Interplay between the kinesin and tubulin mechanochemical cycles underlies microtubule tip tracking by the non-motile ciliary kinesin Kif7

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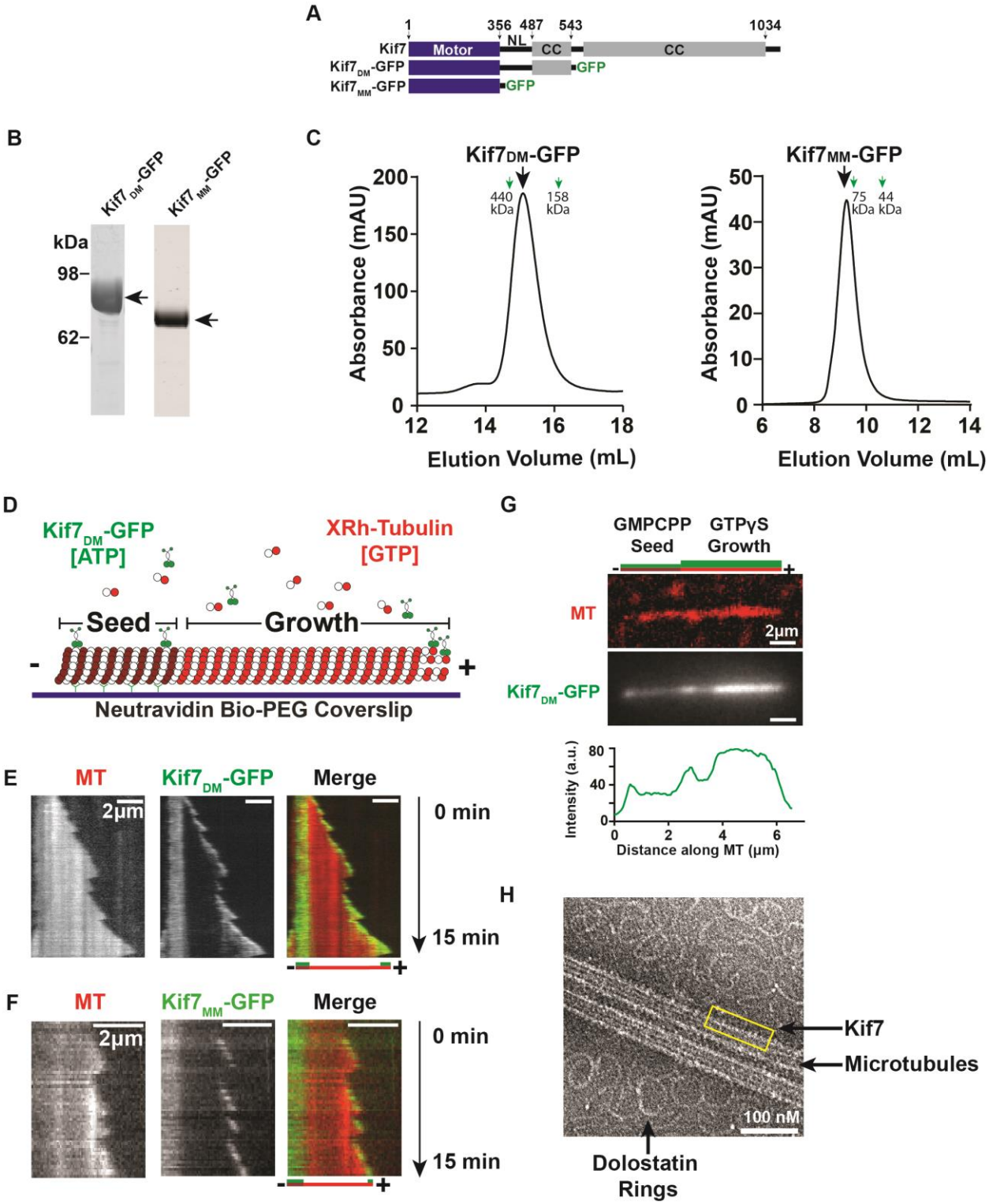

A
1
356 487
543
1034
NL
Motor
CC
CC
Kif7
Kif7DM-GFP
Kif7MM-GFP
GFP
B
Kif7DM-GFP
Kif7MM-GFP
kDa
98
62
C
Kif7DM-GFP
440 kDa
158 kDa
Absorbance (mAU)
Elution Volume (mL)
Kif7MM-GFP
75 kDa
44 kDa
Absorbance (mAU)
Elution Volume (mL)
D
Kif7DM-GFP [ATP]
XRh-Tubulin [GTP]
Seed
Growth
Neutravidin Bio-PEG Coverslip
G
GMPCPP Seed
GTPγS Growth
MT
2μm
Kif7DM-GFP
Intensity (a.u.)
Distance along MT (μm)
E
MT
Kif7DM-GFP
Merge
2μm
0 min
15 min
F
MT
Kif7MM-GFP
Merge
2μm
0 min
15 min
H
Kif7
Microtubules
100 nM
Dolostatin Rings

Figure S1

Figure S1. *Related to Figure 1*

A. Domain organization indicating the motor domain, neck-linker (NL) and coiled-coil (CC) of full length Kif7 and the constructs used for fluorescence microscopy assays.

B. SDS-PAGE analysis of purified Kif7$_{DM}$-GFP (left) and Kif7$_{MM}$-GFP (right). Arrows as indicators.

C. Chromatograms from size exclusion chromatography for Kif7$_{DM}$-GFP (left) (Superose 6 increase 10/300 GL) and Kif7$_{MM}$-GFP (right) (Superdex 75 10/300 GL). Green arrows indicate elution volume of molecular weight markers.

D. Schematic of the dynamic microtubule assay used to examine Kif7$_{DM}$-GFP localization on growing microtubules. X-rhodamine-labeled GMPCPP-stabilized microtubule 'seeds' (brown) were immobilized on a glass coverslip via a neutravidin-biotin linkage. Kif7 localization on the dynamic microtubule was examined by incubating the seeds with X-rhodamine-labeled tubulin (red), 1 mM GTP, Kif7$_{DM}$-GFP (green) and 1 mM ATP.

E. Representative kymograph of the microtubule, GFP and merged channels showing Kif7$_{DM}$-GFP localization at the tip of the growing microtubule over 15 minutes. The schematic below the image indicates the position of the seed (brown), growth (red) and the associated Kif7$_{DM}$-GFP signal (green).

F. Representative kymographs of the microtubule, GFP and merged channels showing Kif7$_{MM}$-GFP localization at the tip of the growing microtubule over 15 minutes. The schematic below the image indicates the position of the seed (brown), growth (red) and the associated Kif7$_{MM}$-GFP signal (green).

G. Representative image of microtubule (top) and associated Kif7$_{DM}$-GFP (middle) and line scan of Kif7$_{DM}$-GFP intensity (bottom) obtained from the experiment described in

28 “Figure 1A’. Assay conditions: 150 nM Kif7$_{DM}$-GFP and 1 mM ATP and 1 mM GTPγS.
29 Microtubule grown only from X-rhodamine labeled tubulin for control. The schematic
30 above the image indicates the position of the seed (brown), GTPγS growth (red) and the
31 associated Kif7$_{DM}$-GFP signal (green).
32 **H.** Electron micrographs of negatively stained Kif7$_{DM}$-GFP binding to straight microtubule
33 and dolostatin induced microtubule rings.

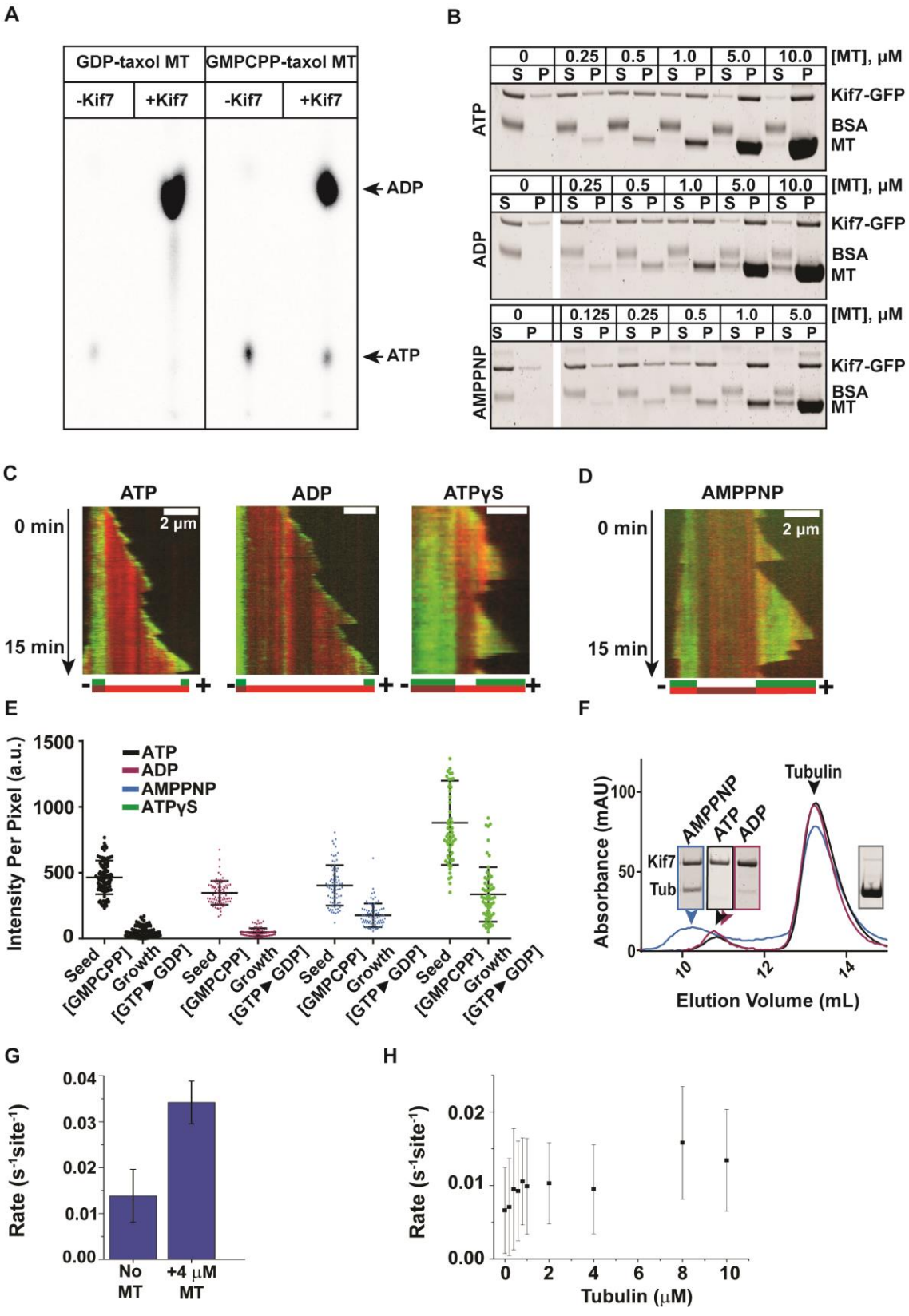

A
GDP-taxol MT
GMPCPP-taxol MT
-Kif7
+Kif7
-Kif7
+Kif7
ADP
ATP
B
ATP
ADP
AMPPNP
[MT], μM
Kif7-GFP
BSA
MT
S
P
C
ATP
ADP
ATPγS
0 min
15 min
2 μm
D
AMPPNP
E
Intensity Per Pixel (a.u.)
ATP
ADP
AMPPNP
ATPγS
Seed
[GMPCPP]
Growth
[GTP▶GDP]
F
Absorbance (mAU)
Tubulin
Kif7
Tub
Elution Volume (mL)
G
Rate (s⁻¹site⁻¹)
No MT
+4 μM MT
H
Rate (s⁻¹site⁻¹)
Tubulin (μM)

Figure S2

Figure S2. *Related to Figure 2*

A. TLC analysis of the adenosine nucleotide content of Kif7 bound to GMPCPP and GTP polymerized taxol stabilized microtubules. >95% of Kif7 associated is in the ADP form.

B. SDS-PAGE analysis of $Kif7_{DM}$-GFP cosedimentation on GDP-taxol stabilized microtubules (0 – 10 µM) in the presence of 1 mM ATP, ADP and AMPPNP. (S: Supernatant, unbound fraction. P: Pellet, bound fraction. BSA: Bovine Serum Albumin, gel loading control).

C. Representative kymographs of $Kif7_{DM}$-GFP localization at the tip of the growing microtubule in the presence of 1 mM ATP, ADP and ATPγS. The schematic under the image indicates the position of the seed (brown), growth (red) and the associated $Kif7_{DM}$-GFP signal (green).

D. Representative merged kymograph showing $Kif7_{DM}$-GFP localization on the growing microtubule in the presence of AMPPNP. The schematic under the image indicates the position of the seed (brown), growth (red) and the associated $Kif7_{DM}$-GFP signal (green).

E. Scatter plot of GFP fluorescence intensity per pixel of $Kif7_{DM}$-GFP on the GMPCPP-seed and GTP►GDP growth regions in the presence of different adenosine nucleotides. Error bars represent standard deviation. Assays were performed under $Kif7_{DM}$-GFP concentrations that resulted in similar average fluorescence intensities on the GMPCPP-seeds in the presence of different nucleotides to allow for normalization. Assay conditions: 150 nM $Kif7_{DM}$-GFP and 1 mM ATP; 250 nM $Kif7_{DM}$-GFP and 1 mM ADP; 10 nM $Kif7_{DM}$-GFP and 1 mM AMPPNP; 50 nM $Kif7_{DM}$-GFP and 1 mM ATPγS. (GMPCPP-seed: ATP: 464.7 ± 127.8; N = 81, ADP: 347.2 ± 90.7; N = 78, AMPPNP: 403.6 ± 153.1; N = 78 and

ATPγS: 879.2 ± 320; N = 61. GDP growth: ATP: 49.9 ± 41.3; N = 81, ADP: 48.0 ± 30.3; N = 78, AMPPNP: 177.4 ± 89.1; N = 78 and ATPγS: 335.9 ± 206.0; N = 61).

F. Size exclusion chromatography (Superdex 200 increase 10/300 GL) and SDS-PAGE analysis (Inset, ATP: black, ADP: purple and AMPPNP: blue) to examine the interaction of $Kif7_{DM}$-GFP (1 µM) with free-tubulin dimer (20 µM) in the presence of 1 mM ATP, ADP and AMPPNP.

G. ATPase assay showing 3-fold enhancement of Kif7 intrinsic ATPase rate in the presence of microtubules. (No microtubule: 0.014 ± 0.006 $s^{-1}site^{-1}$ and +4 µM Microtubule: 0.03 ± 0.005 $s^{-1}site^{-1}$).

H. ATPase assay showing no simulation of the basal ATPase rate of Kif7 by soluble tubulin.

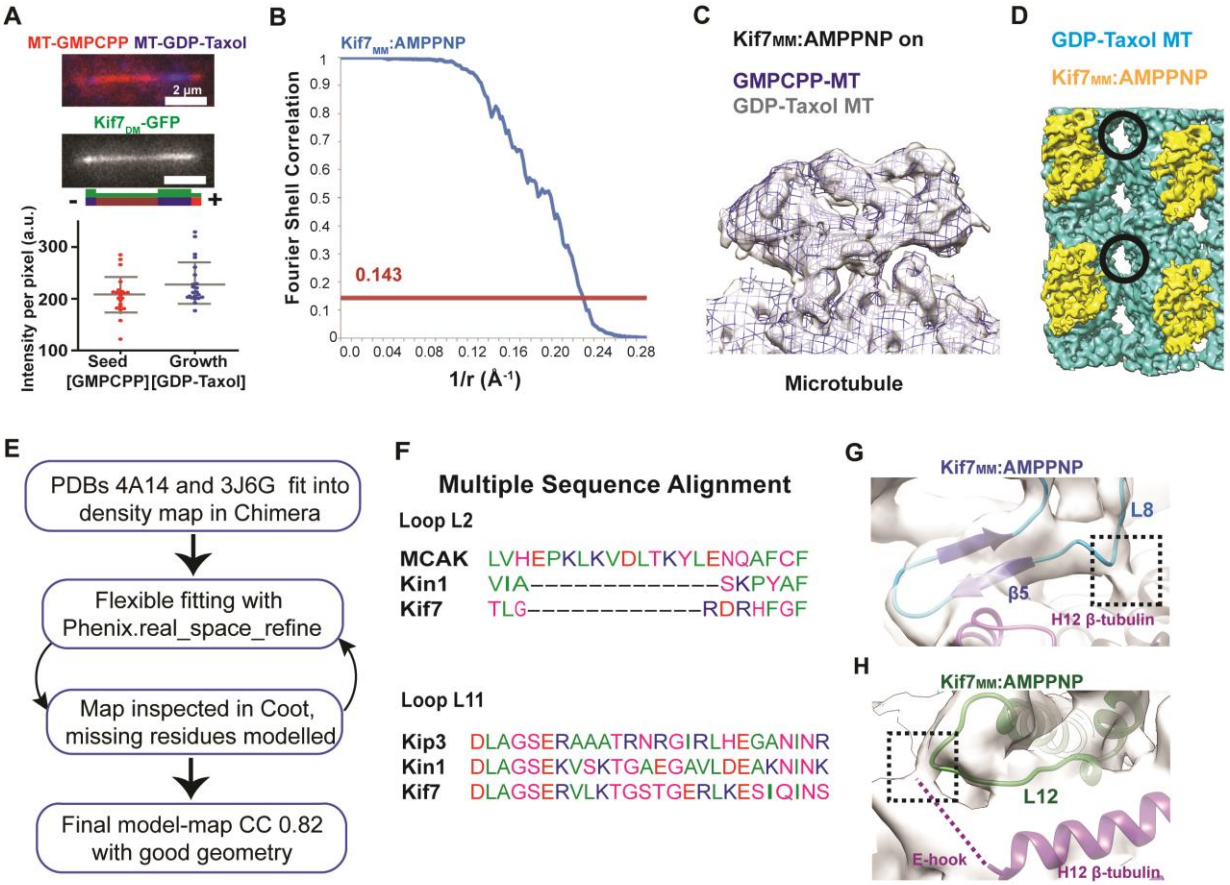

A
MT-GMPCPP MT-GDP-Taxol
2 μm
Kif7DM-GFP
Intensity per pixel (a.u.)
Seed
Growth
[GMPCPP][GDP-Taxol]
B
Kif7MM:AMPPNP
Fourier Shell Correlation
0.143
1/r (Å-1)
C
Kif7MM:AMPPNP on
GMPCPP-MT
GDP-Taxol MT
Microtubule
D
GDP-Taxol MT
Kif7MM:AMPPNP
E
PDBs 4A14 and 3J6G fit into density map in Chimera
Flexible fitting with Phenix.real_space_refine
Map inspected in Coot, missing residues modelled
Final model-map CC 0.82 with good geometry
F
Multiple Sequence Alignment
Loop L2
MCAK LVHEPKLKVDLTKYLENQAFCF
Kin1 VIA----------SKPYAF
Kif7 TLG----------RDRHFGF
Loop L11
Kip3 DLAGSERAAATRNRGIRLHEGANINR
Kin1 DLAGSEKVSKTGAEGAVLDEAKNINK
Kif7 DLAGSERVLKTGSTGERLKESIQINS
G
Kif7MM:AMPPNP
L8
β5
H12 β-tubulin
H
Kif7MM:AMPPNP
L12
E-hook
H12 β-tubulin
I
Absorbance mAU
Elution Volume (mL)
Kif7DM-GFP
Kif7DM-L12 4K-GFP
Kif7DM-L12 3A-GFP
Kif7DM-L8 KHC-GFP
Kif7DM-L10 KHC-GFP
kDa
98
62
J
[MT], μM
Kif7DM-GFP
Kif7DM-L12 4K-GFP
Kif7DM-L12 3A-GFP
Kif7DM-L8 KHC-GFP
Kif7DM-L10 KHC-GFP
BSA
MT
K
MT
Kif7DM-L12 3A-GFP
Merge
2μm
0 min
15 min

Figure S3

Figure S3. *Related to Figure 3*

A. Representative fluorescence image (top) and intensity analysis (bottom) of $Kif7_{DM}$-GFP associated on segmented microtubules containing a GDP-taxol stabilized growth region (blue). Assay conditions: 150 nM $Kif7_{DM}$-GFP and 1 mM ATP and 1 mM GDP-taxol. The schematic below the fluorescence image indicates the position of the seed (brown), growth (blue), cap (red) and the associated $Kif7_{DM}$-GFP signal (green). (GMPCPP-seed: 206.9 ± 36.4; N = 21. GDP-Taxol growth: 229.3 ± 41.1; N=21).

B. FSC curve for $Kif7_{MM}$:AMPPNP on GDP-taxol microtubules.

C. Superposition of cryo-EM reconstructions of $Kif7_{MM}$:AMPPNP bound to GDP-Taxol stabilized microtubules (gray solid) and GMPCPP-microtubules (blue mesh)

D. Electron density of $Kif7_{MM}$ (yellow) in complex with AMPPNP, bound on the GDP-taxol microtubule lattice (blue). Circle shows the canonical EB1 binding site in the inter-protofilament region.

E. Schematic of the procedure for model building and fitting into the EM reconstruction. The initial models used were PDB 4A14: crystal structure of the Kif7 motor domain and PDB 3J6G: EM-derived structure of α- and β-tubulin from GDP-taxol stabilized microtubule.

F. Multiple sequence alignment of Loops L2 and L11 in Kif7 with other kinesins. The structure-based sequence alignment of L2 between MCAK, Kinesin-1 and Kif7 (PDB IDs: 4UBF, 1BG2 and 4A14, respectively), and the sequence alignment of L11 between Kip3, Kinesin-1 and Kif7 (Uniprot IDs: P53086, P33176, Q2M1P5) are shown.

G. & H. Enlarged view of structural elements of $Kif7_{MM}$:AMPPNP with insertions in sequence compared to Kin1:ATP. Helix H12 and E-hook (dotted line) of β-tubulin are

shown in purple. The black boxes highlight the insertions in **(F)** Loop L8-strand β5 (blue) of $Kif7_{MM}$:AMPPNP **(G)** Helix α4 and loop L12 (green) of $Kif7_{MM}$:AMPPNP**.**

I. Purification of all $Kif7_{DM}$-GFP mutant constructs used in the microtubule co-sedimentation assays. Size exclusion chromatograms (left) of constructs: $Kif7_{DM}$-GFP (black), $Kif7_{DM\text{-}L12}{}^{4K}$-GFP (light green), $Kif7_{DM\text{-}L12}{}^{3A}$-GFP (dark green), $Kif7_{DM\text{-}L8}{}^{KHC}$-GFP (blue) and $Kif7_{DM\text{-}L10}{}^{KHC}$-GFP (orange) through a Superose 6 increase 10/300 GL column. Arrow indicates Kif7 elution peak. SDS-PAGE of purified proteins is shown on the right.

J. SDS-PAGE analysis of cosedimentation experiments of $Kif7_{DM}$-GFP mutants on GDP-taxol stabilized microtubules in the presence of 1 mM ATP. (S: Supernatant, unbound fraction. P: Pellet, bound fraction. BSA: Bovine Serum Albumin, gel loading control).

K. Representative kymograph of the microtubule, GFP and merged channels showing $Kif7_{DM\text{-}L12}{}^{3A}$-GFP localization at the tip of the growing microtubule in the presence of ATP. The schematic under the image indicates the position of the seed (brown), growth (red) and the associated $Kif7_{DM\text{-}L12}{}^{3A}$-GFP signal (green).

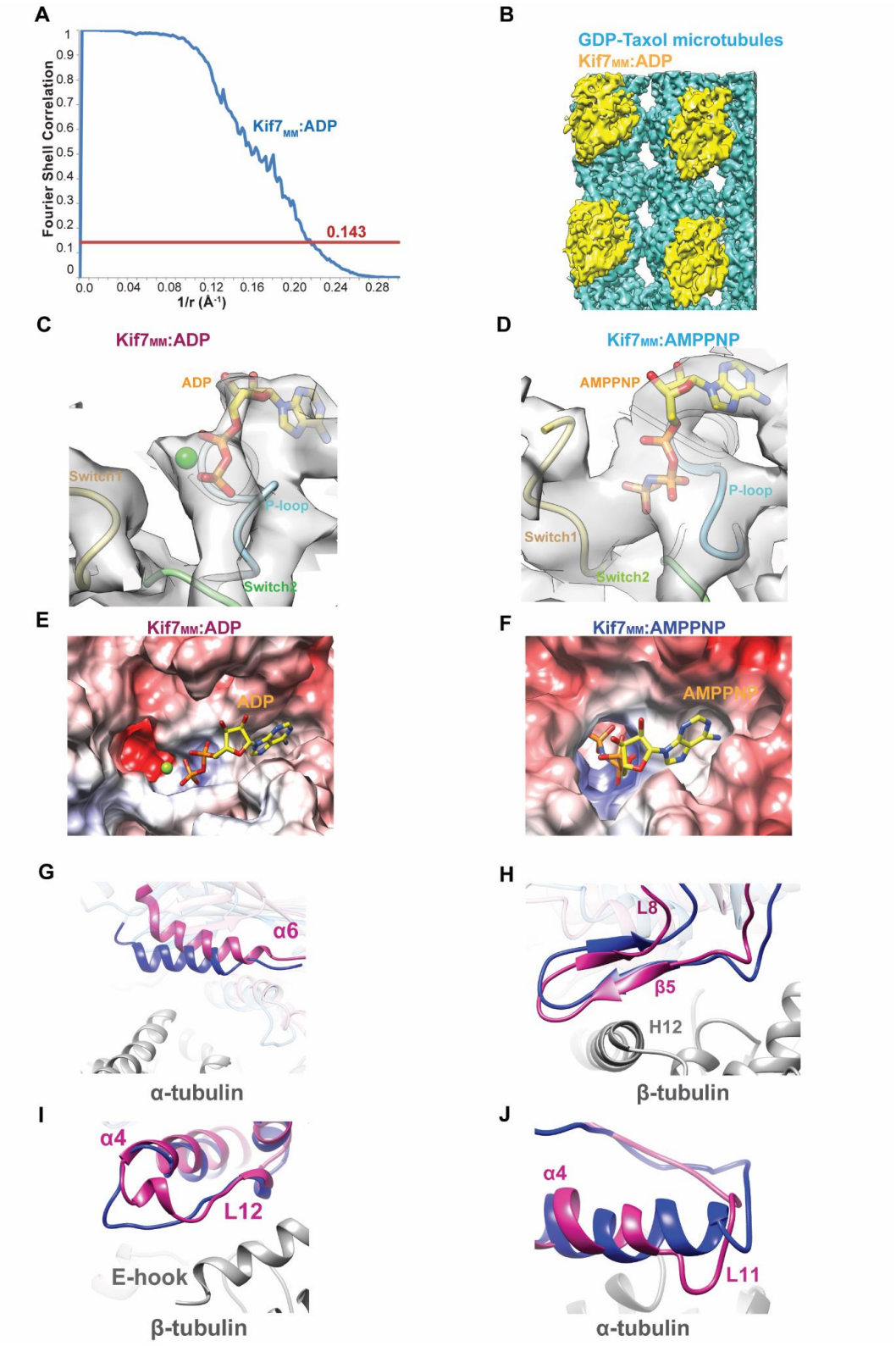

A
Fourier Shell Correlation
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.0
0.04
0.08
0.12
0.16
0.20
0.24
0.28
1/r (Å⁻¹)
Kif7MM:ADP
0.143
B
GDP-Taxol microtubules
Kif7MM:ADP
C
Kif7MM:ADP
ADP
Switch1
P-loop
Switch2
D
Kif7MM:AMPPNP
AMPPNP
P-loop
Switch1
Switch2
E
Kif7MM:ADP
ADP
F
Kif7MM:AMPPNP
AMPPNP
G
α6
α-tubulin
H
L8
β5
H12
β-tubulin
I
α4
L12
E-hook
β-tubulin
J
α4
L11
α-tubulin

Figure S4

Figure S4. *Related to Figure 4*

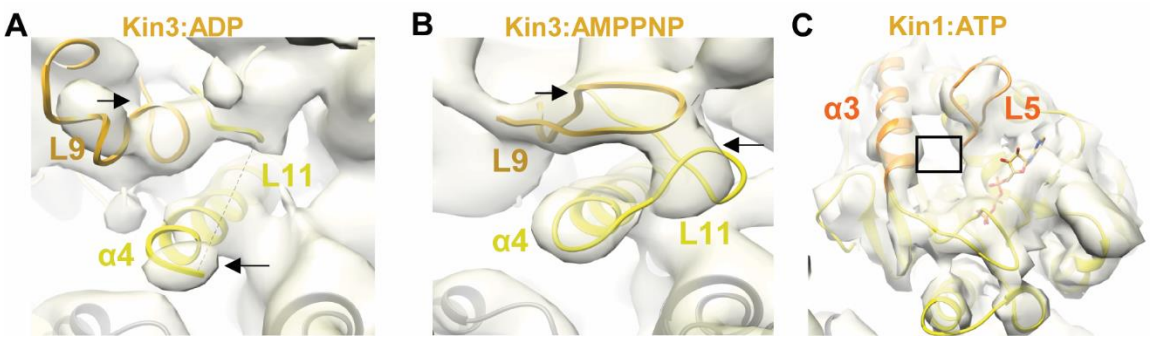
A. FSC curve for Kif7$_{MM}$:ADP on GDP-taxol microtubules.

B. Electron density of Kif7$_{MM}$ (yellow) in complex with ADP, bound on the GDP-taxol microtubule lattice (blue).

C&D. Enlarged view of the NBP for **(C)** Kif7$_{MM}$:ADP and **(D)** Kif7$_{MM}$:AMPPNP. Nucleotides are rendered as yellow sticks. Structural elements flanking the NBP are Switch1 (khaki), Switch2 (green) and P-loop (light blue).

E&F. Electrostatic surface representation of the NBP in **(E)** Kif7$_{MM}$:ADP and **(F)** Kif7$_{MM}$:AMPPNP. Nucleotides are rendered as yellow sticks. Surface potential ranges from -7 (red) to +7 (blue) KT/e.

G-J. Enlarged views showing the superposition between the different secondary structure elements in Kif7$_{MM}$:AMPPNP (blue) and Kif7$_{MM}$:ADP (pink). **(G)** Helix α6, **(H)** Loop L8 and strand β5. **(I)** Helix α4 and loop L12. **(J)** Helix α4 and loop L11 of Kif7. α- and β-tubulin are shown as gray ribbons.



130

131 **Figure S5**

132

Figure S5. *Related to Figure 5*

A&B. Enlarged views of the pincer-like movement of loops L9 and L11 during the ADP to AMPPNP transition in Kinesin-3 (Kin3). **(A)** Kin3:ADP (PDB:4UXS, EMD: 2768), **(B)** Kin3:AMPPNP (PDB:4UXP, EMD: 2766). Arrows highlight the movement of loops L9 and L11.

C. Enlarged view of the α3 and L5 region in Kin1:ATP (PDB:3J8Y, EMD: 6188). Black box highlights the absence of density connecting α3 and L5.

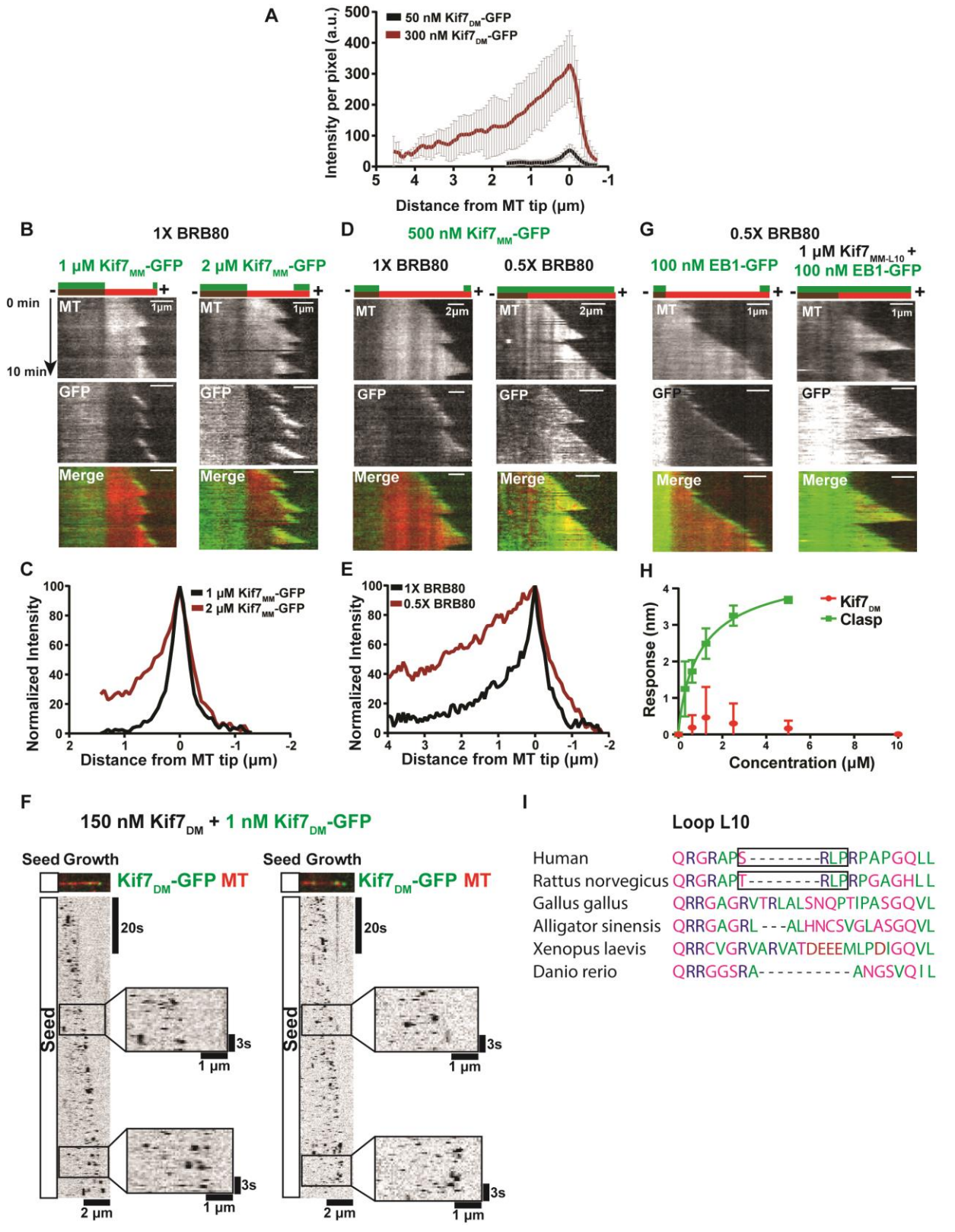

A
50 nM Kif7DM-GFP
300 nM Kif7DM-GFP
Intensity per pixel (a.u.)
Distance from MT tip (μm)
B
1X BRB80
1 μM Kif7MM-GFP
2 μM Kif7MM-GFP
0 min
10 min
MT
GFP
Merge
D
500 nM Kif7MM-GFP
1X BRB80
0.5X BRB80
G
0.5X BRB80
100 nM EB1-GFP
1 μM Kif7MM-L10 + 100 nM EB1-GFP
C
Normalized Intensity
Distance from MT tip (μm)
E
H
Response (nm)
Kif7DM
Clasp
Concentration (μM)
F
150 nM Kif7DM + 1 nM Kif7DM-GFP
Seed Growth
Kif7DM-GFP MT
Seed
20s
3s
1 μm
2 μm
I
Loop L10
Human QRGRAPS--------RLPRPAPGQLL
Rattus norvegicus QRGRAPT--------RLPRPGAGHLL
Gallus gallus QRRGAGRVTRLALSNQPTIPASGQVL
Alligator sinensis QRRGAGRL---ALHNCSVGLASGQVL
Xenopus laevis QRRCVGRVARVATDEEEMLPDIGQVL
Danio rerio QRRGGSRA----------ANGSVQIL

Figure S6

Figure S6. *Related to Figure 6*

A. Averaged GFP fluorescence intensity profile of Kif7$_{DM}$-GFP along the growing microtubule-ends at 50 nM (black; N=25) and 300 nM (brown, N=25). Error bars represent standard deviation.

B. Representative kymograph of the microtubule, GFP and merged channels showing Kif7$_{MM}$-GFP localization on the growing microtubule at 1 µM and 2 µM concentrations. The schematic above the image indicates the position of the seed (brown), growth (red) and the associated Kif7$_{MM}$-GFP signal (green).

C. Normalized GFP fluorescence intensity profile of Kif7$_{MM}$-GFP along the growing microtubule-ends at 1 µM (black; N=20) and 2 µM (brown, N=20) concentrations.

D. Representative kymograph of the microtubule, GFP and merged channels showing 500 nM Kif7$_{MM}$-GFP localization on the growing microtubule at 1X BRB80 and 0.5X BRB80. The schematic above the image indicates the position of the seed (brown), growth (red) and the associated Kif7$_{MM}$-GFP signal (green).

E. Normalized GFP fluorescence intensity profile of 500 nM Kif7$_{MM}$-GFP along the growing microtubule-ends at 1X BRB80 (black; N=20) and 0.5X BRB80 (brown, N=20).

F. Snapshot of spiked single-molecule tracking experiment during Kif7 expansion on the growing microtubule lattice. 1 nM Kif7$_{DM}$-GFP (green) and 150 nM Kif7$_{DM}$ on growing X-rhodamine labeled microtubules (red) at 150 nM concentrations where expansion is visible (top). Representative kymograph generated from the microtubule in **(E)** showing the localization of Kif7$_{DM}$-GFP at microtubule-ends (bottom). Inset shows enlarged regions of the single-molecule tracking.

G. Representative kymograph of the microtubule, GFP and merged channels showing 100 nM EB1-GFP localization on the growing microtubule alone and in the presence of 1

μM Kif7$_{MM-L10}$. The schematic above the image indicates the position of the seed (brown), growth (red) and the associated Kif7$_{MM}$-GFP signal (green).

H. Biolayer interferometry assay to quantitatively examine the binding affinity of Kif7$_{DM}$ (red; 0-10 μM) and Clasp (green; 0-5 μM) to EB1. Error bars represent standard deviation. The plots of BLI response of Kif7$_{DM}$ and Clasp bound versus concentration were fit to a Hill equation to determine the equilibrium dissociation constants at different concentrations. (K_d for Kif7$_{DM}$: No Binding and Clasp: 1.1 ± 0.3 μM).

I. Multiple sequence alignment of loop L10 of vertebrate Kif7 homologues from human, rat (*Rattus norvegicus*), domestic chicken (*Gallus gallus*), Chinese alligator (*Alligator sinensis*), *Xenopus laevis* and zebrafish (*Danio Rerio*) [Uniprot IDs: Q2M1P5, D4A9P0, F1P446, A0A1U7SD20, A0A1L8H0X0 and Q58G59, respectively]. [S/T]x[I/L]P motifs are highlighted by a black box.

Table S1-Related to Figures 3 and 4

Data collection and reconstruction

	$Kif7_{MM}$:AMPPNP (15 protofilaments)	**$Kif7_{MM}$:AMPPNP (14 protofilaments)**	**$Kif7_{MM}$:ADP (15 protofilaments)**	**$Kif7_{MM}$:ADP (13 protofilaments)**
Data collection				
Microscope	Titan Krios (FEI)	Titan Krios (FEI)	Titan Krios (FEI)	Titan Krios (FEI)
Voltage (kV)	300	300	300	300
Nominal magnification*	22,500X	22,500X	22,500X	22,500X
Cumulative exposure dose ($e^- Å^{-2}$)	37	37	44	44
Exposure rate (e^-/pixel/sec)	4.7	4.7	4.7	4.7
Detector	K2 Summit	K2 Summit	K2 Summit	K2 Summit
Pixel size (Å)*	1.31	1.31	1.31	1.31
Defocus range (μm)	0.7-4.24	0.7-4.24	0.86-2.91	0.86-2.91
Average defocus (μm)	2.31	2.31	1.6	1.6
Micrographs Used	428	428	697	697
Total extracted helical segment (no.)	79,125	79,125	50,409	50,409
Refined helical segment (no.)	25,402	44,295	15,919	19,035
Reconstruction				
Final helical segments (no.)	25,402	37,220	15,919	13,488
Symmetry imposed	HP	HP	HP	HP
Resolution (global) FSC 0.143	4.2	4.3	4.2	6.5

Table S2-Related to Figures 3 and 4

Atomic model refinement statistics

	$Kif7_{MM}$:AMPPNP	**$Kif7_{MM}$:ADP**
Refinement protocol	Phenix	Phenix
Resolution for refinement	4.3	4.2
RMS bonds (Å)	0.011	0.006
RMS angles (°)	1.37	1.30
All-atoms clashscore	19	14
Rotamer outliers (%)	3	2
Ramachandran allowed (%)	12	10
Ramachandran favored (%)	88	90
Ramachandran outliers (%)	0	0
Model-map real-space correlation coefficient	0.81	0.83