

# Which way to the centre? Structural and evolutionary basis of substrate transport in epoxide hydrolases

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Epoxide hydrolases belong to the wide-spreaded and multifunctional superfamily of  $\alpha/\beta$ -hydrolases. They have eight-stranded antiparallel structure with the central  $\beta$ -strands surrounded by  $\alpha$ -helices. Their active site is buried between the core and the cap domains and connected with the environment by tunnel(s).

To investigate the basis of substrate transport pathways in epoxide hydrolases, we combined the structural and evolutionary analysis. We ran multiple classical molecular dynamics simulations and analysed the flow of water molecules within the protein structure and the catalytic pocket. We used AQUA-DUCT software to investigate the internal architecture of the epoxide hydrolases. Moreover, we compared the obtained data with the information about the size and shape of the substrates catalyzed by analysed proteins.

To investigate the variability of each protein residue and functionally important regions of investigated enzymes, we used BALCONY (Better ALignment CONsensus analYsis), an R package dedicated for analysis of the evolution of amino acids dispersed in sequence. We identified multiple tunnels in all analysed epoxide hydrolases structures, however only one of them is conserved in most cases. The presented approach shed light into evolution of the access pathways leading to the buried active site and provided information useful for drug design and/or protein re-engineering.