

Selective modulation of the Farnesoid X Receptor using structure-based methods

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The Farnesoid X Receptor (FXR) is a bile acid-responsive nuclear receptor protein that regulates several pleiotropic target genes; many of which, are pertinent to various metabolic and immune defence pathways. It is highly expressed in the liver and intestines and since its orphanisation in the early 2000s, it has garnered significant pharmaceutical interest as a potential drug target for diseases such as Non-Alcoholic Fatty Liver Disease (NAFLD), which is thought to affect 25% of the world's population [1]. Structurally, FXR is very similar to other nuclear receptor superfamily members, maintaining the classic modular architecture. It comprises a highly disordered *N*-terminal domain, a structurally conserved DNA-binding domain (DBD), and a flexible ligand-binding domain (LBD) at the *C*-terminus. To date, research efforts have focussed on the LBD and the design of synthetic compounds that are structurally analogous to the endogenous ligands, bile acids, in addition to the development of novel ligand scaffolds. Due to the challenges associated with the non-discriminate effects of full FXR activation, research has now moved towards the development of small molecule compounds which act as partial agonists or modulators for the receptor, in addition to finding other approaches for regulating FXR's target genes in a more selective manner [2].

Previous work by our group utilised *in silico* methods to probe the LBD for additional druggable pockets and identified a flexible cavity, adjacent to the classic ligand binding pocket, which could be targeted for allosteric activation of the receptor. A library of bile acid-derived compounds, with extended side chains and different backbone modifications, was screened by *in silico* molecular docking. In combination with cell-based assays, docking results helped to identify 2 lead compounds which sufficiently occupied both the canonical and secondary pockets. Co-crystal structures of the FXR LBD and both compounds supported docking results and identified novel binding mechanisms with residues and helices not engaged by endogenous ligands. Changes in the ligand binding pockets were shown to alter the association with co-regulator peptides, by way of conformational changes at the recruitment site on the LBD protein surface. Furthermore, these compounds were shown to differentially regulate FXR target genes both *in vitro* and *in vivo*. As such, the novel compounds developed here present opportunities to therapeutically target the FXR LBD in a more gene-selective manner.

Further work being done by our group includes using computational and experimental methods to investigate the structure of the DBD and its recognition of specific DNA sequences. A better understanding of how the DBD interacts with DNA motifs will help to elucidate the full extent of FXR's transcriptional control. Moreover, it may allow us to identify and develop novel DBD-targeted pharmacological approaches to selectively modulate FXR and circumvent some of the problems associated with global FXR activation.

[1.] Younossi, Z. *et al.* Global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* **15**, 11–20 (2018).

[2.] Massafra, V., Pellicciari, R., Gioiello, A. & van Mil, S. W. C. Progress and challenges of selective Farnesoid X Receptor modulation. *Pharmacol. Ther.* **191**, 162–177 (2018).