

## Investigation of bacterial adhesion mediated by a curli amyloid binding network using AFM

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The Bacterial cell wall plays a significant role in maintaining cellular structure and resisting turgor pressure. It changes during growth and division and also opens a pathway to transfer information from the outer environment into the cell, suggesting that the cell wall is dynamic and its mechanical properties are of significant importance. Biological atomic force microscopy (Bio-AFM) is the tool of choice for detailed microbial studies, because it allows for investigating living microbial organisms in their natural environment at the nano-scale. As the microbial outer membrane interacts with the extra-cellular environment or other surfaces directly, characterizing its membrane structures and binding capacities provides crucial information for understanding fundamental processes such as bacterial adhesion, surface recognition, and initial attachment to abiotic or biotic surfaces. Bio-AFM is also capable of measuring the cell wall stiffness. Analysis of AFM force-indentation curves yield physical properties of the cellular surface such as Young's modulus, internal turgor pressure, and the stretching modulus of the bacteria. Moreover, using a nanomechanical force-sensing approach, we obtained real-time information about the distribution of molecular bonds involved in the adhesion of curled bacteria to fibronectin. We found that a dense collective network of bonds is formed between curli and fibronectin fibers, which results in tight bacterial binding to cell surfaces. Nanomechanical force recognition measurements revealed that approximately 10 bonds were disrupted either sequentially or simultaneously. For a single RGD/CsgA bond, we attained a force of 51 pN and a short lifetime of about 0.85 s. The work required to dissociate a whole *E. coli* cell from the fibronectin surface was 2750  $k_B T$ . Thus amyloid formation of *E. coli* surfaces leads to multi-bond structural components of fibrous nature that explains the strong mechanical binding of curled bacteria to hosts and unveils the functions of these proteins in bacterial internalization and invasion. Overall we demonstrate the potential of single-molecule and single-cell force spectroscopy for revealing accurate dynamic and statistical information about the nanomechanical behavior of multiple bonds involved in collective network formation during cellular adhesion.

### References

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Acknowledgements. This work was supported by an APART (Austrian Programme for Advanced Research and Technology) fellowship of the Austrian Academy of Science (to Y. Oh)