Microtubule Assembly Dynamics at the Nanoscale

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Observation of microtubule assembly at the nanoscale.

(A) Schematic top view of the experimental geometry. A dynamic microtubule extension polymerizes from a bead-linked microtubule seed. The bead is held in the optical trap (magenta) such that when the growing microtubule contacts the barrier (gray), it polymerizes against the force of the trap. (B) Differential interference contrast micrograph of an experiment showing the bead, microtubule, and barrier. (C) Applying a force clamp allows an approximately constant load to be maintained at the microtubule tip.
Simulation of microtubule assembly at the nanoscale

At time (A), the microtubule tip has a multiprotofilament extension with a relatively long and spatially-distributed GTP cap (magenta) of 35 subunits on average. The leading protofilaments then depolymerize and recap, resulting in a growth-phase shortening event, as shown in (B). Note that the GTP cap remains intact, never being less than 20 GTP-tubulin subunits during the shortening phase. Subsequently, the microtubule polymer continues growth, as shown in (C). Catastrophe occurs after 10 sec, as shown in (D-E), at which point the GTP cap has only 3 subunits on average.
**Microtubule assembly at the nanoscale.** (A) Experimental and simulated individual traces of microtubule plus-end assembly behavior at 1.5 pN clamp force are shown as compared to GMP-CPP control traces. Slow net assembly at the microtubule tip is highly variable both experimentally and in simulation. There are clearly retreats during microtubule assembly that are larger than a single layer of tubulin subunits, or even a few layers. (B) To quantify the variability in microtubule assembly, growth and shortening excursions are defined as the total number of consecutive displacements in either the positive or the negative direction (shown by green and red arrows, respectively).
Microtubule assembly is highly variable, with frequent growth-phase shortening events.

A histogram of microtubule growth-phase excursion lengths for 0-1 pN force clamp as compared to GMP-CPP controls. Excursion lengths are defined in this analysis as sequential negative or positive microtubule length changes (no fitting), as shown in previous slide. Growth-phase shortening events are large compared to GMP-CPP controls, indicating that experimental noise cannot account for the observed behavior. The model qualitatively accounts for the extent of positive and negative excursions (red).
Lateral Cap model simulations as compared to nanoscale experimental microtubule assembly data. The single-layer lateral GTP-cap model is unable to account for the large fluctuations in nanoscale microtubule assembly that are observed experimentally.
Single time increment sampling demonstrates single subunit addition

Single Time Increments (0.1 sec): 0-2.5 pN Clamp Force

Single 0.1 second time increment sampling demonstrates that tubulin dimers add as single subunits, not oligomers. Single time microtubule length increments are summarized for individual time steps during assembly. Both GTP microtubules and GMP-CPP control microtubules show small, Gaussian-distributed length fluctuations at single time steps, indicating that tubulin oligomer addition and loss is highly unlikely. The increments in the presence of GTP are larger than in GMP-CPP controls, and the model accounts for the extent of fluctuations observed experimentally while assuming that tubulin addition occurs via single subunits only.
Growth velocity depends weakly on force. Microtubule growth velocity is weakly dependent on force both in simulation and experiment. The high variability is the result of variability in assembly at the nanoscale.
Conclusions

• Microtubule assembly observed at unprecedented temporal and spatial resolution reveals highly variable assembly behavior at the nanoscale.

• Microtubule shortening excursions of multiple GTP-tubulin layers are common during assembly, suggesting that the commonly accepted “single layer cap” model for microtubule dynamic instability requires reevaluation.

• Simulations that rely on an exponentially distributed GTP-Cap qualitatively reproduce the experimentally observed variability in microtubule growth.

• Simulations that rely on a single-layer GTP-Cap do not reproduce the experimentally observed variability in microtubule growth.

• Microtubule assembly variability is not due to incorporation and loss of tubulin oligomers. Single time-step addition and loss events correlate to single tubulin subunit addition and loss.

• Microtubule growth velocity is weakly dependent on force.