

## Autonomous Swinging of a Lipid Tubule under Stationary Irradiation by a $\text{Nd}^{3+}$ :YAG Laser

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A micrometer-sized lipid tubule exhibits oscillatory swinging motion under stationary irradiation by a  $\text{Nd}^{3+}$ :YAG laser ( $\lambda = 1064$  nm). By choosing an appropriate optical path through an objective lens, the laser can be split into dual beams focused on the same position. Using this splitting, a lipid tubule can be shown to exhibit bistability with regard to the orientation of trapping. Driven by a temperature gradient induced by local photon heating, the trapped lipid tubule shows oscillatory motion between two possible orientations. This oscillatory phenomenon of the lipid tubule is thought to represent the breaking of time-translational symmetry under thermodynamically open conditions.

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Under thermodynamically equilibrium conditions, any kind of collective motion of atoms and molecules will degenerate into random thermal motion, according to the equipartition principle. On the other hand, under thermodynamically open conditions, a minor fluctuation can develop into large-scale collective motion [1]. A rich variety of actual examples of the latter phenomenon can be found in living organisms. All living organisms on Earth maintain their lives under thermodynamically open conditions by using energy from the sun and by dispersing thermal energy into their environment. One of the essential features of life is the breaking of time-translational symmetry, e.g., a beating heart, circadian rhythm, cell cycle, etc. In this context, a fundamental study of the breaking of such symmetry, accompanied by energy flow, would be of scientific value. It has been established that a focused laser beam creates a local attractive potential in  $\mu\text{m}$ -scale space, i.e., laser tweezers [2]. When the medium absorbs photons, the laser induces local heating in the system. Since both the profile of the attractive potential and the degree of heating by the laser beam can be controlled, it is tempting to conduct experiments on nonlinear dynamical motion under constant energy flow in a  $\mu\text{m}$ -scaled world [3]. In the present Letter, we describe spontaneous oscillation in a  $\mu\text{m}$ -scaled lipid tubule under stationary illumination by a focused laser.

A lipid tubule was prepared by a slight modification of the method used to form giant unilamellar liposomes [4]. A  $\text{CHCl}_3/\text{MeOH}$  solution of 10 mM neutral phospholipids, egg-yolk PC or dioleoyl-phosphatidylcholine (DOPC), was sucked into a glass capillary ( $\phi$  1 mm). After vacuum evaporation for 6 h, a dry lipid film formed on the inner wall of the glass tube. Buffer solution (10 mM HEPES containing 10 mM  $\text{MgCl}_2$ ,  $\text{pH} = 7.1$ ) was then transferred to the capillary. After incubation for 10 min at room temperature, the contents of the capillary were pushed out onto a glass slide. The resulting lipid tubule is made of multilamellar phospholipid bilayers ("myeline form" [5]) and is typically 1–3  $\mu\text{m}$  in diameter. An optical trapping beam was obtained by introducing a  $\text{Nd}^{3+}$ :YAG laser (SL902T, cw in TEM<sub>00</sub> mode at a wavelength of

1064 nm, Spectron) to an inverted microscope (TE-300, Nikon) through the objective lens [Nikon Plan Fluor, 100 $\times$ , numerical aperture (NA) 1.30] and focused on the sample solution. The spatial distribution of the laser intensity was observed by a beam analyzer (13SKP503, Melles Griot KK). All of the measurements were performed at  $26 \pm 1$  °C. The motion of the tubule in the sample solution was monitored with a CCD camera (CS8310, Tokyo Electric Industry) and recorded by VCR (WVD-9000, Sony). The pictures were captured and analyzed on a PC using an image processor (Cosmos, LIBRARY).

Figure 1A (a) shows bright-field microscopic images of a phospholipid tubule of egg-yolk PC lying parallel to the glass slide, i.e., perpendicular to the optical axis ( $Z$  direction) of the laser (in Fig. 1B, the object indicated by the broken line). The change in the tubule orientation after illumination by the single-mode laser is shown in Fig. 1A (b)–(e), with a time interval of 2 s, where (b) shows just after focusing on the end of the tubule, (c) and (d) show a gradual tilting upward, and finally (e) shows standing up

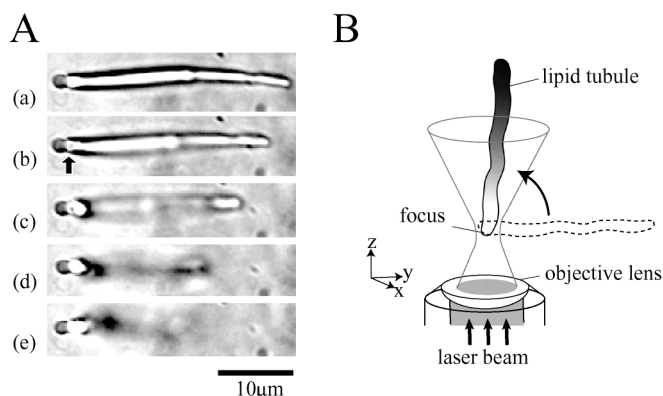


FIG. 1. Alignment of a lipid tubule along the optical axis. A: (a) A lipid tubule observed by light microscopy. (b)–(e) The lipid tubule stands up under laser illumination. The arrow shows the laser focus. The time interval between the images is 2 s. B: Schematic image of an optically trapped lipid tubule. The tubule stands along the  $z$  axis under stationary laser illumination.

parallel to the optical axis. A similar effect on the orientation of an ellipsoidal cell parallel to the optical axis has been reported [6] and was attributed to the profile of the attractive potential together with photon pressure.

Figure 2A shows the intensity profile of the “dual beams” observed by the beam analyzer, as represented by a cross section of a horizontal plane  $10\ \mu\text{m}$  above the focal plane. This split dual beam is obtained by tilting a parallel beam with a galvano mirror by about  $2 \times 10^{-5}$  rad toward the optic axis, which is located  $50\ \text{cm}$  from the focus [7]. With the dual beam, the lipid tubule can lie in either direction, i.e., the tubule orientation becomes bistable.

Figure 2B shows sequential images of the oscillatory motion of the optically trapped lipid tubule at laser power =  $50\ \text{mW}$ , where the “tail” of the oscillating tubule appears as a dark object. The “root” of the tubule is pinned on the focal position.

Figure 3A shows time traces of the brightness at a position  $5\ \mu\text{m}$  to the left of the focus at different laser powers. Figure 3B shows the change in frequency with a change in laser power, together with the apparent length of the tubule, or length of projection on the horizontal plane. When the laser power is below  $50\ \text{mW}$ , the tubule lies parallel to the glass plate in the micrograph. At  $50\ \text{mW}$ , the tubule stands up, as shown in Fig. 1A, in the direction of either of the dual beams (see Fig. 2A). Above  $50\ \text{mW}$ , the tubule exhibits spontaneous oscillating motion. The frequency of this oscillation increases almost linearly with an increase

in laser power. Above  $150\ \text{mW}$ , the periodicity becomes rather irregular, as shown in the top trace of Fig. 3A.

Let us briefly discuss the mechanism of optical trapping. Although the lipid tubule is larger than the wavelength of the laser, the actual substrate that scatters photons is thin phospholipid layers that are on the order of  $5\ \text{nm}$  thick. Thus, the optical force should be described in terms of an electromagnetic wave (Rayleigh region) instead of ray optics (Mie region) [8]. The potential  $U(\mathbf{r})$  exerted by the laser is

$$U(\mathbf{r}) \approx -\alpha \langle \mathbf{E}(\mathbf{r})^2 \rangle, \quad (1)$$

where  $\alpha$  is a positive constant. Based on this consideration, the beam intensity profile in Fig. 2A explains the bimodal energy profile for the direction of the tubule.

To examine the effect of heat generation due to photon absorption by the solvent  $\text{H}_2\text{O}$ , we performed an experiment in  $\text{D}_2\text{O}$  solution. It is well known that the absorption of photons in  $\text{D}_2\text{O}$  is almost negligible for a  $1064\ \text{nm}$  laser [9]. We found that the tubule is trapped in either direction without any oscillatory motion (data not shown).

Next, we tried to evaluate the spatial effect of local heating by the laser. We used the temperature-dependent phosphorescence intensity of the rare-earth chelate europium (III) thenoyltrifluoro-acetonate (Eu-TTA) [10]. At room temperature, the quantum efficiency of Eu-TTA phosphorescence declines rapidly with increasing temperature

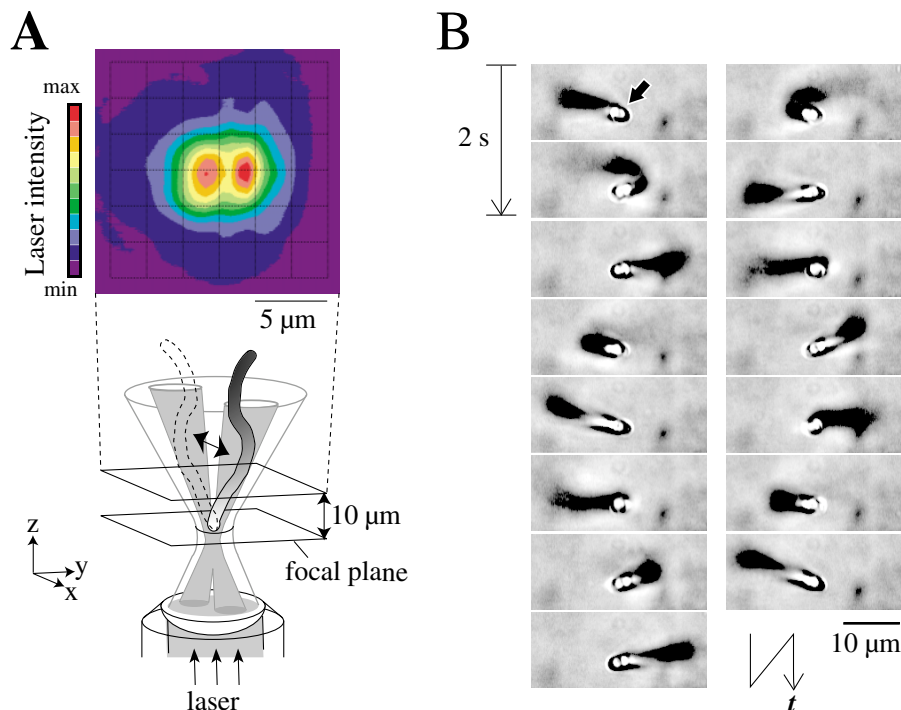


FIG. 2 (color). A: Upper: Spatial distribution of the laser intensity at  $10\ \mu\text{m}$  from the focal plane [7]. Lower: Schematic image of lipid tubule oscillation. The tubule oscillates between the two stable regions of maximum laser intensity. B: Sequential images of lipid tubule oscillation at a laser power of  $50\ \text{mW}$ . The interval between images is  $2\ \text{s}$ .

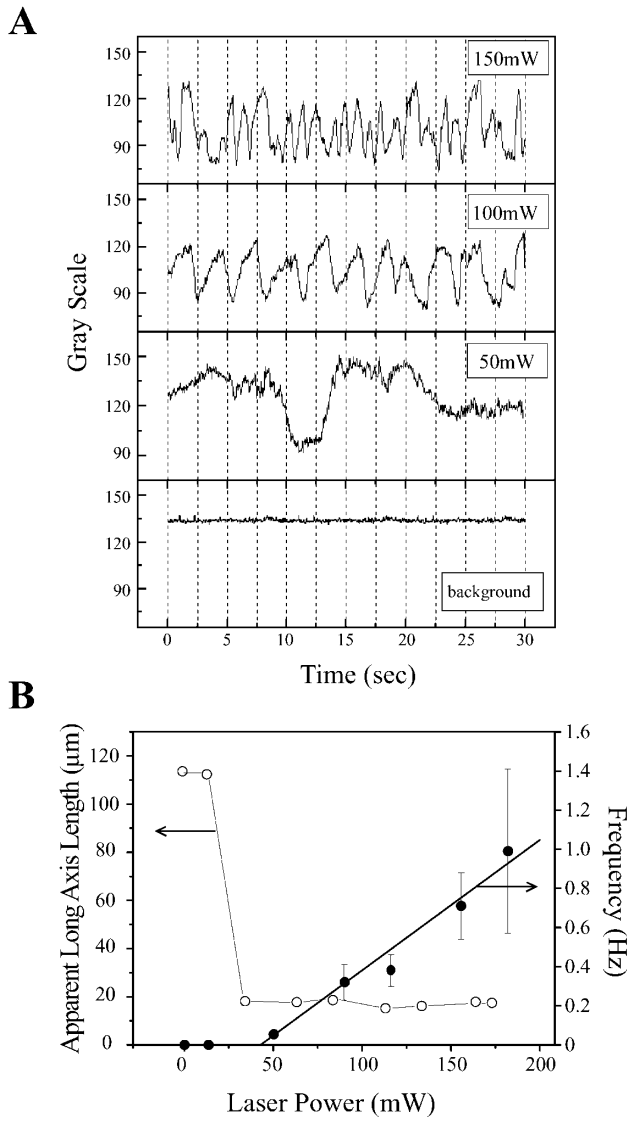


FIG. 3. Time series of tubule oscillation. A: Time series of the oscillation of an optically trapped lipid tubule. Graphs show time-course changes in optical intensity (8-bit gray scale). A low intensity reflects the presence of the tubule in the area. The period becomes shorter when the incident laser power increases. B: Dependence of the period of oscillation on laser power. With a low laser power ( $\sim 30$  mW), oscillation is not observed, while the regularity of oscillation is disrupted at a high power (150 mW).

because of competition with nonradiative processes and coupling through molecular vibrations between the electronic energy levels and the environment [11]. Figure 4A (red line) shows a cross section of the phosphorescence intensity of the liposome surface with Eu-TTA [12]. When the laser tweezers irradiated the liposome, phosphorescence declines near the focus. In this figure, the gray scale reflects phosphorescence intensity and the high-temperature region is depicted as dark. The temperature profile is unimodal, whereas the laser beam profile shows bimodality (green line). The “hot” region is distributed within a diameter of  $1.5 \pm 5 \mu\text{m}$  (at 50 mW) from the

laser focus center, and the size of this region increases with an increase in laser power (e.g.,  $4 \pm 5 \mu\text{m}$  in diameter at 150 mW).

The unimodality of the temperature profile is explained as follows. Considering the stationary illumination by the laser, the temperature ( $T$ ) profile is given by the usual diffusion equation,

$$D\nabla^2 T = -h\langle E^2 \rangle, \quad (2)$$

where  $h$  is a positive constant. The thermal diffusion constant  $D$  is  $0.6 \text{ W}/(\text{m} \cdot \text{K})$  in aqueous solution at room temperature, since the distance between the centers of the double-beam focal points is less than on the order of  $1 \mu\text{m}$ , for the region directly involved with the mechanism of motion (see Fig. 4B). The solution of Eq. (2), represented using a Green function, would give a unimodal profile on  $T$ , as shown experimentally.

Inhomogeneous temperature profile affects isotropic tension ( $F$ ) of the bilayer membrane as follows (at constant surface area) [13]:

$$dF = -KdT, \quad (3)$$

where  $K$  is a positive constant. Thus, the isotropic tension created within a tubular membrane is opposed by changes in temperature.

Summarizing the above results, the mechanism of tubule oscillation is schematically shown in Fig. 4B. First, a lipid tubule is trapped in either direction due to the bistable attractive potential. The tubule is then locally heated by photon absorption, as depicted in Fig. 4A. The resulting temperature gradient around the tubule loses tension on the proximal side of the tubule. The imbalance in tension between the proximal and distal sides drives the tubule toward the other side of the bistable potential. A similar effect causes the tubule to swing back in an autonomous manner. Then, let us try to graph the essence of rhythmic phenomena in terms of nonlinear dynamical system. As explained earlier, the orientation of the tubule can be described by a bistable potential profile. By taking the tilting angle  $\theta$  from the perpendicular axis,

$$U(\mathbf{r}) \approx (\theta - \beta)^2(\theta + \beta)^2. \quad (4)$$

When the tubule tilts in a certain direction, it experiences stress  $\tau$ , the tension differential between the proximal and distal sides of the trapped tubule, due to the temperature gradient deduced from Eq. (3). This explains the destabilizing effect on the stationary positioning of the tubule. From consideration above, the simplest model of the oscillatory orientation of the tubule is given as

$$\begin{aligned} \frac{\partial \theta}{\partial t} &= -A(I) \frac{\partial U}{\partial \theta} + B\tau, \\ \frac{\partial \tau}{\partial t} &= -C(I)\theta, \end{aligned} \quad (5)$$

where  $A(I)$  and  $C(I)$  are approximately linear functions of laser intensity  $I$ .  $B$  is a positive constant. The temperature

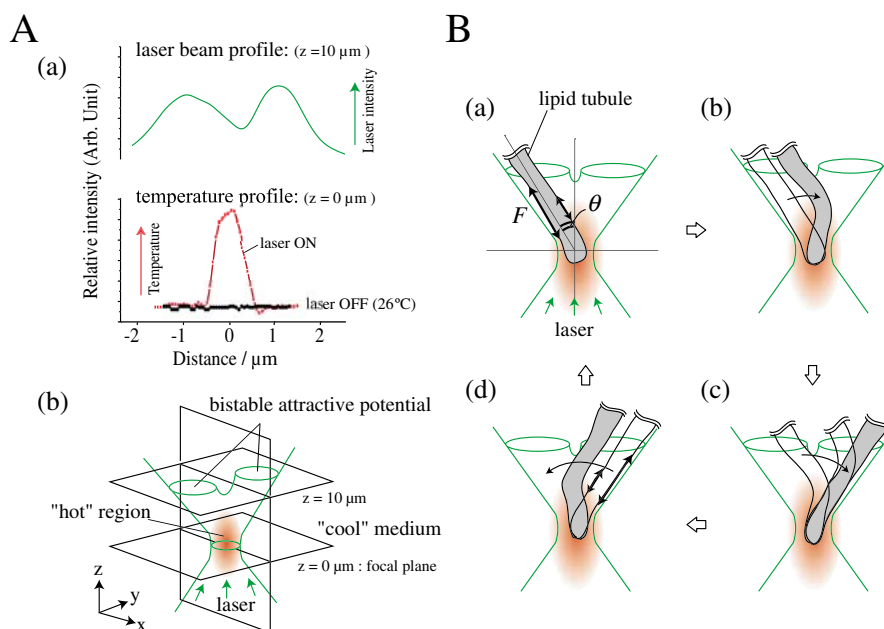


FIG. 4 (color). A: (a) Spatial distribution of temperature under a bimodal intensity profile deduced using the Eu-complex method [12]. The laser power was 50 mW. (b) Schematic illustration of the system. B: Scheme of an optically trapped lipid tubule oscillation. (a) First, the tubule is trapped in either direction due to the bistable attractive potential, with the tilting angle  $\theta$ . The tubule is then locally heated as depicted in A. The resulting temperature gradient around the tubule loses tension ( $F$ ) on the proximal side of the tubule. (b),(c) The imbalance in tension between the proximal and distal sides drives the tubule toward the other side of the bistable potential. After transient swinging, the distal side cools (d), and the cycle repeats.

is considered as a hidden variable [14]. This system clearly exhibits a stable oscillation, i.e., a limit cycle, consistent with the present observation.

Recently, we have shown that single giant DNA molecules exhibit a rhythmic change in conformation under thermodynamically open conditions with the dissipation of absorbed energy into the environment [3]. That model and the system described here demonstrate that limit-cycle motion is driven by a mechanism involving the accumulation and dissipation of energy. A next target would be to construct an isothermal motor driven by a difference in chemical potential. Studies along these lines should shed light on the essence of microscale biological motion.

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