Structure of the dynein-2 complex and its assembly with intraflagellar transport trains

Katerina Toropova1, Ruta Zalyte2, Aakash G. Mukhopadhyay1, Miroslav Mladenov1, Andrew P. Carter2 and Anthony J. Roberts1,*

Dynein-2 assembles with polymeric intraflagellar transport (IFT) trains to form a transport machinery that is crucial for cilia biogenesis and signaling. Here we recombinantly expressed the 1.4-MDa human dynein-2 complex and solved its cryo-EM structure to near-atomic resolution. The two identical copies of the dynein-2 heavy chain are contorted into different conformations by a WDR60–WDR34 heterodimer and a block of two RB and six LC8 light chains. One heavy chain is steered into a zig-zag conformation, which matches the periodicity of the anterograde IFT-B train. Contacts between adjacent dyneins along the train indicate a cooperative mode of assembly. Removal of the WDR60–WDR34–light chain subcomplex renders dynein-2 moneric and relieves autoinhibition of its motility. Our results converge on a model in which an unusual stoichiometry of non-motor subunits controls dynein-2 assembly, asymmetry, and activity, giving mechanistic insight into the interaction of dynein-2 with IFT trains and the origin of diverse functions in the dynein family.

Cilia and flagella are constructed by an intricate motor-protein-driven process of IFT1. Dynein-2, the ubiquitous motor for retrograde IFT, is crucial for cilia biogenesis2–5 and the essential roles of these organelles in cell propulsion, signaling, and sensing environmental stimuli6. Dynein-2-powered movement of cargo along the cilium’s microtubules enables dynamic remodeling of the ciliary proteome. For example, dynein-2 exports G-protein-coupled receptors out of the cilium, enabling proper signal transduction7,8, and also has emerging roles9,10, including in the organization of the ciliary transition zone11,12. Loss of dynein-2 is embryonically lethal in mammals13, and its mutation is associated with a group of developmental disorders arising from defects in ciliary signaling, encompassing short-rib thoracic dysplasias with or without polydactyly14–22. The most striking features of dynein-2 are its stoichiometry and assembly with IFT trains and the origin of diverse functions in the dynein family.

Dynein-2 operates in the context of IFT ‘trains’: linear polymers of IFT-A and IFT-B complexes, which bind cargoes either directly9 or via associating factors such as the BBSome8. Dynein-2 assembles with IFT trains at the ciliary base, moves to the tip in an inhibited state under the power of kinesin-II, then restructures to drive retrograde transport back to the cell body34–37. How the fundamental recognition event between dynein-2 and the IFT train at the base of the cilium is achieved is unclear. Dynein-2 is one of the three major classes of dynein. The other two major classes drive intracellular transport (cytoplasmic dynein-1) and ciliary beating (multiple axonemal isoforms)38,39. Biochemical studies indicate that dynein-2 is a >1-MDa complex with a distinctive composition, incorporating three types of light chain (LC) shared with other dyneins40–44 (DYNLRB, DYNLL, DYNLT; referred to here as RB, LC8, and TCTEX) and five components specific to dynein-2: the motor-domain-containing heavy chain45–47 (DYN2H1; here ‘DHC2’), light intermediate chain48,49 (DYN2LI1; here ‘LIC3’), light chain50–52,54–56 (TCTEX1D2), and two different intermediate chains53,54,57 (WDR60 and WDR34) (Supplementary Table 1). In the absence of structural information, the roles of the multiple dynein-2 subunits in regulation of motor activity and IFT train recognition have been unclear58.

Here, we recombinantly expressed the complete dynein-2 complex and determined its structure to near-atomic resolution using cryo-EM. We show how an unusual stoichiometry of non-motor subunits brings together two copies of the dynein-2 heavy chain, controls their activity, and contorts them into an architecture with extreme asymmetry that matches the periodicity of the IFT-B train. Together, these results give insight into the molecular mechanism of IFT and the origin of diverse functions in the dynein family.

Results

Dynein-2 cryo-EM structure determination. We purified the human dynein-2 complex by co-expressing all of its subunits from a single baculovirus in insect cells (Supplementary Fig. 1a–d) and determined its structure using cryo-EM (Fig. 1). Initial EM analysis revealed that the dynein-2 ‘tail’ domain flexed by as much as 50° and 250 Å relative to its motor domains, displaying a continuum of positions with a favored kink to one side (Supplementary Fig. 1e,f and Supplementary Video 1). Despite this large-scale flexibility, we found that focused classifications on the tail excluding the motor domains yielded detailed averages (and vice versa) (Supplementary Fig. 2), suggesting that the tail and motor domains pivot largely as rigid bodies about a hinge point near their junction. We were therefore able to determine cryo-EM structures of the tail and motor domains at average resolutions of 4.5 Å and 3.9 Å, respectively, with varying local resolution (as depicted in Supplementary Fig. 2f,g). Further local refinements within the tail yielded maps at 4.4 Å. We built a model of the dynein-2 tail using Rosetta comparative modeling11–44 and de novo tracing (Table 1 and Supplementary Fig. 3), and we refined the crystal structure of the motor domain as a monomer41 into the dimer conformation (Supplementary Fig. 3c), thus revealing the architecture of the dynein-2 complex.

Dynein-2 has subunit copy numbers ranging from one to six. The most striking features of dynein-2 are its stoichiometry and asymmetry (Fig. 1 and Supplementary Video 2). The subunit

1Institute of Structural and Molecular Biology, Birkbeck, University of London, London, UK. 2Medical Research Council Laboratory of Molecular Biology, Division of Structural Studies, Cambridge, UK. *e-mail: a.roberts@mail.cryst.bbk.ac.uk

NATURE STRUCTURAL & MOLECULAR BIOLOGY | VOL 26 | SEPTEMBER 2019 | 823–829 | www.nature.com/nsmb
copy number in the complex ranges from one to six, contrasting with dynein-1, which is built from homodimers. The two copies of DHC2 span the complex, each comprising a compact N-terminal domain, an elongated tail region, and a C-terminal AAA+ motor domain. Whereas one copy of DHC2 is straight in the tail region, the other is in a zig-zag conformation. Both copies of DHC2 bind a LIC3 subunit. The two intermediate chains, WDR60 and WDR34, form a heterodimer. Their C-terminal β-propeller domains bind DHC2, and their N-proximal regions are held together by an array of light chains consisting of one RB dimer and three LC8 dimers. We attribute a flexible, weaker density (Supplementary Fig. 4) to the TCTEX−TCTEX1D2 dimer identified in biochemical studies. The presence of three LC8 dimers bridging WDR60 and WDR34 is unexpected, as the binding motifs for only one dimer had been predicted using the most sensitive computational searches. Indeed, the additional motifs deviate from the consensus sequence (Supplementary Fig. 4a), suggesting that LC8 binding to these sites is individually weak and enhanced by avidity in the complex. Whereas the stoichiometry of dynein-2 is unexpected, its structural organization is in agreement with previous studies highlighting DHC2−LIC3 and WDR60−WDR34−RB−LC8−TCTEX−TCTEX1D2 as separable, interacting subcomplexes (Fig. 1).

Two identical heavy chains of dynein-2 adopt different conformations. The two copies of DHC2 show a contrasting mixture of symmetric and asymmetric regions (Fig. 2a). The motor domains...
intrinsically exists in a regulated state ready for anterograde trans-

...suggesting that the dynein-2 complex DHC2TAIL are composed of a series of

...port. The compact N-terminal domains also have C2 symmetry. In

...pack against each other with 180° rotational (C2) symmetry, forming the autoinhibited conformation first visualized in 2D and at ~40°-resolution in situ, suggesting that the dynein-2 complex intrinsically exists in a regulated state ready for anterograde transport. The compact N-terminal domains also have C2 symmetry. In contrast, the intervening tail region (DHC2TAIL) is highly asymmet-

...structure. The propensity of DHC2 to dissociate from dimer to monomer is

...extracts, suggesting that weak dimerization is a conserved property of the dynein-2 heavy chain. DHC2TAIL was flexible in ΔIC–LC, adopting a range of different curvatures (Supplementary Note 3). Motor activity of ΔIC–LC was unregulated, whereas the holoenzyme bound microtubules weakly and exhibited slow (~140 nm/s) microtubule gliding, consistent with the majority of complexes being in an autoinhibited state, ΔIC–LC drove rapid (~530 nm/s) microtubule movement, similar to the isolated motor domain and the velocity of retrograde IFT in mammalian cells (Fig. 3e and Supplementary Video 3). Together,
these data indicate that the IC–LC block (i) brings together two copies of DHC2; (ii) stabilizes their autoinhibited form; and (iii) breaks their symmetry, sculpting their tails into straight and zig-zag conformations.

Asymmetric structure of dynein-2 matches the periodicity of the anterograde IFT-B train. How do dynein-2’s subunits enable it to assemble with IFT trains? To address this question, we docked our structures of the dynein-2 complex into a subtomogram average of the anterograde IFT-B train from *C. reinhardtii* at 37-Å resolution. The distinctive shape of the dynein-2 tail and motor domains yielded an unambiguous fit. The angle between the dynein-2 tail and motor domains in the IFT-train-binding configuration lies within the range of angles observed in the isolated molecule. Docking successive dynein-2 complexes along the long axis of the train revealed how they interdigitate and interact with one another. For example, there is a major contact between LIC3 of one dynein-2 complex and the motor domain of its neighbor. The principal contacts between dynein-2 and the train all involve the heavy chain, DHC2, rather than the non-motor subunits. Moreover, they specifically involve the zig-zag heavy chain (DHC2-B), which matches the periodicity of the IFT-B polymer.

Fig. 3 | A block of intermediate and light chains controls dynein-2 asymmetry, oligomerization, and activity. a, Ribbon diagram of the intermediate chains WDR34 and WDR60, whose N-proximal extensions are held together by a block of RB and LC8 light chains. Other subunits have been omitted for clarity. The translation and rotation relating the WDR34 and WDR60 β-propeller domains are indicated. A schematic (lower right) shows the LC8-binding motifs of WDR34 and WDR60. LC8 is known to be promiscuous in the peptides it can bind, but a glutamine (Q)—typically flanked by small hydrophobic or polar residues—is important for binding. All six of the LC8-binding motifs in WR60 and WDR34 contain this glutamine but are otherwise divergent in sequence. b, Exploded view of asymmetric interactions between the IC-LC block and the two copies of DHC2. Interacting residues predicted by Rosetta modeling are connected by lines and colored according to IC-LC subunit type. c, Enlargements of the boxed regions in b highlighting interactions between DHC2-A and the N-proximal region of WDR60. The cryo-EM map and fitted structural model are shown. d, Negative-stain EM class averages of the dynein-2 holoenzyme (Hol) and a mutant lacking the intermediate chains and light chains (ΔIC-LC), with corresponding cartoons. e, Plot of microtubule gliding velocity as a function of microtubule (MT) length for dynein-2 Hol (gray circles; n = 130 microtubule gliding events from three separate experiments) and ΔIC-LC (red triangles; n = 308 microtubule gliding events from three separate experiments).
Our work shows how dynein-2 uses a mixed stoichiometry of non-motor subunits to generate a radically asymmetric structure that matches its cognate partner, the IFT train. We propose a model (Fig. 4c) in which the IC−LC block brings together two copies of DHC2−LIC3 and sculpts them into different conformations. The zig-zag heavy chain recognizes the periodicity of the assembling IFT train at the ciliary base28. In mutants lacking intermediate or light chains, the heavy chain’s flexibility may enable it to reach its IFT-train-binding shape stochastically, albeit inefficiently, contributing to the impaired but non-null cilia phenotypes observed11,55.

Binding of one dynein-2 complex to the train creates extra binding surface for the next, enabling cooperative loading of dynein-2 along the train, before the entire assembly is imported into the cilium by kinesin-II28. In this model, IFT train disassembly at the ciliary tip24 would destroy the multivalent binding site for inhibited dynein-2, allowing dynein-2 to reconfigure for retrograde motility25,29. The IC−LC block stabilizes the autoinhibited state of dynein-2. It must therefore either reconfigure dramatically or dissociate33,56 during dynein-2 activation at the ciliary tip. Our structure of the dynein-2 complex may help to dissect this event. The structural principles illuminated here—namely, modular use of non-motor subunits to sculpt the large, flexible dynein heavy chains into a configuration matching their function—may be used widely in the dynein family, as axonemal dyneins powering ciliary beating are also likely to contain heteromeric intermediate chains and light chain arrays7.
Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41594-019-0286-y.

Received: 15 April 2019; Accepted: 24 July 2019; Published online: 26 August 2019

References
1. Rosenbaum, J. L. & Witman, G. B. Intraflagellar transport. Nat. Rev. Mol. Cell Biol. 3, 813–825 (2002).
2. Pedersen, B. et al. DYNC2LI1 mutations broaden the clinical spectrum of C. elegans elegans cilia. Nat. Commun. 8, 14591 (2017).
3. Tajkhorshid, E., Prevo, B., Oswald, F., Mangeol, P. & Peterman, E. J. G. The cryo-EM structure of intraflagellar transport reveals how dynein is inaccurately to inactivate unidirectional anterograde movement in cilia. Nat. Cell Biol. 20, 1250–1255 (2018).
4. Arana, M. A., Guttman, D., Stepanek, D. R., Stoppani, L. & Piggins, G. The cytoplasmic dynein-2 intermediate chain is required for retrograde intraflagellar transport in Chlamydomonas. Mol. Biol. Cell 10, 693–712 (1999).
5. Cao, M. et al. Uni-directional ciliary membrane protein trafficking by a cytoplasmic dynein-2 primed for its power stroke. Mol. Biol. Cell 29, 1628–1639 (2018).
6. Patel-King, R. S., Hsu, Y. H., Witman, G. B. & King, S. M. WD60/FAP16 intermediate chain with TCTEX1D2 light chain of the dynein-2 complex is a dynein intermediate chain required for retrograde intraflagellar transport in cilia. Mol. Biol. Cell 24, 2668–2677 (2013).
7. Kessler, M. et al. The Chlamydomonas reinhardtii BBSome is an IFT particle movement of the centrosomal cilia. J. Cell Biol. 217, 1701–1707.e2 (2018).
8. Lechtreck, K.-F. et al. The Chlamydomonas reinhardtii BBSome is an IFT motor activity in vitro. FEBS Lett. 585, 2543–2551 (2011).
9. Lechtreck, K.-F. et al. The Chlamydomonas reinhardtii BBSome is an IFT motor activity in vivo. FEBS Lett. 585, 2543–2551 (2011).
10. Rosenthal, J. et al. Single-molecule turnarounds of intraflagellar transport at the ciliary tip. Cell Reports 25, 1701–1707.e2 (2018).
11. Vuolo, L., Stevenson, N. L., Heesom, K. J. & Stephens, D. J. Dynein-2 intermediate chains play crucial but distinct roles in primary cilia formation and function. eLife 7, e39655 (2018).
12. Lechtreck, K.-F. et al. The Chlamydomonas reinhardtii BBSome is an IFT motor activity in vivo. FEBS Lett. 585, 2543–2551 (2011).
13. King, S. M. Axonemal dynein arms. Cold Spring Harb. Perspect. Biol. https://doi.org/10.1101/cshperspect.a028100 (2016).
14. Chien, A. et al. Dynamics of the IFT machinery at the ciliary tip. eLife 6, e28606 (2017).
15. Toropova, K., Mladenov, M. & Roberts, A. J. Intraflagellar transport dynein is autoinhibited by trapping of its mechanical and track-binding elements. Nat. Struct. Mol. Biol. 24, 461–468 (2017).
16. Yi, P., Li, W.-J., Dong, M.-Q. & Ou, G. Dynein-driven retrograde intraflagellar transport is triphasic in C. elegans sensory cilia. Curr. Biol. 27, 1448–1461.e7 (2017).
17. Mijalkovic, I. et al. The role of the dynein light intermediate chain in the Hippo pathway. Nat. Cell Biol. 20, 2668–2677 (2013).
18. Deck-Peterson, S. L., Redwine, W. B., Vale, R. D. & Carter, A. P. The cytoplasmic dynein-2 transport machinery and its many cargoes. Nat. Rev. Mol. Cell Biol. 19, 382–398 (2018).
19. Rompolas, P., Pedersen, L. B., Patel-King, R. S. & King, S. M. Dynein-2 intermediate chain is required for ciliogenesis. Dev. Cell 28, 828 (2010).
20. Rosenbaum, J. L. & Witman, G. B. Intraflagellar transport. Nat. Rev. Mol. Cell Biol. 828 (2007).
21. Jensen, V. L. et al. Role for intraflagellar transport in building a functional cilia. Am. J. Physiol. Cell Physiol. 292, C1001–C1008 (2007).
22. Wang, D.-J. et al. Intraflagellar transport in Chlamydomonas. Sci. Rep. 8, 754–759 (2018).
23. Taschner, M. & Lorentzen, E. The intraflagellar transport machinery. Cold Spring Harb. Perspect. Biol. https://doi.org/10.1101/cshperspect.a028692 (2016).
24. Chien, A. et al. Dynamics of the IFT machinery at the ciliary tip. eLife 6, e28606 (2017).
25. Toropova, K., Mladenov, M. & Roberts, A. J. Intraflagellar transport dynein is autoinhibited by trapping of its mechanical and track-binding elements. Nat. Struct. Mol. Biol. 24, 461–468 (2017).
54. Perrone, C. A. et al. A novel dynein light intermediate chain colocalizes with the retrograde motor for intraflagellar transport at sites of axoneme assembly in chlamydomonas and Mammalian cells. *Mol. Biol. Cell* **14**, 2041–2056 (2003).

55. Tsurumi, Y., Hamada, Y., Katoh, Y. & Nakayama, K. Interactions of the dynein-2 intermediate chain WDR34 with the light chains are required for ciliary retrograde protein trafficking. *Mol. Biol. Cell* **30**, 658–670 (2019).

56. Li, W., Yi, P. & Ou, G. Somatic CRISPR-Cas9-induced mutations reveal roles of embryonically essential dynein chains in *Caenorhabditis elegans* cilia. *J. Cell Biol.* **208**, 683–692 (2015).

57. Blisnick, T. et al. The intraflagellar transport dynein complex of trypanosomes is made of a heterodimer of dynein heavy chains and of light and intermediate chains of distinct functions. *Mol. Biol. Cell* **25**, 2620–2633 (2014).

**Acknowledgements**

We thank H. Mitchison, C. Moores, S. Webb, and G. Zanetti for comments on the manuscript; Diamond Light Source for cryo-EM facilities at the UK national electron bio-imaging centre (eBIC) supported by the Wellcome Trust, MRC and BBSRC; N. Lukoyanova, J. van Rooyen, A. Siebert and D. Clare for help with cryo-EM data collection; and D. Houldershaw for computational support. This work was funded by Wellcome Trust and Royal Society (104196/Z/14/Z), BBSRC (BB/P008348/1), and Royal Society (MC_UP_A025_1011) to A.P.C; and Wellcome Trust (079605/Z/06/Z) and BBSRC (BB/L014211/1) grants supporting cryo-EM equipment at Birkbeck.

**Author contributions**

K.T: investigation, methodology, visualization, writing of original draft. R.Z: investigation, methodology. M.M: investigation, methodology. A.G.M: investigation, writing - review and editing. A.P.C: investigation, methodology, funding acquisition, supervision, writing - review and editing. A.J.R: conceptualization, investigation, methodology, funding acquisition, supervision, visualization, writing of original draft.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Supplementary information is available for this paper at https://doi.org/10.1038/s41594-019-0286-y.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to A.J.R.

Peer review information: Katarzyna Marcinkiewicz was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature America, Inc. 2019
Methods

Expression of the dynein-2 complex. To generate a plasmid for expression of the human dynein-2 complex, the following genes were synthesized with codon optimization for insect cells (Epoch): the dynein-2 heavy chain (DNCH21), two intermediate chains (WD60 and WD345), light intermediate chain (DYNL2M1), light chains (DYNL8R1/2, DYNLI1/2, DYNLI1/3, and TCTEX1D1), and one component (LC8-like; LOC92067) that proved to not be part of dynein-2 and thus served as a control for the specificity of the complex. Alternate subunit nomenclature is in Supplementary Table 1. Using Gibson assembly, we inserted each subunit into a family of plasmids derived from pAGEBac1 (Genevat Biotech)34. Each plasmid had an expression cassette containing a polH promoter and an SV40 terminator, followed by a Swl site. Using flanking Pmel sites, expression cassettes were excised from the parent plasmid and inserted into the Swl site of another plasmid in the family using Gibson assembly. Using this strategy in a pyramid fashion (Supplementary Fig. 1a), we assembled a plasmid containing both the dynein-2 heavy chain and LC8 tag. A construct lacking the Dynein-2 complex.

Purification of the dynein-2 complex.−80 °C.

PBS, flash frozen in liquid nitrogen, and stored at

ratio of 1% (v/v) for protein production. Three days after infection, cell pellets were

DNA (2 μg), and FuGene HD transfection reagent (3 μl) were mixed and incubated for 15 min, then added drop-wise to 1 x 10^9 S9 cells growing adherently in a six-well plate with 2 ml of medium. After 3 d, the efficiency of transfection was assessed by monitoring YFP expression from EMBacY using a Countess II FL cell counter with an EVOS light cube (Thermo Fisher Scientific). The supernatant (Virus), which was added to a 50-ml S9 culture and incubated for 3 d. The resulting supernatant (Virus) was stored at 4°C and used to infect 0.25- to 4 l cultures at a ratio of 1% (v/v) for protein production. Three days after infection, cell pellets were harvested via centrifugation, washed in 1x PBS, flash frozen in liquid nitrogen, and stored at −80°C.

Purification of the dynein-2 complex. All steps were performed at 4°C. In a typical preparation, frozen cell pellets from 41 of S9 culture were resuspended in purification buffer (50 mM HEPES (pH 7.5), 100 mM KCl, 150 mM K-acetate, 2 mM Mg-acetate, 1 mM EGTA, 10% (v/v) glycerol, 1 mM DTT, 0.2 mM Mg-ATP, 1 mM EDTA, 100 μM PMSE, Roche Complete EDTA-free Protease Inhibitor Cocktail) to a total volume of 200 ml. Cells were lysed in batches using a Dounce homogenizer with 10–20 strokes with a tight clearance pestle. Lysates were clarified via centrifugation, washed in 1x PBS, flash frozen in liquid nitrogen, and stored at −80°C.

Image pre-processing. Movies were aligned, dose-weighted and summed using MotionCor2 (ref. 43). Micrographs were then visually screened, and mistargeted images containing thick carbon support in more than ~50% of the field of view and empty areas were discarded. Gctf64 was used to determine CTF parameters. Three further datasets were collected on a Titan Krios instrument equipped with a K2 Summit direct electron detector and Gatan Quantum energy filter (slit width 20 eV) (Gatan, Inc.). Micrographs were collected in counting mode with a nominal magnification of 125,000× (1.39 × pixel sampling), 18 s exposures, and 60-frame movies. The total dose per movie was 47.5 e/Å^2. Three further datasets were collected on a Titan Krios instrument equipped with a K2 Summit direct electron detector and Gatan Quantum energy filter (slit width 20 eV) at the eBIC imaging facility (Diamond Light Source, Didcot). Data collection parameters are shown in Table 1.

| www.nature.com/nsmb | NATURE STRUCTURAL & MOLECULAR BIOLOGY | www.nature.com/nsmb | NATURE STRUCTURAL & MOLECULAR BIOLOGY |

- 100 μM PMSE, Roche Complete EDTA-free Protease Inhibitor Cocktail
- to 2D classification using Relion v2.1 (ref. 60). Flexibility between the tail and motor domains was further analyzed using multivariate statistical analysis in ImageJ41.
- A total of 4,465 particles were aligned and classified with a mask encompassing the entire molecule. By inspection of the 2D class averages, a subset of particles that corresponded to the major tail view was selected and realigned based on the motor domain region. The aligned particles were then subjected to further classification using a mask that encompassed all observed tail positions but excluded the motor domains. To quantify the tail positions in the resulting 27 class averages (1,261 particles), the angle between the C2 symmetry axis in the motor domains and the dimerization domain in the tail was measured using ImageJ42. A polar plot histogram of tail angles was generated using Matlab R2017b.
- Particles of the ΔIC–LC sample were picked manually and subjected to 2D classification using Relion v2.1. To analyze flexibility in the tail, ImageJ was used to align and classify 2,827 ΔIC–LC particles based on the tail region. By inspection of the 2D class averages, a subset of particles corresponding to the major tail view was selected and subjected to further classification to analyze tail flexibility (25 class averages, 1,116 particles).

Cryo-EM. Immediately after gel filtration, the dynein-2 complex was diluted to 60–70 nM and vitrified using a Vitrobot Mark IV system (Thermo Fisher Scientific) set to 4°C and 95% humidity. A total of 2,336 particles were picked manually, divided into two 15-ml plastic conical tubes. Each tube was supplemented with 400 μl TEV buffer (as purification buffer, lacking KCl), and transferred to empty areas were discarded. Gctf64 was used to determine CTF parameters. To analyze flexibility in the tail, Imagic was used to ‘over pick’ the micrographs, yielding 757,402 initial picks, from which automated particle picking of Datasets 2 and 3. Permissive parameters were used to 2D classification using Relion (v2.0 or v2.1, used for all subsequent image processing steps unless stated). Five of the resulting 2D averages were used as templates for automated particle picking using Goutomatch (http://www.mrc-lmb.cam.ac.uk/kzhang/). The resulting 21,191 picks were subjected to 2D classification, and 11,995 particles from well-defined averages were subjected to 3D classification, using a 60-A low-pass-filtered dynein-1 tail map (EMDB-3370) as a starting model, leading to a 10-Å resolution initial model.

Projections of this 10-Å tail map were generated using SPIDER45, and eight views were used as templates for automated particle picking of Krios Dataset 1 using Goutomatch. Class averages from Dataset 1 were used as templates for automated particle picking of Datasets 2 and 3. Permissive parameters were used to ‘over pick’ the micrographs, yielding 757,402 initial picks, from which 461,684 putative particles were isolated. Following extensive rounds of 2D and 3D classification, 68,623 particles were refined to yield a 4.5 Å resolution tail reconstruction, which was sharpened using a B factor of −125 Å.

Further focused refinements were used to determine maps of three tail subregions with improved local density (Supplementary Fig. 2h–j). The first, encompassing DHC2 TAIL-A, LC3-A, LIC3-A, WD60, WDR34, and LC8, refined to 4.4 Å resolution. The second, encompassing DHC2 TAIL-A (bundles 1–5), DHC2 TAIL-B (bundles 1–3), WD60, WDR34 and RB subunits, also refined to 4.4 Å resolution. The third, encompassing DHC2 TAIL-A (bundles 1–5), DHC2 TAIL-B (bundles 1–3), LIC3-A and –B, and LC8 subunits, was subjected to 3D classification, revealing flexibility in the position of TCTEX/TCTEX1D2 density (Supplementary Fig. 4c). Particles from class 1 containing the best defined TCTEX/TCTEX1D2 and DHC2 TAIL-A densities were refined to 7.5 Å resolution. The half maps, refined maps, sharpened and masked maps, and mask used have been deposited to the EMDB.
Motor domain reconstruction. A model of the autoinhibited state of dynein-2 motor domains, derived from PDB 4RI7 (ref. 43), was low-pass filtered and projected in SPIDER. Eighty distinct views were used as templates for automated particle picking of the initial Polara dataset. The resulting particles were picked to 2D classification using Relion, and 8,313 particles from well-defined averages were selected for 3D classification. Particles from the best-defined 3D class were refined to give an 11–Å resolution initial map of the motor domains from 5,133 particles.

Six 2D averages from the Polara dataset were used as templates for automated particle picking of Krios Datasets 1–3 (Table 2), from which 229,969 putative particles were isolated. After extensive rounds of 2D and 3D classification, 57,265 particles were refined with C2 symmetry to yield a 3.9–Å resolution motor domain reconstruction, which was sharpened using a R factor of −100 Å. The half maps, refined map, sharpened and masked map, and mask used have been deposited to the EMDB.

Local resolutions in the tail and motor domain maps were calculated using Relion. Map visualization was carried out in UCSF Chimera and UCSF Chimera X.

Model building. Tail domain. RosettaCM4, COOT45, iMOD fit46, SWISS-MODEL47, RaptorX Contact48, and PSI-PRED49 software was used to build an atomic model of the tail, as detailed in Supplementary Table 2. RosettaCM was used for density-guided rebuilding, completion, and refinement of homology models. Template-target alignments were generated using HHpred50, and templates were aligned to the cryo-EM density using Chimera’s ‘Fit in Map’ command. A total of 100–300 Rosetta models were generated, and the conformation with the lowest energy (including fit-to-density energy) was used. Coordinates for all subunits were combined in the final tail model and subjected to rounds of relaxation and the density using Rosetta, real-space refinement using Phenix (phenix.real_space_refine, default parameters) and manual adjustments.

For deposition to wwPDB, the tail model was truncated to polyaniline (phenix pdbtools) to denote that the majority of side chains positions are not experimentally determined. Register shifts cannot be excluded in the peripheral lower-resolution regions of the cryo-EM map (Supplementary Fig. 2f), and none of our conclusions rest on the sequence register in these regions.

Motor domains. The crystal structure of the dynein-2 motor domain as a monomer (PDB 4RI7) was separated into rigid bodies and docked into the motor domain EM density using UCSF Chimera’s ‘Fit in Map’ command, then manually adjusted using COOT. A second copy of the motor domain was then generated and fit into the second motor domain EM density using ‘Fit in Map’. Real-space refinement was performed using Phenix (phenix.real_space_refine, default parameters).

Holoenzyme. The dynein-2 tail and motor domain structures were fitted into the subtomogram average of anterograde IFT-B trains from C. reinhardtii cilia51 using Chimera’s ‘Fit in Map’ command. The two DHC2 bundles connecting the tail and motor domain were modeled using SWISS-MODEL (template PDB 3VKG, Chain A) and RaptorX Contact (deposition as UNK).

Microtubule gliding assays. Dynein-2 holoenzyme and ΔDIC–LC samples were biotinylated for microtubule gliding assays via their N-terminal SNAPf tag as ΔDynein-2 holoenzyme and LC samples were biotinylated for microtubule gliding assays via their N-terminal SNAPf tag as ΔDynein-2 holoenzyme and biotinylated for microtubule gliding assays via their N-terminal SNAPf tag as ΔDynein-2 holoenzyme and biotinylated for microtubule gliding assays via their N-terminal SNAPf tag as ΔDynein-2 holoenzyme and biotinylated for microtubule gliding assays via their N-terminal SNAPf tag as ΔDynein-2 holoenzyme and biotinylated for microtubule gliding assays via their N-terminal SNAPf tag as ΔDynein-2 holoenzyme. Microtubule gliding velocities were calculated from kymographs generated in FIJI52. Graphing was performed in Prism5 (GraphPad).

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

Data availability
Cryo-EM maps are available from the EMDB under accession codes EMD-4918 (dynein-2 tail domain) and EMD-4917 (dynein-2 motor domains). Coordinates are available from the RCSB Protein Data Bank under accession codes PDB 6RLB (dynein-2 tail domain), PDB 6RLA (dynein-2 motor domains), and PDB 6SC2 (dynein-2, docked into subtomogram average of the anterograde IFT-B train53). All other data supporting the conclusions of this manuscript are available from the corresponding author upon reasonable request.

References
58. Vijayachandran, L. S. et al. Gene gymnastics: synthetic biology for baculovirus expression vector system engineering. Bioengineering 4, 279–287 (2017).
59. Schlager, M. A., Hoang, H. T., Urnavicius, L., Bullock, S. L. & Carter, A. P. In vitro reconstitution of a highly processive recombinant human dynein complex. EMBO J. 33, 1855–1868 (2014).
60. Scheres, S. H. W. RELION: implementation of a Bayesian approach to cryo-EM structure determination. J. Struct. Biol. 180, 519–530 (2012).
61. van Heel, M., Harauz, G., Orlova, E. V., Schmidt, R. & Schatz, M. A new generation of the IMAGIC image processing system. J. Struct. Biol. 116, 17–24 (1996).
62. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675 (2012).
63. Zheng, S. Q. et al. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. Nat. Methods 14, 331–332 (2017).
64. Zhang, K. Gctf: Real-time CTF determination and correction. J. Struct. Biol. 193, 1–12 (2016).
65. Ludl, S. J., Baldwin, P. R. & Chiu, W. EMAN: semiautomated software for high-resolution single-particle reconstructions. J. Struct. Biol. 128, 82–97 (1999).
66. Frank, J. et al. SPIDER and WEB: processing and visualization of images in 3D electron microscopy and related fields. J. Struct. Biol. 116, 190–199 (1999).
67. Petersen, E. F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612 (2004).
68. Goddard, T. D. et al. UCSF ChimeraX: Meeting modern challenges in visualization and analysis. Protein Sci. 27, 14–25 (2018).
69. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta Crystallogr. D Biol. Crystallogr. 66, 486–501 (2010).
70. Lopéz-Blanco, J. R. & Chacón, P. iMODFIT: efficient and robust flexible fitting based on vibrational analysis in internal coordinates. J. Struct. Biol. 184, 261–270 (2013).
71. Waterhouse, A. et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 46, W296–W303 (2018).
72. Wang, S., Sun, S., Li, Z., Zhang, R. & Xu, J. Accurate de novo prediction of protein contact map by ultra-deep learning model. PLoS Comput. Biol. 13, e1005324 (2017).
73. Jones, D. T. Protein secondary structure prediction based on position-specific scoring matrices. J. Mol. Biol. 292, 195–202 (1999).
74. Zimmermann, L. et al. A completely reimplemented MPI bioinformatics toolkit with a new HHpred server at its core. J. Mol. Biol. 430, 2237–2243 (2018).
75. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D Biol. Crystallogr. 66, 213–221 (2010).
76. Kon, T. et al. The 2.8 Å crystal structure of the dynein motor domain. Nature 484, 345–350 (2012).
77. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682 (2012).
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- 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 | Cryo-EM data collection: Seria/EM v3.5.8 64-bit [in-house Polara], EPU [EBIC, Diamond facility]. Microtubule gliding assays. NIS-Elements AR Software v4.60.00 64-bit [Nikon]. |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data analysis   | MotionCor2, gCTF v1.06, RELION v2.0 and v2.1, SPIDER v2.10, IMagic v140107 and v160504, EMAN v1.9, gautommach v0.53, IMOD fit v1.44, UCSF Chimera v1.13.1, UCSF ChimeraX v0.6.0, Phenix v1.13-2998 and v1.14-3260, RosettaCM v3.9, COOT v0.8.7, SWISS-MODEL (web server: https://swissmodel.expasy.org), RaptorX Contact [web server: http://raptorx.uchicago.edu/ContactMap/], PSI-PRED (web server: http://bioinf.cs.ucl.ac.uk/psipred/), Fiji v2.0.0-rc-65/1.52a, GraphPad Prism v5. |

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/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

Cryo-EM maps are available from the EMDB under accession codes EMDB-4918 (dynein-2 tail domain) and EMDB-4917 (dynein-2 motor domains). Coordinates are available from the RCSB Protein Data Bank under accession codes 6R1B (dynein-2 tail domain), 6R1A (dynein-2 motor domains), and 6SC2 (dynein-2, docked into subtomogram average of the antergrade IFT B train [EMDB-4303]). All other data supporting the conclusions of this manuscript are available from the corresponding author upon reasonable request.
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 Sample sizes were not pre-determined. Cryo-EM data was collected until a sufficiently high resolution to answer our biological question was achieved.

Data exclusions Mis-targeted cryo-EM micrographs that contained empty areas or thick carbon in more than ~50% of the field of view were excluded. During 2D and 3D classification, particles that did not yield detailed images/structures were excluded.

Replication No attempt was made to replicate cryo-EM data which involves averaging of tens of thousands of individual protein molecules.

Randomization Randomization was not relevant to the methods of this study, not a clinical/animal study.

Blinding Blinding was not relevant to the methods of this study, not a clinical/animal study.

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
☒ Animals and other organisms
☒ Human research participants
☒ Clinical data

Methods

n/a Involved in the study

☒ ChiP-seq
☒ Flow cytometry
☒ MRI-based neuroimaging