Supplemental Material: Stress-Induced Dinoflagellate Bioluminescence at the Single Cell Level

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I. EXPERIMENTAL SETUP

The experimental setup, shown schematically in Figure S1, consists of a microscope for visualization and positioning systems to control the two pipettes. All experiments were conducted in a darkened room. The white light illumination of the microscope (Nikon TE2000) was kept to a minimum and sent through a red long-pass filter (620 nm, Knight Optical, UK) to avoid disruption of the night phase of the dinoflagellates and to allow a greater dynamic range in capturing the bioluminescence. That background intensity was controlled in all experiments for uniformity.

Pipettes were positioned with 3D micromanipulators (Patchstar, Scientifica). For small deformation rate experiments, we used a Thorlabs 1D Direct Drive Linear Stage (DDS220/M) to control the motion. All stages were programmed with their native software. The pipettes were connected to syringes with stiff tubing and fluid flow through them could also be used to position the cells (see below). In the flow experiments, we used a syringe pump (PHD2000, Harvard Apparatus) at a constant rate. The test section was a chamber whose top and bottom were coverslips, held apart by ~ 2 mm plastic spacers (see inset of Fig. S1). As *P. lunula* has a very characteristic three dimensional geometry, for consistency, we held the cells the same orientation within the chamber in all experiments.

As cells of *P. lunula* are negatively buoyant, they settle to the bottom coverslip of the sample chamber. Cells were positioned manually with the use of joystick controllers and gentle suction of the flow, as illustrated in Figure S2. The main bioluminescence experiments were recorded with a Prime 95B sCMOS camera (Photometrics). The high sensitivity of the camera allowed for measurements at low light condition but relatively high recording speed. For the PIV and particle tracking experiments, we used a high-speed camera (Photom v311). Figure 1 of the main text was captured using a Nikon D810 DSLR with Differential Interference Contrast (DIC) microscopy.



FIG. S1: Schematic of experimental setup to study bioluminescence produced by single dinoflagellates under controlled stresses.



FIG. S2: Manual positioning of a cell prior to main measurements. (a) The cell is initially drawn up from the bottom cover glass using gentle flow suction. It nearly always aspirated from one of its pointy ends. The cell flashes once in this process (b) and the light decays (c). The pipettes are raised within the sample chamber to be far from the top and bottom chamber surfaces. (d) Using the joystick controllers of the micromanipulators, the cell is placed on the other pipette and then held using gentle suction (e). (f,g) The cell is rotated so that the largest area is exposed to the camera. (h) Finally, by placing the cell between the pipettes, indentation experiments can be performed.

II. SOLUTION OF THE MODEL

Equations 1, 3a and 3b are linear ODEs which can be solved exactly. As described in the main text, we take here the simplest case in which the light flash occurs within the ramp period, and therefore confine the discussion to times $t < t_f$, during which the rate of strain $\dot{\varepsilon}$ is constant. From the three time constants (τ_r, τ_e, τ_a) we find τ_a to be by far the largest, and thus define the two ratios $\lambda, \rho < 1$,

$$\lambda = \frac{\tau_e}{\tau_a} \,, \quad \rho = \frac{\tau_r}{\tau_a} \,. \tag{S1}$$

Then, from (1) the signal is

$$s(t) = \dot{\varepsilon}\tau_e \left(1 - e^{-t/\tau_e}\right) \,. \tag{S2}$$

As $t \to 0$, $s \sim \dot{\varepsilon}t + \cdots$, while at long times s approaches $\dot{\varepsilon}\tau_e$. If we set $t = t_f$ and note that $t_f \equiv \varepsilon_f/\dot{\varepsilon}$, we obtain (2) in the main text. Substituting (S2) into (3b) and solving for h, we find

$$h = \frac{\dot{\varepsilon}\tau_e}{1-\lambda} \left[1 - e^{-t/\tau_a} - \lambda \left(1 - e^{-t/\tau_e} \right) \right] \,, \tag{S3}$$

which varies as $\dot{\varepsilon}t^2/2\tau_a$ as $t \to 0$ and, as with s, approaches $\dot{\varepsilon}\tau_e$ for long times.

Finally, the light intensity is

$$I(t) = \frac{\dot{\varepsilon}\tau_e}{1-\lambda} \left[\frac{1}{1-\rho} \left(e^{-t/\tau_a} - e^{-t/\tau_r} \right) - \frac{\lambda}{\lambda-\rho} \left(e^{-t/\tau_e} - e^{-t/\tau_r} \right) \right],\tag{S4}$$

which behaves as $I \sim \dot{\varepsilon} t^2/2 \tau_r$ as $t \to 0$. At large times, with $\tau_a > \tau_e \sim \tau_r$, the dominant term in (S4) is $I \propto e^{-t/\tau_a}$, so $\dot{I} \sim -I/\tau_a$, a relationship seen in Figs. 3b&c of the main text. Figure S3 shows plots of the solutions above.

To find the time scales τ_r , τ_e and τ_a , we employed a least squares analysis on the average signal from all experiments. The values obtained, $\tau_e \approx 0.027 \text{ s}$, $\tau_r \approx 0.012 \text{ s}$, and $\tau_a \approx 0.14 \text{ s}$, yield the ratios $\lambda \approx 0.19$ and $\rho \approx 0.09$. Thus, the prefactors within square brackets in (S4) are $1/(1-\rho) \approx 1.1$ and $\lambda/(\lambda-\rho) \approx 1.9$. Figure S3c compares the theoretical curve for the flash intensity with the experimental data used in Figure 3 of the main text.



FIG. S3: a) Plots of s, h, and I for $\tau_r = 0.012$, $\tau_a = 0.14$, $\tau_e = 0.027$, and $\dot{\delta} = 500 \mu \text{m/s}$. The inset shows the phase portrait of the intensity signal. b) The same as (a) in a linear-log scale to highlight the early time behaviour. c) Intensity signals corresponding to the results shown in figure 3. Black and blue thin lines show the raw data for fixed $\dot{\delta}$ (3b) and δ_f (3c), respectively, and the yellow lines show their average values. The red line shows the average value of all the raw data. The black line is the same theoretical curve shown in panels a and b, normalized by its maximum value.

III. THRESHOLD MEASUREMENTS BY FLUID FLOW STIMULATION

In Figs. 2a-f of the main text the second pipette is placed at a distance $H \sim 50 \,\mu$ m from the organism with no flow. The flow rate is then stepped up abruptly to approximately 1 ml/h. The organism then flashes once the cell wall is sufficiently deformed. Typically, this happens within 1s. The threshold stress depends slightly on the perturbation rate [S2]. In what follows, we have explain a procedure to keep the same timescale and measure a quantity —the pipette-organism distance H —that can be easily related to the stress experienced by the organism. The choice of 1 ml/h was found to be important. Lower flow rates were often insufficient to trigger bioluminescence, even for very small H, while higher flow rates trigger bioluminescence for large H, with the drawback that this configuration is more sensitive in that the larger the distance, the more difficult the control of the impact zone of the jet. For this reason we fix the flow rate and vary H.

The experimental procedure is as follows. Micropipettes were fabricated to have a prescribed inner diameter of $25 \pm 5\mu$ m. The micropipette is initially placed far from the dinoflagellate and laterally offset so flow does not reach the organism. The micropipette is then moved perpendicularly to the direction of the flow (Fig. S4) and passes across the dinoflagellate in approximately 2 s. As the two pipettes are in the same plane, the jet passes across the centerline of the dinoflagellate. If bioluminescence is observed, the distance H between the micropipette and the closest part of the dinoflagellate in vertical alignment is recorded. If not, the micropipette is brought closer by a few microns and a new sweep is performed. And so on. Among all the observed events, only the ones in which the micropipette is well aligned with the center of symmetry of the dinoflagellates were kept for analysis. A total of 35 independent events were recorded with distances H varying between 9 and 71 μ m, for a mean of $\bar{H} = 32 \pm 17 \,\mu$ m. While Most of the data were recorded with the dinoflagellate held at its center, other attachment points were also studied and required similar distances to trigger bioluminescence.



FIG. S4: Image sequence of a typical flow experiment triggering bioluminescence. The micropipette is moved perpendicularly to the main direction of the flow.



FIG. S5: (a) Numerical simulation of the fluid flow around an organism, modeled as a sphere: (left) mesh, (right) velocity magnitude. (b) Histogram of the threshold force for light production.

IV. FINITE ELEMENT COMPUTATIONS

Computations were performed to estimate the force required for light production. The steady-state axisymmetric Navier-Stokes equations were solved numerically with the finite element software COMSOL [S1] to obtain the flow from a pipette impinging on a cell. Figure S5a shows a close up of the geometry employed. The geometry of the dinoflagellate is simplified to a sphere of radius \mathcal{R} , positioned at a distance H from the outlet of the pipette. The computational domain was chosen to be sufficiently large that the presence of the domain boundaries did not affect the calculations. The inner diameter of the micropipette nozzle was set to $25 \,\mu\text{m}$, with a flow rate $Q = 1 \,\text{ml/h}$, resulting in a fluid exit speed from the micropipette of $U \sim 0.6 \,\text{m/s}$. As a heuristic check on the computational results for fluid forces, we expect the rate of momentum injected toward the cell should be $F_f \sim \rho QU \sim 10^{-7} \,\text{N}$, in agreement with results obtained below.

Based on the actual size of the organisms and the experimental distance from the pipette, the computations were performed with the average value $\mathcal{R} = 30 \,\mu\text{m}$. The results were found to be insensitive to changes in \mathcal{R} within our experimental values. As in experiment, the values of H varied between 9 and 71 μm .

PIV measurements of the flow created by the submerged jet in the absence of the dinoflagellate were compared to the flow field computed within COMSOL, and found to be in good agreement. After this validation, the fluid flow in the presence of the sphere was determined (see Fig. S5) along with the mechanical forces exerted on the surface of the dinoflagellate (sphere) by integrating the stress over its surface. By synthesizing these results with the experimental thresholds for light production we obtain in Figure S5b the probability distribution of the threshold force for bioluminescent flashes. The distribution peaks at $F_f \sim 0.1 \,\mu$ N.

Figure S6 summarizes the main results of the computations. The normal and tangential stresses at the surface of the sphere are plotted as functions of the arc length l in the top-right quadrant of the sphere (Fig. S5a). For a typical pipette distance of $H = 32 \,\mu$ m, the normal component is maximal at l = 0 (denoted σ_n) and is a decreasing function of arc length, reaching half its peak value at an arclength denoted by l_n . The tangential component exhibits a maximum σ_t at arclength $l_t \simeq l_n$. With these definitions l_n and l_t are typical length scales of the stress variations along the surface. The resulting forces along the vertical dimension z are the pressure F_n , frictional force F_t and total fluid force $F_f = F_n + F_t$.

As expected, the smaller the distance H, the larger the forces and stresses. Whatever the value of H the normal stress σ_n is systematically larger than the tangential stress σ_t . For $H = 32\mu$ m, $\sigma_n \sim 200$ Pa and $\sigma_t \sim 60$ Pa. The lengths l_n and l_t are always strongly correlated and nearly identical, and do not vary significantly $(8 - 13 \,\mu\text{m})$ in the range explored). For $H = 32\mu$ m, we find $l_n \sim l_t \sim 9\mu$ m. One can take twice $l_{n,t}$ is an estimate of the perturbation size, in agreement with $\xi \sim 30\mu$ m measured on the images. Computations were also performed to probe the effect of the nozzle diameter. Varying this quantity in the range $20 - 30 \,\mu$ m shows that there is about a factor of 1.5 in the total force and a factor of 2 in the stresses compared to those obtained from the average diameter of $25 \,\mu$ m. The characteristic lengths l_n and l_t were essentially unaffected.



FIG. S6: Results of numerical computations. (a) Normal stress and tangential stress along the surface of the sphere as functions of arclength l ($H = 32 \,\mu$ m in this example). Data are plotted along the top-right quadrant ($l \in [0 - \pi R/2]$). The maximal stresses are σ_n and σ_t and the characteristics arc lengths l_n and l_t denote the half-maximal stress values. (b) Forces, stresses and characteristic arc lengths as functions of H.

V. PERTURBATION STRESS VERSUS PERTURBATION AREA (FIG. 5)

The data in Figure 5 of the main text were taken from the present experiments and the literature as follows:

- 1. AFM experiments, from Tesson and Latz [S2]: sphere diameter 10 μ m and force 3.2 14.5 μ N. $\mathcal{A} = \pi$ (sphere diameter/2)² = 80 μ m². $\Sigma = Force/\mathcal{A} = (0.4 2) \times 10^5$ Pa.
- 2. Micropipette-held cells, from the present study: $l = 9 \,\mu\text{m}$ within the range $8 13 \,\mu\text{m}$ and $\mathcal{A} = \pi l^2 = 250 \,\mu\text{m}^2$ in the range $200 530 \,\mu\text{m}^2$. Maximal stress at the surface $\Sigma = 200$ Pa within the range 80 500 Pa from finite element computations.
- 3. Large-scale flow experiments, from Cussatlegras and Le Gal [S3]. Shear stress $\Sigma = 0.5 1.5$ N/m². Perturbation size ξ equals the organism size: 50 μ m in diameter and 200 μ m in length. $\mathcal{A} = \pi (\xi/2)^2$ in the range 2,000 30,000 μ m².

[[]S1] COMSOL, Multiphysics v. 5.3., COMSOL AB, Stockholm, Sweden.

[[]S2] B. Tesson, and M. I. Latz, Mechanosensitivity of a rapid bioluminescence reporter system assessed by atomic force microscopy, Biophys. J. 108, 1341 (2015).

[[]S3] A.-S. Cussatlegras, and P. Le Gal, Variability in the bioluminescence response of the dinoflagellate Pyrocystis lunula, J. Exp. Marine Biol. Ecol. 343, 74 (2007).