Structural investigation into steroid recognition and conversion by the novel testosterone-hydroxylase CYP109E1 from *Bacillus megaterium*

Jóźwik, IK; [1] Kiss, FM; [2] Gricman, Ł; [3] Abdulmughni, A; [2] Brill, E; [2] Zapp, J; [2] Pleiss, J; [3] Bernhardt, R; [2] and Thunnissen, AMWH[1]

[1] Laboratory of Biophysical Chemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands, a.m.w.h.thunnissen@rug.nl; [2] Department of Biochemistry, Saarland University, Saarbrücken, Germany, ritabern@mx.uni-saarland.de; [3] Institute of Technical Biochemistry, University of Stuttgart, Stuttgart, Germany, juergen.pleiss@itb.uni-stuttgart.de

The newly identified CYP109E1 from *Bacillus megaterium* is the first wild-type bacterial P450 monooxygenase catalysing 16β-hydroxylation of testosterone with high regio- and stereoselectivity. Steroids like corticosterone, with bulky C17 substituents, also bind to CYP109E1, but without being converted. To explore the structural basis of selective steroid recognition and hydroxylation, we have determined high resolution crystal structures of both steroid-free CYP109E1 and of complexes with testosterone and corticosterone. The structures revealed a dynamic active site pocket, which is spacious and widely open in the steroid-free enzyme, but undergoes substantial narrowing upon binding single steroid molecules. In addition, a crystal structure was determined of CYP109E1 with four corticosterone molecules simultaneously occupying the open distal heme pocket. Combined structural analysis and data acquired from molecular dynamics simulations suggest that steroid molecules bind in two distinct, approximately 180° reversed orientations relative to the heme-iron (productive and unproductive binding modes). Our structural results together with mutagenesis and enzymatic data provide unique insights into the molecular basis of CYP109E1 activity, substrate specificity, regio- and stereoselectivity.