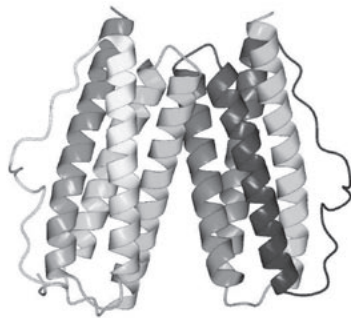


P04.22.428*Acta Cryst.* (2008). A64, C364**Crystal structure of a conserved hypothetical protein, Rv2844, from *Mycobacterium tuberculosis***Li-Wei Hung¹, Minmin Yu², Evan H Bursey², Teresa Woodruff¹, Chang-Yub Kim¹, Tim Lakin³, Brent W Segelke³, Thomas T Terwilliger¹¹Los Alamos National Laboratory, PO Box 1663, Los Alamos, NM, 87545, USA, ²Lawrence Berkeley National Laboratory, 1 Cyclotron Rd. Berkeley, CA 94720, USA, ³Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, CA 94550, USA, E-mail : lwhung@lanl.gov

Rv2844 is a conserved hypothetical protein from *M. tuberculosis*. Among the 4.5 million protein sequences in the non-redundant (NR) sequence database, only 12 proteins share sequence homology with Rv2844, and none of them has a known function. The crystal structure of Rv2844 was determined by Se-SAD experiments and refined at 2.0Å resolution. It revealed a fold of a 4-helix bundle closely related to the Ferritin-like iron storage and electron transport proteins. It is quite possible that Rv2844 defines a novel class of 4-helix bundle metal-binding proteins. High-throughput ligand analysis (Roberts and Kim, to be published) suggested that Rv2844 strongly interacts with s-adenosylmethionine (SAM), a coenzyme involved in more than 40 metabolic reactions in cells. Structural study of Rv2844-SAM complex is underway to understand the nature of their interaction. Since Rv2844 has no sequence homologs in higher organisms, and since experimental data strongly indicate Rv2844's relationship to proteins critical to cell's metabolic pathways, we are in the process of evaluating Rv2844's potential as a drug target. Updated results will be presented.



Keywords: structural genomics, structural biochemistry enzymology, protein structure and function

P04.22.429*Acta Cryst.* (2008). A64, C364**Two men and a genome: A poor man's approach to structural genomics**Ronny C Hughes¹, Miranda L Byrne¹, Damien Marsic², Joseph D Ng^{1,2}¹University of Alabama in Huntsville, Laboratory for Structural Biology, 301 Sparkman Dr., Huntsville, AL, 35899, USA, ²ExtremoZyme Inc, HudsonAlpha Institute for Biotechnology, 601 Genome Way, Huntsville, AL, 35806, USA, E-mail: ron618_331@yahoo.com

A non-automated procedure has been developed to rapidly clone and express the proteins of targeted coding regions from the genome sequence of a novel anaerobic hyperthermophilic archaeon, *Thermococcus thio-reducens*. Selected open reading frames were identified and used for PCR (Polymerase Chain Reaction) amplification in 96 well configuration or used to guide whole gene synthesis. The amplified products were cloned into expression vectors without the use of restriction digest or ligation. Protein expression trials were performed on all clones, and those observed to show overexpression were used for large scale protein

production. Protein crystallization trials were executed on purified proteins by high throughput screening methods exploring hundreds of crystallization conditions at a time. By incorporating proven strategies developed from large structural genomic centers with practical innovations, we have constructed a mini-pipeline in which a small group consisting of as few as two people can survey 1500 open reading frames for cloning, expression, crystallization and structure determination for less than \$200,000. The development of a mini-pipeline structural genomics was supported in part by NSF STTR-05605 and NSF-EPSCoR (EPS-0447675). Crystallographic data was collected at APS on the SERCAT beamline. Hughes, R. and Ng, J.D. (2007). Can small laboratories do structural genomics? *Crystal Growth and Design* 7:2226-2238.

Keywords: thermococcus thio-reducens, high throughput cloning, protein crystallization

P04.22.430*Acta Cryst.* (2008). A64, C364**The PSI structural genomics knowledgebase**Helen M Berman¹, Paul Adams², Andrei Kouranov¹, Rajesh Nair¹, Wendy Tao¹, Torsten Schwede³, Raship Shah¹, John Westbrook¹¹Rutgers, The State University of New Jersey, Chemistry and Chemical Biology, 610 Taylor Road, Piscataway, NJ, 08854, USA, ²Berkeley Structural Genomics Center, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA, ³Division of Bioinformatics, Biozentrum, University of Basel, CH-4056 Basel, Switzerland, E-mail : berman@rcsb.rutgers.edu

The Protein Structure Initiative (PSI) has been successful in producing over 3000 protein structures in a high throughput manner. The strategies used for target selection by PSI centers have resulted in the determination of significantly more novel structures than from structural biology in general. In addition, new technologies for all aspects of the structure determination and analysis pipeline have been developed. The PSI Structural Genomics Knowledgebase (PSI SG KB) is designed to turn the products of the PSI effort into knowledge that is important for understanding living systems and disease. The PSI SG KB Portal allows access to the structures, annotations, technologies, and models that are the products of the PSI. A description of how this resource will enable further biological discovery will be given. The PSI SG KB is funded by the NIH.

Keywords: structural genomics, biosciences databases, structural biological function

P04.22.431*Acta Cryst.* (2008). A64, C364-365**Bridging the gaps in high throughput crystallography: Upstream and downstream developments for ACTOR**Angela R Criswell, Amber Dowell, John Ziegler, Bret Simpson, Charlie Stence, John Truong, Russ Athay, Jim W Pflugrath
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Advances in high throughput methods for crystallography have evolved to automate the full process from crystallization setup to correctly traced electron density maps. Enabling these methods is a pipeline of reliable hardware and software developed to work with limited human intervention. One such tool, the ACTOR.system, has been in use since 2001 and eliminates many of the time-consuming

sample manipulation tasks required in routine crystal screening and data collection. Supplemental ACTOR software for ranking, data collection strategy determination and image processing further increases the value of ACTOR by way of an intelligent framework for automated X-ray diffraction experiments. More recent ACTOR developments incorporate upstream tools used during sample harvesting as well as state of the art sample tracking mechanisms. Additionally, ACTOR software expands downstream to automate structure solution using both 'bind and grind' and SAD methods. Altogether these additions function to enhance and expand the protein crystallographer's toolkit. In this paper we will describe these new hardware and software developments and evaluate their efficacy and reliability for high throughput crystallography.

Keywords: automated data collection, protein crystallography, robotics

P04.23.432

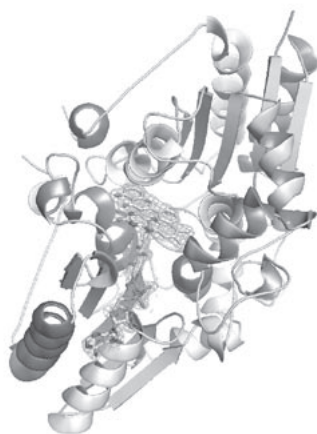
Acta Cryst. (2008). A64, C365

Crystal structure of quinone reductase 2 in complex with the selective inhibitor 5-hydroxyflavone

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Flavonoids, which are obtained from the diet, are generally considered selective inhibitors for the flavo-enzyme quinone reductase 2 (NQO2). Several studies have reported the therapeutic importance of NQO2 as a target for anti-cancer and anti-malarial agents. Here, the crystal structure of NQO2 in complex with 5-hydroxyflavone is reported. This crystal structure complex resolved at 1.87 Angstrom atomic resolution has provided an explanation of the selective inhibition activity of this particular compound towards NQO2. Binding interactions created by 5-hydroxyflavone with amino acids of NQO2 binding site is solely influenced by the chemical structure of this compound. These interactions enforce the molecule to adopt a certain binding mode which is responsible to exert the compound inhibitory activity towards NQO2. Superimposition studies can be carried out with other structure complexes of NQO2 such as NQO2 - resveratrol crystal structure. This can be exploited for future drug design studies with NQO2.



Keywords: crystal structure, NQO2 inhibitor, 5-hydroxyflavone

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A structural studies of free methionine-(R)-sulfoxide reductase from staphylococcus aureus

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The reduction of methionine sulfoxide (MetO) is mediated by methionine sulfoxide reductases (Msr). The MsrA(E.C.1.8.4.11), msrB(E.C.1.8.4.12) families can reduce free MetO and MetO within a peptide or protein context. This process is stereospecific with the S- and R-forms of MetO repaired by MsrA and MsrB, respectively. Cell extracts from an MsrA-B- knockout of Escherichia coli have several remaining Msr activities. It has identified an enzyme specific for the free form of Met-(R)-O, fRMs, through proteomic analysis. The recombinant enzyme exhibits the same substrate specificity and is as active as MsrA family members. The crystal structure of E.coli fRMs was previously determined. In this study, The X-ray diffraction data of Staphylococcus aureus fRMs with DMSO were collected to 1.9Å resolution using synchrotron radiation. The crystals belonged to the hexagonal space group $P6_122$ with cell parameters of $a = b = 89.451$, $c = 88.768$ Å, and $\alpha = \beta = 90^\circ$ $\gamma = 120^\circ$. The asymmetric unit contained one molecules of fRMs, giving a crystal volume per protein mass (V_m) of $0.4 \text{ \AA}^3 \text{ Da}^{-1}$ and a solvent content of 52.13%. The crystal structure of fRMs from Staphylococcus aureus was determined by molecular replacement. The refined model of fRMs gave R factor and R_{free} values of 21% and 23%. The structural similarity of the E.coli and Staphylococcus aureus proteins suggests that most fRMs use three cysteine residues for catalysis and formation of a disulfide bond to enclose a small active site cavity.

Keywords: methionine sulfoxide, free methionine-(R)-sulfoxide reductase, asymmetric unit

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New insight into catalytic mechanism of serine proteases from ultra-high resolution X-ray studies

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Serine proteases have been the subject of intensive studies for many years and many aspects of the mechanism of their action have already been revealed. Yet a number of questions still remains, especially concerning hydrogen bonds within the catalytic triad. One of them focuses on the exact nature of the bond between the catalytic histidine and aspartate. Also the role of a conserved hydrogen bond formed by one of the histidine's carbon atoms is unresolved. Ultra-high resolution X-ray structures of alcalase® - a naturally occurring variant of subtilisin Carlsberg - in complexes with chymotrypsin inhibitor 2 (CI-2) wild type and its reactive site mutant M59P, which binds to the enzyme with 10^{-4} lower affinity, have been obtained (at 100K) and analysed. Additionally, an atomic resolution structure of alcalase with native CI-2 at physiologically relevant temperature is discussed. Having structures representing different snapshots of the enzymatic reaction is a standard approach for gaining insight into the catalytic mechanism. When the standard uncertainty of the coordinates is low, as it is at 1Å resolution, even small structural changes can be reliably measured. Such resolution also allows for direct observation of hydrogen atoms in the electron density. A great benefit comes from the possibility to compare their positions