Cryo Electron Tomography Studies of Bacteria Cell Division and Chromosome Organization.

We use cryo electron tomography to obtain three-dimensional images of *Caulobacter* cells at successive stages of cell division. Our tomographic reconstructions show that the late stages of constriction and separation of the *Caulobacter* inner and outer membrane are separate events occurring one after the other and distinctly separated in time and space, consistent with FLIP assay results. A model for this process is advanced based on a series of 17 tomographic reconstructions. In *Caulobacter*, division proceeds by gradual constriction of the cylindrical part of the cell, and there is no septum as in *B. subtilis* or *E. coli*. The FtsZ ring forms at the division plane about 90 min into a 180 min cell cycle; about 30 min later, constriction of the cell is visible. Many proteins involved in cytokinesis are known in *Escherichia coli* and *Bacillus subtilis* and to a lesser extent in *Caulobacter crescentus*. In each of these species, the widely-conserved tubulin-like FtsZ protein initiates cell division by polymerizing into a ring at the future division site. Preliminary cryo electron tomography data shows for the first time the FtsZ ring in its intact native state. The details of the function of the FtsZ ring in late stages of bacterial cytokinesis are both less understood than earlier stages and more variable across different species. An important part of our work aims at the elucidation of these questions.
We are also working with *Deinococcus radiodurans*, and the related *Deinococcus grandis*. These bacteria constitute a target of great relevance because of their unusual ability to repair DNA damage caused by ionizing radiation and other environmental stress. One goal of particular interest in studying these bacteria is to better understand the mechanical packaging of the DNA, which has been reported to be in a highly condensed, toroidal form. This in turn could help to further elucidate the mechanism of DNA repair in prokaryotes. This form of DNA packaging may be a general model for DNA protection under stress, and preliminary data shows the loose toroidal arrangement of *Deinococcus grandis* DNA. We are also studying the organization of the cell membrane separating individual cells in the dyads and tetrads, since it has been proposed that the exchange of material across these boundaries plays an important role in their unusual radiation resistance.
Another part of our efforts targets the fully automation of data collection, tomographic reconstruction and feature extraction from finished tomograms. We present the first step in the development of a fully automatic membrane segmentation tool.