



Fig. S1: Histogram of the PF distribution for MTs in different states.

Table S1: Data collection and processing statistics. The resolution number for each reconstruction was estimated by calculating the Fourier Shell Correlation (FSC) of one ‘protofilament’ containing 3 adjacent tubulin dimers from the odd and even maps, using a FSC 0.143 criterion. For the GMPCPP-EB3 state, the two resolution numbers are for the 14-PF MT and correspond to class 1 and 2, respectively. We did not pursue a reconstruction for the 13-PF MT due to the limited number of segments.

Dataset / MT States	GMPCPP	GDP	GMPCPP-K	GTP γ S	GMPCPP-EB3	GMPCPP
Location of Data collection	UCSF	UCSF	UCSF	UCB	UCB	WUSTL
Microscope	Titan Krios	Titan Krios	Titan Krios	Titan (low base)	Titan (low base)	Titan Krios
calibrated pixel size	1.079	1.079	1.079	1.330	1.330	1.096
# of frames	25	25	25	20	20	40
exposure time / frame	0.2	0.2	0.2	0.3	0.3	0.2
Total exposure time (sec)	5	5	5	6	6	8
Dose / frame	1.4	1.4	1.4	1.44	1.44	1.33
Total dose	35	35	35	28.8	28.8	53.2
K2 camera mode	super-resolution	super-resolution	super-resolution	counting	counting	counting
Data collection software	SerialEM	SerialEM	SerialEM	Leginon	Leginon	EPU
# of Micrographs used	405	695	346	616	182	1176
# of Particles (13PF / 14PF)	6,465 / 28,882	24,482 / 48,097	5,716 / 29,927	9,071 / 49,145	1,666 / 13,159	25,729 / 110,127
Resolution of symmetrized maps (13PF / 14PF) (Å)	3.97 / 3.62	3.54 / 3.30	3.82 / 3.50	4.05 / 3.49	(NA / NA) / (4.32 / 4.13)	3.43 / 3.11
Resolution of C1 maps (13PF / 14PF) (Å)	4.86 / 4.34	4.12 / 3.90	4.94 / 4.06	5.61 / 4.21	(NA / NA) / (7.05 / 4.89)	4.08 / 3.57

Table S2: Lattice parameters of MTs in different states. The values of the inter-dimer and intra-dimer distances were calculated from the atomic models of each state, using the mean of the C α distances between α - and β -tubulin for each pair of corresponding residues. The dimer rise and dimer twist were measured from the C1 reconstructions of MTs in different states using the *relion_helix_toolbox* program in RELION v2.1, which gave results consistent with those obtained with the *hsearch_lorentz* program in the IHRSR package that we previously used. To assess the robustness of our measurements, we split the whole dataset for each microtubule sample into three, non-overlapping subsets. The three sets of helical parameters measured from the reconstruction of each subset are then used to calculate the mean value and standard deviation of the helical parameters. For each measurement of dimer rise and dimer twist, the standard deviation is less than 0.02.

MT States	Intra-dimer Distance	Inter-dimer Distance	Dimer Rise	Dimer Twist
GDP 13-PF	41.44	40.30	81.76	0.08
GDP 14-PF	41.50	40.25	81.74	-0.48
GDP-K 13-PF	41.38	40.36	81.64	0.09
GDP-K 14-PF	41.40	40.20	81.63	-0.46
EB3-GDP 13-PF	41.28	40.24	81.54	-0.13
EB3-GDP-K 13-PF	41.35	40.32	81.59	-0.11
GTP γ S 13-PF	41.35	40.45	81.93	-0.12
GTP γ S 14-PF	41.32	40.40	81.70	-0.56
EB3-GTP γ S 13-PF	41.29	40.32	81.60	-0.25
EB3-GTP γ S-K 13-PF	41.31	40.51	81.96	-0.27
GMPCPP 13-PF	41.53	42.43	83.95	0.23
GMPCPP 14-PF	41.56	42.45	83.96	-0.33
GMPCPP-K 13-PF	41.45	42.00	83.30	0.21
GMPCPP-K 14-PF	41.42	41.94	83.29	-0.32
EB3-GMPCPP 13-PF	41.34	40.47	81.98	-0.13
GMPCPP-TPX2 13-PF	41.67	42.61	84.33	0.40
GMPCPP-TPX2 14-PF	41.67	42.56	84.31	-0.23
GMPCPP-EB3 14-PF (class 1)	41.41	40.70	82.25	-0.69
GMPCPP-EB3 14-PF (class 2)	41.55	42.08	83.57	-0.45