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Supplementary Materials for

Adaptation of Patterns of Motile Filaments

under Dynamic Boundary Conditions

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This supplementary information file includes:

Supporting discussion, References

Figs. S1 to S19

Movie legends for Movies S1 to S8

Other Supporting Online Material for this manuscript includes the following:

Movies S1 to S8

Supplementary Discussion

Vicsek model-based simulation (microtubule/microtubule interaction, without stretching):

The importance of the nematic interaction between microtubules in the generation of patterns upon application of stretching is supported by a simulation based on the Vicsek model (Figures S6, S7). To understand the mechanism behind the enhancement of stretch induced orientation of microtubules forming streaming flow, we simulated the time-dependent change of alignment of gliding microtubules forming streaming flow in the presence of a certain number of pre-oriented microtubules toward perpendicular direction against horizontal axis without stretching. To simply understand the role of nematic interaction between microtubules in their orientation in the presence of applied pre-oriented microtubules which might bias global orientation direction of microtubules, we did not apply stretching to microtubules in this simulation. The simulation was done on an updated version of our real-time simulation system implementing the Vicsek model.¹ In our simulation, microtubules are represented by a chain of particles connected by a spring force for longitudinal tension and compression, along with a bending restoring force. Then, all microtubule segment particles within the system are under the effect of a Lennard-Jones like potential for repulsive particle-particle interactions. Motor proteins were represented by motor shaped particles randomly distributed over the surface of rectangular simulation area representing an elastomer substrate in wet experiments.

In the simulation, 6 million particles are used to represent 40,000 microtubules ranging from 140 to 160 particles per microtubule, and 4.06 million motor objects are used. Each particle represents about 140 nm of the microtubule length resulting in an average microtubule length of 21 μm , which is almost equal to the experimental value. Each motor object represents the combined effort of 60 motor proteins. The resulting simulation densities are 0.063 microtubules and 400 motor proteins per square micrometre, which correspond to the wet experiment. Each simulation was carried out for 4 h. After initiating movement of microtubules with a velocity of 110 nm s^{-1} , streaming flows of

microtubules emerged in the simulation. The elasticity of the microtubule in our model is represented by a bending restoring force which is defined by the force which pulls the middle of three consecutive particles in the line with the outer two particles. An elasticity coefficient E is introduced to adjust the magnitude of the restoring force where $E = 0$ means no bending restoring force and $E = 1$ means solid-rod like microtubules. Above $E = 0.3$, simulated microtubules formed the streaming flow observed in the wet experiment. To more precisely set the E value, we calculated the path persistence length (L_{path}) of the microtubule trajectory, which is a measure of the straightness of movement of microtubules, using the following equation to quantitatively compare our model to wet experiment findings²

$$\text{Cos}(\theta_{t+\Delta t} - \theta_t) = \exp(-\Delta t/t_p)$$

where $(\theta_{t+\Delta t} - \theta_t)$ represents the time course of the moving direction of microtubules from t sec to the time after a time interval of Δt sec and t_p is the time which gives the L_{path} . The L_{path} was calculated by multiplying the t_p with the time average velocity of microtubules. At $E = 0.7$ gave L_{path} of 27.5 ± 3.5 μm and 410 ± 30 μm with dense and sparse microtubule tests, respectively. The sparse test matches closely with the L_{path} in the wet experiment demonstrated at the sparse condition of microtubules on a kinesin coated surface (386 ± 80.7 μm), which is also close to that of microtubules in the Brownian dynamic simulation (320 ± 37 μm). The L_{path} was estimated by using 250 frames (125,000 data points) selected from a 2 hours simulation using the position of microtubule head segments tracked every 30 seconds.

In the Vicsek model-based simulation, a small fraction of oriented microtubules in a streaming flow was shown to influence all gliding microtubules and determine the direction of global alignment (Figures S7a to 7d, Movie S7). The role of this small number of entities is important for maintaining the alignment of microtubules through self-organization.³ With increase in the number of pre-oriented microtubules, the global orientation of microtubules was enhanced, which is corresponding to the *in vitro* experiment to demonstrate microtubules orientation at different size of stretching strains (Figure

S4). The simulation also suggested that there is a critical number of pre-oriented microtubules to cause amplification of microtubule orientation (20% of total number of simulated microtubules). Probably, this critical number of oriented microtubules is close to the one at stretching strain of 20% which is the critical stretching strain since microtubules were never reorganized at this strain. Although single-step stretching below critical strain can't exceed the critical number of microtubules, the application of such a small stretching in a cyclic manner may accumulate small change of orientation and eventually be able to increase the number of oriented microtubules above the critical value. Since the nematic interaction between microtubules can be observed in microtubules forming zigzag pattern (Figure S12), it may also catalyse zigzag pattern formation. In fact, in the absence of the nematic interaction at low density of microtubules below the density to form streaming flow, the orientation of microtubules with characteristic orientation angles were not clearly observed in *in vitro* experiment although there were small peaks (Figure S13). Thus, stretch induced pattern formation is strongly maintained by collective behaviour of entities in active system.

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Supplementary Figures

Figure S1. Experimental setup for applying mechanical stretching stimuli to streaming flows of gliding microtubules. (a) Design of the newly developed stretch chamber. The chamber is equipped with two computer-controlled stepping motors (SGSP-13ACT-B0; Sigma-Koki) to apply stretching stimuli on a silicone elastomer substrate along the horizontal direction. The adjusting screw installed on the top of the chamber allows controlling the focus of the field of view under a fluorescence

microscope. The stretch chamber enables us to perform experiments maintaining an inert atmosphere around the specimen by passing humid nitrogen gas. The flow rate of the gas was controllable by using two ball valves fixed at the inlet and outlet of the chamber. (b) Image of the stretch chamber used to apply stretching stimuli to microtubules. Two stepping motors were installed to control stretch strain and strain rate using a computer program. Scale bar: 25 mm. (c) Image of movable frame of the stretch chamber where the silicone elastomer substrate was fixed. Scale bar: 10 mm. (d) (i) A schematic diagram showing the application of the sample to the substrate fixed to the movable frame of the stretch chamber and (ii) the way to initiate microtubule movement by applying ATP buffer.

Figure S2. Visualization of change in orientation of individual microtubules before and after single-step stretching. To visualize individual microtubules, a small number of green fluorophore-labelled microtubules were mixed with microtubules labelled with red fluorophore. It was observed that just after stopping stretching, a limited number of microtubules were oriented toward perpendicular direction against stretch axis although most of the microtubules maintained their original alignment. Scale bar: 25 μm . White arrows show representative microtubules orienting toward perpendicular direction against stretch axis just after stopping stretching. Stretching strains: $\epsilon = 75\%$, stretching strain rate: $\dot{\epsilon} = 0.012 \text{ s}^{-1}$. After 120 min of stretching most of the microtubules were found oriented to a direction perpendicular to the stretch axis.

Figure S3. Disappearance of the perpendicular orientation of microtubules against the stretch axis at low density of microtubules. Fluorescence microscopy images showing the orientation of microtubules at low-density condition ($\rho = 5 \times 10^4$ filaments per mm^2) just after the application of uniaxial single step stretching at $\epsilon = 75\%$ and $\dot{\epsilon} = 0.012 \text{ s}^{-1}$ (left) and 1 min after the stretching (right). Scale bar: $10 \text{ }\mu\text{m}$.

Figure S4. Analysis of the orientation of microtubules over time for different uniaxial stretching strains and strain rates. (a) The time course of the nematic order parameter, S for different stretching strains, $\epsilon = 10\%, 20\%, 30\%, 50\%$ and 75% , at a stretching strain rate of $\dot{\epsilon} = 0.012 \text{ s}^{-1}$. At stretching strains of $\epsilon = 30\%, 50\%$ and 75% , microtubules did not show preferential orientation just after application of stretching, but soon they are gradually organized into perpendicular orientation against the stretch axis. At the strains of $\epsilon = 10\%$ and 20% , the microtubules did not respond to the applied stretching stimuli. (b) The S of the microtubules was measured for five different stretching strains at 120 min after application of stretching. Here ϵ_c indicates the critical stretching strain which is required to reorganize the streaming flow of microtubules under single-step stretching stimulus. The strain rate was fixed at 0.012 s^{-1} in these experiments. (c) Time course of S at three different strain rates, $\dot{\epsilon} = 0.012 \text{ s}^{-1}, 0.04 \text{ s}^{-1}$ and 0.2 s^{-1} ; the stretching strain was fixed at $\epsilon = 20\%$ in these three experiments.

Figure S5. Orientation of microtubules at lower density upon single-step stretching of the substrate.

(a) Fluorescence microscopy images of microtubules before and after application of single-step stretching at stretching strain of 130% and strain rate of 0.006 s^{-1} . Scale bar: $10 \mu\text{m}$. (b) The S of microtubules at different stretching strain (0, 10, 20, 30, 50, 100 and 130%). The upper schematic diagram is showing the orientation of microtubules under low and high stretching strains. The red lines indicate perpendicularly oriented microtubules against stretching axis (horizontal axis) and the purple ones represent non-oriented microtubules. With increase in the strain, the number of perpendicularly oriented microtubules increased. The images and the figure were adapted from our previous paper with modification².

Figure S6. Streaming flow formation by microtubules in Vicsek model-based simulation at different rigidity of microtubules. A range of tests are performed using spherical linear interpolation values ranging from $E=0.1$ to 0.8.

Figure S7. Amplification of microtubule orientation by the nematic interaction between microtubules. (a) The emergence of streaming flow of microtubules in the Vicsek model-based simulation from a random state. (b) Reorganization of streaming flows in the presence of pre-oriented microtubules, which is mimicking perpendicularly oriented microtubules in response to stretching in *in vitro* experiments (Note: In this simulation, stretching was not applied to microtubules. We reproduced the microtubule alignment just after stopping stretching.). At the beginning of the simulation, 60% of the microtubules (green) were randomly oriented and the remaining 40% of microtubules (red) were pre-oriented perpendicular to the horizontal direction. The merged state of these microtubules is shown by the bottom images. (c) Time course of the nematic order parameter, S , of randomly aligned microtubules (green colour filaments in Figure S7a, b) for different percentages of pre-oriented microtubules. (d) The order parameter S at steady state after 90 min of initiation of the simulation for different population of pre-oriented microtubules. The global orientation of microtubules was increased remarkably by a small number of pre-oriented microtubules, when the percentage of pre-oriented microtubules was above 20% of the population.

Figure S8. Microtubules gliding on a kinesin coated glass substrate under shear flow. (a) Schematic diagram of the system used to apply shear flow on gliding microtubules. The flow was applied to microtubules by moving the glass substrate back and forth, fixed to the movable frame of the stretch chamber, as done during the application of single-step stretching at the strain of 75% and strain rate of $\dot{\epsilon} = 0.012 \text{ s}^{-1}$. (b) Comparison of nematic order parameter, S between before and after application of the flow. The S was measured after 120 min of initiation of motility and 120 min of application of the flow which is sufficient time for microtubules to reach a steady state. Student t-test; $p > 0.05$.

Figure S9. Correlation between stretching frequency and time evolution of the reorganization of microtubule streaming flow under cyclic stretching. (a-d) (i) Fluorescence microscopy images showing a change in the orientation of microtubules with time at different stretch frequencies of 0.03 Hz, 0.1 Hz, 0.5 Hz and 1.0 Hz which correspond to strain rates of $\dot{\epsilon} = 0.012 \text{ s}^{-1}$, 0.04 s^{-1} , 0.2 s^{-1} and 0.4 s^{-1} , respectively. In these experiments, the stretching strain was fixed at $\epsilon = 20\%$. Scale bars: 50 μm . (ii) Circular histograms showing the distribution of the orientation angle of microtubules after the application of cyclic stretching stimuli at four different stretch frequencies.

Figure S10. Half wavelength of zigzag tracks at low and high density conditions of microtubules. The wavelength was quantified by measuring the distance from one bending point to the next bending point of the zigzag tracks. The measurement was done at both the high and low density region of microtubules.

Figure S11. Comparison of distribution of dense microtubules region between before (red) and after 30 min of cyclic stretching at 0.5 Hz, $\epsilon = 20\%$ (blue). White arrows are showing shifting of the initial microtubule stream to dense zigzag track. The position of dense zigzag tracks was corresponding to that of initial microtubule streaming flow. Scale bar: 50 μm .

Figure S12. Movement of a single microtubule along the tracks of the zigzag pattern. Time-lapse fluorescence microscopy images showing the movement of a single microtubule (a green microtubule pointed with a white arrow) at the curved part of zigzag tracks. Here TAMRA and Alexa488-labelled microtubules were mixed at a molar ratio of 7.3:1. Once the zigzag pattern was formed, microtubules kept moving along the tracks of the zigzag pattern as shown by the movement of the green microtubule. Scale bars: 10 μm .

Figure S13. The weak response of microtubules to mechanical stress at a low filament density condition under cyclic stretching. (a) Fluorescence microscopy image of gliding microtubules after application of cyclic stretching at a low-density condition of microtubules below the critical density required for formation of stream pattern. Scale bar: 200 μm . The white double-headed arrows are showing the stretching axis. (b) Circular histogram showing orientation angle of microtubules at low density condition in the absence (b) and presence (c) of cyclic stretching, respectively. Uniaxial cyclic stretching stimuli were applied to microtubules with a stretching strain of 20% and a stretching frequency of 0.1 Hz. Angle distributions were measured after application of cyclic stretching for 300 s (30 cycles).

Figure S14. Estimation of the surface kinesin distribution before and after stretching of the silicone elastomer substrate. Fluorescence intensity of GFP-kinesin on the substrate before and after single-step stretching (a) and cyclic stretching (b). For single-step stretching the intensity was measured before and just after stretching of the substrate at the strain of 75% and a stretching rate of 0.012 s^{-1} . For the cyclic stretching the intensity was measured after 30 min of stretching of the substrate at the strain of 20% and a stretching frequency of 0.5 Hz. The measurement was done at 10 different positions on the surface of the substrate. No significant difference in the intensity of GFP-kinesin was detected due to single-step stretching. For cyclic stretching experiment, the intensity was significantly decreased by 8% after stretching, which implies that a small number of kinesins detached from the surface after cyclic stretching. Student t-test; **** $p \leq 0.0001$. (c) Comparison of the intensity profile of GFP-kinesins on the substrate obtained before and 30 min after application of cyclic stretching. (d, e) Histogram of intensity of GFP-kinesin on the substrate before (d) and 30 min after cyclic stretching

(e) obtained from Figure S14c. Despite the detachment of 8% of kinesin, no heterogeneity in intensity distribution was observed even after cyclic stretching (skewness=0).

Figure S15. Change in Poisson's ratio, ν of the silicone elastomer substrate upon increasing the strain. The Poisson's ratio decreased non-linearly from ideal value ($=0.5$) with increase in the strain. At 20% strain, which was used for cyclic stretching experiment, the ν was 0.43. For the 75% strain, used in the single-step stretching experiment, the Poisson's ratio was 0.2.

Figure S16. Brownian dynamics simulations of buckling of microtubules during cyclic stretching. The images are showing three representative microtubules aligning to 0°, 66° and 90° against stretching (horizontal axis). White arrows indicate the point of buckling. The cyclic stretching was applied to the microtubules with stretching frequency at 0.1 Hz and the strain of 20%.

Figure S17. Transition of microtubule orientation from a zigzag pattern to a perpendicular direction against stretch axis upon further stretching. (a) Fluorescence microscopy images showing the reorganization of the zigzag pattern of microtubules formed after cyclic stretching with $\epsilon = 20\%$ and 1.0 Hz upon further application of cyclic stretching with 40% strain at 0.5 Hz. The zigzag pattern was destabilised and the microtubules preferentially aligned perpendicular to the stretching axis. Scale bar: 50 μm . (b) Circular histograms showing the distribution of the orientation angle of microtubules ($0^\circ \leq \theta \leq 180^\circ$) before (left) and after (middle and right) destabilisation of the zigzag pattern. The zigzag pattern was further reorganized into the perpendicularly oriented flow against the stretch axis. This result indicates the flexibility of the pattern and its ability to adapt to changes in its boundary conditions.

Figure S18. Visualization of deformation of an indented silicone elastomer substrate. (a) The images showing deformation of the silicone elastomer substrate with grid pattern. Scale bar: 10 mm. Each grid size is $1.5 \times 1.5 \text{ mm}^2$. The whole size of the substrate is $15 \times 15 \text{ mm}^2$. Indentation depth is 10 mm. (b) 3D projection of the indented silicone substrate obtained from a grid displacement in the ‘side view’ image of (a). (c) Strain distribution of the indented substrate measured in ROI enclosed in red rectangular area in (a).

Figure S19. Global view of a large vortex pattern of microtubules. The fluorescence microscopy image showing a vortex pattern of microtubules that emerged after 30 min of application of the radial stretching. Scale bar: 1 mm.

Movie legends

Movie S1

Streaming flow formation of gliding microtubules. Streaming flow, which is a display of pattern formation of gliding microtubules, were observed within 30 min after applying ATP. Scale bar: 50 μm . The movie is 1830 times faster than the original speed.

Movie S2

Perpendicular orientation of microtubule streaming flow after application of single step uniaxial stretching stimulus. After applying a uniaxial stretching stimulus (strain, $\varepsilon = 75\%$, strain rate $\dot{\varepsilon} = 0.012 \text{ s}^{-1}$), the streaming flow of microtubules were destabilised and gradually reorganized into perpendicular orientation against the stretch axis. Scale bar: 50 μm . The movie is 1830 times faster than the original speed.

Movie S3

Origin of long-lived stability of perpendicular orientation of microtubules. Individual microtubules inside global orientation of microtubules formed after application of stretching stimulus (strain, $\varepsilon = 75\%$, strain rate $\dot{\varepsilon} = 0.012 \text{ s}^{-1}$) by dual-colour imaging using TAMRA and Alexa488 labelled microtubules. Scale bar: 50 μm . The movie is 900 times faster than the original speed.

Movie S4

Derailment of a microtubule from global perpendicular alignment. Observation of derailment of an individual microtubule from the perpendicular orientation of microtubules after application of stretching stimulus (strain, $\varepsilon = 75\%$, strain rate $\dot{\varepsilon} = 0.012 \text{ s}^{-1}$). Scale bar: 10 μm . The movie is 30 times faster than the original speed.

Movie S5

Enhancement of perpendicular orientation of microtubules in simulation. Simulation of reorganization of streaming flow of microtubules was initiated by mixing randomly oriented microtubules and perpendicularly pre-oriented microtubules to the horizontal direction at the beginning of simulation with different mixing ratio. The movie is 460 times faster than the original speed.

Movie S6

Time dependent formation of a zigzag pattern of microtubules under cyclic stretching.

Microtubule streaming flow formed unique zigzag pattern, by maintaining $\sim 60^\circ$ and $\sim 120^\circ$ steady alternating angles against the stretching axis under uniaxial cyclic stretching stimuli ($\varepsilon = 20\%$, stretching frequency of 0.5 Hz). Scale bar: 50 μm . The movie is 100 times faster than the original speed.

Movie S7

Gliding microtubules at the bent part of a zigzag pattern. Observation of gliding microtubules inside zigzag patterns formed under uniaxial cyclic stretching stimuli (strain, $\varepsilon = 20\%$, stretching frequency of 1.0 Hz) by dual-colour imaging. Scale bars: 50 μm (left), 25 μm (trajectory). The movie is 56 times faster than the original speed.

Movie S8

Large vortex pattern formation under radial stretching. Large vortex pattern emerged from streaming flow of microtubules after application of radial stretching stimulus with indentation depth of 10 mm at a rate of 0.08 mm s⁻¹. Scale bar: 1 mm. The movie is 2400 times faster than the original speed.