Supplemental Material: Swimming, Feeding and Inversion of Multicellular Choanoflagellate Sheets

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This file contains additional experimental results on flagellar dynamics and geometry of *C. flexa*.

I. VIDEO IMAGING AND ANALYSIS

A. Supplementary Videos

C. flexa sheets were imaged in FluoroDishes (World Precision Instruments FD35-100) by differential interference contrast (DIC) microscopy using a 40× (water immersion, C-Apochromat, 1.1 NA) Zeiss objective mounted on a Zeiss Observer Z.1 with a pco.dimax cs1 camera.

Flagellar characteristics reported in Table I were obtained as follows. Beat frequencies were determined by averaging over five cycles for each of twenty randomly selected cells. All other measurements are averages over ten randomly selected cells. The comparatively large wavelength for *flag-out* sheets may be due in part to the fact that the flagellar waveform in that state is not sinusoidal, and its wavelength is thus less well defined than in the *flag-in* state.

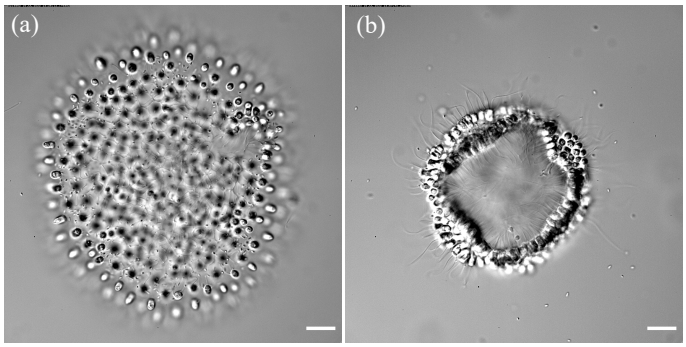

FIG. S1. Flagellar dynamics in *C. flexa* colonies. (a) Snapshot from Video 1, a high speed recording of a *flag-in* sheet used to determine flagellar characteristics reported in Table I. Movie is set to play at 17× slower than real time. (b) As in (a), but for the *flag-out* state in Video 2. Scale bars are 10 μm.

conformation	beat frequency f	length $2L$	amplitude d	wavelength λ
flag-in	45 ± 4 Hz	26 ± 5 μm	2.4 ± 0.6 μm	9 ± 3 μm
flag-out	43 ± 8 Hz	23 ± 4 μm	2.2 ± 0.7 μm	15 ± 3 μm

TABLE I. Measurements of flagellar characteristics for the *flag-in* and *flag-out* sheets in Videos S1 and S2. Uncertainties reported are standard deviations.

B. Estimating the propulsive force from the flagella

In the fluid mechanics model of the *C. Flexa* raft, we approximate the flagella beating as an effective propulsive point force $\mathbf{F}$ acting in the direction $\hat{\mathbf{n}}$. The magnitude of this force F can be approximated using the resistive force theory [S1] as

$$F = 2L\left(\zeta_\perp - \zeta_\parallel\right)(1-\beta) f\lambda \tag{S1}$$

where $2L$ is the flagella length, f the beat frequency and λ the projected wavelength in the direction of the traveling sinusoidal wave (i.e. $\hat{\mathbf{n}}$), the values of which are listed in Table I. Meanwhile,

$$\zeta_\perp = \frac{4\pi\mu}{\ln(2L/r)} \quad \text{and} \quad \zeta_\parallel = \frac{2\pi\mu}{\ln(2L/r)} \tag{S2}$$

are the transverse and longitudinal drag coefficients of a cylindrical filament of radius r, approximated using the resistive force theory, and β is a coefficient that depends on the flagella waveform. Although the flagella waveform is not necessarily sinusoidal, in the absence of better measurements, the value of β is approximated, assuming the flagella takes a sinusoidal waveform $f(x)$ with wavelength λ and amplitude d (Table I), as

$$\beta = \frac{\int_0^\lambda \left(1+f'(x)\right)^{-1/2} dx}{\int_0^\lambda \left(1+f'(x)\right)^{1/2} dx}, \tag{S3}$$

which can be found by numerically. In the limiting of $2\pi d/\lambda \ll 1$, $\beta \approx 2\pi^2 (d/\lambda)^2$.

II. CONFOCAL IMAGING

Sheets in figures S2 and S3 were fixed and stained with FM1-43FX or with Alexa 488-phalloidin as in [S2]. Sheets were imaged on Zeiss LSM 880 with AiryScan using a 63x, 1.4 NA C Apo oil immersion objective (Zeiss). Z-projections were generated with Fiji [S3]. Packing fraction was estimated by projecting cell bodies located within the same plane in a locally flat portion of the sheet and by manually outlining the border of the colonies (red dotted line in S2).

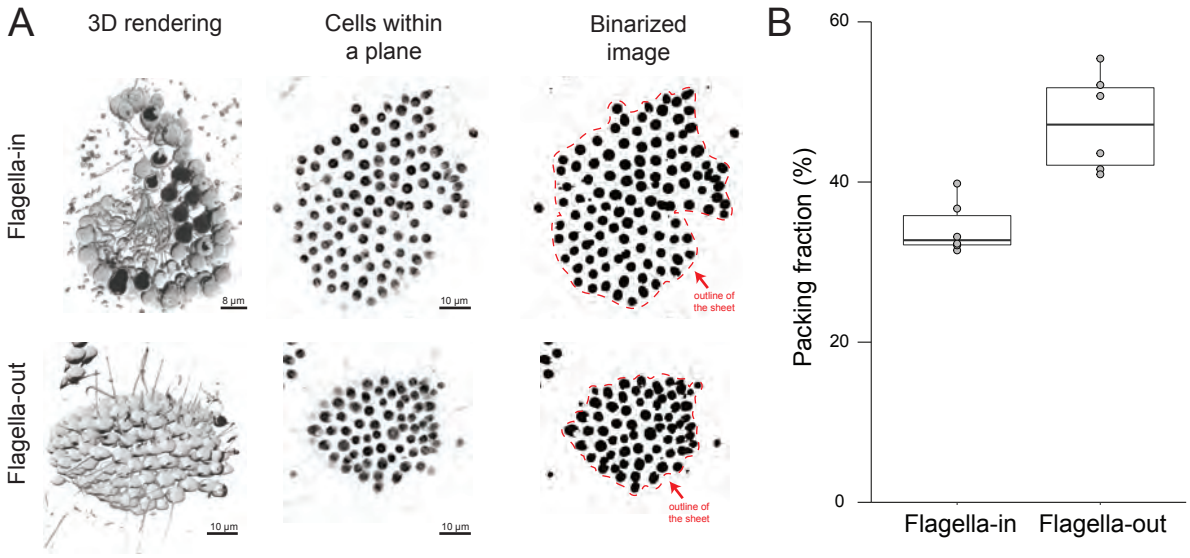

FIG. S2. Packing fraction in flagella-in and flagella-out *C. flexa* colonies. (a) 3D stacks (left column) of sheets stained with the fluorescent membrane marker FM 1-43 FX were imaged by confocal microscopy to determine sheet morphology. Packing fraction was computed by doing a Z-projection of a locally flat portions of individual sheets (middle column) and generating a binarized image in which the area occupied by individual cells appears black (right column). Packing fraction is the ratio of the area occupied by cells to the total colony area within that plane (area within the red dotted line). (b) Boxplot depicting packing fraction values for 6 sheets with flagella out and 6 sheets with flagella-in. p=0.2% by the Mann-Whitney test.

Packing fraction was then computed as the ratio of the area occupied by cells (black area in binarized image in figure S2) to the total area occupied by the colony (area within the red dotted line in figure S2). Polygonal collar borders in figure S3 were manually outlined and colored with Adobe Illustrator 27.3.1 (2023). Hexagonal and pentagonal outlines were counted in 9 colonies and counts are reported in table II.

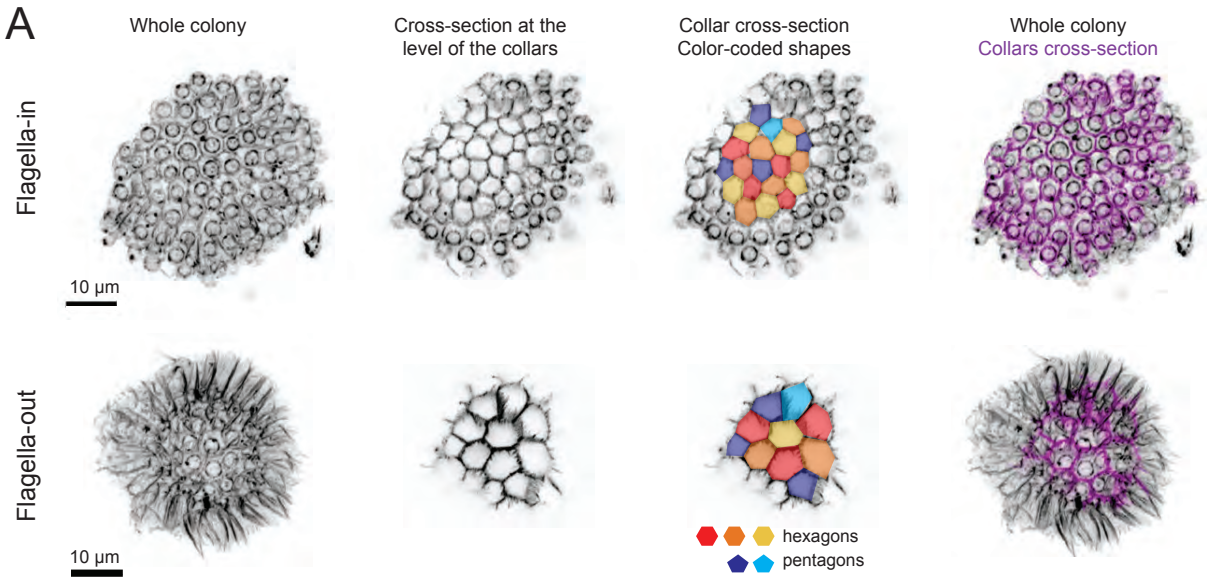

FIG. S3. Hexagonal and polygonal collar outlines within flagella-in and flagella-out *C. flexa* colonies. F-actin within colonies was stained with fluorescent phalloidin, which outlines the microvillous collars linking cells, as well as the actin cytoskeleton within the cell body. Left column: whole-colony Z-projections. Middle left: Z-projections of a few planes intersecting many collars, showing the polygonal outlines of collar contacts. Middle right: color-coded polygons showing a majority of hexagons and a minority of pentagons. Right: whole-colony Z-projection with the plane containing most collars outlined in purple.

TABLE II. Numbers of hexagons and pentagons in representative sections of 9 colonies of *C. flexa*.

colony #	1	2	3	4	5	6	7	8	9	total
hexagons	4	4	2	8	8	15	6	6	8	61
pentagons	4	0	1	2	2	7	2	3	2	23

[S1] E. Lauga, *The Fluid Dynamics of Cell Motility* (Cambridge University Press, Cambridge, UK, 2020), §7.1.3-7.1.4.
[S2] T. Brunet, B.T. Larson, T.A. Linden, M.J.A. Vermeij, K. McDonald, and N. King, Light-regulated collective contractility in a multicellular choanoflagellate, Science **366**, 326–33 (2019).
[S3] J. Schindelin, I. Arganda-Carreras, E. Frise et al. Fiji: an open-source platform for biological-image analysis. Nat Methods **9**, 676–682 (2012).