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*

STEVEN KELLY

on Wednesday, September 14, 2022 at 1:30 p.m. (online)

Thesis Title: **Characterization of the biosynthesis of *Klebsiella pneumoniae* O-antigen polysaccharides and the origin of ribofuranose in bacterial polysaccharides**

Examination Committee:

Dr. Jaideep Mathur, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Matthew Kimber, Dept. of Molecular and Cellular Biology
Dr. Anthony Clarke, Dept. of Chemistry and Biochemistry, Wilfrid Laurier University
Dr. Matthew Sorbara, Dept. of Molecular and Cellular Biology
Dr. Helge Dorfmueller, Dept. of Molecular Microbiology, University of Dundee (External Examiner)

Advisory Committee:
Dr. Chris Whitfield (Advisor)
Dr. Matthew Kimber
Dr. Rebecca Shapiro
Dr. Anthony Clarke

Abstract: *Klebsiella pneumoniae* is a Gram-negative opportunistic bacteria pathogen of global concern due to extensive and growing antibiotic resistance. New therapeutics to combat resistant infections are needed and immunotherapeutic strategies are being pursued. Cell surface polysaccharides can be used to produce glycoconjugate-based immunogens for vaccines or production of prophylactic antibodies. Lipopolysaccharide O-antigen polysaccharides (O-PSs) are candidates, but the use of O-PS in glycoconjugate vaccine production requires a detailed understanding of the structures of O-PSs. In addition, *in vivo* approaches to generate protein-conjugated immunogenic molecules depend on a full understanding of underpinning biochemical activities used to produce them. This research aims to fill some important gaps in current understanding. The *K. pneumoniae* O2a O-PS is prevalent in clinical isolates but is diversified by various structural modifications to generate different antigenic forms. In one example, the O2c antigen is a structurally and antigenically distinct polysaccharide added to the non-reducing terminus of O2a chains. A three gene locus (*wbmVWX*) is implicated in the biosynthesis of the O2c antigen, but the precise biochemical activities encoded by these genes are unknown. Here I show that WbmV and WbmW are glycosyltransferase enzymes sufficient to produce the O2c disaccharide repeat-unit structure. WbmV catalyzes addition of a β-1,5-linked *N*-acetylglucosamine, while WbmW performs the addition of a β-1,3-linked galactofuranose. Together, WbmV and WbmW interact to form the active, membrane associated O2c biosynthesis complex. This work provides the first comprehensive biochemical insight into an unusual form of polysaccharide modification. The O-PSs of *K. pneumoniae* O4 and O7 serotypes share ribofuranose in their repeat units, but the identity of the activated donor for ribofuranose

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residues in any bacterial polysaccharide is unknown, as are the corresponding ribofuranoslytransferase enzymes. Here I unequivocally show the activated donor is 5′-phospho-D-ribosyl-α-1-diphosphate (PRPP) and characterize the first known ribosyltransferase, a bifunctional protein containing a glycan phosphoribosyltransferase (gPRT) and a phosphoribose phosphatase (PRP). The crystal structure of a thermophilic ortholog of these enzymes revealed a novel glycosyltransferase fold shared by a well distributed collection of ribofuranoslytransferases. Using the PRP domain as a probe for bioinformatics, I identified four groups of ribofuranosyltransferase enzymes differing in the sequence of the gPRT components and enzyme modularity. While 3 groups transfer ribofuranose to cytoplasmic O-PS biosynthetic intermediates, one system represented a periplasmic based modification system for the addition of α-linked ribofuranose side chains. This has established several new and novel prototype systems for further investigation of the principles of glycosylation machinery and provide crucial insight and tools for glycoengineering-based immunotherapeutic production.

Curriculum Vitae: Steven completed his Bachelor of Science (Hons.) Biological & Pharmaceutical Chemistry Co-op at the University of Guelph in December 2017. He began his Ph.D. work in the lab of Dr. Chris Whitfield in January 2018.

Awards: NSERC CGS-M (2018); NSERC PGS-D (2019); CSM Student Symposium Competition Finalist (2021); Donald R. Phillips Molecular and Cellular Biology Scholarship (2021).

Publications:

Kelly, S.D., Williams, D.M., Nothof, J.T., Kim, T., Lowary, T.L., Kimber, M.S., Whitfield, C. The biosynthetic origin of ribofuranose in bacterial polysaccharides. *Nature Chemical Biology.* 18:530-537 (2022).

Whitfield, C., Williams, D.M., **Kelly, S.D.** Lipopolysaccharide O-antigens - bacterial glycans made to measure. *Journal of Biological Chemistry.* 295**:**10593-609 (2020).

Clarke, B.R., Ovchinnikova O.O., Sweeney, R.P., Kamski-Hennekam, E.R., Gitalis, R., Mallette, E., **Kelly, S.D.**, Lowary, T.L., Kimber, M.S., Whitfield, C. A bifunctional O-antigen polymerase structure reveals a new glycosyltransferase family. *Nature Chemical Biology.* 16**:**450-457 (2020).

Mann, E., **Kelly, S.D.**, Al-Abdul-Wahid, M.S., Clarke, B.R., Ovchinnikova, O.O., Liu, B., Whitfield, C. Substrate recognition by a carbohydrate-binding module in the prototypical ABC transporter for lipopolysaccharide O-antigen from *Escherichia coli* O9a. *Journal of Biological Chemistry.* 294**:**14978-90 (2019).

Kelly, S.D., Clarke, B.R., Ovchinnikova, O.O., Sweeney, R.P., Williamson, M.L., Lowary, T.L., Whitfield, C. *Klebsiella pneumoniae* O1 and O2ac antigens provide prototypes for an unusual strategy for polysaccharide antigen diversification. *Journal of Biological Chemistry.* 294**:**10863-76 (2019).

Clarke, B.R., Ovchinnikova, O.O., **Kelly, S.D.**, Williamson, M.L., Butler, J.E., Liu, B., Wang, L., Gou, X., Follador, R., Lowary, T.L., Whitfield, C. Molecular basis for the structural diversity in serogroup O2-antigen polysaccharides in *Klebsiella pneumoniae*. *Journal of Biological Chemistry.* 293:4666-79 (2018).