PS04.02.37 CRYSTALLIZATION OF NITRIC OXIDE REDUCTASE CYTOCHROME P450NOR FROM *FUSARIUM OXYSPORUM*. Park S.-Y., Shimizu H, Adachi S, Udai P. S., Iizuka T., Shiro Y., *Shoun H., #Nakagawa A., #Tanaka I., The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351 - 01, Japan, *Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan, #Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060, Japan

Cytochrome P450 nitric oxide reductase purified from *Fusarium oxysporum* (P450nor, *Mr* = 46 kDa) is an unique heme enzyme that catalyzes the reduction of nitric oxide with electrons directly transfer from NADH. The cytochrome P450nor was crystallized with the vapour diffusion method using the sitting drop technique. The crystals were grown in 100 mM MES buffer at pH 5.5 using PEG 4000 as precipitant. The crystal belongs to the space group $P2_12_12_1$ in an orthorhombic system. The cell dimensions are determined a = 55.02 Å, b = 82.42 Å and c = 87.06 Å The asymmetric unit contains one molecule of P450nor protein with a corresponding crystal volume per protein mass (*Vm*) of 2.17 $Å^3$ Da^{-1}. X-ray data collection were carried out at station BL6A of the Photon Factory, Japan. After data reduction (processing package DENZO and SCALEPACK), native data set consists of 80156 measurements of 23943 unique reflections with an *R* merge 5.2 %. The completeness of the data set is 87.1 % for the 100 - 2.0 Å range. *Position of the Fe atom from anomalous dispersion;* Data collection were performed with a Weissenberg camera for macromolecular crystallography and imaging plates as detector of the Photon Factory (BL6A, λ = 1.70 Å). A total of 58480 observations corresponding to 13386 unique reflections were collected to 2.5 Å resolution with *R* merge 5.1 %. Completeness of the data set is 97.3 % for the 100 - 2.5 Å range. No serious radiation damage to the crystal was detected during the data collection. Bijvoet anomalous Patterson map shows clear Fe-Fe self vectors on the Harker sections. We intend to make use of the anomalous-dispersion effect of iron atom combined with multiple isomorphous replacement for structure determination. Preparation of heavy atom derivatives for phase determinations are in progress.

PS04.02.38 PROPOSED MECHANISTIC DETAILS OF THE NICKEL METALLOENZYME UREASE. Matthew A. Pearson[1], Linda O. Michel[2], Robert P. Hausinger[2], P. Andrew Karplus[1], [1]Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853, USA, [2]Departments of Microbiology and Biochemistry, Michigan State University, East Lansing, MI 48824-1101, USA

High resolution structural studies of wild type urease from *Klebsiella aerogenes* as well as several site directed mutants provide insight into possible roles for active site residues proposed to be involved in urea binding and hydrolysis. Urease is a multisubunit enzyme (total molecular weight 249kD) that hydrolyzes urea into ammonia and carbon dioxide. The active site contains two nickel ions that are bridged by carbamylated Lys217 and further ligated by His134 and His136 (Ni-1) and His246, His272, and Asp360 (Ni-2). The structure of wild type urease at a resolution of 1.6 Å, near the diffraction limit of these crystals, provides details of the nickel containing active site, leading to a proposed position for binding of urea. Besides interacting with the binickel site, urea is proposed to form hydrogen bonds with several residues, including His219, His320, and Cys319. Structures of several site directed mutants at these positions have been solved at a resolution of 2.2-2.0 Å. The structures also suggest a position for the tetrahedral transition state of urea hydrolysis, which occurs as an activated water attacks the carbonyl carbon of urea. This proposed transition state is stabilized by a similar set of hydrogen bonds with the previously mentioned residues as well as by interactions with the two nickel ions. The positions for substrate and transition state lead to a detailed proposal for the urease mechanism.

PS04.02.39 TUNING THE REDOX POTENTIAL OF AN ELECTRON TRANSFER PROTEIN: ANALYSIS OF THE HIGH POTENTIAL P80I PSEUDOAZURIN C.A. Peters-Libeu[1], E.T. Adman[1], S. Turley[1], T. Beppu [2] and M. Nishiyama[2]. [1]Department of Biological Structure, University of Washington, Box 35742, Seattle, WA. , USA 98195. [2]Department of Biotechnology and Biotechnology Research Center, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113. Japan

The P80I mutant of *Alcaligenes faecalis* pseudoazurin has the largest redox potential change (+150mV) observed for a non-ligand single site cupredoxin mutant. In pseudoazurin, the copper is in a distorted tetrahedral coordination with two histidines, a cysteine and a methionine. Reduction of native and P80A pseudoazurin at physiological pH induces a lengthening of the methionine copper bond which results in a more trigonal coordination of the copper. Structural analysis of the oxidized and reduced forms of the P80I mutant has revealed that **in both oxidized and reduced P80I pseudoazurin the copper methionine bond length is equivalent to the copper methionine bond lengths observed for reduced native and P80A pseudoazurin The flattened copper center in oxidized P80I pseudoazurin is very similar to the copper center found in the oxidized form of the very high potential cupredoxin Rusticyanin.** The large increase in the redox potential observed for the P80I mutant may be due to a decrease in the reorganization energy of the copper center required for reduction. The ability of the copper center in P80I pseudoazurin to adopt this stable flattened coordination appears to result from an alteration of the hydrogen bond network surrounding the copper site. In cupredoxins, this hydrogen bond network has been suggested to stabilize the copper center in its characteristic geometry.

PS04.02.40 CRYSTALLOGRAPHIC STRUCTURES OF DIFERRIC AND APO DUCK OVOTRANSFERRINS. A. Rawas, H. Muirhead and J. Williams, Department of Biochemistry and Molecular Recognition Centre, University of Bristol, Bristol BS8 1TD, UK.

The structures of Diferric Duck Ovotransferrin (DOT) and Apo Duck Ovotransferrin (APODOT) have been determined by the molecular replacement method. The main differences between the known diferric transferrin structures lie in the relative orientations of the N- and C-lobes with respect to each other. In the DOT structure the large aromatic side chain Phe322 in the N-lobe packs against the conserved residue Gly387 in the C-lobe. This interaction is at the centre of the interface between the two lobes and could play a crucial role in determining their relative orientation.

The DOT molecule can be described as three rigid bodies; the N1 and C1 domains as one rigid body forming the static core of the molecule and the N2 and C2 domains forming two other rigid bodies which move away from the N1 and C1 domains (the static core of the molecule) to form the open structure of APODOT. The structure of APODOT shows that both the N- and the C-lobes are in the open form, where the N2 and C2 domains undergo large rotations as rigid bodies of 51.6° and 49.9° respectively relative to the N1 and C1 domains.