Atherton, Joe and Luo, Y. and Xiang, S. and Yang, C. and Rai, A. and Jiang, K. and Stangier, M. and Vemu, A. and Cook, A.D. and Wang, S. and Roll-Mecak, A. and Steinmetz, M.O. and Akhmanova, A. and Baldus, M. and Moores, Carolyn A. (2019) Structural determinants of microtubule minus end preference in CAMSAP CKK domains. Nature Communications 10 (1), ISSN 2041-1723.

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Structural determinants of microtubule minus end preference in CAMSAP CKK domains

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CAMSAP/Patronins regulate microtubule minus-end dynamics. Their end specificity is mediated by their CKK domains, which we proposed recognise specific tubulin conformations found at minus ends. To critically test this idea, we compared the human CAMSAP1 CKK domain (HsCKK) with a CKK domain from Naegleria gruberi (NgCKK), which lacks minus-end specificity. Here we report near-atomic cryo-electron microscopy structures of HsCKK- and NgCKK-microtubule complexes, which show that these CKK domains share the same protein fold, bind at the intradimer interprotofilament tubulin junction, but exhibit different footprints on microtubules. NMR experiments show that both HsCKK and NgCKK are remarkably rigid. However, whereas NgCKK binding does not alter the microtubule architecture, HsCKK remodels its microtubule interaction site and changes the underlying polymer structure because the tubulin lattice conformation is not optimal for its binding. Thus, in contrast to many MAPs, the HsCKK domain can differentiate subtly specific tubulin conformations to enable microtubule minus-end recognition.

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The involvement of the microtubule (MT) cytoskeleton in numerous processes in eukaryotic cells is enabled by the diverse and adaptable properties of individual MTs. MTs act as tracks for molecular motors, while growing and shrinking MTs can be used to generate force. MTs can also act as signalling hubs, such that specific tubulin conformations within particular regions of the polymer stimulate recruitment of distinct MT-binding partners. The molecular basis of these effects, mediated by the conformational adaptability of tubulin dimers, is only just beginning to be understood and represents a key topic in the cytoskeleton field.

The ends of MTs are important sites of conformational diversity and are often points of communication between the MT cytoskeleton and other cellular components, such as membranes, organelles, centrosomes and chromosomes. The exact conformation(s) of MT ends is an ongoing source of debate, but current evidence points to their being composed of zones with distinct and dynamic tubulin conformations. MT minus ends were long thought to be static and structurally homogeneous, capped by γ-TuRCs and buried at MT organizing centres. More recently, however, the discovery and characterisation of CAMSAP (calmodulin-regulated spectrin-associated proteins)/Patronin family members has revealed that control of non-centrosomal MT minus-end dynamics, and their interaction with specific cellular regions, are vital in numerous aspects of cell physiology. CAMSAP/Patronins are centrally involved in diverse activities including promoting cell polarity, regulation of neuronal differentiation and axonal regeneration, and definition of spindle organization and asymmetry, thereby highlighting the importance of regulation of MT minus-end dynamics in these varied contexts.

At the molecular level, CAMSAPs/Patronins stabilise uncapped MT minus ends and support MT minus-end growth. CAMSAP/Patronins are large, multi-domain proteins with many cellular binding partners. However, the family is defined by the presence of a CKK domain (originally identified in CAMSAP1, KIAA1078 and KIAA1543), which is necessary and sufficient for MT minus-end binding in many CAMSAP/Patrons. Previously, we showed that CAMSAP/Patronin CKK domains preferentially bind to a zone behind the extreme MT minus end, which corresponds to a region where the lattice undergoes a transition to gently curved tubulin sheets. Subnanometer resolution single particle cryo-EM showed that CKK domains bind on the MT lattice between two tubulin dimers on adjacent protofilaments. Mutagenesis of residues at the MT-binding interface in the CKK domain disrupted lattice and minus end binding, showing that the same regions of the CKK domain that contact the MT are also involved in binding to the minus-end zone. Taking these data together, we proposed a model for CAMSAP/Patronin MT minus end recognition, which is mediated by sensitivity of the CKK domain to a curved sheet-like conformation of tubulin exclusive to MT minus ends. Specifically, the model suggested that the tighter CKK interaction with β-tubulin favours binding at MT plus ends while the looser α-tubulin contacts preferentially accommodate tubulin curvature at minus ends. This interaction also can occur on the MT lattice, but CKK binding induces distortion of the non-optimal binding site configuration, manifesting as protofilament skew within the polymer.

Despite these findings, several critical questions relating to the structural basis of this recognition mechanism remain unanswered: How is the CKK-induced MT lattice distortion accommodated, and what can this tell us about minus-end recognition? Can CKK binding to different MT protofilament architectures shed further light on the mechanism of minus end recognition? Intriguingly, we also previously identified CKKs in the amoeboid-flagellate *N. gruberi* and the potato blight fungus *P. infestans* that lacks the binding preference for MT minus ends that was proposed to be present in CKK domains from the last eukaryotic common ancestor. Can a comparison of CKK domains with and without minus-end binding specificity also provide insight into MT minus-end recognition? Since discrimination between different MT lattice zones can depend on relatively subtle structural differences, high resolution information is needed to address these questions. It is currently not possible to image MT minus ends directly at the necessary resolution to observe these conformational variations. However, our previous work showed that CKK lattice binding can be used as a proxy for minus end binding, and cryo-EM studies of the lattice could yield near-atomic resolution information about the CKK-tubulin complex.

We therefore developed a RELION-based image-processing pipeline that enabled us to study the CKK-MT interaction at better than 4 Å resolution. Using this, we determined reconstructions of MT-bound CKK domains on both 13- and 14-protofilament pseudo-helical MTs, allowing visualisation of a wider repertoire of tubulin conformations. We also investigated the CKK domain from *Naegleria gruberi* (NgCKK), which does not show minus-end binding preference. The direct comparison of tubulin binding by NgCKK with that of human CAMSAP1 CKK (HsCKK)—which has minus end binding preference—allowed us to probe our previous model of minus-end recognition. We found that NgCKK, like HsCKK, binds between two tubulin dimers in neighbouring protofilaments. However, NgCKK MT binding is shifted relative to HsCKK, resulting in a modified interaction with tubulin including even smaller contacts with α-tubulin, contrary to our previous prediction. Thus, the comparison between NgCKK and HsCKK shows that looser contacts with α-tubulin compared to β-tubulin are important but not sufficient for minus end recognition. NgCKK binding does not induce protofilament skew, reinforcing that induction of skew is a structural signature for minus-end binding capability. NMR studies revealed a remarkable structural rigidity of both the HsCKK and NgCKK domains. The ability of HsCKK to remodel MTs thus results from a combination of intrinsic structural properties and the precise mode of interaction with its MT-binding site. HsCKK-induced skew arises within the MT lattice from the tilting of entire protofilaments coincidental with contraction of the MT diameter. These multi-disciplinary data reveal that the surprising structural plasticity of MTs, which is distinct from nucleotide-dependent modulations of the MT lattice, forms the basis for minus-end recognition by CAMSAP proteins.

**Results**

**Canonical lattice binding by a CKK domain from *N. gruberi***. The presence of CKK domains in diverse organisms presents a unique resource that can shed light on the conserved or divergent properties of these domains. Our previous analysis suggested that a CKK domain from *N. gruberi* did not exhibit MT minus-end binding preference. We confirmed this using TIRF experiments, showing that, in comparison to the well-characterised MT minus-end preference of HsCKK (Fig. 1a, left panel), NgCKK strongly bound along the entire MT lattice and exhibited no MT minus-end preference on dynamic MTs at a range of concentrations (Fig. 1a, right panels).

To investigate this distinctive behaviour further, complexes formed by either NgCKK or HsCKK and taxol-stabilised MTs were imaged using cryo-EM for structure determination. Our previous work showed that CKK MT binding includes interactions with the C-terminal tails of tubulin (CTTs). To facilitate visualisation of this interaction, we used MTs assembled from tubulin purified from a human tsA201 cell line for our reconstructions. These MTs contain only two β-tubulin...
isoforms and one α-tubulin isoform, have no detectable post-translational modifications as indicated by mass spectrometric analyses\(^{29}\), and are thus much more homogenous than the brain tubulin we previously used. To visualise CKK binding at higher resolution, we also developed an image-processing pipeline for pseudo-helical MTs and different protofilament architectures in RELION (see methods in ref.\(^{28}\)). The resulting unsymmetrised (C1) reconstructions for both NgCKK and HsCKK showed distinct CKK intradimer, interprotofilament densities every 8 nm along the MT axis and an absence of CKK density at the seam (Fig. 1b, c). This validates the accuracy of the pipeline and is consistent with our previous work\(^{2}\), while revealing the MT-bound NgCKK and HsCKK complexes at substantially higher resolutions now for both 13- and 14-protofilament MTs. The C1 reconstructions all have resolutions of 4.7 Å or better, and the symmetrised reconstructions have resolutions of 3.8 Å or better (Supplementary Fig. 1). This allowed us to build atomic models of the NgCKK-MT and HsCKK-MT complexes (Fig. 1d–g, Supplementary Fig. 1i–l, Supplementary Table 1).

While the structures of mammalian CKK domains have previously been determined, our NgCKK reconstructions now reveal the near-atomic resolution structure of a non-mammalian CKK domain (Fig. 1h). It has a typical CKK fold, but sequence differences compared to HsCKK (Supplementary Fig. 2a) are reflected in structural differences in several loop regions (Supplementary Fig. 2b). NgCKK’s loop4, which faces away from...
the MT surface (Fig. 1d, black arrows), is 34 amino acids longer than in HsCKK. There is no extra density in the cryo-EM reconstructions corresponding to this insert even at low thresholds (Fig. 1d), suggesting it is highly flexible/disordered and unlikely to be involved in MT binding. However, there are also structural differences in regions closer to the MT surface: specifically, loop3, the C-terminal single-turn helix and the beta-hairpin leading into loop7, all show backbone RMSDs >2.5 Å (Supplementary Fig. 2c).

The reconstructions show that both NgCKK and HsCKK form an intradimer interprotofilament wedge (Fig. 1d–g), contacting both α- and β-tubulin subunits in a tubulin dimer pair (with constituent monomers numbered α1, β1, α2 and β2). Both CKK domains form MT contacts mainly via a set of surface exposed loops (Fig. 1d–g, described below). The N-terminal extension of each CKK domain also contacts the MT but in distinct ways. The NgCKK N-terminus forms an ordered density that is associated with the surface of β2-tubulin, although the density was not sufficiently defined to allow accurate modelling (Fig. 1d).

Conversely, the HsCKK N-terminus closest to the CKK core forms a distinct interaction with β2-tubulin that was built into the atomic model, whereas density corresponding to its most N-terminal part was hardly visible on the surface of β2-tubulin (Fig. 1f). Furthermore, there is no clear contact between NgCKK and the β1-tubulin CTT (Fig. 1d). This is in contrast to HsCKK, where density corresponding to an additional ~5 residues of β-tubulin’s CTT were visualised in our structures (Fig. 1f, purple) contacting both the CKK core and its C-terminus (Supplementary Fig. 3a). The visualisation of β-tubulin’s CTT interaction with HsCKK is likely facilitated by the limited sequence variability (Supplementary Fig. 3b) and lack of post-translational modifications in the CTT tails in tsA201 β-tubulin compared to brain tubulin.

Overall, these structures show that the core of CKK domains with and without minus-end binding preference have the same protein fold and interact with MTs in similar although not identical ways. Differences in the tubulin interactions are also seen at both their N- and C-termini. Our previous work showed that although these regions contribute to HsCKK MT affinity, neither the N- nor C-terminal extensions define its minus-end binding specificity. However, to test the hypothesis that the absence of minus-end recognition by NgCKK is mediated by its position (i.e. that all CKK cores have minus end recognition properties), we studied a set of CKK N- and C-termini truncations and chimeras using TIRF microscopy (Supplementary Fig. 4). Removal of either or both termini, and swapping the N-terminus of NgCKK for that of HsCKK, drastically reduced the overall affinity of these proteins for MTs. Crucially, the chimera in which the C-terminus of NgCKK was substituted with that of HsCKK retained MT binding but also showed no minus end recognition, clearly arguing against the above gain-of-function hypothesis. Intriguingly, however, the chimera with both N- and C-termini of HsCKK grafted onto the NgCKK core has a slight minus-end preference, albeit significantly less than HsCKK. This hints at modulatory mechanisms of the unstructured termini—in particular the N-terminus on the core CKK domain. Nevertheless, the behaviour of most of these engineered constructs, together with the observation of similar binding sites for NgCKK and HsCKK, and with our previous characterisation of HsCKK mutants, suggests that differences in CKK minus-end recognition behaviours are primarily due to structural differences in the interaction between tubulin and the CKK core. The near-atomic resolution of all our reconstructions allowed us to investigate the mechanistic basis of these effects.

Differences in MT interaction between NgCKK and HsCKK.

To visualise how NgCKK’s MT interaction differs from HsCKK, each CKK-tubulin model was aligned on the tubulin parts of the complex, thereby revealing differences in CKK positioning relative to the MT lattice (Fig. 2a, top). The tubulin dimers in both models readily superimpose (Supplementary Fig. 5), as do helices α1, α2 and loop7 in each CKK domain (Fig. 2a, top). However, NgCKK loop3, loop8 and its C-terminus—which all contact the MT—are displaced compared to HsCKK (Fig. 2a, bottom). While some of these variations are due to structural divergence in NgCKK, this is insufficient to explain all the differences in CKK positioning relative to tubulin. In fact, NgCKK is rotated away from the MT around the apex of loop7 relative to HsCKK (Supplementary Movie 1).

As a result of this shift, even when the sequences in each CKK domain are conserved, our reconstructions show that some NgCKK residues engage differently with the MT lattice compared to equivalent residues in HsCKK. For example, HsCKK loop3 residues R1535 and R1528 contribute significantly to MT affinity. With the improved resolution of our current reconstructions, R1528 is observed extending close to α1-tubulin’s residues H393 on H11, whilst R1535 reaches to contact the β1-tubulin CTT (Fig. 2b, top). On the other hand, in NgCKK loop3—which is one residue longer and positioned differently with respect to the MT surface compared to HsCKK—the residue equivalent to R1528 (R678) interacts with loop H10-β7 of β1-tubulin, possibly hydrogen bonding with E345 (Fig. 2b, bottom). In addition, instead of interacting with α1-tubulin, NgCKK residue R686...
(equivalent to R1535) also interacts with β1-tubulin, extending to within hydrogen bonding distance of E345.

Altogether, the subtle differences within the NgCKK sequence, fold and positioning of the domain relative to tubulin result in a different binding footprint on the MT surface compared to HsCKK (Fig. 2c,d). Both the new HsCKK structure and the NgCKK structure exhibit the previously described larger interface with β-tubulin compared to α-tubulin (Fig. 2e) proposed to be important for minus end recognition2. Intriguingly, however, the differences between HsCKK and NgCKK contacts are most striking on α-tubulin, with the HsCKK C-terminus/loop3 and loop8/N-terminus forming closer contacts with α1- and α2-tubulin, respectively, compared to NgCKK. Indeed, the overall NgCKK footprint is smaller on both α1- and α2-tubulin compared to HsCKK (Fig. 2e). These data thereby highlight that the role of contacts with α-tubulins in mediating the minus end specificity of HsCKK is more sophisticated than was previously proposed (see Discussion).

Sensitivity of HsCKK to microtubule lateral curvature. In binding between two tubulin dimers, CKK domains are well placed to sense changes in inter-tubulin lateral curvature, which affects the distance and angle between adjacent protofilaments.
Previously, we proposed that sensitivity to lateral curvature was an important facet of HsCKK binding minus-end specificity. Since MTs with different protofilament numbers exhibit different lateral curvature, comparison of CKK binding in our 13- and 14-protofilament reconstructions allowed us to investigate this effect. Superposition of a single tubulin dimer from each of the 13- and 14-protofilament atomic models for NgCKK and HsCKK reconstructions shows that the interprotofilament lateral angle in 13- compared to 13-protofilament MTs is ~2° shallower (Fig. 3).

In response, NgCKK is only slightly altered in its binding site on 13-protofilament MTs (Fig. 3a, b, Supplementary Movie 2), but HsCKK experiences a larger displacement out of the interprotofilament cleft on 14-protofilament MTs (Fig. 3c, d, Supplementary Movie 3). Even though the HsCKK interface with α-tubulin remains larger than that of NgCKK on 14-protofilament MTs (Fig. 2e), the comparison between different MT architectures suggests that in the context of decreased lateral curvature, HsCKK is more prone to being squeezed out of its binding site than NgCKK. This is presumably because it binds deeper between protofilaments compared to NgCKK (Fig. 3).

Since our model of minus-end recognition predicts that lateral flattening of adjacent α-tubulin pairs would favour HsCKK binding, the outward displacement of HsCKK from its binding site on slightly flatter 14-protofilament MTs initially appears counter-intuitive. In the lattice, however, lateral flattening affects α- and β-tubulins equally, whereas our model suggested that both (i) lateral flattening in α-tubulin towards the MT minus end is particularly important and furthermore that (ii) preferential lateral flattening in adjacent β-tubulin pairs at MT plus ends would disfavour HsCKK binding. Thus, the potential enhancement of α-tubulin contacts in 14-protofilament MTs is balanced by tightening at the already tight β-tubulin interface. This emphasizes the sensitivity to subtle differences in tubulin conformation encoded by HsCKK that supports its MT minus-end binding preference, in particular the importance of conformational asymmetry in α- and β-tubulins at this site.

### CKK protofilament skew correlates with minus end specificity.

In addition to comparison of the NgCKK and HsCKK binding sites, the overall architecture of the decorated MTs can be compared to shed further light on HsCKK minus-end preference. We previously described the ability of HsCKK to induce positive (right-handed) protofilament skew in MTs polymerized from mammalian brain tubulin. Raw images (Fig. 4a) and particle alignment parameters (Fig. 4b) from our new HsCKK-MT dataset support this observation on tsA201 cell-tubulin MTs. 13-protofilament MTs usually have unwskewed protofilaments, running straight along the MT wall (Fig. 4c), whereas HsCKK binding causes right-handed protofilament skew (Fig. 4a–c). Furthermore, 14-protofilament MTs usually have negatively skewed protofilaments but HsCKK binding caused these protofilaments to lie parallel to the MT wall with no skew (Fig. 4a, b). In other words, we observed induction of right-handed protofilament skew by HsCKK in both types of MT architectures. Intriguingly—and in contrast to the HsCKK—the intrinsic protofilament skew in both 13- and 14-protofilament MTs was unperturbed by NgCKK binding (Fig. 4a–c). This is consistent with the idea that protofilament skew induction correlates with MT minus-end specificity.

Protofilament skew can arise either from a stagger of individual subunits along a protofilament perpendicular to the long axis of the protofilament, or from tilt of the whole-protofilament relative to the MT axis. The higher resolution of our new reconstructions allowed us to probe how protofilament skew is structurally accommodated in HsCKK-bound MTs and thereby shed light on requirements for tubulin plasticity to support HsCKK minus-end recognition. To do this, we aligned and compared the HsCKK and NgCKK C1 cryo-EM reconstructions; this is because although these structures have slightly lower resolutions than the symmetrised reconstructions, the fact that they have not been symmetrised means they more closely reflect the overall polymer structure. When protofilaments of NgCKK and HsCKK models fitted into their corresponding C1 reconstructions are then overlaid, a positive skew of HsCKK protofilaments relative to NgCKK protofilaments is observed (clockwise rotation viewed from the outer surface the MT, Fig. 4d, left). This skew is reflected in increasing RMSD between the two models along the helical axis (shown in Fig. 4d, right). However, when the models of individual protofilament from NgCKK and HsCKK structures are directly aligned, this produces only a small RMSD (~1 Å) along the whole protofilament (Fig. 4e), i.e. the structures of protofilaments from each reconstruction are essentially the same. This comparison shows that, rather than rearrangements within protofilaments, protofilament skew in HsCKK-bound MTs results from a tilt of whole protofilaments relative to the pseudo-helical axis (Fig. 4f).
resolution in our ssNMR spectra compared to the wild-type CKK (Supplementary Fig. 6a). Importantly, this gain in spectral resolution allowed us to conduct fast magic angle spinning (MAS), $^1$H detected ssNMR experiments to further elucidate the CKK conformations in complex with MTs (Supplementary Fig. 6b–d). The chemical-shift perturbations observed upon MT-binding agreed with the previous reported CKK-MT interface while providing a more quantitative and residue-specific description of the behaviour of the domain (Fig. 5a, left). Importantly, we mainly observed chemical-shift perturbations on the amide $^1$H and $^{15}$N atoms, while no significant chemical-shift perturbations were detected for the Cα atoms, indicating that the secondary structures of HsCKK did not alter upon binding to MTs (Supplementary Fig. 6c, d).

In this context, we speculated that despite their overall conserved fold, HsCKK and NgCKK may have intrinsically different structural properties. To obtain additional insight into the structural properties of these CKK domains, we probed residue-specific dynamics of several free CKK domains using solution-state NMR. Specifically, we conducted Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion$^{31,32}$ and Chemical Exchange Saturation Transfer (CEST) experiments$^{33}$ to reveal...
Fig. 3 HsCKK is more sensitive to MT lateral curvature than NgCKK. a A transverse slice, viewed from the MT plus end, through 3 protofilaments from the NgCKK-MT 13- and 14- protofilament (pf) models superimposed on the central protofilament. This shows that the adjacent protofilaments adopt a shallower relative angle in 14-protofilament MTs (orange arrows) and reveals the response of NgCKK bound in the interprotofilament valley to this change in lateral protofilament curvature. Top, view of the superimposed protofilament backbone models from the 13- and 14-protofilament structures superimposed on the central protofilament (dotted pink outline); small blue arrows indicate the relatively small displacement of NgCKK from its binding site on 14-protofilament MTs. Bottom, backbone RMSD between the 13- and 14-protofilament models shown on the 13-protofilament model; b schematic depicting the effect of MT protofilament architecture on NgCKK binding. c A transverse slice, viewed from the MT plus end, through three protofilaments from the HsCKK-MT 13- and 14- protofilament models superimposed on the central protofilament. This again shows how the adjacent protofilaments adopt a shallower relatively angle in 14-protofilament MTs (orange arrows) and reveals the response of bound HsCKK to this change in lateral protofilament curvature. Top, view of the superimposed protofilament backbone models from the 13- and 14-protofilament structures superimposed on the central protofilament (dotted pink outline); large blue arrows indicate the larger displacement of HsCKK from its binding site on 14-protofilament MTs. Bottom, backbone RMSD between the 13- and 14-protofilament models shown on the 13-protofilament model; d schematic depicting the effect of MT protofilament architecture on HsCKK binding.

possible millisecond time-scale conformational exchange processes of CKK domains. The CPMG profiles of HsCKK_N1492A, HsCKK, the CKK domain from mouse CAMSAP3 and NgCKK all showed no exchange in these CPMG time ranges (50 Hz -1.5 kHz) (Fig. 5a, right and Supplementary Fig. 6e). This is particularly noteworthy in NgCKK given the large, flexible loop4 insertion compared to HsCKK (Supplementary Fig. 2a). From our data, we can conclude that motions of loop 4 (which most likely occur on the nanosecond scale) do not influence the milliseconds time-scale motional profile of the rest of the protein backbone that is probed in our CPMG and CEST experiments.

Similarly, the results of additional CEST experiments using HsCKK_N1492A speak against slow milli-second time-scale motion in free CKK domains, for example, in residues T1553 and S1585, which exhibited significant chemical-shift changes upon complex formation (Supplementary Fig. 6f). Similar results were obtained for CAMSAP3 CKK (Supplementary Fig. 6g) and NgCKK (Supplementary Fig. 6h). Taken together, our NMR experiments suggest that the 3D structures of CKK domains—whether they recognize minus-ends or not—are remarkably rigid and, in contrast to many other MAPs, do not undergo structural changes upon MT binding.

HsCKK remodels its binding site causing MT remodelling. We then wanted to know how protofilament skew was brought about by HsCKK binding and accommodated by the MT lattice. The geometric constraints that define MT architecture while maintaining longitudinal and lateral tubulin contacts are captured in the lattice accommodation model13,35, and we investigated in turn the set of interconnected structural parameters this model describes.

The helical set and monomer repeat distances were not significantly different in MTs (13- or 14-protofilament) bound by HsCKK or NgCKK (Supplementary Table 2). However, protofilaments in HsCKK-MTs are closer together (Fig. 5b), giving these MTs a ~4 Å smaller diameter than NgCKK MTs with the same protofilament number. The diameter shrinkage occurs because the centres of mass of all neighbouring B-lattice dimers in a single 3-start helical turn are closer by around 0.4 Å in HsCKK—compared to NgCKK-MTs (Fig. 5c); such a difference is not observed in the equivalent comparison between half-maps within each dataset (Supplementary Fig. 7a). This inward protofilament positioning is not symmetrical around the MT, with a range of 0.4-1.5 Å relative shifts observed in both 13- and 14-protofilament architectures (Fig. 5b), and with the biggest deviations seen at and opposite of the seam. Again, such a difference is not observed in the equivalent comparison between half-maps within each dataset (Supplementary Fig. 7b). The small compression between lateral neighbouring tubulin dimers in the lattice can be observed by superimposing the ones from the atomic models of NgCKK-MT and HsCKK-MT (Fig. 5d, top). This analysis also reveals a longitudinal displacement of adjacent tubulin dimers in the HsCKK model relative to the NgCKK model; this is indicative of shearing of adjacent protofilaments as they skew. There are, however, no detectable differences in interprotofilament lateral contacts (small RMSDs, Fig. 5d, bottom; Supplementary Fig. 7c, d). Rather, small adjustments across the outer tubulin surface—where HsCKK binds—flexibly accommodate shifts in the MT architecture due to HsCKK binding (Fig. 5d, bottom, larger RMSDs in the tubulin on the left). In summary, relative to NgCKK, HsCKK induces small conformational changes in tubulin at its binding site which, in the context of whole MTs, induces protofilament tilt, shear, lateral compression and a reduction in MT diameter (Fig. 5e).

Discussion
To shed light on the MT minus-end binding preference of CAMSAPs, we have structurally compared a CKK domain that does not bind MT minus ends—NgCKK—with the CKK domain from human CAMSAP1 (HsCKK), which mediates CAMSAP1’s MT minus-end binding preference. Little is known about the native MT ultrastructure of Naegleria, so it is possible that NgCKK could recognise MT minus ends on Naegleria MTs36. However, for the purposes of our current study, NgCKK has proven an invaluable tool for evaluating MT minus end binding mechanisms on mammalian MTs. To allow a near-atomic resolution investigation of the subtle mechanism(s) at work, we studied NgCKK and HsCKK MT lattice binding in the context of different MT architectures and used these structures to explain the differences in their MT minus-end recognition properties (Fig. 6).

In this study, we found that NgCKK and HsCKK share the same protein fold, they bind at the same intradimer interprotofilament site and, as revealed using a combination of NMR methodologies, share the same intrinsic structural rigidity. This shows that the presence/absence of MT minus-end recognition is not due to large conformational changes in the domain or significant alterations in the CKK binding site. Although the distinct MT interactions by the flexible regions adjacent to the CKK core have a large effect on MT affinity (Supplementary Fig. 4), the biggest difference between these two domains is that HsCKK forms a more extended interface with the α-tubulin pair at its binding site than NgCKK and sits deeper within the interprotofilament groove. We found that HsCKK is more displaced from its binding site on 14- compared to 13-protofilament MTs, squeezed outwards by the flatter lateral curvature of adjacent protofilaments in the higher protofilament number MT architecture. In addition, HsCKK binding induces positive protofilament skew in both 13- and 14-protofilament MTs while NgCKK does not; our reconstructions show that, in inducing
protofilament skew, HsCKK brings the tubulin dimers to which it binds closer together. While neither intra- nor interdimer longitudinal interfaces are perturbed in HsCKK-bound MTs, their global lattice architecture alters to accommodate the local remodelling at the HsCKK binding site: compared to NgCKK-MTs, the diameter of HsCKK-MTs are smaller and adjacent protofilaments exhibit compression, shear and skew while conserving the B-lattice MT architecture with a single seam predicted by the lattice accommodation model.

The first important aspect of the MT minus-end recognition mechanism by HsCKK revealed by our current data is that the CKK domain itself does not flexibly respond to different tubulin
conformations. Rather, its rigidity is consistent with its sensitivity to, and affinity for, the conformation(s) of polymerized tubulin it encounters. Second, we confirmed that the ability to induce significant protofilament skew in fully decorated MTs correlates with MT minus-end recognition activity. This was previously observed in the HsCKK-N1492A mutant but is now confirmed in the comparison of HsCKK with NgCKK. Third, we previously speculated that skew induction reflects the non-optimally geometry for HsCKK binding of tubulin dimers within the MT lattice compared to minus ends. Our new reconstructions show that this is indeed the case, and that skew arises in response to HsCKK forcing the two tubulin dimers it contacts closer together. Consistent with this idea, the CKK binding site on neighbouring tubulin dimers are predicted to be laterally closer in the transition zone to gently curved tubulin sheets near MT minus ends which HsCKK prefers. Fourth, a key prediction of our previous model is that end specificity by HsCKK is mediated by the asymmetric curvature of tubulin at the minus end, with the α-tubulins less laterally curved relative to the β-tubulins. Conversely, at plus ends—with the β-tubulins less laterally curved—HsCKK binding is inhibited. We previously hypothesised that looser contacts between HsCKK and α-tubulins compared to β-tubulins are an important aspect of MT minus-end recognition. In fact, this difference in contact area between tubulins is seen in both HsCKK and NgCKK structures (Fig. 2e). Thus, while this aspect of the model provides a logical explanation for the reduced affinity of HsCKK for MT plus ends, we now show that, by itself, tighter contact between β-compared to α-tubulin is insufficient to explain HsCKK minus-end preference. However, HsCKK forms different and more extensive contacts with α-tubulins compared to NgCKK; the looser interactions between NgCKK and the α-tubulins suggest that sensitivity to tubulin configuration is thereby absent (Fig. 6) and explains why this protein does not specifically bind MT minus ends. Therefore, our new data show that, while looser than the contacts with β-tubulin, the HsCKK contacts with α-tubulins are critical in enabling it to recognise and bind to the relatively flattened configuration of α-tubulins at minus-ends.

Structural studies of MT-bound MAPs typically reveal conformational changes in the MAP on interaction with the MT lattice. In the most extreme cases, unstructured proteins such as members of the tau/MAP2 family and the mitotic regulatory protein TPX2, become at least partially ordered when in contact with MTs37,38. A recent study of the plant MAP Companion of Cellulose synthase 1 also showed similar behaviour in its disordered N-terminus on binding MTs39. Folded MT binding domains in a number of MAPs—for example, kinesin motor domains40,41, CH domains in EB proteins42, the p150glued CAP-Gly domain43—often undergo some conformational changes and/or ordering of otherwise disordered loop regions on formation of the MT-bound complex. Although the termini adjacent to the CCK become ordered on MT binding, we show that the core of HsCKK, which is essential for minus end recognition, is sufficiently rigid that it does not undergo conformational changes on MT interaction, but rather the MT lattice is remodelled in response to HsCKK binding. In the case of HsCKK, this is because the main MT lattice is not the preferred binding substrate for CKK. However, this behaviour—in which a structurally invariant MAP is exquisitely sensitive to the precise conformation of the underlying tubulin—is likely to be shared by other proteins.

The availability of increasing numbers of MT structures bound to a range of ligands has also emphasised that, far from being an inflexible, structurally invariant cylinder, the MT lattice supports surprising structural plasticity. Lattice compaction at the interdimer interprotofilament tubulin contacts in response to the tubulin GTPase is well documented in MTs polymerized from mammalian tubulin30,42,44–46. End Binding (EB) proteins bind at the corner of four tubulin dimers adjacent to the tubulin GTPase site47 and their preference for the sleeve of GDP.Pi compacted tubulins that dynamically evolves as MTs grow mediates their tip-tracking activity42,47–49. EB binding itself induces a small left-handed protofilament skew by introducing a slight interdimer stagger along the protofilament, the mechanistic significance of which is not yet understood. CKKs bind MTs 4 nm away from the EB binding sites and are insensitive to nucleotide-dependent conformational changes in the lattice2. We show here that HsCKK binding induces right-handed protofilament skew via tilt and shear of entire protofilaments, a completely different mechanism than seen for EBs. Thus, our characterization of the HsCKK-MT interaction also highlights the extent of structural plasticity that can be accommodated in the MT lattice. Small conformational effects induced by one MAP could have substantial consequences for binding of other MT-biding factors. We previously demonstrated that CKK binding at MT ends can sterically compete with kinesin-13 at MT minus ends5, thereby protecting them from depolymerisation21–23. Our current work also suggests that, beyond direct steric competition, different MAPs may exert allosteric control over each other’s MT binding by modifying the conformation of the MT lattice30. This was also suggested by a recent study of axonemal dynein50. Taken together, our data support the idea that MTs can act as allosteric signalling platforms, in which the precise configuration of polymerized tubulins are influenced by their dynamic state and binding partners6,51. In the case of CAMSAPs/Patronins,
sensitivity to structural variations in tubulin is essential for MT minus end recognition. These insights will inform future mechanistic investigations of conformational signalling arising from the MT cytoskeleton.

**Methods**

**Protein expression and purification for TIRF microscopy.** Strep-GFP-tagged human CAMSAP1 CKK (residues 1474-C), *Naegleria gruberi* CKK (residues 612-C, reference sequence XM_002675733.1) and chimeric or truncated CKK constructs were prepared as described previously. Briefly, proteins were expressed in HEK293T cells using a modified pTT5 expression vector (Addgene no. 44006), purified using StrepTactin beads (GE). After incubation with the cell lysate, beads were washed five times with high salt wash buffer (50 mM HEPES, 1.5 M NaCl and 0.01% Triton X-100), and proteins were eluted in elution buffer (50 mM HEPES, 150 mM NaCl, 1 mM MgCl₂, 1 mM EGTA, 1 mM dithiothreitol (DTT), 2.5 mM d-Desthiobiotin and 0.05% Triton X-100, pH 7.4). Purified proteins were snap-frozen and stored at −80 °C. Truncated constructs were as follows: NgCKK ΔN.
**Fig. 5** HsCKK domain rigidity mediates local remodelling of its MT-binding site. 

a Left, chemical-shift perturbations, coloured by differences in ppm, arising from HsCKK_N1492A binding to MTs mapped on the structure of CKK-MT complex (PDB: 5M5C); the CKK domain is depicted in a ribbon representation while the MT surface is shown as a space-filling model; Right, changes of transverse relaxation rates obtained from solution-state NMR CPMG experiments plotted on the 3D structure of the free HsCKK domain; source data are provided as a Source Data file. 

b HsCKK MT binding is accompanied by contraction of the MT diameter; a single turn of models docked within the aligned 13-protofilament HsCKK and NgCKK C1 reconstructions are shown viewed from the minus end; arrows indicate the irregular shift of individual protofilaments. 

c Contraction of MT diameter is caused by shrinkage of the distance between adjacent dimers; the distance between the centre of masses of each pair of adjacent β-lattice dimers was measured in 13- and 14-protofilament C1 HsCKK and NgCKK models; all data points are plotted and bars represents mean ± SD; differences between HsCKK and NgCKK models are statistically significant ($p < 0.0001$, t-test); source data are provided as a Source Data file. 

d Top, alignment of a single dimer from the NgCKK-tubulin and HsCKK-tubulin C1 models shows HsCKK induces compression and shear between the dimers at its binding site; bottom, RMSD of backbone positions in top panel. 

e Schematic summarising modifications imposed by HsCKK but not kinesin or NgCKK binding on MT architecture; modifications are exaggerated for clarity.

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**Fig. 6** NgCKK and HsCKK comparison illuminates the CAMSAP MT minus end recognition model. The schematic of a stable/growing 13-protofilament MT on the left shows different zones within the polymer in which the tubulins adopt subtly different conformations. Towards MT ends, there is a region of transition from the cylindrical lattice to curved sheet-like regions in which interprotofilament connections are maintained but which exhibit decreasing lateral curvature and increasing longitudinal curvature away from the MT lattice. Beyond this transition zone, protofilaments gradually terminate and separate at each MT end. On the right, the interaction of NgCKK and HsCKK with the unique tubulin dimer pair conformation in each zone is compared. On the MT lattice (middle, cyan boxed zone), as visualised in our cryo-EM reconstructions, NgCKK and HsCKK bind at the same site, but HsCKK remodels its binding site by compressing the tubulin dimer pairs, inducing protofilament skew. At the plus end lattice-end transition (top, pink boxed zone), we hypothesise that NgCKK is insensitive to the plus end specific tubulin sheet curvature found here, whereas HsCKK binding is actively disfavoured because of the enhanced lateral flattening in the β-tubulin pair that is specific to the MT plus end. At the minus end lattice-end transition (bottom, orange boxed zone), we again hypothesise that NgCKK is insensitive to the minus end specific sheet curvature. In contrast, HsCKK binding is favoured because, in the context of its particular tubulin interaction, the specific asymmetrically curved configuration of the tubulin pair—in particular the enhanced lateral flattening in the α-tubulin pair—is the preferred binding site conformation for HsCKK.
corresponded to N. gruberi CKK core and C-terminal extension (633–788), NgCKK AC to the N. gruberi NT-terminal extension and CKK core (612–784) and NgCKK D to the N. gruberi NT-terminal extension (633–784) followed by the HsCKK C-terminal extension (1600–1613), the NgCKK Swap N + C construct consisted of N. gruberi CKK core (633–784) preceded by the HsCKK NT-terminal extension (1463–1483) and followed by the HsCKK C-terminal extension (1600–1613). Primer sequences used to prepare these constructs using Gibson assembly are provided in Supplementary Table 3.

**TIRF microscopy analysis of CKK binding to dynamic MTs.** TIRF microscopy was performed on an inverted research microscope Nikon Eclipse Ti-E (Nikon) with the perfect focus system (PFS) (Nikon), equipped with a Nikon CFi Apo TIRF X100 1.49 NA objective (Nikon) and a Photometrics Evolve 514 EMCCD (Roper Scientific) camera or a Photometrics Prime BSI camera, and controlled with MetaMorph 7.7 software (Molecular Devices, CA). Images were projected onto the chip of an Evolve 512 camera with an intermediate ×2.5 lens (Nikon C mount and followed by the Perfect Focus System (PFS) (Nikon), equipped with a Nikon CFI Apo TIRF microscopy). The chip of an Evolve 512 camera with an intermediate ×2.5 lens (Nikon C mount and followed by the Perfect Focus System (PFS) (Nikon), equipped with a Nikon CFI Apo TIRF microscopy).

**Cryo-EM data collection and processing.** Low dose movies were collected manually on a K2 direct electron detector (Gatan) installed on a FEI Tecnai G2 Polara operating at 300 kV with a Quantum post-column energy filter (Gatan), operated in zero-loss imaging mode with a 20-eV energy-selecting slit. A defocus range of 1300 nm was acquired in the same conditions as described previously2. Briefly, the perfect focus system was used to prepare these constructs using Gibson assembly are provided in Supplementary Table 3.

**Cryo-EM sample preparation.** MTs were polymerized using using 5 μg/ml tsa201 cell tubulin at 37°C for 45 min in BRB80 containing 1 mM GTP, 1 μM paclitaxel, and then tubulin eluted at ~80°C. Protein quality and identity were analyzed by SDS-PAGE and mass spectrometry, respectively. tsa201 cell tubulin was purified from tsa201 cell cultures as described previously2,34,35. Briefly, tubulin was isolated from cell lysates via immobilized TOC1 affinity, then tubulin N-ethylated with 0.5 M ammonium sulfate. Tubulin was then buffer exchanged into BRB80 buffer (80 mM PIPES, 2 mM MgCl2, 1 mM EGTA, 1 mM DTT, pH 6.8) with 10% glycerol, and 20 μM GTP and flash frozen in liquid nitrogen. The tubulin was further purified by cycling36 then buffer exchanged into BRB80 with 20 μM GTP and flash frozen in liquid nitrogen.
differences and incorporating taxol from the structure of tubulin-taxol zinc sheets (PDB 1JF6). NgCKK and HsCKK domain starting models were created via homology-modelling of the X-ray and NMR CAMSAP CKK domain (PDBs 5L2N and 1UGJ) (unpublished)) using Modeller. Starting models of HsCKK or NgCKK domains were then fitted into density alongside tubulin and merged into single starting models constructed of six tubulin dimers and four CKK domains. Cryo-EM images were prepared using UCSF Chimera.  

Protein preparations for NMR. Human CAMSAP1 N1492A CKK (residues 1474–1613) and mouse CAMSAP3 CKK (residues 1112–1252) were cloned into a pET28a vector as for WT CAMSAP1 CKK (above). For sample preparation of low MAS ssNMR, uniformly [13C,N]-labeled CAMSAP1 N1492A CKK was produced in E. coli strain Rosetta 2 in M9 minimum medium supplemented with 125 mM 13C-glucose and 15N-NH4Cl. When OD600 reached 0.6, 0.3 mM IPTG was added.  

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The signal linewidth in 1H and 15N dimensions were determined to amount to 0.1 and 0.6 ppm, respectively. CPMG data were processed as follows: For each residue, the standard deviation and the average of signal intensities with different CPMG frequencies were calculated. The ratio of these two values was plotted for every residue on the structures.

For determining chemical-shift changes between free and MT-bound CAMSAP1 CKK and CAMSAP1 CKK N1492A, we transferred solution-state NMR shifts obtained on the free variants to ssNMR experiments on the complexes assuming spectral proximity in all three independent dimensions (HN, N, Ca). With this strategy, we were able to transfer 48 backbone assignments as demonstrated in Supplementary Fig. 6b.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The 13-protofilament and 14-protofilament HsCKK and NgCKK-MT models along with their corresponding electron density maps are deposited in the PDB. The PDB codes are as follows: 13-protofilament HsCKK-MT, PDB: 6QUS [https://www.rcsb.org/structure/6QUS], 14-protofilament HsCKK-MT, PDB: 6YQV [https://www.rcsb.org/structure/6YQV], 13-protofilament NgCKK-MT, PDB: 6QUT [https://www.rcsb.org/structure/6QUT]. The EMDB codes (C1 reconstruction and symmetrised asymmetric unit) are as follows: 13-protofilament HsCKK-MT, EMDB-4643, 14-protofilament HsCKK-MT, EMDB-4654, 13-protofilament NgCKK-MT, EMDB-4464, 14-protofilament NgCKK-MT, EMDB-4650. The source data underlying Figs 2e, 4b, 5a–c, Supplementary Figs 6e–h, 7a are provided as a Source Data file. Other data are available from the corresponding authors upon reasonable request.

Code availability
The ImageJ plugin KymoResliceWide v0.4 (https://github.com/ekatrulka/KymoResliceWide) was used for generating kymographs illustrating the life history of MT dynamics. The cryo-EM image-processing scripts for use with RELION and user instructions are available at https://github.com/moores-lab/MiRP.

Received: 13 March 2019; Accepted: 23 October 2019; Published online: 20 November 2019

References
1. Akhmanova, A. & Steinmetz, M. O. Control of microtubule organization and minus-end minus-end organization. Nat. Rev. Mol. Cell Biol. 16, 711–726 (2015).

2. Atherton, J. et al. A structural model for microtubule minus-end recognition and protection by CAMSAP proteins. Nat. Struct. Mol. Biol. 24, 931–943 (2017).

3. Atherton, J., Stouffer, M., Francis, F. & Moores, C. A. Microtubule architecture in vitro and in cells revealed by cryo-electron tomography. Acta Crystallogr. D. Struct. Biol. 74, 572–584 (2018).

4. McIntosh, J. R. et al. Microtubules grow by the addition of bent guanosine diphosphate. J. Struct. Biol. 175, 2691–2708 (2018).

5. Brouhard, G. J. & Rice, L. M. Microtubule dynamics: an interplay of biochemistry and mechanics. Nat. Rev. Mol. Cell Biol. 19, 451–463 (2018).

6. Creniten, D., Fuller, S. D. & Karsenti, E. Structure of growing microtubule ends: two-dimensional sheets close into tubes at variable rates. J. Cell. Biol. 129, 1311–1328 (1995).

7. Martin, M. & Akhmanova, A. Coming into focus: mechanisms of microtubule minus-end organization. Trends Cell Biol. 28, 574–588 (2018).
9. Akhmanova, A. & Hoogenraad, C. C. Microtubule minus-end-targeting proteins. *Curr. Biol.* 25, R162–R171 (2015).
10. Poonpattanaphant et al. CAMSAP3 maintains neuronal polarity through regulation of microtubule stability. *Proc. Natl. Acad. Sci. USA* 115, 9750–9755 (2018).
11. Toya, M. et al. CAMSAP3 orients the apical-to-basal polarity of microtubule arrays in epithelial cells. *Proc. Natl. Acad. Sci. USA* 113, 332–337 (2016).
12. Yu, X. Q. et al. Microtubule minus-end binding protein CAMSAP2 controls axon specification and dendrite development. *Neuron* 82, 1058–1073 (2014).
13. Noordstra, I. et al. Control of apico-basal epithelial polarity by the microtubule minus-end-binding protein CAMSAP3 and spectraplakin ACF7. *J. Cell Sci.* 129, 4278–4288 (2016).
14. Chung, M. et al. The microtubule minus-end-binding protein patronin/PTENP1 is required for axon regeneration in *C. elegans.* *Cell Rep.* 9, 874–883 (2014).
15. Wang, H., Brust-Mascher, I., Civelekoglu-Scholey, G. & Scholey, J. M. Patronin mediates a switch from kinesin-13-dependent poleward to anaphase B spindle elongation. *J. Cell. Biol.* 203, 35–46 (2013).
16. Derivery, E. et al. Polarized endosome dynamics by spindle asymmetry during asymmetric cell division. *Nature* 520, 280–285 (2015).
17. Khanal, I., Elbedowy, A., Diaz de la Loza, M. D. C., Fletcher, G. C. & Thompson, B. J. Correction: Slot and Patronin polarise microtubules to direct membrane traffic and biogenesis of microvilli in epithelia. *J. Cell Sci.* 129, 2651–2659 (2016).
18. Wang, S. et al. NOCA-1 functions with gamma-tubulin and in parallel to microtubule minus-end polarity by the microtubule minus-end-binding protein CAMSAP3 and spectraplakin ACF7. *Nat. Commun.* 10:5236 (2019).
19. Akhmanova, A. & Hoogenraad, C. C. Microtubule minus-end-targeting proteins. *Curr. Biol.* 25, R162–R171 (2015).
20. Marcette, J. D., Chen, J. J. & Nonet, M. L. The *Caenorhabditis elegans* homeolog, promotes microtubule function in *Caenorhabditis elegans* neurons. *Nat. Commun.* 10:5236 (2019).
21. Jiang, K. et al. Microtubule minus-end stabilization by polymerization-driven protecting microtubule minus ends. *Nat. Commun.* 10:5236 (2019).
22. Hendershott, M. C. & Vale, R. D. Regulation of microtubule minus-end stability by polymerization-driven minus-end lattice seam location. *Nat. Commun.* 10:5236 (2019).
23. Goodwin, S. S. & Vale, R. D. Patronin regulates the microtubule network by dynamics by CAMSAPs and Patronin. *Proc. Natl. Acad. Sci. USA* 111, 5860–5865 (2014).
24. Baines, A. J. et al. The CCK domain (DUF1781) binds microtubules and defines the CAMSAP/Atp4 family of animal proteins. *Mol. Biol. Evol.* 26, 2005–2014 (2009).
25. Zivnov, J. et al. New tools for automated high-resolution cryo-EM structure determination in RELION-3. *eLife* 7, e42166 (2018).
26. He, S. & Scheres, S. H. W. Helical reconstruction in RELION. *J. Struct. Biol.* 198, 163–176 (2017).
27. Scheres, S. H. W. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *J. Struct. Biol.* 180, 519–530 (2012).
28. Cook, A.D., Manka, S.W., Wang, S., Moores, C.A. & Atherton, J. A microtubule RELION-based pipeline for cryo-EM image processing. *J. Struct. Biol.* https://doi.org/10.1016/j.jsb.2019.10.004 (2019).
29. Vemur, A., Atherton, J., Spector, J. O., Moores, C. A. & Roll-Mecak, A. Tubulin isoform composition tunes microtubule dynamics. *Mol. Biol. Cell.* 28, 3564–3572 (2017).
30. Zhang, R., LaFrance, B. & Nogales, E. Separating the effects of nucleotide and EB binding on microtubule structure. *Proc. Natl. Acad. Sci. USA* 115, E6191–E6200 (2018).
31. Palmer, A.G., Kroenke, C.D. & Patrick Loria, J. in Methods in Enzymology, Vol. 339. (eds. James, T.L., Dötsch, V. & Schmitz, U.) 204–238 (Academic Press, 2001).
32. Korzhnev, D. M. & Kay, L. E. Probing invisible, low-populated states of protein molecules by relaxation dispersion NMR spectroscopy: an application to protein folding. *Acc. Chem. Res.* 41, 442–451 (2008).
33. Vallarapalli, P., Bouvignies, G. & Kay, L. E. Studying “invisible” excited protein states in slow exchange with a major state conformation. *J. Am. Chem. Soc.* 134, 8148–8161 (2012).
34. Chretien, D. & Wade, R. H. New data on the microtubule surface lattice. *Bioil Cell.* 71, 161–174 (1991).
35. Wade, R. H., Chretien, D. & Job, D. Characterization of microtubule protofilament numbers. How does the surface lattice accommodate? *J. Mol. Biol.* 212, 775–786 (1990).
36. Gonzalez-Robles, A., Cristobal-Ramos, A. R., Gonzalez-Lazo, M., Omana-Molina, M. & Martinez-Palomino, A. Naegleria fowleri: light and electron microscopy study of mitosis. *Exp. Parasitol.* 122, 212–217 (2009).
37. Kellogg, E. H. et al. Near-atomic model of microtubule-tau interactions. *Science* 360, 1242–1246 (2018).
69. Szeverenyi, N. M., Sullivan, M. J. & Maciel, G. E. Observation of spin exchange by two-dimensional fourier transform 13C cross polarization-magic-angle spinning. *J. Magn. Reson.* (1969) 47, 462–475 (1982).

70. Bloembergen, N. On the interaction of nuclear spins in a crystalline lattice. *Physica* 15, 386–426 (1949).

71. Baldus, M. Correlation experiments for assignment and structure elucidation of immobilized polypeptides under magic angle spinning. *Prog. Nucl. Magn. Reson. Spectrosc.* 41, 1–47 (2002).

72. Baldus, M., Petkova, A. T., Herzfeld, J. & Griffin, R. G. Cross polarization in the tilted frame: assignment and spectral simplification in heteronuclear spin systems. *Mol. Phys.* 95, 1197–1207 (1998).

73. Zhou, D.H. et al. Solid-state NMR analysis of membrane proteins and protein aggregates by proton detected spectroscopy. *J. Biomol. NMR* 54, 291–305 (2012).

**Acknowledgements**

This work was funded by grants from the Medical Research Council, U.K. (MR/R000352/1), the Biotechnology and Biological Sciences Research Council, U.K. (BR/L001421/1) and the Wellcome Trust (079605/Z/06/Z) to C.A.M., from the Dutch Science Foundation NWO (VENI grant 722.016.002 to S.X., NWO-Groot (no. 175.010.2009.002) and TOP-PUNT (no. 718.015.001) grant to M.B.) and uNMR-NL, National Roadmap Large-Scale NMR Facility of the Netherlands (grant 184.032.207), supported by a China Scholarship Council scholarship to C.Y., the intramural program of the National Institute of Neurological Disorder and Stroke (NINDS) and National Heart, Lung and Blood Institute (NHLBI) for A.R.M., and funding from the Swiss National Science Foundation (31003A_166608) to M.O.S.

**Author contributions**

J.A., Y.L., A.A., M.B., C.A.M. designed the research. M.S., A.V., A.C., S.W., A. R-M., M.O.S. supplied reagents and analytical methods. J.A., Y.L., S.X., C.Y., A. R., M.S. performed the research. J.A., Y.L., S.X., C.Y., K.J., M.S., A.A., M.B., C.A.M. analysed the data, and J.A., Y.L., M.B., C.A.M. wrote the manuscript, with contributions from all authors.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Supplementary information is available for this paper at https://doi.org/10.1038/s41467-019-13247-6.

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Peer review information Nature Communications thanks Gregory Alushin, Loren Andrews and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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