PROTEIN LABELING WITH FLUOROGENIC AND TRANSIENT PROBES FOR CONVENTIONAL AND SUPER-RESOLUTION MICROSCOPY

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KEY WORDS: fluorogens, living cells, transient labeling, PAINT, SMLM, STED

Live-cell super-resolution of proteins labeled with genetically encoded fluorescent tags is a challenging task because of the deterioration of the labeling quality in the course of the experiment. Unfinished maturation of the covalently attached fluorescent marker, incomplete photoconversion, and photobleaching further complicate prolonged live-cell superresolution imaging and reduce effective labeling density. The general principles of PAINT (Point Accumulation for Imaging in Nanoscale Topography) resolve most of these challenges by replenishing the photobleached or defective dye from the pool of undamaged molecules. We have recently shown the Protein-PAINT with specific transient binding of cell-permeable fluorogenic dyes to genetically encoded protein tags, engineered from bacterial lipocalins [1]. Here we further extend the range of compatible fluorogenic dyes and test other applications of the labeling system, including FRET. We then show the Protein-PAINT implementation based on fluorescent protein and the transient heterodimerization of artificial coiled coils. Similarly to the Protein-PAINT with fluorogens, we confirm the improvement in the signal photostability and the labeling density in confocal and super-resolution regimes.

[1] Bozhanova N.G., Baranov M.S., Klementieva N.V., Sarkisyan K.S., Gavrikov A.S., Yampolsky I.V., Zagaynova E.V., Lukyanov S.A., Lukyanov K.A., and Mishin A.S. “Protein Labeling for Live Cell Fluorescence Microscopy with a Highly Photostable Renewable Signal.” *Chem. Sci.*, **8**, 7138–42. (2017)

This work was supported by the Russian Science Foundation grant 16-14-10364