

Announcement: The Department of Molecular and Cellular Biology, in the College of Biological Science, invites all interested members of the university community to attend the Final Oral Examination for the degree of **Master of Science** of

ZIAD MARROUSHI, on Tuesday, August 1, 2017 at 1:00 p.m. in SSC 2315

(Advisor: Dr. Lucy Mutharia)

Thesis Title: **Functional analysis of nucleoside diphosphate kinase from *Mycobacterium avium* subspecies *paratuberculosis***

Examination Committee:

Dr. J. Vessey, Dept. of Molecular and Cellular Biology (Chair)

Dr. L. Mutharia, Dept. of Molecular and Cellular Biology

Dr. J. Lam, Dept. of Molecular and Cellular Biology

Dr. S. Seah, Dept. of Molecular and Cellular Biology

Abstract: *Mycobacterium avium* subspecies *paratuberculosis* (*Map*), the causative agent of Johne's disease, is an intracellular pathogen capable of surviving and replicating within macrophages for extended periods with minimal activation of innate immune responses. The mycobacterial secreted protein nucleoside diphosphate kinase (Ndk) altered signalling pathways for macrophage activation, subverted host innate immunity, and disrupted the maturation of membrane-bound phagosomes, enabling the intracellular survival of mycobacteria. Rab small GTPase proteins, Rab5 and Rab7, play a major role in phagosome maturation, facilitating the transition from early phagosomes to phagolysosomes where bacteria are degraded. Ndk from *Mycobacterium tuberculosis* inactivated Rab5 and Rab7 proteins, leading to evasion of innate immunity by interfering with phagosome maturation. Ndk is also a housekeeping enzyme, important in the maintenance of nucleoside triphosphate pools, with histidine at position 117 (H117) involved in autophosphorylation, phosphotransfer, and nucleoside triphosphate-synthesizing activity. We have cloned and expressed putative recombinant *Map* Ndk in *E. coli* and generated anti-Ndk polyclonal antibodies in rats. Native *Map* Ndk was detected as a cellular and extracellularly secreted protein by immunoprecipitation using anti-Ndk antibodies. The immunogenicity of recombinant *Map* Ndk in naturally *Map*-infected cattle, as well as its interaction with bovine macrophage proteins (Rab5/Rab7) and other *Map* proteins were examined. In contrast to studies with *M. tuberculosis*, no interactions were observed between recombinant *Map* Ndk and Rab5 or Rab7. Recombinant *Map* Ndk-H117 catalytic mutants were constructed and will be discussed. Future work examining the potential role of *Map* Ndk to disrupt proinflammatory responses by interfering with the purinergic pathway will also be covered.

Curriculum Vitae: Ziad obtained his Bachelor of Science (Honours) at the University of Toronto in 2011, and began his M.Sc. in the lab of Dr. Lucy Mutharia in the January 2015.