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*

# ALISON BEREZUK

**on Tuesday, March 27, 2018 at 9:30 a.m. in SSC 1511**

**Thesis Title**: **Investigating the role of divisome protein FtsK as an essential checkpoint during bacterial cell division.**

**Examination Committee:**

Dr. A. Bendall, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. C. Khursigara, Dept. of Molecular and Cellular Biology
Dr. M. Kimber, Dept. of Molecular and Cellular Biology
Dr. R. Shapiro, Dept. of Molecular and Cellular Biology
Dr. H. Erickson, Cell Biology, Duke University School of Medicine

**Advisory Committee:**

Dr. C. Khursigara (Adv)
Dr. J. Wood
Dr. M. Kimber

**Abstract:** Bacterial cell division is an essential and fundamental process required to sustain life. In *Escherichia coli*, division requires the recruitment and assembly of approximately thirty soluble and membrane-bound proteins, ten of which are essential (FtsZ, FtsA, ZipA, FtsK, FtsQ/B/L, FtsW/I, FtsN). Complete assembly of this macromolecular complex relies on formation of a dynamic ring-like structure known as the Z-ring. Following initial formation and stabilization of the Z-ring, cells must complete segregation of the bacterial chromosome and remodel the cell envelope to allow septum formation. One key modulator linking early (Z-ring) and late (cell envelope remodeling) division complexes is the essential protein FtsK. FtsK is a bifunctional transmembrane protein that coordinates chromosome segregation with its C-terminus ($FtsK_C$) and cell division with its membrane-anchored N-terminal domain ($FtsK_N$). Although the structure and function of $FtsK_N$ during division is unclear, it is suggested that FtsK acts as a checkpoint to ensure DNA is properly segregated before septation can begin. In this capacity, we hypothesize that FtsK must modulate septum formation during division through the formation of dynamic and essential protein interactions with both the Z-ring and late stage division machinery.

Drawing on advanced molecular techniques and imaging technologies, this thesis refines the membrane topology of $FtsK_N$ using site-directed fluorescence labeling, and elucidates several protein interaction partners that are critical for its role as an essential division checkpoint. Our revised topology revealed a novel functional periplasmic loop of $FtsK_N$ that, when mutated, produces cellular voids. We extensively characterized this novel cell division defect by fluorescence microscopy and high-resolution transmission electron microscopy, which exposed a novel role for $FtsK_N$ in linking cell envelope septation events. In

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addition, UV cross-linking and a genomic suppressor screen each uncovered potential interaction partners of $FtsK_N$ involved in both cell elongation and division. Two of these proteins, rare lipoprotein A (RlpA) and FtsA, were confirmed as direct $FtsK_N$ protein interactors by *in vitro* pull-down assays. Together, these findings provide critical evidence on how $FtsK_N$ may mediate the transition between cell elongation and septation in *E. coli*, and significantly advances our understanding of what is necessary for bacteria to replicate and survive.

**Curriculum Vitae:** Alison obtained her Bachelor of Science (Honours) at the University of Guelph in 2012. In the fall of 2012, she entered directly into the Ph.D. program with Dr. Cezar Khursigara as her advisor.

**Awards:** Ontario Graduate Scholarship (2016 – 2017); Dr. Donald Robert Phillips Scholarship (2015); Pharmacia Molecular and Cellular Biology Graduate Prize (2015); NSERC CGS D (2013 – 2016); Roche Molecular Biochemical Award of Excellence (2013); General Graduate Studies Fund (GGSF) Travel Scholarship (NSERC) (2013); NSERC CGS M (2012 – 2013); Cystic Fibrosis Canada (CFC) Studentship (2012); U of G Deans' Tri-Council Scholarship (2012 – 2016)

## Publications:


**Berezuk, A.M.**, and Khursigara, C.M. (2017) The LPS transport pathway: a new target for the development of Gram-negative antibiotics, in *Antibiotic Drug Discovery: New Targets and Molecular Entities* (Firestine, S., and Lister, T., eds), Royal Society of Chemistry, Cambridge, UK

Ding, Y., **Berezuk, A.**, Khursigara, C.M., and Jarrell, K.F. (2017) Bypassing the need for the transcriptional activator EarA through a spontaneous deletion in the BRE portion of the *fla* operon promoter in *Methanococcus maripaludis*. *Front. Microbiol.* **8**, 1329

Ding, Y., **Berezuk, A.**, Khursigara, C.M., and Jarrell, K.F. (2017) Phylogenetic distribution of the Euryarchaeal archaellum regulator EarA and complementation of a *Methanococcus maripaludis* Δ*earA* mutant with heterologous *earA* homologues. *Microbiology* **163**, 804-815

Ding, Y., Vrionis, H.A., Scheider, J., **Berezuk, A.**, Khursigara, C.M., and Jarrell, K.F. (2016) Complementation of an *algB* mutant of *Methanococcus maripaludis* with heterologous oligosaccharyltransferases. *PLoS One* **11**, e0167611

Ding, Y., Nash, J., **Berezuk, A.**, Khursigara, C.M., Langelaan, D.N., Smith, S.P., and Jarrell, K.F. (2016) Identification of the first transcriptional activator of an archaellum operon in a Euryarchaeon. *Mol. Microbiol.* **102**, 54-70

Roach, E.J., Wroblewski, C., Seidel, L., **Berezuk, A.M.**, Brewer, D., Kimber, M.S., and Khursigara, C.M. (2016) Structure and mutational analysis of *Escherichia coli* ZapD reveals charged residues involved in FtsZ filament bundling. *J. Bacteriol.* **198**, 1683-1693

Ding, Y., Lau, Z., Logan, S.M., Kelly, J.F., **Berezuk, A.**, Khursigara, C.M., and Jarrell, K.F. (2016) Effects of growth conditions on archaellation and N-glycosylation in *Methanococcus maripaludis*. *Microbiology* **162**, 339-350

Ding, Y., Uchida, K., Aizawa, S.-I., Murphy, K., **Berezuk, A.**, Khursigara, C.M., Chong, J.P., and Jarrell, K.F. (2015) Effects of N-glycosylation site removal in archaellins on the assembly and function of archaella in *Methanococcus maripaludis*. *PLoS One* **10**, e0116402

**Berezuk, A.M.**, Goodyear, M., and Khursigara, C.M. (2014) Site-directed fluorescence labeling reveals a revised N-terminal membrane topology and functional periplasmic residues in the *Escherichia coli* cell division protein FtsK. *J. Biol. Chem.* **289**, 23287-23301