Tau Regulation of Kinesin Motility During Axonal Transport – Gabrielle Anderson

Tau is a microtubule associated protein (MAP) largely found in nerve cells that supports the construction and stability of microtubules in the neuronal axon. *In vitro* studies have found that tau also acts to prevent the movement of the motor protein kinesin, which transports intracellular cargo along microtubule tracks in nerve cells. This raises the question as to how can kinesin effectively move its cargo along microtubules *in vivo* in the presence of tau, which exists at high concentrations (~5 μM) in the axon of neurons? The answer is critical because axonal transport activity that occurs in neurons enables organelles, proteins and small molecules to be delivered from the cell body to other sites via the neuronal axon. If any protein component of this transfer process is defective, neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's, and ALS ensue. In order to accurately observe the effect of tau on kinesin motility in an *in vivo* setting, we took advantage of an intact axoplasm preparation isolated from the giant axon of the Atlantic squid (loligo pealeii). Recombinant kinesin previously expressed with a C-terminal biotin tag in *E. coli* and complexed with strepavidin coated red (655 nm) quantum dots in physiological X/2 buffer was used to perfuse the axoplasm. Kinesin/quantum dot motility in the axoplasm was imaged by total internal reflection fluorescence (TIRF) microscopy using a 488 nm argon laser for excitation and avalanche photodiode detectors for single molecule detection. Experiments were then repeated in the presence of physiological levels (~5 μM) of recombinantly expressed tau fluorescently labeled with Alexa-488 and imaged simultaneously along with the kinesin-quantum dot complexes. We observed a dramatic (3.6-fold) reduction of kinesin binding events in the axoplasm's peripheral microtubules with no discernable effect on transport velocity. Despite the reduction of binding events we observed that, in contrast to the previous *in vitro* studies, kinesin is capable of walking on microtubule tracks decorated with tau in a physiologically relevant axoplasm model system.