MS15 THE BIG QUESTION IN STRUCTURAL GENOMICS: GETTING FUNCTION FROM STRUCTURE
Chairpersons: Sung-Hou Kim, Edward Neill Baker

MS15.25.1
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Crystal Structure of Rv0813c Reveals a New Family of Putative FABPs
William Shepard[a], A. Haouz[b], A. Buschiazzo[b], J.M. Betton[b], S.T. Cole[b], P. Alzari[b], [a]*ESRF, 6 rue Jules Horowitz, BP 220, 38043 Grenoble cedex 9, France,* [b]*Institut Pasteur 25 rue du Dr. Roux, 75724 Paris cedex 15, France.* E-mail: shepard@esrf.fr

Rv0813c is a protein of unknown function that we have selected as a target for crystallographic studies in the context of a structural genomics effort on tuberculosis. The crystal structure of Rv0813c, a conserved protein in *M. tuberculosis*, reveals a new family of putative fatty acid binding proteins (FABPs). Rv0813c adopts a 10-stranded beta barrel fold, which closely resembles those of the FABPs found in eukaryotes. This is in fact the first FABP-like protein to be found in prokaryotes. However, Rv0813c lacks the double helix insert of FABPs that covers the entry to the binding site. The beta barrel forms a deep cavity, where a small ligand, which appears to be a morpholine, binds to the phenol hydroxyl group of Tyr192. This tyrosine corresponds to a RxY motif, which forms part of the binding site in FABPs. Furthermore, a network of H bonds, hydrophobic residues and an internal salt bridge surround the binding site and define the shape of the cavity. Most of these residues are well conserved in homologous proteins. Phylogenetic studies show that this family of FABP-like proteins is represented in GC-rich prokaryotes. The structural analysis of Rv0813c suggests that this cytoplasmic protein may have a role in fatty acid transport, storage or signaling. This work supports the notion that high resolution structural studies can provide strong leads as to the biochemical function(s) of the protein.
Keywords: structural genomics, mycobacteria, fatty acid binding protein

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Chasing the Function of Hypothetical Proteins
Osnat Herzberg[a], Gary Gilliland[b], John Orban[a], Andrew Howard[c], John Moult[a], [a]*Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, MD, USA.* [b]*Center for Advanced Research in Biotechnology, National Institute for Standards and technology, Rockville, MD, USA.* [c]*Biological, Chemical, and Physical Sciences, Illinois Institute of Technology, Chicago, IL, USA*. E-mail: osnat@carb.nist.gov

The focus of the Structure2Function program at CARB during the first five years was on prokaryotic proteins of unknown function, mostly from *Heamophilus influenzae* and *E. coli*. The goal was to leverage the 3-D structural information to gain insight into the biochemical function. Approximately 75% of the new structures exhibit folds that have been seen before, and in many cases we were able to make new discoveries about function by utilizing standard assays and mining genome context. The remaining 25% structures defined new folds, a somewhat higher fraction of new folds compared with structures entering the PDB. In some of these cases, the function became known around the time the structure was determined, while in other cases we were able to make broad predictions about the function.
Keywords: structural genomics, hypothetical proteins, crystallography

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Functional & Structural Proteomics of SARS: Defining a Rational Response to Emerging Diseases
Jeremiah Joseph[a], Alexei Brooun[a], Benjamin Neuman[a], Enrique Abola[a], James Stevens[a], Kumar Saikatendu[a], Margaret Johnson[a], Michael Recht[b], Michelle Kraus[a], Mike Nelson[a], Renaud Burrer[a], Sophie Coon[a], Vanitha Subramanian[a], Weizhong Li[c], Adam Godzik[c], Ian Wilson[a], Kurt Wuthrich[a], Mike Buchmeier[a], Raymond Stevens[a], Richard Bruce[b], Ron Milligan[a], Peter Kuhn[a], [a]*The Scripps Research Institute, La Jolla, CA, USA.* [b]*Palo Alto Research Center, Palo Alto, CA, USA.* [c]*The Burnham Institute, La Jolla, CA, USA.* E-mail: jjoseph@scripps.edu

Rapid rational therapeutic and prophylactic responses are crucial when faced with new infectious diseases. The emergence of the coronavirus responsible for the Severe Acute Respiratory Syndrome (SARS) tested the utility of post-genomic technologies to characterize and combat this virus. While virus identification and complete genome sequencing took mere weeks, they have been tough acts to follow for drug and vaccine development. We have undertaken a multi-pronged initiative to understand and address precisely this bottleneck using a structural and functional proteomics approach involving bioinformatics, structural biology (X-ray crystallography, NMR, cryo-electron microscopy), genetic approaches (site-directed mutagenesis, antisense functional mapping, microarray-based functional mapping), and macromolecular interaction studies (nano-calorimetry, ligand-fishing techniques, mass spectrometry) to generate a structure-function-interaction map of the entire proteome of the SARS-CoV and its interactions with the host cell. This presents an exciting and comprehensive set of targets for rational, structure-based drug and vaccine design, defining a paradigm adoptable for *any* emerging infectious disease.

We designed multiple constructs of the 28 SARS-CoV ORFS for expression in *E. coli*, baculovirus and mammalian systems. Over 150 constructs have been processed by high-throughout protein expression and purification. Of the 31 expressing constructs, nano-volume crystallization has produced crystal hits for 5. One crystal structure and one NMR structure has been determined. Cryo-electron microscopy has characterized the packing arrangement of the S, M and N proteins in the virion. The challenge is that most SARS proteins are involved in intimate protein-protein, protein-membrane, or protein-RNA interactions which must be understood for a complete description of its biology.
Keywords: structural genomics, viral structure and function, intermolecular interactions

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Whole-Cell Project of *Thermus thermophilus* HB8 toward Atomic-Resolution Biology
Seiki Kuramitsu[a,b,c], Akio Ebihara[a], Mayumi Kanagawa[a], Noriko Nakagawa[a,c], Ryoji Masui[a,c], Kazutaka Murayama[b], Takaho Terada[a,b], Mikako Shirouzu[a,b], Kunio Miki[a,d], Shigeyuki Yokoyama[a,e], [a]*RIKEN Harima Institute at SPring-8.* [b]*RIKEN Genomic Sciences Center.* [c]*Department of Biology, Graduate School of Science, Osaka University.* [d]*Department of Chemistry, Graduate School of Science, Kyoto University.* [e]*Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Japan.* E-mail: kuramitu@bio.sci.osaka-u.ac.jp

In order to interpret the whole biological phenomena of the cell, we selected a model organism, *Thermus theromphilus* HB8. Its genome size is about 2 Mbp, and the number of its open reading frames (ORFs) is about 2,200 (http://www.thermus.org). Two-thirds of the ORFs are common to the genomes of most organisms including the human, and one-third of the ORFs are hypothetical proteins.

Plasmid construction for protein production has been completed for 2,000 ORFs. *In vivo* protein-production system of *E. coli* could successfully overproduced about 81% of the proteins. More than 85% of the purified proteins were successfully crystallized. For approximately 40% of the purified proteins, their diffraction data sets are of sufficient quality such that structural analysis is possible.

For the hypothetical proteins, structural analysis predicted the function with the success rate of about 60%. The function of the rest of the hypothetical proteins was estimated from transcriptome

analysis, metabolome analysis, gene disruption experiments, and other methods as necessary.

Keywords: structural genomics, functional genomics, model organism

MS15.25.5

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Structural Proteomics : a Rich Source of Purified Proteins for Functional Assays

Aled Edwards, E. Kuznetsova, M. Proudfoot, S. A. Sanders, C. F. Gonzalez, O. V. Litvinova, A. V. Savchenko, C. H. Arrowsmith, A. F. Yakunin, *Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada M4G 1H1*. E-mail: aled.edwards@utoronto.ca

Structural proteomics efforts generate 2-3 times more purified proteins than structures. We have developed general enzymatic assays to screen individually purified proteins for enzymatic activity. The assays have relaxed substrate specificity and are intended to identify sub-subclasses of enzymes (phosphatase, phosphodiesterase, esterase, protease, dehydrogenase, and oxidase) to which the unknown protein belongs. Further biochemical characterization of proteins is facilitated by the application of secondary screens with natural substrates (substrate profiling). We demonstrated the feasibility and merits of this approach for hydrolases and oxidoreductases, two very broad and important classes of enzymes and identified over 40 new enzymes (phosphatases, phosphodiesterases, esterases). The screens were also applied to quickly characterize the large family of unknown proteins in *E. coli*, the haloacid dehalogenase (HAD)-like hydrolases.

Keywords: structural proteomics, enzyme screens, phosphatases

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MS16 HIGH RESOLUTION X-RAY INELASTIC SCATTERING

Chairpersons: Gian Carlo Ruocco, Alfred Baron

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Dynamics of Glassy Materials by High Resolution Inelastic X-ray Scattering

Daniele Fioretto, *Dipartimento di Fisica, Università di Perugia. INFM - CRS Soft, Università di Roma La Sapienza.* E-mail: fioretto@fisica.unipg.it

The nature of short wavelength excitations in glassy materials is highly debated in the physics of disordered materials. What is the length scale beyond which the continuous homogeneous medium approximation breaks down in glasses? What is the microscopic origin of sound attenuation in strong and in fragile glasses? Is there any relationship between propagating acoustic modes and the boson peak?

The development of high resolution inelastic x-ray scattering technique allowed us to experimentally address these problems by measuring the dynamical structure factor $S(Q,E)$ of glassy materials in the mesoscopic region between 1 and some tens nm^{-1}, both varying the energy (E) at fixed exchanged wave vector (Q) and varying Q at fixed E [1]. A review is here reported, together with a comparison with results obtained by complementary techniques like Brillouin light scattering [2] and inelastic ultra-violet scattering [3].

[1] Sette F., Krisch M.H., Masciovecchio C., Ruocco G., Monaco G., *Science*, 1998, **280**, 1550. [2] Fioretto D., Mattarelli M., Masciovecchio C., Monaco G., Ruocco G., Sette F., *Phys. Rev. B*, 2002, **65**, 224205. [3] Masciovecchio C., Gessini A., Di Fonzo S., Comez L., Santucci S.C., Fioretto D., *Phys. Rev. Lett*.,2004, **92**, 247401.

Keywords: glasses, X-ray scattering, light scattering

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Collective Dynamics of Liquid Metals: from Simple to Extremely Non-Simple

Shinya Hosokawa, *Faculty of Engineering, Hiroshima Institute of Technology, Japan.* E-mail: hosokawa@cc.it-hiroshima.ac.jp

Recent developments in high-resolution inelastic X-ray scattering using third-generation synchrotron radiation facilities allow one to investigate the collective dynamics of a wide variety of liquid metals. The IXS studies have started from simple liquid metals such as the first experiment of liquid Li [1], Na, and Mg, and encompassed to several non-simple metals such as liquid Ga, Ge, and Si [2]. The experimental results revealed characteristic common features in the collective dynamics: 1) A clear indication for propagating modes, and 2) a positive deviation of the collective excitations by about 20 % from the hydrodynamic value. In addition, an indication of a short time (sub-picosecond) retaining of the nearest-neighbour correlation is visualized from the quasielastic line of some non-simple liquid metals [2]. A generalized Langevin formalism with a memory function containing two viscoelastic decay channels [3] is commonly used for analyzing the above IXS data.

In this paper, we review the experimental technique of IXS for liquid metals, and then the common feature of the collective dynamics of liquid metals in detail. Some of them are discussed in connection with results of ab initio molecular dynamic simulations.

[1] Sinn H., et al., *Phys. Rev. Lett.*, 1997, **78**, 1715. [2] Scopigno T., et al.,*J. Phys.: Condens. Matter*, 2000, **12**, 8009. [3] Hosokawa S., *et al.*, *J. Phys.: Condens. Matter*, 2003, **15**, L623. [4] Levesque D., et al., *Phys. Rev. A*, 1973, **7**, 1690.

Keywords: X-ray inelastic scattering, phonons, liquid metals

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Vibrational Dynamics of Iron in Proteins

J. Timothy Sage, *Department of Physics, Northeastern University.* E-mail: jtsage@neu.edu

High-resolution x-ray measurements near the nuclear resonance reveal the complete vibrational spectrum of a Mössbauer nucleus. I will illustrate novel opportunities that this site-selective method provides for characterizing the vibrational dynamics of ^{57}Fe at the active sites of heme proteins, iron-sulfur proteins, and related model compounds. (1) Quantitative data on the frequency, the amplitude, and in some cases, the direction of all iron vibrations provide a uniquely detailed benchmark for modern quantum chemical vibrational predictions, with which they can be directly compared on an absolute scale. (2) Measurements on oriented single crystals of iron porphyrins reveal low-frequency out-of-plane vibrations that we identify with the long-sought heme “doming” mode, similar to the motion that takes place on oxygen binding to heme proteins. Moreover, the experimental data provide a direct experimental estimate of the force constant for Fe displacement normal to the heme plane and suggest that this Fe motion is an important element in protein control of biological reaction energetics. (3) Comparisons with calculations and with independent Raman isotope shift measurements probes the extent to which active site vibrations couple to global protein motions.

Keywords: heme proteins, Mössbauer spectroscopy, vibrational spectroscopy

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High-Resolution Inelastic X-ray Scattering of Materials of Geophysical Interest

Guillaume Fiquet, *IMPMC, UMR CNRS 7590. University Pierre et Marie Curie, Paris, France.* E-mail: fiquet@lmcp.jussieu.fr

Inelastic X-ray scattering (IXS) has progressively arisen as one of the major spectroscopic tools with the advent of bright X-ray sources of 3rd generation. It offers the unique opportunity to investigate the phonon or electronic properties *in situ*, at various conditions of pressure and temperatures, and is thus very well suited to the study of the composition and dynamics of the Earth and planetary interiors.

The elasticity and the sound wave anisotropy of hcp-metals, namely iron and cobalt have been investigated at high-pressure by very high resolution (meV) IXS. I will address the case of hcp-iron, the main constituent of the Earth's inner core, and report the direct experimental determination of the anisotropy in the propagation of longitudinal acoustic waves in textured sample above 100 GPa. Hcp-cobalt, here chosen a proxy for iron, has also been studied with the advantage to be