Structural Biology Programme | Kinases, Protein Phosphorylation and Cancer Junior Group

We established 4 main research lines in 2019, and at the same time the lab expanded with the arrival of 2 ‘CNIO Friends’ fellowship recipients: Rubén Julio Martínez Torres (postdoctoral contract) and Moustafa Ahmed Shehata (Carmen Gloria Bonnet predoctoral contract).

→ Structural and molecular determinants of RET catalytic activity and signalling, both in cis by intrinsic elements and in trans by effector kinases and adaptor proteins. We paid special attention to the crosstalk between RET and non-receptor tyrosine kinases (NRTKs).

→ Structure-function studies of RET oncogenic variants, i.e. point mutations targeting the kinase domain and oncogenic fusions generated by DNA-rearrangements, with a special emphasis on the latter in the context of aggressive types of cancers.

→ Structure-based drug-discovery for new (allosteric) RET inhibitors.

→ Histidine phosphorylation and structure-function studies of histidine kinases.

We also focused on less known phospho-specific modifications such as histidine phosphorylation and the regulation of histidine kinases. The current understanding of this type of phosphorylation is poor. Together with T. Schirmer’s group at the Biozentrum (University of Basel), we directly contributed to the first crystal structure of a full-length hybrid histidine kinase and the molecular dissection of its full catalytic cycle (FIGURE). This work was recently published in PNAS (Dubey et al., 2020).

Figure
Crystal structure of full-length hybrid histidine kinase ShkA. (A) Domain organisation (colour coded) of hybrid histidine kinase ShkA. Crystal structure representations, front-back and top-bottom views of AMP-PNP-bound protein, depict a compact dimeric and catalytic incompetent arrangement of the multidomain protein, from Dubey et al. PNAS 2020. (B) Functional evaluation of dual histidine kinase and phosphatase activities. Western blots (WBs) of ShkA wild-type (WT) and phosphotransfer deficient mutant (D430) in the presence or absence of second messenger c-di-GMP using histidine phospho-specific antibodies, upper panel. Phosphate sensor analyses capturing phosphatase activity (i.e. phosphate release) of ShkA WT and D430A, lower panel, from Plaza-Menacho et al. unpublished.

“Understanding protein kinase function and inhibition for better cancer therapeutics.”