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Supporting Material

A tactile response in <I>Staphylococcus aurues</I>

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AFM force-volume control experiments. To confirm the stringency in our detection of specific binding events, force volume imaging was also performed with unbaited tips (i.e., clean silicon nitride tip). The resulting force-separation profiles rarely exhibited sawtooth signatures with consistent contour lengths. However, we did observe attractive forces, consistent with general adhesion forces such as van der Waals interactions occurring near the region of tip-sample contact (see Fig. S1). The presence of this attractive, non-specific adhesion force suggests that the biomolecules localized to the bacterium-substrate interface are sticky/adhesive in nature. The absence of well-defined sawtooth signatures observed using these unbaited tips supports the stringent detection of fibronectin-specific binding events observed when using tips baited with fibronectin.

Regarding the Effect of Contact Area on Avidity Maps. To create the avidity maps we defined the work required to separate the tip from the sample surface (i.e., integrate force with respect to distance). The specific binding force between the tip and sample is directly proportional to the number of proteins undergoing simultaneous extension. In a system with uniform distribution of both binding proteins on the cell surface and fibronectin on the tip surface, the number of binding events is in turn proportional to the contact area between the two interacting surfaces. We thus expect large affinity (or avidity) where there is large contact area.

Although the increased contact area between the cell and the pyramidal AFM tips does enhance the apparent affinity at the cells' perimeter, the increased contact area alone does not account for the highly localized avidity observed. It is difficult to directly quantify the effect of contact area between the cell and tip. However, we can make the following arguments in support of our claim that the highly localized avidity results from true localization of adhesins, rather than from artifacts of the varying tip-cell contact area.

The strongest indication for true adhesin localization is the sharp localization of high avidity at the cell edges. Based on the observation that the cells are deformed into slightly pyramidal shapes (see Fig. 5C in the main text), the contact area between the pyramidal tip and compliant cell should roughly vary as the overlapping area between two inverted triangles sliding apart, the intersection of which varies gently as $(h-x)^2$ and some trigonometric factors (Fig. S2). If adhesins were uniformly distributed and contact area were the only cause of the apparent edge avidity, there should be a gentle decrease in avidity as the tip moves from the cell edge to the cell top surface. This effect, however, is not observed (see Fig. 5B in the main text) even though the instrumental resolution (signal:noise) would certainly allow detection of smaller separation forces. The abrupt decrease in cell-edge avidity thus negates the possibility of uniform distribution of fibronectin binding proteins over the cell surface.

Additionally, the lack of consistent asymmetry in all avidity maps supports the presence of true adhesin localization. In our AFM, the cantilever is canted at 11.5 degrees (standard in commercial AFMs), which should result in consistent and reproducible asymmetry in contact area on the two sides of the cell. The front AFM tip facet touches the right side of the cell, and the back facet touches the left side of the cell. The canting of the pyramidal tip results in different effective contact areas with the front and back facets. In the presence of uniformly

distributed binding proteins, this asymmetry in contact area should consistently manifest itself as asymmetry in avidity, e.g. with one side of the cell exhibiting higher avidity than the other. Asymmetry in avidity has not been consistently observed across the many force volume images collected. Thus, the presence of uniform distribution of binding proteins across the cell surface is very unlikely. The occasional observations of asymmetry in avidity can be attributed to differences in the cells' physiological response (as seen with the cells on fibronectin-coated glass, shown in the main text), rather than artifacts of tip/cantilever orientation.

Two experimental checks were also performed as supplemental evidence of true protein localization to a cell's perimeter. In typical operation, the cantilever is scanned at 0 degrees in a direction such that the facets on the AFM tip (i.e., the flat sides of the pyramidal tip) approach the cell, allowing for large contact area. Changing the scan angle by 45 degrees allows the edge (between facets) of the pyramidal tip to approach the cell and significantly decreases the contact area along this orientation. Although the magnitude of the sawtooth events does decrease (suggesting fewer bonds in parallel), the specific binding events still occur near the bacterium-substrate interface, thus supporting true localization of binding proteins.

Finally, flattened tips of much greater contact area (~100 to 200 nm on a side) were also coated in fibronectin and used to probe the cell surface. Although the flat "tip" is a much larger surface area than the standard tip (radius of curvature less than 60 nm), there was no accompanying increase in fibronectin affinity on the tops of the cells. Since the large fibronectin-coated surface could not "find" adhesins to bind on the cell top surfaces, it is likely that the binding proteins are localized at the edges or under a cell (i.e., at the bacterium-substrate interface).

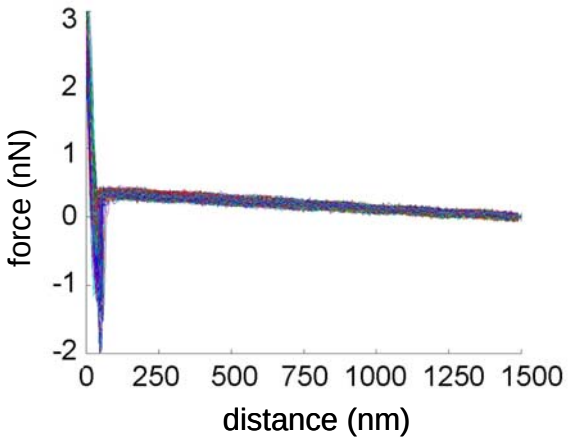

Fig. S1. AFM retraction curves collected with an unbaited tip (i.e., a clean tip) on *S. aureus*.

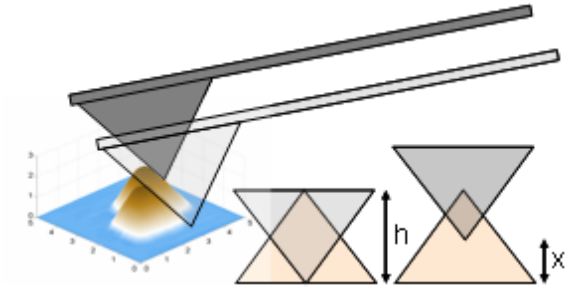

Fig. S2. Schematic of AFM tip on a cell. Drawn to approximate scale.