**P-1.14**

**Exploration of Haloalkane Dehalogenase Variants Created by Directed Protein Evolution**

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Haloalkane dehalogenases (HLDs) are hydrolytic enzymes, which catalyze the cleavage of the halogenated compounds. HLDs belong to the α/β-hydrolase superfamily and are composed of two domains – α/β-hydrolase domain and the cap domain. The active site is in the cavity at the interface of the domains and is composed of the catalytic pentad of the amino acids and two access tunnels. We exploit Halo Tag technology in selection of HLDs, which are represented by enrichment of the genes encoding HLDs using ribosome display (RD). After the 4th round of the RD, we obtained variants of input DhaA_115 protein, RD4-32 and RD4-37. Circular dichroism and differential scanning calorimetry showed that evolved variants are properly folded and stable, even the thermal stability of both DhaA variants is decreased. On the other hand, analysis of their catalytic activities showed that RD4-37 variant possesses increased catalytic activity in comparison with DhaA_115 protein. Preliminary structural analysis suggests that the observed changes in activities of evolved variants are caused by the mutations in the access tunnels. This work was supported by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00112 and by the grant agency of the Ministry of Education, Science, Research, and Sport of the Slovak Republic (grant no. VEGA 1/0074/22).