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Structural study of *Neisseria meningitidis* iron-regulated protein FrpD

Ekaterina Sviridova,[a] Ladislav Bumba,[b] Pavlina Rezacova,[c,d] Peter Sebo,[b,e] Ivana Kuta Smatanova,[a,f] [a]*Institute of Physical Biology USB CB, Zamek 136 Nove Hrady (Czech Republic).* [b]*Institute of Microbiology AS CR, Videnska 1083 Prague (Czech Republic).* [c]*Institute of Organic Chemistry and Biochemistry, Flemingovo nam. 2 Prague (Czech Republic).* [d]*Institute of Molecular Genetics AS CR Flemingovo nam. 2 Prague (Czech Republic).* [e]*Institute of Biotechnology AS CR, Videnska 1083 Prague (Czech Republic).* [f]*Institute of Nanobiology and Structural Biology GCRC AS CR Zamek 136 Nove Hrady (Czech Republic).* E-mail: sviridova@ufb.jcu.cz

FrpD is a highly conserved lipoprotein of *Neisseria meningitidis* anchored to the bacterial outer membrane. The *frpD* gene sequence contains two translation initiation sites, which give rise to production of the full-length FrpD protein ($FrpD_{271}$) that harbours N-terminal signal peptide promoting FrpD export across the cytoplasmic membrane by Sec translocase, and the truncated FrpD protein ($FrpD_{250}$) that lacks the signal peptide and remaining in cytoplasm of the bacteria. The exported $FrpD_{271}$ precursor is processed to its mature form on the periplasmic side of the cytoplasmic membrane, sequentially modified by a lipid molecule at Cys_{25} residue, and sorted to the outer bacterial membrane [1]. The biological function of FrpD appears to be linked to the FrpC protein, since FrpD was found to bind the N-terminal part of FrpC with very high affinity (K_d = 0.2 nM) [1]. However, mechanism of FrpD-FrpC interaction is unknown due to the absence of any structural information on these proteins. Moreover, the primary amino acid sequence of FrpD does not exhibit any similarity to known protein sequences of other organisms, and therefore, a new type of protein fold could be expected. We found out that the full version of $FrpD_{250}$ protein couldn't be crystallized. Therefore, we performed a specific truncation of 21 amino acid residues from N-terminus of $FrpD_{250}$ protein. The native and Se-Met substituted variants of recombinant, truncated version (lacking the first 21 amino acid residues from N-terminus) $FrpD_{43\text{-}271}$ protein were prepared and crystallized using the sitting-drop vapour-diffusion method. The crystals of native $FrpD_{43\text{-}271}$ protein belong to the hexagonal space group $P6_2$, while the crystals of Se-Met substituted $FrpD_{43\text{-}271}$ protein belong to the primitive orthorhombic space group $P2_12_12_1$ [2]. Crystal structure of Se-Met substituted $FrpD_{43\text{-}271}$ was determined using the single anomalous diffraction (SAD) method. The calculated structure was used as a search model in molecular replacement to determine the structure of native $FrpD_{43\text{-}271}$ protein. Currently, the analysis of the Se-Met substituted $FrpD_{43\text{-}271}$structure and comparison with calculated native $FrpD_{43\text{-}271}$ protein structure are underway.

[1] K. Prochazkova, R. Osicka, I. Linhartova, P. Halada, M. Sulc, P. Sebo, J *Biol Chem* **2005**, *280*, 3251-3258. [2] E. Sviridova, L. Bumba, P. Rezacova, K. Prochazkova, D. Kavan, K. Bezouska, M. Kuty, P. Sebo, I. Kuta Smatanova, *Acta Cryst F* **2010**, *66*, 1119-1123

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Structures of methylthioadenosine phosphorylase (MTAP) from *S. Mansoni*

Juliana Roberta Torini de Souza, Ricardo DeMarco, Humberto D'Muniz Pereira. *Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos-SP (Brazil).* Email:jutorini@ursa.ifsc.usp.br

The schistosomiasis is a chronic parasitic illness, caused by the parasite *Schistosoma mansoni* that affects approximately 200 million people worldwide. Schistosomiasis is treated by the use of drugs that are not-in fact effective for the eradication of the disease, and although their efficiency cause serious side effects. The *Schistosoma mansoni* parasite does not posses the *de novo* pathway for purine bases biosynthesis and depends entirely on salvage pathways for its purine requirement. Thus this pathway can be identified as a potential target for the development of specific drugs to combat schistosomiasis. The MTAP enzyme (EC 2.4.2.28) is a component of this pathway, using 5'-deoxy-5'-methylthioadenosine (MTA) as it substrate. MTAP catalyze reaction: S-methyl-5'-thioadenosine + phosphate = adenine + S-methyl-5-thio-α-D-ribose 1-phosphate.

The MTAP amplification was performed using a cDNA library from enriched mRNA of the adult worm. The MTAP gene was cloned in pET28a vector. Expression was done using *E. coli* BL21-CodonPlus (DE3) in 2XTY medium, induced with 100μM/mL IPTG. The protein was purified with affinity chromatography technique using Talon resin (Clonetech). The purified protein was dialyzed with 20mM Tris pH 7.4, 200mM NaCl and 5mM β-mercaptoethanol and submitted to a robotic crystallization trials using Honeybee 939 robot. The MTAP was purified in large amounts with a high level of purity as monitored by SDS PAGE. The yielding was 60mg per liter of 2XYT medium. The protein was crystallized in 100mM Bis-tris pH 6.1-6.5 and 14-18% PEG 3350. Diffraction data was obtained in the MX2 beamline of the LNLS, Campinas-Brazil. Two data sets were collected at 2.0Å and 2.1 Å.

The crystals of MTAP belong to the space group $P2_1$. The first in complex with adenine (MTAP-Ade), has cell dimensions of *a*=81.31Å, b=82.60Å, c=150.30Å and γ=100.6°. The second in the form of apoenzyme (MTAP-apo) has cell dimensions of *a*=80.62Å, b=82.40Å, c=150.28Å and γ=101.5°. The MTAP-ade complex was solved by molecular replacement (MR) employing human MTAP as a search model (PDB id 1CBO). The MTAP-apo was solved by molecular replacement (MR) again, but using MTAP-ade as a search model. Both were solving using Phaser and refinement has been carried using the programs Coot and Phenix. The partially refined resulted for MTAP-ade is R_{work}(%) = 19.8 and a R_{free}(%) = 24.6 and for the MTAP-apo is R_{work}(%) = 18.0 and a R_{free}(%) = 23.5.

The active site of each monomer is located near the interface between subunits. The trimeric arrangement of subunits is very similar to that seen in mammalian PNP. In MTAP, the active site of a given subunit is composed entirely of residues from that subunit. The contacts polar in site are between adenine and the residues Asp230 and Asp232. The H_2N of the adenine does interaction with two residues simultaneously. The adenine interact with two water molecules, one of them does polar contact with a Ser188 and Asp232. In the MTAP-apo, the Phe231 occupies the active site, an active site conformational change induced by adenine was observed. The Phe231 residue leaves the site opening space for the adenine. In overlapping structures, is also observed a conformational change in secondary structure, in the MTAP-ade, the fifth and seventh α-helixes are displaced toward the ligand.

Supported by Fapesp and CNPq.

Keywords: phosphorylase; MTAP; Schistossoma mansoni.

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Crystal structure of a metallo-β-lactamase from a multiresistant strain of *A. baumannii*

Fabiola R. Calviño, Antonio Romero. *Department of Chemical and Physical Biology, Centro de Investigaciones Biológicas, CSIC, Madrid (Spain).* E-mail: frcalvinho@cib.csic.es

Acinetobacter baumannii is one of the species most commonly isolated from clinical specimens and it can be the cause of severe and sometimes lethal, nosocomial infections. It colonizes patients in Intensive Care Units and contaminates inanimate hospital surfaces [1].

Antibiotic resistance to clinically employed β-lactam antibiotics currently is recognized as the most important risk factor for multiresistance. Described resistance mechanisms include hydrolysis by β-lactamases, alterations in outer membrane proteins and penicillin-binding proteins, and increased activity of efflux pumps.

β-lactamases can be divided into four classes (A, B, C, and D) according to their sequence similarities [2]. On the basis of their different catalytic mechanisms, two groups have been established. β-lactamases of classes A, C and D own in its active site serine groups, while class B β-lactamases are metalloproteins that require zinc for their activity. Metallo-β-lactamases often have mosaic structures with additional domains but in general all of them are small enzymes sharing a common four-layer αββα motif with a central β-sandwich and two α-helices on either side. This motif has an intrinsic metal-binding site located at an edge of the β-sandwich and it is occupied by a divalent zinc ion having a tetrahedral array of three histidines and a water molecule. The importance of the zinc ion to β-lactam substrate binding is unquestionable [3].

We present the three-dimensional structure of a new zinc metallo-hydrolase enzyme, extracted from a clinically epidemic multidrug-resistant *A.baumannii* strain. Crystals were grown using the vapour diffusion technique. They belong to space group P1, with cell dimensions a=60.04, b=64.55, c=69.37 Å; α=82.12, β=81.46, γ=75.89 º and four molecules in the asymmetric unit, which diffracted beyond 2.4 Å. We solved the phases by a SAD experiment. Our goal is to understand the role of the two metals ions in the catalytic pathway and get some insights into the mechanism of metallo-β-lactamase hydrolysis. This information could serve as a starting point for the development of new antimicrobial agents given that there is clearly a specific need for new potent class B β-lactamase inhibitors to bridge the existing therapeutic void.

[1] M.L. Joly-Guillou. *Clin Microbiol Infect* **2005**, *11*, 868–73. [2] R.P. Ambler. *Phil Trans R Soc London B* **1980**, *289*, 321–331. [3] J.F. Fisher, S. O. Meroueh, S. Mobashery. *Chem Rev* **2005**, *105*, 395-424.

Keywords: A. baumannii, β-lactamase, zinc.

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Fragment-based drug discovery applied to heat shock protein 90 (HSP90)

Puja Pathuri, Gianni Chessari, Maria G. Carr, Miles Congreve, Joseph E. Coyle, Philip J. Day, Lynsey Fazal, Martyn Frederickson, Brent Graham, Jonathan Lewis, Rachel McMenamin, Christopher W. Murray, Alistair M. O'Brien, Sahil Patel, Glyn Williams, Andrew J. Woodhead, Alison J.-A. Woolford, *Astex Therapeutics Ltd, 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, (United Kingdom).* E-mail: p.pathuri@astex-therapeutics.com

Heat shock protein 90 (HSP90) is a chaperone which guides the folding of proteins into functional configurations affecting stabilisation and activation. Many of these proteins are oncogenes regulating tumour cell growth, survival and apoptosis. Therefore, inhibitors of HSP90 are potentially useful as chemotherapeutic agents in cancer. This poster describes an application of Astex Pyramid™ fragment screening to the ATPase domain of HSP90. The screening identified an aminopyrimidine with affinity in the high micromolar range and subsequent structure-based design allowed its optimisation into a low nanomolar series with good ligand efficiency. A phenolic chemotype was also identified with affinity close to one millimolar. This fragment was optimised using structure based design into a resorcinol lead which has sub-nanomolar affinity for HSP90, excellent cell potency, and good ligand efficiency. This fragment to lead campaign improved affinity for HSP90 by over 1,000,000 fold with the addition of only 6 heavy atoms. Subsequent lead optimisation of the resorcinol lead produced AT13387, a compound that is now in clinical trials for the treatment of cancer.

Keywords: drug, fragment, chaperone

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Structure and binding analysis of active site inhibitors targeting HIV-1 RNase H

Eric B. Lansdon, Qi Liu, Stephanie A. Leavitt, Mini Balakrishnan, Jason K. Perry, Candra Lancaster-Moyer, Nilima Kutty†, Xiaohong Liu, Neil H. Squires, William J. Watkins, Thorsten A. Kirschberg
Gilead Sciences, Inc., 333 Lakeside Dr., Foster City, CA 94404. E-mail: eric.lansdon@gilead.com

Human immunodeficiency virus (HIV-1) reverse transcriptase (RT) is a multifunctional enzyme containing both polymerase and RNase H functionalities. RNase H is required to generate specific RNA primers and subsequently break down intermediate RNA/DNA hybrids during reverse transcription. This activity is dependent on binding of two divalent metal ions in the active site. Pyrimidinol carboxylic acid and *N*-hydroxy quinazolinedione inhibitors were designed to coordinate these metal ions at the active site. High resolution (1.4Å-2.1Å) crystal structures were determined with the isolated RNase H domain and RT that permit accurate assessment of the metal and water environment. The geometry of the metal coordination suggests that the inhibitors mimic a substrate state prior to phosphodiester catalysis. The inhibitors also engage His539 *via* π stacking interactions and form a polar interaction with Arg557. Surface plasmon resonance studies confirm metal-dependent binding to RNase H and demonstrate that the inhibitors do not bind at the polymerase active site of RT. Comparative analysis of human RNase H1 bound to RNA/DNA substrate suggests that there are differences in the position of the equivalent residues at His539 and Arg557 in human RNase H1. This provides a rational for why these inhibitors selectively target the HIV enzyme over the human enzyme. Additional evaluation of the active site reveals an open protein surface with few additional interactions to optimize inhibitors.

Keywords HIV, antiviral therapy, reverse transcriptase

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Structural basis of fosmidomycin's action on *Plasmodium falciparum*

Nobutada Tanaka,[a] Tomonobu Umeda,[a] Yoshio Kusakabe,[a] Masayuki Nakanishi,[b] Yukio Kitade,[c] and Kazuo T. Nakamura,[a]
[a]*School of Pharmacy, Showa University, Tokyo, (Japan).* [b]*College of Pharmaceutical Sciences, Matsuyama University, Matsuyama, (Japan).* [c]*Faculty of Engineering, Gifu University, Gifu, (Japan).* E-mail: ntanaka@pharm.showa-u.ac.jp

The human malaria parasite *Plasmodium falciparum* is responsible for the death of more than a million people each year. The emergence