Phospholipid cavity insight of Pa_LOX.

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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 PH1510 and PH1511 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, Lys138), and specifically cleaves the C-terminal hydrophobic domain of PhSto (1510-N) is a serine protease with a catalytic Ser-Lys dyad (Ser97, Lys138), and specifically cleaves the C-terminal hydrophobic domain of PhSto (1510-N) is a serine protease with a catalytic Ser-Lys dyad (Ser97, Lys138). It is partly similar in structure to the band-7 domain of stomatin. It was determined the crystal structure of the core domain of stomatin PH1511p (residues 56-234, designated as PhSto) at 3.2 Å resolution [2]. And we determined the crystal structure of 1510-N K138A mutant at 2.3 Å resolution [3].

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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 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.