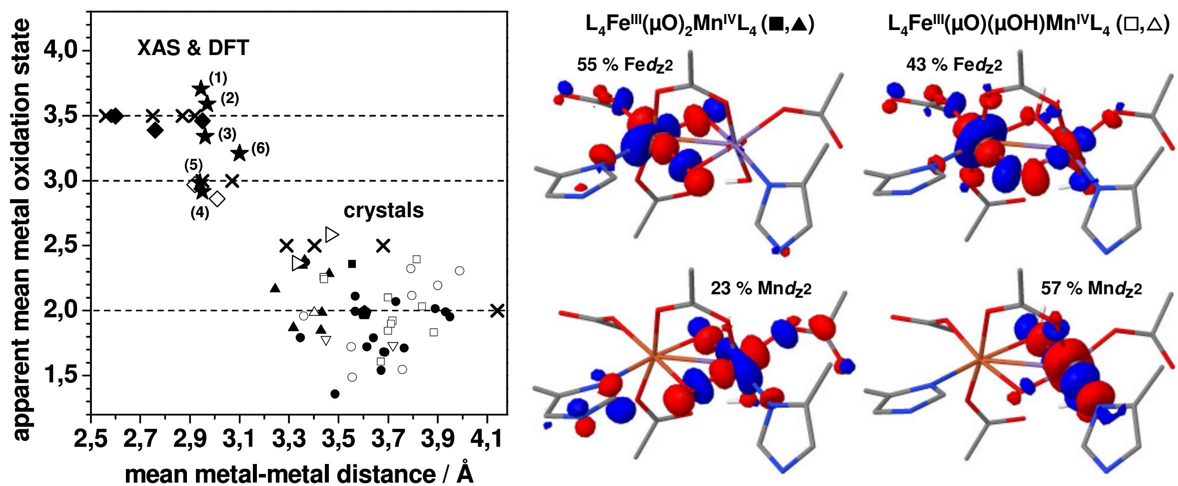
X-ray spectroscopy to characterize intermediates in the catalytic cycle of MnFe and FeFe ribonucleotide reductases



Comparison of metal oxidation states and distances from XAS/DFT and crystallography in R2-type proteins (left) and DFT calculations on FeFe and MnFe cofactor models (right).

Summary:

Ribonucleotide reductases (RNRs) in all organisms convert ribonucleotides to deoxyribonucleotides essential in DNA synthesis. In the “classic” RNRs, the catalytic reactions involve a binuclear iron center (FeFe) and a nearby tyrosine radical in the R2 subunit and proton-coupled electron transfer between R2 and the R1 subunit which carries the nucleotide binding site.

In the RNRs of certain human pathogenic bacteria (*Chlamydia trachomatis*), the tyrosine is absent and R2 houses a novel manganese-iron (MnFe) center. Characterization of intermediates of the MnFe center during the activation of molecular oxygen and the redox reactions was the central topic of this project.

Advanced synchrotron-based X-ray spectroscopy techniques (XAS, XES, RIXS) were employed (1) to determine the atomic structure of the metal sites (metal-metal distances, metal-ligand bond lengths) and its changes in different redox states, (2) to follow changes in the electronic configuration (oxidation state, spin state) of the metal ions during the catalytic cycle, and (3) to track the binding to the metal ions of O_2 and its cleavage products; (4) high-resolution site-selective methods were used for comparative analysis of Mn-Fe and Fe-Fe RNRs; (5) results from X-ray spectroscopy were compared to crystallographic data.

The goal was to obtain atomic-resolution models of all relevant intermediates in the catalytic cycle on the structural, electronic, and functional levels, to determine the mechanism of MnFe RNRs.