Structural organization of the dynein–dynactin complex bound to microtubules

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Cytoplasmic dynein associates with dynactin to drive cargo movement on microtubules, but the structure of the dynein–dynactin complex is unknown. Using electron microscopy, we determined the organization of native bovine dynein, dynactin and the dynein–dynactin–microtubule quaternary complex. In the microtubule-bound complex, the dynein motor domains are positioned for processive unidirectional movement, and the cargo-binding domains of both dynein and dynactin are accessible.

Cytoplasmic dynein-1 (hereafter dynein) interacts with its cofactor dynactin to transport a variety of cellular cargos toward microtubule (MT) minus ends, supporting events critical for cell division and survival1. Although the structure and the mechanical behavior of simplified versions of dynein's catalytic motor domain have been elucidated2,3, the mechanism by which the dimeric dynein holoenzyme powers movement of intracellular cargos over long distances has remained undefined, primarily owing to a lack of structural information for the dynein–dynactin–MT complex.

Dynein is a dimeric multisubunit complex comprising a pair of ∼500-kDa heavy chains (HCs) that contain the motor domain. Each HC also binds a light intermediate chain (LIC) and an intermediate chain (IC) that is complexed with three light chains (LCs; LC7, LC8 and Tctex); together, these components form the tail domain that binds cargo4. We performed negative-stain EM studies of vertebrate dynein, which can be observed as V-shaped dimers (Supplementary Fig. 1). Traditional two-dimensional (2D) analysis of whole particles yielded averages with limited resolvable structural detail (Supplementary Fig. 1b), owing to the extreme conformational flexibility of the head–tail link5–7. To overcome this challenge, we developed a ‘divide and conquer’ image-processing approach (Online Methods and Supplementary Fig. 2a) to reveal the organization of the dynein holoenzyme in unprecedented detail.

In the class averages, two tail-dimerization sites are visible: one at the distal end of the tail and a second positioned about 13 nm closer to the motor domains. The distal mass has long been ascribed to the three LCs8. The second mass, which to our knowledge has never been described, is positioned between donut-shaped lobes (Fig. 1) corresponding to the IC β-propeller domains9. The organization of the three LCs on the IC polypeptide has been established biochemically10, and the proximity of this second dimerization density to the WD40 domains suggests that it corresponds to the LC7 dimer (Fig. 1). Focused 2D analysis of the region extending beyond the putative LC7 dimer shows two

Figure 1 Proposed architecture of native vertebrate cytoplasmic dynein. (a,b) Left, negative-stain class averages of the purified dynein dimer. Middle, atomic coordinates for known dynein subunits (yellow, head domain, PDB 3VKH; orange, LIC, PDB 3W7G; green, LC7, PDB 3L9K) and a seven-bladed β-propeller (blue), filtered to simulate low-resolution EM density and overlaid on the class averages. Right, cartoon representations of the proposed subunit architecture in the class averages. The locations of the HC dimerization domain and IC WD40 domain were recently established11. (c) IC-associated densities, identified through focused 2D analysis of the region surrounding the putative LC7-dimer density (white dashed circle). Densities proposed to correspond to the LC8 and Tctex dimers project away from the LC7 dimer at a range of orientations relative to the dynein tail (shown on right) and do not appear to make any contact with the HC. (d) Crystal structures for LC7 (green, PDB 3L9K), LC8 and Tctex (magenta and orange, respectively, PDB 2PG1), superimposed on the 2D averages in c. (e) Cartoon representation of the proposed organization of dynein subunits. Scale bars, 10 nm.
densities that probably correspond to the LC8 and Tctex dimers that bind the IC N terminus. This mobile IC-LC domain does not appear to interact with any portion of the HC. These data lead to a new refined model of dynein tail organization in which the distal portion comprises exclusively the HC, in agreement with a recent report. Beyond the IC WD40 domains, a small crescent-shaped density is bound to each HC. We attribute these to the LICs, which bind to residues 650–800 of the HC, between the IC-binding site and motor domain. Beyond the LIC, the HC exhibits a dramatic kink that has not been observed in previous studies. The function of the kink, which we observed in 100% of the dynein averages (Supplementary Fig. 2b), is unclear, but its location between the tail and heads suggests that it may serve as a hinge that allows motions associated with the mechanochemical cycle of the head to occur without disrupting tail-cofactor-cargo interactions.

Dynein-based movement of cellular cargos depends upon dynactin, a multiprotein assembly containing distinct domains that bind dynein, MTs and cargos. The dynactin assembly consists of an actin-like polymer of the actin-related protein Arp1, with distinct protein complexes attached at either end. A large structure (‘shoulder’) containing a dimeric assembly of p24, p50 and p150Glued projects from the side of the Arp filament near its barbed end. The pointed end of the Arp filament binds a complex of Arp11, p62, p27 and p25, subunits that contribute to dynactin–complex stability and cargo binding. Although models, based on biochemical analysis and low-resolution EM, exist for dynactin subunit organization, the detailed architecture of this complex has only recently been described.

We determined the structure of native dynactin purified from bovine brain as previously described, resolving the Arp filament to 6.5 Å by cryo-EM. Because the shoulder readily detaches during vitrification, it is not present in the reconstruction. We determined the organization of the entire vertebrate dynactin complex by fitting the 6.5-Å cryo-EM structure of the filament into a 24-Å resolution negative-stain structure of the intact complex (Fig. 2 and Supplementary Figs. 3 and 4). The two-stranded helical organization of the Arp polymer (four copies in one strand, five in the other) is obvious in the structures (Fig. 2a). Dynactin’s single actin protomer lies at the pointed end of the four-strand polymer. At the barbed end of the filament, helices corresponding to the α- and β-tentacles of CapZ-α and CapZ-β are seen bound to the first Arp1 in each filament strand (Supplementary Fig. 4c). Arp11 contacts protomers of both filament strands at the opposite end. The remainder of the pointed-end domain is not as well resolved, owing to intrinsic flexibility, but densities corresponding to the p25 and p27 subunits are discernible as two paralleloid prisms (Fig. 2b). The remaining unaccounted density, which lies between Arp11 and the p25–p27 dimer, is attributed to the p62 subunit.

The p150Glued dimer, a component of the shoulder, contains an N-terminal coiled-coil domain that is absent in the three-dimensional (3D) reconstruction, owing to its flexibility. Focused 2D analysis of the shoulder region reveals a mobile coiled-coil extension with a globular density at its end (Supplementary Fig. 3c). The globular structure that we observe is much larger than what would be expected for the MT-binding CAP-Gly domain located at the p150Glued N terminus.

Furthermore, the position of the density 20 nm from the shoulder is incompatible with the predicted 50-nm length of the p150Glued coiled coil. This domain probably corresponds to the recently described p150Glued40-kDa intercoil domain. The globular density may contribute to dynactin regulation in heretofore-undefined ways, perhaps by governing the mobility of the coiled-coil region to accommodate and facilitate simultaneous interactions of dynactin with both MTs and dynein.

Vertebrate dynein is not processive in the absence of dynactin but instead undergoes diffusive bidirectional movement. Dynactin binding converts this behavior into longer-range, unidirectional motion, particularly when the N-terminal fragment of cofactor bicaudal D homolog 2 (BicD2N) is added. Attempts by other groups to determine the structural arrangement of the dynein–dynactin–BicD2N (DDB) complex were inconclusive, presumably owing to the conformational heterogeneity of dynein.

Because MT binding is expected to limit the conformational flexibility of dynein and the DDB complex, we prepared native dynein–dynactin complexes stabilized with BicD2N and bound to MTs (DDB–MT) (Supplementary Fig. 5a,b). In negative-stain micrographs, the DDB complexes are discernible as structures of ~45 nm in length, attached at a range of angles to the MT surface but tilted in a single direction, consistently with directional movement (Supplementary Fig. 5c). We produced detailed class averages of the DDB–MT complex with the same focused classification strategy used for dynein (Supplementary Fig. 5d,e).

In the DDB–MT complex, dynein is aligned between dynactin and the MT with the distal part of the dynein tail near the dynactin pointed end. The dynein tail associates with the short strand of the dynactin Arp filament, on the side opposite the shoulder (Fig. 3), consistently with the recently determined structure of the isolated dynein tail–dynactin–BicD2N complex. Densities that cannot be attributed to either dynactin or dynein probably correspond to the BicD2N coiled coil. Notably, the centers of the dynein heads are ~17 nm away from the MT, and the MT-binding stalks are oriented at an acute angle (Supplementary Fig. 5d,e). Unlike the motor domains in free DDB complexes, which exhibit a range of orientations and separation,
those in DDB–MT complexes are in proximity to each other (about 7 nm apart) but are not locked into a single orientation relative to each other (Supplementary Fig. 5e–g). The adjacent HC kink may provide a flexible ‘shock absorber’ that allows the motor domains to undergo the structural changes that underlie stepping without interfering with dynactin and cargo interactions. The organization of dynein and dynactin in the DDB–MT complex also elucidates the arrangement of cargo-binding domains. Using the isolated-dynein class averages for comparison, we found that densities corresponding to the IC-LC dimerization domain and the LICs (Fig. 3c) are apparently exposed in the DDB–MT complex. Given the importance of these regions for interactions with dynactin and other binding partners, it makes sense that these parts of the tail remain accessible.

The dynactin shoulder position on the opposite side of the DDB complex from the MT is intriguing, given that the projecting p150\textsuperscript{Glued} arm contains a MT-interacting domain. Although we did not observe a MT-associated density that might correspond to this domain, we observed a thin filamentous extension emerging from the shoulder in certain averages (Fig. 3d). The extension curves around the dynactin Arp filament and appears to make contact with the IC dimerization domain but cannot be traced further. We propose that this extension is the C-terminal portion of the p150\textsuperscript{Glued} coiled coil, which may engage with dynein to help secure it to dynactin during processive movement (Fig. 3e).

This analysis provides the first visualization, to our knowledge, of the dynein–dynactin complex bound to microtubules, revealing an organization in which the HCs are oriented with the motor domains aligned to favor unidirectional movement. At the same time, the complex is structured in a way that favors cargo recruitment (Fig. 3c). The insights provided here will serve as a paradigm for understanding the dynein–dynactin interactions that are essential for a wide range of minus end–directed motile phenomena. Although more detailed structural information will certainly improve understanding of the mechanism of dynein–dynactin–dependent movement, high-resolution determination of this entire structure will be extremely challenging because of the large size of the DDB complex and the heterogeneity of its link to the MT surface. The organizational framework of the DDB complex presented here provides the foundation for future work, supplying a new structural context for interpreting decades of biophysical and biochemical studies aimed at deciphering the mechanics of this fundamental and ancient cellular transport system.

**METHODS**

**Methods and any associated references are available in the online version of the paper.**

**Accession codes.** Density maps of the negative-stain and cryo-EM dynactin reconstructions have been deposited in the Electron Microscopy Data Bank under accession codes EMD-6290 and EMD-6289, respectively.

**Note:** Any Supplimentary Information and Source Data files are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

S.A.K. prepared the isolated dynein and dynactin. S.C. prepared the MT–DDB complex and performed all electron microscopy, image analysis and reconstructions. All authors contributed to the experimental design and assembly of the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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**Online Methods**

**Purification of dynein and dynactin, and analysis of MT binding.** Dynein and dynactin were purified from bovine brain (purchased from a local butcher) by a combination of sucrose-gradient centrifugation and ion-exchange chromatography, as described in ref. 23 but with the following changes. Dynein and dynactin were eluted from a MonoQ 10/100 column with a linear salt gradient. Then each pool (identified by SDS-PAGE) was rechromatographed separately on a MonoQ 5/50 column and eluted with a salt bump. Between 2 and 5 ml of the peak dynein and dynactin fractions was supplemented with 1 mM TCEP and microconcentrated (without desalting or dilution) to ~0.5 ml to yield a final concentration of 1–3 mg/ml. Proteins were kept on ice until use. The appearance of these complexes in negative-stain EM was indistinguishable from that of those isolated with AMP-PNP–MT binding from chick embryo brain, as described previously24. AMP-PNP–dependent binding of bovine dynein to MTs (Supplementary Fig. 5a) was analyzed with a method described previously24, except purified dynein was used, and nucleotide conditions in the MT-copelling step were varied.

**Expression and purification of BicD2N.** A plasmid encoding mouse BicD2 aa 25–400 (BicDNSh; hereafter BicD2N) in pET28a (kanamycin resistant) with an N-terminal His6 and StrepII-superfold GFP tag21 (generously provided by R. McKenney) was transformed into BL21(DE3) cells containing a pACYC-Duet-1 (chloramphenicol resistance) plasmid with rare tRNAs for codon usage in *Escherichia coli*. Cells were then grown at 30 °C in 2xLB medium25 containing 35 µg/ml chloramphenicol and 50 µg/ml kanamycin until cell density reached OD<sub>600nm</sub> = 0.4. The temperature was then dropped to 18 °C, and cultures were induced with 1 mM IPTG (Sigma Aldrich). Cells were grown overnight at 18 °C and harvested, and the cell pellet was flash frozen in liquid nitrogen and stored at −80 °C for later purification.

The frozen cell pellet was thawed on ice and resuspended in lysis buffer containing 50 mM Na<sub>2</sub>PO<sub>4</sub>, pH 8.0, 300 mM NaCl, 10 mM imidazole, and 1× EDTA-free protease inhibitor (G Biosciences). After the cells were lysed by ultrasonication, the lysate was cleared of insoluble cell debris by centrifugation at 35,000 g for 45 min, passed through a 0.2-µm syringe filter and incubated with HisPur Ni-NTA resin (Fisher Scientific) for 2 h at 4 °C. The Ni resin was washed extensively with 50 mM Na<sub>2</sub>PO<sub>4</sub>, pH 8.0, 300 mM NaCl, and 20 mM imidazole, and BicD2N was eluted with the same buffer containing 250 mM imidazole. The His tag–purified BicD2N was concentrated and passed over a Superose 6 gel-filtration column (GE Healthcare) equilibrated with 50 mM Na<sub>2</sub>PO<sub>4</sub>, pH 8.0, 300 mM NaCl and 1 mM TCEP. Pure BicD2N fractions identified by SDS-PAGE were pooled, and glycero1 was added to a final concentration of 5% (v/v). The pooled fractions were then concentrated to 18 µM, flash frozen in liquid nitrogen and stored at −80 °C for later use.

Purification of dynein–dynactin–BicD2N complex bound to MT from mouse brain. 5 g of fresh brain tissue from a 3-month-old female C57BL/6J mouse was washed with ice-cold PMEE buffer containing 4 mM MgSO<sub>4</sub>, 1 mM GTP, 4 mM AMP-PNP, and 2% (w/v) uranyl formate solution. Then each pool (identified by SDS-PAGE) was rechromatographed separately on a MonoQ 10/100 column and eluted with a salt bump. Between 2 and 5 ml of the peak dynein and dynactin fractions was supplemented with 1 mM TCEP and microconcentrated (without desalting or dilution) to ~0.5 ml to yield a final concentration of 1–3 mg/ml. Proteins were kept on ice until use. The appearance of these complexes in negative-stain EM was indistinguishable from that of those isolated with AMP-PNP–MT binding from chick embryo brain, as described previously24. AMP-PNP–dependent binding of bovine dynein to MTs (Supplementary Fig. 5a) was analyzed with a method described previously24, except purified dynein was used, and nucleotide conditions in the MT-copelling step were varied.

**Sample preparation for cryo-EM analysis.** Purified dynein was applied to freshly glow-discharged 400-mesh C-Flat grids (Protochips) containing 2-µm-diameter holes spaced 2 µm apart. Immediately before application of protein sample on the grid, 0.025% (w/v) amphotil A8-35 (Anatrace) was mixed with the sample to aid in dispersing dynactin particles across the holes in the carbon. 4 µl of the dynactin sample was applied to the grid, excess sample was manually blotted with filter paper for ~5–7 s, and the sample was immediately vitrified by plunge freezing in liquid-ethane slurry at −179 °C. The entire procedure was carried out at 4 °C and 98% humidity.

**Data acquisition.** All negative-stain and cryo-EM data were acquired with the Leginon automated data acquisition system26. Data acquisition for negative-stained samples was performed on a Tecnai Spirit (FEI) transmission electron microscope operating at 120 kV. Images were collected at a nominal magnification of 52,000× on a F416 CMOS 4K × 4K camera (TVIPS) with a pixel size of 2.05 Å/pixel at specimen level. All micrographs were collected with an electron dose of 20 electrons/Å<sup>2</sup> with a defocus range from 0.3 µm to 1.5 µm.

**Cryo-EM data for dynactin were collected on a Titan Krios (FEI) electron microscope operating at 300 kV, with a Gatan K2 Summit camera operated in counting mode at a dose rate of ~10 electrons/pixel/s. Each movie comprised 30 frames acquired over 6 s, with a cumulative dose of ~35 electrons/Å<sup>2</sup>. Imaging was performed at a nominal magnification of 22,500×, with a pixel size of 1.31 Å/pixel at specimen level with a defocus range from 0.8 µm to 4 µm.

**Dynein image processing.** 1,200 micrographs of negatively stained dynein samples were collected for 2D analysis (Supplementary Fig. 1). All image preprocessing and initial 2D analysis were performed with the Appion image-processing pipeline27. The contrast transfer function (CTF) of each micrograph was estimated with CTFind3 (ref. 28), and template-based automated particle selection was performed with FindEM27. Templates for particle selection were generated from 2D class averages from a small set of manually picked particles. Phases for each micrograph were corrected with EMAN28, and particles were extracted with a 640 pixel × 640 pixel box. The data were binned by a factor of 2 for faster computation. Individual particles were normalized by elimination of pixels with values above or below 4.5 σ of the mean pixel value with the normalization function in the XMIPP package31.

An initial stack of 120,000 particles was subjected to five rounds of iterative multivariate statistical analysis (MSA)23 and multireference alignment (MRA) in Appion to remove any nonparticle features and aggregates that were erroneously picked by automated particle picker. To speed up this process, the particle stack was further binned by a factor of 4 before analysis. Particles belonging to classes that represented nonparticles or aggregates were eliminated, thus resulting in a stack of 100,000 dynein particles. This particle stack was subjected to one generation of reference-free 2D alignment and classification with ISA23, a module in...
the EMAN2/SPARX package\textsuperscript{34,35}, after application of a low-pass and high-pass filter of 15 Å and 630 Å, respectively. The stable averages obtained from ISAC had the overall V-shaped feature of dynein with two circular features at the two free ends (Supplementary Fig. 1b). Owing to the conformational heterogeneity of dynein, the class averages lacked structural details. In order to improve the resolvable details, we focused on subregions of the molecule (Supplementary Fig. 2). For a given class average, soft-edged masks were created to isolate the tail and individual head domains. These masks were applied to the hundreds of aligned particles belonging to the class average. This resulted in three stacks: one with only the tail region visible, another containing the first head domain, and another containing the second head domain. Each group of particles was individually subjected to three generations of reference-free classification and alignment with ISAC, with pixel error values between 3 and 8 and particles per group ranging from 250 to 100 for different generations. The stable averages obtained from these classifications showed markedly improved structural detail.

In order to generate a representative average of the full dynein molecule, the classification and alignment parameters for each individual classification were applied to the unmasked stack of particles. Although the details outside of the focused region of interest were not well resolved after unmasking, structural features such as the kinked HC domain were discernible in many of the tail- and head-domain averages. These common structural features were used to overlay the head and tail classes in order to reassemble a composite view of the entire dynein molecule. For each set of class averages, the kinked region of one of the head domains was positioned in the center of the image, and the kinked region in the corresponding arm of the tail was also positioned in the center. A rotational and translational search with a limited search radius was used to align the kinked region in both class averages, and the averages were then themselves averaged together. This process was repeated for the second head and tail arm, to result in a composite class average of the complete dynein molecule. These class averages not only revealed detailed structural features of dynein but also showed the wide range of conformational heterogeneity of this complex in solution.

A similar masking approach was used to identify the dynein LCs. The unmasked dynein-tail averages (Supplementary Fig. 2a) in which the LC7 dimer was clearly visible were selected, and a soft mask was applied to the aligned particles around the area next to and including the LC7 dimer (dotted circle in Fig. 1b). These masked particles were subjected to ISAC 2D alignment and classification, and the resulting class averages were unmasked as described earlier.

**Dynactin image processing.** Preprocessing and initial 2D analysis of the negative-stain dynactin data set were performed in the same manner as described for dynein. From 1,978 micrographs, 86,434 automatically selected particles were extracted with a box size of 480 pixels. After several iterations of particle cleaning by MSA/MRA 2D classification, we obtained 46,734 dynactin particles that included the shoulder domain, which were used for 3D reconstruction. We used a 60-Å low-pass–filtered negative-stain reconstruction of dynactin (EMD-2716)\textsuperscript{36} as our initial model for 3D refinement by iterative projection matching with EMAN2 and SPARX libraries\textsuperscript{34,35}. Refinement of the initial model began generating forward projections at an angular increment of 20°, which decreased incrementally to 1°. The refinement would not proceed to the next angular step size until 95% of the particles had converged to a pixel error of less than 1 pixel. Forward projections of the refined volume showed excellent correlation with reference-free class averages, thus supporting the quality of reconstruction, free from model bias (Supplementary Fig. 3d). The resolution of the reconstruction was estimated by splitting the particle stack into odd and even halves and calculating the Fourier shell correlation (FSC) between the resulting volumes. The estimated resolution of the reconstruction at 0.5 FSC was 24 Å (Supplementary Fig. 3e).

Owing to its flexibility, the p150\textsuperscript{Glu} coiled-coil arm was not resolved in the 2D averages or 3D reconstructions of the dynactin complex. With a soft mask to perform focused analysis on the region surrounding the shoulder domain, we were able to visualize the p150\textsuperscript{Glu} side arm. A thin filament could be seen extending away from the shoulder domain with a large globular density at the end, ~20 nm from the shoulder. The class averages show that this domain can undergo a wide range of motion (Supplementary Fig. 3b).

A total of 2,421 cryo-EM images of dynactin were collected and analyzed for a higher-resolution structure. K2 movie frames were aligned with the GPU frame-alignment program described by Li et al.,\textsuperscript{36} which is incorporated into the Appion pipeline. A frame offset of 5 and B factor of 1,000 pixels (ref. 2) were used for the frame-alignment step. CTF parameters were estimated with CTFFind3, and images reporting a confidence value of less than 90% were discarded. Particles were picked with an automated template picker, as described earlier for negatively stained dynactin data. 133,558 particles were extracted from the aligned micrographs with a box size of 512 pixels, which were binned by a factor of 2 for subsequent processing. As described previously, MSA/MRA 2D classification was used to discard false or damaged particles. The shoulder domain, which we have observed to readily dissociate from the dynactin complex, was absent in almost all the particles in the cryo-EM data set.

After removal of junk particles, a stack of 59,538 particles remained, whose coordinates were imported into Relion\textsuperscript{37} for 3D classification and reconstruction. Particles were extracted with a box size of 512 pixels and scaled to 256 pixels, with 2.62 Å/pixel values, in order to reduce computational demand. 3D classification into three classes was performed, with the 24-Å negative-stain reconstruction of dynactin filtered to 60 Å as an initial model. After 25 iterations of 3D classification, 33,305 particles belonging to the most well resolved class average were used for further refinement. Refinement by projection matching in Relion resulted in a 9.5-Å-resolution reconstruction of dynactin (by gold-standard FSC at a cutoff of 0.143), although the pointed end was not resolved at subnanometer resolution, owing to the intrinsic flexibility of this region. A soft 3D mask was generated from the 9.5-Å-resolution dynactin reconstruction, and the pointed end of the complex was removed from the mask. Applying this mask during the refinement improved the alignment of the particles and accordingly improved the resolution of the 3D reconstruction to 7.5 Å (by gold-standard FSC at 0.143). The particle–polishing methodology in Relion was then used to improve the resolution of the complex to 6.5 Å. The local resolution map shown in Supplementary Figure 4d was calculated from the half volumes with the ‘blocres’ function in the Bsoft package\textsuperscript{38}.

The individual components that make up the Arn filament of dynactin were clearly discernible, and the map was segmented with the volume-tracer tool in UCSF Chimera\textsuperscript{39}. The cryo-EM reconstruction of the Arn filament could be fit with high precision into the 24-Å-resolution negative-stain dynactin reconstruction. By low-pass filtering of the cryo-EM reconstruction to a comparable resolution and with ‘diffmap’ from the N. Grigorieff laboratory (http://grigoriefflab.janelia.org/diffmap/), it is possible to isolate density for the shoulder domain from the negative-stain structure.

Nine copies of a homology model of Arn1, based on an F-actin as a template (as obtained by ref. 20), were individually fit into the segmented cryo-EM map of the filament with UCSF Chimera\textsuperscript{39}. A homology model for Arn1 in the Arn filament could be docked with moderate confidence into the segmented EM density for Arn1 at the pointed end of dynactin. The overall shape of the Arn model corresponded to the shape of the density for Arn1. Two copies of the prism-shaped crystal structure of P27 (PDB 3TV0) could be docked into two hollow cylindrical densities at the pointed end, thus suggesting this density to be a p25*p27 dimer. Because no homology model was available for p62, any density not attributed to Arn1 or the p25*p27 dimer was ascribed to p62. UCSF Chimera\textsuperscript{39} was used to generate all surface renderings of the dynactin EM density.

**DDB–MT image processing.** In order to accumulate sufficient particles for 2D analysis, 24,778 micrographs of negatively stained DDB-bound MTs were collected. 30,758 MT-bound DDB particles were manually selected and extracted from the micrographs with a box size of 480 (Supplementary Fig. 5c). Particles were binned by a factor of 2 for processing. The signal contributed by the MT dominated the 2D reference-free alignment of the particles, thus resulting in averages of the MTs in which the DDB complex was indistinguishable. To overcome this issue, a soft-edged mask was applied to the particles such that the majority of the MT was excluded, leaving only the DDB complex and the MT surface. 2D alignment and classification of these particles revealed the overall shape of the complex, which contained an elongated region, corresponding to the dynactin and dynein tail, with circular densities closer to the MT surface, corresponding to the dynein motors. However, these averages still lacked sufficient structural detail for interpretation. With a methodology similar to that used to examine the isolated dynein dimers, we generated masks to focus on the elongated and circular regions separately; this was followed by reassembly with correlated common features. To generate the histograms of the angles between the elongated region of DDB and the MT surface (Supplementary Fig. 5f), we used a technique described previously\textsuperscript{41}.  

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