1. [1] Narayan K, Prosa T J, Fu J, Kelly T F and Subramaniam S 2012 Chemical mapping of mammalian cells by atom probe tomography Journal of Structural Biology 178 98-107
2. [2] Kelly T F, Nishikawa O, Panitz J A and Prosa T J 2009 Prospects for nanobiology with atom-probe tomography MRS bulletin 34 744-50

24

Using quantitative elemental X-ray mapping to determine the cellular distribution of calcium and phosphorus within leaves of Australian and South American Proteaceae

Patrick E. Hayes[2, 1], Peta L. Clode[2], Hans Lambers[1]

1. *School of Plant Biology, The University of Western Australia, Perth, Western Australia, Australia*
2. *Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, Perth, Western Australia, Australia*

Due to its ubiquitous role as a signalling element in plant cells, the cytosolic concentration and cell-type distribution of calcium (Ca) must be tightly regulated in all cells. It is currently thought that most dicots accumulate Ca in mesophyll cells and phosphorus (P) in epidermal cells; this separation of Ca from P is necessary to avoid the deleterious precipitation of calcium phosphates. However, in several Australian Proteaceae species from severely P-impoverished soils, we discovered that P is actually accumulated in photosynthetically active mesophyll cells which is likely a strategy to increase P-use efficiency, by preferentially storing P where it is most needed. However, this shift in P-accumulation may result in the formation of deleterious precipitates with Ca. This project investigates if all Proteaceae accumulate P in mesophyll cells and if this results in a shift in Ca-accumulation away from P. The distribution patterns and concentrations of Ca and P were determined in a range of Proteaceae from both Australia and South America. Leaves of Proteaceae from south-western Australia, Chile and Brazil were dissected and rapid-frozen in liquid nitrogen before being cryoplaned and analysed in the fully frozen-hydrated state. Quantitative X-ray mapping and elemental analysis were performed on transverse leaf sections using a Zeiss Supra 55 FESEM, with an Oxford X-Max80 SDD X-ray detector. The accumulation of P in mesophyll cells was only found in Australian Proteaceae from severely P-impoverished soils, indicating that it is not a family-wide trait and is likely a strategy to increase P-use efficiency. Patterns of Ca-accumulation were not consistent with current understandings, instead showing significant variation, even between closely related species. Such variation in the patterns of Ca-accumulation within a single family and genus is unprecedented and requires further investigation into what factors influence Ca-regulation in leaf cells.

25

Stable isotope labelling with high-resolution imaging mass spectrometry

Matt R. Kilburn[1], Paul Guagliardo[1], Haibo Jiang[1], Laure Martin[1], Heejin Jeon[1]

1. *University of Western Australia, Perth, WA, Australia*

Mass spectrometry traditionally requires material to be extracted in bulk from samples, at the expense of information about the complex spatial relationships of the individual components. However, to understand large-scale phenomena, such as ore-producing hydrothermal systems, or nutrient trafficking in terrestrial and marine ecosystems, researchers are increasingly looking at the chemical processes occurring at the micro-to nano-scale.

Secondary Ion Mass Spectrometry (SIMS) provides chemical and isotopic analysis on micro-volumes of sample, in situ, while NanoSIMS allows imaging at the sub-micron scale. Combining stable isotope labelling with NanoSIMS allows us to directly visualise the distribution of labelled components within an experimental system, without changing its chemical nature. For example, ^{15}N and ^{13}C labels can be attached to specific molecules used in biological systems (nutrient tracking, drug delivery), and deuterated-^{18}O labelled water may be used to investigate mineral-fluid interactions. Furthermore, isotopic labels can be conjugated to specific antibodies to identify proteins, or to oligonucleotides to identify specific species of bacteria.

26

Synchrotron infrared micro-spectroscopy of single cells at the Australian Synchrotron

Keith R. Bambery[1], Mark J. Tobin[1], Ljiljana Puskar[1], Danielle Martin[1], Jitraporn Vongsvivut[1]

1. *Australian Synchrotron, Clayton, VIC, Australia*

Infrared Microspectroscopy is increasingly revealing valuable bio-chemical information of biological and biomedical systems beyond the tissue level at the single cell level. At the Australian Synchrotron Infrared Microscopy beamline, FTIR spectroscopy provides sensitive molecular fingerprinting for tissues and cells without the need for sample pre-treatment with stains or external markers. Due to the brightness of a synchrotron source, good signal to noise at high spatial resolution (diffraction limited) can routinely be performed at the single cell level.

In the study of live microbiological systems the principal restriction on the application of infrared microspectroscopy is the strong absorbance by water in the region of 1650 cm^{-1}, overlaying the Amide I absorption band of proteins. The combination of a highly focused synchrotron beam with liquid cells constructed with microfabricated spacers of 6 to 8 microns in thickness have enabled complete mid-IR spectra to be obtained of single live cells under aqueous media within short scan times.

Some applications include analysis of spectral changes in normal single living cells, diagnosing different disease states, discrimination of cell types and monitoring the effects of drug treatment at the single cell level. Details of these studies conducted at the infrared microscopy beamline at the Australian Synchrotron are presented.