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Cécile Morlot

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

Cecile MORLOT, Institut de Biologie Structurale, CNRS/CEA/UGA, Grenoble, FRANCE

The cell wall 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 stress exerted by the environment. Cell wall 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 cell wall 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 cell wall 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 cell wall labeling (using click chemistry) and super-resolution fluorescence microscopy (dSTORM) in the ovoid-shaped human pathogen *Streptococcus pneumoniae*. Our nanoscale-resolution data (~ 30 nm) unraveled unprecedented spatio-temporal features of cell wall assembly and fate along the cell cycle. It provided geometrical and kinetic parameters of cell wall synthesis that we further used 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 revealed into cell wall synthesis and morphogenesis in ovococci.