Auracyanin A and Auracyanin B: Spectra, Structures and Functions of Two 'Blue' Cu Proteins from a Primitive Photosynthetic Bacterium, *Chloroflexus aurantiacus*,

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We report an X-ray crystal structure analysis of auracyanin A, one of two 'blue' Cu proteins produced by the primitive photosynthetic bacterium, *Chloroflexus aurantiacus.* The overall fold of the auracyanin A molecule is closely similar to the (published) fold of auracyanin B (sequence identity = 38%). The Cu-site dimensions in the two proteins are identical within the limits of precision of the crystal structure analyses. XAS measurements confirm that the structural differences between the Cu sites in auracyanins A and B are indeed very small.

The lack of a significant difference between the coordination geometries in auracyanins A and B is surprising. First, the polypeptide loop where three of the four Cu-binding residues are located is one residue shorter in auracyanin A than in auracyanin B. Second, new measurements of the Cu-site electronic spectra confirm that auracyanin A has a rhombic EPR X-band spectrum consistent with $\varepsilon_{450}/\varepsilon_{600} \approx 0.31$, whereas auracyanin B has an axial EPR X-band spectrum consistent with $\varepsilon_{450}/\varepsilon_{600} \approx 0.12$. We conclude that the difference between these distinctive spectroscopic signatures arises from a very small difference between the energies of two ground-state electronic configurations.

Differences between important surface features of auracyanins A and B suggest that the two proteins interact with different electron-transfer partners. This hypothesis is supported by the sequences of the complete gene products. The molecules described here as auracyanins A and B are actually soluble domains, which are tethered to a membrane by an N-terminal tail. The fact that the N-terminal tails of auracyanins A and B differ both in length and composition suggests that the molecules are associated with different types of membrane site, consistent with the hypothesis that they have different functions in the organism.