Elucidating weak protein-protein interactions and transport dynamics of neuronal membrane proteins

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Neuronal communication relies on the fusion of synaptic vesicles at nerve terminals. The active zone protein Munc13-1 plays a central role in regulating synaptic vesicle docking and priming by catalyzing the opening of the 'closed' conformation of the SNARE protein syntaxin-1 bound to Munc18-1. Syntaxin-1 opening is a central event for neurotransmission, but the underlying molecular mechanism has remained unsolved for decades due to the weak nature of the Munc13-1-syntaxin-1 interactions. We addressed this question by tricking AlphaFold into the prediction of the open conformation of Syntaxin-1 bound to Munc13. In the closed conformation of Syntaxin-1, its SNARE motif interacts extensively with the H_{abc} domain. Upon removing half of the SNARE motif to eliminate many of the interactions, AlphaFold predicted an exciting model of the open conformation of Syntaxin-1 bound to the MUN domain of Munc13-1. We further verified it experimentally by highly sensitive paramagnetic NMR spectroscopy. Our results revealing the Syntaxin-MUN interaction sites can be useful in designing molecules that specifically stimulate or inhibit the binding of Syntaxin to Munc13-1, thereby controlling neurotransmitter release.

Moreover, I will also show an ongoing method development process for performing Single-molecule fluorescence resonance energy transfer (smFRET) of excitatory amino acid transporter 2 (EAAT2) in native membranes. EAAT2 is the predominant glutamate transporter in the brain, playing a vital role in neurotransmission. Defining the dynamics of EAAT2 is essential to understanding transport mechanisms and to testing the effects of various transporter activators. smFRET is a well-established method for characterizing these conformational dynamics. However, conventional purification methods for isolating proteins for smFRET studies require detergent solubilization, which removes their native membrane environment. As an alternative, we purified cell-derived membrane vesicles highly enriched in EAAT2. To label the protein in native system, we are employing genetic code expansion to incorporate a tetrazine-bearing non-canonical amino acid (tet-ncAA) into EAAT2. The ter-ncAA will be labeled by transcyclooctene (TCO) containing FRET pair using click chemistry. Preliminary results indicate successful incorporation of tet-ncAA into EAAT2 and its labeling with a trans-cyclooctene (TCO)-derivatized fluorophore. Next, we plan to optimize expression efficiency and labeling using a TCO-conjugated FRET pair of fluorophores and $(PEG)_{11}$-biotin for immobilization on a streptavidin-coated surface in smFRET experiments. These new methods will allow us to probe EAAT2 dynamics in a native-like context, opening new opportunities for mechanistic insights.