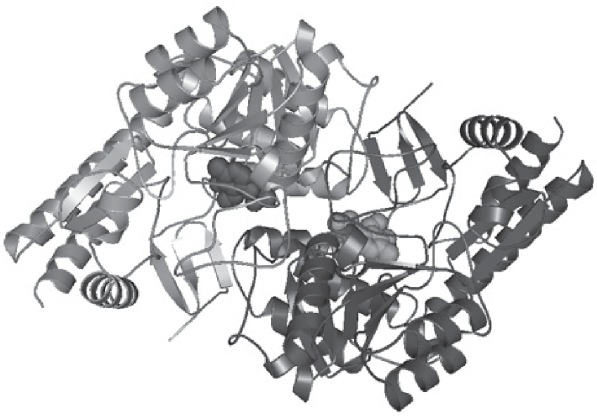
s1.m8.p32 **Crystal Structure of Glutamate-1-Semialdehyde-Aminomutase from *Thermosynechococcus elongatus*.** Jörg Schulze,[a] Wolf-Dieter Schubert,[a] Jürgen Moser,[b] Dieter Jahn[b] and Dirk Heinz[a], [a]*Department of Structural Biology, German Research Centre for Biotechnology (GBF), Germany, and* [b]*Institute of Microbiology, Technical University Braunschweig, Germany. E-mail: jos@gbf.de*

Keywords: Tetrapyrrole biosynthesis; Pyridoxal-5'-phosphate ; Conformational changes

Archaea, plants, and most bacteria synthesize 5-aminolevulinic acid (ALA), the precursor molecule of all tetrapyrroles, from glutamyl-tRNA in a two-step reaction. In the first step, glutamyl-tRNA reductase catalyzes the NADPH-dependent reduction of tRNA-bound glutamate to glutamate-1-semialdehyde. Subsequent transamination by the pyridoxamine-5'-phosphate-dependent enzyme glutamate-1-semialdehyde-aminomutase (GSAM) produces ALA. We determined the three-dimensional structure of GSAM from the thermophilic cyanobacterium *Thermosynechococcus elongatus* by X-ray crystallography. The α_2-dimeric enzyme was heterologously produced in *E. coli.* The structure was solved by molecular replacement.



The overall fold is that of aspartate aminotransferase and related vitamin B_6 enzymes. The structure shows a fully symmetrical dimer. Although pyridoxamine-5'-phosphate (PMP) and pyridoxal-5'-phosphate (PLP) were present during crystallization, the structure contains only PLP, covalently bound as a Schiff base in the active site. Whereas the GSAM structure from Synechococcus [1] contains PMP in one subunit and PLP in the other. This induces conformational differences in both subunits, especially in a loop controlling access to the active site and led to the assumption [1] that GSAM is controlled by negative cooperativity. Our structure from *Thermosynechococcus*, however, reveals that both subunits are able to bind PLP simultaneously, suggesting that they may act independently from each other. GSAM also crystallizes without cofactors and with PMP alone in different crystal morphologies, suggesting conformational changes in the different catalytic states. Determinations of these structures are currently under way.

[1] Hennig, M., B. Grimm, et al. (1997) *PNAS* **94**: 4866-4871

s1.m8.p33 **Receptor Recognition by the Flexible Peroxisomal Targeting Signal Type 1 of Sterol Carrier Protein 2.** Will A. Stanley,[a] Fabian V. Filipp,[b] Petri Kursula,[a] Dmitri I. Svergun,[a] Michael Sattler[b] and Matthias Wilmanns[a], [a]*EMBL-Hamburg, Germany, and* [b]*EMBL-Heidelberg, Germany. E-mail: stanley@embl-hamburg.de*

Keywords: Receptor recognition; Protein-ligand interactions; Supramacromolecular structure

The majority of proteins destined for the peroxisome carry a peroxisomal targeting signal type 1 (PTS1) - a C-terminal tri-peptide with consensus -[S/A/C]-[K/H/R]-[L/M]-CO_2-. Pex5p is the cytosolic receptor for PTS1 proteins. The C-terminal tetratricopeptide repeat (TPR) domain of Pex5p specifically recognises PTS1 proteins and conducts them to the peroxisomal membrane for subsequent transfer [1]. One intriguing feature of PTS1-mediated import is that folded, liganded or oligomeric proteins can be transferred to the peroxisome lumen [2]. In one case, that of sterol carrier protein 2 (SCP2), it has been speculated that the protein must be loaded with a lipid ligand in order to present its PTS1 for Pex5p recognition [3] - thus, ligand-dependent targeting is proposed, implying a sorting mechanism for folded and functional proteins. We have synergistically used a combination of biophysical and structural biology techniques to demonstrate that recognition of SCP2 by the TPR domain of Pex5p is not ligand-dependent but is ligand-tolerant. The PTS1 of SCP2 displays considerable conformational flexibility to facilitate recognition by the TPR domain. This feature allows different isoforms of SCP2 to interact comparably with the TPR domain while the overall fold of SCP2, and it's fatty acyl CoA binding function, remain intact. A crystal structure of SCP2 in complex with the TPR domain of Pex5p is presented. The structure amply accounts for the tolerant binding mode. Further, we identify novel interactions between non-PTS1 residues in SCP2 and non-TPR residues in Pex5p, with important implications for both peroxisomal targeting and for more generalised interactions between TPR containing proteins and their ligands.

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[3] Lensink, M.F., A.M. Haapalainen, J.K. Hiltunen, T. Glumoff, and A.H. Juffer, Response of SCP-2L domain of human MFE-2 to ligand removal: binding site closure and burial of peroxisomal targeting signal. *J Mol Biol*, 2002. **323**(1): p. 99-113.