Rapid Assembly and Collective Behavior of Microtubule Bundles in the Presence of Polyamines

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SUPPLEMENTARY MATERIALS

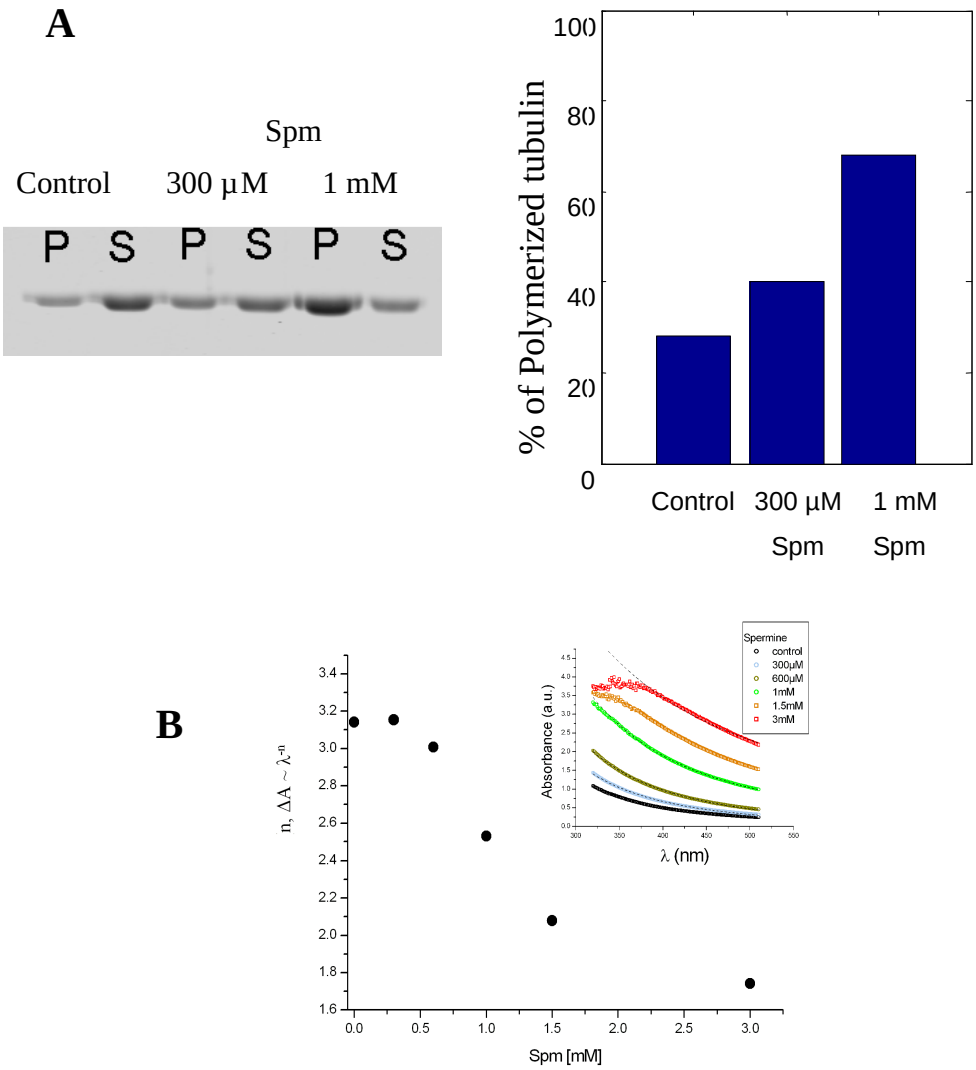
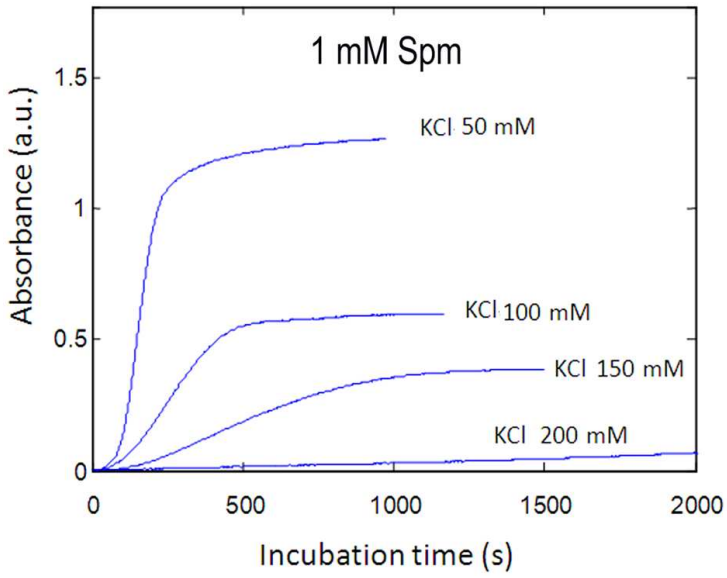


Figure S1 (A): SDS/PAGE analyses of MT assembly in the absence or presence of Spm. After completion of MT polymerization, the samples were centrifuged at 25,000 × g for 20 min to separate MTs (pellet, P) and free tubulin (supernatant, S). In the presence of Spm, the mass of polymerized tubulin increases at the expense of free tubulin. For 1 mM Spm, the mass of MTs increases by factor of 2. This cannot account for the more significant increase of the plateau value observed in figure 3A (8 times for 1 mM Spm).

(B): Power law dependence of the sample absorbance on light wavelength, *n*, versus Spm concentration. Tubulin assembly assays were performed in the presence of Spm at 37°C for 30 min (polymerization was complete). Inset: curves showing the absorbance variations versus wavelength for various Spm concentrations, were used to estimate *n*. As *n* decreases with Spm concentrations, larger structures (MT bundles) than thin rods (MTs) most probably diffracted light under such conditions.

A



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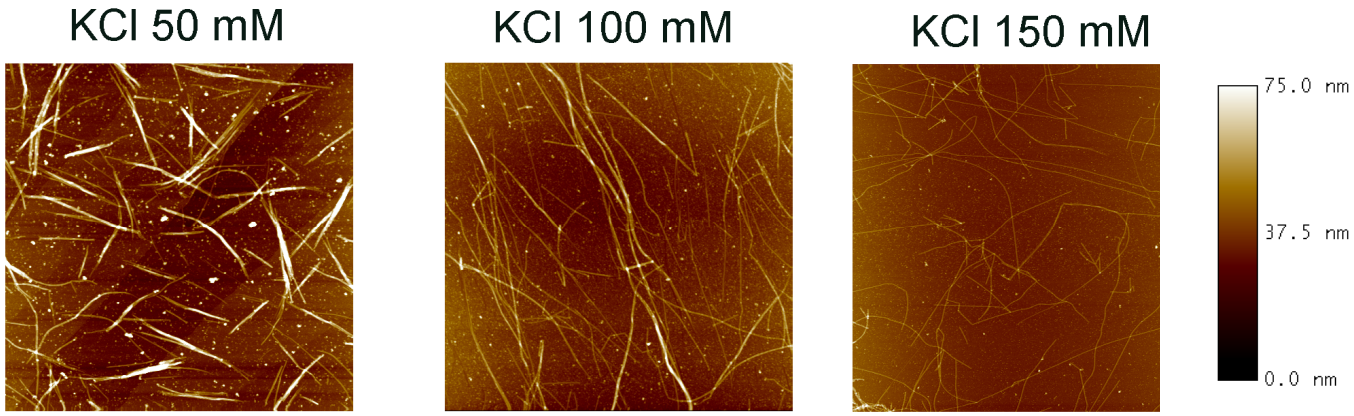
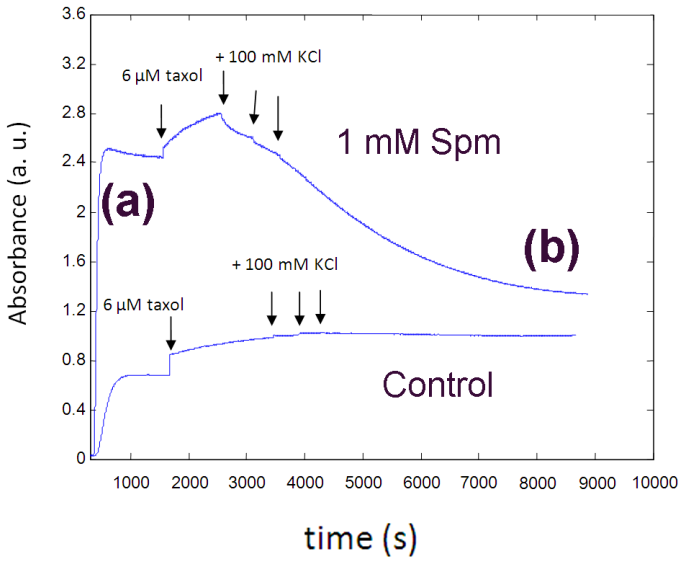


Figure S2: (A) Tubulin assembly (16 µM tubulin) in the presence of 1mM Spm at various KCl concentrations in 50 mM MES-KOH pH 6.8, 0.5 mM EGTA, 4 mM $MgCl_2$, 0.6 mM GTP, 20% glycerol. (B) AFM images of corresponding samples observed after reaching the plateau value. Scanned area: 15×15 µm².

Increasing ionic strength leads to the formation of thinner and longer bundles. For KCl concentrations higher than 100 mM, only isolated MTs were detected.

A



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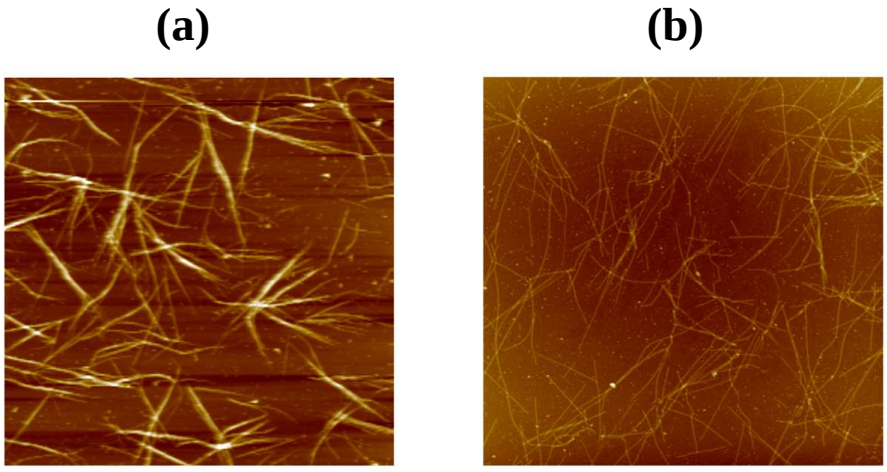
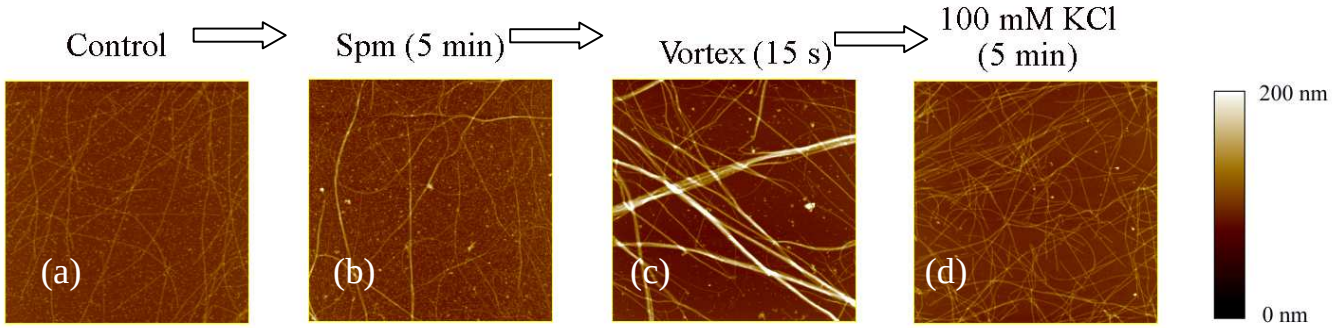


Fig. S3: MTs can be released from bundles upon successive additions of KCl.
(A) Tubulin samples (40 µM tubulin) were first assembled in the presence or absence of Spm and then taxol-stabilized to prevent MTs depolymerization upon addition of KCl. 100 mM KCl was added in successive steps at indicated times (300 mM total). We note that, in contrast with control (same procedure without Spm), the absorbance value decreases upon addition of salt, as expected from the release of MTs from bundles into bulk solution.
(B) AFM imaging of MT bundles resulting from tubulin assembly with 1 mM Spm **(a)** and after the end of the dissociation process **(b)**, as noted in (A). At the end of the process **(b)**, bundles were no longer observed and, instead, isolated MTs were detected thus attesting the MT release from bundles. Scanned area: 15×15 µm².

(A)



(B)

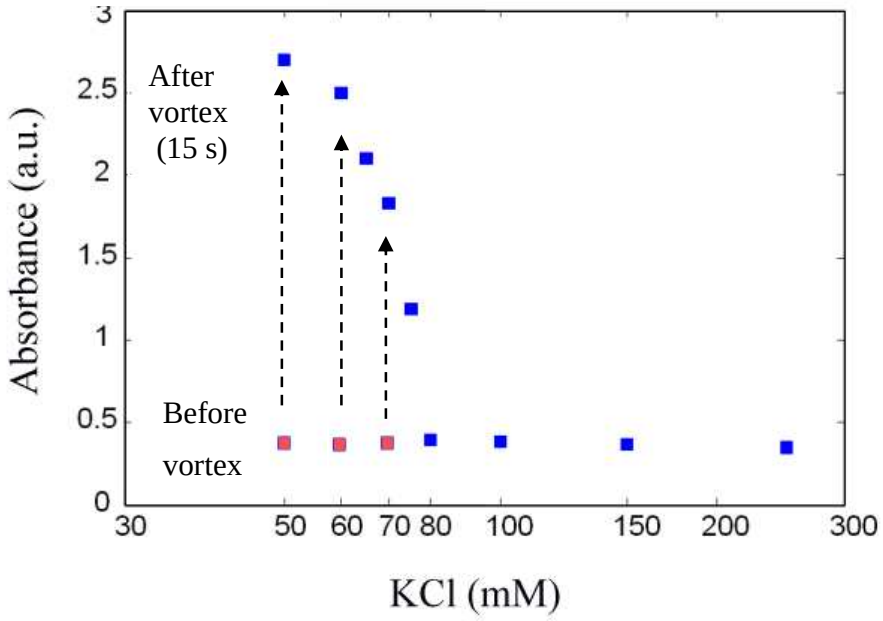


Figure S4: Vortexing promotes MT bundling of preformed MTs
(A) (a) Taxol-stabilized MTs were preformed (5 µM taxol and 30 µM tubulin); (b) Spm (3mM) was added to the sample. We observed a tendency for MTs to form bundles but this process is hindered, the rotational diffusion of long MTs being blocked by the 3D MT network; (c) Samples were then vortexed for 15 s and thick MT bundles were then detected by AFM. Vortexing thus favors MT mobility (or MT breakage which, in turns, increases diffusion) and thus the formation of MT bundles; (d) Addition of 100 mM KCl leads to the disappearance of MT bundles, which indicates that the bundling process is reversible and of electrostatic origin.
(B) Bundle formation assessed by turbidimetry (λ=370 nm) with 3 mM Spm and 30 µM tubulin. Taxol-stabilized MT were first preformed in the absence of Spm. 3 mM Spm and KCl at the indicated concentrations were added to the sample without vortexing. Whatever the concentration of KCl, we do not observe a significant increase of absorbance after 10 min incubation. The samples were then vortexed for 15 s and a large increase in absorbance was then observed for the lowest concentrations of KCl (<80 mM). MT diffusion is then a major obstacle for MTs to form thick bundles.

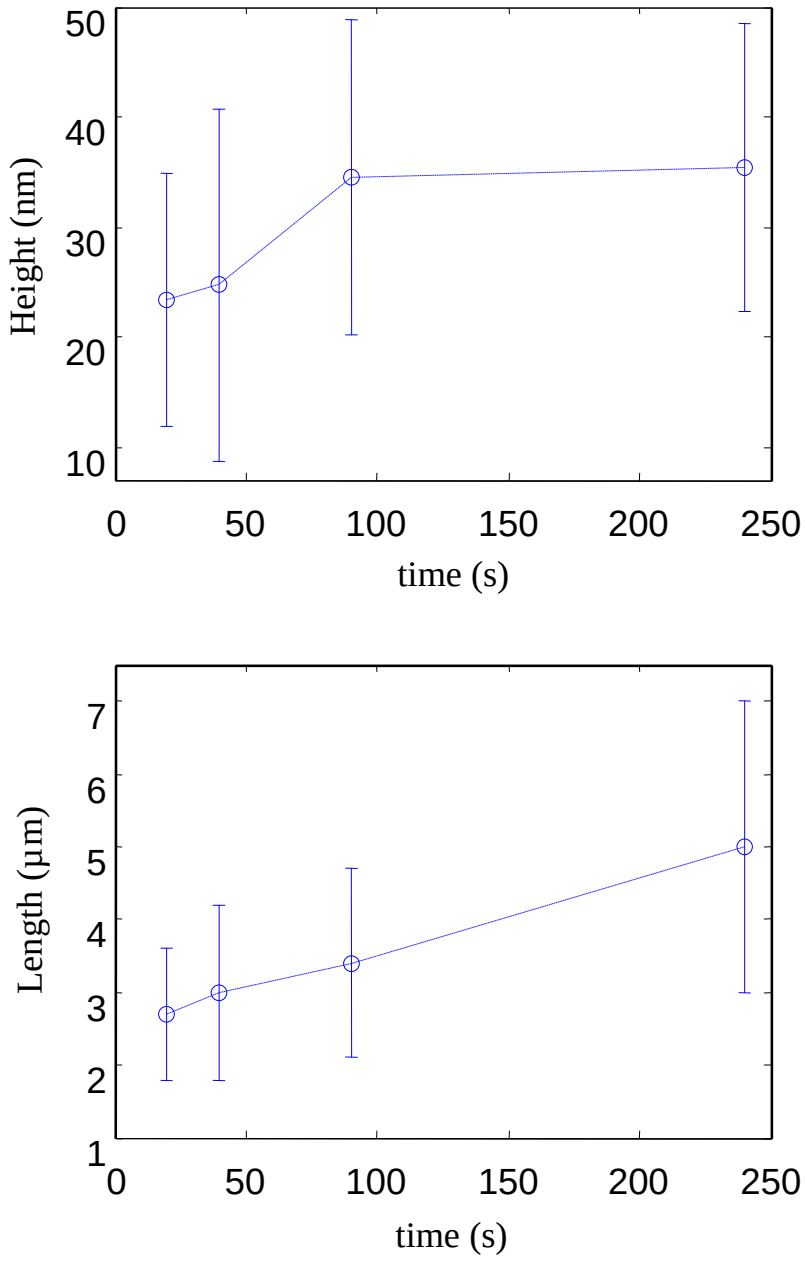


Figure S5: Mean value of bundle height and length versus incubation time extracted from the experimental data presented in fig.4B. Error bars: standard deviations.

TEXT 1: interaction of polyamines with tubulin and MTs

An electrostatic attraction between two anionic bodies may be at first sight counterintuitive since like-charged bodies rather tend to experience self-repulsion. Self-attraction requires the presence of cationic partners which, in the case of small and highly mobile multivalent cations like polyamines, form a liquid phase of strongly correlated counterions on anionic surfaces (1) like that of DNA (2), actin (3) or MTs (4). When two anionic bodies approach each other, new counterion correlations take place at the interface between them and thus lead to an energy gain, such mechanism being more generally viewed as a sharing of counterions. There are many examples for its existence, among them DNA condensation (5) and bundling of cytoskeletal structures like MTs (4) and actin filaments (6). Whereas DNA could be considered as homogenously charged, which simplifies the theoretical treatment of the attraction force, MTs present some peculiarities that need to be taken into account. Indeed, αβ-tubulin heterodimers, the building blocks of MTs, have two highly negatively charged C-terminal tails, one for each monomer. At least 40 % of the whole tubulin charge is concentrated in the last stretch of the C-terminal tail (7, 8), leading to a linear charge density of about 12 e^- /2-3 nm. Such a concentration of negative charges is highly favorable to generate a self-attraction force in the presence of polyamines, while the remaining negative charges of the tubulin body, quite homogenously distributed on the surface, contribute to a lesser extent to this attraction force (9). Consequently, the energy gain, ΔE_e, at the origin of the attraction is due to the sharing of polyamines between two C-terminal tails. As the spacing between the αβ C-terminal tails of a single heterodimer is too large (~ 4 nm) to allow a significant overlapping between them, this interaction can take place only between two different heterodimers. ΔE_e is negative (energy gain) for trivalent or higher valence cations but, for divalent ones, both a higher entropic cost and a lower correlation energy can render ΔE_e positive (energy penalty) (9). We then decided to conduct this study using the tetravalent polyamine, Spm. Such a choice is also important in order to perform the experiments at physiological ionic strengths (I > 0.1 M) since tetravalent cations are better competitors for tubulin neutralization than trivalent or divalent cations and, then, are not easily replaced by monovalent ones (K^+ in this study).

Spm cannot induce tubulin aggregation at physiological ionic strength.

Since self-attraction between tubulin dimers is mediated by only two anchors per heterodimers i.e. the two C-terminal tails, there is little chance of tubulin forming large aggregates in the presence of polyamines. As shown in fig. 1A and B, we rather expect self-attracting tubulin to form, due to geometric reasons, linear chains containing a few tubulin dimers or simple tetramers (two dimers). Let us explain this statement. If we consider that there is an entropic loss of K_BT for a new arriving tubulin dimer to join a tubulin chain or to associate with another free tubulin dimer, the variation of energy, ΔE_T, resulting from the association of n tubulin dimers into a chain is $(\Delta E_T)_{chain} = 2n\Delta E_e + (n-1)K_BT$ whereas, when only an association between two tubulin is possible, we obtain $n/2$ tubulin tetramers, $(\Delta E_T)_{tetra} = 2n\Delta E_e + (n/2)\ K_BT$. The energy difference between the two configurations is then: $(\Delta E_T)_{chain} - (\Delta E_T)_{tetra} = ((n/2)-1)\ K_BT$, which indicates that long tubulin chains are non favorable owing to the increasing entropy penalty with n. Thermal agitation should then preclude the formation of long chains of tubulin dimers unless $|\Delta E_e|$ is considerably larger than K_BT. The value of $|\Delta E_e|$, due to the screening of electrostatic force at physiological ionic strengths, should not be significantly larger than K_BT (9) and is at least lower than the energies of longitudinal or lateral bounds for tubulin dimers in MTs (-6.8 to -9.4 and -3.2

to -5.7 K_BT respectively (10)). This last remark indicates that tubulin self-attraction cannot compete with the incorporation of tubulin in MTs and thus does not prevent MT polymerization.

Spm strongly promotes MT bundling.

When incorporated into MTs, tubulin dimers are no longer free to move, which reduces the entropic penalty of association in bundles compared to the formation of aggregates from free tubulin. In addition, polymerized tubulin dimers have their C-terminal tails pointing outward the MT cylinder and are thus available for an electrostatic interaction mediated by polyamines. MT bundling should then be strongly promoted by the sharing of polyamines between two parallel MTs (fig.1C). In addition, in MT bundles, MTs can interact with many MT neighbors, which results in the formation of hexagonal or necklace bundles (4).
Free tubulin dimers can also interact with MTs by using the array of available C-terminal tails on MTs as anchors. This aspect has been already described in details in the model of facilitated diffusion (9).

References of Text 1

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TEXT 2: Reactivity of isolated MTs to a brief cold exposure

Under control conditions, MT samples are more reactive to a brief cold shock at higher than at lower tubulin concentrations (fig. 6A,C). Indeed, the relative drop of absorbance induced by the cold shock increases with tubulin concentration (fig. 6B). A rational explanation is that destabilization of a given MT may increase the probability for the nearest MTs to be destabilized. As already proposed (1), a cloud of GDP-tubulin released from MT destabilization can pollute the ends of the nearest MTs. We then wonder whether GTP-tubulin dimers have enough time to reach this distance before their regeneration into GTP-tubulin. If we consider, *L*, the mean MT length (about 10 µm), C_C, the critical concentration of tubulin (~18 µM here), and *n*=1640, the number of tubulin dimers per µm of MTs, the mean distance between the nearest MT ends is:

$$l \sim \left(\frac{nL}{C_{tub}-C_C}\right)^{1/3} \qquad (1)$$

We obtain *l*~1 µm for 40 µM tubulin. With a diffusion constant D_{tub} ~ 40 µm^2/s (2), it takes only $t \sim \frac{l^2}{D_{tub}}$ ~25 ms to reach the nearest MT ends, which is significantly shorter than the GDP-tubulin lifetime (5 s for a regeneration rate of 0.2 s^{-1}(3)). GDP-tubulin has then enough time to reach the nearest MT before regeneration into GTP-tubulin. Another point is that rapid diffusion of free GDP-tubulin may lead to its significant dilution in the bulk solution. The relative concentration of freshly released GDP-tubulin to that of free GTP-tubulin at the nearest MT ends, *v*, scales like:

$$v \sim \frac{J_s}{lD_{tub}} \Big/ C_C \qquad (2)$$

, where J_s is the dissociation rate of tubulin dimers during shortening phases (20 µm/min, corresponding to 546 dimers per s). For 40 µM tubulin, we obtain *v*~10^{-3}. There is therefore little chance for one MT undergoing catastrophe to force its nearest neighbors to depolymerize via the shuttling of GDP-tubulin. In addition, *v* weakly varies with C_{tub} ($(C_{tub}-C_C)$ to the power 1/3) and, thus, we can hardly explain the marked dependence of isolated MT reactivity on tubulin concentration. As the lifetime of GDP-tubulin is rather short (5 s), an accumulation of free GDP-tubulin in the bulk solution due to MT depolymerization is also excluded i.e. a significant portion of MT-polymerized GDP-tubulin cannot be released into the solution during such a small amount of time. However, as already advanced to explain the phenomenon of MT oscillations (4, 5), the lifetime of GDP-tubulin oligomers in the form of tubulin rings is significantly longer than that of free GDP-tubulin (1 min for GDP-tubulin rings > 5s for free GDP-tubulin (4)). As a result, GDP-tubulin rings may serve as storage units of GDP-tubulin in the bulk solution. For 40 µM tubulin and Cc ~18 µM, if we arbitrary set that one tenth of MTs during 1 min exposure to cold undergo catastrophe, 2.2 µM GDP-tubulin may accumulate in the bulk solution, which could be sufficient to promote catastrophe (10% of *Cc*). Interestingly, the formation of GDP-tubulin rings increases with tubulin concentration (4), which may explain the effect of increasing tubulin concentration on reactivity to cold exposure. Moreover, at lower tubulin concentrations, the concentration of polymerized tubulin decreases (C_{tub} –*Cc* = 2 µM for 20 µM tubulin). Consequently, if we again consider the same portion of MT destabilization (one tenth), the concentration of GDP-tubulin in the form of rings, 0.2 µM, is two orders of magnitude lower than the C_C , which may not be sufficient to promote MT catastrophe. This would explain why, at lower tubulin concentrations, isolated MTs are less sensitive to brief cold exposures.

References of Text 2

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