**MS06-2-14 Shortcut to hypusine. Structural characterization of putative bifunctional deoxyhypusine synthase from Trichomonas vaginalis**
**#MS06-2-14**

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**Abstract**
Deoxyhypusine synthase (DHS) is a transferase catalysing the formation of deoxyhypusine, which is the first, rate-limiting step of unique post-translational modification: hypusination. During the first step, DHS catalyses the transfer of 4-aminobutyl moiety of spermidine to a specific lysine of eIF5A precursor in a NAD-dependent manner and emergent deoxyhypusine is further hydroxylated to hypusine by deoxyhypusine hydroxylase (DOHH). This modification occurs exclusively on only one protein: eukaryotic initiation factor 5A (eIF5A) and it is essential for cell proliferation and enzymes involved in hypusination are highly conserved in eukaryotes. However, in 2016 Quintas-Granados and colleagues identified that deoxyhypusine synthase from Trichomonas vaginalis (Tv-DHS) has bifunctional activity and it is able not only to form deoxyhypusine, but also to further hydroxylate it to hypusine.
The presented study aimed to investigate putative bifunctional deoxyhypusine synthase from Trichomonas vaginalis using biochemical and structural biology methods.
Bifunctional Tv-DHS and its substrate Tv-eIF5A were expressed and purified. Our crystallization attempts lead us to high-resolution crystal structures determination allowing for detailed analysis and comparison to the human proteins. Based on the Tv-DHS crystal structure we identified putative additional active site, which was further confirmed by site-directed mutagenesis studies. Additionally, we determined binding affinity of Tv-DHS for small molecules ligands and protein substrate. In contrast to the human enzyme, Tv-DHS seems to be less polyamine-specific as spermine activates it to almost the same extent as the preferred substrate – spermidine.
Availability of high-quality structural and biochemical data can significantly advance our understanding of this unique posttranslational modification and probably will aid the design of novel tvDHS inhibitors for potential applications in sexually transmitted diseases therapy.

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