Electronic Supplementary Material (ESI) for Soft Matter

Supporting Information

Growth of Ring-Shaped Microtubule Assemblies through Stepwise Active Self-Organisation

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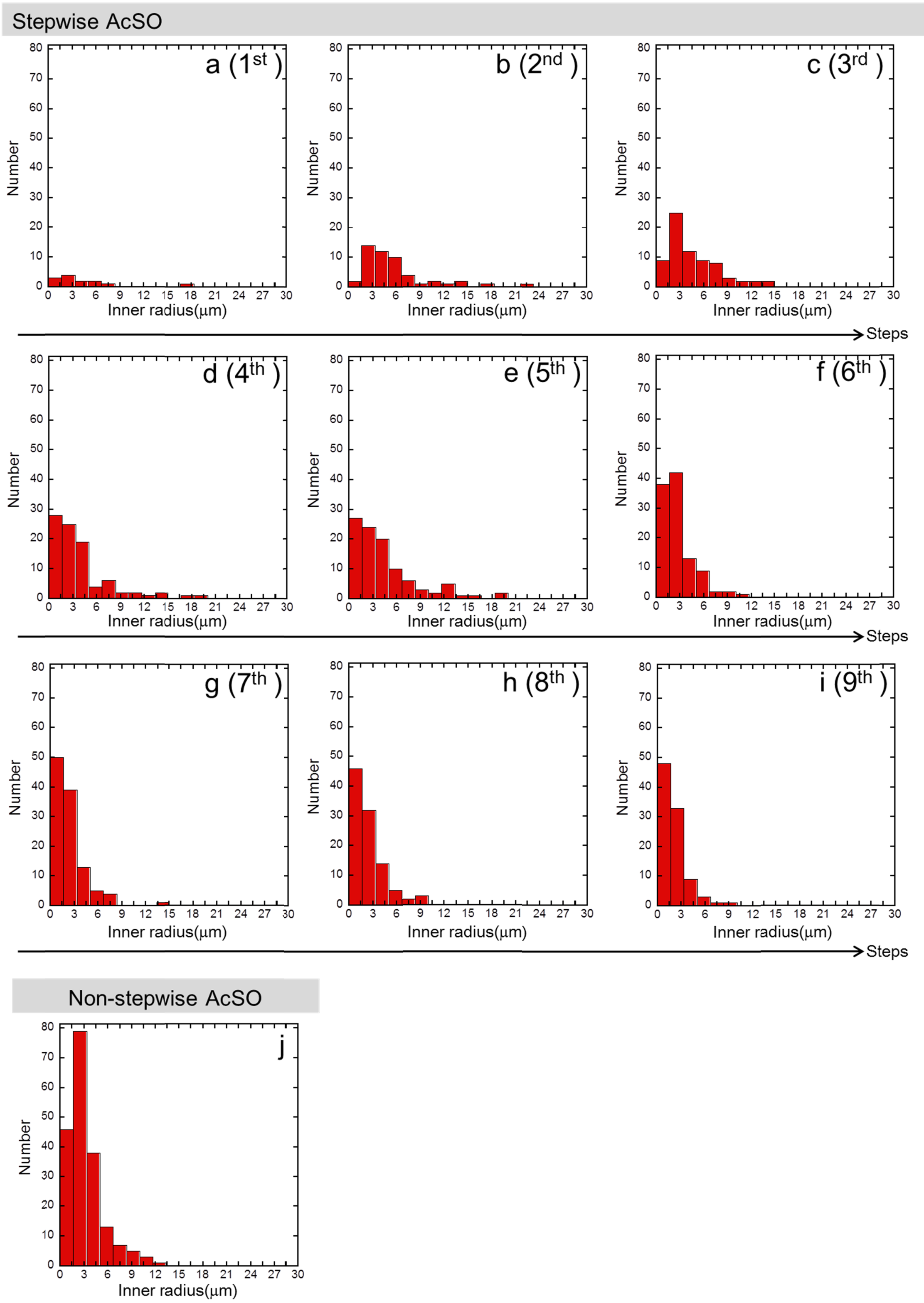
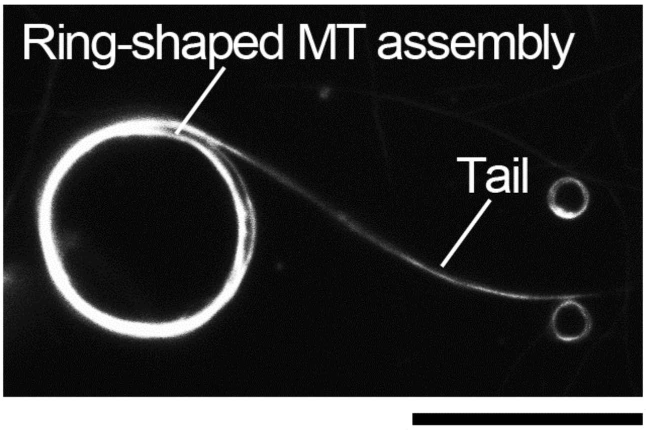


Supporting Figure 1: Inner radius distribution of ring-shaped MTs observed after performing the $1^{st} \sim 9^{th}$ stepwise AcSOs (a~i) and the non-stepwise (j) AcSO.



Supporting Figure 2: Representative fluorescence image of ring-shaped MT assembly with tails observed after performing stepwise AcSO. Scale bar; 25 μm.

Theoretical model of stepwise AcSO

We would like to discuss the theoretical model of stepwise AcSO both prior to and after reaching a thermodynamically stable steady state.

First, we would like to consider the model for the former situation that is based on Supporting Figure 3, which illustrates Supporting Figure 2 in more detail. The figure shows that the MT bundles and the ring-shaped MT assemblies with tails were randomly distributed on a surface. Before discussing stepwise AcSO, we define the length of the time between the addition of new MT filaments and the nucleation of rings as $t_{1/2}$, the average velocity of the MT filaments as v, the distance at time $t = 0$ between the centre of a MT filament and the i-th ring shaped MT assembly as x_i, the size of the i-th ring-shaped MT assembly including the tail as R_i, the plane angle of the i-th ring-shaped MT assembly from the centre of the MT as θ_i and $i = 1, 2, 3, \cdots, i, i+1, \cdots$.

In the 1st step, we assume that the number of added MTs and ring-shaped MT assemblies at the 1st AcSO are n_1 and $N_{\text{1stAcSO}}^{\text{1st step}}$, respectively. If individual MTs form rings independently, then we obtain the following relation

$$n_1 = N_{\text{1stAcSO}}^{\text{1st step}} \tag{S1}$$

This relation corresponds to the area of the oval in the 1st step in Supporting Figure 4.

In the 2nd step, some ring-shaped MT assemblies of the 1st AcSO interact with the newly added MT filaments (probability; $p_{\text{2nd AcSO}}^{\text{2nd step}}$), and the others remain separate. In the scheme of the 2nd step in Supporting Figure 4, the overlapping area corresponds to the number of interacting ring-shaped MT assemblies. Then, if we add MT filaments (n_2), the number of the ring-shaped MT assemblies including those from the 1st ($N_{\text{1st AcSO}}^{\text{2nd step}}$) and 2nd ($N_{\text{2nd AcSO}}^{\text{2nd step}}$) AcSO is $N_{\text{all AcSO}}^{\text{2nd step}}$:

$$N_{\text{all AcSO}}^{\text{2nd step}} = n_1 + n_2 - N_{\text{2nd AcSO}}^{\text{2nd step}} \tag{S2}$$

$$= \left(n_1 - N_{\text{1st AcSO}}^{\text{2nd step}}\right) + \left(n_2 - N_{\text{2nd AcSO}}^{\text{2nd step}}\right) + N_{\text{2nd AcSO}}^{\text{2nd step}} \tag{S3}$$

$$= \left(n_1 - p_{\text{1st AcSO}}^{\text{2nd step}} n_2\right) + \left(n_2 - p_{\text{2nd AcSO}}^{\text{2nd step}} n_2\right) + p_{\text{2nd AcSO}}^{\text{2nd step}} n_2 \tag{S4}$$

where

$$N_{\text{2nd AcSO}}^{\text{2nd step}} = p_{\text{2nd AcSO}}^{\text{2nd step}} n_2 \tag{S5}$$

The first and second terms in eq. (S3) are the number of ring-shaped MT assemblies of the 1st and 2nd AcSO, respectively. The third term is the ring-shaped MT assemblies of the 2nd AcSO, representing the overlapping area. Assuming that $n_1 = n_2$ for simplicity,

$$N_{\text{all AcSO}}^{\text{2nd step}} = 2n_2\left(1 - p_{\text{2nd AcSO}}^{\text{2nd step}}\right) + p_{\text{2nd AcSO}}^{\text{2nd step}} n_2 \tag{S6}$$

$$= N_{\text{1st AcSO}}^{\text{2nd step}} + N_{\text{2nd AcSO}}^{\text{2nd step}} \tag{S7}$$

$$= n_2(2 - p_{\text{2nd AcSO}}^{\text{2nd step}}) \tag{S8}$$

where

$$N_{\text{1st AcSO}}^{\text{2nd step}} = 2n_2\left(1 - p_{\text{2nd AcSO}}^{\text{2nd step}}\right) \tag{S9}$$

Ideally, it is possible to determine $p_{\text{2nd AcSO}}^{\text{2nd step}}$ from the observed $N_{\text{all AcSO}}^{\text{2nd step}}$, n_1 and n_2.

At this point, it is required to consider $p_{\text{2nd AcSO}}^{\text{2nd step}}$. This value would be proportional to the number of collisions of newly added MTs filaments with the ring-shaped MT assemblies of the 1st AcSO, and the collision probability is closely related to the total cross section area around a MT filament or a bundle in the range of $vt_{1/2}$. This probability is proportional to both the number and size of ring-shaped MT assemblies. Now, we adopt the sum of the plane angles of the ring-shaped MT assemblies to describe the probability of collision as

$$p_{\text{2nd AcSO}}^{\text{2nd step}} = \sum_i \frac{\theta_i}{2\pi} \tag{S10}$$

where i and θ_i are directly related to the number and the size of ring-shaped MT assemblies, respectively.

$$\theta_i = \frac{R_i}{2\pi x_i} \tag{S11}$$

Because $p_{\text{2nd AcSO}}^{\text{2nd step}}$ in eq. (S10) is the sum of the length ratios (diameter of the ring-shaped MT assemblies to the circumference of the circle $2\pi x_i$), it is also equal to the sum of the square root of the area ratios of the ring-shaped MT assemblies to the circle in the range of $vt_{1/2}$ (dotted line).

$$p_{\text{2nd AcSO}}^{\text{2nd step}} \sim \sum_i \sqrt{\frac{\pi(R_i/2)^2}{\pi(vt_{1/2})^2}} = \sum_i \frac{R_i}{2vt_{1/2}} \tag{S12}$$

Eq. (S12) explains why higher-order AcSOs are performed in a stepwise manner prior to reaching a thermodynamically stable steady state rather than when reaching equilibrium. As shown in Supporting Figure 2, the lengths of the tails are longer than the diameters of the ring-shaped MT assemblies. Therefore, it is expected that the probability increases by several times or more above that of the ring-shaped MT assemblies without tails.

In the 3rd step, some of the newly added MT filaments interact with some of the ring-shaped MT assemblies of both the 1st AcSO (the probability of formation of new ring-shaped MT assemblies of 2nd AcSOs is $p_{\text{new 2nd AcSO}}^{\text{3rd step}}$) and the 2nd AcSO (the probability of formation of that of 3rd AcSO is $p_{\text{new 3rd AcSO}}^{\text{3rd step}}$), while other rings do not interact From the scheme of the 3rd step in Supporting Figure 4, the total number of ring-shaped MT assemblies in the 3rd step, $N_{\text{all AcSO}}^{\text{3rd step}}$, is

$$N_{\text{all AcSO}}^{\text{3rd step}} = N_{\text{1st AcSO}}^{\text{2nd step}} + N_{\text{2nd AcSO}}^{\text{2nd step}} + n_3 - N_{\text{new 2nd AcSO}}^{\text{3rd step}} - N_{\text{new 3rd AcSO}}^{\text{3rd step}} \quad \text{(S13)}$$

$$= \left(N_{\text{1st AcSO}}^{\text{2nd step}} - N_{\text{new 2nd AcSO}}^{\text{3rd step}}\right) + \left(N_{\text{2nd AcSO}}^{\text{2nd step}} - N_{\text{new 3rd AcSO}}^{\text{3rd step}}\right) + n_3 \quad \text{(S14)}$$

$$= \left(N_{\text{1st AcSO}}^{\text{2nd step}} - p_{\text{new 2nd AcSO}}^{\text{3rd step}} n_3\right) + \left(N_{\text{2nd AcSO}}^{\text{2nd step}} - p_{\text{new 3rd AcSO}}^{\text{3rd step}} n_3\right) + n_3 \quad \text{(S15)}$$

$$= N_{\text{1st AcSO}}^{\text{3rd step}} + N_{\text{2nd AcSO}}^{\text{3rd step}} + N_{\text{3rd AcSO}}^{\text{3rd step}} \quad \text{(S16)}$$

where

$$N_{\text{new 2nd AcSO}}^{\text{3rd step}} = p_{\text{new 2nd AcSO}}^{\text{3rd step}} n_3 \quad \text{(S17)}$$

$$N_{\text{new 3rd AcSO}}^{\text{3rd step}} = p_{\text{new 3rd AcSO}}^{\text{3rd step}} n_3 \quad \text{(S18)}$$

and

$$N_{\text{1st AcSO}}^{\text{3rd step}} = N_{\text{1st AcSO}}^{\text{2nd step}} + n_3 - p_{\text{new 2nd AcSO}}^{\text{3rd step}} n_3 - p_{\text{new 3rd AcSO}}^{\text{3rd step}} n_3 \quad \text{(S19)}$$

$$N_{\text{2nd AcSO}}^{\text{3rd step}} = N_{\text{2nd AcSO}}^{\text{2nd step}} + p_{\text{new 2nd AcSO}}^{\text{3rd step}} n_3 - p_{\text{new 3rd AcSO}}^{\text{3rd step}} n_3 \quad \text{(S20)}$$

$$N_{\text{3rd AcSO}}^{\text{3rd step}} = p_{\text{new 3rd AcSO}}^{\text{3rd step}} n_3 \quad \text{(S21)}$$

The sum of Eqs. (19) ~ (21) is equal to eq. (S15). The probability of collision in the 3rd step, $p^{\text{3rd step}}$, corresponds to the sum of $p_{\text{1st AcSO}}^{\text{3rd step}}$ and $p_{\text{2nd AcSO}}^{\text{3rd step}}$.

$$p^{\text{3rd step}} = \sum_i \frac{R_i}{2vt_{\frac{1}{2}}} = p_{\text{2nd AcSO}}^{\text{3rd step}} + p_{\text{3rd AcSO}}^{\text{3rd step}}$$

$$= \left[\frac{N_{\text{1st AcSO}}^{\text{2nd step}}}{N_{\text{all AcSO}}^{\text{2nd step}}} + \frac{N_{\text{2nd AcSO}}^{\text{2nd step}}}{N_{\text{all AcSO}}^{\text{2nd step}}}\right] p^{\text{3rd step}} \quad \text{(S16)}$$

Using eqs. (S5), (S8) and (S9), we obtain

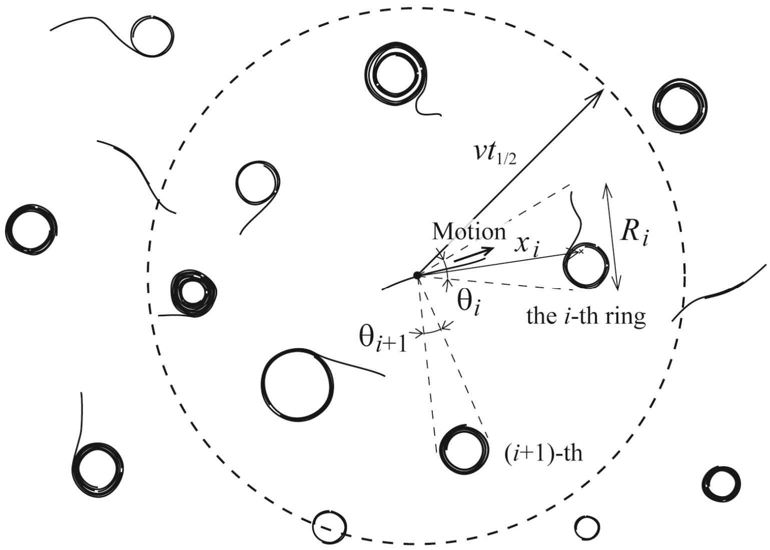
$$p^{\text{3rd step}} = \left[\frac{2\left(1-p_{\text{2nd AcSO}}^{\text{2nd step}}\right)}{\left(2-p_{\text{2nd AcSO}}^{\text{2nd step}}\right)} + \frac{p_{\text{2nd AcSO}}^{\text{2nd step}}}{\left(2-p_{\text{2nd AcSO}}^{\text{2nd step}}\right)}\right] p^{\text{3rd step}} \quad \text{(S17)}$$

$p^{\text{3rd step}}$ could be determined under ideal conditions. Because the number of ring-shaped MT assemblies with tails increases in a stepwise manner, i.e., due to the increase in $\sum_i R_i$ in eq. (S10), the probability of collision also increases.

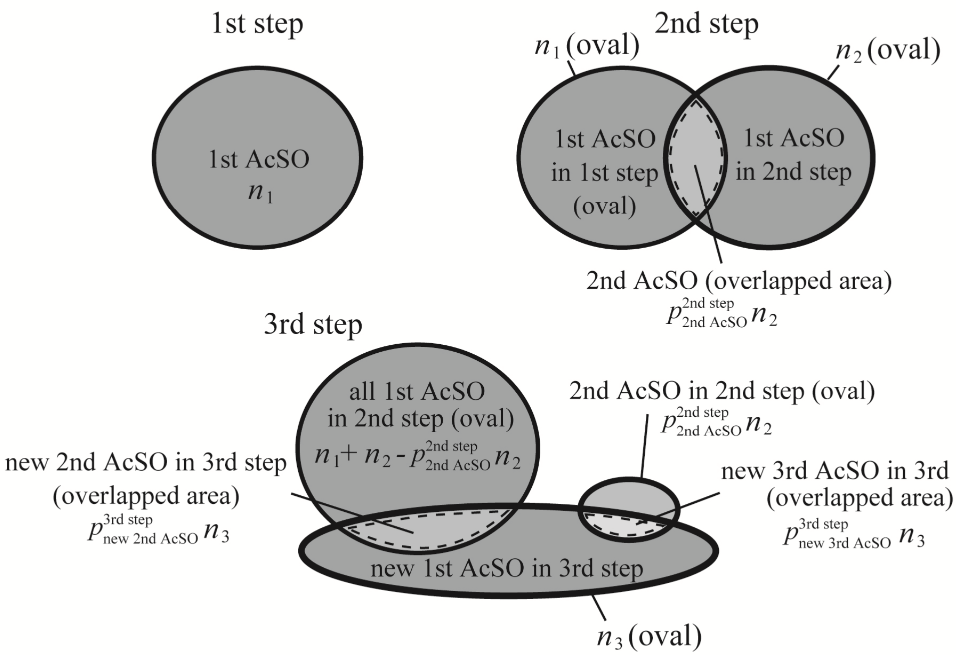
$$p^{\text{3rd step}} > p_{\text{2nd AcSO}}^{\text{2nd step}} \quad \text{(S18)}$$

Obviously, this scenario roughly explains our experimentally observed trend that thicker ring-shaped MT assemblies are formed by stepwise AcSO prior to equilibrium.

Thus far we have discussed the model until the 3rd step, and extending the model for further stepwise AcSOs is possible but is not shown here.



Supporting Figure 3: Schematic representation of stepwise AcSO prior to reaching a stable steady state.



Supporting Figure 4: Scheme for illustrating the formation of thicker ring-shaped MT assemblies in stepwise AcSO. The overlapping area represents the generation of thicker ring-shaped MT assemblies from the interactions between newly added MT filaments or newly formed MT bundles and preformed ring-shaped MT assemblies.

At this point, we further discuss the explanations for the following: ring-shaped MT assemblies are more easily formed from MT bundles than from single MT filaments; thicker ring-shaped MT assemblies cannot be formed in non-stepwise AcSO; higher-order AcSOs are not formed in a stepwise manner at equilibrium; and thicker ring-shaped MT assemblies are easily formed in a stepwise manner prior to reaching a stable steady state.

To highlight the essence of the explanation, we discuss the reasons that are based on the free-energy profile of a first-order phase transition, i.e., the nucleation and growth mechanism from the Ginzburg-Landau[1] (GL) theory using an order parameter, in which two stable states corresponding to the dispersed and condensed state are separated by an energy barrier of ΔE in height, as shown in Supporting Figure 5. We adopt the normalised density of MTs, $\bar{\rho}$, as the order parameter, which is roughly defined as

$$\bar{\rho} = \frac{d_{\mathrm{ring}}^2}{R^2} \tag{S19}$$

where R and d_{ring} are the size of a MT (a bundle, ring-shaped MT assembly, or intermediate state, such as a ring-shaped MT assembly with a tail) and the diameter of the assembly, respectively. $\bar{\rho}\sim 1$ is for ring-shaped MT assemblies, $\bar{\rho}\sim 0$ is for MT bundles, and $0 < \bar{\rho} < 1$ for the intermediate state. In the intermediate state, the $\bar{\rho}$ of the bundle interacting with the assembly is slightly larger than the $\bar{\rho}$ of the nucleus. Therefore, self-assembly occurs spontaneously. The nucleation rate J is

$$J = J_0 \exp(-\Delta E / k_B T) \tag{S20}$$

where J_0 is the prefactor, which reflects the collision probability, and ΔE is the height of the energy barrier. J_0 could be described in terms of eqs. (S10) and (S12) as

$$J_0 \propto \sum_i \frac{\theta_i}{2\pi}, \sum_i \frac{R_i}{2vt_{1/2}} \tag{S21}$$

The characteristic time scale $t_{1/2}$ can be related to J as

$$t_{1/2} \sim J^{-1} \propto \exp(\Delta E / k_B T) \tag{S22}$$

These equations indicate that the time during which new MT filaments are added is short if ΔE is small. In the experiments, we observed that MT bundles more easily formed ring-shaped MT assemblies than did single MT filaments. This finding can be explained by ΔE, which is determined by the competition between the surface and volume energies. In the assemblies formed from fine bundles, the surface energy is less than that of the single MT filaments; therefore, ΔE is small. In contrast, thicker ring-shaped MT assemblies were not formed in the non-stepwise AcSO because the collisions among bundles and the assemblies occurred simultaneously within $t_{1/2}$. Supporting Figure 5(a) explains the above discussion.

Thicker ring-shaped MT assemblies were not observed when new MT filaments were introduced at steady state because the J_0 of the ring-shaped MT assemblies without tails is small, as discussed in eqs. (S10) and (S12), making collisions within $t_{1/2}$ difficult, which is illustrated in Supporting Figure 5(b). In contrast, the thicker ring-shaped MTs assemblies form prior to reaching steady state because the ring-shaped MTs with tails enhance the probability of collision with MT filaments in a finite time period, which is illustrated in Supporting Figure 5(c).

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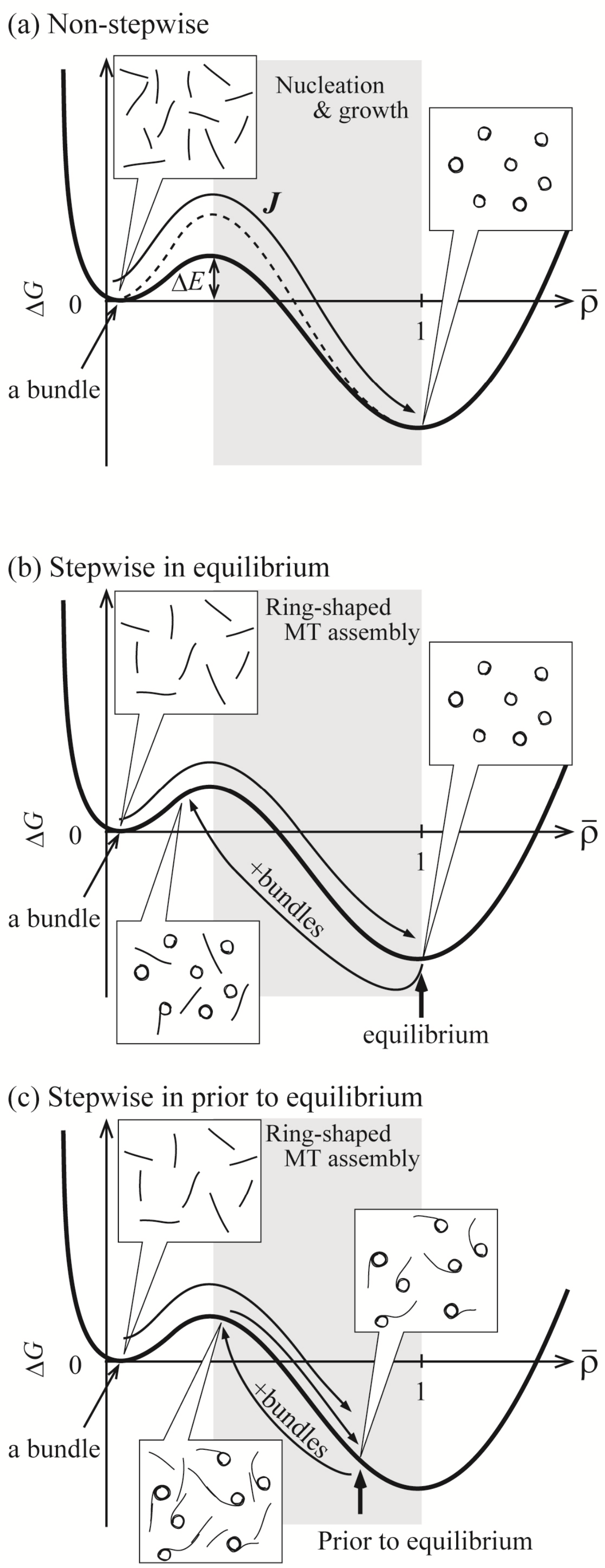

Supporting Figure 5: Schematic representations of the free-energy profiles of nucleation and growth in a plot of the free-energy difference ΔG vs. the normalised density $\bar{\rho}$ from the Ginzburg-Landau theory. The profiles of the AcSOs in the non-stepwise manner (a), stepwise manner in equilibrium (b) and prior to equilibrium (c) are illustrated. The solid and dashed profiles in (a) indicate the profiles from a single MT filament to a ring-shaped MT assembly and from a MT bundle to a ring-shaped MT assembly, respectively. The shaded areas are the parameter conditions that are suitable for nucleation and growth of ring-shaped MT assembly.

Reference:

1. V. L. Ginzburg and L. D. Landau, *Zh. Eksp. Teor. Fiz.* 1950, **20**, 1064.