# CHARACTERIZATION OF THE CATALYTIC ACTIVITY OF THE HGXPRT FROM PYROCOCCUS HORIKOSHII

Dantas, D.S.[1,2;] Pereira, G.A.G[2,] ; Medrano, F.J[1.]

[1]CeBiME, LNLS, Campinas, SP., [2]Laboratorio de Genômica e Expressão, UNICAMP, Campinas, SP.

Purine phosphoribosyltransferases are involved in the salvage of purine bases. They catalyse the reversible transfer, dependent of magnesium, of a phosphoribosyl group from 5-phospho-α-D-ribosyl 1-pyrophosphate to a purine base releasing pyrophosphate, resulting in the correnponding nucleotide monophosphate. HPRT from P. horikoshii shows significant differences in key residues in three of the four catalytic loops. Here, with the study of this protein from a hyperthermophilic microorganism we would like to get some insights into the sequence rearrangements that a protein should go through to gain the necessary stability to perform its activity at high temperature. We have cloned the gene coding for the HPRT from P. horikoshii in the pET15b expression vector, and expressed in E. coli. The protein was purified in two steps: heating at 75ºC (1 hour) followed by centrifugation to eliminate all the precipitated material and a second step of affinity chromatography. We have carried out steady-state kinetic assays to discover the specificity of this protein. The reaction is more efficient with hypoxanthine, followed by guanine and xanthine, with a great dependence of the catalytic constant with temperature, being more efficient at higher temperatures. We show that this protein is able to salvage hypoxanthine, guanine and xanthine. Further studies will give us insights on the effect of sequence rearrangements on the final structure of the protein.

**Supported by**: FAPESP
**Keywords:** Pyrococcus horikoshii, phosphoribosyltransferase, structural characterization.