MOLECULAR MODELING TO PREDICT THE LIGAND BINDING KEY EVENT

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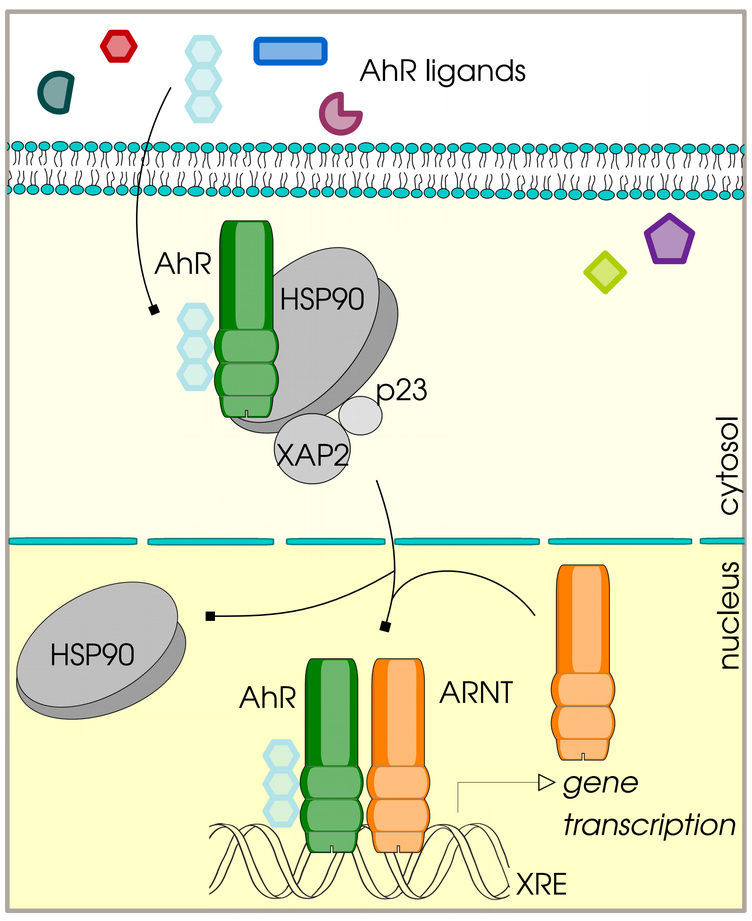
Toxic effects of a molecule can be explained by the Adverse Outcome Pathway model (AOP); the molecular initiating event (MIE) can be the interaction of the molecule with a biological target, a protein for example. After the initial interaction many Key Events (KE) can link the MIE to the Adverse Outcome (AO)[1]. It is useful to describe also the **Key Event Relationship** to assess the causal nature between two measurable biological events[2]. Our focus of study is the Aryl hydrocarbon Receptor (**AhR**), a PAS-containing 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]. Our in-depth study is on the MIE, the ligand binding.

MIE	KE1	KE2	KE3	KE4	KE	Adverse Outcome
						tissue- organ- species- specific
ligand bindng	nuclear translocation	AhR:ARNT dimer formation	AhR:ARNT DNA binding	gene expression (*cyp1a1*)	unknown mechanisms	death, wasting syndrom, oxidative stress, immuno&development toxicity, liver tumor, etc.
		molecular				cellular, organ, individual

mechanism of action



The mechanism is initiated by ligand binding to the cytosolic AhR, then it translocates into the nucleus where it heterodimerizes with its homologous protein ARNT. The dimer binds the xenobiotic-responsive element (XRE) and finally activates the transcription of some target genes (ex. *cyp1a1*)[4].

The AhR domain responsible for ligand binding is the PASB, unfortunately no experimental information is available for this domain.

Anyway it is possible to achieve a structural model by **homology modeling**, *i.e.* a technique that exploits the structural information contained into homologous proteins. The Hypoxia-Inducible Factor 2α (**HIF2α**) dimerizes with the same partner (ARNT) and shows nearly 30% of sequence identity with the mAhR PASB. It was used as template for developing a model with *MODELLER*.

A set of **different AhR agonists** (including toxic and endogenous compounds) were docked into the homology models with *Glide XP*. To include protein flexibility we used the **ensemble docking** approach, *i.e.* docking to multiple protein conformations.

The obtained poses were subjected to **molecular dynamics** simulation to relax the complex. In order to get the most stabilizing contributions we calculated the binding free energy (**ΔGbind**) with the MM-GBSA approach implemented in *Amber* and the ΔGbind was decomposed into **per-residue contributions**.

computational protocol

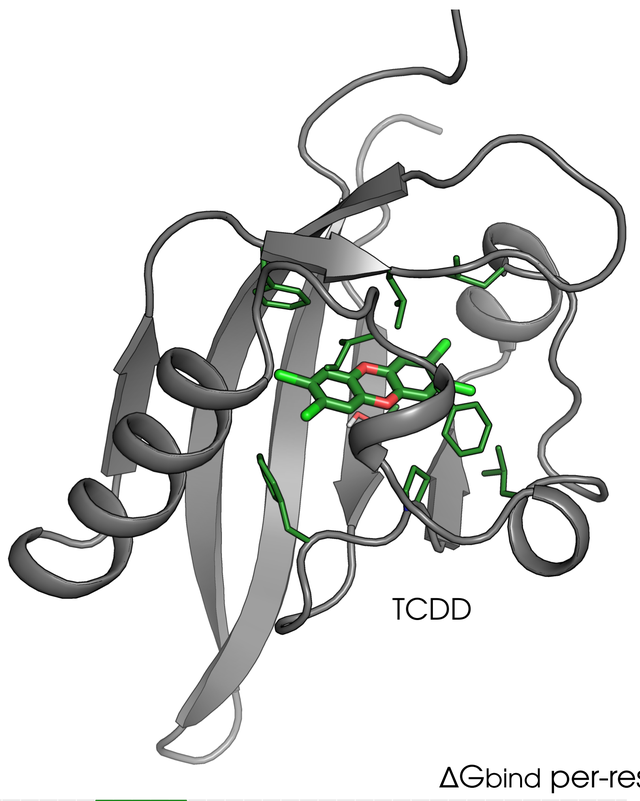
Homology Modeling *modeller*

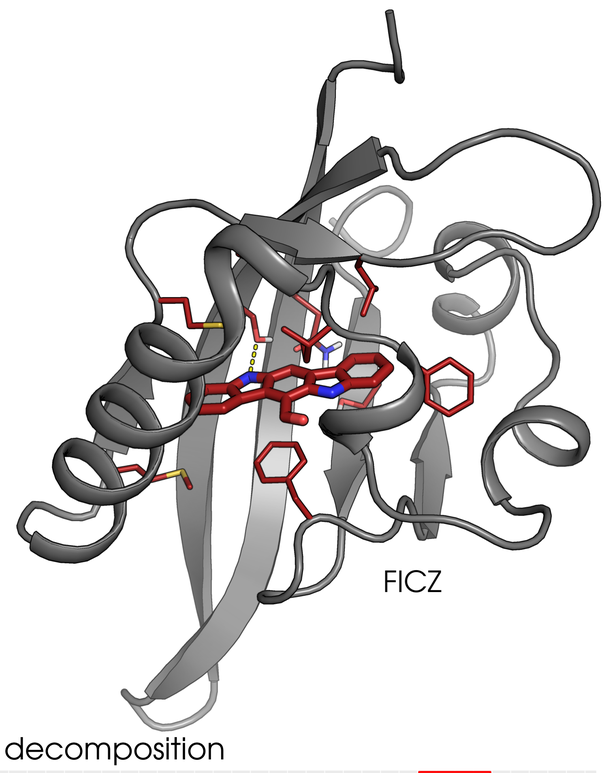
↓

Ensemble Docking *glide XP*

↓

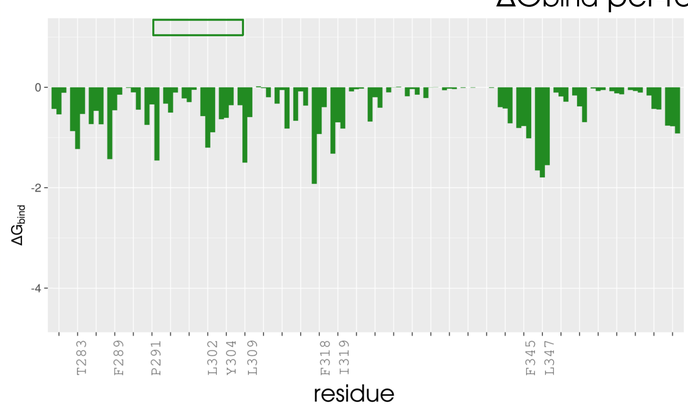
Molecular Dynamic *amber*

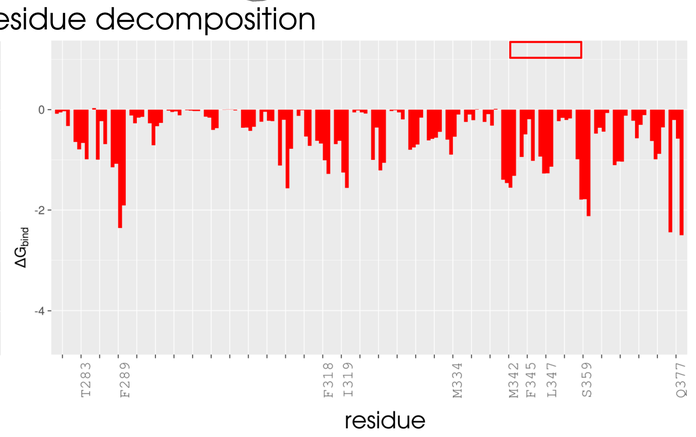




Docking poses and most stabilizing residues. TCDD is the most toxic AhR ligand, and FICZ is an endogenous compound that binds AhR without causing toxicity.

ΔGbind per-residue decomposition





The analysis of specific binding interactions allowed us to identify a subregion of the cavity typical for each group of ligands.

For example TCDD reaches the bottom of the cavity, FICZ establishes an hydrogen-bond at the entrance of the cavity.

Molecular Modeling already allowed a deep comprehension of the key molecular events regulating the mechanism of ligand-dependent and ligand-specific AhR activation[4].

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references

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