

Roles of microtubules and LIS1 in dynein transport machinery assembly

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Cytoplasmic dynein-1, a microtubule (MT)-based motor protein, requires dynactin and a coiled-coil adaptor to form the processive dynein–dynactin–adaptor (DDA) complex^{1,2}. The roles of MTs and dynein regulator lissencephaly-1 (LIS1) in DDA assembly have remained elusive. Here we use cryo-electron microscopy to determine the structural basis of MT- and LIS1-mediated DDA assembly. We show that an adaptor-independent dynein–dynactin complex spontaneously forms on MTs with an intrinsic 2:1 stoichiometry in a highly efficient manner, driven by parallel alignment of dynein tails upon MT binding. Adaptors can wedge into and exchange within the assembled MT-bound dynein–dynactin complex; these processes are enabled by relative rotations between dynein and dynactin and facilitated by the dynein light-intermediate chains that assist the adaptor ‘search’ mechanism. Although LIS1 is dispensable for efficient DD(A)–MT assembly, its presence expands the conformational landscape of DD(A) assemblies on MTs. Cryo-electron microscopy reveals that LIS1 bridges dynactin p150^{glued} and dynein in both the closed Phi-like and open prepowerstroke states, stabilizing low-MT-affinity intermediates that tether dynein molecules in proximity to MTs and prime them for subsequent DD(A) assembly through alternative pathways. These findings demonstrate the dynamic adaptability of the dynein transport machinery and the coordinated roles of MTs and LIS1 in DDA assembly.

Cytoplasmic dynein-1 is a microtubule (MT)-based motor that drives diverse cellular processes, including long-range cargo transport, mitotic spindle organization and maintenance of cell architecture². Dynein is autoinhibited and requires assembly with dynactin and a coiled-coil-containing adaptor for processive motility (into a dynein–dynactin (DD)–adaptor (DDA) complex)^{1–4} (Fig. 1a).

The prevailing model for dynein activation indicates that the DDA complex assembles before engaging with MTs^{1–6}. It has been proposed that dynactin–adaptor binding to dynein activates motility by forcing the dynein amino-terminal tails to adopt a parallel alignment^{3,5,7}. The resulting conformational signal propagates to the motor domains, aligning them in a motility-competent arrangement. Recent cryo-electron microscopy (cryo-EM) structures of MT-bound DD–BICDR1 (DDR–MT)⁸ and DD–JIP3–LIS1 (DDJL–MT)⁹ support this model, as both possess parallel, aligned motors. These and other structures^{3,5} also indicate that select adaptor-bound dynactin complexes (including BICDR1 and JIP3, but BICD2 to a lesser extent) can recruit up to two dynein dimers.

Previous studies also imply that DDA assembly in solution is an inefficient process, as adaptors must be present in large excess (more than ten-fold) relative to dynein, whereas the ionic strength must be kept unnaturally low^{3,5}. In fact, structural studies have often relied on chemical cross-linking and further enrichment of weakly assembled complexes using gel filtration^{3,5}. These observations suggest that either DDA assembly *in vivo* is inefficient, or our current understanding of this process is incomplete.

Observations in cultured cells suggest that dynein-driven long-distance transport relies on an adaptor ‘handoff’ mechanism in which adaptors can be exchanged within a motile DDA complex¹⁰. Such a process would be inefficient under the prevailing model, which requires full disassembly and reassembly of the complex with a new adaptor. These observations raise the possibility that an alternative DDA assembly and activation pathway may exist in cells.

MTs are ubiquitous in eukaryotic cells¹¹, yet their potential influence on the assembly or activation of the dynein transport machinery has not been directly assessed. In this study, we find that MTs play a large part in DD complex assembly, and do so with remarkable efficiency, independent of adaptors. Moreover, we find that lissencephaly-related protein LIS1 does not enhance the final yield of DD(A) complexes but potentially stimulates the kinetics of DD(A) assembly by linking MT-bound and low-MT-affinity complexes, presumably priming the latter for activity. Together, these results provide an improved understanding of the roles of MTs and LIS1 in shaping the assembly pathways of the dynein transport machinery.

Adaptor-free DD assembly on MTs

Although it has been proposed that adaptors mediate DDA assembly and define DD stoichiometry^{1,5,12,13}, other studies have indicated that dynein and dynactin can interact without adaptors¹⁴. To reconcile these

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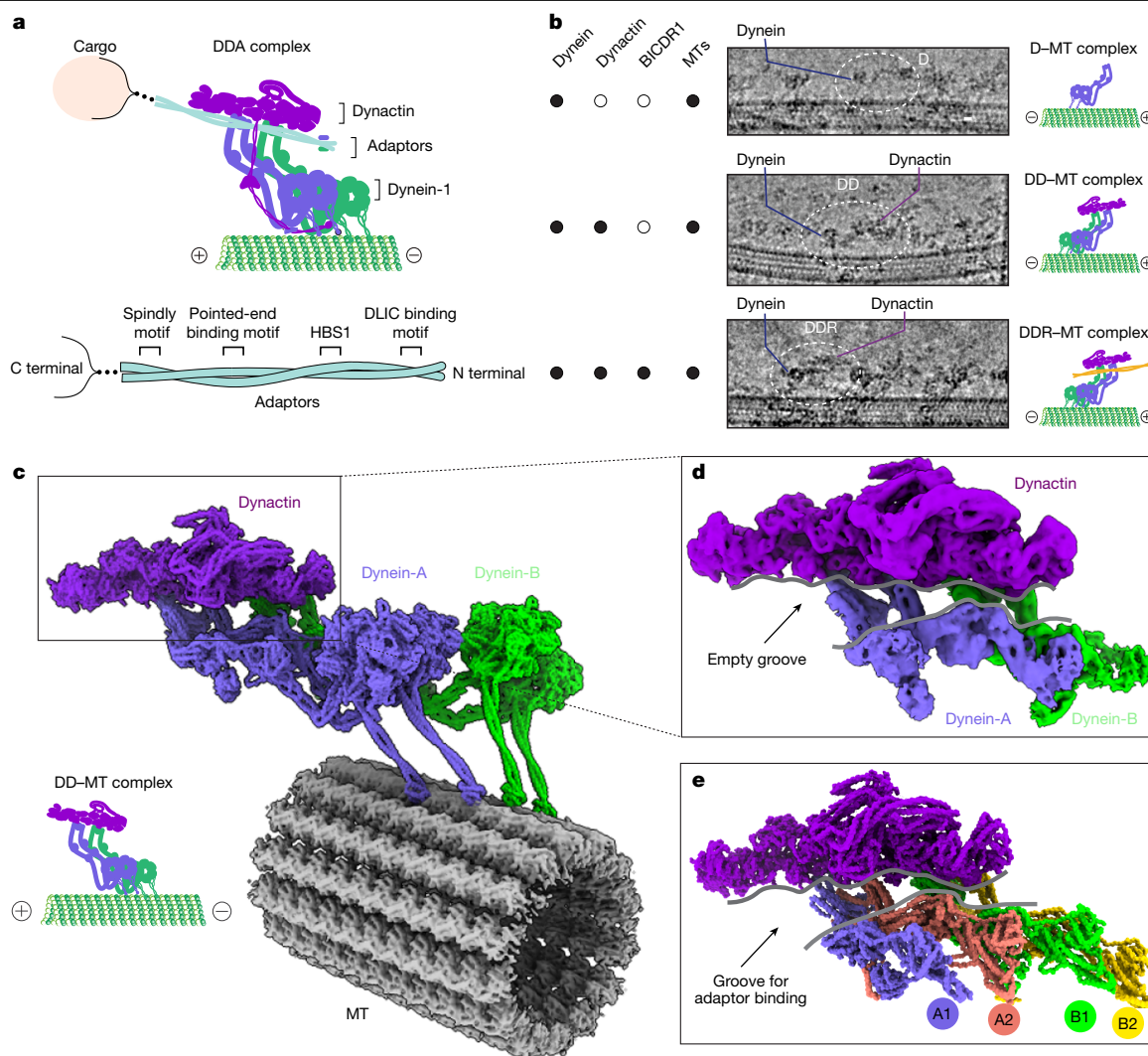


Fig. 1 | Cryo-EM structure of DD-MT complex. **a**, Schematic representation of the dynein transport machinery, illustrating dynein, dynactin and adaptor interactions with cargo (top); and detailed domains of a classical adaptor interacting with dynein and dynactin components, including the spindly motif, pointed-end binding motif, HBS1 and DLIC binding motif (bottom). **b**, Representative Fig. 1). MT binding by DD complexes showed a nucleotide dependency similar to that of binding by dynein alone, with approximately 75% of total dynein associated with MTs in the presence of AMPPNP (Extended Data Fig. 1d,e). We noted a similar pattern for MT binding with bovine dynactin and recombinant human dynein-1 (Extended Data Fig. 1f,g), which are routinely used for DDA assembly assays⁵. Negative-stain electron microscopy (nsEM) showed that the porcine DD-MT complex closely resembled the corresponding DDR-MT complex⁸ (Fig. 1b), suggesting that the former could indeed assemble in an adaptor-independent manner.

apparently conflicting models, we investigated whether dynactin could interact with dynein directly on MTs without adaptors and, if so, in what ratio.

MT copelleting assays with native porcine proteins (Extended Data Fig. 1a) showed that dynein and dynactin could coassemble on MTs in the presence of AMPPNP (Extended Data Fig. 1b and Supplementary Fig. 1), even at physiological ionic strength (150 mM KCl; Extended Data Fig. 1c). MT binding by DD complexes showed a nucleotide dependency similar to that of binding by dynein alone, with approximately 75% of total dynein associated with MTs in the presence of AMPPNP (Extended Data Fig. 1d,e). We noted a similar pattern for MT binding with bovine dynactin and recombinant human dynein-1 (Extended Data Fig. 1f,g), which are routinely used for DDA assembly assays⁵. Negative-stain electron microscopy (nsEM) showed that the porcine DD-MT complex closely resembled the corresponding DDR-MT complex⁸ (Fig. 1b), suggesting that the former could indeed assemble in an adaptor-independent manner.

complexes. **c**, Overall architecture of the DD-MT complex (62,404 particles). **d, e**, Density map (**d**) and molecular model (**e**) of the dynein tail interacting with dynactin, with an empty groove indicated; grey curves highlight the adaptor binding groove. Unless stated otherwise, dynein and dynactin were purified from porcine brain. All adaptor proteins were obtained from a mammalian expression system, except for BICD2, which was expressed and purified from *Escherichia coli*. Scale bar, 20 nm.

DD interactions define 2:1 stoichiometry

To gain insight into the nature of the DD-MT complex, we determined its cryo-EM structure (Extended Data Fig. 2 and Extended Data Table 1). After three-dimensional (3D) classification, we obtained only a single class of MT-bound DD, which revealed a complex that resembled the previously described DDR-MT complex⁸ (Fig. 1c). Even without an adaptor, the MT-bound DD complex was stabilized by extensive contacts between the dynein tail domains and the ARP1 filament of dynactin (Fig. 1d,e and Supplementary Video 1). Notably, this class contains two dynein complexes per dynactin, in contrast to previous in-solution observations in which such stoichiometry was adaptor-dependent^{3,5}.

MT-induced motor alignment

To examine whether and how dynactin binding influenced the morphology of MT-bound dynein, we determined cryo-EM structures of dynein alone on MTs in the presence of AMPPNP. Of the particles, 73.7%

possessed one visible motor domain, with a second motor that was poorly resolved owing to the relative motion of the motors, whereas the remaining 26.3% had two clearly aligned motors with ordered tail and linker densities (Extended Data Fig. 3, Extended Data Table 1 and Supplementary Video 2). The arrangement of the two motor domains closely resembled that of outer-arm dynein bound to doublet MTs¹⁵, indicating that MT binding is sufficient to align the motors independent of dynactin or adaptors (Extended Data Fig. 3c–e).

This MT-induced alignment of the motor domains induced the dynein tails to adopt a parallel orientation (Extended Data Fig. 3e), a state that is amenable to dynactin binding^{3,12}. This observation suggests that MT binding by dynein facilitates DD assembly, contrary to prevailing models in which adaptor-mediated tail alignment triggers motor alignment³. Although 73.7% of MT-bound dynein molecules remained conformationally heterogeneous, the remaining fraction adopting the parallel conformation represented a marked increase relative to free dynein, which possesses almost no such particles³. Consequently, the accessibility of parallel dynein tails to dynactin is greatly enhanced on MTs, leading to efficient DD complex formation. Furthermore, cryo-EM analyses showed that dynactin binding stabilized and further enforced this alignment, shifting the conformational equilibrium towards the parallel state, such that nearly all dynein molecules adopted aligned motor domains in the DD–MT complex (Fig. 1c and Extended Data Fig. 2). These results indicate that the association of dynein with MTs and dynactin may be a cooperative process on MTs.

Adaptor access through dynamic door opening

The unexpected observation of a stable DD–MT structure prompted us to re-evaluate the role of adaptors in DD interactions. In canonical DDA complexes, adaptor binding is mediated by electrostatic complementarity, with negatively charged adaptor surfaces engaging the positively charged adaptor binding groove in the DD interface (Supplementary Figs. 2–4). The absence of adaptors in the DD–MT complex raised the question of whether adaptors could access and activate the preassembled DD–MT complex, despite the adaptor binding groove's appearing to be a narrow region that could hinder adaptor entry (Fig. 1d,e).

To address this, we first analysed the structural dynamics of the DD–MT complex by extensive 3D classification. The adaptor binding groove between the dynein tail and dynactin exhibited notable flexibility in the absence of adaptors (Extended Data Fig. 4a and Supplementary Video 3) but was stabilized upon BICDR1 binding, as observed in our DDR–MT structures and previous studies⁸ (Extended Data Fig. 4b and Supplementary Video 4). The measured width of the narrow region (heavy-chain binding site 1 (HBS1)-A2) varied from 18.8 Å to 24.5 Å, closely matching the approximately 20-Å diameter of coiled-coil adaptors, whereas the second site (HBS1-A1) showed even greater variation (Extended Data Fig. 4c). These observations indicate that the adaptor binding groove transiently opens, creating a dynamic 'doorway' that permits adaptor entry, thereby enabling activation of preassembled DD–MT complexes.

To test the accessibility of the DD–MT complex to adaptors, we performed MT copelleting assays by adding adaptors at different stages of complex formation (Fig. 2). In the first condition ('Adaptor before MT'), dynein and dynactin were mixed with one of several adaptors and incubated for 20 min before addition of MTs, followed by pelleting of the assembled complex. Under these conditions, all adaptors tested assembled into intact DDA complexes on MTs (Fig. 2a and Extended Data Fig. 4d–g). In the second condition ('Adaptor after MT'), dynein and dynactin were first incubated with MTs to enable preassembly of DD–MT complexes, after which an adaptor was added immediately before pelleting and analysis. Notably, all adaptors tested were still able to assemble into DDA–MT complexes, indicating that the dynamic adaptor binding groove of the DD–MT complex remained accessible to incoming adaptors (Fig. 2b).

Inefficient DD assembly off MTs

We next used nsEM to assess the capacity of DDA formation with various full-length adaptors in solution. All adaptors tested except for BICDR1 and HOOK3 failed to promote complex formation under these conditions⁵ (Fig. 3a and Extended Data Fig. 5a,b). Consequently, DDA assembly off MTs was extremely inefficient, with only around 3% of total dynein molecules incorporated into DDR or DD–HOOK3 complexes, despite a ten-fold molar excess of adaptors relative to dynein (Extended Data Fig. 5a,b).

To understand the low efficiency of DDA assembly off MTs, we predicted the structures of known adaptors using AlphaFold¹⁶ (Supplementary Fig. 3). Almost all adaptors appeared to be autoinhibited, either through carboxy-terminal segments blocking the dynactin- and HBS1-binding regions (for instance, Spindly)¹⁷ or through an internal helix occupying the DLIC binding motif (for instance, TRAK2, JIP3)⁹ (Supplementary Fig. 4a). By contrast, BICDR1 and HOOK3 adopted open conformations, consistent with their ability to assemble DDA complexes in solution. These findings support a model in which some adaptors require a further activation step, perhaps mediated by cofactors or cargoes, to promote complex formation^{6,9,17,18}. Nevertheless, adaptor autoinhibition alone cannot fully account for the inefficiency of MT-independent DDA assembly.

LIS1 weakly promotes DD assembly off MTs

LIS1 has a critical role in regulation of dynein activity^{19–22} and has been proposed to participate in DDA assembly^{9,23}. To test whether and to what extent LIS1 could promote DD(A) formation, we performed mass photometry and cryo-EM studies under a series of conditions. Our mass photometry analysis (Fig. 3b and Extended Data Fig. 5c) indicated that LIS1 exhibited very little, if any, effect on DD(A) assembly in solution. Dynein alone assembled into higher molecular weight species that overlapped with the expected molecular weights for DDA and D₂DA, which showed only minor increases upon addition of either BICDR1, HOOK3 and/or LIS1.

We next performed cryo-EM analyses of DD alone, DD–LIS1 (DDL), DD–TRAK2 (DDK), DDK–LIS1 (DDKL), DDR and DDR–LIS1 (DDRL) (Extended Data Fig. 6). We used multireference 3D classification, followed by 3D refinement and *ab initio* two-dimensional (2D) averaging to validate each class. Only free dynein and dynactin were detected in the conditions containing TRAK2, LIS1 or both, whereas approximately 2.6% DDR complexes were formed in solution with BICDR1, a proportion that increased slightly to 3.7% in the presence of LIS1 (Fig. 3c). Even in the best case (DDRL), the extent of DDR assembly remained far less than that observed on MTs (Extended Data Fig. 1d,e). Therefore, LIS1 exhibits a modest effect on DD(A) assembly off MTs.

DD forms on MTs before adaptor binding

Our results so far having suggested that DD complex binding to MTs was favoured over adaptor engagement, we tested this by comparing the relative affinities of the DD complex for MTs and adaptors. Using nsEM, we first measured the affinity of the DD complex for adaptor BICDR1 in the absence of MTs. As the conformation of dynein is linked to its nucleotide state, we examined whether nucleotide conditions affected adaptor binding and found no significant impact in solution (Fig. 3d). In the presence of excess BICDR1 (66-fold), approximately 12% of dynein formed DDR complexes, corresponding to a dissociation constant (K_d) of around 5 μ M for binding of BICDR1 binding to the DD complex in AMPPNP (Fig. 3e). A mass photometric analysis of complex assembly also showed that DDA assembly in solution was very inefficient (Fig. 3b and Extended Data Fig. 5c); we observed little to no assembly in this assay.

We next quantified DD binding to MTs using MT copelleting assays under different nucleotide conditions. Binding affinities ranged from

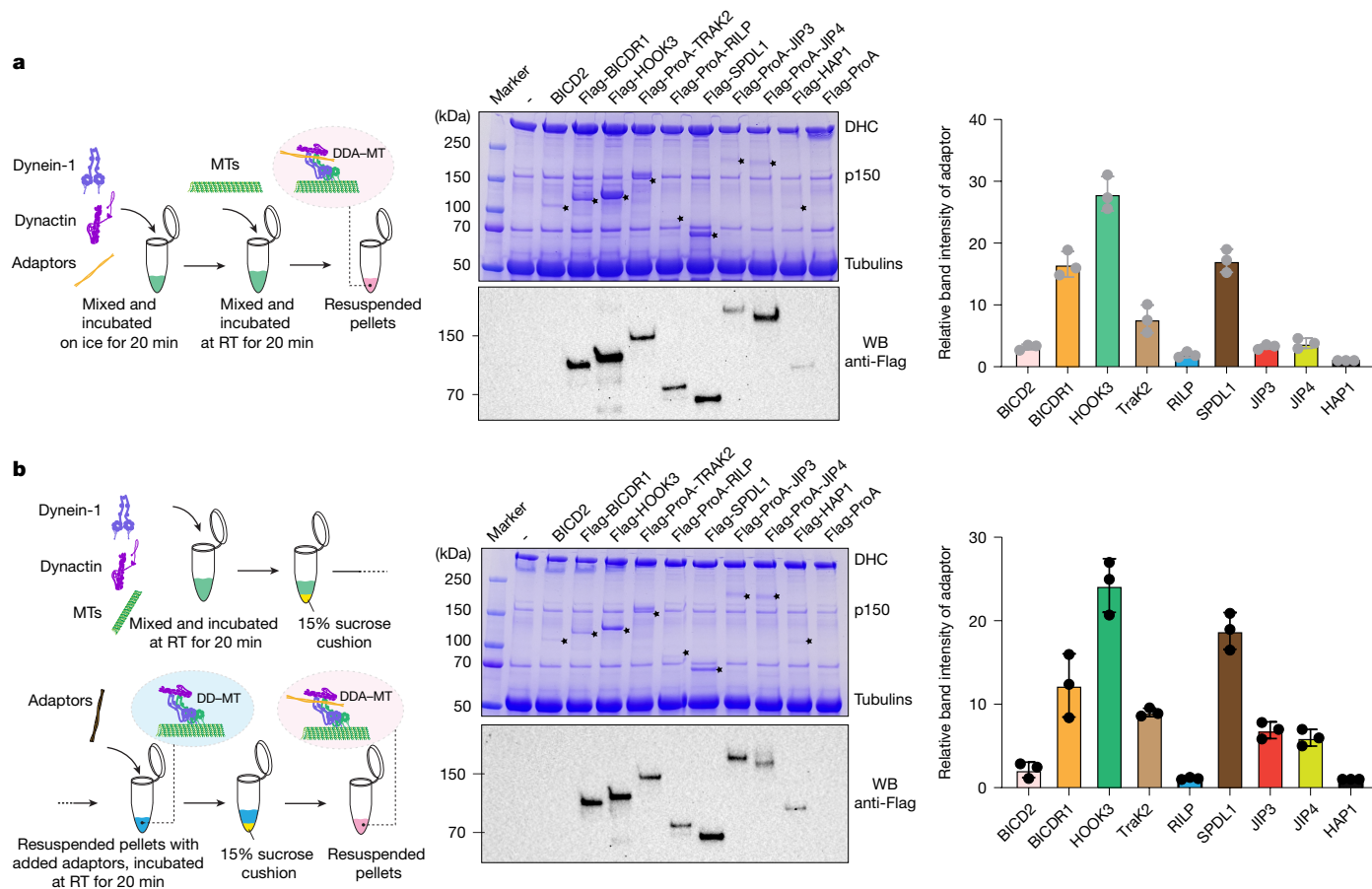


Fig. 2 | Adaptor binding groove in the DD-MT complex is dynamically open to accommodate all adaptors. a, b, MT pelleting assay measuring the binding of DD complex to various adaptors in the ‘Adaptor before MT’ (a) and ‘Adaptor after MT’ (b) conditions. Left, schematic representations of the ‘Adaptor before MT’ and ‘Adaptor after MT’ processes; upper middle, SDS-PAGE gels stained

with simple blue; lower middle, western blotting with anti-Flag; right, relative quantification of adaptor binding to the DD-MT complex (right). Untagged BICD2, a well-characterized adaptor, and Flag-tagged protein A were used as negative controls. Gels are representative of $n = 3$ independent experiments. For gel source data, see Supplementary Fig. 1e–h. RT, room temperature.

0.7 μ M to 1.6 μ M in AMPPNP, ADP and apo conditions, which triggered high MT affinity, with maximal binding values ($B_{\max}$) of 50–75% (Fig. 3f and Extended Data Fig. 7). Thus, the apparent affinity of DD for MTs is approximately five times higher than for adaptor proteins, indicating a clear preference for MT binding. Even in ATP and ADP-Vi conditions, which triggered lower MT affinity, we observed moderately good DD–MT binding ($K_d \approx 2.3 \mu$ M; $B_{\max} \approx 20\%$).

Given that autoinhibited adaptors (for instance, BICD2, JIP3 (ref. 9)) efficiently assembled into DDA complexes only in the presence of MTs, MT binding by the DD complex probably promotes adaptor activation (Figs. 2 and 3a). Considering that tubulin abundance exceeded that of adaptors by two to four orders of magnitude in cells (Fig. 3g), we propose that dynein and dynactin first assemble into the DD–MT complex, which then recruits adaptors, particularly those that are autoinhibited.

DLIC^{helices} help adaptor recruitment

Comparison of the DD–MT and DDR–MT complexes showed that the adaptor makes direct contacts with two conserved helices on the dynein light-intermediate chains (DLIC^{helices}) from both dyneins, specifically A2 and B1 (Supplementary Fig. 2a). Given that these two helices are not engaged in the DD–MT interaction, they are likely to be dispensable for DD–MT assembly. However, previous studies have highlighted their essential role in dynein processivity^{24–26}. We speculate that these interactions stabilize the DDA complex and facilitate adaptor recruitment. We confirmed this with BICDR1, HOOK3 and TRAK2 mutants that lacked

the DLIC binding motif, all of which exhibited reduced binding to DD complexes (Extended Data Fig. 8a–c). Notably, removal of the internal helix (Supplementary Fig. 4) of TRAK2 enhanced DD–MT binding (Extended Data Fig. 8c), suggesting that the internal helix autoinhibits the adaptor, thereby precluding adaptor–DLIC^{helix} engagement during DDA assembly on MTs. This aligns with recent findings that TRAK2 mutation disrupting the interaction between the internal helix and CCI enhances DDA assembly and motility in vivo²⁷. Together, these findings indicate that the paired DLIC^{helices} function as adaptor-capturing elements that facilitate adaptor docking and subsequent reorganization required for stable DDA formation (Extended Data Fig. 8d and Supplementary Video 5).

Adaptor competition and exchange in DD–MT

Our observations suggest that the MT-bound DD complex functions as a preinitiation complex, potentially explaining its diffusive migration along MTs while awaiting the arrival of an activating adaptor^{28,29}. This also implies potential competition among the various adaptors encountering this MT-bound complex in cells. To test this, we conducted an on-MT adaptor competition assay, in which we first assembled DDA–MT using one adaptor and then examined the final components of DDA complexes on MTs after addition of another adaptor to the system. We found that BICDR1 efficiently displaced preassembled TRAK2 in the DDK–MT complex, whereas TRAK2 was unable to displace preassembled BICDR1 in the DD–MT complex (Extended Data Fig. 8e).



Fig. 3 | DD preferentially binds to MTs over adaptor proteins. **a**, Statistical analysis of interactions between DD and various adaptor proteins using nsEM. **b,c**, Mass photometry (**b**) and cryo-EM (**c**) analysis of DD, DDA and DDAL. We observed species corresponding to 2× and 3× dyneins in our mass photometry data for dynein alone in the absence of any other. The masses of these species (approximately 2,900 and 4,300 kDa, respectively) coincided with the approximate expected masses for DDA and D₂DA complexes, respectively. **d**, Ratio of DDR complex to total dynein in different nucleotide-binding states using nsEM. **e**, Binding affinity of DD to BICDR1, with a K_d of 5.107 μM measured

We further tested the presumably uninhibited TRAK2 variant with deletion of the internal helix described above (TRAK2^{ΔIH}). As noted above, deletion of the internal helix increased TRAK2 accessibility to the DD complex, thereby reducing the ability of BICDR1 to outcompete TRAK2 (Extended Data Fig. 8f). These findings indicate that the internal helix

using nSME. **f**, Measurements of the binding affinity of DD to MTs across various nucleotide-binding states using MT pelleting assay, with K_d values listed for each state. For gel source data, see Supplementary Fig. 1m–q. Data in **a–f** are mean $\pm$ s.d. from $n = 3$ independent experiments. **g**, Average expression levels of tubulin β ($n = 66$), dynein heavy chain ($n = 66$), p150 ($n = 66$), LIS1 ($n = 66$) and adaptor proteins (BICDR1, $n = 5$; BICD2, $n = 48$; SPDL1, $n = 13$; TRAK1, $n = 16$; TRAK2, $n = 25$; JIP3, $n = 21$; JIP4, $n = 55$; CCDC88B, $n = 38$; RILP, $n = 26$; HOOK3, $n = 57$; CRACR2A, $n = 26$; RFP13, $n = 15$) in human tissues and cells; data are derived from ProteomicsDB, shown in $\log_{10}$ ppm and presented as mean $\pm$ s.d.

domain of TRAK2 (and probably that of other adaptors) modulates adaptor-DD interactions.

To visualize adaptor competition directly, we analysed the corresponding cryo-EM structures. We first assembled DDK-MT and DDR-MT complexes and determined their structures as control groups.

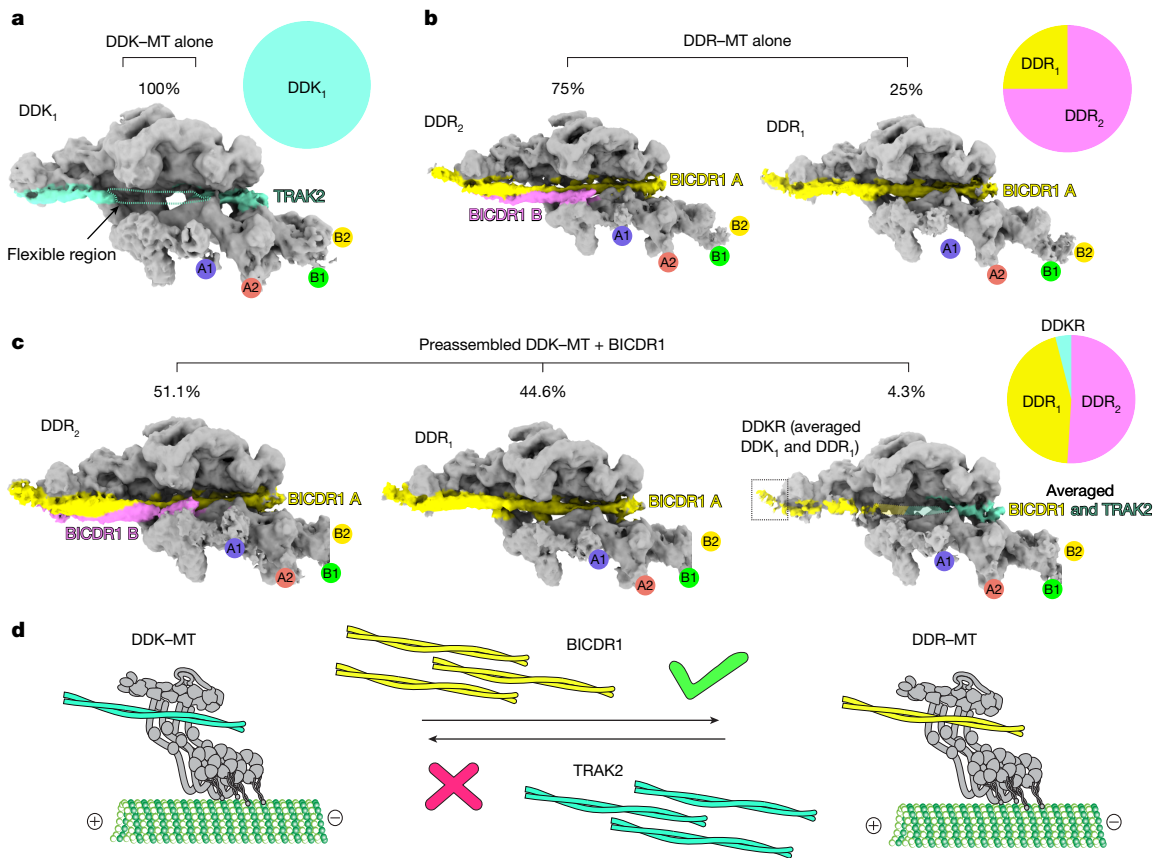


Fig. 4 | Adaptors compete for binding to the DD-MT complex. **a**, Density map of the dynein (tail)-dynactin-TRAK2 complex (DDK-MT, 52,920 particles), showing 100% occupancy by TRAK2 (steel blue). **b**, Density maps of the dynein (tail)-dynactin-BICDR1 complex (DDR-MT, 42,951 particles), showing a distribution of 75% DDR₂ and 25% DDR₁. BICDR1-A and BICDR1-B are coloured yellow and magenta, respectively. DDR₂ and DDR₁ indicate DDR complexes containing two BICDR1 dimers and one BICDR1 dimer, respectively. **c**, Density

maps illustrating the results of the competition assay in which excess BICDR1 was incubated with the preassembled DDK-MT complex. The binding distribution was 51.1% DDR₂ (33,721 particles), 44.6% DDR₁ (29,431 particles), and 4.3% averaged binding of DDK₁ and DDR₁ (2,837 particles). The pie chart summarizes the proportions of these complexes. **d**, Schematic representation of adaptor competition, showing that BICDR1 can compete with TRAK2 for binding to the DD complex.

Cryo-EM 3D classification identified only a single TRAK2 dimer within the adaptor binding groove (Fig. 4a), with the TRAK2 density exhibiting a flexible segment between the HBS1 and pointed-end binding motifs, consistent with AlphaFold predictions (Supplementary Fig. 4a). By contrast, DDR-MT complexes were classified into two states: a dominant class (DDR₂, approximately 75%) with two BICDR1 dimers; and a minor class (DDR₁, approximately 25%) with one BICDR1 dimer (Fig. 4b and Extended Data Fig. 9a). We then assembled the DDK-MT complex and incubated the sample with BICDR1 before vitrification. Nearly all TRAK2 adaptors were replaced by BICDR1, resulting in approximately 51% DDR₂-MT and 45% DDR₁-MT (Fig. 4c and Extended Data Fig. 9b). The remaining proportion of around 4%, termed DDKR-MT, represented an average density map of a small mixed population containing either BICDR1 or TRAK2 (Extended Data Fig. 9b). Together, these results demonstrate that adaptors can compete and exchange within preassembled DD-MT complexes without requiring disassembly and reassembly of the dynein transport machinery (Fig. 4d).

LIS1 expands DD(A) conformations on MTs

To test whether LIS1 could further promote DD(A) formation in combination with MTs, we performed MT copelleting assays and cryo-EM analyses. The former showed that LIS1 did not markedly increase the amount of DD complexes formed on MTs (Extended Data Fig. 10a). We noted that LIS1 copelleted with DD-MT complexes, in spite of our previous observations that LIS1 is unlikely to associate with dynein molecules

that are stably bound to MTs^{30,31}. Consistent with our biochemical data, cryo-EM analysis of this mixture showed that LIS1 did not significantly alter the proportion of DD-MT. However, extensive 3D classification revealed previously unobserved intermediate conformations in addition to the canonical DD-MT structures observed in the absence of LIS1 (Extended Data Fig. 10b–d and Extended Data Table 2). These new species were derived from DD complexes positioned near but not fully assembled on the MT, consistent with the notion that LIS1 binding is largely incompatible with MT-bound dynein^{30,31}.

The first LIS1-bound intermediate adopted a Phi-like state (Phi^L; as we observed recently¹⁹) that included direct contacts between the LIS1 N terminus and p150^{CC1} (Fig. 5a–c). Although this complex did not make direct contact with the MT, it occupied a proximal position around the MT (Supplementary Fig. 5), consistent with its low MT affinity. Structural comparison with our recently reported Phi^L-LIS1 complex¹⁹ showed very minor differences (root mean square deviations of 0.472 Å and 1.440 Å in the motor and tail domains, respectively; Supplementary Fig. 6a–d), confirming their structural equivalence. Surprisingly, subclassification identified no independent classes corresponding to isolated Phi, Phi^L-LIS1 or Phi^L-p150 species, indicating that the Phi^L-LIS1-p150 complex represents a highly stable intermediate tripartite complex captured during DD(A) assembly on MTs.

The second intermediate, termed pre-LIS1-p150, featured an open dynein in a prepowerstroke state, in which one dynein motor domain interacted with LIS1 and p150 through the AAA2-3 region (Fig. 5d–f). This architecture resembled the previously reported DDJL-MT

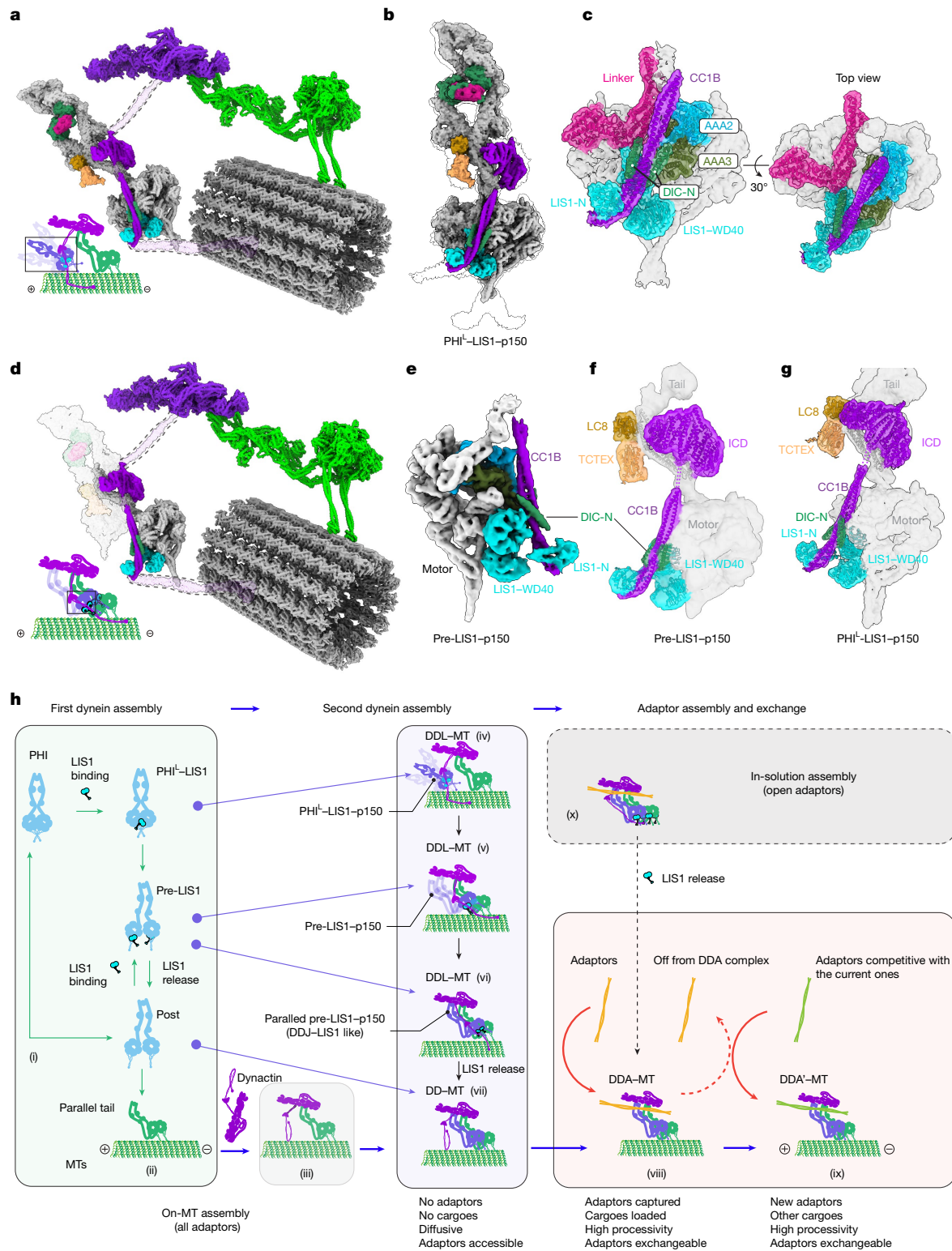


Fig. 5 | Cryo-EM structures of DDL-MT complex and a proposed model illustrating dynein transport machinery assembly and adaptor binding on MTs. **a**, Overall structure of DDL-MT with dynein-A in Φ^I state. **b, c**, Density maps of Φ^I dynein-A interacting with LIS1 (**b**) and CC1B (**c**) (90,591 particles). **d**, Overall structure of DDL-MT with dynein-A in prepowerstroke state. **e**, Density map of dynein-A motor domain in prepowerstroke interacting with LIS1 and CC1B (37,109 particles). **f, g**, Density maps of pre-LIS1-p150 (**f**) and Φ^I -LIS1-p150 (**g**). The DDL-MT complex was reconstituted using recombinant human dynein. **h**, The general pathway for all adaptors is shown in (i)–(ix) (this study, blue arrows). In (i) and (ii), MT is available to bind to open dynein transitioning from

a Φ^I -particle state in the presence or absence of LIS1. In (ii), dynein adopts a parallel tail conformation upon binding to MTs. In (iii), dynactin is recruited, forming a pseudointermediate DD-MT complex. (iv)–(vii) show LIS1-mediated intermediate states of the DDL-MT complex and LIS1-dissociated fully assembled DD-MT. In (viii)–(ix), adaptors compete for binding to DD-MT for cargo transport. Green, purple and red arrows indicate the assembly of the first dynein, assembly of the second dynein, and adaptor binding and exchange on MTs, respectively. The minor pathway (previous studies) is shown in (x): the DDA complex forms off MTs and becomes fully activated after MT binding.

structure but differed in that the two dynein motors were fully separated and flexible and lacked direct contact with MTs in the absence of an adaptor (Fig. 5f,g).

In both intermediates, dynein-A engaged LIS1 and p150 with similar contacts, as reported previously⁹ (Supplementary Fig. 6e–h), but the tails remained unbound from the dynactin ARP1 filament and thus probably represent conformations with low dynactin affinity. Such states were virtually absent in solution but became markedly enriched in the presence of MTs, suggesting that LIS1 remodels the conformational landscape of DD(A) by stabilizing otherwise transient intermediates on MTs. In contrast to previous reports that LIS1, when recruited by NDEL1, promotes DDA assembly³², we found that LIS1 alone did not increase the final yield of DD(A) complexes in the absence of NDEL1, either in solution or on MTs. Instead, it substantially expanded the range of assembly intermediates available on MTs, providing alternative DDA assembling pathways that potentially accelerate this process (Fig. 5a–g).

Discussion

Our study demonstrates a role of MTs in the assembly of dynein transport machinery, leading to a revised model (Fig. 5h). Upon MT binding, the two dynein motor domains become aligned in a parallel conformation that promotes efficient dynactin recruitment (Fig. 5h, (i)–(iii)). This enables subsequent recruitment of a second dynein to the MT-bound complex (Fig. 5h, (iv)–(vii)). The DD complex can efficiently assemble on MTs independently of adaptors and LIS1. However, LIS1 both facilitates the initial binding of dynein to MTs by relieving the autoinhibited phi conformation^{20,22,23,33} (Fig. 5h, (i)) and promotes the recruitment of a second dynein through its interaction with p150 (Fig. 5h, (iv)–(vii)). The existence of stable assembly intermediates Phi¹–LIS1–p150 and pre-LIS1–p150, suggests that LIS1 broadens the conformational landscape of dynein and potentially accelerates the kinetics of DD assembly on MTs despite the final yield of DD complexes not being increased.

Subsequent to promoting their assembly, LIS1 dissociates from DD–MT complexes³¹ before or coincident with formation of the mature DD–MT complex with both dynein motors attached to MTs in a parallel conformation. Our data indicate that p150 also needs to be displaced from dynein upon MT binding, as our extensive cryo-EM classification did not identify any p150 density for the MT-bound dyneins. This potentially enables p150 to be recycled for recruitment of a third dynein³⁴ or to seize dynein while it is detaching from the MT during a processive movement. In the latter case, the released LIS1 can be rapidly recaptured by p150 and a MT-unbound dynein motor domain in the prepowerstroke state, thereby retaining LIS1 during transport, but not by the MT-bound dyneins (Fig. 5h, (vii)). This agrees with previous evidence that LIS1 preferentially binds dynein in the prepowerstroke state off MT^{31,35} and that DDA complexes can transport LIS1 over long distances^{36,37}.

The DD–MT complex probably serves as an inactive preinitiation complex. This complex uses two DLIC^{helices} to assist in the search for a potential adaptor (Fig. 5h, (viii) and Extended Data Fig. 8d). For long-range transport of cargos that require multiple adaptors¹⁰, an incoming adaptor with a higher binding affinity can replace prebound ones, allowing continuous transport without complete disassembly and reassembly of DDA (Fig. 5h, (viii)–(ix)).

The prevailing model proposes that an activated DDA complex assembles in the cytoplasm before landing on MTs to initiate cargo transport^{2,3,9} (Fig. 5h, (x)). Although this is likely to be the case in some physiological contexts (for instance, kinetochore-mediated recruitment of DD complexes³⁸), our results support a model whereby cytosolic DDA assembly represents a minor pathway. Although possible according to our own and previous studies^{2,9,21}, delivery of such large complexes to MTs by diffusion is likely to be inefficient^{2,39}. By contrast, DD complexes exhibit a markedly higher affinity for MTs than for adaptors (Fig. 3e,f), and tubulin is two to four orders of magnitude more

abundant than adaptor proteins (Fig. 3g). Thus, DD complex formation on MTs is a more efficient route for generating motile DDA complexes (Fig. 5h, (i–ix)).

Recent studies have found that adaptor-free DD complexes can indeed assemble in solution³² and on MTs¹⁴. However, such complexes are inactive and exhibit mostly diffusive and/or stationary behaviour on MTs⁴⁰. Single-molecule imaging of DD in live cells has also shown that these complexes colocalize on MTs and are predominantly in a paused state on MTs during retrograde transport, suggesting that most of the dynein transport machinery is inactive *in vivo*^{28,29}. This allows us to speculate that the DD–MT complex exists in a paused, adaptor-free state on MTs in cells and awaits adaptor-bound cargo binding to initiate transport (Fig. 5h, (viii)).

This idea is supported by studies in fission yeast suggesting that dynein binds to and diffuses along MTs before binding to presumed adaptor protein Mcp5, which stimulates minus-end movement⁴¹. Furthermore, neuronal autophagosomes undergo progressive maturation as they move from the distal axon towards the soma, with distinct dynein effectors engaged at different stages: JIP1 initiates transport at the distal axon, HAP1 and Huntingtin support motility in the mid-axon, and JIP3 regulates mature autolysosomes¹⁰. This maturation-linked effector relay ensures that transport and cargo processing remain tightly coordinated to maintain axonal health. Notably, such stepwise recruitment of dynein regulators is highly compatible with our proposed model of a preinitiation DD complex on MTs, in which adaptor engagement occurs in a regulated and stage-specific manner to trigger productive motility.

Adaptors may act as molecular ‘stickers’ that wedge into the binding groove, thereby stabilizing the tripartite DDA complex and enabling processive transport without disassembling the complex. It has been reported that disease-associated BICD2 mutations (including S107L, I189F and N188T) enhance DD binding and increase processivity¹⁸. However, the Q77* loss-of-function mutation, which retains only the DLIC binding domain, preserves DD binding but produces phenotypes resembling LIS1 deficiency⁴². Similarly, HOOK3 truncations containing the HOOK domain maintain DD binding but almost completely abolish processivity⁴³. Thus, an adaptor containing all functional domains is required for processivity (Fig. 1a). Together, these findings support our hypothesis that the preinitiation DD complex assembled on MTs is a necessary prerequisite for subsequent processive motility, and that its full functionality critically depends on the adaptor binding state.

Our findings suggest that recruitment of two dynein molecules is essential for efficient adaptor engagement. The DLIC^{helices} from dynein A2 and B1 probably play a central part by competing with the internal helix of autoinhibited adaptors such as TRAK2, thereby relieving the blocked interfaces and enabling docking on to the DD–MT complex. This requirement for DLIC^{helices} would explain why adaptor binding occurs efficiently only on MTs, where both dynein molecules are properly positioned and aligned, but remains inefficient in solution, especially for autoinhibited adaptors.

We propose that the initial recruitment of the first adaptor depends on these two DLIC^{helices}. Incorporation of a second adaptor occurs most readily with non-autoinhibited adaptors such as BICDR1. By contrast, autoinhibited adaptors, in which the internal helix competes with the DLIC binding motif, can interact with only one DLIC^{helix} (from dynein A1), explaining why complexes such as DDJL–MT⁹ and DDK–MT lack a second adaptor. Even for uninhibited adaptors, only a subset of DD–MT complexes accommodate a second adaptor (Fig. 4b,c).

Finally, adaptor exchange probably begins with competition between the DLIC binding motifs of an incoming adaptor and the currently bound adaptor for access to the DLIC^{helices}. When the incoming adaptor has higher affinity, replacement proceeds efficiently; otherwise, the exchange is incomplete (Fig. 4d). It is worth noting that other adaptor–DD interfaces could also contribute, and that the adaptor exchange mechanism necessitates further investigation.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-026-10153-y>.

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Methods

Purification of dynein and dynactin from porcine brain, and expression and purification of human dynein

The purification of endogenous dynein and dynactin from porcine brain involved a series of precise and systematic steps to isolate these protein complexes with high purity, as previously described⁴⁴. Expression and purification of human dynein were performed as previously described⁴.

Expression and purification of adaptors and LIS1

Full-length BICDR1, HOOK3 and LIS1 fused to a protein A tag were produced in Sf9 insect cells or Expi293 mammalian cells and purified by means of a procedure similar to that used for human dynein, except that ATP was omitted from the GF150 buffer. Both Expi293 (cat. no. A14527) and Sf9 (cat. no. 11496015) cells were purchased from Thermo Fisher Scientific and maintained under sterile culture conditions following standard protocols; no additional authentication or mycoplasma testing was performed. Full-length BICD2 was expressed in BL21 *E. coli* cells and subjected to a purification process as previously detailed⁴⁵. Plasmids encoding the full-length versions of JIP3, JIP4, RILP and TRAK2 were acquired from Addgene. GFP-JIP3/4 was a gift from M. Cookson (Addgene plasmids 164624 and 164620)⁴⁶; pTRE2-Bla (HA-RILP-Flag) was a gift from S. Weinman (Addgene plasmid 102424)⁴⁷; and GFP-Trak2 was a gift from J. Kittler (Addgene plasmid 127622)⁴⁸. The genes encoding HAP1 and Spindly were synthesized by Twist Bioscience. These genes were then inserted into a mammalian expression vector—specifically, a pCAG-Flag-protein A (or pCAG-Flag-MBP) plasmid—designed to facilitate protein expression and purification.

Following the cloning process, the constructs were introduced into Expi293 cells, which are optimized for high-efficiency transfection and protein production through transfection. The expressed adaptor proteins were then isolated using anti-Flag agarose gel, leveraging the affinity of the Flag tag for a straightforward purification process. Subsequently, the proteins were eluted from the gel using a 3×Flag peptide, which competes with the bound protein for binding sites on the agarose gel, effectively releasing the protein. All adaptor proteins were collected, concentrated and stored in a buffer containing 25 mM HEPES pH 7.4, 150 mM NaCl, 1 mM dithiothreitol and 10 % glycerol.

MT pelleting assay

To conduct the pelleting assay, a 20- μ l reaction system was prepared. Initially, 15 μ l of dynein and associated complexes were mixed thoroughly on ice for 15 min to form a preincubation mixture. This mixture was then allowed to equilibrate to room temperature over 5 min, ensuring a gradual transition to prevent any thermal shock that could affect the activity of the protein. Then, an MT mixture comprising 5 μ M tubulin and 20 μ M Taxol was gently added, followed by mixing. The combined mixture was incubated at room temperature for a further 15 min to facilitate binding of dynein or dynein-associated complexes to the MTs.

To efficiently distinguish between MT-bound and unbound components, the reaction mixture was layered over a cushion solution containing 15% sucrose in a specific buffer. This step aids the separation process during centrifugation. The sample was then centrifuged at 20,000g for 8 min, a condition optimized to pellet the MT-protein complexes effectively while leaving unbound proteins in the supernatant. After centrifugation, the supernatant was carefully removed, and the pellet containing the MT-bound fraction was washed twice with a wash buffer to remove any non-specifically bound proteins. Finally, the pellet was resuspended in a buffer tailored to ensure that the MT-bound proteins were in an optimal state for further examination, including biochemical assays and structural studies.

Immunoblotting

For immunoblot analysis, 1 μ l of sample from the corresponding adaptor before MT and adaptor after MT mixtures was collected. Proteins were separated on precast 4–20% SDS-PAGE gels (Bio-Rad) and transferred on to polyvinylidene fluoride membranes (Merck Millipore) preactivated with methanol. The transfer was carried out at 100 V for 90 min in transfer buffer (48 mM Tris, 39 mM glycine, 1.3 mM SDS, 10% methanol). Membranes were blocked for 1 h at room temperature in 5 ml of 5% non-fat milk in TBST (150 mM NaCl, 20 mM Tris-HCl pH 8.0, 0.1% Tween-20), then incubated overnight at 4 °C with mouse anti-Flag M2 antibody (1:2,500; Sigma). After three washes with TBST, membranes were incubated for 1 h at room temperature with HRP-conjugated goat anti-mouse secondary antibody (1:10,000; Pierce), washed again three times with TBST and developed using ECL Western Blotting Substrate (Pierce). Chemiluminescence was captured with a ChemiDoc Imaging System (Bio-Rad). All experiments were repeated independently three times with similar results.

Adaptor competition assay

After the MT pelleting assay had been completed, the first adaptor-assembled DDA complex was formed and purified using a sucrose cushion. Subsequently, the DDA complex was resuspended and incubated with an excess amount of a second adaptor at room temperature for 20 min before being subjected to a second sucrose cushion. Both the supernatant and pellet were then collected separately and analysed by SDS-PAGE.

Negative staining analysis

The DDA complex was assembled by incubating dynein, dynactin and adaptor proteins at a molar ratio of 2:1:20 (adjusted if required) on ice for 30 min. The assembled complexes were applied to copper grids for negative staining and imaged on a Talos 120 transmission electron microscope at $\times 36,000$ magnification, corresponding to a pixel size of 4.2 Å. For each DDA sample, approximately 100 micrographs were collected. Image processing was carried out in CryoSPARC⁴⁹ using a standard workflow. Particles were automatically picked from the micrographs, extracted and subjected to 3–5 rounds of reference-free 2D classification. Classes corresponding to individual dynein, dynactin and intact DDA complexes were selected and refined through further iterative 2D classification to confirm particle identities and remove misassigned or low-quality particles.

Protein expression analysis

Expression levels of tubulin- β , dynein (DHC), dynactin (p150), LIS1 and various adaptors were obtained from ProteomicsDB (<https://www.proteomicsdb.org>), a comprehensive mass-spectrometry-based proteomics database. We systematically curated all available datasets from ProteomicsDB, including tissues, biological fluids, and cell types that expressed either tubulin- β , DHC, p150, LIS1 or adaptor proteins, ensuring a broad representation of biological contexts for our analysis. Protein expression levels were quantified using MS1-based intensity-based absolute quantification, a robust and widely accepted method for estimating absolute protein abundance in label-free proteomics studies. This approach leverages the intensity of peptide signals in MS1 spectra, normalized by the number of theoretically observable peptides per protein, to provide a reliable measure of protein expression levels across diverse samples.

D-MT, DD-MT and DDL-MT sample preparation

Preparation of D-MT and DD-MT samples was followed by MT pelleting assays with a scale-up volume and resuspension with a purposed volume for cryo-EM analysis. The preparation process used for the DDL-MT complex was similar to that used for the DD-MT sample, with minor

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modifications. Human or porcine dynein, dynactin and LIS1 dimer were mixed at concentrations of 0.46 μM , 0.30 μM and 4.6 μM , respectively, and incubated before addition of MTs. The reaction mixture was scaled up appropriately for subsequent cryo-EM grid preparation.

For cryo-EM grid preparation, 3.5 μl of sample was deposited on to glow-discharged Quantifoil R2/1 gold grids (300 mesh) that had been treated for 45 s at 25 mA in a GloQube glow discharge system (Quorum Technologies). Grids were blotted for 2.5–4.5 s under 100% humidity at room temperature and immediately plunge-frozen in liquid ethane using a Vitrobot Mark IV (Thermo Fisher Scientific).

Cryo-EM data collection

All cryo-EM grids were screened at the Yale ScienceHill-CryoEM facility using a Glacios microscope (Thermo Fisher Scientific). Subsequent cryo-EM data were collected at two different sites: Yale ScienceHill-CryoEM facility with a Glacios microscope operated at 300 keV with a K3 detector; and the Laboratory for BioMolecular Structure at BNL with a Krios microscope operated at 300 keV with a K3 detector and a Bioquantum Energy Filter. Automatic data collection was facilitated by either SerialEM⁵⁰ or EPU software. In total, 6,110, 10,660, 3,000 and 8,369 videos were acquired for dynein–MT, DD–MT, DD–BICDR1–MT and DDK–MT, respectively; 15,841 videos were collected at Yale ScienceHill-CryoEM facility using a Glacios microscope (Thermo Fisher Scientific) operated at 200 keV and equipped with a K3 detector for DDL–MT sample. Videos were recorded in counting mode with a nominal magnification of $\times 45,000$ corresponding to a calibrated pixel size of 0.868 Å, and a total electron exposure of $40\text{ e}^- \text{Å}^{-2}$ fractionated into 40 frames. Defocus values ranged from $-1.5\text{ }\mu\text{m}$ to $-2.7\text{ }\mu\text{m}$. Detailed data collection parameters can be found in Extended Data Tables 1 and 2.

Cryo-EM image processing of dynein–MT dataset

Preprocessing steps, including motion correction, contrast transfer function (CTF) estimation and particle picking, were conducted either in cryoSPARC Live⁴⁹ or with an in-house script using MotionCor2 (ref. 51), GCTF⁵² and Gautomatch. Cryo-EM scripts for real-time data transfer and on-the-fly preprocessing are available for download at <https://github.com/JackZhang-Lab/EM-scripts>.

For cryo-EM image processing of the dynein–MT dataset, we used a pipeline previously developed for MT tracing and MT signal subtraction^{53,54} (Extended Data Fig. 3). Initially, MT particles were detected using template matching with a distance cutoff of 8 nm, with a relatively low cross-correlation score set to include MT particles with low contrast or located at cross-over locations. Following three rounds of 2D classification, false-picked MT particles, including carbon edges, and miscentred MTs were rejected. The selected coordinates were recentred and subjected to multi-curve fitting. After MT signal subtraction, the original micrographs were replaced by MT-signal-subtracted micrographs.

Subsequently, a blob-picker was used to detect motor domains in the new micrographs, and 2D classification was used to remove junk particles and residual MT particles, after which the selected particles underwent *ab initio* reconstruction. All original particles were used for initial heterogeneous refinement. After several rounds of 3D and 2D sorting, classes with clear motor features were selected and subjected to local refinement, global and local CTF refinement.

For reconstruction of full-length dynein bound to MT, particles were re-extracted using a large box size covering two motor domains. The particles were directly reconstructed into a volume featuring the weak density of the second motor. Subsequently, this map was low-pass filtered to 60 Å for heterogeneous refinement, in which some classes exhibited enhanced density for the second motor. We then manually fitted the motor map into the weak density to create a two-motor map. This map, together with previous reconstructions, was used for another

round of heterogeneous refinement. Ultimately, three main states were predominantly observed for each dynein–MT dataset: (i) two stable and parallel heads; (ii) one stable leading head; and (iii) one stable trailing head.

Cryo-EM image processing of DD–MT dataset

For cryo-EM image processing of the DD–MT dataset, preprocessing was performed as for the dynein–MT dataset. For particle picking, a previous published dynein tail and dynactin map (EMD-4177)⁵ was projected and used for template picking. The picked particles were directly subjected to heterogeneous refinement with eight classes (Extended Data Fig. 2). The class with clear dynactin and dynein tail density was selected for rounds of reference-free 2D classification. High-quality particles were selected for high resolution reconstruction.

For reconstruction of the dynein motor region, particles were recentred on the motor region and re-extracted. After 3D reconstruction and low-pass filtering to 30 Å, the 4 motor domains were clearly visible but with smeared density. Rounds of heterogeneous refinement were used to improve the motor domain, and two main classes were identified: aligned and staggered, as previously reported⁸. A composite DD map was generated in ChimeraX⁵⁵ by merging local refined maps. DDA datasets were processed similarly.

Cryo-EM data collection and image processing for 2D classification of DD(A)(L)

Dynein and dynactin were assembled with LIS1 and, where indicated, adaptor proteins BICDR1 or TRAK2. Complexes corresponding to six conditions, DD, DDL, DDR, DDRL, DDK and DDKL, were prepared at a molar ratio of 2:1:10:4 (dynein/dynactin/adaptor/LIS1) with dynein at a final concentration of 1.0 mg ml⁻¹. The mixtures were incubated on ice for 30 min before vitrification.

For vitrification, 3.5 μl of assembled sample was applied to glow-discharged Quantifoil R2/1 gold grids (300 mesh, Quantifoil) treated for 45 s at 25 mA using a GloQube glow discharge system (Quorum Technologies). The grids were blotted for 2.5–4.5 s under 100% humidity at 4 °C and plunge-frozen in liquid ethane using a Vitrobot Mark IV (Thermo Fisher Scientific).

Cryo-EM data were collected on a Glacios (Thermo Fisher Scientific) operated at 200 kV, equipped with a K3 electron detector. Videos were recorded in counting mode with a magnification of $\times 45,000$ corresponding to a pixel size of 0.868 Å and a total electron exposure of $40\text{ e}^- \text{Å}^{-2}$ fractionated into 40 frames. Defocus values ranged from $-1.2\text{ }\mu\text{m}$ to $-2.4\text{ }\mu\text{m}$.

In total, six cryo-EM datasets were collected: DD (2,000 micrographs), DDL (2,000 micrographs), DDK (3,232 micrographs), DDKL (3,368 micrographs), DDR (3,296 micrographs) and DDRL (4,419 micrographs). Each dataset was processed independently in CryoSPARC Live for motion correction and CTF estimation. Particle picking was performed using templates derived from the DDR tail and dynein motor domains. Extracted particles (box size, 512 pixels, downsampled to 256 pixels; pixel size, 1.736 Å) were subjected to iterative rounds of heterogeneous refinement, using the DDR tail, dynactin and dynein motor as references. High-quality particles corresponding to DD(A), dynein motor or dynactin were then reassigned to the original micrographs from all six datasets and subjected to 2D classification for statistical analysis (Extended Data Fig. 7).

Cryo-EM image processing of DDL–MT dataset

Image processing of the DDL–MT dataset followed a workflow similar to that used for the DD–MT dataset. After MT signal subtraction, the density map of the DD tail from the DD–MT dataset was used as a template to pick corresponding DD tail particles from the DDL–MT dataset. The extracted particles underwent three rounds of heterogeneous refinement to remove junk particles. Three types of DD complex were identified through iterative classification. Sequential refinement and

classification yielded a dynein-A–dynactin complex (8.63 Å, 32,376 particles) and dynein-B–dynactin complex (9 Å, 40,849 particles). Particles of DD tail with two dyneins bound to dynactin were then re-extracted with a box size of 768 pixels downsampled to 500 pixels (pixel size, 1.33 Å), followed by heterogeneous, homogeneous, CTF and local refinements, yielding a 4.07-Å map of the DD tail. These particles were subsequently re-centred on the motor region to reconstruct the dynein motor, resulting in two conformations similar to those identified in the DD–MT dataset.

To further refine motor conformations, templates corresponding to the dynein motor domain in postpowerstroke (EMD-44691) and prepowerstroke states with or without LIS1 (EMD-44683, EMD-22782) were used for particle picking. Three rounds of heterogeneous refinement were performed, producing three distinct motor conformations. After homogeneous, CTF and local refinements, the postpowerstroke (Post1) dynein motor achieved a resolution of 3.92 Å. The prepowerstroke dynein motor bound to p150-CC1B, DIC-N and LIS1 (pre-LIS1–p150-CC1B) reached 4.26 Å, whereas the Phi-like state bound to the same components (Phi^L–LIS1–p150-CC1B) reached 3.93 Å after re-extraction (pixel size 1.16 Å) and 3D refinement. Recentring of pre-LIS1–p150-CC1B particles revealed a further p150-ICD density at the neck region of the tail, resulting in reconstruction at 8.46 Å resolution (pre-LIS1–p150-CC1B-ICD).

To resolve the tail region of the Phi^L–LIS1–p150-CC1B complex, corresponding motor particles were re-centred on to the tail region. Two rounds of heterogeneous refinement yielded a consensus tail map at 8.57-Å resolution. Local refinements using masks for four tail segments improved the map quality, and the resulting submaps were stitched together into a composite tail map. The composite tail and the motor domain maps were subsequently assembled to generate the full-length Phi-like dynein–LIS1–dynactin–p150 complex.

Model building and refinement

For model building and refinement, we used two previously reported dynein and dynactin structures (Protein Data Bank (PDB) 7Z8F⁸, 6ZNL⁵⁶) as initial models. Local regions were rigidly docked into the cryo-EM map in UCSF ChimeraX. Subsequently, Namdinator⁵⁷, a molecular dynamics flexible fitting tool, was used to further refine the model into the cryo-EM map. Manual inspection and adjustments of the model were carried out in COOT v.0.9.5 (ref. 58).

All models underwent iterative refinement using Phenix real-space refinement 1.21rc1_5190 and manual rebuilding in Coot. The quality of the refined models was assessed using MolProbity⁵⁹ integrated into Phenix⁶⁰, with statistics reported in Extended Data Table 1. For model building of structures in the DDL–MT dataset, previously reported structures from the PDB (9E13, 9BMV, 8PTK and 7Z8F) were used as initial templates for the Phi^L–LIS1–p150 complex (full-length or motor domain), pre-LIS1–p150 (with CC1B or CC1B-ICD), Post1 and the DD tail.

The individual components used for reconstruction of the motor or tail domains of Phi^L–LIS1–p150, pre-LIS1–p150-CC1B or CC1B-ICD, including the phi-like dynein motor domain, LIS1 and p150-CC1B or p150-CC1B-ICD, were extracted from PDB entries 9E13 and 8PTK. The dynein motor in the Post1 state was derived from PDB 9BMV, and the DD tail under DDL–MT conditions was reconstructed on the basis of PDB 7Z8F. These segments were manually fitted into the corresponding cryo-EM maps and assembled using UCSF ChimeraX. The resulting models were further rebuilt in Coot and refined in real space with PHENIX. Model quality was validated using MolProbity in PHENIX, and the corresponding statistics are provided in Extended Data Table 2.

Reporting summary

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

Data availability

All atomic coordinates and cryo-EM maps have been deposited in the PDB and Electron Microscopy Data Bank under accession codes 9DGQ/EMD-46844 (D–MT, overall structure), 9DGP/EMD-46843 (D–MT, dynein motor domain), 9DGR/EMD-46845 (composite DD–MT structure), 9DGS/EMD-46846 (DD tail of DD–MT complex), 9DGT/EMD-46847 (DDR1–MT, DD tail with one dimeric BICDR1), 9DGU/EMD-46848 (DDR2–MT, DD tail with two dimeric BICDR1s), 9DGV/EMD-46849 (DDK–MT, DD tail with one dimeric TRAK2), 9YNG/EMD-73178 (DD tail of DDL–MT dataset), 9YNH/EMD-73179 (composite full-length Phi^L–LIS1–p150), 9YNC/EMD-73173 (Phi^L–LIS1–p150-CC1B, motor domain of Phi^L–LIS1–p150), 9YND/EMD-73174 (pre-LIS1–p150-CC1B, dynein single motor in prepowerstroke state bound with p150-CC1B domain), 9YNE/EMD-73178 (pre-LIS1–p150-CC1B-ICD, dynein motor in prepowerstroke state bound with p150-CC1B-ICD domain), 9YNF/EMD-73176 (Post1, dynein motor in Post1 state).

Code availability

Code used in general cryo-EM data processing and structure determination of dynein bound to MTs is available at GitHub (<https://github.com/JackZhang-Lab/multi-curve-fitting>; <https://github.com/JackZhang-Lab/tubulin-lattice-subtraction>).

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Author contributions Q.R. and J.Y. prepared protein samples, performed biochemical characterizations, MT copelleting assays and immunoblot analyses; collected and analysed negative-stain data; and prepared cryo-EM samples. Q.R., P.C. and K.Z. collected and processed

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cryo-EM data for dynein-MT, DD-MT, DDR-MT, DDK-MT and DDA-MT after on-MT adaptor competition assays. J.Y., Q.R. and K.Z. performed cryo-EM studies of DD, DDL, DDR, DDK, DDKL, DDRL, DDL-MT and DDRL-MT complexes. Q.R. and J.Y. built and refined the structural models. S.M. performed mass photometry experiments. Q.R., J.Y., P.C., S.M. and K.Z. analysed the structures and wrote the manuscript. K.Z. conceived and supervised the project and acquired funding.

Competing interests The authors declare no competing interests.

Additional information

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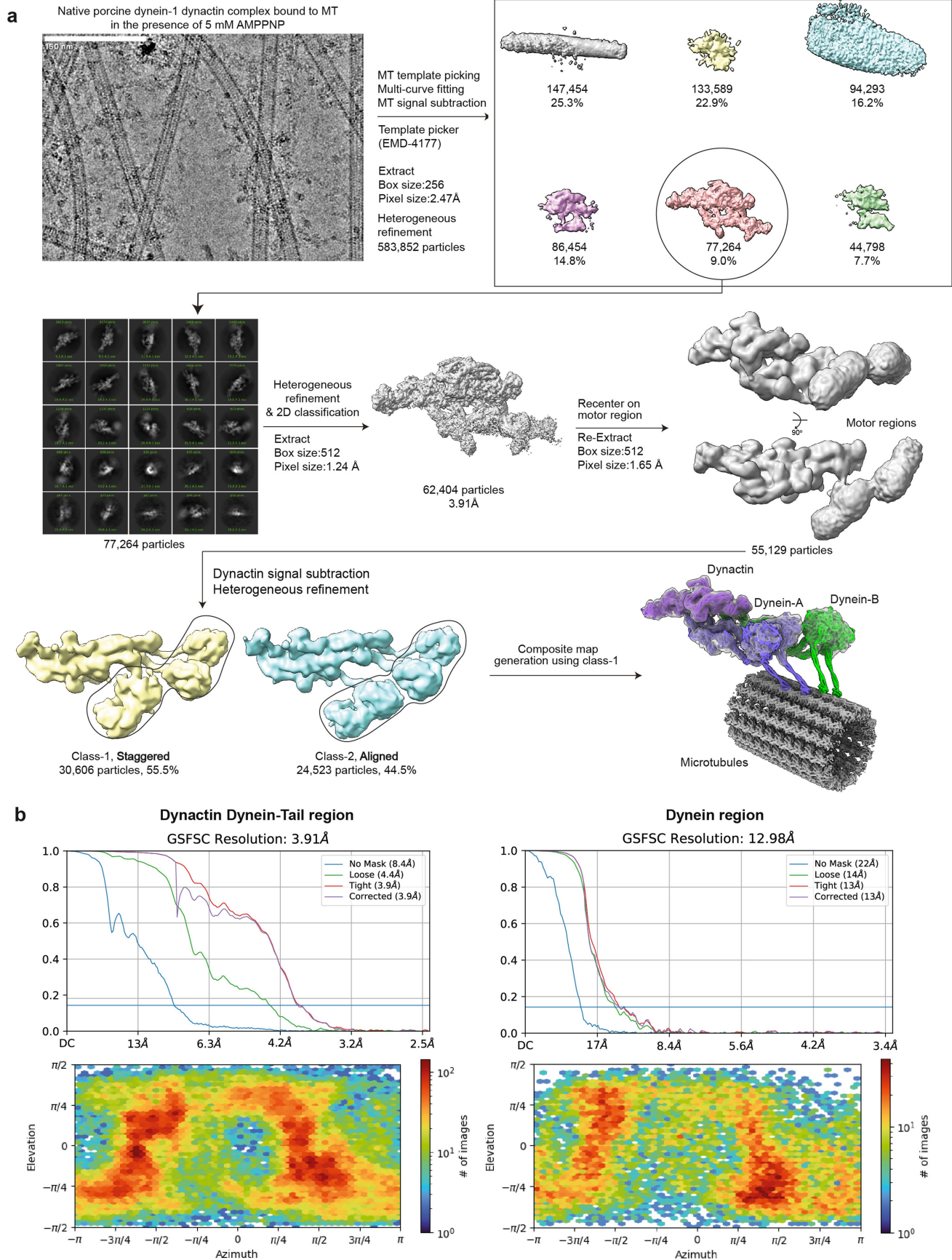
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Extended Data Fig. 1 | Microtubule pelleting assay reveal the formation of the DD-MT complex. (a) Diagram of the microtubule pelleting assay procedure. (b) SDS-PAGE gel of dynein, dynactin, and dynein-dynactin complex bound to MTs in the presence of AMPPNP. (c) SDS-PAGE gel of dynein-dynactin complex bound to MTs under the salt concentration ranging from 50 mM to 1 M in the presence of AMPPNP. (d) SDS-PAGE gel of dynein and dynein-dynactin complex bound to MTs in different nucleotide binding states. (e) Statistical analysis of the amount of dynein bound to MTs relative to dynein alone bound to MTs in the presence of ADP·Vi. Data are presented as mean $\pm$ s.d., analyzed by unpaired

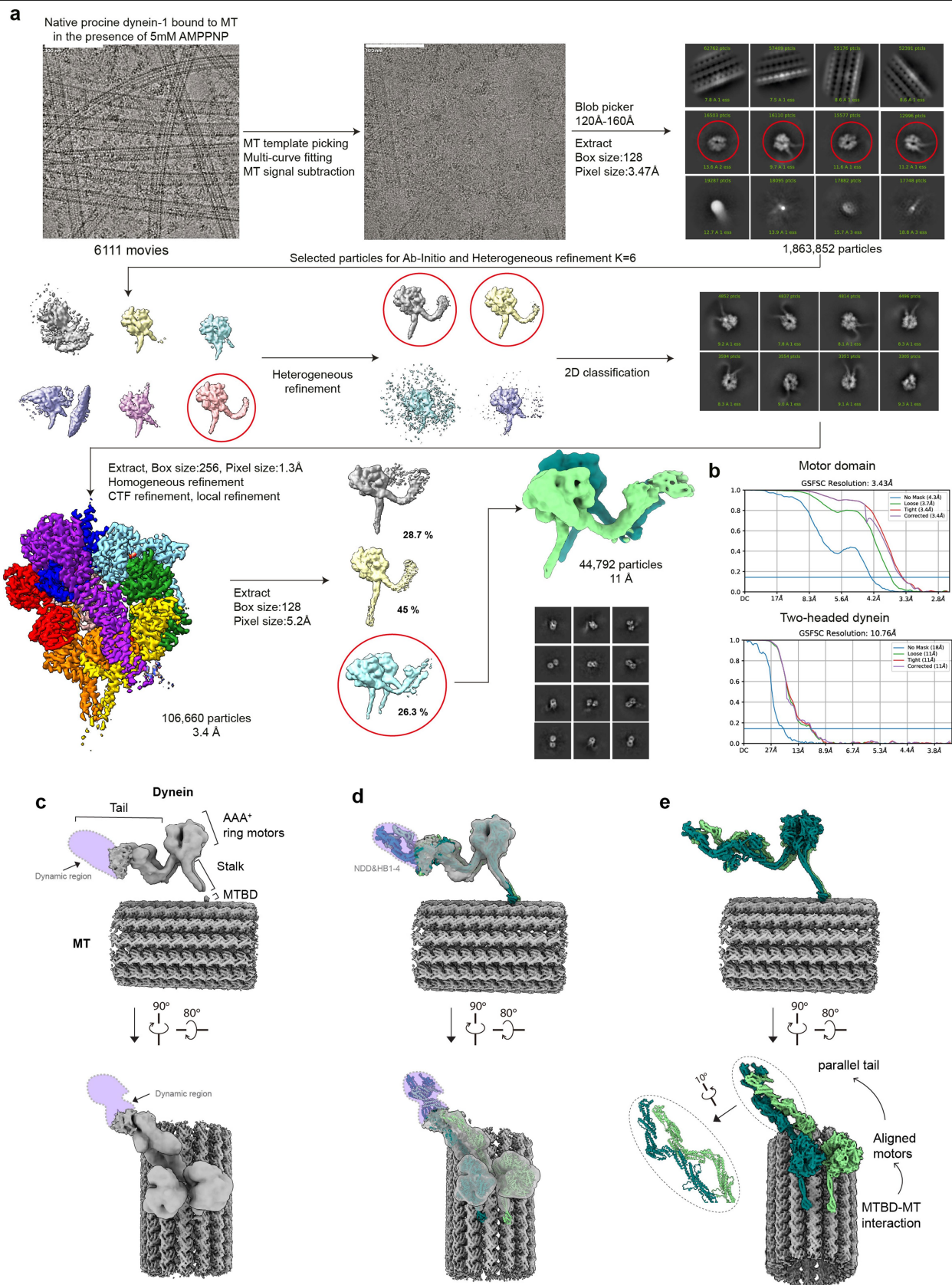
t-test with Welch's correction. (f) SDS-PAGE gel showing dynein and dynein-dynactin complex from different species (pig, bovine, human) bound to MTs in the presence of AMPPNP. (g) Statistical analysis of the amount of dynein bound to MTs relative to dynein alone bound to MTs. Data are presented as mean $\pm$ s.d., analyzed by unpaired *t*-test with Welch's correction. (p, pig; b, bovine; h, human). All SDS-PAGE gels are stained with simple blue. Gels are representative of *n* = 3 independent experiments in **b, c, d, f**. For gel source data, see Supplementary Fig. 1a–d.



Extended Data Fig. 2 | Data processing flow chart of DD-MT.

(a) Representative image of dynein-dyneactin bound to MTs and workflow of cryo-EM image processing. **(b)** FSC curves of dynein tail and dynactin region,

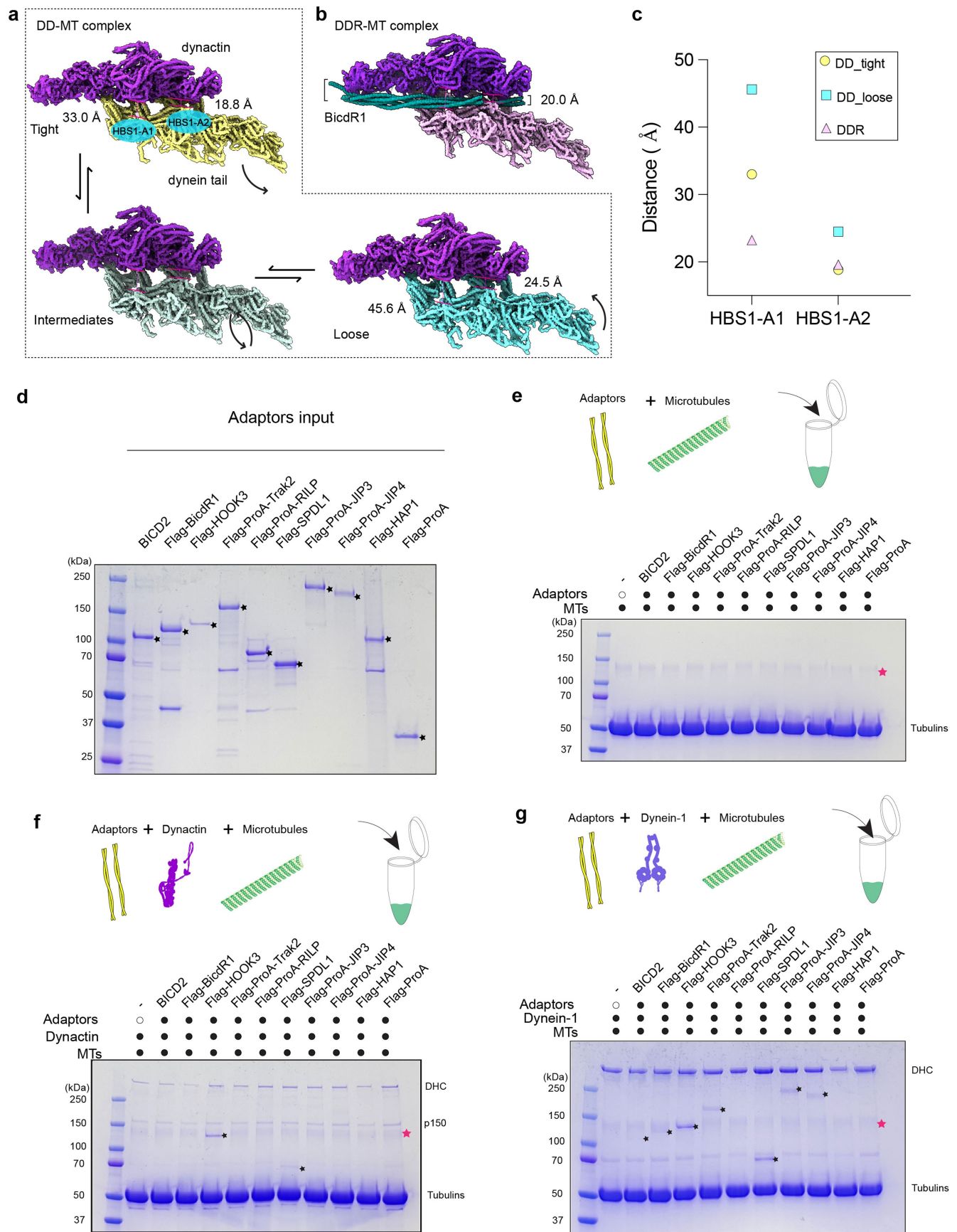
and dynein motors region reconstruction. Dynein-dyneactin with adaptors (BicdR1, Trak2) datasets were processed similarly.



Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | Data processing flow chart of D-MT and cryo-EM structure of D-MT with two aligned motors. (a) Representative image of dynein bound to MTs and workflow of cryo-EM image processing. (b) FSC curves of motor domain and two-headed dynein reconstruction. (c) Two different views of the density map of the D-MT complex are shown (44,792 particles). The side view shows the dynein tail with a dynamic region, the aligned AAA+ ring motors, the stalk, and the microtubule-binding domain (MTBD) of dynein. The dynamic region of the tail is highlighted in light purple. (d) A molecular

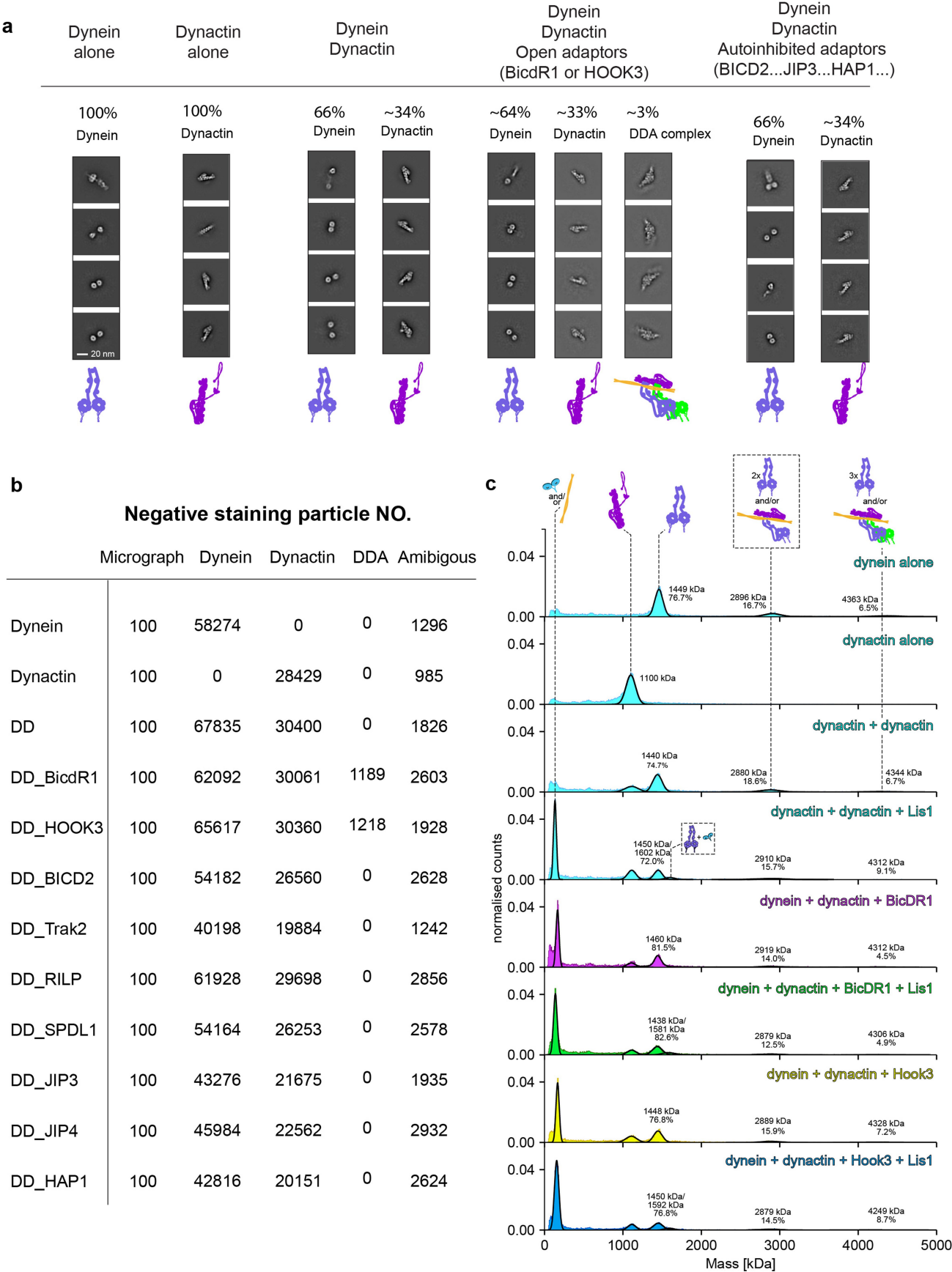
model of dynein-B, extracted from the DDR-MT complex (PDB: 7Z8F), is fitted into the cryo-EM structure of the D-MT complex (c) using rigid-body fitting. The NDD and HB1-4 domains of the dynein heavy chain contribute to the dynamic region. (e) The MT is displayed as a density map, while dynein is represented as a molecular model. A top view shows the parallel tails and the direction of conformational signal transmission, as indicated by arrows, upon interaction between the MTBD and MT.



Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | Dynamics analysis of adaptor binding groove and binding affinity analysis of adaptors, adaptor–dynactin, and adaptor–dynein complexes to microtubules. (a) A series of dynein–dynactin complex structures showing the dynamic adaptor-binding groove, ranging from tight to loose. The distances surrounding two HBS1 sites, HBS1-A1 and HBS1-A2, which mediate the interaction between the dynein heavy chain and the HBS1 of adaptors to regulate adaptor entry, were measured. (b) Dynamics analysis revealing a stable adaptor-binding groove within the DDR-MT complex. (c) Statistical analysis of the HBS1-A1 and HBS1-A2 distances in DDR-MT

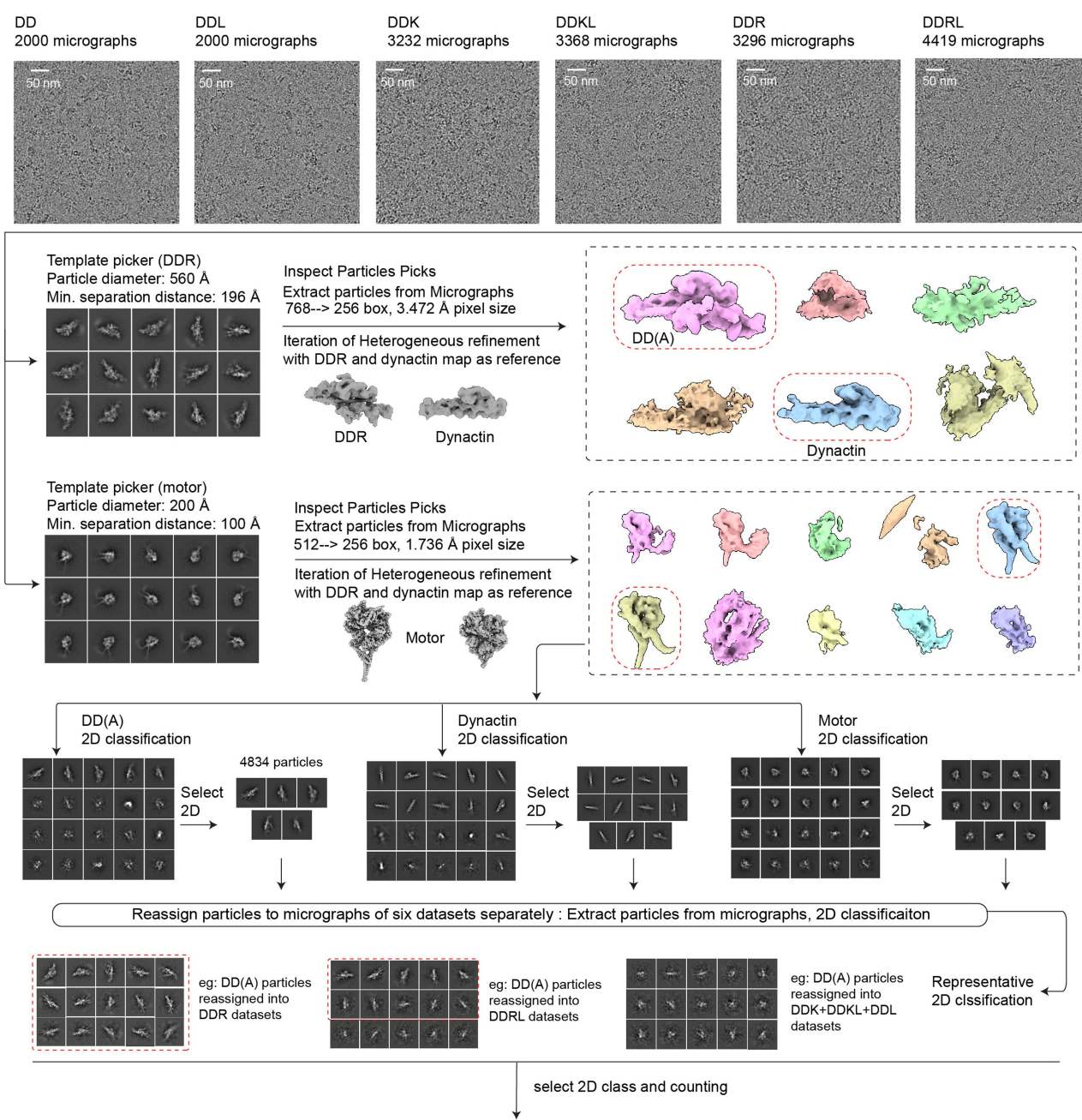
complex, and DD-MT complex in tight and loose states. SDS-PAGE gel of adaptor proteins (input) for all pelleting assay (d). SDS-PAGE analysis of adaptor proteins (e), adaptor–dynactin complexes (f), and adaptor–dynein complexes (g) co-sedimented with MTs in the presence of AMPPNP. Schematic diagrams illustrating the microtubule co-sedimentation assays for each group are shown to the upper of the corresponding gels. Each gel is representative of $n = 3$ independent experiments. The black and red stars indicate adaptors and undissolved tubulin dimers, respectively. For gel source data, see Supplementary Fig. 1i–l.



Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5 | Negative-stain EM and mass photometry analyses of dynein, dynactin, dynein–dynactin, and dynein–dynactin–adaptor complexes. (a) Representative negative-stain EM 2D class averages of each sample, with corresponding populations indicated. Percentages denote the relative proportions of dynein, dynactin, and dynein–dynactin–adaptor

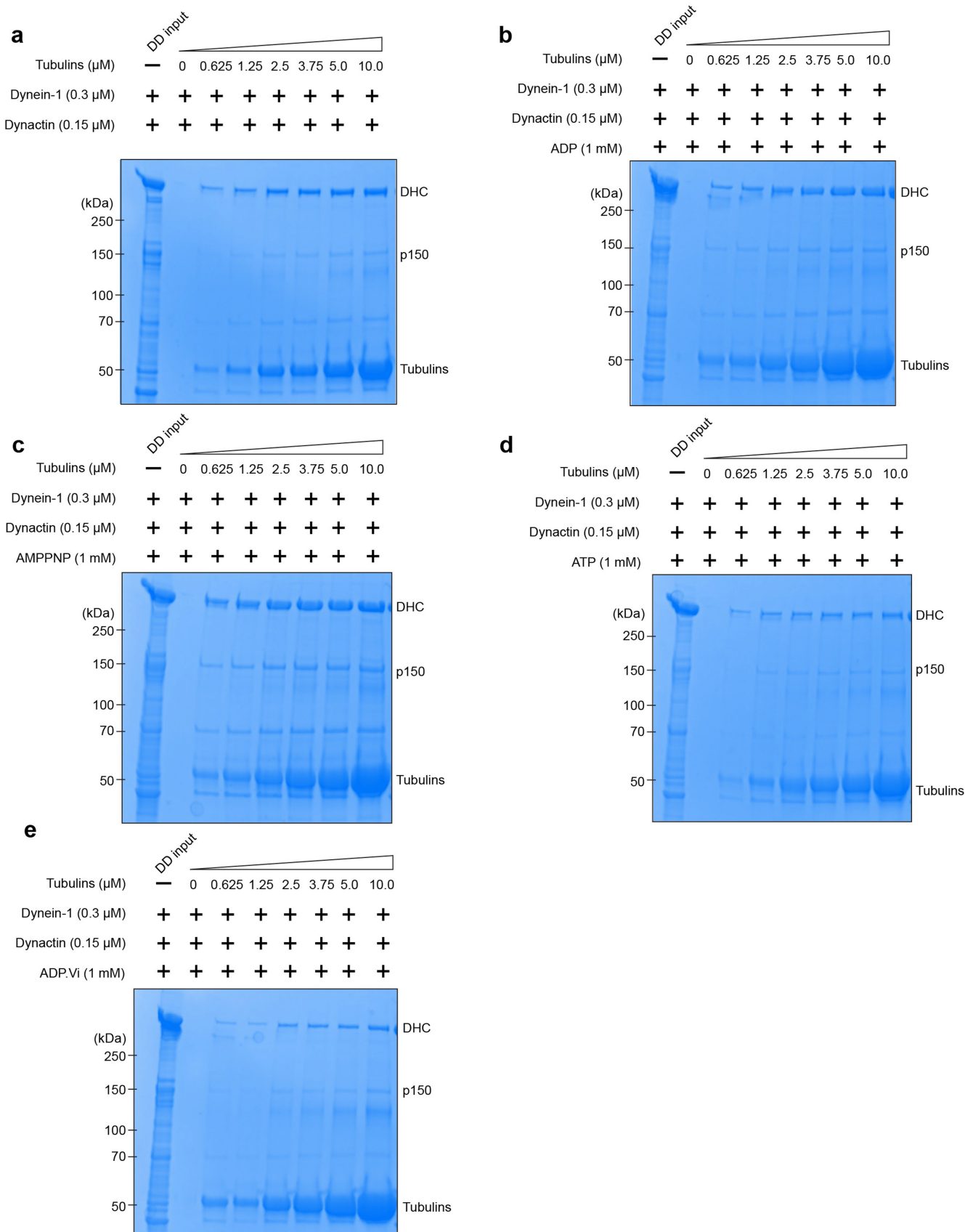
complexes under each condition. Scale bar, 20 nm. (b) Quantitative analysis of particle numbers corresponding to the data shown in (a). (c) Mass photometry analysis of dynein–dynactin and dynein–dynactin–adaptor complex formation in solution, in the presence or absence of LIS1.



b

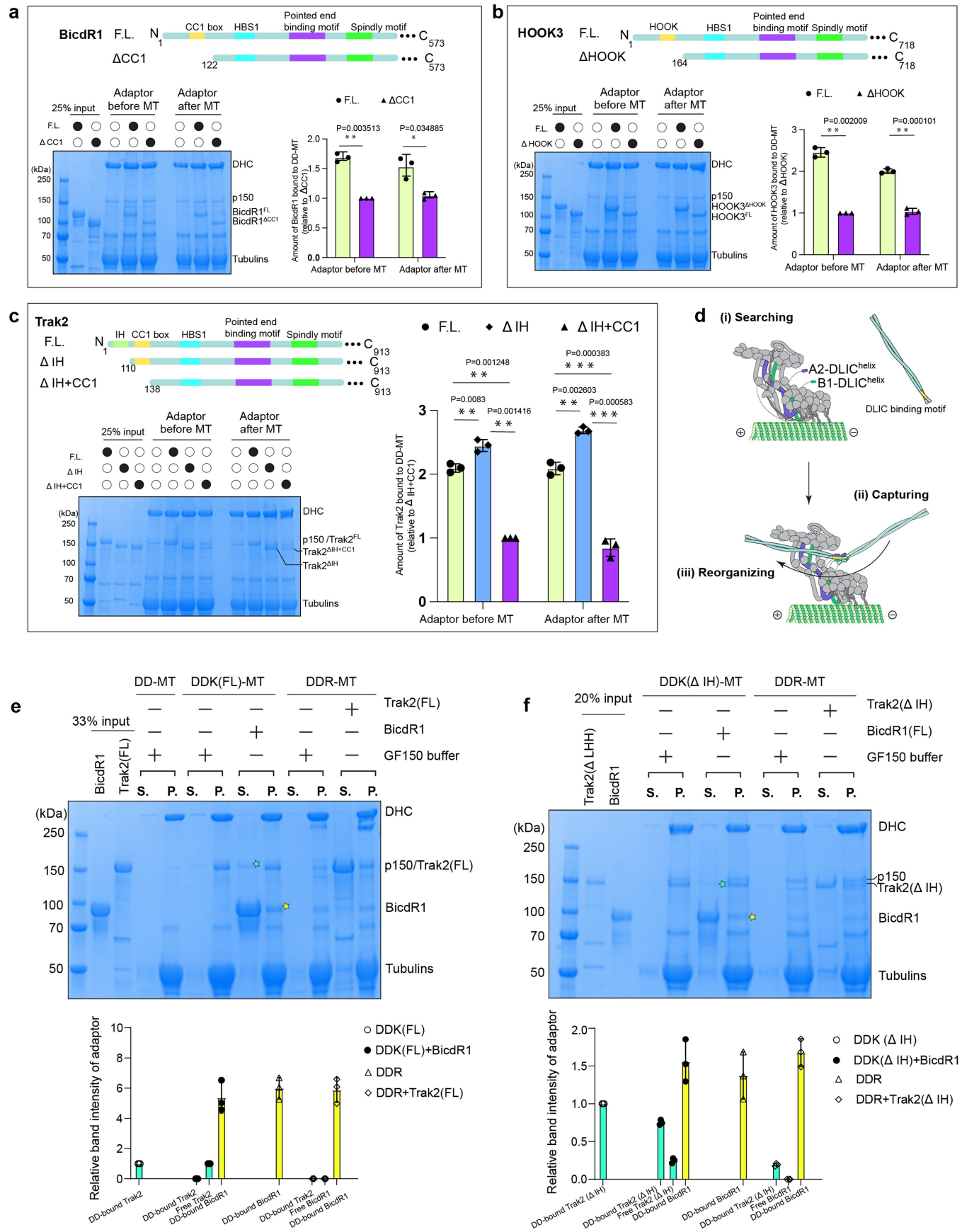
	DD	DDL	DDK	DDKL	DDR	DDRL (Particles NO. , %)
DDA	0	0	0	0	908 (2.6%)	1872 (3.7%)
Free dynactin	7524 (32%)	6967 (35%)	9803 (30.1%)	6816 (34.5%)	10476 (30.0%)	14368 (28.4%)
Free dynein (Minus the motors from DDA and divided by 2)	15989 (68%)	12939 (65%)	22765 (69.9%)	12942 (65.5%)	23119 (66.2%)	34960 (69.1%)

Extended Data Fig. 6 | Cryo-EM analysis of the effect of LIS1 on DD and DDA complex assembly. (a) Cryo-EM data processing workflow for DD, DDL, DDK, DDKL, DDR, and DDRL complexes. (b) Quantitative analysis of particle distributions corresponding to dynein, dynactin, and DD/DDA complexes.



Extended Data Fig. 7 | Binding affinity measurement of DD complex to MTs in different nucleotide binding states. SDS-PAGE gels of dynein-dynactin complex bound to MTs in various nucleotide binding states, (a) Apo, (b) ADP, (c) AMPPNP, (d) ATP, (e) ADP.Vi, stained with simple blue. All dynein samples

used in this assay are in a nucleotide-free condition (Apo). Gels are representative of $n = 3$ independent experiments. For gel source data, see Supplementary Fig. 1m–q.

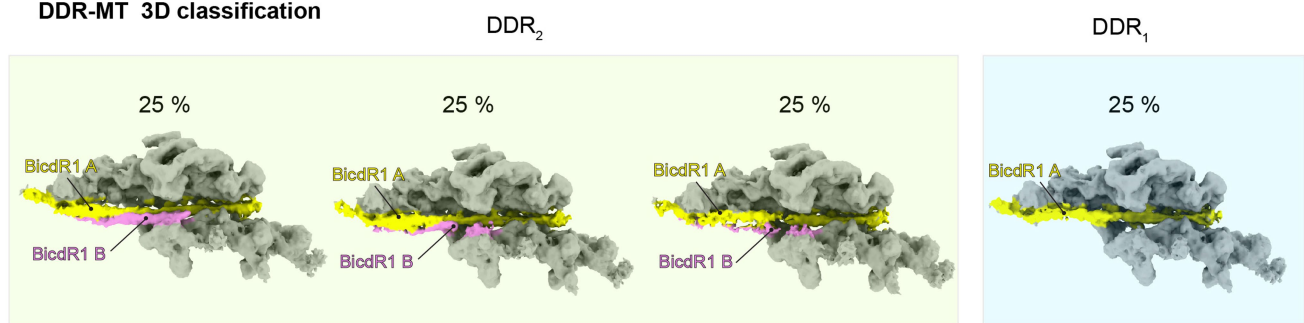


Extended Data Fig. 8 | See next page for caption.

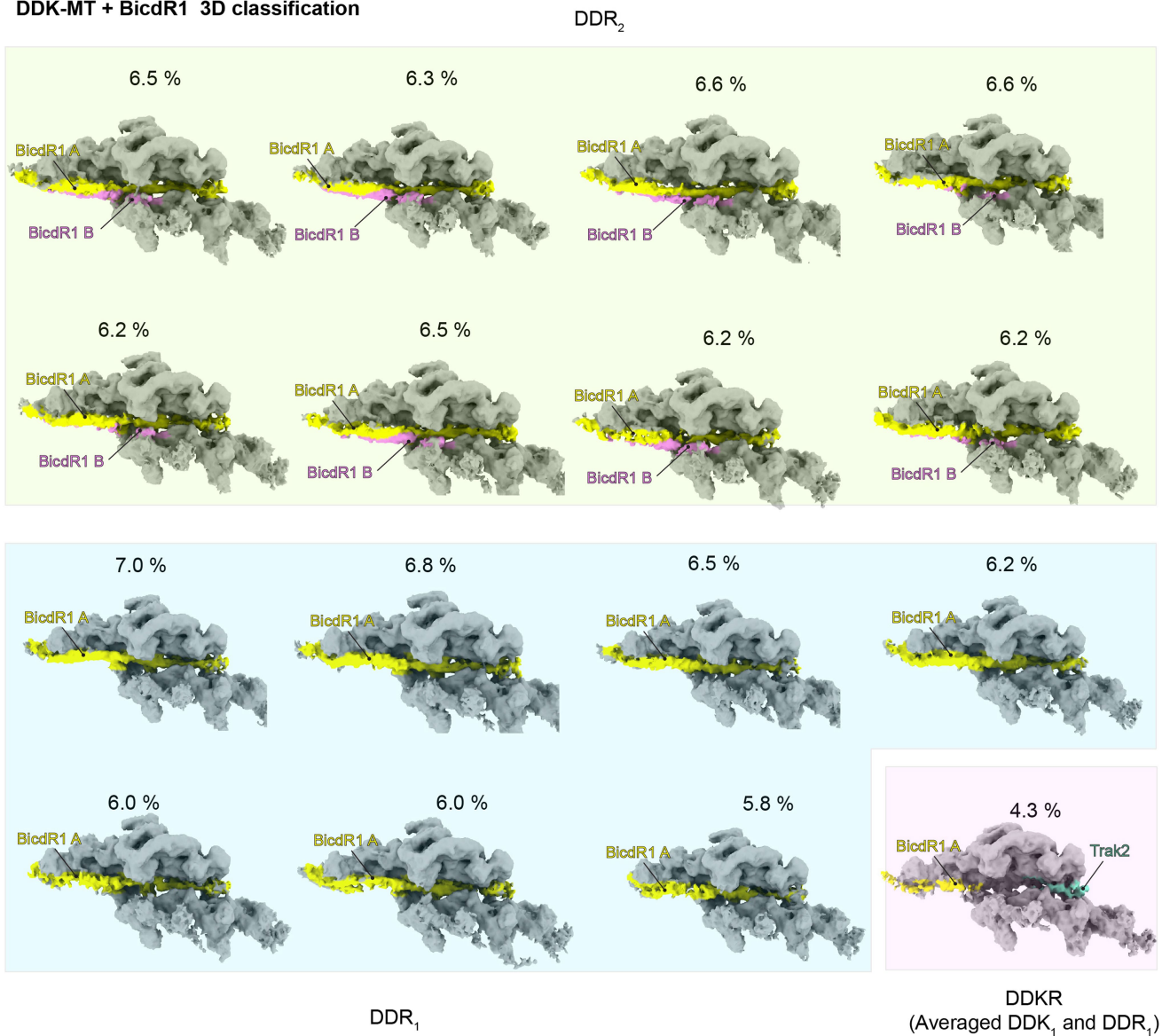
Extended Data Fig. 8 | DLIC-binding motif of adaptor is essential for adaptor recruitment and competition. (a-c) Schematic representations of BicdR1, HOOK3, and Trak2 adaptors showing their key domains and deletion constructs. Microtubule pelleting assays compare the binding of full-length (F.L.) and deletion constructs (Δ CC1, Δ HOOK, Δ IH, Δ IH + CC1) to the dynein-dynactin complex in adaptor before MT and adaptor after MT conditions. SDS-PAGE gels stained with simple blue, quantified in bar graphs with statistical significance indicated (right panel). The Y-axis represents the relative amount of adaptor protein compared to each deletion construct: Δ CC1 for BicdR1, Δ HOOK for HOOK3, and Δ IH + CC1 for Trak2. Gels are representative of $n = 3$ independent experiments, data are presented as mean $\pm$ s.d., and analyzed by unpaired t -test with Welch's correction. For gel source data, see Supplementary

Fig. 1r-t. (d) A proposed model illustrating the stages of adaptor recruitment by DD complex. BicdR1 competes with Trak2 in pre-assembled DDK-MT complex. The adaptor competition assay showing BicdR1 competing with the pre-formed DDK-MT and Trak2 competing with the pre-formed DDR complex. SDS-PAGE gels stained with simple blue show the results of these competition assays. Competition assay with full-length Trak2 (Trak2(FL)) (e), and truncated Trak2 (Trak2(Δ IH)) (f). The quantification of adaptor binding to the DD-MT complex for each competition condition is presented beneath the representative gel image. S, supernatant; P, pellet. The Trak2 competed off from the DDK-MT complex is marked with green stars, while the newly bound BicdR1 in the DDKR-MT complex is marked with yellow stars. Gels are representative of $n = 3$ independent experiments. For gel source data, see Supplementary Fig. 1u-v.

a DDR-MT 3D classification

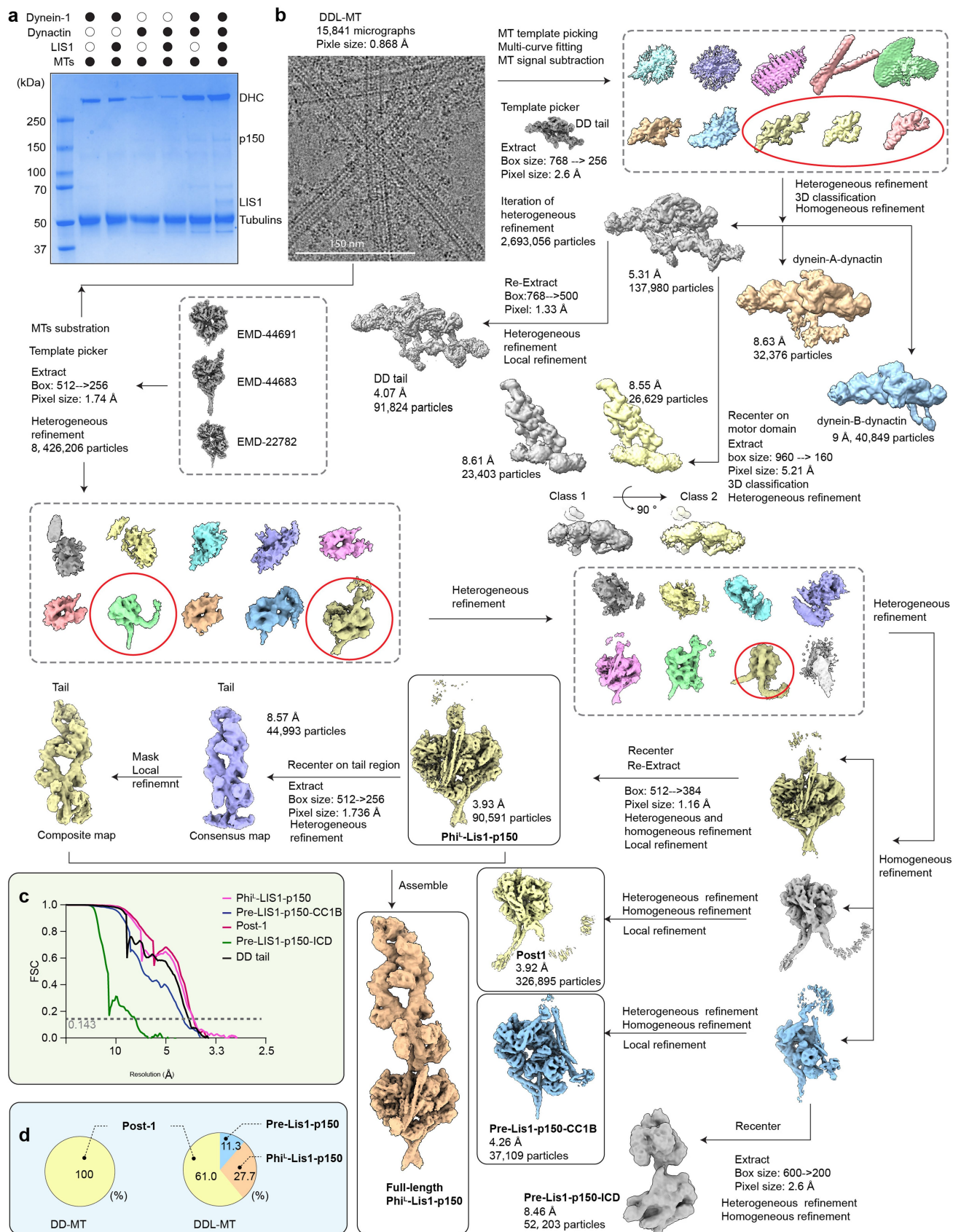


b DDK-MT + BicdR1 3D classification



Extended Data Fig. 9 | 3D classification analysis of DDR-MT and DDKR-MT complexes. (a) 3D classification of DDR complexes showing two classes: DDR_2 and DDR_1 , each representing 25% of the total particles. (b) 3D classification of DDKR complexes showing multiple subclasses of DDR_2 and DDR_1 , with

percentages indicating the proportion of each subclass. The averaged structure of DD-Trak2/BicdR1 is also shown, representing 4.3% of the total particles.



Extended Data Fig. 10 | Cryo-EM analysis of the DDL-MT complex.

(a) Microtubule co-sedimentation assay showing that LIS1 associates with the DD-MT complex to form the DDL-MT complex. Recombinant human dynein was used for DDL-MT assembly. Gel is representative of $n = 3$ independent experiments. For gel source data, see Supplementary Fig. 1w. (b) Data processing

workflow for the DDL-MT complex. (c) FSC curves for the Phi^L-Lis1-p150, pre-LIS1-p150-CC1B, pre-LIS1-p150-ICD, post-1, and DD tail reconstructions. (d) Pie charts summarizing the conformational states of dynein-A within the DD-MT (left) and DDL-MT (right) complexes.

Extended Data Table 1 | Cryo-EM data collection, refinement, and validation statistics of DD-MT and D-MT complexes

	#1, Dynein-MT Overall EMD-46844 PDB-9DGQ	#2, Dynein-MT Motor domain EMD-46843 PDB-9DGP	#3, DD-MT Overall EMD-46845 PDB-9DGR	#4, DD-MT Dynactin-Dynein-Tail EMD-46846 PDB-9DGS	#5, DDR-MT One BicdR1 EMD-46847 PDB-9DGT	#6, DDR-MT Two BicdR1 EMD-46848 PDB-9DGU	#7, DDK-MT EMD-46849 PDB-9DGV
Data Collection and Processing							
Facility	Yale ScienceHill-Cryo-EM facility		Laboratory for BioMolecular Structure in BNL		Yale ScienceHill-Cryo-EM facility		BNL
Microscope	Glacios		Titan Krios		Glacios		Titan Krios
Voltage (kV)	200		300		200		300
Camera	K3		K3		K3		K3
Magnification	45k		105k		45k		105k
Pixel Size (Å)	0.434 (super resolution)		0.4125 (super resolution)		0.434 (super resolution)		0.4125 (super resolution)
Total Electron Exposure (e-/Å²)	40		40		40		40
Defocus Range (µm)	1.5-2.7		1.5-2.7		1.5-2.7		1.5-2.7
Symmetry Imposed	C1	C1	C1	C1	C1	C1	C1
Num of mics	6111		10660		3000		8369
Initial Particles	1 863 852		583 852		390 773		924 770
Final Particles	44 792	106 660	16 372	62 404	10 750	10 576	52 920
Refinement							
Initial models	7z8f	7z8f	7z8f, 6znl	7z8f, 6znl	7z8f, 6znl	7z8f, 6znl	7z8f, 6znl
Map pixel size	5.2	1.302	3.3	1.23	1.736	1.736	3.3
Map Resolution (Å) (FSC 0.143)	10.8	3.4	15	3.9	7.2	7.1	8.8
Map sharpening B-factor (Å²)	NA	66	NA	43.1	NA	NA	NA
Model Composition							
Non-hydrogen atoms	32970	24594	131722	85495	89407	93353	86558
Protein residues	8153	3038	28258	11586	12054	12537	11800
Ligands	MG: 2 ANP: 4 ADP: 2 ATP: 2	MG: 4 ANP: 2 ADP: 1 ATP: 1	MG: 1 ZN: 3	ADP: 9 ATP :1 ZN: 3	ADP: 9 ATP :1 ZN: 3	ADP: 9 ATP :1 ZN: 3	ADP: 9 ATP :1 ZN: 3
Model vs. Data							
FSC Map to Model (Å) (FSC 0.5)	NA	3.7	NA	6.4	NA	NA	NA
Correlation coefficient (mask)	NA	0.77	NA	0.78	0.74	0.72	0.61
B factors (Å²)							
Protein	76.33	68.82	464.7	300.6	328.4	348.55	308.78
Ligand	51.63	39.34	196.63	211.5	211.5	211.5	211.5
R.m.s deviation							
Bond length (Å)	0.01	0.007	0.007	0.004	0.004	0.005	0.003
Bond angles (°)	1.513	1.022	1.309	0.97	0.701	0.983	0.63
Validation							
Molprobability score	0.87	1.53	1.95	1.95	2.04	2.06	1.95
Clashscore	0.98	10.25	10.52	16.02	19.7	21.59	16.21
Rotamer outliers (%)	0	0.11	0	0.14	0.27	0.18	0.14
Ramachandran plot							
Outliers (%)	0.02	0	0.4	0.1	0.1	0.1	0.12
Allowed (%)	2.33	1.33	5.78	3.56	3.6	3.4	3.51
Favored (%)	97.65	98.67	93.82	96.34	96.29	96.5	96.37
Rama-Z (whole)	1.21	0.91	-0.83	0.53	0.35	0.29	0.52

Extended Data Table 2 | Cryo-EM data collection, refinement, and validation statistics of DDL-MT complex

	#8, Dynein-dynactin tail EMD-73178 PDB-9YNG	#9, Composite Full- length Phi ^L -LIS1-p150 EMD-73179 PDB-9YNH	#10, Phi ^L -LIS1- p150-CC1B EMD-73173 PDB-9YNC	#11, Pre-LIS1- p150-CC1B EMD-73174 PDB-9YND	#12, Pre-LIS1-p150- CC1B-ICD EMD-73175 PDB-9YNE	#13, Dynein-motor- Post1 EMD-73176 PDB-9YNF
Data Collection and Processing						
Facility	Yale ScienceHill-Cryo-EM facility					
Microscope	Glacios					
Voltage (kV)	200					
Camera	K3					
Magnification	45k					
Pixel Size (Å)	0.434 (super resolution)					
Total Electron Exposure (e-/Å ²)	40					
Defocus Range (µm)	1.5-2.7					
Symmetry Imposed	C1	C1	C1	C1	C1	C1
Num of mics	15,841					
Initial Particles	2,693,056			8,426,206		
Final Particles	91,824	44,993	90,591	37,109	52,203	326,895
Refinement						
Initial models	7z8f, 6znl	9e13, 8ptk	9e13, 8ptk	8ptk	8ptk	9bmv
Map pixel size	1.33	1.736	1.157	1.736	2.604	1.736
Map Resolution (Å) (FSC 0.143)	4.07	5.5	3.93	4.26	8.46	3.92
Map sharpening B- factor (Å ²)	63.0	-	71.6	73.9	656.8	125.6
Model Composition						
Non-hydrogen atoms	83,380	62,504	56,882	32,118	29,128	24,588
Protein residues	11,583	12,571	7,224	4,160	5,858	3038
Ligands	ZN:3 ADP: 9 ATP: 1	MG: 4 ADP: 6 ATP: 2	MG: 4 ADP: 6 ATP: 2	MG: 2 ADP: 3 ATP: 1	MG: 2 ADP: 3 ATP: 1	MG: 1 ADP: 2 ATP: 1 AMPPNP: 1
Model vs. Data						
FSC Map to Model (Å) (FSC 0.5)	4.7	4.6	4.3	4.6	15.9	4.1
Correlation coefficient (mask)	0.78	0.54	0.86	0.82	0.53	0.81
B factors (Å ²)						
Protein	282.17	246.39	207.07	189.90	529.25	66.61
Ligand	153.59	222.75	169.82	142.70	494.11	46.84
R.m.s deviation						
Bond length (Å)	0.003	0.002	0.002	0.002	0.003	0.003
Bond angles (°)	0.584	0.457	0.570	0.556	0.786	0.564
Validation						
Molprobability score	1.97	1.99	1.70	1.75	1.82	1.58
Clashscore	15.66	11.42	11.56	12.27	6.52	9.24
Rotamer outliers (%)	0.04	0.02	0	0	0	0
Ramachandran plot						
Outliers (%)	0.08	0.03	0	0.02	0.02	0
Allowed (%)	3.91	6.20	2.60	2.76	7.26	2.42
Favored (%)	96.01	93.77	97.40	97.22	92.72	97.58
Rama-Z (whole)	0.80	0.52	2.02	1.45	-2.18	1.56

Reporting Summary

Nature Portfolio 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 Portfolio policies, see our [Editorial Policies](#) 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.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Serial EM4.1, DigitalMicrograph 3.5, EPU 2.6.1
Data analysis	Relion 4.0, MotionCor2, Gctf 1.08, Gautomatch 0.60, Cryosparc 4.12, Coot 0.9.8.1, Molprobit 4.5.2, PyMOL 2.5.4, ChimeraX 1.5, Phenix 1.20.1, AlphaFold 2 & 3, Clustal Omega 1.2.4, http://github.com/JackZhang-Lab .

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All atomic coordinates and cryo-EM maps have been deposited in the Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB) under accession codes 9DGG/46844 (D-MT, overall structure), 9DGP/46843 (D-MT, dynein motor domain), 9DGR/46845/ (composite DD-MT structure), 9DGS/46846 (dynactin-dynein tail of DD-MT complex), 9DGT/46847 (DDR1-MT, dynactin-dynein tail-one dimeric BicdR1), 9DGU/46848 (DDR2-MT, dynactin-dynein tail-two dimeric BicdR1),

9DGV/46849 (DDK-MT, dynactin-dynein tail-one dimeric Trak2), 9YNG/73178 (dynactin-dynein tail of DDL-MT dataset), 9YNH/73179 (composite full-length Phil-LIS1-p150), 9YNC/73173 (Phil-LIS1-p150-CC1B, motor domain of Phil-LIS1-p150), 9YND/73174 (Pre-LIS1-p150-CC1B, dynein single motor in pre-powerstroke state bound with p150-CC1B domain), 9YNE/73175 (Pre-LIS1-p150-CC1B-ICD, dynein motor in pre-powerstroke state bound with p150-CC1B-ICD domain), 9YNF/73176 (Post1, dynein motor in post1 state).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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](https://www.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	No sample-size calculation or pre-specification of sample size was used in our experimental design. The final sample size was chosen by available electron microscopy time and how many usable particles were present on the micrographs obtained during each dataset collection.
Data exclusions	All micrographs were checked manually and unusable ones were excluded on the estimated drift parameters, defocus ranges, estimated usable information by contrast transfer functions, ice thickness, 2D average qualities, 3D classification and reconstruction results using GCTF and cryoSPARC.
Replication	Cryo-EM structures were lowpass filtered and re-refined, which generated consistent reconstructions. All classified particles were re-processed to generated reference-free 2D class average that are consistent multiple cycles of classification, demonstrating the replication of our classification approaches.
Randomization	All particles were randomly split for the purposes of estimating the resolutions based on the 0.143 criterion of the Fourier Shell Correlation. Otherwise randomization was not relevant to these studies.
Blinding	Blinding was not relevant to this study as no populations were preassigned to experimental groups.

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

Methods

n/a	Involvement in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

n/a	Involvement in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Mouse anti-FLAG M2 (SIGMA; product number: F1804)
Validation	ANTI-FLAG M2 antibody, Mouse monoclonal recognizes the FLAG sequence (DYKDDDDK) at the N-terminus, Met N-terminus, and C-terminus. For highly sensitive and specific detection of FLAG fusion proteins by immunoblotting, immunoprecipitation (IP), immunohistochemistry, immunofluorescence and immunocytochemistry. Optimized for single banded detection of FLAG fusion proteins in mammalian, plant, and bacterial expression systems. For more details: https://www.sigmaaldrich.com/SG/en/product/sigma/f1804

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Expi293F cells were used only for protein production, originally purchased from ThermoFisher Scientific (cat no. A14527).
Authentication	No cell line authentication was used.
Mycoplasma contamination	Cells were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Plants

Seed stocks	N.A.
Novel plant genotypes	N.A.
Authentication	N.A.