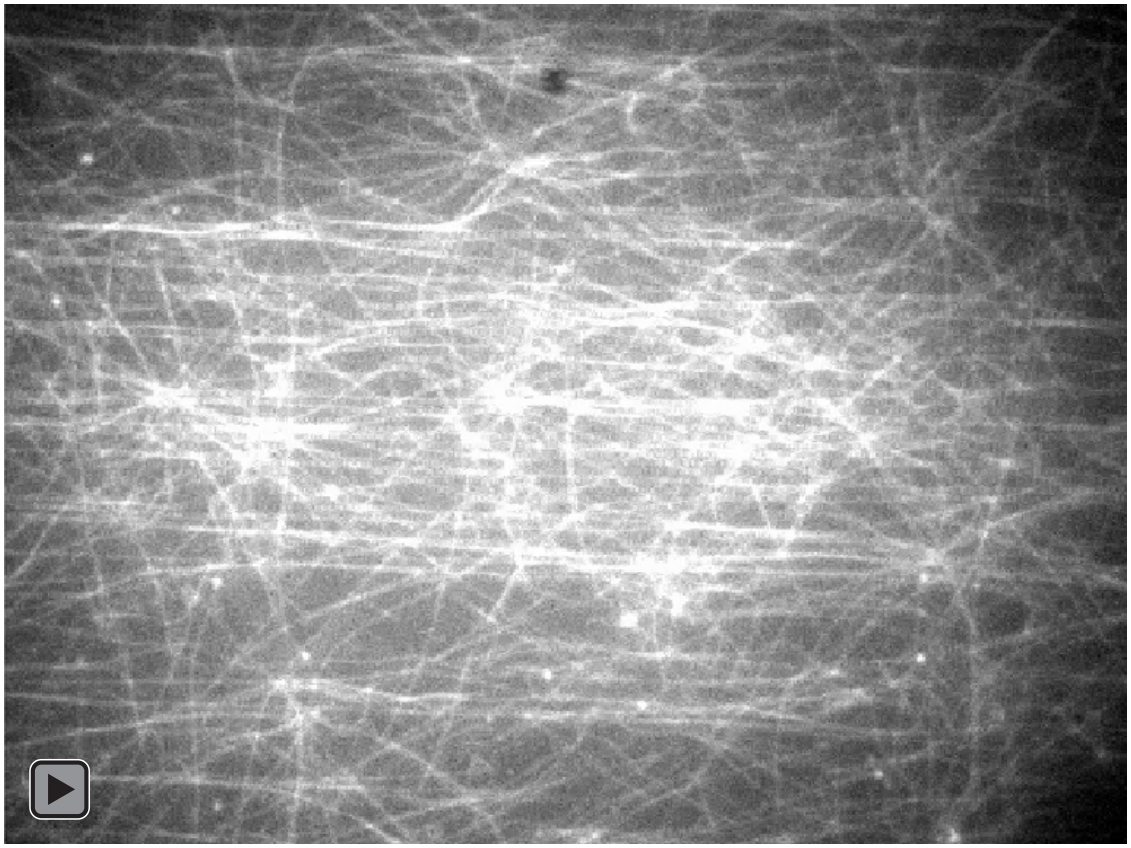
Electronic Supplementary Material (ESI) for RSC Advances.

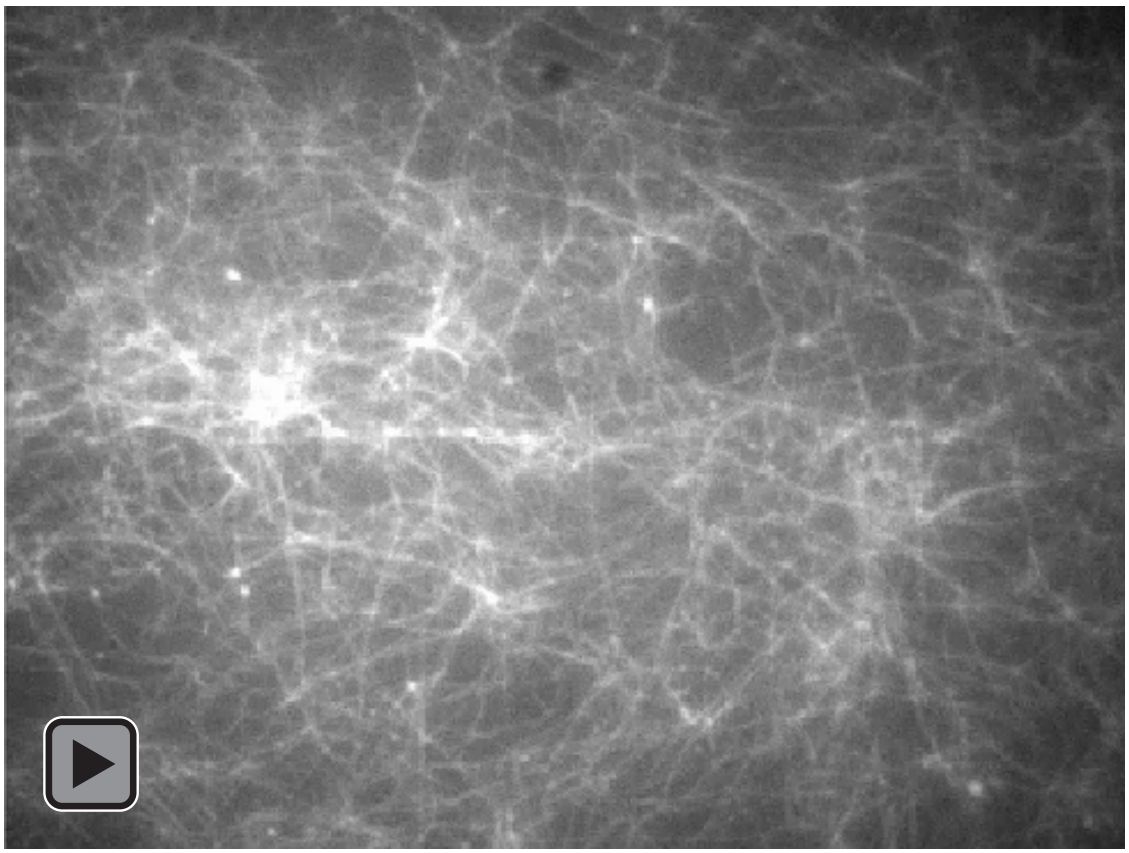
Chemically cross-linked microtubule assembly shows enhanced dynamic motions on kinesins

*Ryuzo Kawamura, Ken-Ichi Sano, Kuniharu Ijiro, and Yoshihito Osada**
*E-mail: osadayoshi@riken.jp

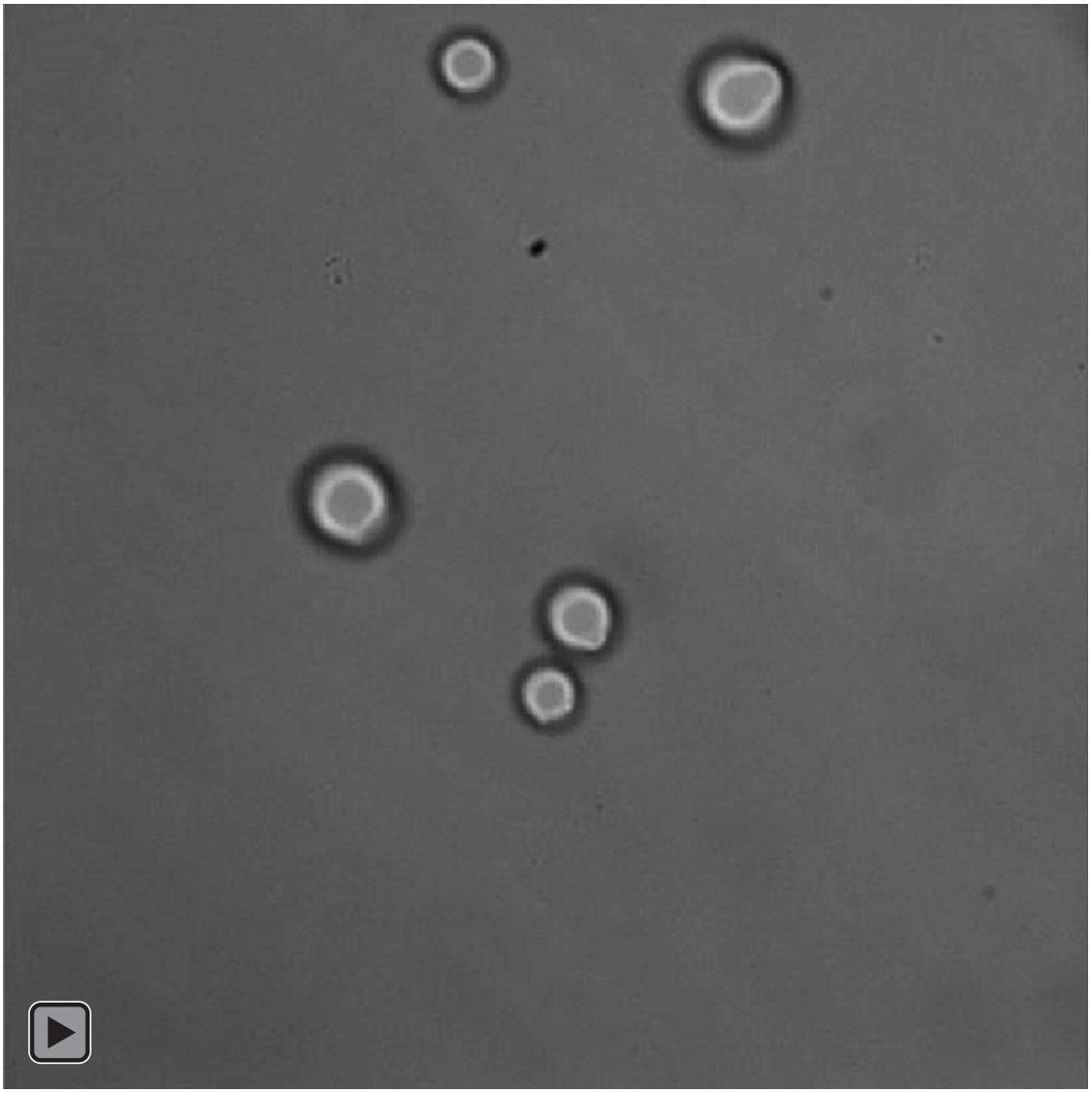
Electronic supplementary information



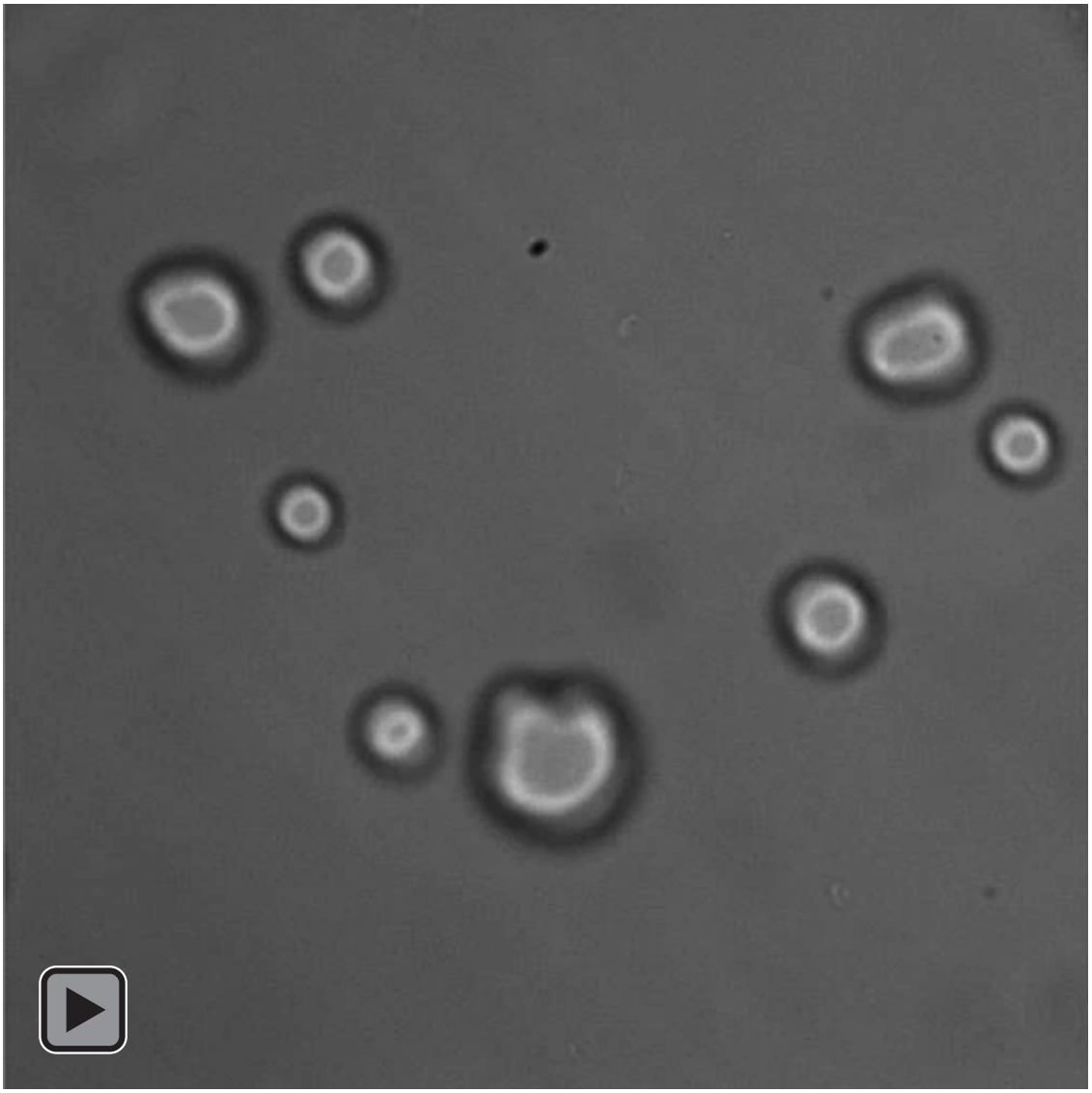
ESI-mov-1: MTs without cross-linker ('0x') were driven by surface-immobilized kinesins. MTs were stabilized and visualized by Oregon green 488 conjugated paclitaxel. This movie is identical to the images of Figure 2a and c. The movie is 20-fold faster than the real time. Image width is 81 μm.



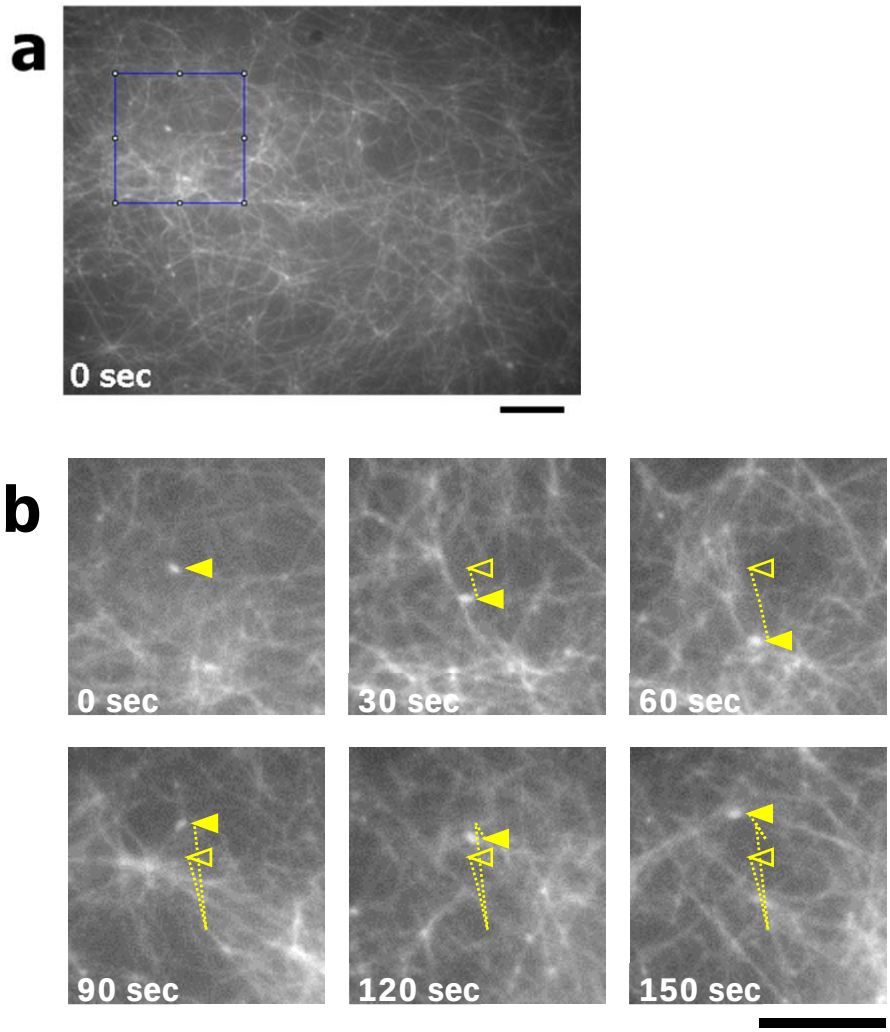
ESI-mov-2: Spread MT gel (‘1x’) is driven on a kinesin-immobilized surface. MTs were stabilized and visualized by Oregon green 488 conjugated paclitaxel. This movie is identical to the images of Figure 2b and d. The movie is 20-fold faster than the real time. Image width is 81 μm.



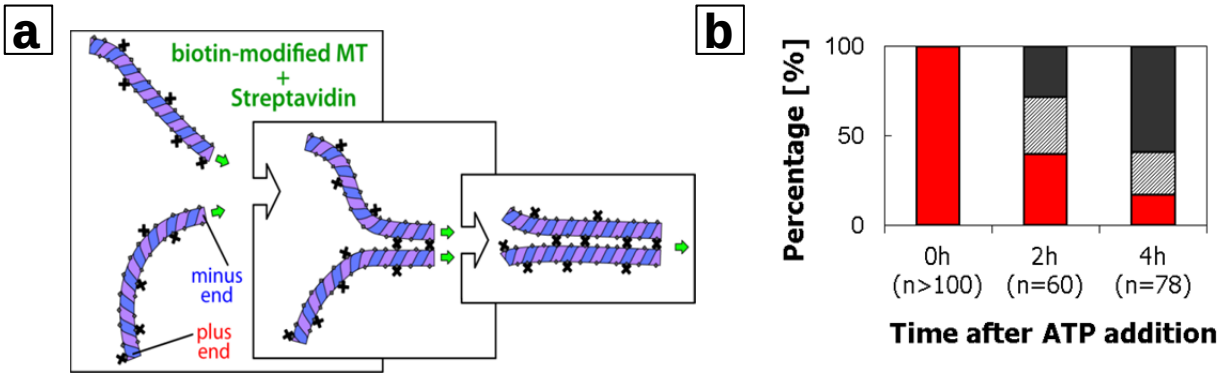
ESI-mov-3: Silica microbeads moving on MTs without cross-linker ('0x'), which are driven by surface immobilized-kinesins. The movie is 20-fold faster than the real time. Image width is 81 µm.



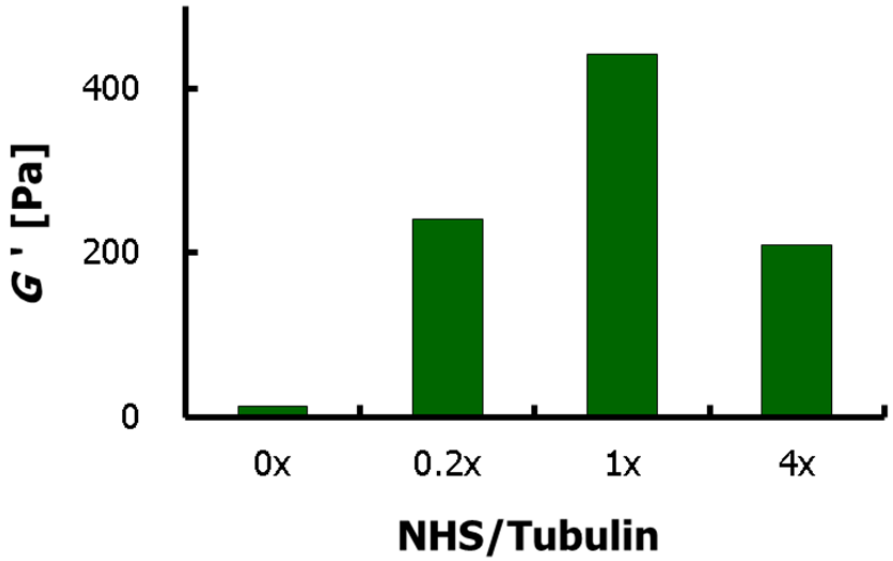
ESI-mov-4: Spread MT gel (‘1x’) is driven on a kinesin-immobilized surface Silica microbeads moving on MT gel (‘1x’) which is driven by surface immobilized-kinesins. The movie is 20-fold faster than the real time. Image width is 81 µm.



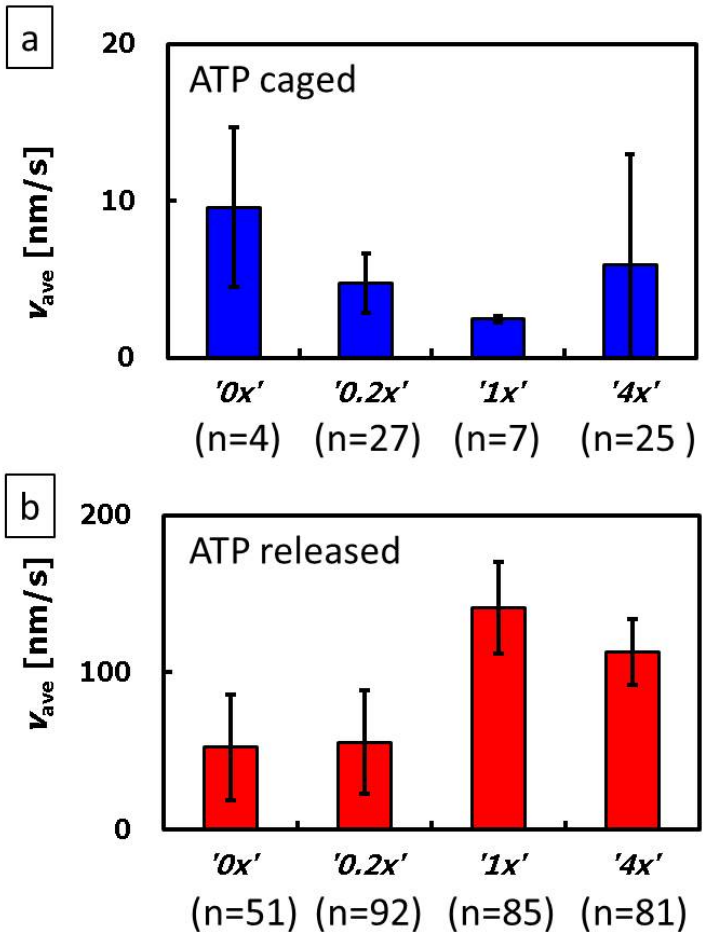
ESI Fig. S1: An example of zigzag motion in MT gel ('1x'). (a) a view field corresponds to ESI-mov-2. (b) the field indicated by blue line in (a) was magnified and a series of the fluorescent images in a time course is shown. The closed arrowheads indicate the position of the tracking bright spot which is a part of MT gel. The open arrowhead indicates the original position at 0 second. The dashed lines are drawn by connecting the arrowhead positions. Scale bars are 10 μm.



ESI Fig. S2: Dynamic formation of MT bundles by biotin-streptavidin binding. The method is briefly depicted as follows.[ref. 29, 30, 37] MTs were formed from tubulins and stabilized with paclitaxel; 45% of tubulins were labeled with biotins and 10% of tubulins were labeled with Alexa 488. In absence of ATP, the MTs were bound to surface-immobilized kinesins and the biotins on MT surfaces were partially covered with streptavidins. By adding ATP, motility assay was started. During the sliding motions, encountered two MTs were bound through the biotin-streptavidin interactions. (a) the zipping up of two parallel MTs are illustrated. (b) percentage of the motile MTs and MT bundles (red), those of one end stacked (gray), and stopped (black) are plotted.



ESI Fig. S3: Elasticity of MT gels. Freshly prepared MT gels with various cross-linker ratios were examined by rheometer as described in our previous report. [ref. 21] The tubulin concentration was 10mg/ml. The elasticity of '1x' showed the highest value among the cross-linker ratios (NHS/tubulin) of 0, 0.2, 1 and 4.



ESI Fig. S4: Average velocities of beads before and after starting the motility assay. X-axis represents the cross-linker ratio of NHS/Tubulin. Since the average velocity of the beads before starting the assay (ATP was kept caged) was 9.6±5.1 nm/s (n=4, '0x') and 2.5±0.2 nm/s (n=7, '1x') the effect of the Brownian motion should be negligibly low compared to those after releasing caged-ATP. Before starting the motility assay, beads were most stable on '1x'. This is convincing with the result of rheometric analysis of the MT gel; '1x' showed the highest storage modulus, indicating that '1x' can retain beads most stably. In contrast to the tendency in the beads average velocity before the ATP activation, the beads on '1x' moved at highest average velocity among the cross-linker ratios. Thus, the most elastic MT network of '1x' is most controllable for beads velocity. [ref. 21]