



# Expanding the Functional Proteome of *Mycoplasma pneumoniae*

A thesis submitted in  
fulfilment of the requirements  
for the degree of  
Doctor of Philosophy:  
Science

2018

**Michael Widjaja**





---

## Certificate of Original Authorship and Declaration

I, Michael Widjaja, 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. This research is supported by an Australian Government Research Training Program Scholarship.

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.

For the following, I, Michael Widjaja, declare that more than 50% of the content in the journal article (Chapter 4) and manuscripts (Chapters 5 and 6) included in this thesis:

- Planning of each chapter was conducted by my supervisors and I.
- Experiments performed and analysed by myself (unless stated).
- The initial draft prepared and written by myself. Any subsequent changes in response to co-authors and editor's reviews were performed by me.

Signature:

Production Note:  
Signature removed prior to publication.

Date:

20/2/2018



---

# Acknowledgements

First, I would like to thank the markers for taking the time to review this thesis. I would also like to thank the Australian Commonwealth Government Department of Industry for the Australian Postgraduate Award Scholarship I received throughout my PhD candidature.

I would like to thank Prof. Duncan Krause and Assoc. Prof. Daniel Brown for donating Mycoplasma antibodies for my research. Thank you to Dr Marie Trussart, Dr Maria Lluch-Senar, and Prof. Luís Serrano for the opportunity to collaborate with your team to investigate the structure of the *M. pneumoniae* chromosome. It was a pleasure to work with you all and I am glad I had the opportunity to do and the outcome. I would also like to extend this gratitude to Dr Roger Dumke and Dr Anne Gründel, thank you both for the Mycoplasma antibodies and the collaboration on Ef-Tu. I hope we can collaborate again in the future.

A big thank you to all the International Organization for Mycoplasmology (IOM) members for the discussions (science related and not), laughs, and fun times. Especially Art Totten, Assoc. Prof. Meghan May, Prof. Mitch Balish (also for the sialic acid column suggestion), Dr Peter Kuhnert, Prof. Joachim Frey, Dr Isil Tulum, Dr Yuhei Tahara, and Steven Distelhorst. I hope to see you all at the IOM 2018!

Back in Australia I would like to thank Dr Cheryl Jenkins for all her help at EMAI with jabbing bunnies and the antibodies. Thanks to the Microbial Imaging Facility at UTS especially Dr Lynne Turnbull for all the troubleshooting, training, and analysis; in addition to Dr Mike Johnson and Assoc. Prof. Cynthia Whitchurch. Like to thank the tech staff (Harry Simpson, Sarah Osvath, Mercedes Ballesteros, and Luke Beebe), because nothing would happen without any of you!

Thank you to the senior proteomic-ists. Jerran for showing me how to model and telling Steve how good of a student I am and to take me on as an Honours student. Thank you Jess for mentoring me at the start of Honours and PhD and being an inspiration in presentations and communication. On the matter of communication, big thank you to Prof. Liz Harry for your support in helping me build my Science Communication experience, I look forward to pursuing this post-PhD.



---

I have of course have to thank my supervisors. Thank you Prof. Steven Djordjevic, for taking me on for Honours and especially for a PhD, and for your supervision in the past six years. Huge thank you to Dr. Matt Padula for training and helping me in the lab, you are a wealth of knowledge and an inspiration to all the students in the lab. To my mentor, friend, guy-that-I-stand-next-to-so-I-look-bigger, gym buddy, and now co-supervisor Dr. Benjamin Armando Bernard Raymond, thank you for everything. My students that I have mentored: Kayla, Angus, Harui, and Andy thank you all for giving me the opportunity to mentor and for your commitment to your projects.

Thanks to my PhD buddies. Ka-tey, was good to have someone to share the PhD pain and stresses with on a daily basis so thank you for relating. Krishy, thank you for the great laughs and all the free food! Thanks to the rest of the PhD cohort for the laughs especially Megan, Jacqueline, Greg, Dan, Em, Nat, and Brendan. Big thanks to Samira, my Metabolic buddy, for talking me up and getting me jobs!

Thank you to mum and dad, without you both I wouldn't be where I am today. Also to Om Ajun, I Nil Nil, Maddy, Dede, and Sam for your support! And to my rabbits, Cookie and Diglett, for always being a source of luck! Thanks to all my Munyaks especially Alex, Shaun, Oz, Nick, Ray, TT, and Baba (also with the image design help). Thank you all for your words of motivation and support of how proud you guys are of me.

Lastly the biggest thank you to Isa, my significant other. Thank you for double checking everything of mine, supporting me, and keeping me in check. I cannot think of anyone else that I would want to have spent these past few years with other than you. 1,2, toilet seat!

If I have missed anyone, apologies and thank you.



# Publications

1. Berry I.J.<sup>†</sup>, Jarocki V.M.<sup>†</sup>, Tacchi J.L., Raymond B.B., **Widjaja M.**, Padula M.P., and Djordjevic S.P. (2017) 'N-terminomics identifies widespread endoproteolysis and novel methionine excision in a genome-reduced bacterial pathogen'. *Scientific Reports* (Submitted).
2. (Chapter 5): **Widjaja M.**<sup>†</sup>, Harvey K.L.<sup>†</sup>, Hagemann L.<sup>†</sup>, Berry I.J., Jarocki V.M., Raymond B.B., Tacchi J.L., Gründel A., Steele J.R., Padula M.P., Charles I.G., Dumke R.<sup>‡</sup>, and Djordjevic S.P.<sup>‡</sup> (2017). 'Elongation factor Tu is a multifunctional and processed moonlighting protein'. *Scientific Reports* (Submitted).
3. Trussart M., Yus E., Martinez S., Baù D., Tahara Y.O., Pengo T., **Widjaja M.**, Kretschmer S., Swoger J., Djordjevic S., Turnbull L., Whitchurch C., Miyata M., Marti-Renom M.A., Lluch-Senar M., and Serrano L. (2017). 'Defined chromosome structure in the genome-reduced bacterium *Mycoplasma pneumoniae*'. *Nature Communications*.
4. Tacchi J.L., Raymond B.B., Haynes P.A., Berry I.J., **Widjaja M.**, Bogema D.R., Woolley L.K., Jenkins C., Minion F.C., Padula M.P., and Djordjevic S.P. (2016). 'Post-translational processing targets functionally diverse proteins in *Mycoplasma hyopneumoniae*'. *Open Biology*.
5. (Chapter 4): **Widjaja M.**, Berry I.J., Pont E.J., Padula M.P., and Djordjevic S.P. (2015). 'P40 and P90 from Mpn142 are targets of multiple processing events on the surface of *Mycoplasma pneumoniae*'. *Proteomes*.
6. Raymond B.B., Jenkins C., Seymour L.M., Tacchi J.L., **Widjaja M.**, Jarocki V.M., Deutscher A.T., Turnbull L., Whitchurch C.B., Padula M.P., and Djordjevic S.P. (2014). 'Proteolytic processing of the cilium adhesin MHJ\_0194 (P123) in *Mycoplasma hyopneumoniae* generates a functionally diverse array of cleavage fragments that bind multiple host molecules'. *Cellular Microbiology*.

<sup>†</sup>shared primary author, <sup>‡</sup>shared principal investigators

# Abbreviations

## Standard abbreviations

Adenine and thymine	A+T
Community Acquired Respiratory Distress Syndrome	CARDS
Dihydrolipoyl dehydrogenase	Pdh-D
Dihydrolipoyllysine acetyltransferase	Pdh-C
Elongation factor Tu	Ef-Tu
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH
Guanine and cytosine	G+C
High Molecular Weight protein	HMW
Isoelectric Point	pI
Liquid chromatography tandem mass spectrometry	LC-MS/MS
Open Reading Frame	ORF
Phosphate Buffered Saline	PBS
Potential of hydrogen	pH
Pyruvate dehydrogenase E1 $\alpha$ subunit	Pdh-A
Pyruvate dehydrogenase E1 $\beta$ subunit	Pdh-B
Short Linear Motifs	SLiMs
Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis	SDS-PAGE

## Unit abbreviations

Base pair	bp	Molar	M
Dalton	Da	Mole percent	Mol%
Gram	g	Percent	%
Hour	h	Percentage weight per volume	w/v
Litre	L	Percentage weight per weight	v/v
Metre	m	Relative centrifugal force	RCF
Minute	min	Second	s



# Table of Contents

<b>Chapter 1. Introduction on <i>Mycoplasma pneumoniae</i> and its interaction with the human host</b> .....	<b>20</b>
1.1 Introduction to <i>Mycoplasma pneumoniae</i> .....	21
1.2 <i>Mycoplasma pneumoniae</i> disease .....	23
1.3 <i>Mycoplasma pneumoniae</i> pathogenicity.....	24
1.4 Structural aspects of <i>Mycoplasma pneumoniae</i> pathogenicity .....	27
1.5 The cytoskeleton and electron dense core of <i>Mycoplasma pneumoniae</i>	29
1.6 Adhesins and accessory proteins of <i>Mycoplasma pneumoniae</i> .....	31
1.7 Moonlighting proteins.....	34
1.8 Thesis aims and Chapter synopses .....	40
<b>Chapter 2. The surface proteome of <i>Mycoplasma pneumoniae</i></b> .....	<b>43</b>
2.1 Preface.....	44
2.2 Introduction .....	44
2.3 Methodology .....	45
2.3.1 Strains.....	45
2.3.2 <i>M. pneumoniae</i> surface labelling with NHS-biotin and avidin affinity purification.....	45
2.3.3 <i>M. pneumoniae</i> enzymatic surface shaving .....	46
2.3.4 Liquid chromatography tandem mass spectrometry (LC-MS/MS).	47
2.4 Results and Discussion.....	47
2.4.1 Biotinylation and purification of the <i>M. pneumoniae</i> surface proteins .....	47

---

2.4.2	The surface proteome of <i>M. pneumoniae</i> .....	50
2.4.3	Bioinformatic analyses on <i>M. pneumoniae</i> surface proteins .....	53
2.4.4	Attachment organelle proteins of <i>M. pneumoniae</i> .....	57
2.4.5	Putative surface proteins of <i>M. pneumoniae</i> .....	59
2.5	Conclusion .....	61
<b>Chapter 3. Characterising potential adhesins of <i>Mycoplasma pneumoniae</i></b> .....		<b>62</b>
3.1	Preface.....	63
3.2	Introduction .....	63
3.3	Methodology .....	64
3.3.1	Strains and cultures.....	64
3.3.2	Host 'Bait' molecules.....	64
3.3.3	'Bait and Prey' affinity chromatography using host 'Bait' molecules .	64
3.3.4	'Bait and Prey' affinity chromatography using heparin as 'Bait' ....	65
3.3.5	'Bait and Prey' affinity chromatography using surface A549 complexes as 'Bait' .....	65
3.4	Results and Discussion.....	66
3.4.1	Host molecule binding proteins of <i>M. pneumoniae</i> .....	66
3.4.2	Potential moonlighting adhesins of <i>M. pneumoniae</i> .....	74
3.4.3	Evidence of cleavage in <i>M. pneumoniae</i> .....	77
3.5	Conclusion.....	81
<b>Chapter 4. P40 and P90 from Mpn142 are targets of multiple processing events on the surface of <i>Mycoplasma pneumoniae</i></b> .....		<b>83</b>
4.1	Preface.....	84





---

4.2	Author contribution.....	84
4.3	Abstract: .....	86
4.4	Introduction .....	87
4.5	Experimental Section.....	90
4.5.1	Strains and cultures and reagents.....	90
4.5.2	Enrichment of <i>M. pneumoniae</i> surface proteins .....	91
4.5.2.1	Biotinylation .....	91
4.5.2.2	Trypsin shaving .....	91
4.5.3	Preparation of <i>M. pneumoniae</i> whole cell lysates for one- and two-dimensional gel electrophoresis.....	91
4.5.4	One- and two-dimensional polyacrylamide gel electrophoresis (PAGE) .....	91
4.5.4.1	1D SDS-PAGE.....	92
4.5.4.2	2D SDS-PAGE.....	92
4.5.4.3	Trypsin Digest.....	92
4.5.5	Heparin affinity chromatography.....	92
4.5.6	Avidin purification of host binding proteins .....	93
4.5.7	Avidin purification of A549 binding proteins.....	93
4.5.8	Liquid chromatography tandem mass spectrometry (LC-MS/MS). .....	93
4.5.9	MS/MS data analysis.....	94
4.5.10	Dimethyl labelling of <i>M. pneumoniae</i> proteins.....	94
4.5.11	Liquid chromatography tandem mass spectrometry (LC-MS/MS): Sciex 5600 .....	95
4.5.12	Liquid chromatography tandem mass spectrometry (LC-MS/MS): Thermo Scientific Q Exactive™ .....	95

---

4.5.13	Bioinformatic analysis of Mpn142.....	96
4.6	Results .....	97
4.6.1	Defining P40 and P90 on the surface of <i>M. pneumoniae</i> .....	97
4.6.2	Bioinformatic analysis of Mpn142.....	104
4.6.3	Cleavage fragments in the N-terminus of Mpn142.....	105
4.6.4	Cleavage fragments residing in the C-terminus of Mpn142.....	106
4.7	Discussion.....	109
4.8	Acknowledgments .....	112
4.9	Author Contributions .....	112
<b>Chapter 5. Elongation factor Tu is a multifunctional and processed moonlighting protein .....</b>		<b>113</b>
5.1	Preface.....	114
5.2	Author contribution.....	114
5.3	Cover page.....	116
5.4	Abstract .....	117
5.5	Introduction .....	118
5.6	Methodology .....	120
5.6.1	Strains, cultures, and reagents.....	120
5.6.2	Enrichment of <i>M. pneumoniae</i> surface proteins .....	121
5.6.2.1	Biotinylation .....	121
5.6.2.2	Trypsin shaving .....	121
5.6.3	Preparation and separation of whole cell lysates for one- and two-dimensional gel electrophoresis.....	121
5.6.3.1	Whole cell lysis preparation.....	121



---

5.6.3.2	1D and 2D SDS-PAGE protein separation .....	121
5.6.3.3	Trypsin Digest.....	122
5.6.4	Affinity chromatography of host binding <i>M. pneumoniae</i> proteins..	122
5.6.5	Liquid chromatography tandem mass spectrometry (LC-MS/MS) and MS/MS data analysis.....	122
5.6.6	Expression and purification of rMpn <sub>Ef-Tu</sub> .....	122
5.6.7	Binding of rMpn <sub>Ef-Tu</sub> .....	123
5.6.7.1	Binding assays.....	123
5.6.7.2	Influence of anti-rMpn <sub>Ef-Tu</sub> on binding .....	124
5.6.7.3	Binding of rMpn <sub>Ef-Tu</sub> to human proteins in ELISA.....	124
5.6.7.4	Microscale thermophoresis .....	124
5.6.8	Binding affinity of rMpn <sub>Ef-Tu</sub> to plasminogen.....	124
5.6.8.1	Effect of NaCl on plasminogen binding .....	124
5.6.8.2	Effect of $\epsilon$ -aminocaproic acid on plasminogen binding.....	124
5.6.8.3	Plasminogen activation and degradation of human fibrinogen and vitronectin.....	125
5.6.8.4	Binding of anti- rMpn <sub>Ef-Tu</sub> antibodies to <i>M. pneumoniae</i> whole cell lysate proteins.....	125
5.6.9	Surface localisation of Ef-Tu on <i>M. pneumoniae</i> .....	125
5.6.9.1	Localisation of Ef-Tu on the surface of <i>M. pneumoniae</i> colonies	125
5.6.9.2	Surface localisation of Ef-Tu on <i>M. pneumoniae</i> cells.....	125
5.6.10	Dimethyl labelling and LC-MS/MS analysis of <i>M. pneumoniae</i> , <i>M. hyopneumoniae</i> and <i>S. aureus</i> proteins .....	125
5.6.10.1	Dimethyl labelling of proteins .....	125
5.6.10.2	LC-MS/MS of dimethyl labelled proteins.....	125

---

5.6.11	Bioinformatic analysis of Ef-Tu .....	126
5.7	Results .....	126
5.7.1	Bioinformatic analysis of Mhp <sub>Ef-Tu</sub> , Sa <sub>Ef-Tu</sub> , and Mpn <sub>Ef-Tu</sub> .....	126
5.7.2	Mpn <sub>Ef-Tu</sub> , Mhp <sub>Ef-Tu</sub> , and Sa <sub>Ef-Tu</sub> are accessible on the bacterial surface and are retained during heparin agarose chromatography .....	128
5.7.3	Mhp <sub>Ef-Tu</sub> , Sa <sub>Ef-Tu</sub> , and Mpn <sub>Ef-Tu</sub> are cleaved on the bacterial cell surface .....	132
5.7.4	Processing events expose new predicted surface macromolecule interaction sites .....	135
5.7.5	Molecular modelling of Ef-Tu .....	136
5.7.6	Mpn <sub>Ef-Tu</sub> and Mhp <sub>Ef-Tu</sub> are potential multifunctional binding proteins....	138
5.7.7	Mpn <sub>Ef-Tu</sub> is a multifunctional adhesin.....	140
5.8	Discussion.....	145
<b>Chapter 6. The P1 adhesin in <i>Mycoplasma pneumoniae</i> is extensively processed and binds multiple host molecules .....</b>		<b>150</b>
6.1	Preface.....	151
6.2	Author contribution.....	151
6.3	Abstract .....	152
6.4	Introduction .....	153
6.5	Methods and Materials .....	155
6.5.1	Strains.....	155
6.5.2	Cell preparation for one dimensional- and two dimensional-SDS polyacrylamide gel electrophoresis.....	156
6.5.3	Liquid chromatography tandem mass spectrometry (LC-MS/MS) and data analysis .....	156



6.5.4	Surface proteome analysis of <i>M. pneumoniae</i> .....	157
6.5.5	Affinity chromatography host binding <i>M. pneumoniae</i> complexes..	157
6.5.6	Affinity chromatography of P1 C-terminal tail binding complexes...	158
6.5.7	Dimethyl labelling of <i>M. pneumoniae</i> and LC-MS/MS analysis ....	158
6.5.8	Immunoblot of <i>M. pneumoniae</i> cell lysates using Anti-P1 serum....	159
6.5.9	Bioinformatic analysis of the P1 adhesin.....	159
6.6	Results .....	159
6.6.1	Bioinformatic analysis of the P1 adhesin.....	159
6.6.2	The P1 adhesin is extensively processed on the cell surface .....	162
6.6.3	Functional analysis of the C-terminal tail of P1.....	166
6.7	Discussion .....	169
6.8	Conclusion.....	174
<b>Chapter 7. Final discussion.....</b>		<b>175</b>
7.1	The surface proteome of <i>Mycoplasma pneumoniae</i> .....	179
7.2	Characterising potential adhesins of <i>Mycoplasma pneumoniae</i> .....	182
7.3	The multifunctional and moonlighting activity of Elongation factor Tu.....	187
7.4	Proteolysis Induced Moonlighting activity of P1, and potentially P90 of <i>Mycoplasma pneumoniae</i> .....	190
7.5	Concluding remarks.....	192
<b>Chapter 8. Appendices.....</b>		<b>194</b>
8.1	Appendix 1: Surface proteome of <i>M. pneumoniae</i> .....	195
8.2	Appendix 2: 'Bait and Prey' affinity chromatography .....	212
8.3	Appendix 3: Potential <i>M. pneumoniae</i> adhesins .....	232
8.4	Appendix 4: Excised gel sections from 1D-SDS PAGE experiments.....	243

---

8.5	Appendix 5: Chapter 5 Supplementary Materials.....	247
8.5.1	Supplementary Figures .....	247
8.5.2	Supplementary Tables.....	254
8.5.3	Supplementary Methodology.....	257
8.5.3.1	Strains and cultures and reagents.....	257
8.5.3.2	Enrichment of <i>M. hyopneumoniae</i> and <i>S. aureus</i> surface proteins..	258
8.5.3.2.1	Biotinylation .....	258
8.5.3.2.2	Triton X-114 phase extraction of biotinylated <i>M. hyopneumoniae</i> proteins .....	258
8.5.3.2.3	Trypsin shaving .....	258
8.5.3.3	Whole cell lysis preparation for one- and two-dimensional gel electrophoresis.....	258
8.5.3.4	Heparin affinity chromatography.....	259
8.5.3.5	Avidin purification of host binding <i>M. hyopneumoniae</i> proteins .	259
8.5.3.6	Extra Peptide search parameters .....	259
8.5.3.7	LC-MS/MS of dimethyl labelled proteins.....	260
8.5.3.7.1	LC-MS/MS (Sciex 5600) of dimethyl labelled proteins .....	260
8.5.3.7.2	LC-MS/MS (Thermo Scientific Q Exactive™) of dimethyl labelled proteins.....	261
8.5.3.8	Supplementary Bioinformatics: Analysis of conservation of amino acids in Mp <sub>nEF-Tu</sub> , Mh <sub>pEF-Tu</sub> , and Sa <sub>EF-Tu</sub> .....	262
	<b>References.....</b>	<b>263</b>



# Table of Figures

Figure 1.1: <i>M. pneumoniae</i> structure. ....	22
Figure 1.2: Scanning electron microscopy of the progress of pathogenicity caused by <i>M. pneumoniae</i> .....	25
Figure 1.3: Immunogold electron microscopy of antibodies targeting CARDS TX on the surface of <i>M. pneumoniae</i> . ....	26
Figure 1.4: Transmission electron micrograph of <i>M. pneumoniae</i> interacting with ciliated epithelium. ....	28
Figure 1.5: Electron micrographs of <i>M. pneumoniae</i> microcolonies .....	29
Figure 1.6: Schematics of the electron dense core of <i>M. pneumoniae</i> and its three components. ....	30
Figure 1.7: Autoradiographs depicting the necessity of surface proteins for the attachment and growth of <i>M. pneumoniae</i> cells. ....	32
Figure 1.8: Schematic representation of the adhesive attachment organelle of <i>M. pneumoniae</i> .....	33
Figure 1.9: Immunogold electron microscopy of the moonlighting proteins on <i>M. pneumoniae</i> . ....	37
Figure 2.1: Western blot <i>M. pneumoniae</i> biotinylated surface proteins. ....	49
Figure 2.2: Venn diagram of <i>M. pneumoniae</i> surface proteins. ....	50
Figure 2.3: Identification of surface exposed proteins from surface shaving. ....	52
Figure 2.4: Shaved peptides of zinc metalloprotease. ....	53
Figure 2.5: Treemap chart of secretion pathway and transmembrane domain predictions of <i>M. pneumoniae</i> surface proteins. ....	55
Figure 2.6: Cellular location predictions of <i>M. pneumoniae</i> surface proteins. ....	56
Figure 2.7: Tryptic sites of the P30 adhesin. ....	58
Figure 2.8: Tryptic sites of the HMW1 accessory protein. ....	58
Figure 2.9: Enzymatic surface shaving of <i>M. pneumoniae</i> cells. ....	60

---

Figure 3.1: Number of <i>M. pneumoniae</i> proteins identified from each ‘Bait and Prey’ affinity chromatography. ....	67
Figure 3.2: Number of <i>M. pneumoniae</i> proteins identified from the six ‘Bait and Prey’ chromatography columns. ....	68
Figure 3.3: Venn diagram of <i>M. pneumoniae</i> surface proteins and affinity host molecules. ....	74
Figure 3.4: Amino acid sequence of the uncharacterized lipoprotein MPN_284 and cleavage fragments derived from this lipoprotein. ....	79
Abstract Figure: Cleavage map of Mpn142 .....	86
Figure 4.1: Peptides identified in surface proteome analysis of Mpn142.....	98
Figure 4.2: Cleavage map of Mpn142.....	103
Figure 4.3: Alignment of the C-terminal sequence of Mpn142 and P1.....	105
Figure 5.1: Bioinformatic analysis of Mpn <sub>Ef-Tu</sub> , Mhp <sub>Ef-Tu</sub> , and Sa <sub>Ef-Tu</sub> .....	128
Figure 5.2: Cleavage map of Mpn <sub>Ef-Tu</sub> .....	131
Figure 5.3: Predicted 3D structures of Mpn <sub>Ef-Tu</sub> .....	137
Figure 5.4: Binding of rMpn <sub>Ef-Tu</sub> to human A549 epithelial cells.....	139
Figure 5.5: Microtitre plate binding assays depicting the interaction of rMpn <sub>Ef-Tu</sub> with human proteins.....	139
Figure 5.6: Mpn <sub>Ef-Tu</sub> resides on the surface of <i>M. pneumoniae</i> . ....	142
Figure 5.7: Microscale thermophoresis output depicting the interaction of rMpn <sub>Ef-Tu</sub> with human molecules.....	143
Figure 5.8: Influence of ions and lysine analog ACA on binding of rMpn <sub>Ef-Tu</sub> to plasminogen and degradation of human fibrinogen and vitronectin by activated plasminogen. ....	144
Figure 6.1: Cleavage map of the P1 adhesin.....	161
Figure 6.2: Immunoblots of cell lysates of <i>M. pneumoniae</i> probed with sera raised against different regions against P1.....	165
Figure 6.3: Affinity chromatography of the P1 peptide.....	168
Figure 7.1: Schematic of the concept of Proteolysis Induced Moonlighting (PIM).....	185





---

8.5.1: Figure 1: Peptides that map to Ef-Tu identified from surface biotinylation and shaving experiments. ....	247
8.5.1: Figure 2: Cleavage map of Mhp <sub>Ef-Tu</sub> . ....	248
8.5.1: Figure 3: Cleavage map of Ef-Tu <sub>Sa</sub> .....	250
8.5.1: Figure 4: Predicted 3D structures of Mhp <sub>Ef-Tu</sub> and Sa <sub>Ef-Tu</sub> . ....	252
8.5.1: Figure 5: Predicted 3D structures of Mhp <sub>Ef-Tu</sub> and Sa <sub>Ef-Tu</sub> . ....	253

# Table of Tables

Table 1.1: Metabolic enzymes and chaperones from <i>M. pneumoniae</i> with moonlighting functions. ....	38
Table 3.1: Proteins identified in all six ‘Bait and Prey’ chromatography experiments. ...	69
Table 3.2: <i>M. pneumoniae</i> moonlighting proteins. ....	73
Table 3.3: Moonlighting proteins previously shown to bind host molecules that were identified in ‘Bait and Prey’ chromatography experiments. ....	76
Table 3.4: Fragments of lipoprotein MPN_284. ....	80
Table 4.1: N-terminal dimethyl labelled and semi-tryptic peptides identified in Mpn142 (Q50341). ....	99
Table 5.1: Dimethyl labelled and semi-tryptic peptides identified in Mpn <sub>EF-Tu</sub> . ....	132
Table 5.2: Putative heparin binding motifs identified in Mpn <sub>EF-Tu</sub> (Uniprot #: P23568). ....	136
Table 6.1: N-terminal peptides in the P1 adhesin identified by LC-MS/MS from dimethyl labelling <i>M. pneumoniae</i> cells. ....	163
Appendix 1: Table 1: Proteins identified in Biotinylation and surface shaving experiments of <i>M. pneumoniae</i> . ....	195
Appendix 1: Table 2: Bioinformatic predictions of the proteins identified on the surface of <i>M. pneumoniae</i> . ....	203
Appendix 2: Table 1: <i>M. pneumoniae</i> proteins identified in elutions from ‘Bait and Prey’ affinity chromatography. ....	212
Appendix 3: Table 1: <i>M. pneumoniae</i> surface proteins also identified in ‘Bait and Prey’ affinity chromatography. ....	232
Appendix 4: Table 1: Avidin affinity chromatography of gel sections and the mass range for each section. ....	243
Appendix 4: Table 2: Actin ‘Bait and Prey’ affinity chromatography of gel sections and the mass range for each section. ....	243
Appendix 4: Table 3: Fetuin ‘Bait and Prey’ affinity chromatography of gel sections and the mass range for each section. ....	243



---

Appendix 4: Table 4: Fibronectin ‘Bait and Prey’ affinity chromatography of gel sections and the mass range for each section.....	244
Appendix 4: Table 5: Plasminogen ‘Bait and Prey’ affinity chromatography of gel sections and the mass range for each section.....	244
Appendix 4: Table 6: Heparin ‘Bait and Prey’ affinity chromatography of gel sections and the mass range for each section.....	245
Appendix 4: Table 7: A549 surface protein complexes ‘Bait and Prey’ affinity chromatography of gel sections and the mass range for each section.....	246
8.5.2: Table 1: Number of binding sites in full length and fragments of Mpn <sub>Ef-Tu</sub> .....	254
8.5.2: Table 2: Dimethyl labelled and semi-tryptic peptides identified in Mhp <sub>Ef-Tu</sub> and Sa <sub>Ef-Tu</sub> . ....	254
8.5.2: Table 3: Putative heparin binding motifs identified in Mhp <sub>Ef-Tu</sub> , and Sa <sub>Ef-Tu</sub> .....	256
8.5.2: Table 4: Number of binding sites in full length and fragments of Mhp <sub>Ef-Tu</sub> .....	257
8.5.2: Table 5: Number of binding sites in full length and fragments of Sa <sub>Ef-Tu</sub> . ....	257

---

# Thesis Preface

The growing incidence of antibiotic resistance globally is a significant public health issue and as previously susceptible bacteria continue to develop resistance, we need to develop novel strategies to counter this trend. *Mycoplasma pneumoniae* is a genome reduced bacteria that is one of the major causes of bacterial pneumonia in close contact settings such as schools and hospitals. Children, the elderly, and the immuno-suppressed are commonly infected due to an under developed or impaired immune system. A successful vaccine against this respiratory pathogen is yet to be developed and treatment options are limited. Additionally, children are limited to one class of antibiotics due to the permanent side effects of other agents.

Antibiotic resistance within *M. pneumoniae* was detected over a decade ago and has now spread to most of the Northern Hemisphere. Though infections are not typically fatal, *M. pneumoniae* can cause secondary co-infections; some of which can be fatal. The work presented within this thesis expands the functional proteome of *M. pneumoniae*, with the goal of discovering potential novel therapeutic or vaccine targets. This was initially achieved by examining the full repertoire of proteins exposed on the surface of *M. pneumoniae*. This thesis then addresses which host antigens these proteins potentially interact with during infection. Although a single protein was not chosen as a vaccine target, the result of the work presented here report a list of potential targets that participate in the colonisation of the respiratory epithelium. This thesis highlights that the interactions between *M. pneumoniae* and host epithelium are complex, and involve a wide range of diverse proteins.

This thesis begins with an introduction to *M. pneumoniae* and what is currently known about the proteins involved during the interaction with the human host.