UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of ***Doctor of Philosophy*** *of*

ASHLEY BROTT

On Tuesday, April 11, 2023 at 9:30 a.m. (online)

Thesis Title: **Inhibition and structural insights into the mechanism of peptidoglycan O-acetylation in *Neisseria gonorrhoeae***

Examination Committee:

Dr. Jaideep Mathur, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Stephen Seah, Dept. of Molecular and Cellular Biology
Dr. Anthony Clarke, Dept. of Chemistry and Biochemistry, Wilfrid Laurier University
Dr. Georgina Cox, Dept. of Molecular and Cellular Biology
Dr. Antoni Planas, Bioengineering Dept., University Ramon Llull (External Examiner)

Advisory Committee:
Dr. Stephen Seah (Co-Advisor)
Dr. Anthony Clarke (Co-Advisor)
Dr. Lynne Howell

Abstract: It is no coincidence that peptidoglycan (PG), which is unique to bacteria, represents a common target for antibiotics and host immune systems, as it fulfills a crucial role in maintaining cellular viability. Bacteria however have evolved to minimize the risk of this "Achilles' heel" through the modification of PG. O-Acetylation of the C-6-hydroxyl of *N*-acetylmuramic acid is commonly found in human pathogens including *Neisseria gonorrhoeae* which was identified by the World Health Organisation as an "Urgent Threat" in a 2019 report on antibiotic resistance. O-Acetylation increases pathogenicity due to its ability to inhibit the activity of lysozyme, a first line of defense used by immune systems. In Gram-negative bacteria it holds additional importance as a means to control endogenous autolysins, and therefore presents an attractive target for the development of new antimicrobials. These would exploit a unique antivirulence strategy in rendering the bacteria susceptible to not only the host immune system but also autolytic activity.

In Gram-negative bacteria, O-acetylation is achieved *via* a two-component system of *O*-acetyltransferases (Pats). PatA, a transmembrane protein, first shuttles an acetyl group across the cytoplasmic membrane which is acquired by PatB that directly modifies the existing peptidoglycan sacculus.

The purpose of this study was to validate PatB from *N. gonorrhoeae* as a novel antivirulence target *via* the identification and characterization of the first inhibitors of a Gram-negative PG *O*-acetyltransferase. To help guide the development of future inhibitors, the structure of PatB was solved using X-ray crystallography. Within the structure, an unpredicted feature was identified which led to additional

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investigation into the association between PatB and the PG sacculus. In addition, the first complete 3D model of PatA from *N. gonorrhoeae* was generated, and a proposed molecular mechanism is presented.

Curriculum Vitae: Ashley completed her Bachelor of Science (Hons.) in Molecular Biology and Genetics at the University of Guelph in 2013. She began her M.Sc. work in the lab of Dr. Anthony Clarke in Fall 2013 and transferred into the Ph.D. program in Fall 2014.

Awards: Canadian Glycomics Symposium Student Poster Competition Winner (2018); Tri-University Protein Symposium Student Seminar Competition Winner (2018); Ontario Graduate Scholarship (2017); Dr. and Mrs. Kenneth F. Gregory Graduate Scholarship (2017); Ontario Graduate Scholarship (2016); Queen Elizabeth II Graduate Scholarship in Science & Technology (2015); Arthur Richmond Memorial Scholarship (2015).

Publications: Danielle L. Sexton, Francesca A. Herlihey, **Ashley S. Brott**, David A. Crisante, Evan Shepherdson, Anthony J. Clarke, Marie A. Elliot. (2020). Roles of LysM and LytM Domains in Resuscitation-Promoting Factor (Rpf) Activity and Rpf-Mediated Peptidoglycan Cleavage and Dormant Spore Reactivation. *J. Biol. Chem*. *295* (27): 9171-9182.

Ashley S. Brott and Anthony J. Clarke. (2019). Peptidoglycan O-Acetylation as a Virulence Factor: Its Effect on Lysozyme in the Innate Immune System. Antibiotics, *8*, 94.

Ashley S. Brott, Carys S. Jones, Anthony J. Clarke. (2019). Development of a High Throughput Screen for the Identification of Inhibitors of Peptidoglycan *O*-Acetylation, New Potential Antibacterial Targets. *Antibiotics*. *8*, 65.

Ashley S. Brott, David Sychantha, Anthony J. Clarke. (2019). Methods Molecular Biology. Bacterial Polysaccharides. Chapter 10: Assays for the Enzymes Catalyzing the O-Acetylation of Bacterial Cell Wall Polysaccharides. Springer Nature. *1954.* ISBN: 978-1-4939-9153-2.

David Sychantha, **Ashley S. Brott**, Carys S. Jones, Anthony J. Clarke. (2018). Mechanistic Pathways for Peptidoglycan *O*-Acetylation and De-*O*-Acetylation. *Front. Microbiol*. *9: 2332.*