

BIOCHEMISTRY SEMINAR

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"Spectroscopic and Mechanistic Studies of a *De Novo* Designed Mononuclear Copper Enzyme for Asymmetric Photobiocatalysis"



De novo enzyme design offers a promising approach to expanding biocatalysis to complex and synthetically valuable reactions beyond those found in nature. NovoCopper exemplifies this approach with a designed copper active site. Together with a ruthenium photocatalyst, it catalyzes asymmetric three-component coupling of organic halides, vinylarenes, and amines. UV-visible and electron paramagnetic resonance (EPR) spectroscopy were used to investigate the copper chemistry underlying this transformation. Spectroscopic characterization revealed a mononuclear type-II Cu(II) center and an EPR-silent Cu(I) state, while competition measurements established picomolar Cu(I) and subnanomolar Cu(II) binding affinities. Substrate-dependent changes in the Cu(II) EPR spectrum supported amine binding at the copper center. Light-induced formation of protein-bound Cu(II) was consistent with electron transfer from Cu(I) to the photoexcited ruthenium catalyst. EPR spectroscopy following spin trapping with 5,5-dimethyl-1-pyrroline N-oxide (DMPO) detected a carbon-centered radical adduct, consistent with radical formation through single-electron reduction of the α -halocarbonyl substrate by the reduced photocatalyst. Together, these findings provide spectroscopic support for substrate binding and a photoinduced electron-transfer pathway connecting copper oxidation to radical formation in asymmetric photobiocatalysis.