Supporting information

Hierarchical Assembly Pathways of Spermine Induced Tubulin Conical-Spiral Architectures

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Figure S1: A slice (3.8 nm thick) through a cryo-electron tomogram of 4 mg/mL tubulin, incubated at 9°C for 48 h with 5 mM spermine, showing bundles of conical-spiral tubules associated in an antiparallel alignment, arranged in a distorted hexagonal lattice. At the bottom left the slicing plane is orthogonal to the long axes of the tubules, whereas in the top right the long axes of the tubules are roughly in the slicing plane. A movie depicting slices of a tomogram of conical-spiral tubule bundles from several directions is deposited (Movie 1). Scale bar is in nm units.
Figure S2: Spermine induced hierarchical tubulin assemblies. a. Azimuthally-averaged buffer-subtracted synchrotron SAXS curves from 4 mg/mL tubulin incubated at 9 °C for 48 h with 1 to 10 mM spermine, as indicated. The solutions were centrifuged (15,700 g, 9 °C, 30 min) and the measurements were performed on the supernatant and the buffer of the sample served as background. Short conical-spirals were present in the samples that contained between 1 and 2.5 mM spermine. At higher spermine concentrations, the concentration of short conical-spirals gradually decreased. b. Azimuthally-averaged supernatant-subtracted synchrotron SAXS curves from 4 mg/mL tubulin incubated at 9 °C for 48 h with 1 to 15 mM spermine, as indicated. The solutions were centrifuged (15,700 g, 9 °C, 30 min) and their supernatant served as background. At 1 mM spermine, there were no significant differences between the sample and supernatant measurements (indicating that the structures in the sample were too small to sediment by the applied centrifugation). Bundles of conical-spiral tubules started to assemble at 1.5 mM spermine. Above 5 mM spermine, inverted tubules started to form and the concentration of conical-spiral tubules decreased. c. Azimuthally-averaged supernatant-subtracted SAXS curves from 4 mg/mL tubulin incubated at 9 °C for 48 h with 20 to 60 mM spermine. Between 20 and 30 mM spermine, hexagonal bundles of inverted tubules formed. Above 40 mM spermine, however, bundles of inverted tubules or other assemblies did not form. We attribute the resolubilization of the assemblies to spermine-induced reentrant condensation. d. Comparison between the concentration-normalized absolute scattering intensity from 4 mg/mL tubulin with 1 mM spermine at steady-state (after 48 h incubation at 9 °C) and 12.4 mg/mL GDP-tubulin (after about 1 h incubation), containing tubulin-rings. A sharper decline in the intensity is observed with 1 mM spermine. The inset compares the expected SAXS curve from a model of the short conical-spiral, containing 18 dimers (red curve), with the expected scattering curve from a tubulin ring, containing 13 dimers. Similar differences are observed.
Figure S3: *Cryo-TEM images of short conical-spirals, conical-spiral tubules, and inverted tubules.* Cryo-TEM images of 4 mg/mL tubulin incubated at 9°C for 48 h with different spermine concentrations, as indicated. Scale bars are in nm units. **a, b.** A short conical-spiral phase (red arrows) was observed in the 1 mM spermine sample and contained mainly short conical-spirals and their fragments (curved tubulin oligomers). **c-f.** The conical-spiral phase (blue arrows) was observed in the 5 mM spermine sample and contained the hierarchical conical-spiral structures, in which antiparallel bundles tubules of conical-spirals were the dominant population. **g-j.** Conical-spirals and inverted tubules (green arrows) coexisted at 10 mM spermine. Base-to-base or top-to-top conical-spiral associations were more abundant than base-to-top association (unlike the 5 mM spermine, in which top-to-base was more abundant). **k, l.** Inverted tubulin phase was observed with 20 mM spermine. This phase was more homogeneous compared with the conical-spiral phase and contained mostly hexagonal bundles of inverted tubules.
Figure S4: Coexistence of hierarchical tubulin assemblies at 9°C. Azimuthally-averaged buffer-subtracted (a, c) or supernatant-subtracted (b) synchrotron SAXS curves (open square symbol), fitted to a linear combination (solid black curves) of experimental scattering curves that mostly contained short conical-spirals (SCS), conical-spirals (CS), conical-spiral tubules (CSTs) or inverted tubules (ITs). The insets show the mass fraction of the contributing assemblies. a. 4 mg/mL tubulin mixed with 1.5 to 2.5 mM spermine and incubated at 9°C for 48 h. The fit was done by a linear combination of the 1 mM spermine signal (rich in short conical-spirals) and the 5 mM spermine signal (rich in conical-spiral tubules). b. 4 mg/mL tubulin mixed with 7.5, 10, or 12.5 mM spermine and incubated for 48 h at 9°C. The fit was obtained by a linear combination of the 5 mM spermine signal (rich in conical-spiral tubules) with the 15 mM spermine signal (rich in inverted tubules). c. Time-resolved SAXS curves measured during the phase transition from bundles of conical-spiral tubules to bundles of inverted tubules, achieved by increasing the concentration of spermine from 5 to 30 mM at 25°C. The fit was done by a linear combination of the signal measured 21 min after the addition of spermine (rich in conical-spiral structures) with the signal measured 22 h after the addition of spermine (rich in inverted tubules).
Figure S5: Slices (3.8 nm thick) through a cryo-electron tomogram of 4 mg/mL tubulin, incubated at 9 ºC for 48 h with 20 mM spermine, showing bundles of inverted tubules, arranged in a hexagonal lattice. The slicing plane is orthogonal to the long axes of the inverted tubules. A movie depicting slices of a tomogram of bundles of inverted tubules from several directions is deposited (Movie 2). Scale bars equal 50 nm.
Figure S6: Spermine induced hierarchical tubulin assemblies at 36°C. 

a. Azimuthally-averaged background-subtracted synchrotron SAXS curves from 4 mg/mL tubulin incubated at 36°C (open red squares) and 9°C (blue curves) for 48 h with 1 to 20 mM spermine, as indicated (1 mM samples were incubated for 5 h). The black curve is a linear combination of the SAXS curves obtained with 2.5 and 20 mM spermine at 36°C. 

b-f. Cryo-TEM images of 4 mg/mL tubulin incubated at 36°C for 48 h with different spermine concentrations, as indicated. Scale bars are in nm units.
Figure S7: Microtubules transformed into conical-spiral tubules and inverted tubules in the presence of spermine.  

a, b. Azimuthally-averaged supernatant-subtracted synchrotron SAXS curves of 8mg/mL tubulin which was polymerized with 4mM GTP or GMPPCP (45min at 36 °C). The polymerized microtubule was then mixed with an equal volume of spermine solutions and incubated for 48h at 9 °C. The final concentration of tubulin was 4mg/mL. The final concentration of GTP (a) or GMPPCP (b) was 2mM, and the spermine concentration was between 1.5 and 20mM, as indicated (in mM units).  

c, d. Azimuthally-averaged supernatant-subtracted synchrotron SAXS curves (open square symbol) from 4mg/mL GTP-tubulin (c) or GMPPCP-tubulin (d) incubated with 7.5 to 12.5 mM spermine, fitted to a linear combination (solid curves) of experimental scattering curves that mostly contained conical-spiral tubules (CSTs) or inverted tubules (ITs). The fit was done by a linear combination of the 5mM spermine signal (rich in conical-spiral tubules) and the 15mM spermine signal (rich in inverted tubules). The insets show the mass fraction of the contributing assemblies.
Figure S8: Time-resolved SAXS measurements. a. Azimuthally-averaged background-subtracted time-resolved synchrotron SAXS curves of 4 mg/mL tubulin with 1, 5, or 20 mM spermine reactions. To collect a sufficiently broad data set, each reaction was repeated several times, as shown on the plots next to the scattering curves. The absolute scattering intensities were normalized to the tubulin concentration. b-d. Comparison between 1 and 20 mM (b), 5 and 20 mM (c), and 1, 5, and 20 mM (d) reactions at the indicated times. The plots show that tubulin assemblies formed at higher spermine concentrations were assembled from intermediates, similar to those observed at lower spermine concentrations. On the right, the approximated averaged intensities at \( q \to 0 \) (\( I_0 \)), are plotted as a function of time. \( I_0 \) is proportional to the average mass of the assemblies and was calculated by averaging the intensities between \( q = 0.05 \) and \( q = 0.06 \) nm\(^{-1}\). The total growth of assemblies was slower in the 1 and 20 mM spermine reactions, compared with the 5 mM reaction.
Figure S9: Time-resolved cryo-TEM images following the assembly of conical-spiral tubules. 4 mg/mL tubulin was mixed with 5 mM spermine at 9°C. Measurements were taken at the indicated times following the onset of the assembly reaction. **32 s to 3 min**: conical-spiral subunits (CSs, white arrows) formed and started to interact with one another in lateral interactions (cyan arrows), vertical (top-to-top (magenta arrows) or base-to-top (in conical-spiral tubule assemblies, black arrows)). **10 min**: conical-spirals assembled into conical-spiral tubules by base-to-top associations. **1.25 h**: conical-spiral tubules assembled into antiparallel bundles (blue arrows). **Between 24 and 48 h**: highly ordered antiparallel bundles of conical-spiral tubules. All scale bars are in nm units.
Figure S10: Time-resolved cryo-TEM images following the assembly of inverted tubules. 4mg/mL tubulin was mixed with 20mM spermine at 9°C. Measurements were taken at the indicated times following the onset of the assembly reaction. 33s: short conical-spirals (SCSs, red arrows) and conical-spiral subunits (CSs, white arrows), formed and started to interact with each other in vertical (top-to-top, magenta arrows) and lateral associations (cyan arrows). 4min: conical-spiral subunits assembled mainly by lateral associations. Between 10 min and 1 h: inverted tubules (ITs, green arrows) formed from conical-spiral subunits. Between 24 and 48 h: only highly ordered hexagonal bundles of inverted tubules were observed. Scale bars are in nm units.
Figure S11: Time-resolved cryo-TEM of the transition between bundles of conical-spiral tubules and hexagonal bundles of inverted tubules. 4 mg/mL tubulin was incubated with 5 mM spermine at 9 °C for 48 h. The solution was then exposed to 30 mM spermine at 25 °C and measurements were taken at different time points after the exposure to 30 mM spermine, as indicated. **Between t = 0 and 32 s:** highly ordered massive antiparallel bundles of conical-spiral tubules (CSTs, blue arrows). **Between 20 min and 6 h:** most of the conical-spiral tubules disassembled into conical-spiral subunits (CSs, purple arrows), which gradually transformed into inverted tubules (ITs, green arrows). **22 h:** the main population was bundles of inverted tubules, whereas some antiparallel bundles of conical-spiral tubules remained trapped. Scale bars are in nm units.
Figure S12: Tubulin assembly in the presence of three other polyamines. a-c. Azimuthally-averaged supernatant-subtracted SAXS curves from 4 mg/mL tubulin incubated for 48 h at 9°C with putrescine (a), spermidine (b), or thermospermine (c). The molecular structures of spermine (as a reference) and each of the other polyamines are also shown. d, e. Cryo-TEM images of 4 mg/mL tubulin incubated for 48 h at 9°C with 5 mM thermospermine, showing conical-spiral subunits and some nonspecific assemblies. Scale bars are in nm units.
Figure S13: Mass fraction distributions of intermediates of short conical-spirals and conical-spirals. **a.** Kinetics and steady-state measurements of 4 mg/mL tubulin with 1 mM spermine (shown as black curves in Figure 4a in the main text) were fitted to the computed scattering curves from atomic models of short conical-spirals, containing 18 dimers, and their fragments, containing 1 and 2 dimers (shown as red curves in Figure 4a in the main text). These models were selected as representative models for relatively large and small tubulin assemblies. We present the best-fitted mass fraction distribution at the indicated time points after mixing tubulin with 1 mM spermine. The results show that before spermine was added, the main population comprised small tubulin oligomers. After spermine was added, the population of dimers decreased and shifted to larger assemblies. **b-d.** The measured concentration-normalized absolute scattering intensity from 4 mg/mL tubulin, 0.39, and 1 s after mixing with 5 mM spermine (**d**). The mass fraction distribution that best-fitted the 0.39 s curve (Figure 4b in the main text) included the short conical-spiral model (18 dimers) and its fragments (1 and 2 dimers) (**b**). The mass fraction distribution that best-fitted the 1 s curve included the conical-spiral subunit model (containing 32 dimers), the short conical-spiral model (18 dimers), and its fragments (1 and 2 dimers) (**c**). **e-g.** The measured concentration-normalized absolute scattering intensity from 4 mg/mL tubulin, 3.28 and 10.10 s after mixing with 20 mM spermine (**g**). The mass fraction distribution that best-fitted the 3.28 s curve (Figure 4c in the main text) included the short conical-spiral model (18 dimers) and its fragments (1 and 2 dimers) (**e**). The mass fraction distribution that best-fitted the 10.10 s sec curve (Figure 4c in the main text) included the conical-spiral subunit model (containing 32 dimers), the short conical-spiral model (18 dimers), and its fragments (1 and 2 dimers) (**f**).
Figure S14: *The tubulin dimer’s orientations in the conical-spiral and inverted tubule that best fitted the scattering data.* We translated the middle-dimer (shown in blue) of the nine-dimer microtubule lattice (PDB ID 3J6F, shown in red) to the origin by $(-10.2 \text{ Å}, 9.74 \text{ Å}, -2.31 \text{ Å})$. We then rotated the dimer about the $z$-axis and then about the $x$-axis, as indicated.
Figure S15: *Fitting the scattering data to alternative models of conical-spiral tubules.* We compare azimuthally-averaged supernatant-subtracted synchrotron SAXS curve from 4 mg/mL tubulin and 5 mM spermine (open symbols) with computed SAXS curves based on alternative atomic models: antiparallel tubules of subunits built from a stack of three rings with varying radii (Stack of Rings, wine curve), antiparallel tubules built from a stack of conical-spiral subunits (Stack of CSs, blue curve), and antiparallel tubules built from continuously connected conical-spiral subunits, connected by a short protofilament, whose length is half a helical-turn (Continuously Connected CSs, red curve). Stack of ring model- three tubulin rings, with radii of 20.55, 17.3, and 15.65 nm (the average radii of conical-spirals), on top of one another (the largest ring at the bottom and the smallest at the top) at vertical displacements of 5.72 nm. To create a tubule, we duplicated the stack of three rings in the vertical direction. The antiparallel alignment was obtained by duplicating one tubule and flipping its direction by 180° vertically and horizontally where the center-to-center distance between the two tubules was 46 nm. Stack of conical-spirals model- the conical-spiral subunit was built from 32 dimers, based on the conical frustum spiral symmetry (see Methods) with a pitch, $p$, of 5.72 nm, the largest radius of 22.3 nm, the smallest radius of 15 nm, and 2.5 helical turns. To create a tubule, we duplicated the conical-spiral subunit in the vertical direction. The antiparallel alignment was obtained by duplicating one tubule and flipping its direction by 180° vertically and horizontally where the center-to-center distance between the two tubules was 44.5 nm. The conical-spiral helical model- see symmetry in the Method section and parameters in the main text.
References
