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* These authors contributed equally

University of Victoria | Graduate Studies

PROGRAMME

The Final Oral Examination
for the Degree of

DOCTOR OF PHILOSOPHY
(Biochemistry and Microbiology)

Michelle Tonkin

2009 Abilene Christian University BSc (Hons)

"Molecular Strategies for Active Host Cell Invasion by Apicomplexan Parasites"

Wednesday, May 28, 2014
1:00pm
Engineering and Computer Science Building, room 108

Supervisory Committee:

Dr. Martin Boulanger, Department of Biochemistry and Microbiology, UVic (Supervisor)
Dr. Caroline Cameron, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Terry Pearson, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Jeremy Wulff, Department of Chemistry, UVic (Non-Unit Member)

External Examiner:

Dr. Lisa Craig, Department of Molecular Biology and Biochemistry
Simon Fraser University

Chair of Oral Examination:

Dr. C. Peter Constable, Department of Biology, UVic

Abstract

Parasites of phylum Apicomplexa cause devastating diseases on a global scale. *Toxoplasma gondii*, the etiological agent of toxoplasmosis, and *Plasmodium falciparum*, the most virulent agent of human malaria, have the most substantial effects on human health and are the most widely studied. The success of these parasites is due in part to a sophisticated molecular arsenal that supports a variety of novel biological processes including a unique form of host cell invasion. Accessing the protective environment of the host cell is paramount to parasite survival and mediated through an active invasion process: the parasite propels itself through a circumferential ring known as the moving junction (MJ) formed between its apical tip and the host cell membrane. The MJ ring is comprised of a parasite surface protein (AMA1) that engages a protein secreted by the parasite into the host cell and presented on the host cell surface (RON2). Thus, through an intriguing mechanism the parasite provides both receptor and ligand to enable host cell invasion. Prior to the studies described herein, the characterization of the AMA1-RON2 association was limited to low-resolution experiments that provided little insight into the functional and architectural details of this crucial binary complex. Towards elucidating the mechanism of AMA1-RON2 dependent invasion, I first structurally characterized *T. gondii* AMA1 bound to the corresponding binding region of RON2; analysis of the AMA1-RON2 interface along with biophysical data revealed an intimate association likely capable of withstanding the shearing forces generated as the parasite dives through the constricted MJ ring. To investigate the role of the AMA1-RON2 complex across genera, species and life-cycle stages, I next characterized the AMA1-RON2 complex from a distantly related genus within Apicomplexa (*Plasmodium*) and from a divergent pairing within *T. gondii*. By combining structural, biophysical and biological data, I was able to generate a detailed model describing the role of AMA1 and RON2 in MJ dependent invasion, which is currently supporting efforts to develop novel vaccines and cross-reactive small molecule therapies.

Awards, Scholarships, Fellowships

Charles S. Humphrey Graduate Student Award (2013–2014)
President's Research Scholarship (2013–2014)
NSERC Alexander Graham Bell Canada Graduate Scholarship - CGS D3 (2011–2014)
Ventura Neale Trust Endowed PEO Scholar Award (2012–2013) [One of 8 Endowed PEO Scholar Awards from Canada and the USA]
Charles S. Humphrey Graduate Student Award (2012–2013)
President's Research Scholarship (2012–2013)
Lindau Award; Canadian Student Health Research Forum (2011) [Recognition of research with greatest novelty and potential, nomination to attend 2014 Nobel Laureate Meeting in Lindau, and selection as the Lindau Lecturer for the 2015 CSHRF]
Julie Payette NSERC Research Scholarship (2010–2011) [Most prestigious Master's level NSERC scholarship awarded to the top 24 applicants]
Mrs. Annie Greskiw Graduate Award (2010–2011)
President's Research Scholarship (2010–2011)
UVic Graduate Fellowship (2009–2010)
Howard E. Petch Research Scholarship (2009–2010)

Presentations

ML Tonkin, MJ Boulanger (2014) Molecular strategies for active host cell invasion by apicomplexan parasites. *University of Victoria Biochemistry and Microbiology Seminar Series*, Victoria, BC.

ML Tonkin, JR Beck, PJ Bradley, MJ Boulanger (2014) Structural characterization of the ISP proteins critical for replication of the apicomplexan parasite *Toxoplasma gondii*. *19th Annual University of Victoria Biochemistry and Microbiology Graduate Student Symposium*, Victoria, BC. **[First Place Talk Award]**

A Poukchanski, HM Fritz, **ML Tonkin**, M Treeck, MJ Boulanger, JC Boothroyd (2013) *Toxoplasma gondii* sporozoites invade host cells using two novel paralogues of RON2 and AMA1. *12th International Congress on Toxoplasmosis*, Oxford, England. **[Elsevier Investigator Award - Best Short Talk/Poster]**

ML Tonkin, SA Arredondo, BC Loveless, JJ Serpa, KAT Makepeace, N Sundar, EV Petrotchenko, LH Miller, ME Grigg, MJ Boulanger (2013) Structural and biochemical characterization of *Plasmodium falciparum* surface proteins *Pf*12 and *Pf*41. *18th Annual University of Victoria Biochemistry and Microbiology Graduate Student Symposium*, Victoria, BC. **[First Place Talk Award]**

ML Tonkin, MJ Boulanger (2012) Building a detailed model of the apicomplexan parasite moving junction complex. *EMBO Practical Course: Structural Characterization of Macromolecular Complexes*, Grenoble, France.

ML Tonkin (2012) Molecular visualization with PyMol and Chimera. *Crystallography Made Crystal Clear Symposium*, Victoria, BC.

ML Tonkin, M Roques, MH Lamarque, M Pugniere, D Douguet, J Crawford, M Lebrun, MJ Boulanger (2011) A structural blueprint for the AMA1-RON2 moving junction complex from *Toxoplasma gondii*. *Canadian Student Health Research Forum*, Winnipeg, MB. **[Gold Award of Excellence (Top 10 Poster); Lindau Award]**

ML Tonkin, E Bruic, MJ Boulanger (2011) Structural and functional characterization of *Toxoplasma gondii* surface antigen glycoprotein SRS2. *11th International Congress on Toxoplasmosis*, Ottawa, ON.

ML Tonkin, E Bruic, MJ Boulanger (2011) *Toxoplasma gondii* host cell attachment: detailing the role of surface antigen glycoprotein SRS2. *16th Annual University of Victoria Biochemistry and Microbiology Graduate Student Symposium*, Victoria, BC. **[First Place Poster Award]**

ML Tonkin, MJ Boulanger (2010) Active invasion by apicomplexan parasites: a structural approach to defining assembly of the moving junction complex. *University of Victoria Biochemistry and Microbiology Seminar Series*, Victoria, BC.

ML Tonkin, E Bruic, MJ Boulanger (2010) Structural and functional characterization of *Toxoplasma gondii* surface antigen glycoprotein SRS2. *Northwest Crystallography Workshop*, Vancouver, BC. **[First Place Poster Award]**

Publications

MH Lamarque, M Roques, M Kong-Hap, **ML Tonkin**, G Rugarabamu, J-B Marq, DM Penarete-Vargas, MJ Boulanger, D Soldati-Favre, M Lebrun (2014) Plasticity and redundancy among AMA-RON pairs ensure host cell entry of *Toxoplasma* parasites. *Nature Comms* Accepted manuscript.

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