

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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Doctor of Philosophy, 2008.

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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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	Collisionally induced/activated dissociation
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 ₄ HCO 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.