MS02.02.05 INTERMEDIATES IN THE REACTION PATHWAY OF CYTOCHROME P450cam. Ilme Schlichting*, Joel Berendzen**, Kelvin Chu**, Ann M. Stock*f*, Matthew Davies♦, Ernest J. Mueller♦, Steven Sligar♦, Robert M. Sweet♣, Dagmar Ringe⊗ & Gregory A. Petsko⊗. *Max Planck Institut für molekulare Physiologie, Abteilung Physikalische Biochemie, Rheinlanddamm 201, 44139 Dortmund and Abteilung Biophysik, Max Planck Institut für medizinische Forschung, Jahnstraße 29, 69120 Heidelberg , Germany; **Los Alamos National Laboratory, Biophysics Group, MS M715, Los Alamos, New Mexico 87545, USA. *f*Center for Advanced Biotechnology and Medicine, Piscataway, NJ 008854-5638, USA; ♣Biology Department, Brookhaven National Laboratory, Upton, New York 11973, USA; ♦Department of Biochemistry and Beckman Institute for Advanced Science and Technology, University of Illinois, Urbana, Illinois 61801, USA; ⊗Rosenstiel Center, Brandeis University, Waltham, Massachussetts 02254, USA

Cytochrome p450 enzymes form a family of ubiquitous heme proteins named after an absorption band at 450nm when complexed to carbon monoxide. P450 enzymes are mixed-function mono-oxygenases. They play a critical role in the synthesis and degradation of many physiologically important compounds and xenobiotics. This makes cytochrome p450s an attractive target for pharmaceutical or environmental industries. The biochemical relevance of the p450 mono-oxygenases in general is their unique ability to catalyze the hydroxylation of non-activated aliphatic or aromatic carbons. The biochemically and structurally best characterized p450 is $p450_{cam}$ from *Pseudomonas putida*, which catalyzes the stereospecific 5-exo-hydroxylation of camphor. Although the structures of the $p450_{cam}$ apoprotein and of complexes of p450 with camphor, various inhibitors and CO have been determined, the structure of the biochemically important p450.camphor.O_2 complex has not been solved yet, as it is unstable due to autooxidation which transforms the enzyme from the ferrous to the ferric form with a rate constant of 10^{-3} s^{-1} at 4 °C in solution. This requires to collect the diffraction data of the relatively short-lived complex either fast or to prolong its life time. Thus, we used Laue and cryocrystallography for the crystal structure determination of intermediates occuring along the reaction coordinate of p450. The methods used, and the structures obtained will be described.

MS02.02.06 TIME-RESOLVED LAUE CRYSTALLOGRAPHY: APPLICATION TO THE PHOTOCYCLE OF PHOTOACTIVE YELLOW PROTEIN. Zhong Ren¶, Kingman Ng¶, Ulrich K. Genick†, Gloria E. O. Borgstahl†, Duncan E. McRee†, Elizabeth D. Getzoff†, Claude Pradervand¶, Wilfried Schildkamp¶ and Keith Moffat¶, ¶Department of Biochemistry and Molecular Biology, University of Chicago, 920 East 58th Street, Chicago, IL 60637, USA and †Department of Molecular Biology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA

Millisecond time-resolved Laue diffraction images obtained during the relaxation of photoactive yellow protein from its photostationary state have been analyzed. Photoactive yellow protein (PYP), a simple, water-soluble, light-sensing, bacterial photoreceptor, undergoes a reversible photocycle: blue light excites the yellow state to produce a red-shifted intermediate that relaxes to form a bleached intermediate which returns to the dark state at a rate of 2-3 s-1. Laue diffraction and simultaneous optical spectroscopy of PYP crystals during their relaxation from a photostationary state reveal the first structure of an intermediate in the photocycle of a biological macromolecule at atomic resolution. In the dark-state structure, the 4-hydroxycinnamyl chromophore (Baca et al., 1994) is buried from solvent exposure by an arginine side chain, which has been proposed to be the gateway for the phototactic signal following light excitation and the proposed trans-to-cis isomerization of the chromophore (Borgstahl et al., 1995). The time-resolved Laue diffraction patterns were analyzed by recently developed data processing algorithms, which incorporate the new concept of resolution-dependent bandpass.

Baca, M., Borgstahl, G.E.O., Boissinot, M., Burke, P.M., Williams, D.R., Slater, K.A. & Getzoff, E.D., (1994). *Biochemistry*, 33, 14369.Borgstahl, G.E.O., Williams, D.R. & Getzoff, E.D., (1995). *Biochemistry*, 34, 6278.

MS02.02.07 STRUCTURE ANALYSIS BY MEANS OF TEMPERATURE JUMP COUPLED WITH THE LAUE METHOD. Hideaki Moriyama, Noriyuki Igarashi, Akira Ikezaki, Nobuo Tanaka. Department of Life Science, Faculty of Biotechnology and Bioscience, Tokyo Institute of Technology, 4259 Nagatsuta, MIDORI-ku, Yokohama, 227 Japan

Temperature jump coupled with the Laue diffraction method has been postulated to analyze crystal structures at a high temperature in a short time period. This method may permit an opportunity of collection of diffraction data prior to destruction of crystal lattice. Rapid increase of crystal temperature was achieved by a laser impact with an infrared-ray irradiation.

The first applications of temperature jump experiments were applied on the structure analysis of 3-isopropylmalate dehydrogenase that isolated from a thermophile (1). The temperature jump device has been developed by a cooperation between Rigaku Co. Ltd. and us. The diffraction experiments in a mode of temperature jump coupled with the Laue method were performed at Beam Line 18B of Photon Factory at Institute of High Energy Physics in Tsukuba, Japan. A crystal was mounted as a usual manner then laser was input for a period of time and white ray was incident by an automated and/or manually linked temperature jump controller. The exposure time was 10 mS. The collected diffraction images on large imagine plates, 400 mm x 800 mm, were processed by the in PF-in-house programs including index, intlaue, lpcor, and lauenorm. The process had been done up to 2.25 Å resolution over the wavelength range between 0.85 and 2.35 Å. The processed data gave R factor, Imean from all measurements for the reflection, at 0.094 in the both data set.

The structure of 3-isopropylmalate dehydrogenase was directly refined using the intact and t-jump Laue data, those refinements gave crystallographic R factor of 0.18 and 0.19, respectively. The temperature factors for the intact and t-jump structures were 22 Å square. The structural r.m.s.d was 0.72 Å and the major differences were found in surface-hydrophilic residues.

1. M. Sakurai, K. Onodera, H. Moriyama, T. Oshima & N. Tanaka, *Protein Engineering*, 8, 763-767 (1995).

MS02.02.08 TIME-RESOLVED LAUE STUDIES FROM DIENELACTONE HYDROLASE AND PORPHOBILINOGEN DEAMINASE. Carr P.D.[1], Robinson A.[1], Ollis D.L.[1], Hädener A.[2], Niemann A.C.[2], Helliwell J.R.[3], Habash J.[3], Cassetta A.[3], Ursby T.[4], Bourgois D.[4], Schotte F[4] and Wulff M. [4]. [1]Res.Sch Chem., Australian National University, Canberra, Australia; [2]Inst. Organic Chem., University of Basel, Switzerland; [3]Dept. Struct. Chem., University of Manchester, UK; [4]ESRF, Grenoble France

Beamline BL3 at the ESRF has been used to collect Laue diffraction patterns from crystals of the enzymes dienelactone hydrolase (DLH) and porphobilinogen deaminase (PD).

Flow cell experiments were performed on both crystal systems using substrates of varying efficacy. Time-dependent intensity changes were observed during the PD experiments that are in broad agreement with solution kinetic measurements (Neimann *et al.*, 1994). Mutant C123S crystals of DLH were used for the flow cell experiments because previous work (Pathak & Ollis, 1990) has shown that wild type crystals are susceptible to deactivation due to oxidation of the active site residue Cys123. Disordering/reordering phenomena were observed when the substrates dienelactone and methyl dienelactone were flowed over these crystals.

In addition to the flow cell experiments data were collected on

wild type DLH crystals to study the time-dependent nature of the oxidation of the active site Cys123 residue. Data were collected both on the in house CCD detector system and image plates. Initial results will be presented from these two studies.

References:
Neimann A, Matzinger P & Hädener A (1994) *Helvetica Chimca Acta* ,77 1791 - 1809
Pathak D & Ollis D.L. (1990) *J. Mol. Biol.*, **214**, 497 - 525

MS02.02.09 LAUE STUDIES ON ACETYLCHOLINESTERASE. Mia L. Raves, (Weizmann Institute of Science), Raimond B.G. Ravelli, Jan Kroon, (Utrecht University), Michel Roth, Dominique Bourgeois, (IBS, Grenoble), Ling Peng, Maurice Goeldner, (Université Louis Pasteur, Strasbourg), Israel Silman and Joel L. Sussman, (Weizmann Institute)

In the last ten years it has become feasible to study dynamic processes in macromolecules at the atomic level. Most of these studies have used the Laue diffraction technique that permits data collection on a second to pico-second time scale by virtue of the polychromatic synchrotron radiation.

It is our aim to do time-resolved experiments on the enzyme acetyl-cholinesterase (AChE). AChE cleaves the neurotransmitter acetylcholine (ACh) in the synapse at a very high turnover rate. The enzyme has a strong dipole moment that draws the positively charged substrate towards the catalytic site, situated at the bottom of a narrow gorge lined with aromatic residues. One of the reaction products, choline, bears the same charge as the substrate, ACh, which raises the problem of its exit route from the active site. In order to attempt to observe the reaction process, photolabile precursors of choline and of carbamylcholine have been synthesized and characterized. These two probes generate choline in different ways, either by direct photocleavage (choline precursor) or by enzymatic hydrolysis of a substrate generated by photocleavage (These tools for the time-resolved crystallographic studies will hopefully permit monitoring of the exit of choline from the active site.

To test the feasibility of the use of the Laue method, trigonal crystals of Torpedo californica AChE soaked with the inhibitor edrophonium were used to collect data at the ESRF in Grenoble, beam line ID9 (BL3) on a CCD detector with an exposure time of 1 msec. The frames were indexed using a new program called Lauecell that is able to determine the relative cell parameters from the Laue pattern semi-automatically. Further processing with the CCP4 Laue software package yielded a data set to 3.1 Å resolution of 80% completeness with 15,000 unique reflections. Constrained refinement of the protein coordinates with X-PLOR gave a difference map in which the density for the inhibitor can be clearly seen.

1. Peng, L. et al. submitted to Biochemistry (1996).
2. Ravelli, R.B.G.et al. accepted in J. Appl. Cryst (199

PS02.02.10 THE DARESBURY LABORATORY LAUE SOFTWARE SUITE. S. Arzt, J.W. Campbell, Q. Hao, D. Nguti, CCLRC Daresbury Laboratory, Daresbury, Washington, WA4 4AD, Cheshire, U.K.; M.M. Harding, University of Liverpool, U.K.; J.R. Helliwell, G. Bradbrook, J. Habash, Y.P. Nieh & E.H. Snell, University of Manchester, U.K.

High quality Laue intensity data can be obtained using synchrotron radiation. The Daresbury Laboratory Laue Software Suite has been developed and 'calibrated' with a series of studies (pea lectin, carbonic anhydrase, concanavalin A, lysozyme, as well as smaller inorganic and organic structure). It is available for distribution from Daresbury (contact Dr. J.W. Campbell) and has been successfully worldwide (e.g. p21 catalysis, Heidelberg; trypsin, NSLS; isocitrate dehydrogenase, Seattle; ESRF Grenoble etc.; see also poster abstract of Carr et al.). The package consists of a series of programs written primarily in FORTRAN, but also using libraries written in 'C' and runs on UNIX and VMS based computer systems. The LAUEGEN program uses an Xwindows based toolkit (XDL_VIEW) also developed at the Daresbury Laboratory. The aim is to exploit the interactive and display facilities available using X-windows but at the same time to provide more automatic procedures for data processing where these are appropriate. A Laue Data Module defines a set of standard parameters describing the crystal, the X-ray detector and the scanned images. It also provides a set of program independent functions for handling these parameters. Other recent developments allow the automatic estimation of sizes and the soft limits λ_{min} and d_{min}. A particular recent emphasis has been to provide new algorithms for deconvolution of the small fraction of multiples; these are important to give fuller coverage at low resolution. A method based on the λ-curve has been implemented and tested with lysozyme (see Campbell et al. (1994), Bull. Mater.Sci. 17, 1-18) and explored in relation to angular sampling collection strategies (see Bradbrook et al. (1995) SPIE 2521, 160-177 for the Röntgen Centennial Celebration).

PS02.02.11 BAYESIAN APPROACH TO THE ANALYSIS OF TIME-RESOLVED PROTEIN LAUE DIFFRACTION DATA. G.P. Bourenkov, A.N. Popov and H.D. Bartunik, Max-Planck Research Unit for Structural Molecular Biology, Protein Dynamics Group, MPG-ASMB c/o DESY, Notkestraße 85, 22603 Hamburg, Germany

A new method of deconvoluting overlapping reflections in protein Laue diffraction patterns solves the problem of the "low resolution hole" without the need for redundancy. It is therefore of particular interest for single-shot time resolved studies. For the first time, non-cyclic reactions may be investigated by Laue diffraction on short time scales. In test application to orthorhombic bovine trypsin, the new method improved the resolution from 1.7 Å to 1.4 Å as compared to standard processing methods. It provided high completeness over the whole resolution range < 7Å. The contrast in electron density maps calculated with the Laue structure factors improved dramatically; the Laue maps are of similar quality as maps that are calculated with monochromatic high-resolution data. The method follows a Bayesian statistical approach. A-priori given information about structure factor amplitudes obeying Wilson's distributions is employed. The (single or redundant) measurement of the intensity of an energetic or spatial overlap yields the normal multivariate probability density function (PDF) of the intensities of the components. This information is associated with the prior PDF through the Bayes theorem. The moments of the resulting posterior PDF give expected values for the component intensities, the structure factor amplitudes and their uncertainties. These moments are always finite and positive, even if the initial normal matrix is degenerate. Due to the nature of the wavelength normalisation curve and the dependence of the scattering power on resolution, accurate estimates are obtained for the structure factors of the components, even in the case of a single observation of an energetic overlap. Furthermore, all data can be processed to the physically relevant wavelength-dependent diffraction limit. No "soft parameters" are involved. The power of the method may be further enhanced, if a (roughly) approximate structural model is available, e.g., of an initial state of a reaction. Then, conditional prior probability density functions may be employed.