MS01.P29

Acta Cryst. (2011) A**67,** C225

Crystallographic analysis at atomic resolution of the N-terminal lectin module of the LBL protein from *Laccaria bicolor*

Iván Acebrón,[a] Blanca de las Rivas,[b] Rosario Muñoz,[b] José Miguel Mancheño,[a] [a]*Department of Crystallography and Structural Biology, Instituto de Química Física "Rocasolano", CSIC, Madrid (Spain).* [b]*Group of Bacterial Biotechnology, Instituto de Ciencia y Tecnología de la Alimentación y Nutrición, CSIC, Madrid (Spain).* E-mail: xivan@iqfr.csic.es

Currently, production of recombinant soluble protein material suitable for structural analyses represents a bottleneck in Structural Biology. Statistics from structural genomics initiatives reveal that more than 80% of the cloned targets cannot be produced in a soluble form (http://targetdb.sbkb.org). With the aim to develop generic protocols aimed at producing soluble recombinant proteins in E. coli with high yield, we have demonstrated that a beta-trefoil lectin from *Laetiporus sulphurous* exhibits properties typical of solubility enhancers and can be used as fusion tag for the efficient production and purification of recombinant proteins. Now, we have found and characterized at atomic resolution a novel beta-trefoil from *Laccaria bicolor* which behaves as an excellent affinity tag.

Recently, we identified the gene 318163 (gene lacc) from the reported sequence of the *L. bicolor* genome [1], that encodes for a protein whose N-terminal region (152 amino acids) presents high sequence identity with the lectin module of the protein LSLa from the fungus *L. sulphureus* that we have already used as a fusion tag for recombinant protein production (Patent: WO 2009/121994 (CSIC) 8.10.2009). We have cloned the complete gene and the region coding for the first 152 amino acids from a cDNA library kindly provided by Dr. F. Martin. Both proteins have been over-expressed and purified in a just a single affinity chromatography step on Sepharose® 4B what reveals that this gene encodes a functional lectin that we have called LBL. Even though it was not possible to produce soluble LBL in *E. coli*, the lectin module (LBL_{152}) has been produced with a high yield (100 mg per liter of culture).

Crystals of LBL_{152} has been prepared in presence of 0.2 M lactose. The crystals grew quickly with a high quality in several different conditions. Best crystals were obtained in 20% (w/v) PEG 6000, 0.2 M ammonium chloride, 0.1 M sodium acetate pH 5.0. Complete diffraction data at 1 Å resolution were collected at 100 K at beamline ID23-2 of the ESRF (Grenoble, France). The subsequent analysis shows that crystals belong to the space group P212121, with a = 52.53 Å, b = 61.27 Å and c = 44.79 Å which indicates that there is only one molecule in the asymmetric unit. The structure has been solved with the molecular replacement method, using the structure of LSL_{150} (N-terminal lectin module of the protein LSLa) [2] as model. As expected, LBL_{152} is a lectin with a beta-trefoil fold.

[1] F. Martin, *et al. Nature* **2008**, *452*, 88-93. [2] J.M. Mancheño, *et al. J Biol Chem* **2005**, *280(17),* 17251-17259.

Keywords: lectin, purification, biocrystallography

MS01.P30

Acta Cryst. (2011) A**67,** C225

Time-resolved analysis of catalytic reaction of copper amine oxidase from *Arthrobacter globiformis.*

Hiroshi Yamaguchi,[a] Misumi Kataoka,[a] Hiroko Oya,[a] Ayuko Tominaga,[a] Masayuki Ohtsu,[a] Toshihide Okajima,[b] Katsuyuki Tanizawa,[b] [a]*School of Science and Technology, Kwansei Gakuin University,* [b]*Institute of Scientific and Industrial Research, Osaka University, (Japan).* E-mail: hiroshi@kwansei.ac.jp

Copper amine oxidases catalyze oxidative deamination of various primary amines to the corresponding aldehydes with concomitant production of ammonia and hydrogen peroxide. The enzyme contains a quinone cofactor, 2,4,5-trihydroxyphenylalanine quinone (topaquinone or TPQ), produced by post-translational modification of the conserved precursor tyrosine residue in the presence of Cu^{2+} ion and O_2. In a previous study, three intermediary structures formed during the TPQ formation have been determined and the detailed reaction mechanism of TPQ biogenesis has been proposed on the basis of their structures [1]. On the other hand, the catalytic reaction of amine oxidase proceeds by a Ping-Pong bi-ter mechanism, consisting of reductive and oxidative half-reactions [2]. In the former reductive half-reaction, a substrate Schiff-base is formed between an amine substrate and the cofactor in the initial oxidized form (TPQ_{ox}), which is finally converted to a semiquinone radical (TPQ_{sq}), yielding an aldehyde product.

To elucidate the structure-based reaction mechanism of copper amine oxidase, the reductive half-reaction catalyzed by the recombinant enzyme from *Arthrobacter globiformis* (AGAO) was analyzed by time-resolved X-ray crystallography. The AGAO crystals were grown anaerobically in a nitrogen-filled globe box. The crystals were soaked in a solution containing its substrate, phenylethylamine, also anaerobically. The crystals were immersed into liquid nitrogen at appropriate time intervals to freeze-trap the reaction intermediates transiently formed in the crystals. Before X-ray data collection, the crystals were subjected to single-crystal microspectrometry for monitoring the absorption spectrum of TPQ that reflects its chemical structure. Diffraction data were collected at 100 K in the BL38B1 and BL44B2 at SPring-8, Japan. Diffraction images were processed by using *HKL2000* and structure refinements were performed by using the program *Refmac5*. Crystal structures of four distinct intermediates formed during the reductive half-reaction have been determined at atomic resolution. Concerted conformational changes of TPQ, several active-site residues located in the substrate-binding pocket and gate residues at the entrance of the substrate channel are observed with the progress of reductive half-reaction.

[1] M. Kim *et al., Nature Struct. Biol.* **2002,** *9,* 591-596. [2] Y.-Ch.Chiu *et al., Biochemistry*. **2006,** *45,* 4105-4120.

Keywords: enzyme, catalytic_reaction, structure

MS01.P31

Acta Cryst. (2011) A**67,** C225-C226

The influence of nickel ions on the photoswitching of the GFP-like protein PDM1-4

Luc Van Meervelt, Ngan Nguyen Bich, Kristof Van Hecke, *Chemistry Department, Katholieke Universiteit Leuven, Celestijnenlaan 200F, B-3001 Leuven (Belgium).* E-mail: luc.vanmeervelt@chem.kuleuven.be

PDM1-4, a reversible photoswitchable fluorescent protein, has been generated by random mutation on Dronpa in order to be more effective in far-field fluorescent nanoscopy techniques. Compared to Dronpa, PDM1-4 exhibits slower switching properties, increasing the resolution in the photoactivated localization microscopy (PALM) technique [1]. We now have determined the crystal structure of both the *on*- and *off*-state of PDM1-4.

Plate-like crystals of PDM1-4 were obtained by the hanging- drop vapor-diffusion method. The *on*-state crystals strongly exhibit green fluorescent emission under ultraviolet light. The *off*-state crystals were

obtained by irradiating the crystals for 10 min. with a blue laser (508 nm). Diffraction data were collected at the PXII beamline of the Swiss Light Source at 100K using a MarResearch CCDC detector.

The structures were solved in space group $P2_12_12_1$ by molecular replacement using the monomer of Dronpa (pdb code 2z1o) as search model. The asymmetric unit contains 12 monomers, associated in three tetramers. The structures were refined at an effective resolution of 3.0 Å for the *on*-state and 3.15 Å for the *off*-state. R/R_{free} values converged at 21.1/25.7% for the *on*-state and 21.3/25.0% for the *off*-state.

PDM1-4 exhibits a β-barrel structure typical for GFP-like proteins. Furthermore, a light-driven *cis-trans* isomerization of the chromophore is observed. From the structures of the *on*- and *off*-states we elucidate that the presence of nickel ions, interacting with His 194 and His 212, decreases the flexibility of the β-strands, resulting in the slower switching kinetics of PDM1-4. The photoswitching mechanism of reversible photoswitchable fluorescent proteins not only arises from the flexibility of the chromophore accompanied by a rearrangement of the proximate residues, but is also influenced by the flexibility of the β-strands.

[1] H. Mizuno, P. Dedecker, R. Ando, T. Fukano, J. Hofkens, A. Miyawaki, *Photochem. Photobiol. Sci.* **2010**, *9*, 239-248.

Keywords: fluorescence, nickel, protein

MS01.P32

Acta Cryst. (2011) A**67,** C226

Crystal Structures of Bacterial Peptidoglycan Amidase AmpD and an Unprecedented Activation Mechanism

Cesar Carrasco-López,[a] Alzoray Rojas-Altuve,[a] Weilie Zhang,[b] Dusan Hesek,[b] Mijoon Lee,[b] Shahriar Mobashery,[b] Juan A. Hermoso,[a] [a]*Departamento de Cristalografía y Biología Estructural, Instituto de Química-Física "Rocasolano", CSIC, Serrano 119, 28006-Madrid (Spain).* [b]*Department of Chemistry and Biochemistry, 423 Nieuwland Science Center, University of Notre Dame, Notre Dame, IN 46556 (USA).* Email: ccarrasco@iqfr.cis.ces

AmpD is a cytoplasmic peptidoglycan (PG) amidase involved in bacterial cell-wall recycling and in induction of *β*-lactamase, a key enzyme of *β*-lactam antibiotic resistance [1], [2]. AmpD belongs to the amidase_2 family that includes zinc-dependent amidases and the peptidoglycan-recognition proteins (PGRPs), highly conserved pattern-recognition molecules of the immune system [3], [4]. Crystal structures of *Citrobacter freundii* AmpD were solved for the apoenzyme, for the holoenzyme at two different pH values, and for the complex with the reaction products, providing insights into the PG recognition and the catalytic process. These structures are significantly different compared to the previously reported NMR structure for the same protein [5]. The NMR structure does not possess an accessible active site and shows the protein in what is proposed herein as an inactive "closed" conformation. The transition of the protein from this inactive conformation to the active "open" conformation, as seen in the X-ray structures, was studied by targeted molecular dynamics simulations, which revealed large conformational rearrangements (as much as 17 Å) in four specific regions representing one third of the entire protein. It is proposed that the large conformational change that would take the inactive NMR structure to the active X-ray structure represents an unprecedented mechanism for activation of AmpD. Analysis is presented to argue that this activation mechanism might be representative of a regulatory process for other intracellular members of the bacterial amidase_2 family of enzymes.

[1] J.T. Park, T. Uehara *Microbiol Mol Biol Rev* **2010,** *72,* 211-227. [2] M. Lee*, et al. J Am Chem Soc* **2009**, *131,* 8742-8743. [3] K.H. Schleifer, O. Kandler *Bacteriol Rev* **1972**, *36,* 407-477. [4] S. Cho*, et al. Proc Natl Acad Sci USA* **2007**, *104,* 8761-8766. [5] E. Liepinsh, C. Genereux, D. Dehareng, B. Joris, G. Otting. *J Mol Biol* **2003**, *327,* 833-842.

Keywords: crystal structure, cell-wall recycling, conformational change

MS01.P33

Acta Cryst. (2011) A**67,** C226

Structure of a novel bacterial small molecule sensor domain with two ligands

Estela Pineda-Molina,[a] Jesús Lacal,[b] José Antonio Gavira,[a] Juan Luis Ramos,[b] Juan Manuel García-Ruiz,[a] Tino Krell,[b] [a]*Laboratorio de Estudios Cristalográficos, IACT CSIC-U. Granada, P.T. Ciencias de la Salud, Granada-18100 (Spain).* [b]*Departamento de Protección Medioambiental, EEZ (CSIC), Granada-18008 (Spain).* E-mail: pineda@iact.ugr-csic.es

Chemoreceptors play important roles in the adaptation of bacteria to changing environmental conditions. For most chemoreceptors the cognate signal molecules are unknown. Receptors can be classified according to the size of their ligand-binding domain (LBD) into cluster I and cluster II. Several structures of cluster I domains are available, but structural information on the larger cluster II domains is lacking. Here we report the structure of *P. Putida* McpS-LBD in complex with two strong chemoattractants, succinate and malate. Crystals were initially grown using the counter-diffusion method to be further improved by the vapor diffusion technique. Malate crystals diffracted up to 1.8 Å and the structure was solved by wavelength Anomalous Scattering (SAS) using Se-methionine derivatized protein. The McpS-LBD structure together with succinate was solved at 1.9 Å resolution by Molecular Replacement (MR). In the solved structures McpS-LBD is a dimer in which each monomer folds as 6 α-helices (2 long and 4 short) organized in two four-helix bundles located at opposite sites or their long bundle axis.These structures are the first examples of cluster II domains and identifies McpS-LBD as a novel small molecule binding domain. Moreover, despite low sequence similarity, both bundles share high structural similarities with *E. Coli* Tar-LBD. In McpS-LBD, as in the case of Tar, the binding of ligand molecules is accomplished by amino acids from both monomers. Site-directed mutagenesis of amino acids involved in ligand binding causes loss of binding activity. It is proposed that Tar-LBD and McpS-LBD are the result of convergent evolution.

[1] M. García-Castro, A. Martín, M. Mena, C. Yélamos, *Organometallics* **2007**, *26*, 408-416.

Keywords: ligand binding domain, chemoreceptor, McpS

MS01.P34

Acta Cryst. (2011) A**67,** C226-C227

Non-catalytic glycogen-binding site and its functional role in glycogen synthase

Adelaida Díaz,[a] Carlos Martínez-Pons,[a] Ignacio Fita,[b] Juan C. Ferrer,[c] Joan J. Guinovart,[a] [a]*Departament of Molecular Medicine, Institute for Research in Biomedicine, Barcelona (Spain).* [b]*Institut de Biologia Molecular de Barcelona, CSIC, Barcelona (Spain).* [c]*Universitat de Barcelona, Barcelona (Spain).* E-mail: adelaida.diaz@irbbarcelona.org

Glycogen is a polymer of α-1,4- and α-1,6-linked glucose residues