

Investigating The Role of a Highly Conserved Tryptophan in The Copper-Binding Site of Bacillus Subtilis YcnI Protein

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Copper is vital for bacterial survival, but it can be detrimental to cells if not properly regulated. *Bacillus subtilis* uses the *ycn* operon to facilitate copper acquisition. Our previous research established that YcnI, one of the three proteins encoded by the operon, binds Cu(II) in its extracellular domain through a distinctive mono-histidine brace motif that coordinates the metal ion with a histidine and a glutamate residue. A highly conserved tryptophan residue in YcnI is also in close proximity to the metal, with a proposed role of cushioning the metal ion. In this study, we constructed a variant where Trp137 was replaced with phenylalanine, a less bulky residue that is occasionally present in similar positions in copper-binding sites of LPMOs. Using X-ray crystallography, we determined the structure of the W137F variant and assessed its copper-binding ability using a bicinchoninic acid assay. Our data indicate that Trp137 is not essential for copper-binding but is structurally significant in stabilizing the copper-binding site.