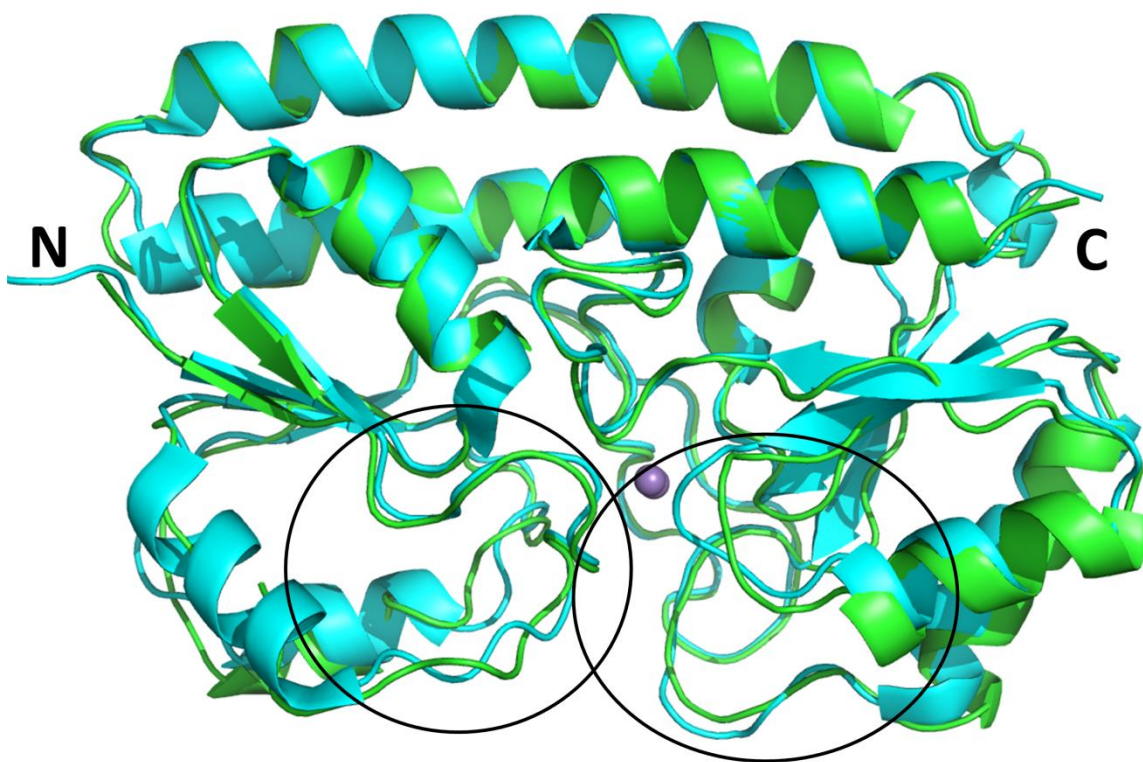
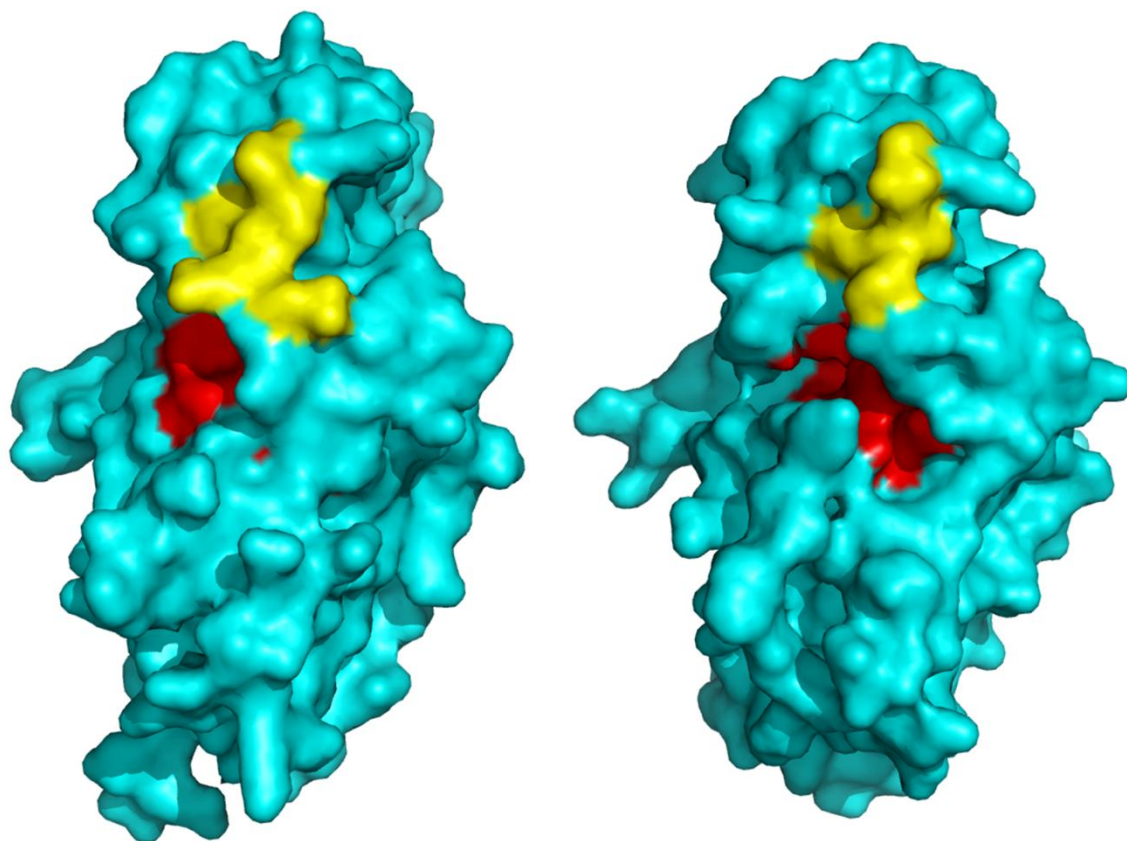


## Supplementary Material



**Supplementary Figure S1.** Superposition of the MnWT and MnR116A structures. Cartoon representations of monomers A from both structures (cyan and green, respectively) were superimposed using the algorithm implemented in Pymol (rmsd = 0.6Å). The black circles show movements in the loops near the entrance to the Mn<sup>2+</sup> ion binding site, while the large  $\alpha$ -helical backbone structures are very similar. The amino and carboxyl termini are denoted with N and C, respectively.



**Supplementary Figure S2.** The entrance to the  $\text{Mn}^{2+}$  binding site is exposed by mutation of R116. The molecular surfaces of the MnWT (left) and MnR116A (right) structures are shown. In order to compare the two structures, the loops missing from the MnR116A structure were also deleted from the calculation of the surface of the MnWT structure. Red indicates the surface covering all atoms that are  $7\text{\AA}$  from the bound  $\text{Mn}^{2+}$  ions. The yellow coloured surface visualizes the loop (residues 242-246) that moves due to the loss of the hydrogen bond between Asn241 and Arg116 in the mutant.

**Supplementary Table S1.** ICP-AES analysis of the  $\text{Mn}^{2+}$  content in the MntC variants.

MntC variant	$\text{Mn}^{2+}/\text{protein}^a$
WT	$0.78 \pm 0.15$
R116A	$0.62 \pm 0.15$

<sup>a</sup>average of 4 measurements