Cryo-EM reveals the structural basis of microtubule depolymerization by kinesin-13s

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Kinesin-13s constitute a distinct group within the kinesin superfamily of motor proteins that promote microtubule depolymerization and lack motile activity. The molecular mechanism by which kinesin-13s depolymerize microtubules and are adapted to perform a seemingly very different activity from other kinesins is still unclear. To address this issue, here we report the near atomic resolution cryo-electron microscopy (cryo-EM) structures of Drosophila melanogaster kinesin-13 KLP10A protein constructs bound to curved or straight tubulin in different nucleotide states. These structures show how nucleotide induced conformational changes near the catalytic site are coupled with movement of the kinesin-13-specific loop-2 to induce tubulin curvature leading to microtubule depolymerization. The data highlight a modular structure that allows similar kinesin core motor-domains to be used for different functions, such as motility or microtubule depolymerization.
Kinesin-13s form a group within the kinesin superfamily of motor proteins that lack motile activity and instead work as microtubule depolymerases. They are important regulators of microtubule dynamics in a variety of eukaryotic cell processes such as mitosis, cytokinesis, axonal branching, and ciliogenesis, and are considered promising anti-cancer therapeutic targets.

Like all kinesins, kinesin-13s possess a highly conserved ATPase motor domain that is essential for their function. The motor domain alone is sufficient to induce microtubule depolymerization. However, an additional stretch of positively charged residues, N-terminal of the motor called the neck domain, is necessary to achieve an activity similar to the full-length protein.

The way that kinesin-13s interact with the microtubule is different from motile kinesins. Motile kinesins couple ATP hydrolysis to unidirectional stepping along the microtubule lattice. Kinesin-13s on the other hand couple ATP hydrolysis to the removal of tubulin subunits at the microtubule ends and when interacting with the microtubule lattice they undergo directional unbiased one-dimensional diffusion. It is thought that kinesin-13s promote microtubule depolymerization by inducing a curved tubulin configuration incompatible with the microtubule tubular structure. Kinesin-13 constructs that include the motor domain form complexes with curved tubulin akin to the ones observed at microtubule ends during depolymerization.

Although much is known regarding the mechanism of action of motile kinesins, how a highly conserved motor domain is adapted in kinesin-13s to interact with the microtubule in such a distinct manner, and induce microtubule depolymerization is still not clear. To address these questions here we use cryo-electron microscopy (cryo-EM) to solve the structures of Drosophila melanogaster kinesin-13 KLP10A protein constructs in complex with curved tubulin protofilaments or bound to the microtubule lattice. Our results show how the kinesin-13-specific loop-2 couple ATP-hydrolysis conformational changes in the motor domain with tubulin bending and microtubule depolymerization.

**Results and Discussion**

**KLP10A in complex with straight or curved tubulin.** In the presence of ATP-like analogs kinesin-13 protein constructs that include the motor domain bind to the microtubule lattice and also form stable and very ordered complexes with curved tubulin. The complexes with curved tubulin consist of curved protofilaments with bound kinesins and can be observed by electron microscopy in isolation, bound at the end of microtubules or wrapped around microtubules forming spirals.

![Fig. 1 Cryo-EM 3D density maps.](image-url)
Examples of these complexes obtained with the KLP10A protein constructs used in this work are shown in Supplementary Fig. 1. On the other hand, in the absence of nucleotides (apo-state), KLP10A does not form complexes with curved protofilaments\(^1\), but binds well to straight tubulin in the microtubule lattice (Supplementary Fig. 1a). We solved the cryo-EM structures of minimal microtubule-depolymerization-capable kinesin-13 constructs used in this work are shown in Supplementary Fig. 1. These elements include: the P-loop, a central \(\beta\)-sheet with flanking \(\alpha\)-helices, and the switch loops (SW1 and SW2). The P-loop, a central \(\beta\)-sheet with flanking \(\alpha\)-helices, and the switch loops (SW1 and SW2) are involved in nucleotide binding and the switch loops sense how they are coupled to the bound tubulin conformation.

Conformational changes in the nucleotide binding pocket. The structure of the KLP10A nucleotide binding site can be described in terms of the common structural elements found in other kinesins and the related NTPases, myosins, and G-proteins\(^21,22\). These elements include: the P-loop, a central \(\beta\) sheet with flanking \(\alpha\)-helices, and the switch loops (SW1 and SW2). The P-loop is involved in nucleotide binding and the switch loops sense the nucleotide species present in the active site by adopting distinct conformations\(^21\). In all three 3D maps, the switch loops are

### Table 1 Cryo-EM data collection, refinement, and validation statistics

|                      | #1 NMMT\textsubscript{apo} (EMDB-7027) (PDB 6B01) | #2 NMMT\textsubscript{AMPPNP} (EMDB-7028) (PDB 6B0L) | #3 CTMT\textsubscript{AMPPNP} (EMDB-7026) (PDB 6B0C) |
|----------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| **Data collection and processing** | | | |
| Nominal magnification | 22,500 | 22,500 | 22,500 |
| Voltage (kV)         | 300 | 300 | 300 |
| Electron exposure (e\(^{-}\)/Å\(^2\)) | 63.8 | 63.5 | 62.5 |
| Defocus range (µm)\(^x\) | 1.3-2.2 | 1.1-2.3 | 1.1-2.5 |
| Pixel size (Å)       | 1.073 | 1.073 | 1.073 |
| Number of images taken | 449 | 1464 | 210 |
| Number of images used | 229 | 202 | 482 |
| Symmetry imposed     | Helical | Helical | Helical |
| Initial particle images (no.\(^b\)) | 23,986 | 30,971 | 21,134 |
| Final particle images (no.\(^c\)) | 10,647 | 6040 | 16,942 |
| Number of asymmetrical units | 159,705 | 90,600 | 254,130 |
| Map resolution (Å)   | 3.78 | 3.98 | 3.51 |
| FSC threshold        | 0.143 | 0.143 | 0.143 |
| Map resolution range (Å)\(^d\) | 2.8-550 | 3.0-550 | 2.6-660 |
| **Refinement**        | | | |
| Initial model used (PDB code) | See note e | See note e | 3J2U |
| Model resolution (Å) | 3.78 | 3.98 | 3.51 |
| FSC threshold        | 0.143 | 0.143 | 0.143 |
| Map resolution range (Å) | 3.78 | 3.98 | 3.51 |
| Map sharpening B factor (Å\(^2\)) | –130 | –20 | –100 |
| Model composition    | Non-hydrogen atoms | 9829 | 9861 | 16,440 |
| Protein residues     | 1238 | 1238 | 2060 |
| Ligands              | 3 | 4 | 6 |
| R.m.s. deviations    | | | |
| Bond lengths (Å)     | 0.011 | 0.012 | 0.009 |
| Bond angles (°)      | 1.38 | 1.33 | 1.22 |
| Validation           | MolProbity score | 1.83 | 1.97 | 1.72 |
|                      | Clashscore | 6.59 | 8.67 | 5.43 |
|                      | Poor rotamers (%) | 0.19 | 0.0 | 0.17 |
| Ramachandran plot    | | | |
| Flavored (%)         | 92.53 | 91.64 | 93.40 |
| Allowed (%)          | 100 | 100 | 99.95 |
| Disallowed (%)       | 0 | 0 | 0.05 |

\(^a\) Range of the average defocus measured on micrographs. The range comprises 90% of the micrographs used (5% of the micrographs defocuses are below and 5% are above this range).

\(^b\) Total number of particles picked (Methods).

\(^c\) Number of 15R particles retained after the second classification round (Methods).

\(^d\) Higher resolution estimate (lowest spacing value) corresponds to the lowest spacing bin containing at least 1% of the voxel boxes of the blocres local resolution distribution (see Methods and Supplementary Fig. 3).

\(^e\) Computationally generated models based on the KIF14 complex with a 15 protofilaments microtubule map (EMD 2609), tubulin PDB file 1JFF, and kinesin PDB file 4OZQ.
resolved (Figs. 1 and 2). In the NMMT\textsubscript{apo} complex, the nucleotide binding pocket is empty and the electron densities associated with switch loops regions are positioned relatively far away from the nucleotide site (Fig. 2a, d, g). In analogy with other NTPases this corresponds to the “open” configuration of the catalytic site. Interestingly, the catalytic site is also in the open configuration in the NMMT\textsubscript{AMP-PNP} structure despite the presence of AMP-PNP in the nucleotide binding pocket (Fig. 2b, e, h). In contrast, in the CTMMT\textsubscript{AMP-PNP} complex the KLP10A nucleotide binding pocket adopts the “closed” configuration (Fig. 2c, f, i). The switch loops move closer to the nucleotide γ-phosphate and α-helix 4 (H4) which is part of SW2 adopts a different orientation relative to the rest of the motor domain (Fig. 2, Supplementary Movies 1-3).

The closed configuration of the nucleotide binding pocket found in the CTMMT\textsubscript{AMP-PNP} complex is similar to the one found in the crystal structures of motile kinesins thought to represent the hydrolysis competent ATP bound form\textsuperscript{23}. It is also similar to the crystal structure reported recently of another kinesin-13 (hMCAK) in complex with curved tubulin\textsuperscript{24}. These similarities indicate that the nucleotide pocket of kinesin-13s and motile kinesins undergo similar conformational changes in response to the nucleotide species in the active site. However, our results also indicate that in kinesin-13s these conformational changes are coupled not only to the nucleotide species in the active site but also to the particular tubulin conformation, straight or curved to which the motor domain is bound. The nucleotide pocket is closed with AMP-PNP only when bound to curved tubulin and it is open when bound to straight tubulin (Fig. 2, Supplementary Movies 1-3).

Near the nucleotide binding pocket we also observed structural differences between KLP10A and other kinesins that are likely related to the distinct functionality of kinesin-13s. Loop-5, near the P-loop, is in a different configuration than the one observed in KLP10A.
in other kinesins, where it is either not resolved or it is in a different configuration than the one observed here (Supplementary Fig. 4). Residues in this area are not highly conserved between kinesin families and it is thought that distinct L5s modulate the ATPase activity of different kinesins. Accordingly, between kinesin families and it is thought that distinct L5s subunit relative to other areas of the motor domain is part of the kinesin.

Movement of the switch regions relative to the nucleotide and P-loop causes a rearrangement of sub-domains within the KLP10A motor domain. The central eight stranded β-sheet, which contains the P-loop and spans several regions within the motor domain, becomes less twisted in the CTMMT AMP-PNP complex (c). P-loop aligned central β-sheets of the three complexes, NMMTapo and NMMTAMP-PNP in light semi-transparent blue and CTMMTAMP-PNP in dark blue. Numbers indicate the strand order in the KLP10A sequence.

**Conformation and position of the KLP10A-neck-domain.**

Thirty six residues of the neck-domain are resolved in the NMMT complexes forming a loop and an α-helix at the back of the motor domain (Fig. 5a, b). A similar structure for this part of the neck domain has been observed in the crystal structures of other kinesin-13s but with a variable orientation of the α-helix relative to the motor domain (e.g., 2GRY, 2HEH, 1V8K, 5MIO). To determine the position of the neck-domain in KLP10A-curved tubulin complexes we performed 2D analysis of the curved tubulin NM complexes. The interacting residues comprise the kinesin-13 class-conserved elongated loop-2 (L2).

The KLP10A–tubulin interface. The KLP10A motor domain putative tubulin binding interface can be subdivided in three areas (Figs. 3, 4): Area I, comprising of interactions with β-tubulin, area II comprising of interactions with α- and β-tubulin at the intra-dimer interface, and area III consisting of interactions with α- and β-tubulin at the inter-dimer interface. Area III is formed by the kinesin-13 class-conserved elongated loop-2 (L2). The interacting residues of the neck-domain in KLP10A-curved tubulin complexes we performed 2D analysis of the curved tubulin complexes and determined the position of the neck-domain. The neck-domain in KLP10A-curved tubulin complexes were performed 2D analysis of the curved tubulin complexes and determined the position of the neck-domain in KLP10A-curved tubulin complexes.

The KLP10A–tubulin interface. The KLP10A motor domain putative tubulin binding interface can be subdivided in three areas (Figs. 3, 4): Area I, comprising of interactions with β-tubulin, area II comprising of interactions with α- and β-tubulin at the intra-dimer interface, and area III consisting of interactions with α- and β-tubulin at the inter-dimer interface. Area III is formed by the kinesin-13 class-conserved elongated loop-2 (L2). The interacting residues comprise the kinesin-13 class-conserved motif KVD at the L2 tip and complementary residues located in α- and β-tubulin (Fig. 4d). This interaction is critical for kinesin-13 function as mutating the residues KVD to alanines abolishes kinesin-13 microtubule depolymerase activity. The position of the L2 tip relative to the other two areas of interaction (I and II) is different in the CTMMTAMP-PNP and the NMMT complexes (Fig. 3a–c). Despite the different L2 relative position, interactions with tubulin are maintained by a concomitant tubulin conformational change going from the straight (NMMT complexes) to the curved configuration (CTMMTAMP-PNP complexes, Fig. 3, Supplementary Movies 4 and 5).

**Fig. 3 Reshaping of the KLP10A–tubulin interface.** a–c Side views highlighting selected areas of the KLP10A motor domain and tubulin interface undergoing conformational changes. Inset at the right of each panel shows a zoomed view the nucleotide pocket. Central β-sheet strands in dark blue; Switch-1 loop in green, Switch-2 and H4 in magenta, L2, L7, L8 and H6 in light blue. The three areas of tubulin–KLP10A interaction (I–III) are indicated. Note the line connecting the three interacting areas highlighting their different relative positions in the NMMTapo and NMMTAMP-PNP (a, b) relative to the CTMMTAMP-PNP complex (c). d P-loop aligned central β-sheets of the three complexes, NMMTapo and NMMTAMP-PNP in light semi-transparent blue and CTMMTAMP-PNP in dark blue. Numbers indicate the strand order in the KLP10A sequence. e, f Superimposed KLP10A model structures aligned on the top β-tubulin subunit (β-1). e NMMTapo, f CTMMTAMP-PNP. Closing of the KLP10A nucleotide pocket is associated with a ~12° rotation of between monomers of the bound tubulin (24° per tubulin heterodimer).
were observed: (1) a pattern where a KLP10A motor domain binds to every tubulin heterodimer (Fig. 5d). This decoration pattern is similar to what is observed with the motor-domain only KLP10A construct. (2) A skipping pattern where the motor domains bind to every other tubulin heterodimer (Fig. 5e). This pattern is similar to the one previously reported for another kinesin-13 construct that also included the neck-domain. The skipping pattern occurring with kinesin-13 constructs that include the neck-domain indicates that this domain may sterically prevent the binding of two contiguous kinesin-13 molecules along a protofilament. Supporting this interpretation class average images of KLP10A NM tubulin complexes with the skipping pattern reveal an elongated density going from the back of the motor domain to the intra-dimer interface of the next tubulin heterodimer (Fig. 5e–f). This 2D density can be well fitted with a model in which the C-terminal half of the neck domain (the part closer to the motor domain) has the same structure observed in the NM complexes (Fig. 5a, b) as the result of this part becoming delocalized when displaced from its tubulin binding site by an adjacent motor domain bound to the microtubule lattice.

The interaction of the neck domain with an adjacent tubulin heterodimer suggests that it may play a role in inducing or increasing tubulin inter-dimer curvature. However, the neck domain is unlikely to be the major driver of tubulin curvature as the M construct can readily form stable complexes with curved tubulin.

**Tubulin conformational changes.** The differences between the curved tubulin protofilament (CTMMTAMP-PNP complex) and the microtubule lattice straight tubulin can be described to a first approximation as rigid body rotation of 12° between tubulin subunits (24° per tubulin heterodimer) and a small displacement between subunits perpendicular to the rotation plane. (Fig. 3e, f and Supplementary Fig. 6). We previously referred to this displacement as shear, but the amount of shear estimated here is less than previously estimated from lower resolution data. The tubulin structure in the new KLP10A-curved tubulin protofilament model is relatively similar to several tubulin crystal structures but with a higher degree of curvature (Supplementary Fig. 6). The higher curvature suggests that KLP10A can bend tubulin protofilaments beyond the typical tubulin curvature observed outside the microtubule lattice. Furthermore, the 24° per dimer curvature observed in the CTMMTAMP-PNP complex (helical path with 23.82° rotation and 11.1 Å rise per tubulin dimer) is not the maximum value we observe. In KLP10A–tubulin complexes not wrapped around microtubules a range of curvatures were observed up to 27° per tubulin heterodimer (Fig. 5c).

The increased tubulin curvature induced
Comparison with other kinesin structures. To our knowledge in all the structures available of kinesin13s motor domains not bound to tubulin (e.g., PDB accession codes: 1V8K29, 2GRY, 2HEH, 1V8J29, 4UBF, 4Y0534, 5XJA35, 5XJB35) the switch loops appear disordered and the nucleotide binding pocket is in the open configuration, even when ATP analogs are bound. This differs from the KLP10A–tubulin complex structures presented here where the switch loops are ordered (Fig. 2g–i), whether the nucleotide binding pocket is open (NMMT complexes) or closed (CTMMTAMP-PNP complex). This indicates that tubulin binding induces ordering of the switch loops. This is readily accounted for by the multiple interactions that SW1 and H4-SW2 make with each other and with tubulin in area II of the KLP10A–tubulin interface (Fig. 4 and Supplementary Fig. 5).

When comparing the structure of KLP10A–tubulin complexes with that of motile kinesin such as kinesin-1 there are many similarities as expected from the high degree of homology within the motor domain in the kinesin superfamily. Areas I and II of the interface with tubulin are very similar with several kinesins conserved residues establishing contacts (Supplementary Fig. 5). The open and closed configurations of the nucleotide binding pocket and associated sub-domains movements within the motor domain36–38 are also very similar (Supplementary Fig. 8). In the case of N-terminal-motor-domain-kinesins, such as kinesin-1, closing of the nucleotide pocket is associated with ATP binding and docking of the neck-linker33,36,37, a structure C-terminal of the motor domain between the motor domain and the dimerization domain (not to be confused with the kinesin-13 neck-domain). Docking of the neck linker of one motor domain biases movement of the partner motor domain towards the microtubule plus-end driving unidirectional movement along microtubules59. It is also thought that the activities of the two motor domains are coordinated by the position of their connecting neck linkers60. In the case of C-terminal-motor-domain-minus-end-directed kinesins, docking of their C-terminal region (corresponding to the neck linker) is associated with a reorientation of the stalk domain (located N-terminus to the
motor domain) in the microtubule minus-end direction\textsuperscript{22,41}. Interestingly, we also observe docking of the neck linker associated with closure of the nucleotide binding pocket (Fig. 6). The KLP10A neck-linker is in a down orientation (pointing toward the microtubule minus end) in the structures where the nucleotide binding pocket is open (Fig. 6a, b) and docks in an up orientation when the nucleotide binding pocket is closed (Fig. 6c–e). As the nucleotide binding pocket closes the ensuing sub-domain re-arrangements provoke conformational changes at the other side of the motor domain where the neck-linker is located. H4 and β-strand-1 move apart creating a hydrophobic pocket where residue L610 at the beginning of the neck-linker plugs into. A hydrophobic residue at the equivalent position of KLP10A L610 is highly conserved among kinesins (Fig. 6f), and a similar hydrophobic interaction between the neck-linker (or C-terminal end in C-terminal kinesins) and a hydrophobic pocket where residue L610 at the beginning of the neck-linker plugs into. Residue K608 colored red and shown as a ball and stick atom representation. e Close up of the image d showing the KLP10A residues forming the hydrophobic pocket where residue L610 plugs into. Residue K608 marks the end of α-helix 6 and the beginning of the neck linker. f Aligned neck linker sequences of six kinesins-13s (DmKLP10A, DmKLP59C, DmKLP59D, HsKIF2A, HsKIF2B, and HsKIF2C) and seven motile kinesins (DmKHC, HsKIF5A, DmKLP61F, HsKIF11, DmKLP64D, and DmNCD). Dm Drosophila melanogaster, Hs Homo sapiens. Asterisk is aligned with DmKLP10A K608. Arrow is aligned with DmKLP10A L610. Structures oriented in a–e with the tubulin plus-end-at the top. H4 α-helix 4, NL Neck-linker, βS1 β-strand 1

Coordination between partner motor domains through their neck-linkers connection allows kinesin-1s and other microtubule plus-end-directed motile kinesins to walk processively along microtubules\textsuperscript{43}. Full length kinesin-13s are also reported to be moderately processive enzymes that on average remove four tubulin dimers per encounter with a microtubule end\textsuperscript{15}. As with N-terminal kinesins such as kinesin-1, the kinesin-13s dimerization domain (or part of it) is located C-terminal to the motor domain\textsuperscript{12,31}. This configuration makes the two neck linkers of the partner motor domains the inter-connecting structural element that provides a communication pathway between the two nucleotide binding pockets. The fact that KLP10A and kinesin-1s show a similar relationship between neck-linker position and nucleotide binding structure (open or close) raises the possibility that similar mechanisms mediate processivity in motile and microtubule depolymerase kinesins.

Microtubule depolymerization mechanism. We propose that the key conformational change leading to tubulin bending and microtubule depolymerization is the movement of the kinesin-13-specific loop-2 relative to the other areas of the kinesin-13–tubulin interface (Fig. 7). The most significant differences between KLP10A and kinesin–1–tubulin complexes are the additional tubulin contacts made by L2 and the relationship between nucleotide species, tubulin binding and nucleotide pocket closure (Supplementary Figs. 5, 8, 9). In the case of KLP10A, nucleotide pocket closure requires ATP binding (as

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**Fig. 6** KLP10A neck-linker docking. The figure shows the structure of the beginning of the neck-linker domain present in the KLP10A protein constructs (six residues at the C-terminal end of the motor domain). a NMNT\textsubscript{apo} b NMNT\textsubscript{AMP-PNP} c CTMMT\textsubscript{AMP-PNP} Neck-linker in red, KLP10A motor domain in blue, β-tubulin in orange, α-tubulin in yellow, corresponding cryo-EM map density as a gray mesh. d CTMMT\textsubscript{AMP-PNP} structure with neck-linker in the up docked position. Core motor domain solvent exclusion surface colored by hydrophobicity with neck-linker shown as ribbon and atom sticks (Kyte and Doolittle hydrophobicity scale with orange to blue representing more to less hydrophobicity). Residue L610 colored red and shown as a ball and stick atom representation.
Proposed model for kinesin-13 microtubule depolymerization and ATPase activity coupling (neck-linker domain). 

**Fig. 7** Kinesin-13 microtubule depolymerization model. a Cartoon representing four distinct kinesin-13 conformations and equivalent states in kinesin-1: apo as in the NMNTtep complex; pre-stroke as in the NMNTAMP-PNP complex; post-stroke as in the CTMMTAMP-PNP complex; unbound as in the structures of several kinesin-13 motor domains not bound to tubulin. The switch regions are represented as green and magenta lines with straight or wavy lines indicating ordered or disordered conformations respectively. The three areas of interaction with tubulin are represented as a square (area I), a circle (area II, H4, SW2), and a trapezoid (area III, L2). The neck-domain is represented as a light blue line. Closing of the nucleotide pocket and reshaping of the tubulin interface is represented as a bending motion between the two halves of the motor domain (blue). SW switch regions, NBP nucleotide binding pocket, ND Neck-domain, NL Neck-linker, T: ATP. b Proposed model for kinesin-13 microtubule depolymerization and ATPase activity coupling (neck-linker on the KLP10A motor domain cartoon representation not shown). See main text for discussion.

In summary, our structural studies provide an atomic level detail of the mechanism of microtubule depolymerization by kinesin-13s, how this process is coupled to ATP hydrolysis and how it relates to the mechanism of translocation by motile kinesins.

**Methods**

**Kinesin proteins expression.** Kinesin proteins were expressed in BL21 cells (BL21 (DE3)pLysS, Thermofisher). The motor construct (M) includes *Drosophila melanogaster* KLP10A residues 279–615 and was expressed as previously reported18. The neck motor construct (NM) includes KLP10A residues 198–615 cloned into pRSET B plasmid with an N-terminal histidine tag. A TEV protease site (ENLYFQG) was introduced between the His tag and the start of the KLP10A sequence. Plasmid transformed cells were grown overnight in LB media containing 200 µg/ml ampicillin and 34 µg/ml chloramphenicol. The overnight culture was used to inoculate 6–8 l of LB media containing 200 µg/ml ampicillin and 34 µg/ml chloramphenicol.
chloramphenicol with an initial OD_{500=600} of 0.2–0.3. The cultures were incubated at 240 rpm, 37 °C until the OD_{500=600} reached 1.2. Protein expression was induced by adding 0.25 mM IPTG and incubating for 7 h at 30 °C and 200 rpm. Cells were spun for 15 min at 3000 x g and the pellet stored at −80 °C. All the purification steps below were performed at 4 °C. Bacteria cells were lysed at 4 °C with a 10% Laemmli buffer exchanged at 4 °C to BRB40 (40 mM K-PIPES, pH 6.8, 1 mM MgCl₂, 1 mM EGTA, 250 mM KCl, 150 µM Mg-ATP, 5 mM β-mercaptoethanol, 80 mM K-PIPES, pH 7.2), adjusted with KOH to attain an extinction coefficient of 600 µl/mg/ml before purification to eliminate aggregates. Microtubule polymerization was initiated with 10 mM imidazole and one tablet of protease inhibitor cocktail (Sigmafast Protease Inhibitor Cocktail Tablets, EDTA-Free, Sigma-Aldrich) per 20 g of cell paste. The lysate was spun for 40 min at 150,000 x g and 4 °C. The supernatant was passed through a NiNTA column (Qiagen) that was previously equilibrated with buffer A supplemented with 10 mM imidazole. The column was washed sequentially with: first, 100 ml of buffer A supplemented with 50 mM imidazole; second, 100 ml of buffer A supplemented with 1 M KCl; third, 8 ml buffer A. Elution was performed by adding and collecting 1 ml at a time of buffer A supplemented with 1 M EGTA, 250 mM KCl, 150 µM Mg-ATP, 5 mM β-mercaptoethanol, 80 mM K-PIPES, pH 7.2. The purified kinesin solution was concentrated, supplemented with 20 % (v/v) sucrose, aliquoted and flash frozen at −80 °C with a final protein concentration of 140–150 µM. KLP10A concentration was estimated by UV absorbance at λ = 280 nm using a NanoDrop (ThermoFisher, MA) and extinction coefficient of 23,300 L/mmol/cm (value estimated from the protein sequence and assuming one ATP molecule bound).

Microtubules. Microtubules were prepared from porcine brain tubulin (Cytokeleton, Inc. CO). Tubulin lyophilized pellets were resuspended in BRB80 (80 mM K-PIPES, 1 mM MgCl₂, 1 mM EGTA, pH 6.8) to 45 µM and spun at 313,000 x g before polymerization to eliminate aggregates. Microtubule polymerization was done in the buffer with 20 % (v/v) sucrose, aliquoted and stored at −80 °C for up to 1 week. Microtubules were polymerized with 20 µM m-paclitaxel in BRB80, with 2 mM AMP-PNP, 2 mM MgCl₂ and 20 µM m-paclitaxel (all final concentrations). The mix was incubated at room temperature for 20 min and spun for 10 min at 28 °C, 30,000 x g. The pellet was resuspended in BRB80 buffer with 20 % (v/v) sucrose, aliquoted and stored at −80 °C for up to 1 week. The solution was layered onto an untreated electron microscope grid (Quantifoil carbon/copper grids 400 mesh R2/4) and incubated 1 min at room temperature. The grid was then placed into a Vitrobot apparatus for plunge freezing into liquid ethane. Vitrobot settings: 100% humidity, 2 s blotting with Whatman #1 paper and −2 mm offset. Microtubules and the KLP10A motor construct-curved tubulin protofilament complexes (CTMMT_{AMP-PNP} Complex) were imaged at a higher defocus (∼3.0 μm).

Helical-single-particle 3D reconstruction. Movie frames were aligned with motioncor2 (v0.1–30–2017) using default parameters, generating both dose-weighted and non-dose-weighted sums. CTF parameters per micrographs were estimated with Gctf (v0.50) on aligned and non-dose-weighted movies average.

Number of images, particle-boxes and asymmetric units included in the 3D reconstructions is as listed in Table 1. A helical-single-particle approach was used to obtain 3D electron density maps of microtubule structures decorated with KLP10A motor construct (NMNMT complexes) and with KLP10A motor construct-curved tubulin protofilament spirals (CTMMT_{AMP-PNP} Complex). The approach consisted of the following steps: Step 1. Microtubules were manually picked on the aligned micrograph averages using a custom script written in the R programming language.

Step 2. Microtubule symmetry identification and selection. Atomic models of microtubule filaments ranging from 11 to 16 protofilaments (A or B, left or right handed protofilament twist) were generated, and converted to densities with EMAN2 [26] pdb2mrc with a voxel size of 4 Å/voxel. The densities were low passed filtered to 15 Å and the Spider [27] operation PQ 3Q was used to generate 200 reference projections per microtubule type. Experimental microtubule image segments were extracted and wired to 4 Å. Spider2 was used to generate a map of each experimental microtubule image subjected to multiple-reference alignment and classification with the set of model microtubule image projections (Spider2 SHC operation) to identify the best matching pairs. A microtubule type was assigned to each experimental filament image as the microtubule type with more matches using the experimental filament image segments. Only filaments for which a majority of the corresponding segments identified as 15R (15 protofilaments with right handed super-twist) were selected for further processing. This procedure was repeated using as reference a 15R microtubule reconstruction generated from the experimental data as explained in step 3. At the end only microtubule segments with at least 10 successive particles identified as 15R were retained for further processing.

Step 3. Initial model. To get an initial model a first round of 3D reconstruction was performed following the procedure described in step 5 but using a single reference map instead of two independent ones. The reference maps were generated by converting to electron densities the PDBs that corresponded to each experimental microtubule image subjected to multiple-reference alignment and classification with the set of model microtubule image projections (Spider2 AP SHC operation) to identify the best matching pairs. A microtubule type was assigned to each experimental filament image as the microtubule type with more matches using the experimental filament image segments. Only filaments for which a majority of the corresponding segments identified as 15R (15 protofilaments with right handed super-twist) were selected for further processing. This procedure was repeated using as reference a 15R microtubule reconstruction generated from the experimental data as explained in step 3. At the end only microtubule segments with at least 10 successive particles identified as 15R were retained for further processing.
matching procedure a new set of axis coordinates was determined. This set was fitted to a spline curve with a maximum degree of freedom of 2, and re-interpolated every 82 Å along the axis to generate a new set of coordinates for the next cycle.

Step 5. Position-orientation refinement and 3D reconstruction. Refinement was done completely independently in two halves of the data set using two different starting models. The data were split in half by dividing each microtubule filament in two halves. For each of the two independent refinements the data was further divided in two halves. These two quarter data sets were used to get an estimate of the resolution to which the new refinement was filtered for the next refinement cycle. Refinement was done in two stages. The first using Spider22 and the second using Frealign35. An exhaustive search of the Euler angles and displacements corresponding to each particle image was performed by multi-reference alignment against projection images of the reference model (Spider operation AP SHC) on 2× binned, CTF corrected particle images (phase filtering only). The orientation and position parameters determined were used to generate a 3D density map by back-projection of the particle-image data set. Helical symmetry was imposed using hispose38. This map was used as the reference model for a new round of multi-reference alignment. Three rounds of the Spider alignment procedure were performed. The resolution was estimated from the FSC curve calculated using the two 3D maps obtained from the two quarters data sets. This resulted in two independently refined 3D maps each with a resolution estimate. After three rounds of alignment the estimated resolution was 6–7 Å. The final cumulative displacement and angular rotations determined in the two Spider refinements were given to Frealign35 to calculate the two reference models used to perform two independent position/angular refinements and 3D reconstructions. Particle-images were extracted every 82 Å along the filament (box size: 1212 pixels for the quarter 2× datasets and 651 × 616 pixels for the CTMMAP-PNP data set). To limit the number of overlapping particle-images in each of the two halves datasets used in each of the two independent Frealign refinements the image/particle stack were reordered (and corresponding position angular information) so that odd and even images corresponded to distinct filament quarters. For CTF correction local CTF parameters were estimated with Ctf85 (v.3-30). Several cycles of Frealign refinement (typically 5) were run limiting the data used to 1/10 Å−1, until no further improvement in resolution was detected. Then additional cycles, limiting the refinement data to 1/8 Å−1, were run until no further improvement in resolution was detected. At the end of the last cycle, the two unfiltered half maps from each independent refinement were averaged to obtain two independently refined maps.

Step 6. Final resolution estimates. Final resolution was estimated using the FSC12 criteria (Supplementary Fig. 2) from the two independently refined maps (gold standard) using masks generated with Relion27 2.0 post-processing. To make these maps the masks were low-pass filtered to 12 Å, thresholded, the resulting boundaries extended by 2 pixels in all direction and smoothed with a raised cosine edge 5 pixels wide. FSC curves for distinct parts of the maps (microtubule, kinesins or curved tubulin protifilament) were generated by masking the corresponding regions of the map with local masks. These local masks were created by converting to density the coordinates of local region models, (EMAN132, 3-pcm low-pass filtering and thresholding them as indicated above. The same mask was used for the two NMMT maps. Local resolution was also estimated from the two raw half maps using the Bsoft50 (v. 1.9.5) function blocwes with a kernel size of 10 pixel (Supplementary Fig. 3).

Step 7. Map post-processing. The two independently refined raw half maps were averaged, corrected for the modulation transfer function of the K2 summit detector and sharpened to restore high resolution contrast with Relion27 2.0 post-processing program. The B-factors used were: −130, −100, and −20 Å2 respectively for the NMMTamp, CTMMAP-PNP, and NMMPAMP-PNP maps. The maps were then locally corrected using Bsoft14 (v.2) and local resolution estimates given by Bsoft blocwes (step 6). Illustration of the 3D density maps and molecular models were prepared using UCSF-Chimera59 and Blender.

2D class averages of CTMMAP-PNP complex. Images of ring-like particles of KLPI0A NM construct in complex with curved tubulin protifilaments in the presence of AMP-PNP (Supplementary Fig. 1d) were aligned and classified using Relion57 (v. 2.0). CTF was estimated using CTFTfind190. First 10,016 ring-like structures were picked manually from 464 cryo-EM images by marking their centers and masking them with a circular mask of 540 Å. This set of particles was subjected to three rounds of alignment classification and averaging with 100 class averages in each round. Only particles assigned to classes showing distinct tubulin rings with resolved tubulin dimers were kept for the next cycle. In each cycle the coordinates of the particles in the original images were recalculated based on the position of the average-image particle. This was done by applying the inverse transformation used to align the particles and any additional displacement necessary to re-center the particle in the class-average-image. The updated coordinates were then used to generate a new set of particles for the next round of alignment classification and averaging. This procedure resulted in three class averages with well centered ring like structures with 15, 14, and 13 tubulin dimers (Fig. 3c). The number of particles in each of these classes were respectively 469, 2171, and 920. We then calculated the coordinates in the original images corresponding to each of two contiguous tubulin dimers in these three class averages. With these coordinate three new sets of particle images (one per each of the three ring-like class average, Fig. 5c) were generated. Each particle-image was masked with a circular mask to included only two tubulin heterodimers (176 Å mask diameter). These three data sets were subjected to three rounds of alignment classification and averaging done as before (centering the particles on the inter-dimer interface of the two tubulin heterodimers approximately at the location where the tip of KLP10A loop-2 would contact tubulin). This procedure resulted in three independently aligned groups of class average images. Two distinct types of class average images could be recognized in each of the three groups. One in which there is a kinesin density associated with each tubulin heterodimer (two kinesins and two tubulin heterodimers) and another with one kinesin per two tubulin heterodimers (Fig. 3d, e). The number of particles associated to each of these two class-average images for each of the three groups (15, 14, and 13 tubulin heterodimers ring like structures) were respectively (954, 1441, 610, 567), and (1109, 530) with the first number in parenthesis corresponding to the class with two motor-dominus bound and the second number corresponding to the class with one motor domain bound.

Model building. Atomic models of the cryo-EM density maps were built as follow. First, atomic models for each of the proteins chains involved in the complex were generated from their amino-acid sequence by homology modeling using Modeller98. Second, models of the complexes were built by manually placing and fitting the polypeptide chain models into the cryo-EM electron density maps using UCSF-Chimera57. At this step N- or C-terminal residues of the chain models falling outside the electron densities (i.e., with no associated density) were deleted from the models. Third, the complex models were flexible fitted into the electron density maps using Rosetta for cryo-EM62. For this step we first generated models using the relax-protocols and picked the models with the best scores (best matching to the cryo-EM density among the lowest 20% energy models). From these models we then generated over 100 models using the iterative-local-rebuilding protocols and picked the ones with the best scores as before. Fourth, we refined the best scored Rosetta models against the cryo-EM density maps using Phenix43 real space refinement tools. Fifth, the Phenix refined models were edited in Coot65 and UCSF-Chimera57 to resolve a few atomic clashes, geometry problems and areas of the model outside the cryo-EM density. The edited models were used as the reference models to 1/8 Å.

Data availability. Data supporting the findings of this manuscript are available from the corresponding author upon reasonable request. Atomic coordinates have been deposited in the Protein Data Bank (PDB) with accession numbers 6B0C, 6B0L, and 6B0T. The corresponding cryo-EM density maps have been deposited in the Electron Microscopy Data Bank under the accession numbers EMD-7026, EMD-7027, and EMD-7028.

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**Additional information**

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