Journal Name

ARTICLE TYPE

Cite this: DOI: 00.0000/xxxxxxxxxx

Electronic Supplementary Information for 'Different binding mechanisms of *Staphylococcus aureus* to hydrophobic and hydrophilic surfaces'

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1 Influence of surface delay time on hydrophilic and hydrophobic surfaces

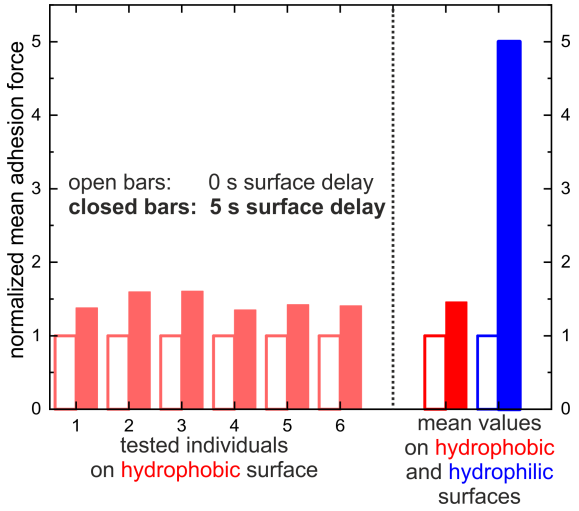

Fig. S1 Relative differences in mean adhesion forces for 6 individual cells on the hydrophobic surface compared to the more than one order of magnitude larger difference on the hydrophilic surface.

Figure S1 shows that for the tested cells on the hydrophobic surface, the increase in adhesion force was on average less than 50 %, while it increases more than 500 % in the hydrophilic case (see also Fig. 1 in the main text). Also, if we compare in how many cases a cell shows distinct adhesion to a surface (indicated by a detectable adhesion force in the retraction curve), this number is 100 % on the hydrophobic surfaces no matter what the surface delay time is. On hydrophilic surfaces, however, about 40 % of cells do not show adhesion for 0 s surface delay time while this value decreases to under 1 % after 5 s of surface delay.

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2 Adhesion of *S. aureus* strains JE2 and N315 to hydrophilic and hydrophobic surfaces

Figure S2 shows the mean force-distance curves and the mean values of the adheion force and rupture lengths for 6 tested individuals each of the clinically relevant *S. aurues* strains, the USA300 CA-MRSA derivative JE2 and the HA-MRSA strain N315, on hydrophilic and hydrophobic surfaces, respectively. As described in the main text for strain SA113, for both strains here, the mean curves on hydrophilic surfaces reach lower forces and have a much larger standard deviation as the curves on hydrophobic surfaces. Although the number of tested individuals is not as large as for strain SA113, the probed cells nicely corroborate our finding discussed in the main text of the manuscript.

3 Model details

Our model extends the work of Thewes *et al.* [1] who described the bacterium as hard sphere decorated with macromolecules that is attached to a moving cantilever to simulate SCFS experiments. For computational purposes only the bottom part up to a certain height is covered by a fixed number of randomly distributed macromolecules. The length fluctuations, as well as mechanical responses to stretching of these macromolecules is modelled by Hooke's law where the stiffness of each macromolecule is drawn random from a distribution. The binding of individual molecules to the surface is modelled by a simple square potential with a given potential depth V and interaction range r. This binding allows the molecules to pull on the bacterium. The pulling forces on the bacterium are balanced by the bending of the cantilever (modelled as the extension of a spring), and the bacterium is moved to equilibrium position, between each step of the cantilever.

We modify this model by using worm-like chain (WLC) polymers with probabilistic parameters. The contour length L is drawn from a Weibull distribution while the Kuhn length B is sampled from a uniform distribution. To compute the response to stretching efficiently we use the well known approximation of the WLC model by Marko and Siggia [2]. For simplicity, we use

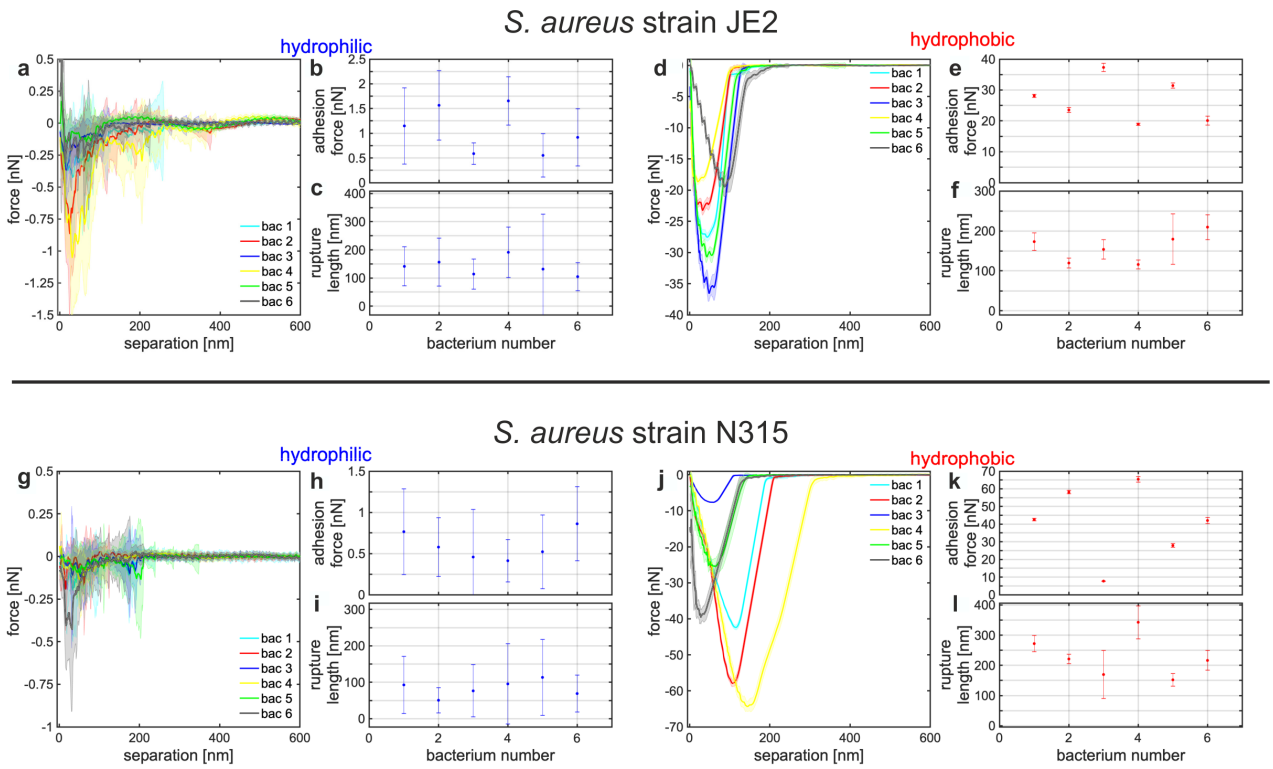

Fig. S2 (a,d,g,j) Mean SCFS retraction curves of 6 exemplary individual bacteria of each strain on hydrophilic (a,g) and hydrophobic surfaces (d,j) (shaded area is standard deviation). (b,c,e,f,h,i,k,l) Mean adhesion forces (b,e,h,k) and rupture lengths (c,f,i,l) extracted from single SCFS retraction curves of the cells (error bars are standard deviation).

the same energy for stretching and length fluctuations. To account for compression of the macromolecules, we introduce a rest length $l_0 = \sqrt{\frac{L \cdot B}{6}}$ below that the response is modelled as Hookean spring. Hence the energy E and force f of a macromolecule with stretch l are determined by

$$E(l) = k_B T \frac{L}{2B} \left(\frac{l - l_0}{L} \right)^2 \begin{cases} 2 + \frac{1}{1 - \frac{l - l_0}{L}} & , l_0 \leq l \\ 3 & , 0 \leq l \leq l_0 \end{cases}$$

$$f(l) = -\frac{k_B T}{2B} \begin{cases} 4\frac{l - l_0}{L} - 1 + \frac{1}{\left(1 - \frac{l - l_0}{L}\right)^2} & , l_0 \leq l \\ 6\frac{l - l_0}{L} & , 0 \leq l \leq l_0 \, . \end{cases}$$

Since the force model is non linear, we have to calculate the equilibrium position of the bacterium numerically, by simple bisectioning. Additional to changing the force response, we do not allow binding to the surface by mere proximity to the surface. If the macromolecule is in the range of the surface, it can bind with probability e^{-H}, where H represents a potential barrier. If the molecule is attached, it decreases it's energy by a binding energy V, and is able to pull on the bacterium.

The model parameters used, if not stated differently, are given in table S1. An overview of the simulation steps is given in Algorithm 1 SCFS-Simulation. In order to avoid artefacts from instantaneous cantilever steps, we subdivided each experimental cantilever step in hundred sub steps inside the simulation.

4 Parameter discussion

Kuhn lengths of proteins are typically in the range of $0.1 - 1$ nm[3–5] . As already mentioned in the main text, forces to unfold binding proteins are typically $0.1 - 0.4$ nN[5–7], and are structure and pulling speed-specific. The experimental estimation for the relevant protein length is difficult: While usually 0.36 nm per amino acid are used to estimate the length of a fully unfolded protein, some proteins detach before fully unfolding. We found that for relevant surface proteins of *Staphylococcus aureus* both behaviours are observed. ClfA for instance has a folded length of 25 nm and unfolds fully to a length of 285 nm before detaching[8] while Cna or SdrC do not unfold fully before detaching[9,10]. SasG is a well studied example which forms fibrils of 53 nm length while it is composed of several domains which, depending on the loading rate, can unfold independently to give a fully unfolded length of 505 nm[7,11,12]. In addition, the proteins are initially anchored in height of the membrane, but it's not clear if they stay or change their height within the membrane due to synthesis of new peptidoglycan. Therefore, it is not possible to deduce how far surface proteins protrude from the cell wall. Hence, rupture lengths do not need to correspond to the lengths of folded or even unfolded proteins. However, it is worth reminding that not just proteins might contribute to the adhesion but also teichionic acids or capsular polycarbonates. For these reasons, we will briefly discuss the influence of different length of macromolecules.

To vary the length of the involved macromolecules, we changed the scale parameter of the Weibull distribution (see Fig. S3, S4). Note that this does not only change the mean, but also the variance of the distribution. Interestingly, an increase in the scale parameter did not lead to a significant change in the initial part of the retraction curves. However, with increasing scale parameter, the minima of the retraction curves shift to higher separations while reaching bigger adhesion forces. Additionally, the rupture lengths increase substantially with increasing scale parameter. That behaviour is observed for all barrier heights, but for high barriers, is overshadowed by the stochastic response. The number of attached macromolecules (see Fig. S4) varies accordingly. This means that with a higher scale parameter more attached molecules are observed. However, for high barriers, the variance increases substantially, leading to overlapping curves. The increase of attached macromolecules with increasing scale parameter has its origin in the short interaction range of the surface. Just when macromolecules are long enough to get into the range of the surface potential, they are able to bind. If the cells have longer macromolecules, more of them can come into the range of the surface potential and can tether. If now more molecules of different lengths are bound to the surface, one would naively expect a change in the initial part of the retraction curve. However, as already mentioned, this is not observed, because the macromolecules show a non-linear WLC behaviour when stretched, and only contribute to the force when they are significantly extended in relation to their length. This lack of strong forces at small extensions is the reason why the initial part of the retraction curves shows no sensitivity to the changing scale parameter although more macromolecules bind to the surface. Because the macromolecules only contribute substantially to the force when they are strongly stretched, and because more and longer macromolecules bind, the adhesion force increases and shifts to higher distances. To conclude, if we compare two cells with longer and short macromolecules, the adhesion force of the cell with longer macromolecules increases and shifts to higher distances.

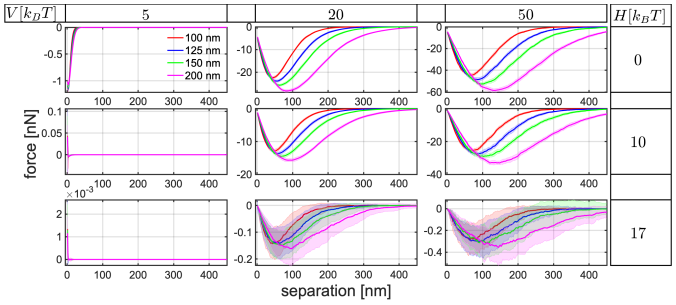

Fig. S3 Mean retraction curves for different potential depth V and barriers H, extracted from the first 10 retractions of 20 simulated cells with surface delay time of 5 s. Shaded area is standard deviation. Different colors correspond to scale parameters as indicated in the first panel.

5 Mean Retraction Plot with Delay Time Dependence

We discuss now the mean retraction curves for 5 s (blue) and 0 s (black) surface delay time belonging to figure 9 (see Fig. S5).

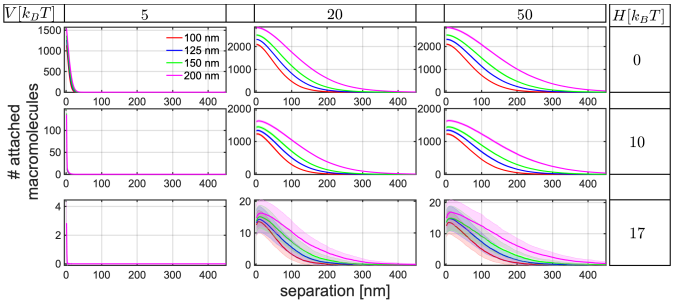

Fig. S4 Ensemble mean of the number of attached macromolecules for different potential depth V and barriers H, extracted from the first 10 retractions of 20 simulated cells with surface delay time of 5 s. Shaded area is standard deviation. Different colors correspond to scale parameters as indicated in the first panel.

The adhesion force, increases with increasing potential depth and drops for higher barriers. For low potential barriers, additional surface delay time increases the adhesion force only marginally. Yet, for high barriers, a substantial increase in adhesion force and stochasticity was observed. This conforms to the change in the number of involved macromolecules as discussed in the main text.

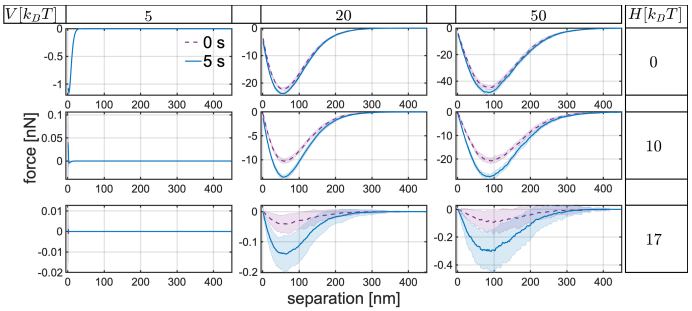

Fig. S5 Mean retraction curves for different potential depth V and barriers H, extracted from the first 10 retractions of 20 simulated cells. Shaded area is standard deviation. While blue solid lines indicate 5 s of surface delay time, purple dashed lines indicate 0 s.

Notes and references

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Algorithm 1 SCFS-Simulation

procedure INITIALIZATION

procedure APPROACH
  while force trigger not reached & step possible **do**
    cantileverHeight ← cantileverHeight − stepSize
    procedure MAINUPDATES
    if wantToMeausure **then**
      procedure TAKE MEASUREMENT
    end
  end

procedure WAITING
  for given number of iterations **do**
    procedure MAINUPDATES
  end

procedure RETRACTION
  while inital cantilverHeight is not reached **do**
    cantileverHeight ← cantileverHeight + stepSize
    procedure MAINUPDATES
    if wantToMeausure **then**
      procedure TAKE MEASUREMENT
    end
  end

procedure MAINUPDATES
  for 200 iterations **do**
    for number of macromolecules **do**
      chose a random macromolecule & propose a random change in the stretch $l \mapsto l + U\Delta l$ where U is uniformly distributed in $[-1, 1]$
      if proposed length and distance to surface > 0 **then**
        accept l according to Metropolis algorithm
      end
      if macromolecule in range of surface potential **then**
        if macromolecule is attached **then**
          bind with probability e^{-H}
        else
          unbind with probability $e^{-(H-V)}$
        end
      else
        unbind
      end
    end
    update bacterium position by force balance of cantilever and all bound macromolecules via bisection and threshold 10^{-4} nm
  end

Table S1 Reference Parameters and Symbols

number of macromolecules N	5000
minimum macromolecule Kuhn-length	0.2 nm
maximum macromolecule Kuhn-length	1 nm
scale parameter for macromolecule length	125 nm
form parameter for macromolecule length	1.5 nm
radius of bacterium	500 nm
decorate area height (from bottom of bacterium)	50 nm
Spring constant of the cantilever	0.6 nN/nm
k_BT	4.1 pN nm
interaction range of surface potential r	2 nm
change of macromolecule length Δl	1 nm
macromolecule stretch	l [nm]
macromolecule Kuhn length	B [nm]
macromolecule contour length	L [nm]
macromolecule rest length	$l_0 = \sqrt{\frac{L \cdot B}{6}}$ [nm]
potential barrier	$H[k_BT]$
potential depth	$V[k_BT]$

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