Structural Insight into the Fluorescence Mechanism of GFP-like Proteins

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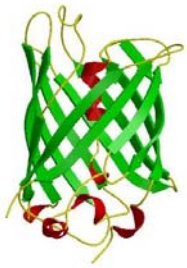
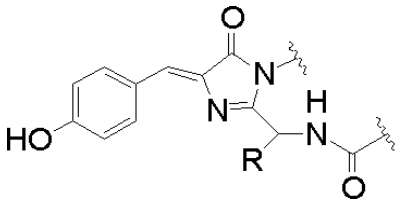
Green fluorescent protein from *Aequorea victoria* (avGFP) is now a ubiquitous tool in the fields of molecular and cellular biology for the visualization of gene expression, protein localization, and protein–protein interactions in living cells. That's why the 2008 Nobel Prize for Chemistry has been awarded to Profs Shimomura, Chalfie and Tsien for the discovery and development of the avGFP.

Jellyfish (*Aequorea Victoria*)



Overall structure of avGFP



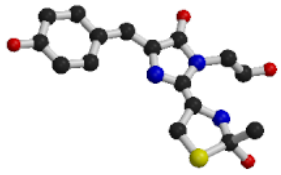
Chromophore of avGFP

In recent years, interest has grown in development of avGFP variants that exhibit efficient optical properties for more localized and precise studies of protein movement *in vivo*. Successful examples include 'Dronpa' showing reversible photochromism and 'Keima' showing a large Stokes' shift. Accordingly, structural study is of particular importance for the understanding the basis of their optical properties.

So far, we have solved dozens of crystal structures of GFP-like proteins and achieved house-made crystallization kits suitable for crystallization of newly cloned GFP-like proteins.

1) Finding of a novel three-ring chromophore

Monomeric Kusabira-Orange (mKO) emits orange light at a peak of 559 nm. We have solved its crystal structure at 1.65 Å resolution and found a novel three-ring chromophore. The conjugated π-electron system of the three-ring chromophore was more extended than that of avGFP, but less extended than that of the *Discosoma* sp. red fluorescent protein (DsRed). The structural finding is sufficient to account for the orange-emitting ability of mKO.

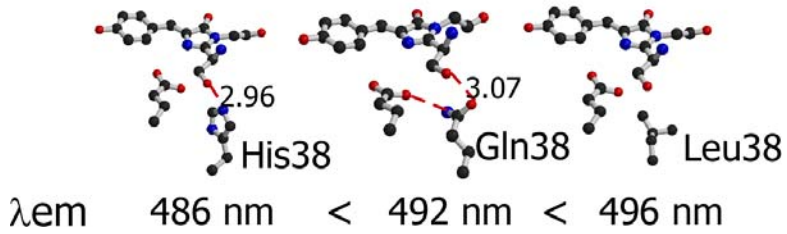


Chromophore of mKO

2) Retinal fine-tuning of emission color

Crystal structures of a cyan-emitting protein (KCy) and its variant have been determined. They revealed that the chromophore (Ser62-OH) of KCy makes a non-covalent hydrogen-bonding interaction with His38 residue, which seems to govern chromophore polarization. On the based of the structures, we successfully constructed the

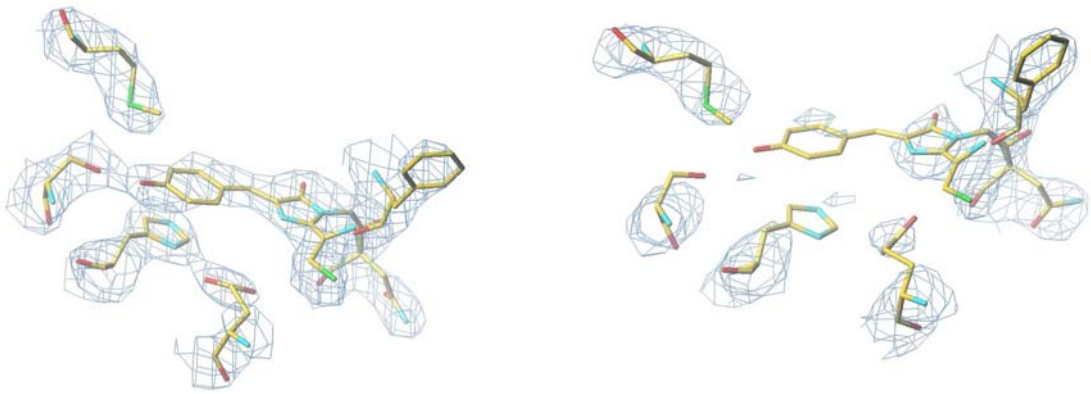
variants that display a small red-shift in emission spectra, compared to the wild-type.



Fine-tuning of peak maximum in fluorescence spectra

3) Structural flexibility

The structural basis for the photochromism in Dronpa was poorly understood because a crystal structure of the dark state has been elusive. Unclear electron density around the chromophore (3.0 Å resolution) of Dronpa in the dark state should indicate disorder, namely, flexibility of the chromophore.



Electron density map of Dronpa of the bright state (left) and the dark state (right) with superimposed atomic model of the chromophore and the surroundings

Therefore, we performed NMR analysis of Dronpa to find structural flexibility of the protein in the dark state. In the study, we have demonstrated that the combination of crystallography and NMR is a powerful approach for studying dynamics in proteins.

4) Factor for Stokes' shift

We have crystallized and subsequently determined the crystal structure of Keima that shows a large shift between the colors of light it absorbs and emits, *i.e.* large Stokes' shift. The possible mechanism for the large Stokes' shift will be discussed.

Acknowledgment

The structural study of GFP-like proteins has been carried on in collaboration with Dr Miyawaki's group in RIKEN BSI.