# BMB Graduate Group PhD Defense

## Friday, June 5 at 10:00 a.m.

Chalmers Hall Room 36 (Johnson Cancer Research Center conference room)

# Zachary Spaulding

Advisor: Dr. Michal Zolkiewski

## Clp ATPases: structure, function, and modulation of their activity

AAA+ ATPases are found across all domains of life and are involved in a broad range of cellular processes, from regulating DNA replication to mediating protein folding and turnover. The Clp/Hsp100 subfamily of AAA+ ATPases are a group of highly conserved chaperones that support cellular protein quality control or proteostasis.

During the course of infection, many host defense mechanisms work to disrupt proteostasis in the infectious microorganisms. These defense mechanisms, including elevated temperatures and increased oxidative stress, lead to the accumulation of proteotoxic aggregates, limiting the viability of the pathogen cell. ClpB, ClpA, and ClpX all work to alleviate proteotoxic stress by physically unfolding proteins and protein aggregates for either subsequent reactivation (in the case of ClpB) or degradation by the protease ClpP. I investigated the application of DBeQ as an inhibitor of *E. coli* Clp chaperones and chaperone-protease complexes using BAP, an engineered variant of ClpB that, like ClpA and ClpX, binds to the protease ClpP, coupling protein unfolding to proteolysis. In this investigation I found that DBeQ is selective for ClpB/BAP and inhibits this protein with a degree of potency that is orders of magnitude greater than either ClpA or ClpX.

Human CLPB, also known as SKD3, is a novel AAA+ protein found in the mitochondrial intermembrane space. Mutations in the *CLPB* gene have been implicated in human disease. I have discovered that CLPB forms nucleotide stabilized dodecamers, a structure that is rarely observed among AAA+ ATPases. Furthermore, using mutations in the Walker A and Walker B motifs of CLPB, I have identified MICU1 as a *bona fide* substrate of this chaperone.