SCIENTIFIC SEMINAR

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NMR Study of the Dynamics of Human Mitogen-Activated Protein Kinase p38α

Mammalian p38 mitogen-activated protein kinases (MAPKs) are essential as signaling molecules for many processes, particularly mediating stress response. The p38 MAP kinase family consists of four isoforms, of which p38α is the best characterized and expressed in most cell types in the human body. p38α is a 42kDa intracellular soluble serine-threonine kinase activated by various extracellular stimuli, including inflammatory mediators, bacterial lipopolysaccharides, and environmental stress (UV radiation, osmotic shock, and heat). However, despite the several existing studies on the crystal structures of p38α, there is a lack of information about the motional processes that can potentially couple allosteric binders with active-site inhibition.

Using innovative labeling schemes, in particular different deuteration levels, in conjunction with MAS solid-state NMR, we aim for the characterization of residues that have been obscured using solution NMR. We, therefore, expressed and purified non-phosphorylated 15N, 13C, and 2H p38α, and micro-crystallized it in the presence of different ligands. We used three labeling schemes for assignments: a fully protonated approach, RAP 50, and perdeuteration with 100% back-exchange. Due to the size of the protein, high overlapped regions are found in 2D H/N correlations. Thus, assignment experiments were recorded in a 4D and 5D fashion using 3D experiments as a base.

Some of the critical dynamic processes, such as enzymatic catalysis, folding, or allostery, occur in the µs-ms regime. Those conformational changes can be observed by measuring relaxation dispersion parameters such as 15N R1ρ. Therefore, here we subjected the dynamics in the µs-ms time scale to Near Resonance Relaxation Dispersion techniques. These 15N NERRD experiments were recorded either in a pseudo-4D fashion or as individual 3Ds using NUS for the partially deuterated samples with and without ligand present in the lipidic pocket. The data obtained hint to a motional coupling throughout the p38 architecture, transducing information between different units of the enzyme.

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EXCELENCIA SEVERO OCHOA

Tuesday
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Atrio 800
12.00H

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