comprehensive venom inventory owing to the external and internal distribution of cnidae. Moreover, the use of transcriptomics alone will only identify putative toxins with homology to known peptides,[9] thereby overlooking novel scaffolds of potential interest as drug leads.

We have examined the utility of incorporating matrix-assisted laser desorption/ionisation imaging mass spectrometry (MALDI-IMS) into a venomics strategy to discern the tissue distribution of peptides and infer biological functions. MALDI-IMS is commonly used in clinical applications to identify protein changes within cancers, detect biomarkers within tissues, and as a tool for drug discovery.[10–12] Recently, the application of MALDI-IMS has been extended to examine peptidome complexity in centipede venom glands.[13,14]

We conducted a pilot MALDI-IMS study using transverse sections of the sea anemone, *Oulactis muscosa*, a species found along the eastern Australian coastline. Regions of interest (ROI) were selected based on biological functions and associated cnidae profile (Fig. 1a–e). External ROI included tentacles (prey capture and immobilisation), acrorhagi and frill (defence), and column (external defence). Internal ROI included the actinopharynx (throat) and mesenterial filaments, both used in prey immobilisation and digestion. Fig. 1 displays the unique individual spectra produced for each ROI, reflecting mass diversity within each tissue region (individual masses for ROI spectra and comparison provided in Supplementary Material, Fig. S1).

Fig. 2 exemplifies the capability of MALDI-IMS, displaying the distribution of three individual peptide masses, from which we can potentially draw inferences linking biological function to morphology. Fig. 2b highlights a putative peptide toxin, as it occurs solely in the ectoderm of the tentacles, where cnidae are densely packed. Fig. 2c shows a peptide with a distribution restricted to muscular tissue, implying a physiological role, and Fig. 2d shows a ubiquitously distributed peptide.

By utilising a venomics strategy that combines transcriptomics and proteomics with MALDI-IMS, we can potentially identify and correlate peptides according to tissue-specific regions. These results will aid our understanding of the functional evolution of sea anemone peptide toxins, while providing a library of novel peptides and scaffolds that may be useful as pharmacological tools or drug leads.

Supplementary Material

Individual masses for ROI spectra and comparison (Fig. S1) are available on the Journal's website.

Conflicts of Interest

The authors declare no conflicts of interest

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