Species-dependent hydrodynamics of flagellum-tethered bacteria in early biofilm development: Supplementary information

1 Methods

1.1 Experimental Imaging

After injection into the flow cell, the bacteria were imaged immediately without flow for 5-20 minutes. The flow cell was attached to a heating stage set to 30°C. High speed movies were recorded using a Phantom V12.1 high speed camera (Vision Research), collecting bright-field images with a 100× oil objective and a 2× multiplier lens on an IX83 Olympus microscope. Unless otherwise stated, videos are recorded at 200 frames per second (fps) and are playing back at 50 fps. Frame numbers at the bottom of each video correspond to the data acquisition frames. Videos have a width of 160 pixels which corresponds to 8 μm.

1.2 Image Analysis

Images were analyzed using a combination of software and algorithms previously described [1, 2] in MATLAB (Mathworks) with the following modifications. Before generating the binary image via Otsu thresholding, we further preprocess and filter the images using two edge detection filters, one to re-enhance the bacteria septa and edges between adjacent bacteria, and one to re-enhance the bright inner part of bacteria. After generating the binary image, we use the MATLAB function 'regionprops.m' to generate instantaneous shape properties, which includes fitting an ellipse to the bacterium shape. Because we are imaging the two-dimensional projection of bacteria, we have to infer their tilt angle via the normalized tilt angle, which is calculated as 1 minus the eccentricity of the fitted ellipse squared. A value that approaches zero corresponds to a bacterium in the horizontal state, while a value that approaches one corresponds to a bacterium in the vertical state. The XY locations of the bacterium's two poles are represented via the two foci of the fitted ellipse. The inner or outer pole corresponds to the bacterium pole that is closer to or farther from the surface, respectively.

2 Summary of model results

We summarise the results of the model in figure S1. The behaviours we see for the parameters chosen here include the following: fast rotations in an upright position; slow rotations while lying horizontal and close to the surface; slow rotations while slowly standing up; quickly standing upright from initial condition close to the surface; spinning about head axis while remaining close to the surface without rotating about normal to the surface; free part of flagellum stands upright and head angle oscillates or aligns parallel to surface; repeatedly sweeping down to the surface then standing up while also rotating about the surface normal.

References

[1] M. L. Gibiansky, J. C. Conrad, F. Jin, V. D. Gordon, D. A. Motto, M. A. Mathewson, W. G. Stopka, D. C. Zelasko, J. D. Shrout, and G. C. L. Wong. Bacteria use type IV pili to walk upright and detach from surfaces. *Science*, 330:197, 2010. doi: 10.1126/science.1194238.

[2] A. S. Utada, R. R. Bennett, J. C. N. Fong, M. L. Gibiansky, F. H. Yildiz, R. Golestanian, and G. C. L. Wong. *Vibrio cholerae* use pili and flagella synergistically to effect motility switching and conditional surface attachment. *Nat. Commun.*, 5:4913, 2014. doi: 10.1038/ncomms5913.

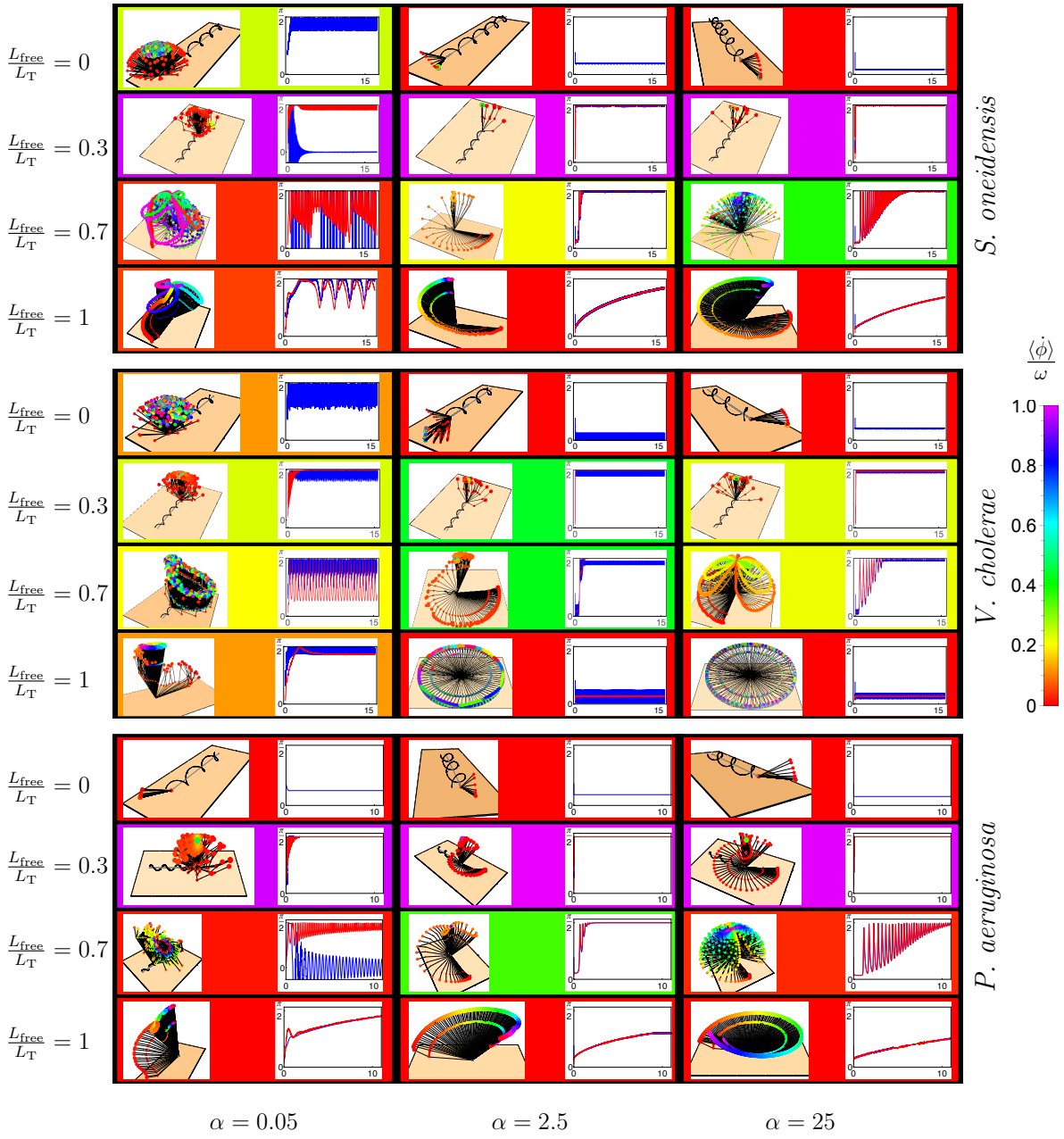

Figure S1: Summary of the results for (a) *S. oneidensis* model, (b) *V. cholerae* model, (c) *P. aeruginosa* model. The background colour of each panel shows the average angular velocity about the $\hat{\mathbf{z}}$ axis, $\langle\dot{\phi}\rangle$ in units of the hydrodynamic frequency ω. The figure on the left of each panel shows the trajectory of the model and the figure on the right of each panel shows the angles between the surface and the free part of flagellum (red) and between the surface and the cell head (blue). α is the ratio of the head spring constant and the flagellar motor torque.