Structural insights into cell septation in *Mycobacterium tuberculosis*

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The cell wall of bacterial pathogens is a complex structure essential for survival and virulence. In *Mycobacterium tuberculosis (Mtb)*, the D,L endopeptidase RipA has emerged as a major peptidoglycan (PG) hydrolase for cell separation in cytokinesis and, although defects in this protein have important implications for *Mtb* virulence, the precise mechanisms by which RipA mediates cell separation remain elusive.

In our previous work [1], we reported the crystal structure of the RipA homologue from *C. glutamicum*, revealing the enzyme in an auto-inhibited conformation. We proposed that RipA activation occurs through its interaction with the transmembrane protein SteB, which is largely conserved in *Mycobacteriales,* **Fig. 1**.

Here, we present genetic and biochemical evidence showing that a second conserved transmembrane protein, SteA, is also involved in RipA regulation. We report structural studies of the SteA/SteB/RipA system in *M. tuberculosis* that allowed us to propose a novel mechanism of PG hydrolysis regulation, different from the FtsEX regulatory system active in Gram-positive bacteria.

Our work provides important insights into the fundamental biological processes and specificities that underlie mycobacterial cell division, with potential clinical relevance as cell wall integrity is directly linked to antibiotic tolerance.

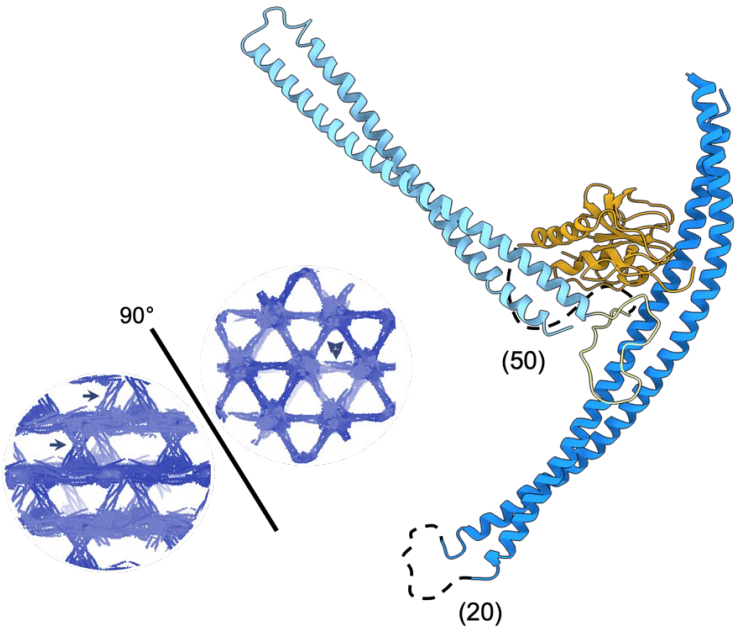

Figure 1. Crystal structure of full-length *Cglu* RipA (Cg1735, PDB 8AUC) and projections of its electron density map. The catalytic domain is depicted in yellow while the coiled-coil domains in blue and light blue.

[1] Gaday, Q., Carloni G., Megrian, D., Martinez, M., Sokolova, B., Ben Assaya, M., Legrand, P., Brûlé, S., Haouz, A., Whenkel, A.M., Alzari, P. (2022) *Proc. Natl. Acad. Sci.* **119,** 50, e2214599119.