Supporting Information

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Fig. S1. Binding of outer arm dynein from different species of sea urchin [(A) *Hemicentrotus pulcherrimus* (B) *Strongylocentrotus intermedius*]. Outer arm dynein molecules were mixed with MTs in the absence of nucleotide, and negatively stained with 1% uranyl acetate containing 20 μg/ml bacitracin. Among the several species of sea urchin we tested, dynein from some species (e.g., *Hemicentrotus pulcherrimus* or *Pseudocentrotus depressus*) tended to bind randomly to MTs (A), often forming clusters (white arrows). In contrast, outer arm dynein from *Strongylocentrotus intermedius* or *Strongylocentrotus nudus* showed patches of regularly arranged dynein molecules between two MTs (B). In one patch, all dynein molecules have uniform orientations, with the tails binding to the same MT of the pair (black arrowheads). In another patch between the same pair of MTs, dyneins can bind in the opposite orientation, with the tails binding to the other MT (white arrowheads). Even when the same species of sea urchin was used, regularity of binding varied between different preparations. (Scale bars, 100 nm.)
Fig. S2. The dynein-MT complex in the no-nucleotide state observed by negative stain EM (A) and cryo-EM without staining (B). (A) The dynein-MT complex was stained with 1% uranyl acetate containing 20 μg/ml bacitracin. Globular head domains and curved tails interacting with the MT below (A-MT; see text for definition) are clearly observed. Extra densities (black arrowheads) on the upper MT (B-MT), probably corresponding to the MT binding domain of the stalks, are visible, but connections between these domains and the heads are difficult to see. (B) The dynein-MT complex was rapidly frozen without staining, and observed by conventional cryo-EM. The minus end of the B-MT is to the right. The contrast of the image is low compared with the negative stain (A) or cryo-positive stain images (Figs. 2 and 4). However, the stalks are still visible for some molecules, and they are tilted toward the MT minus end (arrows). (Scale bar, 50 nm.)
Fig. S3. Comparison with the tomographic structure of axonemes. Our averaged image in the no-nucleotide state (A) was compared with a 2D projection of the cryoelectron tomography structure of *Chlamydomonas* axonemes (B), reported by Ishikawa et al. (2007) [Ishikawa T, Sakakibara H, Oiwa K (2007) The architecture of outer dynein arms in situ. *J Mol Biol* 368:1249–1258]. Because wild-type outer-arm dynein from *Chlamydomonas* has 3 heads, a two-headed mutant (oda11) was used for comparison. When a section from the tomography 3D map was viewed in the orientation indicated by an arrow in the transverse section (D), the resulting image (B) agreed well with our averaged images (A). The red dotted lines indicate the position of the section. (C) Merged view of A and B. The extra density seen between the heads and the A-tubule of the tomography map (red arrows) probably corresponds to the docking complex, which is not included in our specimens. In D, the approximate positions of the heads of β and γ heavy chains, respectively, are indicated in cyan and green. The β and γ heavy chains of *Chlamydomonas* outer arm dynein are thought to correspond to βi and α heavy chains in sea urchin, respectively. Note that, in this viewing direction, the two dynein heads are superimposed. The plus end is to the left in A–C. The section in D is viewed from the proximal (minus) end. (Scale bar, 20 nm.)
Fig. S4. A comparison with the QFDE-EM images of axonemes. (A and B) Our cryo-positive stain images of the dynein-MT complex in the no-nucleotide (A) and ADP·Vi (B) states. (Insets) Class-averaged images, shown in Fig. 5A, #1 and 5B, #1 and #2. (C and D) QFDE images of sea urchin axonemes in the absence of nucleotide (C) or in the presence of ATP (D), from figures 8 and 10 of Sale et al. (1). They reported that a single, large head is visible in the absence of ATP, but two domains are visible with ATP. Our images are consistent with these QFDE images in both nucleotide states. (Scale bar, 50 nm.) [Reproduced with permission from ref. 1 (Copyright 1985, The Rockefeller University Press).]

Movie S1. Motility of the dynein-MT complex. Dynein was mixed with Tetramethylrhodamine-labeled MTs [Hyman A, et al. (1991) Preparation of modified tubulins. Methods Enzymol 196:478–485] to give final concentrations of 50 μg/ml and 500 μg/ml, respectively, in a motility buffer containing 0.5% BSA, 1 mM DTT and 20 μM Taxol, and incubated for 10 min at room temperature. The mixture was introduced into the flow chamber, allowed to stand for 10 min at room temperature, and washed with a motility buffer containing 0.5% BSA, 1 mM DTT, 20 μM Taxol, and an oxygen scavenger (200 μg/ml glucose oxidase, 30 μg/ml catalase, and 3 mg/ml glucose). After adding 1 mM ATP (final concentration), sliding of the MTs was observed using a fluorescence microscope (BX60; Olympus) equipped with UplanApo 100× NA 1.35 oil-immersion lens (Olympus). The images were projected onto a WAT-120N CCD camera (Watec) and were contrast enhanced with Image-J software. The movie shows two MTs sliding along and out from the dynein-MT complex in sequence. (Scale bar: 2 μm.)

Movie S1 (AVI).