

# Efficient Tagging of Endogenous Proteins in Human Cell Lines for Structural Studies by Single Particle Cryo-EM

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Structural analysis of endogenous proteins has long been a dream; however, it requires complicated case-by-case biochemistry as well as time-consuming antibody/nanobody generation. A promising alternative would be to introduce the affinity tags on the protein of interest by CRISPR/Cas9. Unfortunately, CRISPR/Cas9-based genome editing methods require expertise and sophisticated equipment, as a result, CRISPR/Cas9-based genome engineering has not shown benefit to structural biology as it has already demonstrated in other fields.

To address this limitation, we designed a set of homology-directed repair (HDR) donor templates, coined plasmid YifanCheng (pYC). This allows us to efficiently introduce affinity tags to the endogenous proteins of interest. The knock-in procedure consists of three straightforward steps: 1) insert homology arms by using multiple cloning sites; 2) the delivery of HDR donor form with CRISPR/Cas9 and sgRNA by transient transfection; and 3) selection of genome-edited cells using a dual selection system. The resulting knock-in stable cell line can be generated in as little as two weeks when applied to HEK293 cells, which are commonly used in structural studies for recombinant over-expression,

Combined with technological advances in single-particle cryogenic electron microscopy, this labelling strategy allows structural studies of endogenous proteins in a temporal and spatial manner. We successfully demonstrated this strategy by tagging of six different human proteins in both HEK293 and Jurkat cells, a commonly used for HIV model system. Using endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an example, we demonstrated temporal and spatial structural dissection of endogenous protein along with its biological phenotype. By tracing the response of GAPDH to chronic oxidative stresses, we found the correlation between the increased number of oxidative catalytic subunit in GAPDH and nuclear translocation. Our results provide new insight into GAPDH nuclear translocation in pathogenic oxidative environments, and further shows the utility of this technique to understanding the molecular mechanisms of proteins in their nature environments.