BIO Web of Conferences **129**, 16009 (2024) https://doi.org/10.1051/bioconf/202412916009
EMC 2024

Multiparametric investigation of bacterial surface structure with correlative atomic force microscopy

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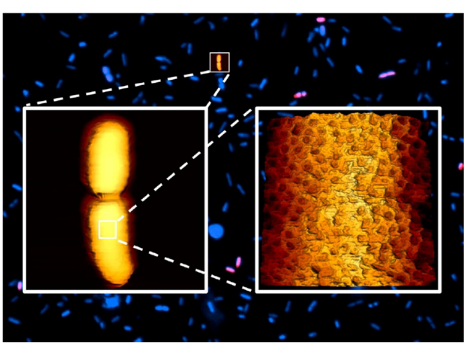
Background incl. aims
Atomic force microscopy (AFM) has become an indispensable tool for high-resolution structural analysis of specimens ranging from single molecules to complex biological systems, while simultaneously being able to correlate topography and mechanics at near native/physiological imaging conditions. In turn, the combination with advanced/customized optics leverages the advantages of immunolabelling techniques for truly correlative microscopy. Specifically, the use of a tip-scanning AFM, as compared to a sample-scanning system, enables simultaneous high-resolution correlation experiments with advanced optical techniques. Studying certain Gram(-) bacterial species have attracted a lot of interest recently as model systems for investigating their outer membrane envelope.

Methods
We have applied fast tip-scanning AFM imaging in combination with confocal microscopy to study the surface structure of bacterial species. We have studied the surface structure of E. coli MG1655 bacteria to gain a deeper insight into the supramolecular organization of their outer membrane protein network.

Results
By additionally exploiting full-fledged sample mechanical mapping we can correlate any substructural surface analysis of the layered bacterial specimen with differences in bacterial stiffness. We demonstrate how the application of dual z-actuator tip-scanning AFM imaging can be exploited for instant high-resolution analysis of tall bacterial samples exceeding 1 µm in height. We will also discuss how the combination of high-speed tip-scanning AFM with antimicrobial agents can be used as a tool to study the bacterial envelope in real-time and potentially develop strategies for fighting microbial resistance.

Conclusion
AFM can be successfully applied to correlate high-resolution structural features and mechanical properties of the outer membrane envelope in MG1655 E. coli bacterial species in combination with advanced optical microscopy.

Graphic:



Correlative Microscopy of MG1655 bacterial species. Confocal laser scanning microscopy was used to localize living bacteria (Hoechst 33342, blue), while high-resolution fast AFM Imaging reveals the pore structure of the *E.coli* surface. NestedScanner AFM topography images acquired at 1 Hz line rate (overview) and 5 Hz line rate (zoom). XYZ ranges of the AFM regions are 4 µm x 4 µm x 1.4 µm (overview) and 200 nm x 200 nm x 15 nm (zoom).

Keywords:

AFM, Correlative Microscopy, Bacterial Imaging