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In vitro High-Resolution Architecture and Structural Dynamics of Bacterial Systems

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Elucidating the molecular architecture of bacterial and cellular surfaces and its structural dynamics is essential to understanding mechanisms of pathogenesis, immune response, physicochemical interactions, and environmental resistance. I will discuss the application of *in vitro* atomic force microscopy (AFM) for studies of high-resolution architecture, assembly and structural dynamics of several microbial systems, including bacteria and bacterial spores. We have demonstrated that strikingly different species-dependent high-resolution structures of the spore coat appear to be a consequence of crystallization mechanisms that regulate the assembly of the spore coat [1-2]. In case of *Clostridium novyi-NT* spores [3], coat layers were found to exhibit screw dislocations typically observed on inorganic and macromolecular crystals. This presents the first case of non-mineral crystal growth patterns being revealed for a biological organism, which provides an unexpected example of nature exploiting fundamental materials science mechanisms for the morphogenetic control of biological ultrastructures. I will present data on the development of an AFM-based immunolabeling technique for the proteomic mapping of macromolecular structures on the bacterial surfaces [4]. I will present data on the direct *in vitro* visualization of the high-resolution structural dynamics of single *Bacillus* and *Clostridium* spores germinating under native conditions [3,5]. Here we show that AFM reveals previously unrecognized germination-induced alterations in spore coat architecture and topology as well as the disassembly of outer spore coat rodlet structures. These results suggest that the spore coat rodlets are structurally similar to amyloid fibrils. The *in vitro* AFM imaging also revealed the porous fibrous cell wall structure of newly emerging and mature vegetative cells, consisting of a network of nanometer-wide peptidoglycan fibers. I will also present data on the direct visualization of stress-induced environmental response of metal-resistant *Arthrobacter oxydans* bacteria to Cr (VI) exposure. These studies demonstrate that *in vitro* AFM can probe microbial surface architecture, environmental dynamics and the life cycle of bacterial and cellular systems at near-molecular resolution under physiological conditions. This work was performed under the auspices of the U.S. DOE by LLNL under contract number DE-AC52-07NA27344.

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