Cytoplasmic dynein is a motor protein that walks along microtubules (MTs) and performs mechanical work to power a variety of cellular processes. It remains unclear how a dynein dimer is able to transport cargos against load without coordinating the stepping cycles of its two heads. Here by using a DNA-tethered optical trapping geometry, we find that the force-generating step of a head occurs in the MT-bound state, while the ‘primed’ unbound state is highly diffusional and only weakly biased to step towards the MT-minus end. The stall forces of the individual heads are additive, with both heads contributing equally to the maximal force production of the dimer. On the basis of these results, we propose that the heads of dynein utilize a ‘load-sharing’ mechanism, unlike kinesin and myosin. This mechanism may allow dynein to work against hindering forces larger than the maximal force produced by a single head.
Cytoplasmic dynein (herein referred to as dynein) motors walk processively towards the microtubule (MT)-minus end and generate forces of several pN \(^1\,^2\). The mechanical work produced by dynein motors has a broad range of cellular functions, including cargo transport, mitotic spindle positioning and organization of the MT network \(^3\). Despite dynein’s central roles in neurobiology and development, the mechanism of its force production remains poorly understood in comparison with other molecular motors, in part because of its large size and complex structure \(^4\).

Dynein is a homodimer of two ~500-kDa heavy chains. In contrast to kinesin and myosin, which have a single ATP-binding site per motor domain, the dynein motor domain (head) contains six AAA\(^+\) ATPase subunits arranged into a hexameric ring (Fig. 1a). Four of the AAA\(^+\) subunits bind nucleotide and the AAA1 subunit serves as the primary site of ATP hydrolysis. The AAA\(^+\) ring connects to a MT via a 15-nm coiled-coil stalk bearing a small MT-binding domain (MTBD), resulting in a ~25-nm separation between the MTBD and the AAA1 site \(^5\,^6\). The two rings dimerize through an N-terminal tail domain, which also serves as the binding site for a number of light chains and adapter proteins \(^7\). Dynein-driven transport requires other components, such as the cofactor dynactin, and regulatory proteins Lis1 and Nud\(e\) \(^8\).

The following model of dynein’s mechanochemical cycle has been proposed to explain how a dynein monomer generates force. ATP binding to the AAA1 site \(^9\) triggers the head’s release from the MT and drives a priming stroke of the linker \(^9\). The linker, a long hinged domain at the base of the tail \(^10\,^11\), undergoes large-scale conformational changes across the face of the AAA \(\mp\) ring in an ATP-dependent manner \(^9\,^12\,^13\) (Fig. 1b). Notably, the linker exits the ring at the AAA4 site in the unprimed state and at the AAA2 site in the primed state. The priming stroke has been proposed to move the stalk and MTBD of the unbound head towards the minus end of the MT \(^9\). After ATP hydrolysis, the head re-binds to MT at a new location and releases inorganic phosphate \(^10\). The linker then undergoes a power stroke, generating tension in the process and returning the monomer to its unprimed state \(^13\). While intramolecular tension has been proposed to play a significant role in dynein motility, the magnitude of this tension remains to be measured directly. The proposed model does not explain how much mechanical work is produced by conformational changes of the linker and how two heads function together in a dimer to walk against a hindering load.

In this study, we use a head-tethered optical trapping geometry \(^14\) to directly observe the force production and stepping of individual heads of a walking dynein dimer. We find that each head relies on diffusion to travel to the next binding site on the MT following its priming stroke. Force is then produced by the power stroke after the head rebinds to the MT. The stall forces of the two heads are approximately additive despite the absence of coordination in their stepping, leading us to propose a load sharing model for dynein-driven cargo transport. These results reveal unique properties of dynein force generation in comparison with other cytoskeletal motors.

**Results**

**Development of head tethered dynein geometry.** Force production of cytoskeletal motors has been studied extensively by attaching an optically trapped bead to the dimerization domain. Such assays directly measure the step size and stall force of the motor’s tail \(^2\), providing detailed information about the load dependence of each head’s stepping kinetics under the assumption that the heads alternately take steps and swap the leading position. However, dynein’s heads can adapt a wide variety of orientations and step independently of each other \(^15\,^16\). Therefore, not much can be learned about the force production and stepping of an individual head from these assays.

In order to characterize the behaviour of a dynein head under load, we linked an optically trapped bead to one of the heads of GST-Dyn1 \(\sim 31 \text{kDa}\), a tail-truncated Saccharomyces cerevisiae dynein dimerized with glutathione S-transferase (GST) (Supplementary Fig. 1). This construct takes steps of the same size and duration as full-length dynein \(^17\), stalls at ~70% of full-length dynein’s stall force \(^2\), and is herein referred to as wild type (WT). Dynein was covalently linked to a DNA tether at its C terminal (Fig. 2a). The labelling efficiency of the DNA tether to dynein was adjusted (Fig. 2b) to be <15% to minimize the likelihood of dual labelling. The length of the tether (74 bp, 25 nm) was sufficiently long to minimize steric hindrance from the trapped bead on the walking molecule. To test the impact of C-terminal DNA tethers on dynein motility in unloaded conditions, we labelled the distal end of the tether with Cy3 (Supplementary Fig. 2a) and observed the motility of DNA-tagged motors in a single-molecule fluorescence assay (Fig. 2c). Labelled motors remained active and processive, walking at
respectively (mean ± s.e.m.; two-tailed t-test, P = 0.28; Fig. 2e,f). These values are in agreement with the tail-tethered force measurements using the same construct (Arne Gennerich, personal communication). Stall forces are not affected by the C-terminal fusion of the HaloTag (HT) domain because a construct with an N-terminal HT fusion stalls at similar forces (Supplementary Fig. 3).

Next, to determine the effect of load on the stepping of a dynein head, we collected and analysed position traces of head- and tail-tethered dynein motors under near-stall (2.5 pN) hindering loads in the presence of saturating (1 mM) ATP (Fig. 3a). The size of steps taken by the DNA-tethered head (12.1 ± 0.7 nm backward and 11.0 ± 0.5 nm forward steps, mean ± s.d.) was indistinguishable (two-tailed t-test, P = 0.67) from the size of steps measured at the tail (12.7 ± 5.7 nm backward and 12.3 ± 5.6 nm forward steps; Fig. 3b). This is consistent with our previous observation that stall forces remain the same between the two geometries because one ATP-driven step is capable of generating a specific amount of work, which is a product of force and step size. Moreover, stepping rates remained similar between the two geometries (10.2 ± 0.5 s⁻¹ head-tethered, 10.6 ± 0.5 s⁻¹ tail, mean ± 95% confidence interval; Fig. 3c). This is in contrast to unloaded conditions, in which the head takes nearly twice the centre-of-mass step size and steps less frequently15,17. Our findings indicate that the DNA-tethered head decreases its step size to match that of tail-tethered dynein under load.

Figure 2 | Measuring forces produced by a single dynein head.
(a) Schematic of the head-tethered optical trapping assay for measuring forces produced by a single dynein head. One head of a GST-dimerized motor (GST shown in orange) is labelled with a DNA tether at the C-terminal HT tag (yellow) and tethered to a trapped bead through a biotin–streptavidin (green) linkage. (b) Cy5-DNA-labelled dynein migrates in two distinct bands in a denaturing gel. Fluorescent image identifies the labelled fraction. Labelling efficiency was 31% in the gel shown here. (c) Kymograph shows that Cy3-DNA-labelled dynein moves processively towards the MT-minus end. Scale bars: 2 μm (horizontal), 10 s (vertical). (d) Measurement of the stall force of head-tethered dynein. Valid stall events are marked with red ticks. Trap stiffness (ktrap) is 0.037 pNnm⁻¹. (e,f) Stall force histograms of head- and tail-tethered dynein motors, respectively (mean ± s.e.m.; N = 97 and 123, respectively).

98 ± 9 nm s⁻¹ (mean ± s.e.m., N = 73), slightly slower than DNA-free motors tagged with green fluorescent protein (GFP; 117 ± 7 nm s⁻¹, N = 64). (Supplementary Fig. 2b,c). These results show that dynein’s motility is not significantly hampered by the presence of a DNA tether. Yet, the tether remains relatively rigid18 and does not introduce excessive noise into the trap recordings. The distal ends of the DNA tethers were attached to polystyrene beads through a biotin–streptavidin linkage.

Effects of load on stepping and force production of a head. Using a similar geometry, it has been shown that a kinesin-1 head takes twice the steps’ size of the tail; however, its stall force halves relative to that of the tail14. This is consistent with the energetics of a kinesin step, which harnesses the hydrolysis energy from one ATP and produces the same amount of work19. Because dynein lacks a regular stepping pattern15,16 and possesses multiple ATP-hydrolysis sites, a dynein head may respond differently to external load, which would in turn affect its measured force production. To test this, we compared the stall force of a DNA-tethered dynein head with the combined force production through the tail domain (Fig. 2d). Unlike kinesin, the head-tethered motor stalls at a force of 3.0 ± 0.1 pN (mean ± s.e.m.), similar to the stall force of the tail-tethered motor (3.1 ± 0.1 pN, mean ± s.e.m.; two-tailed t-test, P = 0.28; Fig. 2e,f). These values are in agreement with the tail-tethered force measurements using the same construct (Arne Gennerich, personal communication). Stall forces are not affected by the C-terminal fusion of the HaloTag (HT) domain because a construct with an N-terminal HT fusion stalls at similar forces (Supplementary Fig. 3).

Measurement of force generated by dynein’s power stroke. The linker swing mechanism is essential for force generation and motility of dynein motors8,13. It has been proposed that force is produced in the MT-bound state, as the linker transitions from the primed to unprimed conformation. On the other hand, the linker undergoes a priming transition as it releases from MT, and this was proposed to drive the movement of the stepping head towards the minus end9. It remains unclear how much work is performed by each of these conformational changes. To address this question, we measured the stall force and step size of a dynein heterodimer in which one head is rendered catalytically inactive. Force production in one head was abolished by introducing a K/A mutation in the Walker A motif of the AAA1 site, preventing ATP from binding20. The mutant (AAA1K/A) head was dimerized with a WT head using N-terminal FKBP12 and FRB (FKBP-rapamycin binding domain) tags (WT/AAA1K/A, Fig. 4a)17. Constructs dimerized via the FRB–rapamycin–FKBP12 chemistry exhibit similar processivity and stepping properties to GST-dimerized dynein17. The stall force of head-tethered FRB–FKBP12-dimerized WT/WT dynein is similar (3.0 ± 0.1 pN, t-test, P = 0.90, N = 13) to the GST-dimerized construct, verifying that heterodimerization tags do not hinder the motor’s force production.

By attaching the DNA tether to either the WT head or the mutant head, we were able to separately investigate the energetics of the two strokes. Because the mutant (AAA1K/A) head remains in an unprimed, tightly bound state and is incapable of undergoing a power stroke12, motility is powered solely by the active (WT) head. High-resolution tracking studies of this
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Primin stroke does not generate substantial work. To test whether the priming stroke of the linker produces mechanical work as the WT head searches for a new MT-binding site, we attached the DNA tether to the WT head of the heterodimer (Fig. 5a). In contrast with our previous finding that mutant head-tethered heterodimers stall at half the stall force of the WT/WT homodimer, we measured the stall force of the live head-tethered construct to be as low as 0.5 ± 0.1 pN (mean ± s.d.; Fig. 5b,c). This construct showed that WT head mostly remains in the lead and drags the mutant head forward21. The force required to pull the mutant head is likely supplied by the power stroke of the WT head and transmitted via linker tension. In contrast, when the tether is directly attached to the sole active head, this head must step forward against load without any assistance provided by its inactive partner. An active dynein head releases from the MT and moves forward as its linker is primed. It is possible that, during this motion, the MT-bound head serves as an anchor that the stepping head actively pushes away from through the priming stroke of its linker2. Therefore, we expect stall force measurements in the WT head-tethered geometry to test whether the priming stroke of the linker produces mechanical work during the stepping of a head.

The WT/AAA1K/A heterodimer walks processively at 13.6 ± 1.2 nm s⁻¹. When the DNA tether was attached to the AAA1K/A mutant head, the motor stalled at half the stall force of the WT/WT homodimer, 1.5 ± 0.4 pN (mean ± s.d., Fig. 4b,c). Because the DNA-tethered head is catalytically inactive, this assay constitutes a measurement of the minimal amount of force generated by dynein’s power stroke. The step size of the mutant head at near-stall (1.5 pN) hindering load was slightly lower than that of the WT head at 2.5-pN-hindering load (9.5 ± 6.2 nm for backward steps and 8.5 ± 5.5 nm for forward steps, Supplementary Fig. 5a,b), and the stepping rate was ~2.5 times lower than that of a WT head at 3.6 ± 0.3 s⁻¹ (Supplementary Fig. 5c). The stall force of a head remained nearly constant across 20 μM to 1 mM ATP (Fig. 4d). The lowest tested ATP concentration of 20 μM is below the published K_M (ATP) of yeast dynein22 and less than half of the K_M (ATP) of the Dyn133 kDa construct used in this study (46 μM, Supplementary Fig. 6). This contrasts with kinesin-1, the stall force of which decreases at limiting ATP concentrations23. Our results are consistent with the observation that the stall force of the dynein centre-of-mass does not depend on ATP concentration2. Furthermore, because a stalled dynein is in equilibrium between forward and backward steps, our finding suggests that both forward and backward steps are governed by the same ATP-dependent rate and slow down proportionately when ATP is limiting.

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force corresponds to an energetic bias of \( \sim 1.5 \text{k_BT} \) at room temperature, assuming an average step size of \( \sim 12 \text{nm}^{17} \). Therefore, a single dynein head is only minimally biased to move forward when it is not bound to the MT and is unable to generate appreciable forces in the unbound state. To rule out the possibility that the extremely low forces produced by the WT head-tethered construct may be caused by a loss of processivity upon DNA attachment, we verified that this construct still walks processively over long distances against sub-stall (0.4 pN)-hindering loads \( (k_{\text{trap}} = 0.008 \text{pN nm}^{-1}) \). Despite having a very low stall force, the live-head-tethered construct is able to move processively over long distances from the MT, a dynein head undergoes a priming stroke. This component of the step is highly diffusional and can be biased backwards by external load. The force-generating step occurs after the head binds to a new site on the MT lattice. The force-generating step occurs after the head binds to a new site on the MT lattice.

**Force–velocity relationship of a dynein head.** To investigate how the overall motility of a dynein dimer is affected by load when one or both heads are able to produce force, we measured the velocities of head-tethered WT/WT and mutant head-tethered WT/AAA1K/A heterodimer under varying loads at saturating (1 mM) ATP. The shape of a motor’s force–velocity (F–V) curve is determined by how forward and backward stepping rates are affected by external load\(^{22,23} \). Our findings differ from the F–V relationship previously reported for full-length dynein in centre-of-mass trapping geometry\(^7 \). The velocities we observe increase continuously as external force transitions from hindering to assisting values. There is no detectable plateau at near-zero forces, suggesting that the principal rate-limiting transition in the step of a dynein head involves substantial movement along the MT axis.

The F–V curve fits well \( (R^2 = 0.975) \) to a simple one-state motor model (Fig. 6a), wherein the movement of a motor is fully described by load-dependent forward and backward stepping rates (see Supplementary Note 1). Interestingly, the best fit...
The results suggest that the different response of forward and backward stepping rates to load arises from the asymmetry of the MT-binding interface. It has been shown that the dynein MTBD behaves as a catch bond under load, as hindering forces slow down its ATP-dependent release from the MT21. The characteristic length arising from our F–V curve may thus correspond to a distance by which the head needs to move in order to release the catch bond of the MTBD.

Discussion

Characterizing the behaviour of both WT and mutant heads under load allowed us to provide a mechanistic description of the force production by the two heads of a walking dimer. The force is generated mainly in the MT-bound state, consistent with the power stroke model26. Our force measurements set a lower bound for the force produced by a power stroke at 1.5 pN (Fig. 4). This force also constitutes a measurement of the maximal force with which one head can pull on its partner head through linker tension. While the priming stroke of the unbound head has been proposed to push against the MT-tethered head and drive motility towards the MT-minus end8, we found that it provides less than 1.5 kBT of directional bias per step and thus cannot account for the mechanical work performed by dynein over one mechanochemical cycle. However, the priming stroke remains vital to dynein’s mechanochemical cycle as it is likely to increase the reach of the diffusional step and prime the linker to enable an effective subsequent power stroke.

We demonstrated that a dynein head is able to generate half the stall force of the dimer. Given dynein’s flexibility and lack of strict interhead coordination, the heads in a dynein dimer can be thought of as a pair of mechanically coupled monomers as opposed to a single cyclic machine akin to myosin V or kinesin-1. We propose that each of dynein’s monomers is capable of independently generating approximately half of the stall force of the dimer, and that each head’s force production contributes to the total output of the dimer. We refer to this framework as ‘load-sharing’ between dynein heads to highlight its similarities with the additive forces observed when a cargo is driven by a low number of processive motors27–29.
Our vision of how load-sharing may be achieved in cytoplasmic dynein is illustrated in Fig. 7. A dynein dimer spends the majority of the time with both heads bound to the MT. Unlike kinesin and myosin, the hindering load is distributed among both heads with their long flexible tails acting as springs (Fig. 7-i). When one head releases from the MT, the entire load is transferred to the bound head (Fig. 7-ii). The bound head functions as an anchor, while the released head performs a diffusional search for a new binding site. During this diffusional search, the tail may briefly move backwards in response to external load; however, we expect this motion to be too rapid to be resolvable, given the ~3-ns time resolution of our assay. When the stepping head rebinds to MT (Fig. 7-iii) and its linker undergoes a power stroke (Fig. 7-iv), load is redistributed between the tails. The tail of the leading head becomes more stretched, as undergoes a power stroke (Fig. 7-iv), load is redistributed between the large and flexible structure of the dynein dimer and can potentially aid the understanding of collective force generation and release (MTBR) protocol was used to remove excess DNA and enrich the population of active motors. For MTBR, freshly prepared MTs were suspended in sodium–phosphate buffer, pH 7.4) to two G-25 columns (GE Healthcare). De-salted tethers, at ~25 μM concentration in DLB, were frozen and stored at ~20°C. A biotinylated DNA oligo was replaced with a 5′ Cy5-modified oligo with an identical nucleotide sequence.

Protein purification. S. cerevisiae cultures expressing the dynein constructs used in the study were grown to an optical density (OD)600 of 1.0–1.5. They were then harvested by centrifugation and the pellets were resuspended in ~3 ml of water per litre of starting culture. The resuspended pellets were frozen by dripping into liquid nitrogen and lysed by grinding in a commercial stainless steel coffee grinder and can potentially aid the understanding of collective force generation among the two partner heads, allowing a dynein dimer to work against hindering forces higher than the maximal force produced by each head. A distinguishing feature of dynein’s motility under load is that the step size and stepping rate of a DNA-tethered head are the same as the size and rate of steps measured when the load is applied to the tail. This unusual finding can be explained by the large and flexible structure of the dynein dimer and tension-dependent changes in dynein’s stepping pattern21 (Supplementary Fig. 8).

Our findings further distinguish dynein from other cytoskeletal motors and provide new clues for understanding how it functions in vivo. Unlike kinesin-1, which readily releases from the MT at near-stall loads23, the dynein MTBD resists large backwards loads21. This may make dynein ideally suited for transportation of end-directed transport by multiple motors.

**Method**

Preparation of DNA tethers. Double-stranded DNA tethers labelled with HT ligand (Promega) on one end and biotin on the other were prepared as follows. A biotinylated DNA oligo (5′-biotin/TTTCGAGATTACCGGGCGAGAGCG TCAGGGCCAGGTTCACAGAGGGCGAGGGTGTTGGCCAGGGAGC) was hybridized with an amine-modified DNA oligo (5′-Cy5 amino/ GTTGGCGGGGTCGGGCTGGCCACCTCGGCTTCGTTGACCTCGGCTG GTCGAGGGGTCGGGCTGGCCACCTCGGCTTCGTTGACCTCGGCTG) in a solution (20 mM HEPES, 50 mM KCl, 1.25 mM MgCl₂, pH = 8.4) at 90°C for 2 min, followed by incubation at 25°C for 40 min. An amine-reactive HT Succinimidyl Ester (O4) ligand (Promega) was then attached to the terminal amine group on the DNA by incubation for 6 h at room temperature. The reaction was then quenched with 1 mM glycine for 10 min to remove excess reactive ligand. The resulting tethers were then passivated by adding powdered BSA to a final concentration of 10% glycerol, pH 7.7) to two G-25 columns (GE Healthcare). De-salted tethers, at ~25 μM concentration in DLB, were frozen and stored at ~20°C.

Conjugation of motors to microspeheres. CARboxyl latex beads (0.9 μm, Life Technologies) were coated with either streptavidin or custom-made rabbit polyclonal cGFP antibodies (Covance) as follows. The beads were washed four times in activation buffer (10 mM MES, 100 mM NaCl, pH 6.0) by vortexing, spinning down at 10,000 g for 5 min and resuspending. One milligram each of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and Sulfo-NHS (N-hydroxysulfosuccinimide) crosslinkers (Pierce Biotechnology), dissolved in 100 μl of dimethylformamide (DMF) were added to the beads. The solution was sonicated for ~5 min and vortexed at low speed until visible clumps disappeared. Afterwards, the beads were washed three times with coupling buffer (100 mM sodium–phosphate buffer, pH 7.4), mixed with streptavidin or antibodies (at ~1 mg of dry streptavidin or antibody per 100 μl of 1% w/v bead stock) and resuspending for 30 min. Then beads were then passivated by adding powdered BSA to a final concentration of 10 mg ml⁻¹ and shaking for 1.5 h at room temperature. Passivated beads were washed three times with wash buffer (50 mM KCl, 1.25 mM MgCl₂, 1 mM EDTA, 10% glycerol, pH 7.4) and can potentially aid the understanding of collective force production by trimeric outer-arm dyneins.32 Finally, it has been reported that multiple dyneins combine their efforts more effectively than do multiple kinesins33. The load-sharing mechanism inherent to a single dimer may explain dynein’s enhanced ability to pull cargos when working in a team. In a system with multiple motors transporting the same cargo, individual motors experiencing a hindering force will tend to remain attached to the track and prevent the cargo from slipping backwards. Meanwhile, motors that are not subjected to a significant hindering force will have a much higher probability of stepping forward, resulting in self-regulating and efficient minus-end-directed transport by multiple motors.

**Methods**

Preparation of DNA tethers. Double-stranded DNA tethers labelled with HT ligand (Promega) on one end and biotin on the other were prepared as follows. A biotinylated DNA oligo (5′-biotin/TTTCGAGATTACCGGGCGAGAGCG TCAGGGCCAGGTTCACAGAGGGCGAGGGTGTTGGCCAGGGAGC) was hybridized with an amine-modified DNA oligo (5′-Cy5 amino/ GTTGGCGGGGTCGGGCTGGCCACCTCGGCTTCGTTGACCTCGGCTG GTCGAGGGGTCGGGCTGGCCACCTCGGCTTCGTTGACCTCGGCTG) in a solution (20 mM HEPES, 50 mM KCl, 1.25 mM MgCl₂, pH = 8.4) at 90°C for 2 min, followed by incubation at 25°C for 40 min. An amine-reactive HT Succinimidyl Ester (O4) ligand (Promega) was then attached to the terminal amine group on the DNA by incubation for 6 h at room temperature. The reaction was then quenched with 1 mM glycine for 10 min to remove excess reactive ligand. The resulting tethers were then passivated by adding powdered BSA to a final concentration of 10% glycerol, pH 7.7) to two G-25 columns (GE Healthcare). De-salted tethers, at ~25 μM concentration in DLB, were frozen and stored at ~20°C.
times with 1 × PBS (pH 7.4) and stored at 4 °C with 0.1% sodium azide and 0.5 mg mL⁻¹ BSA.

**Sample preparation.** In FRB-FKBP12 heterodimer experiments, dynein monomers (of which only one was previously labelled with a biotinylated double-stranded DNA tether at the C-terminal HT) were dimerized with 200 nM rapamycin for 10 min at room temperature before being diluted to the final concentration and mixed with streptavidin-coated beads. In tail-tethered GST homodimer experiments, dynein dimers were diluted to the final concentration and mixed with streptavidin-coated beads (when working with the GFP-GST-Dyn1,314-HT construct) or streptavidin-coated beads (when working with the biotinylated HT-GST-Dyn1,314-GL construct; Supplementary Fig. 1). The final concentration was determined experimentally for each preparation such that no more than 35% of beads exhibited any activity when brought into contact with the axoneme, ensuring that >95% of observing events can be attributed to the actions of single motors. Dynein was allowed to bind to the beads for 10 min at 4 °C before proceeding with sample preparation. The sample chamber was loaded by first flowing Cy5-labelled axonemes in low-salt DLB buffer (300 mM HEPES, 2 mM MgCl₂, 1 mM EDTA, 10% glycerol, pH 7.2), waiting 30 s for the axonemes to adsorb to the glass surface, and then chasing with a solution of dynein-coated beads in motility buffer (assay DLB as specified above with the addition of 35 μg mL⁻¹ protocatechuc acid (PCD), 2.5 mM protocatechuate-3,4-dioxygenase (PCAL), 10 mM DTT and 1 mg mL⁻¹ casein and 1 mM ATP unless specified otherwise). The ends of the sample chamber were then sealed with nail polish to prevent liquid evaporation.

**Optical trapping assay.** All trapping experiments were performed on a fully automated optical trap, custom-built in an acoustically isolated and temperature-controlled (± 0.1 °C) room around the body of a Nikon Ti-E inverted microscope. Dynein-coated beads were trapped with a 2-W 1,064-nm laser (Coherent), tightly focused in the image plane with a × 100 1.49 N.A. apochromat objective (Nikon). The trap was steered with a pair of perpendicular acousto-optical deflectors (AA Opto-Electronic), placed in a plane conjugate to the back-focal plane (BFP) of the objective. Power in the trap was controlled throughout the experiment with a half-wave plate on a motorized rotary mount (New Focus). Position of the bead relative to the centre of the trap was monitored using BFP interferometry, by imaging the BFP of a 1.4 N.A. oil-immersion condenser (Nikon) on a position-sensitive detector (First Sensor Inc.) Raw data from the detector were digitized by an NI 9215 analogue input module (National Instruments), connected to an NI cRIO-9114 reconfigurable chassis (National Instruments). Signals were acquired and saved to disk at either 5 or 20 kHz, and position feedback was performed at up to 200 Hz using the field-programmable gate built into the cRIO-9114 chassis. Detector response was calibrated by rapidly raster-scanning the laser across a trapped bead with the acousto-optical deflectors and fitting the resulting curve to a cubic polynomial (Supplementary Fig. 4c) and trap stiffness was obtained from the Lorentzian fit to the power spectrum of a trapped bead. The temporal resolution of optical trap data is fundamentally limited by the stiffness of the trap, as Brownian noise dominates the recordings at frequencies higher than the corner frequency of the Lorentzian power spectrum. The trap stiffness values used in this work resulted in corner frequencies of up to 400 Hz, corresponding to a temporal resolution of ~2.5 ms. Owing to the compliance of the dynein motor and the motor-bead linkage, the smallest step size that could be reliably detected by our step-finding algorithm was ~2 nm. The accuracy of position and stiffness calibrations were independently verified by measuring the trap force of kinesin-1 to be 5.6 ± 0.8 pN, in good agreement with previously published values. Axoneme tracks, labelled with Cy5, were excited with a 633-nm laser (Coherent), visualized with a monochrome camera (The Imaging Source) and published values. Axonemes were excited with a 30-mW 633-nm laser (Coherent) and imaged with a × 100 1.49 N.A. apochromat oil-immersion objective (Nikon) on an iXon EMCCD camera (Andor). Assay preparation was identical to the optical trap sample preparation described in a previous section, except without the addition of latex beads.

**Data analysis.** Position data were fit to steps using a fully automated, maximum likelihood-based step finding algorithm46, implemented in MatLab. Because the algorithm assumes white noise with no autocorrelations, data were downsampled to ~50% of the corner frequency of the trap before fitting. Force-clamp runs that lasted shorter than 1 s, covered less than 50 nm, or included instantaneous jumps larger than the steps were rejected. To analyze the otherwise uncontrolled output of the step-finding algorithm was accepted as is, without any further modifications. Motor velocities were determined by fitting individual runs with a straight line and calculating the slope of the line.

To generate dynein force histograms, position data from fixed-trap recordings were downsampled to 500 Hz for ease of visualization and stall events were manually selected. To qualify as a stall, the position trace had to reach a plateau and remain stationary (with the mean deviations of less than ±10 nm) for at least half a second before terminating in a ‘rip’. A ‘rip’, indicating that the motor fully released from the MT, had to constitute a rapid (<2 ms) jump of at least 50 nm, larger than the maximum step a dynein molecule can be expected to take. For kinesin-1, the definition of stall was the same except the minimum duration of the plateau at the top of the stall was decreased to 0.1 s, to account for kinesin’s faster stepping rate and shorter near-stall dwells.

F curves were fit to the velocity expression for a one-state motor model25, \[ V(\eta) = V_\infty - a(1 - F/(a + F/\eta)) \] where \( F \) is the external force, \( V_\infty \) is the unloaded velocity because of forward stepping only, \( a \) is the energetic bias provided by ATP hydrolysis, \( \eta \) is a characteristic distance and \( a \) is a dimensionless parameter that defines the partitioning of load dependence between the forward and backward stepping rates. The net unloaded velocity \( V_\infty \) can be expressed as \( V_\infty = V_{\infty,0}(1 - e^{-\gamma/\eta}) \). See Supplementary Note 1 for detailed description of the model.

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**Author contributions**

V.B. and A.Y. designed experiments; V.B. performed the biochemical experiments; V.B., N.L.H. and A.C. performed optical trapping assays, V.B., N.L.H. and A.C. analysed the data and V.B. and A.Y. wrote the manuscript.

**Additional information**

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