# The development of proteomic techniques to study the Australian Paralysis Tick, *Ixodes holocyclus.*

The application of proteomic technology to an organism with poor bioinformatic information.

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## CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student

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## Acknowledgments

This work was started in February, 2000, performed on a part-time basis and submitted in November, 2008. During that time I was a laboratory manager for the Immunobiology Unit (2001), then one of the laboratory managers for the Institute for the Biotechnology of Infectious Diseases (IBID) (2002-2005) and finally a technical specialist for the Proteomics Technology Centre of Expertise (PTCE) (2006-present). For 18 months in the middle I was assisting in the relocation of the Faculty of Science from Gore Hill to the Broadway campus. As one would expect, over this considerable period of time, I met and had dealings with many, many people. Some of these deserve special mention on the following pages.

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## Figures and tables.

## Abbreviations.

| | |
|---|---|
| 1-D | One-dimensional |
| 2-DGE | Two-dimensional gel electrophoresis |
| ASB-14 | Amidosulfobetaine-14 |
| BLAST | Basic local alignment search tool |
| CDS | Coding determining region |
| CID/CAD | Collisonally induced/activated disocciation |
| DTT | Dithiothreitol |
| ESI | Electrospray ionisation |
| EST | Expressed sequence tag |
| ETD | Electron transfer dissociation |
| IEF | Isoelectric focusing |
| IPG | Immobilised pH gradient |
| LC | Liquid chromatography |
| LC/MS/MS | Liquid chromatography coupled with tandem mass spectrometry |
| LDS | Lithium dodecyl sulphate |
| MALDI | Matrix Assisted Laser Desorption Ionisation |
| MCE | Multi-compartment electrolyser |
| MES | 2-(N-morpholino)ethanesulfonic acid |
| MS | Mass spectrometry |
| MS/MS | Tandem mass spectrometry |
| MudPIT | Multi-dimensional Protein Identification Technology |
| MW | Molecular weight |
| NCBI | National Centre for Biotechnology Information |
| $NH_4HCO_3$ | Ammonium hydrogen carbonate |
| PBS | Phosphate buffered saline |
| PDB | Protein Data Bank |
| PIR | Protein Information Resource |
| PRF | Protein research foundation |
| PTM | Post-translational modification |
| QTOF | Hybrid Quadrupole Time-Of-Flight mass spectrometer |
| SDS | Sodium dodecyl sulphate |
| SDS-PAGE | Sodium dodecyl sulphate polyacrylamide gel electrophoresis |
| Swiss-Prot | Swiss Institute of Bioinformatics protein database |
| TBP | Tributylphosphine |
| TFE | 2,2,2 – Trifluoroethanol |
| Tris | Tris(hydroxymethyl)methylamine |
| UTC7 | 7M urea, 2M thiourea, 1% C7BzO |

## Abstract.

The Australian paralysis tick, *Ixodes holocyclus*, is representative of the majority of organisms studied in biology in that the bioinformatic information available (genome sequence, annotated coding regions and protein sequences) are far from complete. The study of well characterised model organisms has shown that proteomics and its associated technologies are able to isolate, identify and characterise individual protein isoforms at femto to attomole amounts of sample. With these model organisms, this can be achieved in either unpurified or partially purified samples (shotgun proteomics) or by high resolution separations using isoelectric fractionation and two-dimensional gel electrophoresis.

In a poorly characterised organism, this is not the case. The work presented in this thesis applies proteomic technologies to characterising the tick proteome in a hypothesis and non-hypothesis driven manner. In the non-hypothesis driven approaches, fractionation and separation methodologies were applied to determine which method or combination of methods provided the greatest number of protein identifications. The results of these studies showed that the resolution of protein isoforms provided by 2-DGE is invaluable for characterising proteins from *I. holocyclus*. This is because the homogenous protein spot can be excised from the gel and characterised by *de novo* sequencing of MS/MS spectra with the knowledge that all peptides are from the same protein. However, successful *de novo* sequencing is reliant on good quality MS/MS spectra, which is partly reliant on intensely stained gel spots, which is determined by the amount of sample loaded onto the gel. It is well documented and demonstrated in this study that overloading of 2-D gels with samples containing high abundance proteins, tick cytoskeletal proteins in this case, can cause spot resolution problems. Fractionation of the sample using a Multi-compartment Electrolyser and equalisation with Proteominer partially addresses this issue, but further refinement is necessary.

The optimised sample preparation methods were then applied in hypothesis driven experiments to characterise specific protein subtypes using Western blots and a novel fluorescent zymogram approach. The analysis identified a number of proteins that will need

further characterisation, using molecular biological and recombinant protein expression techniques, to determine their suitability as vaccine candidates.