

The 6-O-methylglucosyl-containing lipopolysaccharides (MGLPs) and the 3-O-methylmannosylcontaining polysaccharides (MMPs) are two unusual polymethylated polysaccharides (PMPS) produced by mycobacteria. Both PMPS localize to the cytoplasm, where they have been proposed to regulate fatty-acid biosynthesis owing to their ability to form stable 1:1 complexes with long-chain fatty acids and acyl-coenzyme A derivatives. In sequestering the products of fatty-acyl synthase I (FAS I), PMPS are thought to facilitate the release of the neo-synthesized chains from the enzyme, thereby not only reopening active sites essential for enzyme turnover but also terminating their elongation. In addition, PMPS have been proposed to serve as general fatty-acyl carriers, the role of which would be to facilitate the further processing of very long and insoluble fatty-acyl CoAs, including mycolic acids, by increasing the tolerance of mycobacteria to high cytoplasmic concentrations of these products while protecting them from degradation. The glucosyl-3-phosphoglycerate synthase (GpgS), is a retaining  $\alpha$ -glucosyltransferase that initiates the biosynthetic pathway of the MGLPs in mycobacteria. The enzyme transfers a Glcp moiety from UDP-Glc to the 3 position of the phosphoglycerate to form glucosyl-3-phosphoglycerate. Here we report new crystal structures of the apo and UDP complex forms of GpgS from *Mycobacterium tuberculosis* at 2.6 and 3.0 Å resolution respectively. The overall structure shows the two-domain organization typical of GT-A GTs. We propose a plausible model for donor and acceptor substrates recognition and catalysis. The implications of this model for the comprehension of the early steps of MGLPs biosynthesis and the catalytic mechanism of other members of the GT-A family are discussed.

**Keywords:** Mycobacteria, glucosyl-3-phosphoglycerate synthase (GpgS)

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#### Crystal structure of cyclophilin-a enzyme from *Azotobacter vinelandii*

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The Peptidyl-Prolyl Isomerases (Cyclophilins, FKBP and Parvulins) catalyze the cis-trans isomerization of peptide bonds preceding prolyl residues, therefore accelerating protein folding [1]. Cyclophilins (Cyp) have been established as a model system in enzymology in terms of extensive efforts of understanding the mechanism of enzyme catalysis in full depth [2]. *Azotobacter vinelandii* is a well known agricultural, aerobic, soil-dwelling bacterium, which fixes atmospheric nitrogen, converting it to ammonia, which is the most ingestible form of the element for the plants. There are two known cyclophilins in *A. vinelandii*: cytoplasmic AvCyPA and periplasmic AvCyPB.

The crystal structure of the cytoplasmic cyclophilin A was determined by molecular replacement at 1.7 Å resolution. In addition, the crystal structure of the protein complexed with the synthetic tetrapeptide succinyl-Ala-Phe-Pro-Phe-p-nitroanilide (sucAFPFpNA) was determined at 2.0 Å resolution. The tetrapeptide sucAFPFpNA was used as a substrate for an assay that confirmed that *A. vinelandii* AvCyPA possesses PPIase activity. The tetrapeptide is bound as a proline cis-isomer and adopts different conformations from those observed in other related structures. Comparisons between the uncomplexed and complexed structures as well as other CypA structures provides additional insights about structure-function relationships of this enzyme. Also structural studies for PPIases from a new organism may complement existing studies and help achieve a better understanding of the link between sequence variation and enzymatic function.

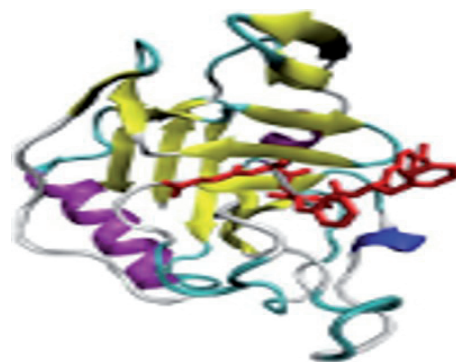


Figure 1: The complex between AvCyPA and synthetic peptide sucAFPFpNA solved at 2.0Å resolution

[1] Wang, J. Heitman, *Genome Biology* **2005**, 6, 226. [2] E.Z. Eisenmesser, O. Millet, W. Labeikovsky, D.M. Korzhnev, M. Wolf-Watz, D.A. Bosco, J.J. Skalicky, L.E. Kay, D. Kern, *Nature* **2005**, 438, 7064, 117-121.

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#### Crystal structure analysis of the genetic encoded photosensitizer KillerRed

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Several methods have been developed to elucidate the protein functions and interactions in living cells up to the present. Among them, methods for losing protein functions derived meaningful data from biochemical and cellular biological experiments. Chromophore-assisted light inactivation (CALI) is one of promising techniques to inactivate target proteins in living cells [1]. In CALI, chromophore molecules are used as photosensitizer, which produce highly reactive free radicals including reactive oxygen species (ROS) by irradiation of intense light. ROS have short lifetime, therefore the damage radius is limited to approximately 3-4 nm [2]. This indicates that inactivation of the protein(s) is limited in short timescales and very small regions, where the inactivation light is exposed. So far some fluorescent small molecules such as malachite green and fluorescein were used as photosensitizer for CALI applications. These photosensitizers should exogenously introduce into living specimen, which is the bottleneck of developing versatile application of CALI. KillerRed is the first genetically encoded photosensitizer, which has notable phototoxicity. KillerRed is developed by protein engineering from the hydrozoan chromoprotein anm2CP, a homolog of GFP [3].

For the farther development of KillerRed, we determined the crystal structure of KillerRed to understand the structural basis for its phototoxicity. The crystal structure of KillerRed was solved by S-SAD at 2.8Å resolution. The data sets were collected using the loopless data-collection method [4] with chromium K $\alpha$  X-rays. The overall structure of KillerRed was 11-stranded  $\beta$ -barrel with an internal  $\alpha$ -helix passing through inside of the barrel, which is characteristic of the fluorescent protein family. The chromophore formed by the autocatalytic cyclization