# LESSONS FROM THE ANCIENT EVOLUTIONARY HISTORY OF THE PEROXIDASE-CATALASE SUPERFAMILY

Zámocký Marcel[1,2], Kamlárová Anna[2], Obinger Christian[1]

[1] *Department of Chemistry, Division Biochemistry, VIBT – Vienna Institute of BioTechnology, University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria*
[2] *Institute of Molecular Biology, Lab of Phylogenomic Ecology, Slovak Academy of Sciences, Dúbravská cesta 21, SK-84551 Bratislava, Slovakia*

*E-mail: marcel.zamocky@savba.sk*

Four heme peroxidase superfamilies (peroxidase-catalase, peroxidase-cyclooxygenase, peroxidase-chlorite dismutase and peroxidase-peroxygenase superfamily) arose independently in evolution, which differ in overall fold, active site architecture and enzymatic activities. The redox cofactor is heme *b* or posttranslationally modified heme that is ligated by either histidine or cysteine. Heme peroxidases are found in all kingdoms of life and typically catalyse the peroxide dependent one- and two-electron oxidation of a myriad of organic and inorganic substrates [1]. In the present study we have focused on the peroxidase-catalase superfamily that represents the most abundant peroxidase superfamily in all domains of life [1]. Phylogenetic analyses show the occurrence of three well separated structural families (Family I, II and III) and novel clades representing hybrid enzymes between them. The typical overall globular fold of all representatives of this superfamily consists of twelve α-helices and was already acquired from the beginning and only slightly modified in the later steps of ongoing divergent evolution.

Bifunctional catalase-peroxidases (KatGs) from planctonic bacteria are probably the most ancient forms of this superfamily having evolved in numerous bacterial and archaeal phyla. KatGs are homodimeric proteins with each protomer being composed of a N-terminal heme-binding domain and an additional C-terminal (heme-free) domain apparently formed by ancient gene duplication. Only the N-terminal domain harbors the essential amino acids for catalysis and binding of the prosthetic group including a distal triad (Trp, His, Arg) and a proximal triad (His, Asp, Trp). A KatG-typical posttranslational modification that is essential for the *catalatic* activity comprises a covalently-linked adduct Trp-Tyr-Met including a mobile Arg that regulates the redox chemistry of peculiar cofactor. From bacterial KatGs eukaryotic bifunctional catalase-peroxidases evolved independently in fungi and among various protists.

During further evolution the mobile arginine was lost together with parts of the C-terminal domain. Further steps included removal of the covalent adduct (Trp, Tyr, Met) resulting in hybrid A peroxidases, which completely lost the *catalatic* activity [2]. After several speciation events resulting genes coded for highly specialized monofunctional peroxidases (either ascorbate, cytochrome *c*, or manganese & lignin peroxidases). Finally, plant secretory peroxidases and fungal hybrid heme B peroxidases have evolved. The latter contain a N-terminal catalytic domain together with a C-terminal sugar-binding domain. We present this phylogenetic analysis together with studies on *in vivo* & heterologous expression of hybrid B peroxidases. Our research was supported by APVV with grant APVV-14-0375 and by the agency VEGA with Grant 2/0021/14.

## References

[1] Zamocky M., Hofbauer S., Schaffner I. et al. (2015) Arch. Biochem. Biophys. 574:108-119

[2] Zamocky M., Gasselhuber B., Furtmüller P.G., Obinger C. (2014) Cell. Mol. Life Sci. 71:4681-4696