Supervisor: Dr. John Allingham

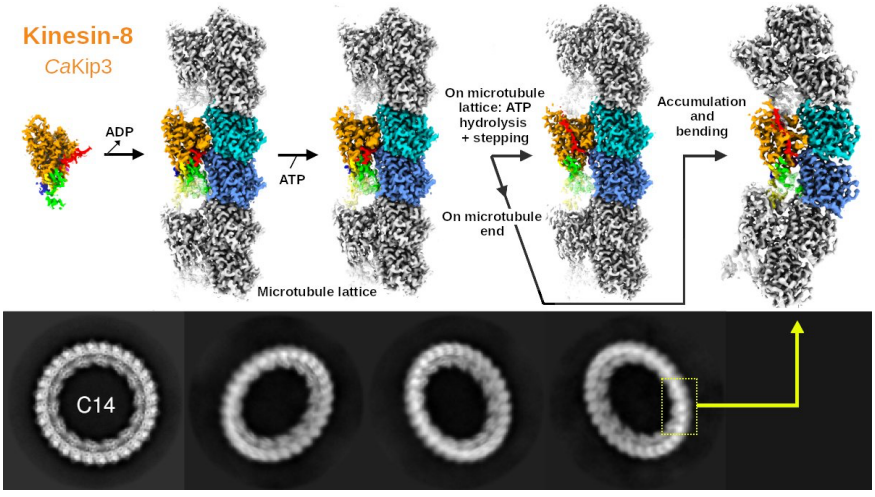
Project #1 Title: Targeting the mitotic kinesin Kip3 in *Candida albicans* as a novel antifungal strategy

Project Outline: Kip3 is a mitotic kinesin motor protein that regulates chromosome positioning during division of *Candida albicans* fungi by controlling the length of their mitotic spindle microtubules[1]. We have shown that Kip3 loss of function mutants display severe mitotic and cell growth defects, suggesting that Kip3 could be a high-value target to control fungal infections. Thus far, only myosin motor proteins have been proven to be clinically actionable as therapeutic targets[2], so additional research is needed to include kinesins in this target class. Toward this unmet need we have identified a small molecule that inhibits the ATPase activity of purified Kip3 protein at low nanomolar doses. Our next objectives are to determine if this Kip3 inhibitor is toxic to cultured *C. albicans* cells, determine whether the toxic effects of the inhibitor are, in fact, the result of Kip3 inhibition, and elucidate the mechanistic basis for inhibition of Kip3 ATPase activity and relate this loss of activity to any observed mitotic and/or cell growth defects.

Project Goals:

1. Culture *C. albicans* cells and quantitatively examine their viability and hyphae-forming abilities in the presence and absence of the Kip3 inhibitor.
2. Use live-cell fluorescence microscopy to observe mitotic spindle assembly, chromosome segregation, and cell division in inhibitor-treated *C. albicans* cells that express fluorescent markers for these events.
3. Measure microtubule-based motility and microtubule depolymerization activities of inhibitor-treated Kip3 proteins and use X-ray crystallography and electron microscopy (cryo-EM) to elucidate high-resolution structures of kinesin-tubulin and kinesin-microtubule complexes containing the inhibitor to determine the mechanistic basis for inhibition of Kip3 ATPase activity.

Experimental Approaches: The student involved in this project will learn research approaches from multiple disciplines, including microbiology, biochemistry, structural biology, and drug design. They will use our extensive library of genetically engineered *C. albicans* strains to microscopically observe spindle dynamics and chromosome segregation in living cells and learn how these processes are affected by drug treatment[3]. The student will gain expertise in protein expression, purification, biochemical activity analysis, and protein structure determination by X-ray crystallography and cryo-EM to elucidate the biochemical and structural basis of kinesin inhibition by the small molecule. They will also learn to perform biophysical studies that assess motility and microtubule depolymerization by purified kinesins within reconstituted microtubule-based systems to understand how kinesin inhibition leads to mitotic errors and growth defects in cells.



References:

1. Hunter, B., Benoit, M., Doubleday, C., Asenjo, A., Trofimova, D., Sosa, H., Allingham, J.S. (2022) Kinesin-8-specific loop-2 controls the dual activities of the motor domain according to tubulin protofilament shape. *Nature Communications*, volume 13, Article number: 4198.
2. Trivedi et al. (2022) A small-molecule myosin inhibitor as a targeted multi-stage antimalarial. *bioRxiv*
3. Shoukat, I., Frazer, C., Allingham, J.S. (2019) Kinesin-5 Is Dispensable for Bipolar Spindle Formation and Elongation in Candida albicans, but Simultaneous Loss of Kinesin-14 Activity Is Lethal. *mSphere* Vol. 4, No. 6.