

hydrophobic patches are present on the surface of AtATG12: one is conserved in both Atg12 and Atg8, while the other is unique to Atg12. Considering that they share Atg7 as an E1-like enzyme, we suggest that the first hydrophobic patch is responsible for the conjugation reaction, while the latter is involved in Atg12-specific functions.

[1] Mizushima N., Noda T., Yoshimori T., Tanaka Y., Ishii T., George M.D., Klionsky D.J., Ohsumi M., Ohsumi Y., *Nature*, 1998, **395**, 395. [2] Sugawara K., Suzuki N.N., Fujioka Y., Mizushima N., Ohsumi Y., Inagaki F., *Genes Cells*, 2004, **9**, 611.

**Keywords:** protein crystallography, ubiquitin system, autophagy

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#### Crystal Structure of Novel Cyan-emitting Fluorescent Protein from *Acropora* Stony Coral

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Variants of green fluorescent protein (GFP) existing in different spectral features with blue, cyan and yellow-green emissions were originally generated from the bioluminescent jellyfish *Aequorea victoria*. We have solved the crystal structure of a novel cyan-emitting fluorescent protein (CFP) from *Acropora* coral to a resolution of 2.0Å. The protein possesses a tyrosine residue in the chromophore, while enhanced CFP, one of mutants of *Aequorea* GFP, has tryptophan residue at this position. In our crystal structure, two protomers pack closely together to form a dimer. The protein fold is in the shape of a cylinder, comprising 11 strands of the  $\beta$ -barrel threaded by an  $\alpha$ -helix running up the axis of the cylinder and short helical segments on the ends of the cylinder. The chromophore is attached inside the cylinders, and it is consistent with the formation of aromatic systems made up of Tyr70 with reduction of its C <sup>$\alpha$</sup>  - C <sub>$\beta$</sub>  coupled with cyclization of the neighboring glutamine (Gln69) and glycine (Gly71) residues. The number of polar groups and structured water molecules are buried adjacent to the chromophore. Also, the structural identification of the dimer contacts may allow mutagenic control of the state of assembly of the protein.

**Keywords:** structural biology, fluorescence, structure and properties

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#### Structural Study of Atg5 and Atg16 Essential for Autophagy

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Atg5 is a 34 kDa protein which is covalently modified by a ubiquitin-like protein, Atg12 by a ubiquitin-like conjugation system [1]. The Atg12-Atg5 conjugate then forms a multimeric complex with Atg16 [2]. Atg12-Atg5/Atg16 complex is essential for autophagy, the main pathway for the degradation of cytoplasmic components such as proteins and organelles in all eukaryotic cells. In autophagy, cytoplasmic components are enclosed by double-membrane structures termed autophagosomes, which subsequently fuse with the vacuole / lysosome. Atg12-Atg5/Atg16 complex is localized to autophagosome precursors (isolation membrane). The localization implies that the complex plays major roles in the development of autophagic isolation membranes into autophagosomes.

We determined the crystal structure of Atg5 in complex with the N-terminal region of Atg16 using methods of multiple isomorphous replacement with anomalous scattering (MIRAS) and multiwavelength anomalous dispersion (MAD). Atg5 consists of two ubiquitin-like domains and a helical domain. The N-terminal region of Atg16 has a long helical structure, which binds to the helical domain of Atg5 via salt bridges and hydrophobic interactions. Biological

analyses of Atg5/Atg16 complex are now in progress.

[1] Mizushima N., Noda T., Yoshimori T., Tanaka Y., Ishii T., George M.D., Klionsky D.J., Ohsumi M., Ohsumi Y., *Nature*, 1998, **395**, 395. [2] Mizushima N., Noda T., Ohsumi Y., *EMBO J.*, 1999, **18**, 3888.

**Keywords:** membrane trafficking, autophagy, ubiquitin system

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#### Crystal Structure of Novel Orange-emitting Fluorescent Protein from Stony Coral

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Green fluorescent protein (GFP) from *Aequorea* and GFP-like proteins are now ubiquitously used as biological markers. The chromophore is formed in an autocatalytic cyclization of encoded tripeptide segment. The fluorescent properties of the proteins should connect to the environment around the chromophore, in which the chromophore interacts with amino acids forming hydrogen-bonds,  $\pi$ - $\pi$  stacking and so on. Thus, to understanding better the physicochemistry of GFP and GFP-like proteins, it is of importance to have 3D-structural information, especially regarding their chromophores.

We have crystallized a novel orange-emitting fluorescent protein from a stony coral, which shows emission peak maxima at 548nm, and subsequently, succeed in determination of the structure to 1.7Å resolution using molecular replacement method. The protein shares  $\beta$  can fold which is specific to the fluorescent proteins. Comparison of the environment around the chromophore with that of the other structural-known GFP and GFP-like proteins have emerged that a cation- $\pi$  interaction between the chromophore and a charged amino acid affects orange-emitting fluorescent property of the protein.

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

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#### Proline Isomerization in Stefin B: a Crucial Step Towards Amyloid Fibril Formation

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For prion proteins as well as cystatins it has been suggested that formation of the 3-dimensional domain swapped dimers is the crucial step in fibril formation process, whereas higher order oligomers have not been characterized so far. One of the mutants of stefin B, the P79S, exhibited a higher stability and a prolonged lag phase of fibrillation. It forms tetrameric oligomers, which we were able to crystallize and determine their structure at 1.4 Å resolution ( $a=120\text{Å}$ ,  $b=31\text{Å}$ ,  $c=51\text{Å}$ ,  $\alpha=\gamma=90^\circ$ ,  $\beta=96^\circ$ , space group C2). The tetramer structure is built from a pair of domain-swapped dimers related by a crystallographic 2-fold axis. The structure comparison with the native stefin B structure revealed that the flip of the Ser72-Leu80 loop is associated with the trans to cis isomerization of the peptide bond of Pro74, which is the only absolutely conserved proline residue in the cystatin family of the cysteine protease inhibitors. The crucial role of the proline peptide bond trans-cis isomerization is further supported by the activation energy needed for stefin B P79S mutant to undergo tetramerization, which corresponds to the energy of proline isomerization. These data suggest that the proline isomerization may be the crucial step governing the kinetics of stefin fibril growth.

**Keywords:** protein crystallography, amyloidogenesis, protein structure and folding