Discovery of Bioactive Proteins from Scorpion Venom using Two Dimensional Mass Spectrometry

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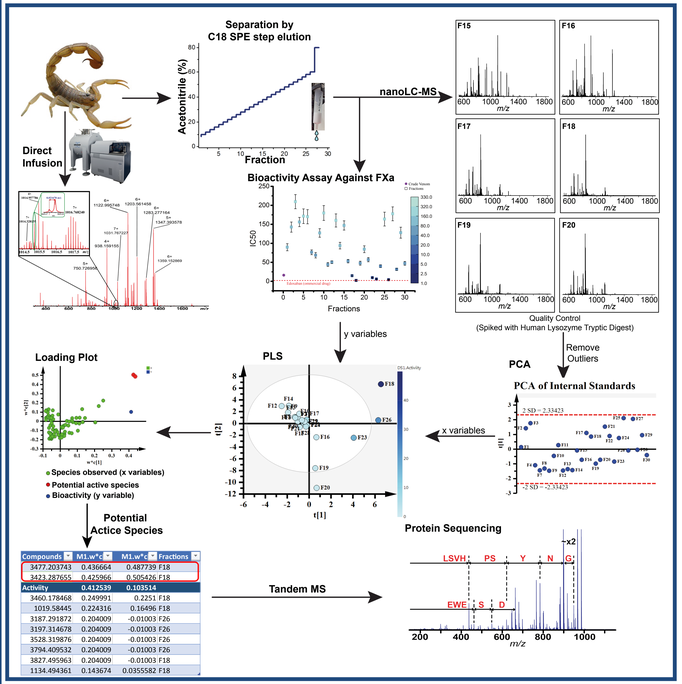
Introduction

The scorpion Mesobuthus Martensii, is a species widely present in China and used in traditional Chinese medicines for thousands of years.[1] Venoms from this type of scorpions contain a highly complex mixture of proteins which have been proven to contain bioactive components.[2,3] These peptides show a diverse variety of pharmaceutical properties for the treatment of many conditions, such as cardiovascular problems, drug dependence, chronic pain, diabetes, and even tumors.[4,5]

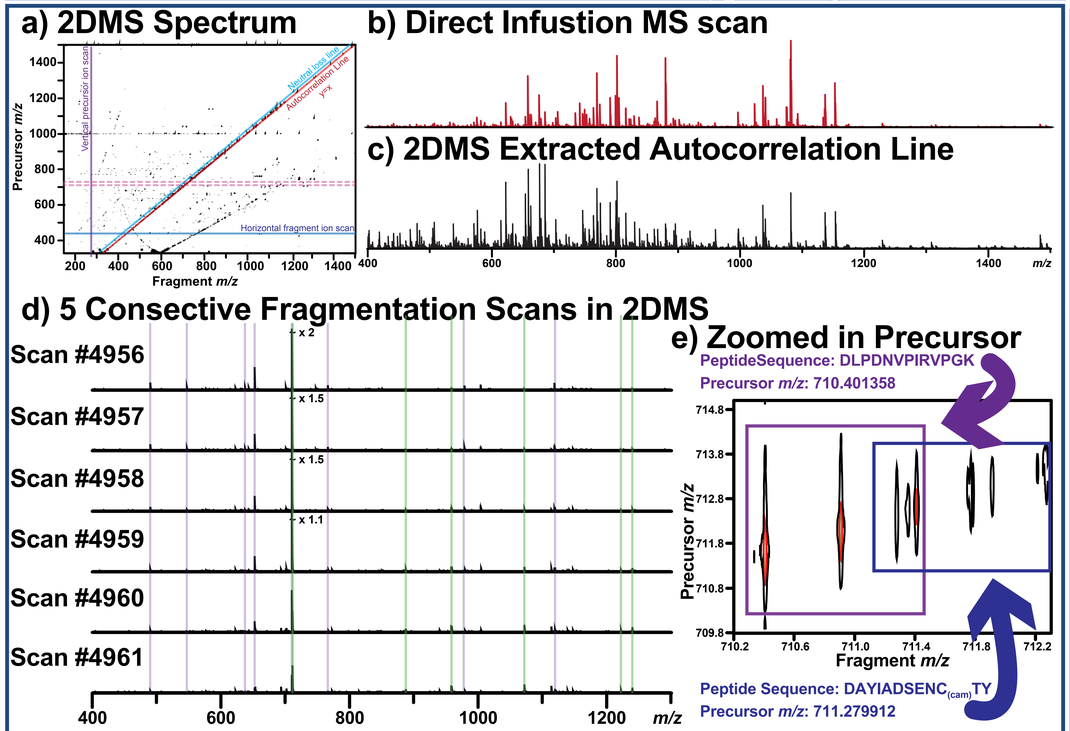
Some proteins have shown in-vitro inhibition of the enzyme factor Xa, which catalyses the conversion of pro-thrombin to thrombin, causing blood coagulation, leading to thromboembolism. These proteins are highly varied in structure and often heavily modified and/or crosslinked with particularly high numbers of disulphide bonds, with only partial genome sequences available, sequencing and tandem MS of these species is extremely challenging.

Crystal Structure of Factor X
PDB:2BOK

Methods



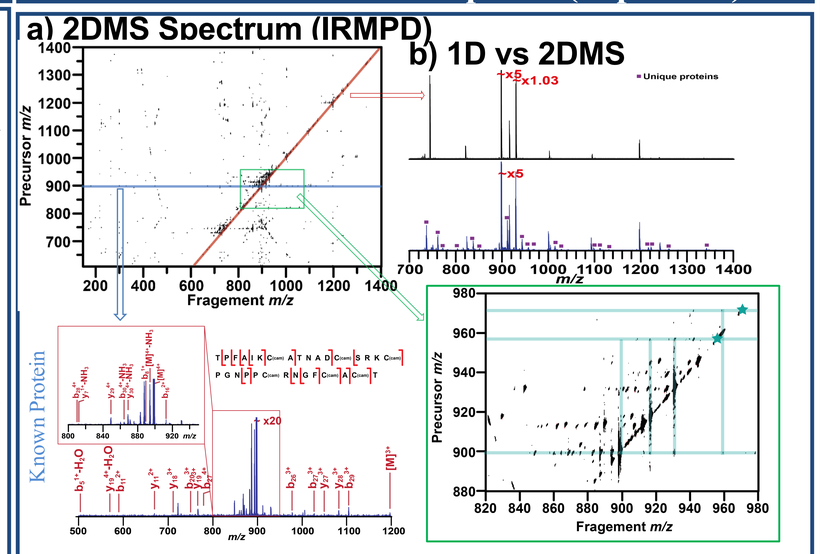
Results: 2D - FTICR-MS of Crude Venom Tryptic Digest



Conclusions

- By utilising standard proteomics methods, it is difficult to find new potential pharmaceutical targets.
- 2D-FTICR-MS can provide additional information to nLC-MS/MS which is essential to de novo sequencing of unknown proteins and peptides.
- Bioactivity assays coupled with statistical methods helps to narrow down potential bioactive species
- Potential, novel FXa inhibitor protein has been sequenced.
- 2DMS allows for easy identification of labile modifications.
- The method is applicable to identifying potential pharmaceutical leads from other natural products.

Results: 2DMS of Bioactive Species (Top-down)



Fragmentation pattern suggest that this is a labile modification

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