Cryo-EM structure of the inhibited (10S) form of myosin II

Myosin II is the motor protein that enables muscle cells to contract and nonmuscle cells to move and change shape. The molecule has two identical heads attached to an elongated tail, and can exist in two conformations: 10S and 6S, named for their sedimentation coefficients. The 6S conformation has an extended tail and assemblies into polymeric filaments, which pull on actin filaments to generate force and motion. In 10S myosin, the tail is folded into three segments and the heads bend back and interact with each other and the tail, creating a compact conformation in which ATPase activity, actin activation and filament assembly are all highly inhibited. This switched-off structure appears to function as a key energy-conserving storage molecule in muscle and nonmuscle cells, which can be activated to form functional filaments as needed—but the mechanism of its inhibition is not understood. Here we have solved the structure of smooth muscle 10S myosin by cryo-electron microscopy with sufficient resolution to enable improved understanding of the function of the head and tail regions of the molecule and of the key intramolecular contacts that cause inhibition. Our results suggest an atomic model for the off state of myosin II, for its activation and unfolding by phosphorylation, and for understanding the clustering of disease-causing mutations near sites of intramolecular interaction.

Cryo-EM structure of 10S myosin II

Class averages of cryo-imaged molecules showed multiple views of the 10S conformation, with evidence of secondary structure in the heads and clear density for all three tail segments (Extended Data Fig. 1; Methods). The refined reconstruction (EMD-22145; resolution range approximately 4–9 Å) confirmed this appearance, revealing secondary structure in the motor domains (including side-chain detail), the light chains and the individual α-helices of the tail (Figs. 1b–d, Extended Data Figs. 1b, 2, Extended Data Table 1). To our knowledge, these features, observed here in intact myosin II, have previously been seen only in X-ray structures of the separate components. The two heads interact with each other through their motor domains. Seg1 of the tail (also known as subfragment 2 or S2) exits the heads at the junction of the two regulatory domains, crosses the BH, reverses direction at hinge 1 (not seen in the map owing to flexibility), where it becomes seg2. Seg2 passes around the edge of the BH and reverses direction at hinge 2, becoming seg3, which crosses the BH parallel to, but resolved from, seg1 (Fig. 1).

We interpreted the structure by rigidly fitting the motor and regulatory domains of a two-headed myosin fragment (Protein Data Bank [PDB] 1I84) independently into the electron microscopy map, and then refining the fit (Fig. 2a; Methods). We fitted and refined the tail

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1Division of Cell Biology and Imaging, Department of Radiology, University of Massachusetts Medical School, Worcester, MA, USA. 2Department of Cellular and Molecular Biology, University of Texas Health Science Center at Tyler, Tyler, TX, USA. 3Present address: Cryo-EM Shared Resources, Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA, USA. 4Present address: Massachusetts Facility for High-Resolution Electron Cryo-microscopy, University of Massachusetts Medical School, Worcester, MA, USA. 5These authors contributed equally to this work. Shixin Yang, Prince Tiwari. e-mail: roger.craig@umassmed.edu

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in a similar way, using the α-carbon backbone of the subfragment-2 coiled-coil (PDB 2FXM). The refined model (PDB 6XE9; Fig. 2a, b, Extended Data Fig. 2c–g) revealed further detail of the heads, the head–tail junction, the tail and the intramolecular interfaces that clamp the molecule in the off state.

Structure of the heads

The reconstruction and fitted model show that the two motor domains are essentially identical in structure (Extended Data Fig. 3a). Similar location and structure of the converter domains suggests that the motor domains are in the same pre-powerstroke, ADP·Pi nucleotide state, in which ATP has been hydrolysed but the products of hydrolysis, ADP and inorganic phosphate, remain bound. By contrast, the regulatory domains make quite different angles with their motor domains, owing to different flexing in the pliant region of the head (near L790; Extended Data Fig. 3b,c). Similar differential flexing of the BH and FH occurs in the thick filament IHM, suggesting that it is a fundamental feature of the motif. It enables the heads, interacting asymmetrically at their motor domain interface, to come together at their C termini and attach to S2 without requiring significant unwinding of its coiled coil (Extended Data Fig. 3d). Our structure does not support a recent low-resolution thick filament model in which BH flexing is much smaller. We also observe another point of flexibility that enables the heads to attach to S2 without requiring its uncoupling: a straightening of the FH regulatory domain between the ELC and RLC, which brings the C termini of the heads about 7 Å closer together (Extended Data Fig. 3e, f; contrast with ref. 23). In each regulatory domain, the density for the α-helical backbone, to which the ELCs and RLCs bind, was continuous, with little evidence for melting in the BH suggesting from flexible fitting to a previous 20 Å resolution filament structure.

Structure of the head–tail junction

Flexing about the head–tail junction is essential for myosin function, but the structure of this region has remained unknown, as its flexibility makes it impossible to study by X-ray crystallography. Our map clearly shows the junction, which starts with an abrupt bend (the ‘hook’) at the C-terminal end of the regulatory domain heavy chain. The hook leads to invariant proline P849 and the start of the coiled-coil tail (Extended Data Fig. 4). The map densities suggest that both hooks are α-helical, with no major melting suggested from low-resolution filament models. The hook angles are similar in both heads (about 90°; Extended Data Figs. 3e, 4), and show no evidence for the differences proposed as a source of compliance facilitating connection to the tail in the IHM. This compliance apparently occurs at the pliant point and between the light chains in the regulatory domains. The α-helices of seg1 lead directly from the hooks, with possible confined melting around P849 (approximately K848–Q852; Extended Data Fig. 4). We see no evidence for dissociation of the first eight heptads of each S2 heavy chain into individual α-helices or their binding to the surface of the heads. Instead, the helices apparently associate with each other from the start of S2 (around V853), though with a slightly longer helical pitch and less order in the first few residues (Extended Data Fig. 5b). This may also contribute to relief of stress in the IHM (see above), and could be important in generating the off state. Lower stability of the coiled-coil at the start of S2 is suggested by less ordering in X-ray crystal structures and in our density map (Extended Data Fig. 2).

Structure and path of the tail

The organization of the tail in 10S myosin has previously been observed only at low resolution. Our high-resolution reconstruction clearly resolves the two α-helical densities, which twist around each other in a left-handed supercoil (Fig. 2, Extended Data Figs. 2, 5). The pitch of the coiled coil varies substantially along its length (Extended Data Fig. 5), as also observed in thick filaments. Whereas some portions have fairly constant crossover distances (approximately 70 Å), the start of seg3 lead directly from the hooks, with possible confined melting around P849 (approximately K848–Q852; Extended Data Fig. 4). We see no evidence for dissociation of the first eight heptads of each S2 heavy chain into individual α-helices or their binding to the surface of the heads. Instead, the helices apparently associate with each other from the start of S2 (around V853), though with a slightly longer helical pitch and less order in the first few residues (Extended Data Fig. 5b). This may also contribute to relief of stress in the IHM (see above), and could be important in generating the off state. Lower stability of the coiled-coil at the start of S2 is suggested by less ordering in X-ray crystal structures and in our density map (Extended Data Fig. 2).
**Interactions producing the off state**

Our map shows details of multiple intramolecular contacts, which we suggest clamp 10S myosin in the inhibited state (Fig. 3, Supplementary Table 1). These occur in the same regions suggested by our previous negative-stain reconstruction, and we use the interaction nomenclature defined in that work. We analysed these putative interactions using UCSF Chimera (Methods). Because of the limited resolution of the reconstruction, we consider these as potential rather than definitive interactions, at least in the lower-resolution regions.

**Head–head interactions**

The density map shows clear contact between the BH and FH motor domains, involving the actin-binding surface of the BH and the catalytic and converter domains of the FH (Figs. 1, 2). Specifically, loop I365–N381 and helix T382–L390 of the BH lie near helix E727–Y734 and loop E735–D748 of the FH converter (interaction BF1; Fig. 3b, Supplementary Table 1). BH helix V395–L403 lies over helix F727–Y734 of the FH converter and helix IL53–D167 of the FH catalytic domain. These contacts broadly support previous work at 20 Å resolution,[21,22] but precisely locate the potential interacting residues. However, we do not observe any contact between the BH motor domain and the FH ELC, which were previously thought to form an interface within the IHM[9] (Fig. 3b, Supplementary Table 1). These BH–FH interactions probably contribute to inhibition by hindering movements of the FH converter required for ATPase activity and by blocking BH binding to actin.[9]

**BH–tail interactions**

The BH appears to be locked down by three interactions with the tail[9]. First, as segment 2 travels from the top of the molecule, it passes through a groove on the edge of the BH, making contact around L1431–D1436 with helix K72–D74 of the SH3 domain and around Q1445–L1452 with R718 and L766 in or near the converter (TB2, TB3; Fig. 3e). Here, seg2 physically blocks movement of the BH converter required for phosphate release (Extended Data Fig. 7a–d), directly explaining the inhibition of ATP turnover by the BH through trapping of ATP hydrolysis products in the active site.[24] Second, the tail (around L1494–L1498) next contacts helix D (E67–A77) of the ELC N-lobe (interaction TB4), potentially stabilizing the 10S conformation (Fig. 3f). Third, after a hairpin bend at hinge 2, seg3 (approximately A1577–E1587) contacts helix E of the BH RLC (E99–A107) (TB5, Fig. 3g) and comes close to the regulatory domain α-helix Q817–V824. It then crosses the BH motor domain, parallel to and contacting seg1 (around L1628–E1647 in seg3 with R910–M925 in seg1; TT2, Fig. 3k). Seg1 and seg3 both appear to ‘hover’ above the surface of the BH (interaction TBI, Fig. 3d), contacting it only between around L1604–E1612 of seg3 and loop L450–F460 of the BH motor domain. It was previously assumed that there are multiple interactions of segments 1 and 3 with the surface of the BH. However, as there is a gap of approximately 8 Å between these tail segments and the BH, there appear to be few contacts. Weak electrostatic interaction (over 5–10 Å [ref. 29]) may occur over these longer ranges, consistent with weak, salt-sensitive binding of S2 to myosin heads in solution[30].

**RLC–RLC interaction**

The RLCs approach within about 4–4.5 Å of each other at the base of their N-terminal lobes, where helix A and the A–B linker in the BH, and helix D and the A–B linker in the FH come together (BF2, Fig. 3c), strengthening previous suggestions of RLC–RLC interaction[21,23,26] which is thought to be important in regulating smooth and skeletal muscle activity[27].

**FH–tail interaction**

The FH contacts the tail at three sites. The CM loop (T404–K420) and loop 2 (K626–T658), in the actin-binding interface, form contacts TF1 and TF2 with seg1 (M925–A941; Fig. 3h, i), as previously proposed for.

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**Fig. 2** Fitting of atomic model to map and locations of mutations.

a, Cryo-EM map from Fig. 1 (contour level 0.0125) fitted with refined atomic model (PDB 6XE9; Supplementary Video 2). b, Refined model (Supplementary Video 3), showing locations of disease-causing mutations in smooth muscle and nonmuscle myosin II (Extended Data Table 2) plotted onto the atomic model (motor domain mutations in blue, tail mutations in yellow). c, Locations of all tail mutations (heads omitted for clarity). Ellipses, mutations that could affect folding at hinges; long bracket, clustering of mutations in the three tail segments presumed to interact with each other in the 10S structure—these may affect stability of the folded molecule. Numbers are amino acid positions: black, ends of the coiled-coil (850–1937), the two hinges (1175, 1535), and the C terminus (1979); amino acids 1938–1979 form a non-helical tailpiece; coloured numbers indicate positions of point, duplication or deletion mutations. See also Extended Data Fig. 9 and Supplementary Video 4.
and 3, is 10 times more active than 10S myosin8, supporting the inhibi-

tion movements, conserving ATP. HMM, which lacks segments 2

ADP.Pi state, with hydrolysis products trapped by inhibition of their

We conclude that these multiple contacts together pin the myosin II mol-

by phosphorylation of S19 on the RLCs, leading to breaking of the inhibitory

interactions. A possible mechanism for activation based on the atomic

model, including visualization of the N-terminal 24 residues of the BH

R731 (numbering based on smooth muscle myosin II), in the interface

with the BH (Extended Data Table 2, Extended Data Fig. 9a), which

might influence head–head interaction; a BH mutation in the inter-

face (K386) may have a similar effect. By contrast, there are multiple

mutation sites in the BH that are in close proximity to seg2. One group

of such mutations stands out, clustered where seg2 wraps around the

BH, particularly in residues in and near the SH3 and converter domains,

several of which are within 10 Å of seg2, and some within 4 Å (Fig. 2b, c,

Extended Data Fig. 9b). While some of these mutations may directly

affect ATPase activity, their proximity to seg2 suggests that they could

alter BH–seg2 interaction and thus stability of the 10S structure. Mutations

in seg2 also occur in this region, and could similarly affect this

contact. If the seg2–converter interaction inhibits BH ATPase activity

(as described above), mutations in seg2 might disrupt this inhibition.

There are also multiple mutations that might affect tail–tail interac-

tions. These include mutations in seg1 that coincide with interaction

site TT2, potentially affecting interaction with seg3 (Fig. 3a, k, Extended

Data Fig. 9a, k). These mutations, which occur in all three segments, may affect

filament formation after activation. Mutation of R1570 in seg3, part of the proposed binding site for the BH

phosphorylation domain (Extended Data Fig. 8b, c, i), could influence

folding or phosphorylation (Extended Data Fig. 9c).

This structure–mutation analysis parallels analysis of the IHM of

cardiac thick filaments in relation to hypertrophic cardiomyopathy, in

which disease correlated with mutations in the head–head interface,

which were proposed to destabilize head–head interaction34,35. Our

analysis suggests that intramolecular interfaces may be important

for diseases associated with smooth muscle and nonmuscle myosin

II, mostly related to interactions involving the tail.

Conclusion

Our cryo-EM reconstruction provides detailed insights into the struc-
ture of intact myosin II in the 10S conformation and the physical basis

of its inhibition, correcting and integrating previous structural and

solution studies. Our model for the off state, and for its activation

thick filaments22, sterically inhibiting FH–actin binding (Extended

Data Fig. 7h–k). Helix A in the N-lobe of the FH RLC fills the gap between

the two α-helices at the origin of seg1 (L850–Q856), with multiple poten-
tial interactions with the tail (TF3, Fig. 3j), probably affecting regulatory

mutations on the basis of their proximity to (and thus potential effect on

interaction with) other regions of the molecule in the folded structure.

Most FH disease mutations have no influence on the 10S conforma-
tion, as they are far from any intramolecular contacts (Fig. 2b, Extended

Data Fig. 9a). They might influence head function directly, independ-
ently of 10S folding. Three exceptions are mutations of R253, R507 and

The 10S structure and myosin mutations

Several human diseases are linked to mutations in the heavy chains of

smooth muscle and nonmuscle myosin II, including breast and

prostate cancer, blood diseases and smooth muscle dysfunction36,37. These

myosins, which both form the 10S structure5,39, have essential

roles in smooth muscle and nonmuscle cellular functions32. We investi-
gated whether alteration of the 10S conformation might contribute to
these diseases by aligning the smooth muscle and nonmuscle myosin

II sequences (Supplementary Table 2) and mapping disease mutations
onto the smooth muscle heavy chain (Extended Data Table 2) and the

10S atomic model (Fig. 2b). We considered the potential effects of
mutations on the basis of their proximity to (and thus potential effect on
interaction with) other regions of the molecule in the folded structure.

Mechanism of inhibition and activation

We conclude that these multiple contacts together pin the myosin II mol-

eule in its 10S conformation. Both heads are in the pre-powerstroke,

ADP.Pi state, with hydrolysis products trapped by inhibition of their

converter movements, conserving ATP. HMM, which lacks segments 2

and 3, is 10 times more active than 10S myosin8, supporting the inhibi-

tory role of these segments3. Actin-interaction loops are blocked in both

heads through head–head (BH) and head–tail (FH) interactions, and

unfolding of the tail is inhibited owing to its multiple intramolecular

interactions. The result is complete inhibition of the molecule in the

10S, dephosphorylated state. The 10S structure is activated by phos-

phorylation of S19 on the RLCs, leading to breaking of the inhibitory

interactions. A possible mechanism for activation based on the atomic

model, including visualization of the N-terminal 24 residues of the BH

RLC, is presented in Extended Data Fig. 8.

Fig. 3 | Intramolecular interactions in the 10S atomic model. a, Cartoon of

10S structure showing locations of interactions. Nomenclature from ref. 7:

B, interaction with BH; F, interaction with FH, T, interaction with tail. Interaction

TB6 is discussed further in Extended Data Fig. 8. b–k, Detail of interactions

BF1 (b), BF2 (c), TB1 (d), TB2 and TB3 (e), TB4 (f), TB5 (g), TF1 (h), TF2 (i), TF3 (j)

and TT2 (k) (see Supplementary Table 1). Colour scheme as in a and Fig. 2a, b. All

views from front of molecule, except d (from bottom) and g (from side); parts

of molecule removed for clarity. Cnv, converter; CD, catalytic domain. See

Supplementary Video 5.

The 10S structure and myosin mutations

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Most FH disease mutations have no influence on the 10S conforma-
tion, as they are far from any intramolecular contacts (Fig. 2b, Extended

Data Fig. 9a). They might influence head function directly, independ-
ently of 10S folding. Three exceptions are mutations of R253, R507 and
and unfolding, provides a framework for further testing of functional mechanisms and for understanding how mutations may cause disease, with implications for drug design.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-3007-0.

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Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Protein preparation

Smooth muscle myosin II was purified in the dephosphorylated state from adult turkey (Meleagris gallopavo) gizzard, obtained from a local turkey farm. Ten-microlitre aliquots (30 μM, 15 mg ml⁻¹) were flash-frozen in liquid nitrogen and stored at −80 °C. Smooth muscle myosin has similar structural and functional properties to nonmuscle myosin II, which both form essentially identical 10S structures.39

Specimen optimization

Negative staining was used to check specimen purity, quality and grid loading. A thawed aliquot of myosin II was diluted to 10 nM in 0.15 M sodium acetate, 1 mM EGTA, 2.5 mM MgCl₂, 0.5 mM ATP, 10 mM MOPS, pH 7.5, generating the 10S structure.7 The 10S conformation is formed by weak interactions that are easily disrupted by electron microscopy preparative conditions.9,43. Molecules were therefore crosslinked in solution at room temperature for 1 min with 0.1% glutaraldehyde followed by quenching with 100 mM Tris pH 8.0, immediately before grid preparation.14,38,39. This stabilizes the 10S conformation, without substantially altering the structure of the molecules.39,43,44. Five microlitres of the diluted and crosslinked sample were applied to a carbon-coated electron microscopy grid and negatively stained with 1% (w/v) uranyl acetate.7,45. Grids,pretreated with UV light to optimize stain spreading, were imaged on an FEI Tecnai Spirit transmission electron microscope at 120 kV with a 2K × 2K CCD camera.

Cryo-EM

A thawed aliquot of smooth muscle myosin was freshly diluted to 0.2 μM and crosslinked for 1 min as described above. A 3-μl droplet was applied to a freshly glow-discharged ultrathin 0.2-nm carbon film on a lacey carbon 300-mesh copper support grid (Ted Pella). Glow discharge was carried out for 60 s at 25 mA on a PECLCO easiGlow (Ted Pella). The grid was blotted for 6 s at pressure setting 5 then vitrified in liquid ethane cooled by liquid nitrogen, using a Vitrobot Mark IV (FEI/ThermoFisher) operated at 10 °C and 95% relative humidity.

Data acquisition

Grids were screened on a Talos Arctica (ThermoFisher) cryo-EM at 200 kV. Optimal regions of selected grids were then imaged (SerialEM 3.8.047) on a Titan Krios transmission electron microscope (Thermo Fisher) at 300 kV, using a K3 direct electron detector (Gatan) in counting mode and a Gatan GIF Quantum energy filter with a slit width of 20 eV. A total of 10,950 movies were collected at a nominal magnification of 105,000× in nanoprobe EFTEM super-resolution mode yielding a physical pixel size of 0.83 Å at the specimen level. Each movie contained 20 frames over 1.6 s exposure time, with a dose rate of 18.45 e−Å⁻²−s⁻¹. The nominal defocus range was set from −1.2 to −3.5 μm.

Data processing

Each frame in a movie was aligned to correct beam-induced motion using MotionCor244 with 5 × 5 patches and a B-factor of 150, and then summed with a binned pixel size of 0.83 Å per pixel. The defocus of each image was determined using CTFIND4.44. The subsequent image processing was carried out using RELION 3.0.40. 10,604 images were selected for further image processing after removing images with defocus larger than 3.5 μm. The 10S myosin molecules consist of a folded tail region, which is flexible, and relatively rigid heads.39

Atomic fitting

A refined atomic model of the interacting heads motif,45 based on chicken gizzard smooth muscle myosin (PDB: 1I84), was docked into the electron microscopy density map by rigid-body fitting using UCSF Chimera 1.14.42. The two motor domains, the two regulatory domain α-helices, and the four light chains were each docked individually. To fit the tail regions of the reconstruction, human β-myosin subfragment 2 (PDB: 2FXM) was used to create a homology model for the turkey gizzard smooth muscle myosin amino acid sequence (residues 853–954) using the SWISS-MODEL server. This model was docked into segment 1 by rigid-body fitting using UCSF Chimera. There is currently no atomic model for segments 2 or 3. These two segments were modelled using the α-carbon backbone (poly-A model) of segment 1 (that is, after removing side chains). The residues were then numbered by assigning E1355 to hinge 2. This assignment is based on measurements made from negative stain 2D-class averages and is therefore subject to uncertainty, which could be 2–3 amino acids (3–4.5 Å) either side of residue 1535.

The resulting model (consisting of the two heavy chains and four light chains) was subjected to multiple cycles of real-space refinement using Phenix 1.11.13 followed by manual modification of the model in Coot 0.8.9.2 EL.54. The map was sharpened using the auto-sharpen map tool in Phenix. The resulting map was used to improve the fitting of the model, as some of the bulky side chains became resolved. A real-space refinement was done with the improved model and the resulting PDB entry was deposited. Figures were made using UCSF Chimera (contour levels are indicated in figure legends) and PyMOL (https://www.pymol.org).

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.
Extended Data Fig. 1 | Imaging and processing of 10S myosin II. a. Raw cryo-EM image. Circles indicate individual molecules; red, face view; blue, edge view. This is one of 10,950 micrographs recorded at 300 kV on the Titan Krios, and is representative of those showing the particles most clearly. A preliminary set of 400 micrographs from a different set of grids was first recorded on a Talos Arctica at 200 kV, producing a similar, initial reconstruction, at 9 Å resolution.

b. Gold-standard Fourier shell correlation (FSC) curve using half maps; global resolution estimate is 4.3 Å by the FSC 0.143 criterion.

c. Typical 2D class averages of 10S myosin (25 of a total of 43 good class averages, representing a total of 260,360 particles). Edge views show poor definition of the longer end of the tail (stars), corresponding to mobility in the upper part of the reconstruction (Extended Data Fig. 2).

d. Enlarged class average showing main features in reconstruction. BH/FH = blocked/free head; Seg = segment number.
Extended Data Fig. 2 | Resolution and atomic fitting of 10S structure.

a, b, Front and rear views of density map (contour level 0.0125), showing estimated local resolution according to RESMAP51. Resolution of the heads is highest in the MDs (especially the BH), and lower in the RDs (especially the FH), corresponding to local regions of varied mobility. Resolution in the tail is best where it is stabilized by contacts with other domains (seg2 with the BH MD and ELC), especially in the specific α-helix making the contact. The tail regions at the top are noisy and of low density (Extended Data Fig. 1c). c, d, Docking of the refined model to the map shown at high contour cutoff (0.025; cf. Fig. 2a), revealing clear secondary structure and quality of fit (front, rear views respectively). e–g, Fitting to show map quality. e, β-strand 249-254 of the BH MD, showing side-chain density. f, α-helix 431-443 in the BH MD, showing side-chain density. g, Coiled-coil in seg2 contacting the BH MD (1425-1491), showing 5.4 Å α-helical pitch (cf. d).
Extended Data Fig. 3 | Comparison of free and blocked heads.

**a**, Superposition of BH (red) and FH MDs (green) using Matchmaker in Chimera. There is an almost perfect match, including the converter domains (Cnv), which show no more than ~5 Å movement, suggesting that the two heads are in the same biochemical state. Orientation of heads is that seen in front view of BH.

**b, c**, Alignment of MDs of BH and FH, oriented as attached to actin in rigor state. There is a large difference in angle between the BH and FH RDs with respect to their MDs in both longitudinal (**b**) and azimuthal planes (**c**) (defined with respect to plane of filament sliding). BH and FH were aligned by superposing their MDs on the MD (not shown) of mammalian actomyosin in the rigor state (PDB 5H53). RLCs and ELCs have been removed for clarity.

**d**, Face-view of IHM shows how C termini of the two heads (P849), at bottom of RDs, come within ~28 Å of each other where they meet S2 (not shown). This proximity depends on the differential flexing of the RDs with respect to the MDs in the two heads. If the FH had the BH RD angle, the C termini in the IHM would be too far apart (~58 Å) to join to the 20 Å-diameter S2 without its substantial uncoiling. The angle of the FHRD is the major structural difference that brings the C termini of the two heads close enough together to make their simultaneous attachment to S2 possible. Comparison of RD angles was made by superposing the BH and FH MDs.

**e**, Comparison of isolated regulatory domain structures in BH and FH (LCs omitted for clarity) after aligning residues K823-P849 (the left half of the molecule). The C-terminal hooks make similar ~90° angles with the RD helix. There is a small difference in angle between the FH and BH helices in the N-terminal half of the RD heavy chain.

**f**, Comparison of regulatory domain structures within IHM. The BH RD was superimposed on the FH RD in the N-terminal half. The straighter course of the FH RD brings the FH and BH C termini that attach to S2 closer together by ~7 Å, facilitating attachment to S2 without any substantial unwinding of the coiled coil. This flexibility in the RD, bringing the FH C terminus closer to the BH, thus aids in formation of the IHM, along with the different angles of the RDs with respect to the MDs seen in **b-d**.
**Extended Data Fig. 4 | Structure of head-tail junction.**

**a**, Atomic model fitted into the electron density map (contour level 0.021) in the region of the head-tail junction. The map shows tubes of density for the two hooks, which form 90° bends with the α-helical heavy chain of the RDs. Such tubular density is characteristic of α-helices in other parts of the structure. P849 is the invariant proline that marks the junction between each head and the tail. View is from front.

**b**, IHM front-view showing hooks at C-terminal end of each RD. ELCs and RLCs removed for clarity.

**c**, Enlargement of hook region showing ~28 Å distance between invariant prolines at the C terminus of each head heavy chain.

**d**, IHM face-view showing α-helical backbone of BH and FH RDs, hooks, and coiled-coil tail regions. Seg1 α-helices (red, green) continue from BH and FH hooks.
Extended Data Fig. 5 | Variations in the coiled-coil of 10S myosin. a, Oblique view of back of reconstruction. Red arrows indicate relatively regular crossovers of coiled-coil (~60-75 Å apart) as segment 2 wraps around the BH MD, and a long, parallel (untwisted) stretch in segment 3 after leaving hinge 2 (~100 Å; white bar). b, Atomic model of tail showing approximate distance between crossovers in coiled coil. Purple residues (numbered) are estimated to be at crossovers as seen in this face view, with distances between them shown. E1535-E1612 is an almost straight, non-coiled region of the tail, especially the green chain. The two heads would connect to the bottom of seg1 at L850. L850-T889 represents the first ~5 heptads of S2 (black star), which appear to associate with each other from the start, though with a slightly longer helical pitch in the first few residues. The two α-helices are shown in different colours for clarity. Their specific connection to the BH or FH is unknown (apart from seg1) due to lack of continuity of density in the top half of the reconstruction. Blue region in seg2 is M1462-K1472 (see text). c, Side-view of reconstruction showing the long stretch of untwisted coiled-coil in segment 3, running over the BH. Yellow star here and in a shows position of skip residue 1592. d, Hinge 2, in face- and end-views, showing continuity of coiling of the two α-helices about each other through this sharp bend, with local melting of α-helices likely at the bend. Glu1535 (spheres) is thought to mark the hinge point, although uncertainty in measurement of negative stain images means that the hinge could occur 2-3 amino acids either side of 1535. For this reason, numbering of amino acids in segs 2 and 3 in the atomic model is uncertain to the same degree. Density maps shown at contour level of 0.016.
Extended Data Fig. 6 | Comparison of segment 1 position in filament and 10S molecule. The different position of seg1 in the IHM of the tarantula filament and the 10S molecule is illustrated by comparing the PDBs of the best fits (tarantula filament, PDB 3JBH; 10S molecule, PDB 6XE9 [this work]).

a, b, Front and rear views of IHM in which the head regions (filament, tan; 10S, blue) have been superposed (using Matchmaker in Chimera). c, d, Same views as for a, b, but with heads removed for clarity. Seg1 is yellow for the filament (bent conformation) and pink for the molecule (straight). The two segments run in different positions, ~ 20 Å apart, centre to centre. Strikingly, the position occupied by seg1 in the filament is taken by seg3 in the 10S molecule (yellow/blue overlap).

e, f, The different seg1 positions are also clear when the filament (red) and 10S (blue) maps are compared. Front and rear views confirm that seg1’s in the two reconstructions are laterally displaced from each other. Similar results are obtained for two independent filament maps: EMD-1950 (shown) and EMD-6512.

[Images of IHM comparisons with filament and 10S structures superposed]
Extended Data Fig. 7 | Mechanisms of converter domain and actin-binding inhibition in BH and FH. a–g, Converter domain inhibition. a, Atomic model of 10S structure. Both heads are in the ADP.Pi state (see text). b, 10S model, with a myosin head in ADP state (PDB 3I5F, yellow heavy chain) superimposed on the BH by matching motor domains. The RD in this nucleotide state is straight and the Cnv (pink) is in a very different location from the BH Cnv (purple), clearly clashing with seg2. c, d, Detail of this clash in front and end views, with other parts of molecule removed. This comparison shows that for the BH to lose its Pi (going to the ADP state), its Cnv will clash with seg2. We conclude that seg2 acts as a physical barrier (reinforced by the mechanical restraint created by the connection of both lever arms to S2), preventing this transition and inhibiting BH ATP turnover. e, 10S model, with ADP-state head (PDB 3I5F) superposed on the FH MD. f, g, Detail of e, with LCs removed for clarity. The ADP-state RD and its Cnv (pink) are to the right (red arrow) of the ADP.Pi-state FH. This comparison suggests that for the FH to lose its Pi, the interaction of its Cnv with the BH MD must be broken. We propose that this is inhibited in the 10S structure by the strength of this interaction (BF), reinforced by FH interactions with the tail (TF1, TF2 and TF3). Together these interactions would prevent the ADP.Pi→ADP transition and inhibit FH ATP turnover. h–k, Actin-binding inhibition. The BH and FH were attached to actin by superposing their MDs on the MD (not shown) of mammalian actomyosin in the rigor state (PDB 5H53), as described in Extended Data Fig. 3. Segments 2 and 3 were removed for clarity. The modelling shows that attachment via both BH and FH is inhibited due to major steric clashes of other parts of the 10S structure with actin. h, Inhibition of binding via the FH. Front view of IHM shows that S2 clashes (dashed circle) with actin (2 monomers shown). i, Rotated 90° around vertical axis with respect to h. j, Inhibition of binding via the BH. Front view of IHM shows that S2 and the FH both clash with actin (dashed circles). k, Rotated 90° around vertical axis with respect to j.
Extended Data Fig. 8 | Proposed mechanism of 10S myosin inhibition and activation, based on the atomic model and MD simulations. Smooth and nonmuscle myosin IIIs are activated by phosphorylation of their RLCs on S19, leading to breaking of the 10S intramolecular interactions, unfolding to the extended structure, and assembly into functional filaments. Our atomic model indicates a possible mechanism. Our previous work suggested that the single interaction most critical to the folded conformation is that occurring between seg3 and the BH RLC, and we noted how proximity of BH RLC S19 might regulate this interaction. Our atomic model suggests that seg3 in fact contacts the BH RLC at two sites. One is the C-lobe (TB5, Fig. 3a, g, and panel e above). The other involves the 24-residue N-terminal extension of the RLC, the phosphorylation domain (PD)\(^1\), containing S19 (interaction TB6, Fig. 3a). The PD is not observed in structures of the myosin head, but has been modelled by molecular dynamics simulations (a(i)), dephosphorylated PD, ribbon and surface charge depictions; red, negatively charged; blue, positively charged; upper box, PD sequence, MLCK binding site green; S19, yellow; N-terminal half positively charged\(^1\). Our EM map reveals significant density (b, red rectangle), extending from F25, that fits this PD (b shows best fit of model from a(i) to BH PD density) and lies over seg3, below TB5 (red rectangles in b, showing fitting; c, model based on fit; e, zoomed-out model). In the atomic model (e), interaction occurs between positively charged residues of the PD N-terminal half and a negatively charged patch (-1560-1572) in seg3\(^2\) (d, red rectangle; surface charge depiction; red, negatively charged; blue, positively charged; see interaction TB6 in Supplementary Table 1), which could strengthen TB5 (e). There is also significant density for a portion of the FHPD (b, green rectangle), which fits residues 20-24, while the positively charged N-terminal half (a) fits weak density near to negatively charged residues of BH RLC helix B (b-e, green rectangles). This would strengthen interaction BF2 between the RLCs. These interactions involving the RLCs, especially the BH PD with seg3, appear to be the key features creating the off state, supported by the other interactions already described. The structural basis of unfolding upon S19 phosphorylation remains unknown due to the absence of the PD in previous structures. The apparent PD densities we observe suggest the following model (h). Phosphorylation appears to occur first on the FH, then the BHF. EPR and molecular dynamics simulations suggest that phosphorylation causes straightening and stiffening of the PD\(^2\) (a: i. dephosphorylated, ii. phosphorylated, iii. transition, dephosphorylated → phosphorylated). When the unphosphorylated PDs (compact in our map; e, h, stage I in the activation sequence) are replaced by the phosphorylated (straightened) conformations (grey helices in f, g, using PD structures from a(ii)), the FHPD interaction with BH RLC helix B is removed (due to straightening and to the reduction in positive charge), which could weaken the RLC-RLC and thus head-head interaction (f, FH RLC phosphorylated, purple rectangle\(^5\)), releasing the FH, while retaining the folded tail structure (h, stage 2). When the BH is also phosphorylated, straightening/stiffening of its PD, and reduction in its positive charge, breaks its interaction with seg3 (g, red arrow, yellow rectangle; h, stage 3). With weakening of these interactions, seg2 could dissociate from the BH MD and ELC, leading to complete unfolding to the 6S structure (h, stage 4). In support of this proposal, replacement of charged amino acids near S19 in the RLC PD showed that unfolding upon phosphorylation may be due to net negative charge reduction of the PDs\(^6\). This physical model suggests that the two PDs with their phosphorylation sites, and the associated regions of seg3, represent a localized structural confluence in which the key events of activation and deactivation take place (the “phosphorylation zone”, e-g). We tested the PD structure suggested by the MD simulations (in the case of the BH) by examining the sharpened map in this region and manually creating a model with the PD sequence to best fit the map using Coot (panel (i) above; viewing angle changed slightly from b to best show density and model features). The density clearly suggests a short helix followed by a loop and a second helix, with density present for the entire length of the PD. This is the first time that the PD has been directly visualized, as it is disordered in isolated myosin heads. We suggest that it is the binding of the PD to seg3 (occurring only in the 10S structure) that makes this visualization possible. The atomic model based on this fitting broadly supports the bent, helix-loop-helix conformation suggested by the MD simulations of the unphosphorylated PD (a(i)). The model (panel b) suggests that basic residues K11, K12, and R13, close to acidic residues D1565 and E1566 in seg3, electrostatically hold seg3 in the folded conformation—the most crucial interaction of the 10S structure—and in close proximity to the regulatory S19. MD simulations suggest that phosphorylation creates a salt bridge between phosphorylated S19 and R16, which causes the PD loop to become α-helical, straightening and stiffening the PD as a whole\(^7\). As discussed above, we propose that it is this straightening, and the reduction in positive charge, that cause the dissociation of seg3 from the PD, leading to unfolding and activation of the 10S structure as a whole (a, g, h). From the model it is not clear whether the BH RLC would be fully available for binding by MLCK in the 10S structure. Importantly, even if sufficiently exposed, the interaction of K11-R13 with seg3 could slow binding by MLCK, as these residues are also involved in MLCK substrate recognition\(^5\). If such hindrance occurs, this would be consistent with the proposal\(^7\) that BH phosphorylation, occurring after FH phosphorylation (b), is the final, required step for activation and unfolding.
Extended Data Fig. 9 | Locations of disease-causing mutations in 10S structure. 

**a.** Overview of 10S molecule showing distribution of mutations, yellow in tail, blue in heads. 

**b-d.** Enlargements of mutation regions. 

**b.** Proximity of BH converter (Cnv, purple) and SH3 domain (pink) to seg2 mutations, and of BH SH3 and MD mutations to seg2. 

**c.** Mutations in segs 1, 2, and 3. V1529 is near hinge 2 and could impact hinge function. R1570 is part of the proposed interaction region of the BH RLC PD on seg3, and could impact RLC function. M860–E866 is a duplication, which could translate seg1, impacting its interactions downstream. 

**d.** Mutations in seg1 coincident with TT2 interaction. See Fig. 2b, Extended Data Table 2.
# Extended Data Table 1 | Cryo-EM data collection, refinement and validation statistics

| 10S smooth muscle myosin  |
|---------------------------|
| (EMDB-22145)              |
| (PDB 6XE9)                |

### Data collection and processing

| Parameter                        | Value               |
|----------------------------------|---------------------|
| Magnification                    | 105,000x            |
| Voltage (kV)                     | 300                 |
| Electron exposure (e-/Å²)        | 43                  |
| Defocus range (μm)               | -1.2 to -3.5        |
| Pixel size (Å)                   | 0.415               |
| Symmetry imposed                 | C1                  |
| Initial particle images (no.)    | 1,765,220           |
| Final particle images (no.)      | 260,360             |
| Map resolution (Å)               | 4.3                 |
| FSC threshold                    | 0.143               |
| Map resolution range (Å)         | 4.1 – 9.5           |

### Refinement

| Parameter                        | Value               |
|----------------------------------|---------------------|
| Initial model used (PDB code)    | 1I84, 2FXM          |
| Model resolution (Å)             | 4.4                 |
| FSC threshold                    | 0.143               |
| Model resolution range (Å)       | 4.0–4.6             |
| Map sharpening B factor (Å²)     | 40                  |
| Model composition                |                     |
| Non-hydrogen atoms               | 22,081              |
| Protein residues                 | 2,949               |
| Ligands                          | 0                   |
| B factors (Å²)                   |                     |
| Protein                          | 257.18              |
| Ligand                           | --                  |
| R.m.s. deviations                |                     |
| Bond lengths (Å)                 | 0.03                |
| Bond angles (°)                  | 0.488               |
| Validation                       |                     |
| MolProbity score                 | 2.19                |
| Clashscore                       | 20.98               |
| Poor rotamers (%)                | 0.77                |
| Ramachandran plot                |                     |
| Favored (%)                      | 94.60               |
| Allowed (%)                      | 5.40                |
| Disallowed (%)                   | 0.00                |
Extended Data Table 2 | Locations of disease-causing mutations in smooth and nonmuscle myosin II

| Mutated residue* | Gene/myosin type† | MYH11 equivalent‡ | Location§ | Potential interaction partner(s)‖ | Ref. |
|------------------|-------------------|------------------|-----------|----------------------------------|-----|
| W33, V34, P35    | MYH9/NMII-A       | W36, V37, P38    | SH3       | BH-Seg2/FH-none                  | 17  |
| N83, A95, S96    | MYH9/NMII-A       | N96, A98, S99    | MD, near SH3 | BH-Seg2/FH-none             | 17  |
| N97              | MYH10/NMII-B      | N96              | MD, near SH3 | BH-Seg2/FH-none             | 55  |
| S120             | MYH14/NMII-C      | S99              | MD, near SH3 | BH-Seg2/FH-none             | 17  |
| S237 (DAN)       | MYH11/SMM         | S245             | Switch 1  | BH-none/FH-none               | 38  |
| R247 (MUS)       | MYH11/SMM         | R253             | Near switch 1 | BH-Seg3/FH-BH CM loop       | 56  |
| K373             | MYH9/NMII-A       | K386             | MD        | BH-FH/FH-none                  | 17  |
| G376             | MYH14/NMII-C      | G363             | MD        | BH-none/FH-none               | 17  |
| R501             | MYH11/SMM         | R507             | Switch 2  | BH-none/FH-BH C-loop          | 57  |
| W512 (DAN)       | MYH11/SMM         | W512             | Relay loop | BH-Seg2/FH-none             | 58  |
| R702, R705, Q706 | MYH9/NMII-A       | R715, R718, Q719 | Near SH1 helix | BH-Seg2/FH-none     | 17  |
| R709             | MYH10/NMII-B      | R715             | Near SH1 helix | BH-Seg2/FH-none     | 55  |
| R718             | MYH9/NMII-A       | R731             | Converter | BH-none/FH-BH C-loop          | 17  |
| L722             | MYH14/NMII-C      | L711             | Near SH1 helix | BH-Seg2/FH-none     | 55  |
| R726             | MYH14/NMII-C      | R715             | Near SH1 helix | BH-Seg2/FH-none     | 17  |
| M847-E853dup     | MYH9/NMII-A       | M860-E866dup     | Seg1      | Seg1-none                     | 17  |
| E908             | MYH10/NMII-B      | E914             | Seg1, ring 1† | Seg1-Seg3/Seg1-FH         | 17  |
| K910             | MYH9/NMII-A       | K923             | Seg1, near ring 1,2‡ | Seg1-Seg3/Seg1-none    | 17  |
| R933             | MYH14/NMII-C      | K922             | Seg1, near ring 1,2‡ | Seg1-Seg3/FH          | 17  |
| V941             | MYH14/NMII-C      | L930             | Seg1, near ring 2† | Seg1-Seg3-Seg1-FH     | 59  |
| L976             | MYH14/NMII-C      | L965             | Seg1      | Seg1-Seg3                     | 17  |
| K1044            | MYH11/SMM         | K1044            | Seg1, upper* | Seg1-Seg2,3***              | 57  |
| K1048-E1054del   | MYH9/NMII-A       | K1061-E1067del   | Seg1, upper# | Seg1-Seg2,3***              | 17  |
| G1055-Q106Bdel   | MYH9/NMII-A       | G1068-Q1081del   | Seg1, upper* | Seg1-Seg2,3***              | 17  |
| E1066-A1072del/dup | MYH9/NMII-A     | E1079-A1085dup   | Seg1, upper# | Seg1-Seg2,3***              | 17  |
| E1084del         | MYH9/NMII-A       | E1087del         | Seg1, upper# | Seg1-Seg2,3***              | 17  |
| V1092-R1162del   | MYH9/NMII-A       | L1105-R1175del   | Seg1, upper# | Seg1-Seg2,3***              | 17  |
| S114             | MYH9/NMII-A       | S1127†           | Seg1, upper# | Seg1-Seg2,3***              | 17  |
| T1155, R1162, R1165 | MYH9/NMII-A    | T1168, R1175, R1178 | Hinge 1# | Segs1,2-Seg3**          | 17  |
| T1162            | MYH10/NMII-B      | T1168            | Hinge 1# | Segs1,2-Seg3**          | 17  |
| L1205-Q1207del   | MYH9/NMII-A       | L1218-Q1220del   | Seg2, upper# | Seg2-Seg2,3***              | 17  |
| L1287 (DAN)      | MYH11/SMM         | L1295            | Seg2, upper# | Seg2-Seg1,3***              | 38  |
| R1400            | MYH9/NMII-A       | Q1413            | Seg2, lower | None                       | 17  |
| D1424            | MYH9/NMII-A       | D1437            | Seg2, lower | Seg2-BH SH3                 | 17  |
| Q1443-K1445dup   | MYH9/NMII-A       | Q1456-K1458dup   | Seg2, lower | Seg2-BH converter          | 17  |
| D1447            | MYH9/NMII-A       | D1460            | Seg2, lower | Seg2-BH converter          | 17  |
| V1516            | MYH9/NMII-A       | V1529            | Hinge 2   | None                       | 17  |
| R1557            | MYH9/NMII-A       | R1570            | Seg3, near BH | Seg3-BH RLC PD       | 17  |
| I186             | MYH9/NMII-A       | I1829            | Seg3, upper# | Seg3-Seg2,3***              | 17  |
| E1841            | MYH9/NMII-A       | D1854            | Seg3, upper# | Seg3-Seg2,3***              | 17  |
| G1924, D1925, P1927 | MYH9/NMII-A     | G1937, N1938, P1940, | Seg3, non-helical tailpiece* | None | 17  |
| V1930, R1933, M1934, | MYH9/NMII-A     | A1944, R1948, M1949, | Seg3, non-helical tailpiece* | None | 17  |
| D1941, E1945     | MYH9/NMII-A       | D1960, E1964     | Seg3, non-helical tailpiece* | None | 17  |

The table lists mutations in nonmuscle myosin II A, Ib, Ic and smooth muscle myosin II that have been associated with disease17,38,55–59, together with regions of the molecule in close proximity to the mutations. The table and Fig. 2b, c demonstrate that many of the mutations occur close to regions we have identified as sites of intramolecular interaction in the 10S molecule. This suggests that mutation could alter the structure or stability of the folded conformation (see text).

*Location of mutation in nonmuscle or smooth muscle myosin II sequence. DAN, zebrafish; MUS, mouse; all others, human.

†Myosin heavy chain gene/myosin type (nonmuscle myosin: NMII-A, B, C; smooth muscle myosin: SMM).

‡Equivalent residue number in chicken smooth muscle myosin (MYH11) after sequence alignment (Supplementary Table 2).

§Location is the approximate domain location (for heads) or position in tail. Mutations can occur in FH and BH and in both chains of the tail.

‖The first symbol shows which head (BH, FH) or segment (1, 2, 3) contains the mutation; the second symbol, after the hyphen, shows the nearby domains/potential interaction partners. After the "/", the same notation applies to the other head or segment. Because the two heads have different environments, some mutations might affect BH but not FH interactions and vice versa. These potential interactions are mapped onto the 10S structure in Fig. 2b, c and Extended Data Fig. 9. BH, FH and segments are colour-coded to correspond to Fig. 2b, c. Our criteria for "nearby domains/potential interaction partners" are distances of 3.5 to ~ 10 Å. ~1/3 of these are in the 3.5-5 Å range; mutation sites >5 Å from a partner (not close enough for actual contact) would still impact local conformation and possibly alter nearby interactions.

¶Rings 1 and 2 refer to negatively charged regions (905-916 and 932-946, respectively) on segment 1.

#Upper region of tail, not included in reconstruction (Figs. 1a, 2c).

**Although the upper regions of segments 1, 2 and 3 are not in the reconstruction, they lie close together in negative stain images‡, and appear likely to interact with each other.
Reporting Summary

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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

- Data collection: SerialEM 3.8.0
- Data analysis: MotionCor2 1.2.2, CTFFIND 4.1.13, RELION 3.0, ResMap 1.95, UCSF Chimera 1.14, Phenix 1.17.1, Coot 0.8.9.2 EL, PyMOL 2.3.4

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data availability. Structural data that support the findings of this study on the structure of 10S myosin II have been deposited in the Worldwide Protein Data Bank (wwPDB) under accession codes EMD-22145 (the EM density map) and PDB 6XE9 (the atomic model). PDB data used to build the initial model were PDB 1J84 and PDB 2FXM.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Statutory tests were not a part of this study and the sample size was not predetermined. Sample size (number of images and particles used) was determined by the time available on the electron microscope. The number of images recorded was 10,950 and the number of particles that went into the final reconstruction was 168,613. This number was sufficient to provide a high resolution (4.3 Å) structure, which was the goal of the study. |
| Data exclusions | The following exclusions were pre-established. Micrographs that had a defocus > 3.5 um were excluded. Particles were removed if their 2D class averages didn't show secondary structure or if their 3D reconstruction had low resolution. |
| Replication | Two imaging sessions were carried out, on different microscopes at different accelerating voltages. The first, on a Talos Arctica, produced a reconstruction at 9 Å resolution. The second, from a new set of grids, was on a Titan Krios and produced a 4.3 Å resolution reconstruction. The latter reconstruction reproduced the former, but with more detail. |
| Randomization | No statistical comparisons were made in this study, and there was only one experimental group: myosin molecules in the 10S conformation. Therefore randomization was not applicable. |
| Blinding | Blinding was not relevant to this study as there were no groups used for comparison. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
| ☒ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology and archaeology |
| ☒ | Animals and other organisms |
| ☒ | Human research participants |
| ☒ | Clinical data |
| ☒ | Dual use research of concern |

Methods

| n/a | Involved in the study |
| ☒ | ChiP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |