Crystal structure of the fluorescent protein from Dendronephthya sp. in both green and photoconverted red forms

Fluorescent proteins (FPs) with spectroscopic properties controlled by light form a distinct class of biomarkers called phototransformable fluorescent proteins (PTFPs). The ability of PTFPs to change their fluorescence upon exposure to light of a specific wavelength made them essential biomarkers for super-resolution optical imaging in living cells. PTFPs comprise of three main groups: photoactivatable fluorescent proteins (PAFPs), photoconvertible fluorescent proteins (PCFPs) and reversibly switchable fluorescent proteins (RSFPs). PAFPs are ideal for regional optical marking in pulse-chase experiments on live cells and tissues. RSFPs can be used in patterned illumination microscopy (for example RESOLFT), which requires markers that are capable of enduring multiple cycles of reversible photoactivation. Controlled phototransformation of PCFPs enables their use in advanced imaging with resolution beyond the diffraction limit of light; they are excellent markers for localization based super-resolution microscopy (for example PALM).

The FP from Dendronephthya sp. (DendFP) is a member of the Kaede-like group of photoconvertible fluorescent proteins with a His62-Tyr63-Gly64 chromophore-forming sequence. The fluorescence of DendFP irreversibly changes from green (506 nm) to red (578 nm) upon irradiation with UV and blue light. We have determined X-ray structures of native green and photoconverted red forms of DendFP and revealed an important role of positions 142 and 193 for photoconversion. Substitutions Ser142Ala and His193Leu caused a moderate red shift of DendFP fluorescence and a considerable increase in the photoconversion rate, making this variant a promising template for generation of novel photoconvertible biomarkers for live-cell super-resolution imaging.

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