The many faces of Cyp106A2: How does rational protein design work

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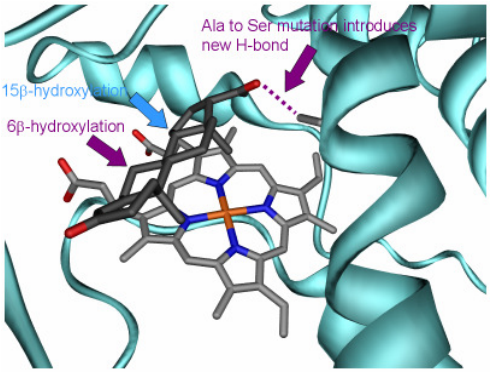
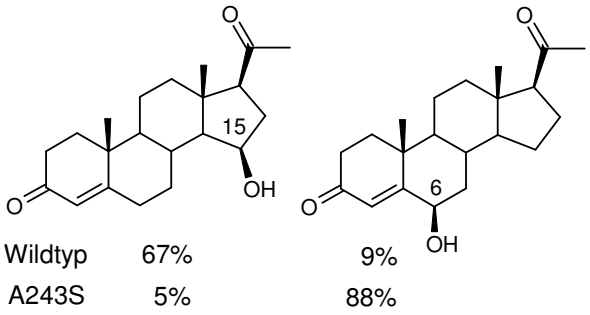
Cytochrome P450 enzymes are not only involved in the metabolism of pharmaceutical drugs, but also in biosynthesis. This can be exploited for the biotechnical production of substances that are otherwise difficult to obtain. CYP106A2 from *Bacillus megaterium* ATCC 13368 is a bacterial steroid hydroxylase that also accepts a variety of terpenoids as substrates. Surprisingly, abietic acid shows a type-II difference UV spectrum, which is typical for inhibitors, and induces a bending of the heme-cofactor. [1] We therefore carried out quantum chemical calculations of the UV/VIS spectrum for bound water and CO as model type-I, respectively type-II ligands. Our results suggest that heme distortion alone causes the unusual spectroscopic behavior.





Progesterone as substrate produces a variety of products whereby 15-OH-progesterone is the major one (67%). [2] To obtain a larger fraction of the minor side product 6β-hydroxy-progesterone we inspected the different docking conformations produced by AutoDock (Version 4.2). [3] Introducing a new hydrogen-bond suggested to stabilize the substrate orientation from which this hydroxylation product was formed. The corresponding Ala243Ser mutant that was subsequently constructed showed 6β-hydroxy-progesterone as selective main product (88%) and a lower fraction of side products (<10%) than the original wild-type form of the enzyme (33%).

[1] S. Janoscha, Y. Carius, M. Hutter, C. Roy D. Lancaster, R. Bernhardt, *ChemBioChem*, **2016**, *17*, DOI:10.1002/cbic.201500524.

[2] T. Sagadin, J.L. Riehm, T. Nikolaus, M.C. Hutter, F. Hannemann, R. Bernhardt, *manuscript in preparation*

[3] G. Morris, R. Huey, W. Lindstrom, M. Sanner, R. Belew, D. Goodsell, A. Olson, *J. Comput. Chem.*, **2009**, *16*, 2785-2791.