

Poster Presentation

MS29.P02

Structure of *B. cereus* Class Ib Ribonucleotide Reductase NrdI-Fe₂-NrdF Complex

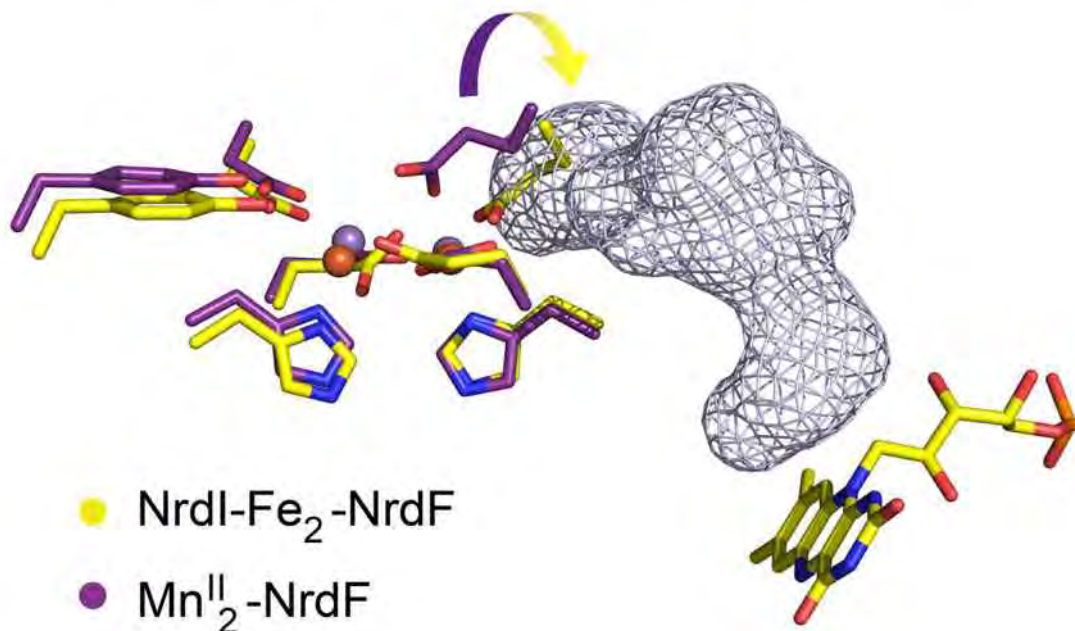
M. Hammerstad¹, H. Hersleth¹, A. Tomter¹, Å. Røhr¹, K. Andersson¹

¹University of Oslo, Department of Biosciences, Oslo, Norway

Class Ib ribonucleotide reductases (RNRs) use a dimetal-tyrosyl radical (Y•) cofactor in their NrdF (beta2) subunit to initiate ribonucleotide reduction in the NrdE (beta2) subunit. Contrary to the diferric tyrosyl radical (FeII₂-Y•) cofactor, which can self-assemble from FeII₂-NrdF and O₂, generation of the MnII₂-Y• cofactor requires the reduced form of a flavoprotein, NrdIh_q, and O₂ for its assembly. Here we report the 1.8 Å resolution crystal structure of *Bacillus cereus* Fe₂-NrdF in complex with NrdI. Compared to the previously solved *Escherichia coli* NrdI-MnII₂-NrdF structure, NrdI and NrdF binds similarly in *Bacillus cereus* through conserved core interactions. This protein-protein association seems to be unaffected by metal ion type bound in the NrdF subunit. The *Bacillus cereus* MnII₂-NrdF and Fe₂-NrdF structures, also presented here, show conformational flexibility of residues surrounding the NrdF metal ion site. The movement of one of the metal-coordinating carboxylates is linked to the metal type present at the di-metal site, and not associated with NrdI-NrdF binding. This carboxylate conformation seems to be vital for the water network connecting the NrdF di-metal site and the flavin in NrdI. From these observations, we suggest that metal-dependent variations in carboxylate coordination geometries are important for active Y• cofactor generation in class Ib RNRs. Additionally, we show that binding of NrdI to NrdF would structurally interfere with the suggested alpha2beta2 (NrdE-NrdF) holoenzyme formation, suggesting the potential requirement for NrdI dissociation before NrdE-NrdF assembly after NrdI-activation. The mode of interactions between the proteins involved in the class Ib RNR system is, however, not fully resolved. [1]

[1] M. Hammerstad, H.-P. Hersleth, A.B. Tomter et al, *ACS Chem. Biol.*, 2014, DOI: 10.1021/cb400757h

Metal ion-dependent carboxylate conformation



Keywords: metalloprotein, tyrosyl radical, protein-protein complex