BCHM 421/422 – 2018/2019

Project Outline: Fungal infections are frustratingly common and costly healthcare problems that have risen sharply in recent years. With a mortality rate of ~40% of people suffering from bloodstream infections, *Candida albicans* poses one of the most menacing fungal threats. Those most at risk for developing these infections are individuals whose immune system is weakened by overuse of antibiotics, autoimmune disorders, organ or stem cell transplants, cancer chemotherapy, poor nourishment, or even high levels of stress. When this happens, *Candida albicans* takes advantage and begins to reproduce, infiltrate the bloodstream, and spread throughout the body. Treatment of these infections is extremely difficult and there are limited drugs that can kill the fungus without having adverse side-effects. Emergence of drug resistance is another major challenge.

My laboratory has identified a group of potentially non-cross-reactive targets in *Candida albicans* that show promise for use in therapeutic intervention development [1]. These include a small group of *Candida albicans*-specific mitotic spindle motor proteins (kinesins) that bind and re-structure mitotic spindle microtubules in order to execute chromosome segregation and help the fungus grow as infectious hyphae [2]. We now aim to understand at a molecular level how each of these motors function in these processes. This will teach us how to target them with inhibitors in ways that will prevent or halt Candida infections, while avoiding harm to treated individuals.

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Project Title: Understanding and targeting kinesin motors in invasive human fungal pathogens

Keywords (3-5):

1. **Infection**
2. **Mitosis**
3. **Kinesin**
4. **X-ray crystallography**
5. **Protein structure**

Project Goals:

1. Determine how *Candida albicans* kinesin ‘Kip3’ controls microtubule length during assembly, orientation, and extension of the bipolar mitotic spindle.
2. Identify which regions of the Kip3 protein specify its cellular locations and functions as a microtubule structure regulator.

3. Determine the overall structure and microtubule interactions of Kip3.

Experimental Approaches: To understand the mechanism of action of *Candida albicans* Kip3 [3-5], you will use a ‘dissect and build’ approach to parse the Kip3 protein into is individual functional domains, and then assess the structure and function of these domains separately, before building a model to describe how this motor protein functions as a whole.

To do this, you will learn to express and purify separate sections of the Kip3 protein from bacteria. You will then be trained to perform *in vitro* biochemical studies of these truncated Kip3 proteins in order to assess their ability to catalyze changes in microtubule polymer dynamics, and their susceptibility to inhibition with a library of small molecules. You will also be trained to produce crystals of these proteins in order to observe their three-dimensional atomic structure using X-ray crystallography, and will learn to assemble large complexes of Kip3 proteins with tubulin (the building block of microtubules) and use Small-Angle X-ray Scattering (SAXS) in order to view the architecture of intermediates in the microtubule-restructuring mechanism.

References:

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