Electronic Supplementary Material (ESI) for Nanoscale Horizons.
This journal is © The Royal Society of Chemistry 2020

Supplementary Information

Fast chemical force microscopy demonstrates that glycopeptidolipids define nanodomains of varying hydrophobicity on mycobacteria

Albertus Viljoen[1], Felipe Viela[1], Laurent Kremer[2,3] and Yves F. Dufrêne[1,4]

[1]Louvain Institute of Biomolecular Science and Technology, UCLouvain, Croix du Sud, 4-5, bte L7.07.06, B-1348 Louvain-la-Neuve, Belgium

[2]Institut de Recherche en Infectiologie de Montpellier (IRIM), Université de Montpellier, CNRS UMR 9004, Montpellier, France.

[3]INSERM, IRIM, 34293 Montpellier, France.

[4]Walloon Excellence in Life sciences and Biotechnology (WELBIO), Belgium

[*]Corresponding author:

Yves Dufrêne: yves.dufrene@uclouvain.be

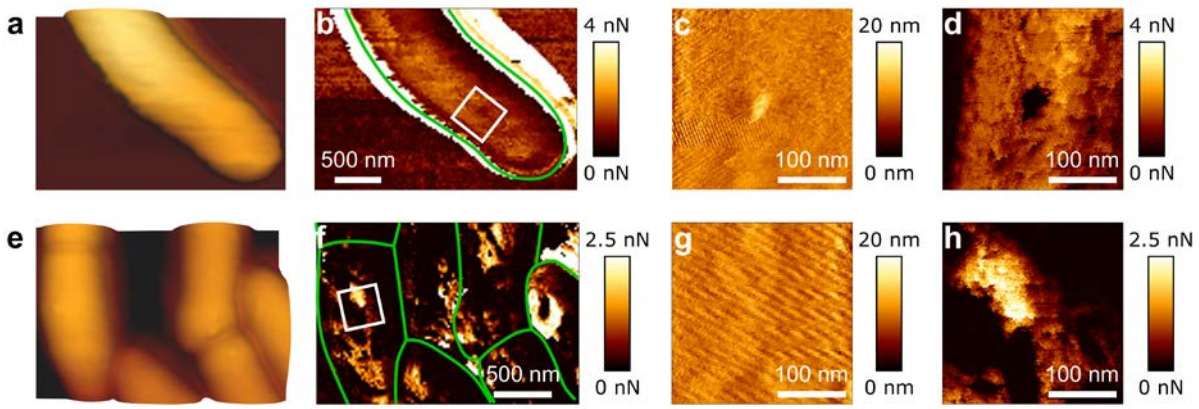

Fig. S1 Hydrophobic tips capture homogenous hydrophobicity on the *Mycobacterium abscessus* R variant (a-d) and hydrophobic nanodomains on the S variant (e-h). (a, e) 3-D projections of height data collected with hydrophobic tips (a, R variant cell, 2.5 µm × 2.5 µm, e, S variant cells, 2 µm × 2 µm) and (b, f) accompanying adhesion maps. Due to an increased contact area between the pyramidal tips and the sharp edges of the bacteria very large adhesion forces were recorded on the cell edges, particularly evident for the R variant. The outlines of bacteria are indicated by green lines. White boxes (300 nm × 300 nm) in b and f demarcate areas on which high-resolution data were collected, shown in c, g (height images) and d, h (adhesion maps), respectively.

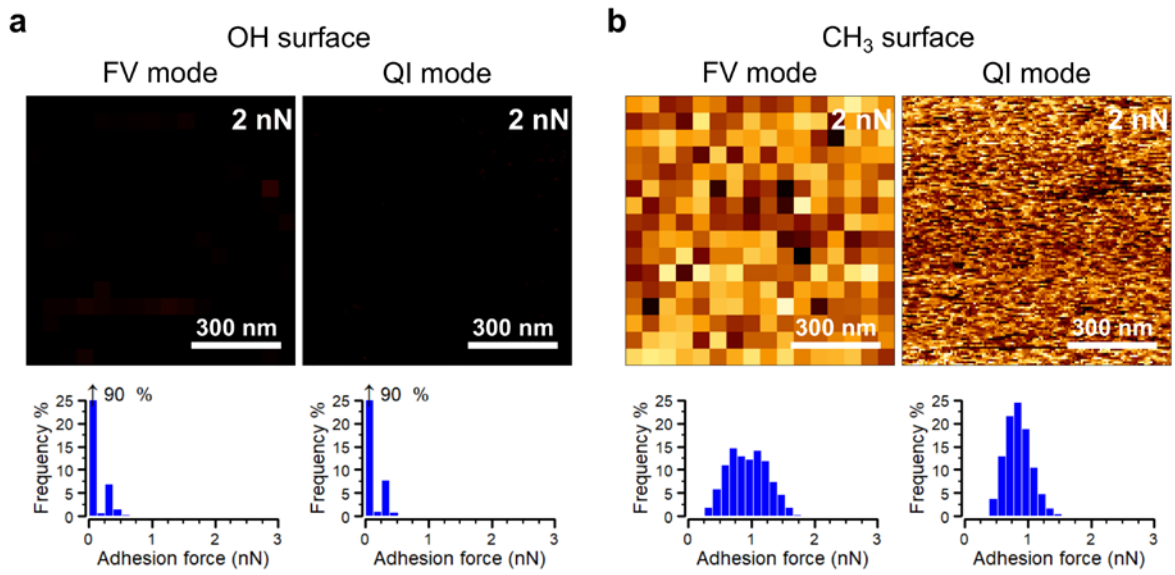

Fig. S2 Hydrophobic tips do not detect adhesive forces on hydrophilic (OH-group exposing) model surfaces, but do detect strong forces on hydrophobic (CH_3-group exposing) surfaces. (a) Adhesion maps (top) and histograms (bottom) for interactions between hydrophobic tips and hydrophilic surfaces (one representative surface-tip combination out of a total of n = 3). (b) Data obtained similarly for interactions between hydrophobic AFM tips and hydrophobic surfaces (out of n = 3). The z-range in all adhesion maps extend from 0 to 2 nN.

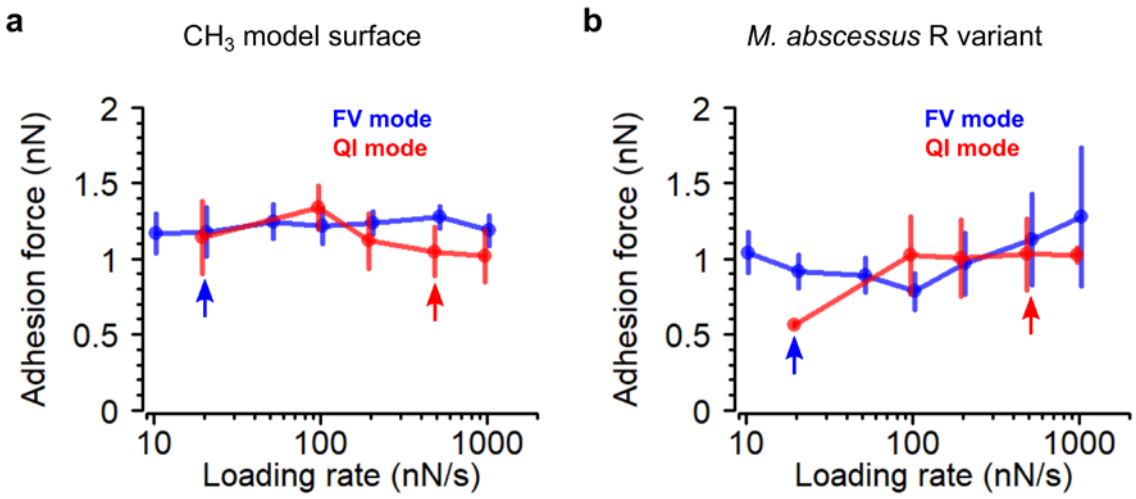

Fig. S3. The high loading rate applied in Quantitative Imaging (QI) does not significantly influence the magnitude of hydrophobic forces. (a) Dynamic force spectroscopy (DFS) plots obtained in Force Volume (FV) and QI modes for hydrophobic tips and hydrophobic model surfaces. (b) DFS plots obtained in FV and QI modes for hydrophobic tips and *M. abscessus* R variant cells. In FV, data were collected on 300 nm × 300 nm (16 × 16 pixel) areas, and in QI on 300 nm × 300 nm (128 × 128 pixel) areas, both on model surfaces and on cells. A blue arrow points to the loading rate usually applied in FV (1 µm/s = 20 nN/s) and a red arrow points to the loading rate that we applied in QI (25 µm/s = 500 nN/s).

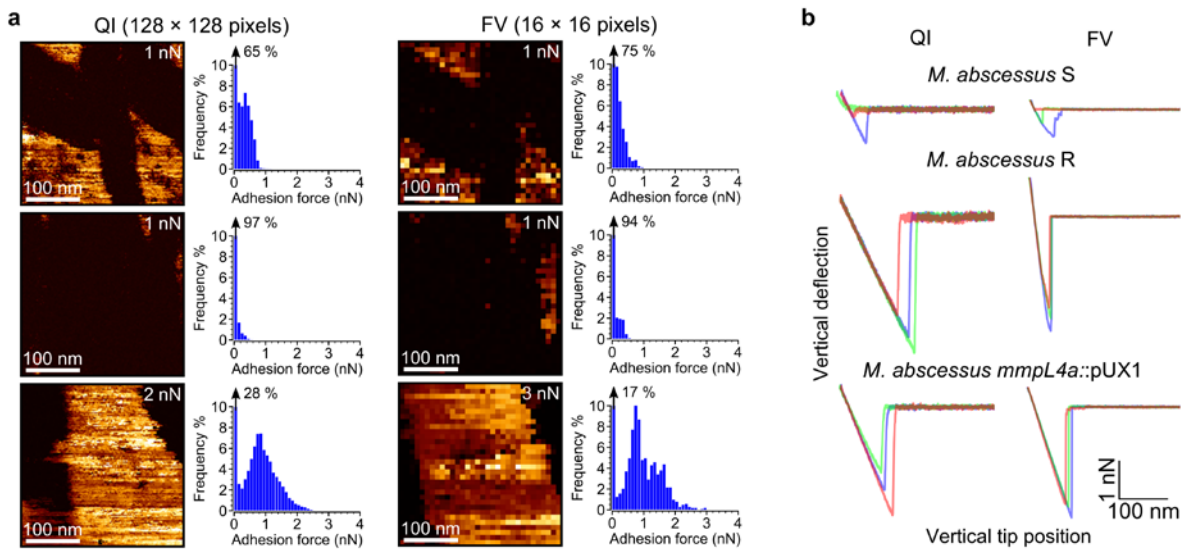

Fig. S4 Adhesion maps on *M. abscessus* S variant cells with hydrophobic tips reveal a heterogenous spread of hydrophobic nanodomains both in FV mode with standard speed parameters (1 µm/s) and in QI mode with increased speed (25 µm/s). (a) Three representative high-magnification force maps produced by QI (left) and FV (right), and their corresponding histogram plots. All maps were produced for the *M. abscessus* S variant. (b) QI and FV force-distance curves obtained on *M. abscessus* S, R and *mmpL4a*::pUX1 surfaces.

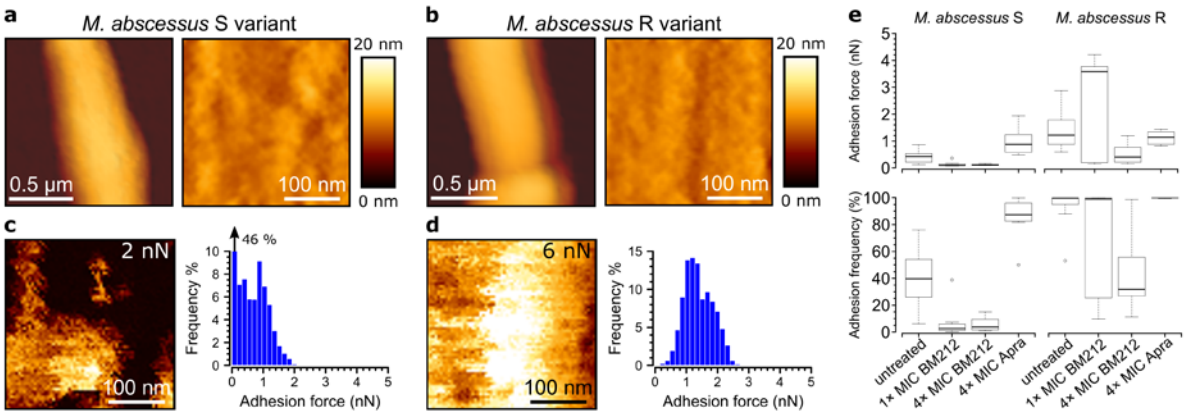

Fig. S5 Effect of an antibiotic that does not directly target cell wall processes on *M. abscessus* S (a, c) and R (b, d) surface topology (a, b) and surface hydrophobicity (c, d). At least eight cells were used for each condition. (e) Boxplots of adhesion forces and binding frequencies obtained on *M. abscessus* S and R cells, either untreated or treated with 2 µg/ml (1 × MIC) or 8 µg/ml (4 × MIC) BM212 or 8 µg/ml (4 × MIC) apramycin.

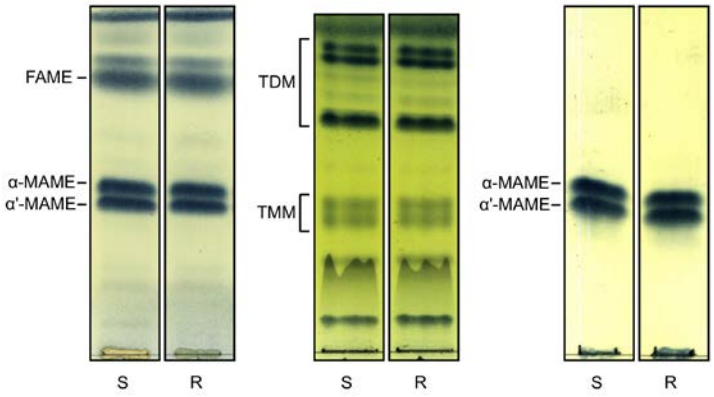

Fig. S6 Thin layer liquid chromatography analysis of the different mycolate containing lipid species in *M. abscessus* shows no differences between S and R variants. (left) TLC of total methyl esters of mycolic acids (MAME) and fatty acids (FAME) obtained from saponified cells. (middle) TLC of the apolar lipid fraction extracted from whole cells (prior to saponification) and containing TMM and TDM. (right) MAME extracted from saponified delipidated cells.