INFLUENCE OF MUTATION OF THE TRANSMEMBRANE-HELIX OF CYP17A1 ON CATALYTIC DOMAIN-MEMBRANE INTERACTIONS AND FUNCTION

Ghulam Mustafaa*[a], P. Nandekara[b], Goutam Mukherjeea[c], Rebecca C. Wade[a,b,c]

[a]Molecular and Cellular Modeling Group, Heidelberg Institute of Theoretical Studies (HITS), [b]Center for Molecular Biology (ZMBH), DKFZ, [c]ZMBH Alliance, cInterdisciplinary Center for Scientific Computing (IWR), Heidelberg University, Germany

Human cytochrome P450 (CYP) enzymes play an important role in the metabolism of drugs, steroids, fatty acids and xenobiotics. A subset of CYPs is responsible for steroidogenesis; of these CYP17 is a major drug target for prostate cancer therapy. Human CYPs are anchored to the endoplasmic reticulum membrane by their N-terminal transmembrane (TM) helix. However, the structural and functional importance of the TM-helix is unclear since, on truncation of the TM-helix or modification of the N-terminal amino acid sequence, CYPs can still associate with the membrane and maintain enzymatic activity [1-3]

In the current study, we investigated the effect of mutations in the first 8 N-terminal TM-helix residues of CYP17, originally modified by Imai et al. (1993) to increase the expression of human CYP17 in *E.Coli*, on the orientation and interactions of the globular domain of CYP17 with the membrane. Coarse-grained and all-atom simulations of CYP17 in a phospholipid bilayer were performed. The mutations in the TM-helix, especially W2A and E3L, resulted in amphipathic helix characteristics which led to an unstable TM-helix and gradual drifting of the TM-helix out of the hydrophobic core of the membrane. This instability of the TM-helix also influenced the membrane interactions and orientation of the globular domain, which was compared with experimental measurements for CYP17 in a nanodisc. In some simulations, the mutations led to the TM-helix obstructing the substrate access tunnel from the membrane to the active site, which could affect enzymatic activity.

[1] Barnes, H. J., Arlotto, M. P. & Waterman, M. R, *Proc. Natl. Acad. Sci.* **1991,** *88,* 5597–5601
[2] Imai, T., Globerman, H., Gertner, J. M., Kagawa, N. & Waterman, M. R, *J. Biol. Chem.* **1993** *268,* 19681–9
[3] Shukla, A., Huang, W., Depaz, I. M. & Gillam, E. M. J, *Xenobiotica.*, ***2009***, 39**,** 495–507