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*

KARL STEFFENSEN

On Wednesday, August 9, 2023 at 1:30 p.m. (online)

Thesis Title: **Actin and reactin: actin allosteric responses are mediated by actin's C-terminus**

Examination Committee:

Dr. Jaideep Mathur, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. John Dawson, Dept. of Molecular and Cellular Biology
Dr. Steffen Graether, Dept. of Molecular and Cellular Biology
Dr. Melanie Alpaugh, Dept. of Molecular and Cellular Biology
Dr. John Allingham, Dept. of Biomedical and Molecular Sciences, Queen's University (External Examiner)

Advisory Committee:
Dr. John Dawson (Advisor)
Dr. Steffen Graether
Dr. Cezar Khursigara

Abstract: Actin plays an essential role in fundamental life processes, interacting with more proteins than any other protein. Actin's structure is dynamic, undergoing structural changes that facilitate interactions with actin binding proteins (ABPs) and their subsequent functions. Many of actin's structural changes and actin:ABP interactions appear to involve actin's C-terminus. Due to its inherent flexibility, actin's C-terminus had not been resolved at high-resolution in actin structures until recently. The lack of structural resolution limited actin C-terminal research over the last 20 years, leaving the details of *how* actin's C-terminus modulates actin structure and functions a mystery. In this thesis, I present evidence of a dynamic allosteric network within actin, modulated by actin's C-terminus. *In vitro* motility assays suggest that removing the inherent flexibility of actin's C-terminus inhibits the generation of cellular forces by myosin, though the inhibition of myosin activity is inconsistent between assays conducted under the same conditions. To explore the structural mechanisms behind actomyosin inhibition, I developed and employed *in silico* methods which has been shown to supplement *in vitro* and *in vivo* studies. *In silico* results presented in this thesis support the suggestion that actin's C-terminus modulates actin structural properties and changes through dynamic allosteric networks, and that the disruption of C-terminal allostery has negative functional effects. It is therefore recommended that actin's C-terminus becomes a focus of actin research to better understand actin's roles in essential cellular functions for life on Earth.

Curriculum Vitae: Karl completed his Bachelor of Science in Mechanical Engineering specializing in Materials Science at Queen's University in Winter 2013. He then completed his Bachelor of Science in Biology at the University of Guelph in Winter 2019. He began his Master of Science in the lab of Dr. John Dawson in Fall 2019 and transferred into the PhD in Molecular and Cellular Biology program in Fall 2020.

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Awards: Graduate Tuition Scholarship (2019)

Publications: Steffensen, KE & Dawson, JF. Actin's C-terminus coordinates actin structural changes and functions. Cytoskeleton, 1-17 (2023) doi.org/10.1002/cm.21757.

Prill, K., Jones, MR., Steffensen, KE., Teng, GT., Dawson, JF. Increasing the calcium sensitivity of muscle using trifluoroperazine-induced manipulations in silico, in vitro and in vivo systems. Arch. Biochem. Biophys, Volume 735, 109521 (2023) doi.org/10.1016/j.abb.2023.109521.