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► **To cite this version:**

Cécile Morlot. Click and Collect at high resolution to unlock the secrets of cell wall synthesis. 2023 EMBO Workshop Great Wall Symposium, Sep 2023, Sintra, Portugal. hal-04870803

HAL Id: hal-04870803

<https://hal.science/hal-04870803v1>

Submitted on 7 Jan 2025

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Click and Collect at high resolution to unlock the secrets of cell wall synthesis

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The peptidoglycan is a three-dimensional sugar and peptide network that surrounds the bacterial cell. It confers a cell shape adapted to the ecological niche of the bacterium and protects it against mechanical stresses. Peptidoglycan synthesis and integrity are thus essential for bacterial proliferation and survival. Despite the importance of these fundamental processes, which constitute sources of antibiotic targets, we still poorly understand how the peptidoglycan is assembled and remodeled in space and time to ensure proper cell division, shape and integrity. This is particularly true for ovoid-shaped bacteria such as streptococci and enterococci, in which two different modes of peptidoglycan synthesis, dedicated to cell division and elongation, are confined to an annular region with nanometric dimensions at midcell.

Fluorescence microscopy is a method of choice to investigate cell wall assembly but it suffers from two major drawbacks. First, the newly synthesized material must be labeled with a probe that will not perturb the physiological process. Second, the physical properties of light limit the resolution to about 250 nm, which approximates the dimensions of the cell wall synthesis region. We have met these two challenges by combining metabolic peptidoglycan labeling (using click chemistry) and super-resolution fluorescence microscopy (dSTORM) in the ovoid-shaped human pathogen *Streptococcus pneumoniae*. Our nanoscale-resolution data (~ 30 nm) unravel unprecedented spatio-temporal features of peptidoglycan assembly and fate along the cell cycle. It provides geometrical and kinetic parameters of peptidoglycan synthesis that we further use to simulate the morphogenesis of the ovoid cell *in silico*.

I will present our methodological strategy and the major insights that our experimental and modeling analyses can reveal into peptidoglycan synthesis and morphogenesis in ovococci.