Invited Lecture

Signalling transduction through RIPK2: how a kinase becomes a scaffold

S. Cusack[1] and E. Pellegrini[1,2]
[1]EMBL, 71 Avenue des Martyrs, 38000 Grenoble,
[2]Current adresse : IBS, 71 Avenue des Martyrs, 38000 Grenoble
epellegrini@ibs.fr

In the innate immune response, activation of the pattern recognition 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 adaptor kinase 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 domain of RIPK2 is an attractive drug target for inflammatory diseases related 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 signalilng 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 RIPK2 by promoting its polymerization and how this event promotes kinase 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.

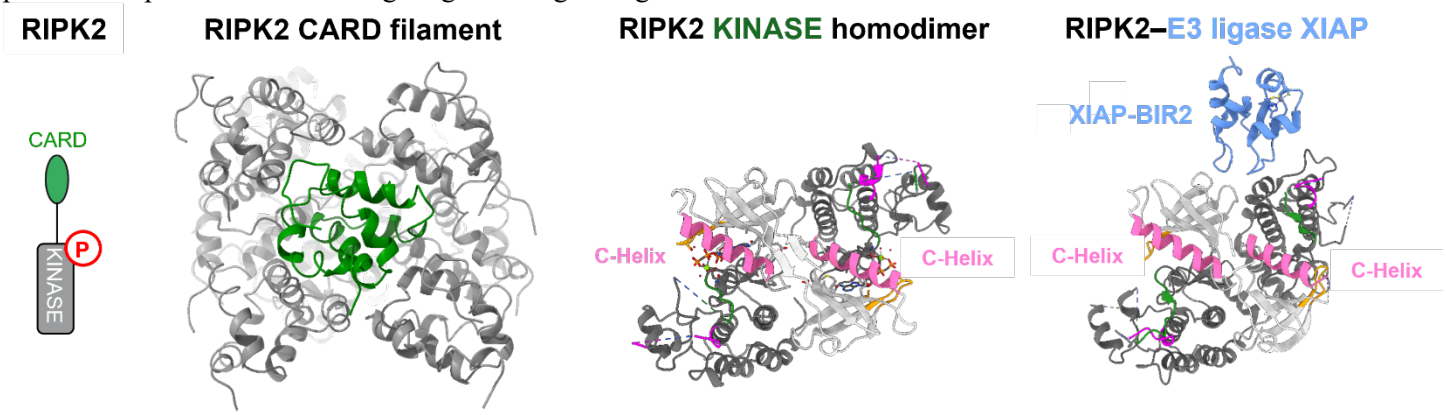

Figure 1. Gallery of the RIPK2 structures, which will be described in the talk.

[1] Pellegrini, E., Signor, L., Singh, S., Boeri Erba, E. & Cusack, S. (2017) *PLoS One.* **12**(5):e0177161.

[2] Pellegrini, E., Desfosses, A., Wallmann, A., Schulze, W.M., Rehbein, K., Mas, P., Signor, L., Gaudon, S., Zenkeviciute, G., Hons, M., Malet, H., Gutsche, I., Sachse, C., Schoehn, G., Oschkinat, H. & Cusack, S. (2018) *Nat Commun.* **9**(1):4043.

[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.* **6**(11):e202201784.