Structure of human cytoplasmic dynein-2 primed for its power stroke

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Members of the dynein family, consisting of cytoplasmic and axonal isoforms, are motors that move towards the minus ends of microtubules. Cytoplasmic dynein-1 (dynein-1) plays roles in mitosis and cellular cargo transport1, and is implicated in viral infections2 and neurodegenerative diseases3. Cytoplasmic dynein-2 (dynein-2) performs intraflagellar transport4 and is associated with human skeletal ciliopathies5. Dyneins share a conserved motor domain that couples cycles of ATP hydrolysis with conformational changes to produce movement6–8. Here we present the crystal structure of the human cytoplasmic dynein-2 motor bound to the ATP-hydrolysis transition state analogue ADP.vanadate9. The structure reveals a closure of the motor’s ring of six AAA+ domains (ATPases associated with various cellular activites: AAA1–AAA6). This induces a steric clash with the linker, the key element for the generation of movement, driving it into a conformation that is primed to produce force. Ring closure also changes the interface between the stalk and buttress coiled-coil extensions of the motor domain. This drives helix sliding in the stalk which causes the microtubule binding domain at its tip to release from the microtubule. Our structure answers the key questions of how ATP hydrolysis leads to linker remodelling and microtubule affinity regulation.

There are four nucleotide-binding sites in the dynein motor, but movement only depends on ATP hydrolysis in the first site (AAA1)7,11,12. When this site is nucleotide free or bound to ADP, the microtubule binding domain (MTBD) binds to the microtubule and the linker adopts the straight post-power-stroke conformation6–8,12–14. Upon ATP binding and hydrolysis, the MTBD detaches from the microtubule and the linker is primed into the pre-power-stroke conformation8,12,13,14 (Fig. 1a). MTBD rebinding leads to a force producing swing of the linker (power stroke) back to the post-power-stroke position and the release of ATP hydrolysis products to reset the cycle12,14–16.

To address how the linker is primed and dynein released from microtubules, we co-crystallized the human dynein-2 motor domain with ADP.vanadate (ADP.Vi) to trap it in a pre-power-stroke state1 (Extended Data Fig. 1 and Extended Data Table 1). The linker in this dynein-2:ADP.Vi structure has a 90° bend (Fig. 1b) consistent with low-resolution studies of pre-power-stroke dynein13,14,17. Dynein’s AAA+ domains are each divided into an αβ ‘large’ subdomain (AAAL, helices H0–H4 and beta strands S1–S5) and an α ‘small’ subdomain (AAAS, helices H5–H9)18. The individual subdomains of dynein-2:ADP.Vi are highly similar to those in post-power-stroke crystal structures of dynein-1 from Saccharomyces cerevisiae19 (dynein-1:APO; Protein Data Bank (PDB) accession number 4AKJ) and Dictyostelium discoideum20 (dynein-1:ADP; PDB accession number 3VKG) (Extended Data Fig. 2 and Supplementary Data 1). This suggests conformational changes between these structures (Supplementary Discussion and Extended Data Fig. 3a) are not related to sequence differences but are caused by the different nucleotide states.

In dynein-2:ADP.Vi, all four nucleotide-binding sites are occupied (Fig. 1c). The AAA1 site, found between AAA1 and AAA2, binds ADP.Vi (Fig. 2a) and Extended Data Fig. 4a–d) with conserved motifs16 (Fig. 2b). The trigonal–bipyramidal vanadate group mimics the ATP γ-phosphate during hydrolysis20. It is surrounded by three important catalytic residues16: the Walker B glutamate (W-B: E1742), the sensor-I asparagine (S-I: N1792) and the AAA2L arginine finger (RF: R2109), suggesting the structure is in the ATP hydrolysis–competent conformation.

In the dynein-1 structures there is a gap between AAA1 and AAA2. The closure of this gap in dynein-2:ADP.Vi (Supplementary Video 1) is driven by the arginine finger–ADP.Vi contact. It is reinforced by additional interactions between AAA1L and AAA2L (Fig. 2c). A pair of conserved inserts in AAA2L20 (the ‘H2 insert’ and the ‘pre-sensor-I’ (PS-I) insert) contact the H2 helix in AAA1L (Fig. 2a, c) and displace H2 and H3 relative to the rest of AAA1L (Extended Data Fig. 5a). The AAA1L sensor-I loop, which varies in position depending on dynein’s nucleotide state (Extended Data Fig. 5b), swings in to contact AAA2L (Fig. 2c).

The other nucleotide-binding sites contain tightly bound nucleotides that co-purify with the motor domain (Extended Data Fig. 4e–j and Supplementary Discussion). The density in AAA2 is consistent with an ATP, as observed in all the dynein structures13,18. As in dynein-1:ADP, the densities in AAA3 and AAA4 suggest the presence of ADP. In the dynein-1:APO structure these sites are empty. In all dynein structures

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The AAA2 and AAA3 nucleotide-binding sites are in a similar closed conformation. This means that the whole AAA2–AAA4 region forms a rigid block (Extended Data Fig. 5c, d).

The linker, which is divided into four subdomains13,18, bends between subdomains 2 and 3 in the pre-power-stroke dynein-2:ADP.Vi structure. Compared with the straight post-power-stroke linker, the mobile subdomains 1 and 2 (Link1–2, helices H5–H9) undergo a rigid-body movement relative to the static subdomains 3 and 4 (Link3–4, helices H11–H18) (Fig. 3a and Supplementary Video 2). The hinge helix (H10), which connects Link1–2 and Link3–4, is forced to adopt a curved conformation. The isolated linker prefers a straight conformation8, suggesting that the whole AAA2–AAA4 region forms a rigid block (Extended Data Fig. 5c, d).

In all dynein structures, the static Link3–4 is connected to the AAA ring via contacts to AAA1 (Extended Data Fig. 6a–c). The closure of the AAA1 site in dynein-2:ADP.Vi establishes two additional interactions (Fig. 3b). The AAA2L PS-I insert contacts the loop between H11 and H12 on Link3 via a backbone interaction. The displacement of AAA1L (Fig. 3b) precludes the AAA1L sensor-I loop contacting AAA2L. Purple spheres represent contacts.

Figure 2 | ADP.Vi binding to AAA1 nucleotide-binding site induces closure of AAA1/AAA2 interface. a. AAA1L, AAA1S and AAA2L enclose ADP.Vi. The AAA2L H2 and PS-I inserts (red) contact AAA1L H2. b. Upper panel: the Mg$^{2+}$-ADP contacts the Walker A (W-A: K1695), Walker B (W-B: D1741) and the sensor-II (S-II: R1867) residues. The trigonal-bipyramidal Vi-group mimics the ATP-hydrolysis transition state and is surrounded by sensor-I (S-I: N1792) and Walker B (W-B: E1742) residues and the AAA2L arginine finger (RF). Lower panel: schematic diagram showing the distances from ADP.Vi to the catalytic residues (in Å). c. AAA1/AAA2 interface closure is reinforced by the AAA1L sensor-I loop contacting AAA2L. Purple spheres represent contacts.

We had anticipated that the movement of the mobile part of the linker would be driven by its interaction with the highly conserved inserts in AAA2L13,14,18. It was therefore surprising to find that these inserts contact only the static part of the linker. How then could AAA1 site closure induce linker bending? To address this question we asked what would happen if the AAA + ring adopted the ADP.Vi state but the linker remained in the straight, post-power-stroke conformation. The rigid-body behaviour of AAA2–AAA4 means that closure of the AAA1 site would lead to a steric clash between the mobile Link1–2 region and the AAA4L PS-I insert (Fig. 3e). This is demonstrated by the overlap between these regions observed in an alignment of the straight linker from dynein-1:ADP onto the dynein-2:ADP.Vi structure (Fig. 3f). The additional contacts between the AAA + ring and the static Link3–4 (Fig. 3f) prevent it moving and mean that the clash can only be relieved by the mobile Link1–2 adopting its pre-power-stroke position (Fig. 3e, g and Supplementary Video 3).

To test this model we used negative-stain electron microscopy to assay linker movement (Fig. 3h, i and Extended Data Fig. 8a, b). In the presence of ADP all dynein-2 motors had an angle between the stalk and linker of $54 \pm 13^\circ$ (mean $\pm$ s.d.) (Fig. 3h). In the presence of ADP.Vi the majority of motors showed a pre-power-stroke conformation with an angle of $145 \pm 20^\circ$. We then tested the ability of dynein-2 mutants to adopt the pre-power-stroke state in the presence of ADP.Vi (Fig. 3i). Removal of the AAA2L inserts abolished the linker movement, consistent with previous data19. It also completely prohibited microtubule gliding activity (Extended Data Fig. 8c). When the AAA4L PS-I insert was deleted only a small percentage of motors attained the pre-power-stroke formation. The isolated linker prefers a straight conformation8, suggesting that the whole AAA2–AAA4 region forms a rigid block (Extended Data Fig. 5c, d).
conformation (Fig. 3i), supporting our model that the AAA4L PS-I insert plays a major role in linker bending. In agreement with this interpretation, the microtubule gliding velocity of this mutant was only 10% of wild type (Extended Data Fig. 8c). In contrast, removal of the Link1–2 contacts with AAA2L and AAA3L had a minimal effect on linker movement (Fig. 3i).

In addition to triggering movement of the linker, ADP.Vi binding to dynein reduces the affinity of its MTBD for microtubules21. Biochemical22,23 and structural14,18,24–26 evidence suggests this involves the helices in the stalk, coiled-coil helix 1 (CC1) and coiled-coil helix 2 (CC2), sliding past each other by one turn of α-helix. The dynein-2:ADP.Vi structure, where the MTBD has low microtubule affinity (Extended Data Fig. 9)

Figure 3 | Linker bending upon closure of AAA1 nucleotide-binding site. a, A 90° bend between linker subdomains 1 and 2 (Link1–2) and 3 and 4 (Link3–4) forces the hinge helix (H10) to curve. b, The AAA2L PS-I insert contacts Link3 and AAA1L R1726 forms a salt bridge with E1420 on the hinge helix. c, Hydrophobic residues (yellow) stabilize the Link2/Link3 bend. d, Link1–2 contacts the AAA2:H2 and AAA3L:H2–S3 inserts. e, AAA1 site (blue/cyan) closure causes a rigid-body movement of AAA2–AAA3–AAA4 (cyan–green–yellow) leading to a clash (black star) with Link1–2 (light pink). To relieve the clash, the linker adopts the pre-power-stroke conformation. f, The straight post-power-stroke linker (grey), aligned via Link3–4 onto dynein-2:ADP.Vi, would clash with the AAA4L PS-I insert (yellow spheres). g, In dynein-2:ADP.Vi the linker moved to avoid the clash. h, In a negative-stain electron microscopy assay, the angle between the stalk (yellow) and green fluorescent protein (GFP)–linker (green/purple) of the dynein-2:ADP motor is 54° ± 13° (mean ± s.d.). With ADP.Vi most dynein-2 motors are in a pre-power-stroke state with an angle of 145° ± 20° (mean ± s.d.). i, Deletion of the AAA2L (AAA2L:H2 + PS-I) or AAA4L inserts (AAA4L:PS-I) hinders the linker adopting the pre-power-stroke conformation with ADP.Vi. Mutation of either AAA3 (AAA3L:H2–S3) or AAA2 (R1413A + E2028A) linker-ring contacts has no effect. Dashed half-circles in h and i mark 20% dynein-2 particles. All negative-stain electron microscopy experiments were done in triplicate. The number of particles used in each experiment are provided in Methods.
and Supplementary Discussion), answers the key question of how the sliding is initiated.

In dynein-2:ADP.Vi the base of the stalk deviates from the symmetrical, regular coiled coil observed in dynein-1:ADP (Fig. 4a). The stalk CC2 helix contains a kink, located near the stalk/buttress interface, which causes it to slip relative to CC1. The resulting asymmetry between the two helices is similar to that observed in the parallel coiled-coil homodimer Acanthamoeba castellanii (AC1)–AC1. A comparison of the dynein-1:ADP and dynein-2:ADP.Vi structures (Fig. 4b) suggests how the movement of the buttress, relative to the stalk, is coupled to the movement of CC2. In dynein-2:ADP.Vi the buttress slides relative to CC1 but moves together with CC2.

The stalk and buttress emerge from AAA4S and AAA5S respectively. Their relative movement is coupled to rearrangements in the AAA+ ring. Closure of the AAA1 site and the rigid body movement of AAA2–AAA4 force the AAA4/AAA5 interface to close and the AAA6L subdomain to rotate towards the ring centre (Fig. 4b and Supplementary Discussion). The AAA5S subdomain rotates as a unit together with AAA6L, and this movement pulls the buttress relative to the stalk (Supplementary Videos 4 and 5).

Unlike myosin and kinesin motors, dynein shares mechanistic similarities with AAA+ proteins that remodel their substrates29. In dynein, one substrate is the linker which is bent by a clash with the AAA+ ring. This bent conformation is stabilized by contacts at the Link2/Link3 interface, the importance of which is highlighted by the fact that a mutation there (G1442D) can cause the human ciliopathy Jeune syndrome30 (Supplementary Discussion). When the AAA1 site reopens, the bent linker reverts to its preferred straight conformation31 and generates force. In addition to the linker, the dynein AAA+ ring also remodels the stalk. Here the motions of AAA+ domains are directly coupled to sliding of helices in the coiled coil (Supplementary Video 6).

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions R.Z. and H.S. screened many dynein species for expression and crystallization. R.Z. expressed human dynein-2 in insect cells, obtained crystals in the presence of vanadate and collected data. H.S. phased the structure and built an initial model. A.P.C. built and refined the structure. R.Z. and H.S. made mutants and performed biochemical assays. L.U. performed negative-stain electron microscopy. H.S. and R.Z. prepared the manuscript.

Author Information Coordinates and structure factors have been deposited in the Protein Data Bank under accession number 4HR7. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.P.C. (cartera@mrc-lmb.cam.ac.uk).

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METHODS
Cloning of constructs. DNA sequence coding for a variant of human cytoplasmic dynein-2 isofrom 1 (GenBank reference number BAB60721), codon-optimized for expression in Spodoptera frugiperda (Sf9) cells, was amplified (coding region D0191–Q4307) using Phusion High-Fidelity DNA Polymerase (New England Biolabs). The primers used for construct amplification contained sites homologous to a pFastBac vector (Invitrogen Life Sciences Technologies) that had been modified to contain a tobacco etch virus protease cleavable tandem Protein A tag for purification followed by a GFP. InFusion (Clontech Laboratories) was used to insert the dynein-2ΔAAA1L–AAA3L#8689 into the pFastBac vector. The final construct used for crystallization, electron microscopy and microtubule gliding assays had an amino (N)-terminal GFP, followed by a glycine (G) serine (S) spacer and dynein-2ΔAAA1L–AAA3L#8689 (GFP–dynein-2ΔAAA1L–AAA3L#8689). All mutants were prepared by standard cloning techniques using GFP–dynein-2ΔAAA1L–AAA3L#8689 in the pFastBac vector background as a template. ΔAAA2L H2 + PS-1 had regions 2022–2030 and 2074–2085 replaced by GG, ΔAAA4L PS-1 had region 2734–2774 replaced by GSGSG, ΔAAA3L H2–S3 had region 2339–2344 replaced by GG and R1413A + E2042A had R1413 and E2042 substituted by alanines. All constructs were sequence verified.

Protein expression in Sf9 cells. The modified pFastBac plasmids were transformed into a DH10 EMBacY Escherichia coli strain which carried a bacmid harbouring the baculovirus genome. Clones containing bacmids in which the pFastBac vector had been fully integrated were selected by blue white screening. Recombinant bacmids were prepared according to standard procedures, transfected into 2 ml Sf9 cells (0.5 × 10^6 cells per milliliter) using FuGENE HD Transfection Reagent (Promega) and incubated at 27 °C for 72 h (P1 virus). A milliliter of P1 virus was subsequently used to infect 50 ml of Sf9 cells (2 × 10^6 cells per milliliter) followed by incubation at 27 °C and 127 rpm for 72 h (P2 virus). Five milliliters of P2 virus were used to infect 500 ml of Sf9 cells (2 × 10^6 cells per milliliter) followed by the incubation procedure described before. Cells were harvested after centrifugation at 4 °C and 2,500g for 30 min. The pellet was washed in ice-cold PBS, snap-frozen in liquid nitrogen and stored at −80 °C.

Protein purification. Frozen pellets were resuspended in lysis buffer (30 mM HEPES pH 7.4, 50 mM KOAc, 2 mM MgOAc, 0.2 mM EGTA, 10% v/v glycerol, 300 mM KCl, 0.2 mM MgATP, 1 mM DTT and 2 mM PMSF). Resuspended cells were lysed manually in a dounce homogenizer. Cell debris and insoluble proteins were removed by ultracentrifugation at 4 °C and 600,000g for 30 min. Dynein constructs were pulled out from the lysate using IgG sepharose beads (GE Healthcare, 5 ml of beads per litre of Sf9 culture). IgG sepharose beads were washed with 15 bead volumes of lysis and tobacco etch virus protease buffer (30 mM Tris HCl pH 8, 150 mM KOAc, 2 mM MgOAc, 1 mM EGTA, 10% v/v glycerol, 300 mM KCl, 0.2 mM MgATP, 1 mM DTT and 2 mM MgATP). Protein was released from the beads during overnight cleavage with tobacco etch virus protease. Size-exclusion chromatography was performed on a Superose 6 column (GE Healthcare) in size-exclusion chromatography buffer (20 mM Tris HCl pH 8.0, 100 mM KOAc, 2 mM MgOAc, 1 mM EGTA, 10% v/v glycerol, 1 mM DTT).

Protein crystallization. Peak fractions of GFP–dynein-2ΔAAA1L–AAA3L#8689 after size-exclusion chromatography were pooled and concentrated to 8 mg mL−1. To lock dynein in its pre-power-stroke state, MgATP (Sigma Aldrich) and Na3VO4 (New England Biolabs) were added at a final concentration of 3 mM each. Crystals were obtained by hanging-drop vapour diffusion at 19 °C. Crystals were collected as described above. Diffraction data were obtained by synchrotron radiation at beamline I02 at the Diamond Light Source. The data were collected at 100 K on beamline I02 at the Diamond Light Source. The data were integrated using MOSFLM and scaled using AIMLESS5. In the case of the anisotropic Native-1 data set, a first round of integration and scaling was performed with a resolution limit of 3 Å. The data were then subsequently analysed with the University of California, Los Angeles Molecular Biology Diffraction Anisotropy Server (http://services.mbi.ucla.edu/anisoscale/), which suggested resolution cutoffs of α = 4.2 Å, β = 4.4 Å and c = 3.4 Å. The second round of data integration and scaling was done with the resolution cutoffs mentioned above (Extended Data Table 1). Phasing was performed with AUTOARAP32 using the multiple isomorphous replacement with anomalous scattering (MIRAS) approach with a low-resolution Native-2 data set and the Na+ [PW12O40] peak and inflection data sets as heavy atom derivatives (Extended Data Table 1). The final electron-density map after density modification was of sufficient quality to identify the location of all dynein-2 subdomains in the asymmetric unit. Homology models for the individual subdomains were obtained by combining PDB accession number 3VKG with the amino-acid sequence of human cytoplasmic dynein-2 isofrom 1 using the PHYRE server35. The homology models were placed in the asymmetric unit followed by iterative rounds of refinement in REFMAC53 against the Native-1 data set, employing the ‘isotype’ secondary structure restraints ‘refinement options, and manual rebuilding in COOT54. Refining the model against the anisotropy-corrected data obtained from the University of California, Los Angeles Molecular Biology Diffraction Anisotropy Server significantly improved the quality of the resulting electron-density maps. The final model was evaluated by calculating a simulated annealing composite omit map in CNS and had 99.9% of the residues in the allowed regions of the Ramachandran plot. All figures were prepared using PYMOL (http://www.pymol.org), IGLPLO7 and Jalview55.

Vanadate-mediated ultraviolet-photo cleavage of dynein-2 crystals. Crystals obtained under the conditions described above were harvested and washed three times in 10 μl reservoir solution followed by exposure to ultraviolet light (254 nm) for 1 h. Crystals were subsequently dissolved in sample buffer, boiled for 10 min at 95 °C and analysed by SDS–polyacrylamide gel electrophoresis.

Nucleotide content analysis. Nucleotides were extracted from dynein-2 samples that had been purified as described above. Nucleotide extraction was performed essentially as described previously39. Briefly, concentrated protein was precipitated by adding HClO4 to a final concentration of 0.5 M. The sample was vortexed and centrifuged for 10 min at 4 °C and 14,000g. The supernatant was mixed with 1 M K2HPO4, 3 M KOH and 100% acetic acid (final concentrations were 125 mM, 375 mM and 0.5 M respectively). Total nucleotide content and protein concentration before extraction were measured using a NanoDrop ND-1000 spectrophotometer.

Negative-stain electron microscopy. Dynein motor domain constructs were purified as described above and diluted into electron microscopy assay buffer (50 mM Tris HCl pH 8, 150 mM KOAc, 2 mM MgOAc, 1 mM EGTA and 0.1 mM DTT) to a final concentration of 30 nM. Negative-stain electron microscopy was performed either in the presence of 3 mM MgADP (ADP) or 3 mM MgATP and 3 mM Na3VO4 (ADP.Vi) on plasma-cleaned carbon film on 400-square mesh copper grids (Electron Microscopy Sciences). The samples were stained with 2% (w/v) uranyl acetate. Electron micrographs (Extended Data Fig. 8a) were recorded on a Gatan Orius SC200B CCD (charge-coupled device) fitted to a FEI Tecnai G2 Spirit transmission electron microscope operating at 120 kV. Data were collected at ~1 nm underfocus, with a pixel size of 3.2 Å and an estimated dose of 20 electrons per square angstrom during 1 s exposures. Automated particle picking was done in aMicroGrid using the Swc641 software (http://www.cryooultramicroscopy.com/software/index.php). Autopicked particles were subjected to two-dimensional classification to identify incorrectly picked particles, which were manually checked and removed from the data set. The remaining particles were classified into ten classes, which was sufficient to represent all observed views. Each class was then subclassified into 50 subclasses. Noisy subclasses were discarded and those remaining contained sufficient signal to noise to identify the stalk and linker–GFP clearly (Extended Data Fig. 8b). The ImageJ azimuthal average plugin (http://rsb.info.nih.gov/ij/plugins/azimuthal-average.html) was used to integrate the intensity values surrounding the outside of the motor domain with 1 bin width. This generated a plot with two peaks corresponding to the intensity for the stalk and the GTPase-binding site of two Gaussian functions (Jgor Pro 6.3, http://www.wavemetrics.com/products/products.htm) was used to measure the angle between all. All experiments were performed in triplicate. The angle distribution, using a 10 bin width, was visualized by either histogram or rose plot. The number of particles used were as follows: WT + ADP: 3151, 9914, 7534; WT + ADP + Vi: 8710, 5793, 5284; AAA2L H2 + PS-1 8664, 4308, 9220; AAAAAL PS-19835, 10799, 9955; R1413A + E2042A: 7994, 2445, 2445, 11581; AAAAAL H2–S3: 6353, 12185, 6399.

Microtubule gliding assays. A microtubule gliding assay was adapted from previously published work29. Briefly, anti-GFP antibody (Roche) was non-specifically bound to the glass surface of a flow chamber. The free surface was blocked with 1% (w/v) HEPES pH 7.2, 2 mM MgOAc, 1 mM EGTA, 10% (v/v) glycerol, 1 mM MgATP and 20 μM mATP. GTP-bound dynein-2 was then applied and after 30 s incubation washed with assay buffer. Finally, motility buffer that contained microtubules, oxygen scavenging system and 1 mM ATP as an energy source was applied and gliding was observed by total internal reflection fluorescence microscopy. All data analysis used ImageJ55.

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Extended Data Figure 1 | Examples of the electron density quality in dynein-2:ADP.Vi.  $2F_o - F_c$ electron density in different parts of dynein-2:ADP.Vi. Amino-acid side-chains are clearly resolved in the linker (a), AAA1 (b), AAA4 (c) and AAA6 (d). Only the main-chain could be traced in the stalk (e) and the buttress (f). The electron density in a–d was map-sharpened. The contour level is $1\sigma$, except for e which was contoured at $0.75\sigma$. 
Extended Data Figure 2 | Structural similarity between individual subdomains of dynein-1 and dynein-2. Alignment of individual subdomains from dynein-2:ADP Vi and dynein-1:ADP (PDB accession number 3VKG). 

**a**, Alignment of AAA+ large (AAA1L-AAA6L) subdomains and the linker subdomains (Link1–2, Link3–4).

**b**, Alignment of individual AAA+ small subdomains (AAA1S–AAA6S) and the C-terminal domain. Dynein-2 subdomains are coloured according to the scheme used in the main text, and shown in the inset cartoons. Dynein-1 subdomains are shown in grey.

Calculated root mean squared deviation (r.m.s.d.) values are shown above each alignment and demonstrate that the subdomains of dynein-2 are structurally highly similar to dynein-1. The AAA+ ring subdomains with the largest r.m.s.d. differences are AAA1L and AAA1S. These subdomains are the most strongly conserved part of the dynein structure and the differences are probably due to the ADP Vi binding. The distortion of AAA1L, by its interaction with the AAA2L inserts, is described in the main text.
Extended Data Figure 3 | Closed interfaces between AAA+ domains of the AAA+ ring in dynein-2:ADP.Vi. a, Gaps in the AAA+ rings of different dynein motor domain crystal structures. In dynein-1:apo (PDB accession number 4AKI) and dynein-1:ADP (PDB accession number 3VKG) there are gaps between AAA1L/AAA2L and AAA5L/AAA6L or AAA4L/AAA5L. In dynein-2:ADP.Vi a smaller gap exists between AAA5L/AAA6L. Gaps are indicated by black arrows. b, Calculated buried surface areas indicate that the interfaces between AAA1/AAA2, AAA2/AAA3, AAA3/AAA4, AAA4/AAA5 and AAA6/AAA1 are tightly closed in dynein-2:ADP.Vi (buried surface areas 1,059–1,706 Å²). The AAA5/AAA6 interface is more open (buried surface area 837 Å²). Nucleotides are shown in stick representation. AAAL, AAA+ large subdomain; AAAS, AAA+ small subdomain.
Extended Data Figure 4 | The four nucleotide binding sites of dynein-2:ADP.Vi. a–c. The AAA1 site contains electron density consistent with an Mg.ADP.Vi molecule. All catalytic amino-acid residues have the correct conformation to support catalysis. d. Photo cleavage of washed dynein-2 crystals upon exposure to ultraviolet light (+UV) produces two bands of 300 and 90 kDa (arrowheads). This suggests crystals contain an ADP.Vi group in AAA1. e, f. The AAA2 site contains density consistent with a Mg.ATP molecule. g, h. The AAA3 and AAA4 sites contain electron density that is best modelled as ADP. In contrast to AAA1, AAA2–AAA4 have lost the catalytic residues necessary for ATP hydrolysis (the Walker B glutamate, the arginine finger, sensor-I and sensor-II motifs). The $F_o - F_c$ electron density (a, e, g, i) is contoured at 3σ. The $2F_o - F_c$ electron density (c) is contoured at 1σ. W-A, Walker A motif; W-B, Walker B motif; S-I, sensor-I; S-II, sensor-II; RF, arginine finger. Magnesium ions (Mg$^{2+}$) are shown as green spheres. The vanadium ion of the vanadate molecule (Van) is shown as a pink sphere.
Extended Data Figure 5 | Changes in conformation within dynein AAA+ ring. 

**a**, Superimposition of the AAA1L domains of dynein-2:ADP.Vi (blue) and dynein-1:ADP (grey) shows that helices H2 and H3 of AAA1L are displaced when the H2-$β$ hairpin insert of AAA2L (red) comes into contact with H2 of AAA1L. **b**, An alignment of the AAA1L domains of dynein-2:ADP.Vi (blue), dynein-1:APO (PDB accession number 4AKI) (pale yellow) and dynein-1:ADP (PDB accession number 3VKG) (grey) shows that the loop containing the sensor-I residue is highly variable between the structures. In the presence of ADP.Vi the loop makes contacts (purple spheres) with AAA2L. **c, d**, Superimposition of AAA2–AAA4 domains between dynein-2:ADP.Vi and dynein-1:ADP (c) or dynein-1:APO (d) shows that AAA2–AAA4 move as a rigid body.
Extended Data Figure 6 | Linker interaction with the AAA+ ring in dynein-2:ADP.Vi, dynein-1:ADP and dynein-1:APO. a–c, Link3–4 interacts with AAA1L in all structures similar. Mainly hydrophobic contacts exist between the linker H11 helix and the H2 helix as well as the S2 β-sheet of AAA1L. In addition the long peptide that connects the linker with AAA1 (yellow) mediates contacts between Link3–4 and AAA1L. d, Link1–2 is stabilized by contacts with AAA2 and AAA3 in dynein-2:ADP.Vi Link1–2, e, by contacts with the AAA2 H2 insert in dynein-1:ADP and f by contacts with AAA5 in dynein-1:APO. Red spheres represent contacts.
Extended Data Figure 7 | Conservation of contact sites between linker and dynein ring. Multiple alignment of cytoplasmic dynein-1 (Cyt-1), dynein-2 (Cyt-2), axonemal inner arm dyneins (IDA) and outer arm α (ODAα) and β (ODAβ) dyneins. Dyneins are from human (Hs), Chlamydomonas reinhardtii (Cr), Tetrahymena thermophila (Tt), D. discoideum (Dd), S. cerevisiae (Sc), Drosophila melanogaster (Dm), Emericella nidulans (En) and Candida albicans (Ca). Residues are shaded by conservation, with dark blue being the most conserved. Red asterisks mark hydrophobic contacts that stabilize the bent linker conformation, black asterisks mark the contact site between AAA2L H2 insert (E2028) and the linker (R1413) and green asterisks mark poorly conserved contacts between the linker and the AAA3 H2-S3 insert.
Extended Data Figure 8 | Characterization of dynein-2 mutants by negative electron microscopy and microtubule gliding assays. 

**a**, Representative micrographs showing the quality of the raw electron microscopy data. Scale bar, 20 nm. 

**b**, Left, histograms showing distribution of angles between the linker and the stalk in three replicate negative-stain electron microscopy experiments (10° bin width); right, representative subclasses used for angle measurement. 

**c**, Mean velocities of dynein-2 mutants in microtubule gliding assays.

GFP–dynein-2<sub>D1091–Q4307</sub> (wild type: WT) glides microtubules at 134 ± 8 nm s<sup>-1</sup> (N = 99). The microtubule gliding velocities for the other constructs are ΔAAA2L H2–PS-I, 59 ± 4 nm s<sup>-1</sup> (N = 79); K1413A + E2028A, 49 ± 2 nm s<sup>-1</sup> (N = 31); and ΔAAA4L PS-I, 14 ± 1 nm s<sup>-1</sup> (N = 121). Microtubule gliding was not observed in case of ΔAAA2L H2 + PS-I. Error bars, s.e.m.
Extended Data Figure 9 | The MTBD in dynein-2 ADP.Vi is in the low microtubule affinity conformation. a, b, Alignment of dynein-2 ADP.Vi MTBD (pale yellow) with dynein-1 MTBDs (grey) in the low microtubule affinity conformation (PDB accession numbers 3ERR and 3WUQ respectively), and c with a dynein-1 MTBD in the high microtubule affinity conformation (PDB accession number 3J1T). The stalk CC1 and the MTBD H1 undergo conformational changes depending on the microtubule affinity of the MTBD. In dynein-2:ADP.Vi the arrangement of these structural elements suggests the MTBD is in the low microtubule affinity conformation. Stalk CC1 and MTBD H1 are coloured blue in low-affinity structures and red in high-affinity structures.
Extended Data Table 1 | Data collection, phasing and refinement statistics

| Dataset | Native-1 | Native-2 | Na₃[PW₁₁O₄₀] |
|---------|----------|----------|---------------|
| Space group | C222₁ | C222₁ | C222₁ |
| Cell dimensions | 136.0, 487.2, 276.5 | 136.2, 487.7, 276.9 | 135.7, 481.9, 276.5 |
| a, b, c (Å) | Peak | Inflection |
| Wavelength (Å) | 0.97949 | 0.97949 | 1.21416 | 1.21476 |
| Resolution (Å) | 56.5-3.40 | 56.1-6.0 | 65.9-6.0 | 69.5-6.0 |
| R_sym or R Qaeda | 10.1 (69.2)* | 6.3 (17.7) | 24.0 (111.6) | 36.1 (170.6) |
| l/σl | 7.6 (1.1) | 207.2 (28.4) | 8.0 (2.4) | 5.9 (1.7) |
| Completeness (%) | 62.2 (1.9)§ | 94.9 (99.6) | 99.8 (99.9) | 99.6 (100.0) |
| Redundancy | 4.1 | 3.6 | 11.7 | 10.0 |
| Refinement | | | |
| Resolution (Å) | 56.6-3.41 | | |
| No. reflections | 74060 | | |
| R_work/R_free | 23.7/28.5 | | |
| No. atoms | 22816 | | |
| Protein | 22697 | | |
| Ligand/ion | 119 | | |
| Water | - | | |
| B-factors | | | |
| Protein | 122.0 | | |
| Ligand/ion | 69.8 | | |
| Water | - | | |
| R.m.s deviations | Bond lengths (Å) | 0.012 | | |
| Bond angles (°) | 1.55 | | |

*Highest resolution shell is shown in parenthesis.

§The low completeness to 3.4 Å is due to the anisotropic diffraction limits (a= 4.2 Å, b= 4.4 Å, c= 3.4 Å). When the data is scaled to a resolution limit of 4.2 Å the overall completeness is 97.9% and the completeness in the highest resolution shell is 78.4%.