binding protein (SBP) component of the high-affinity manganese ABC-type transport system from the cyanobacterium Synechocystis sp. PCC 6803 (Syn) to 2.9 Å by combined MAD/molecular replacement. The metal ion binding site containing Mn2+ has a distorted tetrahedral geometry, with Glu220 and Asp295 situated closer to the ion than His89 and His154. This geometry may be due to a disulfide bond between Cys219 and Cys268.

Sequence homology comparisons show that only putative cyanobacterial manganese SBPs contain these conserved cysteines, suggesting the MntC has a special role in manganese mobilization into the photosynthetic apparatus. We show that reduction of the disulfide bond in vitro releases bound manganese. We propose that in vivo reduction of the disulfide bond by a redox active protein, alters the position of Glu220 thereby modifying the affinity towards the bound metal. We have identified a homologous gene from the thermophilic cyanobacterium *T. vulcanus*. The full length clone (GenBank accession code AAV65297) was sequenced and found to be 54% homologous with the *Syn mntC* and it contains the conserved cysteines. The gene was cloned into an expression vector and the expressed protein has been purified and crystallized. Preliminary ICP-MS measurements show that this protein binds Mn2+, and we thus propose that this gene encodes for the MntC homolog in these species. We are now in the process of fine tuning the crystallizing conditions of this protein in order to determine its crystal structure.

**Keywords:** ABC transporter system, photosynthesis, redox

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**P.04.01.6**


**3D Structure Determination of the Cpn60-2 Protein from Mycobacterium tuberculosis**

Anat Shahar a, E. Dobrovetsky b, M. Melamed-Frank b, Y. Kashi b, N. Adir b, 1Department of Chemistry and Institute of Catalysis Science and Technology, Technion, ISRAEL; 2Department of Biotechnology and Food Engineering, Technion – ISRAEL. E-mail: manat@tx.technion.ac.il

Heat shock proteins (HSP) are a large super-family of proteins which are highly conserved throughout evolution and are necessary for the correct folding of proteins inside the cell. Cpn60-2 from *Mycobacterium tuberculosis* (Mt) belongs to the HSP60 family which is also called Chaperonins. These proteins are involved in folding of a large number of proteins in an ATP dependent manner. In addition, Cpn60-2 is one of the most immunogenic of all Mt proteins, eliciting a significant immune response when whole cells are used in vaccination. Due to its high immunogenicity, Cpn60-2 has a medical importance.

We have isolated Cpn60-2 by over expression of the cloned gene encoding for Cpn60-2 into pQE60 vector to enable metal chelate affinity purification. The recombinant protein was shown to protect *E. coli* cells from heat shock stress. Crystals of His-Cpn60-2 grow in 2-14 days and were improved by different methodologies. The full length clone (GenBank accession code C172) was sequenced and found to be 54% homologous with the *Syn mntC* and it contains the conserved cysteines. The gene was cloned into an expression vector and the expressed protein has been purified and crystallized. Preliminary ICP-MS measurements show that this protein binds Mn2+, and we thus propose that this gene encodes for the MntC homolog in these species. We are now in the process of fine tuning the crystallizing conditions of this protein in order to determine its crystal structure.

**Keywords:** protein crystallography chaperones, stress, bacteria

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**P.04.01.7**


**Crystal Structure of OXA-24, a Novel Class D β-lactamase with Carbapenemase Activity**

Elena Santillana a, Germán Bou b, Antonio Romero b, "Centro de Investigaciones Biológicas, CSIC; Ramiro de Maetzu, 9, 28040-Madrid (Spain); "Complejo hospitalario a universitario Juan Canalejo; A Coruña (Spain). E-mail: esh@cib.csic.es

One of the main concerns in Medicine is the presence of microorganisms causing infections which harbour antibiotic resistance mechanisms. Among the different mechanisms associated with antibiotic resistance, much attention is currently being focused on the presence of β-lactamases. Oxacillinases are Ambler class D β-lactamases that possess active site serine groups like class A and class C β-lactamases. These enzymes are characterized by their hydrolytic activity for isoxazolyl, penicillins, methicillin and aztreonam significantly, sparing most extended-spectrum cephalosporins.

Six oxacillinases with carbapenem-hydrlyzing activity have been sequenced from *Acinetobacter baumannii*. OXA-24 shares 40% identity with a group of oxacillinases consisting of OXA-5, -7, -10 and -11. Despite these similarities, some interesting and differing features exist between previous oxacillinases and OXA-24. Thus, OXA-24 lacks hydrolytic activity against oxacillin, cloxacillin, and methicillin but displays a moderate level of resistance to carbapenem. Crystals of OXA-24 from *A. baumannii* were grown using the vapour diffusion technique. They belong to space group P41212, with cell dimensions a=b=102.2 Å, c=86.1 Å and one molecule in the asymmetric unit, which diffracted beyond 2.5 Å. It was possible to locate the position of the enzyme in the unit cell using molecular replacement with the coordinates of OXA-10 as a search model. The three dimensional structure of OXA-24 could establish the molecular basis to explain the relevance of the substitutions in its hydrolytic activity. The structure is currently undergoing refinement.

**Keywords:** β-lactamases, antibiotic resistance, protein crystallography