SUPPLEMENTARY MATERIAL

SUPPLEMENTAL FIGURE, TABLE, AND MOVIE LEGENDS

Figure S1, related to Figure 1 and Table 1: **High-resolution cryo-EM of microtubules.** A) Micrograph of GMPCPP microtubules decorated with kinesin. Bar, 100 nm. B) Left, micrograph showing crystalline ice due to poor vitrification. Bar, 100 nm. Right, power spectrum of the Fourier transform of the image shows a strong signal at 3.7 Å. C) Left, reference-free class average calculated from 48 aligned microtubule segments. Right, power spectrum of the Fourier transform of the image shows layer lines to ~6.7 Å resolution

Figure S2, related to Figure 1, Table 1**: High-resolution three-dimensional reconstructions of microtubules.** A) Schematic of the IHRSR procedure adapted for microtubule specimens with a dimer repeat. The microtubule segments are associated with 13 or 14 protofilament reconstructions by multi-model projection matching. After each round of projection matching, an asymmetric back projection is generated from each group (13pf and 14pf) of aligned segments, and the 3-start helical parameters (rise and twist) describing the monomeric tubulin lattice are calculated. These helical parameters are used to generate and average 13 or 14 symmetry-related copies of the asymmetric reconstruction. In the symmetrized volume, there is only one “good” protofilament that does not have α-and β-tubulin mis-aligned due to be presence of a seam. This single good protofilament is extracted from the microtubule with a wedge-

shaped 3D mask, and the same helical parameters are used to generate a new microtubule density that contains a seam. The resulting models are used for projection matching during the next round of refinement. A final refinement of the microtubule segment alignment parameters is performed in FREALIGN without further refinement of helical parameters. B) Fourier Shell Correlation (FSC) curves for microtubule reconstructions were used to estimate resolutions of each reconstruction using a cutoff of 0.143.

Figure S3, related to Figure 2, Table 2: **Assessment of Rosetta atomic models.** A) Low energy models after the final iteration of refinement for each of the three ligand-bound states are converged in terms of energy and structure. The red line indicates the cut-off for the 1% lowest energy structures. B). Each of the models in the low energy ensembles were compared with the three consensus models for GMPCPP, GDP, and GDP–taxol. The RGB values for the color of each point correspond to normalized x,y,z coordinates; the distinct, homogeneous colors present in each cluster indicate that the low energy ensembles are highly internally similar and distinct from one another.

Figure S4, related to Figure 2**: Additional views of the GMPCPP model fit to the experimental density map.** Views of the energy minimized all-atom GMPCPP consensus model in the segmented cryo-EM density map, colored as in Fig. 2, of A) α-tubulin H7 B) α-tubulin H4, C) the α-tubulin C-terminal domain,

D) the β-tubulin nucleotide-binding domain sheet, E) α-tubulin H6, and F) β-tubulin H9.

Figure S5, related to Figure 3: **Observed conformational changes are robust to reference frame at the ensemble and single-model level.** Vector plots of the GMPCPP to GDP transition. The left panel is equivalent to Figure 3B, except that all vectors are displayed. The right panel shows difference vectors calculated between the consensus models aligned on the bottom α-tubulin subunit rather than β-tubulin. The nearly identical vector field suggests that the conformational transitions we observe are not sensitive to the chosen frame of reference and represent robust differences between the ensembles.

Figure S6, related to Figure 5**: Taxol binding results in a distinct β-tubulin conformation.** Vector plots of the GDP to Taxol (left) and GMPCPP to Taxol (right) transition in β-tubulin. Elements that directly bind taxol are colored yellow, elements adjacent to the E-site are colored pink, and elements adjacent to the N-site are colored blue. Taxol is colored yellow, E-site nucleotide pink, N-site nucleotide orange. Vectors are colored by direction. The similar patterns demonstrate that taxol binding results in a conformation of β-tubulin distinct from both the GDP and GMPCPP bound states.

Table S1a, Related to Figure 1, and Figures S1 and S2**: Summary of cryo-EM data.** The number of films collected for each dataset, the number of microtubules

selected and used for the reconstruction, and the estimated number of asymmetric units contributing to the final reconstruction are listed.

Table S1b, related to Figure 2**: Correlation coefficients of consensus models vs. reconstructions.** The correlation of the refined model against its map is higher than the correlation to alternative maps for all cases, except for the GDP–taxol model, for which the correlation to the GDP–taxol and GMPCPP reconstructions are equivalent. This is probably due to the comparatively higher resolution of the GMPCPP map (4.7 Å) with respect to the GDP–taxol map (5.6 Å). Real-space correlations were computed using the 'measure correlation' function in UCSF Chimera for the 3 x 3 lattice containing the consensus model of each state vs. each map.

Table S1c, related to Figure 2**: Cross-correlation analysis of models demonstrates that model-map fit is general.** Real space cross-correlation was used to compare a single dimer extracted from consensus models to 14pf GMPCPP maps (used for structure refinement) and to 13pf GMPCPP maps (independently determined map). Simulated maps are computed to 5.0 Å resolution, based on consensus model backbone. The electron crystallographic, straight tubulin structure (PDB 1JFF) and a X-ray crystallographic, bent tubulin structure (PDB 1SA0) were also compared to both maps. The relatively high cross correlation score of 1JFF suggests that the slight yet consistent variations

between the scores of different states is due to an improved, state dependent fit into the maps. Cross-correlations were measured using UCSF Chimera.

Table S1d, related to Figures 3 and 5**: RMSD analysis of models and 1JFF.** RMSD analysis is used to compare the consensus models from each nucleotide state to one another as well as to 1JFF, demonstrating that 1JFF is more dissimilar to all three models than the models are to each other. Either the β-subunit or the entire dimer of GMPCPP or GDP was used as a superposition frame of reference. The RMSD reported is computed over either the β– or α– subunit of the corresponding reference structure.

Movie S1, related to Figures 1 and 3: **Morph between the GMPCPP and GDP reconstructions.** View is from inside the microtubule lumen. □-tubulin density is green and alternating layers of β-tubulin density are colored in different shades of blue to highlight tubulin dimers.

Movie S2, related to Figures 1 and 5: **Morph between the GDP–taxol and GMPCPP reconstructions**. View and colors as movie S1, but comparing the GDP–taxol and GMPCPP reconstructions. Taxol density is yellow.

Movie S3, related to Figure 1**: Morph between the kinesin-free GMPCPP and GDP reconstructions.** View and colors as movie S1, but comparing the control GMPCPP and GDP reconstructions in the absence of kinesin. Note that

longitudinal remodeling is still evident, but is no longer attributable to the E-site due to averaging of α- and β-tubulin

Movie S4, related to Figure 3**: Morph between GMPCPP and GDP atomic models and maps at the E-site demonstrates significant structural changes.** α-H8 (orange), α-T7 (purple), β-T3 (blue), β-T4 (salmon), and β-T5 (navy) exhibit correlated movement, downwards towards the minus end and inwards towards the lumen, upon nucleotide hydrolysis.

Movie S5, related to Figure 3**: α-tubulin intermediate strands (S7-S10) move significantly between the GMPCPP and GDP states, reminiscent of the structural transition between straight (1JFF) and bent (1SA0) tubulin.** Strands S7-S10 of the α-subunit are observed to shift approximately 1.28 Å towards the plus end, in unison with the α-H7 helix. While the change is reminiscent of the structural change between straight and bent tubulin, no rotation is associated with the intermediate strands due to lattice constraints.

Movie S6, related to Figure 4**: Structural elements on the microtubule surface that interact with associated factors move significantly upon GTP hydrolysis.** Movement of α-tubulin C-terminal helices (H11 in red, H12 in magenta) appears coupled to the movement of the intermediate strands (S7-S10). There is a large movement of H10 in α-tubulin (light pink) with respect to

H3 in b-tubulin across the lateral interface (green). These two elements define the binding site for the EB family of +Tips proteins.

Movie S7, related to Figure 5**: Structural changes upon GTP hydrolysis and taxol binding on E-site loops β-T5 and β-T7, and on helix H7 of both α- and β-subunits.** Nucleotide hydrolysis causes a distortion of β-H7, β-T5, and β-T7 that may propagate into the α-subunit through α-tubulin H7 and the α-tubulin strands of the intermediate domain. Binding of taxol may acts as a wedge, pushing up on β-H7 and reversing the structural changes observed upon hydrolysis.

EXPANDED EXPERIMENTAL PROCEDURES

Kinesin and microtubule preparation for cryo-EM

Human monomeric kinesin K349 cys-lite was expressed and purified as previously described (Rice et al., 1999) and desalted into Storage Buffer (25 mM Tris-Cl, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2mM DTT, 100 μM ATP). For grid preparation, the kinesin at 10 mg/ml was rapidly thawed and diluted 1:4 into EM buffer (80 mM PIPES, pH 6.8, 1mM EGTA, 1mM $MgCl_2$, 1mM DTT, 0.05% Nonidet P-40) and immediately desalted into EM buffer using a Zeba Spin desalting column (Pierce). ATP was added to the desalted sample to 100 μM, and aggregates were removed by ultracentrifugation at 80,000 RCF for 15 minutes at 4ºC in a Beckman TLA-100 rotor. Kinesin being prepared for dynamic

microtubule decoration was also supplemented with GTP to 2mM before ultracentrifugation.

Taxol-stabilized GDP microtubules (MTs) were prepared as described previously (Alushin et al., 2010). GMPCPP microtubules were prepared by polymerizing 10 mg/ml porcine brain tubulin (Cytoskeleton Inc.) in CB1 buffer (80mM PIPES, pH 6.8, 1mM EGTA, 1mM $MgCl_2$, 1mM GTP, 10% glycerol) for 1 hour at 37ºC. The MTs were then pelleted using a tabletop microcentrifuge spinning at 17,000 RCF for 20 minutes. The supernatant was removed, and the pellet resuspended in 13ul of cold EM buffer and left on ice for 20 minutes. This solution was clarified by ultracentrifugation at 80,000 RCF for 10 minutes at 4ºC in a Beckmann TLA-100 rotor. 10 µl of 1mM GMPCPP was added to the GDP-tubulin sample and allowed to exchange for one hour on ice. 65 µl of pre-warmed EM buffer was added and GMPCPP-MTs polymerized during a 2-hour incubation at 37ºC.

Dynamic (primarily GDP) microtubules were prepared by polymerizing 10 mg/ml tubulin in CB1 buffer at 37°C for 30 minutes. The microtubules were then pelleted in a tabletop microcentrifuge at 17,000 RCF for 15 minutes. The pelleted microtubules were maintained at 37ºC for 15-60 minutes until grid preparation.

Cryo-EM sample preparation

All MT samples were all prepared on 400-mesh C-flat grids (Protochips, Inc) containing 1.2 µm holes separated by 1.3 µm spacing. Grids were glow-

discharged at 1 Amp for 60 seconds in an Edwards Auto 306 Carbon Evaporator prior to sample application. All cryo specimens were prepared using a Vitrobot (Maastricht Instruments), with the humidity chamber set to 100%, a blot offset of -1 mm, and a blotting time of 3 seconds with Whatman No. 1 filter paper. The chamber was maintained at 25°C for GMPCPP and GDP-taxol specimens, and 37°C for dynamic microtubules. Grids and tweezers were also pre-warmed to 37°C for the dynamic microtubule specimens.

Diluted kinesin was placed in a 37ºC water bath for 2 to 3 minutes prior to grid preparation. Taxol and GMPCPP MT cryo specimens were prepared in an identical fashion: microtubules were diluted to 0.25 mg/ml in EM buffer (supplemented with 20 μM taxol for the taxol specimen) and 4μl was applied to the grid. The MTs were allowed to adsorb to the grid for 1 minute, then 2 μl of warm 2.5 mg/ml kinesin were applied and incubated for 30 seconds. To increase the occupancy of microtubule binding, 4 μl of the sample mixture was then removed from the grid, and an additional 2 μl of kinesin was applied. The sample was incubated for 30 seconds before blotting and plunging into liquid ethane.

Pelleted dynamic GDP-microtubules were resuspended into 60 μl of warm 2.5m/ml kinesin in EM buffer supplemented with 2mM GTP. 4 μl of this MT-kinesin mixture was added to the EM grid and allowed to incubate for 1 minute before blotting and vitrification. All samples were maintained at liquid nitrogen temperature until they were loaded into the electron microscope.

Electron Microscopy

Cryo grids were loaded into a 626 single tilt cryo-transfer system (Gatan) and inserted into a Titan electron microscope (FEI) operating at 300keV. A grid atlas was acquired and squares exhibiting appropriate ice thickness were targeted using the Leginon data collection software (Suloway et al., 2005). Low-dose exposures (25 $e^-/Å^2$) were acquired at 72,000X magnification using the Tecnai low dose kit on Kodak SO163 film. Data were collected using an underfocus ranging from 1.4 to 3.5 µm. Films were developed for 12 minutes in full strength Kodak D19 developer, fixed for 5 minutes, and subsequently digitized at 0.87 Å/pixel using a Nikon Super CoolScan 8000. The pixel size at the specimen was calibrated from power spectra of micrographs of negatively stained catalase crystals imaged under identical conditions. The number of films that were digitized for each sample is shown in Table S1a.

Image processing

All processing of the digitized data leading to three-dimensional reconstruction of each sample was performed within the Appion processing environment (Lander et al., 2009) in the same manner. The contrast transfer function (CTF) was estimated using CTFTILT (Mindell and Grigorieff, 2003), and micrographs whose averaged power spectra indicated excessive drift, thick ice, or beam-induced motion were excluded from further processing. Microtubules were manually selected by drawing a line through the center of each filament, and segments were extracted at a spacing of 80Å, such that the center of each "particle" contained a unique layer of tubulin dimers in projection. Segments were

excised from the micrograph using a box size of 768x768 pixels, normalized, and any pixels whose values were above or below 4.5 sigma of the mean pixel value were replaced with values close to the mean drawn from a modeled shot noise distribution using XMIPP (Sorzano et al., 2004).

For reference-free 2D classification, each segment was phase-flipped using applyctf from the EMAN package (Ludtke et al., 1999), using defocus values estimated from its position in the micrograph. The particle stack was then binned by a factor of 2, and all but the central 200Å of each MT segment was then excluded with a soft mask whose orientation was determined by the orientation of the line drawn to pick the filament relative to the raw micrograph. The particle stacks were subjected to 4 rounds of iterative multivariate statistical analysis (MSA) and multi-reference alignment (MRA) using the CAN (Ogura et al., 2003; Ramey et al., 2009) and IMAGIC (van Heel et al., 1996) software packages. A final MSA and particle clustering was performed on the aligned particles using IMAGIC, generating class averages each containing approximately 50 particles. Particles which were members of classes that did not clearly show kinesin density (for kinesin-bound samples), or whose power spectrum did not exhibit a 10 Å layer line, were excluded from further processing, as were classes containing particles that appeared to be damaged or did not belong to 13 or 14 protofilament (pf) MTs.

Three-dimensional reconstruction

Undecorated 13 and 14 pf MT densities were scaled to the appropriate pixel size and used as initial models for a preliminary reconstruction of a 20,000 segment kinesin-bound GDP-MT + Taxol dataset. Phase-flipped particle segments were subjected to a cross-correlation based multi-model projection matching routine, followed by asymmetric back-projection of the sorted particles. Upon completion of 5 rounds of projection-matching and back-projection, kinesin density was visible on the surface of both the 13 and 14 pf MT reconstructions. The protofilament that subjectively exhibited the highest quality density was extracted from the reconstruction and used to generate 13 and 14 pf microtubule maps with seams. These preliminary densities served as starting models for refinement of complete datasets using a multi-model iterative helical real space reconstruction (IHRSR) approach.

The modified IHRSR reconstruction schema for microtubules is summarized in Figure S2. Multi-model projection matching of 13 and 14 pf MTs and asymmetric back-projection of the aligned particles was performed using libraries from the SPARX (Hohn et al., 2007) and EMAN2 (Tang et al., 2007) software packages. For each model, the 3-start helical parameters associated with the tubulin monomer lattice were calculated from the asymmetric reconstruction with the “hsearch_lorentz” function from the IHRSR program package (Egelman, 2007). Based on these helical parameters, 13 or 14 symmetrically-related copies of the asymmetric reconstruction were generated and averaged together. In the symmetrized volume, there is only one “good” protofilament that does not have α-and β-tubulin mis-aligned due to the presence

of a seam. This single good protofilament was extracted from the density with a wedge-shaped 3D mask, and the searched helical parameters are used to generate a new volume that contains a seam. This new map is then used for the next round of refinement. The angular increment used to create forward projections for matching decreased incrementally from 4 to 1 degree. A final refinement of the alignment parameters was performed for only the 14pf particles in FREALIGN (Grigorieff, 2007) without further refinement of helical parameters, using a modified script that incorporated the MT seam regeneration after each round.

The final resolution for each reconstruction was estimated by calculating the Fourier Shell Correlation (FSC) of a single dimer extracted from the even and odd volumes. For each odd and even map, a 34x40x74 voxel (59.2 x 69.6 x 128.8 $Å^3$) rectangular box of density centered on a single tubulin dimer was segmented out from the 3D reconstruction. This box of density was then multiplied by a soft-edged rectangular mask of the same size and then padded to 96x96x96 voxels. The FSC was calculated between the masked tubulin dimer densities from the odd and even maps. Inclusion of the kinesin density in this calculation worsened the estimated resolution, likely due to its flexibility relative to the tubulin dimer surface. The final resolutions of kinesin-bound samples at FSC=0.143 are 4.7, 4.9, and 5.5 for the GMPCPP, GDP, and GDP-Taxol microtubules, respectively (Figure S2B). For visualization of higher resolution features and refinement of atomic models, a negative B-factor of 400 was applied to the GMPCPP-kinesin MT and 450 to the GDP-kinesin and GDP-Taxol- kinesin

MT reconstructions using the program BFACTOR (http://grigoriefflab.janelia.org/bfactor), with the high-resolution cutoff determined by the FSC 0.143 criterion.

Atomic model building and refinement with Rosetta

Initial models of the microtubule lattice were obtained through rigid-body docking the electron crystallographic structure of tubulin (1JFF) into the resulting density maps using Chimera. A stretch of 8 residues was not modeled (residues 40-47 in the original PDB) due to disorder in both the initial crystal structure as well as the electron density map. The lattice was modeled in a 3 x 3 arrangement using symmetry operations as previously described (DiMaio et al., 2011), using the refined symmetry parameters from IHRSR. Additional runs were performed allowing lattice parameters and structure to optimize simultaneously, but no change in lattice parameters was observed in low energy models.
Rosetta's structure refinement protocol incorporates a variety of data to produce low-energy models consistent with the electron density map (Song et al., 2013). Fragments of crystal structure templates, are chosen with equal probability and are recombined to produce new models. To prepare the templates for refinement, crystal structures of previously solved tubulin structures, PDB IDS 3RYI (only for GDP) and 3RYH (only for GMPCPP) (Nawrotek et al., 2011), as well as 4I4T, 4I50, and 4I55 (Prota et al., 2013), were minimized into the electron density map to improve global alignment. Structures 3RYI and 3RYH, were crystallized with the corresponding ligand being modeled (3RYI with GDP and 3RYH with

GMPCPP) and provided accurate local structure in the vicinity of the nucleotide binding sites. Additionally, 3 and 9- residue fragments, picked according to sequence similarity and secondary structure propensity (Gront et al., 2011) were used to re-build regions corresponding to poor fits into the experimental maps. Ligand conformations were copied from the corresponding crystal structures and were held fixed throughout refinement.

Each round of refinement consists of coarse-grain refinement, in which the templates and fragments are recombined to produce full-length models, followed by all atom energy minimization(Song et al., 2013). Model-map agreement is quantified by addition of an energy term to the score function (DiMaio et al., 2009) and is present at all stages of refinement. During coarse-grain refinement (with centroid atoms representing side-chain conformations) the electron density weight has a weight of 2; during full-atom refinement the weight is increased to 20. Weights are determined based on structure prediction benchmarks (DiMaio et al., 2013). We performed three rounds of iterative refinement; after each round, in which approximately 4000 models are generated, the ensemble of low-energy models was assessed for fit to the map on an average, per-residue basis. The final ensemble of models were selected based on the lowest 1% of models according to total energy (Rosetta energy + electron density 'energy'). Convergence was assessed by the quality of the energy funnel (Figure S3A) and by the similarity of models within low-energy ensembles (Figure S3B). In all cases, the spread of energies and RMSDs of the low-energy models was small, on the range of 10 Rosetta Energy Units (REU) for the asymmetric unit (the

dimer), and average 0.56 Å RMSD within ensembles, indicating strong convergence. Additional tests to assess model specificity were performed in which the correlation of representative models to their respective maps were measure as well as correlation to alternative maps (Table S1b). The representative models had the highest correlation to their respective maps, with the exception of GDP–taxol. The GDP–taxol representative structure correlates equally with the GMPCPP and the GDP–taxol map, which is probably due to the lower resolution of the GDP–taxol map. The robustness of model-map fit was tested through cross-correlation of the models against an independent 13pf GMPCPP map. As observed in correlation measurements to the 14pf map (used in structure refinement), the GMPCPP consensus model has the highest correlation score for the 13pf GMPCPP map (not used for refinement), indicating that the model-map fit we observe is robust. As a control, we calculated the cross-correlation for crystal structures 1JFF (straight tubulin) and 1SA0 (bent tubulin). As expected, we observe a lower correlation score for the 1JFF to the GMPCPP map compared to the refined consensus models, and an even lower score for 1SA0

To produce energy-minimized consensus models, sidechain rotamers were optimized based on the consensus models. Constrained-minimization was subsequently employed for backbone and sidechain coordinates of the lowest energy model. All energy minimization is performed in the context of the experimental maps, with a map weight of 20.

Molecular graphics

All structural figures were generated with UCSF Chimera (Goddard et al., 2007; Pettersen et al., 2004). Displacement vectors were generated with a Python program that generates marker (.cmm) files which are viewable and editable in Chimera. The volume of the taxol-binding pocket was measured using POVME (Durrant et al., 2011).

Rosetta: https://www.rosettacommons.org/software/

Chimera: http://www.cgl.ucsf.edu/chimera/

POVME: http://nbcr.ucsd.edu/wordpress2/?page_id=2087

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