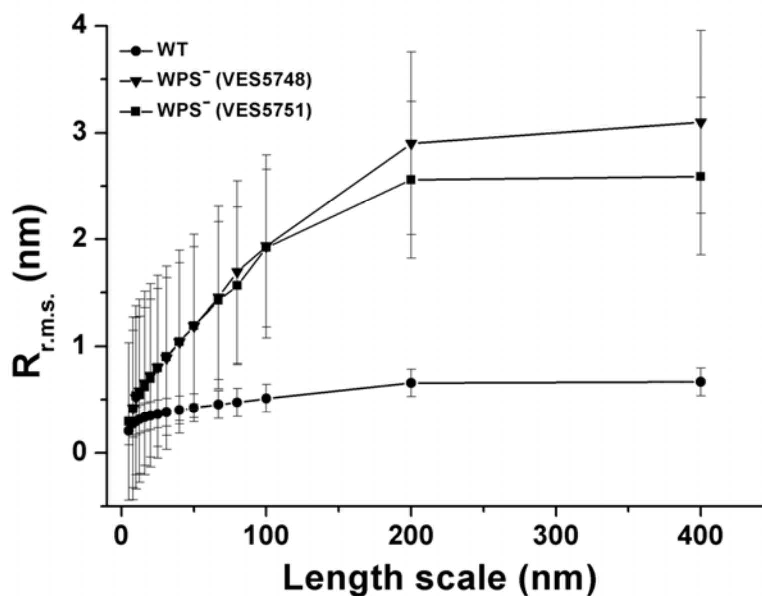
Supplementary information

Imaging the nanoscale organization of peptidoglycan in living *Lactococcus lactis* cells

Guillaume Andre, Saulius Kulakauskas, Marie-Pierre Chapot-Chartier, Benjamine Navet, Marie Deghorain, Elvis Bernard, Pascal Hols, and Yves F. Dufrêne



Supplementary Figure S1. Statistical analysis of topographic images. Variation of the root mean square roughness (R_{rms}) constructed from the power spectral density (PSD) analysis as a function of the length scale, for *L. lactis* WT (●), VES5748 (▼) and VES5751 (■) WPS$^-$ mutants. Each data point represents the mean ± standard deviation of four or five images obtained from independent cells.

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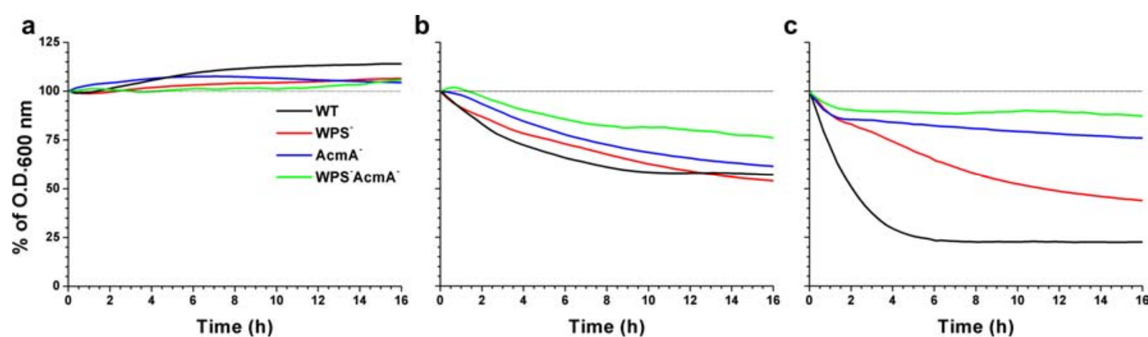
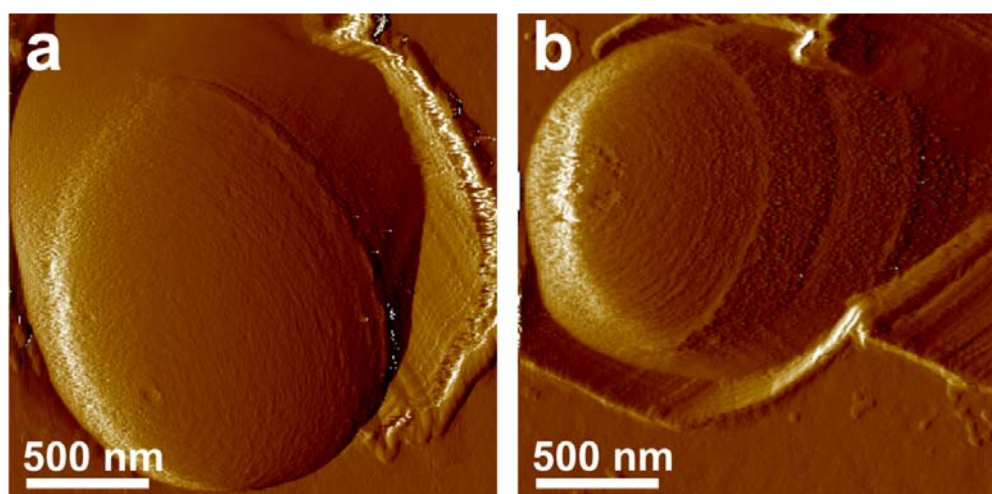


Supplementary Figure S2. Autolysis assays. Evolution with time of the cell population - i.e. optical density at 600 nm (O.D.$_{600\ nm}$) - of the wild-type strain, and of the VES5748 WPS$^-$, AcmA$^-$ and VES1876 WPS$^-$AcmA$^-$ mutants resuspended in three different buffers: sodium acetate buffer (150 mM, pH 5.2) which is known to limit hydrolysis[46-48] (a), phosphate buffer (50 mM, pH 7.0) which activates autolysins, thus hydrolysis[46-48] (b) and phosphate buffer (50 mM, pH 7.0) with Triton X-100 (0.05 %), which induces autolysis by membrane permeabilization[49] (c).



Supplementary Figure S3. Ridges are observed in different environmental conditions. AFM deflection images (applied force of 250 pN) of VES5748 WPS$^-$ cells incubated for 2 hrs in phosphate buffer (a) or in ultrapure water (b).

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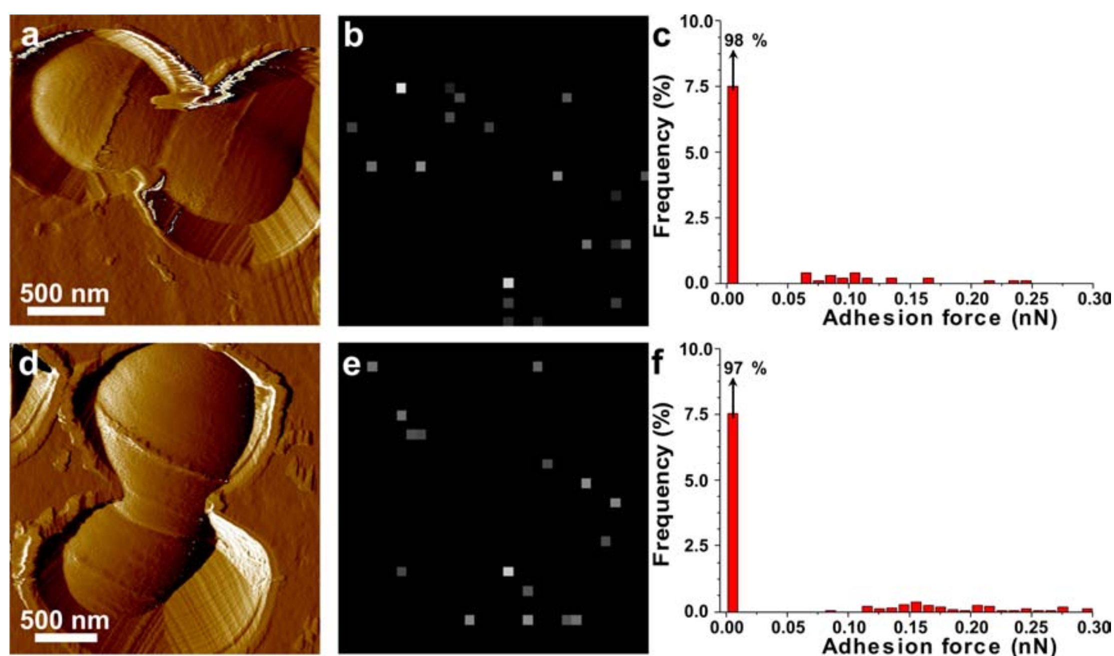
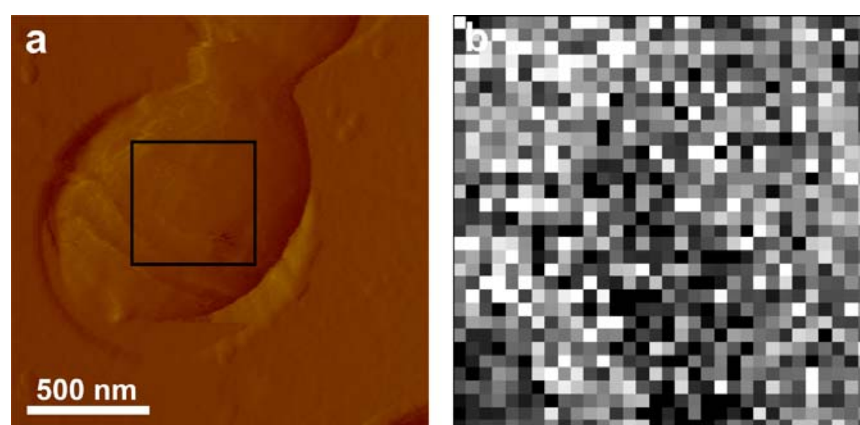


Supplementary Figure S4. Control experiments demonstrating the specificity of the single-molecule recognition measurements. (a) Deflection image, (b) adhesion force map (500 nm x 500 nm) and (c) adhesion histogram (n = 2048 curves from 2 different maps) recorded with a silicon nitride tip on the VES5748 WPS$^-$ mutant. (d) Deflection image, (e) adhesion force map (500 nm x 500 nm) and (f) adhesion histogram (n = 4096 curves from 4 different maps) recorded with a LysM tip on the VES5748 WPS$^-$ mutant, in the presence of 10 μg.ml^{-1} peptidoglycan.



Supplementary Figure S5. Single-molecule recognition imaging of trichloroacetic acid (TCA)-treated cells reveals dense and homogeneous peptidoglycan localization. (a) AFM deflection image of a VES5748 WPS$^-$ mutant cell after treatment with TCA, documenting a smooth surface in which ridges are no longer visible. (b) Adhesion force map (500 nm x 500 nm) recorded with a LysM tip in the square area shown in the deflection image, using a maximum applied force of 250 pN.

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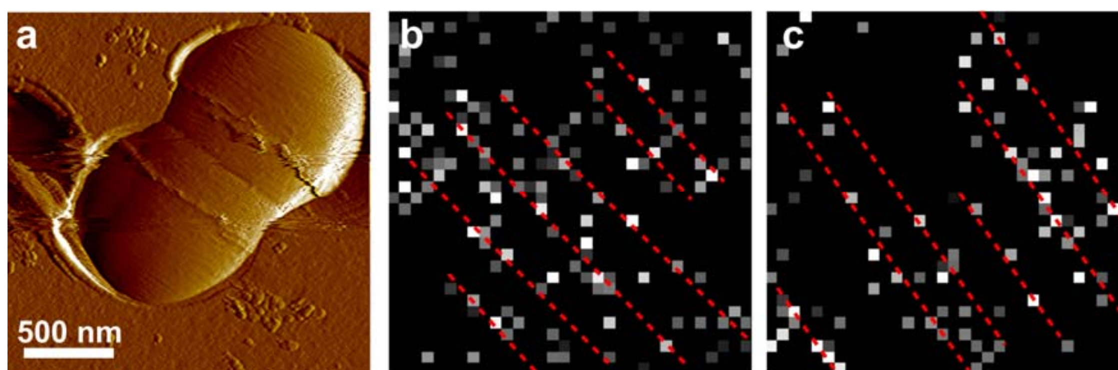

Supplementary Figure S6. Recognition imaging of peptidoglycan residues using lectin tips. (a) Deflection image recorded with a silicon nitride tip on the VES5748 WPS⁻ mutant. (b, c) Adhesion force maps (500 nm x 500 nm) recorded on top of the cell with AFM tips functionalized with either wheat germ agglutinin (b) or *Griffonia simplicifolia* lectin II (c). Many of the detected residues (bright pixels) were arranged as lines running parallel to the short cell axis (red lines).

References

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