fragments attributable to alternative linearizations are detected and assigned, opening the possibility to **quantify the relative contribution of different fragmentation pathways** (e.g., branching ratios) within MS² spectra.

The **timsOmni–OSc combination is general and transferable** and has been successfully applied to additional cyclic peptide derivatives, demonstrating its broader applicability.

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Conflicts of Interest
JG, AS, MK, DS, GT, DP and MG are employees of Bruker, which develops and manufactures advanced mass spectrometry platforms for life science applications. This presentation includes data and findings related to Bruker's new timsOmni product.