Torque Induced on Lipid Microtubules with Optical Tweezers

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Abstract. Chiral Phospholipids are found self-assembled into cylindrical tubules of 500 nm in diameter by helical winding of bilayer stripes under cooling in ethanol and water solution. Theoretical prediction and experimental evidence reported so far confirmed the modulated tilt direction in a helical striped pattern of the tubules. This molecular orientation morphology results in optically birefringent tubules. We investigate an individual lipid microtubule under a single optical trap of 532 nm linearly polarized laser. Spontaneous rotation of a lipid tubule induced by radiation torque was observed with only one sense of rotation caused by chirality of a lipid tubule. Rotation discontinued once the high refractive index axis of a lipid tubule aligned with a polarization axis of the laser. We further explored a lipid tubule under circularly polarized optical trap. It was found that a lipid tubule was continuously rotated confirming the tubule birefringent property. We modified the shape of optical trap by cylindrical lens obtaining an elliptical profile optical trap. A lipid tubule can be aligned along the elongated length of optical trap. We reported an investigation of competition between polarized light torque on a birefringent lipid tubule versus torque from intensity gradient of an elongated optical trap.

1. Introduction
Self-assembly of lipids generates a varieties of three-dimensional morphologies such as micelles, vesicles and tubules which may be used to encapsulate and carry drugs or molecules of various sizes. These aggregated-lipid structures are formed depending on temperature, molecular composition and additives. A number of applications of these morphologies have been explored extensively due to their biodegradable and biocompatible properties. Since 1984, lipid tubules formed by self assemble of phospholipids composed of diacetylene components[1] have attracted considerable attention due to their unique structures of hollow cylinders with opened ends, assembled by rolling up bilayer films. Recent development on the applications of inner nanospace and outer space of lipid tubules have been reviewed comprehensively including encapsulation of metal ions or molecules, their ability of controlled release and hierarchical structuring of nanotubes[2]. Fabricating ordered array of well-aligned lipid tubules is a significant development in integrating them with novel devices. Several techniques have been demonstrated to achieve these arrangements. A few techniques to obtain lipid tubule ordered arrays implementing patterned substrate and microfluidics were presented by Fang [3] including the use of microfluidic network and liquid crystal fiber with an advantage of being a single-step process. An alternative for ordering these lipid tubules can be achieved with light by means of optical tweezers. Here we present the use of optical tweezers to align lipid tubule in various condition of light.

Optical tweezers have been employed as a powerful tool to manipulate microscopic objects without any direct contact. Force due to radiation pressure of focused laser causes motion of transparent dielectric medium[4] and dielectric micron sized particles[5]. A tightly focused laser beam, typically...
through high numerical aperture microscope objective, incident on a microscopic particle can trap the particle at the focal point. Translation and positioning of a particle are possible as a result of the electric field intensity gradient of a laser. This force is generated from the transfer of momentum from light to a particle under the law of momentum conservation. Optical tweezers applications have been demonstrated in a wide variety of fields especially in biological systems such as living cells[6], organelle[7] and DNA[8].

Other than translation of microscopic objects, optical tweezers can also rotate these objects if they are optically active. In recent years, it was discovered that torque on a transparent birefringent particle can be induced under linearly polarized laser tweezers and the alignment of object along the polarization plane is possible[9, 10]. Polarized light changes its state of polarization from linear to circular or elliptical states once light passes through a birefringent object given that neither fast nor slow axis of an object lies along polarization direction. Conservation of angular momentum of the system plays a role in rotation of an object. Continuous rotation was also investigated on an optically trapped birefringent object under circularly or elliptically polarized laser[9, 10]. Clockwise or counterclockwise rotation can be controlled by changing handedness of circularly polarized light. These rotations result from transfer of spin angular momentum from light to an object.

2. Material and Method

Lipid tubules were prepared by controlled cooling of R-enantiomer 1,2-bis-(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine (DC8,9PC) (Avanti Polar Lipids, Alabaster, AL) at a concentration of 5 mg/ml in ethanol/water (70:30, v:v). The mixture was heated with active stirring to 60 °C to dissolve all lipids, and was allowed to cool down to 25 °C at a rate 0.5 °C per minute. Lipid tubules were obtained suspended in ethanol and water with diameter 0.5 - 1 μm and their length varies from 10 to 100 μm. DC(8,9)PC tubule lengths depend upon the solvent composition[15] and upon the cooling rate[16] during the formation process. 254 nm-UV-polymerization was performed on lipid tubule suspension for 20 minutes at room temperature to strengthen their structure. An AFM image in Figure 1 shows bundles of lipid tubules after drying in air for a few minutes. The image revealed helical winding of lipid bilayer stripes forming hollow cylindrical structure as discussed in[17]. For Optical tweezers study, lipid tubules were dispersed in deionized water and shaken to ensure even distribution. The suspension was filled between two microscope glass cover slips with fixed spacers of 10 μm separating them. The sample was then inserted into microscope translation stage for optical trapping experiment.

Our optical Tweezers setup is illustrated in Figure 2. An upright microscope (Nikon LV100) was used with laser trapping path directed in through an additional objective lens underneath the sample. A 532 nm DPSS laser (power adjustable upto 1W) was used as an optical tweezers light source. The beam passed through Glan Thompson polarizer first to obtain completely polarized light. A half waveplate was used to rotate the direction of linear polarization. To change light from linearly polarized light to circularly polarized light, a quarter waveplate was inserted in place of a half waveplate for continuous rotation of the polarization of light. Two spherical lenses were used as a beam expander to match the beam size to back aperture of an objective lens. This spherical beam expander could be replaced by...
two cylindrical lenses to generate a line optical trap. In our experimental setup (Figure 2), the laser beam was directed to eyepieces in transmission mode and to a CCD camera. A 532 nm notch filter was used in the beam path to reduce laser intensity, however, the beam was still quite intense. To be able to observe lipid tubules behavior, the experiment was done by blocking off the beam at a certain frequency so that lipid tubule images can be recorded on the CCD camera. The experiment was divided into two parts: optical trap with circular profile beam and optical trap with line profile beam. Lipid tubule motion under these optical traps was examined.

![AFM image of lipid tubule formed by controlled cooling of DC8,9PC in ethanol and water.](image1)

**Figure 1** AFM image of lipid tubule formed by controlled cooling of DC8,9PC in ethanol and water.

![Experimental setup of an optical tweezers system. The inset illustrates cylindrical lens set for replacing spherical lens set to obtain line profile laser beam.](image2)

**Figure 2** Experimental setup of an optical tweezers system. The inset illustrates cylindrical lens set for replacing spherical lens set to obtain line profile laser beam.
3. Result and discussion

3.1 Optical Trap with circular profile beam

Circular Gaussian profile optical trap was created with 532 nm laser (10 mW) directed to 100X N.A. Plan Fluor objective lens underneath the microscope. The beam was polarized by a Glan Thompson polarizer and its polarization direction was controlled by a half waveplate. It was possible to trap and rotate an individual lipid tubule under this optical trap as shown in Figure 3. The lipid tubule rotation continued until the long axis of a tubule aligned with polarization direction of laser. Thus, it was possible to control an orientation of a lipid tubule with polarized light. Rotation of lipid tubule under different states of laser polarization is shown in Figure 3: for polarization making an angle 45° to x-axis (Figure 3(a)) and -45° to x-axis (Figure 3(b)). Lipid tubules experienced clockwise rotation for both cases. We investigated further for the sense of rotation of lipid tubules and found that in all attempts (~ 100 repeats) lipid tubule underwent clockwise rotation. This clockwise rotation is due to the chirality of lipid tubules. These tubules were self assembled from R-enantiomers and, thus, absorbing left circularly polarized light. Their optically active property was evidenced from circular dichroism (CD) study of lipid tubules as previously reported. [18, 19] Our lipid tubules studied showed positive CD signal [20] implying that plane polarized light passing through lipid tubules will emerge with its polarization rotating counterclockwise viewing toward the light source.[21] Angular momentum of the system needs to be conserved resulting in rotation of lipid tubules in opposite direction to emerging light, giving total angular momentum of system to be zero similar to that of incident light.

Additionally, we examined these lipid tubules for their birefringence behaviour by putting them under circularly polarized light. The circularly polarized light was created by replacing half waveplate in the beam path with quarter waveplate and setting polarization direction of incident laser to 45° with respect to quarter waveplate fast axis. We can create both right circularly polarized light and left circularly polarized light. It was found that the sense of rotation of lipid tubules followed the sense of rotation of polarization of light confirming the birefringent property of lipid tubules. The experiment was first demonstrated and described by Beth[22] on rotation of suspended birefringent quartz waveplate due to changing of polarization state of light. A few reports demonstrated rotation due to spin angular momentum of light on fabricated birefringent particles using optical tweezers, e.g., in [9, 10].

As stated by Friese et al. [9], when light with specified polarization state incident onto birefringent material of thickness d, reaction torque per unit area can be written as

$$\tau = -\frac{\mu}{2\omega} E^2 \sin(kd(n_o - n_e)) \cos(\phi) \sin(2\theta) + \frac{\mu}{2\omega} E^2 \left(1 - \cos(kd(n_o - n_e))\right) \sin(2\phi)$$

with $\theta$ as the angle of linearly polarized light direction with respect to the fast axis of birefringent material and $\phi$ as the degree of ellipticity of light: $\phi = 0$ and $\phi = \pi/4$ represent linearly polarized light and circularly polarized light respectively. The first term is torque resulted from linearly polarized light and the second term is caused from circularly polarized light with detailed explanation explained in [9]. We further examined another aspect on alignment of self assembled lipid tubules by distorting the beam shape of laser in optical tweezers.
3.2 Optical Trap with line profile beam

The beam profile was altered by two cylindrical lenses set in place of spherical beam expander as shown in Figure 2. Elliptical beam imitating a line profile was obtained as shown in Figure 4(a) with the length of semi-major axis $\approx 28 \mu m$ and the length of semi-minor axis $\approx 7 \mu m$. Polarization direction of the beam was controlled by half wave-plate in the setup of Figure 2. $\beta$ is denoted to specify the angle between polarization direction and semi-major axis of the elliptical beam. The beam is applied to a lipid tubule with its initial position shown in the left most image of Figure 4(b). Lipid tubule rotation was recorded while under optical trap from its initial position ($t=0$ s) until the tubule aligned with the long axis of the beam at time specified in the last image (Figure 4(b)). However, it was difficult to detect angle of a tubule while under optical trap due to high laser intensity. Thus, beam blocking is applied at a certain interval to detect tubule direction until it aligned with semi-major axis of the beam. Angular velocity was investigated for different angle $\beta$. We are interested in simultaneous effect of torque due to polarization of laser and torque due to elliptical potential well of laser intensity. The initial position of lipid tubule is at angle $\alpha \approx 30^\circ$-$40^\circ$ with respect to the semi-major axis of the elliptical beam. The angular velocity was examined for both counterclockwise (Figure 4(b)) and clockwise (Figure 4(c)) rotations by varying $\beta$ from 0$^\circ$ to 90$^\circ$ with 15$^\circ$ step. The result is illustrated in the plot of Figure 5. The overall trend of the plot demonstrated clearly that clockwise rotation is faster than counterclockwise rotation. The result intensifies the observation above when all rotations observed for alignment of lipid tubules along polarization under circular beam were clockwise. This effect due to chirality accelerates the clockwise rotation and decelerates counterclockwise rotation under elliptical beam profile. It is clearly evidenced from the plot that at 15$^\circ$ a quick drop in angular velocity appeared for both clockwise and counterclockwise rotation. This can be interpreted that when tubule rotate until it reaches 15$^\circ$ where laser polarization is, it falls into a potential well of the polarization which is comparable to the potential well of laser intensity profile. These two forces were competing, however, in the end lipid tubule slowly rotated toward the semi-major axis of the elliptical beam thus the final images in Figure4(b) and Figure4(c) were obtained. For $\beta$ other than 15$^\circ$, the potential well of laser polarization is easily overcome due to the force from laser intensity along the long axis of elliptical beam profile.

Figure 3 Rotation of a lipid tubule under linearly polarized laser with (a) polarization at an angle $+45^\circ$ to the x-axis and (b) polarization at an angle $-45^\circ$ to the x-axis.
Figure 4 (a) Schematic drawing of elliptical beam profile with its polarization set at an angle $\beta$ with respect to long axis of the beam. This beam was employed to rotate a lipid tubule undergoing (b) counterclockwise rotation and (c) clockwise rotation for $\beta = 15^\circ$.

Figure 5 Plot of angular velocity of a lipid tubule rotating counterclockwise (CCW) and clockwise (CW) under optical trap versus angle $\beta$ (Figure 4(a)) between long axis of line profile and laser polarization direction.

4. Conclusion
We found that under linearly polarized optical trap, lipid tubule will continue its rotation until its long axis aligned with the polarization direction. The rotation only occurs in clockwise direction due to chirality effect. By applying circularly polarized light, lipid tubule will continue its rotation in the same sense as the handedness of circularly polarized light. Further investigation on line optical trap showed that it was possible to rotate lipid tubule to align along the long axis of the line profile. The competition between potential well of polarization trap and potential well of laser intensity profile trap on a lipid tubule was studied showing that laser intensity profile has stronger influence on aligning the
lipid tubule. Additionally, the tubule chirality also provides noticeable effect on angular velocity showing clockwise rotation faster than counterclockwise rotation. Further understanding of all these aspects regarding optical torque of lipid tubules will allow their expansion for future applications.

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6. References

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