## Structural Basis for Cyan-Emitting Mechanism in a Cyan Fluorescent Protein

<u>Akihiro Kikuchi</u><sup>a</sup>, Eiko Fukumura<sup>a</sup>, Satoshi Karasawa<sup>b</sup>, Atsushi Miyawaki<sup>b</sup>, Yoshitsugu Shiro<sup>a</sup>, *aRIKEN Harima Institute at SPring-8, Kouto, Mikazuki-cho, Sayo-gun, Hyogo 679-5148, Japan. bBrain Science Institute, RIKEN, Hirosawa, Wako city, Saitama 351-0198, Japan.* E-mail: kikuchi@spring8.or.jp

Green fluorescent protein (GFP) from *Aequorea victoria* has become an important tool in molecular and cellular biology and spectral variant proteins with blue, cyan and yellow emissions have been generated from wild-type GFP. They have expanded the range of colours available for application of GFP-based techniques. As far, substitution of tryptophan for tyrosine at the second amino acid of the chromophore-forming tripeptide was the only procedure for generating cyan-emitting fluorescent proteins (CFPs). On the other hand, some CFPs that possess a tyrosinyl-chromophore have been recently cloned from Anthozoa. To understand cyan-emitting mechanism in the CFPs from Anthozoa, it is imperative to have structural information regarding their chromophore environment.

We have cloned and subsequently solved the crystal structure at 1.4 Å resolution of a new CFP from *Fungia* coral. The structure reveled that the chromophore is formed by cyclization reaction of three residues (Ser-Tyr-Gly), which is the same as that of GFP, and that the direction of –OH group in the Ser residue should be of importance for the nature of cyan-emitting property. Theoretical calculation supports the proposed mechanism.

Keywords: fluorescent proteins, structural biochemistry, structure-properties relationships