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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: COMPLEX AND EMERGING TECHNIQUES IN STRUCTURAL BIOLOGY

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Abstract P-44: Dimer Structure of Highly Thermostable Archaeal Beta-Galactosidase Revealed by Cryo-EM

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Background: Studies on beta-galactosidases (EC3.2.1.23) are of a great value for the optimization of industry production of low-lactose or lactose-free dairy products. A new 975 amino acid *Da*-β-gal (proposed to be a GH35 family member) from hyperthermophilic archaeon *Desulfurococcus amylolyticus* possesses high thermoactivity and thermal stability at temperatures closed to 100°C.

Methods: Negative staining TEM with 1% uranyl acetate was performed for sample characterization prior to the cryo-EM studies. Preliminary Cryo-EM dataset was collected using Titan Krios (Thermo-Fisher, USA) equipped with the direct electron detector Falcon II at 300kV accelerating voltage. Movie stacks were preprocessed using Warp. All further processing steps were performed in CryoSPARC and Relion 3.0. Constructed full-atom model of dimer formed by similar mesophilic beta-galactosidase from *Trichoderma reesei* (*Tr*-β-gal), PDB entry 3OGV, was refined by molecular dynamics (MD) simulations in explicit water box with periodic boundary conditions in Amber16 (ff14SB, TIP3P, PME, 1 atm, 300K, 100 ns).

Results: The *Da*-β-gal enzyme, expressed in *Escherichia coli* cells and purified to homogeneity by column chromatographies, was subjected to negative contrast microscopy and Cryo-EM. Analysis of electron density distributions in collected set of 30K appropriately classified individual particle images allowed us to solve the structure of the enzyme at 7Å resolution. The symmetry of obtained structure has revealed that in utilized buffer conditions the enzyme exists as a dimeric form. MD simulations of *Tr*-β-gal hypothetical dimer, have shown stability of the complex along 100 ns trajectory. As estimated from the model the protomer of *Tr*-β-gal buries about 7.5% of solvent accessible surface area into dimer interface.

Conclusion: The preliminary CryoEM data in concert with molecular modeling identifies dimeric quaternary structure of a 111-kDa *Da*-β-gal that can be assumed as a structural basis for high temperature stability of the enzyme.

Key Words: beta-galactosidase • protein 3D structure • thermostable enzymes • cryo-EM

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