

## **Ru(bpy)<sub>2</sub>(mbpy)-Adx(1-108) Complex: Photoreduction and Crystal Structure**

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Bovine adrenodoxin (Adx) is a [2Fe-2S] protein. The truncated form Adx(1-108) was covalently modified with the ruthenium(II) bipyridyl complex ((Ru(bpy)<sub>2</sub>(mbpy))) to test the “shuttle” hypothesis of the electron transfer mechanism.

The crystal structure of the Ru(bpy)<sub>2</sub>(mbpy)-Adx(1-108) complex was solved by molecular replacement at 1.5 Å resolution. Ru(bpy)<sub>2</sub>(mbpy) is covalently bound to Adx(1-108) and exposed to solvent. Two chiral components of Ru(bpy)<sub>2</sub>(mbpy) cause two alternative conformations of the side chain of Cys95 of Adx(1-108).

Activity assays suggest that labeling might affect intermolecular electron transfer between redox-protein partners. The dye-associated photoreduction and chemical reduction of Adx is accompanied by a two-electron transfer. However, spin quantification points out that just one of the two iron atoms of the reduced Adx is in the Fe<sup>2+</sup>-state.

Adx(1-108) can be photoreduced *via* the ruthenium compound, as confirmed by EPR. Ru(bpy)<sub>2</sub>(mbpy)-Adx(1-108) does not display new *g* values. The electron transfer rate depends on the concentration of the complex, indicating intermolecular transfer to take place. Extrapolation to Adx concentration of zero gives the intramolecular rate constant. Possible electron transfer pathways calculated based on the 3D-structure are in the physiological range and could be related to the calculated intramolecular rate constant.

**Keywords:** metallo-enzymes, electron transfer, crystal structure