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