Speaker: Shuwa Xu

Lab: Zinn lab

Title: Using protein engineering guided by biophsics to study affinity requirements for cell surface proteins during nervous system synaptic wiring.

Abstract: The study of the mechanisms by which neurons connect to each other to form functional networks is an important area of neuroscience. In the developing CNS, each neuron makes contact with many possible partners but forms synapses on only a few, suggesting that a complex cell recognition code controls synaptic targeting. Past studies have demonstrated the roles of cell surface recognition molecules in this process. Cell recognition proteins bind to their partners with a wide range of affinities, but the significance of these variations is poorly understood. Here we provide a systematic characterization of the *in vivo* consequences of altering CAM affinity.

Ig superfamily proteins Dpr (21 proteins) and DIP (11 proteins) form a complex interaction network. One DIP can bind different Dprs with affinities varying over 100 folds. In the medulla of the fly visual system, most neurons express a single DIP, and tend to be postsynaptic to neurons expressing Dprs to which that DIP binds *in vitro*. Interactions between DIP and Dpr partners are essential for wiring of neuronal circuits. Loss of DIP and Dpr functions lead to multiple developmental defects, including neuronal cell loss due to apoptosis and mis-targeting to a different visual processing layer. DIP-α binds to Dpr10 with relatively stronger affinity among DIP::Dpr interactions. Using protein engineering, we designed DIP-α and Dpr10 mutant proteins spanning a wide range of binding affinities. We further measured mutant proteins' binding affinity by *in vitro* SPR experiments, showing affinities variance from 10-fold stronger than wild-type to 50-fold weaker. We modified the *DIP-α* and *dpr10* genomic loci to endogenously express affinity mutant forms of each protein. These mutant proteins were all expressed *in vivo* in normal patterns. We show that reducing affinity causes graded loss-of-function phenotypes affecting both cell death and synaptic targeting, while increasing affinity inhibits natural apoptosis of Dm4 neurons. In addition, cell survival and synaptic targeting have different affinity requirements, and there is an affinity threshold for proper targeting. When overexpressed, even the lowest-affinity mutants of *DIP-α* or *dpr10* can rescue cell death in the respective null mutants. Affinity reduction can be compensated for by increasing avidity through changes in gene copy number. Our data suggest that for DIP-α-expressing neurons to accomplish different cellular tasks, different thresholds of interaction affinities with Dpr10-expressing neurons are required: low-affinity interactions are sufficient to maintain cell survival, while higher affinity is required for correct synaptic targeting. Every neuron expresses a combination of cell recognition molecules with varying affinities, and may use different interactions for different cellular functions.