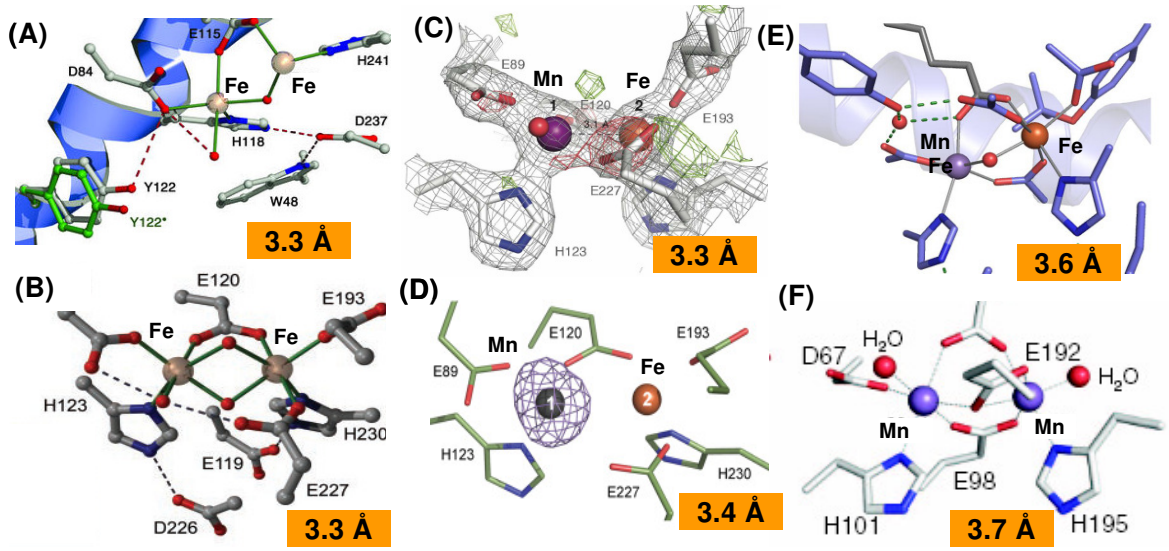
High-valent FeFe, MnFe, and MnMn cofactors in R2-homologue proteins from human pathogens: XAES-DFT investigations on molecular structures and electronic configurations



Examples of crystal structures of prototypic FeFe, MnFe, and MnMn cofactors in R2 proteins of ribonucleotide reductases (orange = metal-metal distances).

Summary:

The iron-oxygen superfamily of enzymes is characterized by a prototypic homo-bimetallic FeFe cofactor, which functions as an O_2-activating catalyst, involving high-valent intermediates in electron transfer (ET) processes. Ribonucleotide reductases (RNRs), essential for DNA synthesis in all organisms, and ligand-binding oxidases, important for challenging conversion reactions, are prominent examples.

Recent results in the field suggest that in the R2-homologue subunits of the human pathogens *Chlamydia trachomatis* (*Ct*) and *Mycobacterium tuberculosis* (*Mt*) a hetero-bimetallic MnFe species forms the native catalyst instead of the standard FeFe cofactor and MnMn sites are also active. In addition, these cofactors can function in the presence and absence of the well-known tyrosyl radical of standard RNRs. This calls for a general re-evaluation of the mechanistic concept of this type of dimetal cofactor. New crystal structures represent significant progress, but contain only low valence states of the cofactors.

We aim at unravelling the molecular structure and electronic configuration of the FeFe, MnFe, and MnMn cofactors in their O_2-activated high-valent states (III_2; III,IV; IV_2) in *Ct* R2 and *Mt* oxidase proteins using advanced X-ray absorption and emission spectroscopy techniques in combination with density functional theory calculations (XAES-DFT). These parameters are related to cofactor assembly routes, metal site protonation, interactions between protein subunits, and initiation and gating of the ET.

Our novel information from spectroscopy and theory will allow to assign prerequisites for the site-selective metal incorporation, compare structure-function relationships in RNR and oxidase enzymes, reveal the roles of cofactor species in the O_2 activation and ET reactions, improve strategies for crystallography on the high-valent protein states, and construct detailed model structures for gaining deeper insight into the mechanisms of this fascinating manifold of dimetal cofactors.