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

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The bacterial cell wall confers shape and protection 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 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 midcell region with nanometric dimensions.

Fluorescence microscopy is a method of choice to investigate cell wall assembly but it suffers from two major drawbacks. First, the cell wall must be labeled with a probe that will not perturb the physiological process. Second, the diffraction of light limits the resolution to about 250 nm, approximating the dimensions of the wall synthesis region. We have met these challenges by combining metabolic cell wall labeling and dSTORM in the ovococcus *Streptococcus pneumoniae*. Our nanoscale-resolution data 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 major insights that it revealed into the spatio-temporal features of cell wall assembly and morphogenesis in ovococci.