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 ***Master of Science*** *of*

SHAWNA ZHU

On Thursday, August 24, 2023 at 9:30 a.m. (SSC 1511)

Thesis Title: **Exploring the roles of drug efflux pumps during the transport of compounds across the *Escherichia coli* cell envelope**

Examination Committee:

Dr. Jaideep Mathur, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Georgina Cox, Dept. of Molecular and Cellular Biology
Dr. Rebecca Shapiro, Dept. of Molecular and Cellular Biology
Dr. Cezar Khursigara, Dept. of Molecular and Cellular Biology

Advisory Committee:

Dr. Georgina Cox (Advisor)
Dr. Rebecca Shapiro

Abstract: In Gram-negative bacteria, the relatively impermeable outer membrane and the exporting activities of drug efflux pumps synergize to prevent the periplasmic and intracellular accumulation of antibiotics. Given the emergence of antibiotic resistance, drug efflux pumps are promising targets for new drug discovery. The *Escherichia coli* drug efflux network is highly complex and comprises 35 drug efflux pumps exhibiting varying degrees of functional redundancy, which has limited the study of efflux pump function. Harnessing a recently developed efflux platform built upon a highly efflux-deficient mutant background, EKO-35, I determined the substrate profiles of each individual drug efflux pump within the *E. coli* drug efflux network and summarized the physicochemical properties affecting the transport of compounds across the outer membrane. Surprisingly, the loss of 35 inner membrane drug efflux pumps affected the susceptibility of *E. coli* to several poorly-characterized synthetic antimicrobial agents to a lesser extent than the inactivation of the major outer membrane channel TolC. This channel forms complexes with numerous multidrug-resistant efflux pumps to facilitate the export of compounds across the outer membrane. Using genetic, phenotypic, and biochemical approaches to investigate the basis of these susceptibility differences, I revealed that one of these synthetic compounds is an inhibitor of MsbA, an essential transporter involved in outer membrane biogenesis. The genetic repression of *msbA* in a Δ*tolC* mutant resulted in increased sensitivity to this compound and subsequently identified a synthetic lethal interaction between MsbA and TolC. A CRISPR interference genetic screen uncovered further instances of synthetic lethal interactions between TolC and other essential cell envelope biogenesis components. Overall, this thesis provides important insight into the physicochemical properties underlying the transport of drugs across the *E. coli* cell envelope and reveals the potential involvement of TolC in cell envelope biogenesis and maintenance.

Continued…

Curriculum Vitae: Shawna completed her Bachelor of Science in Biology at the University of Waterloo in April 2021. She began her Master of Science program in Molecular and Cellular Biology in Dr. Georgina Cox's lab in September 2021.

Awards: Ontario Graduate Scholarship (2021-22); Canada Graduate Scholarship - Master's (2022-23).

Publications: Goetz, J.A., Kuehfuss, N.M., Botschner, A.J., **Zhu, S.**, Thompson, L.K., and Cox, G. (2022). Exploring functional interplay amongst *Escherichia coli* efflux pumps. Microbiology *168*. 10.1099/mic.0.001261.

Teelucksingh, T., Thompson, L.K., **Zhu, S**., Kuehfuss, N.M., Goetz, J.A., Gilbert, S.E., MacNair, C., Geddes-MacAlister, J., Brown, E.D., and Cox, G. (2022). A genetic platform to investigate the functions of bacterial drug efflux pumps. Nat. Chem. Biol. 10.1038/s41589-022-01119-y.