Quantitative 3D Imaging by Cryo-EM

(Shapes, membranes, and mapping of subcellular structures in whole bacteria)

Luis R. Comolli[1], Kenneth H. Downing[1]

Michael J. Fero[2], Harley McAdams[2]

[1]Lawrence Berkeley National Laboratory (LBNL),
Berkeley, CA 94720

[2]Department of Developmental Biology, Stanford School of Medicine, Stanford University, Stanford CA 94305

The shape of the bacteria cell wall during division and the deformation at the division plane are a function of the material properties of the cell wall, the growth rate, and the force due to the FtsZ ring. We have obtained 23 cryo-EM tomographic reconstructions through a time course following the cell division process in *Caulobacter crescentus*. We have also acquired over 300 images of the process by high resolution cryo-EM. The cell membranes have been segmented by an in-house developed utility, and represented on a canonical triangular mesh. These cell membrane surfaces provide us local curvatures; together with independently determined elastic parameters for the membrane, these data allow us to estimate the local forces exerted during the process. Our current models support the conclusion that cell growth and cell contraction occur on similar timescales, and that the contractile force at the division midplane is minimal, serving to direct the growth of the peptidoglycan mesh very near the division plane so as to eventually pinch and isolate the two halves of the dividing cell. The potential energy stored in the peptidoglycan mesh under the strain induced by the FtsZ ring is always minimal while insertion of new cell wall material near the division midplane at the point of inflection in the dividing cell is essential. During the late stages of the *Caulobacter crescentus* cell division process, the inner membrane (IM) separates from the cell wall and proceeds to compartmentalize the cytoplasmic spaces of mother and daughter cells. The outer membrane (OM) and S-layer lag behind forming a narrow tubular structure that keeps both cells connected until the IM process is completed. The shape of the IM surface evolves in a seemingly independent process. We compare the modeled membrane surfaces obtained from our data with simulated evolutions of close membrane surfaces, with realistic elastic parameters, to try to provide a first quantitative estimate of the work involved in this process.

We also illustrate the combined use of cryo electron tomography and spectroscopic difference imaging in the study of sub-cellular structure and subcellular bodies in whole bacteria. We limit our goal and focus to bodies with a distinct elemental composition that is in sufficiently high concentration to provide the necessary signal-to-noise level at the relatively large sample thicknesses of the intact cell. This combination proves very powerful, as demonstrated by the characterization of a phosphorus-rich body in *Caulobacter crescentus*. We also confirm the presence of a body rich in carbon, demonstrate that these two types of bodies are readily recognized and distinguished from each other, and provide for the first time to our knowledge structural information about

them in their intact state. In addition we also show the presence of a similar type of phosphorus-rich body in *Deinococcus grandis*, a member of a completely unrelated bacteria genus. Cryo electron microscopy and tomography allow the study of the biogenesis and morphology of these bodies at resolutions better than 10 nm, while spectroscopic difference imaging provides a direct identification of their chemical composition.