# Ultrahigh-resolution crystal structure of Class 3 L-asparaginase

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L-Asparaginases are a diverse group of enzymes that catalyze the hydrolysis of L-asparagine to L-aspartate and ammonia. Beyond their metabolic roles, L-asparaginases are important pharmaceuticals for treating acute lymphoblastic leukemia or as potential antibacterial and antifungal targets in agriculture.

A revised classification system [1] divides L-asparaginases into three distinct classes: Class 1 (bacterial-type), 2 (plant-type), and 3 (*Rhizobium etli*-type). The Class 3 enzymes constitute a structurally distinct group found in bacteria and fungi. They are poorly studied, as the first structure was deciphered only recently [2], in contrast to enzymes from Class 2 and especially from Class, which have been studied for decades. The active site of Class 3 asparaginases has a characteristic architecture based on two Ser-Lys tandems and a nearby zinc cation. Understanding of its catalytic mechanism remains a high challenge and, especially that one of the active-site Ser residues appears in the crystal structures to be tightly hydrated by three water molecules or bearing a covalent modification.

Here, we present the sub-atomic crystal structure of the Class 3 L-asparaginase from the pathogenic fungus Botryosphaeria parva (BpA), solved and refined to the unprecedented resolution of 0.78 Å. This is the highest resolution reported for any L-asparaginase structure to date, exceeding even typical resolutions expected for small-molecule crystal structures. This exceptional resolution reveals a host of subtle structural features, fully supported by the high-quality electron density maps. These include the configuration of the active site, conformation of flexible regions, hydration by conserved water molecules, and the identity of bound ligands. The ultra-high resolution is particularly important in the active site region, where interpretation based on lower-resolution maps had been previously challenging or inconclusive. This level of quality and detail allowed definite elucidation of the covalent modification of the nucleophilic serine residue. While similar modifications were observed in previous, lower-resolution structures [2, 3], the poorer quality of the electron density maps has limited the interpretation to a tentative modeling with three tight water molecules near the nucleophilic hydroxyl group. Furthermore, the extraordinary resolution allows us to use methods and algorithms typical for small-molecule crystallography, such as fully anisotropic least-squares refinement or calculation of the estimated standard uncertainties of the model parameters with the *SHELXL* program. The current refinement has converged at *R* < 10%.

The unprecedented resolution of BpA sets a new benchmark for protein structural studies and is essential for structure-based functional analyses aimed at development of potential novel therapeutics or biotechnological applications.

#### [1] Loch, J. I. & Jaskolski, M. (2021) *IUCrJ* **8**, 514.

#### [2] Loch, J.I. et al. (2021) *Nat. Commun.* **12**, 6717.

#### [3] Loch, J. I. et al. (2023) *Acta Cryst.* D**79**, 775.

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