# BCHM 421/422 – 2022/2023

**Project Title:** Dissection of the ATPase cycle of kinesin-8 at the microtubule plus end

**Project Outline:** Kip3 belongs to the kinesin-8 family, a unique group of microtubule-binding motor proteins that are both motile and able to destabilize microtubules. Kip3 binds and hydrolyses ATP within its motor domain during each of these activities, and recent studies have determined how its ATPase activity catalyzes its movement along the microtubule lattice toward the plus end. However, it is unclear how and when ATP hydrolysis happens in Kip3 when it is removing tubulin subunits from the microtubule end. The main objective of this project is to understand how ATP hydrolysis-mediated conformation changes in Kip3 are coupled to the microtubule depolymerization reaction by Kip3. This knowledge will provide a complete picture of how these motors localize to the ends of cytoplasmic or spindle microtubules, from where they regulate the positioning and size of the mitotic spindle during mitosis.

**Supervisor:** Dr. John Allingham

**Keywords:** Kinesin, microtubule, tubulin, ATPase, motor protein, structural biology

**Project Goals:**

1. Design, express, and purify Kip3 motor domain proteins that lack ATP binding or hydrolysis activity.
2. Compare microtubule depolymerization activities of wild-type and ATPase-defective Kip3 mutants.
3. Screen for crystals of Kip3-tubulin complexes trapped in each intermediate state of ATP hydrolysis.
4. Perform X-ray crystallography and electron microscopy (cryo-EM) to determine high-resolution structures of kinesin-tubulin complexes to identify ATPase activity-induced conformational changes that disrupt tubulin lattice interactions leading to microtubule depolymerization.

**Experimental Approaches:** To address the objective of this research, assays have been developed to simultaneously quantify the depolymerization and ATPase activities of Kip3. Because the motor domain of Kip3 houses the ATPase region, and is sufficient to bind and depolymerize microtubules, this research can be completed in vitro using recombinant proteins comprising just this part of the protein. Also available are high resolution structures of Kip3 bound to microtubules and to nucleotides representing each intermediate state of ATP hydrolysis that relate to motility. These structures will be used to guide site-specific mutational studies aimed at disabling Kip3's ATPase activity so that it will be possible to identify stages of the depolymerization reaction that do or do not require ATP hydrolysis. ATPase activity of Kip3 will be measured using biochemical assays that detect release of the hydrolysis products: ADP or Pi. Depolymerization activity will be determined by ultracentrifugation and fluorescence microscopy. High-resolution images of depolymerization reaction intermediates will be visualized by X-ray crystallography and cryo-EM.

**Impact:** These studies will ultimately lead to a working model that illustrates how an ATP hydrolysis cycle can be tuned to either drive movement of a motor along a tubulin lattice or disrupt the lattice itself.

**References:**


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