Supporting Information

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SI Materials and Methods

Design of the CENP-E Dimeric Construct. The full-length *Xenopus* CENP-E DNA (kindly provided by Y.M.) was used as a template for PCR to create a 392-aa dimer (CENP-E 392). The forward primer was GGAGAATATACATATGTCCGAGGGAGATGCAGTTAAAGTGTGTG, and the reverse primer was GTGCTCGAGCTCTTTGTGTAGTTGTTTGATTTCAGCTAGC. The resulting fragment was inserted into the bacterial expression vector pET21a (Novagen) between the NdeI and XhoI sites. To construct a CENP-E dimer with a Gcn4 sequence at the carboxyl terminus (ATGAAACAGCTTGAGGACAAAGTAGAGGAGCTGCTGTCCAAGAATTACCATCTTGAAAATGAGGTTGCGAGACTTAAGAAGCTTGTCGGGGAGCGA), the CENP-E sequence was extended to amino acid 396, followed by a KpnI restriction site and a 1-aa linker (AGAGAAGATAGAGGTACCCAC). The linker was included to render the coiled-coil structure of the Gcn4 sequence in frame with the predicted coiled-coil structure of the CENP-E. The Gcn4 sequence was ligated to the carboxyl terminus of the extended CENP-E between the KpnI and SalI sites and placed into the pET21a expression vector by GenScript. All DNA constructs were confirmed by sequencing.

Protein Expression and Purification. CENP-E 392 was expressed in Bl21 (DE3) RIL cells (Stratagene). Nine liters of culture were grown to an OD_{590} of 0.4–0.6 and induced with 0.5 mM isopropyl β-D-thiogalactoside (IPTG) at 18°C for 20–24 h. Cells were pelleted and frozen at −20°C. Pelleted cells were suspended in lysis buffer (50 mM Tris, 40 mM imidazole, 10% sucrose, 0.3 M NaCl, 5 mM $MgCl_2$, 0.1 mM ADP, 1 mM PMSF, 2 μg/ml leupeptin, 1 μg/ml pepstatin A, 2 μg/ml aprotinin, 1 mg/ml lysozyme, pH 7.9) and incubated for 30 min with 1 mg/ml lysozyme (Sigma). The lysed cells were sonicated and treated with ribonuclease A (10 μg/ml) and dexoyribonuclease I (5 μg/ml) for 15 min. Cellular debris was pelleted, and the clarified extract was serially filtered (0.8-μm, 0.45-μm, and 0.2-μm pore size). The extract was incubated at 4°C overnight after the addition of a 10-ml bed volume of Ni-NTA agarose (Qiagen). The agarose beads were washed twice with wash buffer (20 mM Tris, 50 mM imidazole, 5% sucrose, 0.5 M NaCl, 5 mM $MgCl_2$, 0.1 mM ADP, 1 mM PMSF, pH 7.9), and the expressed fusion protein was eluted by affinity chromatography using an AKTA FPLC model P-920 (GE Healthcare) and a gradient of 50 mM to 1 M imidazole. Fractions containing the protein were dialyzed overnight (25 mM Hepes, 100 mM KCl, 5% sucrose, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 0.1 mM ATP, 1 mM PMSF, pH 7.5). The fusion protein was further purified by Q Sepharose column chromatography with a gradient of 50–500 mM KCl, dialyzed overnight, and frozen at −80°C. Microtubules were prepared by polymerization of tubulin (Cytoskeleton) in the presence of taxol in the buffer consistent with the experiment.

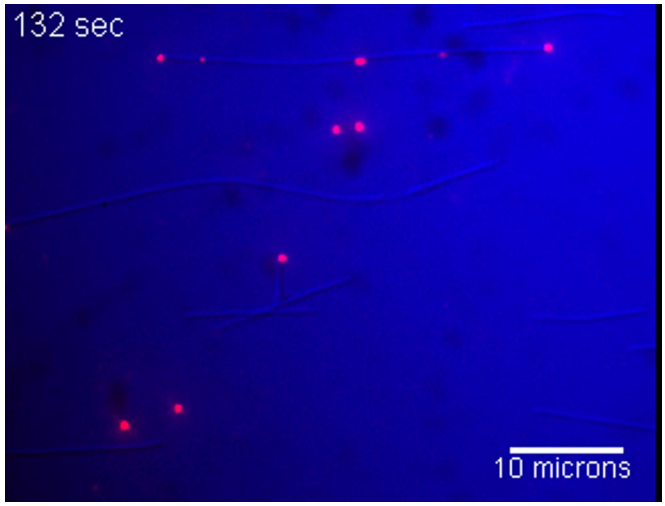
TIRF Microscopy. Quantum dots and rhodamine-labeled microtubules were observed on an objective-type total internal reflection setup (1) by using an inverted microscope (IX-70; Olympus) equipped with 60× or 100× oil objective (N.A. = 1.45; Olympus) and 1.5× magnification unit. A 532-nm Nd-YAG laser (CrystaLaser) is used for excitation of quantum dots and fluorescent microtubules. Axonemes were visualized through differential interference contrast microscopy using an oil condenser. Images were acquired with an Andor iXon back-illuminated electron-multiplying CCD camera (Andor Technology) at a rate varying between 2 and 30 Hz.

Optical Trapping Apparatus. Trapping of polystyrene beads was achieved by a near-infrared laser (Nd:YVO_4, 1,064 nm; Spectra-Physics) through an 60× water objective (N.A. = 1.20; Nikon) implemented on a Nikon Eclipse 2000U inverted microscope. A halogen light source (Optical Analysis) was used for bright-field imaging to visualize beads and axonemes. Stage translation was done by using a micropositioning system (MS-2000; Applied Scientific Instruments) for capturing beads and positioning them near an axoneme. The fine position adjustment was accomplished with a piezoelectric stage (Nano-LP; Mad City Labs). The bead position was determined simultaneously by recording bright-field images with an Andor CCD camera and focusing trap laser on a quadrant photo diode (QPD) (Current Designs). The data acquisition from QPD was done via custom software written in LABVIEW.

Position calibration of QPD was done by either scanning beads stuck to the surface with the piezoelectric stage or comparing QPD voltage data with position determined by bright-field images of moving beads. The stiffness for each trapped bead was measured by fitting a Lorentzian to the power spectrum of Brownian motions (2). The drag coefficient was corrected for proximity to the surface. A drag method for calibrating stiffness was also used to confirm stiffness values from the power spectrum method and to determine the linearity of the trap. For calibrating stiffness with the drag method, the stage was translated at a constant velocity and the displacement of the bead from the trap center was measured. Stiffness values from both measurements agreed within 5%, and the linearity of the trap force was verified within the maximum bead displacements in our stall force measurements. Trap stiffness values for stall force measurements were between 0.06 and 0.1 pN/nm.

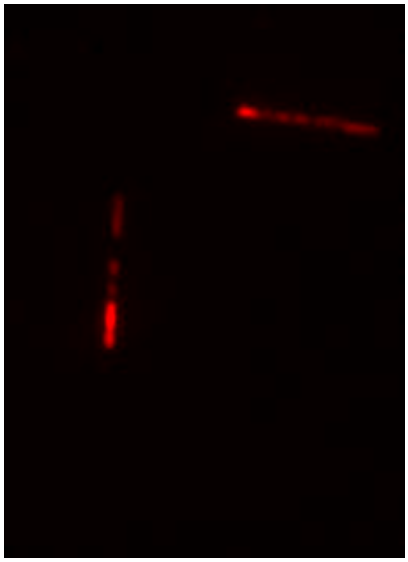
Data Analysis. Single-molecule tracking of quantum-dot-labeled proteins was achieved by fitting the fluorescence intensity profile to a 2-dimensional Gaussian function. For run length measurements, the distance moved by each molecule is determined starting from its landing on an axoneme until dissociation. Average distance traveled was calculated through a single exponential fit to a histogram of all runs. Average velocity at each ATP concentration was determined by fitting a Gaussian distribution to a histogram of velocities that were weighted by run length. Michaelis constant for ATP was calculated by fitting the ATP dependence of velocity to the Michaelis–Menten equation $V = (V_{max} \cdot [ATP])/(K_M + [ATP])$. The positions of steps were determined by a Student t test program developed in IDL.

1. Yildiz A, *et al.* (2003) Myosin V walks hand-over-hand: Single fluorophore imaging with 1.5-nm localization. *Science* 300:2061–2065.
2. Svoboda K, Block SM (1994) Biological applications of optical forces. *Annu Rev Biophys Biomol Struct* 23:247–285.



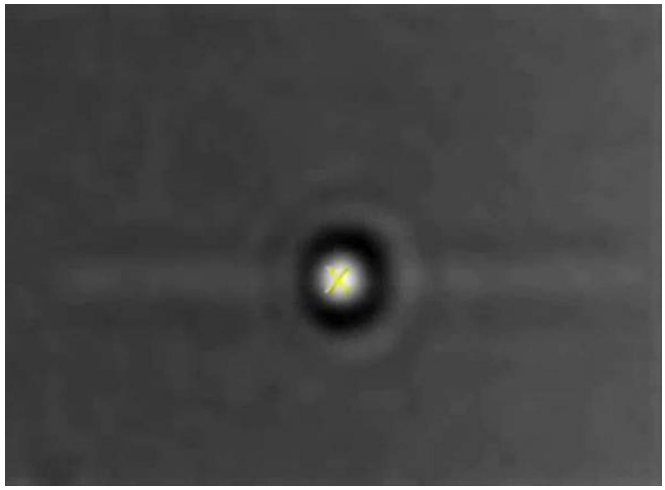
Movie S1. Processive movement of quantum dot-labeled CENP-E on axonemes.

Movie S1 (MOV)



Movie S2. Microtubule gliding assay with surface-immobilized CENP-E using polarity-marked microtubules. Bright sides indicate microtubule minus ends.

Movie S2 (MOV)



Movie S3. Transport of a 0.44-μm-diameter polystyrene bead by CENP-E on an axoneme displaying a number of stall events. Yellow mark shows approximately the position of trap center.

Movie S3 (MOV)