PUP-IT: a novel proximity-tagging system for protein-protein interaction study

With the support by the National Natural Science Foundation of China and ShanghaiTech University, the research team led by Prof. Zhuang Min (庄敏) at the School of Life Science and Technology, ShanghaiTech University has developed a novel proximity-tagging system that can be used to identify weak and transient protein-protein interactions (PPIs). This method is particularly useful when used to discover the PPIs on cell membrane as demonstrated in their research paper published recently in *Nature Methods* (2018, 15: 715—722).

Membrane protein-protein interactions are important to mediating cellular signaling, cell-cell communications as well as ligand-receptor interactions. However, it is challenging to identify the interacting proteins of a membrane protein since the harsh membrane protein extraction procedures usually disrupt the interaction. In recent years, proximity-tagging methods, such as BioID and APEX have been developed to assist the discovery of transient and weak protein-protein interactions. The general principle for the proximity tagging is to genetically fuse an enzyme to the bait protein, which allows the interacting prey protein to be labeled with biotin containing molecules. Different proximity tagging systems employ different proximity labeling enzymes.

The new proximity-labeling system reported by Zhuang's group takes the advantage of a prokaryotic protein conjugation system. A small protein Pup is covalently linked to the lysine on the substrate protein via a Pup ligase PafA. They first showed that PafA is a proximity-labeling enzyme with promiscuous modification sites but specific to proximal located proteins. Then they fused PafA with a transmembrane protein CD28, a co-stimulatory receptor in T cell signaling, and identified new CD28 interacting candidates as well as known CD28 interacting proteins. The new method is named PUP-IT (pupylation based interaction tagging). Since PafA-mediated target labeling occurs on site where other proximity methods are based on the diffusion of reactive substrates, PUP-IT potentially has a smaller labeling radius. More importantly, the enzyme PafA and the labeling molecule Pup can be genetically encoded, suggesting a potential future application of PUP-IT *in vivo*.

In this paper, Liu et al. also demonstrated the principle of extracellular application of PUP-IT. By using the FKBP/FRB dimerization system, they linked PafA to either the extracellular domain of CD28 or a protein cytokine IL2. They showed that the receptor-fused PafA can label the ligand and *vice versa*. Altogether, PUP-IT provides an alternative approach to study membrane protein-protein interactions and has a great potential for future applications.

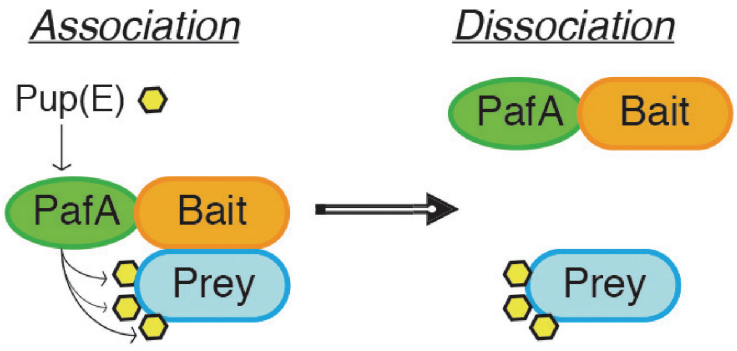

Figure The PUP-IT design.