Supplemental Material

Nano-scale Cell Wall Deformation Impacts Long-range Bacterial Adhesion Forces to Surfaces

Yun Chen, Akshay K. Harapanahalli, Henk J. Busscher, Willem Norde, and Henny C. van der Mei*

University of Groningen and University Medical Center Groningen, Department of Biomedical Engineering

Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands.

Control experiments to demonstrate effective bacterial probe preparation. Effective attachment of a staphylococcus on a poly-L-lysine coated cantilever was demonstrated by comparing force-distance curves between a staphylococcal probe and a poly-L-lysine coated cantilever *versus* a glass surface (see Fig. S1).

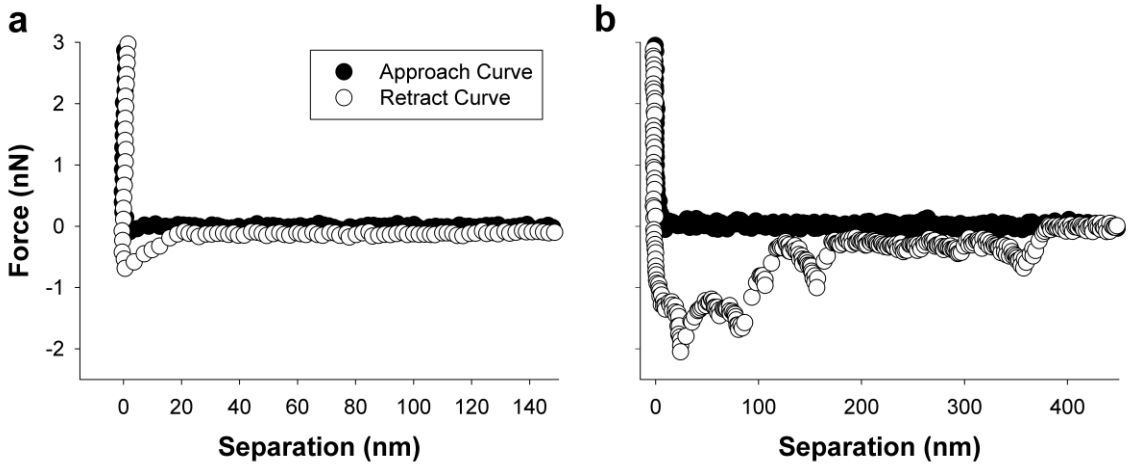

FIG S1 Examples of force-distance curves recorded for a poly-L-lysine coated cantilever (a) and a staphylococcal probe (*S. aureus* NCTC 8325-4) (b) on a glass surface taken in 10 mM potassium phosphate buffer (pH 7.0) under a maximal loading force of 3 nN. Note that the X-axes have different scales.

The poly-L-lysine coated cantilever adheres weakly to the glass surface with a single, narrow, adhesion force in the retract curve, while the staphylococcal probe shows a stronger adhesion force with multiple peaks upon retract.

A second control involves the possible disturbance of the bacterial cell wall upon air-drying the staphylococci to the cantilever, which might be especially important for the *Δpbp4* mutants with their weakened cell wall. In Fig. S2, it can be seen that drying times

up to 3 min do not systematically affect the force-distance curves, neither of the wild-type, parent strains nor of the *Δpbp4* mutants within the reproducibility of the experiments. In neither case do the force-distance curves resemble those of a cantilever without bacteria.

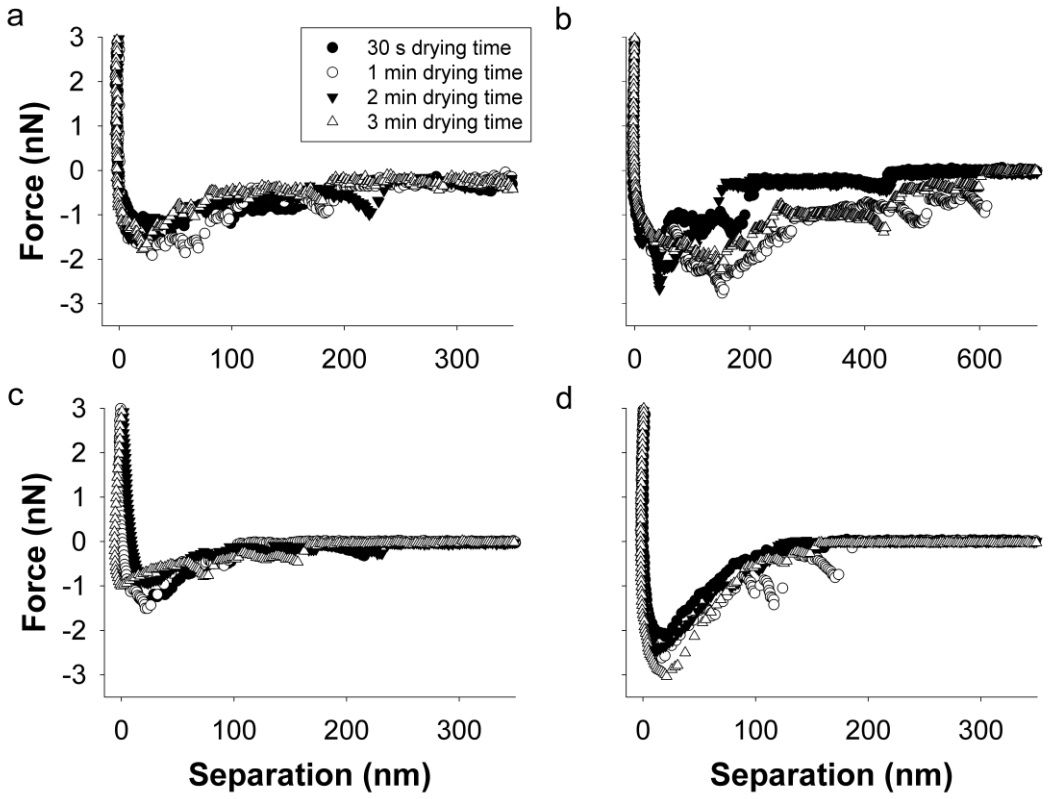

FIG S2 Retract force-distance curves for staphylococcal probes prepared of *S. aureus* NCTC 8325-4 (a), *S. aureus* NCTC 8325-4 *Δpbp4* (b), *S. aureus* ATCC 12600 (c) and *S. aureus* ATCC 12600 *Δpbp4* (d) after different drying times. Note that panel b has a different X-axis scale than the other three panels.

Replicate force-distance curves for a staphylococcal probe and influence of the loading force. Force-distance curves between staphylococci and glass surfaces were generally reproducible (see Fig. S3), showing clear effects of the loading force (see Fig. S4).

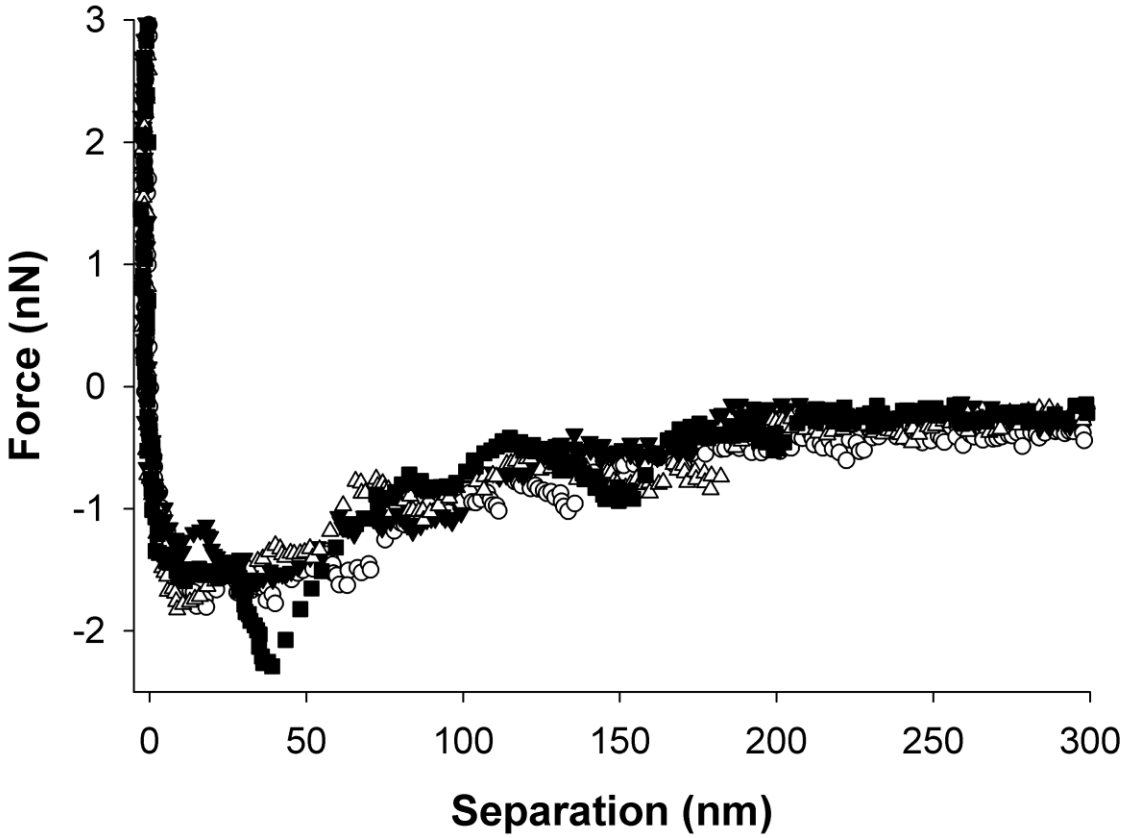

FIG S3 Five replicates of retract force-distance curves recorded for a bacterial probe of *S. aureus* NCTC 8325-4 under a loading force of 3 nN at a same spot on a glass surface in 10 mM potassium phosphate buffer (pH 7.0). Different symbols represent five different replicates.

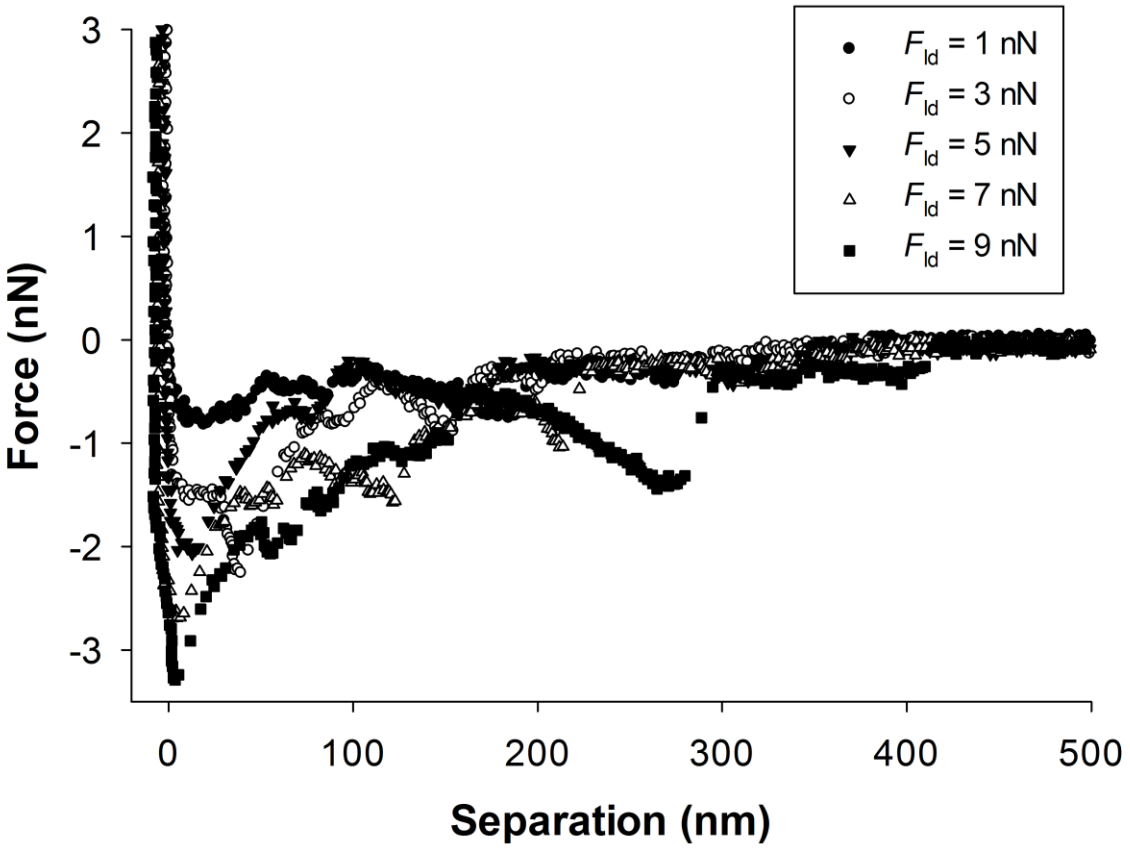

FIG S4 Retract force-distance curves for a bacterial probe of *S. aureus* NCTC 8325-4 on a glass surface under loading forces F_{ld} of 1, 3, 5, 7 and 9 nN in 10 mM potassium phosphate buffer (pH 7.0).

Influence of centrifugation and sonication on the hydrodynamic radii of planktonic staphylococci. In order to verify whether centrifugation and sonication affected the hydrodynamic radii of the staphylococci in their planktonic state, three additional harvesting protocols were applied other than the standard protocol described in the Materials and Methods section. Their hydrodynamic radii R_0 and polar radii $r_{\text{Height Image}}$ were determined using DLS and AFM PeakForce-QNM mode, respectively:

- PROTOCOL 1: staphylococci were harvested by a single centrifugation at 5000 × g for 5 min and directly suspended in 10 mM potassium phosphate buffer.
- PROTOCOL 2: 10 s sonication at 30 W was carried out intermittently for three times for bacteria harvested using Protocol 1, while cooling the suspension in a water/ice bath.
- PROTOCOL 3: the bacteria were harvested and suspended as described in the standard protocol, but no sonication was conducted afterwards.

Fig. S5 summarizes the hydrodynamic radii R_0 (Fig. S5a) the polar radii $r_{\text{Height Image}}$ (Fig. S5b) of bacterial cells prepared by different protocols. Two-sided, one-way ANOVA indicated no significant differences in polar radii of staphylococci harvested according to different protocols ($p > 0.05$).

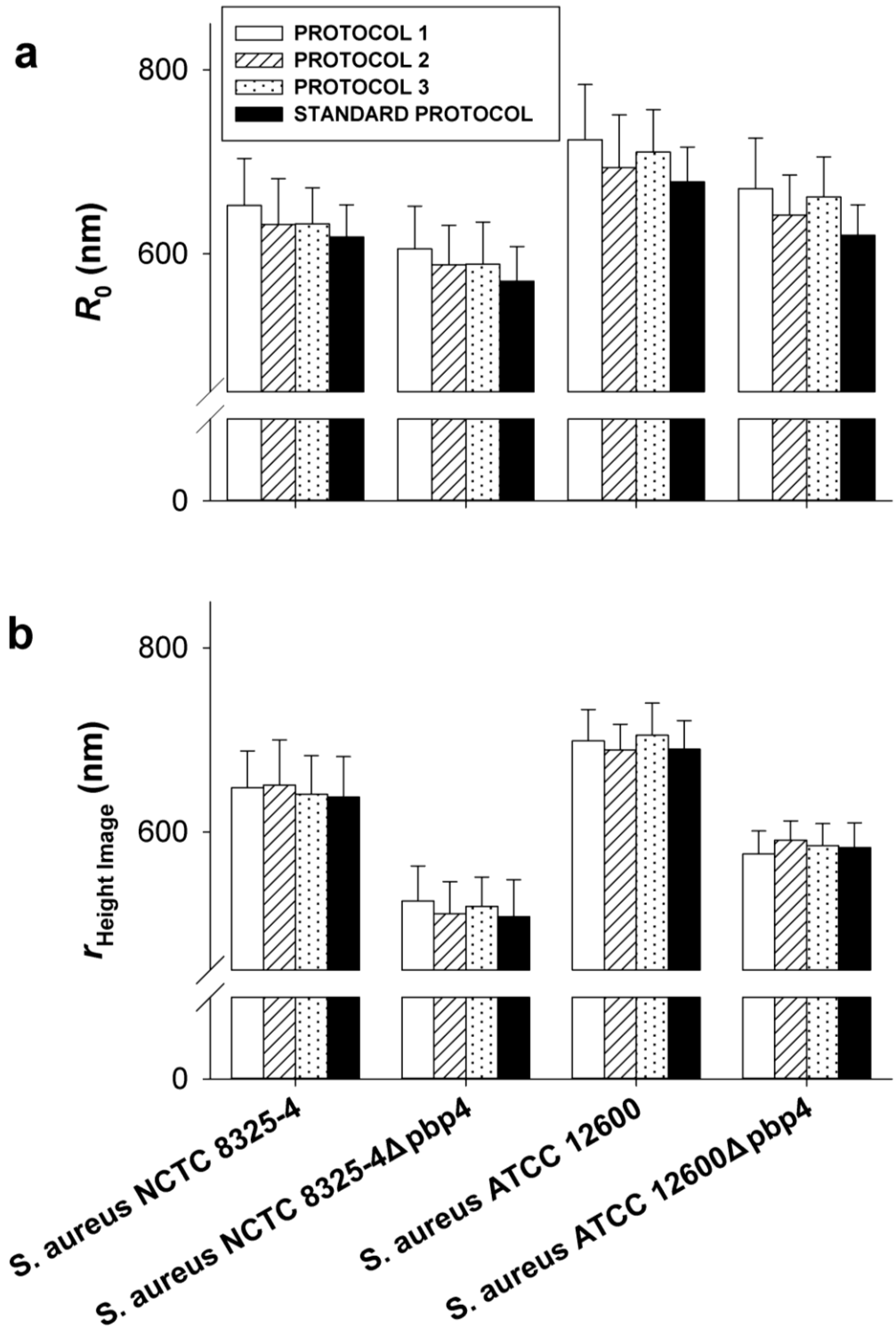

FIG S5 Hydrodynamic radii R_0 measured by DLS (a) and polar radii $r_{\text{Height Image}}$ determined using AFM imaging (b) for staphylococci harvested according to different

protocols. Error bars in panel a denote the standard deviations over nine aliquots taken from three separate bacterial cultures of each strain, and error bars in panel b denote the standard deviations over at least 60 staphylococci taken from three separate bacterial cultures of each strain.