

Supplementary Information

Construction of artificial cilia from microtubules and kinesins through a designed bottom-up approach

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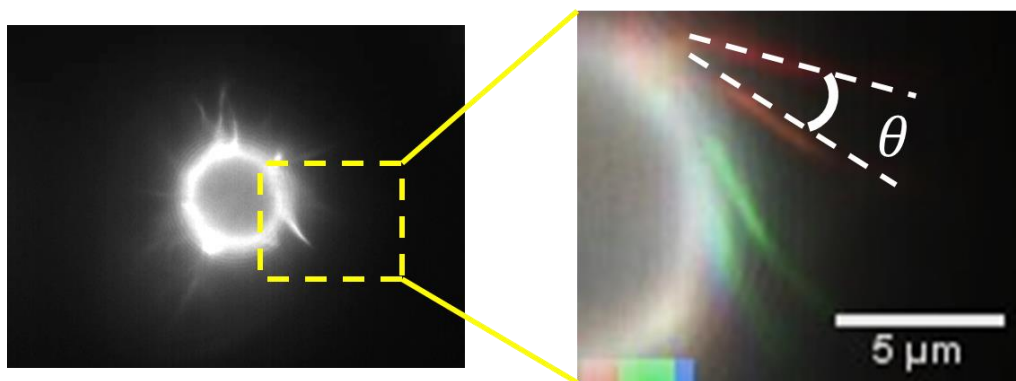
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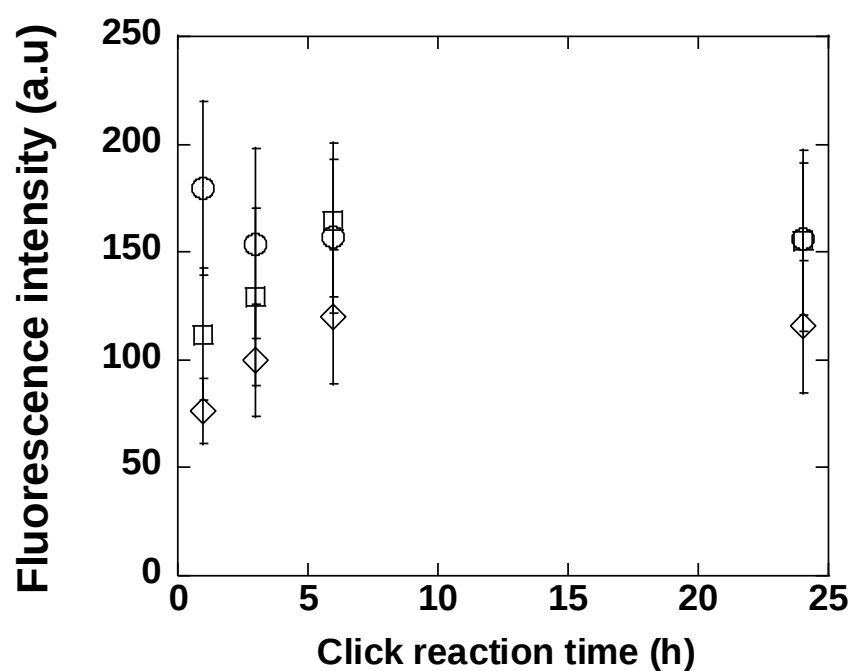
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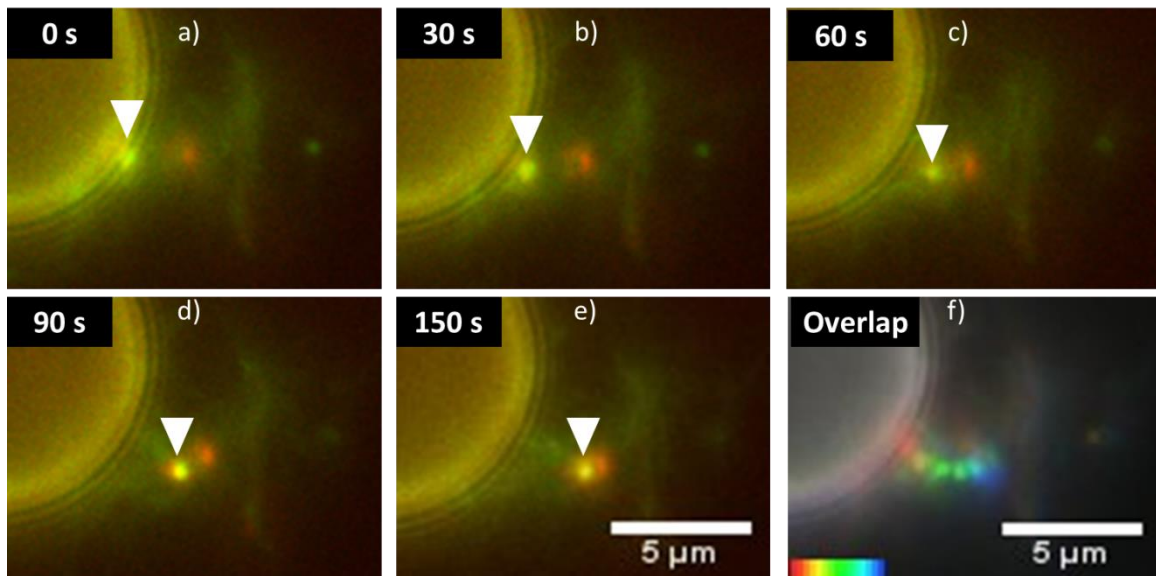
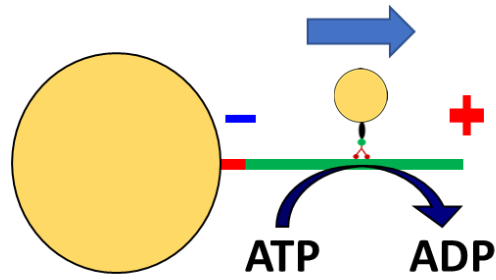
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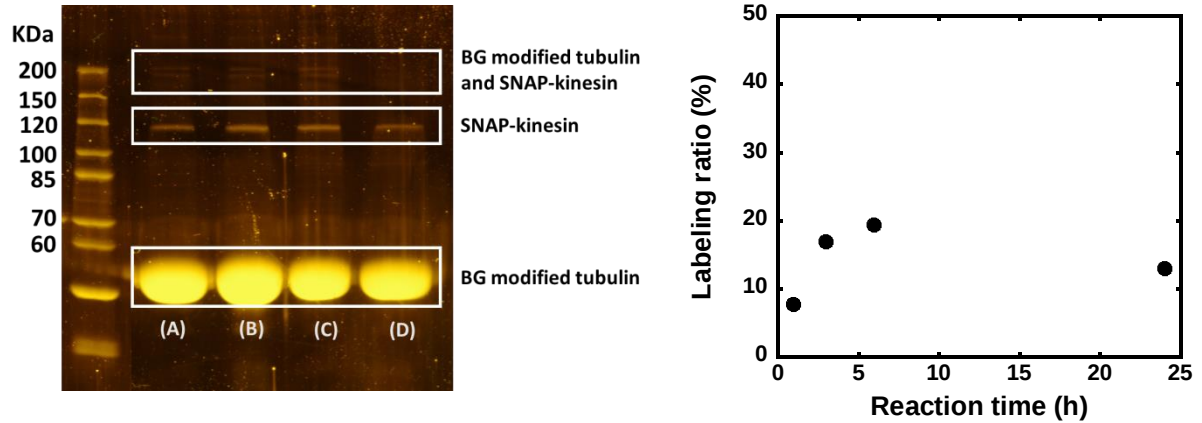
Supplementary Figure 1: Measurement of the angular velocity of artificial cilia. The change in angle (θ) for every 5 sec was measured manually using ImageJ plugin ‘color footprint’. Here the red and blue color indicates the initial and final position of the microtubule bundle respectively.



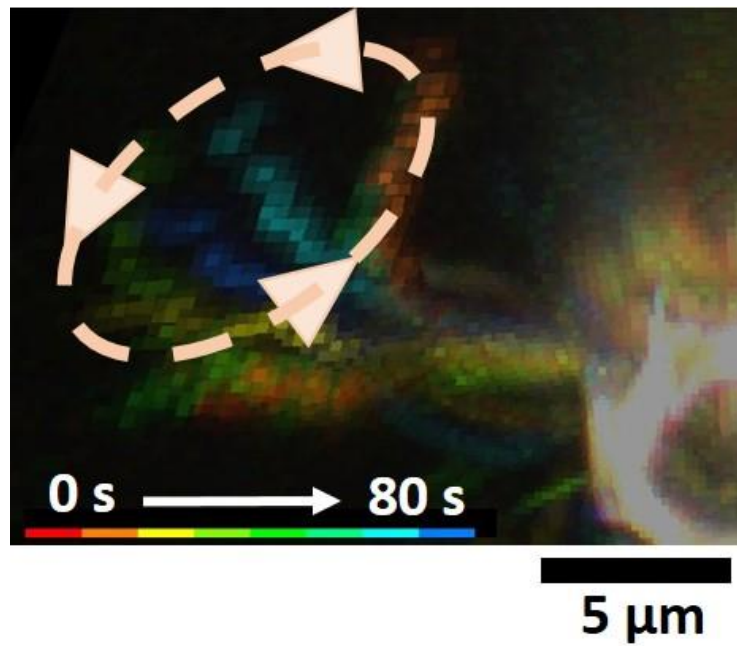
Supplementary Figure 2: Effect of the click reaction time on the attachment of the seed microtubules to the polystyrene beads. Different symbols represent results for beads with different sizes. Here, the circle, square and diamond stand for the beads with diameter of 3.36, 6.72 and 11.2 μm respectively. Based on these results the beads with diameter of 6.72 μm were used for the experiments where the click reaction time was fixed at 6 h. Error bar: standard deviation.



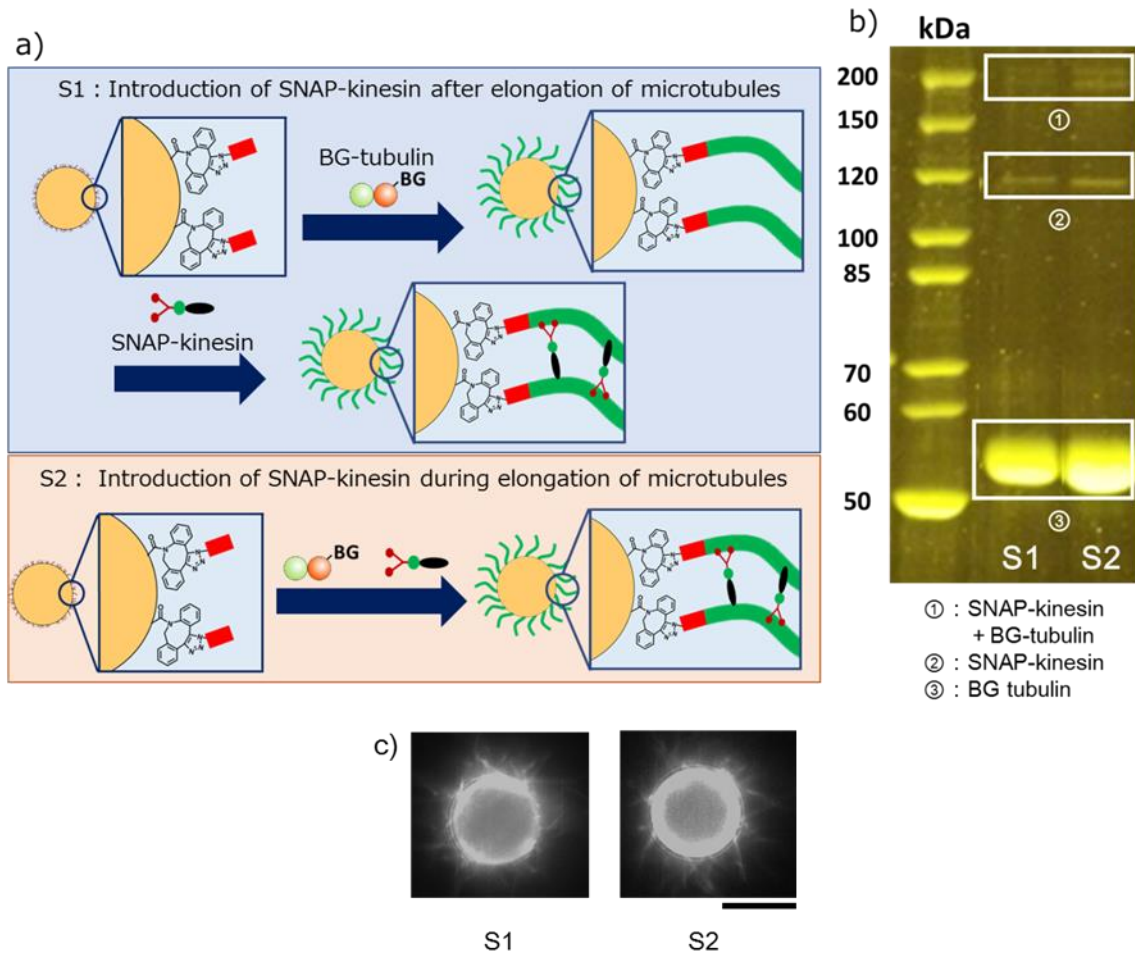
Supplementary Figure 3: Confirmation of polarity of microtubules grown from polystyrene beads. A schematic representation shows the design of this experiment (top). Time lapse fluorescence microscopy images (a-e) showing the movement a kinesin conjugated bead, indicated by the white arrow, towards the free end of microtubules grown from the surface of the bead. The overlapped color footprint image (f) indicates the movement of a kinesin driven bead, where red and blue colors stand for the initial and final position of the bead respectively.



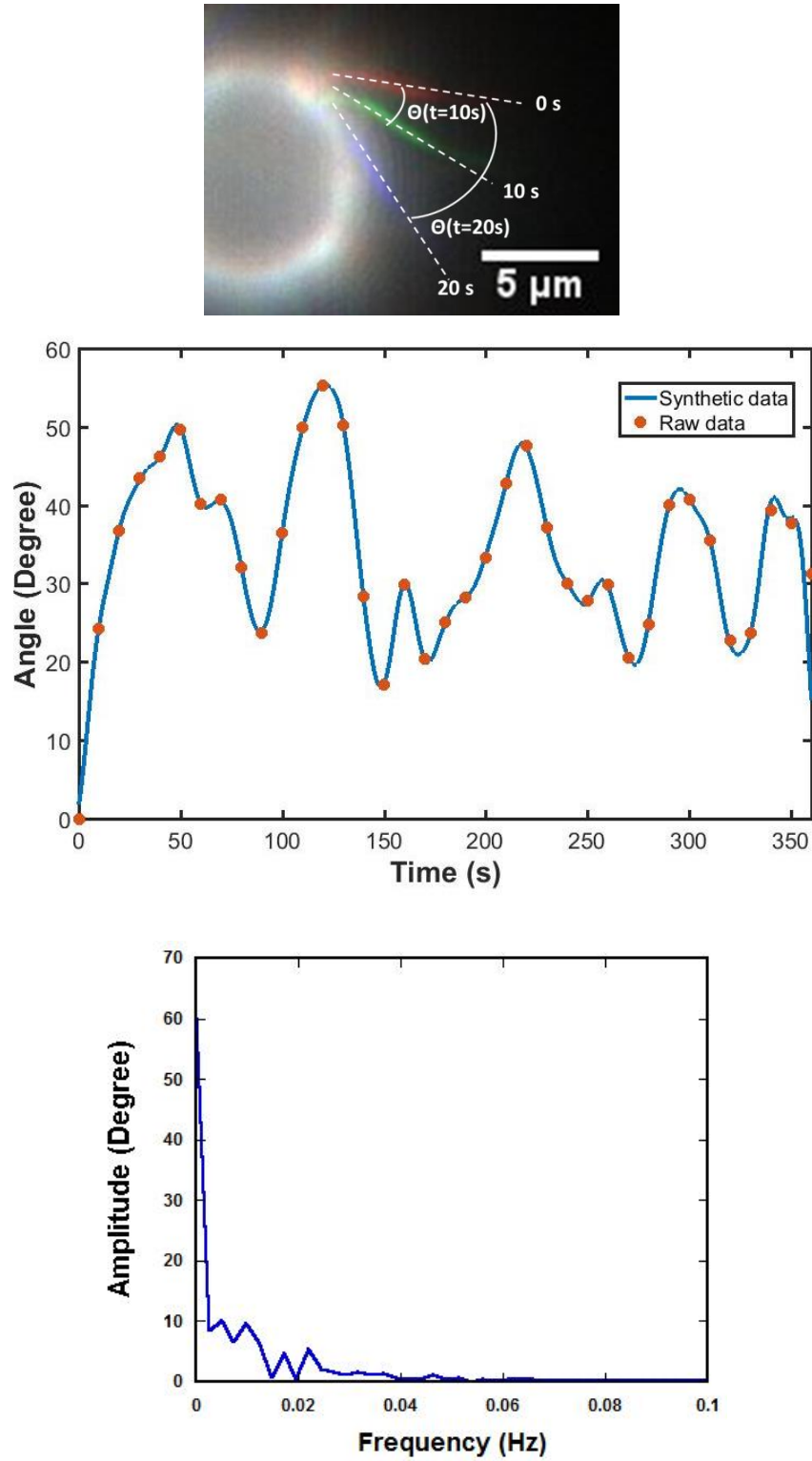
Supplementary Figure 4: Labeling ratio of SNAP-kinesins to microtubules (tubulins) was estimated from results of the SDS-PAGE (left). SNAP-kinesins were mixed with a mixture of BG modified tubulins and Alexa488 modified tubulins during microtubule polymerization and incubated at 37 °C for different prescribed time periods: (A) 1 h, (B) 3 h, (C) 6 h, (D) 24 h. From the intensity of the bands corresponding to 200 KDa, and 55 KDa labeling ratio of the SNAP-kinesins to tubulins were found to be: 0.75%, 1.05%, 2%, 0.51% for the 1 h, 3 h, 6 h, and 24 h incubation time respectively. From the intensity of the bands corresponding to 200 KDa and 120 KDa, the ratio of kinesins conjugated to tubulins with respect to the kinesins applied in feed were estimated, which were found to be 7.7%, 16.89%, 19.4% and 12.9% for 1 h, 3 h, 6 h and 24 h incubation time respectively (right). In each case the concentration of kinesin applied in feed was 200 nM. In the measurement of the labeling ratio using densitometry, it was assumed that overloading of tubulins did not affect the linearity in measurements; however a possibility of overestimation of the labeling ratio could not be ruled out, which needs to be addressed properly in future works.



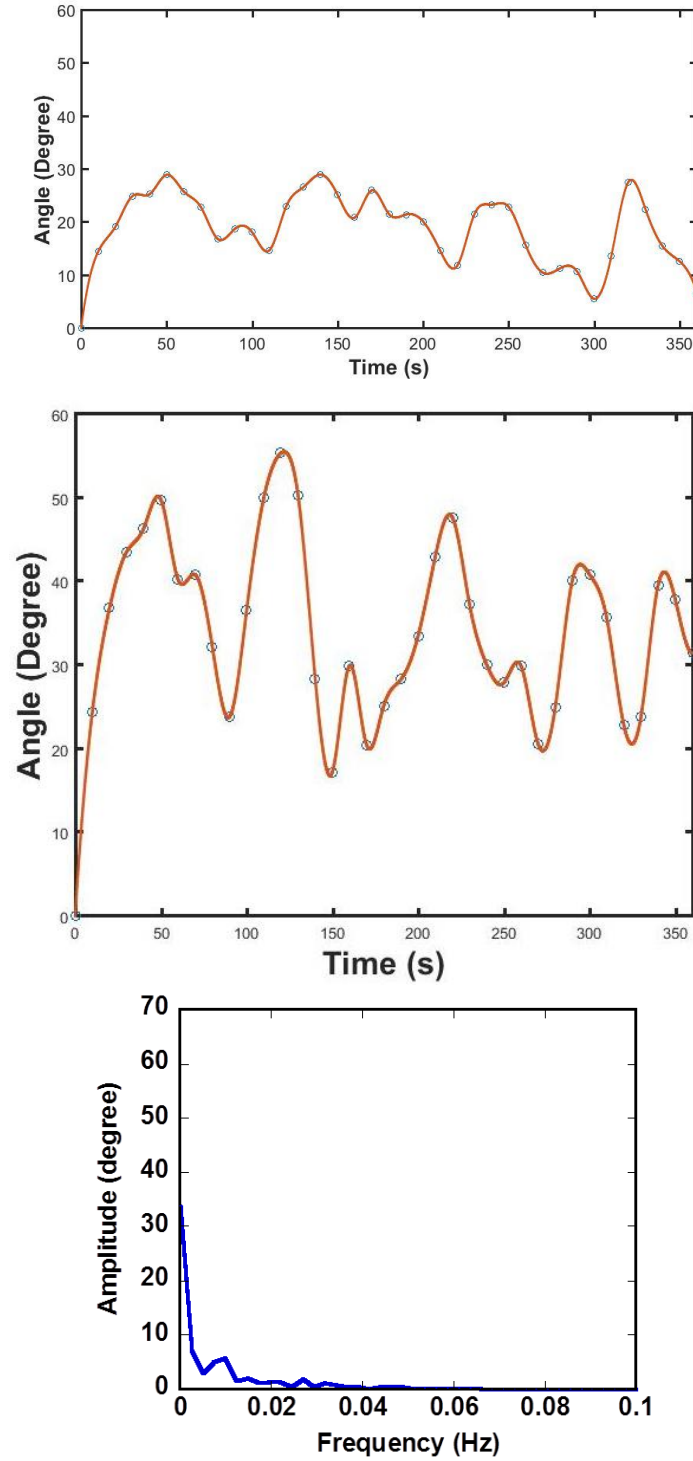
Supplementary Figure 5: Time lapse color footprint image showing the beating of a microtubule bundle with rotational motion captured under a confocal laser scanning microscope. The arrows indicate the direction of rotation of the microtubule bundle.



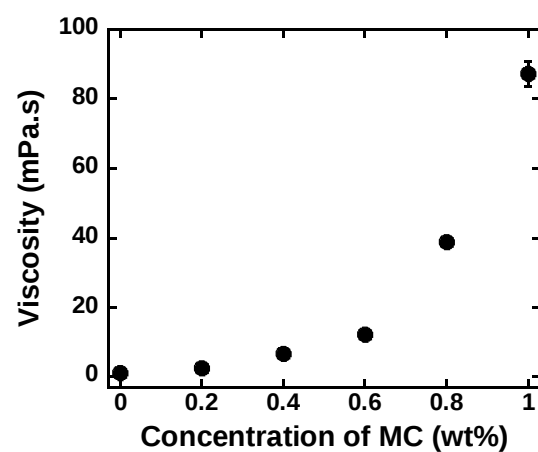
Supplementary Figure 6: Estimation of the labeling ratio of the kinesin to microtubules for two different approaches. a) In one case, the SNAP-kinesins were introduced after the elongation of the seed microtubules (S1). In the other case, the SNAP-kinesins were introduced at the time of microtubule elongation by mixing them with the polymerization buffer (S2). b) The labeling ratio of the kinesin to microtubules, estimated from the result of the SDS-PAGE, was much lower for S1 (~0.25%) than that of S2 (~2%). Here the kinesin concentration was 200 nM and the incubation time of the mixture of SNAP-kinesin and tubulin was 6 h in each case. c) Fluorescence microscopy images of microtubules elongated from polystyrene beads following the two methods. Scale bar: 5 μm .



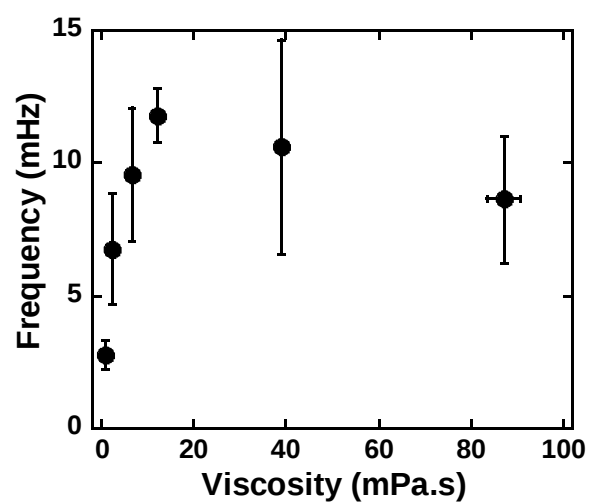
Supplementary Figure 7: The change in angle with time at an arbitrary position of the artificial cilia (top and middle) and its Fast Fourier Transformation (blue line) show periodicity in the beating of the artificial cilia. From the power spectrum (bottom) the beating frequency is found to be 0.01 Hz.



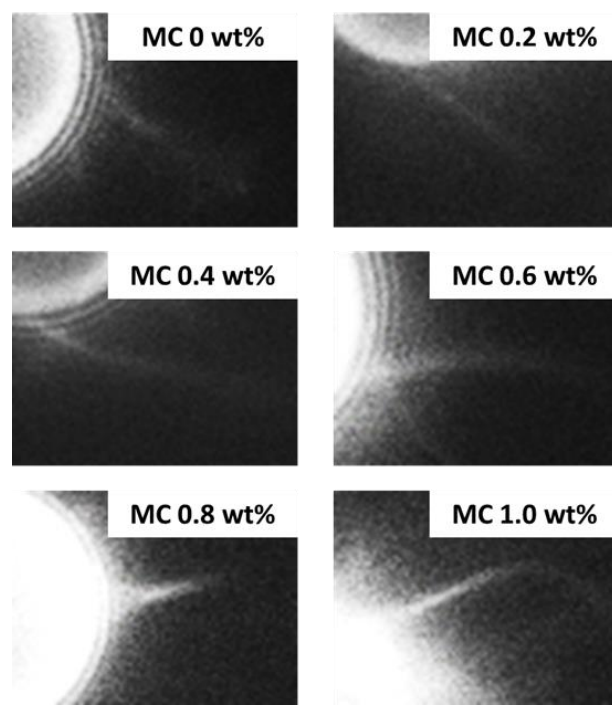
Supplementary Figure 8: Periodic change in angle with time at an arbitrary position of an artificial cilia and corresponding Fast Fourier Transformation (solid line) for 100 nM (top) and 200 nM (middle) kinesin concentration, and the power spectrum for the case of 100 nM kinesin (bottom).



Supplementary Figure 9: Change in the viscosity of the buffer with the change in the concentration of methylcellulose.



Supplementary Figure 10: Dependence of the beating frequency of artificial cilia on the viscosity of buffer. Error bar: standard deviation.



Supplementary Figure 11: Representative fluorescence microscopy images showing the change in thickness (fluorescence intensity) of microtubule bundles upon changing the concentration of the methylcellulose (MC). Scale bar: 5 μm .

Legends for the supplementary movies

Supplementary movie 1: Beating with circular motion exhibited by an artificial cilia (in the presence of ATP), constructed on the surface of a polystyrene bead with diameter of 6.72 μm . The movie was captured under a confocal laser scanning microscope. The area of view is $(15 \times 20) \mu\text{m}^2$. The speed is 100 time faster than the real time speed.

Supplementary movie 2: Artificial cilia shows no beating or rotational motion in the absence of ATP. The artificial cilia were constructed on the surface of a polystyrene bead with diameter of 6.72 μm . The movie was captured under a confocal laser scanning microscope. The area of view is $(15 \times 20) \mu\text{m}^2$. The speed is 100 time faster than the real time speed.