# Unraveling Dimerization Interfaces of PAS Domain Dimers: Lessons from the AhR/ARNT Complex.

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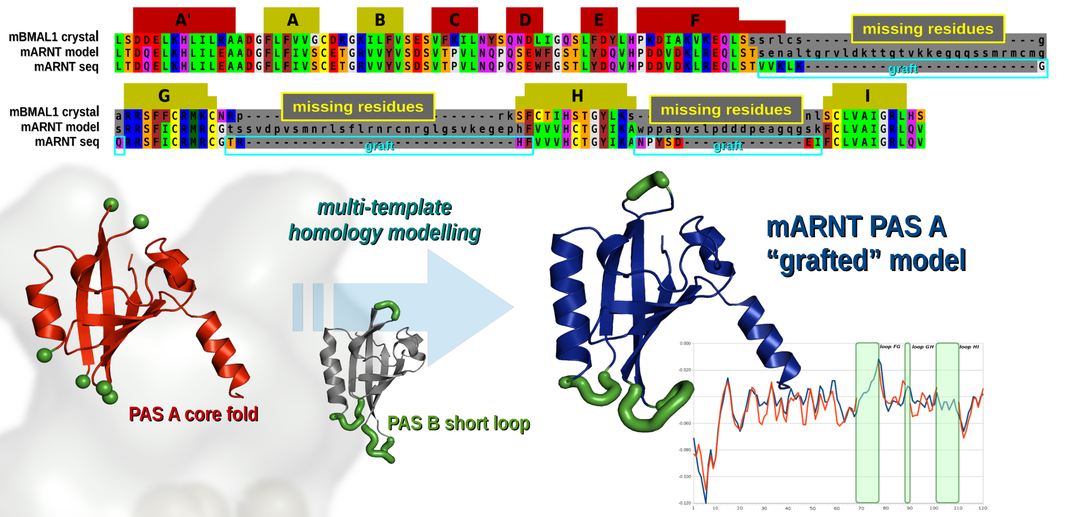
*The Aryl hydrocarbon Receptor (AhR) is a trancription factor that belongs to the bHLH-PAS family. It is activated by binding to a wide range of xenobiotics, including polycyclic- and halogenated-aromatic hydrocarbons. Upon ligand binding, it dimerizes with the bHLH-PAS partner protein AhR Nuclear Translocator (ARNT) and initiates a detoxification pathway by inducing the expression of the related genes The characterization of the molecular mechanisms on how AhR can trigger such pathways requires the structural characterization of the Per-Arnt-Sim (PAS) region, composed by a tandem repeat of two PAS domains (PAS-A and PAS-B), that is involved in both ligand binding and dimerization. Unfortunately, these domains have so far proved difficult to produce in large-scale expression studies and therefore they have been analysed using homology modelling techniques.*

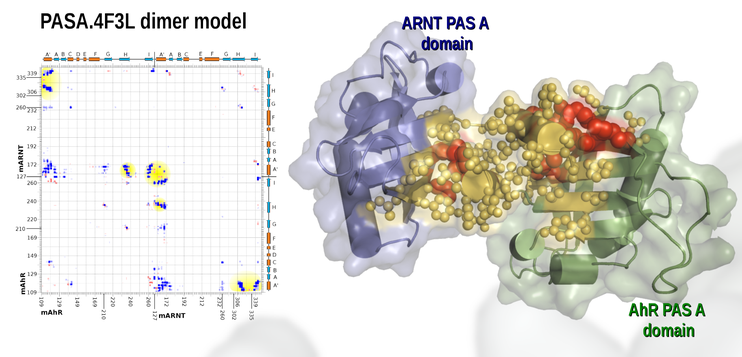
## Model Building and PAS Dimer Assembly

The PAS-B AhR model was already proposed and validated in our previous work [1], the PAS-B ARNT domain was modeled from a crystallographic template completely resolved. By contrast the avalaible templates for both the PAS-A domains show missing regions related to very extended loops that may introduce high structural noise using an *ab-inito* loop refinement strategy. Considering the highly conserved structural folds between PAS-A and PAS-B domains, the shorter topologically equivalent PAS-B loops were grafted onto the PAS-A domains The DOPE profile of the models well overlaps the profile of the ones of the original template structures, indicating that the inserted loops do not perturb the overall fold of the models. Subsequently, the whole PAS-A and PAS-B dimer models were assembled from the individual domain models. The dimer models were assembled on four alternative scaffolds: the murine CLOCK/BMAL1 heterodimer, including the PAS-A and the PAS-B dimerized domains (PDB 4F3L), the murine AhR homodimer (PDB 4M4X) and the human HIF2α/ARNT heterodimer (PDB 3F1P). In the following, such models will be termed ***PASA.4F3L, PASA.4M4X, PASB.4F3L, PASB.3F1P***, according to the structural template adopted.The extension of the dimerization interface was evaluated through the calculation of variation in total Solvent Accessible Surface Area (ΔSASA).




## Dimerization Interface and Energy Decomposition Analysis

The evaluation of the dimerization interfaces (residues as yellow spheres) reveals that both the PAS-A dimer models share similar extension, involving similar subset of secondary structural elements. By contrast the PAS-B dimer models show really different dimerization interfaces, in which the PAS-B AhR domain offer two opposite sides for binding to ARNT, accounting for the altrenative dimerization modes proposed by CLOCK/BMAL1 and HIF2α/ARNT complexes, respectively.

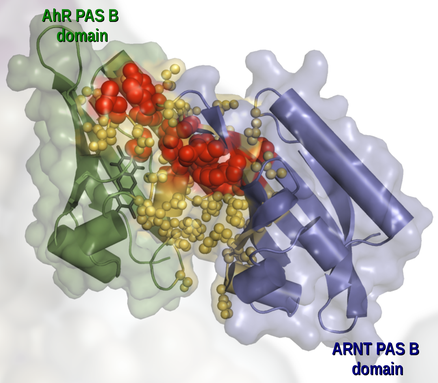


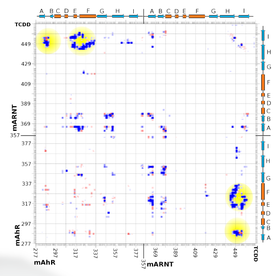


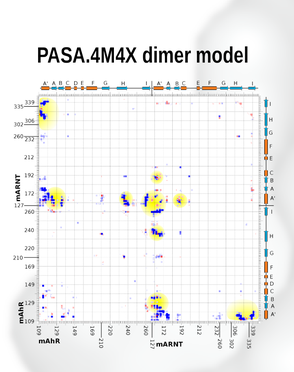


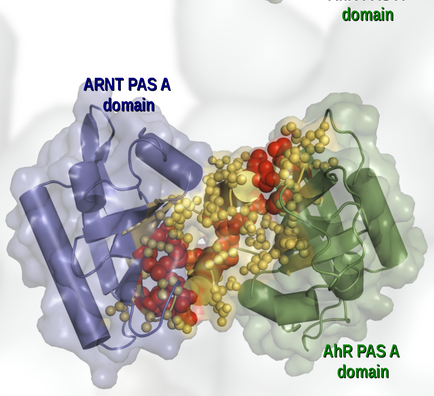
### PAS-A dimer models

The binding free energy of each dimer model was evaluated by means of the MM-GBSA method. In order to detect the energetic couplings that mainly contribute to the definition of the overall $\Delta G_{binding}$ the approach of Energy Decomposition Analysis was performed [2]. In PAS-A dimer models the energetic couplings (residues as red spheres) mainly involve the A' α helix of AhR and the H, I ß strands of ARNT (and viceversa). In the case of **PASA.4M4X** dimer model the more compact interface emphasizes energetic coupling between the A' α helices of both domains.



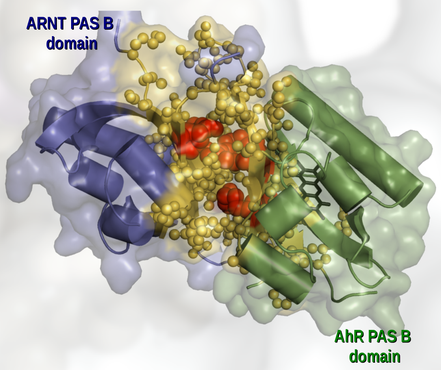



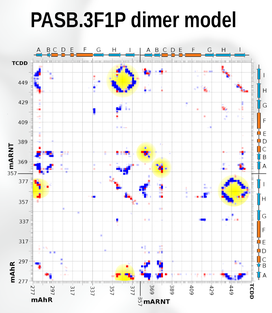




### PAS-B dimer models

The energetic coupling of the model **PASB.4F3L** seems to span the overall interface, where the HI loop of ARNT take contact with the AB loop and the E, F α helices. The dimerization interface of the model **PASB.3F1P** mainly involve the A, H, I ß strands of both the domains, but few stabilizing couplings seem mapped in the most buried portion of the interface. Also, the interaction energy matrix depicts sparse regions that define a destabilizing contribution to the definition of the overall $\Delta G_{binding}$ (red spots on the matrix).




## Rank Products and PPI Hot Spot Prediction

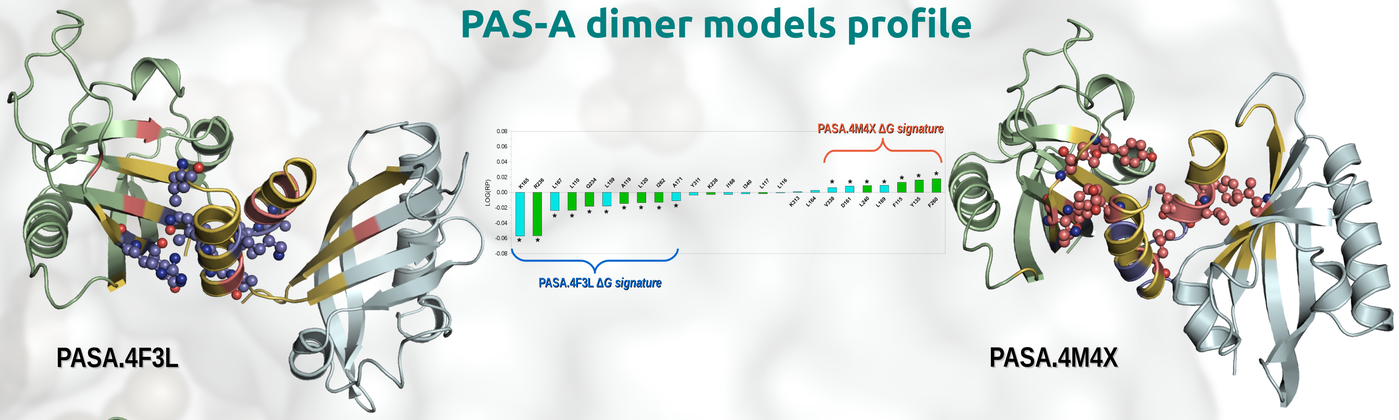
The per-residue contributions to the $\Delta G_{binding}$ of the alternative dimerization modes of PAS-A or PAS-B dimer models were directly compared each other, using a novel approach based on the *rank products* algorithm [3]. Each dimer model is characterized by a Δ*G signature* that emphasizes the pattern of residues which contribution to the binding free energy is sgnificantly different.

The adoption of PPI hot spots prediction algorithms further refined these patterns by highlighting residues that may have a disrupting effect on dimerization if mutated. The PPI hot spot prediction was obtainedobtained from tools based on *in silico* alanine scanning (Robetta[4]), machine learning approach (KFC2[5]), or potential contact scoring function (HotPoint[6]).

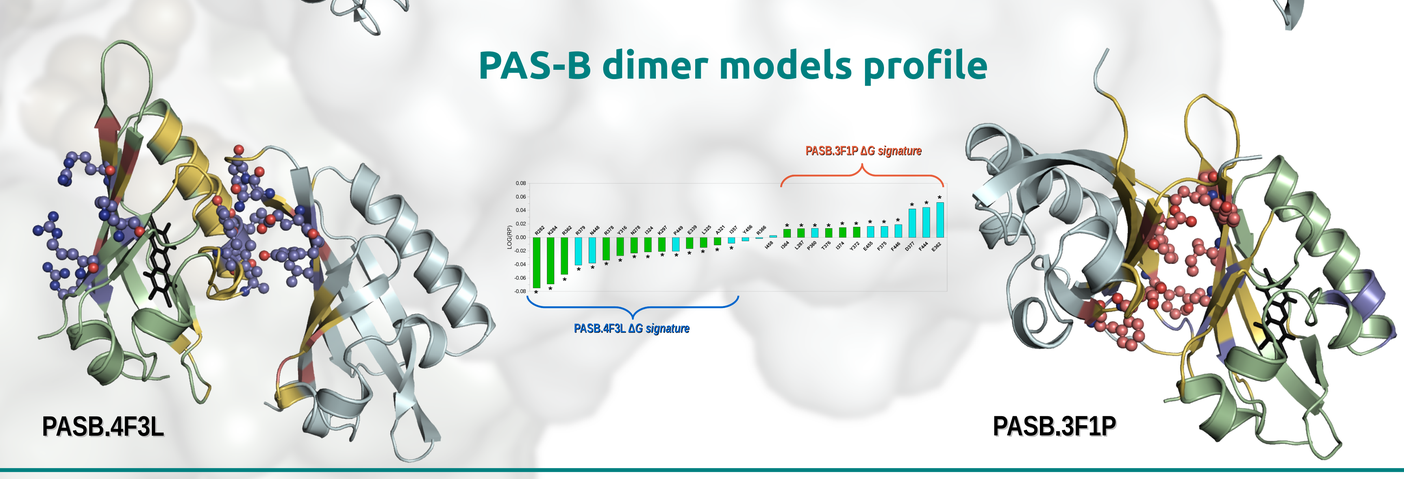
Residues that are predicted as PPI hot spots and belong to a Δ*G signature* define topological positions that can selectively affect the stabilization of a specific dimer model. This is particobtainedularly relevant to discriminate between alternative dimer models that share similar interfaces, such as the PAS-A dimer models.

On this basis a list of 23 residues was found, whose stabilizing effect is peculiar for each dimerization interface. In this perspective, a set of experimental mutagenesis assays is planned to identify and validate which of the proposed alternative dimerization modes is the most reliable.

### PAS-A dimer models profile




### PAS-B dimer models profile

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