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.