LETTERS

Insights into microtubule nucleation from the crystal structure of human γ-tubulin

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Microtubules are hollow polymers of αβ-tubulin that show GTP-dependent assembly dynamics and comprise a critical part of the eukaryotic cytoskeleton. Initiation of new microtubules *in vivo* requires γ-tubulin, organized as an oligomer within the 2.2-MDa γ-tubulin ring complex (γ-TuRC) of higher eukaryotes[1–3]. Structural insight is lacking regarding γ-tubulin, its oligomerization and how it promotes microtubule assembly. Here we report the 2.7-Å crystal structure of human γ-tubulin bound to GTP-γS (a non-hydrolysable GTP analogue). We observe a 'curved' conformation for γ-tubulin–GTPγS, similar to that seen for GDP-bound, unpolymerized αβ-tubulin[4]. Tubulins are thought to represent a distinct class of GTP-binding proteins, and conformational switching in γ-tubulin might differ from the nucleotide-dependent switching of signalling GTPases. A crystal packing interaction replicates the lateral contacts between α- and β-tubulins in the microtubule[5], and this association probably forms the basis for γ-tubulin oligomerization within the γ-TuRC. Laterally associated γ-tubulins in the γ-TuRC might promote microtubule nucleation by providing a template that enhances the intrinsically weak lateral interaction between αβ-tubulin heterodimers. Because they are dimeric, αβ-tubulins cannot form microtubule-like lateral associations in the curved conformation[5]. The lateral array of γ-tubulins we observe in the crystal reveals a unique functional property of a monomeric tubulin.

The X-ray crystal structure of a human γ-tubulin–GTPγS complex was determined by molecular replacement at 2.7-Å resolution (see Methods) (Fig. 1a, b and Supplementary Table 1). This is the first structure of a monomeric tubulin and the highest resolution structure of any tubulin to date. We also determined the essentially identical structure of a γ-tubulin–GTP complex at somewhat lower resolution (see Supplementary Methods), providing strong evidence that the γ-tubulin–GTPγS structure represents a bona fide GTP-bound conformation of γ-tubulin. These are the first tubulin structures with GTP or a GTP analogue bound at an exchangeable site.

As expected from the high sequence conservation within the tubulin superfamily, the overall structure of γ-tubulin is similar to previously reported α- and β-tubulin structures[4,6]. Relative to α- and β-tubulin, there are several sites at which γ-tubulin has insertions or deletions that are highly conserved across species (see Supplementary Fig. 1). Two of these, the insertion two Gly residues at position 99 in the T3 loop and the Asp insertion at position 175, are located in regions of disorder in the γ-tubulin structure, but are located on a presumptive longitudinal contact surface and might therefore help to determine γ-tubulin-specific longitudinal interaction properties (nomenclature and interaction surfaces are defined as in refs. 7, 8. Briefly, longitudinal interactions occur along a protofilament; lateral interactions occur between protofilaments, around the microtubule wall). His 107 is also lacking in γ-tubulin; this deletion restores a more ideal α-helical register to helix H3′ and removes a bulge from this region of the structure (see Supplementary Fig. 1c). Our structure reveals that removal of this bulge changes a lateral interaction surface of γ-tubulin, causing the amino-terminal end of helix H3 to protrude (see Supplementary Fig. 1c). Several other features unique to γ-tubulin sequences reside on surfaces equivalent to those that make longitudinal or lateral contacts between α- and β-tubulin in microtubules (see Supplementary Fig. 1a, b). It is difficult to determine the relative importance of some of these γ-tubulin-specific features, because the molecular interactions underlying lateral interactions in microtubules have yet to be visualized in atomic detail. Nevertheless, it is striking that these and other unique features of the γ-tubulin family map to known tubulin–tubulin interaction surfaces. These residues might collectively dictate γ-tubulin interaction strengths distinct from those of α- or β-tubulin.

In contrast to these local surface differences, many of the elements responsible for GTP binding are shared between α-, β-, and γ-tubulin[4,6,7]. Similar to β-tubulin, the guanine base of GTP in γ-tubulin is sandwiched between Phe 225 and Cys 13, with Asn 229 and Asn 207 contributing important hydrogen bonds (Fig. 1b). With the exception of conserved Ser 140 and Thr 145 residues, most of the phosphate contacts are made by backbone amides: Cys 13 to O1α, Gln 12, Gly 144, Gly 146 and Thr 145 to O1β, and Gly 144 and Thr 145 to O3γ. We have placed an Mg^{2+} ion in electron density near the β- and γ-phosphates, making contacts with phosphate oxygens O2β and O3β, and with Asp 68, Glu 70 and a water molecule.

Not all features of the nucleotide binding pockets are identical between γ- and β-tubulin, however. In addition to the disordered T3 loop mentioned above, the T5 loop is also disordered in γ-tubulin. In αβ-tubulin, the T3 and T5 loops participate in longitudinal contacts, so it is possible that they only become ordered and form interactions with GTP/GDP upon longitudinal tubulin association. We performed comparative nucleotide binding studies to determine the degree to which structural similarity between the nucleotide-binding pockets of γ- and β-tubulin extends to shared GTP-binding properties (Fig. 1c). γ- and β-tubulin both bind GTP with very similar affinity (~60 nM). Competition experiments using GDP show that both γ- and β-tubulin have a similar preference for GTP over GDP. Together with strong conservation of sequence and structure, the essentially identical nucleotide-binding affinities of γ-tubulin and β-tubulin indicate that they probably share common nucleotide-binding energetics that are largely unaffected by structural differences in the T3 and T5 loops. This also makes it likely that, analogous to its action on β-tubulin, α-tubulin can stimulate the GTPase activity of γ-tubulin upon longitudinal association.

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Two distinct conformations of αβ-tubulin have been characterized structurally. The differences between the two conformations involve small but significant domain rearrangements within and between both tubulin subunits. The 'straight' αβ-tubulin conformation has only been observed in microtubules and in the protofilament-containing sheets induced by polymerization in the presence of zinc[5,6]. A 'curved' conformation has been observed in unpolymerized αβ-tubulin bound to the stathmin-like domain of the microtubule-destabilizing protein RB3. One feature that distinguishes these two tubulin conformations is the arrangement of the segment containing helices H6 and H7 (ref. 4). Another is the relative orientation between the structurally conserved, rigid N-terminal domain and the more variable intermediate domain[4]. Notably, superposition of the straight and curved β-tubulin conformations onto the γ-tubulin structure, using the structurally well-conserved N-terminal domain, reveals that the arrangement of indicative domains in the γ-tubulin–GTPγS structure more closely resembles that of the curved conformation (Fig. 2, Table 1 and Supplementary Fig. 2). Although the conformational match is extensive, it is not perfect. The surface-exposed helices H9 and H10 of γ-tubulin are distinctively arranged, differing from the arrangement seen in the two previously determined αβ-tubulin structures (Fig. 2, Table 1 and Supplementary Fig. 2). However, most secondary structural elements in γ-tubulin are much closer to their curved αβ-tubulin counterparts. Using this superposition, atoms from helices H6 through H7 in γ-tubulin have an r.m.s. coordinate deviation of 2.46 Å to the straight conformation of β-tubulin, but only 1.38 Å to the curved conformation of β-tubulin. Similarly, atoms in the largely buried β-sheet of the intermediate domain of γ-tubulin have an r.m.s. coordinate deviation of 2.31 Å to the straight conformation and only 1.18 Å to the curved conformation. Comparisons to the two known α-tubulin conformations yield similar results (Table 1 and Supplementary Fig. 2).

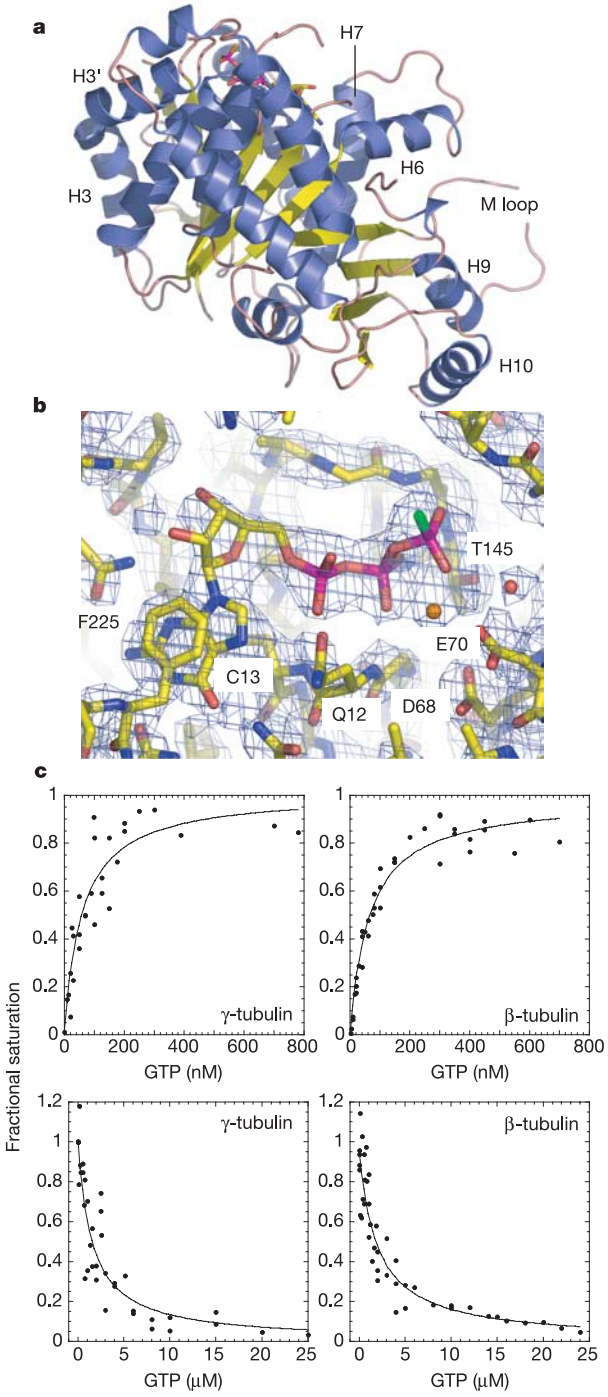

Figure 1 | Structure and nucleotide binding-properties of γ-tubulin. **a**, Cartoon representation of the γ-tubulin structure. **b**, Representative electron density from a complete annealed σ_A-weighted $2mF_o - DF_c$ map computed from the final model. GTP-γS and interacting residues are shown. **c**, Nucleotide binding studies reveal that γ-tubulin (left) and the exchangeable site on β-tubulin (right) have similar GTP/GDP binding properties. The affinities of γ-tubulin and β-tubulin for GTP are 58.4 ± 12.6 nM and 64.5 ± 6.3 nM (respectively) and GDP affinities are 1.13 ± 0.2 μM for γ-tubulin and 1.55 ± 0.25 μM for β-tubulin.

When bound to either GTP-γS or GTP, γ-tubulin adopts a curved conformation with a characteristic arrangement of helices H6 and H7 and the intermediate domain. The similarity between this conformation and that seen for both curved α- and β-tubulin in the unpolymerized, RB3-bound state (Fig. 2, Table 1 and Supplementary Fig. 2) suggests that this is a functionally relevant tubulin conformation. The arrangement of helices H6 and H7 in the curved conformation of α- and β-tubulin is incompatible with straight longitudinal interactions[4] like those presumed to occur between the γ-TuRC and a microtubule (see below). Thus, our unexpected observation of a curved, GTP-bound conformation represents the first structural evidence that curved-to-straight transitions might occur in γ-tubulin at the minus-end of a microtubule, and that they might be substantially nucleotide-independent. This would distinguish conformational switching in γ-tubulin from that in signalling GTPases, consistent with the observation that tubulins represent a distinct class of GTP-binding proteins[8]. In addition, several recent structures of the monomeric prokaryotic tubulin homologue FtsZ in different nucleotide states all show essentially the same curved conformation[9].

One of the observed crystal packing interactions bears a striking similarity to the homotypic lateral interactions previously only seen to occur in the microtubule lattice[5] (Fig. 3). The γ-tubulin interaction lacks the lateral curvature of the cylindrical microtubule lattice, but uses a virtually identical contact region and buries a comparable surface area (Fig. 3). The γ-tubulin structure thus shows that, at the level of a tubulin monomer, a curved conformation can form microtubule-like lateral interactions. In contrast, the curved conformation of the αβ-tubulin heterodimer discourages microtubule growth for two reasons: it disrupts the parallel presentation of α- and β-tubulin lateral interaction surfaces within a heterodimer, and the positions of helix H6 and the H6–H7 loop are incompatible with straight longitudinal assembly between heterodimers[4]. By virtue of being monomeric, γ-tubulin acquires a unique functional property—the ability to self-associate laterally while in the curved conformation.

Lateral interactions between αβ-tubulins must be sufficiently flexible to permit microtubules formed *in vitro* to contain between 9 and 16 protofilaments, and to allow the formation of flat sheets that

have been observed as intermediates in microtubule elongation[10]. The intrinsic flexibility of the structurally conserved lateral interaction, together with local sequence conservation (see Supplementary Fig. 1b, c), makes it likely that lateral interactions like those observed in the crystal form the basis for cylindrically curved arrays of γ-tubulin such as those found in the γ-TuRC. It also suggests that components of the γ-TuRC other than γ-tubulin have important roles in establishing a specific ring-like geometry.

Our results provide insight into the possible mechanisms and regulation of γ-tubulin-mediated microtubule nucleation. The low stability of lateral associations between αβ-tubulin heterodimers is believed to be the weak link in microtubule assembly. Appropriately spaced lateral assemblies of γ-tubulin, analogous to those we observe in the crystal (Fig. 3), and such as we propose exist within the γ-TuRC, have the potential to make strong longitudinal contacts with multiple αβ-tubulins. These assemblies profit from the unique propensity of monomeric γ-tubulin to form stable lateral interactions, with spacing and pitch coincident with those of the

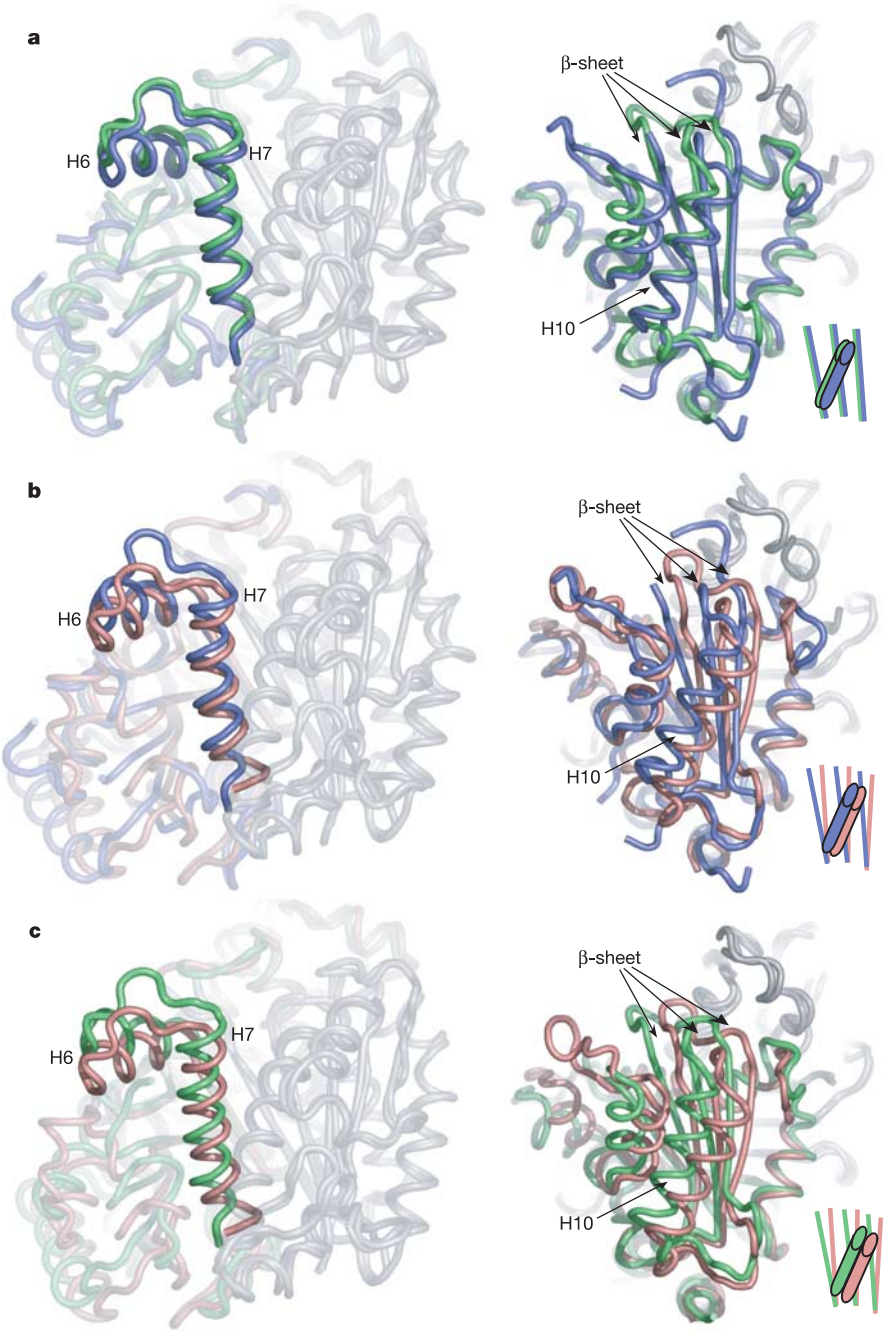

Figure 2 | γ-tubulin adopts a curved conformation. Structural superposition of β-tubulin conformations onto γ-tubulin using the rigid N-terminal domain. **a**, The γ-tubulin structure (blue) and the curved β-tubulin structure (green) share a similar arrangement of the H6–H7 segment (left) and of intermediate domains (right). **b**, The γ-tubulin structure (blue) and the straight β-tubulin structure (pink) show characteristic differences in the orientation of the H6–H7 segment (left) and the intermediate domain (right). **c**, Comparison between the curved (green) and straight (pink) β-tubulin conformations, illustrating the characteristic differences in the H6–H7 segment (left) and the intermediate domain (right).

Table 1 | Statistics for superposition of α- and β-tubulin conformations onto γ-tubulin

Tubulin (α or β); straight (S) or curved (C)	Intermediate β-sheet				H10				H6–H7 segment				N-terminal domain			
	α (S)	α (C)	β (S)	β (C)	α (S)	α (C)	β (S)	β (C)	α (S)	α (C)	β (S)	β (C)	α (S)	α (C)	β (S)	β (C)
r.m.s. deviations (Å)																
γ-tubulin	2.18	1.14	2.31	1.18	3.68	2.65	4.23	3.20	1.98	0.99	2.46	1.38	1.09	1.21	1.36	1.15
α-tubulin (S)	–	2.11	1.04	2.43	–	4.51	1.83	3.95	–	1.82	1.06	2.77	–	1.01	0.92	1.01
α-tubulin (C)	–	–	2.27	1.26	–	–	5.16	2.75	–	–	2.29	1.78	–	–	1.35	1.12
β-tubulin (S)	–	–	–	2.68	–	–	–	4.28	–	–	–	3.24	–	–	–	1.10

All known α- and β-tubulin conformations (straight, PDB code 1JFF; curved, PDB code 1SA0) were aligned to the γ-tubulin structure using the N-terminal domain (residues 1–175). Pairwise r.m.s. coordinate deviations for indicated secondary structural elements of the intermediate domain were calculated for these aligned models. The N-terminal domain r.m.s. deviations (rightmost four columns) are included as a control to show that this domain aligns well for all sequences and conformations. The intermediate β-sheet includes residues 267–273, 313–320, 352–358 and 373–38. Helix H10 includes residues 322–340. The H6–H7 segment includes residues 205–240. Deviations between curved and straight conformations of different sequences are significantly greater than deviations between like conformations and like sequences.

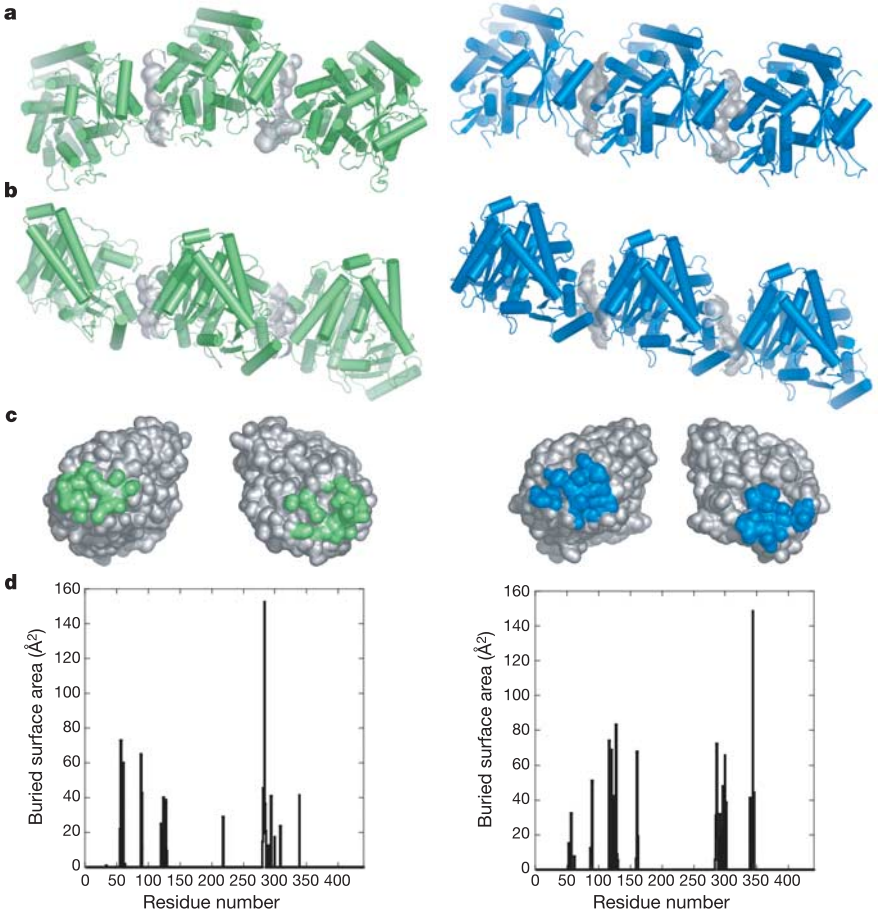

Figure 3 | Lateral interactions between γ-tubulins resemble lateral interactions in the microtubule lattice. a, 'Minus end' views of laterally interacting β-tubulins in the microtubule lattice (green) (K. Downing, personal communication), and laterally interacting γ-tubulins in the crystal (blue). Contact regions between monomers are indicated by the grey surfaces on the central monomer. **b**, Comparative 'outside' views of the same interactions, showing a similar pitch for both. **c**, Lateral interaction regions of β-tubulin in the microtubule lattice (green) and γ-tubulin in the crystal (blue) are indicated on the molecular surface. Microtubule and γ-tubulin crystal interaction footprints are very similar. **d**, Comparison of the buried surface area (in Å^2) at each position for β-tubulin lateral interactions in the microtubule lattice (left) and γ-tubulin crystal interactions (right). Virtually identical regions of the structure are involved in both interactions.

microtubule lattice. Presumably, these lateral assemblies of γ-tubulin promote microtubule assembly by providing a template that directly enhances lateral interactions between adjacent γ-TuRC-bound αβ-tubulins. Whether γ-tubulin within the γ-TuRC is present in a curved or straight conformation remains to be determined. However, the significant increase in intermolecular contact surface area observed when αβ-tubulin straightens in the microtubule lattice argues that γ-tubulin is also likely to switch conformations, either in response to α-tubulin binding or through the action of other γ-TuRC components. By analogy to β-tubulin, GTP binding and hydrolysis on γ-tubulin might regulate its affinity for α-tubulin, potentially providing a mechanism for the observed release of microtubules from the centrosome[11] or tuning of the nucleating activity of γ-TuRC.

METHODS

Protein expression, crystallization and GTP/GDP binding experiments. See the Supplementary Information for details of expression, purification, GTP/GDP binding experiments, and protein crystallization.

Structure determination. Diffraction data were collected at beamline 8.2.1

Table 2 | X-ray statistics

Refinement	GTP-γS	GTP
Resolution (Å)	2.7	3.0
R_{work}/R_{free}	24.0/29.2	24.7/30.9
Number of atoms		
Protein	3145	3078
Ligand/ion	33	33
Water	22	0
B-factors		
Protein	52.8	49.4
Ligand/ion	44.8	46.4
Water	41.1	N/a
r.m.s deviations		
Bond lengths (Å)	0.0085	0.0086
Bond angles (°)	1.67	1.95

(HHMI) at the Advanced Light Source in Berkeley, California. Thin, radiation sensitive crystals required the merging of data from two crystals in order to obtain reasonably complete data sets at resolutions higher than 3.1-Å. Data processing and reduction were carried out using the HKL2000 package[12]. Molecular replacement searches and subsequent refinements were carried out using CNS[13], and model building was performed using O[14]. Initial phases for the GTP-γS data were determined by molecular replacement using a β-tubulin search model[4] (PDB accession number 1SA0) with side chains truncated to alanine and cofactors (GDP, Mg^{2+}, colchicine and waters) removed. Regions of the search model not confirmed by the initial electron density map were deleted, and side chains with clear electron density in the initial map were added. As the model phases improved, additional side chains and loops were added when there was interpretable electron density. GTP-γS was added only after the electron density clearly indicated the presence of the γ-phosphate. Over the course of model refinement and rebuilding, complete annealed omit maps were used extensively to help avoid model bias. The lower resolution GTP-bound structure was solved using the GTP-γS structure stripped of cofactors as a search model, and subsequently refined in a similar manner to the GTP-γS structure. Refinement statistics are presented in Table 2. Figures were made using PyMOL[15].

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information Coordinates and structure factors have been deposited in the Protein Data Bank under accession numbers 1Z5V and 1Z5W. Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to D.A.A. (agard@msg.ucsf.edu).