

## MS84.P02

*Acta Cryst.* (2011) A67, C737**Investigation of the Mosaicity of a  $\text{Li}_2\text{SO}_4\cdot\text{H}_2\text{O}$  Crystal**

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No single crystal is perfect; a real crystal can be approximated by mosaic blocs of small perfect crystals. This approach is the basis for extinction analysis in X-ray structure analysis.

Extinction is the weakening of the diffracted X-ray intensity due to multiple scattering in the crystal. Perfect crystals scatter according to the dynamical theory ( $I \sim |F|^2$ ), but no real crystal is perfect. Ideal imperfect crystals, on the other hand, scatter according to the kinematical theory ( $I \sim |F|^2$ ).

In most cases, the measured intensities of real crystals are in between both cases and an extinction correction is needed to fulfil the kinematic approach.

Present theories dealing with extinction corrections are based on the approach of a mosaic crystal and describe x-ray scattering in terms of kinematic approach using certain "correction terms" to implement the structure of a real crystal [1, 2, 3]. Most experimentalists use the extinction as black box. However, for a real structure refinement the parameters used for a certain extinction correction are typically not verified by the experiment.

The mosaic blocs within a real crystal are misorientated to each other and are affected by lattice strain. In addition both 3D shape and size of the blocs are not known. All these parameters can be determined by high-resolution x-ray diffraction techniques as known from semiconductor research performing  $\omega$ - and  $\omega$ - $2\theta$ -scans through certain reciprocal lattice points.

Our approach is to verify the validity of existing extinction theories by independent diffraction experiments with high resolution. In our present experiments we used a  $\text{Li}_2\text{SO}_4\cdot\text{H}_2\text{O}$  crystal plate. The measurements were performed at beamline D3 at Doris, HASYLAB at DESY. Several reflections in Laue and Bragg geometry were measured.

The  $\omega$ -scan is a rotation of the crystal by  $\Delta\theta$ , while the detector is fixed at  $2\theta$  and the  $\omega$ - $2\theta$ -scan is a rotation of the crystal by  $\Delta\theta$  and simultaneously a movement of the detector by  $2\Delta\theta$ . The determined parameters from the FWHMs of these scans can be used to determine extinction.

Finally the evaluated parameters are used for structural refinement and are compared with solutions achieved without the knowledge of the real structure.

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**Keywords:** extinction, mosaicity, crystal

## MS85.P01

*Acta Cryst.* (2011) A67, C737**The structure of SapA lipoprotein discs**Konstantin Popovic,<sup>a</sup> John Holyoake,<sup>b,c</sup> Régis Pomès<sup>b</sup> and Gilbert G. Privé,<sup>a,c</sup> <sup>a</sup>Department of Medical Biophysics, University of Toronto, Toronto, Ontario, (Canada). <sup>b</sup>Hospital for Sick Children, Toronto, Ontario, (Canada). <sup>c</sup>Ontario Cancer Institute, Toronto, Ontario, (Canada). E-mail: kpopovic@uhnres.utoronto.ca

Sphingolipid activator protein SapA is an essential non-enzymatic cofactor required for the breakdown of galactosylceramide by -galactosylceramidase within the lysosome. A defective SapA fails to activate the degradation of galactosylceramide, which in turn leads

to globoid-cell leukodystrophy (Krabbe disease). The 1.9 Å crystal structure of the SapA in complex with 40 internally-bound detergent (LDAO) molecules provides a high-resolution view of a discoidal lipoprotein particle. In this complex the acyl chains of the detergent molecules form a highly ordered bilayer-like hydrophobic core surrounded by two copies of a protein belt. Saposin A lipoprotein discs exhibit limited selectivity with respect to the incorporated lipid, and can solubilize phospholipids, sphingolipids and cholesterol into discrete, monodisperse particles with mass of approximately 27 kDa. These discs may be the smallest possible lipoprotein structures that are stabilized by lipid self-assembly. The saposin A-lipid disc is most likely the effective substrate-presenting particle in galactosylceramide hydrolysis. We propose that saposin A activates the galactosylceramide hydrolysis reaction by solubilizing the target lipid in 3.2 nm particles, thus increasing the accessibility of the lipid headgroup to the enzyme active site.

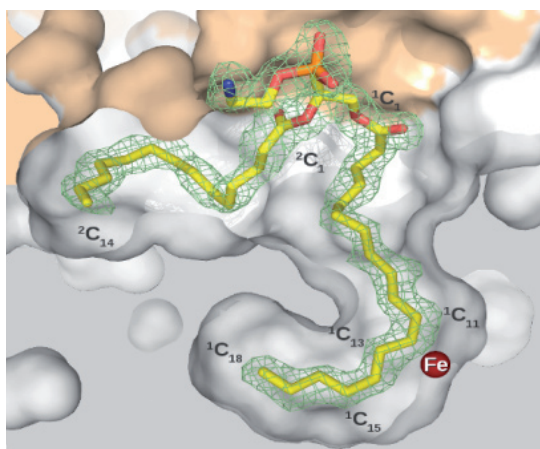
**Key words:** lipoproteins, saposin, X-ray crystallography

## MS85.P02

*Acta Cryst.* (2011) A67, C737-C738**First prokaryotic LOX structure: interactions with phospholipids and membranes**Xavi Carpena,<sup>a</sup> Albert Garreta,<sup>a</sup> Silvana Pompeia do Val de Moraes,<sup>b</sup> Queralt Garcia,<sup>a</sup> Antonio Ortiz,<sup>c</sup> Montserrat Busquets,<sup>d</sup> Àngels Manresa,<sup>b</sup> Betty Gaffney,<sup>c</sup> Ignasi Fita,<sup>a</sup> <sup>a</sup>Institute for Research in Biomedicine (IRB-Barcelona) and Institut de Biologia Molecular (IBMB-CSIC), Barcelona, (Spain). <sup>b</sup>Facultat de Farmàcia. Universitat de Barcelona, Barcelona, (Spain). <sup>c</sup>Facultat de Veterinària. Universidad de Murcia, Murcia, (Spain). <sup>d</sup>Facultat de Biologia. Universitat de Barcelona, Barcelona, (Spain). <sup>e</sup>Department of Biological Science. Florida State University, Tallahassee, (USA). E-mail: xcvcri@ibmb.csic.es

The biological role of lipoxygenases, non-heme iron dioxygenases responsible for a myriad of essential functions in eukaryotes, remains unexplained in prokaryotes, which generally lack polyunsaturated fatty acids. The first crystal structure of a prokaryotic lipoxygenase (Pa\_LOX) from *Pseudomonas aeruginosa*, shows major differences with respect to eukaryotic enzymes such as the lack of the PLAT domain and the insertion, in the catalytic domain, of a pair of long antiparallel  $\alpha$ -helices. An enlarged binding pocket showed a complete phosphatidylethanolamine molecule (with chains of 18 and 14 carbons in length). Five carbons, of the longest chain, approach the catalytic iron suggesting how the pentadienyl moiety is stabilized within the substrate binding pocket. Several crystal forms from the native Pa\_LOX and two inactive mutants confirm the high affinity of the N-terminus towards the phospholipid polar moiety. In turn, the variability of the disposition of the two helices insertion suggests the exposure of the hydrophobic upper part of the substrate binding pocket towards the membrane during the capture of phospholipids. Measurements, performed using phospholipid containing vesicles by means of ITC and fluorescence spectroscopy, show a clear interaction of Pa\_LOX with certain model membranes.

Eukaryotic lipoxygenases perform a crucial role in the pathways involved in inflammatory processes against pathogen infection. In addition, for these enzymes it has also been reported a high degradation activity against the membranes of some cellular organelles. All this, and taking into account the capability of Pa\_LOX to interact directly with membrane phospholipids, without any further facilitator, invites to hypothesize that Pa\_LOX was preserved as a secreted enzyme to interfere with the host immune response and/or to assist in the degradation of membrane barriers.



Phospholipidic cavity insight of Pa\_LOX.

**Keywords:** lipoxygenases, membrane interaction

### MS85.P03

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#### Crystal structures of lipid-raft protein stomatin and its specific protease

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Stomatin is a major integral membrane protein of human erythrocytes, the absence of which is associated with a form of hemolytic anemia known as hereditary stomatocytosis. It is reported that stomatin regulates the gating of acid-sensing ion channels in mammalian neurons. However, the function of stomatin is not fully understood. In the genomic sequence of the hyperthermophilic archaeon *Pyrococcus horikoshii*, the putative operon-forming genes PH1511 and PH1510 encode stomatin and STOPP (stomatin operon partner protein), respectively. The N-terminal region of PH1510p (1510-N) is a serine protease with a catalytic Ser-Lys dyad (Ser97 and Lys138), and specifically cleaves the C-terminal hydrophobic region of stomatin PH1511p [1]. It is reported that stomatin forms higher-order oligomers. Stomatin is thought to act as an oligomeric scaffolding protein in lipid rafts. The cleavage of stomatin by STOPP might be involved in a certain regulatory system, but little is known about its physiological role. We determined the crystal structure of the core domain of stomatin PH1511p (residues 56-234, designated as PhSto<sup>CD</sup>) at 3.2 Å resolution [2]. And we determined the crystal structure of the 1510-N K138A mutant at 2.3 Å resolution [3].

PhSto<sup>CD</sup> forms a novel homotrimeric structure. Three  $\alpha/\beta$  domains form a triangle of about 50 Å on each side, and three  $\alpha$ -helical segments about 60 Å in length extend from the apexes of the triangle. The  $\alpha/\beta$  domain of PhSto<sup>CD</sup> is partly similar in structure to the band-7 domain of mouse flotillin-2. Flotillin is reported to be involved in endocytosis [4]. While the  $\alpha/\beta$  domain is relatively rigid, the  $\alpha$ -helical segment shows a conformational flexibility, adapting to the neighboring environment. One  $\alpha$ -helical segment forms an anti-parallel coiled coil with another  $\alpha$ -helical segment from a symmetry-related molecule. The  $\alpha$ -helical segment shows a heptad repeat pattern, and mainly hydrophobic residues form a coiled-coil interface. The determined structure shows a novel trimeric fold, and the coiled-coil fold observed in the crystal probably contributes to the self-association.

1510-N preferentially degrades hydrophobic substrates. According

to the structure of 1510-N, each active site around Ser97 is rich in hydrophobic residues, and is therefore suitable for hydrophobic substrates. According to the gel-filtration analysis, 1510-N forms a dimer. The determined structure of 1510-N K138A shows two possible sets of dimer from the symmetry-related molecules. One stable dimer shows a contact surface area (1,498 Å<sup>2</sup>) larger than that of another dimer (1,180 Å<sup>2</sup>). In the stable dimer, the 19-residue-long loop mainly constitutes the interface of the dimer, and is significantly kinked at around Ala138, which is mutated from Lys. Thus catalytic Lys138 probably has an important role on the conformation of the loop.

In order to understand the mechanism how 1510-N protease degrades the C-terminal region of stomatin, a structural analysis of 1510-N in complex with its ligand stomatin would be needed.

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**Keywords:** membrane\_protein, protease, structure

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#### Gram-positive bacterial conjugation: new structural insight on plasmid pIP501

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Conjugative plasmid transfer is an important mean for horizontal gene spread (e.g. of antibiotic resistance) [1]. The model host of our Gram-positive multiple antibiotic resistance plasmid pIP501 is *Enterococcus faecalis*, which presents an important nosocomial pathogen. The plasmid conjugation process in Gram-negative bacteria has been studied in detail, whereas little information is available about the corresponding mechanisms in Gram-positive bacteria [2]. pIP501 has the broadest known host range for plasmid transfer in Gram-positive bacteria and is the first system for which also stable replication in Gram-negative bacteria was shown.

The transfer region of pIP501 is organized in an operon encoding fifteen putative transfer proteins. Three of these Tra proteins show significant sequence similarity to *Agrobacterium tumefaciens* T-DNA transfer system proteins: an ATPase (ORF5 homologue to VirB4) [3], a coupling protein (ORF10 homologue to VirD4) and a lytic transglycosylase (ORF7 homologue to VirB1) [4].

One priority of the project is to determine the structure of ORF11 and ORF14, two members of the T4SS for which no homologues exist in the *Agrobacterium tumefaciens* T-DNA transfer system. 7xHis-fusion proteins of both candidates have already been successfully expressed, purified, biochemically characterized and used in crystallisation- and optimization screens.

Recently, the structure of ORF14 was solved, using selenomethionine anomalous data for phasing. The 1.4 Å structure revealed an internal dimer fold, consisting of anti-parallel beta sheets in the middle and a "helix-turn-helix" like motif on both ends. Together with previous EMSA results, these findings support the assumption that ORF14 is a DNA binding protein. To acquire detailed insight into this interaction, ORF14 has been set up with dsDNA oligos in co-crystallisation experiments.