

Breaking the limits of light microscopy by creation of smart variants of a photoconvertible fluorescent protein - mEos3.2 through directed evolution

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Advancement in fluorescent protein engineering complemented with high-end microscopy is the progressive milestone for detailing intra-cellular components. In spite of a wide array of fluorescent proteins are available, developing photo-changeable fluorescent proteins (PCFP) is pre-requisite for super-resolution microscopy. PCFP shows change in emission spectra from green to red after activation at 405 nm. This Change in emission spectra is attributed to chromophore modification of the protein. Photo-changeable fluorescent proteins facilitate much brighter images with higher resolution and higher contrast in conventional as well as super-resolution microscopy. Our study focuses on modification of one such PCFP - mEos3.2 protein possessing comparatively lower brightness. In this study, we aim to improve the brightness of mEos3.2 using method called Directed Evolution, a three-step process comprising mutagenesis, screening, and analysis. In confocal screening we found few mutants showing significant brightness improvement. We created recombinant proteins for each of these mutants and validated that all other fluorescent properties remain unchanged. To verify the efficacy of these mEos3.2 mutants, we epitope-tagged them with B-actin and transfected them separately into mammalian cells. Out of all the mutants tested, three showed significantly brighter actin filaments as compared to native mEos3.2 tagged B-actin. We further characterized these three novel mutants using super-resolution microscopic study (PALM). So in conclusion, we successfully generated three mutant variants of mEos3.2 which are significantly brighter than the earlier version of the mEos3.2 protein.

References:

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