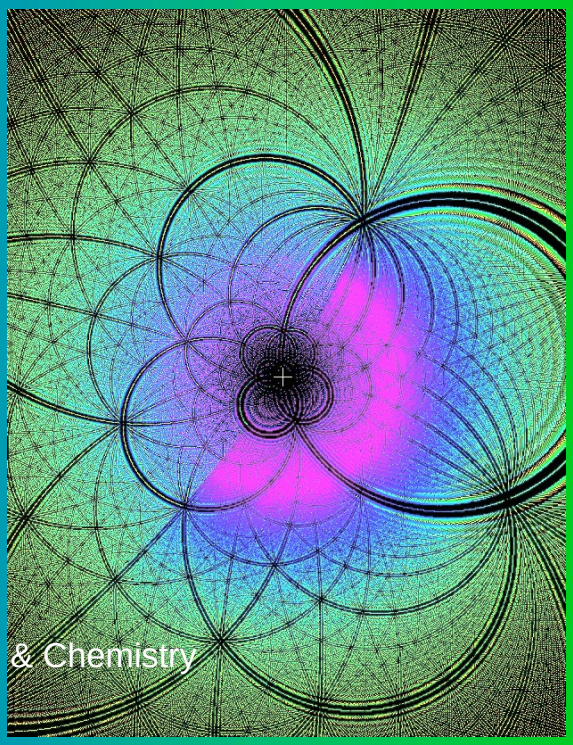
Workshop on Time-resolved and Laue X-ray Crystallography

May 6-8, 2006
BioCARS / APS
Argonne, Illinois USA



- Methods in Time-resolved Macromolecular Crystallography
- Small-molecule Crystallography
- Spectroscopy
- Experiment and Techniques
- Applications: Photoreceptors, Enzymes & Chemistry
- Computational Methods
- Poster Session
- "Meet the Experts"

Partial List of Confirmed Speakers:

Shin-ichi Adachi (Japan)
Philip Anfinrud (USA)
Dominique Bourgeois (France)
Lin Chen (USA)
Eric Collet (France)
Philip Coppens (USA)
Maria Hrmova (Australia)
Hyotcherl Ihee (South Korea)
Ulrich Nienhaus (Germany)
Benoit Roux (USA)
Ilme Schlichting (Germany)
Marius Schmidt (Germany)
Cynthia Stauffacher (USA)
Carrie Wilmot (USA)

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Web Site:

http://www.aps.anl.gov/Users/Meeting/TR_Workshop/

BioCARS
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Image of computer generated Laue diffraction pattern courtesy of Zhong Ren, Renz Research, Inc.

Workshop on Time-resolved and Laue X-ray Crystallography

Organizer: BioCARS, The University of Chicago

The aim of this workshop is to present the current capabilities, applications and future prospects of this technique to the biology and also chemistry communities. This workshop will bring together experts from the various fields to discuss experimental details, data analysis methods as well as scientific applications and will also offer a hands-on demonstration of Laue data processing.

BioCARS is presently upgrading its time-resolved facility with new x-ray optics and advanced laser systems; operation is expected to resume with monochromatic and Laue experiments during the APS Run 2006-3. This workshop is intended to stimulate the interest in time-resolved phenomena and expand the user community in this field.

Saturday, 6-May-2006

Science Sessions -- APS Bldg. 402 Auditorium

Session 1: Methods – Introduction and Overview

8:30 AM Welcome
Keith Moffat, The University of Chicago, Chicago (USA)

8:45 AM Observing proteins at work in the crystal
Dominique Bourgeois, Institut de Biologie Structurale & ESRF, Grenoble (France)

9:30 AM Structure determination of reaction intermediates by trapping approaches
Ilme Schlichting, Max Planck Institute for Medical Research, Heidelberg (Germany)

10:15 AM Break, Atrium and Gallery

Session 2: Methods – Experimental Details

10:30 AM Capabilities for Laue and time-resolved experiments at BioCARS
Reinhard Pahl, BioCARS, The University of Chicago, Chicago (USA)

11:00 AM Implementation of time-resolved experiments at the ESRF
Friedrich Schotte, Laboratory of Chemical Physics, NIH-NIDDK, Bethesda (USA)

Session 3: Applications – Photoreceptors

11:30 AM TBA
Richard Vierstra, University of Wisconsin, Madison (USA)

12:00 Noon Lunch – APS Bldg. 402, Gallery Lower Level
Poster Setup

1:00 PM Static and time domain structures of integral membrane proteins determined at the APS
James Norris, The University of Chicago, Chicago (USA)

1:30 PM Early intermediates of the PYP photocycle probed by picosecond Laue x-ray crystallography
Hyotcherl Ihee, KAIST, Daejeon (South Korea)

2:00 PM Allosteric action in real time: Time-resolved crystallographic studies of a cooperative dimeric hemoglobin
William E. Royer, University of Massachusetts Medical School, Worcester (USA)

2:30 PM A new photo switch - the structural basis of the E/Z isomerization in biliproteins
Marius Schmidt, Technische Universität München, Garching (Germany)

3:00 PM Break, Atrium and Gallery

Session 4: Methods – Computational Approaches

3:15 PM Protein dynamics from computer simulations and time-resolved x-ray crystallography
Gerhard Hummer, NIH/NIDDK, Bethesda (USA)

3:45 PM Potassium Channels - Ion conduction, selectivity, gating, inactivation, and blockage
Benoit Roux, The University of Chicago, Chicago (USA)

4:15 PM Cluster analysis of time-dependent crystallographic data: Identification of structural intermediates
Konstantin Kostov, University of Illinois at Chicago, Chicago (USA)

4:45 PM Gas migration inside proteins - methods and mechanisms
Jordi Cohen, University of Illinois at Urbana-Champaign, Urbana-Champaign (USA)

5:30 PM End of Session

Poster Session and Reception *-- APS Bldg. 402, Gallery Lower Level*

6:00 PM – open end

Sunday, 7-May-2006

Science Sessions -- APS Bldg. 402 Auditorium

Session 5: Methods & Applications – Chemical Crystallography

8:30 AM Time-resolved experiments in daily single bunch mode: Current status of beamline NW14A at the Photon Factory Advanced Ring
Shin-ichi Adachi, Photon Factory, KEK, Tsukuba (Japan)

9:15 AM Photoinduced 1D exciton-string preceding macroscopic switching evidenced by 100ps diffuse scattering
Eric Collet, Universitee Rennes 1, Rennes (France)

10:00 AM Break, Atrium and Gallery

10:15 AM High resolution time-resolved diffraction
Philip Coppens, State University of New York at Buffallo, Buffalo, (USA)

11:00 AM Taking molecular snapshots in photochemical reactions
Lin X. Chen, Chem. Div. Argonne National Laboratory, Argonne (USA)

Session 6: Methods & Applications – Enzymes and Large Molecules

11:30 AM Molecular odysseys in mapping enzyme reaction coordinates by x-ray crystallography
Andrew D. Mesecar, University of Illinois at Chicago, Chicago (USA)

12:00 Noon Lunch – APS Bldg. 402, Gallery Lower Level

1:00 PM Through the Looking Glass: Examining enzyme mechanism and inhibition with time-resolved x-ray crystallography of HMG-CoA reductase
Cynthia V. Stauffacher, Purdue University, West Lafayette (USA)

1:30 PM Mechanism of product/substrate exchange in plant glycoside hydrolases: Conventional and Laue crystallographic approaches
Maria Hrmova, The University of Adelaide (Australia)

2:00 PM On your mark – Get set – Planning time-resolved studies of protocatechuate 3,4-dioxygenase
Douglas Ohlendorf, University of Minnesota, Minneapolis (USA)

2:30 PM Efficient data collection for the structure determination of an icosahedral virus by Laue crystallography
Tianwei Lin, The Scripps Research Institute, La Jolla (USA)

3:00 PM Break, Atrium and Gallery

Session 7: Methods & Applications – Spectroscopy

3:15 PM Structural and spectroscopic studies of photo-induced conformational changes in heme proteins and GFP-like proteins
Ulrich Nienhaus, Universität Ulm, Ulm (Germany)

4:00 PM Time-resolved studies of protein function: From infrared to x-rays
Philip A. Anfinrud, Laboratory of Chemical Physics, NIH-NIDDK, Bethesda (USA)

4:30 PM X-rays, Action, Camera! The heartache of redox enzyme photoreduction during catalytic movie production
Carrie Wilmot, University of Minnesota, Minneapolis (USA)

5:15 PM End of Session

Reception & Dinner at The Morton Arboretum

6:30 PM – open end

Monday, 8-May-2006

Science Sessions -- APS Bldg. 402 Auditorium

Session 8: Methods – Data Analysis

8:30 AM Introduction
Vukica Srajer, BioCARS and The University of Chicago, Chicago (USA)

8:45 AM Laue data collection and data processing - General issues
Zhong Ren, Renz Research Inc., Westmont (USA)

9:45 AM User-friendly Singular Value Decomposition for time-resolved crystallography
Marius Schmidt, Technische Universität München, Garching (Germany)

10:15 AM Laue data analysis with TReX
Friedrich Schotte, Laboratory for Chemical Physics, NIH-NIDDK, Bethesda (USA)

10:45 AM Break, Atrium and Gallery

Session 9: Software

11:00 AM Demonstration of the software package Precognition
Zhong Ren, Renz Research Inc., Westmont (USA)

12:00 Noon Lunch, APS Bldg. 402, Gallery Lower Level

Hands-On Sessions -- APS Bldg. 402, Rm. E1100

Session 10: Hands-On Data Analysis

1:00 PM Precognition

3:00 PM Break, Atrium and Gallery

3:15 PM LaueView

5:00 PM Discussion – End of Workshop

Workshop on Time-resolved and Laue X-ray Crystallography

Abstracts for Science Sessions

Session 1

Observing proteins at work in the crystal

Dominique Bourgeois, Institut de Biologie Structurale & ESRF, Grenoble (France).

Biological molecules constantly breathe around their average shape, wandering in a conformational landscape at the basis of their activity. Static 3D structures solved by "standard" crystallography generally poorly account for such plasticity. However, many proteins remain fully functional in the crystalline state, making "kinetic" crystallography a tool that, beside structure, can address mechanism [1]. By initiating biological turnover in the crystal, transient structural species form which may be filmed on-the-fly by fast (Laue) diffraction or captured by trapping methods. This talk will provide a basic introduction to kinetic crystallography. It will mostly focus on Laue diffraction that has now reached an amazing level of sophistication and has found a "niche of excellence" in the study of cyclic, ultra-fast, light-triggered reactions. The importance of single-crystal spectroscopy as a tool to design, interpret and validate kinetic crystallography experiments will also be emphasized.

[1] Bourgeois, D., Royant, A. (2005) *Advances in Kinetic Crystallography.* Curr. Opin. Struc. Biol. 15, 1-10.

Structure determination of reaction intermediates by trapping approaches

Ilme Schlichting, Max Planck Institute for Medical Research, Dept. of Biomolecular Mechanisms, Heidelberg (Germany).

Knowledge of the three-dimensional structure of reaction intermediates is indispensable for a thorough understanding of reaction mechanisms. Due to their fleeting nature, intermediates cannot be crystallized but need to be generated in the crystal. Reaction initiation needs to be fast, gentle and efficient. Different methods for triggering reactions exist that include diffusion, illumination by light, temperature jumps and X-ray radiolysis. The pro-and cons will be discussed and illustrated by examples, as will be means to characterize the reaction in the crystalline state. Once the reaction kinetics has been characterized, appropriate data collection schemes need to be devised. Depending on the reaction, data collection under steady state and single turn over conditions may be better suited. When working under single turn over conditions, the major decision will be whether to collect the data in real time as the reaction proceeds (time-resolved crystallography [1]), using for example, the Laue method, or whether to slow the decay step of the intermediate in question, thereby trapping it (kinetic crystallography [2]). Different trapping approaches will be described.

[1] Moffat, K. (2001) *Time-Resolved Biochemical Crystallography: A Mechanistic Perspective.* Chem. Rev. 101(6); 1569-1582.
[2] Schlichting, I. (2000) *Crystallographic Structure Determination of Unstable Species.* Acc. Chem. Res. 33(8); 532-538.

Session 2

Capabilities for Laue and time-resolved experiments at BioCARS

Reinhard Pahl, BioCARS, The University of Chicago, Chicago (USA).

Current status and future capabilities of the user facility for time-resolved studies at BioCARS, a NIH/NCRR funded Synchrotron Structural Biology Resource at the Advanced Photon Source, will be presented. Using the Laue x-ray diffraction technique snapshots are taken of molecules in action [1]. During the past years a continuously growing user community has developed in this field; projects under investigation include light and chemically triggered reaction mechanism. BioCARS is presently enhancing the technical capabilities for time-resolved diffraction experiments and spectrophotometry; the technical upgrades will improve resources for complementary optical monitoring of reactions in crystals, update the laser systems, and most importantly improve the x-ray optics to enable experiments utilizing a single x-ray pulse.

[1] Schmidt, M., Pahl, R., Ihee, H., and Šrajer, V. (2005) Protein ligand interaction probed by time-resolved x-ray structure determination. In: Methods in Molecular Biology, Vol. 305, Protein-Ligand Interactions: Methods and Applications (G.U. Nienhaus ed.). Humana Press, Totowa, NJ, pp. 115-154

Session 3

Static and time domain structures of integral membrane proteins determined at the APS

Richard H.G. Baxter, Nina Ponomarenko, Brandon Seagle, and James Norris, The University of Chicago, Chicago (USA). Elmars Krausz, Australian National University, Canberra (Australia).

The bacterial photosynthetic reaction center (RC) of *Blastochloris viridis* is a 140 kDa integral membrane protein that was first crystallized by Hartmut Michel and its structure determined to atomic resolution by Johann Deisenhofer, for which they received the Nobel Prize in 1988 along with Robert Huber. Integral membrane proteins remain difficult to crystallize since to date (4–28–2006) only 108 unique structures of integral membrane proteins are known even though approximately one third of all proteins are membrane bound.

Because the photosynthetic RC protein performs a series of electron transfer steps that are believed to be accompanied by structural changes, we have explored the photochemistry of the RC of *B. viridis* and *Rhodobacter sphaeroides* at the APS. When the RC is excited by light, a series of rapid electron transfer events occur resulting in the arrival of an electron at the primary quinone acceptor Q_A with a time constant of about 200 ps. Next, the electron is transferred from the primary quinone acceptor Q_A to a secondary quinone acceptor Q_B, Finally, the original electron donor, a special pair of bacteriochlorophylls, is re–reduced by a bound cytochrome subunit.

Q_A has been reported to exist in two locations, proximal and distal, and that switching between these sites is controllable by light. However, refinement of our monochromatic x-ray diffraction data finds Q_A bound only in the proximal binding site. In addition, single crystals of RC protein from *B. viridis* were analyzed by time resolved Laue diffraction, in the dark and 3 ms after illuminating the crystal with a pulsed laser (630 nm, 3 mJ/pulse, 7 ns duration). No significant structural differences were observed between the light and dark datasets; in particular, no Q_A movement was detected by time-resolved Laue diffraction. Using monochromatic x-rays and repeating the freeze trapping procedure in the presence and absence of light irradiation also found no significant structural changes for the *R. sphaeroides* RC.

To examine the extent of photo activation in these optically dense crystals, we investigated the photochemistry of the bacterial RC of *B. viridis* by near-infrared spectroscopy. The overall extent of photo activation was 50%, which demonstrates that the time-resolved crystallographic method can be applied to optically dense crystals. The optical measurement of the charge–recombination rate suggests

the presence of a long-lived charged state within the crystal. This set of experiments on the bacterial photosynthetic RC protein will be discussed in terms of possible future time resolved experiments.

Early intermediates of the PYP photocycle probed by picosecond Laue x-ray crystallography

Hyotcherl Ihee, KAIST, Daejeon (South Korea).

Sub-nanosecond structural dynamics studies of photoactive yellow protein (PYP) in a single crystal using time-resolved Laue crystallography are presented. A comprehensive set of Laue data during the PYP photocycle was previously collected over forty-seven time points (1ns – 1 s) involving laser pulses with a duration of several nanoseconds at 485 nm used to initiate the photocycle. Analysis of this data allowed us to track the positions of all atoms in PYP during its photocycle and directly observe how p-coumaric acid chromophore absorption of a blue light photon triggers a reversible photocycle. A complex chemical mechanism is proposed from five distinct structural intermediates. Structural changes at the chromophore in the early, red-shifted intermediates are transduced to the exterior of the protein in the late, blue-shifted intermediates through an initial "volume-conserving" isomerization of the chromophore and the progressive disruption of hydrogen bonds between the chromophore and its binding pocket. Recently, we have achieved sub-nanosecond time resolution by using 100 ps long laser pulses at 418 nm for excitation. The femtosecond laser pulses were purposely stretched up to 100 ps because time-resolved optical studies of PYP in protein crystals suggested that a stretched optical pulse would increase the yield of the photogenerated PYP intermediates. The stretching was achieved by passing the femtosecond optical pulse through a pair of Brewster-cut fused silica rods, with additional stretching achieved by passing through multimode optical fiber. This is the first time sub-nanosecond structural changes of the chromophore have been clearly visualized and allows us to propose a novel isomerization mechanism connecting the ground pG structure to the earliest intermediate structure.

Allosteric action in real time: Time-resolved crystallographic studies of a cooperative dimeric hemoglobin

William E. Royer, and James E. Knapp, University of Massachusetts Medical School, Worcester (USA).

Vukica Srajer, and Reinhard Pahl, The University of Chicago, Chicago (USA).

The ability of allosteric protein molecules to alter their structures in response to ligand binding provides an important regulatory mechanism. Despite the availability of static structures of different states in a number of allosteric proteins, information about the kinetic pathway between such alternate states is limited. Time-resolved crystallographic analysis provides tools to obtain direct time-dependent structural information at high resolution on the entire protein molecule. We have carried out nanosecond time-resolved diffraction experiments on single crystals of *Scapharca* dimeric hemoglobin, a protein whose alternate states show strong functional differences, despite relatively localized transitions that are compatible with the crystal lattice. Our results provide an unprecedented view of global allosteric protein transitions as they proceed from 5ns to 80μs. Within 5ns of the photolytic release of ligands, an intermediate forms as R-state protein subunits respond to the presence of unliganded heme groups. Transition to this intermediate involves structural changes in the heme groups, neighboring residues and interface water molecules. The intermediate changes very little during the ns time-domain and lays a foundation for apparently concerted tertiary and quaternary structural changes that occur on a microsecond time scale and are associated with the transition to a low affinity T-state structure. Persistence of a T-state structure even after ligands rebind suggests a slow T to R transition that may result from the greater dimeric stability in the T-state.

A new photo switch – the structural basis of the E/Z isomerization in biliproteins

Marius Schmidt, Anamika Patel, and Yi Zhao, Technische Universität München, Garching (Germany).

Wolfgang Reuter, Ludwig-Maximilians Universität München (Germany).

Linear tetrapyrrols, so called bile chromophores, are found in phycobiliproteins and phytochromes. Phycobiliproteins are located in the phycobilisoms of photosynthetic algae and the phytochromes are photoreceptors in plant and other organisms. Phytochromes as well as one single phycobiliprotein, the phycoerythrocyanin (PEC), are found to be photoactive. They may isomerise from a stable *Z* to a stable *E* configuration. Using light of proper wavelength one can switch between the two configurations in a reversible fashion. However, a structural basis of this transition does not exist. We have successfully crystallized an isolated phycobiliprotein subunit, that of α_E-PEC of *M. laminosus*, with the phycoviolobilin chromophore in the *E*-configuration [1]. The structure of the α_E -PEC can be compared with the phycoviolobilin in its *Z*-configuration found in the functional, trimeric PEC [2]. With this the structural basis of the isomerization is revealed. When going from *Z* to *E*, ring D of the chromphore rotates around the C15=C16 double bond and orients approximately perpendicular to the plane formed by the other rings. The effective length of the conjugated system of π-electrons is reduced. This explains the large absorption shift associated with the *Z* to *E* transition. In the *E*-isomer the chromophore ring A interacts with the backbone of a flexible loop and ring D is bound to a tyrosin. This stabilizes the isomers. We anticipate that the results hold for all photo-active biliproteins including the phytochromes. Moreover, the *Z*/*E* isomerization in PEC may shine light on protein-chromophore interactions in general.

[1] Schmidt, M., Patel, A., Zhao, Y., Reuter, W. (2006) The structural basis of the photoactivity in biliproteins. *Z*/*E*-Isomerization of Phycoerythrocyanin. *In preparation*.
[2] Schmidt, M., Krasselt, A., Reuter, W. (2005) Local Flexibility of a Protein Substructure as a Prerequisite for Reversible Chromophore Isomerization of α-Phycoerythrocyanin. BBA 1764, 55-62.

Session 4

Protein channels – Ion conduction, selectivity, gating, inactivation, and blockage

Benoit Roux, The University of Chicago, Chicago (USA).

Recent progress in the determination of the structure of biological ion channels by x-ray crystallography gives a fresh impetus to efforts directed at understanding the fundamental principles governing ion permeation in molecular terms. Nonetheless, static structures alone may not be sufficient. The complexity of ion channels is such that information extracted from computational models can contribute to refine our understanding of ion channels. In recent years, molecular dynamics simulations based on atomic models have been increasingly used to investigate the function of ion channels. Such computer simulations represent one of the most powerful approaches to study ion permeation at the microscopic level. I will describe our recent results on the ion conduction, selectivity, channel inactivation, gating and the action of channel blockers.

Cluster analysis of time-dependent crystallographic data: Identification of structural intermediates

Konstantin Kostov, University of Illinois at Chicago, Chicago (USA).

Keith Moffat, The University of Chicago, Chicago (USA).

A time-resolved macromolecular crystallography experiment is a series of time-dependent difference electron density maps that display the time-dependent changes in underlying structure as a reaction progresses. The goal is to interpret such data in terms of a small number of crystallographic refinable, time-independent structures each associated with a reaction intermediate; to establish the pathways and

rate coefficients by which these intermediates interconvert; and thereby elucidate a chemical kinetic mechanism. One strategy towards this goal is to use cluster analysis, a statistical method that groups objects based on their similarity. If the difference electron density at a particular pixel in the time-dependent electron density (TDED) maps is sensitive to the presence of a particular intermediate, then the evolution of its difference electron density will parallel the time concentration profile of that intermediate with time. The rationale is to cluster pixels with respect to the shapes of their TDEDs, so that each group or cluster of pixels corresponds to one structural intermediate. Clusters of pixels whose TDEDs reflect the presence of two or more specific intermediates can also be identified. From such groupings one can then infer the number of intermediates. In this way the time-independent difference density characteristics of each intermediate can be obtained, and its structure refined. We review the principles of cluster analysis and clustering algorithms, and describe the application of the method to simulated and experimental data for the photocycle of photoactive yellow protein (PYP).

Gas migration inside proteins – methods and mechanisms

Jordi Cohen, University of Illinois at Urbana-Champaign, Urbana-Champaign (USA).

Many proteins need to interact with gas molecules, such as oxygen and carbon monoxide, to perform their function. Unlike for most other ligands, the reactive sites for gases are often buried deep inside the proteins with no obvious entry pathways. Describing the locations of the gas pathways and the mechanism of gas migration is an essential step in understanding how proteins such as globins, oxygenases, and many others work. This talk will focus on the gas migration pathways and processes inside hydrogenase, which has important engineering applications, and myoglobin, which is well-studied experimentally and theoretically. Various computational methodologies will be described to comprehensively find and describe the gas migration pathways inside a given protein, even in the absence of visible gas channels in their crystal structures.

Session 5

Time-resolved experiments in daily single bunch mode: Current status of beamline NW14A at the Photon Factory Advanced Ring

Shin-ichi Adachi, Shigeru Yamamoto, Kimichika Tsuchiya, Tasuro Shioya, Takeharu Mori, Hiroshi Sawa, Hiroshi Kawata, and Shin-ya Koshihara, Photon Factory, KEK, Tsukuba (Japan). Shunsuke Nozawa, Ryoko Tazaki, Jun-ichi Takahashi, Laurent Guérin,Jiro Itatani, and Masahiro Daimon, ERATO, Tsukuba (Japan). Tokushi Sato, Ayana Tomita, and Matthieu Chollet, Tokyo Institute of Technology, Tokyo (Japan).

Photon Factory Advanced Ring (PF-AR) is a 6.5-GeV storage ring operated in single-bunch mode for ~5000 hours/year. NW14A is a new insertion device beam line at PF-AR aiming for sub-nanosecond-resolved x-ray diffraction and XAFS experiments. NW14A has options for various time-resolved setups, and we will present some examples of time-resolved x-ray diffraction and XAFS experiments which are feasible at NW14A: 1. time-resolved monochromatic diffraction experiment with a CCD, an IP, or a fast scintillation counter (mounted on Huber), 2. time-resolved Laue diffraction experiment with a CCD or an IP, 3. time-resolved XAFS experiment with a fast scintillation counter and Boxcar/Gated Integrators.

Table 1. Parameters and instruments for time-resolved experiment at NW14A

X-ray repetition rate	794 kHz (single bunch) or 945 Hz (s.b. with X-ray pulse selector)
X-ray band width	monochromatic (Si (111), 5 – 25 keV) or pink (5 – 25 keV)
Mirrors	Bent-cylindrical mirror, Double-flat mirrors for high-energy cutoff
Detectors	marccd165 (mounted on mardtb, 2048x2048 pixels), RIGAKU Rapid (cylindrical IP), fast scintillation counter, avalanche photodiode, ionization chamber
Lasers	Spectra Physics Tunami + Spitfire (150 fs), OPA8000, Continuum PowerLite8000 Nd :YAG (3-5 ns)
Others	Huber 7-axis diffractometer, XAFS Table SRS Boxcar/Gated Integrator, DG535

Photoinduced 1D exciton-string preceding macroscopic switching evidenced by 100ps diffuse scattering

Eric Collet, Johann Hébert, Marylise Buron, and Hervé Cailleau, Universitee Rennes 1, Rennes (France).

Laurent Guérin, Ayana Tomita, Tokushi Sato, Ryoko Tazaki, Shunsuke Nozawa, and Shin-ichi Adachi,

High Energy Accelerator Research Organization (KEK), Tsukuba (Japan).

Within the emerging field of ultra-fast x-ray science and the development of next generation sources, time-resolved diffuse scattering opens new opportunities to watch the generation, correlation and propagation of photoinduced excitations between the constituent molecules of solids. The investigation of such precursor phenomena is illustrated here by the detection, by 100 picosecond x-ray diffuse scattering, of cooperative charge-transfer charge-transfer exciton-strings of one-dimensional nature, in the first step of the photoinduced transition in the molecular crystal TTF-CA.
The time-dependence of the intensity of Bragg reflections on the 100 fs, triggered by the generation of coherent atomic oscillations in a solid, was reported in different materials. X-ray diffuse scattering experiments on solids open the unique opportunity to get key information on local excitations and on the nature of the cooperative interactions between the constituent molecules of crystals. In that, they are very different from diffuse scattering from solution. We show here that such local photoinduced phenomena, which may be the precursor effects driving real photoinduced phase transitions, can be detected by this technique.

High resolution time-resolved diffraction

Philip Coppens, State University of New York at Buffalo, Buffalo (USA).

Knowledge of the geometry of molecular excited states at atomic resolution is crucial for a full understanding of photo-chemically induced processes. Time-resolved pump-probe X-ray diffraction methods are now capable of providing the geometry of excited states of molecular complexes in crystals [1], and can lead to a unique assignment of the states responsible for observed spectroscopic features.
We will present several results on metal-metal bond shortening on excitation to microsecond lifetime triplet states in organometalic solids. Advanced chemical theory provides a qualitative interpretation of the results, but often does not reproduce the quantitative aspects of the observed geometry. The energy difference between the experimental results and the predicted theoretical geometry may be small even when the two geometries differ significantly, indicating that matrix effects in the crystal may have a pronounced influence [2].
The monochromatic technique used in our experiments provides the necessary accuracy, but has disadvantages in terms of the length of the experiment and the accumulated laser-photon flux on the

sample. The application of Laue techniques in conjunction with the Precognition software [3] will be explored in upcoming experiments. A fast shutter and the possibility for single pulse selection in regular operating modes will be briefly discussed [4]. The ultimate goal is the monitoring of chemical reactions in solids at picosecond or shorter lifetimes. Supramolecular solids, in which reactive molecules are embedded in an inert host matrix are ideally suited for such studies. Examples will be presented.

[1] Coppens, P., Vorontsov, I.I., Graber, T., Gembicky, M., Kovalevsky, A.Yu. (2005) Acta Crystallogr. A 61, 162.
[2] Novozhilova, I., Coppens, P. (2006) To be published.
[3] Z. Ren, Renz Research, Inc., http://renzresearch.com.
[4] Gembicky, M., Oss, D., Fuchs, R., Coppens, P. (2005) J. Synch. Rad. 12, 665-669.

Taking molecular snapshots in photochemical reactions

Lin X. Chen, Chem. Div. Argonne National Laboratory, Argonne (USA).

Although kinetics, energetics and coherence of photoexcited molecules have been revealed by ultrafast laser spectroscopy, structures of excited states are virtually unexplored due to the lack of appropriate pulsed x-ray sources. The unprecedentedly high photon flux and brilliance of x-ray pulses from current synchrotron sources enable recent studies of photoexcited molecular structures in solution with a time resolution of 30-100 ps using the laser pulse pump, x-ray pulse probe approach. A number of photoexcited molecular structural studies will be presented, including excited state metalloporphyrins and their structural changes in photocatalytic reactions, metal-to-ligand-charge-transfer excitation of transition metal complexes, and triplet state formation of organometallic building blocks. These studies aim at capturing the transient molecular structures that are crucial to the outcome of the photochemical reactions, such as the correlation of reorganization energy of the excited states with their lifetimes in photoinduced charge separation. Combining results from excited state structural studies with those from ultrafast optical studies and DFT calculations gives new insight into the photochemical pathways and provides guidance in synthesizing molecules with targeted functions. Future prospective with much shorter x-ray pulses using XAFS and WAXS will be discussed.

Session 6

Molecular odysseys in mapping enzyme reaction coordinates by x-ray crystallography

Andrew Mesecar, University of Illinois at Chicago, Chicago (USA).

Our lab is currently working on trying to understand the relationships between protein dynamics and enzyme catalysis i.e. the role of conformational change in orienting substrates for catalysis. Our working hypothesis is that enzyme catalytic power does not stem from some single “enzymagical” property, but originates through the coordinated and integrated use of different and explicable physical-chemical, structural and dynamic properties. We have been using a combination of experimental approaches, including monochromatic and Laue x-ray crystallography coupled with different chemical and physical trapping methods, to experimentally address the contribution of orientation and dynamics to enzyme catalytic power. Although we are still working on developing methods to visualize the reaction intermediates in the isocitrate dehydrogenase reaction, which has been our model enzyme system for a number of years, we are attempting to develop new enzyme systems that we hope will be amenable to time-resolved crystallographic studies. These enzyme systems include: trihydroxytoluene dioxygenase (THTDO), an extradiol dioxygenase from Burkholderia sp; and organophosphorus hydrolase (OPH) from D. radiodurans, which is an enzyme which degrades chemical weapons. For THTDO, we have determined the monochromatic and Laue x-ray structures to high resolution, we have designed and synthesized a series of caged-catechol substrates with heavy-atom tracking substituents (HATS), and we have constructed and characterized mutant enzymes that are designed to bottleneck reaction intermediates. For

OPH, we have determined a series of monochromatic and Laue x-ray structures, and we have completed an initial time-resolved Laue experiment of the reaction. The time-resolved experiment was performed on a single OPH crystal that was mounted in a capillary flow-cell. The reaction was triggered by the addition of a very slow substrate that contained a heavy-atom tracker (HAT), and the Laue structures were determined at times of 0, 30 and 60 minutes. Our current progress on these three enzyme systems will be presented and discussed.

Through the Looking Glass: Examining enzyme mechanism and inhibition with time-resolved x-ray crystallography of HMG-CoA reductase

Cynthia V. Stauffacher, Chandra Duncan, Tim J. Schmidt, Calvin N. Steussy, and John W. Burgner, Purdue University, Lafayette (USA). Jeremy Weitgenant, Olaf Wiest, and Paul Helquist, University of Notre Dame, (USA).

Our laboratory is pursuing a multifaceted research project to understand the molecular mechanism of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the first committed step in the biosynthesis of isoprenoids, and so has been the target for the highly successful statin drugs used to control cholesterol. From the structures of the two type members of this enzyme family, the human (Class I) and Pseudomonas mevalonii (Class II) HMG-CoA reductases in binary and non-productive ternary complexes, a catalytic mechanism was proposed that can now be investigated in molecular detail. With the discovery of a slow substrate, we have been able to construct crystalline complexes that provide a series of snapshots of trapped intermediates along the reaction pathway. In these experiments, the Pseudomonas mevalonii enzyme has been shown to be fully active in the crystal, which opened the possibility of observing the structural changes during catalysis using time-resolved Laue diffraction. Changes observed in these complexes show a substantial substrate rearrangement, shifts in domain orientation of the enzyme and the closing of a fifty residue flap over the active site. Initial Laue data collected from the cubic I4132 native crystals indicated that we could collect sufficient data in each frame at a high enough resolution that a full time-resolved experiment was possible. Trial data collection using a caged NAD to run full and partial reactions has indicated that these crystals are amenable to the time-resolved Laue approach. Development of potential new caged NAD reagents and further refinement of the kinetics of the natural and slow substrate dithio-HMG-CoA are being done to address the particular challenges in the coordination of the unidirectional two-stage catalytic reaction in this enzyme for time-resolved crystallography.

Mechanism of product/substrate exchange in plant glycoside hydrolases: Conventional and Laue crystallographic approaches

Maria Hrmova, and Geoffrey Fincher, The University of Adelaide (Australia). Joseph Varghese, Victor Streltsov, and Alys Peisley, CSIRO-MHT, Melbourne (Australia). Hugues Driguez, CNRS-CERMAV, Grenoble (France). Brian Smith, WEHI, Melbourne (Australia).

A glucose molecule, which represents the final hydrolytic product of a plant β-d-glucan glucohydrolase, is bound in the active site until a new, incoming substrate molecules approaches [1, 2]. If we could synchronize dissociation of glucose from the active site throughout a crystal, time-resolved X-ray Laue crystallography could be used to monitor simultaneously the glucose product diffusing away and the new substrate entering the enzyme's active site. We have prepared a photo-caged, non-hydrolyzable substrate analogue, methyl-O-thio-gentiobioside (G6sG-OMe) for synchronizing the release of the bound glucose, following laser-mediated removal of a blocking caged group. Preliminary photolytic and Laue crystallographic studies indicated that with collimated light directed through a Xenon-lamp at 350 nm and delivered through a fibre during a 2 min illumination period, we could achieve photolytic cleavage of the

caged non-hydrolyzable substrate analogue G6sG-OMe. Further, with Laue polychromatic light (1.05-1.40 Å) we have collected several data sets with the native crystals of β-d-glucan glucohydrolase, in the presence and absence of the caged and free G6sG-OMe ligands. The experimental X-ray data resolved to 2.8 Å at 10 oC, through 90o, and with 1.5o oscillations per frame. The data are now ready for structural refinements, and during structure solution of populations of intermediates we will use information from several stable intermediates along the hydrolytic pathway of the β-d-glucan glucohydrolase [2-5].

[1] Varghese, J.N., Hrmova, M., Fincher, G.B. (1999) Structure 7, 179.
[2] Hrmova, M., Varghese, J.N., DeGori R, Smith, B.J., Driguez, H., Fincher, G.B. (2001) Structure 9, 1005.
[3] Hrmova, M., De Gori, R., Smith, B.J., Vasella, A., Varghese, J.N., Fincher, G.B. (2004) J Biol Chem 279, 4970.
[4] Hrmova, M., Streltsov, V., Smith, B.J., Vasella, A., Varghese, J.N., Fincher, GB (2005) Biochemistry 44, 16529.
[5] Hrmova, M., De Gori, R., Smith, B.J., Driguez, H., Varghese, J.N., Fincher, G.B. (2002) Plant Cell 14, 1033.

On your mark – Get set – Planning time-resolved studies of protocatechuate 3,4-dioxygenase

Douglas H. Ohlendorf, C. Kent Brown, R. Gosu, and Cathleen A. Earhart, University of Minnesota, Minneapolis (USA).

Protocatechuate 3,4-dioxygenase (3,4-PCD) uses a nonheme iron cofactor to catalyze the cleavage of protocatechuate (3,4-dihydroxybenzoate, PCA) between the vicinal catechols by O2. PCA is one of 3 key intermediates used by nature to degrade aromatic compounds. The binding of PCA has been visualized at 2.1 Å using anaerobic crystals of 3,4-PCD soaked in a range of inhibitors and substrate. These studies have produced a mechanistic hypothesis of iron center neutrality. This hypothesis will be tested through the use of neutron diffraction. To visualize O2 binding, O2 attack on substrate, and subsequent reaction steps we propose using x-ray Laue techniques. The feasibility of these experiments has been demonstrated by (1) collecting and analyzing a Laue data set for unbound 3,4-PCD, and (2) testing the ability of a laser pulse to releasing O2 from a caged O2 compound.

Efficient data collection for the structure determination of an icosahedral virus by Laue crystallography

Tianwei Lin, The Scripps Research Institute, La Jolla (USA).

Since the cryogenic methods are not always applicable to virus crystals, the data collection from these crystals is inefficient and labor intensive. We exploited the Laue crystallography of an icosahedral virus, Cowpea mosaic virus. The virus crystallizes in a cubic I23 space group with a=317Å. Despite the large unit cell dimensions, the data can be obtained efficiently by the Laue method and a single exposure was sufficient for the structure determination. An application of the Laue method is in the field of high-pressure crystallography, in which the diffraction from CPMV crystal was dramatically improved.

Session 7

Structural and spectroscopic studies of photo-induced conformational changes in heme proteins and GFP-like proteins

Gerd Ulrich Nienhaus, Department für Biophysik, Universität Ulm, Ulm (Germany) and Department of Physics, University of Illinois at Urbana-Champaign, Urbana (USA).

For many years, myoglobin has served as a model system for the study of structure-dynamics-function relationships in proteins. Its ligand binding reaction is a comparatively simple biological process; yet it is

exceedingly complicated when examined in detail. A multitude of kinetic features have been observed using time- and temperature-resolved optical and vibrational spectroscopy on carbonmonoxy myoglobin (MbCO). In recent years, cryocrystallography and time-resolved crystallography studies under photolyzing conditions have associated these features with different photoproduct structures that are characterized by CO ligands residing in different internal cavities of the protein. We have investigated the functional role of these cavities in the ligand binding reaction of wild-type MbCO and a large number of mutants by using infrared and visible spectroscopy. Each metastable location of CO within the protein is associated with its individual CO infrared spectrum, which enables us to observe ligand migration between cavities, ligand dynamics within a cavity, and conformational changes of the protein.
I will also present some of our recent investigations on GFP-like proteins, fluorescent marker proteins that are of enormous importance for live-cell imaging. Of particular interest are photoactivatable proteins, for example EosFP, a protein that switches its fluorescence emission from green to red upon irradiation with visible light.

Time-resolved studies of protein function: From infrared to x-rays

Philip Anfinrud, Laboratory of Chemical Physics, NIH/NIDDK, Bethesda (USA).

Understanding how a particular protein functions requires not only knowledge of its static structure, but also of its conformational evolution as it executes its function. Myoglobin (Mb), a ligand-binding heme protein found in our muscle tissue, has long served as a model system for investigating the relations between protein structure, dynamics, and function. Flash photolysis of the carbon monoxide adduct (MbCO) liberates CO from the heme binding site, promptly producing a well-defined reactive state. Femtosecond time-resolved optical studies of the heme absorption reveal spectroscopic changes that arise from electronic relaxation, heme cooling, protein conformational relaxation, and ligand rebinding. Femtosecond time-resolved polarized IR spectroscopy reveals spectroscopic changes of the CO vibrational stretch, which informs on the time-dependent orientation of CO, the presence of a docking site that mediates the transport of ligands to and from the active binding site, as well as the dynamics of ligand binding and escape. While spectroscopy taught us much about CO dynamics in Mb, much less was known about the structural evolution that accompanies CO transport. To probe the structural evolution, we have pursued picosecond time-resolved X-ray studies of MbCO on the ID09B beamline at the ESRF. Three-dimensional electron density maps, reconstructed from time-resolved X-ray diffraction "snapshots," have been stitched together into movies that unveil, with <2-Å spatial resolution and 150-ps time-resolution, the correlated protein motions that accompany and/or mediate ligand migration within the hydrophobic interior of the protein. Through these studies, we have mapped out majority pathways for ligand migration and escape, and have uncovered structural explanations for dynamical differences observed in various mutants of MbCO. The ability to witness correlated protein motion in real time with near-atomic resolution deepens significantly our understanding of the relationships between protein structure, dynamics, and function.

X-rays, Action, Camera! The heartache of redox enzyme photoreduction during catalytic movie production

Carrie M. Wilmot, University of Minnesota, Minneapolis (USA).

X-rays are oxidizing, leading to the production of photoelectrons from within the sample, primarily generated from solvent molecules. Despite the low temperatures (100K or below) now used routinely by macromolecular crystallographers, the photoelectrons often end up reducing chemical groups within the sample. Photodamage is prevalent at high intensity synchrotron sources, and can occur on a much faster timescale than the time needed to complete data collection from a single crystal.
Redox enzyme active sites, which are optimized to promote redox chemistry, are particularly prone to rapid photoreduction. Luckily many redox active centers in proteins have UV/visible spectral features that define their electronic state. These can be used to measure the rate of photoreduction within oxidized

redox centers during X-ray exposure. A practical strategy is presented in which multiple crystals are used to build up a true and complete image of an oxidized catalytic intermediate. The rates of photoreduction at multiple oxidized centers are measured and used to define a maximum length of time a crystal can be exposed to X-rays before significant photodamage occurs. UV/visible spectral data are collected during X-ray exposure by a microspectrophotometer, which in the example presented is mounted on the goniometer of BioCARS 14-BM-C at the Advanced Photon Source. Challenges associated with single crystal spectroscopy are also discussed, including anisotropy and prism effects.
Using this strategy, the resulting X-ray crystal structure "snapshots" of redox enzyme catalytic intermediates can be placed along the reaction co-ordinate to build up a "movie" of catalysis at the molecular level.

Session 8

Laue data collection and data processing – General issues

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Laue data collection is unique due to its motionless geometry. However, coverage of reciprocal space is a common concern of all diffraction data collection techniques, such as Laue, monochromatic oscillation, and Weissenberg, since data completeness and redundancy depend on it directly. Laue data collection strategy must also consider an uneven coverage of reciprocal space, and more rapid radiation damage in time. This spatial unevenness is further multiplied by the intensity unevenness of incident beam at all wavelengths. Data processing refers to a computational analysis of diffraction images that results in fully-reduced structure factor amplitudes. In general, data processing is like an indexing-integration-scaling dance. Different diffraction geometries have distinctive styles. A harmonic dance requires precision in each step and tolerance to errors from the previous steps. So does data processing. Step 1 must establish an accurate geometric model of each diffraction pattern in a dataset. This is achieved by auto-indexing, refinement of various parameters of crystal and experimental apparatus, and corrections for detector distortion. Step 2 integrates and isolates the intensity, *i.e.* diffraction power, of each predicted reflection, some easily observable by eye, some not, and some sharing the same detector pixels with each other, known as spatial overlap. Step 3 compares all integrated intensities together in order to reveal therefore to reduce systematic errors, and to identify therefore to reject random noise. The most significant systematic correction in Laue data processing is normalization of incident beam intensity at all wavelengths.

User-friendly Singular Value Decomposition for time-resolved crystallography

Marius Schmidt,and Yi Zhao, Technische Universität München, Garching (Germany).

Singular Value Decomposition (SVD) is a technique which has been established to work successfully with time-resolved crystallographic data [1-2]. SVD may act as a noise filter and determines the main-components found in the time-dependent difference maps of a time-resolved experiment. The biggest advantage, however, is the fact that the relaxation times of the kinetics are faithfully found in a global way in the right singular vectors. These relaxation times may be used to select and fit a chemical, kinetic mechanism to the data. With this, interpretable difference electron densities of the intermediates can be extracted and the structures of the intermediates can be modeled [3]. The existing program, "SVD4TX", which performs such a decomposition enabled the kinetic analysis of the photocycle of the photoactive yellow protein from nanoseconds to seconds [4-6]. The relaxation times as well as the structures of the intermediates were found. In addition, compatible chemical-kinetic mechanisms were determined by "posterior analysis" which exploits the absolute scale of the crystallographic data.
The existing version of "SVD4TX" was reprogrammed in C++. Three major parts were improved. (i) A convenient GUI for user-friendliness was implemented. (ii) To calculate the concentrations of the intermediates, a general approach to solve a series of coupled differential equations is now used. This approach diagonalizes the coefficient matrix which describes the chemical-kinetic mechanism. A fit to the

data which uses this approach is more stable and yields faithful results. (iii) “Posterior analysis” will be performed in a user friendly fashion within the same GUI. The object-oriented design of the programs allows for easy implementation of new chemical-kinetic mechanisms with an arbitrary large number of intermediates and new features in this program.

[1] Schmidt, M., Rajagopal, S., Ren., Z., Moffat, K. (2003) *Application of Singular Value Decomposition to the Analysis of Time-Resolved Macromolecular X-ray Data*. Biophys. J. 84, 2112.
[2] Rajagopal, S., Schmidt, M., Anderson, S., Ihee, H., Moffat, K. (2004) *Analysis of experimental time-resolved crystallographic data by singular value decomposition*. Acta Cryst. D60, 860.
[3] Schmidt, M., Pahl, R., Ihee, H., Šrajer, V. (2005) *Protein ligand interaction probed by time-resolved X-ray structure determination*. In: *Methods in Molecular Biology*, Vol. 305, Protein-Ligand Interactions: Methods and Applications (G.U. Nienhaus ed.). Humana Press, Totowa, NJ, 115.
[4] Schmidt, M., Pahl, R., Anderson, S., Srajer, V., Ren, Z., Moffat, K. (2004) *Protein Kinetics: Structures of Intermediates and Reaction Mechanism by Time-Resolved X-ray Crystallography*. PNAS 101, 4799.
[5] Rajagopal, S., Anderson, S., Srajer, V., Schmidt, M., Pahl, R., Moffat, K. (2005) *A structural pathway for signaling in the E46Q mutant of photoactive yellow protein*. Structure 13, 55.
[6] Ihee, H., Rajagopal, S., Srajer, V., Pahl, R., Schmidt, M., Schotte, F., Anfinrud, P.A., Wulff, M., Moffat, K. (2005) *Visualizing Chromophore Isomerization in Photoactive Yellow Protein from Nanoseconds to Seconds by Time-Resolved Crystallography*. PNAS 102, 7145.

Laue data analysis with TreX

Friedrich Schotte, Laboratory for Chemical Physics, NIH/NIDDK, Bethesda (USA).

Workshop on Time-resolved and Laue X-ray Crystallography

Abstracts for Poster Session

Saturday, 6-May-2006

TR1

Proteins in Action: Following the Dissociation of Carbon Monoxide from Wild Type and L29F Myoglobins in the Picosecond to Millisecond Time Range using Time-resolved Crystallography

Roman Aranda IV, Elena J. Levin, and George. N. Phillips Jr., University of Wisconsin, Madison (WI), USA. Friedrich Schotte and Philip A. Anfinrud, NIH-NIDDK, Bethesda (MD), USA
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Time-resolved crystallographic data of carbon monoxide-bound (CO) wild type (WT) and L29F myoglobin (Mb) crystals were obtained by breaking the CO-iron bond with a laser pulse. Laue diffraction data was collected from 100 ps to 31 ms after photolysis of the WT Mb at a resolution of 2.00 Å and from 100 ps to 10 ms after photolysis of the L29F Mb at a resolution of 2.05 Å. Using difference refinement, coordinate models were developed for each time point and quantitative data were extracted to create a more explicit representation of the photo-dissociation process. Both the WT and L29F proteins progress from the CO-bound to the deoxy state after photolysis and both return to the CO-bound state within the ms time range. Due to the introduction of phenylalanine in the heme pocket and the strained conformation it takes after photolysis, L29F Mb forces the photolyzed CO from the docking site faster than WT Mb. As both proteins reach the deoxy state, a water molecule occupies the area above the heme iron in the distal portion of the pocket and remains until it is replaced by the rebinding of CO and the return of the proteins to the CO-bound state. This work demonstrates the relationship between structure and function in Mb, explains the kinetic differences between the WT and L29F Mb, and follows CO as it traverses through the various binding sites in the proteins before rebinding.

TR2

Illuminating the mechanism of HMG-CoA reductase: a road paved by monochromatic and polychromatic crystallographic techniques

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HMG-CoA reductase (HMGR) is the first committed step in isoprenoid biosynthesis. It converts HMG-CoA to mevalonate with the oxidative power from two hydride transfers supplied by two molecules of the cofactor NADH. The use of standard monochromatic x-ray crystallographic techniques on dead-end complexes of HMGR (*P. mevalonii*) along the reaction pathway have resulted in static pictures of the different conformations the protein and ligand can adopt. However, these complexes have lead to questions regarding the timing of events that must occur in order to get from one step to another and what these intermediate structures look like. To answer these questions, time-resolved Laue crystallography has been employed and initial data has been collected, processed, and has subsequently been analyzed. Initial results indicate that HMG-CoA reductase is amenable to this technique and while the details of the active site are still under investigation, general movement of the domains within the protein have been observed.

TR3

Mechanism of product/substrate exchange in plant glycoside hydrolases: Conventional and Laue crystallographic approaches

Maria Hrmova, The University of Adelaide (Australia)
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A glucose molecule, which represents the final hydrolytic product of a plant β-D-glucan glucohydrolase, is bound in the active site until a new, incoming substrate molecules approaches [1, 2]. If we could synchronize dissociation of glucose from the active site throughout a crystal, time-resolved X-ray Laue crystallography could be used to monitor simultaneously the glucose product diffusing away and the new substrate entering the enzyme's active site. We have prepared a photo-caged, non-hydrolyzable substrate analogue, methyl-*O*-thio-gentiobioside (G6sG-OMe) for synchronizing the release of the bound glucose, following laser-mediated removal of a blocking caged group. Preliminary photolytic and Laue crystallographic studies indicated that with collimated light directed through a Xenon-lamp at 350 nm and delivered through a fibre during a 2 min illumination period, we could achieve photolytic cleavage of the caged non-hydrolyzable substrate analogue G6sG-OMe. Further, with Laue polychromatic light (1.05-1.40 Å) we have collected several data sets with the native crystals of β-D-glucan glucohydrolase, in the presence and absence of the caged and free G6sG-OMe ligands. The experimental X-ray data resolved to 2.8 Å at 10 ^{o}C, through 90^{o}, and with 1.5^{o} oscillations per frame. The data are now ready for structural refinements, and during structure solution of populations of intermediates we will use information from several stable intermediates along the hydrolytic pathway of the β-D-glucan glucohydrolase [2-5].

[1] Varghese, J.N., Hrmova, M., Fincher, G.B. (1999) *Structure* 7, 179.
[2] Hrmova, M., Varghese, J.N., DeGori, R., Smith, B.J., Driguez, H., Fincher, G.B. (2001) *Structure* 9, 1005.
[3] Hrmova, M., De Gori, R., Smith, B.J., Vasella, A., Varghese, J.N., Fincher, G.B. (2004) *J Biol Chem* 279, 4970.
[4] Hrmova, M., Streltsov, V., Smith, B.J., Vasella, A., Varghese, J.N., Fincher, GB. (2005) *Biochemistry* 44, 16529.
[5] Hrmova, M., De Gori, R., Smith, B.J., Driguez, H., Varghese, J.N., Fincher, G.B. (2002) *Plant Cell* 14, 1033.

TR4

Toward Time-Resolved Laue Diffraction of Mitochondrial Aldehyde Dehydrogenase

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Human mitochondrial aldehyde dehydrogenase (ALDH2) has two important cellular functions: detoxication of acetaldehyde derived from ethanol metabolism, and bioactivation of nitroglycerin administered to treat heart disease.
ALDH2 catalyzes the oxidation of aldehydes via covalent linkage to an active-site cysteine, hydride transfer to NAD^+, and subsequent deacylation to release the acid product. ALDH2 also possesses esterase activity, which does not require coenzyme. Both the dehydrogenase and esterase reactions are activated by divalent metal ions. The mechanism of nitroglycerin bioactivation by ALDH2 is not clear, although it likely shares features of the esterase reaction.
Previously, we solved x-ray structures of ALDH2 in complex with NAD^+ and NADH. These structures show that the oxidized and reduced cofactor occupy two conformations when bound to ALDH2. The dominant conformation observed for NAD^+ is ideal for hydride transfer but would impair deacylation, whereas the only conformation observed for NADH is ideal for deacylation. Importantly, we see no conformational change of the protein backbone when comparing the apo, NAD+ and NADH structures. Additionally, structures obtained in the absence of magnesium ions show that Mg^{2+} binding to the pyrophosphate group is important for stabilizing the two cofactor positions. These structures support the hypothesis that isomerization of cofactor occurs after hydride transfer, and suggest that ALDH2 activation by divalent metal ions could be due to acceleration of cofactor isomerization.

To further test this hypothesis, it would be invaluable to observe the cofactor during catalysis using time-resolved Laue diffraction. We report that initial analysis of static Laue data on apo ALDH2 crystals suggests that time-resolved studies with this enzyme are feasible. In parallel, we are using enzyme kinetics to investigate the effect of magnesium ions on a partial reversal of the dehydrogenase reaction, with potential application for time-resolved studies.

TR5

The allosteric pathway of HbI explored by atomic resolution and time-resolved crystallography

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The dimeric hemoglobin (HbI) from the clam *Scapharca inaequivalvis* is an exceptional system to study allostery because its cooperative ligand-binding mechanism is mediated by mainly tertiary and not quaternary structural changes, permitting this reaction to be observed within a crystalline lattice. The high ligand affinity of the R-state in part occurs because of a hydrogen bond formed between His 69 and the ligand. This hydrogen bond is stronger in HbI than in sperm whale myoglobin (Mb) based on the comparison on the comparison of the CO stretching frequency observed by infrared (IR) spectroscopy of equivalent mutations made at positions 37 (B10) and 69 (E7). This is consistent with 0.82 Å data in which the epsilon nitrogen and not the delta nitrogen is protonated. The low oxygen affinity T-state form of HbI occurs because the distal His sterically hinders oxygen binding, and by the reduced reactivity of the heme iron due to the presence of Phe97 within the proximal pocket. Time-resolved crystallographic analysis shows that ligand release from the R-state form of HbI results in the formation of a transient intermediate by 5ns. This unliganded R-state intermediate persists for about 1μs before it undergoes an apparent concerted transition to form a tertiary T-state structure. Mutagenesis results show that the stabilization of the unliganded R-state structure will lower the oxygen affinity. The destabilization of this intermediate has the opposite effect.

TR6

Cluster analysis of time-dependent crystallographic data: Identification of structural intermediates.

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A time-resolved macromolecular crystallography experiment is a series of time-dependent difference electron density maps that display the time-dependent changes in underlying structure as a reaction progresses. The goal is to interpret such data in terms of a small number of crystallographically refinable, time-independent structures each associated with a reaction intermediate; to establish the pathways and rate coefficients by which these intermediates interconvert; and thereby elucidate a chemical kinetic mechanism. One strategy towards this goal is to use cluster analysis, a statistical method that groups objects based on their similarity. If the difference electron density at a particular pixel in the time-dependent electron density (TDED) maps is sensitive to the presence of a particular intermediate, then the evolution of its difference electron density will parallel the time concentration profile of that intermediate with time. The rationale is to cluster pixels with respect to the shapes of their TDEDs, so that each group or cluster of pixels corresponds to one structural intermediate. Clusters of pixels whose TDEDs reflect the presence of two or more specific intermediates can also be identified. From such groupings one can then infer the number of intermediates. In this way the time-independent difference density characteristics of each intermediate can be obtained, and its structure refined. We review the principles of cluster analysis and clustering algorithms, and describe the application of the method to simulated and experimental data for the photocycle of photoactive yellow protein (PYP).

TR7

Towards time-resolved X-ray crystallography of a high-fidelity DNA polymerase

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DNA polymerases replicate DNA by successively adding nucleotides to a growing primer strand in a template-directed fashion. This complex reaction requires binding of a nucleotide and divalent cations and coordinated movement of template and primer oligonucleotides. The large fragment of DNA polymerase I from a strain of Bacillus stearothermophilus is capable of catalyzing this reaction processively inside a crystal [1]. We have recently observed the incorporation and translocation of up to 12 nucleotides inside the crystal. The polymerase retains its ability to discriminate between correctly and incorrectly paired nucleotides in the crystal. In addition, we can capture structures of reaction intermediates by chemical trapping [2]. These structures show that the polymerase undergoes large-scale conformational changes during catalysis. We are now attempting to capture additional reaction intermediates by time-resolved X-ray crystallography. Caged deoxyribonucleotide analogs have been synthesized in an attempt to synchronize the reaction in the crystal. The ability to observe processive, high-fidelity replication directly in a crystal establishes the Bacillus polymerase as a powerful model system for time-resolved structural studies of DNA synthesis.

[1] Kiefer et al. (1998) *Nature* 391, 304-307
[2] Johnson et al. (2003) *Proc. Natl. Acad. Sci. USA* 100, 3895-900

TR8

Structural Studies of a Novel Phosphotriesterase capable of degrading soman using Polychromatic and monochromatic X-rays

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Phosphotriesterase (OPH) is an enzyme that is capable of hydrolyzing organophosphorus neurotoxins such as those commonly found in a variety of insecticides and chemical warfare agents. This enzyme is naturally found in a variety of organisms including bacteria, squid, insects and humans. We have cloned, expressed, purified and determined the x-ray structure of an OPH enzyme isolated from an extremophile that has increase thermostability and solubility compared to the most commonly studied enzyme from *Pseudomonas diminuta*. More over, our enzyme has increased activity toward soman gas. Unfortunately, none of the enzymes studied to date have activity toward the most lethal and abundant chemical warfare agent on earth, Russian VX-gas. For this reason, we are looking to re-engineer our enzyme to broaden its substrate specificity range by means of site-directed and saturation mutagenesis, as well as other directed evolution approaches.
In order to be successful, we are using x-ray crystallography to map the reaction coordinate of the enzyme and to identify residues that play important roles in catalysis. We have determined the high resolution structure of OPH in complex with an intermediate analog by using monochromatic x-rays. We have also used polychromatic x-ray methods to determine the structures of 3 separate time points (T = 0, 30, 60 minutes) on a single crystal that was subjected to a slow-reacting substrate in a flow cell. All data sets were taken at BioCARS at the Advanced Photon Source (Argonne National Laboratory). The final structures and progress in analysis of the data will be presented.
This research is supported by Office of Naval Research award N000140210956.

TR9

Studies of heme proteins by time-resolved crystallography: allosteric action and structural relaxation

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Marius Schmidt and Fritz Parak, Technische Universität München, Garching, Germany
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Time-resolved macromolecular crystallography is reaching a mature phase with demonstrated ability to detect small structural changes on ns and sub-ns time scale [1-6] and with important advances in the analysis of time-resolved crystallographic data, such as the use of Singular Value Decomposition (SVD) method to determine the structures of intermediates and elucidate the reaction mechanism [3-4]. We present results of ns time-resolved crystallographic studies of heme proteins: allosteric action in real time in cooperative dimeric hemoglobin and structural relaxation processes in myoglobin [1]. These pump-probe experiments were conducted at the BioCARS beamline 14-ID at the Advanced Photon Source (USA).

[1] Schmidt et al. (2005) *PNAS* 102 (33) 11704-11709.
[2] Ihee et al. (2005) *PNAS* 102 7145-7150.
[3] Rajagopal et al. (2005) *Structure* 13, 55-63.
[4] Schmidt et al. (2004) *PNAS*, 101, 4799-4804.
[5] Schotte et al. (2003) *Science* 300, 1944.
[6] Šrajer et al. (2001) *Biochemistry* 40, 13802.

TR10

New Biology and New Software for Time-Resolved Crystallography

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Wolfgang Reuter. Max Planck Institut für Biochemie, Martinsried, Germany
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Biology: The L29W mutant of myoglobin is one of the slowest CO rebinder known. After photolysis of the CO a large time-window is available to investigate the initial relaxations of the protein without interference from rebinding. Time-resolved crystallographic experiments on this mutant show that the initial relaxation phase is non-exponential [1]. This interesting behaviour will initiate new experiments with the fastest time-resolution possible. These experiments will be described by "physical kinetics" which includes diffusion processes. Investigations at longer times on this mutant could be compared with similar experiments on the wild-type myoglobin. The results lead to the determination of the migration pathway of CO in the wild-type myoglobin.
α-Phycoerythrocyanin is a photoactive protein which contains a linear tetrapyrrol. Two stable isomeric states with configurations *Z* and *E* are generated by shining visible light of wavelength 500 nm or 580 nm, respectively, onto the protein. The absorption spectra of these two states differ largely and can be reversibly switched into each other. This new optical switch has been investigated by conventional crystallography in both isomeric states. In the *Z*-isomer the chromophore is in its extended form. In the *E*-form ring D of the tetrapyrrol has isomerized about the C15=C16 double bond and has been rotated 90 degrees out of the plane of the B/C rings. The large absorption change can be explained by the effective length of the conjugated π-system of double bonds. The effective length of many bilin chromophors scales linearly with their absorption maximum. In both configurations α-PEC fits perfectly onto this line.

Using microspectrophotometry we demonstrate that the spectra in solution and in the crystal are identical and the isomerization takes place also in the crystal.
Software: The existing software package "SVD4TX" [2] which performs a singular value decomposition on time-resolved X-ray data has been reprogrammed in C++. All features which characterize "SVD4TX" are kept and a single GUI for easy user guidance through the entire SVD-process was developed. In addition, a new routine diagonalizing the coefficient matrix which describes putative chemical kinetic mechanisms was implemented. With this more stable fits are possible. "SVD-flattening" and "Posterior analysis" will also be available from the same GUI for easy usage.
For more localized features the software "ProMsk" was developed which uses a mask to integrate the time-dependent difference electron density of distinct protein moieties. This program was used to obtain the results for the L29W myoglobin [1].

[1] Schmidt, M., Nienhaus, K., Pahl, R., Krasselt, A., Nienhaus, U., Parak, F., Šrajer, V. (2005) *Kinetic Analysis of Protein Structural Relaxations -A Time-Resolved Crystallographic Study*. PNAS USA 13 11704-11709.
[2] Schmidt, M., Rajagopal, S., Ren, Z., Moffat, K. (2003) *Application of Singular Value Decomposition to the Analysis of Time-Resolved Macromolecular X-ray Data*. Biophys. J. 84 2112-2129

TR11

Studies of ultrafast fs-laser generated strain fields with coherent X-rays

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In its new 324 bunch mode of operation, the APS has opened new avenues of fs-laser science and techniques to be developed. In this new mode, if one uses the tightly focused low pulse energy (nJ), high repetition rate fs-laser Ti:Sapphire oscillator (88 MHz) in 7-ID-D, every laser pulse and x-ray bunches can be overlapped and delayed with respect to each other, resulting in high repetition rate pump-probe experiment which uses all the APS x-rays! This poster describes an example of how coherent x-ray experiments may be used to study laser generated strain fields in semiconductors. With an oscillator beam focused to 7 micron onto GaAs, we have observed the coherent x-ray diffraction pattern with a high resolution CCD camera. We have developed two techniques to observe the strain field, a topographic technique, and a coherent diffraction technique. The topographic technique is quite useful to spatially overlap the laser and x-ray beams. The coherent diffraction technique is far more spatially sensitive and easier to interpret. This experiment may help to develop techniques that will be used at the future Free Electron Laser sources.

TR12

Time-resolved crystallography and optical studies of single crystals – Present capabilities and future directions at BioCARS

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Time-resolved crystallography is a unique technique for determining the structures of intermediates and excited states in biomolecular and chemical reactions. Using the Laue x-ray diffraction technique at the high-brilliance third-generation x-ray sources (ESRF, APS, SPring-8, etc.) snapshots are taken of molecules in action with a time resolution of about 100ps, the typical duration of a single x-ray pulse at these synchrotron sources [1]. We present the status of the user facility for time-resolved studies at BioCARS, an NIH/NCRR funded Synchrotron Structural Biology Resource at the Advanced Photon Source [2]. During the past years a continuously growing time-resolved user community has developed;

projects under investigation include light and chemically triggered reaction mechanism. An overview will also be given on the current efforts in enhancing the technical capabilities for time-resolved diffraction experiments and spectrophotometry at BioCARS. The technical upgrades will improve resources for complementary optical monitoring of reactions in crystals, update the laser systems, and most importantly improve the x-ray optics to enable experiments utilizing a single x-ray pulse.
BioCARS, Sector 14, is supported by the National Institutes of Health, National Center for Research Resources, under grant number RR07707. Use of the Advanced Photon Source is supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.

[1] Schmidt, M., Pahl, R., Ihee, H., and Šrajer, V. (2005) Protein ligand interaction probed by time-resolved x-ray structure determination. In: Methods in Molecular Biology, Vol. 305, Protein-Ligand Interactions: Methods and Applications (G.U. Nienhaus ed.). Humana Press, Totowa, NJ, pp. 115-154.
[2] http://cars9.uchicago.edu/biocars/index.html

TR13

The Frontier of X-Ray Pulse Selection

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High speed X-ray pulse selectors are used in combination with a probing laser for time resolved experiments at ESRF, APS, SPring-8 and PF-AR. A fast rotating triangular shaped disk with a beam channel grooved along one of the disk sides serves as the shutter element. Open times of the x-ray beam channel in the range of 500 ns and +/-2ns phase stability with respect to the bunch clock are realized with the present systems. The maximum open sequence of about 1000 Hz is limited by the tolerable rotation speed of the shutter rotor which is made from high-strength titanium alloy. The design criteria of a new disk, which was proposed by Michael Wulff, Philip Anfinrud, Friedrich Schotte et al. will be presented. This advanced disk will have tilted entrance and exit portions of the beam channel so as to compensate for the disk distortion under the centrifugal stress at the 1 kHz operation speed. This makes a minimum aperture of the trapezoidal beam tunnel possible. Optimized open times below 300 ns are expected, which are necessary to isolate single bunches from the 24-bunch mode at the APS or the 16-bunch mode at the ESRF. For some experiments faster sequences of the x-ray pulses are desirable in order to cope with the improved speeds of the recent laser generation. This feature will be offered by tunnel-less chopping with the beam passing at close distance along all three sides of the highly precise machined disk. Pulse frequencies of up to 3 kHz are feasible in this way. For pink beam chopping a step profile is provided as beam bypass along one of the disk sides. The different options for x-ray pulse selection can be adjusted by shifting the device. The modified disks are scheduled to replace the present shutters of the x-ray pulse selectors at the ESRF beamline ID9 and BioCARS beamline 14-ID at the APS in the coming months.

Workshop on Time-resolved and Laue X-ray Crystallography

Organizer: BioCARS, The University of Chicago

May 6 – 8, 2006
Argonne, Illinois USA

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