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The role of tubulin-tubulin lattice contacts in the mechanism of microtubule dynamic instability

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ABSTRACT

Microtubules form from longitudinally and laterally assembling tubulin α/β -dimers. The assembly induces strain in tubulin, resulting in cycles of microtubule catastrophe and regrowth. This so-called dynamic instability is governed by GTP hydrolysis that renders the microtubule lattice unstable, but it is unclear how. We used the human microtubule nucleating and stabilising neuronal protein doublecortin and high-resolution cryo-EM to capture tubulin's elusive hydrolysis intermediate GDP.Pi state, alongside the pre-hydrolysis analogue GMPCPP state, and the post-hydrolysis GDP state with and without an anti-cancer drug Taxol®. GTP hydrolysis to GDP.Pi, followed by Pi release, constitute distinct structural transitions, causing unevenly distributed compressions of tubulin dimers, thereby tightening longitudinal and loosening lateral inter-dimer contacts. We conclude that microtubule catastrophe is triggered because the lateral contacts can no longer counteract the strain energy stored in the lattice, while reinforcement of the longitudinal contacts may support generation of force.

INTRODUCTION

Microtubules (MTs) are key players in cell division, migration and signalling and are thus targets for herbicides, fungicides and in the treatment of many human diseases, most notably cancer. Their cylindrical lattice is built through longitudinal (head-to-tail) and lateral (side-by-side) supramolecular assembly of the tubulin α/β -heterodimer. In solution this heterodimer favours a 'bent' conformation, but the lattice geometry imposes and temporarily stabilizes a tense 'straight' conformation¹⁻³ (Fig. 1a). Such spring-like conformational strain lies at the heart of MT dynamic instability and is vital for MT function^{4,5}. The crucial switch between MT growth and shrinkage – known as 'catastrophe' – is driven by the β -subunit GTPase cycle, but its molecular basis has been unclear. The long-standing question is how the strain energy stored in the lattice is released to depolymerise MTs and do work in the cell.

α - and β -tubulin subunits are structurally very similar. Each comprises three

38 functional domains^{6,7} determining MT polarity: 1) a GTP-binding N-terminal (N)
39 domain, located towards the faster growing MT +end, 2) an intermediate (I) domain,
40 facing the MT -end, and 3) a C-terminal (C) domain protruding from the MT wall (Fig.
41 1a). Tight 'head-to-tail' dimerisation of α/β -tubulin buries the GTP-binding site of the
42 α N domain at its interface with the β I domain⁷, making it a non-exchangeable site (N-
43 site), always occupied by GTP. The corresponding region in the β -subunit is
44 exchangeable (E-site), and when occupied with GTP, it mediates analogous, but
45 metastable 'head-to-tail' association with α I domain of another dimer, forming
46 protofilaments (PFs). Individual PFs are compatible with the relaxed (energetically
47 favoured) 'bent' tubulin conformation, but the lateral association of tubulin via specific
48 interlocking of N and I domains gradually straightens tubulin dimers as well as the PFs
49 formed by them^{2,8} (Fig. 1a). Thus, the mature MT geometry imposes and temporarily
50 stabilises the straight tubulin conformation^{1-3,5}, and the MT wall integrity relies on N
51 and I domain involvement in both longitudinal and lateral lattice contacts (Fig. 1a). In
52 vitro, MTs polymerise with a range of PF numbers, but in cells most MTs have a 13-PF
53 architecture. This architecture necessitates that at one site called the seam, α - and β -
54 tubulin form heterotypic lateral contacts, breaking the otherwise helical symmetry of
55 an MT cylinder. Thus 13-PF MTs are pseudo-helical.

56 As described above, tubulin strain is inherent to building of an MT wall and is
57 like a time bomb waiting for the trigger for disruption. This trigger involves GTP
58 hydrolysis at the inter-dimer interface: the β -subunit's GTP is hydrolysed due to
59 recruitment of the α -subunit's catalytic E254 residue⁷ during MT polymerisation. The
60 equivalent residue within the intra-dimer interface is a non-catalytic β K254, hence the
61 N-site GTP is never hydrolysed. E-site GTP hydrolysis somehow renders the MT wall
62 unstable. Recent high-resolution cryo-EM structures using GTP analogues show^{9,10}, in
63 line with earlier studies^{11,12}, that hydrolysis is accompanied by a longitudinal inter-
64 dimer lattice compaction (~ 2 Å), but it is elusive how this rearrangement is linked to
65 MT catastrophe. How is the strain energy stored in the lattice released to depolymerise
66 MTs? The vital structure of the GDP.Pi intermediate has remained unknown, although
67 GTP γ S has been thought to mimic it^{10,13-15}.

68 The imbalance between subunit strain energy and lattice binding energy leading
69 to catastrophe would be predicted to result from perturbation of lattice contacts during
70 and/or after lattice compaction. To detect these likely subtle (in the range of ~ 2 Å)
71 changes in lattice contacts it is necessary to compare high-resolution MT structures
72 representing sequential steps in the GTPase cycle in a uniform MT architecture. This
73 has not been achieved to date. We used doublecortin (DCX), a unique MT-associated
74 protein (MAP) and a perfect tool for this work, as it nucleates and stabilises a
75 physiological 13-PF MT architecture by binding between 4 tubulin dimers in all
76 nucleotide states (Fig. 1a, d) without perturbing known GTPase-linked transitions^{16,17}.
77 Crucially, DCX's robust MT nucleation activity allowed rapid (~ 30 sec) sample
78 preparation, such that we could capture the earliest stages of DCX-MT polymerisation
79 (Fig. 1b), including the long-sought GDP.Pi transition state. By focusing on only the

80 13-PF MT architecture in the presence of only one MT binding partner – in contrast to
81 previous work^{9,10} – our structures shed new light on the structural transitions that drive
82 dynamic instability.

83 RESULTS

84 Determination of 13-PF structures of DCX-MTs in different nucleotide states

85 To elucidate the mechanism of MT catastrophe, we analysed conformational changes
86 in tubulin through its GTPase cycle with single particle cryo-EM, and assessed the
87 impact of these changes on longitudinal and lateral lattice contacts. Overall, we
88 determined 13-PF structures of 4 nucleotide states of DCX-MTs: 1) a GTP-like lattice
89 (4.4 Å resolution) stabilised by GMPCPP, a slowly hydrolysable GTP analogue¹⁸; 2) a
90 GDP.Pi lattice (4.2 Å) and 3) a control GTPγS lattice (4.4 Å), both of which show clear
91 density equivalent to the γ-P of GTP, but no density for the magnesium ion¹⁰; and 4) a
92 GDP lattice (3.8 Å) (Fig. 1c, Table 1, Supplementary Figs 1-3 and Supplementary Table
93 1). We calculated the lattice spacing and refined atomic models of tubulin for each
94 reconstruction (Fig. 1c, Table 1 and Supplementary Table 1). The pre-hydrolysis
95 GMPCPP lattice has an extended axial repeat (83.74 ± 0.20 Å), whereas the GDP.Pi,
96 GTPγS and GDP lattices are compacted (81.74 ± 0.06 , 82.10 ± 0.07 and 81.90 ± 0.06 Å,
97 respectively). The compaction of the lattice together with the absence of magnesium
98 density support the interpretation that we have captured the post-hydrolysis, GDP.Pi
99 state. Even 10-20 dimer-long multi-PF MT precursor assemblies (DCX-preMTs)
100 exhibit a compacted lattice (Fig. 1b), which emphasizes the speed of GTP hydrolysis
101 and that the MT wall does not need to be complete for it to occur, at least with DCX
102 bound at inter-dimer vertices (Fig. 1d). The overall tubulin conformation in GTPγS-
103 DCX-MTs is in fact more similar to that in GDP-DCX-MTs than in GDP.Pi-DCX-MTs
104 (Supplementary Fig. 3) despite the presence of γ-P/γ-S (Supplementary Fig. 2). This
105 explains the slow MT growth observed with GTPγS and highlights the unusual
106 properties of this nucleotide analogue¹⁴.

107 GTP hydrolysis in β-tubulin leads to uneven compression of α-tubulin

108 To characterise the origin of the lattice compaction in our reconstructions, we
109 compared the different MT states by superposition on the β-subunit, which itself does
110 not show large conformational changes¹⁰. This comparison reveals conformational
111 rearrangements in the nucleotide-binding βT5 loop that likely cause changes in the E-
112 site hydrogen bond network (βN domain) in the GDP.Pi state relative to the GMPCPP
113 state (transition 1) (Fig. 2a, b and Videos 1, 2). The βT5 loop interfaces with the
114 adjoining tubulin subunit via αS9 strand of the αI domain's central β-sheet. This β-sheet
115 is sandwiched between two inter-dimer anchor points: A1) a hydrophobic contact
116 between the C-terminal end of helix αH8 plus αH8-S7 loop and helix βH11¹⁰; and A2)
117 a hydrogen bond between K336 side chain at the C-terminus of helix αH10 and K174
118 backbone carbonyl in βT5 loop (Fig. 2a-c). Thus, the conformational change in the βT5
119 loop leads to a ~1 Å shift of the αI domain towards the β-subunit. The αN domain,

120 connected to the α I domain via α S6-S7 strands, undergoes a more prominent
121 displacement (up to ~ 2.2 Å) towards both the MT -end and lumen, together with a part
122 of the C domain associated with it. This results in an uneven compression of the whole
123 α -subunit, globally manifested as lattice compaction. After Pi release (transition 2), the
124 α -subunit slightly twists (by a further ~ 1 Å; Fig. 2a and Video 1), further narrowing the
125 gap between the dimers.

126 **Uneven compression of α -tubulin reinforces longitudinal lattice contacts**

127 Transitions 1 and 2 strengthen the longitudinal inter-dimer interface (Fig. 2c, d,
128 Supplementary Fig. 4 and Video 3). While the core of the interface is conserved among
129 all states, transition 1 results in additional interactions between α N-terminus and β T2
130 loop, α T7 loop and helix β H1, and more extended interaction of strand α S9 with helix
131 β H11 (Fig. 2c), burying ~ 165 Å² ($\sim 10\%$) more of the solvent accessible surface area
132 (SASA) and increasing the predicted amount of energy needed to dissociate the
133 complex (ΔG^{diss}) by ~ 0.8 kcal/mol ($\sim 15\%$) (Fig. 2d). Transition 2 further increases the
134 interface area by ~ 110 Å² and ΔG^{diss} by ~ 1.1 kcal/mol due to additional interactions of
135 α H3-S4 loop with β S3-H3' loop and α T7 loop with helices β H1 and β H2 (Fig. 2c, d).

136 **Uneven compression of α -tubulin weakens lateral lattice contacts**

137 It seems paradoxical that the polymer becomes unstable while longitudinal contacts
138 become tighter after lattice compaction. This draws attention to the lateral contacts.
139 The N and I domains are connected in the lattice by both the longitudinal and the lateral
140 interfaces, enabling transmission of structural information between PFs. Due to the
141 uneven compression of the α -subunits during transition 1, the lattice compaction not
142 only tightens the inter-dimer spaces, but also perturbs the lateral contacts; this is
143 because the α N domains move further towards the MT -end and lumen than the
144 laterally connected α I domains from the neighbouring PFs (Fig. 3 and Video 4). Thus,
145 in our GTP state model, the conformation of the lateral contacts between the α -subunits
146 are close enough (< 4 Å) to support multiple points of connectivity (e.g. Gly57-Glu284,
147 His88-His283, His88-Glu284, Glu90-Lys280, Gln128-Gln285). After hydrolysis, two of
148 these distances (the Gly57-Glu284 and His88-His283 pairs) increase > 4 Å as measured
149 in our models of the GDP.Pi and GDP states. For example, α Gly57- α Glu284 are ~ 3.8 Å
150 apart in the GTP state model, ~ 4.6 Å apart in the GDP.Pi state model (Fig. 3b and
151 Supplementary Fig. 5) and ~ 5.6 Å apart in the GDP state model. Transition 2 also
152 results in noticeable perturbation of lateral contacts between the β -subunits. Out of
153 several putative connections (< 4 Å) in our models of the GTP and GDP.Pi states (e.g.
154 Ala55-Arg282, Lys58-Gln280, Gln83-Tyr281, Arg86-Tyr281 and Glu125-Lys336),
155 separations of > 4 Å are observed between the β Ala55- β Arg282 and β Lys58- β Gln280
156 pairs in the GDP state model, while density corresponding to the side chain of β Lys58
157 appears distinct in the GDP state reconstruction compared to the GDP.Pi state
158 reconstruction, indicating that this residue is no longer involved in the lateral contact
159 after Pi release (Fig. 3b, Supplementary Fig. 5 and Video 4).

160 **DCX-MT lattice skew varies slightly with nucleotide state**

161 The GTPase-dependent lattice transitions also manifest as changes in PF skew. The
162 extended GMPCPP lattice shows a slightly right-handed skew ($0.16 \pm 0.08^\circ$). Transition
163 1 straightens PFs ($0.00 \pm 0.08^\circ$), and transition 2 restores some of the initial skew (0.08
164 $\pm 0.07^\circ$) (Supplementary Fig. 6). The direct influence of DCX on this property is not
165 known, but MTs bound by the end binding (EB) protein have a left-handed PF skew¹⁰,
166 reaching $0.25 \pm 0.01^\circ$ in the GTP γ S-EB-MT, whereas our GTP γ S-DCX-MT is almost
167 straight ($-0.02 \pm 0.08^\circ$) (Supplementary Fig. 6 and Supplementary Table 1). Thus, PF
168 skew is influenced by several variables, including the tubulin nucleotide state and
169 bound MAPs, but it is not obvious from all these data that it is intrinsic to the
170 mechanism of dynamic instability.

171 **The role of lateral contacts in MT integrity supported by Taxol[®] stabilisation**
172 **mechanism**

173 The blockbuster MT-stabilising drug Taxol[®] binds near the β -subunit lateral contacts⁶
174 and inhibits catastrophes. It has been suggested that it stabilises MTs by allosterically
175 counteracting lattice compaction⁹. We investigated this idea using GDP-DCX-MTs and
176 found that Taxol[®] binding to these pre-stabilised MTs does not reverse the GTPase-
177 driven conformational changes. Rather, it locally displaces fragments of the β M and the
178 β S9-S10 loops ($\leq 1.7 \text{ \AA}$) that it directly contacts (Fig. 4). Therefore, an alternative
179 explanation of Taxol[®]'s MT-stabilising effect is that, while tubulin lateral contacts are
180 loosened by GTP hydrolysis (Fig. 3b), Taxol[®] locks the β M lateral loops in the lattice-
181 constrained conformation and thereby prevents PF peeling. Such stabilisation of the
182 otherwise labile GDP-MTs may also explain why the drug renders MTs flexible^{19,20}.
183 Since it stabilises every other lateral contact along each PF (assuming saturation),
184 Taxol[®] probably keeps the PFs only loosely associated within the GDP-MT lattice.
185 Presumably as a consequence, we find MTs pre-stabilised with Taxol[®] to be poorly
186 decorated with DCX in vitro (Supplementary Fig. 1b). The same is true for cellular
187 MTs²¹, providing further evidence for looseness of the Taxol[®]-stabilised lattice, which
188 DCX can override if it binds first.

189 **DISCUSSION**

190 Our data suggest that a portion of GTP hydrolysis energy is absorbed by the lattice,
191 triggering a 2-step loosening of its lateral contacts, first between the α -subunits
192 (transition 1) and then between the β -subunits (transition 2), which coincides with
193 strengthening of inter-dimer longitudinal interfaces (Fig. 5). This explains why whole
194 PFs peel away from MTs after rapid induction of catastrophe¹. Our data suggest that
195 catastrophe happens because the lateral contacts become too weak to counteract the
196 intrinsic strain of the straight tubulin dimers. The role of homotypic lateral contacts in
197 dynamic instability has been discounted in previous studies^{9,10}, but in comparing MTs
198 stabilised by a single protein ligand (DCX) and in capturing the bona fide GDP.Pi state

199 of the tubulin GTPase, we have identified their likely crucial role in contributing to MT
200 dynamics. Very small differences in the energetics of lateral contact formation due to
201 isoform-specific sequences could be an important mechanism for regulating MT
202 dynamics in particular physiological settings²². As tubulin relaxes (bends), the
203 disassembling PFs curl outward generating force^{3,23}. The developing curvature in turn
204 weakens the inter-dimer longitudinal contacts (Supplementary Fig. 7a-c), allowing
205 complete PF breakdown. So, the fine-tuned MT dynamics seem to only require
206 perturbation of a few connections per dimer to tip the balance towards MT catastrophe.
207 In our GDP-DCX-MT, catastrophe is prevented by DCX stabilisation.

208 These results explain why only GTP- or GMPCPP-tubulin spontaneously
209 nucleate MTs, since only the extended lattice forms favourable lateral contacts,
210 apparently balancing the energetic cost of tubulin straightening. To counteract tubulin
211 bending, the longitudinal contact is also larger in the lattice ($\sim 1533 \text{ \AA}^2$) compared to X-
212 ray structures of curved PFs ($832\text{-}1026 \text{ \AA}^2$)²⁴⁻²⁸ (Supplementary Fig. 7c). Only this
213 lattice-imposed straight conformation brings the catalytic α E254 residue sufficiently
214 close to the E-site GTP to complete the GTPase machinery (Supplementary Fig. 7d).

215 The specific flexibility of Taxol[®]-MTs makes sense when the role of lateral
216 contacts in dynamic instability is taken into account. Thus, where Taxol[®] is added to
217 MTs after polymerisation has been allowed to proceed, our work suggests that the
218 lateral contacts in these MTs would be loose due to the majority of tubulin being bound
219 to GDP. Taxol[®] works by holding the β -tubulin lateral contacts in place, but the entirety
220 of the lattice is presumably rather flexible, explaining the biophysical properties of these
221 MTs²⁹ and the documented knock-on effects on binding by microtubule regulators,
222 including EBs and DCX^{21,30}. Further, in the case of experiments where Taxol[®] is added
223 at polymerisation initiation, it seems likely that its binding may alter or trap the
224 GTPase-linked structural transitions in some more complex, time-dependent and
225 structurally heterogeneous way. A previous study⁹ proposed that Taxol[®] prevents or
226 reverses GTPase-dependent MT lattice compaction, and that MT stabilisation by the
227 drug relies on preservation of the extended (GTP-like) lattice state. In our study and in
228 another recent publication¹⁹, Taxol[®] binding was found to not induce lattice expansion.
229 Similarly, other MT stabilising agents may be expected to also work by stabilizing lateral
230 contacts in lattice-like conformations³¹.

231 The stochastic nature of GTP hydrolysis events in the lattice⁴ would be predicted
232 to cause lateral contact mismatches between neighbouring pre- and post-hydrolysis
233 dimers. These by themselves could cause tensions in the lattice, destabilising it and/or
234 synchronising hydrolysis, presumably contributing to the multi-step nature of
235 catastrophe³². The MT seam is an inbuilt source of post-hydrolysis lateral mismatch¹⁰,
236 but not the only source of MT instability, because MTs do not simply unzip at the seam
237 - rather their individual PFs depolymerise completely. Our analysis shows that the
238 irreversible lattice transitions cause a global MT catastrophe, liberating tubulin to
239 exchange its nucleotide and start the cycle again.

240 The intrinsic polarity of the tubulin dimers themselves² and of the

241 conformational changes we have described here (Figs 2, 3 and 5), likely contribute to
242 the intrinsic but mechanistically poorly understood differences in MT dynamics at the
243 MT + and -ends. Our data highlight the conformational plasticity of α -tubulin at the
244 longitudinal tubulin interface. If this conformational plasticity is recapitulated at the
245 MT -end, we predict that this would provide a previously unanticipated conformational
246 barrier to stable addition of the incoming tubulin dimers. At the +end, in contrast, while
247 we predict that α -tubulin retains its conformational plasticity in the incoming free
248 tubulin dimers, the polymer-held β -tubulin would be predicted to provide a more stable
249 platform/template for dimer docking, a stability that is reflected in faster +end growth.

250 With a multitude of MAPs, polymerases, depolymerases and modifying
251 enzymes in vivo, MT dynamics are unlikely to be governed by GTP hydrolysis alone,
252 but by a complex network of regulators. For example, recent work has indicated the
253 importance of acetylation around the lateral contacts as cellular means to prolong MT
254 longevity^{33,34}; this reinforces our conclusions about their role in MT stability. Here we
255 show that the majority of MTs - built from GDP.Pi or GDP tubulin - are structurally
256 poised for immediate catastrophe and force generation. However, overriding cellular
257 regulators can ensure that tubulin strain energy remains stored in the lattice until
258 required, prompted by cell physiology.

259

260 **ACCESSION CODES**

261 Our maps and coordinates were deposited in EMDB/PDB with the following accession
262 codes: GMPCPP-DCX-MT, EMD-3961 and PDB ID 6EVW; GDP.Pi-DCX-MT, EMD-
263 3962 and PDB ID 6EVX; GTP γ S-DCX-MT, EMD-3963 and PDB ID 6EVY; GDP-
264 DCX-MT, EMD-3964 and PDB ID 6EVZ; GDP-DCX-Taxol[®]-MT, EMD-3965 and PDB
265 ID 6EW0.

266

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271

272 **AUTHOR CONTRIBUTIONS**

273 S.W.M. conceived experimental strategies, designed and carried out experiments and
274 computations, processed data, interpreted results and wrote the manuscript; C.A.M.
275 proposed and supervised research, interpreted results and wrote the manuscript.

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277 **COMPETING FINANCIAL INTEREST STATEMENT**

278 **Competing interests.** None to declare.

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384

385 **FIGURE LEGENDS**

386 **Figure 1 | Tubulin GTPase cycle and MT structures stabilised by DCX.** **a**, DCX binds
387 between 4 tubulin dimers, nucleating and stabilizing 13-PF 3-start MTs. In its absence,
388 MTs undergo catastrophe once GTP hydrolysis and Pi release outpace MT growth.
389 Then tubulin bends, whole protofilaments (PFs) peel away and depolymerise, and the
390 cycle resumes. **b**, Top, representative cryo-micrograph showing the earliest tubulin
391 assemblies (bent PFs), DCX-MT precursors (DCX-preMTs) and a mature 13-PF DCX-
392 MT. Bottom, averaged power spectra and the layer line profile plots of DCX-MTs
393 (n=16) and preMTs (n=9) showing compacted lattice spacing. **c**, Lumenal views of the
394 cryo-EM reconstructions of MTs in GMPCPP, GDP-Pi and GDP states with fitted
395 backbone traces of the refined atomic models of tubulin and close-up views of
396 nucleotide densities (GTP and GMPCPP, yellow; GDP.Pi, orange; GDP, red) with
397 selected residue side chains (coloured by heteroatom) around them at the exchangeable
398 (E) and non-exchangeable (N) sites. N and I tubulin domains are outlined for one
399 subunit. **d**, Front view of DCX binding site. N, I and C tubulin domains are outlined
400 for one subunit.

401

402 **Figure 2 | GTPase-dependent structural transitions strengthen the longitudinal MT**
403 **lattice contacts.** **a**, Backbone front view and angled close-up cut-away view
404 comparisons of different MT nucleotide states based on superposition on the β 1-
405 subunit (underlined); coloured by the degree of displacement, heteroatom or as
406 follows: GMPCPP, red; GDP.Pi, orange; GDP, red; Mg^{2+} , green. Tubulin domains are
407 outlined and their overall relative movements around A1 and A2 anchor points are
408 indicated with straight arrows. Curved arrows indicate how these transitions influence
409 tubulin in the context of MT lattice. **b**, Conformational change of the T5 loop and the
410 likely rearrangement of local hydrogen bonds (solid lines) shown with experimental
411 densities and atomic models coloured as in **a**. **c**, +End view on the longitudinal inter-
412 dimer interface; the relevant α -tubulin regions (colour-labelled) are shown as backbone
413 traces superposed on top of the β -tubulin model-derived atomic surfaces of the whole
414 β -subunits (white), with interfacing regions (black-and-white labels) coloured by
415 element (C, grey; N, blue; O, red); nucleotides are shown as sticks; dotted ovals mark
416 the interface expansion at each step. **d**, Plotted PDBePISA (www.ebi.ac.uk/pdbe/pisa/)
417 calculations of the solvent-accessible surface areas (SASA) and the dissociation
418 energies (ΔG^{diss}) of the longitudinal inter-dimer interfaces in different nucleotide states.
419 The error bars represent 3 averaged inter-dimer interfaces generated with parallel
420 refinements of non-crystallographic symmetry (NCS) mates in 6-dimer MT wall
421 models (see Methods).

422

423 **Figure 3 | GTPase-dependent structural transitions weaken the lateral MT lattice**
424 **contacts.** **a**, Backbone luminal cut-away view at lateral contacts between two adjacent
425 PFs in different nucleotide states, aligned and coloured as in Fig. 2a. Tubulin domains
426 are outlined and their transitions around A1 and A2 anchor points are indicated with
427 curved arrows. **b**, MT +end view of the α 2-subunits undergoing the first transition
428 (top) and a luminal view of the β 2-subunits undergoing the second transition; close-
429 up views of selected connections (solid lines) compromised (dotted lines) during the
430 transitions. Atomic models are coloured as in Fig. 2a or by heteroatom and are
431 presented with experimental densities; tables summarise the likely affected lateral
432 bonds according to measurements performed in the models. The interacting atoms are
433 named (parentheses) according to the PDB convention.

434

435 **Figure 4 | Effect of Taxol® binding to GDP-DCX-MT.** **a**, Luminal backbone view of
436 GDP-DCX-MT structure with Taxol® superposed on the GDP-DCX-MT structure
437 without Taxol®, coloured by the degree of displacement or as follows: Taxol®, green
438 sticks; GTP, yellow sticks; GDP, red sticks. Regions showing the largest displacement
439 are limited to loops directly contacting the drug (arrows). **b**, Unchanged β E-site before
440 (grey ribbon and the nucleotide) and after (green ribbon and red/heteroatom coloured
441 nucleotide) addition of Taxol®, shown in the density of the MT lattice containing
442 Taxol®; selected residues are shown as sticks coloured by heteroatom. **c**, Close-up on
443 Taxol® binding site in GDP-DCX-MT reconstruction with Taxol® (green map) and
444 without Taxol® (grey map), focusing on local structural changes. The models are
445 coloured as in **a**; the most displaced regions are shown as sticks where density is present,
446 and pointed with arrows. Reconstruction without Taxol® has weaker density for R276
447 side chain (not visible at this density threshold), suggesting that Taxol® somewhat
448 orders this residue in the Taxol®-bound MT. Taxol®'s close interaction with the β M loop
449 – a main contributor to MT lateral contacts – explains how the lateral contacts are
450 locked to promote MT lattice stability upon Taxol® binding.

451

452 **Figure 5 | Mechanisms and implications of MT catastrophe and the tubulin GTPase**
453 **cycle.** The specific area of this work (framed) is presented together with other stages of
454 GTPase cycle discussed in the paper. Free GDP-bound tubulin exchanges E-site GDP
455 to GTP and starts MT nucleation by assembling bent PFs, which gradually straighten
456 as MT lattice assembly progresses. Completion of MT lattice leads to strain
457 accumulation by imposing an unfavourable straight conformation on tubulin. GTP-
458 hydrolysis enables release of this strain in stages, which lead to MT catastrophe: during
459 the first transition, the α -subunits undergo uneven compression, weakening lateral,
460 while tightening longitudinal lattice contacts. The second transition further tightens
461 longitudinal contacts and weakens the lateral contacts between β -subunits. The strain
462 overcomes the looser lateral contacts causing outward peeling of PFs and force
463 generation (lightening). The developing curvature eventually breaks the longitudinal
464 inter-dimer contacts, liberating tubulin to allow nucleotide exchange and resumption
465 of the cycle.

466

467

468

469

470 **TABLES**

471

472 **Table 1 | Cryo-EM data collection, refinement and validation statistics**

	GMPCPP-DCX- MT (EMDB- 3961) (PDB 6EVW)	GDP.Pi-DCX- MT (EMDB- 3962) (PDB 6EVX)	GTP γ S-DCX- MT (EMDB- 3963) (PDB 6EVY)	GDP-DCX-MT (EMDB-3964) (PDB 6EVZ)	GDP-DCX- Taxol®-MT (EMDB-3965) (PDB 6EWO)
Data collection and processing					
Magnification	35,971	35,971	35,971	35,971	35,971
Voltage (kV)	300	300	300	300	300
Electron exposure (e ⁻ /Å ²)	25	25	25	25	25
Defocus range (μm)	-0.4 to -2.5	-0.4 to -2.5	-0.4 to -2.5	-0.4 to -2.5	-0.4 to -2.5
Pixel size (Å)	1.39	1.39	1.39	1.39	1.39
Symmetry imposed ^a	12-fold	12-fold	12-fold	12-fold	12-fold
Initial particle images (no.)	11,552	7,727	9,395	32,256	18,651
Final particle images (no.)	3,244	6,591	6,037	30,434	17,626
Map resolution (Å)	4.4	4.2	4.4	3.8	3.8
FSC threshold ^b	0.143	0.143	0.143	0.143	0.143
Map resolution range (Å)	4.1 – 5.8	4.0 – 5.8	4.0 – 5.8	3.2 – 4.8	3.2 – 4.8
Refinement					
Initial model used (PDB code)	6EVZ	6EVZ	6EVZ	3JAR	6EVZ
Model resolution (Å)	4.5	4.2	4.4	3.8	3.8
FSC threshold	0.143	0.143	0.143	0.143	0.143
Model resolution range (Å)	200 – 4.5	200 – 4.2	200 – 4.4	200 – 3.8	200 – 3.8
Map sharpening <i>B</i> factor (Å ²)	-137	-167	-156	-163	-99
Model composition					
Non-hydrogen atoms	40,536	40,896	40,890	40,866	41,238
Protein residues	5,112	5,166	5,166	5,166	5,166
Ligands	24	24	18	18	24
<i>B</i> factors (Å²)					
Protein	110	101	93	58	110
Ligand	143	86	82	64	103
R.m.s. deviations					
Bond lengths (Å)	0.00	0.00	0.01	0.00	0.01
Bond angles (°)	0.71	0.65	0.72	0.67	0.76
Validation					
MolProbity score	1.66	1.46	1.41	1.32	1.55
Clashscore	5.74	3.73	3.32	2.58	4.39
Poor rotamers (%)	0	0	0	0	0
Ramachandran plot					
Favored (%)	94.98	95.69	95.26	95.98	95.79
Allowed (%)	5.02	4.31	4.74	4.02	4.21
Disallowed (%)	0	0	0	0	0

^aDCX does not bind at the seam of the 13-PF MTs, thus pseudo-helical symmetry was applied across 12 PFs between which DCX binds. ^bGold standard resolution estimation according to Chen et al³⁵

473 **METHODS**

474 **Protein preparation.**

475 Human doublecortin isoform 2 (DCX, residues 1-360) was cloned into pNic28Bsa4
476 vector (Structural Genomics Consortium, Oxford, UK), which adds a tobacco etch
477 virus (TEV) protease-cleavable N-terminal His tag to the protein. After expression in
478 BL21 Star (DE3) *E. coli* cells (Invitrogen), the cells were sonicated in the lysis buffer (50
479 mM Na₂HPO₄ pH 7.2, 300 mM NaCl, 10 mM imidazole, 10% glycerol, 2 mM DTT)
480 supplemented with protease inhibitor cocktail (cOmplete Cocktail Tablet,
481 Roche/Sigma Aldrich), and the lysates were clarified by centrifugation. Clear lysates
482 were passed through nickel HisTrap HP column (GE Healthcare) and His-DCX was
483 eluted with 10-250 mM imidazole gradient. To remove the His tag from the DCX
484 protein, we used a His-tagged TEV protease expressed in-house and then removed both
485 His-TEV and the cleaved tag by a passage through nickel beads (GE Healthcare). DCX
486 was then captured on a HiTrap SP HP ion exchange column (GE Healthcare)
487 equilibrated in BRB80 buffer (80 mM PIPES [piperazine-N,N' -bis(2-ethanesulfonic
488 acid)] pH 6.8, 1 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-
489 tetraacetic acid], 1 mM MgCl₂, 1 mM DTT [dithiotreitol]) and eluted with NaCl
490 gradient (15-300 mM). DCX was then finally purified and desalted by gel filtration
491 through Superdex 200 size exclusion column (GE Healthcare) equilibrated in the
492 BRB80 buffer.

493 Lyophilised bovine brain tubulin was purchased from Cytoskeleton and
494 reconstituted to 100 μM concentration in BRB80 supplemented with either 1 mM GTP
495 for dynamic MTs (GTP-tubulin) or one of the nucleotide analogues: GMPCPP
496 [Guanosine-5'-[(α,β)-methylene]triphosphate] (Jena Biosciences) (GMPCPP-tubulin)
497 or GTPγS [Guanosine 5'-O-(3-thiotriphosphate)] (Roche) (GTPγS-tubulin).

498 **Preparation of cryo-EM samples.**

499 To obtain GMPCPP-DCX-MTs with high GMPCPP occupancy, 30 μM GMPCPP-
500 tubulin was cycled twice through: 30 min polymerization at 37 °C in BRB80 buffer
501 containing 1 mM GMPCPP, pelleting and depolymerisation in cold buffer. In the third
502 polymerisation round, 5 μM GMPCPP-tubulin was co-polymerised with 3.5 μM DCX
503 under the same conditions. This polymerisation strategy was required to obtain a fully
504 extended 13-PF MT lattice.

505 GTPγS does not nucleate MTs, hence it cannot be enriched in the lattice
506 through tubulin polymerisation and depolymerisation cycles and its occupancy is not
507 known. For GTPγS-DCX-MTs 5 μM GTPγS-tubulin was co-polymerised for 30 min
508 with 3.5 μM DCX at 37 °C in BRB80 buffer containing 2 mM GTPγS.

509 For GDP-DCX-MTs 5 μM GTP-tubulin was co-polymerised for 30 min with
510 3.5 μM DCX at 37 °C in BRB80 buffer containing 1 mM GTP. For GDP-DCX-Taxol®-
511 MTs 1 mM Taxol® was added after 30 min of polymerisation and the sample was
512 incubated for further 30 min at 37 °C.

513 In all the above MT preparations, sub-stoichiometric concentration of DCX
514 versus tubulin was used to nucleate MTs with a desired 13-PF architecture, while
515 keeping MT bundling minimal. These MTs were applied directly to glow-discharged
516 Lacey grids (Agar) and incubated for 30 sec at room temperature. Then the grids were
517 briefly blotted and 50 μM DCX solution in BRB80 buffer was added to maximise MT
518 decoration with DCX. The grids were then transferred to Vitrobot (FEI/Thermo Fisher
519 Scientific) and incubated there for 1 min at 30 °C and 95 % humidity, before finally
520 blotting and plunge freezing in liquid ethane.

521 For GDP.Pi-DCX-MTs 10 μM GTP-tubulin was mixed with 50 μM DCX in cold
522 BRB80 buffer containing 1 mM GTP and immediately applied to a glow-discharged
523 Lacey grid for rapid polymerisation directly on the grid inside the Vitrobot set to 30 °C
524 and 95 % humidity. After 30 sec incubation the grid was blotted and vitrified as before.

525 **Cryo-EM data collection.**

526 Micrographs were acquired on a 300 kV Polara microscope (FEI) combined with a K2
527 Summit camera (Gatan) operated in counting mode after energy filter with a 20 eV slit.
528 The magnification at the specimen plane was 35,971x resulting in a pixel size of 1.39 Å.
529 The dose rate was ~ 5 e-/pixel/sec, corresponding to ~ 2.6 e-/Å²/sec. The total dose on
530 the specimen was ~ 23.4 e- collected over 9 sec exposures fractionated into 36 movie
531 frames (0.25 sec/frame). We used SerialEM software
532 (<http://bio3d.colorado.edu/SerialEM/>) to manually collect exposures with -0.4 to -2.5
533 μm defocus range.

534 **Image processing and 3D reconstruction.**

535 We used MotionCorr³⁶ to globally and locally (25 tiles/image) align movie frames.
536 Using EMAN 1 Boxer³⁷ we picked MT segments from these drift-corrected image sums.
537 Boxes of 652 x 652 pixel size spanned ~ 11 tubulin dimers and were cut along MTs with
538 ~ 8 dimer overlap. These segments were subsequently treated as single particle input to
539 Chuff^{38,39}, a custom-designed multi-script processing pipeline using Spider⁴⁰ and
540 Frealign⁴¹. The initial seam finding alignment was done in Spider by projection
541 matching to a synthetic 13-PF DCX-MT reference filtered to 30 Å. The contrast transfer
542 function (CTF) parameters were estimated with CTFFIND3⁴², and the CTF correction
543 was performed during local refinement within Frealign, producing isotropic 3D
544 reconstructions with pseudo-helical symmetry applied 12 times. Independently
545 processed half maps were combined in Relion 1.4⁴³ and subjected to its standard post-
546 processing routine, involving: (1) estimation of map resolution based on Fourier Shell
547 Correlation (FSC) between the two half maps, (2) computation of the average B-factor
548 based on Guinier plot using the EMBfactor program⁴⁴, and (3) map sharpening using
549 the computed B-factor value. The resolutions of the final maps were estimated using
550 0.143 FSC cut-off criterion and the absence of over-fitting was confirmed with high-
551 resolution noise substitution test³⁵ (Table 1, FSC_{true}). Side chains of acidic residues are
552 mostly missing, likely due to their exceptional susceptibility to radiation damage, unless

553 they are stabilised by an interaction (e.g. D177 H-bonding with GMPCPP, Fig. 1c).
554 Reconstructions using data collected with the first 4 e- did not recover these vulnerable
555 side chains.

556 **Atomic model refinement.**

557 We used a high-resolution cryo-EM 6 GDP-tubulin dimer model (PDB code: 3JAR¹⁰,
558 devoid of EB3 chains) as a starting point for refinement in all of our cryo-EM density
559 maps. Nucleotides were substituted as necessary with structures downloaded from the
560 Grade Server (<http://grade.globalphasing.org/cgi-bin/grade/server.cgi>) and DCX
561 density was masked away and excluded from the refinements by zoning maps around
562 tubulin structures in UCSF Chimera⁴⁵. Each isolated map was placed in a new unit cell
563 with P1 space group. Ten macro-cycles of refinements in real space were carried out at
564 each round using phenix.real_space_refine (<http://phenix-online.org/>) with default
565 settings (Ramachandran plot, C-beta deviations, rotamer and secondary structure
566 restraints). Non-crystallographic symmetry (NCS) group definitions were manually
567 provided as constraints for the related tubulin chains. The program automatically
568 determined weight between data and the restraints to achieve RMS deviations for
569 covalent bonds not greater than 0.01, and for angles not greater than 1.0. Model
570 geometry was evaluated by MolProbity⁴⁶ after each round of refinement, and
571 problematic regions in the models were manually corrected in Coot⁴⁷. This process was
572 repeated for every structure until satisfactory level of model:map agreement with
573 excellent model stereochemistry were accomplished (Table 1).

574 **Estimation of PF skew in different MT lattices.**

575 We calculated the average difference between the assigned ϕ -angles (rotation angle
576 around MT axis) of the consecutive DCX-MT segments cut along individual MTs in
577 each lattice state. These individual values of skew were then averaged over > 50 MTs
578 per lattice state (Supplementary Fig. 6). To determine statistical significance between
579 the means we used one-way analysis of statistical variance (ANOVA), since standard
580 deviations across all datasets were not significantly different by both Brow-Forsythe and
581 Bartlett's tests, according to Prism 6 (graphpad.com). The degree of freedom within
582 each group (residual) amounted to 355 and between the groups to 4, resulting in the F
583 ratio of 68.67 and the significance level (P value) of < 0.0001.

584 **Estimation of DCX occupancy in different MT lattices.**

585 A Fourier-transformed image of any DCX-MT shows a $\sim 1/4$ nm layer line
586 corresponding to ~ 4 nm tubulin subunit repeat and an $\sim 1/8$ nm layer line
587 corresponding to ~ 8 nm DCX repeat. To estimate the relative DCX occupancy in each
588 lattice state we averaged power spectra of all the DMX-MT segments in a given state
589 using EMAN³⁷ and calculated intensities of the $1/8$ nm layer lines in relation to the $1/4$
590 nm layer lines. To calculate the error of that ratio for each lattice state we divided each

591 dataset into 3 approximately equal subsets and calculated the average ratio and its
592 standard deviation (Supplementary Fig. 1b).

593 **Figure and video preparation.**

594 Molecular visualisations in all figures and videos (Supplementary Information) were
595 prepared using UCSF Chimera⁴⁵ .

596

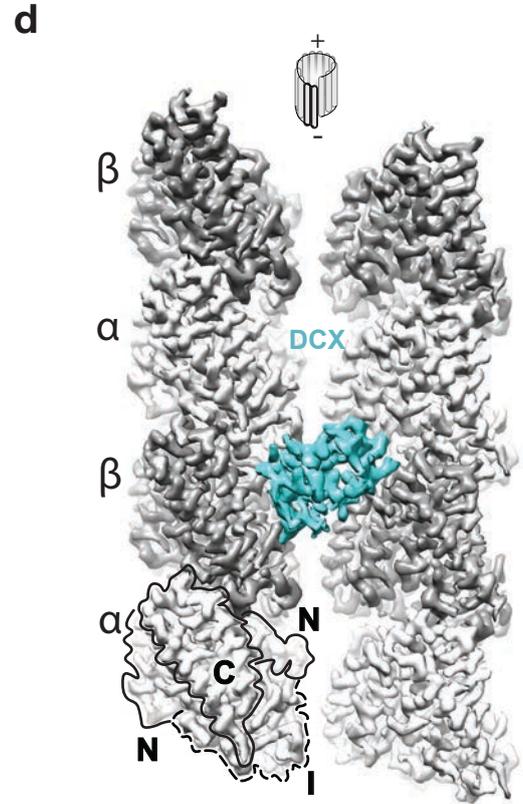
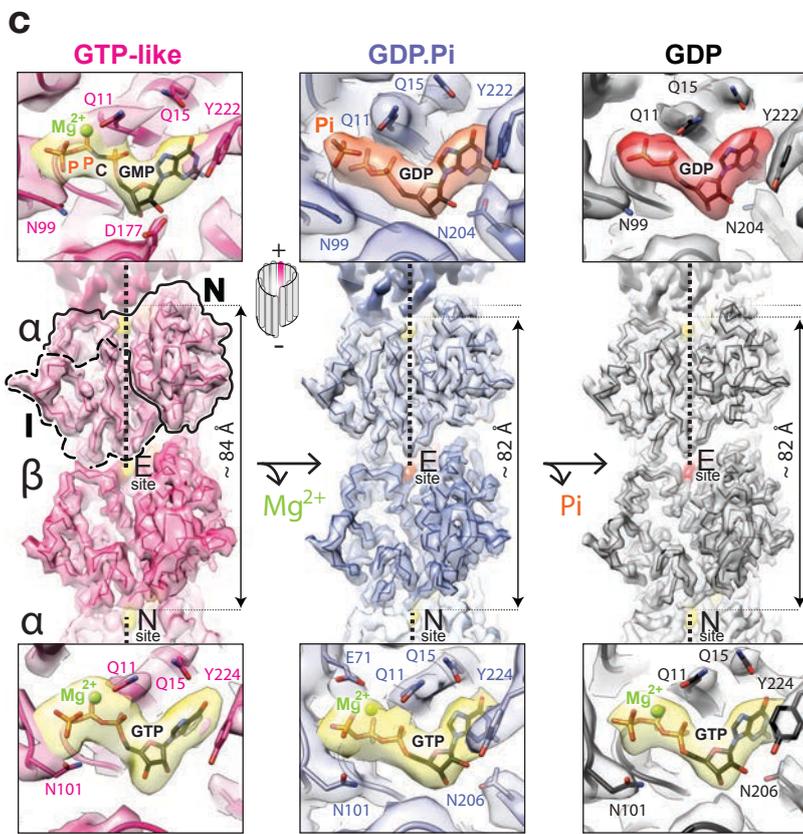
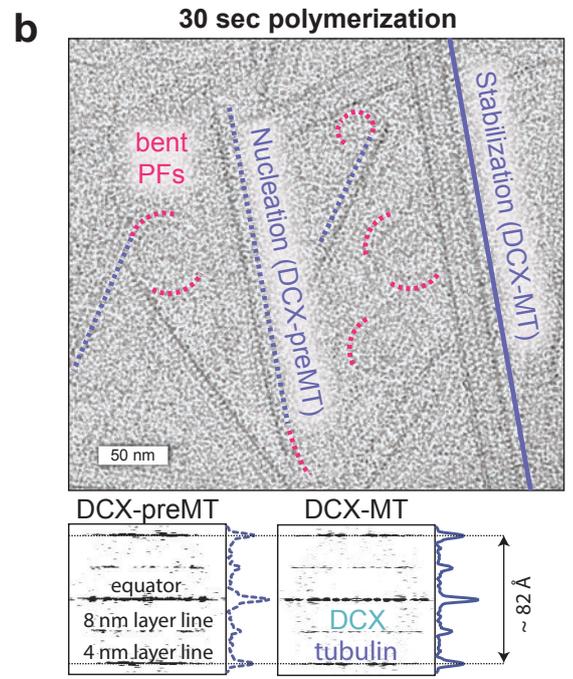
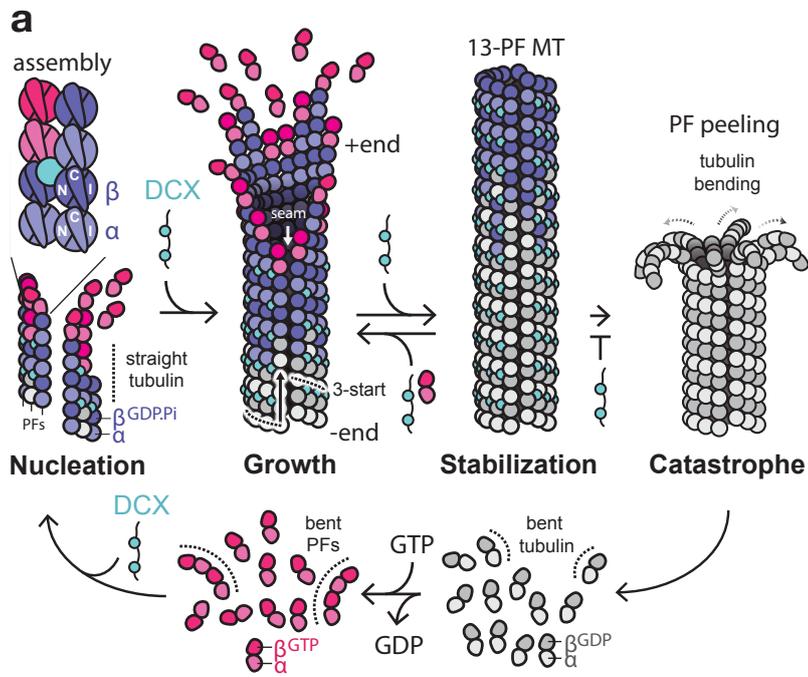
597 **Data availability.**

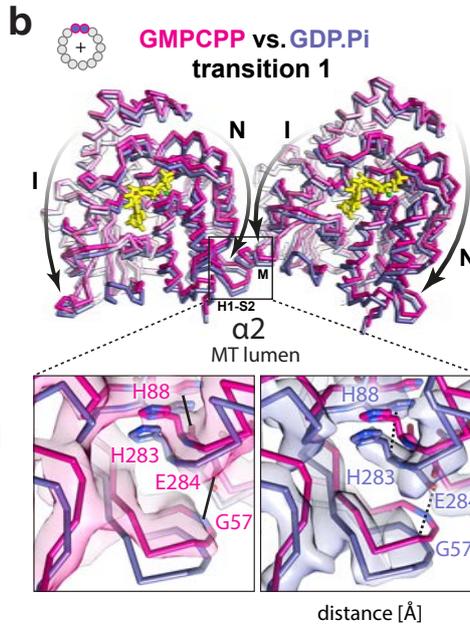
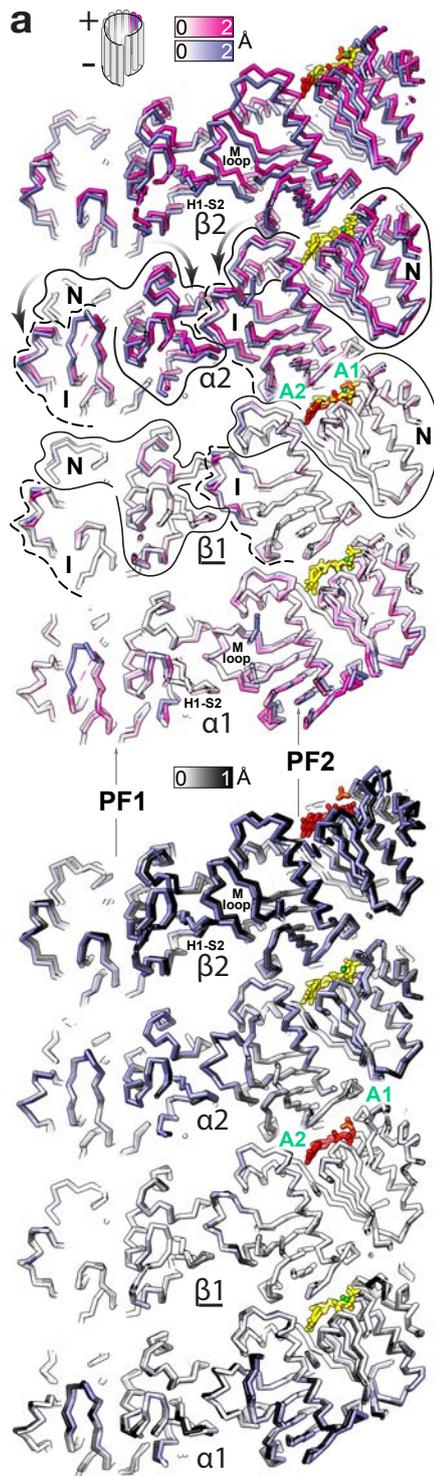
598 Our maps and coordinates were deposited in EMDB/PDB with the following accession
599 codes: GMPCPP-DCX-MT, EMD-3961 and PDB ID 6EVW; GDP.Pi-DCX-MT, EMD-
600 3962 and PDB ID 6EVX; GTP γ S-DCX-MT, EMD-3963 and PDB ID 6EVY; GDP-
601 DCX-MT, EMD-3964 and PDB ID 6EVZ; GDP-DCX-Taxol[®]-MT, EMD-3965 and PDB
602 ID 6EW0.

603 **REFERENCES**

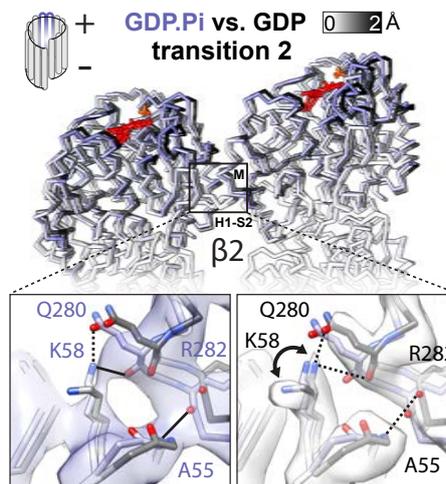
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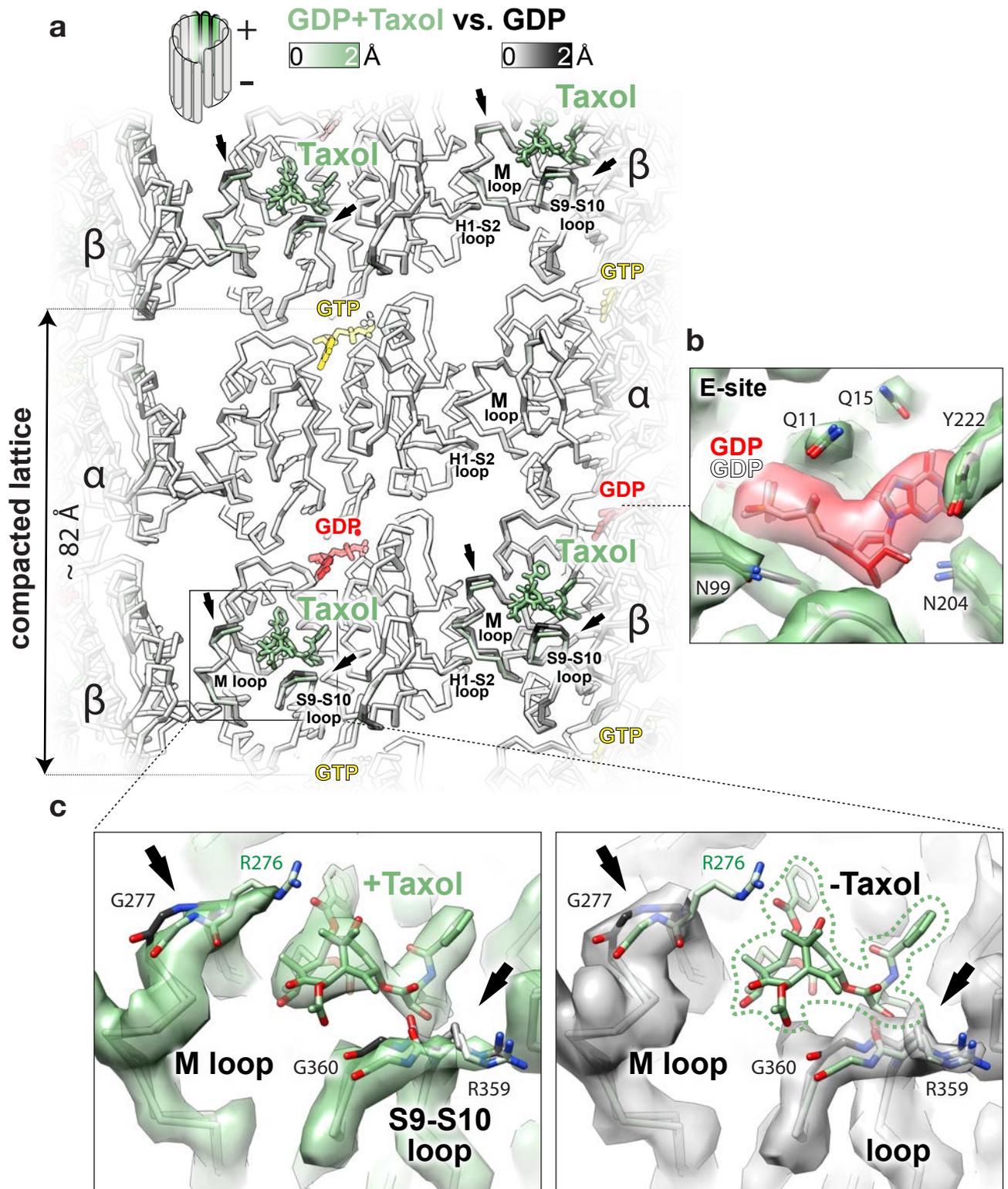


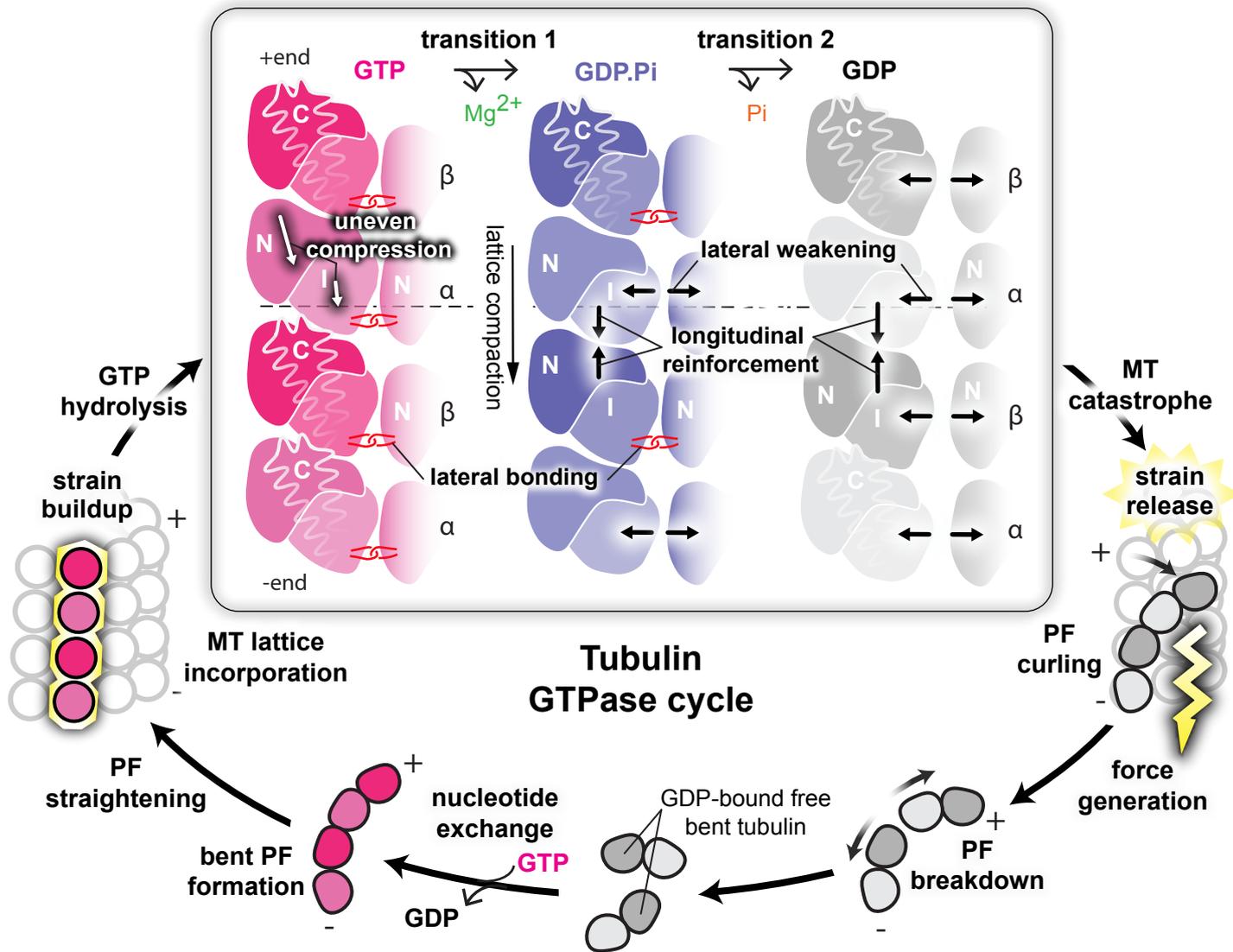


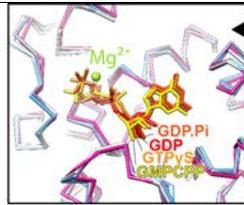
	GMPCPP	GDP.Pi
α		
G57 (N) - (O) E284	~ 3.8	~ 4.6
H88 (ND1) - (O) H283	~ 3.6	~ 4.6



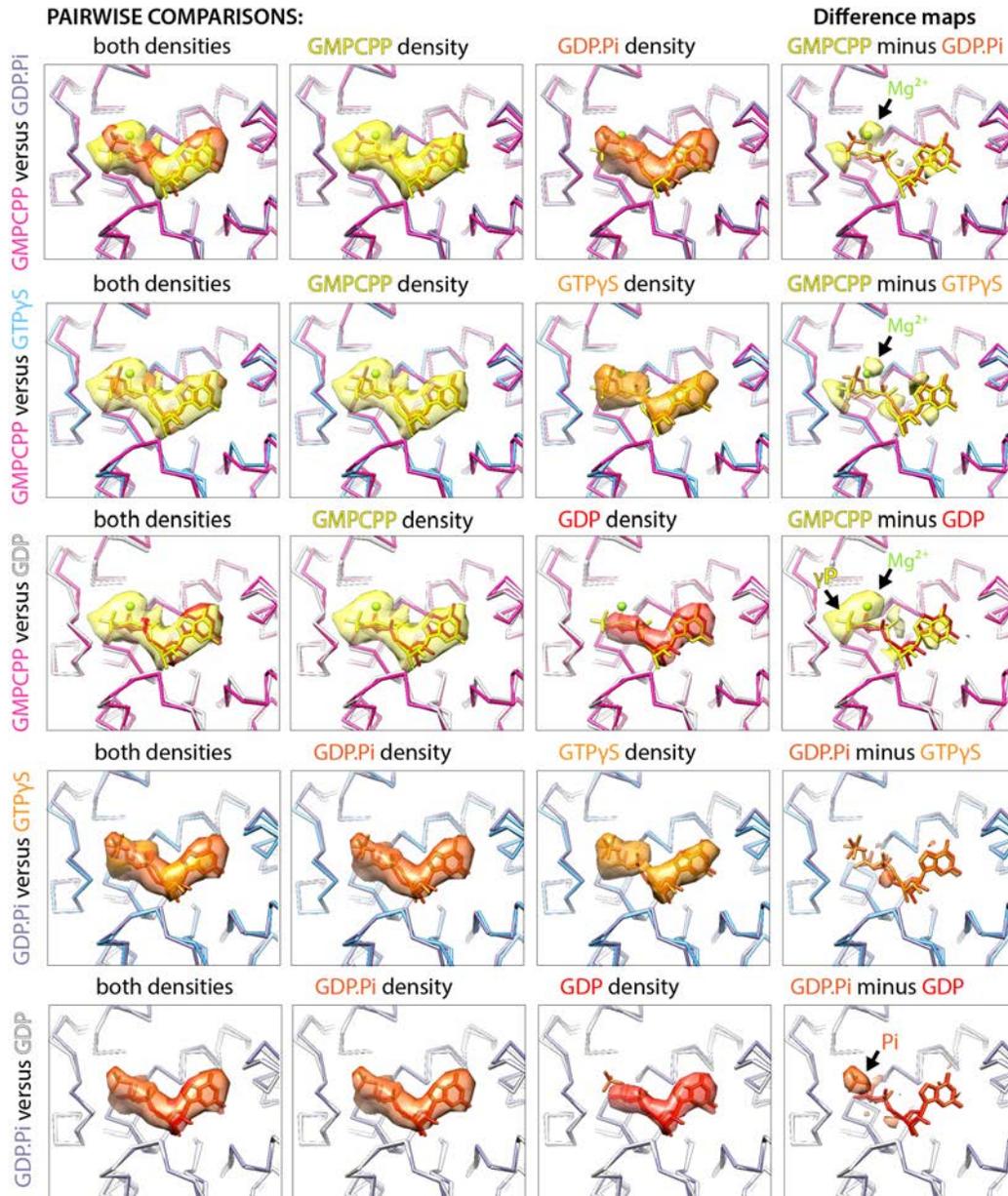
	GDP.Pi	GDP
α		
G57 (N) - (O) E284	~ 4.6	~ 5.6
H88 (ND1) - (O) H283	~ 4.6	~ 4.3
β		
K58 (NZ) - (O) Q280	~ 2.6	~ 4.5
A55 (N) - (O) R282	~ 3.7	~ 4.4







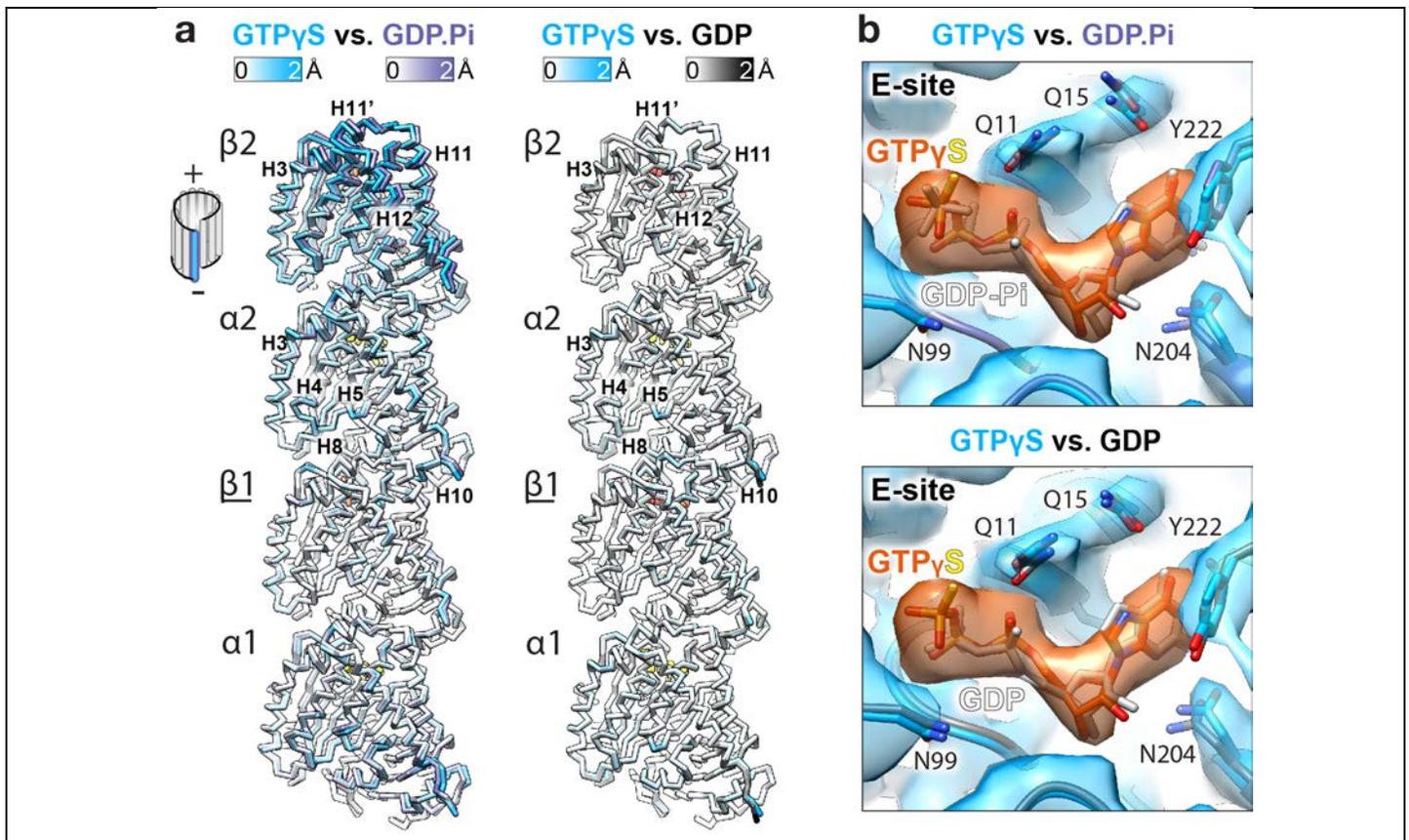
OVERVIEW: E-site of all states aligned on β -tubulin subunit
 protein: GMPCPP state, GDP.Pi state, GTPyS state, GDP state
 ligand: GMPCPP, GDP.Pi, GTPyS, GDP, Mg^{2+}



Supplementary Figure 2

Difference mapping between ligand densities in different nucleotide states

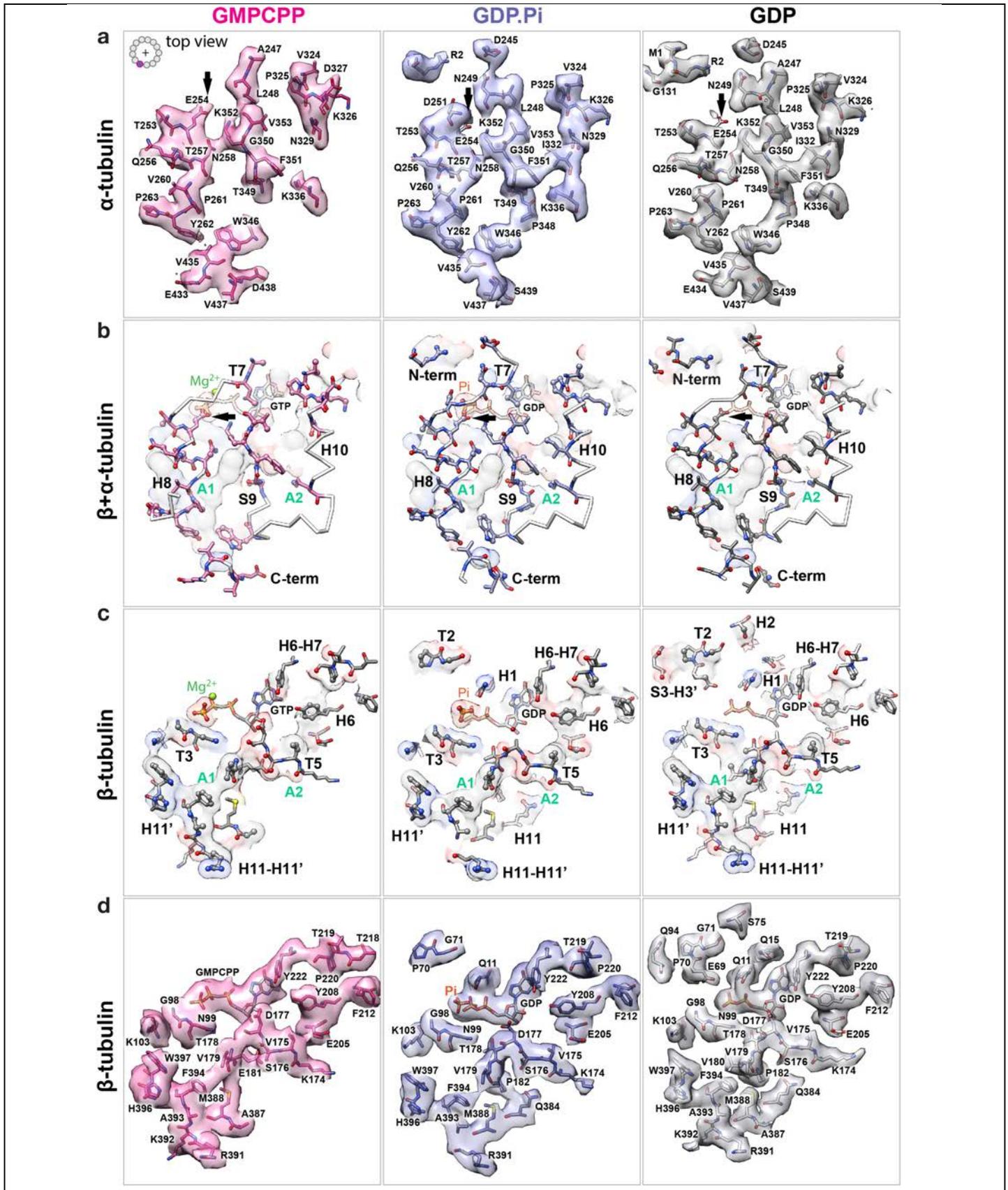
Pairwise comparison of nucleotide binding sites in different reconstructions provide additional support for conclusions about the nucleotide states captured in this study, including the loss of the metal ion between GMPCPP and GDP.Pi. The GDP.Pi vs GTPyS comparison shows the similarity of the density corresponding to these nucleotides despite their different effects on MT conformation (Supplementary Fig. 3).



Supplementary Figure 3

Tubulin conformation in the GTP γ S-DCX-MT resembles that in the GDP-DCX-MT

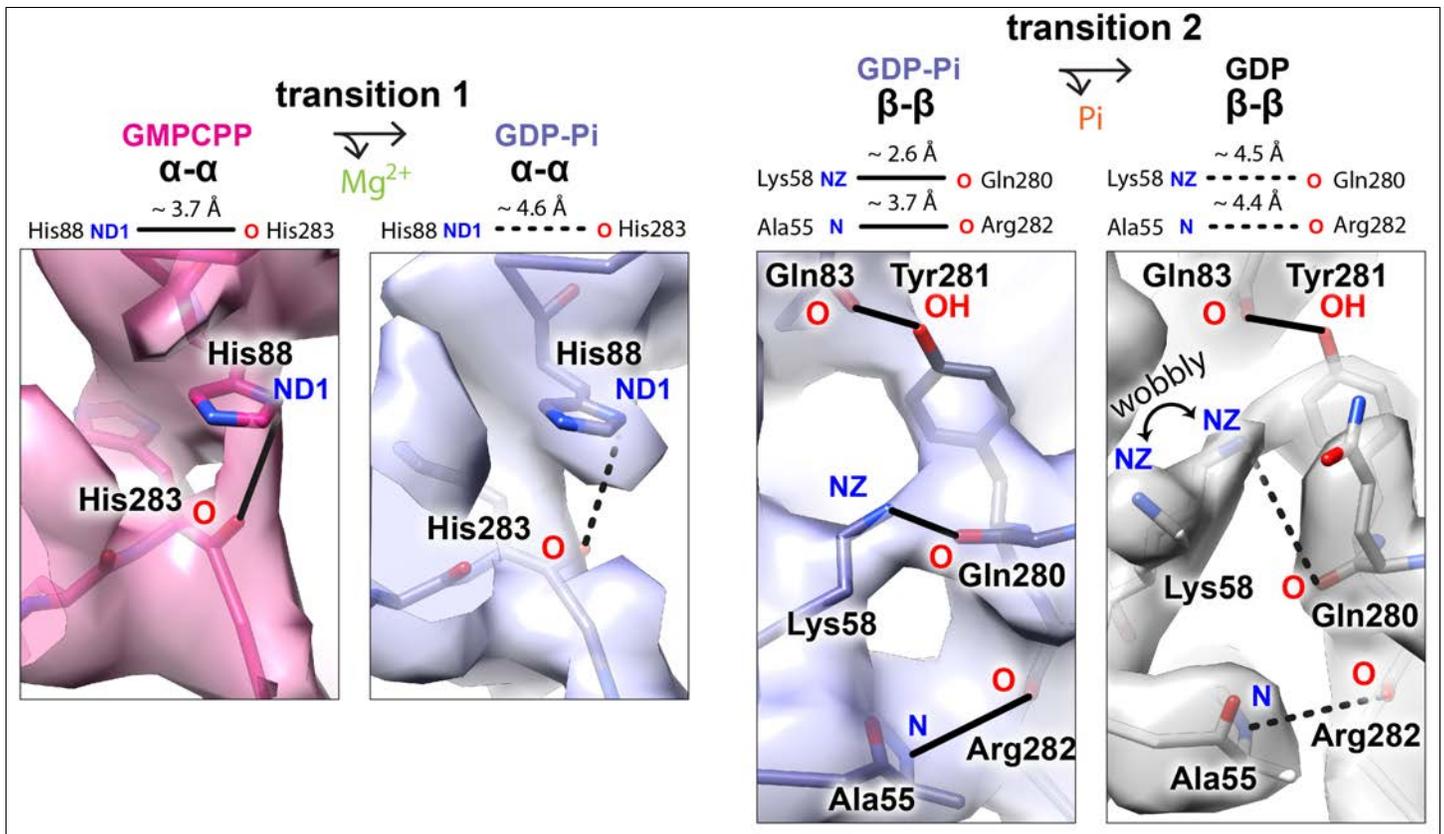
a, Backbone front view comparisons of tubulin conformation in the GTP γ S lattice against those in the GDP.Pi or GDP lattices, based on pairwise superposition on the β 1 subunit (underlined); coloured by the degree of displacement or as follows: GTP γ S, orange; GDP.Pi, orange, GDP, red. **b**, β 1 E-site comparisons in the cryo-EM map of the GTP γ S lattice: tubulin density, sky blue; nucleotide density, orange. Tubulin model chains are shown as ribbons: GTP γ S state, sky blue; GDP.Pi state, slate blue; GDP state, grey, and their associated nucleotides (GTP γ S, orange/heteroatom; GDP.Pi and GDP, light grey) and selected side chains (coloured by heteroatom) are shown as sticks.



Supplementary Figure 4

Details of the longitudinal inter-dimer interface in different DCX-MT lattices

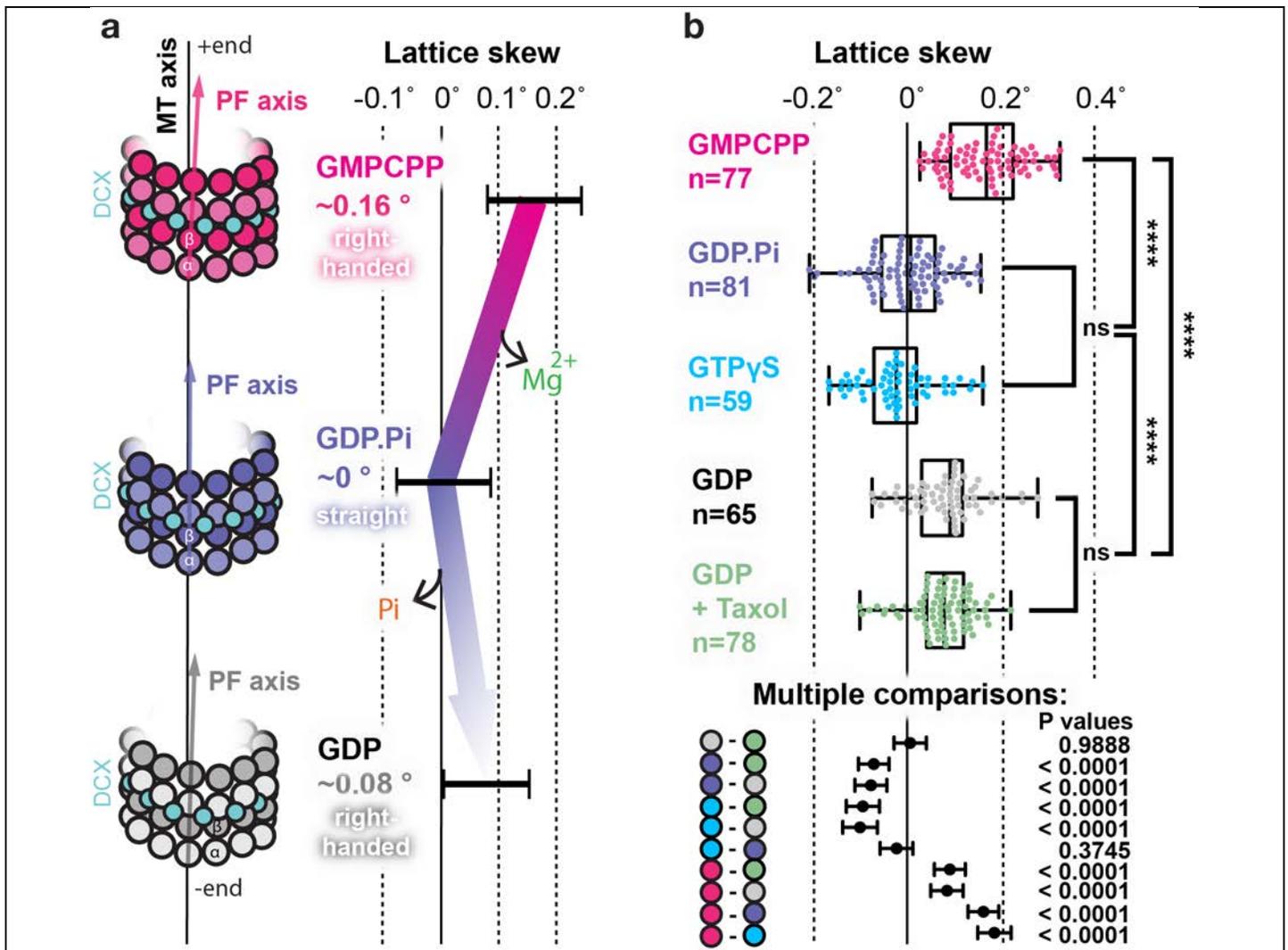
View from MT +end at: **a**, α -tubulin residues having at least one atom within 4 Å distance from any atom in the interfacing β -tubulin, shown as sticks coloured by heteroatom in their cryo-EM density. The density for the catalytic E254 residue is captured only in the GMPCPP state (arrows); **b**, superposition of both α and β faces of the interface shown in different representations with two anchor points (A1 and A2) indicated, to aid relating positions of the interfacing residues; α -face residues (shown in **a** with IDs) are connected by white backbone, to visualize secondary structural context (labelled); the atoms within 4 Å distance from the interfacing β -tubulin are shown as spheres; E254 residues are pointed with arrows; β -face atoms within 4 Å distance from the interfacing α -tubulin are shown as model-derived surfaces coloured by element (C, grey; N, blue; O, red); **c**, β -tubulin residues having at least one atom within 4 Å distance from any atom of the bound α -tubulin; the residues are shown as sticks coloured by element (see **d** for ID labels), and the atoms within 4 Å distance from the interfacing α -tubulin are shown as spheres together with model-derived surface coloured by element; secondary structures to which particular residues belong and two anchor points (A1 and A2) are indicated; **d**, cryo-EM density and ID labels for the residues shown in **c**.



Supplementary Figure 5

Select views at particular changes in the lateral contacts of an MT lattice during the GTPase transitions

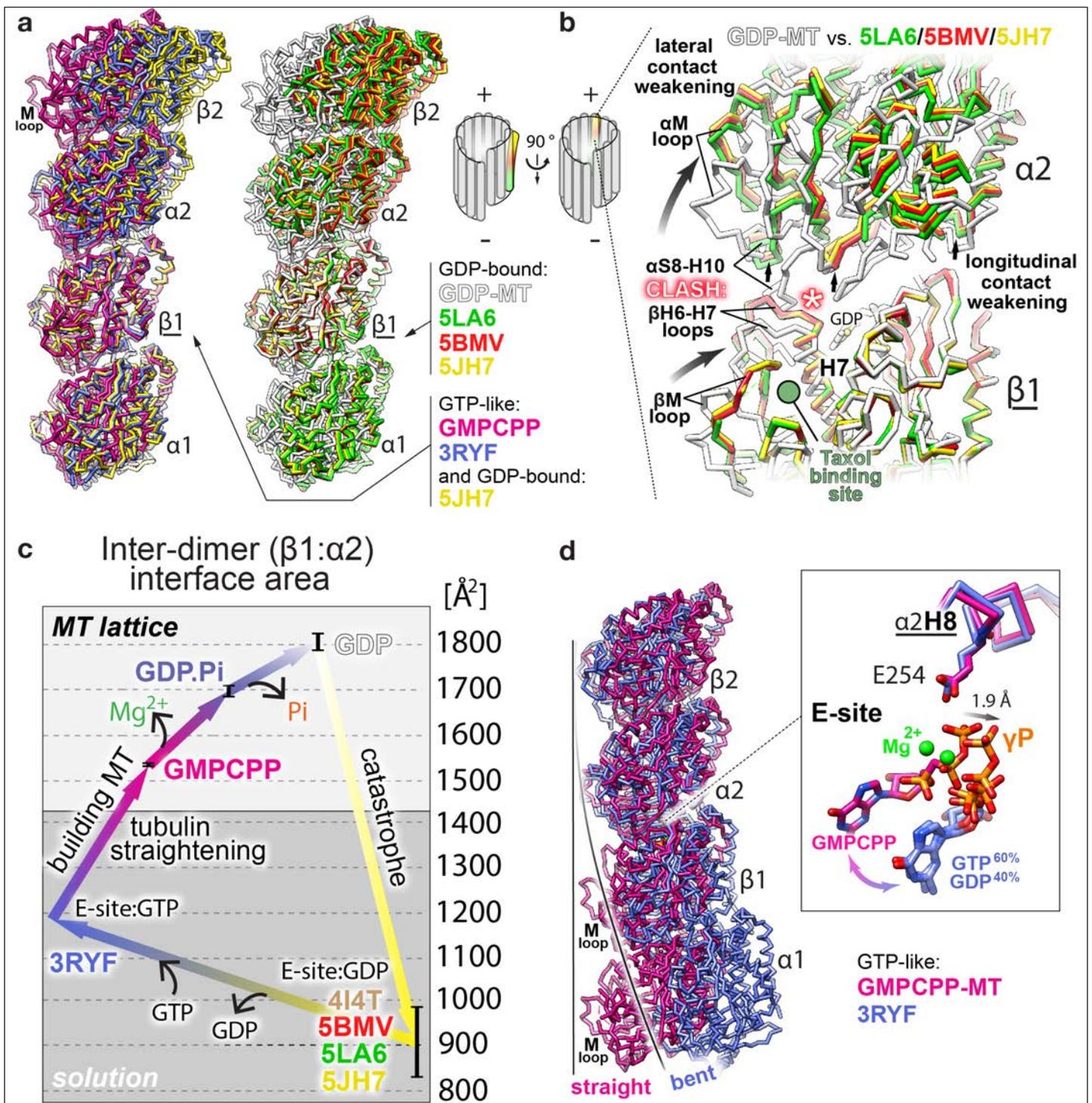
Related to and extending Fig. 3 to provide additional depiction of selected stable and vulnerable connections, according to measurements performed on our models of MT nucleotide states. Top panels focus on one inter- α -subunit connection (solid line) likely to be affected (dotted line) from the first transition. Bottom panels focus on one inter- β -subunit connection likely to be preserved (solid lines) and two connections likely to be affected (dotted lines) during the second transition. Atomic models are shown with experimental densities and coloured accordingly or by heteroatom. The interacting atoms are named according to the PDB convention.



Supplementary Figure 6

Changes in PF skew accompanying nucleotide state transitions in the DCX-MT lattice

a, Cartoon illustration of PF skew in selected DCX-MT lattices and the plot showing mean value (–) of the skew angle measured as described in Methods. Error bars represent standard deviations. **b**, Statistical analysis of the skew angles of all DCX-MT lattices by ordinary one-way ANOVA using Prism 6 software (see Methods). Top, box and whiskers plot combined with scatter dot plot showing all *n* data points collected for each lattice (*n* = sample size). The whiskers are defined by the minimum and the maximum value, the box shows the interquartile range (between the 25th and 75th percentile) and the central line indicates the median value (50th percentile). Significance has been verified through multiple comparisons (see below): **** - significant, *p* < 0.0001; ns - not significant. Bottom, Multiple comparisons: comparing every mean with every other mean by Tukey-Kramer method, which is suitable for unequal sample sizes between compared groups. Plotted are the differences between the mean skew angle values of the compared lattices (colour-coded circles) together with 95 % confidence intervals (CIs) for the difference between the two means. Only the CIs of the two not significantly different pairs of values (*p* > 0.05) contain 0 value. Multiplicity adjusted P values (accounting for multiple comparisons) are also reported.



Supplementary Figure 7

Comparisons of bent (solution) and straight (MT lattice-associated) tubulin conformations and their mechanistic consequences

a, Backbone side view of β 1-subunit (underlined) superposition of different bent tubulin X-ray structures with our straight tubulin structures. Left, comparison of tubulin conformation in the extended GTP-like MT lattice (GMPCPP) with that of a less bent tubulin-RB3 stathmin-like domain complex, 60% occupied by GTP (PDB code: 3RYF), and a more bent, GDP-bound tubulin-eribulin complex (5JH7). Right,

comparison of tubulin conformation in the compacted GDP-MT lattice with bent, GDP-bound conformations in: tubulin-pironetin complex (5LA6), tubulin-stathmin-TTL-vinblastin complex (5BMV), and 5JH7. **b**, Close-up lumenal view of the superposition shown on the right in panel a. During the bending of GDP-tubulin after catastrophe, the M-loops - main contributors to MT lateral contacts - become drastically displaced and incompatible with forming MT lattice-like contacts. Taxol® binding (at the indicated site) prevents M-loop displacement in the context of the lattice and therefore stabilises it (see Fig. 4 for details). Moreover, the conformation of helices β H6, β H7 and their connecting loop in the bent tubulin would clash with α S8-H10 loop in the straight tubulin (asterisk). Thus, bending of tubulin dimers weakens not only lateral, but also longitudinal inter-dimer interactions (small arrows). **c**, Inter-dimer interface area changes during tubulin GTPase cycle computed by PDBePISA (www.ebi.ac.uk/pdbe/pisa/). The areas from 4I4T \approx 1026 \AA^2 (crystal structure of tubulin-RB3-TTL-Zampanolide complex), 5BMV \approx 898 \AA^2 , 5LA6 \approx 877 \AA^2 and 5JH7 \approx 832 \AA^2 were averaged ($908 \pm 83 \text{\AA}^2$) as GDP-bound (Esite:GDP) bent tubulin structures, and the area of 3RYF \approx 1188 \AA^2 provided a single value for the only available 60% GTP-bound (Esite:GTP) tubulin structure. Results for "MT lattice" are as shown in Fig. 2d. Error bars represent standard deviation of the mean where applicable. **d**, Superposition of tubulin in the extended GTP-like MT lattice state (GMPCPP) with 60% GTP-bound bent tubulin (3RYF) on helix α H8, to visualise the increased distance between the catalytic E254 residue and the γ -P of GTP in the bent tubulin conformation, compared to the straight conformation. The acidic side chain of E254 polarises water molecule making it ready for nucleophilic attack on the positively charged γ -P. Tubulin straightening in the MT lattice seems to facilitate (catalyse) GTP hydrolysis, by shortening the distance between the polarised water and its target; conversely, tubulin bending seems to result in a conformation suboptimal for GTP hydrolysis, potentially preventing premature hydrolysis in the associating bent PFs (MT precursors).