Deciphering the AhR:ARNT Dimerization Process: How to Assembly the Functional Puzzle of Interacting Interfaces.

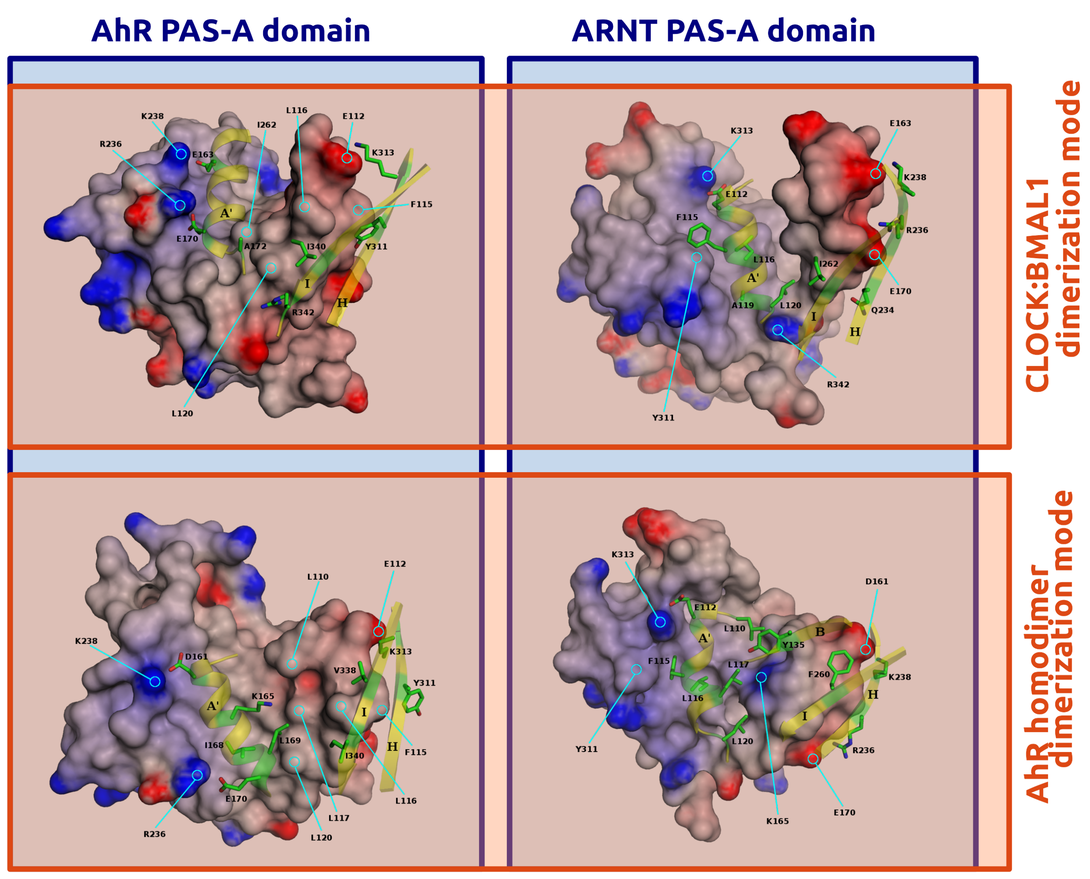
Corrada D[1], Soshilov AA[2], Denison MS[2], Bonati L[1] dario.corrada@unimib.it

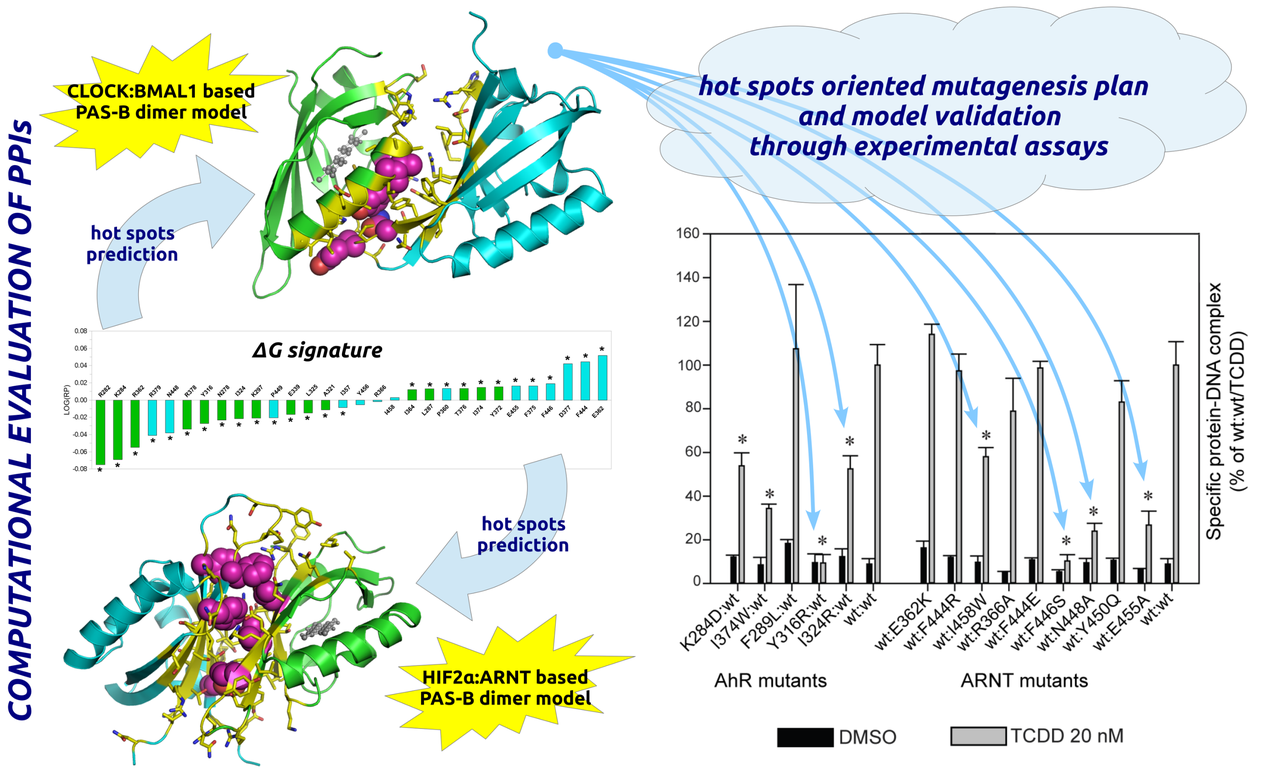
[1] *Department of Earth and Environmental Sciences - University of Milano-Bicocca, Milan, Italy*
[2] *Department of Environmental Toxicology - University of California, Davis, California, USA*

*The Aryl hydrocarbon Receptor (**AhR**) is a transcription factor that belongs to the bHLH-PAS protein 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 protein partner AhR Nuclear Translocator (**ARNT**) and promotes a **detoxification** pathway by inducing the expression of the related genes. The elucidation of the molecular mechanisms on how AhR can trigger such cellular response requires the **atomistic characterization** of the complex. Our full-length structural models offer a solid computational framework for the exhaustive comprehension of the **Protein-Protein Interactions** (PPIs) involving the interplay between AhR and ARNT and open new avenues for further studies on the molecular basis of transformation and transactivation of this transcription factor.*

HOMOLOGY MODELLING

Theoretical models based on **comparative studies** have been realized for the individual PAS domain dimers (PAS-A and PAS-B). The PPI interfaces exhibit a high degree of both geometric and electrostatic complementarity, justifying the potential reliability of these models .



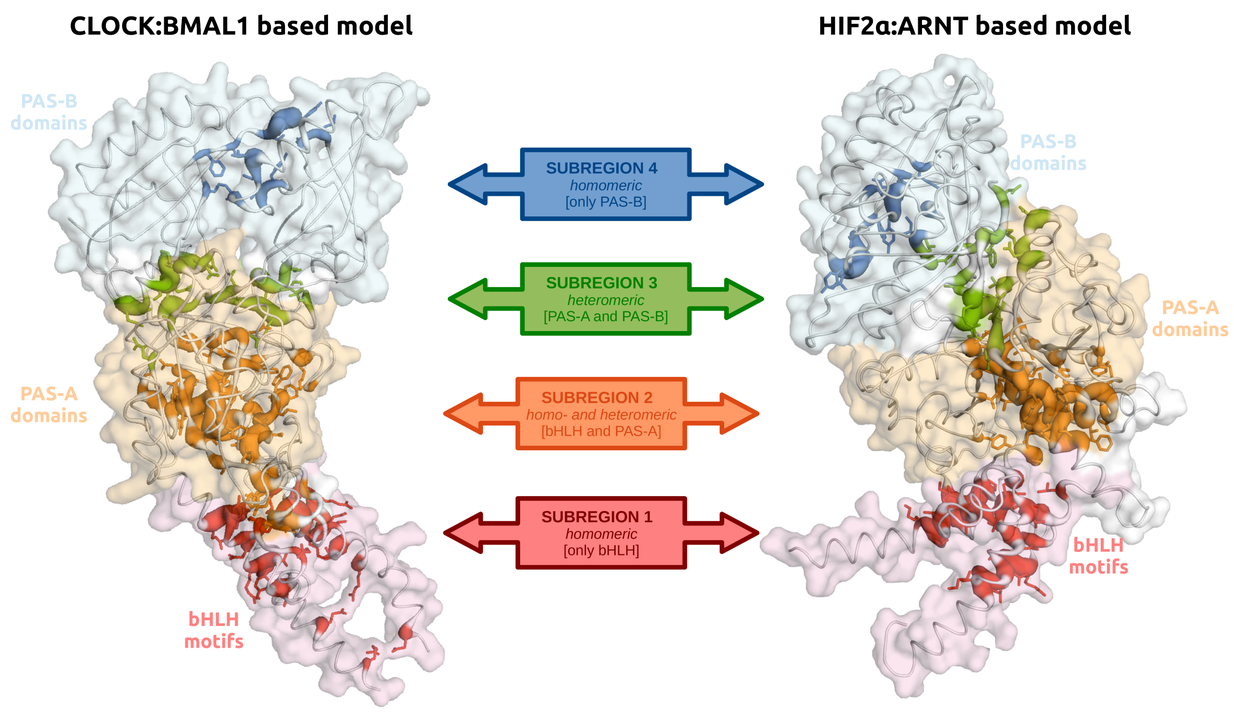


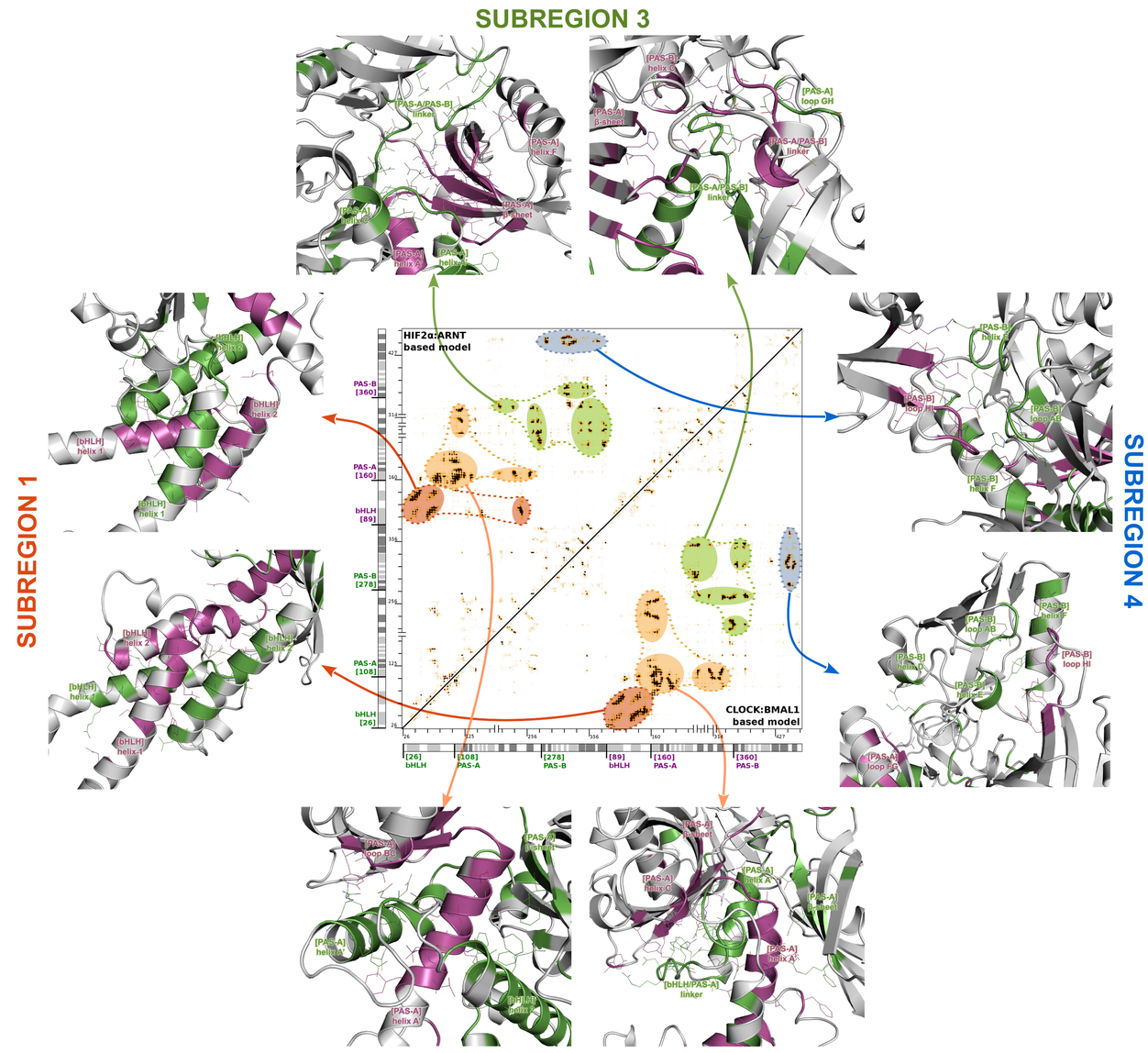
INTERFACE VALIDATION

We have proposed two **alternative dimerization modes** for each PAS domain dimer model. Each pair was compared in order to establish **specific patterns** of interacting residues using the two sample Rank Products algorithm [1] and we have inferred a panel of positions that could have the most disrupting effect upon mutation. As a result, **experimental mutagenesis assays** were planned to select and **validate** which of the proposed structural models is the most **reliable** [2].

SCAFFOLD ASSEMBLY

The recent availability of full-length X-ray structures of homologous bHLH-PAS complexes led us to achieve the overall architecture of the complete N-terminal region of the AhR:ARNT complex. The dimerization interfaces of the **full-length homology models** were determined by the analysis of variations in Solvent Accessible Surface Area [3]. Even if the quaternary scaffold of the templates used show relevant differences, it is possible to outline a common **continuum of PPIs** spanning from bHLH motifs to PAS-B domains.





IDENTIFYING NETWORKS

The Energy Decomposition Analysis [4] approach further dissects the interfaces with the aim of identifying the most relevant energy couplings that could primarily drive the dimerization process. First of all, it is possible to delineate four distinct **subregions** where the main PPIs are clustered together.

The topological distribution of the most stabilizing contributions greatly overlaps and only minor peculiar islets, characteristic of the two models, can be found. The critical role of several positions along the dimerization interfaces agree with the effective disrupting mutations collected by literature.

References

[1] Koziol J. (2010) *FEBS Lett.* 584, 4481-84
[2] Corrada D, Soshilov AA, Denison MS, Bonati L. (2016) *PLoS Comput Biol.* 12, e1004981
[3] Kleinjung J, Fraternali F. (2015) *Nucleic Acid Res.* 33, W342-6
[4] Corrada D, Morra G, Colombo G (2013) *J Phys Chem B.* 117, 535-52

This research is supported by

The National Institute of Environmental Health Sciences - USA (Grant R01 ES007685)