

## THE DISCOVERY AND CHARACTERIZATION OF TUNGSTEN INSERTASE IN TUNGSTEN COFACTOR BIOSYNTHESIS

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The tungsten cofactor (Wco) plays a crucial role in the activity of tungstoenzymes which are vital for essential biological processes. In the biosynthesis of Wco, the pathway overlaps with that of molybdenum cofactor (Moco) until the step of metal insertion. While significant research has been conducted on molybdenum insertion enzymes, there has been limited investigation on tungsten insertase (W-insertase). In this study, we identified the metal insertase MoeA1 from *Methylobacterium extorquens* AM1 as the key enzyme responsible for tungsten insertion in the synthesis of Wco. Isothermal titration calorimetry demonstrated the exclusive binding of tungstate to MoeA1, and X-ray crystal structure revealed a distinct metal-binding region which is critical for tungstate selectivity and efficient incorporation. These findings significantly advance the understanding of Wco biosynthesis, particularly the specific recognition and integration of tungstate by W-insertase.