

# The basal activity of constitutive androstane receptor (CAR) - Homology modelling versus crystallisation

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The constitutive androstane receptor (CAR) belongs to the superfamily of nuclear hormone receptors that function as ligand-activated transcription factors. CAR plays an essential role in the metabolism of xenobiotics and is involved in drug-drug interactions which makes it an interesting pharmaceutical target. In contrast to most other known nuclear receptors CAR shows constitutive activity for which the structural basis was unknown.

When starting our project no 3D information for CAR was available. Therefore, a homology model of the CAR ligand binding domain (LBD) was established based on the related pregnane X (PXR) and vitamin D receptor (VDR). Molecular dynamics (MD) simulations were carried out to validate the models and to examine the molecular basis for constitutive activity.

Our studies revealed a tyrosine residue to be essential for basal activity of CAR [1]. Docking of known agonists and site-directed mutagenesis (in vitro and in silico) supported the hypothesis of a "molecular mimicry" in which a tyrosine side-chain mimics a bound agonist. Additionally a hydrogen bond was predicted to be essential for constitutive activity which could be verified by experimental studies [2].

Very recently the X-ray structures of human and mouse CAR complexed with various ligands have been solved and published [3]. This gave us the opportunity to verify the quality of the generated homology model. The CAR X-ray structures and the homology model show a nearly identical structural organisation. This is reflected by a low root mean square deviation (1.7 Å) for all atoms forming the ligand binding pocket (LBP). Other relevant stereochemical parameters are also almost identical.

In contrast to the homology model the X-ray structures of CAR show an additional helix (helix X) that has been proposed as main feature for constitutive activity. In addition a specific salt bridge was proposed to be essential for CAR function. The actual role of these features for basal activity is unclear. VDR does not show constitutive activity but also possesses an additional helix X in complex with agonists. Therefore a ligand induced formation of helix X cannot be excluded. MD simulations of the X-ray structures revealed an instability of the salt bridge. The hydrogen bond predicted by homology modelling could not be found in the X-ray crystals but was established during MD simulations.

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## References

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