Electronic Supplementary Information (ESI)

Effect of length and rigidity of microtubules on the size of ring-shaped assemblies obtained through active self-organization

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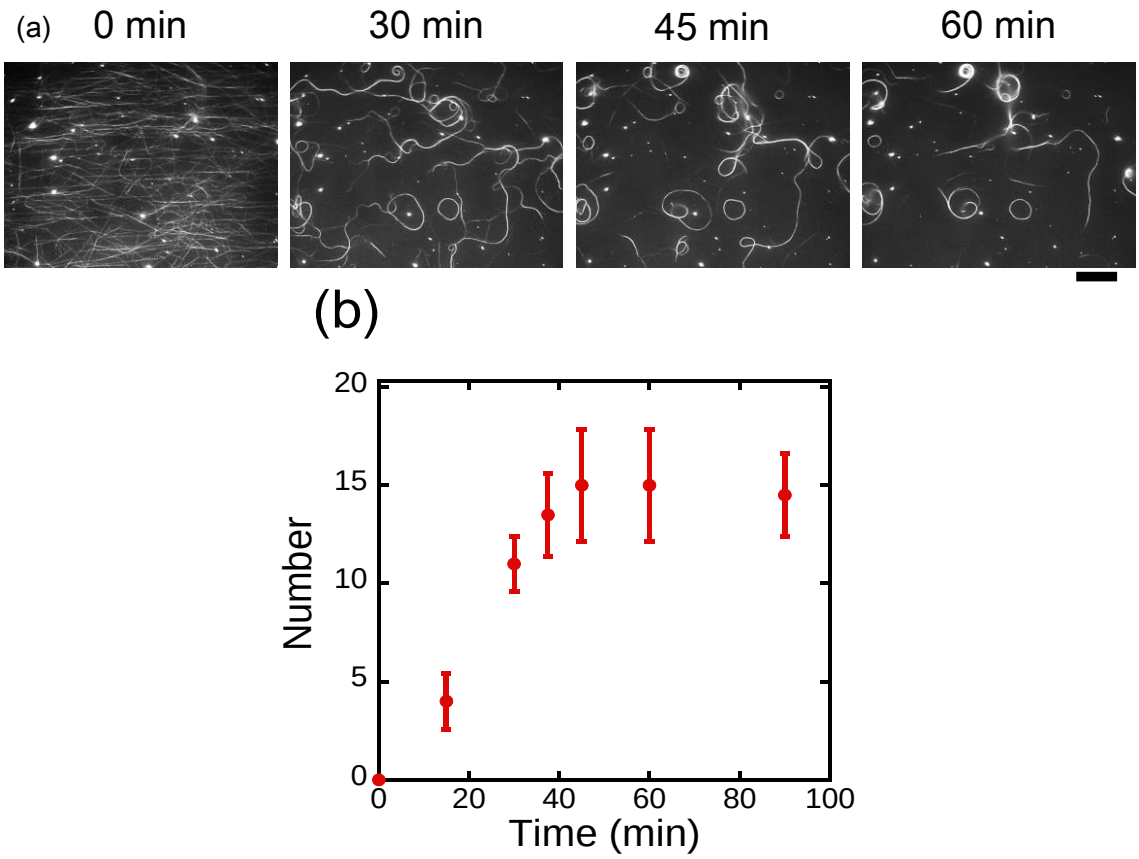

Fig. S1: (a) Fluorescence microscopy images showing the time course of AcSO of GTP-MTs that finally formed stable ring-shaped MT assemblies by ~45 min. (b) The change in the number of ring-shaped MT assemblies with time in a specific area when GTP-MTs were employed in the AcSO. Error bar: standar deviation and scale bar: 20 μm.

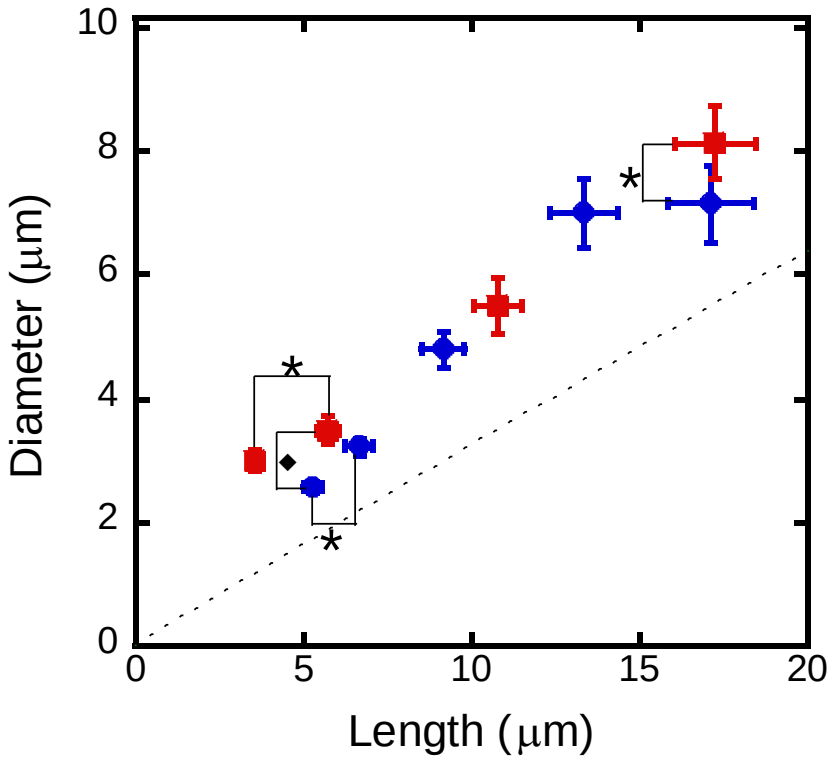

Fig. S2: Average diameter of ring-shaped MT assemblies obtained from the AcSO of length tuned GTP-MTs on substrates coated with K560 (blue circle) and K573 (red square). Dotted line represents the theoretically calculated diameter obtained from corresponding average MT length considering one MT filament forms a ring-shaped assembly whose circumference is equal to the length of the MT filament. From the statistical analyses (student's t-test), the differences in size of ring-shaped MT assemblies due to change in the length of MTs employed in the AcSO were found to be statistically significant (*P<0.01). The difference in size of ring-shaped MT assemblies due to change in the length of kinesin in the AcSO were not statistically significant (◆P>0.05), although for longer MTs the difference was considerable (*P<0.01). Error bar: standard error of mean.

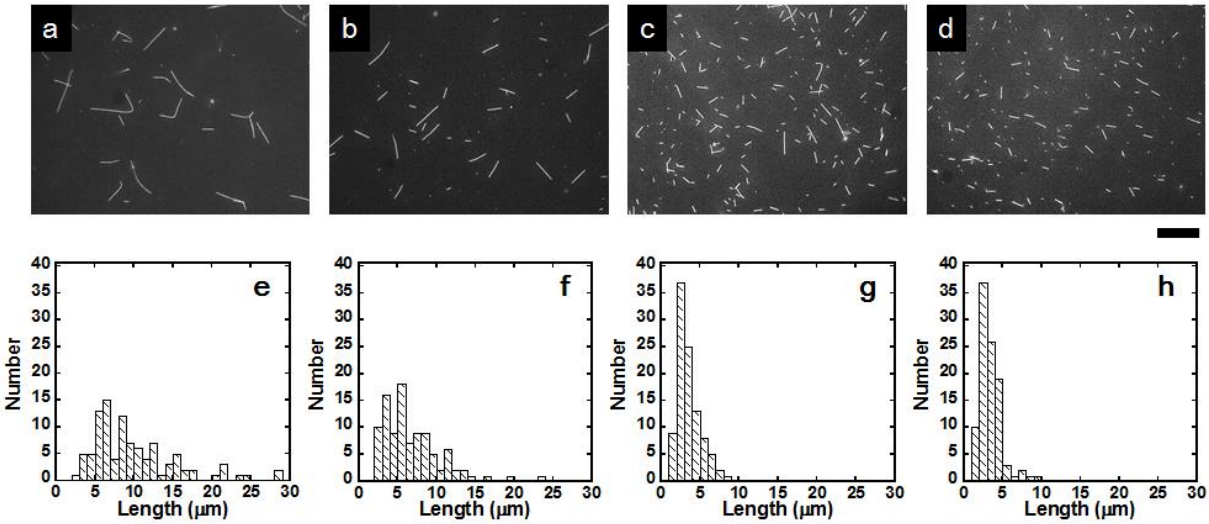

Fig. S3: Fluorescence microscopy images of GMPCPP-MTs (in the absence of taxol): (a) before and (b-d) after shear treatment. For shearing MT solution was passed back-and-forth (b) one, (c) three and (d) five times through a syringe-mounted needle by manual operation of the syringe. Histograms show the distribution of GMPCPP-MTs length at different conditions: (e) no shearing, shearing treatment for (f) one, (g) three and (h) five times. The average length of MTs decreased from 10.0±5.5 μm to 7.1±4.5, 3.5±1.5 and 3.4±1.4 μm after one, three and five times shear treatment respectively. Number of MTs considered for analyses was 100 in each case. Scale bar: 20 μm.

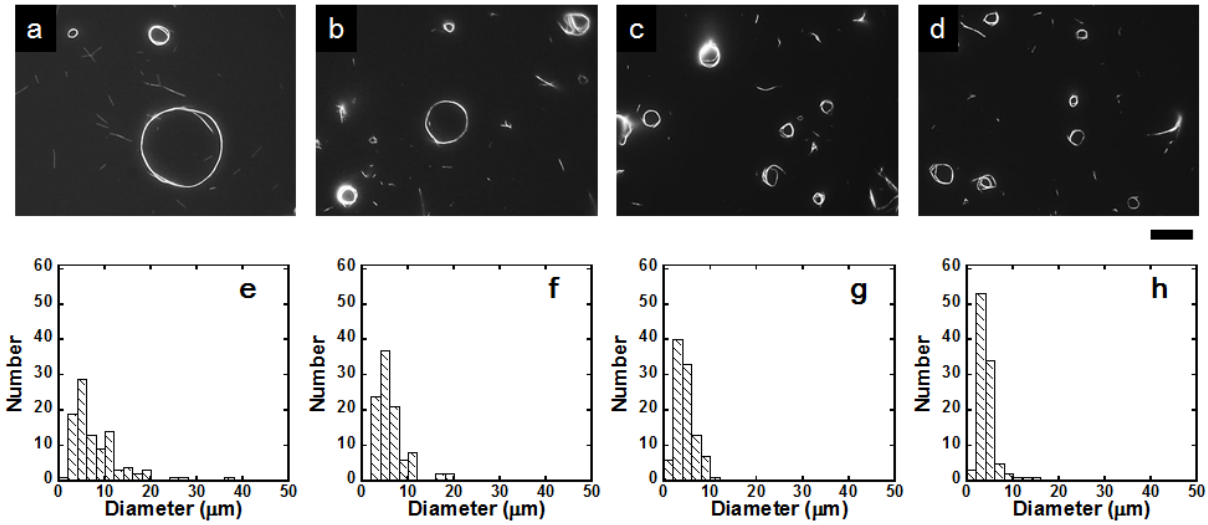

Fig. S4: Fluorescence microscopy images of ring-shaped assemblies on the K573 coated substrate obtained from the AcSO of GMPCPP-MTs (in the absence of taxol) with different lengths shown in the Fig. S3 (a-d). As seen from these images and also from the histograms of size distribution of ring-shaped MT assemblies (e-h), with the decrease of MT length due to shearing, size (inner diameter) of ring-shaped MT assemblies decreased. Histograms were prepared by analyzing the inner diameter of ring-shaped assemblies obtained from the AcSO of GMPCPP-MTs (no taxol) that underwent: (e) no shearing, (f) one, (g) three and (h) five times shearing before being employed in the AcSO. The average diameter decreased from 8.2±5.7 μm to 6.2±3.4, 4.6±2.1 and 4.3±2.1 μm after one, two, three and five times shear treatment of MT filaments respectively. Scale bar: 20 μm.

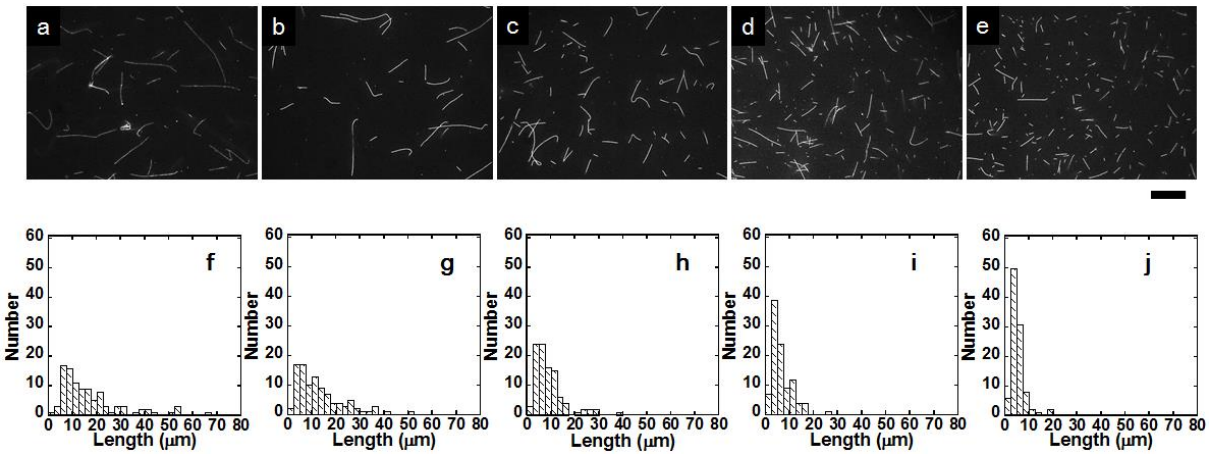

Fig. S5: Fluorescence microscopy images of GTP-MTs: (a) before and (b-e) after shear treatment. For shearing treatment, MT solution was passed back-and-forth (b) one, (c) two, (d) three and (e) five times through a syringe-mounted needle by manual operation of the syringe. Histograms show the distribution of GTP-MT length at different conditions: (f) no shearing, shearing treatment for (g) one, (h) two, (i) three and (j) five times. The average length of GTP-MT decreased from (f) 17.1±12.8 μm to (g) 13.4±9.9, (h) 9.2±6.4, (i) 6.7±4.2 and (j) 5.3±2.9 μm after one, two, three and five times shear treatment respectively. Number of MT considered for analyses was 100 in each case. Scale bar: 20 μm.

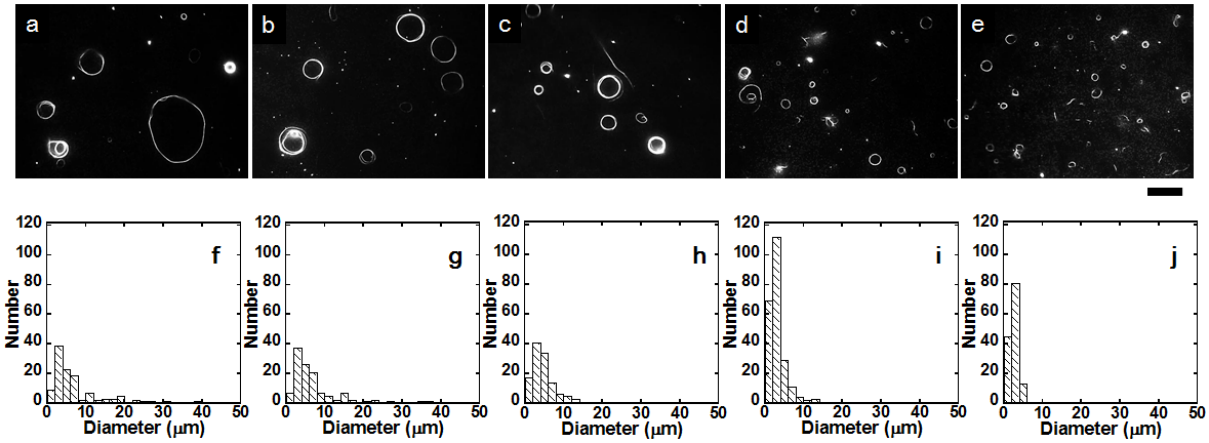

Fig. S6: Fluorescence microscopy images (a-e) of ring-shaped assemblies obtained from the AcSO of GTP-MTs with different lengths shown in the Fig. S5 (a-e). The AcSOs were performed on the K560 coated substrate. As seen from these images and also from the histograms of size distribution of ring-shaped MT assemblies (f-j), with the decrease of MT length due to shearing treatment the size (inner diameter) of ring-shaped MT assemblies also decreased. Histograms were prepared by analyzing the inner diameter of ring-shaped assemblies obtained from the AcSO of GTP-MTs that underwent: (f) no shearing, (g) one, (h) two, (i) three and (j) five times shearing before being employed in the AcSO. The average diameter of ring-shaped MT assemblies decreased from (f) 7.2±6.7 μm to (g) 7.0±6.1, (h) 4.8±3.3, (i) 3.2±2.1 and (j) 2.6±1.1 μm due to one, two, three and five times shear treatment of MT filaments respectively. Scale bar: 20 μm.