Structural investigations of arginyltransferases

Verna Van[1], Nna-Emeka Ejimogu[2], Aaron Smith[3]

[1]University of Maryland, Baltimore County (UMBC) Dept. of Chemistry [2]UMBC, [3]UMBC

vernavan1@umbc.edu

Arginyltransferases (ATE1s) are a class of essential eukaryotic enzymes that catalyze arginylation, the post-translational transfer of Arg from an aminoacylated tRNA to a range of protein targets. This modification typically occurs at N-terminal acidic residues, though ATE1 can also covalently attach Arg to mid-chain residues. Arginylation may have either degradative or non-degradative effects. For example, some arginylated proteins are processed through the Arg N-degron pathway, which marks these post-translationally modified proteins for degradation by the ubiquitin-proteasome system. In contrast, arginylation may also manifest non-degradative effects such as thermodynamic stability, subcellular relegalization, or functional changes. The diversity of ATE1's targets confers its role as a global regulator, influencing functions such as cardiovascular development, neurological processing, and even the stress response. However, a lack of ATE1 structural knowledge has limited the determination of its three-dimensional fold, how it is regulated, and how it recognizes its substrates. Using a combination of X-ray crystallography, cryo-EM, and size-exclusion chromatography-coupled small angle X-ray scattering (SEC-SAXS), our lab has successfully solved the structure of Saccharomyces cerevisiae ATE1 (ScATE1). The three-dimensional structure of ScATE1 reveals a bilobed protein containing a canonical GCN5-related N-acetyltransferase (GNAT) fold. Structural superpositions and electrostatic analyses indicate this domain as the location of catalytic activity and tRNA binding. Furthermore, the structure reveals the spatial connectivity of the N-terminal domain, which we previously showed binds an [Fe-S] cluster, to the enzymatic active site, hinting at the cluster's regulatory influence. As the first atomic-level structure of any ATE1, this achievement brings us closer to answering pressing questions regarding the molecular-level mechanism of eukaryotic post-translational arginylation.