DOCKING TO HOMOLOGY MODELS HIGHLIGHTS THE MOLECULAR DETERMINANTS OF LIGAND BINDING TO THE AHR

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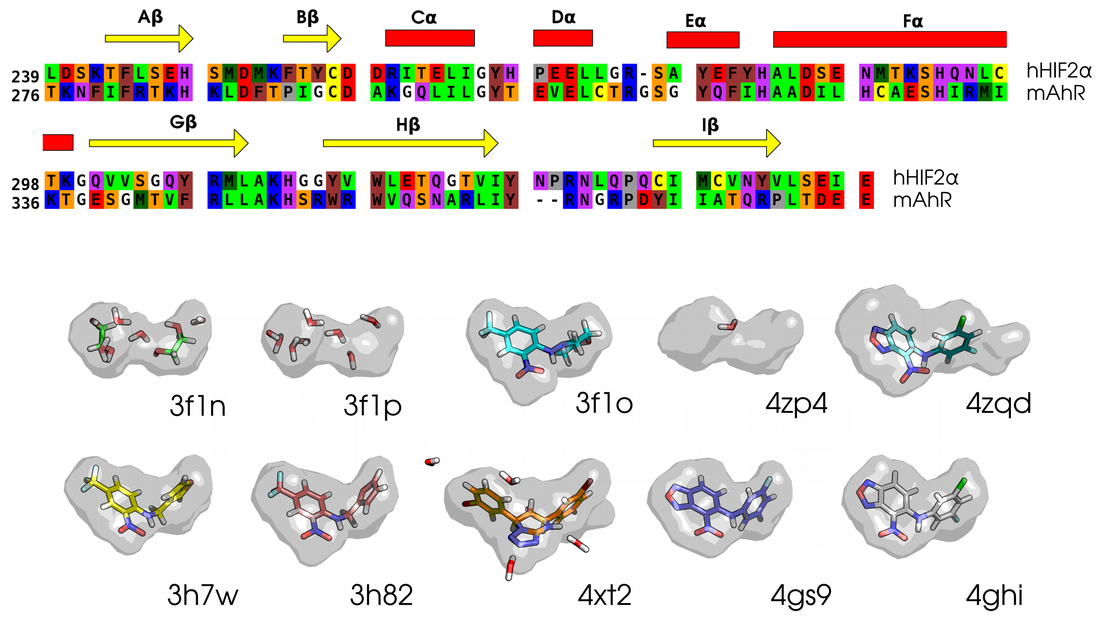
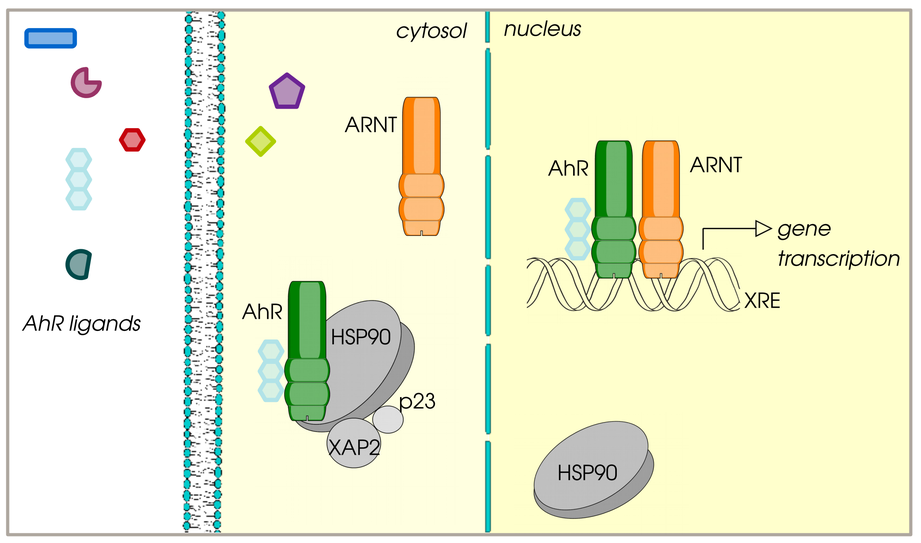
Molecular docking studies can shed light into the molecular determinants of ligand binding. When no experimental structures are available, docking can also be applied to homology models. Given that the model quality, especially in the binding site, greatly affects the accuracy of docking predictions[1], development of strategies able to include protein flexibility[2] may help in the effective use of docking to homology models. In this work, ligand binding of structurally different ligands to the mouse Aryl hydrocarbon Receptor (mAhR) homology model is analyzed.

HOMOLOGY MODELING



No experimental information is available for the AhR LBD but since last years the X-ray structures of many homologous PAS domains have been determined, in particular the Hypoxia-Inducible Factor 2α (HIF2α) shows nearly **30%** of **sequence identity** with the mAhR LBD.
We developed 10 homology models of the mAhR LBD using MODELLER, each one based on a different HIF2α template structure in order to describe the **flexibility** of the **binding cavity**. All the ligands and solvent molecules in the HIF2α internal cavity were maintained during the modeling and refinement stages.

MECHANISM OF ACTION



AhR is a ligand-dependent transcription factor that responds to exogenous and endogenous chemicals with the induction of gene expression and production of diverse biological and toxic effects[3].
The **mechanism is initiated** by **ligand binding to the AhR**, which is present in the cytosol as a multiprotein complex, and the PAS-B domain acts as ligand binding domain (LBD).

RESULTS

Protein **flexibility** and **plasticity** were introduced in both the ensemble docking to multiple homology models and the post-docking MD simulations to obtain an **accurate description** of binding of diverse chemicals within the binding cavity of the receptor. The ΔG_{bind} analysis allowed us to discriminate the **molecular determinants** for binding of the different ligands.

We identified **three main arrangements** within the binding cavity:

1. hydrophobic molecules interact mainly with residues located at the bottom of the cavity (**TCDD**);
2. others occupy the whole cavity and in some cases are stabilized by hydrogen bonds with residues in the middle (**FICZ**);
3. small and polar ligands stay at the entrance (**leflunomide**).

These poses will be validated by a mutagenesis study focused on the key stabilizing residues predicted for each ligand by the computational protocol here presented.

PROTOCOL

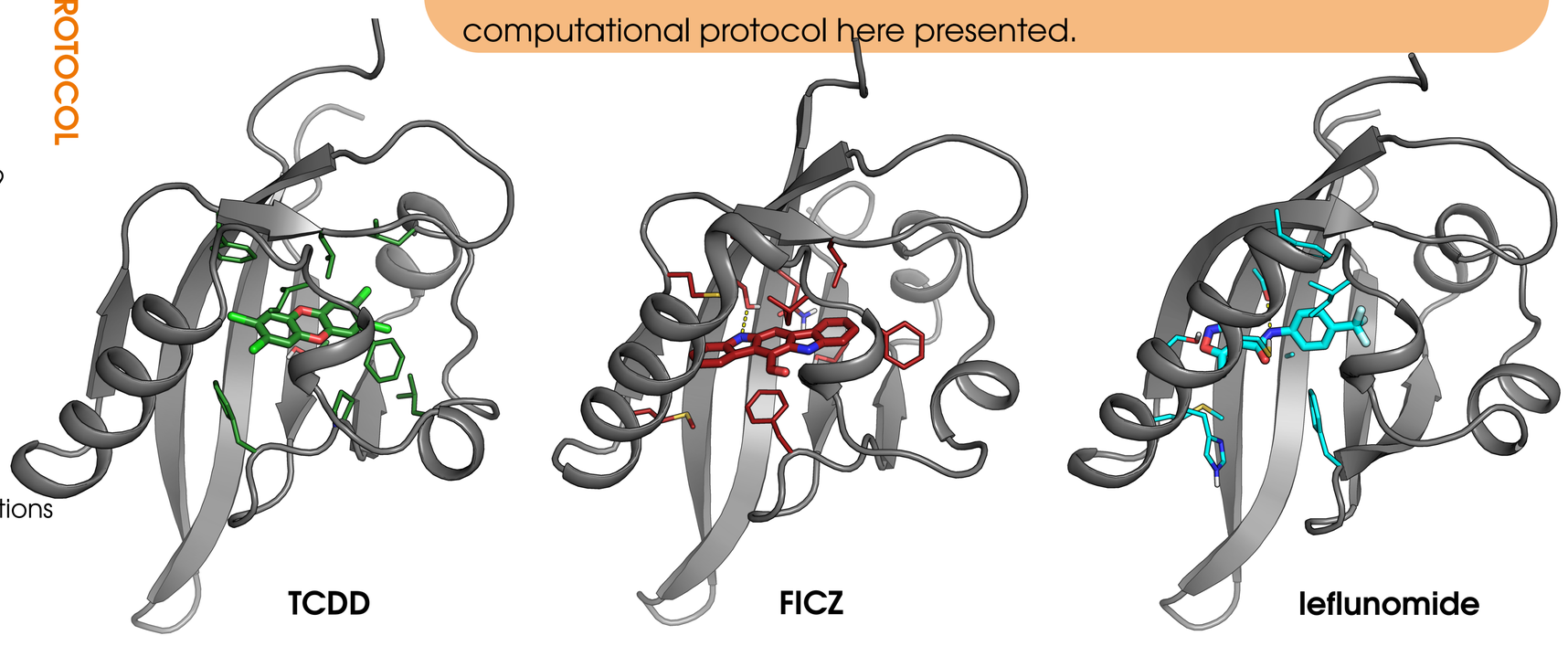
Homology Modeling
Modeller with BLK function
Templates: 3f1n 3f1p 3f1o 4zp4 4zqd 3h7w 3h82 4xt2 4gs9 4ghi
500 models automodel routine
1000 models loopmodel routine
loops: res. num. 310-314+365-369
DOPE score to select the best model

Model Refinement
Macromodel
Internal sidechains refinement.
200 kcal mol^{-1} constraint backbone

Ensemble Docking
Glide XP
docking to different protein conformations
flexible ligand

Pose Refinement
AMBER
for each pose 10 ns or 20 ns
Force field: FF14SB
water model: tip3p

ΔG_{bind} calculation
MM-GBSA implemented in AMBER
single trajectory
per-residue decomposition analysis



REFERENCES

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2. Fan H, Irwin J & Webb B. Molecular docking screens using comparative models of proteins. J Chem Inf Model 2512–2527 (**2009**)
3. Bonati L, Corrada D, Giani Tagliabue S & Motta S. Molecular modeling of the AhR structure and interactions can shed light on ligand-dependent activation and transformation mechanisms. Curr Opin Toxicol 2, 42–49 (**2017**)

This research was supported by the National Institute of Environmental Health Sciences (USA)(R01ES007685)