Electron diffraction of *Stenotrophomonas maltophilia* nuclease SmNuc1

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Stenotrophomonas maltophilia is a gram-negative bacterium from class *Gammaproteobacteria*. It belongs to causative agents of hospital-acquired infections and is multidrug-resistant. SmNuc1, a nuclease from *Stenotrophomonas maltophilia*, belongs to the S1-P1 family of nucleases (enzymes cleaving nucleic acids). S1-P1 nucleases occur in some multi-drug resistant bacteria; however, with a poorly understood role.

SmNuc1 expression, purification and characterization has been described [1]. Its seven X-ray crystal structures (free, mutated forms and complexes with RNA cleavage products) have been recently published [2]. SmNuc1 is a zinc dependent enzyme. Its fold consists mostly of α-helices. The active site, a trinuclear zinc cluster coordinated by nine residues, is placed on SmNuc1 surface cleft.

To better understand the cleavage mechanism of SmNuc1, we set a goal to also solve SmNuc1 crystal structures based on neutron diffraction and electron diffraction. This contribution will focus on our efforts to get electron diffraction data of SmNuc1.

SmNuc1, 13.7 mg/ml, was prepared in buffer 50 mM Tris pH 7.5, 150 mM NaCl. Small needles of SmNuc1 were crystallized in a sitting drop containing 10 µl of protein + 10 µl of reservoir solution. Crystal solutions were pipetted to glow-discharged golden 300 Quantifoil grids. The grids were vitrified using Vitrobot: the solutions were equilibrated using 5 s waiting time and blotted for 5 s. Then, the grids were immersed into liquid ethan and then stored in liquid nitrogen.

The grids were checked using transmission electron microscope FEI Tecnai G2 20. They contained sufficient quantities of needle-like crystals of size around 0.3x2x10 µm. The crystals diffracted with the best resolution of ca. 3 Å. The data were indexed in PETS2 [3]. The unit-cell parameters 43.6 Å, 72.4 Å, 82.1 Å, 90°, 103°, 90° are similar to unit-cell parameters of the previously solved X-ray structures of the protein.

[1] Hustáková, B., Trundová, M., Adámková, K., KovaĺT. Dušková, J. & Dohnálek, J. (2023). *FEBS Lett.* **597**, 2103.

[2] Adámková, K., Trundová, M., Koval, T., Hustáková, B., Kolenko, P., Dušková, J., Skálová, T. & Dohnálek, J. (2025). *FEBS J.* **292**, 129.

[3] Palatinus, L., Brázda, P., Jelínek, M., Hrdá, J., Steciuk, G & Klementová, M. (2019). *Acta Crystallogr. B* **75**, 512.

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