Structural and Functional Characterization of PAS Domain Dimers: the Aryl Hydrocarbon Receptor Paradigm

Dario CORRADA, Laura BONATI dario.corrada@unimib.it

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

MOTIVATION

The Aryl hydrocarbon Receptor (AhR) is a transcription factor activated by a wide range of xenobiotics. Upon binding to such ligands, it dimerizes with the AhR Nuclear Translocator (ARNT) and induces the expression of genes involved in the detoxification pathway.

Unraveling the molecular mechanisms on how AhR can trigger such pathways requires the structural characterization of the two Per-Arnt-Sim domains in which it is composed (PAS-A and PAS-B). Together, the PAS domains patrol the AhR biological activity regulating the dimerization process.

These domains have so far proved difficult to produce in large-scale expression studies and therefore they have been analysed using single domain homology models supported by mutagenesis studies. The recent depositions of X-ray structures of PAS domain dimers provided us with an opportunity to start a new research direction that involves modeling and characterization of the AhR::ARNT PAS domain dimer.

Two different dimerization models were obtained for both the PAS-A and PAS-B, on the basis of the different template structures used for modeling (PDB ID: 4F3L and 4M4X, for the PAS-A dimer; 4F3L and 3F1P, for the PAS-B dimer).

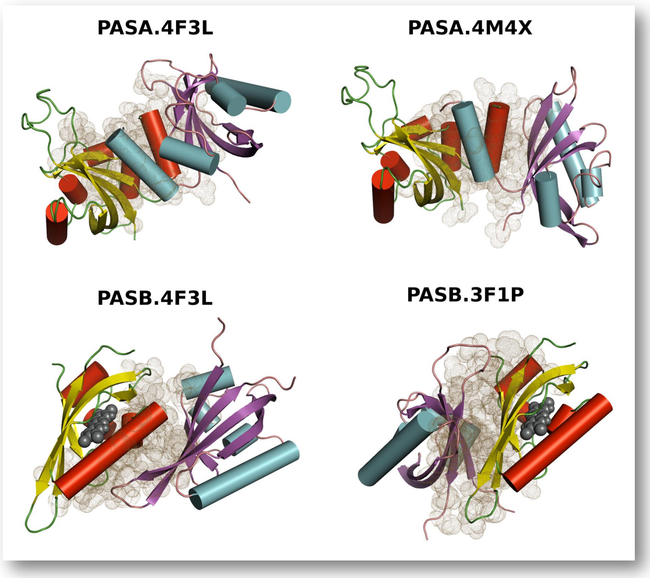

Figure 1: PAS-A and PAS-B dimer models, the PPI interface is highlighted in dots.

METHODS

The structures presented herein were obtained following an homology modeling strategy. From each structural alignment 100 putative models were produced and ranked according to the DOPE distance dependent statistical potential [1]. The PPI interfaces were assessed by submitting the models to the PISA web service [2].

The electrostatic potential surface and ΔSASA were calculated in order to inspect the complementarity of the related protomer interfaces. For each dimer the $\Delta G_{binding}$ values were estimated by means of the MM-GBSA method and the Energy Decomposition Analysis was performed in order to detect those energy couplings that are relevant for the binding process [3].

The PAS dimer models were characterized through the definition of those residues whose sidechains are predicted to significantly destabilize the binding mode along the dimerization interface, if mutated. The predictions of these hot spots were collected using different tools for the PPI evaluation based on *in silico* alanine scanning procedure (Robetta [4]), machine learning approach (KF2C [5]) and the potential contact scoring function (HotPoint [6]).

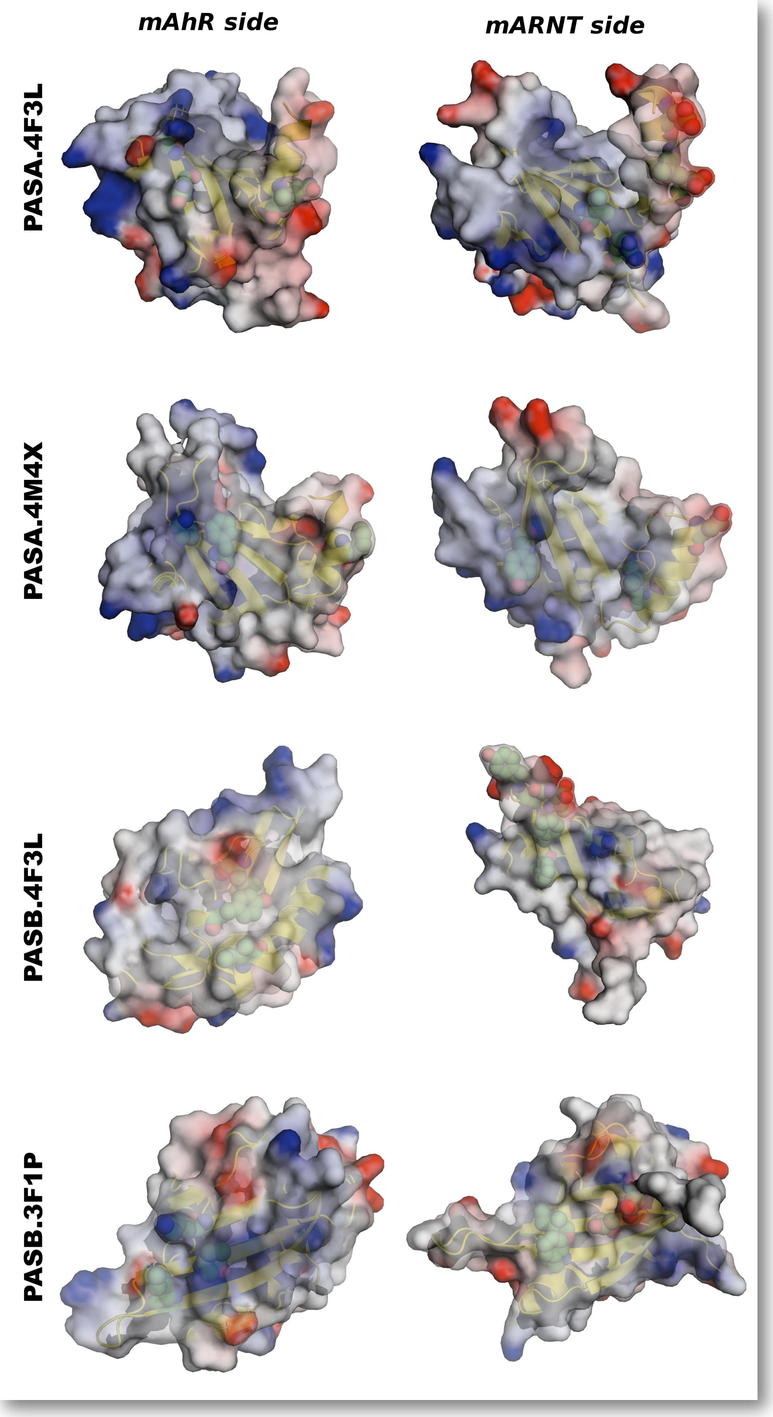

Figure 2: electrostatic potential surface of binding interfaces.

RESULTS AND NEXT STEPS

The PAS-A dimerization interfaces are quite similar, both in terms of $\Delta G_{binding}$ and ΔSASA. From a visual inspection, slight differences emerge due to the reciprocal spatial orientation of the individual protomer. The PASA.4M4X model shows a more closed interface than PASA.4F3L. In particular, the helices A' are more tightly packed against the β-sheet of the related protomers.

The PAS-B dimerization interfaces are very different, where the helical bundle (PASB.4F3L) or the β-sheet side (PASB.3F1P) of the AhR protomer appear involved in the dimerization. From the $\Delta G_{binding}$ values, the PASB.4F3L model clearly appears more favorite than PASB.3F1P. It should be noted that, in PASB.3F1P, the AhR protomer exhibits a charged interface towards the mainly neutral interface of the ARNT protomer.

The energy contribution provided by each secondary structure element was effectively dissected by Energy Decomposition Analysis. In particular, a restricted subset of residues have been identified as the key determinants of the binding. This subset was furtherly refined and complemented with the hot spot list provided with the PPI prediction tool.

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 interfaces will be the most reliable.

The dimer models will be further investigated through full atom MD simulations, in order to explore the conformational space. Collective coordinated motions will be inferred by means of the Essential Dynamics approach. In this context, the identification of long-range effects that extend far away the interface could improve our understanding of the AhR activation mechanism.

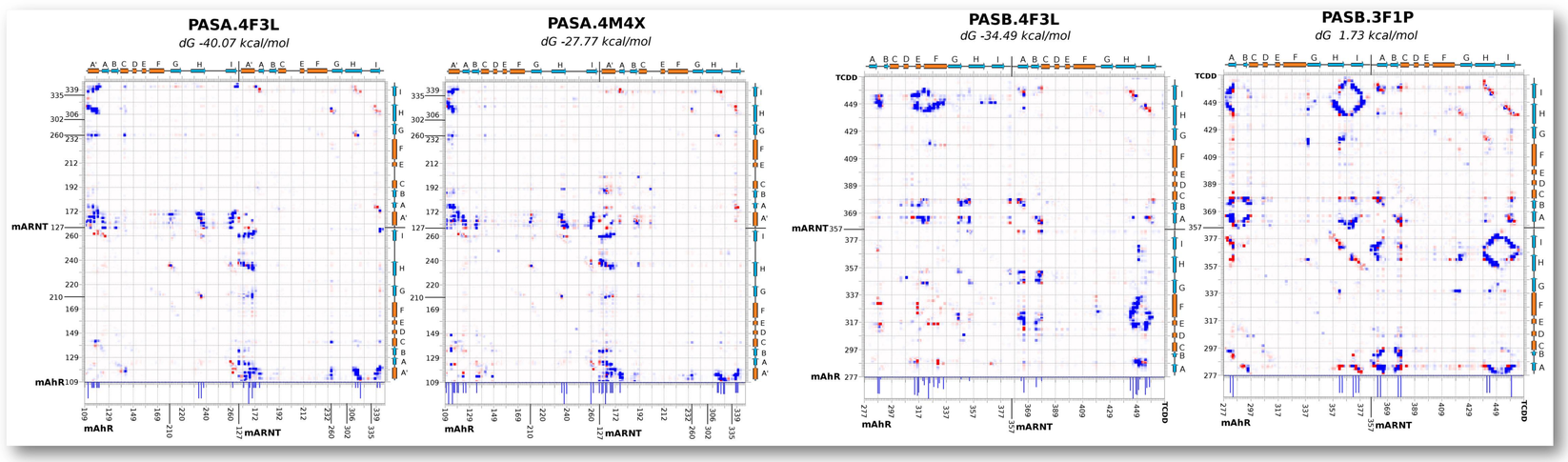

Figure 3: the $\Delta G_{binding}$ is calculated by means of the MM-GBSA method, and it is decomposed into individual energy couplings.

REFERENCES

[1] Shen M., Sali A., *Protein Sci.*, **2006**, 15, 2507-24
[2] Krissinel E., Henrick K., *J. Mol. Biol.*, **2007**, 372, 774-97
[3] Tiana G., Simona F., De Mori G. M. S., Broglia R. A., Colombo G., *Protein Sci.*, **2004**, 13, 113-24
[4] Kortemme T., Baker D., *Proc. Natl. Acad. Sci. USA*, **2002**, 99, 14116-21
[5] Zhu X., Mitchel J.C., *Proteins*, **2011**, 79, 2671-83
[6] Tunchbag N., Keskin O., Gursoy A., *Nucleic Acids Res.*, **2010**, 38, W402-6

This research is supported by the National Institute of Environmental Health Sciences - USA (Grant R01 ES007685)