Structural Studies of Hyperthermophilic Enzymes from *Pyrococcus horikoshii*

<u>Sergei Pletnev</u>^a, Sergei Kozlov^b, Alexander Wlodawer^a, ^aMacromolecular Crystallography Laboratory, National Cancer Institute at Frederick, Frederick, MD USA. ^bCancer and Developmental Biology Laboratory, National Cancer Institute at Frederick, Frederick, MD USA. E-mail: svp@ncifcrf.gov

Pyrococcus horikoshii is hyperthermophilic archaeva that grow at temperatures between 88°C and 104°C with the optimal growth temperature of 98°C. The proteins synthesized by this organism have exceptional heat resistance properties and thus, may be used in different industries including pharmaceutical, food, chemical, paper and others. Structural genomics approach has been applied to determination of crystal structures of a number of these enzymes.

Gene fragments that encode target proteins have been amplified by PCR from cDNA of *P. horikoshii* OT3, complemented with N- or C-terminal His-tags and integrated into pET30a expression plasmid. The resulting constructs have been transformed into *E. coli* strain Rosetta-gami B (DE3) for protein production.

Four out of total nine enzymes have good expression levels. Purification protocols based on metal affinity and size exclusion chromatography have been developed. Typically 50 mg of pure protein suitable for crystallization can be produced from 2 liters of culture. Crystallization trials using nanotechnology robotics have produced encouraging results. Progress on the project will be reported. **Keywords: p. horikoshii, thermophilic enzymes, structural genomics**