ESRF	**Experiment title: Ribonucleotide Reductase subunit R2 from E. coli**	**Experiment number:** 01-02-51
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Report:

The crystal structure of an Azide Complex of the Diferrous R2 Subunit of Ribonucleotide Reductase Displays a Novel Carboxylate Shift with Important Mechanistic Implications for Diiron-Catalyzed Oxygen Activation

Andersson, M:E., Högbom, M., Rinaldo-Matthis, A., Andersson, K.K., Sjöberg, B.-M., and Nordlund, P. (1999). *J. Am. Chem. Soc.* **121**, 2346-2352.

The di-nuclear Fe-center in the R2 protein of ribonucleotide reductase catalyzes oxygen activation chemistry leading to generation of the essential stable tyrosyl radical. Related oxygen reactions occur in several other di-iron containing enzyme systems where highly oxidative reaction intermediates are required to activate the substrates. Two such examples are methane monooxygenase and Δ^9 stearoyl-acyl carrier protein desaturase where oxygen activation takes place at Fe-centers whose structures are similar to the Fe-center in R2. In an attempt to structurally characterize the nature of the dioxygen cleavage reaction performed by these proteins we have determined the crystal structures of two different forms of the di-

ferrous R2 protein in the presence of azide, a potential Fe ligand. In crystals of the wt protein no azide binding was detected. The mutant protein F208A/Y122F has a larger hydrophobic pocket around the Fe-center and in the structure of this protein azide bind as an η^1-terminal ligand to Fe2, the Fe ion farthest away from the tyrosine residue to be oxidized in the radical generation reaction. Glu 238, the Fe ligand most exposed into the hydrophobic pocket, coordinates the Fe-center in a novel μ-(η^2,η^1) bridging mode with one of the carboxylate oxygen atoms forming a bridge between the two iron ions and the other oxygen being coordinated to Fe2. Through this bridging the Fe-Fe distance is shortened to about 3.4 Å as compared to 3.9 Å for the structure of the reduced wt protein. Based on the novel carboxylate shift and recent data on the spectroscopic properties of the key intermediate X we propose a unique structure for intermediate X and a detailed mechanism for dioxygen cleavage. This mechanism suggests an asymmetric oxygen cleavage with a terminal oxo/hydroxo group as the major species responsible for substrate activation.