



Title	Dynamic self-organization and polymorphism of microtubule assembly through active interactions with kinesin
Author(s)	Tamura, Yoshiki; Kawamura, Ryuzo; Shikinaka, Kazuhiro et al.
Citation	Soft Matter, 7(12), 5654-5659 https://doi.org/10.1039/c1sm05413a
Issue Date	2011-06-21
Doc URL	https://hdl.handle.net/2115/48991
Rights	Soft Matter, 2011, 7(12), 5654-5659 - Reproduced by permission of The Royal Society of Chemistry (RSC)
Type	journal article
File Information	Supplementary_information.pdf, Supplementary information



Supporting Information for Publication

Polymorphism of MT assembly through a dynamic self organization

Yoshiki Tamura¹, Ryuzo Kawamura^{1,†}, Kazuhiro Shikinaka^{1,§},

Akira Kakugo^{1,2}, Yoshihito Osada^{1,†}, Jian Ping Gong^{1,}*

Hiroyuki Mayama^{3,}

¹Department of Biological Science, Graduate School of Science, Hokkaido University,
Sapporo 060-0810, Japan

²PRESTO, Japan Science and Technology Agency, Sapporo 060-0810, Japan

³Nanotechnology Research Center, Research Institute for Electronic Science, Hokkaido
University, Sapporo 0001-0021, Japan

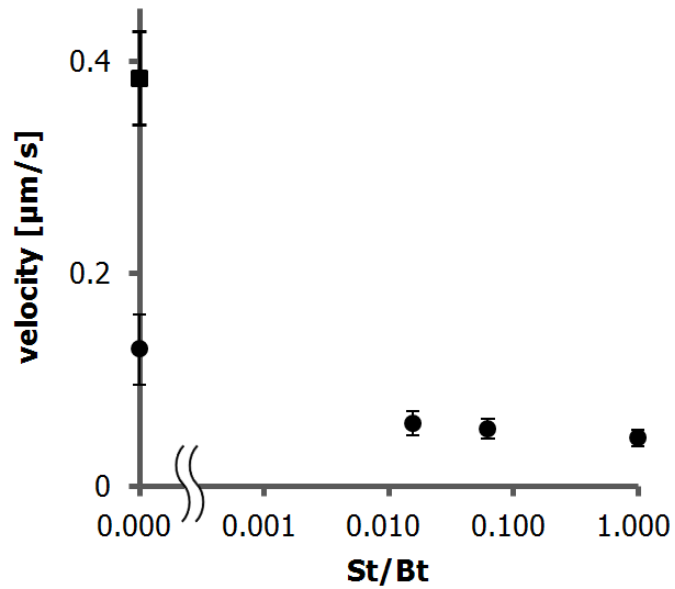
[§]Present address: Institute of Symbiotic Science and Technology, Tokyo University of
Agriculture and Technology, Koganei 184-8588, Japan

[†]Present address: Riken, Saitama 351-0198, Japan.

Sliding velocity of MTs

Figure S1

Average velocities of the MTs were plotted against the various St/Bt ratio. Error bar is standard deviation. ■: $C_{\text{Tub}} = 672 \mu\text{M}$, $\text{Bt/Tub} = 0$ ($n = 40$). ●: $C_{\text{Tub}} = 672 \mu\text{M}$, $\text{Bt/Tub} = 1/1$; $\text{St/Bt} = 0$ ($n = 58$), $1/64$ (50), $1/16$ (50) and $1/1$ (50).

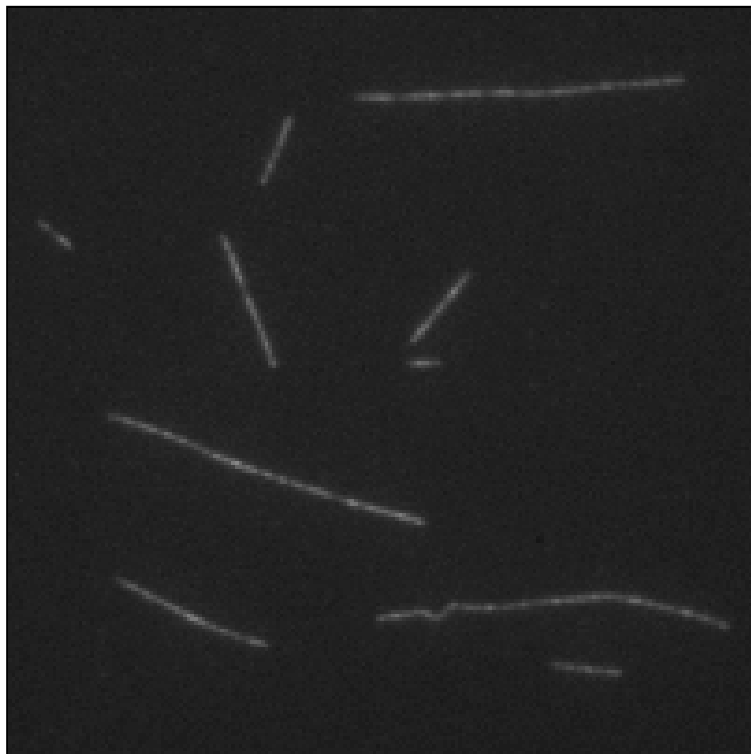


Descriptions of the Movies

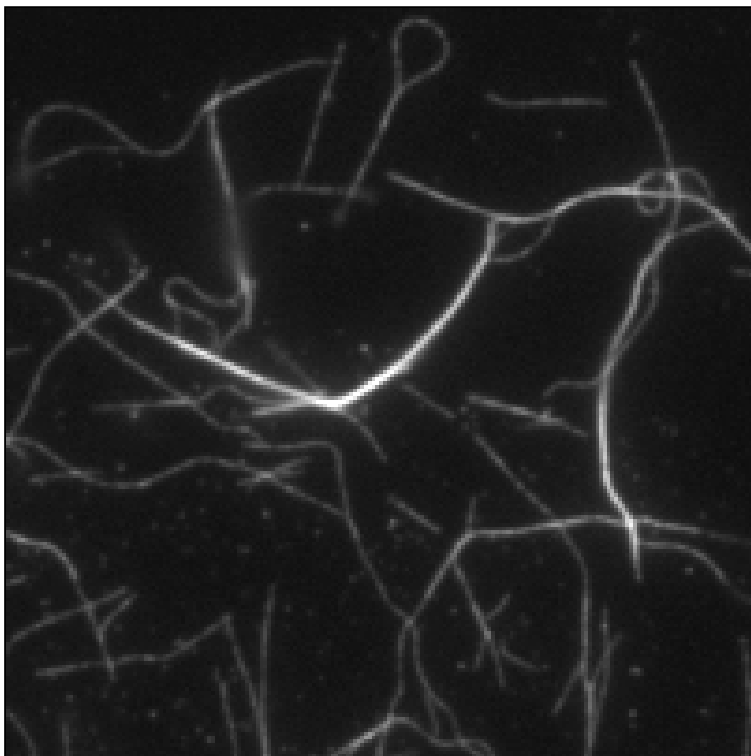
Movie S2(a)–(c)

Time-lapse movie showing the motility of the MT assembly formed through the AcSA process; the images were obtained using a fluorescence microscope. (a) $C_{\text{Tub}} = 13.4$ nM, $Bt/Tub = 1/16$, $St/Bt = 1/2$. (b) $C_{\text{Tub}} = 672$ nM, $Bt/Tub = 1/1$, $St/Bt = 1/2$. (c) $C_{\text{Tub}} = 3360$ nM, $Bt/Tub = 1/1$, $St/Bt = 1/2$. The full width of the view is $53\text{ }\mu\text{m}$. The movies 1(a), 1(b) and 1(c) correspond to the still images of (I), (II) and (III) in Figure 3(b), respectively. The images were recorded at 10s-interval. The time of the movie is accelerated 100-fold (1 s movie equals 100s experiment time).

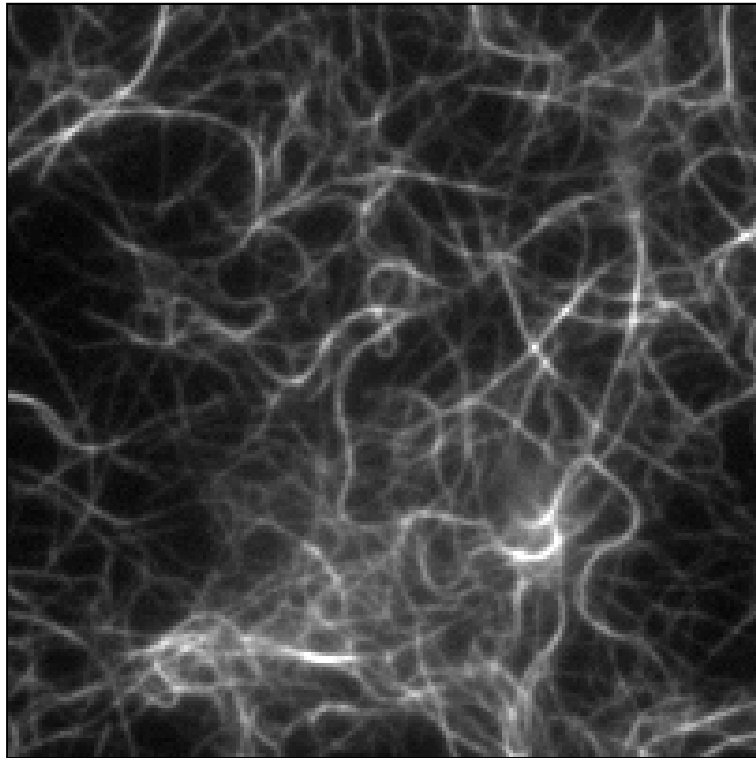
S2(a)



S2(b)



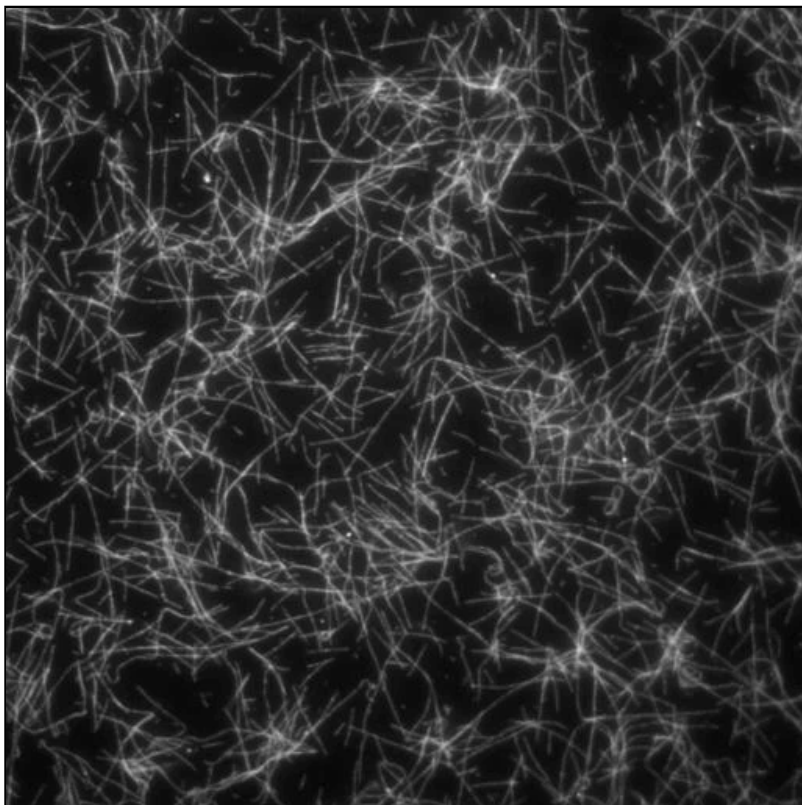
S2(c)



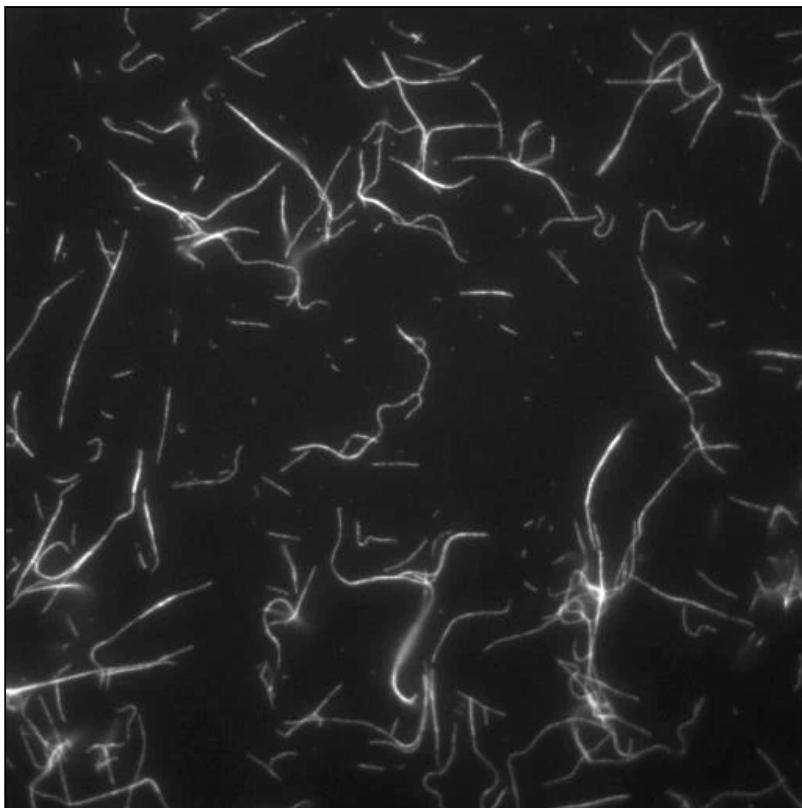
Movie S3(a)–(d)

Time-lapse movie showing the motility of the MT assembly formed through the AcSA process; the images were obtained using a fluorescence microscope. (a) $St/Bt = 1/256$. (b) $St/Bt = 1/16$. (c) $St/Bt = 1/8$. (d) $St/Bt = 1/64$. C_{Tub} and Bt/Tub are kept at a constant value as 672nM and 1/1, respectively. The full width of the view is 135 μm . The movies 2(a), 2(b), 2(c) and 2(d) correspond to the still images of (I), (II), (III) and (IV) in Figure 4(b), respectively. The time of the movie is accelerated 100-fold (1 s movie equals 100s experiment time).

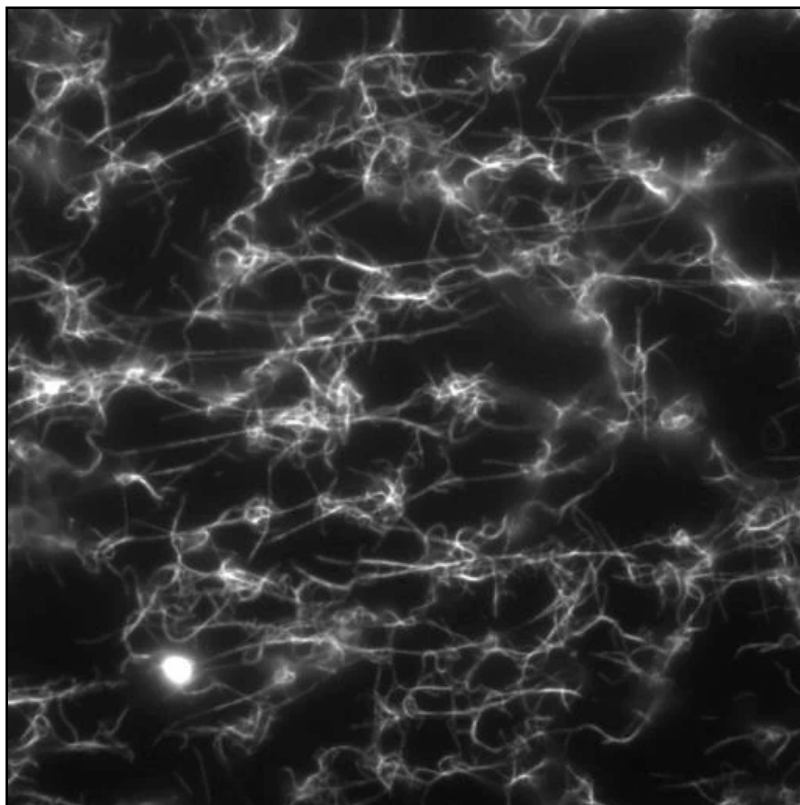
S3(a)



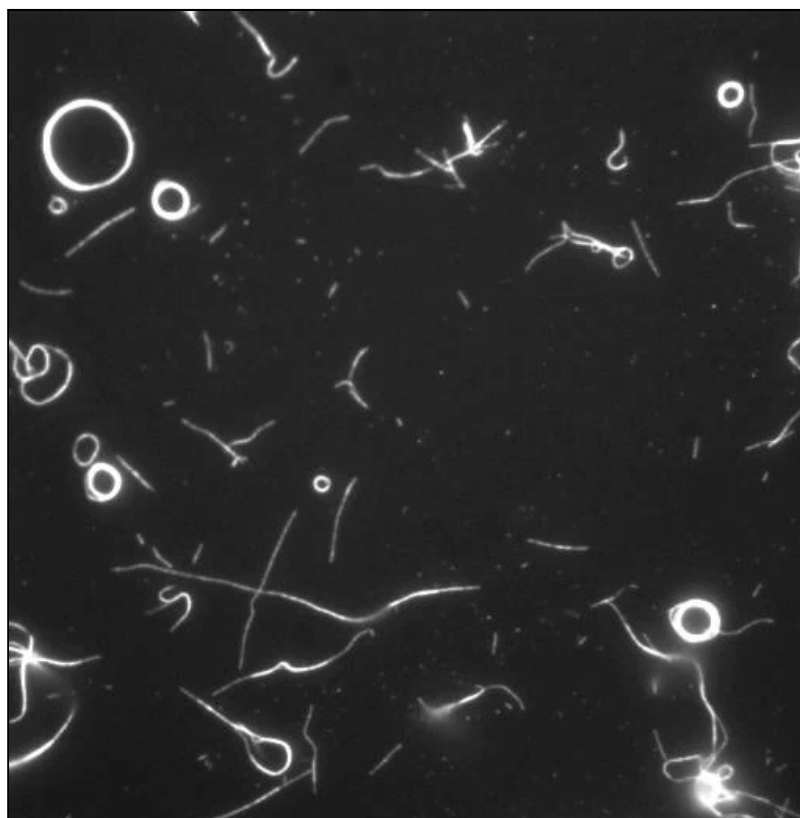
S3(b)



S3(c)



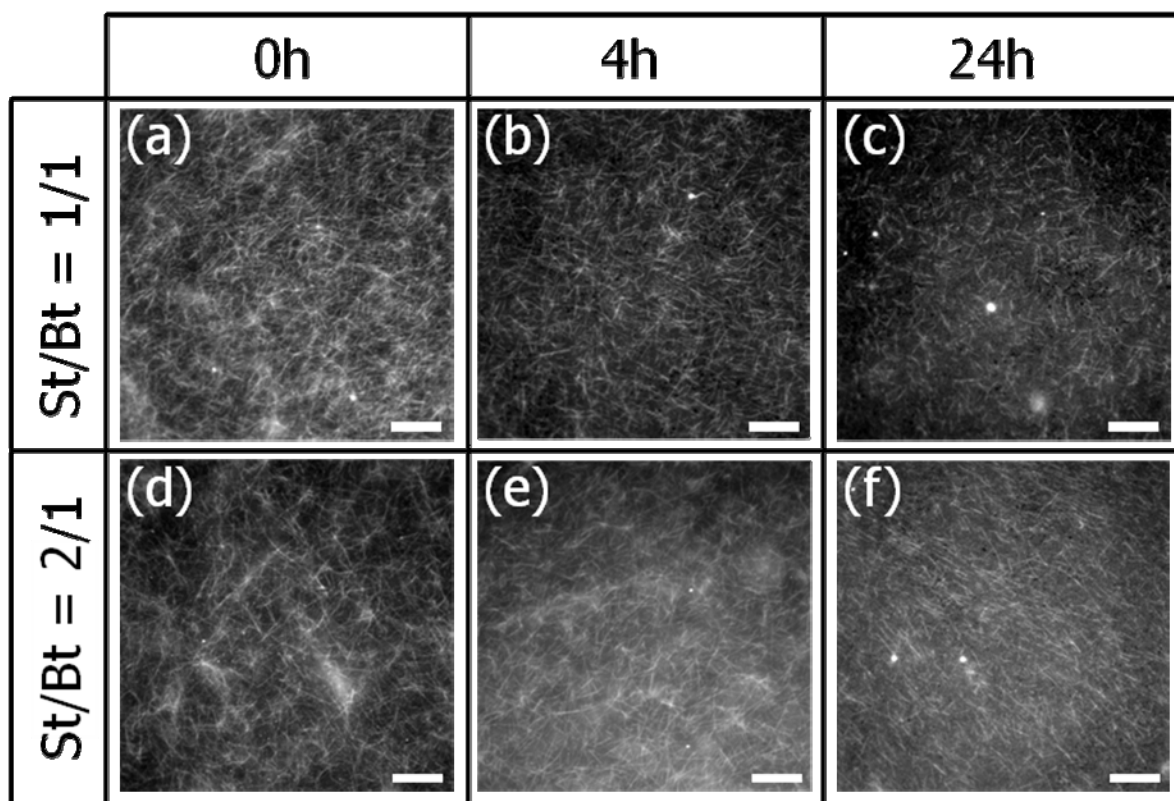
S3(d)



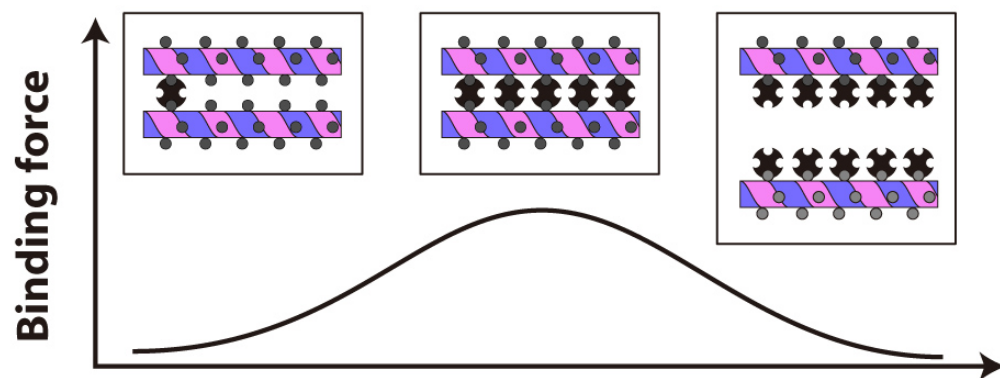
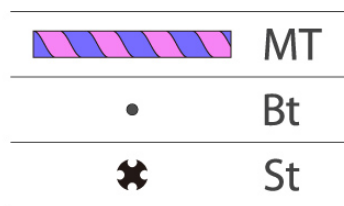
Passive self-assembly at St/Bt ratio of 1/1 – 2/1

Figure S4(a)–(g)

(a)–(f) Time evolution of MT assembly obtained through PaSA with high St/Bt ratio. The images were obtained using a fluorescence microscope. St/Bt ratios were 1/1 and 2/1 for (a–c) and (d–f). C_{Tub} and Bt/Tub are kept at a constant value as 3360 nM and 1/1, respectively; this is the same as the those conditions in Figure 2. Scale bar = 20 μm . (g) Predicted effect of St/Bt on the binding force. The axis of St/Bt of Fig. 5(Binding Force) was extended based on the result shown in (a–f). At St/Bt ratio of 1/1 or higher, no bundle was formed suggesting that the existence of peak in the binding force against St/Bt ratio.



(g)



Discussion of the bundle formation mechanism in the kinesin-St/Bt-MT system

To understand the formation of MT assembly under the competition between the attractive interaction (St/Bt) and the driving force (kinesins), we discuss the formation mechanism based on a general scheme of nucleation and growth process. As shown in Figures 3(a) and 4(a), there exists a critical MT concentration or St/Bt ratio for the formation of MT bundles. Usually, an increase in the concentration of components or binding energy between the components facilitates nucleus formation. After the formation of the nucleus, the thermodynamically favorable growth process proceeds. To discuss the bundle formation in a free energy argument, we can consider that a bundle has length L and radius R consisting of N MTs with length L and radius r . Therefore, the free energy for bundle formation, ΔG , can be expressed as follows:

$$\Delta G = -\alpha V + \beta S \quad (1)$$

where α is the energy gain due to bundle formation of MTs per volume and β is the surface energy density. As discussed later, α is related to the number of St and Bt sites, and β is assumed to be constant. V and S are the volume and surface of the bundle, respectively. Here, V and S are described as follows:

$$V = \pi R^2 L \approx N \pi r^2 L \quad \text{and} \quad (2)$$

$$S = 2\pi R L + 2\pi R^2 \approx 2\pi R L = 2\pi N^{1/2} r L \quad (3)$$

where R is the radius of the bundle that is related to the radius r of MT as $R \approx N^{1/2} r$. Here, the end effect of the bundle is neglected. Therefore, ΔG is derived as follows:

$$\Delta G \approx -\alpha N \pi r^2 L + 2\beta \pi N^{1/2} r L \quad (4)$$

The critical N , N^* , is determined from $\partial(\Delta G)/\partial N = 0$ as follows:

$$N^* = \left(\frac{\beta}{\alpha r} \right)^2 \quad (5)$$

On the other hand, the critical radius R^* and the height of the energy barrier ΔG^* can be obtained as follows:

$$R^* \propto (N^*)^{1/2} = \frac{\beta}{\alpha r} \quad \text{and} \quad (6)$$

$$\Delta G^* \approx \frac{\pi \beta^2}{\alpha} L \quad (7)$$

Next, we should consider the term α . First, as discussed in Figure 5, the binding force for forming MT bundles is correlated with the St/Bt ratio, and this part would be roughly described as follows:

$$\alpha_{\text{binding}} \approx \varepsilon n x^2 \quad (8)$$

where ε and n are the binding energy per St/Bt site and the density of the binding sites, respectively, and $x = \text{St/Bt}$. This relationship is maintained within the range of $x < 1/2$. On the other hand, as shown in Figure 2(d–f), growth of the MT bundles was observed even after 24 h of PaSA, and this resulted in thicker bundles compared to the bundles obtained through AcSA. This result indicates that the contribution of the kinesin driving force exerted on the MT may not be negligible. In fact, it was observed that thin bundles were split during sliding on the substrate. Thus, the driving force arising from kinesins would disturb bundle formation. The contribution of the driving force for disturbing bundle formation can be described as follows:

$$\alpha_{\text{motor}} \approx -\delta m (x - 1/2)^2 \quad (9)$$

where δ is a positive constant and m is a density of motor interacting on MT. At $x \sim 0$,

the contribution of the driving force becomes maximal, while it decreases with increased St/Bt ratios. At $x = 1/2$, the contribution becomes minimal. Therefore, α could be expressed as the sum of α_{binding} and α_{motor} as follows:

$$\alpha \approx -\delta m (x - 1/2)^2 + \varepsilon n x^2 \quad (10)$$

Thus, ΔG^* can be calculated as follows:

$$\Delta G^* \approx \frac{\pi \beta^2}{-\delta m (x - 1/2)^2 + \varepsilon n x^2} L \quad (11)$$

Eq. (11) explains the dependence of ΔG^* on St/Bt. Here, ε is assumed to be almost comparable or slightly larger than δ^{25} . When $x \sim 0$, the denominator becomes $\sim \delta m$, and then ΔG^* should be much larger than $k_B T$, resulting in suppressed bundle formation. On the other hand, when $x > 0$, the denominator becomes larger with increase of x , and there is the condition in which ΔG^* becomes comparable to or smaller than $k_B T$. The factors ε and δ will be experimentally determined in the near future.