ROLE OF DYNAMICS IN LIGAND-PROTEIN INTERACTIONS: MODELLING OF BINDING TO THE PREGNANE X RECEPTOR

Lara **CALLEA**, Stefano **MOTTA**, Sara **GIANI TAGLIABUE** and Laura **BONATI**
University of Milano-Bicocca, Department of Earth and Environmental Sciences, Milan, Italy
l.callea@campus.unimib.it

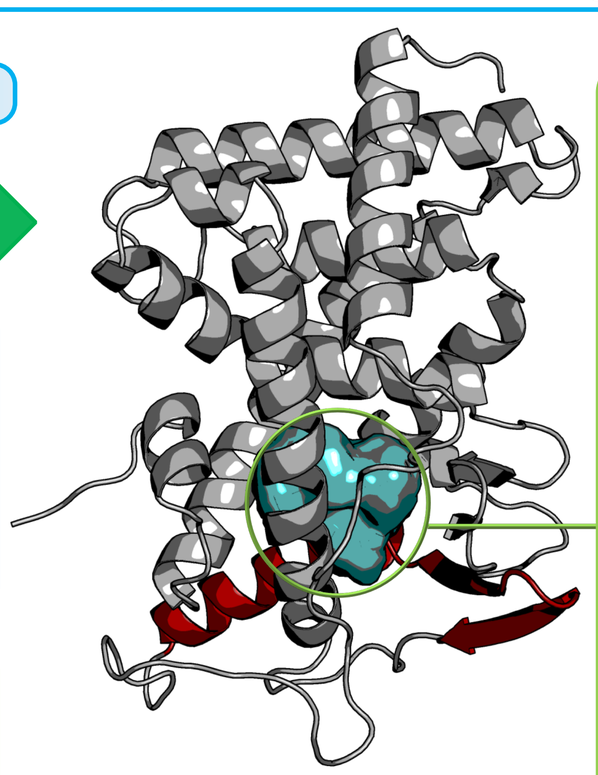
Modelling ligand-protein interactions considering the dynamical behaviour of the system is a challenging task due to the high degree of conformational freedom involved. The study-case here investigated is the binding of a set of ligands to the **Pregnane X Receptor (PXR)**[1], a nuclear receptor activated by a wide spectrum of diverse ligands and involved in the regulation of drug metabolism. The study of ligand binding to PXR is very complex because the ligand binding domain (LBD) is characterized by a large, buried and flexible cavity able to accommodate ligands with different geometries and properties. To this aim we tested different computational methods by using a set of X-ray structures of ligand-PXR complexes as references.

METHODS

DOCKING → ENSEMBLE DOCKING → MOLECULAR DYNAMICS

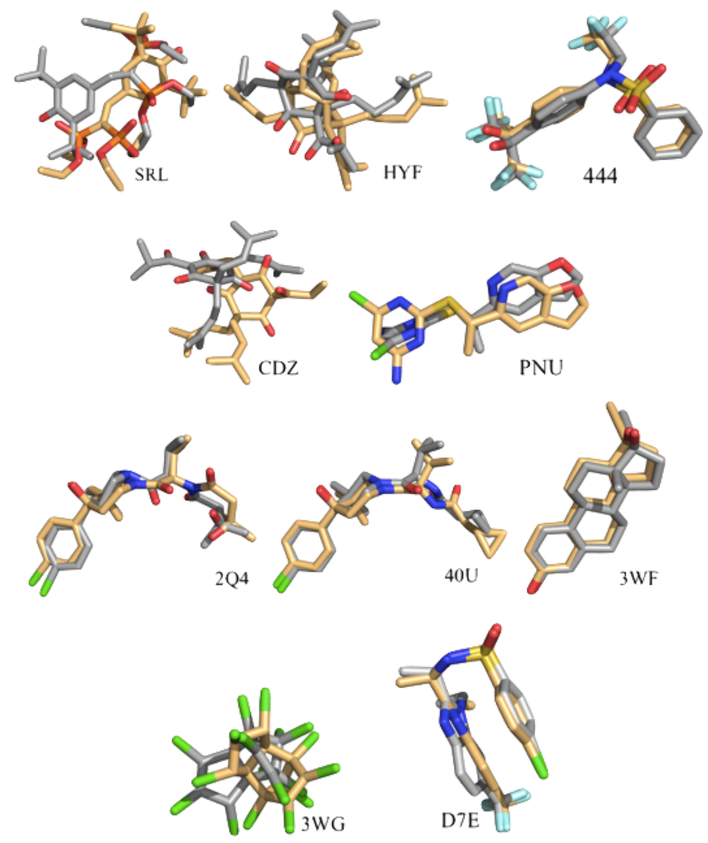
We compared different computational methods that include protein flexibility at different levels: **molecular docking** in which protein is maintained rigid, **ensemble docking**[2] that performs docking to different protein conformations, and methods based on **molecular dynamics** implemented in BiKi[3] software: MD-binding and BiKi Netics tools.

The results obtained from the **docking** calculations, using the apo structure, showed high RMSD values and a poor reproduction of the X-ray geometries. As expected, the limits related to the inclusion of protein flexibility were evident.

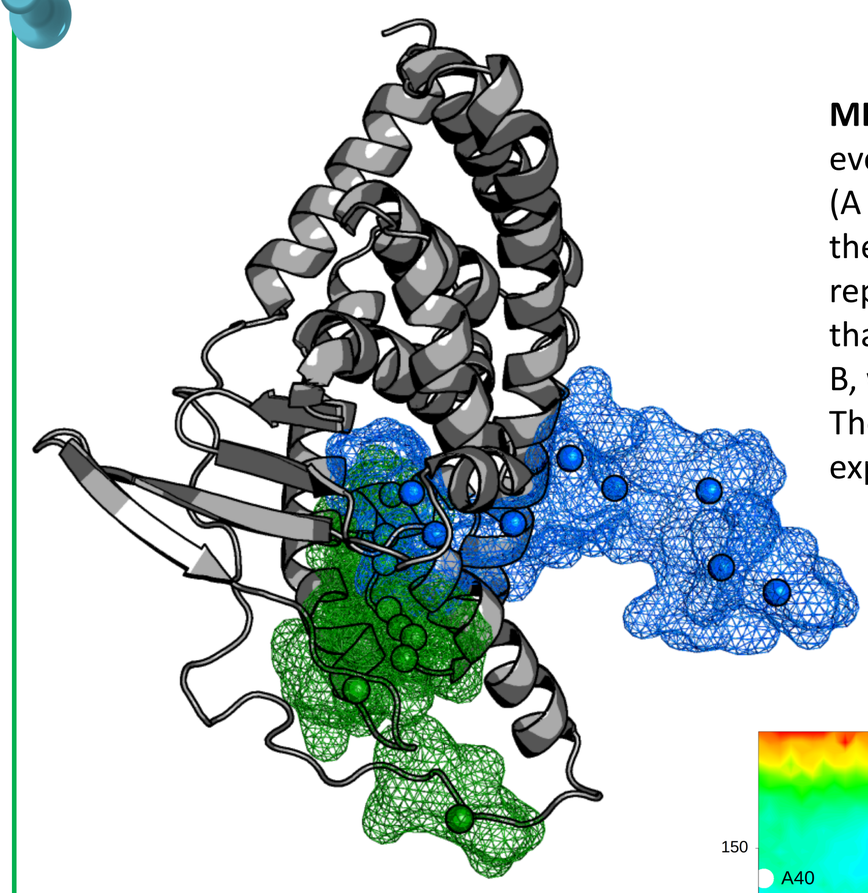


RESULTS

The **ensemble docking** was performed on five X-ray confomations.

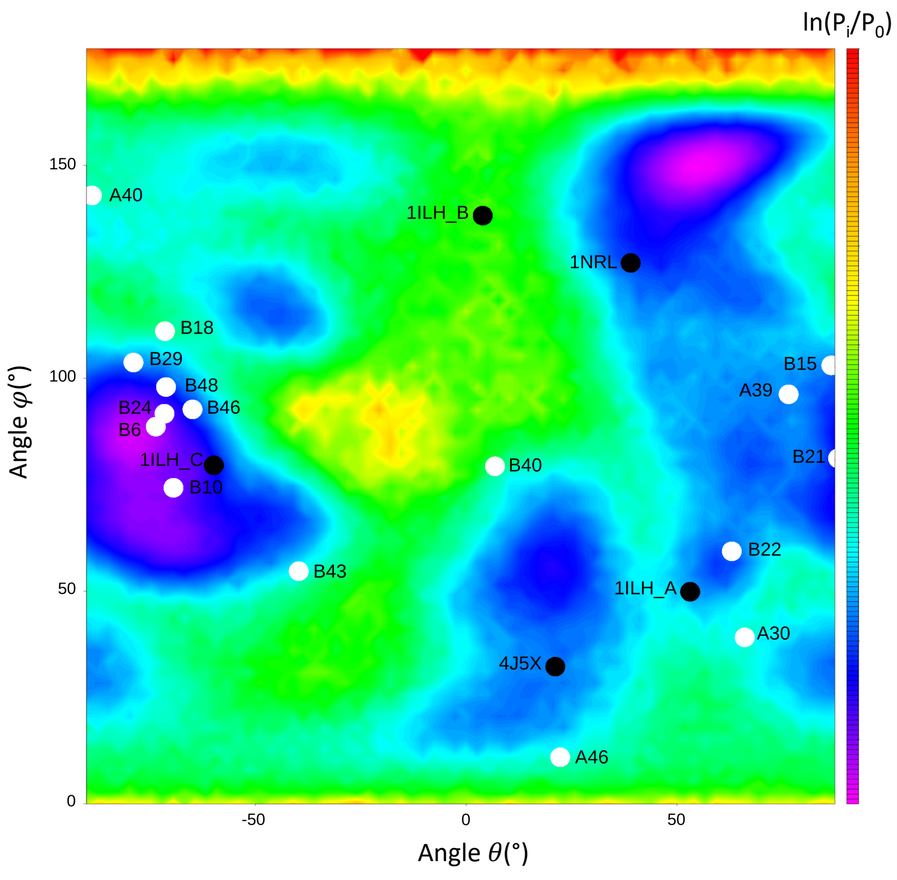


The best poses (light orange) only in some cases well reproduced the X-ray structures (grey).



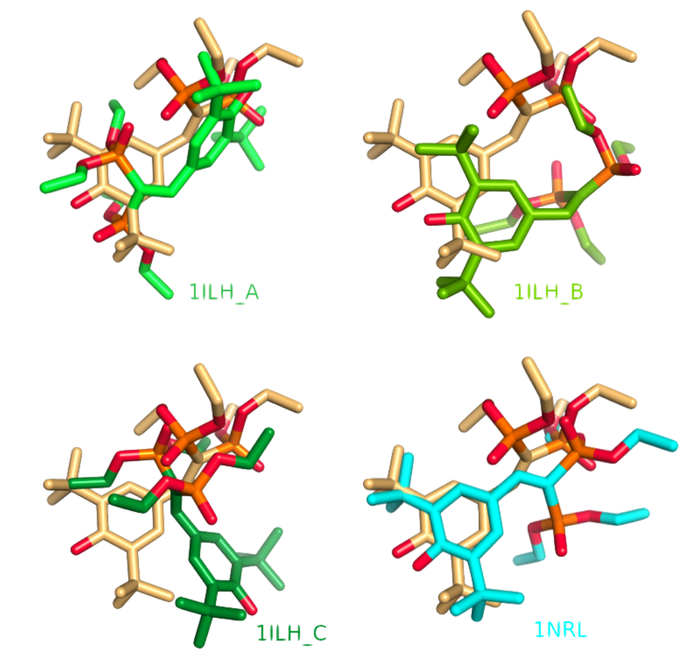
MD-binding[4], used to simulate the binding event, allowed to identify two possible paths (A in green and B in blue) for the entrance of the ligand inside the cavity. For each path, 50 replicas were performed. The results showed that in 84% (42/50) of cases the preferred is B, while only in 16% (8/50) the preferred is A. The sampling was not sufficient to reach the experimental minima (see map).

Starting from the most promising poses obtained with MD-binding, accelerated MD simulations were performed using the **BiKi Netics**[5] tool to extend sampling of the different binding modes and compare their stability.
Cluster analysis on the sampled conformations showed that, starting from different geometries, most of the simulations move close to the most stable 1NRL X-ray structure.
The second cluster confirmed the MD-binding results by approaching the 1ILH_C structure.



Most promising poses obtained with MD-binding (white points) compared to the crystallographic geometries (black points) in a map representing different ligand orientations in the binding geometries. The most sampled regions by BiKi Netics are colored in purple.

Our interest was focused on the ligand SRL.
SRL is present in multiple X-ray structures with different binding modes:



The best pose obtained for SRL in 4J5X (light orange) reproduced the binding mode of the 1NRL structure (cyano) with a RMSD < 1Å.

Conclusions

The use of the three computational methods allowed a deeper understanding of the binding mechanism of the ligands to PXR, by providing complementary information.

References

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