



References:

- [1] Poppe L. & Rétey J. (2005) *Angewandte Chemie* 24:3668-3688
[2] Ritter H. & Schulz G.E. (2004) *Plant Cell* 16: 3426-3436
[3] Zs. Bata et al. (2017) *Advanced Synthesis and Catalysis* 12: 2109-2120

SUPPORTED BY THE ÚNKP-17-3-III NEW NATIONAL EXCELLENCE PROGRAM OF THE MINISTRY OF HUMAN CAPACITIES

Keywords: catalytic mechanism, ligand binding, enzyme engineering

MS10 Hydrogen-bonding & weak interactions studied by neutrons and X-rays

Chairs: Prof. Marta E. G. Mosquera, Dr. Matthew Blakeley

MS10-O1

An aminophenothiazine inhibitor of the NCS-1/Ric8a complex regulates synaptic function in fragile X Syndrome

Maria Jose Sanchez-Barrena¹, Alicia Mansilla², Antonio Chaves-Sanjuán¹, Nuria Campillo³, Ourania Semelidou⁴, Loreto Martínez-González³, Efthimios Skoulakis⁴, Alberto Ferrús², Ana Martínez³

1. Departamento de Cristalografía y Biología Estructural. Instituto de Química Física Rocasolano. CSIC, Madrid, Spain
2. Dpto. de Neurobiología del Desarrollo. Instituto Cajal. CSIC, Madrid, Spain
3. Dpto. de Biología Química y Física. Centro de Investigaciones Biológicas. CSIC, Madrid, Spain
4. Division of Neuroscience, Biomedical Sciences Research Centre Alexander Fleming, Vari, Greece

email: xmjose@iqfr.csic.es

The protein complex formed by the Ca²⁺ sensor neuronal calcium sensor 1 (NCS-1) and the guanine exchange factor protein Ric8a co-regulates synapse number and probability of neurotransmitter release, emerging as a potential therapeutic target for diseases affecting synapses such as Fragile X syndrome (FXS), the most common heritable autism disorder [1]. Using crystallographic data and the virtual screening of a chemical library [1,2], we identified a set of heterocyclic small molecules as potential inhibitors of the NCS-1/Ric8a interaction. The aminophenothiazine FD44 interferes with NCS-1/Ric8a binding and it restores normal synapse number and associative learning in a *Drosophila* FXS model [3]. The crystal structure of NCS-1 bound to FD44 and the structure-function studies performed with structurally close but inactive analogues explain the FD44 specificity and how this small compound can inhibit such big protein-protein interface: FD44 stabilizes NCS-1 in a conformation that impedes Ric8a recognition [3]. Our study demonstrates the druggability of the NCS-1/Ric8a interface and uncovers a suitable region in NCS-1 for development of additional drugs of potential use on FXS. In fact, we have combined the structural information obtained by X-ray crystallography, together with advanced computational methodologies to understand the basis of FD44 affinity and selectivity and find drug-like molecules with improved activity. Since other neuronal disorders share with FXS the synaptic density and morphology abnormalities, we believe that our compounds would be also useful for a whole range of synaptopathies, such as Rett Syndrome, autism, schizophrenia or bipolar disorder.

References:

-
- [1] Romero-Pozuelo, J. et al. (2014) *Journal of Cell Science* 127, 4246-4259.
 [2] Baños-Mateos, S. et al. (2014) *Acta Crystallographica A* 70, 530-534.
 [3] Mansilla, A. et al. (2017) *PNAS* 114(6), E999-E1008.
-

Keywords: calcium sensor, molecular recognition, protein-protein interaction

MS10-O2

Selective protonation and stereoelectronic effects govern catalysis in a pyridoxal-5'-phosphate-dependent enzyme

Andrey Kovalevsky¹

1. Neutron Scattering Division, Oak Ridge National Laboratory, Oak Ridge, United States of America

email: kovalevskyay@ornl.gov

Pyridoxal-5'-phosphate (PLP, vitamin B₆ derivative) is one of the most ubiquitous co-factors in biological systems. At least 140 different types of biochemical transformations are catalyzed by PLP-dependent enzymes, comprising ~4% of all classified enzymatic activity. PLP-dependent enzymes perform some of the most difficult chemical reactions involving amines and amino acids, including transamination, racemization, phosphorylation, decarboxylation, aldol cleavage, elimination, and replacement reactions. PLP-dependent enzymes are categorized into five recognized fold-types (I-V), each performing characteristic chemistry. Despite decades of research, the physical determinants that govern specificity of and catalytic enhancement by PLP-dependent enzymes remain largely unresolved. Fold-type I is the most prevalent, mainly promoting transamination and decarboxylation reactions. Two major hypotheses have been developed to understand the different types of chemistry in PLP-dependent enzymes, namely 1) stereoelectronic control and 2) electronic modulation through selective protonation. An enzyme active site local environment can promote different protonation profiles of PLP during the catalytic cycle. We report the first neutron crystal structure of a Fold I PLP-dependent enzyme, aspartate aminotransferase (AAT). In crystal, AAT was captured in both the internal and external aldimine states after soaking with substrate analog α -methyl-D-aspartic acid. The neutron structure illustrates the protonation state differences between the reactant (internal aldimine) and intermediate (external aldimine) states, and sheds new light on the catalytic mechanism of transamination. We are using QM/MM calculations and MD simulations based on the correct models derived from the neutron structure, and neutron vibrational spectroscopy for deeper understanding of the enzyme mechanism and to uncover the contribution of low-energy protein vibrations to catalysis.

Keywords: protonation, catalysis, dynamics