Title:
Zn Differentially Regulates Microtubule Interactions and Motor Protein Behavior on Microtubules

Purpose of Study:
Zinc (Zn) is an essential mineral involved in intracellular signaling, enzyme activity regulation, and gene regulation. Disruptions in its equilibrium have been linked to disease states including tau-mediated Alzheimer's, worsened outcomes from traumatic brain injury, more severe epileptic seizures, and exacerbated depression. It is now beginning to be understood that Zn also plays a crucial role in the binding of microtubule associated proteins (MAPs) to microtubules, including tau and the microtubule motor proteins dynein and kinesin. Such molecules play critical roles during various neuronal processes. For example, a recent study has shown Zn's ability to inhibit neuronal axonal transport by reducing motor protein activity, and halting the movement of mitochondrial and lysosomal cargos. However, the exact mechanisms by which Zn affects cargo transport and MAP-microtubule binding are unknown. The goal of our research is to directly assess the role of Zn on a suite of MAPs (e.g. tau, DCX, MAP9, kinesin, dynein, etc).

Methods Used:
To assess Zn's effect on MAPs, we employed an in vitro reconstitution approach. To this end, we purified various fluorescently-tagged MAPs and used florescence microscopy-based in vitro assays to measure microtubule binding.

Summary of Results:
Our results thus far show that the ability of MAPs to bind microtubules is greatly impacted by Zn. However, the specific response is unique to each MAP, with tau and dynein demonstrating microtubule binding relationships that are inverse to Zn concentration, while kinesin shows a biphasic response in which low concentrations stimulate microtubule binding, but high levels disrupt binding. Single molecule motility assays with kinesin revealed that the low concentrations of Zn that stimulate microtubule binding are not conducive to motility and in fact lead to the stalling of kinesins along the microtubule. We also find that the effect of Zn on kinesin is rapidly reversible upon addition of a Zn chelator.

Conclusions:
Our observations suggest a model for Zn regulation of cargo transport in which different proteins exhibit differential microtubule-binding behavior in response to Zn and further suggest that Zn can directly impact the net directionality of motor protein motility. Thus, stimulation of a Zn channel such as transient receptor potential mucolipin 1 (TRPML1) can be a key regulator of cargo movement. Our work provides insight into the properties of Zn to modulate microtubule binding and influence the motility of motor proteins.