P04.22.428

Crystal structure of a conserved hypothetical protein, rv2844, from *Mycobacterium tuberculosis*

Li-Wei Hung[1], Minmin Yu[2], Evan H Bursey[2], Teresa Woodruff[1], Chang-Yub Kim[1], Tim Lekin[3], Brent W Segelke[3], Thomas T Terwilliger[1]

[1]Los Alamos National Laboratory, PO Box 1663, Los Alamos, NM, 87545, USA, [2]Lawrence Berkeley National Laboratory, 1 Cyclotron Rd. Berkeley, CA 94720, USA, [3]Lawrence 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.
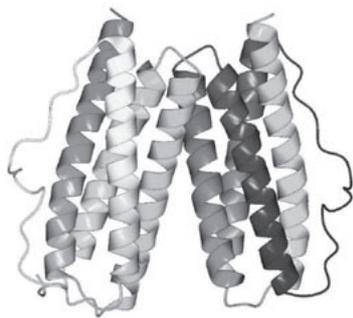
Keywords: structural genomics, structural biochemistry enzymology, protein structure and function

P04.22.429

Two men and a genome: A poor man's approach to structural genomics

Ronny C Hughes[1], Miranda L Byrne[1], Damien Marsic[2], Joseph D Ng[1,2]

[1]University of Alabama in Huntsville, Laboratory for Structural Biology, 301 Sparkman Dr., Huntsville, AL, 35899, USA, [2]ExtremoZyme 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 thioreducens. 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 thioreducens, high throughput cloning, protein crystallization

P04.22.430

The PSI structural genomics knowledgebase

Helen M Berman[1], Paul Adams[2], Andrei Kouranov[1], Rajesh Nair[1], Wendy Tao[1], Torsten Schwede[3], Raship Shah[1], John Westbrook[1]

[1]Rutgers, The State University of New Jersey, Chemistry and Chemical Biology, 610 Taylor Road, Piscataway, NJ, 08854, USA, [2]Berkeley Structural Genomics Center, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA, [3]Division 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

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
Rigaku Americas Corporation, 9009 New Trails Dr, The Woodlands, TX, 77381, USA, E-mail：angela.criswell@rigaku.com

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