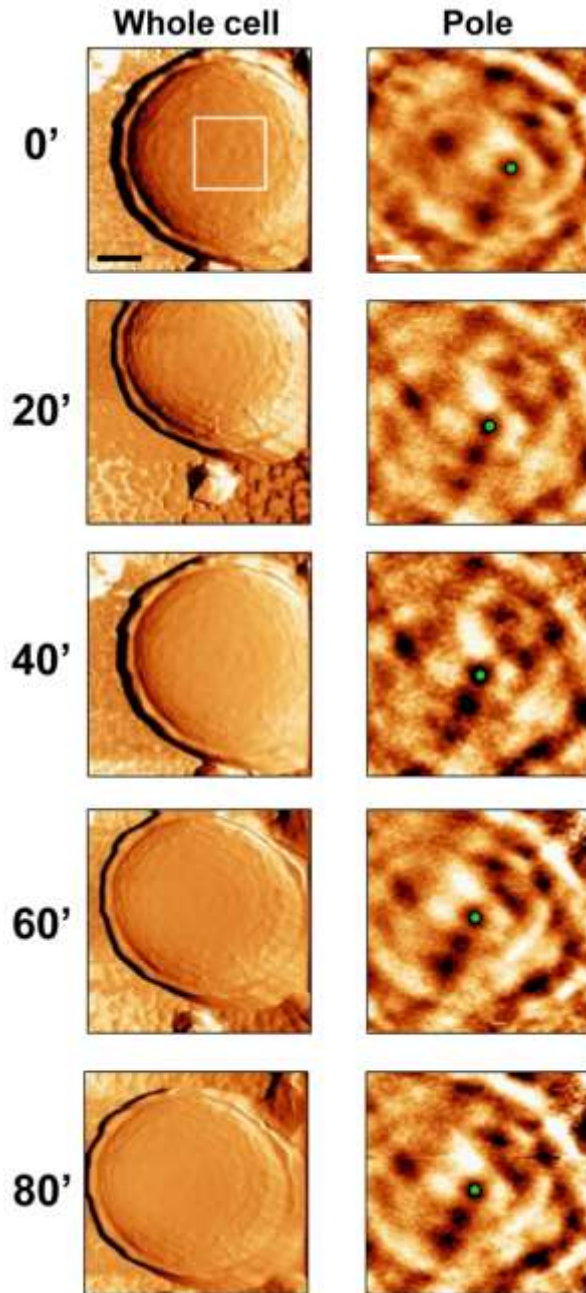
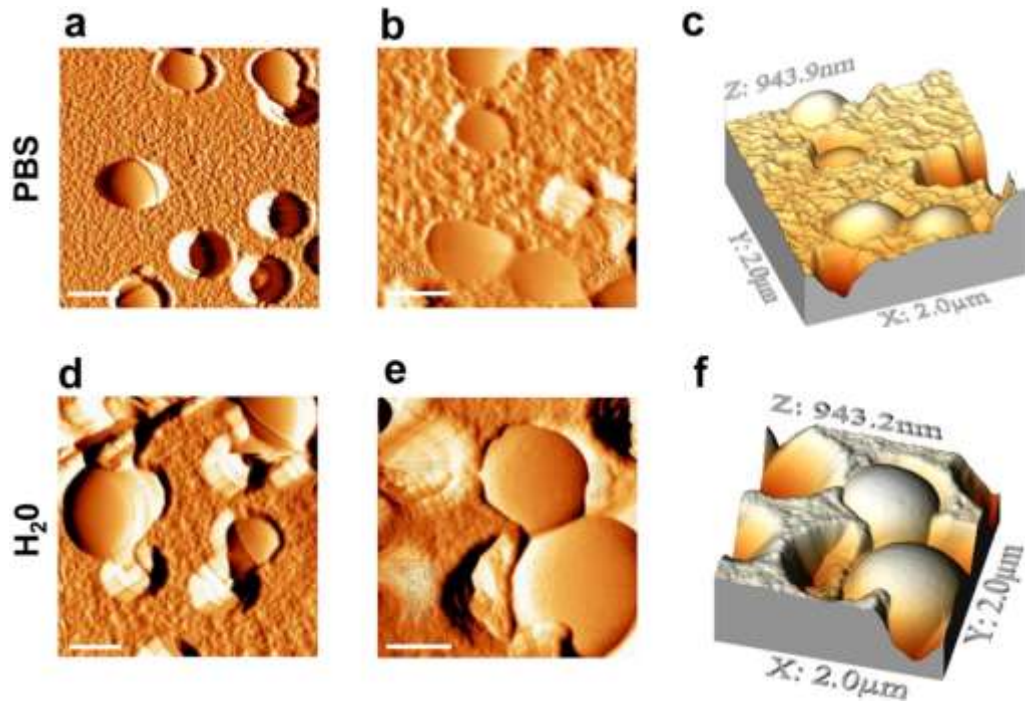


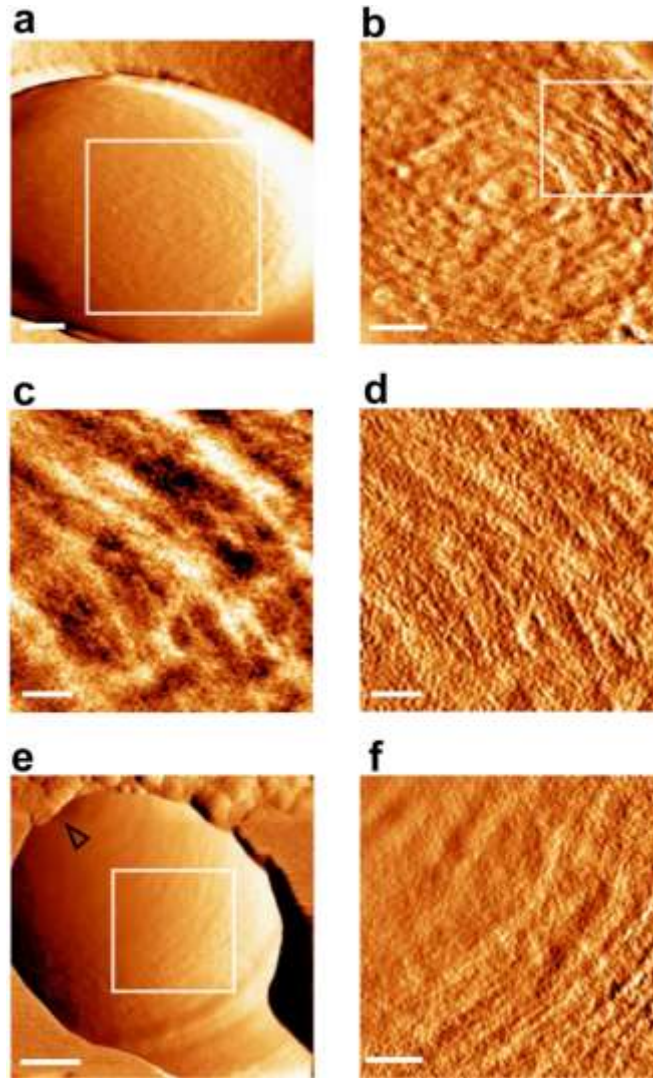
Supplementary Figure 1. The influence of different peak-force scans on surface architecture. (a-c) Peak-force error mode images of whole bacteria (scale bars, 200 nm). (d-f) Height mode images of the cell's top show a banded architecture only when a 1 nN imaging force is used (scale bars, 95 nm; height scale, 4.2 nm; profile of bands from f is at the right). (g-i) Elastic modulus images are sensitive to surface properties only when a 1 nN force is used (color scale, 0-4 MPa;). Force curves recorded from the cell (black) and filter (gray) from spots marked in c (approach is full line and retraction is dashed line).



Supplementary Figure 2. Scanning bacteria using 1 nN force does not alter the surface topography during multiple scanning cycles. (a) Representative force-error images from an experiment in which single cells were scanned repeatedly in ultrapure water (scale bar, 200 nm; 1,024 pixels/ μm ; 1 Hz rate). (b) High-magnification height mode images show the surface topography does not significantly change with time (scale bar, 100 nm; height, 4.1 nm; green dot marks the center of the pole).



Supplementary Figure 3. Bacteria imaged in ultrapure water are significantly swollen. (a, b) Representative force-error image of pore-trapped GBS imaged in PBS (scale bars, 600 nm and 500 nm, respectively). (c) 3D-representation of (b). (d, e) Representative force-error image in ultrapure water (scale bars, 600 nm and 500 nm, respectively). (f) 3D-representation of (e).



Supplementary Figure 4. Single strands in stationary *srtA* mutant. (a, e) Representative force-error image of stationary *srtA* mutant (scale bars, 150 nm and 200 nm, respectively). (b) Magnified force-error image of the pole region from a (scale bar, 75 nm). (c, d) Height and corresponding force-error images, respectively (scale bar, 25 nm; height, 1.3 nm). (f) Magnified force-error image of the side-wall region from e (scale bar, 25 nm).

Supplementary Table 1. Bacterial strains used in this study

Strains	Relevant genotype and characteristics ^a	Source
<i>S. agalactiae</i>		
NEM316	WT strain, serotype III, ST23	1
<i>lgt-lsp</i>	NEM316 <i>lgt</i> Δ <i>aphA-3</i> <i>lsp</i> Δ <i>aad6</i> ; Km, Sm	2
<i>cpsD</i>	NEM316 <i>cpsD</i> Δ <i>aphA-3</i> ; Km	3
<i>srtA</i>	NEM316 <i>srtA</i> Δ <i>aphA-3</i> ; Km	4

^a*aphA-3*, 3'-aminoglycoside phosphotransferase type III; *aad6*, 6-adenylyltransferase; Km and Sm, enzymatic resistance to kanamycin and streptomycin, respectively; Str, streptomycin-resistant mutant.

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