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*

KURT SCHROETER

On Friday, January 5, 2024 at 9:30 a.m. (SSC 1511)

Thesis Title: **Characterization of bacterial catabolic enzymes involved in sterol and bile acid side chain degradation**

Examination Committee:

Dr. Steffen Graether, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Stephen Seah, Dept. of Molecular and Cellular Biology
Dr. Tariq Akhtar, Dept. of Molecular and Cellular Biology
Dr. Siavash Vahidi, Dept. of Molecular and Cellular Biology
Dr. Geoff Horsman, Dept. of Chemistry, Wilfrid Laurier University (External Examiner)

Advisory Committee:
Dr. Stephen Seah (Advisor)
Dr. Tariq Akhtar
Dr. Matthew Kimber
Dr. Anthony Clarke

Abstract: Certain Actinobacteria and Proteobacteria have the rare ability to catabolize eukaryotic steroids. The linear steroid side chain is catabolized via a mechanism analogous to fatty acid β-oxidation, where repeated reaction cycles shorten the side chain by two-three carbon atoms. These organisms have significant biotechnology applications including pharmaceutical synthesis and bioremediation.

Steroid degradation gene clusters in various Actinobacteria encode multiple enoyl-CoA hydratases that catalyze the hydration of enoyl-CoA esters during side chain catabolism. Steady state kinetic parameters of enzymes from *Mycobacterium tuberculosis*, *Rhodococcus jostii* RHA1, and *Thermomonospora curvata* towards steroid substrates indicate they exhibit distinct side chain length specificity, suggesting the hydration reaction in each β-oxidation cycle is catalyzed by distinct enzymes.

Steroid degrading proteobacteria encode a distinct MaoC hydratase: Steroid HYdratase (Shy). Steady state kinetic parameters of Shy from *Comamonas testosteroni* indicate it efficiently hydrates C_5 steroid side chains. The structure of Shy was determined via X-ray crystallography, revealing a homodimer of MaoC hydratase domains with two active sites. The substrate binding mode of Shy was further elucidated via modelling and mutagenesis studies. This differs from the actinobacterial hydratases which lose one active site to accommodate bulky steroid substrates.

In Actinobacteria the hydrated C_5 side chain is subsequently oxidized by a 3-hydroxyacyl-coenzyme A dehydrogenase. Steady state kinetic assays and mass spectrometry analysis of ChsB1 from *M. tuberculosis* and Hsd4A from *T. curvata* revealed that these enzymes are oxidoreductases that catalyze

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the oxidation of the hydrated steroid side chains, while also exhibiting secondary 17-hydroxy steroid dehydrogenase activity.

Curriculum Vitae: Kurt completed his Bachelor of Science (Honours) in Microbiology at the University of Guelph in Winter 2018. He started a direct-entry PhD in Molecular and Cellular Biology in Fall 2018 under the supervision of Dr. Stephen Seah.

Awards: Larry Calvert CNC/IUCr Trust Fund Award (2019); Queen Elizabeth II Graduate Scholarship in Science and Technology (2020-22).

Publications: **Schroeter, K. L.**, Abraham, N., Rolfe, N., Barnshaw, R., Diamond, J., Seah, S. Y. K. (2022) Bacterial Hydratases Involved in Steroid Side Chain Degradation Have Distinct Substrate Specificities. J. Bacteriol. *204* (9), e00236-22.

Abraham, N., **Schroeter, K. L.**, Zhu, Y., Chan, J., Evans, N., Kimber, M. S., Carere, J., Zhou, T., & Seah, S. Y. K. (2022). Structure-function characterization of an aldo-keto reductase involved in detoxification of the mycotoxin, deoxynivalenol. Sci. Rep. *12* (1), 14737.

Aggett, R., Mallette, E., Gilbert, S. E., Vachon, M. A., **Schroeter, K. L.**, Kimber, M. S., and Seah, S. Y. K. (2019). The steroid side-chain-cleaving aldolase Ltp2-ChsH2$_{DUF35}$ is a thiolase superfamily member with a radically repurposed active site. J. Biol. Chem. *294* (31), 11934–11943.