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Bacterial division and morphogenesis in high definition

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Ovococci represent a bacterial morphological group that includes several human pathogens (enterococci and streptococci). Their shape results from cell wall synthesis and remodeling processes that allow cell division and elongation. These processes are performed by multi-protein complexes that assemble into ringlike structures scaffolded by the cytoskeletal protein FtsZ at midcell. While most elongation and division proteins are known, we still barely understand how they assemble into functional machineries in vivo, and how they synthesize and remodel the cell wall to ensure proper cell division and morphogenesis. Singlemolecule localization microscopy offers very powerful techniques to study cellular processes at resolutions ten times higher than conventional fluorescence microscopy. However, the use of these nanoscopy techniques in bacteria remains challenging because their cellular features (small size, cell envelope, low copy number of proteins) add up to the complexity of single-molecule data collection and processing. In the human pathogen Streptococcus pneumoniae, we implemented PhotoActivated Localization Microscopy (PALM) to observe the cellular positioning and architecture of cell wall synthesis machineries, and we set up a combination of bioorthogonal labeling (using copper-free click chemistry) and dSTORM (direct STochastic Optical Reconstruction Microscopy) techniques to visualize their enzymatic activity. I will present our approaches and discuss how the nanoscale localization data they generate allow revisiting the mechanisms of cell wall synthesis in ovococci.