# Unprecedented nuclease activity explained by structure – where one bond matters

## J. Dohnálek1, K. Adámková1, M. Trundová1, B. Husťáková1, J. Dušková1, P. Kolenko1,2, T. Skálová1, T. Kovaľ1

### 1Institute of Biotechnology, Czech Academy of Sciences, Průmyslová 595, 25250 Vestec 2Czech Technical University in Prague, Břehová 7, 115 19, Prague

### dohnalek@ibt.cas.cz

S1-P1 nucleases are coded for by fungi, trypanosomatids, plants and some pathogenic bacteria [1]. *Leishmania amazonensis* 3’-nucleotidase is most likely a virulence factor, the plant enzymes have a role in growth and cell death, and the role of bacterial enzymes from this family is not clear. These 25-40 kDa enzymes are typically zinc-dependent. The active site relies on the metal cluster and the nucleobase-binding site 1 stabilizing the –1 nucleotide with respect to the cleaved O3’-P3’ bond. The enzymes are universal with respect to the cleaved substrate, which enables their application in biotechnologies. They cleave DNA, RNA, single strands, double strands, viroids, some modified nucleotides, oligonucleotides and genomic DNA, substrates which are structured or unstructured, without any significant sequence preference [1].

While their fold does not change across the species, their activity profiles differ dramatically. Some representatives preferentially cleave ssDNA with negligible activity towards double strands, other process RNA, and the plant enzymes have comparable activity towards all types of nucleic acids.

Our studies of S1-P1 nucleases from plants, fungus, and two bacterium species [2-5], including crystal structures, mutagenesis, numerous product/ligand complexes helped us better understand the structure-function questions, such as active site remodelling, sensitivity to metal replacement, and key mobility elements in the active site. Recently, we have identified a “supernuclease” SmNuc1 from opportunistic pathogen *Stenotrophomonas maltophilia* capable of previously unseen rates for this enzyme class, uncovered the key region for RNA/DNA preference, which enables activity optimization, and discovered its high activity towards cyclic-di-GMP, the bacterial second messenger [6]. Our crystallographic studies answered key questions regarding non-specificity of S1-P1 nucleases and brought us closer to understanding protonation details of the protein-nucleic acid interactions.

1. T. Koval, J. Dohnalek *Biotechnol. Adv.* (2017) Epub 2017 Dec 14, 10.1016/j.biotechadv.2017.12.007.
2. T. Kovaľ T, L.H. Østergaard, J. Lehmbeck, *et al.* *PLOS One* 11, (2016), e0168832.
3. M. Trundová, T. Kovaľ, R.J. Owens, *et al.* *Int J Biol Macromol* **114**, (2018), 776.
4. K. Adámková, T. Koval', L.H. Østergaard, *et al.* *Acta Crystallogr D***78,** (2022), 1194.
5. B. Husťáková, M. Trundová, K. Adámková, et al. *FEBS Lett* **597**, (2023), 2103.
6. K. Adámková, M. Trundová, T. Kovaľ, B. Husťáková, *et al.* *FEBS J.*, **292**, (2025), 129.

This work was supported by the Czech Science Foundation (25-17546S), and by the Czech Academy of Sciences (86652036). CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042 and European Regional Development Fund-Project No. CZ.02.01.01/00/23\_015/0008175 is acknowledged for providing access to all facilities at CMS in BIOCEV for this project.