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2 **Structural-mechanical remodelling of GDP-microtubules by kinesin**

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4 Daniel R. Peet<sup>1,2</sup>, Nigel J. Burroughs<sup>2,3</sup>, Robert A. Cross<sup>1\*</sup>

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6 <sup>1</sup>Centre for Mechanochemical Cell Biology, Warwick Medical School, Coventry, CV4 7AL, UK.

7 <sup>2</sup>Warwick Systems Biology Centre, University of Warwick, Coventry, CV4 7AL, UK.

8 <sup>3</sup>Mathematics Institute, University of Warwick, Coventry, CV4 7AL, UK.

9 **Kinesin-1 is a nanoscale molecular motor that walks towards the fast growing (plus) ends**  
10 **of microtubules (MTs), hauling molecular cargo to specific reaction sites in cells. Kinesin-**  
11 **driven transport is central to the self-organisation of eukaryotic cells and shows great**  
12 **promise as a tool for nano-engineering<sup>1,2</sup>. Recent work hints that kinesin may also play a**  
13 **role in modulating the stability of its MT track, both *in vitro*<sup>3-5</sup> and *in vivo*<sup>6</sup>, but results are**  
14 **conflicting<sup>7-9</sup> and mechanisms are unclear. Here we report a new dimension to the**  
15 **kinesin-MT interaction, whereby strong-state (ATP-bound and apo) kinesin-1 motor**  
16 **domains inhibit the shrinkage of GDP-MTs by up to 2 orders of magnitude and expand**  
17 **their lattice spacing by ~1.6%. Our data reveal an unexpected new mechanism by which**  
18 **the mechanochemical cycles of kinesin and tubulin interlock, allowing motile kinesins to**  
19 **influence the structure, stability and mechanics of their MT track.**

20 During stepping, kinesin motor domains cycle through a series of nucleotide-specific  
21 conformations<sup>10,11</sup>. We tested different nucleotide states of kinesin-1 motor domains to  
22 quantify their influence on MT stability. We attached fluorescent MT 'seeds' to the inside of  
23 a flow chamber via biotin-NeutrAvidin linkages and flowed in GTP-tubulin, causing dynamic  
24 MTs to grow from the seeds (Fig. 1a). We then initiated MT depolymerisation by washing  
25 out tubulin, whilst simultaneously flowing in kinesin-1 motor domains. We used a kinesin  
26 motor domain mutant (T93N) as a stable homologue of the nucleotide-free (apo) state of  
27 the motor<sup>12</sup>, and compared this to wild-type (WT) motor domains (K340) in the presence of  
28 different nucleotides. Strong-state kinesin binds tightly and stereospecifically to MTs<sup>13</sup>. We  
29 found that T93N reduced MT shrinkage to 1% of the control rate (Fig. 1b,c) and that  
30 AMPPNP-WT kinesin had a similar effect, whilst weak-state (ADP-bound) kinesin had no  
31 detectable effect (Fig. 1c). We conclude that strong-state kinesin powerfully inhibits the  
32 shrinkage of GDP-MTs.

33 Next, we bound MTs to a kinesin-coated coverslip in a flow chamber, triggered  
34 depolymerisation by washing out residual GTP-tubulin, and again observed MT shrinkage.  
35 Geometric constraints suggest that in this arrangement, at most 5 protofilaments can bind  
36 to the kinesin surface (Fig. 2a). Despite this, entire MTs were stabilised (Fig. 2c). We then  
37 flowed solutions through the channel in 2 steps (Fig. 2b). First, ADP was flowed in, reducing  
38 the fraction of kinesins in a strong state and increasing the MT shrinkage rate (Fig. 2c,

39 Supplementary Movie 1). By titrating the ADP concentration, we found that MT shrinkage  
40 rates could be fine-tuned over 2 orders of magnitude (Fig. 2d, Supplementary Table 1).  
41 Comparing the inhibition of MT shrinkage by kinesin in solution (maximally  $0.21 \pm 0.02$  (25)  
42 dimer  $\text{PF}^{-1} \text{ s}^{-1}$  (mean  $\pm$  SEM ( $n$ ))) with that of the kinesin surface ( $0.06 \pm 0.01$  (6) dimer  $\text{PF}^{-1} \text{ s}^{-1}$   
43 in the presence of 400 nM ADP) shows that surface immobilisation enhances the stabilising  
44 effect of kinesin, despite its binding being restricted to only a subset of protofilaments.  
45 Frequently, faint fluorescent trails were visible on the kinesin-coated surface in the wake of  
46 retreating MT tips. These shrank endwise upon addition of ADP, suggesting that their tubulin  
47 is still assembled into protofilaments (Fig. 2c, Supplementary Movie 1). Trails are tapered,  
48 and fluorescence intensity analysis (Fig. 3a, Supplementary Methods and Supplementary Fig.  
49 1-2) indicates that at their tips they contain 2-3 protofilaments (Fig. 3b). On average, trails  
50 can shrink faster than their MT stem because they appear transiently, typically forming,  
51 lengthening and retracting multiple times during the shrinkage of each surface-attached MT  
52 (Supplementary Fig. 3). As a final step in these experiments, we flowed in a buffer containing  
53 taxol and ATP, triggering kinesin-driven sliding to reveal the MT polarity.

54 Why does a kinesin-coated surface stabilise MTs but also cause them to split? Taxol-  
55 stabilised MTs have recently been shown to split on a kinesin-coated surface<sup>14</sup>. However,  
56 ATP-driven kinesin motility was essential to this process, which is not the case for our GDP-  
57 MTs. Several strands of evidence suggest that kinesin binding can change the lattice  
58 conformation and mechanics of MTs. A kinesin-coated surface has been reported to reduce  
59 the Young's modulus of taxol-stabilised MTs<sup>15</sup>. Structural changes have also been reported  
60 following kinesin binding to taxol-stabilised<sup>16</sup> and GMPCPP-bound MTs<sup>17</sup>. Furthermore, the  
61 longitudinal compaction of the MT lattice that accompanies GTP hydrolysis is reduced in  
62 kinesin-bound MTs<sup>18</sup>, suggesting that kinesin influences the longitudinal spacing between  
63 tubulin subunits in the MT lattice. We therefore hypothesised that kinesin binding modifies

64 the axial spacing between GDP-tubulin subunits in the MT lattice. Binding kinesins to one  
65 side of a MT, as in our surface assay, would then change the lattice spacing on that side but  
66 not the other, causing shear stress that could facilitate splitting.

67 To test the idea that kinesin binding stabilises a distinct conformation of the MT lattice, we  
68 used hydrodynamic flow to bend tethered dynamic MTs, thereby expanding the MT lattice  
69 on the convex side and compacting it on the concave side (Fig. 4, Supplementary Movie 2).  
70 We supplemented T93N into the flow and observed the mechanical response of MTs upon  
71 stopping the flow. In the absence of kinesins, the MTs quickly recoiled to a straight  
72 conformation and rapidly depolymerised. Remarkably, low concentrations of T93N (15-30  
73 nM) blocked this recoil, effectively locking the GDP-MTs in a curved conformation as well as  
74 inhibiting their shrinkage. These data heavily suggest that indeed strong-state kinesins  
75 preferentially bind and stabilise a distinct longitudinal lattice spacing of GDP-MTs.

76 We noticed that in the presence of higher concentrations of kinesin (> 50 nM), the curved  
77 MTs tended slowly to re-straighten. To explain this observation, we speculate that strong-  
78 state kinesins bind preferentially *but not exclusively* to one side of curved MTs. At high  
79 kinesin concentrations, the favoured side of the MT would then quickly become fully  
80 occupied, while binding would continue more slowly on the unfavoured side, ultimately  
81 driving the MT back into a straight conformation (Fig. 4b). Kinesins have previously been  
82 reported to bind preferentially to GTP-MTs, which have a longer lattice spacing than GDP-  
83 MTs<sup>19</sup>. In light of this, we postulated that the binding of strong-state kinesins drives an  
84 increase in the lattice spacing of GDP-MTs.

85 In order to directly test this model, we grew dynamic MTs from surface-tethered fluorescent  
86 seeds as previously but this time we capped their exposed tips with fluorescent GMPCPP-  
87 tubulin (Fig. 5a). We aligned the MTs using pressure-driven microfluidics. The GDP-MTs and  
88 their stabilised caps were fluorescently labelled in different colours and imaged using TIRF

89 microscopy. The microfluidics system allowed us to maintain a constant flow rate whilst  
90 introducing either 200 nM of WT kinesin motor domains or 1 mM ADP into the flow. When  
91 apo-kinesin was introduced, the GDP-bound segment of the MT lengthened as predicted  
92 (Fig. 5b, Supplementary Movie 3).

93 TIRF microscopy visualises an optical section ~100 nm deep and MTs remained visible  
94 throughout the experiment, indicating that the flow constrained them within this 100 nm  
95 thick section. Strikingly, the MTs briefly crinkled upon switching from ADP to kinesin, causing  
96 them to dip in and out of the TIRF illumination (Supplementary Movie 4). This transient  
97 crinkling can also be seen in our initial MT bending experiments (Supplementary Movie 2).  
98 The crinkles progressively straightened (within 10-30 s) to reveal that the MT had expanded.  
99 Switching to ADP caused MTs to quickly recoil to their original length (<4 sec, 2 frames in our  
100 data) as the kinesin unbound (Fig. 5c, d). Quantification reveals that apo-kinesin binding to  
101 GDP-MTs increases their length by 1.6%, via a uniform axial expansion of the MT lattice  
102 (Supplementary Animation 1). The kinesin-induced lattice expansion appears to be fully  
103 reversible and the expand-and-recoil cycle can be repeated multiple times (Fig. 5d).

104 We additionally performed experiments where we used methylcellulose rather than flow to  
105 encourage the MTs to remain in focus, introducing a flow only intermittently to exchange  
106 buffers. Under these conditions MTs tended to become 'stitched' to the coverslip at sparse  
107 interaction sites. Introducing kinesin then caused the MTs to bow locally between these  
108 sites (Fig. 5f, Supplementary Movie 5), emphasising the expansion of the lattice. By  
109 measuring the change in contour length between the two stabilised MT caps, we confirmed  
110 that this bowing accommodates a 1.6% expansion of the GDP-MT lattice (Fig. 5g). MTs  
111 became more densely 'stitched' to the surface if multiple cycles were performed but this did  
112 not influence the measured expansion (Supplementary Fig. 4). When ADP was flowed  
113 through the channel, the MTs again recoiled to their original lengths (Fig. 5f).

114 Our data show that strong-state kinesin stabilises the GDP-lattice of dynamic MTs, and  
115 concomitantly increases their axial lattice spacing by 1.6%. Kinesins are known to bind to the  
116 intra-dimer interface of  $\alpha\beta$ -tubulin, away from the inter-dimer contacts of the MT  
117 lattice<sup>10,11,17,18,20</sup>. This suggests to us that kinesin binding allosterically modifies the  
118 conformation of GDP-tubulin, giving it properties more similar to GTP-tubulin. A 1.6%  
119 expansion equates to approximately 1.3 Å per 80 Å tubulin dimer, similar to the 1.7 Å  
120 difference observed by cryo-EM between GMPCPP-MT-kinesin and GDP-MT-kinesin  
121 structures<sup>20</sup>. Strong-state kinesins have previously been reported to alter the structure of  
122 both taxol-GDP-MTs<sup>16</sup> and GMPCPP-MTs<sup>17</sup>. Moreover, a long-range, ATP-dependent  
123 cooperative effect has been described whereby the first few kinesins that bind facilitate  
124 subsequent binding events in the same region of the MT, again suggestive of a kinesin-  
125 induced conformational change<sup>21</sup>.

126 We envisage that the ability of strong-state kinesin to stabilise GDP-MTs by inducing a  
127 conformational change in their tubulin subunits provides at least a partial mechanistic  
128 explanation for the surface-bound depolymerisation trails and the bend-locking  
129 phenomenon reported here. Thus, a MT landing on and binding to a kinesin-coated surface  
130 would likely become stretched on its surface-bound side. This stretching would create shear  
131 stress in the lattice and potentially contribute towards formation of the trails observed in  
132 our kinesin-clamp experiments. Similarly, for the MT bend-locking, expanding the  
133 longitudinal MT lattice spacing by 1.6% exclusively on one side of the MT would be more  
134 than sufficient to account for the observed kinesin-stabilisation of curvature. Indeed, full  
135 occupancy on one side with zero occupancy on the other would produce a radius of  
136 curvature of 1.6  $\mu\text{m}$ , far tighter than we observe in any of our post-flow data.

137 We have worked with kinesin-1, the best-studied kinesin, but it is possible that the  
138 mechanism we report here is common to other kinesins. Kif14 is a slow kinesin that binds to

139 MTs in a rigor-like conformation and inhibits their shrinkage<sup>22</sup>. Kinesin-5 is reported to  
140 stabilise protofilament assemblies during MT growth<sup>23</sup>, potentially due to its strong state  
141 stabilising the polymer. Kip2<sup>24</sup> and Kip3<sup>25</sup> are also reported to dwell at MT ends and  
142 influence MT stability.

143 Our work reveals a specific action of strong-state kinesins in stabilising the GDP-lattice of  
144 dynamic MTs. MT activated ADP release creates a strong (apo) state and this process is  
145 affected by the tubulin and kinesin species<sup>26</sup>, by post-translational modifications<sup>27</sup> and by  
146 the nucleotide state<sup>28</sup> of the MT. Importantly, the residency of kinesin in the strong states is  
147 also influenced by mechanical force<sup>29</sup>. Such forces will arise *in vivo* wherever kinesins do  
148 mechanical work, for example at kinetochores, in MT bundles<sup>30</sup>, at cortical attachment  
149 sites<sup>31</sup>, and during the transport of vesicles against a resistance<sup>32</sup>. It will be important now to  
150 understand the role of these various effects in determining how kinesin motility may feed  
151 back on MT dynamics.

152 In conclusion, our data reveal a novel mechanism that allows kinesin-1 to feed back on the  
153 structure and stability of its MT track. Recent advances in the remote control of kinesin  
154 motility, such as photo-switchable fuels<sup>33</sup>, suggest the potential for precise spatial control of  
155 these effects.

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## 158 **Methods**

### 159 **Proteins and biochemical reagents**

160 Tubulin was purified from pig brains as previously<sup>3</sup> with additional steps as follows. Tubulin  
161 was polymerised in 50 mM PIPES, 1.2 mM MgSO<sub>4</sub>, 1 mM EGTA, 1mM GTP, 1 mM  
162 dithiothreitol (DTT) and 186 mg ml<sup>-1</sup> glutamic acid and incubated for 60 min at 37 °C MTs  
163 were centrifuged in a TLA 100.3 rotor at 85,000 rpm for 20 min at 35 °C resuspended in K-  
164 PEM with 1 mM GTP, 1 mM MgSO<sub>4</sub> and 1 mM DTT, cooled to 4 °C and centrifuged in a TLA  
165 100.3 rotor at 85,000 rpm for 20 min. The supernatant was run through a Hiprep 26/10  
166 desalting column into K-PEM buffer (100 mM PIPES, 1 mM MgSO<sub>4</sub>, 2 mM EGTA (Fisher);  
167 adjusted to pH 6.9 with KOH) and 20 µM GTP. Tubulin concentrations were determined  
168 using  $E_{280} = 105,838 \text{ M}^{-1} \text{ cm}^{-1}$ .

169 X-rhodamine labelled tubulin was purchased from Cytoskeleton Inc. (USA). Alexa Fluor 488  
170 (Molecular Probes) labelled tubulin was prepared using standard protocols<sup>34</sup>.

171 Kinesin was purified as previously<sup>35</sup>. Kinesin concentrations were determined using  $E_{280} =$   
172  $15,300 \text{ M}^{-1} \text{ cm}^{-1}$ .

173 Nucleotides were from Jena Biosciences (Germany). Other reagents were from Sigma (UK)  
174 unless stated otherwise.

### 175 **Bead-mPEG crosslinking**

176 0.5 µm yellow-green carboxylated FluoSpheres (Thermofisher) were diluted to 1% solids and  
177 activated using 10 mg/ml 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) in pH 6  
178 MES buffer and mixed gently at room temperature for 30 min. Beads were then centrifuged,  
179 resuspended in 10 mg/ml of methoxypolyethylene glycol amine 750 (mPEG-amine) in pH 7.4  
180 PBS and mixed gently at room temperature for 2 h. Adding 90 mM glycine quenched the



181 reaction. After 30 min the beads were centrifuged and resuspended in 0.1% Tween20 in K-  
182 PEM 5 times and stored at 4 °C.

### 183 **Flow chamber assembly (for manual flow-through)**

184 Flow chambers were assembled from 22x22 mm no. 1.5 glass coverslips (Menzel, Germany)  
185 and 76x26 mm 1-1.2 mm thickness glass slides (Menzel, Germany). Double-sided Scotch  
186 tape was sandwiched between the glass surfaces to form a 2 mm wide channel. The  
187 periphery of the chamber was further secured using nail polish excluding the channel ends,  
188 which were left open. Solutions were drawn through the channel by using grade 1 Whatman  
189 filter paper.

### 190 **Microfluidics**

191 Microfluidic flow chambers were assembled by stacking the following: a 50x22mm no. 1.5  
192 glass coverslip (Menzel, Germany), cleaned using the same protocol as the tubulin depletion  
193 assays; 81 µm thick double-sided adhesive tape (ArCare 90445, kindly provided by Adhesives  
194 Research), with a Y-shaped channel (two 8x0.75 mm inlets joining a 22x1.5 mm channel) cut  
195 out using a Silhouette Portrait plotter cutter; a 40x22mm cyclo-olefin polymer (COP)  
196 coverslip (ZF14-188, Zeon) with portholes cut out using a plotter cutter. Ports were  
197 assembled from ring magnets (8.16 mm OD x 3.5 mm ID, First4magnets) pressed into a 3D-  
198 printed ABS magnet holder that aligns the magnets with the portholes in the COP coverslip,  
199 which was then cast in polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning) to fill the  
200 core of the ring magnets and form a 0.8 mm thick cushion to form the interface between the  
201 magnets at the COP coverslip. A 1.25 mm biopsy punch was used to bore holes through the  
202 PDMS-filled core of the magnets. 127 µm ID x 1.59 mm OD polyether ether ketone (PEEK)  
203 tubing was then inserted into the holes, forming a tight seal. The flow chamber was placed  
204 on a custom made ferromagnetic stainless steel (grade 430) microscope stage, which sealed  
205 the interface with the tubing by attracting the magnets and held the sample in position.

206 Flow was driven using an MFCS-EZ, inlets were closed and opened using an L-switch, and the  
207 flow was monitored on the outlet using a size M flow unit (Fluigent). A function was written  
208 in Matlab (Mathworks) to provide fully integrated control of the microfluidics with the  
209 microscope.

#### 210 **Tubulin depletion and MT bending assay**

211 Coverslips were sonicated (600 W bath, Ultrawave) in 3% Neutracon detergent (Decon  
212 Laboratories, UK) for 30 min at 60 °C before undergoing extensive wash-sonication cycles in  
213 ultrapure water (18.2 M $\Omega$  resistivity). A flow chamber was then assembled, filled with 0.2  
214 mg ml<sup>-1</sup> PLL-PEG-biotin (SuSoS, Switzerland) and incubated for 30 min. The chamber was  
215 washed with K-PEM before adding 1 mg ml<sup>-1</sup> NeutrAvidin (Thermo Fisher Scientific) for 5 min  
216 and washing again. MT seeds (polymerised by incubating 26  $\mu$ M of 15% labelled Alexa488-  
217 tubulin and 1 mM GMPCPP in K-PEM at 37 °C for 25 min) were pelleted, diluted to ~ 60 nM,  
218 injected into the chamber and incubated for 5 min. After washing the chamber with K-PEM,  
219 dynamic MT extensions were grown from the seeds by flowing through with 15  $\mu$ M tubulin,  
220 1 mM GTP, GOC oxygen scavenger (4.5 mg ml<sup>-1</sup> glucose, 0.2 mg ml<sup>-1</sup> glucose oxidase, 35  $\mu$ g  
221 ml<sup>-1</sup> catalase, 0.5% (v/v)  $\beta$ -mercaptoethanol), 1 mg ml<sup>-1</sup>, 1 mg ml<sup>-1</sup> BSA and 0.1% (v/v)  
222 Tween20 in K-PEM. MTs were allowed to grow for > 15 min at 25 °C prior to imaging with  
223 epifluorescence and dark-field illumination. Tubulin was then depleted by flowing through  
224 pre-warmed (25 °C K-PEM, supplemented with kinesin motor domains and nucleotides as  
225 described in the main text. MT bending was achieved by rapidly drawing solutions through  
226 the channel using Whatman filter paper.

#### 227 **Kinesin-clamp assay**

228 Fluorescence controls (colour-segmented stabilised MTs) were prepared by incubating 5  $\mu$ M  
229 30% labelled Alexa Fluor 288 tubulin and 0.2 mM Guanosine-5'-[( $\alpha,\beta$ )-

230 methyleno]triphosphate (GMPCPP) in K-PEM at 37 °C for 60 min and pelleted in an airfuge  
231 (Beckman Coulter) at 25 psi for 10 min. The supernatant was discarded and the pellet  
232 resuspended in pre-warmed 5 µM 30% labelled X-rhodamine tubulin and 0.2 mM GMPCPP  
233 in K-PEM. MTs were left to anneal at room temperature then diluted 50-fold before use.

234 Coverslips were sonicated (600 W bath) at room temperature in a 1:1 solution of methanol  
235 and HCl for 30 min, then sonicated for 4 × 5 min in ultrapure water. Coverslips were then  
236 sonicated in 0.2 M KOH for 60 min, then sonicated for 5 × 5 min in ultrapure water. The  
237 coverslips were then spun dry using a Spin Clean (Technical video), incubated at 100 °C for  
238 30 min and plasma-cleaned (PLASMA clean 4, ILMVAC) for 5 min. Coverslips were then  
239 silanised by immersion in 0.05% dimethyldichlorosilane in trichloroethylene for 60 min,  
240 washed in methanol, sonicated for 5 × 5 min in methanol and spun dry.

241 A flow chamber was assembled using a silanised coverslip and filled with 0.1 mg ml<sup>-1</sup> anti-  
242 6xHistidine antibodies (372900) for 10 min. The surface was then blocked by filling the  
243 chamber with 0.5 mg ml<sup>-1</sup> α-casein and incubating for 5 min. 75 nM K340 was then  
244 introduced and incubated for 10 min, after which the chamber was washed with 10 chamber  
245 volumes of K-PEM. Stabilised segmented MTs were then introduced. Unbound MTs were  
246 washed out immediately with K-PEM. Dynamic MTs, polymerised by incubating 50 µM 30%  
247 labelled Alexa Fluor 488 tubulin (same stock as used for fluorescence controls) and 1 mM  
248 GTP in K-PEM for 45 min at 37 °C were diluted 20-fold in warm (37 °C K-PEM and  
249 immediately flowed through the chamber. Next, 10 chamber volumes of warm K-PEM were  
250 flowed rapidly through the chamber. The sample was then imaged using epifluorescence on  
251 the dark-field microscope and ADP in K-PEM was introduced at the desired concentration.  
252 Once MTs had shortened sufficiently, 10 µM taxol and 200 µM ATP in K-PEM was flowed  
253 into the chamber.

254 **MT expansion assay with microfluidics**

255 0.2 mg ml<sup>-1</sup> PLL-PEG-biotin was pipetted into a microfluidic flow chamber and incubated for  
256 > 15 min before connecting to the pump and washing through each inlet with 50 µl of K-  
257 PEM. Dynamic MTs were then polymerised following the same initial steps as the tubulin  
258 depletion assay, except 20 µM of 10% x-rhodamine-labelled tubulin was used to grow the  
259 dynamic extensions, which were left for 1 h to polymerise, and buffer exchanges were  
260 performed using the microfluidic pump. MTs were then capped by incubating with 6 µM of  
261 15 % Alexa-488 tubulin + 1 mM GMPCPP for 20 min before switching to 0.1% Tween20 + 2  
262 mg/ml BSA for 5 min. The two pressure outlets of the pump were calibrated to achieve the  
263 same user-defined flow rate immediately before imaging with TIRF microscopy. K-PEM with  
264 1X GOC, 0.1% Tween20, 0.02% methylcellulose (1500 cP) and 2 mg/ml BSA was then flowed  
265 through with 1 mM ADP + 0.01% mPEG-beads or 200 nM K340, switching between the two  
266 every 80 frames, cycling 3 times. Frames were acquired every 2 s. Beads were additionally  
267 flowed through immediately after the experiment ended and frames were acquired every  
268 0.2 s for measuring the flow velocity (tracked manually). Images shown had the background  
269 subtracted in the green imaging channel by subtracting the median and applying a rolling  
270 ball ( $r=200$ ) to each frame using Fiji/Imagej.

#### 271 **Surface-stitched MT expansion assay**

272 Coverslips were incubated in 1 M HCl at 50 °C for 12-15 hours, rinsed with ultrapure water  
273 twice, sonicated in ultrapure water for 30 min, sonicated in ethanol for 30 min, rinsed in  
274 ethanol and dried by spinning or spraying with nitrogen gas. MTs were then polymerised as  
275 in the MT expansion assay with microfluidics, except the MT seeds and caps were labelled to  
276 30% with Alexa488 and the dynamic extensions were grown using unlabelled tubulin. MTs  
277 were capped for 10 min before washing the chamber with 100 µl of 0.1% Tween in K-PEM.  
278 Hereafter, each buffer contained GOC, 0.1% Tween20 and 0.02% methylcellulose (4000 cP)  
279 in K-PEM. The chamber was washed with 30 µl of buffer before imaging. During imaging, 40

280  $\mu$ l of 200 nM K340 was flowed through manually, followed later by 40  $\mu$ l of 1 mM ADP.  
281 Flowing through with 100  $\mu$ l of buffer depleted the ADP, after which a new field of view  
282 could be imaged using epifluorescence and dark-field illumination and the kinesin and ADP  
283 flows repeated. We imaged no more than five times in a single flow channel.

#### 284 **Dark-field/epifluorescence microscopy**

285 Images were captured by an EM-CCD camera (iXon<sup>EM</sup>+DU-897E, Andor) fitted to a Nikon  
286 E800 microscope with a Plan Fluor 100x NA 0.5-1.3 variable iris objective. A custom-built  
287 enclosure with an air heater (Air-Therm ATX, World Precision Instruments) was used to keep  
288 samples at 25 °C. Dark-field illumination was achieved using a 100 W mercury lamp  
289 connected to the microscope via a fibre optic light scrambler (Technical video), cold mirror,  
290 500-568 nm band-pass filter (Nikon) and a dark-field condenser (Nikon). A stabilised mercury  
291 lamp (X-cite exacte, Lumen Dynamics) provided illumination for epifluorescence, connected  
292 to the microscope with a light pipe. Motorised filter wheels (Ludl Electronic Products)  
293 housed the fluorescence excitation and emission filters: 485/20 and 536/40 for Alexa-488  
294 and 586/20 and 628/32 for X-rhodamine (Chroma). Combined dark-field and fluorescence  
295 imaging was achieved using an FF505/606-Di01-25x36 dichroic mirror (Semrock) and  
296 electronic shutters to switch between illumination modes. The shutters, filter wheels and  
297 camera were controlled using Metamorph software (Molecular Devices).

#### 298 **TIRF microscopy**

299 Images were captured by an EM-CCD camera (iXon<sub>3</sub> 888, Andor) fitted to a Warwick Open-  
300 Source Microscope (WOSM; wosmic.org), which was equipped with 473 nm (Cobolt) and  
301 561 nm (Obis) laser lines and a Nikon 100x NA 1.49 TIRF objective. Acquisition was triggered  
302 using a Matlab script, which in turn used Micromanager for capturing images, launched  
303 WOSM macros for all other microscope functionality, and controlled and monitored the  
304 microfluidics. Pixels were 130 nm. Data were acquired at 23 °C.

### 305 **Analysis of MT shrinkage rates**

306 Data were analysed in Matlab (Mathworks). Each MT image was aligned horizontally, using  
307 the function *imrotate* to rotate the image according to a hand-drawn line, before averaging  
308 columns of 11 pixels spanning the MT to generate an intensity profile. This was repeated for  
309 the same region of interest (ROI) for every frame in an image stack. Kymographs were  
310 produced by vertically concatenating the intensity profiles. Shrinkage rates were measured  
311 by manually tracing kymographs using the *impoly* function, extracting coordinates and  
312 calculating the slope. Time and distance calibration was automated by extracting the image  
313 metadata. Rates quoted in this paper assume a conversion of 125 dimer PF<sup>-1</sup> = 1 μM.  
314 Quantitative fluorescence analysis of MTs in a kinesin-clamp is presented in Supplementary  
315 Methods. Graph plotting and statistical tests were also carried out using Matlab.

### 316 **Analysis of MT expansion assay with microfluidics**

317 Kymographs were generated using the Fiji/ImageJ plugin KymoResliceWide and the  
318 subsequent analysis was performed using Matlab. The expansion of the MT was measured  
319 for each time point as shown in Supplementary Figure 5. These measurements were then  
320 analysed for each ADP or kinesin interval: the mean value was taken for each ADP interval,  
321 whereas a logistic curve was fitted (using bisquare robust fitting) to each ADP-kinesin  
322 transition to estimate the kinesin-driven expansion (as shown in Fig. 5d).

### 323 **Analysis of surface-stitched MT expansion assay**

324 Coordinates of MTs were extracted from dark-field images using the semi-automated  
325 Fiji/ImageJ plugin, JFilament<sup>36</sup>. The coordinates were then mapped onto the fluorescence  
326 channel and used to generate a 5-pixel-wide line scan. The fluorescence profiles of the cap  
327 and the seed were each fitted with a Gaussian error function in Matlab. The length of the  
328 GDP-bound section of the MT is then given by the distance between the point of inflection

329 on each curve. The MT length change was then assessed by taking the mean length of the  
330 manually identified plateaus, as shown in Fig. 5c. Points deviating by greater than 5% from  
331 the median length in these intervals were discarded prior to fitting. Long MTs were  
332 selectively chosen for the analysis to improve precision, with the average length of kinesin-  
333 free GDP-MT segments being 41  $\mu\text{m}$ .

334

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431

## 432 **Author contributions**

433 D.R.P. and R.A.C. designed experiments. N.J.B. provided mathematical insight. D.R.P.  
434 collected and analysed the data, and produced the manuscript and figures. All authors  
435 contributed towards the discussion and interpretation of results, and editing the manuscript.

436

## 437 **Competing financial interests**

438 The authors declare no competing financial interests.

439 **Figure legends**

440 **Supplementary Movie 1 | A kinesin-clamp assay.** The image data (*top*) corresponds to the  
441 kymograph in Fig. 2c (*bottom*). A minus-end trail is clearly seen in the no nucleotide phase.  
442 Addition of ADP causes the MT tips to shrink. In this case, the minus-end trail is retained  
443 during shrinkage. MTs are re-stabilised upon addition of taxol and ATP, and the resulting  
444 kinesin-driven MT gliding reveals the MT polarity.

445

446 **Supplementary Movie 2 | Strong-state kinesin can lock the curvature of GDP-MTs.** For  
447 each concentration of T93N, images are sorted according to the MT orientation. The marked  
448 MTs in each row (*orange asterisks*) fall into the orientation range depicted by the protractor  
449 diagrams (*left*). MTs are straight and dynamically unstable at the beginning of the movie.  
450 Arrows (*top*) highlight the presence and direction of hydrodynamic flow, which causes MT  
451 bending. In the absence of kinesin, stopping the flow causes the MTs to re-straighten and  
452 continue to depolymerise. MT curvature is preserved at low concentrations of T93N but not  
453 at high concentrations. MTs also transiently crinkle when T93N is flowed through at high  
454 concentrations.

455

456 **Supplementary Movie 3 | Kinesin reversibly expands MTs under constant hydrodynamic**  
457 **flow.** The movie corresponds to the MT shown in Fig. 5b. As kinesin and ADP are alternately  
458 introduced into the flow, the MT visibly expands and contracts, most obviously seen by the  
459 downstream MT tip visibly shifting right and left. Both surface-free and surface-snagging  
460 behaviour is shown in this movie, and the MT expands to the same extent in each case (Fig.  
461 5d).

462

463 **Supplementary Movie 4 | MTs briefly crinkle when kinesin is introduced to the flow.** An  
464 ADP-to-kinesin transition is shown, during which the MT crinkles and thereby dips in and out

465 of the TIRF illumination. Most of the MTs are visibly free from the surface for the duration.

466

467 **Supplementary Movie 5 | Kinesin increases the lattice spacing of surface-stitched GDP-**

468 **MTs.** The movie corresponds to the MT shown in Fig. 5f. Part way through the movie, 200

469 nM of monomeric kinesin (K340) was flowed through the channel and the MT extends and

470 bows so as to follow a longer path length. Flushing with 1 mM ADP triggers kinesin

471 unbinding, and the MT reverts to its original length. After washing the sample thoroughly

472 with buffer, the process can be repeated. After the first cycle, the MT becomes tethered to

473 the surface at a greater number of interaction sites. During the second kinesin flow-through,

474 part of the MT briefly goes out of focus but it is recruited back into the optical plane,

475 demonstrating that our protocol restricts motion in the z-axis to permit reliable

476 quantification. Scale bar is 10  $\mu\text{m}$ .

477 **Supplementary Animation 1 | Kymograph profile matching for measuring MT expansion.**

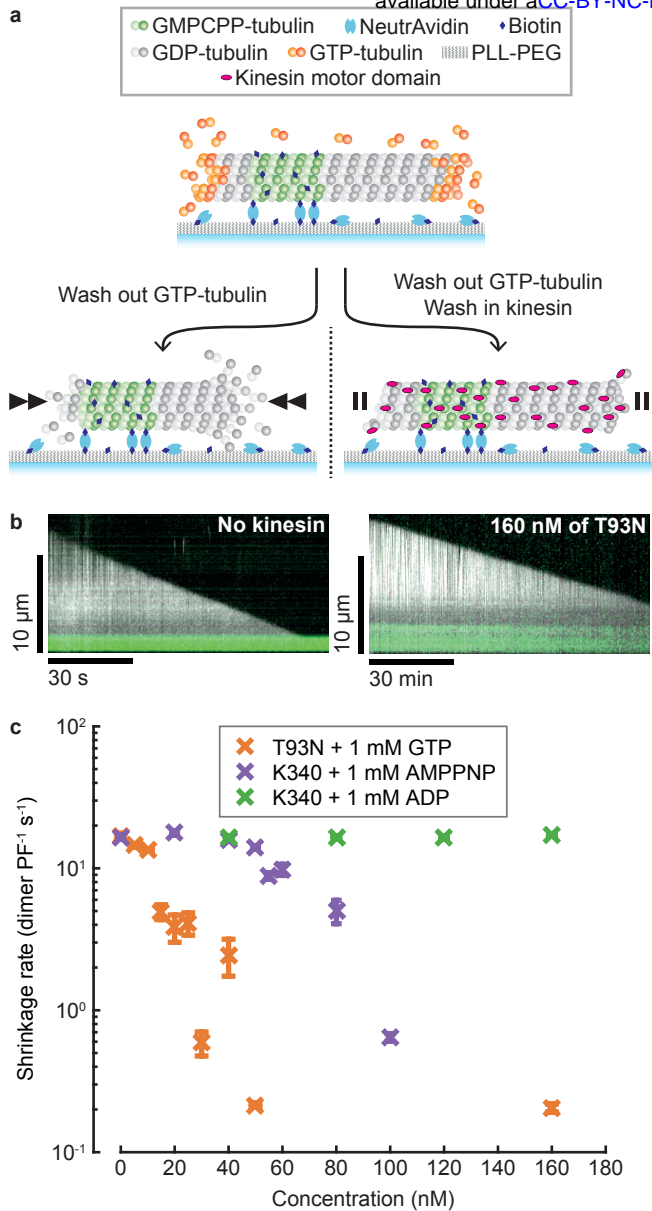
478 The 'original' kymograph shown is the same as in Fig. 5b. The 'transformed' kymograph is

479 the same image but each row has been compressed by the values shown in Fig. 5d and then

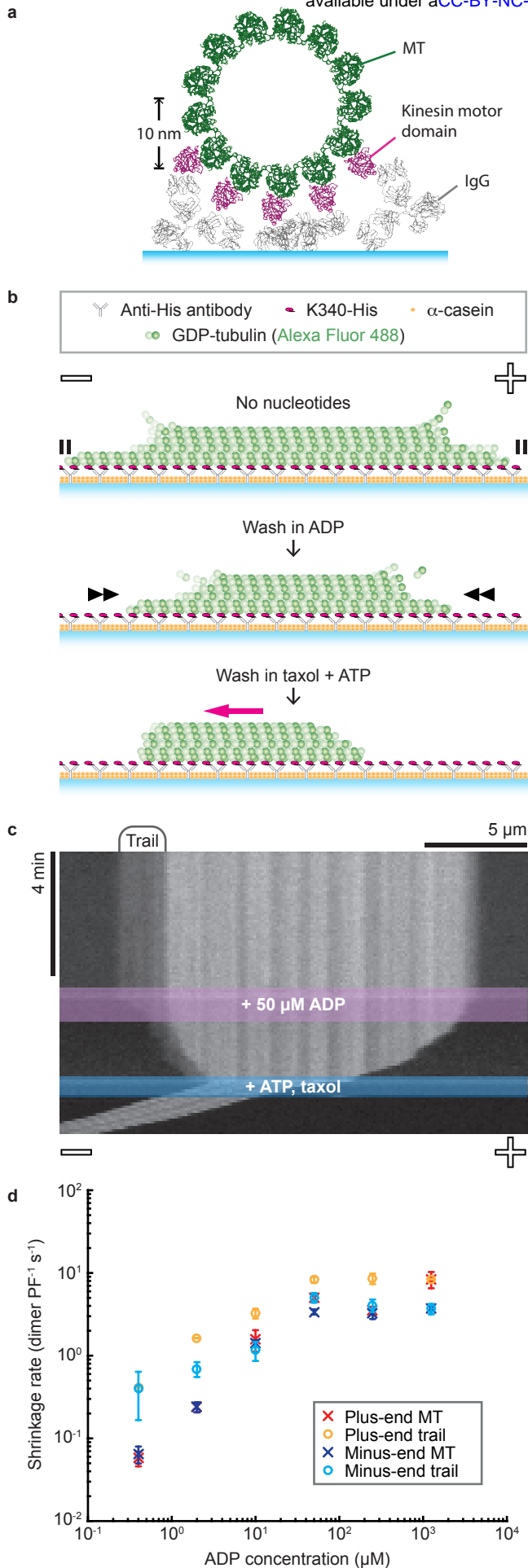
480 the magenta imaging channel was used for alignment. This highlights the expansion that

481 occurs when kinesin binds to the MT.

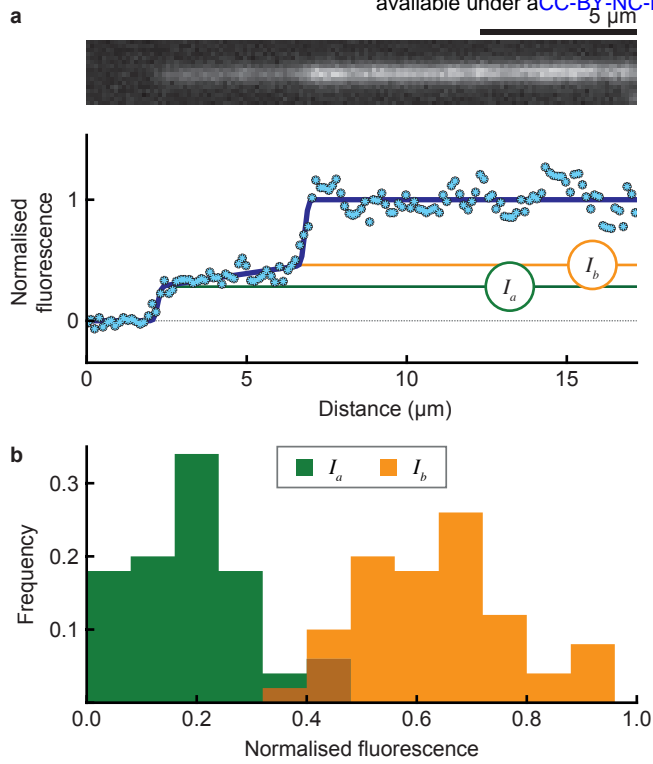
482



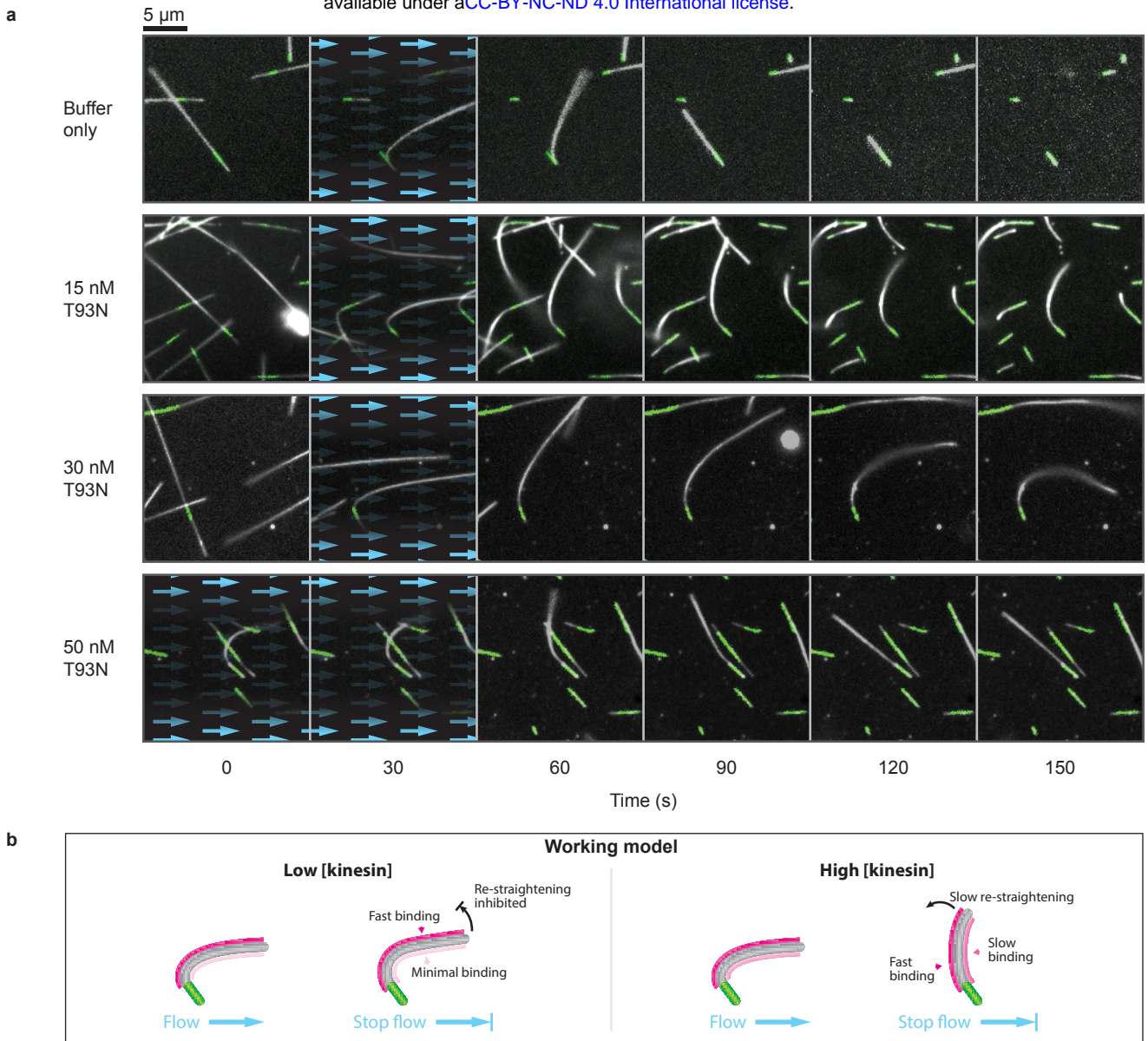
**Figure 1 | Strong-state kinesins inhibit GDP-MT shrinkage. a,** Schematic representation of a tubulin depletion assay. Dynamic MTs shrink rapidly when GTP-tubulin is depleted (left) unless bound to strong-state kinesins (right). **b,** Representative kymographs of MTs shrinking in the absence (left) and presence (right) of T93N. Note the different time scales. Dynamic MTs are shown in white (dark-field) and fluorescent seeds in green (epi-fluorescence). **c,** Shrinkage rates of MTs bound to kinesins in distinct nucleotide states. GTP was included with T93N only. Error bars are mean  $\pm$  SEM.  $14 \leq n \leq 53$  for all conditions.



**Figure 2 | MTs are stabilised when kinesins bind to one side of the lattice.** **a**, Cross-sectional view of a kinesin-clamp assay, showing IgG (PDB:1IGY) and kinesin-bound MT (PDB:4UXT) structures to provide scale. **b**, Schematic of a kinesin-clamp assay. **c**, Representative kymograph of a MT in a kinesin-clamp. **d**, Average shrinkage rates of MTs and their trails. Error bars are mean  $\pm$  SEM, reflecting inter-MT variability. *n*-values are given in Supplementary Table 1.

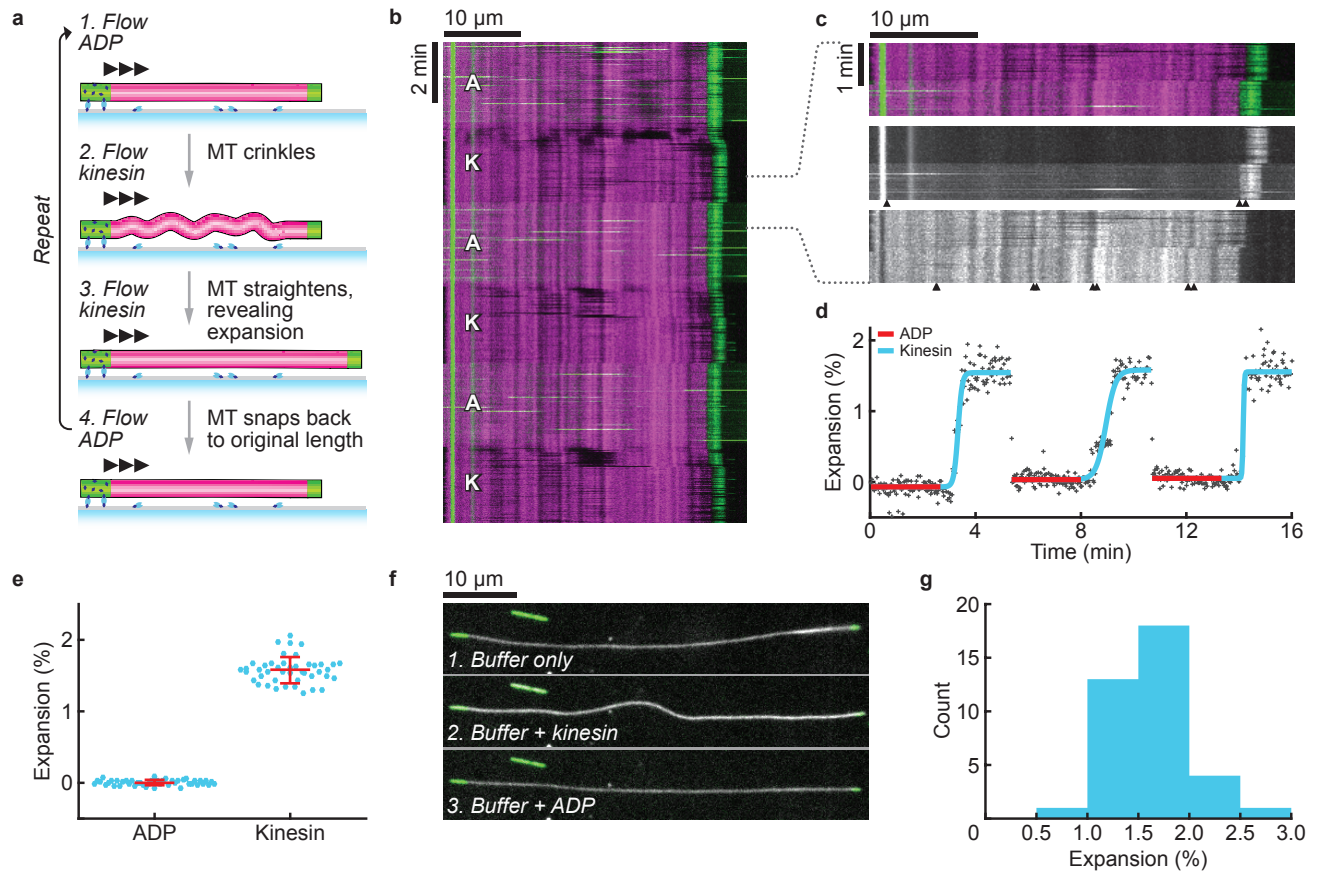


**Figure 3 | Subsets of protofilaments are stabilised by a kinesin-coated surface.** **a**, Model fit to the intensity profile of a MT tip (bottom) with the associated fluorescence image (top). ( $I_a$ ) is the intensity at the tip and ( $I_b$ ) at the base of the trail. **b**, Histogram of ( $I_a$ ) and ( $I_b$ ) values for ( $n = 50$ ) MTs, normalised to the intensities of their parent MTs in the no-nucleotide phase of the experiment. Mean  $\pm$  SD is  $0.19 \pm 0.11$  and  $0.64 \pm 0.13$  for  $I_a$  and  $I_b$ , respectively.



**Figure 4 | Nucleotide-free motor domains can bend-lock MTs. a**, Time-lapse images of MT bending experiments for a range of kinesin concentrations. Blue arrows highlight the presence and direction of fluid flow. Dynamic MTs appear white (dark-field) and fluorescent seeds are marked in green (epi-fluorescence). Each condition was tested twice on independent occasions. MTs shown here have been selected for having similar orientations. A more extensive selection is given in Supplementary Movie 2, which shows a complete range of orientations and lengths. **b**, Working model. We propose that kinesin binds preferentially to the stretched (convex) side of the MT and also stabilises this expanded region of the MT lattice. At high kinesin concentrations, the convex side of the MT would quickly saturate. Binding would also occur slowly on the concave side, causing this side to expand, progressively restraightening the MT.





**Figure 5 | Kinesin increases the lattice spacing of GDP-MTs.** **a**, Fluorescently labelled GDP-MTs (magenta) were grown from surface-bound GMPCPP-tubulin seeds (green) and then capped with GMPCPP-tubulin. Buffer was flowed through the channel at a constant rate and while switching between ADP and kinesin containing solutions. MTs transiently crinkled when kinesin was added, before straightening to reveal they had lengthened. Addition of ADP caused immediate recoil of the MTs to their original lengths. **b**, Representative kymograph of a MT changing length as the flow switched between ADP (A) and kinesin (K) containing buffers. A constant volumetric flow rate was maintained throughout the experiment ( $74.3 \pm 0.3 \mu\text{l}/\text{min}$ ; mean  $\pm$  SD, 480 time points). This translated to a flow velocity of  $248 \pm 43 \mu\text{m}/\text{s}$  (mean  $\pm$  SD,  $n=43$ ) near to the surface, measured by tracking fluorescent beads that were included in the ADP buffer. **c**, Magnified region from panel **b** highlighting the contraction observed when switching from kinesin-containing to ADP-containing solutions. *Top*: merge. *Middle*: Green imaging channel (GMPCPP-tubulin and beads). Buffer exchange is visible due to the high background provided by the fluorescent beads (after  $\sim 50$  sec). Arrowheads highlight the graduated movement along the MT during the exchange. The untethered tip retracts in  $<4$  s (2 frames) upon switching to ADP. *Bottom*: Magenta imaging channel (GDP-tubulin). Fiducial markings reveal the expansion along the length of the MT. **d**, Expansion measured for the MT shown in panel **b**, obtained by scaling the fluorescence intensity profile of each time point (in the magenta imaging channel) to match the average profile when ADP is present. **e**, MT lattice expansion, showing 44 measurements from 16 MTs. Values are  $0.00 \pm 0.04$  for ADP (control) and  $1.58 \pm 0.18$  for kinesin (mean  $\pm$  SD). **f**, Sequential images of a surface-stitched GDP-MT (dark-field image shown in white). Flow in this experiment was intermittent and images shown were taken in the absence of flow. When kinesin was added, the MT bowed to accommodate expansion of the MT lattice between the surface-bound points. The MT straightened and shortened upon addition of ADP. **g**, Expansion of surface-stitched GDP-MTs, given by the ratio of GDP-MT contour lengths with and without kinesin. Mean  $\pm$  SD is  $1.64 \pm 0.39$  ( $n=37$ ).