Supplementary Information For:

Title: Molecular structure of the intact bacterial flagellar basal body

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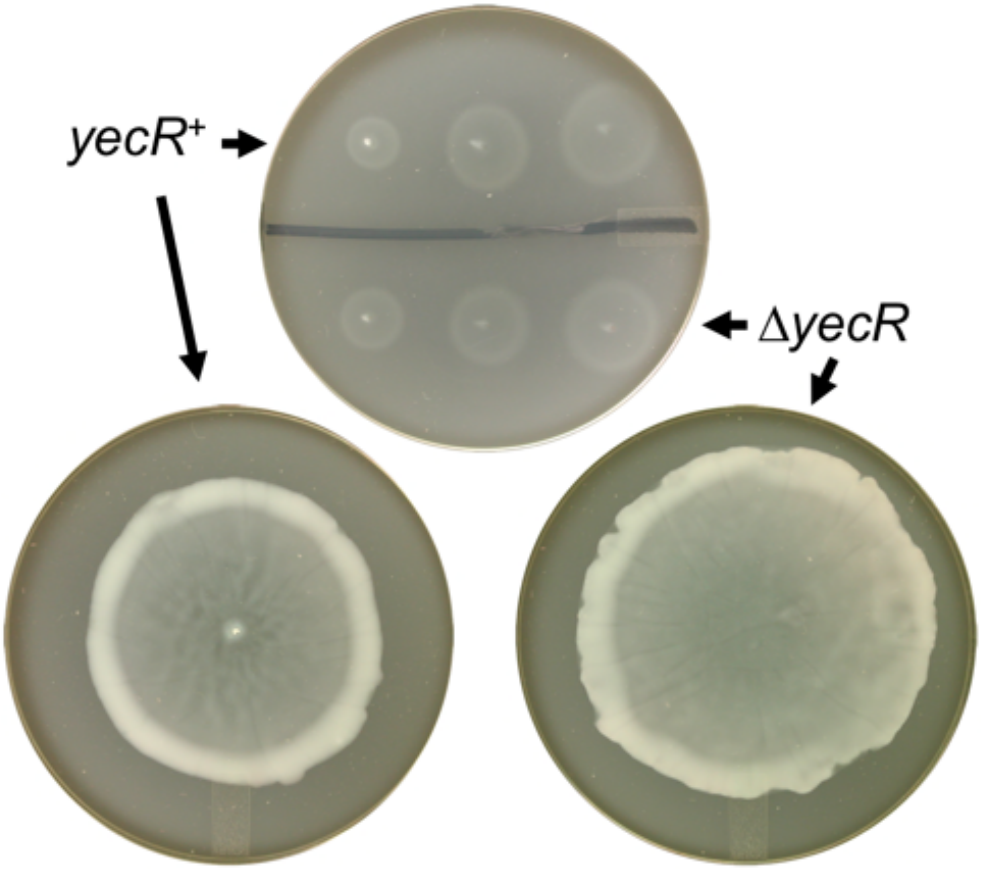
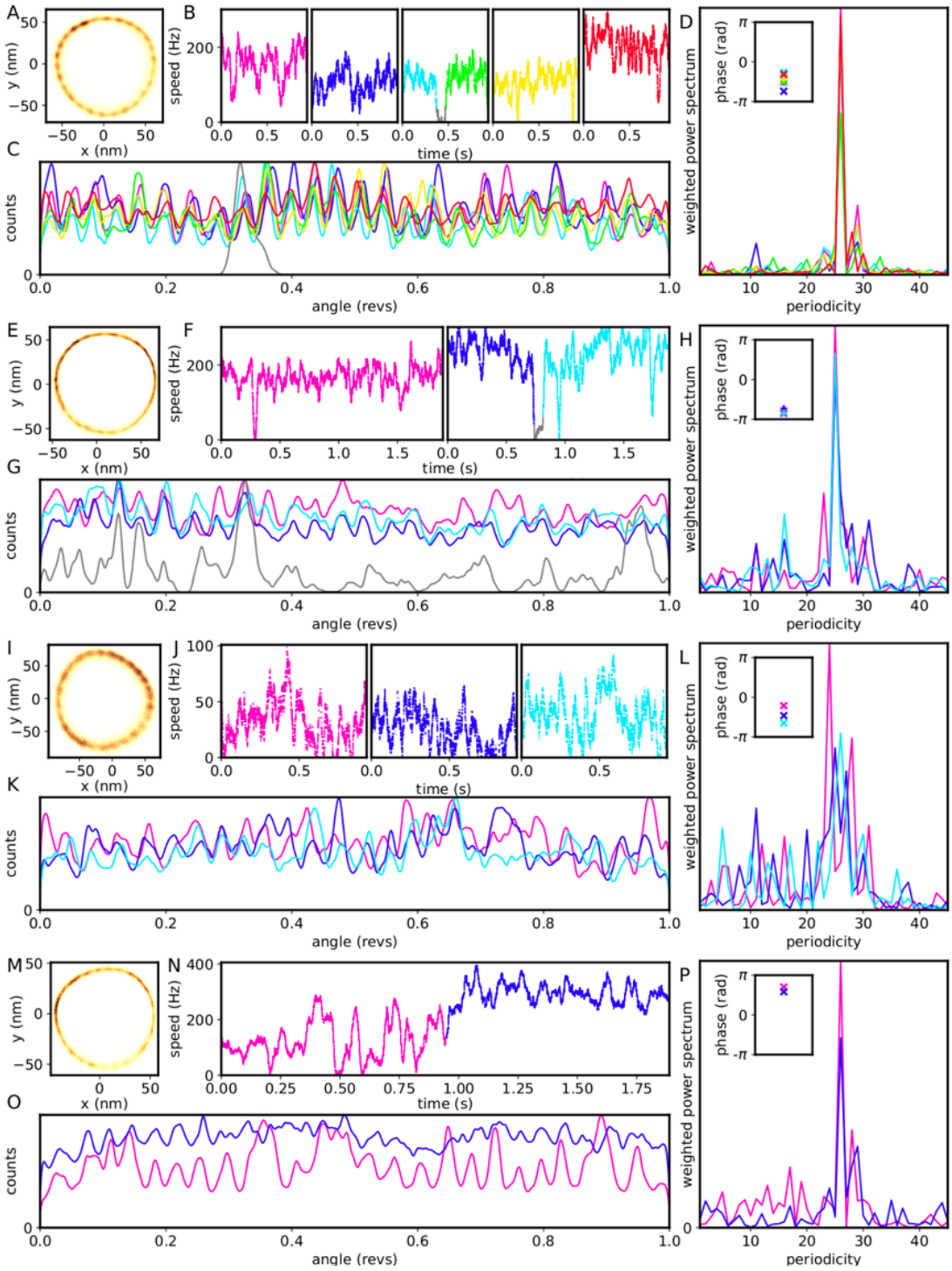
Inventory:

Supplementary Figures 1-3, Supplementary References

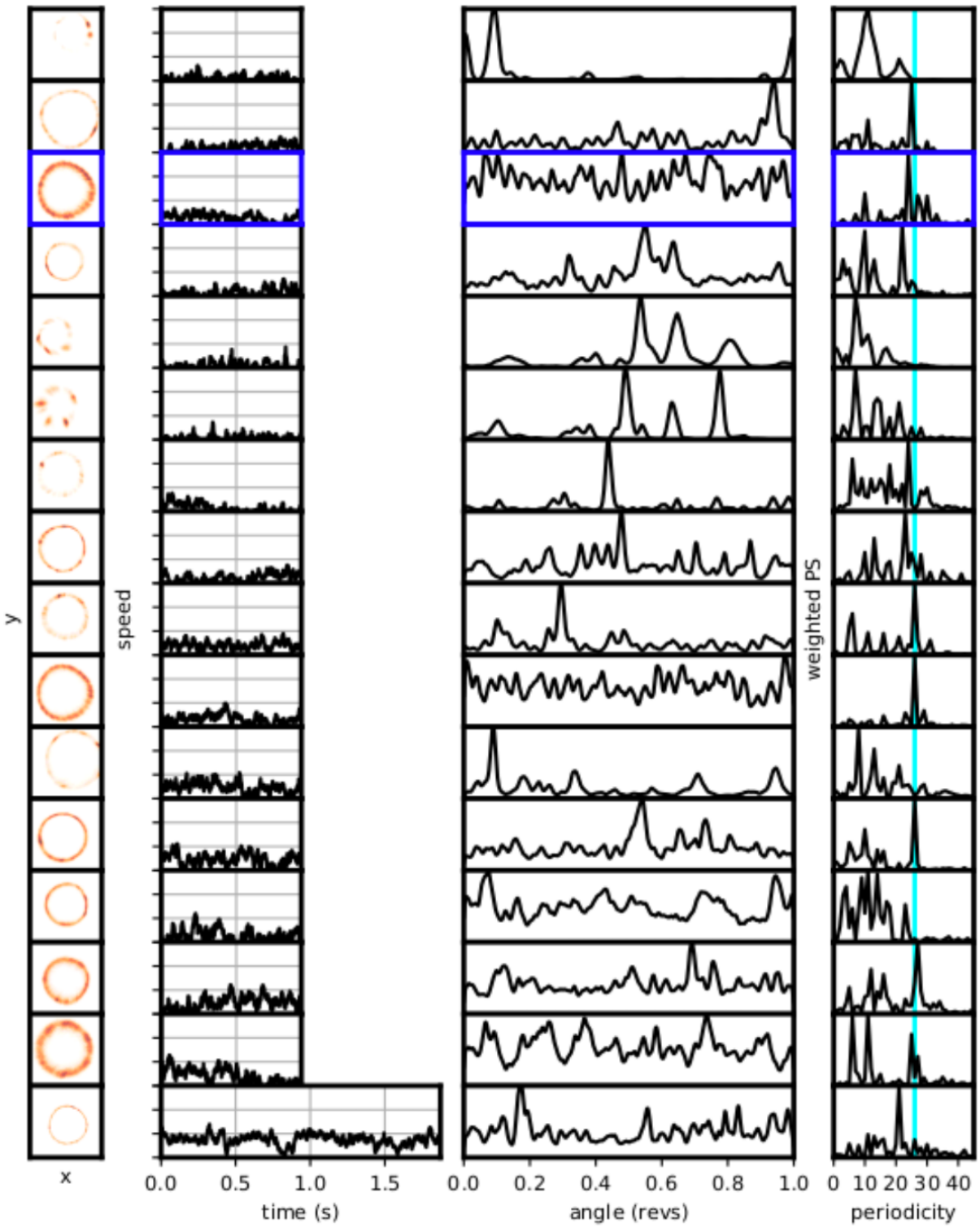


Supplementary Figure 1. Swimming (top) and swarming (bottom) motility assays are shown for a strain deleted for the *yecR* locus as compared to an isogenic wild-type (yecR$^+$) strain. Swimming motility was assayed in motility agar (per liter: 10g tryptone, 5g NaCl and 3g Bacto Agar). Top: three independent colonies for each strain were taken by toothpick from cells grown overnight on L agar plates and poked into the motility agar plate and incubated for 3 hours at 37°C. Bottom: a 5 µl aliquot from overnight liquid cultures grown in L medium was used to inoculate the center surface of individual swarm agar plates prepared as described [1] and incubated at 37°C for 9 hours.



Supplementary Figure 2: All recordings from 4 out of 27 different motors in which both 26-fold periodicity and discrete speed changes, presumably corresponding to stator protein exchange [2,3], were observed. The location (phase) of the 26-fold potential in each motor changes very little; neither with discrete speed changes, presumably corresponding to stator protein exchange [2,3], nor over time gaps which are long with respect to the expected stator exchange rate [4]. (A-D) additional recordings of the motor outlined in magenta in Supplementary Figure 3. A) 2D histogram of the x,y position of a 100nm bead attached to a rotating motor over 5 1s recordings. B) The corresponding angular speed, (median

filtered, 20 ms window). Each break in the x axis represents a new recording and a time gap of about 30 s. C) Kernel density estimation of the angular position, colors corresponding the recordings shown in (B). D) Corresponding weighted power spectra. The inset shows the phase of the 26-fold Fourier component for each segment. E-H) The same plots as (A-D) for another motor. The second recording is split into two segments separated by a pause (grey), likely corresponding to the loss of the stator unit. I-L) Another example, with three recordings of a third motor. M-P) Another example, of a fourth motor, where the recording is separated into two segments, likely demarcating a change from 1 to 2 stators. The motors of (E-H), (I-L), and (M-P) are outlined in blue in Supplementary Figure 3.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1.0
1.5
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angle (revs)
weighted PS
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30
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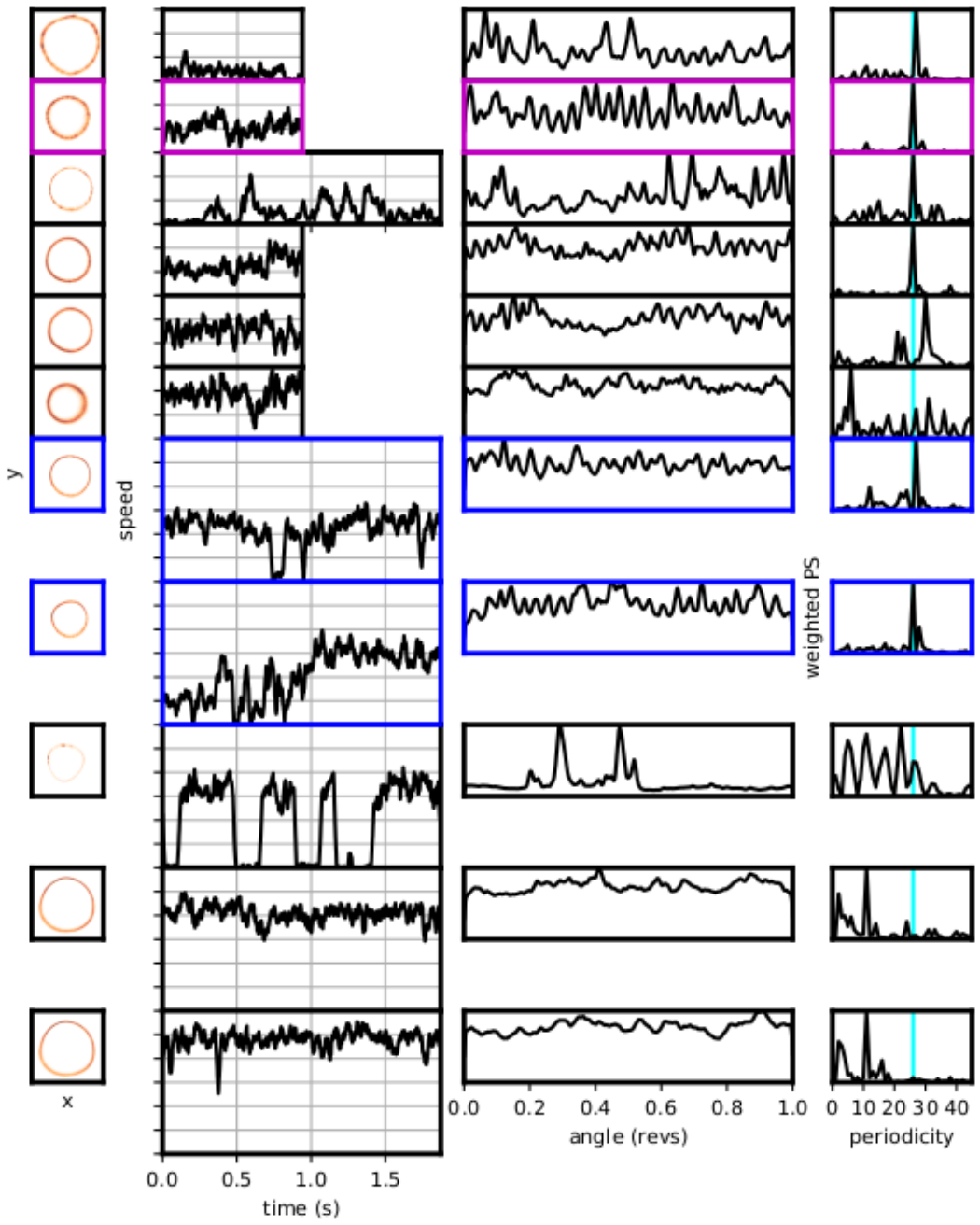
periodicity



Supplementary Figure 3: All 27 traces that went into the weighted power spectrum shown in Figure 1 and Extended Data Figure 3. From left to right, the subplots show: 2D x,y histogram, 100 nm x 100nm; speed (Hz) versus time (s) over 1-2 s (median filtered, 20 ms window), gridlines marked at 0.5 s and 100 Hz intervals; kernel density of the angular position; weighted power spectrum, cyan line marks a periodicity of 26 to guide the eye. The trace outlined in magenta is that shown in Figure 1 of the manuscript and Extended Data Figure 3. The traces outlined in blue correspond to traces of Supplementary Figure 2, as stated in the legend. The wide range of observed speeds is due to variations in sodium-motive force (54 - 187 mV) as well as likely differences in the number of stator units [6].

Supplementary References

1 Wozniak, C. E., Chevance, F. F. & Hughes, K. T. Multiple promoters contribute to swarming and the coordination of transcription with flagellar assembly in Salmonella. *J Bacteriol* **192**, 4752-4762, doi:10.1128/JB.00093-10 (2010).

2 Leake, M. C. *et al.* Stoichiometry and turnover in single, functioning membrane protein complexes. *Nature* **443**, 355-358, doi:10.1038/nature05135 (2006).

3 Nord, A. L., Sowa, Y., Steel, B. C., Lo, C. J. & Berry, R. M. Speed of the bacterial flagellar motor near zero load depends on the number of stator units. *Proc Natl Acad Sci U S A* **114**, 11603-11608, doi:10.1073/pnas.1708054114 (2017).

4 Nord, A. L. *et al.* Catch bond drives stator mechanosensitivity in the bacterial flagellar motor. *Proc Natl Acad Sci U S A* **114**, 12952-12957, doi:10.1073/pnas.1716002114 (2017).