

## DEALING WITH DIFFICULT PROTEINS – APPLICATION OF PROTEOMICS TO THE WOOL KERATIN FAMILY

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Keratin proteins are ubiquitous in nature, being found in such places as skin, cell nuclei and wool and hair fibres. Two major types of keratin proteins are found in the latter: the  $\alpha$ -helical intermediate filament proteins (IFPs) and the amorphous keratin associated proteins (KAPs) of the matrix, in which these IFPs are embedded. One of the goals of the protein chemistry group at Canesis is to combine proteomic with genomic approaches to aid in the location of markers for wool quality traits. Previous studies have shown that some families of proteins show a high degree of inter- and intra-breed variation [1,2]. In addition we are in the process of determining the differential expression of proteins in the cells of the wool fibre to understand the processes involved in assembly of intermediate filaments as a first step in the process of developing new biocomposite materials. As part of this process, robust methods are required for identification of proteins from wool extracts separated on 2DE gels.

Keratins present unique problems when it comes to the mass spectrometric identification of protein material from separated spots on two-dimensional electrophoretic (2DE) gels. They are noted for their high degree of homology, 92% in the case of the wool Type I IFPs; 95% among the KAP1 high sulphur proteins (HSPs), and this requires a high degree of sequence coverage in order to find sequences unique to individual proteins [3]. In addition, the proteins are noted for their high concentration of cysteine, 22 moles% in the case of the KAP1 HSPs, and low concentrations of acidic and basic proteins. This has two effects: the proteins, particularly the HSPs, are difficult to extract from the gels, even after tryptic digestion, and they yield a small number of high molecular weight peptides, resulting in their poor detectability by MALDI-TOF MS. The relatively few basic groups has meant that identification of the KAP1s has relied on the detection of only one or two low abundance, high molecular weight peptides using the peptide mass fingerprinting approach [4]. Because of these problems, we have been exploring the use of different staining technologies that minimise or eliminate the fixing step as a way of improving identification of proteins separated by 2DE. In addition, MALDI-TOF data was combined with high-resolution nanoLC-MS/MS data to search for complementary information using two different ionisation methods, MALDI and nano-electrospray, on a quadrupole time-of-flight instrument.

### References

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