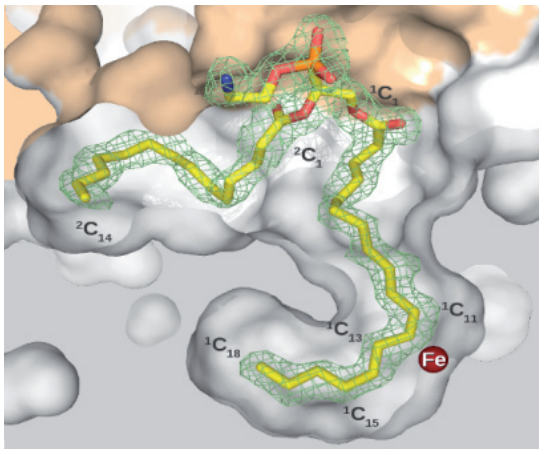

Phospholipidic cavity insight of Pa_LOX.

Keywords: lipoxygenases, membrane interaction

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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 $PhSto^{CD}$) at 3.2 Å resolution [2]. And we determined the crystal structure of the 1510-N K138A mutant at 2.3 Å resolution [3].

$PhSto^{CD}$ forms a novel homotrimeric structure. Three α/β domains form a triangle of about 50 Å on each side, and three α-helical segments about 60 Å in length extend from the apexes of the triangle. The α/β domain of $PhSto^{CD}$ 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 α/β domain is relatively rigid, the α-helical segment shows a conformational flexibility, adapting to the neighboring environment. One α-helical segment forms an anti-parallel coiled coil with another α-helical segment from a symmetry-related molecule. The α-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 $Å^2$) larger than that of another dimer (1,180 $Å^2$). 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.

[1] H. Yokoyama, I. Matsui, *Journal of Biological Chemistry* **2005**, *280*, 6588-6594. [2] H. Yokoyama, S. Fujii, I. Matsui, *Journal of Molecular Biology* **2008**, *376*, 868-878. [3] H. Yokoyama, S. Hamamatsu, S. Fujii, I. Matsui, *Journal of Synchrotron Radiation* **2008**, *15*, 254-257. [4] O. O. Glebov, N. A. Bright, B. J. Nichols, *Nature Cell Biology* **2006**, *8*, 46-54.

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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 Gramnegative 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.