Improved site-specific labeling for smFRET measurements using non-natural incorporation

Dr. Chattrakun Kanokporn ("Tu")

Tennessee Tech University

Single-molecule Förster Resonance Energy Transfer (smFRET) is a powerful technique for probing biomolecular dynamics with nanometer spatial resolution. Precise site-specific labeling of proteins with donor and acceptor fluorophores is essential for accurate smFRET measurements. However, traditional labeling approaches often suffer from heterogeneity, low efficiency, or perturbation of protein function. Incorporation of non-natural amino acids (nnAAs) provides a versatile and orthogonal strategy for site-specific labeling, enabling high labeling fidelity and minimal structural interference.

In this seminar, I will present recent advances in improving the incorporation efficiency of nnAAs into proteins expressed in *E. coli* for smFRET applications using a redesign approach. I will discuss the optimization of a new plasmid construct that allows for an improved efficiency of non-natural amino acid incorporation. Evidence of a protein model showing such improvement leads to significantly increased labeling efficiency and reproducibility in smFRET experiments, thereby improving the resolution and interpretability of dynamic structural data. These developments pave the way for more reliable and broadly applicable smFRET studies in complex biological systems.