Supplemental information

EB3-informed dynamics of the microtubule stabilizing cap during stalled growth

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Dynamics of the stalled microtubule cap

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Supplemental Figures and Movies
Supplemental Figure 1

A) Montage of a microtubule-barrier stalling event in the presence of 15µM tubulin and 20nM EB3. During barrier contact microtubule polymerization halts, the EB3 signal at the microtubule plus end diminishes as GTP hydrolysis progresses, followed by a catastrophe. The position of the barrier is indicated by the small vertical white lines. Scale bar denotes 5 µm.

B) In order to increase the signal to noise and remove light scattering at the edge of the overhang, a minimum projection of the image stack is subtracted from each pixel. The kymographs show the data before and after correction of the event in (A).
C) Determination of the position of the microtubule tip is obtained by manual tracking (left). Due to the loss of the EB signal during stalling and insufficient signal-to-noise of the microtubule signal, automatic tracking was not possible. The intensity of the EB3 comet is obtained from a region surrounding the manual trace with a width of 10 pixels or 1.1 μm (middle). The mean EB3 signal at the microtubule lattice is obtained from the region between the seed and the comet (right).

D) EB3 signal during microtubule-barrier contact of the event in (A), obtained by subtracting the mean lattice intensity from the EB3 signal at the tip at different time points (indicated by the colour coding). The total contact duration is 3.75 seconds.
Figure S2
To find a \( D_{tip} \) and \( k_{hyd} \) parameter pair that best describes the experimental datasets, we first compare the experimental and simulated distributions for both microtubule lifetimes and contact durations. Over the range \( D_{tip} = \{1600, 1800, \ldots, 4000\} \) and \( k_{hyd} = \{0, 0.01, \ldots, 0.6\} \) we simulate 500 microtubule growth events for each parameter pair and calculate the mean distribution. The mean simulated distribution for each parameter pair is then compared to the experimental distribution with a Kolmogorov-Smirnov test, yielding a similarity parameter.

Next, the comet decay rate that best describes the experiment, being a single value and not a distribution, is obtained by considering the absolute difference between the simulated rate for each parameter pair and experimental rates. The similarity parameters are subsequently collected in three 2D heatmaps (three left panels), which are normalized between 0 and 1 to treat each experimental distribution with equal weight. A value closer to 1 indicates a higher similarity between the simulated and experimental datasets. To reduce the number of parameter pairs to be simulated, cubic interpolation is applied on the heatmaps. Evaluating the product of the three heatmaps (shown in the fourth heatmap) results in a region of parameter pairs of \( D_{tip} \) and \( k_{hyd} \) best describing the experimental dataset. The highest score in that region is used as the best fit. On the right, 25 bootstrapped simulated distributions based on the thus determined best fitting parameter set are plotted together with the experimental distributions for microtubule lifetimes and contact durations.
Supplemental Figure 3

A) Mean normalized GTP/GDP-Pi trace of 1000 simulated stalling events aligned on the moment of catastrophe (mean ± SE). The simulation parameters for 0, 20, 50, and 100 nM EB3 are found in Fig. 5C.

B) Mean experimental EB3 intensity traces during microtubule stalling aligned on the moment of catastrophe (mean ± SE). The intensity is normalized with the mean of the steady-state intensity prior to barrier contact. Number of stalling events analysed: 20 nM, n = 151, 50 nM, n = 104, and 100 nM EB3, n = 92.

C) Simulated position of the microtubule tip during microtubule stalling prior to catastrophe for 0, 20, 50, and 100 nM EB3 (mean ± SE).
D) Duration of pausing (left) and microtubule shrinkage (right) before the onset of catastrophe after barrier contact based on Fig. S3C. Microtubule pausing is defined as the time between the moment that the growth velocity is reduced to 10% of the steady-state value and the moment of catastrophe. Both the pausing duration and the shrinkage before the onset of catastrophe depend on the mean duration of hydrolysis.
Supplemental Figure 4

Previously unpublished data of the barrier contact duration using the barrier design from (Kalisch et al., 2011). The barriers were composed of SiO with a height of approximately 1.5 μm and without an overhang. Microtubules were nucleated from GMPCPP-seeds towards the barriers in the presence of 15 μM tubulin alone (n = 17), and with addition of 200 nM Mal3-Alexa488 (n = 30), and with the combined addition of 200 nM Mal3-Alexa488, 8 nM Tea2, and 50 nM Tip1 (n = 34).
Supplemental Figure 5

A) Schematic of calculating the probability of finding a sequence of $N \geq N_{\text{unstable}}$ GDP subunits at a distance $x$ from the tip. The microtubule lattice is treated as a series of independent Bernoulli trials with probability $p(x)$ of finding a GTP/GDP-Pi subunit and probability $q(x) = 1 - p(x)$ of finding a GDP subunit. The probability of finding a sequence of 3 GDP subunits at position $x$ is $p(x)q(x)^3$. 

B) Graph showing the cumulative fraction of position of $N_{\text{GDP}} \geq N_{\text{unstable}}$ (nm) for $N_{\text{unstable}} = 3$. The graph compares experimental data with simulation and analytical solutions.

C) Graph showing MT length (μm) and velocity (nm s$^{-1}$) versus time (s) for MT tip and Cap end. Cross-correlation analysis is also shown with a lag time (s).

D) Table showing EB3 concentration ([μM]) experiment simulation theory.

| [μM] | Lifetimes experiment | Simulations experiment | Simulations theory |
|------|----------------------|------------------------|-------------------|
| 0    | 155 ± 15             | 164 ± 7.4              | 143               |
| 20   | 94 ± 3.7             | 71.9 ± 3.0             | 73                |
| 50   | 57 ± 3.5             | 46.3 ± 1.9             | 52                |
| 100  | 54 ± 2.4             | 39.9 ± 1.8             | 60                |

| [μM] | Lifetimes | Simulations | Simulations | Simulations |
|------|-----------|-------------|-------------|-------------|
| 7.2  | 180 ± 20  | 168 ± 20    | 125         |
| 10   | 220 ± 20  | 202 ± 15    | 182         |
| 15.2 | 320 ± 40  | 314 ± 41    | 286         |
| 20   | 360 ± 50  | 367 ± 51    | 352         |
| 28   | 550 ± 100 | 560 ± 98    | 587         |
B) The location of $N_{GDP} \geq N_{unstable}$ in the 1D microtubule lattice follows a Gaussian cumulative density distribution. The analytical solution holds well for the entire range of $N_{unstable}$ values explored in the simulations.

C) Correlation between the simulated growth fluctuations and the position of the cap end, as defined by $N_{unstable}$. Example of a simulated microtubule growth event showing the position of the microtubule tip and cap end, based on the parameters for 0 nM EB in Fig 5C. (left, top). The velocity of the cap end is correlated with the growth fluctuations of the microtubule tip (left, bottom). The cross-correlation between the tip and cap end velocity has a characteristic delay, which is approximately equal to $k_{hyd}^{-1}$.

D) Table with the microtubule lifetimes (median ± SEM) based on the EB-dependent dataset in Fig. 5C (top) and on the dataset in Fig. 7B (bottom) (Janson et al., 2003). We find that the experimental, simulated, and theoretical lifetimes are in good agreement.
**Supplemental Figure 6**

A) Table with experimental microtubule ageing parameters. The shape and rate parameters were obtained by fitting the lifetime distributions for freely growing microtubules with a Gamma distribution (mean ± 95% CI).

| EB3 [nM] | Lifetime [s] | Shape | Rate [s⁻¹] |
|----------|--------------|-------|------------|
| 0 nM     | 155 ± 15     | 1.9 ± 0.23 | 0.0095 ± 0.001 |
| 20 nM    | 94 ± 3.7     | 1.9 ± 0.12 | 0.018 ± 0.001  |
| 50 nM    | 57 ± 3.5     | 1.6 ± 0.12 | 0.024 ± 0.002  |
| 100 nM   | 54 ± 2.4     | 1.7 ± 0.10 | 0.027 ± 0.002  |

B) Time-dependent tip fluctuations are sufficient to introduce microtubule ageing. The tip fluctuations increase according to $D_{\text{tip}}(t) \propto 1 - e^{-0.025t}$, with $D_{\text{tip}}(0) = \frac{1}{2} D_{\text{tip}}$ and $D_{\text{tip}}(\infty) = \frac{5}{4} D_{\text{tip}}$.

C) Table with simulated microtubule ageing parameters. The shape and rate parameters were obtained by fitting the lifetime distributions for freely growing microtubules with a Gamma distribution (mean ± 95% CI).

| EB3 [nM] | $k_{\text{ageing}}$ [s⁻¹] | Shape | Rate [s⁻¹] |
|----------|----------------------------|-------|------------|
| 0 nM     | 0.025                      | 1.7 ± 0.19 | 0.0085 ± 0.001 |
| 20 nM    | 0.025                      | 1.9 ± 0.22 | 0.019 ± 0.003  |
| 50 nM    | 0.025                      | 2.0 ± 0.24 | 0.029 ± 0.004  |
| 100 nM   | 0.025                      | 2.1 ± 0.24 | 0.027 ± 0.004  |

D) Theoretical shape of an experimentally measured microtubule tip. Fluctuations of the microtubule could generate a blurred during frame acquisition with TIRF. However, the calculated blurring is on the order of 20 nm, too small to be detected by TIRF microscopy.
Supplemental Movies

Movie S1, related to figure 1. Microtubules polymerizing towards the barrier in the presence of tubulin alone.

Microtubules are growing towards the microfabricated barriers in the presence of 15 μM HiLyte488-tubulin. The GMPCPP seeds are labelled in magenta and the tubulin in green. Images were collected with TIRF microscopy at a 500 ms interval. Video is sped up 120 times. Time is shown in the format min:sec.

Movie S2, related to figure 1. Microtubules polymerizing towards the barrier in the presence of GFP-EB3.

Microtubules are growing towards the microfabricated barriers in the presence of 15 μM rhodamine-tubulin (magenta) and 20 nM GFP-EB3 (green). Images were collected with TIRF microscopy at a 250 ms interval. Video is sped up 240 times. Time is shown in the format min:sec.