MS02.07.06 XAS STUDIES OF THE Cu4 CENTERS OF CYTOCHROME c OXIDASE: A UNIQUE BINUCLEAR COPPER CLUSTER. Ninian J. Blackburn*, Simon de Vries†, Robert A. Scott†, James Fee†, Yi Lu‡, Chris Dennison† and Gerard Cantor†.†Oregon Graduate Institute of Science and Technology; †Delphi University of Technology, ‡University of Georgia; ‡University of California, San Diego; ‡University of Illinois, Urbana-Champaign; ‡Leiden Institute of Chemistry

The Cu4 centers of cytochrome c oxidases are unique examples of a new type of binuclear copper cluster. X-ray crystallography of enzymes from beef heart, Paracoccus, and the engineered cyoA fragment of the quinol oxidase of E. coli have provided a structure which closely resembles that of the cytochrome c oxidase. In all cases, the two Cu atoms are bridged by two cysteine ligands, and there is an extremely short Cu-Cu distance of ~2.4 Å. X-ray absorption spectroscopy, which had previously predicted the short Cu-Cu distance, has been used to further refine the structural details of the site, in both the oxidized and reduced forms. Subtle changes are detected in the metrical parameters of the oxidized versus reduced proteins which suggest that the short distance may be the result, in part, of a weak metal-metal bond. The dinuclear copper site may be explained by a copper binding site that is solvent exposed, and the fact that the copper is held in a nearly tetrahedral geometry by a strong interaction with the Cys ligand.

MS02.07.07 STRUCTURE OF SUBUNIT II OF A QUINOL OXIDASE WITH REENGINEERED Cu4 SITE. Matthias Wilmanns, Kristina Djinovic, Pekka Lappalainen, Mark Kelly, Elisabeth Sauve-Deriksson & Matti Saraste, EMBL, Heidelberg, Germany

The crystal structures of the periplasmic fragment from the wild-type CyoA subunit II of the Escherichia coli quinol oxidase and of a mutant with a reengineered dinuclear copper centre ("purple cyoA") have been solved at 2.7 and 2.5 Å, respectively. Quinol oxidases belong to the superfamily of cytochrome oxidases. This enzyme is a member of the protein complex that catalyses the reduction of molecular oxygen to water and utilizes the free energy of this reaction to generate a transmembrane proton gradient during respiration. The electron entry site in subunit II is a mixed-valence dinuclear copper in the enzymes which oxidize cytochrome c. This centre has been lost during the evolution of the quinol-oxidizing branch of blue copper oxidases.

CyoA is folded as a β-stranded, mostly antiparallel sandwich followed by three α-helices. The dinuclear copper centre is located at the loops between strands 85-86 and 89-910. The two copper ions are 2.5 Å distance and symmetrically coordinated to the main ligands which are two bridging cysteines and two terminal histidines. The residues that are distinct in cytochrome c and quinol oxidases are around the dinuclear copper centre. A recent structure of CyoA with reduced dinuclear copper centre shows a virtual identical arrangement of the two copper ions except for increased distances between the two terminal histidines and the copper ions. Structural comparison suggests a common ancestry for subunit II of cytochrome oxidase and blue copper proteins.

PS02.07.08 CRYSTAL STRUCTURE OF CUCUMBER STELLACYANIN AT 1.7 Å RESOLUTION. P. John Hart*, Aram N. Nersissian†, Joan Selverstone Valentine, and David Eisenberg†. UCLA-DOE Laboratory of Structural Biology and Molecular Medicine, Box 951570, UCLA, Los Angeles, CA 90095. †Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024.

Stellacyanins are blue (type I) copper glycoproteins that differ from other cupredoxins (such as plastocyanin and azurin) in many of their properties. They have an unusual copper ligand (Gln instead of Met found in other mononuclear blue copper proteins), they perform more rapid long-range electron transfer, and they exhibit pH-dependent, reversible EPR and electronic absorption spectra. Until now, stellacyanins have eluded structure determination. Here we report the refined three-dimensional crystal structure at 1.7 Å resolution of stellacyanin from cucumber peelings.

The overall fold of the cucumber stellacyanin copper-binding domain is organized in two β-sheets, one of three β-strands and one of four. Two α-helices are found in loop regions between β-strands. One side of the molecule is predominantly negatively-charged, and provides a possible interaction site for redox partners. The characteristic spectroscopic properties and electron transfer reactivity of stellacyanin, relative to other well characterized blue copper proteins, may be explained by a copper binding site that is solvent exposed, and the fact that the copper is held in a nearly tetrahedral geometry by a strong interaction with the Gln ligand.

PS02.07.09 XAFS AND CRYSTALLOGRAPHIC STUDIES OF AN AZURIN AND A BLUE COPPER NITRITE REDUCTASE FROM A DENTRIFYING BACTERIUM. S. Samar Hasman1,2, Fraser Dodd1,2, Richard Strange1, Gunter Grossmann3, Lorreta Murphy1, Zelda Abraham1, Robert Early1, Barry Smith1,2, Molecular Biophysics Group, Daresbury Laboratory, Warrington, WA4 4AD, 2School of Applied Sciences, De Montfort University, Leicester LE1 9BH, 1John Innes Centre, Norwich Science Park, Norwich, UK

Results from a high resolution (~1.7 Å) crystallographic studies of oxidised and reduced azurin crystals are compared to the XAFS studies of this new azurin.

Crystal structure study of the first blue copper nitrite reductase will be reported. Results from substrate and ligand binding studies will be presented and the chemical (structural) changes associated with such binding will be discussed in terms of reaction mechanism. Background references:
1. 'X-ray scattering provides direct evidence for a trimeric structure in solution of Nitrite Reductase from Alcaligenes xylosoxidans.', J. G. Grossmann et al., Biochemistry, 32, 7360-7366 (1993)

PS02.07.10 CRYSTALLIZATION AND PRELIMINARY X-RAY STUDIES OF AZURIN-I AND AZURIN-II FROM DENTRIFYING BACTERIUM ALCALIGENS XYLOSOXIDANS GIFT 1051. Chumin Li*, Tsuyoshi Inoue*, Masaharu Gotowda*, Kazuyuki Hamada*, Nobuya Nishio*, Shinichiro Suzuki**, Kazuyuki Yamaguchi***, and Yasushi Kii*. 1Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita 565, 2De¬partment of Chemistry, Faculty of Science, Osaka University, Toyonaka 560, Japan.

Azurins are the small copper-containing proteins that function as electron transfer agents in the redox systems of some bacteria. It has been known for a long time that only one azurin is obtained from one species of bacteria, except for the case of Methylobacterium. Recently, two azurins were found instead of the single previously identified one in both Alcaligenes xylosoxidans NCIB 11015 and GIFT 1051. Here we present our recent work on the crystallization and preliminary X-ray studies of Azurin-I and Azurin-II from Dentrifying Bacterium Alcaligenes XYLOSOXIDANS GIFT 1051. Both azurins were crystallized by the hanging drop