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Three-dimensional Structure of Human FKBP52
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FKBP family proteins are immunophilins which process peptidyl-prolyl isomerase (PPIase) domain and they can all bind FK506, a macrolide immunosuppressant. FKBP52 is a FKBP protein, which can be separated into four domains. The first (FK1) and the second (FK2) domains are similar with FKBP12. The third domain includes three tetratricopeptide repeat (TPR) motives and the forth domain contains calmodulin binding-site.

We have overexpressed and purified FKBP52 full-length and three segments of FKBP52, including FKBP52-FK1, N(1-260) and C(145-459). The crystals of FKBP52-FK1, N(1-260), C(145-459) have been obtained, as well as the complex of C(145-459) and a C-terminal pentapeptide MEEVD from Hsp90. The three dimensional structure of FKBP52 has been defined based on the crystal structures of N(1-260) and C(145-459). The structures have indicated the pattern of natural substrates binding to the active site of PPIase and the reason why the FKBP52-FK506 complex is not able to inhibit calcineurin activity, and has interpreted why FK2 has no PPIase activity. The functional differences between FKBP52 and FKBP51 have been clarified by comparing their structures. A hormone-signaling model based on FKBPs / Hsp90 / hormone receptor complex assembly has been proposed and FKBP51 is regarded as a negative feedback factor of FKBP52 in hormone signaling.
Keywords: FKBP52, PPIase, hormone receptor complex

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Structural Investigations of GFP-like Proteins
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Green Fluorescent Protein (GFP) from *A.Victoria* is an 11 stranded β-barrel protein with a cyclic tri-peptide chromophore. Encoded by a single gene, GFP is self folding and has an autocatalytic mechanism of chromophore formation.

The intrinsic pigmentation and fluorescence properties of GFP-like proteins arise from their all-protein chromophores, with their differing spectral properties due to their unique chromophore structures and environments of each protein. Understanding GFP-like protein structure and related function is of fundamental interest.

The GFP-like proteins under investigation include a highly fluorescent, moderately fluorescent and two non fluorescent proteins, eqFP611, HcRed, KFP and Rtms5 respectively. All of which have strong sequence and structural homology to both GFP and a red fluorescent protein from *Discosoma* coral (DsRed). I have solved high resolution crystal structures of KFP, eqFP611 and HcRed. From structural comparisons between previously solved structures, Rtms5, DsRed and GFP.

By the detailed investigation of several GFP-like proteins through their structural determination and characterization I am gaining an understanding of the dynamic nature of the GFP-like protein family. It is anticipated that we will expand the current understanding of chromophore structure in conjunction with related protein fluorescence, subsequently aiding the development of novel GFP-like protein applications.
Keywords: GFP, protein cyrstallography, chromophore structure

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Crystal Structure of Heme Binding Enolase P46 from *Bacteroides fragillis*
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The anaerobic gram-negative bacterium *Bacteroides fragilis* is frequently found in intestines and is known to cause intra-abdominal infections. A 46 kDa heme binding protein (P46) from *B. fragilis* was found to be induced in iron restricted condition [1]. The sequence of P46 shows it to be an enolase, an enzyme in glycolysis. Enolase forms dimers or octamers and the crystal structure of enolase from human, yeast, *Escherichia coli* and *Streptococcus pneumoniae* are known. But their structures do not explain heme binding to P46.

To investigate heme binding mechanism, we solved the crystal structure of P46 at 2.6 Å resolution. P46 forms an octamer in the crystal structure and solution. Furthermore, we measured the affinity of P46 for heme using surface plasmon resonance and found that the dissociation constant is 2.65 μM.

[1] Otto B.R, et al., *Infect Immun*., 1996, **64**, 4345.
Keywords: enolase, heme binding protein, bacteroides

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Structural Studies of the Disulfide Oxidoreductases DsbA from *Xylella fastidiosa*
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The first member of the disulfide oxidoreductases (DsbA) family was identified and characterized in *Escherichia coli* as a periplasmic protein involved in disulfide bond formation. It was also shown that the DsbA protein assists the correct folding of exported proteins containing disulfide bonds and in *Vibrio cholerae* a member of this family is required for the functional maturation of secreted virulence factors. *Xylella fastidiosa* is a phytopathogenic bacterium that causes serious diseases in a wide range of economically important crops. *X. fastidiosa* genome analysis revealed the presence of two members of the DsbA family (from now named DsbA1 and DsbA2). Furthermore, a sequence alignment showed that the active site regions of DsbA1 and DsbA2 differ from each other by one residue, usually considered important for the enzymatic activity. The purified proteins DsbA1 and DsbA2 were submitted to crystallization trials. Crystals of DsbA1 were obtained and X-ray diffraction data were collected at the Brazilian Synchrotron Light Laboratory. Best crystals diffracted to 2.2 Å resolution and belong to space group C2 with unit cell parameters a= 200.06 Å, b= 41.24 Å, c= 79.97 Å and β= 96.17°. DsbA1 crystals were also obtained after protein incubation with a reducing agent and diffracted up to 1.9 Å. The quick cryo-soaking technique was applied and some data sets were collected from heavy atom-derivative crystals. Attempts to solve the structure using the SIRAS and MIRAS methods are in progress. Circular Dichroism and Fluorescence experiments are being performed in order to obtain complementary structural data.
Financial Support: FAPESP, LNLS, CNPq
Keywords: xylella fastidiosa, disulfide oxidoreductase, X-ray diffraction data

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Structure of MntC from Cyanobacteria
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We have determined the crystal structure of the MntC solute

binding protein (SBP) component of the high-affinity manganese ABC-type transport system from the cyanobacterium *Synechocytis* sp.PCC 6803 (*Syn*) to 2.9Å by combined MAD/molecular replacement. The metal ion binding site containing Mn^{2+} 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. vulcanu*s. The final full length clone (GenBank accession code AAV65297) was sequenced and found to be 54% homologous with the *Syn mntC* and it contains the conserved cysteins. 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 Mn^{2+}, 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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3D Structure Determination of the Cpn60-2 Protein from *Mycobacterium tuberculosis*

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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 immunogenity, 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 crystallization conditions are 10% 2-propanol, 20% PEG 4K, 0.1M Hepes pH 7.5. Crystallographic analysis shows the crystals to be monoclinic ($P2_1$) with unit cell parameters of a=58.460Å, b=112.209Å, c=77.5Å, β=95.482º and containing a dimer in the asymmetric unit. We have collected a complete 2.75Å data on ESRF beamline ID14-1. The structure has been solved by the molecular replacement method using a lower resolution model recently published. At present, the structure of the Cpn60-2 has been refined to R/R_{free} factors of 23.69/31.79%.

Keywords: protein crystallography chaperones, stress, bacteria

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

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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 aztreonan significantly, sparing most extended-spectrum cephalosporins.

Six oxacillinases with carbapenem-hydrolyzing 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 carbapenemes. Crystals of OXA-24 from *A. baumannii* were grown using the vapour diffusion technique. They belong to space group $P4_12_12$, 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

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Crystallographic Studies of Human Methionine Adenosyltransferase (MAT)

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Methionine adenosyltransferase (MAT) catalyzes the formation of the key enzymatic cofactor, S-adenosylmethionine (AdoMet), from ATP and methionine. AdoMet is important because of it's involvement in various biochemical pathways including polyamine synthesis as well as the methylation of nucleic acids and lipids. MAT activity, in mammals, is regulated by a β subunit which lowers the Km of MAT for L-methionine and renders the enzyme more susceptible to feedback inhibition by AdoMet. This regulatory subunit has been modeled and is currently in crystallization trials. Furthermore, to better understand the role of the β-regulatory subunit, the three dimensional structure of the human MAT complexed to its β subunit will be determined through crystallographic studies.

[1] LeGros H.L. Jr., Halim A.B., Geller A.M., Kotb M., *J Biol Chem*. 2000 , **75(4)**, 2359. [2] Kotb M., Geller A.M., *Pharmacol Ther*. 1993, **59(2)**, 125.

Keywords: methionine adenosyltransferase, S-adenosylmethionine, L-methionine

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Optimization of Crystallization of the Flavoprotein WrbA by using Additives

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Tryptophan (W)-repressor binding protein A, WrbA, is an *Escherichia coli* stationary-phase protein. Its predicted influence on the binding interaction between DNA and the tryptophan repressor (TrpR) wasn't proved [1] and thus its physiological function remains unclear. According to sequence analysis and homology modelling, WrbA was identified as the founding member of a new protein family, sharing the open, twisted α/β fold typical for flavodoxins [2]. The biochemical and biophysical studies of purified WrbA apoprotein [1]