PhD Studentship Proposal, Prof. Peter O'Connor, University of Warwick

Title: New approaches to mass spectrometry analysis of cyclic and bicyclic peptides.

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Cyclic peptides are common active compounds in nature, including Cyclosporin A, Gramicidin S, Valinomycin, conotoxins, defensins, cyclotides, octreotide, and Bacitracin. All of these compounds have potent and varied biological activities along with increased chemical stability compared to linear peptides due to their tightly folded structure and lack of termini which confer resistance to enzymatic degradation. Additionally, such naturally inspired cyclic peptides generally confer conformational rigidity, changes to membrane permeability, versatility in synthesis and modifications providing a diversity of chemical space, improvements in oral bioavailability, and mimicry of protein-protein interactions. From a pharmaceutical perspective, these properties make cyclic peptides attractive candidates for drug development, particularly for challenging targets that are not easily addressed by small molecules or large biologics. Their unique characteristics position them as a promising class of therapeutics in various areas, including oncology, immunology, and infectious diseases.

ANALYTICAL CHALLENGE. Despite their substantial interest and impact as pharmaceutically active compounds, cyclic peptides represents a significant unsolved analytical problem. The core of the problem is that tandem mass spectrometry fragmentation analysis of cyclic peptides requires at least two backbone cleavages. Since the first cleavage is (mostly) random, this event creates an isomeric mixture of ring-opened fragments which have not changed in mass from the precursor mass at all. Thus, the second cleavage generates a chimeric mixture of all fragments from each of those primary cleavages, generating an extremely complex mixture of fragment masses which are incredibly difficult to differentiate. Further molecular analysis challenges include:

Fragmentation complexity. Cyclic and bicyclic peptides have a closed-ring structure, which makes traditional linear peptide sequencing methods less effective. Their cyclic nature can lead to complex fragmentation patterns that are difficult to interpret using standard mass spectrometry techniques.

Lack of distinct termini. Unlike linear peptides, cyclic peptides lack distinct N- and C-termini, which are often used as reference points in sequencing. This absence complicates the process of determining the amino acid sequence.

Multiple fragmentation pathways. The cyclic structure allows for multiple fragmentation pathways, leading to a more complex mass spectrum that can be challenging to decipher.

Structural isomers. Cyclic peptides can have structural isomers with identical mass but different sequences or cyclization points, making it difficult to distinguish between them using mass alone.

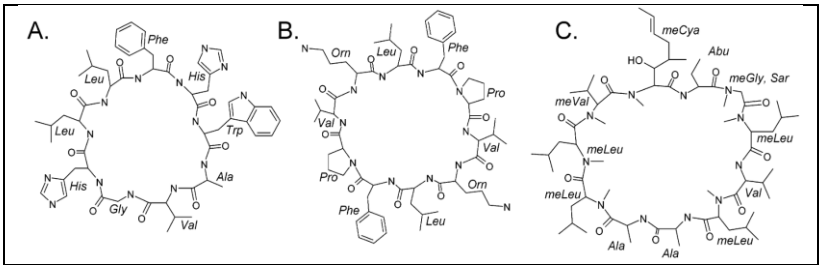

Figure 1. Cyclic peptide structures of three biologically active cyclic peptides including gramicidin S and Cyclosporin A.[1]

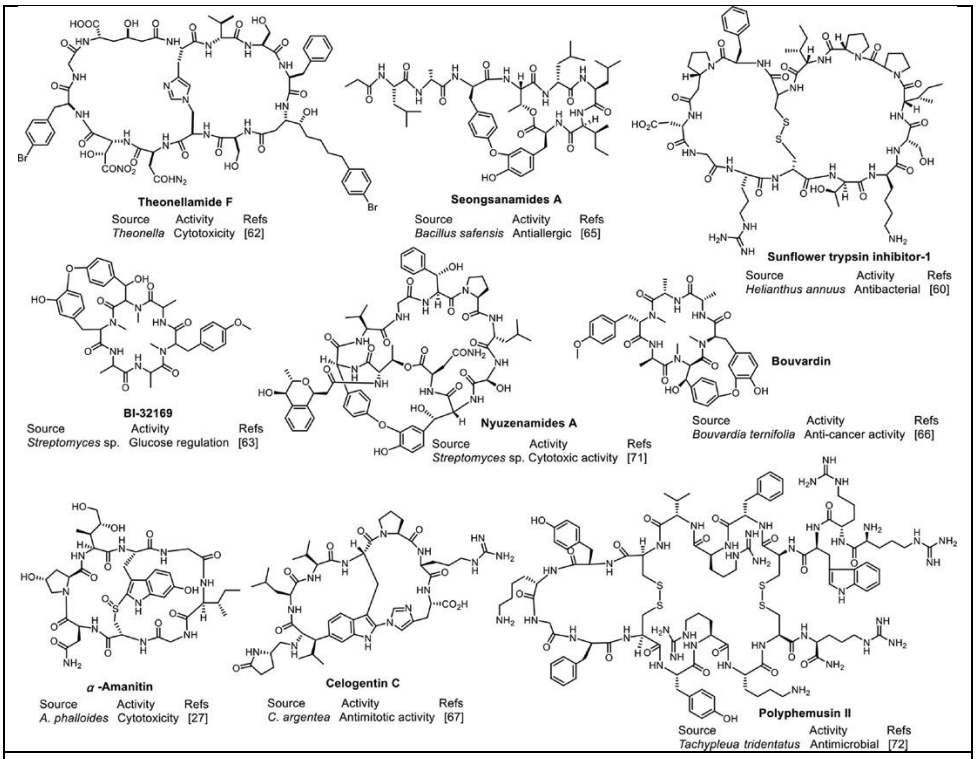

Figure 2. Other bioactive cyclic peptides.[2]

Post-translational modifications. Identifying and localizing post-translational modifications on cyclic peptides can be more challenging due to the cyclic structure and complex fragmentation patterns.

Three-dimensional structure. Determining the three-dimensional structure of cyclic and bicyclic peptides using mass spectrometry techniques can be particularly challenging due to their compact and constrained nature.

Limited fragmentation. Some cyclic peptides may be resistant to fragmentation, making it difficult to obtain sufficient structural information.

Overall, these analytical challenges have limited the literature on cyclic peptide mass spectrometry analysis to very simple small cyclic peptide structures like those in Figure 1.

Metabolites and low abundance contaminants. As long-lived bioactive molecules cyclic and bicyclic peptides, metabolites and chemical modifications can accumulate and often need to be analysed. Methods like ion mobility can be used to enhance signals for such low abundance additional components.

OPPORTUNITIES. This PhD student will use all of the tools available in modern, advanced mass spectrometry to attempt to address these analysis challenges. These methodologies include:

Multiple fragmentation methods. Combining different fragmentation techniques like Electron Capture Dissociation (ECD), Infrared Multiphoton Dissociation (IRMPD), and Ultraviolet Photodissociation (UVPD) to obtain complementary structural information

High-resolution Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometry. This technique provides ultra-high mass accuracy and resolution, which can be crucial for distinguishing between closely related structures and identifying modifications

Two-dimensional mass spectrometry (2DMS). This technique, which Prof. O'Connor has been developing, could provide more comprehensive fragmentation data for complex cyclic structures

Trapped ion mobility spectrometry can be used to separate precursor molecules, even within isomeric mixtures based on variance in their collision cross-section in a background gas. The Warwick research group has TIMS on both a time-of-flight mass spectrometer as well as on an FTICR mass spectrometer.

Chemical labelling of individual moieties on the cyclic/bicyclic peptides allow mass tagging of individual species giving the analyst a way to differentiate and distinguish distinct new

endgroups which when compared to the standard spectrum should allow sequencing and PTM analysis.

A PhD student working to solve these problems would benefit significantly from working on the projects mentioned. Here are some key advantages:

Cutting-edge research experience: The student would be involved in developing and applying advanced mass spectrometry techniques, particularly in Fourier Transform Ion Cyclotron Resonance (FTICR) and two-dimensional mass spectrometry (2DMS).

Interdisciplinary exposure: The projects span instrumentation development, fundamental research, and various applications in chemistry, biochemistry, and environmental science.

Skill development: The student would gain expertise in Mass spectrometry instrumentation and techniques, data analysis and interpretation, programming (e.g., Python for data analysis), and experimental design and optimization

Collaboration opportunities: Prof. O'Connor's work involves partnerships with industry (e.g., Bruker) and other academic institutions, providing networking opportunities.

Funding experience: The student would be exposed to the grant writing and funding process, given Prof. O'Connor's successful track record in securing research grants.

Publication potential: The diverse range of projects offers numerous opportunities for high-impact publications.

Industry relevance: Experience with the spin-out company, Verdel Instruments Ltd., could provide insights into commercializing research.

The PhD student will benefit from the following:

Access to state-of-the-art facilities: The student would work with advanced mass spectrometry equipment at the University of Warwick.

Mentorship: Prof. O'Connor has supervised numerous PhD students and postdoctoral fellows, indicating a strong mentorship environment.

Diverse application exposure: Projects range from proteomics and metabolomics to environmental analysis and pharmaceutical research.

International exposure: Opportunities for attending international conferences and collaborating with researchers worldwide.

Career development: The skills and experience gained would prepare the student for careers in academia, industry, or entrepreneurship in the field of analytical chemistry and mass spectrometry.

References

(1) Leymarie, N.; Costello, C. E.; O'Connor, P. B. Electron capture dissociation initiates a free radical reaction cascade. *Journal of the American Chemical Society* **2003**, *125* (29), 8949-8958.

(2) (1) Feng, D.; Liu, L.; Shi, Y.; Du, P.; Xu, S.; Zhu, Z.; Xu, J.; Yao, H. Current development of bicyclic peptides. *Chin. Chem. Lett.* **2023**, *34* (6), 108026.