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Crystal Structure of a Cyanobacterial BLUF Protein, Tll0078

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The sensor proteins for blue-light using the FAD (BLUF) domain belong to the third family of the photoreceptor proteins using a flavin chromophore, where the other two families are phototropins and cryptochromes. We have determined the crystal structure of the Tll0078 protein from *Thermosynechococcus elongatus* BP-1, which contains a BLUF domain bound to FAD, at 2 Å resolution.

The crystals belonged to space group $P2_12_12_1$ with cell dimensions of a=89.5 Å, b=109.9 Å, and c=169.9 Å. The asymmetric unit contains 10 monomers of Tll0078 (one decamer). Five Tll0078 monomers are located around the non-crystallographic five-fold axis to form a pentamer, and two pentamers related by two-fold noncrystallographic symmetry form a decameric assembly. The monomer consists of two domains, the BLUF domain at the N-terminal region and the C-terminal domain. The overall structure of the BLUF domain consists of a five-stranded mixed β-sheet with two αhelices running parallel to it. The isoalloxazine ring of FAD is accommodated in a pocket formed by several highly-conserved amino acid residues in the BLUF domain.

Keywords: flavoprotein structure, photosensors, protein crystallography

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SR Beamline for Macromolecular Assembly Crystallography Operated by IPR at SPring-8

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Biological macromolecular assemblies play significant roles in many biological reactions systems. To reveal the function of the macromolecular assemblies at atomic level, three-dimensional structure of the complex molecules is essential. A synchrotron radiation beamline for biological macromolecular assemblies at SPring-8 is operated by the Institute for Protein Research, Osaka University. Since crystals of biological macromolecular assemblies are often x-ray radiation sensitive and extremely weak diffraction power, it is essential to use high-brilliance and highly paralleled synchrotron radiation for diffraction data collection. This beamline is designed to collect high resolution and high quality diffraction data from macromolecular assembly crystals with large unit cells. A newly designed detector, DIP6040, which is a hybrid-type of image plates and a CCD, is installed.

Diffraction data from crystals with large unit cell (a=b=630, c=350Å) has been collected above 3.6 Å resolution. The present status and recent results of the beamline will be presented.

Keywords: biological macromolecular crystallography, synchrotron radiation crystallography, biological macromolecular assemblies

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Calculation of Biological Units from Protein Crystallography Data

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Solution of protein structures by means of X-ray diffraction delivers crystal structure and protein coordinates that form an asymmetric unit of the crystal. These coordinates do not necessarily identify the biological unit, or protein assembly that performs a certain physiological function. However, it is reasonable to expect that biological units last out to the crystallization and therefore it may be possible to find their structure and composition from the crystal data.

We propose a method for the calculation of protein assemblies from crystal structures, which is different from previously published [1,2] in that it is based on general principles of chemical thermodynamics. Our method employs graph techniques for the identification of all potential assemblies in a crystal, represented as a periodic graph, and each assembly is then analyzed for chemical stability on the basis of protein affinity and entropy change upon dissociation. As found for structures with experimental evidence of their oligomeric states, our method achieves 89% of correct predictions, which is higher then previously reported [1,2].

The developed software has been made available to public by setting up a web service that can take uploaded PDB and mmCIF coordinate files for analysis. The service also provides protein interfaces and protein assemblies precalculated for all PDB entries of structures solved by X-ray diffraction.

[1] Henrick K., Thornton J., *Thrends Biochem. Sci.*, 1998, **23**, 358. [2] Ponstingl H., Kabir T., Thornton J., *J. Appl. Cryst.*, 2003, **36**, 1116.

Keywords: protein assembly, protein interactions, protein crystals

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X-ray Structure of Recombinant Core 8D of the Nuclear Chaperone Nucleoplasmin

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The efficient assembly of histone complexes and nucleosomes involves the participation of molecular chaperones. The histone chaperone nucleoplasmin represents the most abundant protein in the *Xenopus* oocyte nucleus. It mediates nucleosome assembly by removing basic proteins from sperm chromatin and exchanging them with histones. This function is modulated by phosphorylation of nucleoplasmin at multiple sites.

The nucleoplasmin core domain structure forms a pentameric arrangement where each monomer consists of two domains: a core, that forms a stable ring-like pentamer, and a tail, which holds a polyglutamic tract and the nuclear localization signal. The lacking of the poly-Glu region, a putative binding site for basic proteins, does not affect its capacity for the binding of the sperm basic proteins and the chromatin decondensation. This activity has been reproduced artificially in a recombinant core domain through mutation of putative phosphorylation sites to aspartate, thus mimicking the charge effect of phosphorylation. The crystallographic studies of this recombinant domain (called CORE8D) at 2.5 **Å** resolution show the presence of these mutations which do not affect the folding of the monomer and so the formation of the pentameric structure, even though they are located in exposed flexible regions. The crystal packing has revealed the formation of a nucleoplasmin-core decamer that could represent its normal biological oligomerisation state. This decamer has localized negative charges near the interface (interactions between the Asp58 and Lys82 from all the opposite monomers), with a network of hydrogen bond waters which serve to maintain together the opposite pentamers.

Keywords: nucleoplasmin, protein, crystallography

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Hinge Peptide and Intersubunit Interface in Domain Swapping

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The domain swapping is a phenomenon, which is observed in an increasing number of proteins. Among the swapped proteins, bovine seminal ribonuclease (BS-RNase) represents a unique example, as the