HIGHLY PHOTOSTABLE FLUORESCENT LABELING OF PROTEINS IN LIVING CELLS BASED ON HETERODIMERIZATION OF ARTIFICIAL COILED COILS

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The genetically encoded covalent binding of fluorescent proteins to target structures is a common visualization method of proteins' dynamics and interactions in living cells. However, this method has several limitations such as low photostability and impossibility of labeling nascent proteins due to slow maturation rate of chromophores.

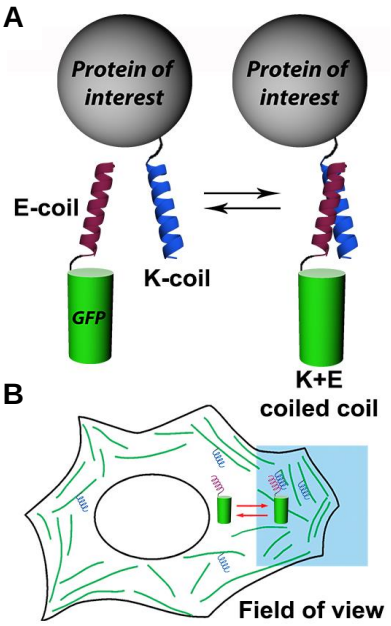

Figure 1. KECs labeling. A) The scheme of target proteins labeling with KECs. B) The continuous replenishment of nascent labels in confocal regime.

Here we present a novel method of labeling of proteins in living cells using transient interactions of small (21-28 residues) helical tags (so-called K/E-coils, KECs) [1]. 'The labeling is achieved by co-localization of K and E coils, one of which (E) is fused to target protein and complementary one (K) is fused to a fluorescent protein (Figure 1A). The ability of K/E-coils to transiently heterodimerize with tunable (from micromolar to nanomolar range) affinity provides continuous exchange and replenishment of tags bound to the target structure with the cytosolic pool of fluorescent labels. Therefore, in the condition of partial cell illumination (i.e. confocal or TIRF microscopy, Figure 1B), the photostability of labeling significantly exceeds that of covalently fused probes. The increased photostability also allowed to achieve more localizations (hence a better-reconstructed image) in super-resolution localization microscopy. In addition, we demonstrated the possibility of using this method for labeling *de novo* synthesized proteins almost immediately after their appearance, since the labeling efficiency of KECs labeling does not depend on the maturation time of chromophore.

To conclude, we developed a labeling method, that is well-suited for visualization of various target proteins, thereby enabling their advanced characterization in time and space.

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[1] M.M. Perfilov; N.G. Gurskaya; E.O. Serebrovskaya; P.A. Melnikov; S.L. Kharitonov; T.R. Lewis; V.Y. Arshavsky; V.P. Baklaushev; A.S. Mishin; K.A. Lukyanov, "Highly photostable fluorescent labeling of proteins in live cells using exchangeable coiled coils heterodimerization". *Cell. Mol. Life Sci.* (2020). https://doi.org/10.1007/s00018-019-03426-5