

Structural Studies of Macromolecular Complexes: Cytochrome b_6f

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Seeing protein complexes in states relevant to their biological function is one of the challenges of macromolecular crystallography. The integral-membrane cytochrome b_6f complex carries out electron and proton transfer reactions in the photosynthetic membrane of oxygen-evolving photosynthetic organisms, and is the electronic connection between photosystems II and I. The 220-kDa dimeric b_6f complex consists of a total of 16 subunits and 14 chromophores. The 3.0-Å crystal structure [1] was solved by isomorphous replacement and anomalous scattering, with reference to previously determined structures of the extrinsic domains of two subunits. Dimer formation creates two central cavities with access to electron transfer sites for exchange of the lipid-soluble substrate. The most significant finding was an unexpected and novel heme group, bound to the protein by a single thioether bond. Motion of one extrinsic domain between electron-transfer sites within the b_6f complex is suggested by the overall organization of subunits.

Preparation of pure, monodisperse complex and its crystallization required a significant period of testing poorly diffracting crystals to optimize purification and crystallization protocols. In general, crystals of complexes are frequently quite small or imperfect. A synchrotron X-ray beam that can be tailored to the size of a small crystal or to a region of a crystal is optimal for such samples. The GM/CA Collaborative Access Team has developed dual undulator beamlines at the Advanced Photon Source to deliver small X-ray beams and the goniometry to orient and visualize small samples [2].

[1] Kurisu G., Zhang H., Smith J. L., Cramer W. A., *Science*, 2003, **302**, 1009-1014. [2] Fischetti R. L. et al., *Abstract 2071 IUCr*, 2005.

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