In-Cell Fluorescence Activation and Labeling of Proteins Mediated by FRET-Quenched Split Inteins

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Abstract:

Protein labeling has facilitated in vitro studies for protein structure, dynamics, protein-protein interaction and so on. However, traditional methods of protein labeling are often inadequate for in vivo studies because they require purification of the protein, chemical labeling, repurification, and reintroduction into cells by invasive methods such as microinjection. The most useful method of protein labeling is attachment of fluorescent protein such as GFP to a target protein. Although GFP variants have proven to be extremely useful for in vivo studies of protein function, their utility is somehow inherently limited because of their relatively large sizes, potential for oligomerization. Another most promising approach for in vivo protein labeling is the use of intein-mediated protein trans-splicing. Intein-mediated labeling of proteins is highly modular, allowing the covalent site-specific incorporation of a myriad of biophysical probes into proteins.

In this seminar, I will introduce in vivo protein labeling methods suppressing cellular background fluorescence.

References: (list the papers you are going to cite in the presentation)

1. Borra R, Dong D, Elnagar AY, Woldemariam GA, Camarero JA. In-cell fluorescence activation and labeling of proteins mediated by FRET-quenched split inteins. *J Am Chem Soc.* **134**, 6344-53, (2012)

2. Schütz V1, Mootz HD. Click-tag and amine-tag: chemical tag approaches for efficient protein labeling in vitro and on live cells using the naturally split Npu DnaE intein. *Angew Chem Int Ed Engl.* **53**, 4113-7, (2014)