FXR-OMICS:
Identification of Structural Requirements for FXR Binding

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Introduction

The farnesoid X receptor (FXR) is a ligand-activated transcription factor belonging to the nuclear receptors superfamily. [1] It is mainly expressed in liver, intestine, adrenal gland and kidney and its main function consists in the regulation of the expression of various genes involved in cholesterol and bile acids synthesis and metabolism. [2] Moreover FXR is also involved in triglyceride synthesis and metabolism, gluconeogenesis and glycogen synthesis; recently another important role of FXR consisting in the downregulation of some genes involved in inflammation has been discovered. [3] Upon ligand binding, FXR first recruits a co-activator peptide, then forms a heterodimeric complex with the retinoid X receptor (RXR) and the dimer FXR-RXR binds to the specific DNA response elements, regulating the transcription of the target genes. Because of this broad spectrum of regulated genes, FXR is considered a promising but challenging target.

The use of full agonists (or antagonists) will result in activating (or silencing) a considerable number of genes, eventually inducing many side effects. Therefore there is a growing interest in the development of FXR gene-selective modulators: these compounds usually behave as FXR partial agonists and induce the recruitment only of specific co-activators, showing no effects on others. [4] During the last years, several crystal structures of FXR have been reported and deposited in the Protein Data Bank, in complex with agonists and partial agonists. All the structures present the same general architecture but some conformational differences exist between them. With our study we wanted to analyze the structural dissimilarities among the available structures to identify the key residues characterizing the binding profile of full and partial agonists, in the attempt to elucidate the binding mode of gene selective modulators.

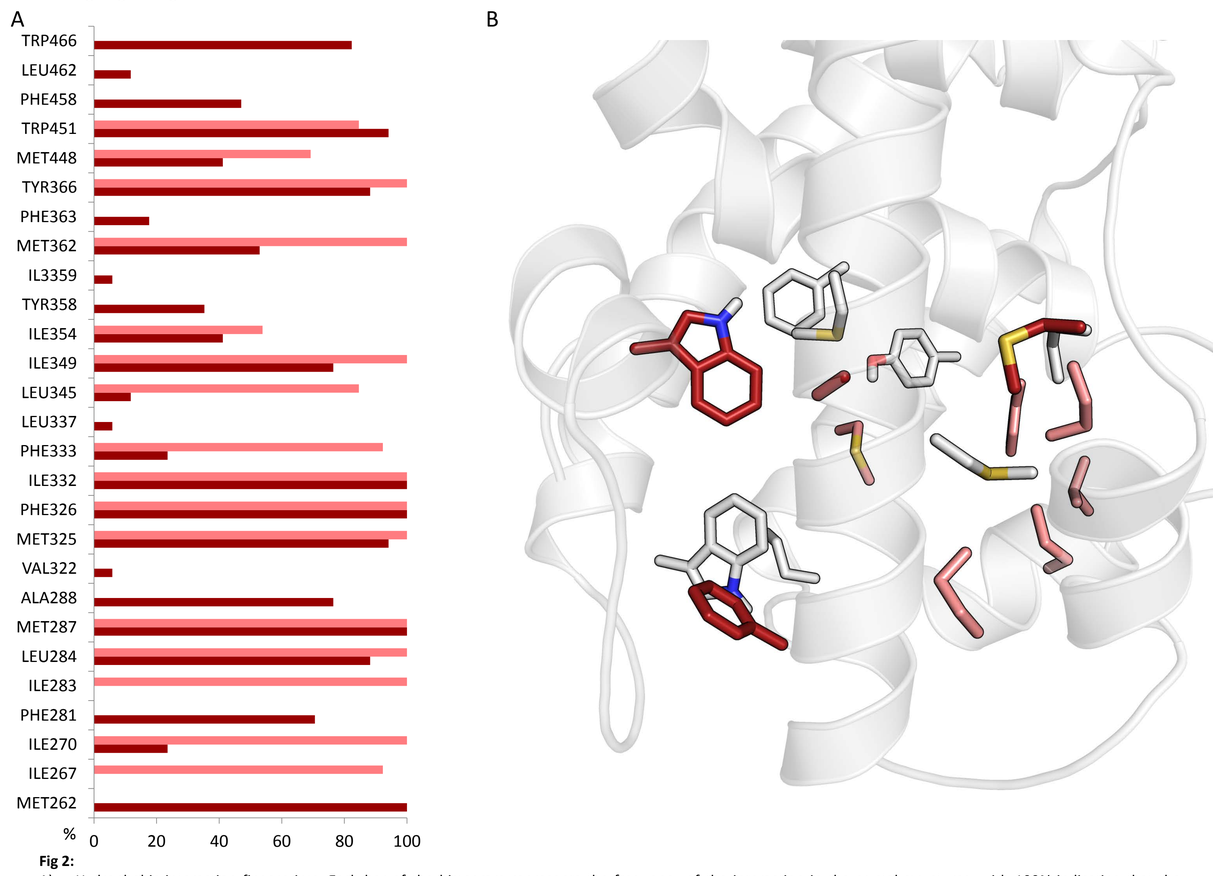


Fig 2:

A) Hydrophobic interaction fingerprints. Each bar of the histogram represents the frequency of the interaction in the crystal structures, with 100% indicating that the interaction is observed in all the crystal structures. In dark red is represented the frequency for agonists, in salmon for partial agonists.

B) Most important residues are highlighted here: in light grey are represented those residues interacting with agonists and partial agonists, in dark red residues interacting only with agonists and in salmon residues interacting only with partial agonists.

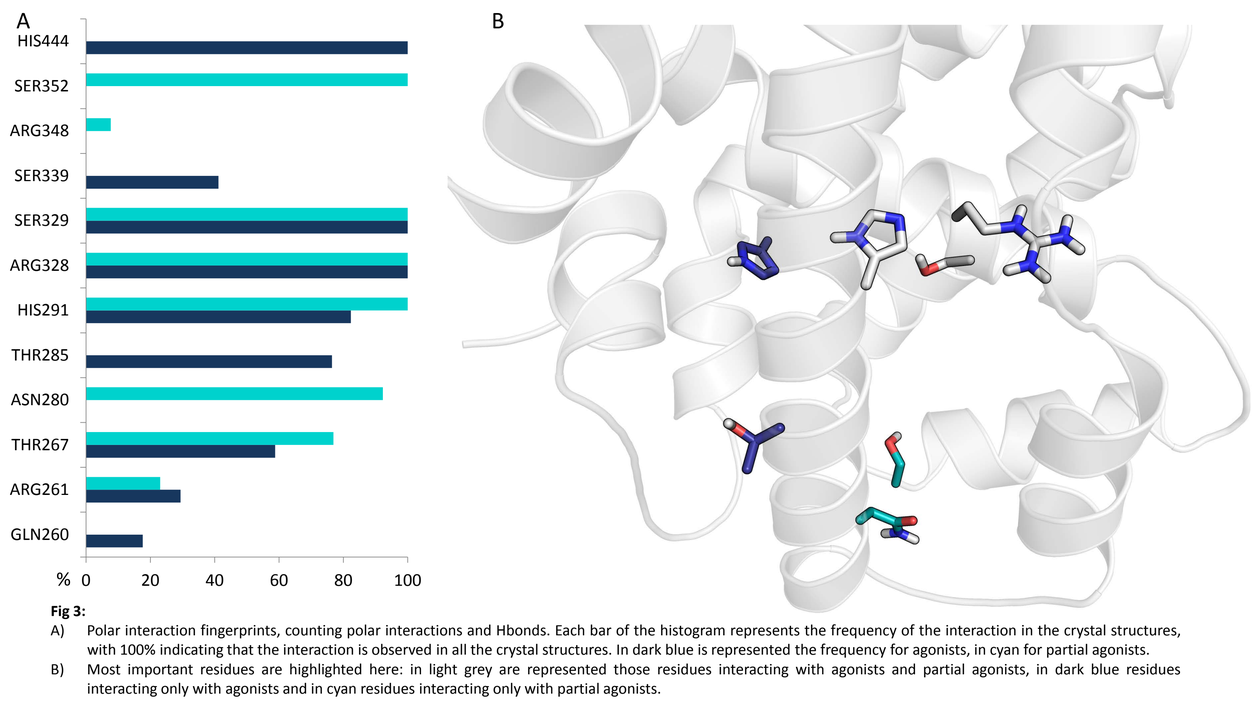


Fig 3:

A) Polar interaction fingerprints, counting polar interactions and Hbonds. Each bar of the histogram represents the frequency of the interaction in the crystal structures, with 100% indicating that the interaction is observed in all the crystal structures. In dark blue is represented the frequency for agonists, in cyan for partial agonists.

B) Most important residues are highlighted here: in light grey are represented those residues interacting with agonists and partial agonists, in dark blue residues interacting only with agonists and in cyan residues interacting only with partial agonists.

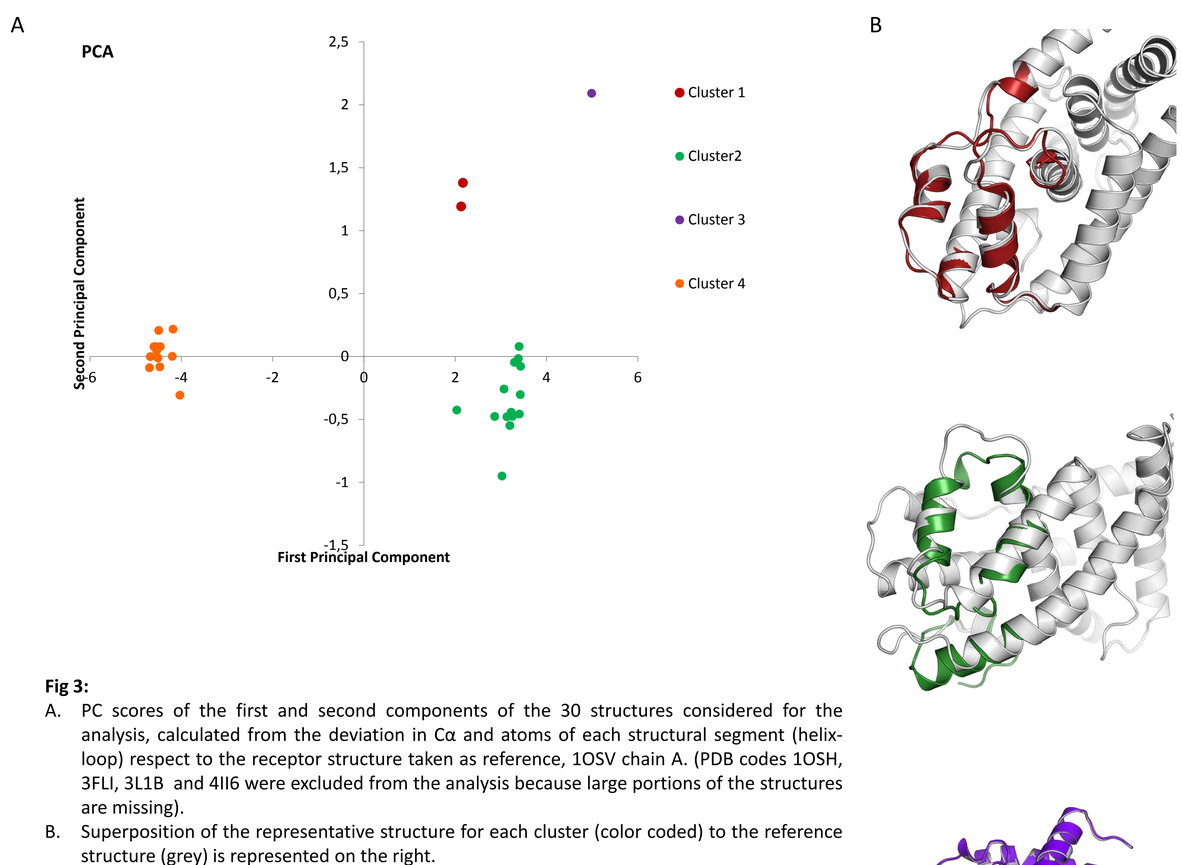
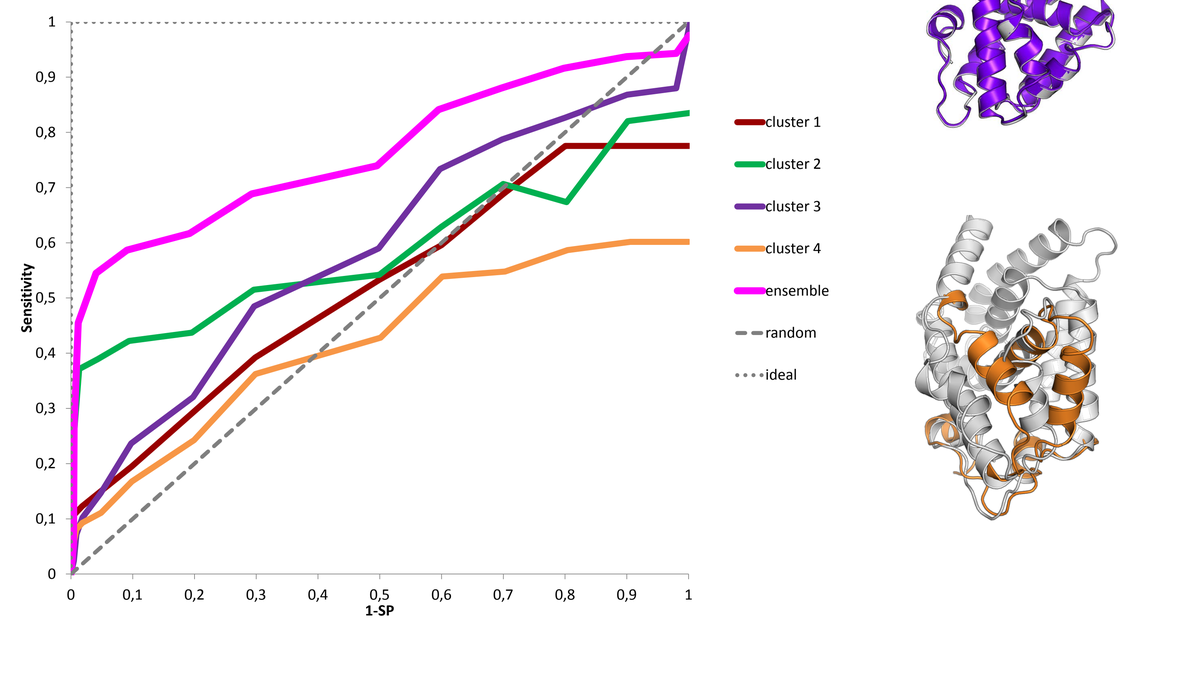


Fig 3:

A. PC scores of the first and second components of the 30 structures considered for the analysis, calculated from the deviation in Cα and atoms of each structural segment (helix-loop) respect to the receptor structure taken as reference, 1OSV chain A. (PDB codes 1OSH, 3FLI, 3L1B and 4II6 were excluded from the analysis because large portions of the structures are missing).

B. Superposition of the representative structure for each cluster (color coded) to the reference structure (grey) is represented on the right.

C. ROC curves for each cluster and for the ensemble.



Results 1

Structural interaction fingerprints (SIFt) have been calculated using Maestro [5] for each crystallographic complex (Fig 2A and 3A). Agonists and partial agonists display some common interactions located in the central region of the binding site, while differ for the interactions established in the distal portions. Both the two classes establish hydrophobic interactions with Met387, Met325, Phe326, Ile332, Ile349, Tyr366 and Trp451 and polar interactions with His291, Arg328 and Ser329. All the co-crystallized agonists preferably occupy the region of the binding site delimited by h3, h11, h12, therefore interacting with residues Phe281, Thr285, Ala288, His444 and Trp466. Partial agonists are located in the region of the binding site defined by h2, h3, h6 and h7 and interact with Ile263, Ile270, Asn280, Ile283, Phe333, Leu345, Ser352, and Met362.

Results 2

PCA and hierarchical cluster analysis allowed us to identify four different clusters: the first one collects the two structures presenting a some structural variation in h2 and h6 compared to the reference structure; the second cluster contains almost all the agonist crystal structures, irrespective of the scaffold of the co-crystallized ligand; the third cluster contains the only structure presenting a 3D structure almost identical to the reference structure and the fourth cluster contains receptors co-crystallized with partial agonists. Crystal structures closer to the center of each cluster were selected to perform a retrospective virtual screening analysis, to evaluate the ability to recognize scaffolds other than the co-crystallized ones and to evaluate the improvement in performances using the ensemble of structures compared to the results using single receptor structures.

Conclusions

✓With this preliminary study we identified which residues are important for FXR binding, and wich residues are crucial for full or partial agonist activity.

✓Partial agonists do not display any interaction with His444 and Trp466 considered to function as the receptor's activation trigger, whereas almost all the crystal structures in the agonist form present interactions with both residues, irrespective of the chemical scaffold of the agonist.

✓We assessed the importance of considering an ensemble of receptor conformations to better describe the conformational space of the binding site.

Future Perspectives

Using MD simulation of FXR bound to agonist, partial agonist and antagonist we are trying to improve the description of the conformational space of the binding site and to further investigate the key residues involved in the binding process of the different ligand classes

References

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