

## **The extracellular polysaccharide Pel makes the attachment of *P. aeruginosa* to surfaces symmetric and short-ranged**

**Benjamin J. Cooley,<sup>a</sup> Travis W. Thatcher,<sup>a</sup> Sara M. Hashmi,<sup>b</sup> Guillaume L'Her,<sup>a</sup> Henry H. Le,<sup>a</sup> Daniel A. Hurwitz,<sup>a</sup> Daniele Provenzano,<sup>c</sup> Ahmed Touhami,<sup>d</sup> and Vernita D. Gordon<sup>\*a</sup>**

<sup>a</sup> Center for Nonlinear Dynamics and Department of Physics, University of Texas at Austin, 2515 Speedway, C1610, Austin, TX 78712, USA

<sup>b</sup> Department of Chemical and Environmental Engineering, Yale University, 9 Hillhouse Avenue, New Haven CT 06510, USA

<sup>c</sup> Department of Biological Sciences and Department of Biomedical Sciences, University of Texas at Brownsville, Brownsville, TX 78520, USA

<sup>d</sup> Department of Physics and Astronomy, University of Texas at Brownsville, Brownsville, Texas 78520, USA

\* Correspondence to: [gordon@chaos.utexas.edu](mailto:gordon@chaos.utexas.edu)

### **Supplementary Materials:**

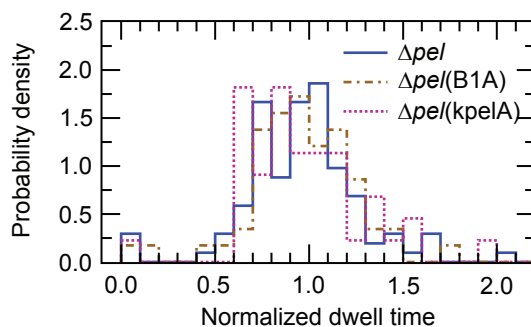
Pel mutants

Flagellum and pili mutants

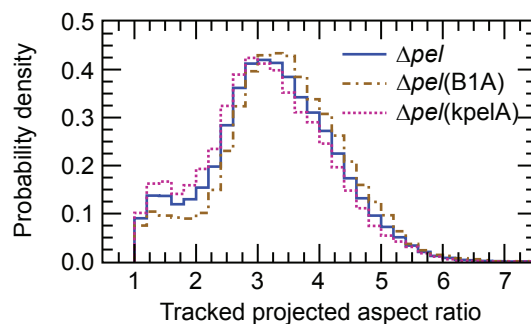
Estimating an average tilt angle

### Pel mutants

Two Pel mutants,  $\Delta pel(B1A)$  and  $\Delta pel(kpelA)$ , were used to test whether a difference was observed between an in-frame deletion and an out-of-frame deletion. No substantial differences were seen between the two mutants. See Figure S1 and S2.



**Figure S1:** Normalized dwell times for  $\Delta pel(B1A)$ ,  $\Delta pel(kpelA)$ , and all  $\Delta pel$  bacteria combined. The combined curve is also shown in Figure 1. Dwell times are manually measured for bacteria present within the first five hours of the experiment and normalized to the mean doubling time for each or both strains. Measured doubling times are  $\Delta pel(B1A)$ : 77.9 min;  $\Delta pel(kpelA)$ : 88.6 min; combined  $\Delta pel$ : 82.5 min.

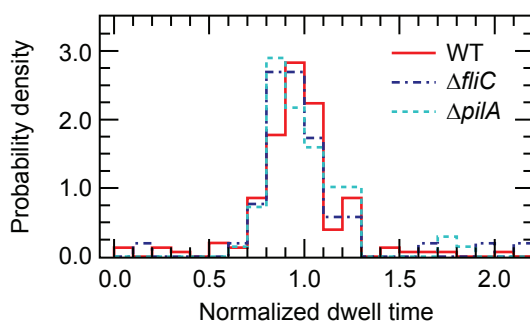


**Figure S2:** Tracked projected aspect ratios of  $\Delta pel(B1A)$ ,  $\Delta pel(kpelA)$ , and combined  $\Delta pel$  bacteria. The combined curve is also shown in Figure 2b.

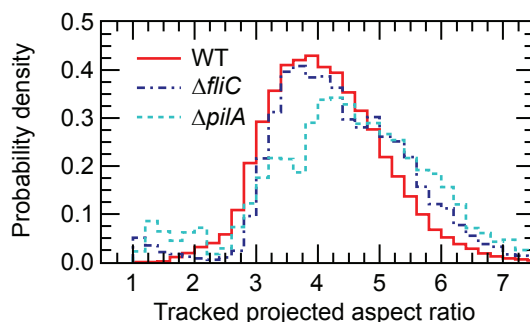
### Flagellum and pili mutants

A flagellum knockout mutant ( $\Delta fliC$ ) and a pili knockout mutant ( $\Delta pilA$ ) were tested as additional controls. The  $\Delta fliC$  bacteria were mobile on the surface. The  $\Delta pilA$  bacteria, however, were not mobile on the surface. As a result, they tend to remain in one place and form very dense clusters after a few division cycles. The normalized dwell times of both mutants were very similar to those of the WT bacteria; all three types typically remain on the surface throughout a doubling cycle. See Figure S3.

The histogram of tracked projected aspect ratios for  $\Delta fliC$  bacteria is very similar to that of the WT. The histogram of tracked projected ratios for  $\Delta pilA$  bacteria is shifted to larger values. This is an artefact of the image processing prior to tracking. The erosion mentioned in the main discussion of aspect ratios is enhanced when bacteria are very close together. Because the  $\Delta pilA$  bacteria pile up close to each other as they grow, their images are eroded more than images of other strains, leading to exaggerated aspect ratios. See Figure S4.



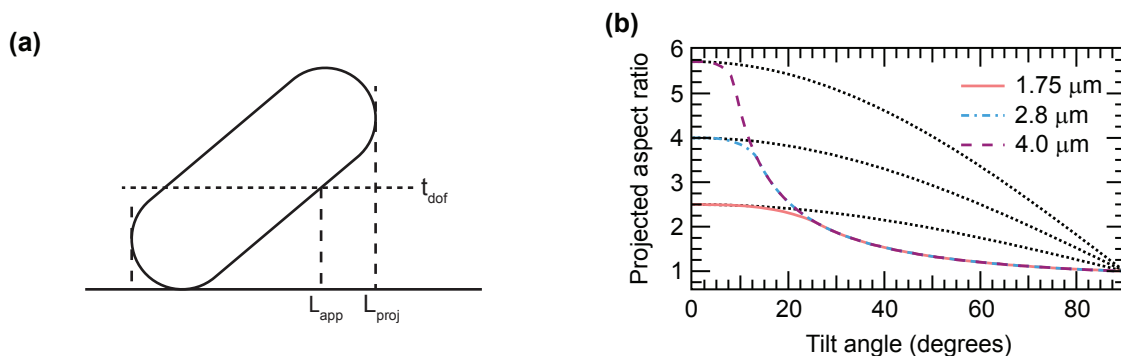
**Figure S3:** Normalized dwell times. Dwell times for WT,  $\Delta fliC$ , and  $\Delta pilA$  bacteria are manually measured for bacteria present within the first five hours of the experiment and normalized to each strain's mean doubling time. Measured doubling times are WT: 59.4 min;  $\Delta fliC$  64.7 min;  $\Delta pilA$  50.9 min.



**Figure S4:** Aspect ratio histograms. The  $\Delta fliC$  aspect ratios are very similar to the WT. The aspect ratios for the  $\Delta pilA$  bacteria show a shift to higher values that is an artefact resulting from the image processing done before tracking.

### Estimating an average tilt angle

Taking the peak of the histogram for projected aspect ratios as a reference, we can attempt to calculate an average angle of tilt from the peak of the other strains' histogram. The simplest approach assumes that the peak of the  $\Delta pel\Delta psl$  histogram represents the average projected length and therefore gives an average angle of  $25^\circ$ . However, this angle is falsely large because the limited depth of field of the microscope objective means that bacteria ends that tip up past the focal depth go out of focus and are lost in the image processing. This optical truncation reduces the tracked aspect ratio,  $L_{app}$ , to one that is smaller than the true geometric projected length,  $L_{proj}$ , for a given tilting angle. A simplified diagram of this situation is shown in Figure S5a. For our microscope objective, the threshold set by the depth of field is at a height of about  $0.5 \mu\text{m}$ . We estimate the apparent lengths and the correct projected lengths for a range of tilting angles for bacteria of lengths  $1.75 \mu\text{m}$ ,  $2.8 \mu\text{m}$ , and  $4.0 \mu\text{m}$ ;  $2.8 \mu\text{m}$  is the approximate peak of the length histogram for the WT bacteria, which we take to be the mean length for both the WT and the mutants. At tilt angles close to zero, the apparent length,  $L_{app}$ , is at first the same as the true geometric projected length,  $L_{proj}$ , but  $L_{app}$  drops by up to a factor of six as the bacterium tilts up through the threshold created by the finite depth of field,  $t_{dof}$ . Plots comparing  $L_{proj}$  and  $L_{app}$  for a range of tilt angles and three lengths of bacterium are shown in Figure S5b. Note that all three curves overlap for tilt angles above about  $24^\circ$ , because the true length of the bacterium is irrelevant once the end is well above the threshold. Given these results, we cannot easily estimate a mean tilt angle for the  $\Delta pel\Delta psl$  bacteria. However, if we take the peak of the aspect ratio histogram ( $\sim 3.5$ ) and assume a bacterial length of  $2.8 \mu\text{m}$ , we can use Figure S5b to read off a tilt angle of  $\sim 14^\circ$ . For  $\Delta pel$ , again assuming a bacterial length of  $2.8 \mu\text{m}$  and taking the peak of the aspect ratio histogram ( $\sim 3.0$ ), we can read off a tilt angle of  $\sim 16^\circ$ . We speculate that this may correspond to the geometry of an attachment in which mediation of adhesion by an appendage is more important than it is in the Psl-expressing strains.



**Figure S5:** (a) Diagram showing apparent length ( $L_{app}$ ) is shorter than the true projected length ( $L_{proj}$ ) as a bacterium tilts above the threshold defined by the depth of field ( $t_{dof}$ ). (b) Plot of  $L_{app}$  vs. tilt angle for three different bacterium lengths, assuming  $t_{dof} = 0.5 \mu\text{m}$ . The dotted black lines represent  $L_{proj}$  for each bacterium length.