MS02.07.06 XAS STUDIES OF THE Cu_A 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 Canters¥. †Oregon Graduate Institute of Science and Technology; §Delft University of Technology, ¶University of Georgia; §University of California, San Diego; #University of Illinois, Urbana-Champaign; ¥Leiden Institute of Chemistry

The Cu_A 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 structural description of the site. The coppers are bridged by two cysteine ligands, and have 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. These studies have been extended to include Cu_A derivatives of the blue proteins azurin and amicyanin produced by "loop-directed mutagenesis", in which the Cu_A-binding sequence has been introduced into the blue copper proteins.

MS02.07.07 STRUCTURE OF SUBUNIT II OF A QUINOL OXIDASE WITH REENGINEERED CuA SITE. Matthias Wilmanns, Kristina Djinovic, Pekka Lappalainen, Mark Kelly, Elisabeth Sauer-Deriksson & Matti Saraste, EMBL Heidelberg, Postfach 102209, D-69012 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 2.3 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 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 cytochrome oxidases.

CyoA is folded as a 11-stranded, mostly antiparallel ß-sandwich followed by three α–helices. The dinuclear copper centre is located at the loops between strands ß5-ß6 and ß9-ß10. The two coppers are at 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 coppers 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.

PS.02.07.08 CRYSTAL STRUCTURE OF CUCUMBER STELLACYANIN AT 1.7 Å RESOLUTION. P. John Hart[1], Aram N. Nersissian[2], Joan Selverstone Valentine[2], and David Eisenberg[1]. [1]UCLA-DOE Laboratory of Structural Biology and Molecular Medicine, Box 951570, UCLA, Los Angeles, CA 90095. [2]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 DENITRIFYING BACTERIUM. S. Samar Hasnain[1,2], Fraser Dodd[1,2], Richard Strange[1], Gunter Grossmann[1], Lorreta Murphy[1], Zelda Abraham[3], Robert Eady[3], Bary Smith[3], [1]Molecular Biophysics Group, Daresbury Laboratory, Warrington WA4 4AD, [2]School of Applied Sciences, De Montfort University, Leicester LE1 9bH, [3]John Innes Centre, Norwich Science Park, Norwich, UK

Results from a high resolution (~1.7Å) 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)
2.'Evidence of two distinct azurins in *Alcaligenes xylosoxidans*'(NCIMB 11015): potential electron donors to nitrite reductase', F E. Dodd et al Biochemistry, 34, 10180-10186 (1995)
3. 'The substrate bindng site in copper nitrite reductase and its similarity to Zn carbonic anhydrase', R. W. Strange et al. Nature-Structural Biology 4, 287-292 (1995)
4. 'Structure of a new azurin from Alcaligenes xylosoxidans at 1.9Å resolution', F. E. Dodd, S. S. Hasnain et al Acta Cryst D51 1052(1995)

PS02.07.10 CRYSTALLIZATION AND PRELIMINARY X-RAY STUDIES OF AZURIN-I AND AZURIN-II FROM DENITRIFING BACTERIUM ALCALIGENS XYLOSOXIDANS GIFU 1051. Chunmin Li*, Tsuyoshi Inoue*, Masaharu Gotowda*, Kazuyuki Hamada*, Nobuya Nishio*, Shinichiro Suzuki**, Kazuya Yamaguchi**, and Yasushi Kai*. *Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita 565, **Department 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 *Methylomonas J.* Recently, two azurins were found instead of the single previously identified one in both *Alcaligenes xylosoxidans* NCIB 11015 and GIFU 1051. Here we present our recent work on the crystallization and preliminary X-ray studies of Azurin-I and Azurin-II from Denitrifing Bacterium *Alcaligenes Xylosoxidans* GIFU 1051. Both azurins were crystallized by the hanging drop