# Challenges and Successes in Determining the Structure of Arginyltransferase 1 (ATE1)

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Eukaryotic post-translational arginylation, mediated by the family of enzymes known as the arginyltransferases (ATE1s), is an important post-translational modification that can alter protein function and even dictate cellular protein half-life. Multiple major biological pathways are linked to the fidelity of this process, including neural development, cardiovascular development, cell division, and even the stress response. Despite this significance, the structural, mechanistic, and regulatory mechanisms that govern ATE1 function have remained elusive. We have recently demonstrated that ATE1s are previously undiscovered [Fe-S] proteins. We have used biochemical, spectroscopic, and analytical methods to decipher the composition and reactivity of this [Fe-S] cluster. Fascinatingly, we find that ATE1 cluster-binding preserves oligomeric homogeneity while increasing arginylation efficacy, demonstrating that this evolutionarily-conserved [Fe-S] cluster regulates arginylation. *In vivo* alterations of the [Fe-S] cluster-binding residues also compromise the ability of yeast (*Saccharomyces cerevisiae*) to respond appropriately to external stressors. To understand the role of the [Fe-S] cluster in ATE1 function, we sought to determine the structure of *S. cerevisiae* ATE1. Despite our ability to generate diffraction-quality crystals of this enzyme, the determination of a structural model from the X-ray data of *S. cerevisiae* ATE1 was a considerable undertaking that included the use of experimental phasing, molecular replacement using Robetta- and AlphaFold-derived models, cryoEM, and small- angle X-ray scattering. The challenges of such a journey will be discussed, as will aspects of the atomic-level structure of ATE1 that underpin our hypothesized mechanism of post- translational arginylation.