



COLLEGE OF BIOLOGICAL SCIENCE
Department of Molecular and Cellular Biology

ANNOUNCEMENT: Interested members of the University Community are invited to attend the Final Oral Examination for the Degree of **Master of Science** of

Alexander (AJ) Stirling

of the Department of Molecular and Cellular Biology
on Thursday, March 30, 2017 at 9:30 a.m. in SSC 1511

Thesis Title: Structure-Function Studies of Acyl-CoA Dehydrogenases Involved in Steroid Degradation

Examination Committee: Dr. F. Brauer, Dept. of Molecular and Cellular Biology (Chair)
Dr. S. Seah, Dept. of Molecular and Cellular Biology
Dr. M. Kimber, Dept. of Molecular and Cellular Biology
Dr. A. Clarke, Dept. of Molecular and Cellular Biology

ABSTRACT

Alexander Stirling, B.Sc. (Hons.)

Advisor: Dr. Stephen Seah

The acyl-CoA dehydrogenases (ACADs), CasL-CasN from *Rhodococcus jostii* RHA1 and Tcur3481-Tcur3483 from *Thermomonospora curvata* DSM 43183, were heterologously expressed and purified. Tcur3481-Tcur3483 has an 18-fold higher specificity constant for the 3-carbon side chain steroid metabolite compared to the five carbon side chain metabolite. Tcur3481-Tcur3483 structure was determined to a resolution of 2.1 Å. The protein adopted an $\alpha_2\beta_2$ structure, similar to the structure of the *Mycobacterium tuberculosis* ACAD FadE26-FadE27. The putative substrate binding site of Tcur3483 is 1.6 times smaller in volume than FadE26 and the catalytic glutamate base is part of a helix while the corresponding residue in FadE26 part of a loop. These differences may contribute to the broader substrate specificity of FadE26. The molecular determinants of substrate specificity were also tested by creating the Ala691Gly variant of the *M. tuberculosis* ACAD, FadE34. The variant gained the ability to utilize 3-carbon isopropyl side chain metabolite as a substrate.

CURRICULUM VITAE:

Alexander (AJ) obtained his Bachelor of Science (Hons.) in Microbiology from the University of Guelph in the spring of 2013, then began his M.Sc. in the lab of Dr. Stephen Seah in September 2014.

Publication:

Ruprecht, A., Maddox, J., Stirling, A.J., Visaggio, N., and Seah, S.Y.K. (2015). Characterization of novel acyl coenzyme A dehydrogenases involved in bacterial steroid degradation. *J. Bacteriol.* 197, 1360–1367.