



CDB SEMINAR

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16:00~17:00 A7F Seminar Room

Versatile protein tagging with split fluorescence protein: a scalable strategy for GFP tagging of endogenous human proteins

Summary

In addition to the fluorescent protein fusion, live cell protein imaging has now seen more application of epitope tags. The small size of these tags enables signal amplification and may reduce functional perturbation. To address their background issue, we adapt self-complementing split fluorescent proteins, super-folder GFP and sfCherry, as epitope tags (hereafter FP11-tags) for live cell protein labeling. Tandem repeats of FP11-tags allows proportional enhancement of fluorescence signal and reduction of photobleaching for live imaging. The small size of FP11-tags also enables an efficient and scalable way to insert them into endogenous genomic loci via CRISPR-mediated homology-directed repair without molecular cloning. By using electroporation of Cas9/sgRNA ribonucleoproteins, we demonstrated scalability by targeting 48 human genes and show that the resulting GFP fluorescence correlates with protein expression levels. Our protocols can be easily adapted for the tagging of a given target with tandem repeats of FP11-tags, critically enabling the study of low-abundance proteins. Finally, we show that our FP11-tagging approach also allows biochemical isolation of native protein complexes for proteomic studies. Together, our results illustrate the versatility of FP11-tag as a labeling tool for both imaging and non-imaging applications, which pave the way for the proteome-wide analysis of protein localization and interaction networks in a native cellular context.

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