IX INTERNATIONAL CONFERENCE

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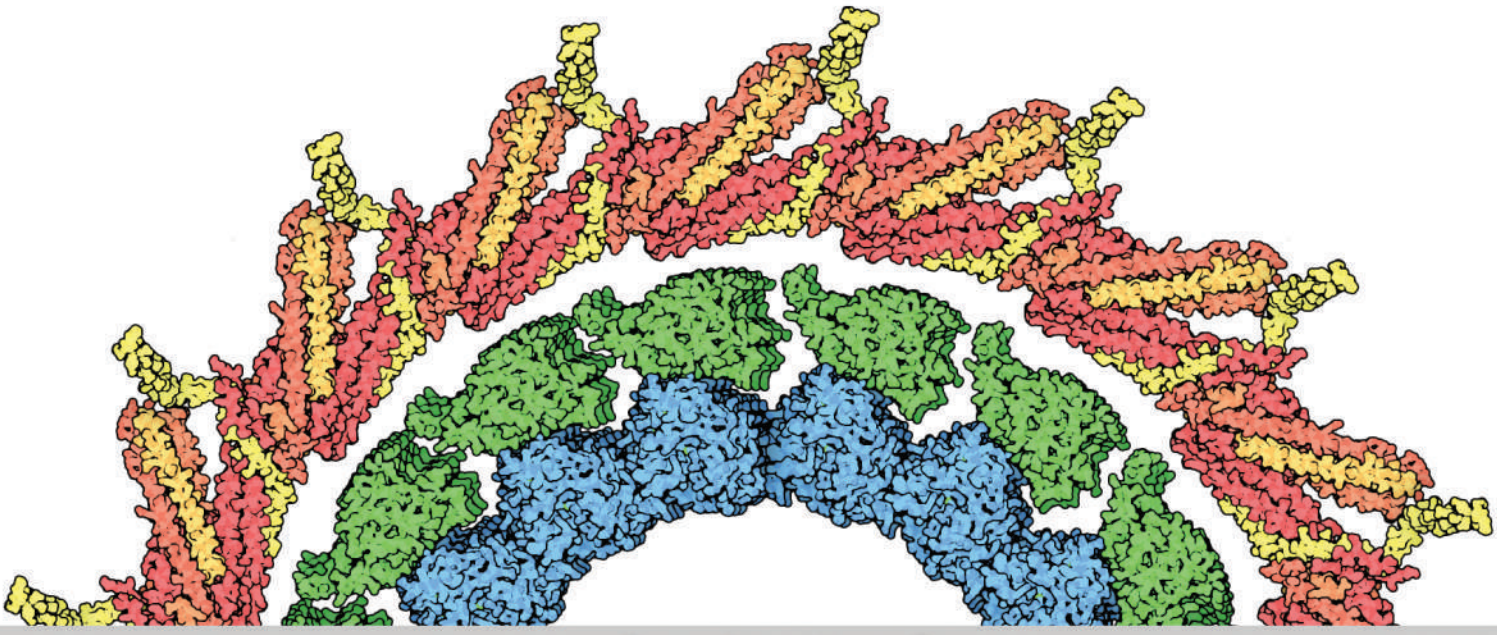
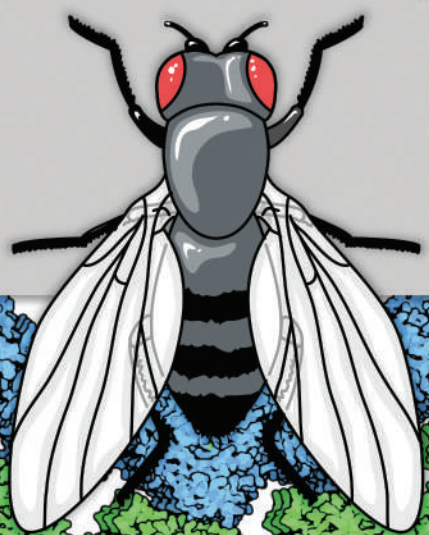
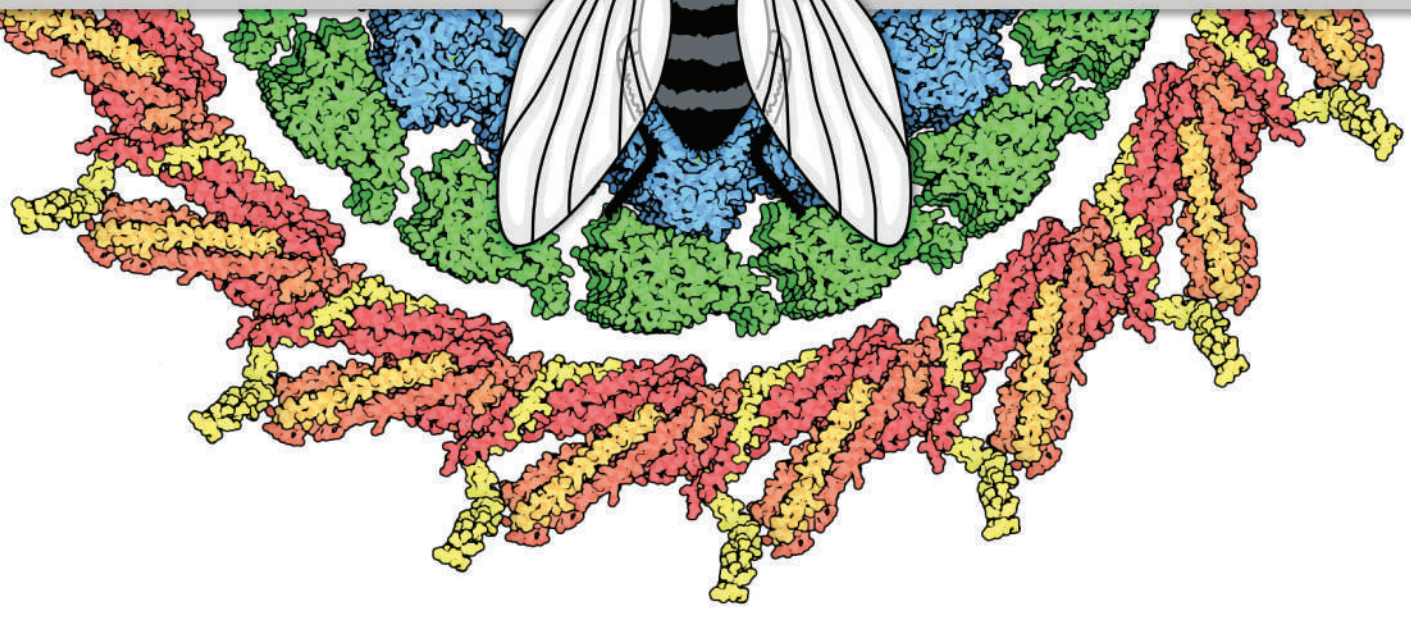
New Challenges in Molecular Biotechnology

Book of Abstracts







Instituto Universitario de Investigación
Biocomputación y Física
de Sistemas Complejos
Universidad Zaragoza

Universidad
Zaragoza
1542

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In Vitro Validation of New PAH Binding Compounds for non-Kuvan Responsive PKU Patients

María Conde-Giménez[1,2], Sandra Salillas[1,2], Juan J. Galano-Frutos[1,2], Alejandro Mahía[1,2], María Galiana[1,2], M. Dolores Díaz-de-Villegas[3], Javier Sancho[1,2,4*]

[1] *Institute of Biocomputation and Physics of Complex Systems (BIFI) - Joint Units IQFR-CSIC-BIFI, and GBsC-CSIC-BIFI, University of Zaragoza, 50018 Zaragoza, Spain.*
[2] *Department of Biochemistry and Cellular and Molecular Biology, University of Zaragoza, 50009 Zaragoza, Spain.*
[3] *Institute of Chemical Synthesis and Homogeneous Catalysis (ISQCH), Department of Organic Chemistry, University of Zaragoza, 50009 Zaragoza, Spain.*
[4] *Aragon Institute for Health Research (IIS Aragon), Center for Research in Biomedicine, 50009 Zaragoza, Spain.*

* Presenter's electronic address: mcondeg-at-unizar.es

Phenylketonuria (PKU) is a rare autosomal recessive disorder caused by mutations in the gene that codifies the phenylalanine hydroxylase (PAH) enzyme. These PAH mutations have been compiled in the International Database of patients and mutations causing PKU (BIOPKUdb) and most of them induce loss of conformational stability and decreased physiological enzymatic activity characterized with increased Phe blood levels and toxic brain levels if it is untreated. Current therapies to treat this disease are not entirely effective so new therapeutic approaches are needed. Kuvan, the natural cofactor of PAH, is prescribed for PKU patients with a mild phenotype and it is believed that acts as a pharmacological chaperone in the enzyme folding equilibrium. Based on different drug discovery strategies, we have identified novel chemicals that bind to the enzyme and we have validated their in vitro properties as a promising pharmacological compounds before PKU animal model testing. These new compounds show a thermal upshift stabilizing effect in fluorescence unfolding curves and a dissociation constant in a μM range by ITC assays. They are also low or moderate toxic in two cell lines (HepG2 and HEK293T). Finally, we test their in vitro efficacy in HEK293T transfected cell lysates with WT PAH variant and different mutant´s plasmids. We evaluate their steady-state PAH protein level fold and PAH enzymatic and specific activity fold for compounds incubated and untreated PAH over-expressing cell lysates. This evaluation will help us to elucidate their potential chaperoning effect in different PAH pathological variants and so different PKU phenotypes.

Keywords

Pharmacological Chaperone | PKU | PAH