Analysis of nudibranch microstructures using ultrathin cryomicrotome sectioning and Mass Spectrometry Imaging allows spatial distribution of molecular species to be determined at nanometer resolution

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< Mass Spectrometry imaging (MSI) analysis of histological sections of biological specimens has, in recent years, enabled the association of specific molecules with the morphological structure of the tissue. MALDI-TOF/TOF instrumentation has been used extensively for these experiments due their speed and high sensitivity. The spatial resolution achievable using MSI, using MALDI as the ionisation process, is limited by the laser size and matrix crystal size. Fundamentally MALDI imaging experiments are 2 dimensional experiments, with explorations into the z stacking realised by analysing serial sections of the tissue. Commonly, spatial resolution of 40-50 m is reported, and under specific conditions, related to the laser and the matrix deposition method, spatial resolution can be reduced to better than 10 m. It should be noted that reduction of the spatial resolution reduces the sample size and ultimately the number of ions reaching the detector. In our current study we have investigated ultrathin cryosections of high-pressure nitrogen frozen tissue by MALDI-TOF. Specifically we have been working with the Mantle Dermal Formations (MDFs) present in the outer rim of C*hromodoris kuiteri*, a nudibranch species found in the coastal waters of Queensland. It has been hypothesised that *C. kuiteri* sequesters latrunculin-A, which is ingested from some of the sponges the nudibranch feeds upon, to the MDF structures found in the outer rim of the organism. Dissected glands containing multiple MDFs were prepared using high-pressure nitrogen freezing, and sectioned at a thickness of 100 nm using a cryo-ultramictrome and placed into conductive ITO cover slips. The analyte of interest in these structures, latrunculin-A, possesses sufficient conjugation to ionise directly, without the need for a matrix. Through optimisation of the reflectron mode MALDI-TOF acquisition method, we were ultimately able to achieve 25 m in the 2 Dimensional MSI experiment, but 100 nm in the Z direction. Subsequent analysis of strips of ultrathin cryo-ultramicrotome sections by MALDI-TOF based imaging experiments enabled us to image our way through the MDF structures with a Z direction resolution of 100 nm.

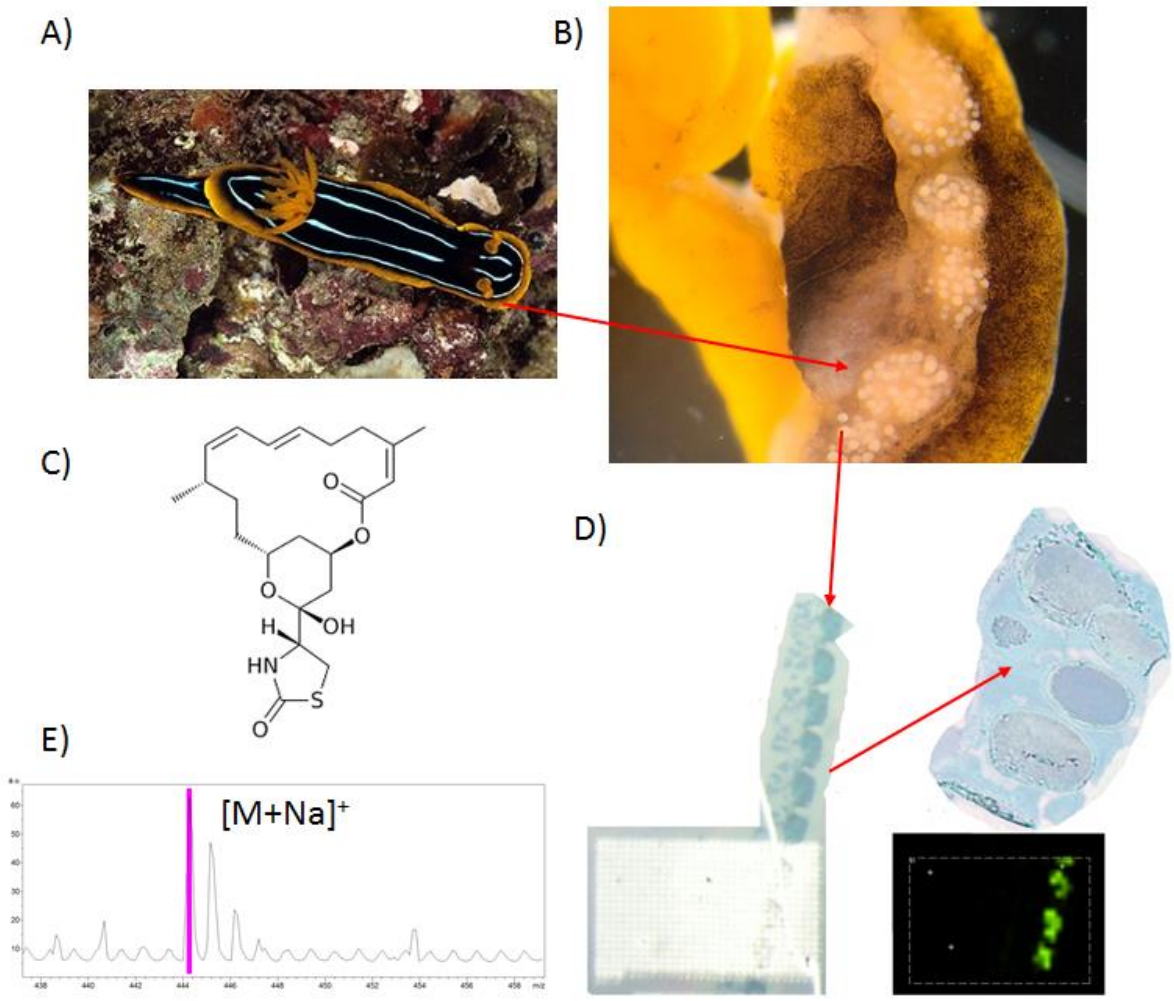

Figure 1. A) C*hromodoris kuiteri,* B) The yellow rim of the nudibranch - dissected to highlight the Mantle Dermal Formations (MDF) to show how they are arranged. C) Structure of Latrunculin-A. D) Optical Image of ultrathin cryosections of the MDF containing gland shown alongside Mass Spectrometry Imaging for the $[M+Na]^+$ adduct of Latrunculin-A. E) Mass Spectrum observed for Latrunculin-A observed from ultrathin sections of MDF containing gland during MALDI MSI analysis.>

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