

1 **Supplemental Information for “Maintenance of motility bias during cyanobacterial**
2 **phototaxis”**

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13 *Running title:* Maintenance of phototactic motility bias

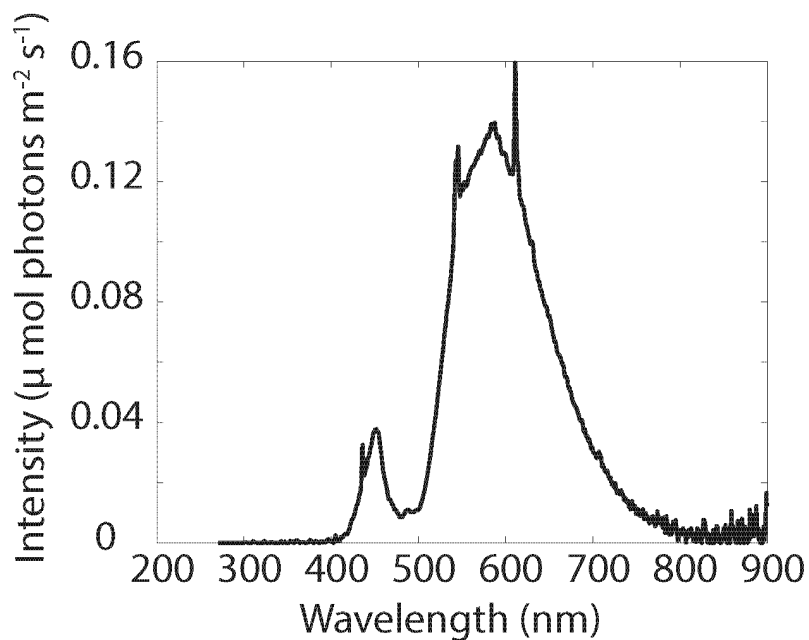
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15 *Keywords:* collective behavior, single-cell imaging, photoreceptor, Type IV pili

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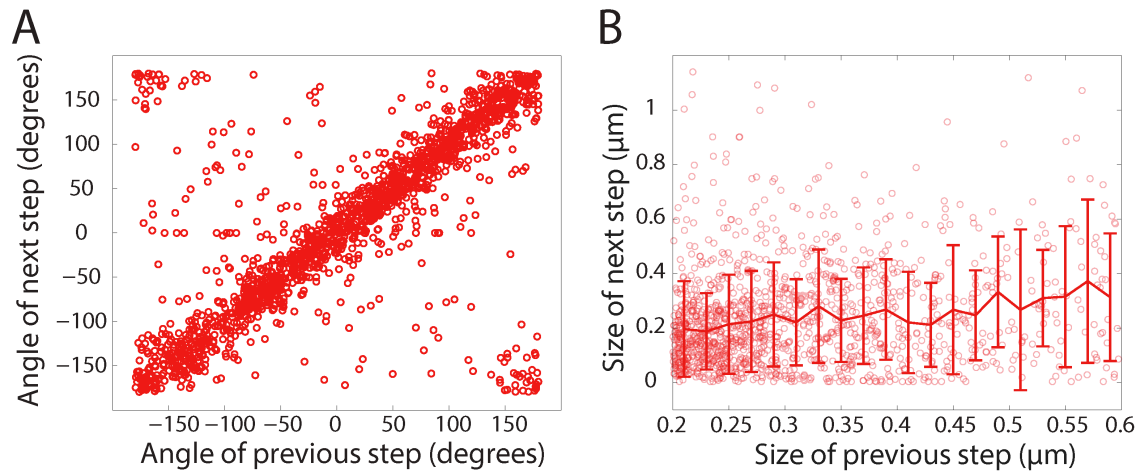
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33 **Supplementary Figures**

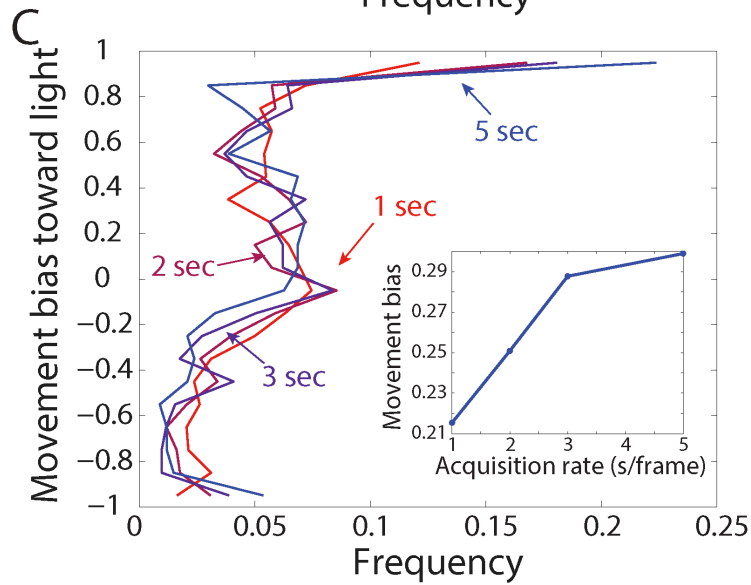
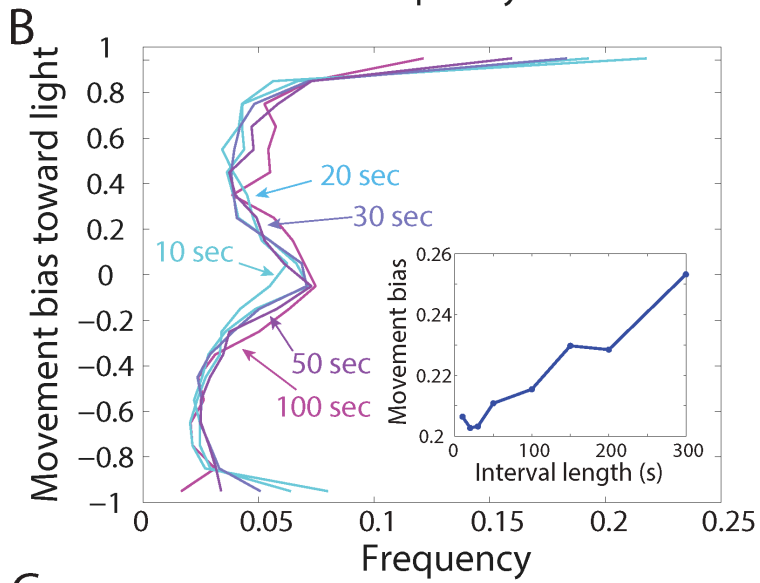
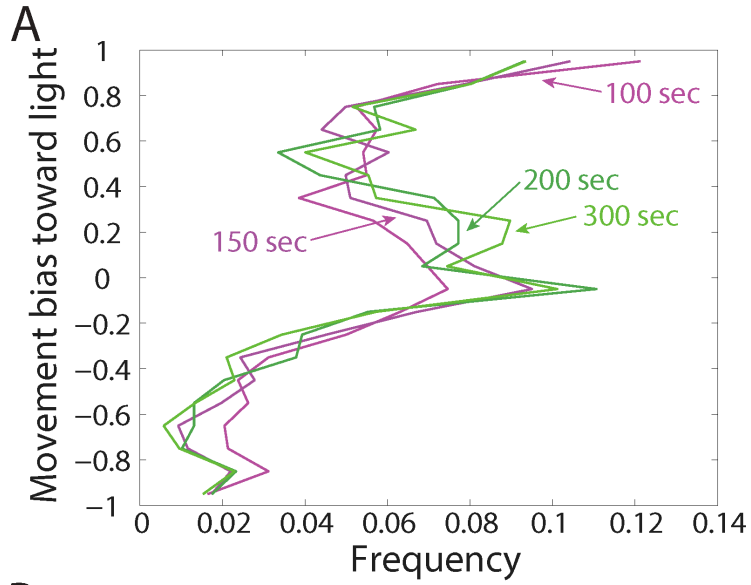


34 **Supplementary Figure 1: Spectrum for the warm-white LED used in our**
35 **experiments.**
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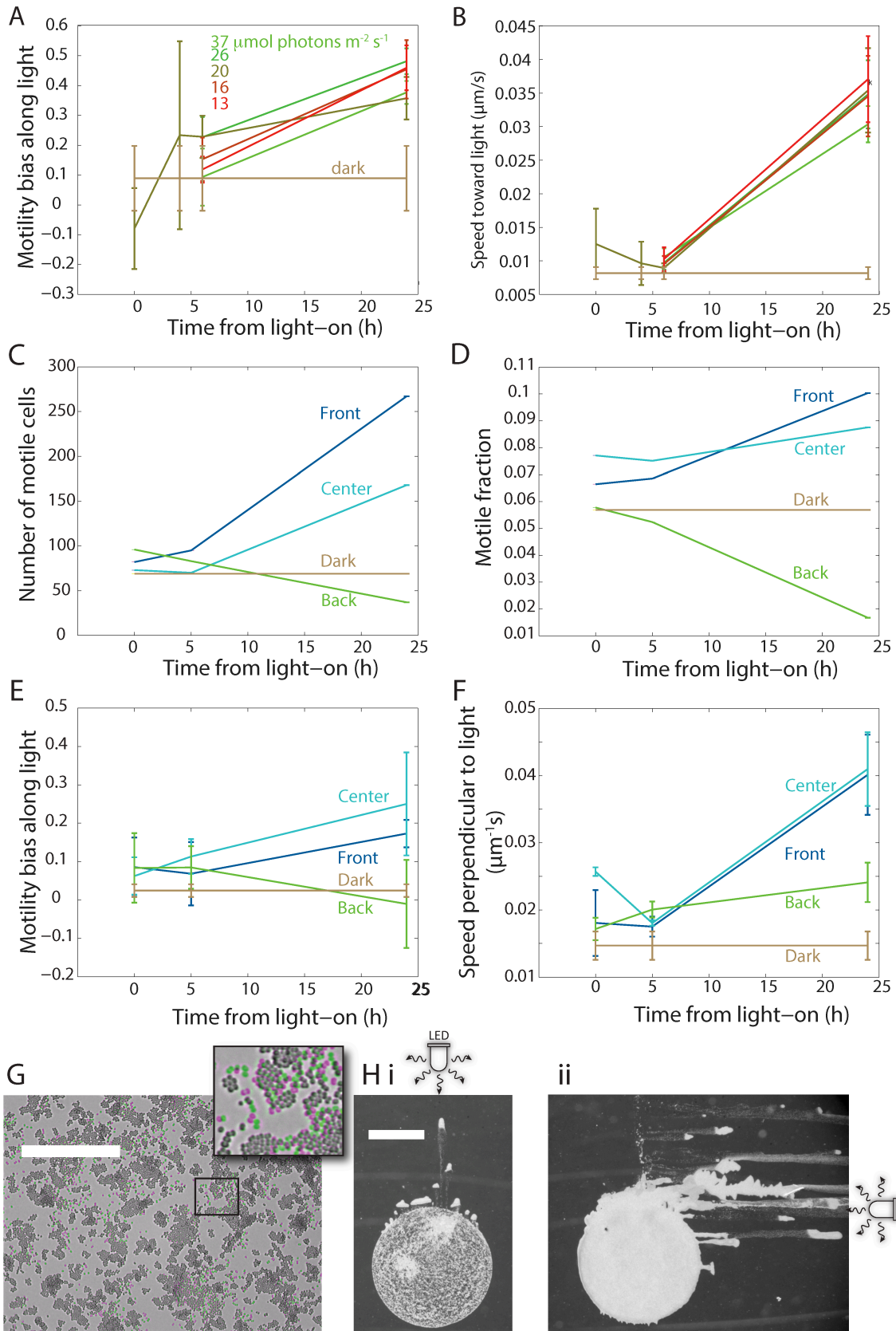


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Supplementary Figure 2: Maintenance of step angles in consecutive steps indicates progressive pilus retraction. (A,B) Size and angle of steps following steps of length 0.2-0.6 μm indicates (A) a high degree of correlation in the direction of movement (correlation coefficient = 0.21, $p < 10^{-15}$), but (B) little correlation in the sizes of successive steps. Data were taken from the midfinger region experiencing the light-off condition in the experiment described in Fig. 3B. In (B), the data are indicated as open circles, with the mean \pm standard deviation shown as a line plot.



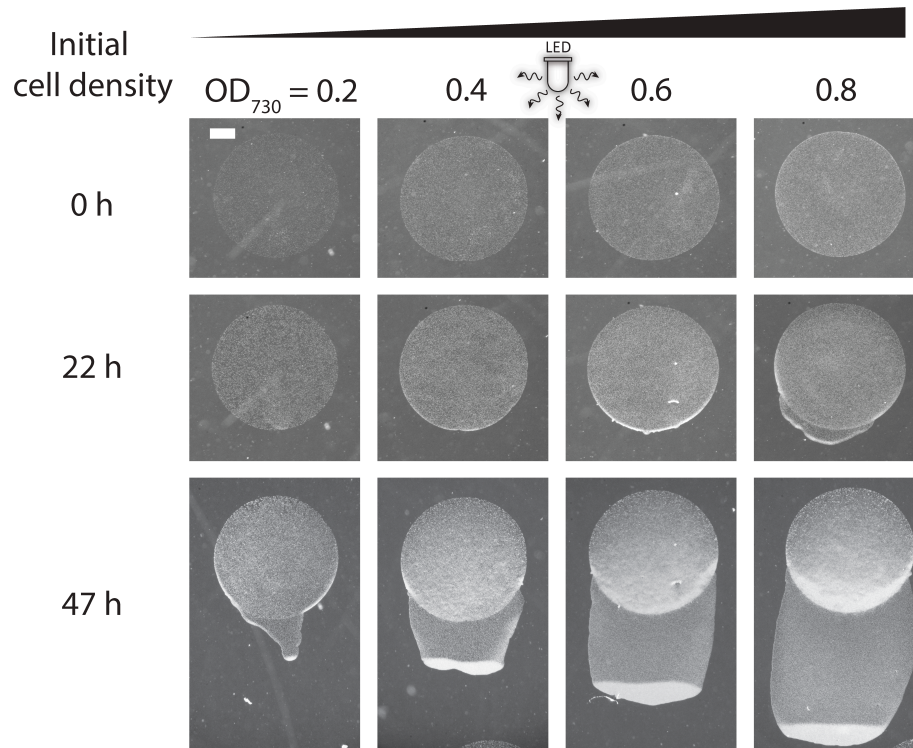
46 **Supplementary Figure 3: Long measurement intervals and high data acquisition**
47 **frequencies are required to accurately measure motility bias.** In each case, we plot
48 the distribution of the motility bias values of cells at the front of an inoculation of wild-
49 type cells, averaged over all measurements obtained from a 600-s movie with frames
50 taken every second. (A) The distribution of bias values for windows ≥ 100 s. (B) Shorter
51 windows for measuring bias resulted in increased fractions of cells moving processively
52 (with bias close to 1). (C) Data acquisition frequencies < 1 frame/s (achieved by ignoring
53 frames from the same movie) resulted in increased fractions of cells with bias close to 1.
54 The average bias for each curve is plotted in the insets in (B) and (C).



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Supplementary Figure 4: Cells at the back of the drop are persistently stationary.

57 (A,B) Over time, the motility bias and speed of cells in the front region of the inoculation
58 were not strongly affected by the intensity of incident light. (C-F) Cells in an inoculation
59 were imaged at $t = 0, 4, 6,$ and 24 h after inoculation, at the back, center, and front
60 regions. Cells were imaged in the dark at $t = 0$ as a control and plotted across time as a
61 reference line. (C) Number of motile cells. (D) Fraction of cells that were classified as
62 motile. (E) Motility bias over time. (F) Speed perpendicular to the light direction. The
63 motility bias differed between the back, center, and front regions, but speed increased
64 over time in all regions. However, the number of motile cells remained the same in the
65 back, while the fraction of motile cells decreased over time due to increased cell numbers
66 from division. Therefore, the increase in motile cell fraction in the center and front cannot
67 be attributed entirely to EPS accumulation. As motile cells moved out of the back of the
68 drop, non-motile cells were left behind. (G) Overlay of two time-lapse images, 10 min
69 apart, of cells in the back region. Cells that have moved over the 10 min are highlighted,
70 with their original position in green and the final position in magenta. Cells that did not
71 move remain in gray-scale. Very few cells were motile in this region. Scale bar = $20 \mu\text{m}$.
72 (H) Demonstration that the cells in the back region were mostly non-motile, by observing
73 the long-term effects of rotating the light source 90° relative to the drop. (i) An
74 inoculation was subject to rotation of the light source 90° relative to the original incident
75 direction. (ii) Ninety-six hours later, new fingers extended from most regions of the
76 original inoculation and existent fingers, except for the back of the drop. Scale bar = 1
77 mm.



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Supplementary Figure 5: *taxDI* cells form a single, wide front across a wide range of initial cell densities. Time-lapse of *taxDI* cells deposited on an agarose surface with increasing initial cell densities. Light was incident from the top of the figure. Cell density was quantified by optical density (OD) at 730 nm. The lack of well-separated, finger-like projections contrasted those observed in communities of wild-type cells (Fig. 1A). Scale bar = 1 mm.