ShT-04.1-1
Abolishing "structural blindness" in metalloproteins: PioC, a NOE-less protein structure

I.B. Trindade[1], M. Invernici[2], F. Cantini[2], M. Piccioli[2], R.O. Louro[1]
[1]*Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Universidade Nova de Lisboa, Lisboa, Portugal,* [2]*Magnetic Resonance Center and Department of Chemistry, University of Florence, Florence, Italy*

Almost half of all known enzymes are metalloproteins where the metal center(s) are essential for catalysis, electron transfer, metal storage/transport, or provide stability and structural properties. NMR is a privileged method for characterizing metalloproteins providing the structure at atomic resolution, information about amplitude and time-scale of internal dynamics, hints on electronic structure and oxidation states in conditions that mimic the physiological context. However, in a significant part of the metalloproteome the metal ion is paramagnetic and, in its vicinity, a "blind sphere" exists where nuclear relaxation is enhanced and signal detection becomes a challenge. This challenge may be circumvented by substituting the paramagnetic metal with a diamagnetic analogue. Yet, this strategy often fails since it leads to unfolded proteins or the diamagnetic analogue may not mimic adequately the native paramagnetic metal. Using recent developments in pulse sequences, here we present a strategy for achieving structure determination in paramagnetic proteins [1]. PioC from *Rhodopseudomonas palustris* TIE-1 is the smallest High Potential Iron-Sulfur Protein (HiPIP) ever isolated. The paramagnetism from the [4Fe-4S] cluster affects 60% of the protein, making it the perfect example of the dual nature of paramagnetic NMR. On one side relaxation precludes signal detection, and on the other it provides unique sets of information. The structure of PioC was determined by NMR using two different sets of restraints, one containing Nuclear Overhauser Enhancements (NOEs) and another containing Paramagnetic Relaxation Enhancements (PREs). These were used independently and then combined revealing that under favorable conditions, PREs can efficiently complement and eventually replace NOEs for structural characterization [1]. 1. Trindade IB, Invernici M, Cantini F, et al (2020) PRE-driven Protein NMR Structures: an Alternative Approach in Highly Paramagnetic Systems. FEBS J https://doi.org/10.1111/febs.15615

Monday 5 July
9:00–11:00, Marmorna Hall A

Neurobiochemistry

S-03.1-1
The nicotinic receptor modulation of higher brain functions: from chemistry to cognition

J.P. Changeux
Institut Pasteur, Paris, France

A critical event in the history of both biological chemistry and neuroscience was the chemical identification of the first receptor for a neurotransmitter. It happened to be the nicotinic acetylcholine receptor. The nicotinic receptor has since then become the founding father of a broad family of brain receptors, paving the way for their identification, including that of the GABAA receptor, of glutamate receptors and subsequently G-protein linked receptors. Moreover, the nicotinic receptor appears as a typical "allosteric machine" which mediates indirect allosteric interactions between a set of topographically distinct sites – the ACh binding site, the ion channel and several allosteric modulatory sites – through a discrete and reversible conformational change of the protein. The model emerging from these studies has led to the conception and development of new pharmacological agents. The knowledge acquired with the nicotinic receptor has been further exploited to reach higher levels of brain organization, including conscious processing. The contribution of nicotinic receptors to the action of nicotine on addiction and on cognitive enhancement is explored, in particular, using a novel experimental strategy that combines nicotinic receptor genes knock-out and stereotaxic gene re-expression in the mouse. The presently available data illustrate that different brain circuits are involved in the dual use of a drug and such is the case of nicotine. Last the specific contribution of nAChRs to conscious access is evaluated in the framework of the Global Neuronal Workspace model developed by Dehaene, Kerszberg, Changeux (1998). These data and relevant theoretical models create a striking landmark in the thinking of brain sciences by causally and reciprocally linking the molecular to the cognitive levels both, within the individual brain and, between brains, in the social and cultural environment, thus suggesting new bridges between brain sciences and the humanities.

S-03.1-3
Modulation of synaptic signalling by adenosine and cannabinoids

A. Sebastiao
Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

Cannabinoid receptors 1 (CB1R) are widely distributed in neurons and astrocytes. Exogenous activation of CB1R inhibit excitatory and inhibitory synaptic transmission and plasticity to disrupt memory. Adenosine is another ubiquitous neuromodulator of synaptic signalling. We focused on (1) understanding the role of endocannabinoids (eCBs) to modulate synaptic plasticity phenomena; (2) understanding how the two neuromodulators, cannabinoids and adenosine, control each other. We found that eCBs have a dual role upon hippocampal long-term potentiation (LTP), inhibiting weak LTP while facilitating strong LTP (Silva-Cruz et al., 2017 - Front Pharmacol. 8:921.eCollection), likely acting as a high pass filter to reduce signal to noise ratio of synaptic strengthening during memory consolidation. Exogenous activation of CB1Rs consistently inhibit LTP at the hippocampus and, probably by disrupting the fine-tune homeostatic control exerted by eCBs upon synaptic plasticity, consistently disrupt memory consolidation and affect brain connectivity between brain areas relevant for memory (Mouro et al., 2018 – J Neurochem 47:71-83). Importantly, adenosine A2A receptor (A2AR) antagonists attenuated the inhibitory action of CB1R agonists upon LTP and prevented memory consolidation impairment caused by acute (Mouro et al., 2017 – Neuropharmacology, 117:316-327) or chronic (Mouro et al., 2019 – Neuropharmacology 155:10–21) intake of CB1R agonists. A1Rs, though present in CB1R positive interneurons (Rombo et al., 2016 – Cereb Cortex 26:1081–1095), do not influence the inhibitory action of exogenous activation of CB1Rs on synaptic transmission