Ensemble and single-molecule dynamics of IFT dynein in Caenorhabditis elegans cilia

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Cytoplasmic dyneins drive microtubule-based, minus-end directed transport in eukaryotic cells. Whereas cytoplasmic dynein 1 has been widely studied, IFT dynein has received far less attention. Here, we use fluorescence microscopy of labelled motors in living Caenorhabditis elegans to investigate IFT-dynein motility at the ensemble and single-molecule level. We find that while the kinesin composition of motor ensembles varies along the track, the amount of dynein remains relatively constant. Remarkably, this does not result in directionality changes of cargo along the track, as has been reported for other opposite-polarity, tug-of-war motility systems. At the single-molecule level, IFT-dynein trajectories reveal unexpected dynamics, including diffusion at the base, and pausing and directional switches along the cilium. Stochastic simulations show that the ensemble IFT-dynein distribution depends upon the probability of single-motor directional switches. Our results provide quantitative insight into IFT-dynein dynamics in vivo, shedding light on the complex functioning of dynein motors in general.

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Microtubule-based intracellular transport of organelles, receptors, messenger RNA and other cargoes is crucial for the functioning of eukaryotic cells. Cytoplasmic dyneins, members of the AAA + ATPase family, are large multi-subunit motor complexes that are the key drivers of minus-end-directed transport along microtubules. Cytoplasmic dynein 1 plays a role in a wide range of cellular processes, including cell organization, mitosis and axonal transport, while cytoplasmic dynein 2 (or IFT dynein) is specifically involved in intraflagellar transport (IFT) in cilia.23. Whereas structure, motility properties and regulation of cytoplasmic dynein 1 have been widely studied14-15, far less is known about IFT dynein. 

Primary cilia project from the surface of most eukaryotic cells and have important roles in sensory perception and signalling3-4. They are built and maintained by the cooperative action of IFT-dynein and kinesin-2 motors in a bidirectional transport process along the axoneme called IFT.5-19. In Caenorhabditis elegans chemosensory cilia, two anterograde motors of the kinesin-2 family, heterotrimeric kinesin-II and homodimeric OSM-3, work together to transport cargo from ciliary base to tip.20 Kinesin-II traverses the base, transition zone and proximal segment as the ‘import’ motor, gradually handing over transport to OSM-3, which drives transport along the rest of the cilium up to the tip.21 IFT dynein is the sole retrograde motor mediating the transport of turnover products from the tip back to the base.22-25

Previous studies of IFT dynein have primarily focused on motor structure and subunit composition23-29. More recently, the role of different IFT-dynein subunits has been investigated in C. elegans and Chlamydomonas reinhardtii.31 Although IFT-dynein velocities and frequencies have been reported in different organisms at the ensemble level26,28,30-34, we are not aware of studies that have addressed IFT-dynein dynamics at the single-molecule level. As a consequence, IFT-dynein motility properties remain relatively poorly characterized.

Here, we investigate IFT-dynein motility parameters at the ensemble and single-molecule level in living C. elegans using sensitive fluorescence microscopy. At the ensemble level, we find that directionality of IFT trains is not modulated by the kinesin-2/dynein ratio, contrary to other tug-of-war, opposite-polarity motility systems. Single-motor trajectories reveal unexpected, distinct features of IFT-dynein motility: diffusive behaviour at the ciliary base, pauses, turns, directed motion and switches between these behaviours. Stochastic simulations allow linking this single-molecule behaviour to the ensemble IFT-dynein distribution along the cilia, revealing that this distribution crucially depends upon the probability of single-motor directional switches.

Results

IFT-dynein ensemble dynamics. Intracellular transport is often driven by groups of motors working together35,36. As such, the exact composition and size of the motor ensembles might affect motility properties and dynamics. To image in vivo IFT-dynein motor dynamics, we generated C. elegans strains with enhanced green fluorescent protein (EGFP) attached to the IFT-dynein light intermediate chain XBX-1 (ref. 28). Disruption of XBX-1 results in defective retrograde IFT in C. elegans cilia28, XBX-1 orthologues of C. reinhardtii (light intermediate chain, LIC) and mammals (dynein 2 light intermediate chain, D2LIC) were found to be associated with the motor heavy chain in co-immunoprecipitation assays, suggesting that XBX-1 is an integral subunit of the IFT-dynein complex.37,38 We generated two C. elegans strains: one expressing genome-integrated, EGFP-tagged XBX-1 using Mos1-mediated single-copy insertion (MosSCI)39 and another with XBX-1::EGFP expressed from an extrachromosomal array (Fig. 1a, Supplementary Tables 1 and 2 and Supplementary Fig. 1a). We first determined the steady-state motor distribution from (time-averaged) fluorescence image sequences recorded with epifluorescence microscopy. In both strains, XBX-1 is highly enriched in the base and the beginning of the transition zone, with relatively constant concentration in the rest of cillum (Fig. 1a and Supplementary Fig. 1b and 1c). In the extrachromosomal array strain, however, about twice as much XBX-1::EGFP is localized at the base and much more in the dendrite (20-30 times), guiding our choice to use the MosSCI strain for our quantitative imaging approach.

To characterize the properties of IFT-dynein ensembles moving together in trains, we recorded fluorescence image sequences and generated kymographs using KymographClear that uses Fourier filtering to separate anterograde and retrograde moving components (Fig. 1a)40. Trajectories were extracted from kymographs using KymographDirect40 and further analysed to obtain position-dependent velocities (Fig. 1b,c), motor numbers (Fig. 1d), frequency (Fig. 1e,f) and flux (Fig. 1g). IFT-dynein-driven trains exhibit relatively constant velocity of ~1.7 μm/s along distal and proximal segments of the C. elegans cilium, similar to previously reported retrograde IFT velocities30,34,41. In the transition zone, however, we observe the slowing down of IFT-dynein trains. A similar deceleration has been observed before for IFT particle B, OSM-3 and kinesin-II and is most likely due to transition zone structures impeding transport21. IFT-dynein motor numbers were calculated from the fluorescence intensities of trains as described before21, assuming that each IFT-dynein dimer consists of two XBX-1::EGFP subunits42. We find that the number of IFT dyneins per train remains relatively constant during transport from transition zone to the tip and vice versa (Fig. 1d). However, whereas anterograde trains are composed of 40-50 IFT-dynein complexes as cargo, retrograde trains contain only 20-30 motors driving motion. In a previous study, we observed a proportionally comparable decrease of kinesin motors and IFT-particle components on retrograde trains compared with anterograde trains41. Electron microscopy studies of C. reinhardtii have shown a similar decrease in train length43. In more recent studies, a difference in size between retrograde and anterograde trains was not observed, but retrograde trains appeared morphologically different compared with anterograde trains44,45. Automated detection of peaks in vertical kymograph intensity profiles (that is, intensity at a given position as a function of time) (Fig. 1e,f) shows that retrograde trains are ~1.6 times more frequent than the anterograde trains. Similarly, in Trypanosoma brucei and C. reinhardtii cilia, retrograde frequency was observed to be higher31,32.

To obtain further quantitative insight into IFT-dynein trafficking, we calculated XBX-1 flux in the cilia. Flux is defined here as the number of motors (calculated from the area under fluorescence intensity versus time profiles such as Fig. 1e,f) multiplied by the local velocity (Fig. 1g). In this way, all IFT dyneins are taken into account, including small trains that might not be discernible in kymographs32. We find that retrograde and anterograde XBX-1 fluxes are very similar (Fig. 1g), implying that the amount of IFT-dynein motors in the cilium is constant in time and that there is no substantial loss of IFT dynein at the ciliary tip. Taken together, our results indicate that retrograde trains are more frequent but smaller than anterograde trains, resulting in equal anterograde and retrograde fluxes.

Directionality is not modulated by kinesin-2/dynein ratio. We next addressed how IFT dynein cooperates with the anterograde kinesin-2 motors. Different modes of opposite-polarity motor cooperation have been proposed in literature, including mechanical-competition models (tug-of-war) and coordinated,
regulated action models. To gain more insight into opposite-polarity motor cooperation in *C. elegans* chemosensory cilia, we generated double-labelled MosSCI strains XBX-1::EGFP OSM-3::mCherry and XBX-1::EGFP KAP-1::mCherry (KAP-1 is a subunit of kinesin-II; Supplementary Table 1). We imaged the strains using two-colour fluorescence microscopy to...
simultaneously visualize IFT dynein and either kinesin-2 motor (Fig. 2a and Supplementary Movie 1). Visual inspection of kymographs alluded that all OSM-3- or kinesin-II-containing trains also carry IFT dynein (Supplementary Fig. 2). To further substantiate this observation, we registered the two colour channels on the camera and determined the Manders’ colocalization coefficients for both directions of motion from the Fourier-filtered, background, bleaching and bleed-through corrected kymographs (Fig. 2a). The Manders’ coefficients M1 and M2 are measures of how well fluorescence intensity of pixels in the first channel colocalize with those in the second channel, and vice versa. To account for the ciliary locations of KAP-1 and OSM-3, we focused, for each kinesin motor, on a specific cilary segment: the distal region (4.0–7.5 μm) for XBX-1::EGFP OSM-3::mCherry and the proximal region (0–3.5 μm) for XBX-1::EGFP KAP-1::mCherry (Fig. 2b). For XBX-1 and OSM-3, both Manders’ coefficients are close to one (M1 = 0.92 ± 0.01; M2 = 0.90 ± 0.01, mean ± s.e.m., n = 24) and equally so for XBX-1 and kinesin-II (M1 = 0.92 ± 0.01; M2 = 0.82 ± 0.02, n = 18). These values are significantly larger than control correlations of two different, same-directionality OSM-3 (M1 = 0.40 ± 0.03; M2 = 0.44 ± 0.02) or kinesin-II kymographs (M1 = 0.43 ± 0.03; M2 = 0.45 ± 0.03, Supplementary Table 3). This analysis shows that most, if not all, IFT trains contain both anterograde and retrograde motors. In addition, whereas kinesin-II is proposed to undergo diffusion in C. reinhardtii flagella, our results show that in C. elegans kinesin-II is actively carried back to base by IFT dynein, in agreement with previous findings.

For tug-of-war motor systems, it has been shown that the transport direction is modulated by the ratio of opposite polarity motors. To test whether such a mechanism might be at play in C. elegans IFT, we determined the ratio of IFT dynein and kinesin-2 motors on anterograde and retrograde IFT trains (Supplementary Fig. 2) by evaluating the ratio of the fluorescence intensities of the two motors (Fig. 2c). On average, the XBX-1/KAP-1 fluorescence-intensity ratio is 2.47 ± 0.13 on anterograde (mean ± s.d. for 67 trains in 20 animals; averaged from 0 to 3.5 μm along the cilium) and 2.63 ± 0.16 on retrograde trains (61 trains in 20 animals). The XBX-1/OSM-3 ratio is 1.55 ± 0.27 on anterograde (95 trains in 24 animals; averaged from 4 to 7.5 μm along the cilium) and 1.71 ± 0.27 on retrograde trains (86 trains in 23 animals). These results reveal that there is no significant difference in dynein/kinesin-2 ratio between retrograde and anterograde trains, which strongly suggests that, in C. elegans IFT, directionality is not modulated by the ratio between opposite-polarity motors.

To further probe the interplay between IFT dynein and the kinesin-2 motors, we generated a double-labelled XBX-1::EGFP and OSM-3::mCherry strain that lacks functional kinesin-II (Supplementary Table 1 and Supplementary Movie 1). Steady-state intensity profiles show that the overall number of XBX-1 and OSM-3 in the cilium is decreased (Supplementary Fig. 3), in agreement with previous experiments on OSM-3 (ref. 21). XBX-1 and OSM-3 motor numbers are also lower on anterograde and retrograde trains (Supplementary Fig. 3). Intriguingly, the IFT dynein/OSM-3 ratios are similar to those in the constructs with functional kinesin-II (Fig. 2c). This observation suggests that IFT dynein/kinesin-2 ratios are tightly controlled in the IFT system, for example by the limited and fixed number of motor binding sites on the IFT particle complex.

**Single-molecule quantification.** To understand how the dynamics of individual IFT-dynein motors result in the overall ensemble behaviour discussed above, we investigated the XBX-1 motility properties at the single-molecule level. To access the individual dynein regime, we generated C. elegans strains expressing XBX-1::paGFP (Supplementary Table 1). After photoactivation of only a limited number of paGFPs, we could visualize individual XBX-1 in living C. elegans for the first time (to our knowledge). Analysis of the recorded image stacks using single-particle tracking yielded 494 single-XBX-1 trajectories from 7 worms. These trajectories were located along the entire cilium, except for the last 1–2 μm close to the ciliary tip. This part of the phasmid is commonly bent with respect to the rest of the cilium and was, as a consequence, out of focus. Careful inspection of the trajectories revealed different single-motor behaviours (Supplementary Movie 2).

Most of the single motors moved unidirectionally in either anterograde (182 trajectories; 37% of all 494) or retrograde...
Figure 3 | Single-molecule quantification reveals novel IFT-dynein behaviour. (a) Quantification of single-motor IFT-dynein trajectories: 37% anterograde motion (magenta), of which 14% with pausing (light magenta); 27% retrograde motion (green), of which 13% with pausing (light green); 16% at the base (blue); 12% stationary (yellow); 8% turnarounds (purple); $n = 494$ trajectories from 7 worms. Only trajectories of 600 ms or longer are taken into account. (b–f) Representative single-motor IFT-dynein traces showing: pausing (light green) in unidirectional trajectories (b), deceleration upon reaching the ciliary base (c), diffusive behaviour at the ciliary base (d) and A-to-R (anterograde to retrograde) directional switches at different positions with corresponding images and kymograph (e,f). (e,f) Time: vertical; scale bar, 2 s. Position: horizontal; scale bar, 2 µm. Arrow indicates turnaround position. The movie corresponding to (f) is shown in Supplementary Movie 2. (g,h) Histograms of IFT-dynein A-to-R turnarounds ($n = 34$) showing their location and pausing time. R-to-A not shown (only four recorded trajectories).
(135 trajectories; 27%) direction (Fig. 3a). A smaller fraction of trajectories showed diffusive behaviour (81 trajectories; 16%; Fig. 3d). These trajectories were primarily located at the ciliary base, congruent with XBX-1 enrichment in this region (Supplementary Fig. 1b). Only a small fraction of the trajectories (58; 12%) corresponded to stationary motors. Further analysis of the retrograde single-molecule trajectories confirmed that IFT dyneins slow down upon approaching the ciliary base, as observed at the ensemble level (Fig. 3c). Collectively, these findings are in agreement with our data on the ensemble motility properties of XBX-1, where kymographs show continuous train movement from base to tip, driven by kinesins, with XBX-1 as cargo and from tip to base, driven by IFT dynein (Fig. 1a).

We then turned our focus to the characteristics of individual IFT dynein motility that cannot be observed at the ensemble level. First, we noticed that both anterograde and retrograde trajectories were sometimes interrupted by pauses (anterograde: 26 trajectories, 14% of the 182 anterograde trajectories; retrograde: 18 trajectories, 13% of the 135 retrograde trajectories; Fig. 3b). The average duration of these pauses was 0.71 ± 0.20 s (s.d.), with the longest pause lasting 1 s. Second and unexpectedly, we observed directional switches of IFT dynein (38 trajectories, 8%). The vast majority (34; 89% of 38 directional switches) were in the anterograde-to-retrograde direction (A-to-R; Fig. 3e,f and Supplementary Movie 2). In these trajectories, IFT dynein appears to switch from being a cargo on a kinesin-driven anterograde IFT train to an active transporter moving in the retrograde direction. A histogram of the locations where A-to-R turnovers took place indicates that there is no A-to-R turnaround hotspot, but rather these directional switches can occur at all locations in the cilium (Fig. 3g). Furthermore, a histogram of pause durations during A-to-R switches (Fig. 3h) reveals that the majority of switches (23; 64%) is faster than 150 ms (the time between subsequent frames in our measurements). To obtain further insight into directional switches, we determined the probability density (probability per µm travelled) of an IFT dynein to switch from A-to-R (P_{AR}) or R-to-A (P_{RA}) by calculating the total number of A-to-R (or R-to-A) turnovers divided by the total anterograde (or retrograde) distance travelled. We found a P_{AR} of 0.14 ± 0.03 µm⁻¹ and P_{RA} of 0.07 ± 0.03 µm⁻¹; that is, the probability density of anterograde IFT dyneins reversing is twice as high as retrograde IFT dyneins. As a consequence, an IFT dynein motor traveling from the base in the anterograde direction has a probability of 28 ± 7% of reaching the ciliary tip (assuming 9 µm ciliary length). For a retrograde-moving IFT dynein the probability to reach the base when it started at the tip is 53 ± 12%. This difference in turnaround probability between IFT dynein moving in anterograde and retrograde direction highlights an important difference between anterograde and retrograde trains; IFT dynein appears more strongly coupled to trains when it is active as driver than when it is passive as cargo.

Stochastic modelling. To connect the observed single-molecule behaviour to the overall IFT-dynein distribution over the cilia, we performed stochastic simulations. In these simulations, we allowed an IFT-dynein motor to take nanometre steps along a 9 µm cilium and stochastically reverse direction. As input parameters we used the experimentally determined, position-dependent IFT dynein train velocities and the turnaround probability densities P_{AR} and P_{RA}. We assumed that a motor turned around upon reaching the ciliary tip (without pause) or base (with an exponentially distributed pause duration t_{PA} base of on average 500 ms, Fig. 4a). The simulations yielded time-averaged motor distributions in the cilium and histograms of the turnaround locations as output (Fig. 4a,b). The experimental histograms and distributions can be reproduced well using this simple model. The simulated turnaround histograms indicate that compliant with our experimental findings, A-to-R and R-to-A turns occur all along the cilium (Fig. 4b). However, they also indicate that most turns must occur at tip and base, which we did not observe in these single-molecule trajectories. For turns at the tip, this can be explained by the spatial orientation of the tip that is not in the same focal plane as the rest of the cilium, therefore making it impossible to detect single molecules around the tip in these experiments. This out-of-focus effect is less of a problem in our ensemble data that indicate efficient and fast turnarounds of XBX-1 at the tip (Fig. 1a), as observed before\(^\text{[9]}\). To obtain deeper insight into turnaround behaviour of XBX-1 specifically at the tip, we recorded single-molecule trajectories in a small subfraction of C. elegans that were oriented such that the distal segment including tip was located in one image plane (Supplementary Fig. 4). From these trajectories we determined the XBX-1 pause duration during tip turnaround and found that the majority of switches were almost instantaneous (<600 ms; 67% of 54 trajectories). At the base, we attribute the failure of experimentally detecting all turns to the pausing and diffusing of motors at this location that substantially reduces the chance of detecting turns in the relatively short trajectories, whose duration is limited by photobleaching. Further simulations with altered turnaround probability densities show that motor distribution is highly sensitive to tuning the pausing time and turnaround probability, with relatively small modifications of the probabilities resulting in substantially altered motor distributions (Fig. 4c and Supplementary Fig. 5). This suggests that the interaction between IFT dynein and the IFT train is an important parameter regulating IFT.

Discussion

IFT dynein is the retrograde motor that drives IFT in cilia and flagella and is essential for ciliary assembly and signalling. Here, we probed its ensemble and single-molecule dynamics in the chemosensory cilia of C. elegans, providing the first single-molecule characterization of IFT-dynein motility in vivo. A key single-molecule observation was that some individual IFT-dynein motors switch direction along the entire length of the cilium. We determined that the turnaround probability densities are 0.14 ± 0.03 µm⁻¹ for A-to-R turns and 0.07 ± 0.03 µm⁻¹ for R-to-A turns, corresponding to effective average anterograde and retrograde run lengths of 7 ± 1 and 14 ± 1 µm, respectively. It is important to note that these in vivo run lengths indicate the distance along which motors remain attached to a train either as cargo or as active driver of transport. We can only observe that XBX-1 is attached to a moving IFT train. On anterograde trains, kinesin is the active motor; IFT dynein is cargo and thus not active as motor. On retrograde trains, at any given time at least a fraction of the IFT-dynein motors must be involved in driving the transport. From in vitro motor stall force measurements, it has been estimated that at least four motors actively drive transport at a given time in C. reinhardtii\(^\text{[34]}\), but the rest might effectively be cargo. Here we cannot distinguish between these two roles. As such, these values do not correspond to run lengths measured in vitro on isolated motors using optical tweezers or single-molecule fluorescence microscopy that have not been determined for IFT dynein, but are typically 1–3 µm for cytoplasmic dynein 1 (refs 6,52,53). Furthermore, these results indicate that IFT dynein is more likely to reverse direction as passenger than as driver. This suggests that the interaction between IFT dynein and the IFT-particle backbone is stronger when IFT dynein drives retrograde transport than when it is carried as anterograde cargo. This could be due to direction-dependent differences in train...
properties or tension-dependent interactions between IFT dynein and particle. Another explanation could be that active motors remain bound to the microtubule after detachment from a train that could allow rebinding to the train.

How do these single-molecule IFT-dynein turnarounds relate to our ensemble findings that trains of IFT dynein appear to switch direction only at base and tip of the cilium? To shed more light on this apparent inconsistency, we performed stochastic simulations of IFT-dynein runs. The simulations confirm that although most motors turn at the base and tip, turnarounds along the entire cilium are necessary to maintain the IFT-dynein distribution. A recent ensemble-level study has shown that most anterograde-to-retrograde XBX-1 turns occur at the ciliary tip. This is consistent with our ensemble data, single-molecule data and simulations that show almost instantaneous XBX-1 turns at the tip. In C. reinhardtii, however, an IFT-dynein light intermediate chain has been shown to pause substantially during tip turnaround (~1.7 s). In addition, C. elegans IFT-dynein components DLYT-3, DLC-1 and DYCI displayed turnaround behaviour different from XBX-1. This raises questions about whether those IFT-dynein components show similar dynamics at the single-molecule level as XBX-1, what role each component plays in retrograde transport and whether there are differences between species. Despite recent progress in identifying IFT-dynein subunits, the exact motor composition remains elusive. To fully understand retrograde transport, it will be vital for future structural and functional studies to uncover which dynein-associated subunits are an integral component of the motor and which act as regulatory, accessory or adapter proteins.

Our findings not only provide insight into dynein dynamics in IFT, but are also valuable in understanding the dynamics of cytoplasmic dyneins in general. While numerous studies have reported characteristics of cytoplasmic dynein 1 motility in vitro, including side and back stepping, response to load and collective behaviour, in vivo studies have been lacking. Further studies will be necessary to discern whether cytoplasmic dynein 1
transposase. Coordinated worms lacking the mCherry signal were selected and injected with a phasmid mix containing the gene of interest, co-injection selection was classified as an event when at least 600 ms long with minimum displacement of 4 frames, were defined as stationary. Stationary behaviour within retrograde trajectories, anterograde trajectories or directional switches was defined as pausing. A directional switch or turnaround event has a local maximum or minimum in the coordinates perpendicular to the spline. A trajectory was classified as an event when at least 600 ms long with minimum displacement of 200 nm. Particles with interframe displacement of <50 nm, consecutively over at least 4 frames, were defined as stationary. Stationary behaviour within retrograde trajectories, anterograde trajectories or directional switches was defined as pausing. A directional switch or turnaround event has a local maximum or minimum in the x coordinate. A minimum of three consecutive steps in the same direction are taken before and after the turn.

Manders’ colocalization coefficients. Pixels in EGF and mCherry channels are aligned post imaging using a custom-written MATLAB algorithm based on kymographDirect and KymographClear developed in our lab. Single-molecule images were analysed using a tracking and linking algorithm in MATLAB (Mathworks). A spline was drawn along the cilium to define the x axis of displacement; y coordinates are perpendicular to the spline. A trajectory was classified as an event when at least 600 ms long with minimum displacement of 200 nm. Particles with interframe displacement of <50 nm, consecutively over at least 4 frames, were defined as stationary. Stationary behaviour within retrograde trajectories, anterograde trajectories or directional switches was defined as pausing. A directional switch or turnaround event has a local maximum or minimum in the x coordinate. A minimum of three consecutive steps in the same direction are taken before and after the turn.

Stochastic simulations of IFT-dynein dynamics. Stochastic simulations were performed using a custom-written algorithm in LABVIEW (National Instruments). Single motors moved repetitively along a 9,000 nm track with 1 nm steps and experimentally measured velocity (10,000 events). Motors turned around stochastically based on experimentally determined probability of turnaround over distance, $P_{Sk}$ (anterograde to retrograde) and $P_{ar}$ (retrograde to anterograde). To account for the overlap of two phasmid cilia in the distal segment, intensities and histograms were multiplied by 2 – $\left(1 - \frac{1}{2}\right)$ (1993).

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Acknowledgements
We thank J. Girard and D. Voersel for help with data analysis, J. van Krugten for his help with the single XBX-1 tip-turn experiments, M. Bos for cloning the XBX-1::paGFP construct, S. Acar and J.M. Scholey for helpful discussions and advice and E. Kroeze for biochemical support. Several strains were provided by the CGC that is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). We acknowledge financial support from the Netherlands Organization for Scientific Research (NWO) via a VICI grant, an NWO-Groot and an ALW Open Program grant, and from NanoNextNL of the Government of the Netherlands and 130 partners.

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Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications
Competing financial interests: The authors declare no competing financial interests.

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How to cite this article: Mijalkovic, J. et al. Ensemble and single-molecule dynamics of IFT dynes in Caenorhabditis elegans cilia. Nat. Commun. 8, 14591 doi: 10.1038/ncomms14591 (2017).

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