Generation and characterization of some UnaG mutant

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Abstract

UnaG protein from Japan eel (Anguilla japonica) is a novel fluorescent protein with binding domain that acquires fluorescence when bound to unconjugated bilirubin (UC-BR). In this study, several point mutations (F17M, N57D, N57E, N57R, L41F, Y99F_Y134W, Y99M_Y134M, and W9F_W103F) were made on the UnaG nucleotide sequence via using a method for sequence and ligation independent cloning (SLIC). The aim of the mutations on UnaG is to figure out the change in fluorescence properties. The new mutagenic vector was transformed into the commercial competent cells (E. coli Mach1) by using heat shock at 42 °C for 2 minutes. Transformed cells were grown on and selected from the LB agar plate with ampicillin. (1:1000). The DNA sequencing results show that all these mutations have done correctly. The expression of the mutant proteins was made in the pTOLT expression system by inducing with IPTG. Cells were collected with high speed centrifugation. Before disrupting the cells, lysozyme enzyme was added to make break up the cells easier, some protease inhibitors (phenylmethylsulfonyl fluoride, benzamidine) were added for the protection from proteases of the protein and DNase and RNase were added on the cell pellet to avoid the DNA and RNA contaminations. Ultracentrifugation was applied on the cell lysate. Ni- NTA affinity chromatography system was used to get the pure mutant proteins from supernatant. SDS-PAGE and semi-dry Western blot were applied on the protein for the qualitative analyse. The pure protein bands were observed on the SDS-PAGE gel image. Additionally, the spectroscopic features of purified mutant proteins were measured after adding fresh UC-BR on fluorescence spectrophotometer. Excitation and emission spectra of the mutant proteins are similar; even so they have different fluorescence intensity at the same concentration. This study suggests that mutant UnaG proteins can be used to detect UC-BR level of cells/tissue.

Fluorescent proteins (FPs) are workhorses in live-cell fluorescence microscopy due to the facile and specific labeling of the target proteins. Likewise, FPs have been extensively used in single-molecule localization microscopy. The first demonstrations of SMLM used photoactivatable fluorescence proteins along with organic dyes. The on–off transition of fluorescence emission is required in the SMLM for the temporal separation of individual molecules within the diffraction-limited area, allowing high precision localization of the individual molecules. The quality of the resultant super-resolution image is determined by two photophysical characteristics of the fluorophores. The photon number emitted from the fluorescent state determines the localization precision of determining the centroid position of a single fluorophore. The number of switching cycles and the fraction of time spent in the fluorescent state, termed as the on–off duty cycle, are related to the labeling density and the Nyquist resolution (Shroff et al., 2008). Most of the FPs offer lower photon numbers than the organic fluorophores, hence resulting in lower spatial resolutions ; Furthermore, FPs often show irreversible fluorescence transition that restricts the spot density or transition between two different emission states that complicates multicolor applications

Recently, we introduced UnaG protein as an efficient SMLM probe for multicolor live-cell imaging. UnaG is a ligand-activatable FP with a fluorogenic ligand, bilirubin. The ligand and apoUnaG protein are non-fluorescent in solution and become fluorescent upon binding to form holoUnaG. Photoswitching of UnaG is mediated by repetitive binding of bilirubin after photooxidation of the ligand followed by detachment of the damaged bilirubin from the protein. UnaG offers the highest photon numbers among blue-absorbing FPs and easily controllable switching kinetics. The off-switching rate is controlled by excitation intensity and oxygen concentration. The on-switching rate is linear to the concentration of bilirubin, whose fluorescence is undetectable up to micromolar concentration for supporting the repetitive recovery of fluorescence.

Here we investigated the photoswitching nature of enhanced UnaG (eUnaG) protein and its application to SMLM. eUnaG is a single-mutated variant of UnaG, in which Val 2 is substituted with leucine . The single mutation near the N-terminus boosts the brightness of bacterial expression to about twofolds. Since UnaG is a

suitable fluorescent protein for SMLM, we measured the single-molecule photophysical characteristics of eUnaG and explored the SMLM imaging capability. We found that lower aggregation tendency of eUnaG leads to the benefit in the morphology and brightness of cellular structures, especially in the case of vimentin filament, whose morphology is sensitive to the fused tags.

Biography:

Numan has started his PhD at Karamalu Mehmetbey University, Turkey and still continues. Also, he is working as a Research Assistant at Bioengineering Department of the same university. He is the part of Tubitak and British Council Newton Katip Celebi Fund bilateral cooperation program with collaborate Newcastle University and Karamanolu Mehmetbey University.

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