# Dynamic Alignment and Millimeter-scale Vortex Formation of Microtubules Driven by Different Types of Dynein

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## ABSTRACT

Experimental systems have long been demanded for the study of collective motion often observed in biology (a flock of birds, a shoal of fish, cell migrations during development etc). *In vitro* motility assays commonly used in biophysical studies on protein-motors now fulfill the demand described above. Using the *in vitro* motility assays, we report collective motion and vortex emergence of microtubules (MTs) driven by some subspecies of axonemal dyneins and find that under some experimental conditions, the collective motion of MTs can display nematic order, millimeter-scale meandering streams or millimeter-scale vortices. To explore the conditions causing such phase-shifts, we examine the effects of mechanical properties of dyneins on the pattern formation.

## Categories and Subject Descriptors

A.0 [***Conference Proceedings***]

## General Terms

Measurement, Design, Experimentation

## Keywords

*In vitro* motility assay, dynein, microtubule, self-organization, nematic interaction

## 1. INTRODUCTION

*In vitro* motility assays, in which individual fluorescently-labeled protein filaments are observed moving on protein motor-coated surface, are simple but versatile assays for studies of protein motor's functions [1-5]. Thus, the assays have contributed to the progress in our understanding of protein motors function. Recently the motility assays have been used as a powerful tool for studies on ensemble behavior of self-propelled particles[6-8]. Advantages of the motility assays are: interaction of individual protein filaments can be precisely characterized and ensemble behavior of a large number of filaments can be observed. Therefore, these *in vitro* motility assays meet demands for the study of collective motion often observed in biology (flocking birds, migrating cells during development, etc). We have expanded the *in vitro* motility assays to the studies of collective motion and found that under various experimental conditions, the collective motion of MTs can display nematic order, millimeter-scale meandering streams or millimeter-scale vortices. To explore the conditions causing such phases, we use different types of dynein (dynein c and g of *Chlamydomonas* flagella), which have distinct mechanical properties and examined the effects of these properties on time course and end-point patterns of MTs.

## 2. MATERIALS AND METHODS

### 2.1 Protein Preparation

The materials we used are inner arm dynein subspecies c and g of *Chlamydomonas* flagella, which are single-headed dynein composed of one heavy chain and two types of light chains, actin and p28 (dyein c) or centrin (dynein g). The purification procedure is described shortly below. Flagella of outer-arm-less mutant, *oda1*, of *Chlamydomonas reinhardtii* (strain 137c) were detached with dibucaine treatment and collected with serial centrifugation. After demembranation of axonemes, crude dynein was extracted with 0.6 M KCl. Highly purified inner-arm dyneins were obtained by two-step anion exchange chromatography using Mono Q and Mini Q columns (both from GE Healthcare) with 120–500 mM KCl gradient in HMDE buffer (30 mM HEPES, 5 mM $MgSO_4$, 1 mM DTT, and 1 mM EGTA, pH 7.4 adjusted with KOH). Since these two columns have distinct chromatographic properties due to different column carriers, contaminants were removed effectively.

Tubulin was obtained from porcine brains. It was purified with two cycles of polymerization and depolymerization followed by phosphocellulose P11 column chromatography, and then stored at –80°C. The procedure of fluorescence labeling of tubulin was described elsewhere [8]. MTs were polymerized from the tubulin stock solution (as the mixture of fluorescent and non-fluorescent tubulin) upon supplementation with 2 mM GTP and incubation for 30 minutes at 37°C. Microtubules were diluted in HMDE buffer containing 20 µM paclitaxel at 25°C.

### 2.2 *In Vitro* Motility Assays

The *in vitro* motility assays were performed as described [8]. Shortly, a flow cell was made of a glass slide (72mm × 18mm) and a cover slip (18 mm × 18 mm) cleaned with non-ionic detergent and rinsed with de-ionized water. Two slivers of polycarbonate film with 50µm in thickness were used as spacers of the flow cell (*ca.* 10 µL in volume). the dynein samples were diluted with 0.5 mg/ml BSA in HMDE buffer. The dynein solutions were kept on ice for 30 minutes and then perfused into the flow cells and let stand for 5 minutes. Then, 0.5 mg/ml BSA

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in HMDE buffer was perfused. Two minutes later, a solution for motility assay (1 mM ATP, 25 µg/ml pyruvate kinase, 1.25 mM PEP, 10 µM paclitaxel, and ca. 10 µg/ml microtubules (MTs) in HMDE, pH 7.4 adjusted with KOH) was perfused. After 3 minutes, MT translocation was recorded with a cooled-CCD camera using a fluorescence microscope.

# 3. RESULTS

## 3.1 MT Sliding on Dynein-coated Surface

These purified dyneins adsorbed on a glass surface at densities higher than 1000 molecules/µm$^2$ were capable of moving MTs on glass surface at velocities of ca. 6 µm/sec (for dynein-g) and ca. 12 µm/sec (for dynein-c) in the presence of 1 mM Mg-ATP at 23 ˚C. Microtubules with length of 24 ± 12 µm (n = 95) did not move in the absence of Mg-ATP but bound to dynein-coated glass surface being slightly aligned by flow. Upon the addition of 1 mM Mg-ATP, MTs started moving smoothly. Even at the low surface densities of MTs, they sometimes collided each other during their sliding movement. At their collisions, MT behavior after the collision depended upon the colliding angles.

## 3.2 Colliding MTs Show nematic Alignment

At the high colliding angles, an MT colliding against the other at its front end often climbed up the other. In the other hand, at low colliding angles, MTs often aligned each other. These interactions between MTs lead to nematic alignment. When the surface densities of MTs increased, the frequency of the colliding increased. Under such circumstances, within a few minutes, streams of moving MTs spontaneously appeared, which showed meandering motions with a very large distance (longer than 400 µm) compared to the microtubule's length and size of a dynein molecule. The number of MTs in a stream increased over time (>1 µm in width and >300 µm in length). Finally, these MT streams grew into vortices within tens of minutes.

## 3.3 Emergence of arrays of MT vortices

Now, we increased the density of MTs on the dynein-coated surface five to ten times as high as in conventional assay and observed wider area than that in conventional assays for longer time. Within a few minutes, streams of moving MTs spontaneously appeared. The number of microtubules in a stream increased over time (>1 µm in width and >300 µm in length).

Finally, these MT streams grew into vortices within tens of minutes. For the surface coated with axonemal dyneins c, the vortices emerged within 10min after addition of ATP (**Figure 1**) while the surface coated dynein g, it took for 10 min or longer to form the vortices. In both cases, the vortices formed centimeter-scale 2D-arrays over the whole surface of the flow cell. The mean diameter of the vortices formed on dynein-c-coated and dynein-g-coated surfaces were 443 ± 64 µm and 398 ± 66 µm (N=209) respectively.

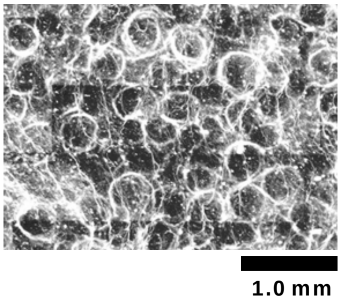

**Figure 1. MT vortex array on dynein-c coated surface**

# 4. SUMMARY

We proposed a model to explain the result in our previous paper [8]. The model predicts that non-white noise trajectories and nematic interactions of self-propelled particles can create the large-scale ordered structures. Motion of lonely MT without collision show persistent curving motion which was confirmed in the case of dynein-c and dynein-g. The curvature of the trajectory shows correlation with length constant. Though the nematic interaction is indispensable for pattern formation, the numerical model suggests the length constant is another key of ensemble behavior of MTs. The variations of time courses of vortex formation and the size of vortices found in different dyneins suggest that the correlation might be derived from the properties of dyneins. Assignment of mechanical properties of dynein into two parameters, correlation distance/time of trajectory curvature and distribution of trajectory curvature awaits further studies.

# 5. ACKNOWLEDGMENTS

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