

W0330

Crystal Structure of Fet3p, a Multicopper Oxidase that Functions in Iron Import. P. John Hart¹, Alexander B. Taylor¹, Christopher S. Stoj², Daniel J. Kosman², ¹Dept. of Biochemistry, The Univ. of Texas Health Science Center, San Antonio, TX 78229, ²Dept. of Biochemistry, State Univ. of New York, Buffalo, NY 14214.

High-affinity iron uptake in yeast requires Fet3p, a 636 residue multicopper-containing protein that catalyzes the oxidation of Fe(II) to Fe(III) by molecular oxygen. After this ferroxidation step, Fet3p traffics Fe(III) to the iron permease Ftr1p for translocation into the cytoplasm. Copper and iron metabolism are thus intimately linked, a fact supported by the observation that *fet3Δ* strains of *S. cerevisiae* are iron-deficient. This was a challenging crystallographic problem, because the secreted protein is heavily glycosylated and crystals are often plagued with twinning problems. After an extensive search, conditions were eventually established that minimized the twinning fraction and X-ray diffraction data were collected to 2.8 Å resolution. Attempts to use the structures of other multicopper oxidases (MCOs) such as laccase in molecular replacement failed, and the structure was ultimately determined using the MAD phasing method with the intrinsic copper centers. Six-fold real-space averaging of the MAD-phased map was required to produce an electron density map suitable for model building. The Fet3p structure reveals ten N-linked glycosylation sites, identifies the likely Fe-coordination residues that confers ferroxidase specificity, and identifies possible structural and electron-transfer H-bonding networks.