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Time-Resolved Nanometer Scale AFM Imaging of Antimicrobial Peptide Activity on Live *Escherichia coli* Cells

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Bacterial membranes have a much more complex structure than mammalian cell membranes. As such, knowledge of bacterial membrane composition and organization, as well as characterization of the molecular-level responses to drug interactions, is critical to the development and assessment of effective antibacterial drug formulations. Cellular drug responses involve highly dynamic processes. However, the ability to image live cells with nanometer resolution on timescales relevant to dynamic cellular events has proven challenging. With traditional AFM systems, the typically longer image acquisition times required to obtain a single high-resolution image (~minutes) has limited the ability to investigate dynamic biological processes. While recent years have shown significant progress in the development of high-speed atomic force microscopy (HS-AFM), the nature of the instrumentation that has been developed has several drawbacks in specimen size, requiring small scan sizes and flat sample surfaces. As such, the majority of biologically-related HS-AFM studies have concentrated on imaging single biomolecules with little focus on using HS-AFM to examine cellular processes.

With the rapidly growing antibiotics crisis, antimicrobial peptides (AmP) are increasingly being investigated as therapeutic alternatives. Key to their success is an understanding of the mechanisms by which AmPs interact with the cell membrane and facilitate cellular death. Using HS-AFM, we have obtained the first high-resolution time sequence images of the native structure of a bacterial outer membrane, obtained directly on the surface of living *Escherichia coli* cells. The increased time resolution of HS-AFM allowed us to observe dynamic changes in the nanoscale structure of the outer membrane in direct response to the AmP CM15, at timescales relevant to the mechanism of AmP-induced cell death. To understand how CM15 interacts with the bacterial inner membrane, we also conducted HS-AFM imaging on supported model membranes that mimic the composition of the inner membrane of *E. coli.* Our results revealed the formation of circular, pore-like defects within specific lipid domains upon exposure to the AmP. The results of these HS-AFM studies have provided the first opportunity to resolve the dynamics of AmP-mediated cell death in a native cell membrane environment in real-time and with nanoscale resolution.

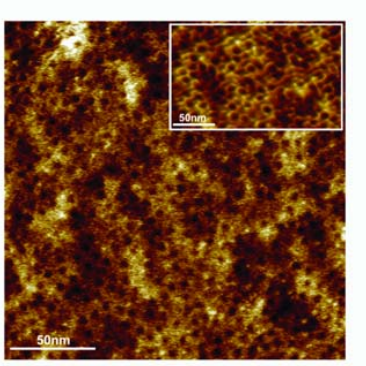

Figure 1. High-resolution AFM imaging of the native outer membrane surface of individual living *E. coli* cells revealed the presence of nanometer-sized features. Fast Fourier Transform filtering of the image (inset) revealed areas of ordered structures believed to be densely packed porin molecules.

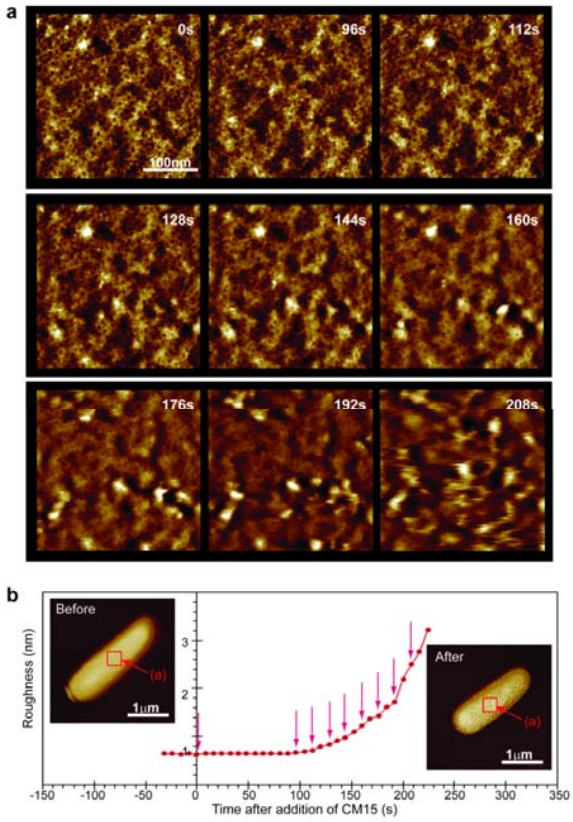

Figure 2. Time series of CM15 activity on the nanoscale structure of the *E. coli* outer membrane. (a) CM15 was added to the imaging fluid at t=0s and images recorded every 8 seconds (Image Size=300nm.). After ~96s incubation, the cell membrane was observed to increase in roughness (b) accompanied by the disappearance of the ordered membrane surface structures, membrane micellization, and the appearance of 'pore-like' lesions. It is important to note that once the effects of CM15 were initially observed, the subsequent changes in the cell surface occurred fairly rapidly over ~224s, at which point imaging of the cell surface became unstable. The inset phase images in the graph (b) show the location on the cell where the high-resolution time-series images in (a) were obtained. The data points on the graph corresponding to the images in (a) are indicated by the arrows.

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