Unraveling PPI hot spots for PAS domains dimerization: the case of Aryl hydrocarbon Receptor.

Dario CORRADA, Laura BONATI

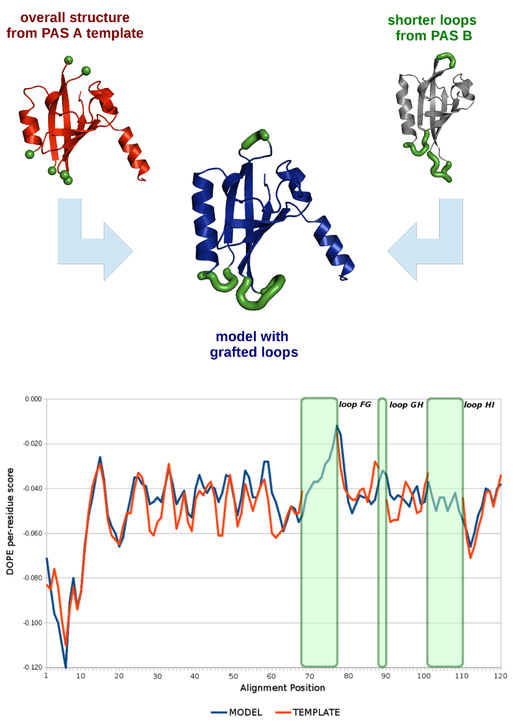
Dipartimento di Scienze dell'Ambiente e del Territorio e di Scienze della Terra
Università degli Studi di Milano-Bicocca, Milano, Italy

MOTIVATIONS

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, which is composed by a tandem repeat of two PAS domains (PAS-A and PAS-B). 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. Modelling of the murine AhR::ARNT dimer models was performed starting from homologous complex of bHLH-PAS proteins: the murine CLOCK::BMAL1 heterodimer, including the PAS-A and the PAS-B domains (PDB 4F3L), the murine AhR homodimer (PDB 4M4X) and the human HIF2a::ARNT heterodimer.

METHODS

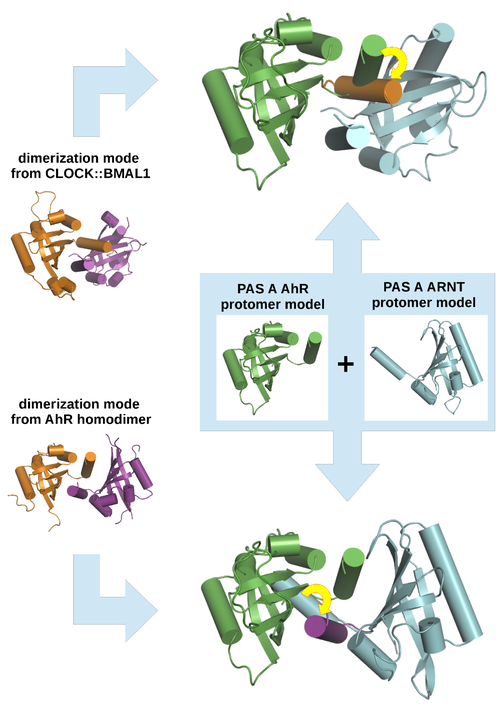
1. Protomers Modelling



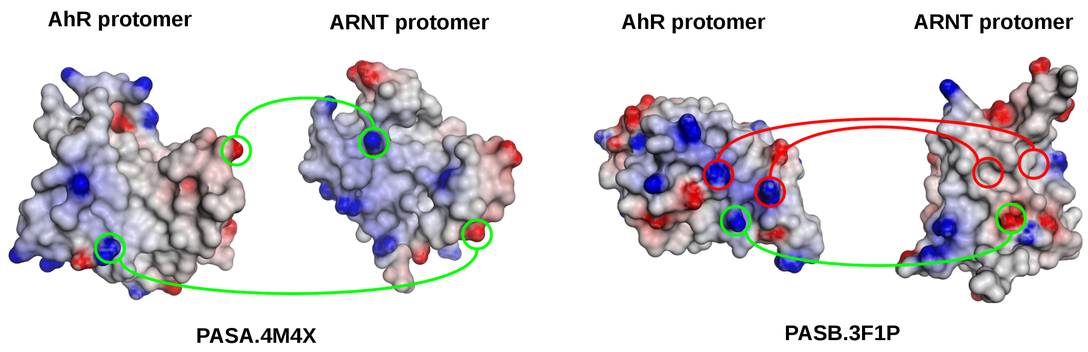
The individual PAS domains were built separately. While the PAS-B domains were built starting from completely resolved structural templates, the experimental structures for modelling the PAS-A domains showed unresolved regions (more than 20 residues) that maps far away from the dimerization interface. Considering the highly conserved structural folds between PAS-A and PAS-B domains, the shorter topological equivalent PAS-B loops were grafted onto the PAS-A domains. The DOPE profile of the models well overlap the profile of the ones of the original template structures, indicating that the inserted loops do not perturb the overall fold of the models.

2. Dimer Models Assembly

The PAS protomers models were orientend each other superposing them onto the 3D structure of the dimer structural templates Two alternative models were produced for the PAS-A dimer (from the CLOCK::BMAL1 complex and the AhR homodimer), and two for the PAS-B dimer (from the CLOCK::BMAL1 and the HIF2α::ARNT complexes). In order to remove steric clashes derived from a suboptimal fitting, between the protomer models and the dimer templates, some secondary structural elements need to be arranged. In the case of the PAS-A dimer models the spatial orientation of the N-terminal α helices A' were adjusted accordingly to the structure of the templates adopted.

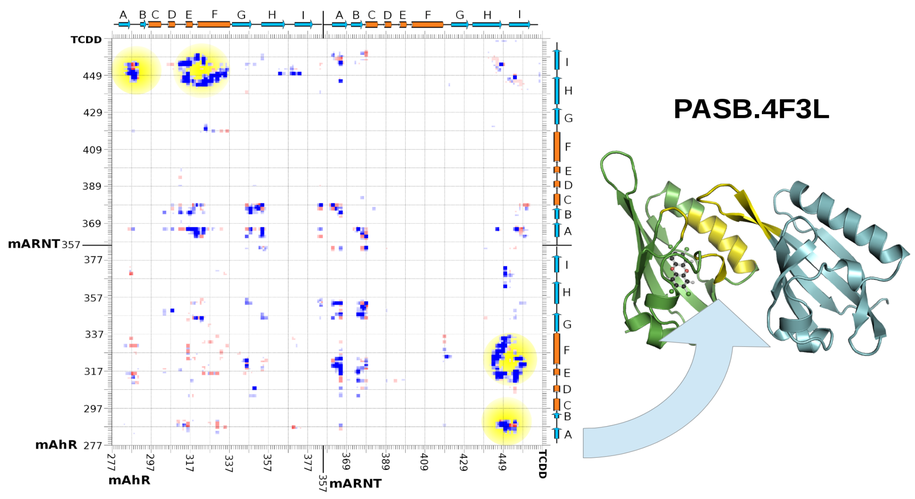


3. Electrostatic Potential Surface

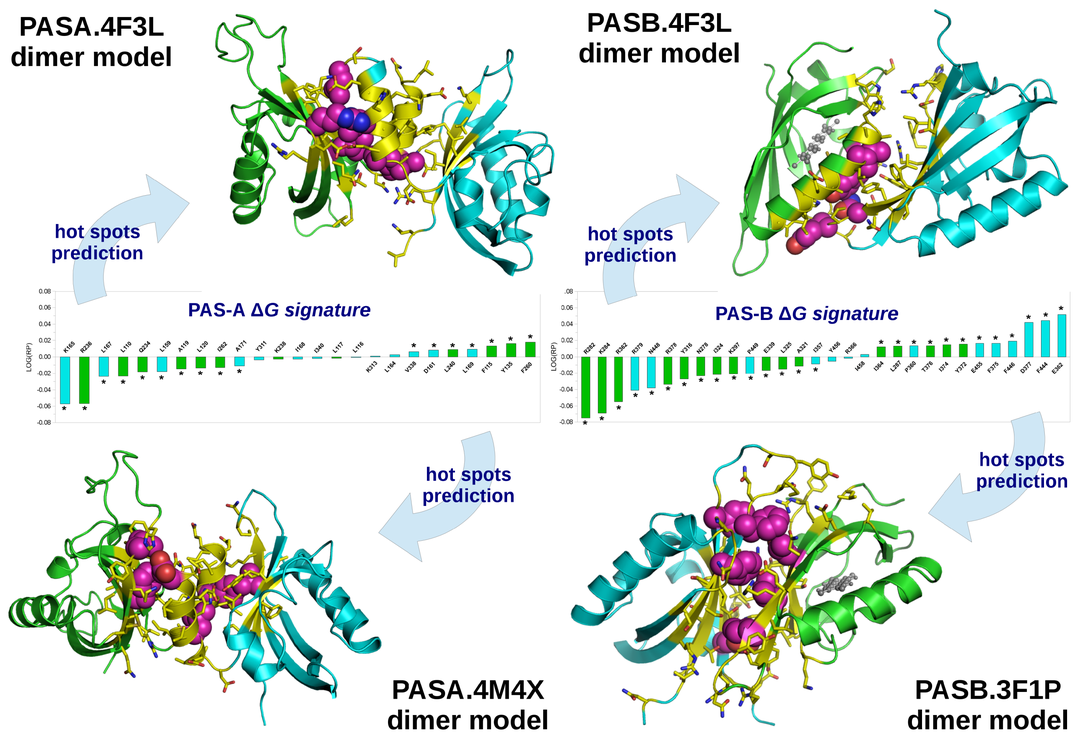


The extension of the dimerization interface was evaluated through the calculation of variation in total Solvent Accessible Surface Area (ΔSASA). The Electrostatic Potential Surface allowed to evaluate the complementarity of charged/neutral regions along the dimerization interface. It should be noted that in the PASB.3F1P dimer model the AhR protomer exhibits a charged interface toward the mainly neutral interface of the ARNT protomer.

4. Energy Decomposition Analysis



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 Δ*Gbinding* the approach of Energy Decomposition Analysis was performed [1]. Even if the relevant differences in the spatial arrangement of the α helices A', the stabilization of the dimerization interfaces of both PAS-A dimer models is governed by energetic coupling that partially overlap.



RESULTS

The per-residue contributions to the $\Delta G_{binding}$ of the alternative dimerization modes of PAS-A or PAS-B dimer model were directly compared each other, using a novel approach based on the *rank products* algorithm [2]. Each dimer model is characterized by a Δ*G signature* that emphasizes the pattern of residues which contributtion to the binding free energy is sgnificantly different.

The adoption of PPI hot spots prediction algorithms further refined these patterns in order to highlight those residues that may have a disrupting effect on dimerization if mutated. The PPI hot spot prediction was obtained from tool based on *in silico* alanine scanning procedure (Robetta[3]), machine learning apprioach (KFC2[4]), or potential contact scoring function (HotPoint[5]).

Residues that are predicted as a PPI hot spots and belong to a Δ*G signature* define those topological positions that can selectively affect the stabilization of specific dimer model, especially in the case of alternative dimer model that share similar interfaces, such as the PAS-A dimer models.

A list of 23 residues was found, whose stabilizing effect is peculiar for each dimerization interface. In this perspective, a set ofexperimental mutagenesis assays is planned to identify and validate which of the proposed alternative interfaces will be the most reliable.

REFERENCES

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