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 structural description of the site. The copper centers 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. The structural description of the site has provided a basis for understanding the function of these proteins.

The crystal structure 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 the respiratory chain.

CyoA is folded as a 11-stranded, mostly antiparallel β-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 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 oxidase 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 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.

Stellacyanin is a blue-type copper-containing protein that differs from other copper proteins 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.