Soft Matter

Paper - supplementary material

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Stochastic binding of *Staphylococcus aureus* to hydrophobic surfaces - supplementary material

Nicolas Thewes,[a] Alexander Thewes,[b] Peter Loskill,[a†] Henrik Peisker,[c] Markus Bischoff,[c] Mathias Herrmann,[c] Ludger Santen[b] and Karin Jacobs*[a]

1 Viability of bacterial probe

Fig. S1 depicts a viable bacterial probe (green) manufactured according to the described method and investigated under a fluorescent light microscope using a live/death staining (Life Technologies GmbH, Germany) after a series of force/distance measurements. Random tests of cell viability showed that our whole procedure does not alter the cell viability.

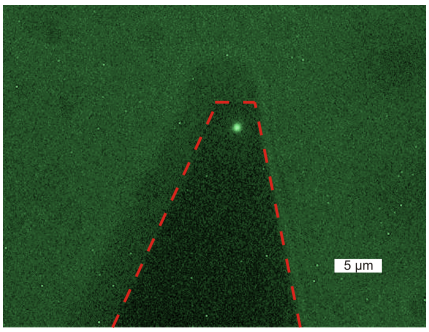

Fig. 1 Fluorescence micrograph (magnification 630x) of a single bacterial probe, after adding a small amount of live/death stain (Life Technologies GmbH, Germany). The cantilever is enframed by dashed red lines to improve visibility.

2 Model details

In our theoretical approach, we implement a reductionist model of the experimental setup.

The cantilever is represented as a linear spring with spring constant k_c and untensioned length $l_0^c = 0$. We model the bacterium as a spherical object with radius R. The cell wall macromolecules are also modelled as linear springs. As a key feature of the model we assign random spring constants k_i and, at least in principle, lengths l_0^i to molecule i. The k_i's are identically distributed random variable chosen from the interval $[k_{min}, k_{max}]$. Such a distribution of k_i's is the only way we found to reproduce the shape of the experimental approach curve. For the simulation results given in the paper the l_0^i is the same for all molecules. We considered a total number of N springs, which may be considerably larger than the actual number of cell wall proteins. The first end of the spring is attached to the cell wall and the second one is pointing towards the substratum. The springs are always parallel to the z-axis.

We considered random spring positions on the cell wall. For the purpose of computational efficiency we decorated only the half of the sphere which is opposite to the substratum, i.e. we restrict the possible z-coordinates of the spring's position at the cell wall to the interval [-R,0] *

In the following we use the coordinate system which is illustrated in Fig. S2: The substratum is located in the x-y-plane at $z = 0$ and the cantilever starts at position $(0, 0, z_c = z_{max})$. The bacterium is attached to the cantilever and its initial distance to the substratum is given by $d = z_{max} - 2R$.

The binding potential between substratum and macromolecules was chosen as a square well potential with a depth of $-14 * k_b * T$ and a range of $3\,nm$. Explicitely, V(z) reads as follows:

$$V(z) = \begin{cases} 0 & z > 3\,nm \\ -14 * k_b * T & 0 < z < 3\,nm \\ \infty & z < 0 \end{cases} \quad (1)$$

According to this, springs that are closer to the substratum than 3nm are considered as bounded, so the energy for a bounded spring is $E_s = \frac{1}{2}(l_i - l_0^i)^2 - 14 k_b T$ and for unbounded springs $E_s = \frac{1}{2}(l_i - l_0^i)^2$, where l_i determines the actual length of spring i.

The following table summarizes the standard parameter setup, which was used for the results in our study.

[a] *Experimental Physics, Campus E2 9, Saarland University, D-66123 Saarbrücken, Germany. E-mail: k.jacobs@physik.uni-saarland.de*
[b] *Theoretical Physics, Campus E2 6, Saarland University, D-66123 Saarbrücken, Germany*
[c] *Institute of Medical Microbiology and Hygiene, Saarland University, D-66421 Homburg/Saar, Germany*
† Present address: Dept. of Bioengineering and California Institute for Quantitative Biosciences (QB3), University of California at Berkeley, Berkeley, California 94720, USA

* Here we give the cell wall position relative to the center of the bacterium.

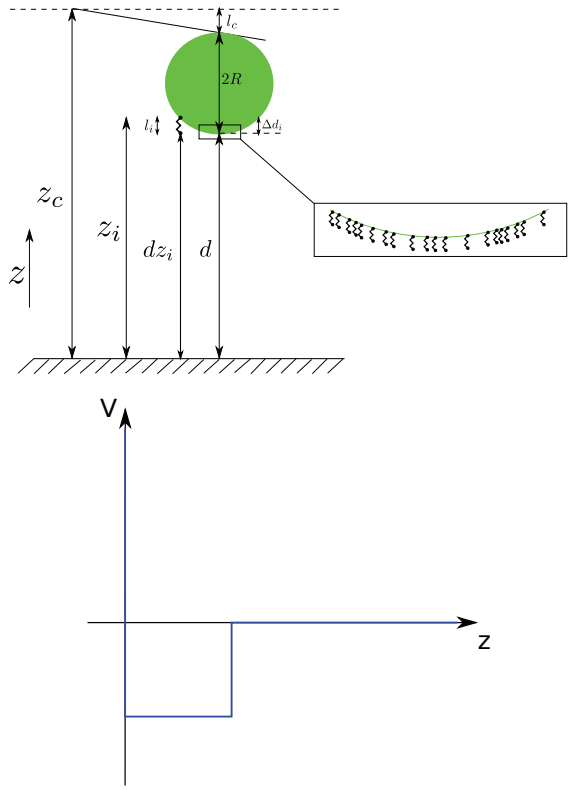

Fig. 2 Upper figure: Schematic picture of the model. Lower figure: Binding potential for the surface macromolecules, the depth of the potential is -14∗k_BT and the width is 3 nm.

Number of proteins N	8000
Untensioned length of proteins l_0^i	30 nm
Maximum spring constant of proteins k_{max}	0.001 nN/nm
Minimum spring constant of proteins k_{min}	$5*10^{-6}$ nN/nm
Untensioned length of the cantilever l_0^c	0 nm
Spring constant of the cantilever	0.03 nN/nm
Radius of the bacterium	500 nm
Range of binding potential	3 nm
Depth of binding potential	$-14*k_b*T$

These values were chosen since they give a good agreement between experiment and simulations.
We carefully checked the influence of changes in the parameters on the simulation results. We found that variations of the parameters only result in quantitative changes of the received curves, the principle shape was preserved. For example, a higher number of proteins or a deeper potential lead to a larger adhesion force, decreasing the untensioned length of proteins results in a smaller snap-in separation.

Given that n springs are bound to the substratum, the equilibrium position of the bacterium, which determines the extension of the bound springs, can be calculated analytically.
The total energy of the system consists of two parts, the bending energy of the cantilever and the energy of the stretched springs:

$$E = \frac{1}{2}\sum k_i(l_i - l_0^i)^2 + \frac{1}{2}k_c(l_c - l_0^c)^2 \tag{2}$$

where the sum runs over all bounded springs.
We suppressed the contribution from the binding potential since this is constant for bounded springs and plays no role when calculating the equilibrium position.

By considering the boundary conditions of the model, one can rewrite the energy as a function of a single variable, which we chose to be the distance of the bacterium to the substratum d.
The position z_c of the cantilever is given by

$$z_c = l_c + 2R + d \tag{3}$$

with l_c = actual deflection of the cantilever. The z-coordinate z_i of the first end of spring i is

$$d + \Delta d_i = z_i = dz_i + l_i \tag{4}$$

with dz_i = distance of the second end of the spring to the substratum, Δd_i = distance of the first end of the i-th spring to the plane $z = d$.
The latter quantity is given by

$$\Delta d_i = R - \sqrt{R^2 - (x_i^2 + y_i^2)} \tag{5}$$

with x_i, y_i = x,y-coordinate of the first end of the i-th spring.

Putting all this together we can write the energy of the system as follows:

$$E = \frac{1}{2}\sum k_i(d + \Delta d_i - dz_i - l_0^i)^2 + \frac{1}{2}k_c(z_c - d - 2R - l_0^c)^2 \tag{6}$$

This form contains only a single variable d, all other quantities are given parameters of the system configuration. Therefore, the equilibrium distance d of the bacterium to the substratum for a given cantilever position and configuration of bound springs reads as:

$$d_E = \frac{k_c(z_c - 2R - l_0^c) - \sum k_i(\Delta d_i - dz_i - l_0^i)}{k_c + \sum k_i} \tag{7}$$

All in all we have three different kinds of degrees of freedom in the model. First, the position of the cantilever, which is updated following the given experimental protocol. Second, the position of the bacterium, which we consider as the equilibrium position for a given configuration of bound springs and position of the cantilever, and, third, the stochastic simulation of the springs, which we carry out at room temperature using standard Metropolis algorithm.

Precisely, our simulation approach, which is closely related to the experimental setup consists of the following steps:

1. Move the cantilever by an amplitude which corresponds to one hundredth of the step length in experiment.

2. Perform 200 MC-sweeps for the springs and the bacterium,

where a sweep is given by

(a) N spring-updates:

i. Chose a spring i and a random displacement r.

ii. Move the second head of spring i by distance r.

iii. If allowed, meaning $dz_i > 0$ and $l_i > 0$, accept the move according to Metropolis algorithm.

iv. Go back to (i) until N spring-updated are completed

(b) Update of the bacterium position

3. Go back to one, after every hundredth step of the cantilever, read out the configuration of the system

Retraction starts if the given force trigger is reached.

3 Protein-modifying treatment

Supplementary material to Fig. 6

To evaluate the macromolecular origin of bacterial adhesion, bacteria have been treated by pronase E and glutaraldehyde. In Fig. S3 force/distance curves of approach (blue) and retraction part (red) are shown, whereas in Fig. 6 only the approach parts have been depicted for clarity. Experimental parameters were optimized to study the approach process. Therefore, relatively soft cantilevers were used. Curves a) and c) are before treatment, curve b) is recorded after pronase E application and curve d) is taken after glutaraldehyde action. For the cases shown, the maximum adhesion forces of the two bacteria of the reference curves are that large that they cannot be evaluated in detail. However, the order of magnitude can roughly be estimated by the extrapolation of the gradients of the retraction curves: For both, it is in the range between -20 nN and -30 nN. Clearly, after any of the protein-modifying treatments, the maximum adhesion force is greatly reduced.

The same holds true for the simulated force/distance curve using the rough model, with only one stiff spring, for describing the effect of the protein-modifying treatment (cf. Fig. S4). Also in the simulated force/distance curve the adhesion force is greatly reduced by using only one stiff spring (Fig. S4b) as compared to a distribution of soft springs (Fig. S4a).

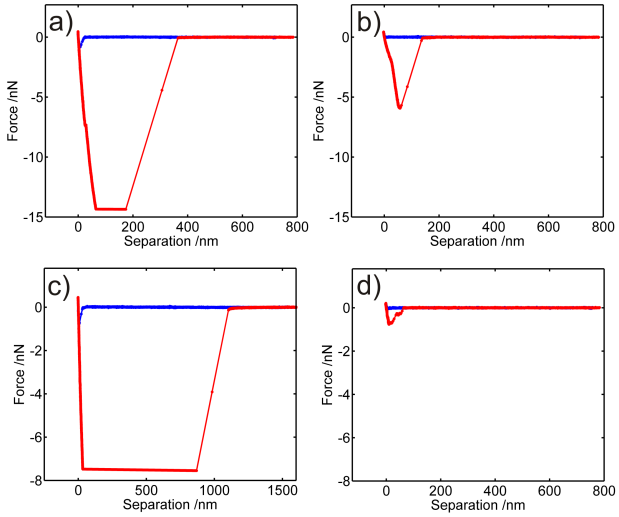

Fig. 3 Force/distance curves of approach (blue) and retraction (red) parts of two individual bacterial probes for testing the influence of enzymatic/chemical treatment on the bacterial adhesion process. Curves in a) and c) each depict the native state, the curve in b) shows the adhesion after pronase E treatment and curve d) after glutaraldehyde action.

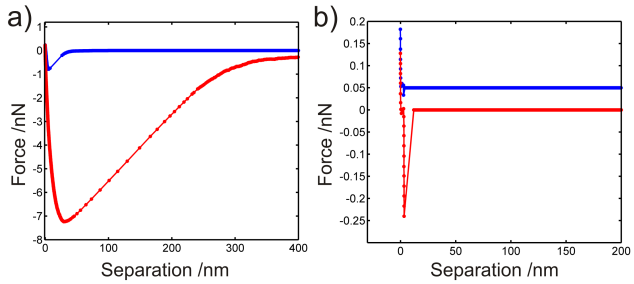

Fig. 4 Simulated force/distance curve with approach (blue) and retraction (red) part. a) MC model force/distance curve using the parameter setup described above and b) simulated force/distance curve that reflects the protein-modifying treatment by using only one stiff spring (for clarity, the blue curve has been shifted by +0.05 nN).