The structure and function of Biotin Protein Ligase:

A focus on *Staphylococcus aureus, Saccharomyces cerevisiae, Candida albicans* and *Homo sapiens.*

Nicole Renee` Pendini, B. Sc. Honours (University of Adelaide)

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University of Adelaide
South Australia

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Abbreviations

ACC: Acetyl CoA Carboxylase

AMP: Adenosine monophosphate

Apo: Unliganded enzyme

ATP: Adenosine triphosphate

BCCP: Biotin Carboxyl Carrier Protein

BirA: Biotin inducible repressor

BPL: Biotin Protein Ligase

Bt: Biotin

BtOH-AMP: Biotinol-5'-adenosine monophosphate

CaBPL: *Candida albicans* Biotin Protein Ligase

EcBPL: *Escherichia coli* Biotin Protein Ligase

HCS: Holocarboxylase synthetase

Holo: Ligand bound enzyme

MCD: Multiple carboxylase deficiency

MR: Molecular replacement

MRSA: Methicillin resistant *Staphylococcus aureus*

PC: Pyruvate carboxylase

PDB: Protein Data Bank

PDBID: Protein Data Bank identification code

PhBPL: *Pyrococcus horikoshi* Biotin Protein Ligase

R.M.S.D: Root mean square deviation

SaBPL: *Staphylococcus aureus* Biotin Protein Ligase

ScBPL: *Saccharomyces cerevisiae* Biotin Protein Ligase

SAXS: small angle X-ray scattering

VRSA: Vancomycin resistant *Staphylococcus aureus*

Abstract and summary of thesis for Nicole Renee Pendini

Biotin Protein Ligase (BPL) is an essential enzyme responsible for the covalent attachment of biotin to a specific lysine residue of biotin-dependent carboxylases, transcarboxylases and decarboxylases. Due to the fundamental processes that these enzymes are involved in such as lipogenesis, amino acid catabolism and gluconeogenesis, much research has been conducted on these enzymes. Studies encompassing structural, mutational and catalytic functions of these enzymes have lead to novel drug developments for the treatment of obesity, diabetes, metabolic syndrome, bacterial and fungal infections.

As BPL is required for activation of these enzymes by biotinylation, it is believed that it too could be targeted in a similar way to produce novel therapeutics. To date, the most characterised BPLs are from the Gram-negative bacteria *Escherichia coli* and the archea *Pyrococcus hirokoshii*. However minimal information is known about other forms of clinically important bacterial species or eukaryotic forms of this important enzyme.

Through my candidature I have compiled a thorough literature review summarised as chapter 1: Introduction. Furthering this literature analysis, a human BPL model was generated with aid of BPL structural co-ordinates already deposited in the protein data bank (PDB), thus allowing focus on human BPL mutations that cause multiple carboxylase deficiency (chapter 2). I have solved the structure of BPL from the clinically important pathogenic bacteria *Staphylococcus aureus.* This was performed in several ligand-bound and non-bound states (chapters 3 and 4). A novel high-throughput assay was

developed to test BPL activity. This assay allow testing of compounds that could potentially inhibit the BPL from *Candida albicans* (a species responsible for invasive fungal infections) (chapter 5). Large amounts of highly purified BPL from *Saccharomyces cerevisiae* allowed for the first structural analysis of a eukaryotic BPL (Chapter 6). The work has been summarised by a general discussion and future directions for the project (Chapter 7).

Thesis layout:

The thesis will be presented as a series of manuscripts either published or intention to be submitted for publication. Each manuscript will form a self-contained chapter with its own references. Included will be an overall general introduction and general discussion to link the information from these papers so as to present a uniform body of research conducted during candidature.

Declaration for thesis containing published work and/ or work prepared for publication

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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The author acknowledges the copyright of published works contained within this thesis including:

Chapter 2:

Nicole R. Pendini, Lisa M. Bailey, Grant W. Booker, Matthew C. Wilce, John C. Wallace & Steven W. Polyak. (2008) Distant relatives of Biotin Protein Ligase aid in understanding multiple carboxylase deficiency. *Biochemica Biophysica Acta – Proteins and Proteomics*, Jul-Aug 2008, 1784(7-8), 973-82. PMID: 18442489

Chapter 3:

Pendini NR, Polyak SW, Booker GW, Wallace JC, Wilce MC (2008) Purification, crystallization and preliminary crystallographic analysis of biotin protein ligase from *Staphylococcus aureus*. *Acta Crystallogr Sect F Struct Biol Cryst Commun.* 2008 Jun 1;64(Pt 6):520-3, 2008 May 23. PMID: 18540065

Chapter 4:

Nicole R. Pendini, Steven W. Polyak, Grant W. Booker, John C. Wallace, & Matthew C. Wilce, Crystal structures of apo and liganded Biotin Protein Ligase from *Staphylococcus aureus*: towards the development of new antibiotic for MRSA. To be submitted for publication.

Chapter 5:

Nicole R Pendini; Lisa M Bailey; Grant W Booker; Matthew C Wilce; John C Wallace; Steven William Polyak (2008) Biotin Protein Ligase from *Candida albicans:* Expression, purification and development of a novel assay, Arch Biochem Biophys. 2008 Nov 15;479(2):163-9. Epub 2008 Sep 11, PMID: 18809372

Chapter 6:
Nicole R. Pendini, Nathan Cowleson, John C. Wallace, Grant W. Booker, Matthew C. Wilce & Steven W. Polyak (2008) Characterisation of the domain structure of yeast biotin ligase and its complexes by small-angle X-ray scattering and molecular modelling. Submitted to JBC tracking number JBC/2009/013490.

Authorisation to publish each paper has been given and provided in print for each chapter containing copyright and co-authored work, including acknowledgement of contribution to the work from each author.

Communications and Presentations

Pendini, N. R., Cowieson, N., Wallace J.C., Booker G.W., Wilce, M.C and Polyak, S.W., The characterisation of the domain structure of yeast biotin ligase and its complexes by small-angle X-ray scattering and molecular modelling (2009), Biomolecular Dynamics and Interactions Symposia: Protein folding, function and assembly. Abs:P6.

Pendini, N. R., Polyak, S.W., Booker G.W., Wallace J.C. and Wilce, M.C., *Staphylococcus aureus* Biotin Protein Ligase as a novel antibiotic target (2009), 34th Lorne Conference on Protein Structure and Function. Abs#157.

Pendini, N. R., Polyak, S.W., Booker G.W., Wallace J.C. and Wilce, M.C., *Staphylococcus aureus* Biotin Protein Ligase as a novel antibiotic target (2008), University of Adelaide, School of Molecular and Biomedical Sciences Research Symposia. **Awarded** $200 for best poster presentation.

Pendini, N. R., University of Adelaide, School of Molecular and Biomedical Sciences Research Symposia, Scientific Image competition: **Awarded** $50 for best image.

Pendini, N. R., Polyak, S.W., Booker G.W., Wallace J.C. and Wilce, M.C., *Staphylococcus aureus* Biotin Protein Ligase as a novel antibiotic target (2008) Melbourne Protein Group meeting, Bio21, Victoria, Australia **Awarded** $50 for best poster presentation.

Pendini, N. R., Polyak, S.W., Booker G.W., Wallace J.C. and Wilce, M.C., Biotin Protein Ligase as a novel antibiotic target (2008), Monash University, Department of Biochemistry and Molecular Biology Annual Postgraduate Research Conference, invited speaker. **Awarded** $200 for best presentation. Abstract S04.

Pendini, N. R., Polyak, S.W., Booker G.W., Wallace J.C. and Wilce, M.C., Biotin Protein ligase as a novel antibacterial target: a focus of *Staphylococcus aureus* (2008) Sicily, Italy, 40th Course for the International School of Crystallography: From Molecules to Medicine: Integrating Crystallography in Drug Discovery, Pos 67. **Awarded** student travel scholarship € 600.00.

Pendini, N. R., Polyak, S.W., Booker G.W., Wallace J.C. and Wilce, M.C., Biotin Protein Ligase as a novel antibacterial target (2008), 33rd Lorne Conference on Protein Structure and Function. Abs#361. **Awarded** student travel award $100.

Pendini, N. R., Polyak, S.W., Bailey, L.M., Booker G.W., Wilce, M.C. and Wallace J.C., Purification and characterisation of Biotin Protein Ligase from *Candida albicans* (2007), Monash University, Department of Biochemistry and Molecular Biology Annual Postgraduate Research Conference.

Pendini, N. R., Polyak, S.W., Bailey, L.M., Booker G.W., Wilce, M.C. and Wallace J.C., Purification and characterisation of Biotin Protein Ligase from *Candida albican* (2007), 32rd Lorne Conference on protein structure and function Abs#238. **Awarded** student travel award $100.

Pendini, N. R., Polyak, S.W., Swift, R., Booker G.W., Wilce, M.C. and Wallace J.C., Probing the importance of the N-terminal region of Human Biotin Protein Ligase (2007), The University of Adelaide Molecular and Biomedical Sciences School Symposia: **Finalist** in the scientific image competition for "BirA crystal images".

Pendini, N. R., Polyak, S.W., Booker G.W., Wilce, M.C. and Wallace J.C., Determination of the Structure of Yeast Biotin Protein Ligase: Implications as a Novel Antifungal Drug Target (2006), Australian Society for Medical Research SA division Scientific Meeting, invited presenter. **Awarded:** $400 for Best oral presentation by an ASMR student member.

Pendini, N. R., Polyak, S.W., Booker G.W. and Wallace J.C., Probing the importance of the N-terminal region of Human Biotin Protein Ligase (2006), 31st Lorne Conference on Protein Structure and Function. **Awarded** student travel award $100.

Pendini, N. R., Polyak, S.W., and Wallace J.C., 2005-Combio
The purification of Eukaryotic Biotin Protein Ligase.

Pendini, N. R., Polyak, S.W., and Wallace J.C., The purification of yeast Biotin Protein Ligase (2005). The East Coast Protein Structure Conference.

Pendini, N. R., Polyak, S.W., and Wallace J.C., (2005), The purification of yeast biotin protein ligase, Australian Society for Medical Research SA division.

Pendini, N. R., Polyak, S.W., Swift, R., and Wallace J.C., A survey of human Biotin domains as a substrate for Biotin Protein Ligase (2005) 30th Lorne Conference on Protein Structure and Function. **Awarded** student travel award $100.

Co-author poster presentations:

Ng, B., Pendini N.R., Tieu W., Kuan K., Morona R., Abell A., Wilce M.C.J., Wallace J.C., Polyak S.W. and Booker G.W. (2008) Discovery of biotin protein ligase as a novel class of antibiotic. University of Adelaide, School of Molecular and Biomedical Sciences Research Symposia, Pos 12

Invited Presentations at National Conferences
Polyak, S.W., Tieu, W., Ng, B., Pendini, N.R., Kuan, K., Morona, R., Booker, G.W., Wilce, M.C., Wallace, J.C., Abell, A.D. (2008) Inhibitors of biotin protein ligase: A novel class of antibiotics for the treatment of *Staphylococcus aureus* Proc. Aust. Health and Medical Research Congress Abstract 153

Ng, B., Pendini N.R., Tieu W., Kuan K., Morona R., Abell A., Wilce M.C.J., Wallace J.C., Polyak S.W. and Booker G.W. (2008) Discovery of biotin protein ligase as a novel class of antibiotic. ComBio2008, Pos WED-024

Ng, B., Pendini, N., Tieu, W., Kuan, K., Morona, R., Wallace, J. C., Wilce, M., Abell, A., Booker, G. W. & Polyak, S. W. (2008) Inhibitor of biotin protein ligase: a novel class of antibiotics for the treatment of *Staphylococcus aureus*. Australian Society for Medical Research SA division Scientific Meeting

Mayende, L., Swift, R. D., Bailey, L. M., Pendini, N. R., Wallace, J. C. & Polyak, S. W. (2008) Domain structure of human holocarboxylase synthetase: evidence of an interaction between the N-terminal and C-terminal halves Proc. Lorne Conference on Protein Structure and Function, Pos 255

Polyak, S. W., Stojkoski, C., Pendini, N. R., Booker, G. W. & Wallace, J. C. (2007) Biotin protein ligase: A novel antibiotic target Proc. Lorne Conference on Protein Structure and Function, Pos 323

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