PAVING THE WAY TO FUNCTIONAL LIVE-CELL NANOSCOPY WITH TRANSIENT PROTEIN-LABELING TAGS

Alexander S. Mishin, Alexey S. Gavrikov, Maxim M. Perfilov,
Nadezhda S. Baleeva, Mikhail S. Baranov
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry
of the Russian Academy of Sciences
Miklukho-Maklaya, 16/10, Moscow, 117997, Russian Federation
E-mail: mishin@ibch.ru

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Live-cell nanoscopy of intracellular proteins is hindered by inevitable photobleaching of genetically encoded fluorescent tags. The use of renewable transient fluorescent tags for protein labeling (protein-PAINT) recently developed in our lab [1,2] partially solve this problem. Here we further exploit the advantages of protein-PAINT for single-molecule FRET (smFRET) measurements in living cells. The substantial fraction of genetically encoded fluorescent sensors rely on intramolecular FRET. The conventional approaches for FRET efficiency estimation, such as acceptor photobleaching are time-consuming and tricky to implement in live-cell SMLM setup. Rapid exchange of the transiently binding FRET acceptor constitutes an alternative way of measuring the FRET. We report on the development of chemically modulated FRET acceptors based on transient interaction of small protein tag and the cell-permeable chromophores, or transient interaction of two protein-based tags. We assess the feasibility of protein-PAINT for smFRET measurements in living cells and report on the perspectives of adaptation of existing genetically encoded fluorescent indicators for functional nanoscopy.

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