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Determination of the nano-scaled contact area of Staphylococcal cells†

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Supporting information

S. aureus adhesion on hydrophobic surfaces

S. aureus cells showed a slowly decreasing adhesive strength on strongly hydrophobic surfaces: Adhesion force and energy decreased linearly with an increasing number of force-distance curves on random positions on an OTS surface (see Figure 1). Notably, this effect was pronounced to a different extent for different individual cells (different slopes of the fits in Figure 1). This phenomenon may be due to the loss of cell wall attached macromolecules that mediate adhesion in course of repeated force-distance curves [1]. Crossing the hydrophobic/hydrophilic interface, we therefore expect a similar effect on the hydrophobic part of the sample. To characterize that linear decrease, the negative slope m of the best-fit lines in Figure 1 is used to interpret the force-distance curves gained while crossing the hydrophobic/hydrophilic interface shown in Figure 6 a and 6 c in the full article.

In contrast to *S. aureus* cells, *S. carnosus* cells featured robust adhesion mechanisms (yet a lower adhesion strength than *S. aureus*) that withstand multiple adhesion events when probed by AFM force spectroscopy, meaning that within the experimental error, adhesion energy and force remained constant when probing only the hydrophobic or hydrophilic surface area. Therefore, the slope of the best-fit line is set to zero for the experiments with *S. carnosus*, see Figure 6 b and 6 d in the full article.

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Size of *S. aureus* and *S. carnosus*

On average, the cell diameters of *S. aureus* and *S. carnosus* cells are very similar, as can be seen in Figure 2, with diameters matching literature values. [2,3] The mean radii of both species differ by less than 6 % and the standard deviation for both species less is than 7 %. However, the radii measured in SEM micrographs characterize the size of the bacteria in the dry state (in vacuum), therefore, the absolute size may not be the one that is relevant for our experiments, yet it can be expected that the size distribution is similar. However, the variation in bacterial cell radius is much smaller than the variation of the radius of the contact area (Figure. 7 in the main paper), which can be over 30 %.

Application of the method to rigid spheres

To illustrate the strength of the experimental setup, we performed the same type of experiments with a polystyrene bead (purchased as colloidal AFM-probe from Sqube, Bickenbach, Germany) with a diameter of 2 µm to determine its contact area. The sphere features an RMS surface roughness of 1.2 nm as determined by AFM. We obtained a radius of the contact area of 94 *pm*4 nm (see Figure3, left). For comparison, the experiments were also performed with a hydrophobized silica sphere of 4 µm radius (sphere from Polyscience, Warrington, USA, glued to an MLCT-0-E cantilever from Bruker Nano, Santa Barbara, USA). Here, we find a contact area radius of 190 ± 8 nm (see Figure3, right). For both relatively rigid spheres, the ratio of the radius of the contact area to the radius of the sphere is substantially smaller than for the bacterial cells. However, for the rigid spheres, the contact mechanics is very different to the one for the soft, macromolecule-covered bacterial cell wall since its interaction forces to the surface is mainly dominated by single asperities of the colloidal probe. [4] Therefore, a deeper study of colloidal con-

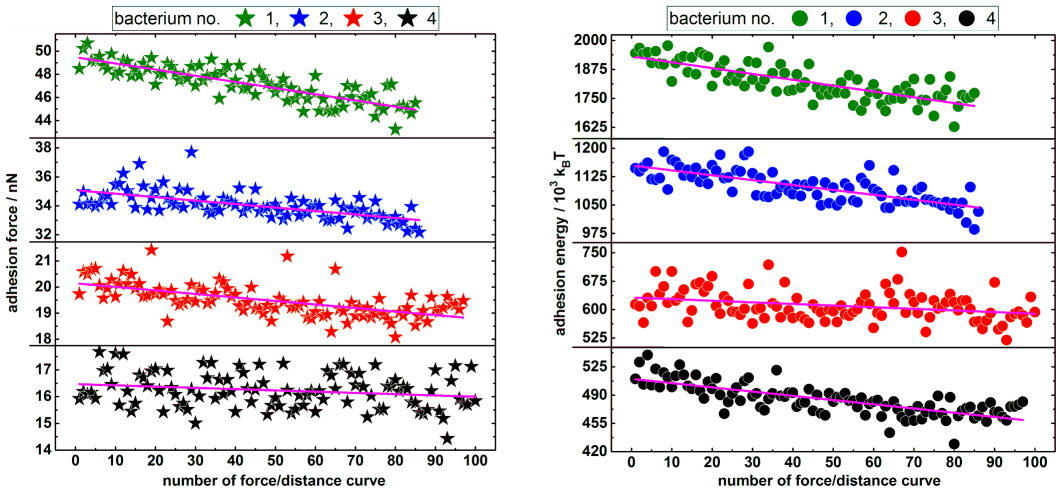

Fig. 1 Adhesion force (left) and energy (right) of four *S. aureus* cells in dependence of the number of recorded force-distance curves on random positions on a strongly hydrophobic OTS-surface. A linear fit of each set of values revealed a systematic decrease in adhesive strength in all cases. The extent of this decrease (slope of the linear fit) is a cell-individual property.

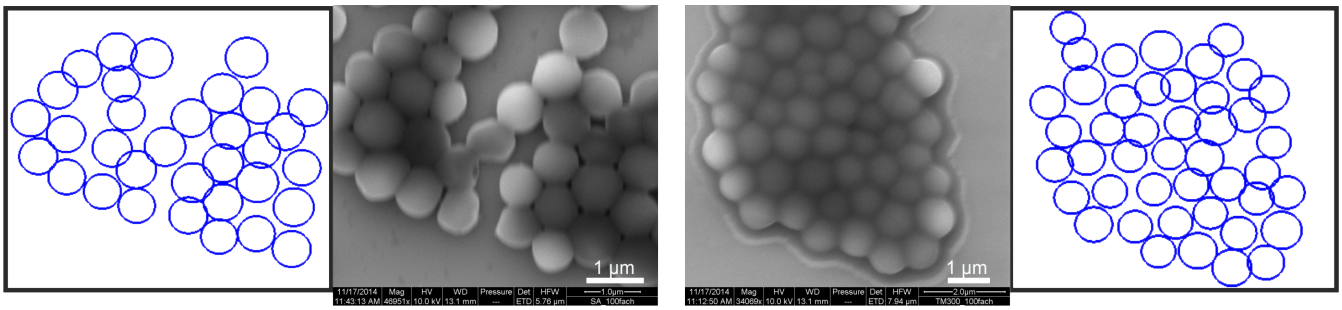

Fig. 2 Scanning electron micrographs of dried *S. aureus* (left) and *S. carnosus* (right) cells. (Note that the scales are not the same.) Cells and their size were automatically recognized with Matlab.

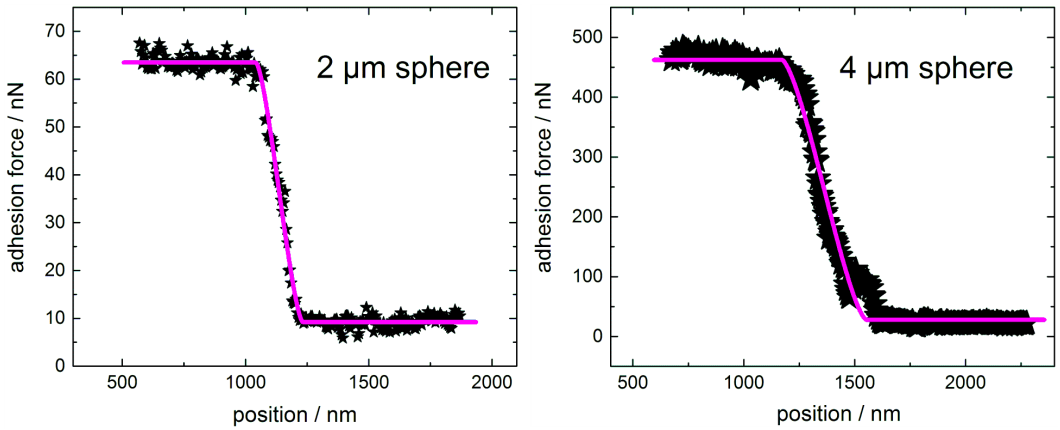

Fig. 3 Adhesion force as a function of the position near the hydrophobic/hydrophilic interface for a 2 µm-polystyrene bead (left) and 4 µm-hydrophobized silica sphere (right).

tact will not pave the way to interpreting our results for bacteria in more detail. However, the colloidal probe experiments show the versatility of our method, which is not restricted to bacterial cells. It moreover corroborates the notion that the size of the bacterial contact area is mainly determined by tethering surface macromolecules which are obviously not present on the colloidal probes.

Influence of the force trigger of radii calculated from force data

The radii of the contact area calculated from adhesion force data for different force triggers displayed in Figure 4 show a similar trend as already shown in Figure 10 in the full article: Radii determined from experiments with a higher force trigger are in the same range or larger than radii recorded with a lower force trigger. This is especially visible when comparing data for *S. aureus* cells calculated from force triggers of 3 nN and 30 nN. Nevertheless, in all cases - and for both tested species - the increase of the contact area does not behave like predicted by the Hertzian model.

Correlation between adhesive strength and contact area – all data

Figure 5 shows adhesion forces and adhesion energies of all tested cells (*S. aureus* as well as *S. carnosus*) in dependence of their contact area for all force triggers used. In all cases, no correlation between adhesive strength and size of the contact area is observed.

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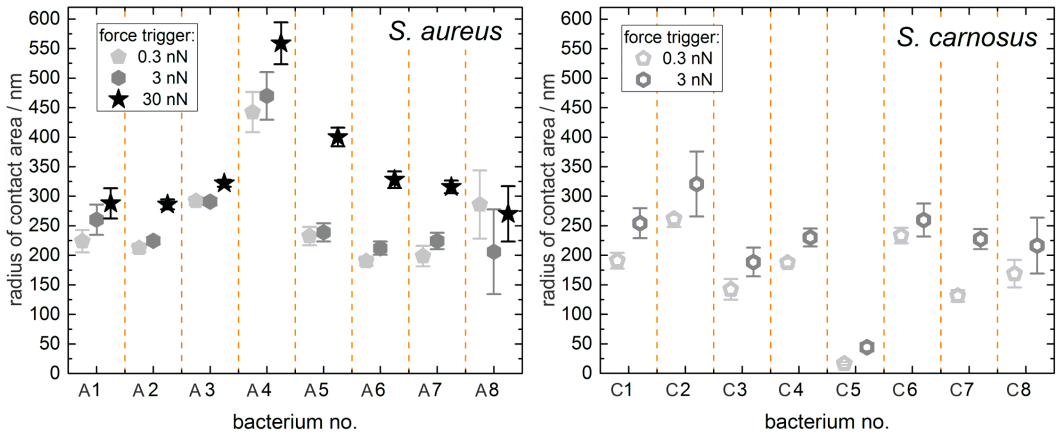

Fig. 4 Radii of the contact area between bacterial cell and surface for eight individuals of *S. aureus* (left) and *S. carnosus* (right). The radii were calculated from adhesion force data that were obtained with force triggers of 0.3 nN (light grey pentagons), 3 nN (dark grey hexagons) and (for *S. aureus* only) 30 nN (black stars).

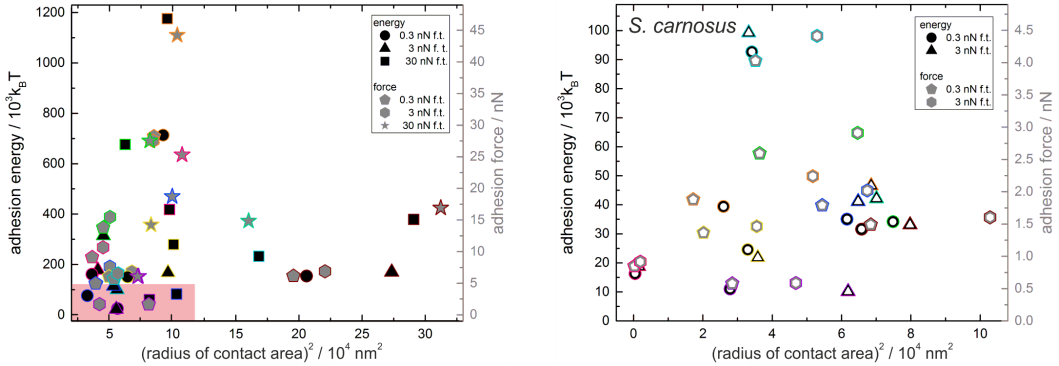

Fig. 5 Adhesion energy (black symbols) and force (grey symbols) in dependence of the contact area for eight different bacterial cells (different edge colors) and all used force triggers (f. t.); *S. aureus* (left) and *S. carnosus* (right). To place emphasis on the lower adhesive strength of *S. carnosus* cells, the colored rectangle in the left diagram represents the scale of the right diagram.