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Signalling transduction through RIPK2, a drug target for IBDs

Abstract: In the innateimmune response, activation of the patternrecognition receptors Nucleotide Oligomerization Domain 1 and 2 (NOD1 and NOD2) triggers a proinflammatory response, which plays a crucial role in both bacterial pathogen detection and gut homeostasis maintenance. Dysregulation of NOD signalling is involved in several genetic and non-genetic inflammatory diseases, such as Inflammatory Bowel Diseases (IBDs), which comprise Crohn's disease and Ulcerative Colitis, increasingly frequent disorders in the Western world.

Activation of NOD by bacterial peptidoglycans leads to the recruitment of the adaptorkinase RIPK2, which,upon auto-phosphorylation, serves as a scaffold for binding downstream effectors, such as XIAP, a key E3 ubiquitin ligase in the NOD signalling pathway. The kinase domainof RIPK2 is an attractive drug target for inflammatory diseasesrelated to NOD2,and inhibition of RIPK2 kinase activity has been shown in vivo to be beneficial as a therapeutic strategy to treat IBDs. However, RIPK2 auto-phosphorylation activity is not required for signalling, questioning the significance of the kinase domain in signalling transduction.

To answer this question, we applied integrative structural biology (X-ray crystallography, cryo-EM, and NMR), supported by biophysical characterization and in-cell validation, to obtain structural snapshots of RIPK2, either alone or in complex with upstream (NOD2CARDs) and downstream (XIAP) proteins in the signalling pathway [1-3]. During this talk, I will guide you through the long journey that took us to obtain these structures and eventually provide a molecular explanation of how NOD2 triggers the active conformation of RIPK2by promoting its polymerization and how this event promoteskinase dimerisation and, therefore, the scaffolding role of the kinase domain (Fig 1).

Our findings not only deepen our comprehension of the NOD signalling but also provide useful data for the design of more potent and specific inhibitors targeting NOD signalling.

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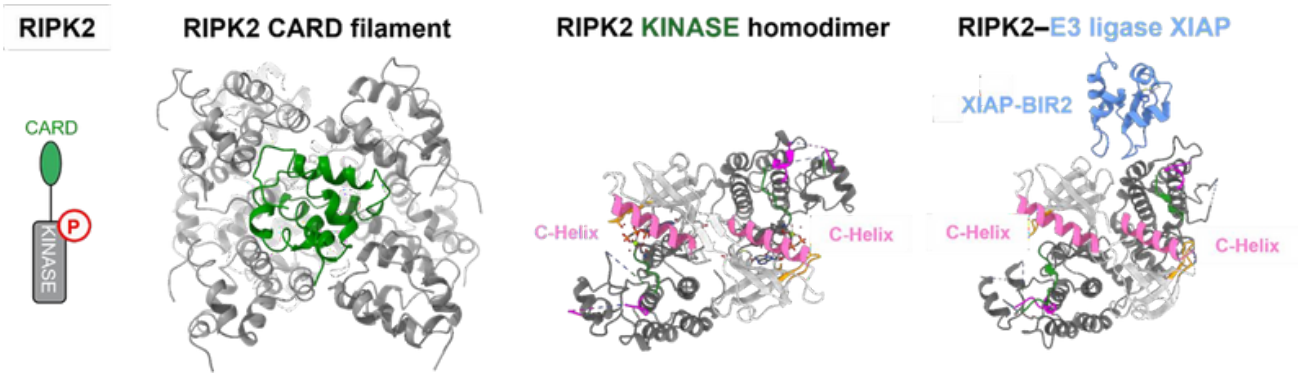

Figure 1. Gallery of the RIPK2 structures, which will be described in the talk.

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[3] Lethier, M., Huard, K., Hons, M., Favier, A., Brutscher, B., Boeri Erba, E., Abbott, D.W., Cusack S. & Pellegrini E. (2023) Life Sci Alliance.
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Biography: Erika Pellegrini's career in the field of structural biology began with a one-year internship during the second year of her M.Sc. in Medical, Molecular, and Cellular Biotechnologies at Università Vita-Salute San Raffaele in Milan, Italy. This internship took place in the Biocrystallographic unit under Dr. Massimo Degano at San Raffaele University, where she received extensive training in protein expression and purification from bacterial sources. Afterward, She moved outside her country for three years PhD (from January 2011 until January 2013), shared between the Structural Biology group at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) and the NMR group of Prof. John Waltho at the University of Sheffield, UK. During the PhD, she acquired and tuned skills in molecular biology, biochemistry, and X-ray crystallography by applying these techniques in studying enzymatic phosphorylation transfer of relevant drug targets: the bacterial mutase β-Phosphoglucomutase (βPGM), the human small-G protein RhoA and the human kinase p38α.

In the subsequent 5 years post-doc in the group of Dr Stephen Cusack at the European Molecular Biology Laboratory, (EMBL, Grenoble; April 2013-June 2018), She successfully applied and extended the acquired PhD knowledge on protein kinases. In July 2018, following her first maternity leave, she was promoted to the position of research scientist (July 2018-November 2022), with dual responsibilities: continuing her research on RIPK2 and participating in the managing of the internal cryo-EM platform (July 2018-September 2020). Since 2018, she has been also collaborating with an industrial partner, Oncodesign Precision Medicine (OPM), Djon, France on the structure optimization of RIPK2 kinase inhibitors targeting IBDs.

In October 2023, she joined the EBEV group, at the Institut de Biologie Structurale (IBS, UMR 5075 CEA-CNRS-UGA Grenoble, France), where she investigated the interaction between CHromatin-Modifying Proteins or CHarged Multivesicular body proteins (CHMPs) and DNA in vitro, and she plan to use her structural biology skills to reveal the mechanism of action of CHMP proteins on chromatin remodeling both in vitro and in a more physiological context.