P018 *In vivo* biotinylation of neuronal ubiquitin substrates

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Ubiquitination has been implicated in regulating axonal growth and path finding, presynaptic growth, postsynaptic density composition, synaptic efficacy, synaptic plasticity, and axonal degeneration. Ubiquitination disorders can also contribute to many neurodegenerative conditions, including diseases such as Parkinson's and Alzheimer's. With a few exceptions, the regulatory mechanisms by which synaptic proteins are targeted for ubiquitination are poorly understood. In order to elucidate the molecular basis of synaptic plasticity, we aim to identify all neuronal proteins that are also ubiquitin substrates.
The isolation of ubiquitin conjugates at a proteomic scale has been successfully achieved only in yeast. We have developed a novel strategy for tissue-specific expression on higher organisms of biotinylated ubiquitin. Ubiquitin conjugates bound *in vitro* to streptavidin can then be purified with very little background.
Our ubiquitin construct is functional as it is incorporated into conjugates when expressed in *Drosophila* embryonic neurons. It is biotinylated in the living embryo with a very high efficiency, and the ubiquitin conjugates bind strongly to the streptavidin beads.
We are planning, in collaboration with Junmin Peng' lab (Emory University, Atlanta), to identify the ubiquitin conjugates isolated this way using mass spectroscopy, and also to determine which of them are no longer ubiquitinated in flies mutant to specific E3 ligases.