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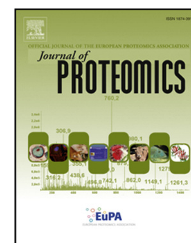

Review

A Sydney proteome story☆

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ABSTRACT

This is the story of the experience of a multidisciplinary group at Macquarie University in Sydney as we participated in, and impacted upon, major currents that washed through protein science as the field of Proteomics emerged. The large scale analysis of proteins became possible. This is not a history of the field. Instead we have tried to encapsulate the stimulating personal ride we had transiting from conventional academe, to a Major National Research Facility, to the formation of Proteomics company Proteome Systems Ltd. There were lots of blind alleys, wrong directions, but we also got some things right and our efforts, along with those of many other groups around the world, did change the face of protein science. While the transformation is by no means yet complete, protein science is very different from the field in the 1990s.
This article is part of a Special Issue entitled: 20 years of Proteomics.

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1. Introduction

1.1. *Protein science in the 1980s and 1990s*

During the early 1980s monoclonal antibodies were becoming the most powerful tool available for not only characterising developmentally regulated proteins but also purifying them at levels that enabled protein primary structure analysis. Marianne Krefft (Max Planck Institut fur Biochemie in Munchen, in Keith Williams' lab), was using monoclonal antibodies to study a model developmental system and she identified a cell surface glycoprotein that would define our course into the field of protein science. In 1984 Williams returned to Macquarie University in Sydney to establish the Biotech programme and introduce a more molecular approach to biology in an academic discipline with strong emphasis on evolution and ecology.

It was not a great time to be a protein scientist as the genomics revolution was in full flight, with concomitant mass migration of technologically oriented biologists into DNA-based experimental programmes.

However, in the same way that the DNA science was being transformed, we were exhilarated by having new tools to probe hitherto intractable problems, such as studying complex cell surface glycoproteins. With monoclonal antibodies as probes for both the peptide backbone and a glycosylated domain, we were able to make considerable progress in characterising a Prespore Specific Antigen (PsA) of *Dictyostelium discoideum*, a key marker for one class of cells in a small multicellular structure. This enabled studies about emergence of the prespore cells and how the pattern of two classes of cells was formed (based on flow cytometry and tissue staining studies). At the biochemical level, we had sufficient material from using affinity columns to extract chemical amounts of the protein. This led to getting limited protein sequence information and matching the gene through studies in Jeff Williams' lab at the University College London, UK. As we understood more, we realised that this cell surface protein was inserted into the membrane by a glycolipid anchor. Characterisation of the protein and its glycoforms helped us reorient our programmes to what became proteomics, while ultimately the glycolipid anchor was determined by Paul Haynes working with Mike Ferguson's group in Dundee, Scotland and the 3D structure of the glycoprotein was done by Bridget Mabbutt and Paul Curmi at UNSW in Sydney.

In all 9 PhD students worked on some aspect of this one protein, and this was a time when genomics researchers were getting confident about working on multiple genes. We realised that protein science needed to change, but the way forward wasn't clear. We had established MUCAB (Macquarie University Centre for Analytical Biotechnology) with a major focus on protein and glycoprotein chemistry and we used standard approaches of the time to characterise proteins that had been purified through laborious techniques. N-terminal Edman sequencing was a key first step in the protein chemistry armoury. Andrew Gooley had spent time in Helmut Meyer's group in Bochum and this upskilled the group dramatically. Meyer was studying Serine and Threonine phosphorylation at the time and, serendipitously, PsA was glycosylated on a Threonine rich region of the protein. A key finding by Andrew was that there was an O-glycosylated repeat spacer domain that was polymorphic in different isolates (with 3, 4 or 5 copies of a glycosylated PTVT repeat).

By the early 1990s we were running technology courses in MUCAB and a wildly enthusiastic New Zealander, Ben Herbert, kept coming across the Tasman. Ben, who worked on wool, was highly skilled at sample preparation and gel technologies, which we recognised as critical roadblocks at that time. Ben was passionate about 2-D gels. What attracted our attention was the ability to array hundreds of proteins in two dimensions so that they were chemically pure. More exciting was Ben's push to increase the sample loadings so that chemical amounts of proteins were arrayed and then by electroblotting to PVDF, hundreds of highly purified, archived proteins were available for characterisation. At this time it was hard to go the next step and actually characterise proteins from PVDF blots of 2-D gels, but it certainly changed the way we were looking at protein science. This was reminiscent of genomics, where large numbers of genes could be studied.

Concurrent with the preparative 2-D gel developments in the mid-1990s, Pappin, Cottrell, Henzel and others defined the simple and elegant idea of using mass spectrometry to identify a subset of tryptic peptides from a tryptic digest of a pure protein. MALDI-TOF MS was now sufficiently developed

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