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Ogut, I., Lankathilaka, W.K.P., Holz, R.C. and Bennett, B. (2020), Elucidating the role of the axial cysteine residue in NHase catalysis and the enzyme maturation. The FASEB Journal, 34: 1-1. doi:[10.1096/fasebj.2020.34.s1.00563](https://doi.org/10.1096/fasebj.2020.34.s1.00563)

### **Abstract**

Nitrile hydratases are metalloenzymes that contain trivalent Fe/or Co ions in their active site, coordinated in an N<sub>2</sub>S<sub>3</sub> ligand environment. The sulfur atoms are post-translationally modified and have three different oxidation states (αCys, Cys-SOH and Cys-SOO<sup>-</sup>) while backbone amines account for the nitrogen ligands and are deprotonated. Theoretical calculations assume that the αCys ligand is a thiolate, making it a strong π-donor ligand and a good nucleophile. A critical outstanding mechanistic question is whether the formation of a transient disulfide bond is essential for catalytic turnover. Such a transient disulfide bond was proposed based on QM/MM calculations between the αCys sulfur and the sulfur of the proposed sulfenic acid cyclic intermediate. However, more recent theoretical studies is self-inconsistent in its conclusions regarding a transient disulfide formation. To examine the role of the αCys108 ligand in catalysis, it was mutated to A, M, S, and H in the Co-type NHase from *Pseudonocardia thermophila* JCM 3095 (*Pt* NHase). ICP-MS demonstrated reduced metal ion content for each mutant: <5% (αC108A), ~44% (αC108M), ~50% (αC108S) and ~55% (αC108H) and catalytic activity also decreased to ~0.3%, ~0.8%, ~1.6%, and ~6.7%, respectively, towards acrylonitrile. The X-ray crystal structure of αC108A confirmed the mutation and the decreased active site metal content. Therefore, the axial thiolate is important to properly tune the electronic properties of the active site metal ion, but is not essential to catalysis. These data provide insight into the critical mechanistic question of whether the axial αCys ligand forms a transient disulfide bond during turnover.

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