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Abstract

MitoNEET is a [2Fe-2S] cluster-containing protein with an unclear function. However, some of the functions are proposed to have a link to the iron sulfur clusters contained within the protein. Within seven Angstroms of the iron-sulfur cluster there are many amino acid residues that are reactive. There are three ligating cysteine residues (Cys72, Cys74, Cys83) and one ligating histidine residue (His87). A lysine residue (Lys55') of the opposite dimer interacts with the ligating histidine through a hydrogen-bonding network involving a structural water molecule. This hydrogen-bonding network activates Lys55 towards reacting selectively with the enzyme cofactor pyridoxial-5-phosphate (PLP). The spectroscopic signatures of free PLP and bound PLP have allowed for the development of a colorimetric assay to detect other small molecules that bind in the same pocket of mitoNEET. Typical ligand binding site determination methods are expensive and involve radioactivity. The goal of this project was to develop a method that is less expensive and does not utilize radioactive materials. The development of this assay paves the way for future high-throughput screening of future drug candidates.

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