Torque force in the kinesin stroke drives coupled yaw axis and orbital rotations of kinesin coated gold nanorods

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ABSTRACT

Kinesin motor domains generate impulses of force and movement that have both translational and rotational components, raising the question of how the rotational component contributes to motor function. We used a new assay in which kinesin-coated gold nanorods (kinesin-GNRs) move on suspended microtubules, for three plus-end-directed kinesins: single-headed KIF1A, dimeric ZEN-4 and single-headed kinesin-1. Polarization of the light scattered by all three types of kinesin-GNRs periodically oscillated as they orbited the microtubule along a left-handed helical trajectory. Our analyses revealed that each kinesin-GNR unidirectionally rotates about its yaw axis as it translocates, and that the period of this yaw-axis rotation corresponds to two periods of its left-handed helical orbit around the microtubule axis. Stochastic simulations suggest that the yaw-axis rotation enhances biased lateral displacement of the kinesin team. Our study reveals biaxial rotation as a new mode of motility in kinesin teams that helps the team to sidestep obstacles.

MAIN TEXT

Kinesins are adenosine 5'-triphosphate (ATP)-driven and microtubule-based molecular motors with multiple mechanical functions, including cargo transport along microtubules, the regulation of microtubule dynamics, and the sliding of bundled microtubules¹⁻³. Recent advances in techniques for 3D particle tracking with simultaneous measurements of force and torque have revealed that kinesins have translational and rotational degrees of freedom on microtubules, such that their motilities consist of both helical⁴⁻⁸ and rotational⁹ motions. For example, single-molecule kinesin-1 dimers unidirectionally rotate their stalk domain as they move processively via a hand-over-hand mechanism, especially at high ATP concentration⁹. Furthermore, mutagenic elongation of the neck linker of kinesin-1 dimers increases the probability of protofilament switching during stepping along the microtubule¹⁰. The neck-linker mutant dimers retain processivity but switch protofilaments more readily, driving left-handed helical motion of multimotor-coated beads around the axis of the microtubule, whereas individual wild-type kinesin-1 dimers faithfully track the microtubule axis by stepping along individual protofilaments⁶. The protofilament-tracking ability of kinesin-1 dimers may derive from their rotary hand-over-hand mechanism⁹ and their neck stability^{6,8}. Wild-type kinesin-2 and -8, which have longer neck-linker domains than kinesin-1, and kinesin-6, which has an atypical neck region, do not track protofilaments. All these kinesins move towards microtubule plus ends along left-handed helical trajectories, as shown in a bead assay^{6,11}, a microtubule surface-gliding assay^{8,11-13}, and a single-molecule tracking assay⁸. Furthermore, teams of non-processive dimeric kinesin-14 Ncd¹⁴⁻¹⁶, which moves towards microtubule minus

ends, as well as teams of truncated single-headed kinesins-1^{17,18}, -3¹⁹, -5^{4,20} and -6¹¹ motors have all been shown to drive corkscrewing motions of microtubules in surface-gliding assays. The handedness of these motions is consistent. Whilst plus-end directed kinesins move along a left-handed helix, minus-ended kinesins move along a right-handed helix. These helical motilities imply that the stroke (the unitary impulse of force and movement generated by the motor domains) has an off axis (torque) component, driven either by conformational changes in the motor domain or by the directionally biased selection of microtubule binding sites^{6,8,11,15,17,19} (or potentially both). The underlying mechanisms and functional relevance of the torque component remain elusive.

To gain further insight, we have developed a novel motility assay in which gold nanorods (GNRs), coated with a team of kinesin molecules, move unhindered along suspended microtubules. Our assay uses light scattered by the kinesin-coated GNRs to track their position, and the polarization of the light that they scatter to report their orientation. We performed the GNR motility assay on three plus-end-directed kinesins: single-headed KIF1A, dimeric ZEN-4 and single-headed kinesin-1.

Results

GNR imaging for particle tracking and polarization measurements. We developed a motility assay based on tracking of kinesin-coated GNRs. Focusing of light scattered by the GNRs allows positional tracking, whilst the ratio of the polarization of this scattered light in orthogonal directions reports the orientation of the GNR as it moves over the microtubule surface (Fig. 1a and Methods). GNR images were obtained by laser dark-field imaging using highly inclined illumination with nearly total internal reflection angle^{21,22} via a perforated dichroic mirror²³. The position of each GNR spot was measured in three dimensions using a cylindrical lens²⁴ (Fig. S1). Polarization measurements of GNRs were performed using a combination of the polarized incident laser beam and a polarizing beam displacer, which two orthogonal polarization signals were projected on the camera, allowing us to measure 0° to 180° GNR orientations in the sample plane (Fig. 1b and Methods). Kinesin constructs were prepared with a biotinylated peptide (Avi-tag) fused to their C-terminus. Biotinylated GNRs (40 nm × 68 nm) were coated in streptavidin and biotinylated kinesin molecules were attached to this streptavidin layer (Methods). Maximum capacity of kinesin molecules attached to the GNR is roughly estimated to be ~100 molecules and a portion of the attached kinesin molecules could generate driving force and torque on the microtubule. Microtubules were suspended on microbeads (0.5 μm) via the antigen–antibody system for β-tubulin, so that kinesin-coated GNRs were free to orbit the microtubules between bead-attachment points (Fig. 1c). Control experiments established that the Z-displacement of GNR in the range of ±200 nm had negligible effect on the GNR polarization measurement in our experimental system

(Fig. S2). Using the GNR motility assay, we performed a comparative analysis of three plus-end-directed kinesins, which have distinctive properties of processivity and oligomeric state: biased-diffusional motile and single-headed human kinesin-3 (KIF1A)²⁵, weakly processive and dimeric *Caenorhabditis elegans* kinesin-6 (ZEN-4)²⁶, and non-processive and truncated single-headed rat kinesin-1 (KIF5B)²⁷ (Methods).

GNR motility assay for single-headed KIF1A. We performed the GNR motility assay for single-headed KIF1A (1-366 amino acid residues (AAs))²⁵ at a saturating ATP concentration (2 mM ATP). GNRs carried multiple KIF1A molecules, allowing GNRs to move processively along microtubules. We found that the light-scattering intensities of each pair of KIF1A-coated GNR (KIF1-GNR) spots, corresponding to orthogonal polarization signals from a single GNR, periodically oscillated in intensity during helical movement, with an increase in each signal corresponding to a decrease in the other (13 particles out of 62 tested KIF1-GNRs) (Fig. 1d and Supplementary movie 1). The degree of polarization (DOP) as a function of the summed-intensity of the two GNR spots depicted an elliptical trajectory, indicating continuous rotations of the polarization (Fig. 1e). The *X–Y–Z*-trajectory of the KIF1-GNR showed steady left-handed helical motion around the microtubule long axis (Fig. 1f). Figure 1g shows that the time trajectories of the *X*- and *Y*-displacements, the degree of polarization and summed-intensity, and the polarization angle as viewed from above the glass substrate. The time trajectory of the polarization angle exhibited continuous counterclockwise rotations during the left-handed helical motion of the KIF1-GNR around the microtubule. The *Y*-displacement and

the polarization signals of the KIF1-GNR exhibited strong cross-correlation and periodicity (Fig. 1h), indicating that the GNR polarization fluctuation was synchronized with the helical motion on the microtubule. We also observed clockwise and oscillatory rotations of the GNR polarization, synchronized with the helical motion of KIF1-GNRs (Fig. 2a and 2b). The translational velocity (parallel to the microtubule axis) was $0.51 \pm 0.12 \mu\text{m s}^{-1}$ (mean $\pm$ standard deviation (SD), $n = 13$ GNRs), which is nearly equal to the microtubule sliding velocity of $0.49 \mu\text{m s}^{-1}$ in the surface-gliding assay (Fig. S3). The pitch of the helical motion measured in the X - Y -trajectory was $0.69 \pm 0.36 \mu\text{m}$ (mean $\pm$ SD, $n = 33$ cycles in 13 KIF1-GNRs), which is slightly shorter than that of the corkscrewing of microtubules on a surface of KIF1A of $0.89 \pm 0.21 \mu\text{m}$ (mean $\pm$ SD, $n = 35$ cycles in 5 microtubules) ($p \ll 0.01$, Welch's t-test). The displacement in the microtubule axis during one cycle of fluctuation of the GNR polarization signals (hereafter referred to as polarization pitch) was $0.69 \pm 0.37 \mu\text{m}$ (mean $\pm$ SD, $n = 33$ cycles in 13 KIF1-GNRs), which was almost equal to that of the helix pitch (Fig. 2c). We note that the measured helix pitch is a combination of the helix pitch exerted by motors on the straight microtubule lattice and any supertwist pitch of the microtubule, with the supertwist depending on the number of the protofilaments. However, when the measured helix pitch is less than $1 \mu\text{m}$, as it is here, the supertwist of the microtubule has negligible influence on the measured helix pitch (Methods and Fig. S4). We therefore report the uncorrected helix pitch unless otherwise mentioned.

GNR motility assay for dimeric ZEN-4 and single-headed kinesin-1. Next, we

performed the GNR motility assay for the weakly-processive dimeric kinesin, ZEN-4 (1-555 AAs)²⁶, again at a saturating ATP concentration (2 mM) (Methods). We again found that the polarization signals of the ZEN4-coated GNRs (ZEN4-GNR) periodically changed, correlated with the *Y*-displacements during steady forward movement (13 particles out of 49 tested ZEN4-GNRs) (Fig. 3a). The analyses revealed the counter-clockwise, clockwise, and oscillatory rotations of these ZEN4-GNRs. The forward velocity of the ZEN4-GNR was $0.39 \pm 0.05 \mu\text{m s}^{-1}$ (mean $\pm$ SD, $n = 13$ ZEN4-GNRs), which is comparable to the velocity in the bead assay¹¹. The polarization pitch of the ZEN4-GNR was $0.67 \pm 0.20 \mu\text{m}$ (mean $\pm$ SD, $n = 34$ cycles in 13 ZEN4-GNRs) in the microtubule axis, which is equal to the helix pitch of $0.67 \pm 0.21 \mu\text{m}$ (mean $\pm$ SD, $n = 34$ cycles in 13 ZEN4-GNRs) (Fig. 3b). Thus, whilst ZEN4-GNRs moved more slowly than KIF1-GNRs, the polarization rotation of ZEN4-GNRs was again phase-locked to their helical motion, as for KIF1-GNRs.

Lastly, we also performed the GNR motility assay for the non-processive truncated single-headed kinesin-1 (sK1) (1-340 AAs)²⁷ and the processive double-headed kinesin-1 (dK1) (1-430 AAs)²⁷ constructs at a saturating ATP concentration (2 mM) for comparison with the experiments of the single-headed and biased-diffusional motile KIF1A and the double-headed and weakly processive ZEN-4. For sK1 GNRs, we again found examples where the light-scattering intensities of each orthogonally polarized pair of spots changed periodically during steady forward movement (5 particles out of 40 tested sK1-GNRs) (Fig. 3c). The forward velocity of sK1-GNRs was $0.21 \pm 0.02 \mu\text{m s}^{-1}$ (mean $\pm$ SD, $n = 5$ sK1-GNRs), which is comparable to previously reported velocities in

the bead assay²⁸. The polarization pitch of the sK1-GNR was $0.83 \pm 0.19 \mu\text{m}$ (mean $\pm$ SD, $n = 11$ cycles in 5 sK1-GNRs), which is again comparable to the helix pitch of $0.80 \pm 0.21 \mu\text{m}$ (mean $\pm$ SD, $n = 11$ cycles in 5 sK1-GNRs) (Fig. 3d). As a control to check whether rotation of the GNR polarization is coupled to helical orbiting of the supertwist of the microtubules or not, we studied GNRs coated with *rat* kinesin dimers dK1-GNRs, which faithfully track protofilaments⁶ rather than side-stepping. Polarization angle of these dK1-GNRs did not exhibit unidirectional rotation along a long-pitch helical path in our observation (Fig. 3e). These data indicate that periodic oscillations of the intensity of polarized light scattered by kinesin-GNRs within the sample plane only occur for motor teams that step along the short-pitch helical path.

Helical and yaw-axis rotations of kinesin-coated GNRs. Our GNR motility assays revealed that the polarization of KIF1-, ZEN4-, and sK1-GNRs rotated periodically whilst moving along a helical trajectory towards microtubule plus ends. Our results are well explained by a model in which a kinesin-coated GNR unidirectionally rotates 180° about its short (yaw) axis in one period of short-pitch helical motion around the microtubule long axis (Supplementary movie 2). In this model, the time trajectories of the GNR angle in the X - Y -plane are given by

$$\arctan[\cos(\omega_{\text{roll}}t) \cdot \tan(\varphi + \omega_{\text{yaw}}t)]$$

where ω_{yaw} and $\omega_{\text{roll}} (= \pm 2\omega_{\text{yaw}})$ are the angular velocities of yawing and rolling of a kinesin-coated GNR on the microtubule, respectively and φ is defined by the relative angle to the microtubule axis when the kinesin-coated GNR is at the top position of its

helical trajectory (Methods). Three types of observed rotations of the polarization angle (counterclockwise, oscillatory, and clockwise rotations) are reproduced by our proposed model depending on the initial-phase angles (φ) of the kinesin-coated GNR. For example, rotations of the GNR polarization, as viewed from above the sample plane, are counterclockwise at $\varphi = 0^\circ$, oscillatory at $\varphi = 45^\circ$ and 135° , and clockwise at $\varphi = 90^\circ$ (Fig. 4 and Fig. S5a. See also Supplementary movie 2 and 3). Note that when the kinesin-coated GNR moves along a helical path without yawing, rotation of the GNR polarization is also oscillatory (Fig. 4 bottom and Fig. S5b. See also Supplementary movie 4). In this model, the GNR yawing direction is also a determinant of apparent rotational patterns of the GNR polarization in the sample plane. Pearson's correlation coefficient between the Y -displacement and the angle of the kinesin-GNR is negative in the case of the clockwise yawing, while the correlation coefficient of the counter-clockwise yawing is positive (Fig. 4 and Fig. S5a). Estimated by the correlation coefficient between the Y -displacement and the polarization angle, 8 out of 13 KIF1-GNRs, 5 out of 13 ZEN4-GNRs, and 3 out of 5 sK1-GNR exhibited clockwise yawing, whilst 5 out of 13 KIF1-GNRs, 8 out of 13 ZEN4-GNRs, and 2 out of 5 sK1-GNR counterclockwise yawing. The directionalities of yawing of these kinesin-coated GNRs were not significantly different from an equal probability using binomial test (p -values > 0.5 for KIF-GNR, ZEN4-GNR, and sK1-GNR).

Our proposed model, in which kinesin-GNRs rotate around their yaw axes as they move along and around the microtubule long axis, predicts that immobilized kinesin-GNRs might drive rotation of an overlying short microtubule about both its long and short axes. To test the rotational motility of the kinesin-GNRs during translation, we performed the

surface-gliding assay for ZEN4-GNRs, in which ZEN4-GNR particles were sparsely attached on the glass surface (3~8 particles per 100 μm^2) (Methods). We found indeed that short microtubules ($< \sim 2 \mu\text{m}$) slid until only their ends were in contact, and then unidirectionally rotated on ZEN4-GNRs (Fig. 5 and Supplementary movie 5). In the geometry of observation images, we observed both counterclockwise rotation ($n = 4$ microtubules) and clockwise rotation ($n = 4$ microtubules). These observations also support a model in which kinesin-coated GNRs moving along a microtubule are driven to undergo both helical and yaw-axis rotations.

Discussion

We developed the GNR motility assay and harnessed it to analyses cargo rotations driven by teams of single-headed KIF1A, dimeric ZEN-4, and single-headed kinesin-1. For all 3 kinesins, ~ 20% of the kinesin-coated GNRs showed periodic fluctuation of the GNR polarization signal, phase locked to the orbit of the GNR around the microtubule, over multiple orbital periods. For the remaining ~80% of records, we did not observe periodic variation in the GNR polarization signals. Where we did observe periodic fluctuation in the GNR polarization signals, we always saw phase-locking to the orbit of the GNR around the microtubule axis. Our observations reveal unidirectional yawing (rotation of the team) as a new mode of motility in three different types of kinesin teams.

We found that the polarization and helix pitches were comparable among KIF1-, ZEN4- and sK1-GNRs (0.7~0.8 μm) ($p > 0.05$ for adjusted pairwise comparisons using Welch's t-test with the Holm method), despite substantial differences in their forward velocities ($p < 0.01$ for adjusted pairwise comparisons using Welch's t-test with the Holm method). The helix pitch is given by $P = 2\pi V_X / \omega_{Helix}$, where P is the pitch size, V_X is the forward velocity, and ω_{Helix} is the angular velocity in the Y - Z -plane. We see that the forward velocities are different among KIF1-, ZEN4-, and sK1-GNRs but the pitches are equal. The ratios of V_X to ω_{Helix} are thus almost equal. This means that the angular velocity of helical motion is directly proportional to the forward velocity for all our kinesin-coated GNRs, suggesting that the off-axis component is intrinsic to each stroke, and independent of the rate of stroking.

This relationship can be well modeled by a 2D noise-driven ratchet mechanism, as proposed by Mitra *et al.* for single-headed KIF1A¹⁹. Based on this mechanism, we now consider how yawing of the kinesin-coated GNRs might influence its motility. Since a kinesin-coated GNR rotated 180° about its yaw axis in one period of a helical trajectory with the helix pitch of about 700 nm, the GNR rotates $2^\circ \approx 180^\circ / (700 \text{ nm} / 8 \text{ nm})$ with 8 nm of forward displacement, which is equivalent to the length of the tubulin dimer. The 2° rotation generates at most $\pm 1 \text{ nm} \approx \pm 20 \sim 34 \text{ nm} \times \tan(2^\circ)$ of lateral displacements of the kinesin molecules bound on the GNR (40 nm in diameter and 68 nm in length) (Fig. 6a). Lateral displacement (d) of 1 nm is equivalent to $0.2 \approx 1 \text{ nm} / 5.1 \text{ nm}^{29}$ in the asymmetry factor (α) toward the off-axis, which would be sufficient to influence the noise-driven ratchet mechanism (Fig. 6b). A recent discussion by Mitra *et al.* indicates that around 1 μm of a helix pitch is reproduced by the 2D Brownian ratchet model with a slight off-axis asymmetry ($\alpha_Y = 0.53$) relative to the on-axis asymmetry ($\alpha_X = 0.27$)¹⁹.

We evaluated the off-axis fluxes of three particles differing in their starting positions for diffusion ($-d, 0, +d$) in the noise-driven ratchet model with asymmetry potentials (α) after $1/\gamma$ units of time³⁰ (Methods):

$$J_0 = \frac{\gamma}{4} \left\{ \text{erfc} \left[\frac{\alpha}{2} \sqrt{\gamma} \right] - \text{erfc} \left[\frac{1-\alpha}{2} \sqrt{\gamma} \right] \right\}$$

$$J_{\pm d} = \frac{\gamma}{4} \left\{ \text{erfc} \left[\frac{\alpha \mp d}{2} \sqrt{\gamma} \right] - \text{erfc} \left[\frac{1-\alpha \pm d}{2} \sqrt{\gamma} \right] \right\}$$

where J_0 represents the flux of particles starting diffusion at 0 and $J_{\pm d}$ represents the flux of particles starting diffusion at $\pm d$. In calculations with $\alpha = 0.53$ and $\gamma = 160 \text{ s}^{-1}$ (Methods), the absolute values of J_{-d} and J_{+d} increased with d along a sigmoidal

curve and the net flux, $J_{-d} + J_0 + J_{+d}$ described the convex downward function of which the value reached a minimum at $d = 0.5$ (Fig. 6c and 6d). Importantly, the slight displacement ($d < 0.2$) is sufficient to cause biased flux because $|J_{-d}|$ is much larger than $|J_{+d}|$ (Fig. 6c, inset). These suggest that unidirectional rotation of a motor-coated cargo enhances the sideward bindings of motor molecules toward the biased direction.

To explore the consequences of combined rotational and helical motion of kinesin-coated GNRs, we performed Monte Carlo simulations of the two-state noise-driven ratchet model. In this simulation, multiple particles, which are linked to a rigid body cargo with a spring, stochastically take biased forward and/or sideward steps on the 2D lattice and generate torque to twist the spring and then rotate the cargo when taking a step (Fig. 7). Based on the force- and moment-balance equations, the cargo moves and rotates around the center of the joint points connecting the particles in the attached state (Fig. 7a and Methods). Additionally, the twist angle ($\Delta\theta$) of the cargo driven by the torque generated by the particles taking a step is given by $\Delta\theta = (N_{\text{step}}\theta_{\text{step}} + b_{\theta})/N_{\text{on}}$, where N_{step} is the number of particles taking a step and generating a torque in a unit of time; N_{on} is the number of particles bound to the rail in the unit of time; b_{θ} is the rotational diffusion of the cargo with a normal distribution with zero mean and a standard deviation of σ_{θ} ; θ_{step} is the twist angle of the spring driven by the torque T_{step} generated by the single particle, assuming that the twist angle θ_{step} is proportional to the torque T_{step} (Methods). Note that the rotational diffusion of the cargo (b_{θ}) is negligible for the particle formation in Figure 7a (Methods).

First, we optimized the parameters for the biased forward and sideward movement of

the cargo without torque generation ($\theta_{\text{step}} = 0$), matching the $\sim 0.8\text{-}\mu\text{m}$ helix pitch of KIF1A and ZEN-4 in the gliding assay, which is the ratio of forward steps to leftward steps ($\sim 100/13$) (Fig. 7b). We note that the simulation without torque generation often showed unidirectional rotation of the cargo, but the period of rotation was irregular and uncorrelated with the 13-lattice period of the Y -displacements modeled as the 13- protofilament microtubule. We then performed the simulation with torque generation. When θ_{step} was approximately -3° to -4° , the cargo exhibited periodical clockwise rotation and the period of rotation closely matched the 13-lattice period (Fig. 7c and Fig. S6). Correlation between counterclockwise rotation and sideward movement of the cargo was also reproduced when θ_{step} was $+4^\circ$ (Fig. S7). The lateral displacement in a calculation time in the presence of torque ($\theta_{\text{step}} = -4^\circ$) increased about 1.3-fold, compared with that in the absence of torque ($\theta_{\text{step}} = 0^\circ$), which is consistent with the analysis by the noise-driven ratchet model indicating that unidirectional rotation of a motor-coated cargo enhances the sideward bindings of motor molecules toward the biased direction (Fig. 6d).

In this simulation, lower number or lower density of particles result in weaker correlations between the Y -displacement and the rotation trajectories (Fig. S8), suggesting that the formation of particles on the cargo are also critical for the rotation motility of a kinesin team. Less frequent observation of the kinesin-GNRs showing periodic fluctuation in the GNR polarization signals might be caused by heterogeneous formations of kinesin molecules on the kinesin-GNRs. Also, in the GNR assay experiments, each kinesin-coated GNR exhibited specific directionality of yawing, which is consistent with previous single-molecule measurements for the kinesin-1 dimer⁹. Although the detailed

mechanism of directional yawing of a kinesin team remains unclear, not only the conformational changes in the motor domain but also the formation of kinesin molecules on the GNR and further the configuration of the kinesin-GNR and the microtubule might be involved.

A recent single-molecule study of the kinesin-1 dimer construct revealed that it unidirectionally rotates the stalk domain by $\sim 1^\circ$ with 8-nm steps at high ATP conditions and in the presence of an external force⁹. Therefore, the estimated θ_{step} of 4° might be reasonable especially in the absence of an external force. Previous structural studies of kinesin-1 and KIF1A revealed that rearrangement in the motor domain from the pre-stroke state to the post-stroke state contains rotational motions of the subdomains^{31–33}. A torque-generation property may be built into the kinesin motor domain (Supplementary discussion). Furthermore, the neck-to-tail region is critical for torque transmission from the head domain to the cargo. In the case of ZEN-4, its neck domain contains a non-canonical long sequence; however, high-speed AFM imaging of a ZEN-4 dimer revealed a distinctive globular mass in the neck region³⁴. Thus, the non-canonical neck-domain of ZEN-4 may be a stiffness component rather than a flexible linker.

Previous studies proposed that sideward stepping of kinesins and dynein allows them to bypass roadblocks on a microtubule^{6–8,11,13,35}. We performed Monte Carlo simulations of bypassing of a roadblock with various multiple-motor motilities, especially changing the two parameters: the off-axis asymmetry, α_Y , and the torque generation, θ_{step} (Fig. 7d and Methods). The simulation results in the case of $\alpha_Y = 0.58$ and $\theta_{\text{step}} = 0$ showed that the sideward movement allowed motors to avoid the roadblock (Fig. 7d, second from

the left), whereas the motors without off-axis asymmetry ($\alpha_Y = 0.5$ and $\theta_{\text{step}} = 0$) failed to bypass it within the calculation time (Fig. 7d, the most left). Moreover, the simulation results in the case of $\alpha_Y = 0.5$ and $\theta_{\text{step}} = -4^\circ$ indicate that the rotational motility also helps motors avoid the obstacle (Fig. 7d, second from the right). Interestingly, the motors with off-axis asymmetry ($\alpha_Y = 0.58$) and torque generation ($\theta_{\text{step}} = -4^\circ$) exhibited a ‘spin move’ (i.e., a combination of spinning and sideward motion), and further increased the probability of bypassing the obstacle (Fig. 7d, the most right). The evasion time by the spin move was 2.6-fold faster than that by only sideward stepping. These simulations thus suggest that the spin move of a kinesin team will help sustained transport of a cargo in situations where the microtubule lattice is crowded with microtubule associated proteins, motor proteins, and other lattice-binders.

In summary, the GNR motility assay of single-headed KIF1A, dimeric ZEN-4, and single-headed kinesin-1 revealed that kinesin-coated GNRs unidirectionally rotated around their yaw axis, phase-locked with their helical orbit of the microtubules. Theoretical analyses with a noise-driven 2D ratchet model and Monte Carlo simulations showed that unidirectional rotation enhances the biased sideward movement of kinesin-bound cargo. Our results reveal that in kinesin teams, the production of torque drives not only orbital motion of the kinesin team around the microtubule axis, but also coupled yaw-axis rotation of the team and its attached cargo. We propose based on quantitative simulations that this combination of properties enables cargos bound to multiple kinesins more efficiently to bypass roadblocks. Our work provides new insights into the structural basis of kinesin motor stepping^{33,36} and into the implications of their translational and

343 rotational degrees of freedom for their cellular function³⁷.

METHODS

Gold nanorod imaging. Figure 1a shows a schematic drawing of the laser dark-field microscope system used for gold nanorod (GNR) imaging. The laser dark-field optical system was constructed on an inverted microscope (Ti; Nikon, Tokyo, Japan), equipped with variable-angle epi-illumination^{21,22} and a custom-built perforated dichroic mirror (PDM) (Sigma Koki, Tokyo, Japan). The PDM had an elliptical hole (short axis = 6 mm; long axis = 8.5 mm) at its center position, which made a circular window when viewed from the optical axis. The scattered light from the GNRs was transmitted through this central circular window. Illumination was performed using a 635-nm diode laser (MRL-III-635L-10mW; CNI laser, Changchun, China), which was further attenuated by neutral density filters (ND) and converted to circularly polarized light by a quarter-wave plate (WPQ-6328-4M; Sigma Koki, Tokyo, Japan). The collimated and circularly polarized incident beam was focused by a lens (L1 in Fig.1a, $f = 220$ mm) onto the back focal plane of the objective lens (PlanApo N, 60 $\times$, NA 1.42; Olympus, Tokyo, Japan). GNR images were obtained by laser dark-field imaging using highly inclined illumination with nearly total internal reflection angle. The back focal plane was focused outside a camera port of the microscope with an achromatic lens (L3 in Fig.1a, $f = 60$ mm) to make an equivalent back focal plane for constructing the optics for polarization measurements and 3D tracking.

For polarization measurements, the scattered light from a GNR illuminated by polarized incident beam was further split into two orthogonally polarized components by a polarization beam displacer (BD40; Thorlabs, Tokyo, Japan), which was set in the

optical path between the relay optics constructed by two lenses (L3 ($f = 60$ mm) and L4 ($f = 120$ mm) in Fig.1a)³⁸. Two separated and polarized light beams were imaged as two spots on a CMOS camera (LDH2500; Digimo, Tokyo, Japan). The orientations of the two orthogonally separated polarization components of the beam displacer were diagonal relative to the transmission axis of the polarization filter. For angular calibration, a linear polarizer was additionally set at the position between L2 and L3 and rotated from 0° to 180° in 10° intervals (Fig. 1b).

For 3D tracking, concave and convex cylindrical lenses (CnC ($f = -200$ mm) and CvC ($f = 200$ mm)) were set in front of the camera²⁴. The interval between CnC and CvC was 13 mm. Calibration of 3D tracking along the Z-axis was performed by moving the objective in 50-nm intervals (Fig. S1), using a custom-built stage equipped with a pulse motor (SGSP-13ACT; Sigma Koki, Tokyo, Japan) and a controller (QT-CM2; Chuo Precision Industrial, Tokyo, Japan). We analyzed the widths of the GNR spots along the X-axis (S_X) and Y-axis (S_Y) obtained by 2D-Gaussian fits. The calibration factor for the z-displacement obtained from the ratio of S_Y to S_X was $0.98 \mu\text{m}/\text{ratio}$.

The temperature of the sample stage was controlled using a custom-built water-circulating temperature control system. Image acquisition in the GNR motility assay was performed at 100 frames/s with the commercial software for the CMOS camera (Digimo, Tokyo, Japan). The data were saved as eight-bit AVI files. The effective pixel size of the detector was 42 nm. Image analysis and further analyses were performed using custom software written in LabVIEW (National instruments, Tokyo, Japan).

Polarization measurements. For polarization measurements, the scattered light from a GNR illuminated by polarized incident beam was split into two polarized components by the polarization beam displacer as mentioned above. Thus, the intensities of two polarized GNR spots as functions of the GNR orientation (θ) are expressed by

$$I_{\text{bottom}} = I_0 \cdot \cos^2(\theta + \beta) \cdot \cos^2(\theta + \beta - \gamma) \cdot + b$$

$$I_{\text{upper}} = I_0 \cdot \cos^2(\theta + \beta) \cdot \cos^2(\theta + \beta - \gamma + 90) + b$$

where I_0 is the amplitude; θ is the GNR orientation in the sample plane; β and γ are phase angles of polarization of the incident beam in the sample plane and the beam displacer, respectively; and b is the background intensity. The observed intensity profiles in Fig. 1b were well fitted by these functions with $I_0 = 127.5 \pm 3.3$, $\beta = 99.3^\circ \pm 0.8^\circ$, $\gamma = 45.3^\circ \pm 0.7^\circ$, and $b = 19.5 \pm 0.9$. GNR angles in the sample plane were obtained by fitting an ellipse function to the plot of the degree of polarization $(I_{\text{bottom}} - I_{\text{upper}})/(I_{\text{bottom}} + I_{\text{upper}})$ as a function of the summed-intensity $(I_{\text{bottom}} + I_{\text{upper}})$, as shown in Fig. 1b.

Kinesin constructs. In all kinesin constructs used in this study, the functional sequence Avi-His, which consists of the Avi-tag (GLNDIFEAQKIEWHE) for biotinylation and the 6×His-tag for histidine-affinity purification, was fused to the C-terminus of truncated kinesin domains. For the monomeric KIF1A construct, the fragment of *human* KIF1A (1–366 amino acid residues (AAs)) obtained from a *human* cDNA library was cloned into eGFP-Avi-His/pColdIII plasmids. eGFP stabilized the solubility of the truncated KIF1A sample. In addition, for the surface-gliding assay of the KIF1A construct, we used the

construct of *human* KIF1A (1–366 AAs) cloned into mRuby-Zdk1-His/pColdIII plasmids. For the dimeric ZEN-4 construct, the fragment of *C. elegans* ZEN-4 (1–555 AAs) obtained from CBD-TEV-ZEN4/pGEX-6p plasmids³⁴ was cloned into Avi-His/pET32a plasmids. For the monomeric and dimeric kinesin-1 constructs, the fragments of *rat* kinesin-1 (1–340 AAs and 1–430 AAs for the monomeric and dimeric constructs²⁷, respectively) were cloned into Avi-His/pColdIII plasmids. All constructs were verified by DNA sequencing.

Expression and purification of kinesin. For expression and purification of the kinesin constructs, the proteins were expressed in *Escherichia coli* strain BL21 Star (DE3) cells using 0.4 mM Isopropyl-β-D-thiogalactopyranoside (IPTG) (Sigma) for 9 to 12 hours at 20 °C. The collected cells were lysed in lysis buffer (50 mM potassium phosphate; pH 7.4, 350 mM NaCl, 1 mM MgCl₂, 10 μM ATP, 1 mM DTT, 0.1% Tween20, 10% glycerol, protease inhibitors, 50 mM imidazole) and sonicated in iced water for 15 minutes. The bacterial lysate was centrifuged for 20 minutes at 260,000g at 4 °C to remove cell debris and insoluble proteins. The clarified supernatant was purified with the His-tag affinity by chromatography with a HisTrap HP Ni²⁺sepharose column (GE Healthcare). The collected peak fraction was further purified through a HiTrap desalting column (GE Healthcare) to exchange the buffer (20 mM potassium phosphate; pH 7.4, 1 mM MgCl₂, 1 mM DTT, 10 μM ATP) containing 80 mM NaCl for the KIF1A and the kinesin-1 constructs, or 250 mM NaCl for the Zen-4 construct.

For the KIF1A construct, we further purified the active proteins based on their affinity

for microtubules³⁹. KIF1A and microtubules were mixed and incubated for 15 minutes in the presence of 1 mM AMPPNP. The mixture was centrifuged for 20 minutes at 260,000g at 25 °C to remove unbound KIF1A molecules. The pellet was resuspended in ATP-containing buffer (20 mM PIPES-KOH; pH 7.4, 5 mM MgCl₂, 10 mM potassium acetate, 250 mM NaCl, 20 μM paclitaxel, 5 mM ATP) and incubated for 15 minutes at 23 °C for detachment of KIF1A molecules from the microtubules. Microtubules were removed by centrifugation (260,000g, 15 minutes, 23 °C).

The purified proteins were flash-frozen and stored in liquid nitrogen. The concentration of proteins was estimated using the Bradford method with the use of BSA as a standard.

Purification of tubulin. Tubulin was purified from porcine brains through four cycles of temperature-regulated polymerization and depolymerization in a high-molarity PIPES buffer to remove contaminating MAPs⁴⁰. The purified tubulin was flash-frozen and stored in liquid nitrogen.

Preparation of Cy5-microtubules. Tubulin stock solution (~6 mg mL⁻¹, 50 μL) and Cy5-labelled tubulin solution (~20 mg mL⁻¹, 1 μL, ~50% labelled) were mixed on ice and then diluted twofold with chilled BRB80 buffer (80 mM PIPES-KOH; pH 6.8, 1 mM MgCl₂, 1 mM EGTA). One microliter each of GTP (100 mM), and MgCl₂ (100 mM) was added to the diluted tubulin solution. The tubulin solution was incubated at 37 °C for 1 hour for polymerization and stabilized with 20 μM paclitaxel. The Cy5-microtubules were further purified by centrifugation³⁹. Ray *et al.* reported that polymerization in PIPES

buffer results in ~60% of 14-protofilament microtubules and ~30% of 13-protofilament microtubules⁴¹.

Preparation of protein-G-coated beads and streptavidin-coated gold nanorods. For preparation of protein G-coated beads, carboxylate-modified fluorescent microbeads (0.5 μ m; Thermo Fisher Scientific, USA) were diluted in the activation buffer (100 mM MES; pH 6.0). EDC and sulfo-NHS were added to the bead solution and incubated for 15 minutes at room temperature. The beads were then washed with 100 mM HEPES buffer at pH 8.0. Protein-G was then added to the bead solution and reacted for 2 hours at room temperature. Any unreacted amine reactive groups were quenched by adding excess glycine. Excess proteins were removed by centrifugation (21,000g, 15 minutes, 23 °C). The beads were resuspended in BRB80 buffer.

For preparation of streptavidin-coated GNRs, biotin-labelled GNRs (5 nM) (C12-40-600-TB; Nanopartz, CO, USA) and streptavidin (5 mg mL⁻¹) were mixed. After 15 minutes' incubation, excess streptavidin was removed by centrifugation (4,000g, 3 minutes, 23 °C) and then the pellet was resuspended in Milli-Q water and sonicated for 2 s. The procedure for removing excess streptavidin was repeated twice.

GNR motility assays. Streptavidin-coated GNR (2 μ L, 5 nM) and kinesin sample (2 μ L, 1–2 μ M) were mixed and incubated for at least 15 minutes at room temperature. For the assay of KIF1A, 2 μ L of 0.2% Tween20 was further added to the mixture of kinesin and GNR. For the assay for ZEN-4, 0.5 μ L of 5 M NaCl and 2 μ L of 0.2% Tween20 were

further added to the mixture of kinesin and GNR.

A flow chamber (sample volume of about 5 μL) was prepared using two coverslips (24 mm $\times$ 32 mm and 18 mm $\times$ 18 mm) stuck by double-sided tape. Protein-G-coated bead solution was nonspecifically absorbed to the coverslip. After 3 minutes' incubation, the flow chamber was washed with 20 μL of 1 mg mL^{-1} BSA and further incubated for 3 minutes. The flow chamber was washed with 20 μL of BRB80. Then, 10 μL of 20 $\mu\text{g mL}^{-1}$ anti- β -tubulin antibody solution (sc-58884; Santa Cruz, USA) was flowed to the flow chamber. After 3 minutes' incubation, the flow chamber was washed with 20 μL of BRB80. Then, 10 μL of Cy5-labelled microtubule solution ($\sim 2 \mu\text{M}$ tubulin concentration) was flowed into the flow chamber such that the microtubules bound to the beads (Fig. 1c). After 3 minutes' incubation, the flow chamber was washed with 20 μL of 1 mg mL^{-1} casein. After 3 minutes' incubation, the flow chamber was washed with 20 μL of the motility buffer (20 mM PIPES-KOH; pH 7.4, 4 mM MgCl_2 and 10 mM potassium acetate) containing the kinesin-coated GNRs ($\sim 100 \text{ pM}$), ATP (2 mM), the ATP regeneration system (0.8 mg mL^{-1} creatine kinase, 10 mM creatine phosphate), the oxygen scavenger system (3 mg mL^{-1} glucose, 50 U mL^{-1} glucose oxidase, 1200 U mL^{-1} catalase), and 1 mg mL^{-1} casein. The GNR assays were performed at 27 $^{\circ}\text{C}$. Image acquisition was performed at 100 frames s^{-1} .

Surface-gliding assays for KIF1A with QD-coated microtubules. Qdot525 (QD)-coated and Cy5-labelled microtubules were prepared as previously described¹⁸. The surface-gliding assays were performed in flow chambers assembled from two coverslips

attached using double-sided tapes. Before the assay, kinesin stock solution was diluted to 1 μM with motility buffer. Five microliters of a 5 mg mL^{-1} solution of protein G (Sigma-Aldrich, Tokyo, Japan) was added to the flow chamber (about 5 μL). After 5 minutes' incubation, the flow chamber was washed with 20 μL of BRB80 buffer, followed by another 5 minutes of incubation with 5 μL of 0.05 mg mL^{-1} anti-His-tag antibody. The flow chamber was washed with 20 μL of BRB80 buffer, and then 5 μL of 0.5 mg mL^{-1} casein solution was added. After 5 minutes' incubation, the flow chamber was washed with 20 μL of BRB80 buffer. Then, 10 μL of KIF1A solution (1 μM) was added to the flow chamber. The flow chamber was then washed with 20 μL of BRB80 after 5 minutes' incubation, and 20 μL of QD-coated microtubule solution (0.05–0.1 μM tubulin) was added to the flow chamber. Finally, the flow chamber was washed with 20 μL of BRB80 buffer after 5 minutes' incubation, and 20 μL of motility buffer containing 3 mM ATP, 20 μM paclitaxel, 0.5% β -mercaptoethanol, 0.25 mg mL^{-1} casein, ATP regeneration and oxygen scavenger systems were added. Fluorescent images of the QD- and Cy5-labelled microtubules were captured using TIRF microscopy at 25 °C. Corkscrewing pitches in the surface-gliding assay were determined by measuring the longitudinal displacement of the QDs in each period of oscillation of the lateral displacement along the microtubule axis.

Surface-gliding assays for ZEN4-GNR. Preparations of Cy5-labelled microtubules and ZEN4-GNR were prepared as described above. Surface-gliding assays were performed in flow chambers assembled from two coverslips attached using double-sided tape. The

ZEN4-GNR solution was diluted to ~50 pM with motility buffer and added to the flow chamber (about 5 μ L). After 5 minutes' incubation, the flow chamber was washed with 20 μ L of BRB80 buffer, followed by 5 minutes' incubation with 10 μ L of 0.5 mg mL⁻¹ casein solution. The flow chamber was then washed with 20 μ L of BRB80 buffer, and 20 μ L of Cy5-labelled microtubule solution (2 mM ATP, Cy5-labelled microtubules, 0.25 mg mL⁻¹ casein, and ATP regeneration and oxygen scavenger systems in motility buffer) were added. The assays were performed at 27 °C. Image acquisition of the Cy5-labelled microtubules was performed at 10 frames s⁻¹.

Quantification and statistical analysis. Polarization and position data of the observed kinesin-coated GNRs were obtained by image analysis with software written in LabVIEW (National instruments, Tokyo, Japan). Velocities of the kinesin-coated GNRs were determined by fitting the trace of the longitudinal displacement along the microtubule axis (*X*-direction) with a linear function. Rotation and helix pitches in the GNR assays were determined by measuring the *X*-displacements in the periods of oscillations of the angle- and *Y*-trajectories, respectively. Statistical hypothesis testing was performed using R statistical software.

Correction of the measured pitch. The measured pitch (P_{Measured}) comprised two helical elements: the helix pitch exerted by motors (P_{Motor}) without supertwist of the microtubule, and the supertwist of the microtubule depending on the number of the protofilaments (P_{MT}). P_{Measured} is given by

$$\frac{1}{P_{\text{Measured}}} = \frac{1}{P_{\text{Motor}}} + \frac{1}{P_{\text{MT}}}$$

The P_{MT} of 12-, 13-, and 14-protofilament microtubules is estimated to be about -3.4 , -24.8 , and $6.8 \mu\text{m}$, respectively (we assign a positive value to the pitch in the left-handed helix)⁴¹. When the absolute value of P_{Measured} is much smaller than the absolute value of P_{MT} ($< \sim 1 \mu\text{m}$), P_{Motor} is not appreciably changed by the supertwist (Fig. S4).

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Mathematical model of helical and rotational motion of a kinesin-coated GNR on a microtubule. The GNR motility assay revealed that KIF1-, ZEN4-, and sK1-GNRs unidirectionally rotate about the GNR's short axis, moving along the left-handed helix track. The helix track of the center position of a GNR is given by

$$h(x, y, z) = (vt, a \cdot \cos(\pi/2 - \omega_{\text{Helix}}t), a \cdot \sin(\pi/2 - \omega_{\text{Helix}}t))$$

where v is the translational velocity along the x -axis, ω_{Helix} is the angular velocity in the Y - Z -plane, and a is the radius of the helix. Assuming that the long axis of the GNR is parallel to the X - Y -plane under the initial conditions, the initial position matrix consisting of the tip, center, and end positions of the GNR is given by

$$I(\text{tip, center, end}) = \begin{pmatrix} r \cdot \cos(\varphi) & 0 & r \cdot \cos(\varphi + \pi) \\ r \cdot \sin(\varphi) & 0 & r \cdot \sin(\varphi + \pi) \\ 0 & 0 & 0 \end{pmatrix}$$

where r is the radius along the long axis of the GNR and φ is its phase angle. Because a kinesin-coated GNR unidirectionally rotates about its short axis and further around the microtubule, the resultant rotation comprises yawing and rolling. We assume that a kinesin-coated GNR would not rotate about the pitch axis. Matrices of yawing and rolling

are given by

$$R_{\text{Yaw}}(\omega_{\text{Yaw}}t) = \begin{pmatrix} \cos(\omega_{\text{Yaw}}t) & -\sin(\omega_{\text{Yaw}}t) & 0 \\ \sin(\omega_{\text{Yaw}}t) & \cos(\omega_{\text{Yaw}}t) & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

$$R_{\text{Roll}}(\omega_{\text{Roll}}t) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos(\omega_{\text{Roll}}t) & -\sin(\omega_{\text{Roll}}t) \\ 0 & \sin(\omega_{\text{Roll}}t) & \cos(\omega_{\text{Roll}}t) \end{pmatrix}$$

where ω_{Yaw} and ω_{Roll} are the angular velocities of yawing and rolling, respectively.

Taken together, the helical and rotational trajectory of a motor-coated GNR is written as

$$R_{\text{Roll}}R_{\text{Yaw}}I + h$$

Thus, the time trajectory of the GNR orientation in the X - Y -plane is given by

$$\arctan[\cos(\omega_{\text{Roll}}t) \cdot \tan(\varphi + \omega_{\text{Yaw}}t)]$$

Our analyses indicate that kinesin-coated GNRs rotate 180° in one period of the helix.

This is the case of $\omega_{\text{helix}} = \omega_{\text{roll}} = \pm 2\omega_{\text{Yaw}}$. The calculated helical and rotational

trajectories of a kinesin-coated GNR and the time trajectories of the GNR orientation with

different phase angles are shown in Fig. 4, Fig. S5, and Supplementary movies 2–4.

Evaluation of particle flux in the noise-driven ratchet model. The previous study by

Astumian and Bier³⁰ clarified the particle flux in the two-state, noise-driven ratchet model.

Following their discussions, we evaluated the particle flux for different start positions,

assuming that sideward translations ($\pm d$) of motors takes place owing to the rotation of

their cargo (see Fig. 6a). In the two-state ratchet model, when the barrier moves to the

down state, the particles start to diffuse. The probability density function of a diffusing

particle at the start position of $+d$ in the X -direction is given by

$$P_{+d}(x; t) = \frac{1}{2\sqrt{\pi t}} \exp \left[-\frac{(x-d)^2}{4t} \right].$$

After $1/\gamma$ units of time, the probability that the particle is located to the right of α is given by $\int_{\alpha}^{\infty} P_{+d}(x; 1/\gamma) dx = \frac{\gamma}{4} \operatorname{erfc} \left[\frac{\alpha-d}{2} \sqrt{\gamma} \right]$ and the probability that it on the left of $-(1-\alpha)$ is $\int_{-\infty}^{-(1-\alpha)} P_{+d}(x; 1/\gamma) dx = \frac{\gamma}{4} \operatorname{erfc} \left[\frac{1-\alpha+d}{2} \sqrt{\gamma} \right]$. The particle flux, J_{+d} is thus given by

$$J_{+d} = \frac{\gamma}{4} \left\{ \operatorname{erfc} \left[\frac{\alpha-d}{2} \sqrt{\gamma} \right] - \operatorname{erfc} \left[\frac{1-\alpha+d}{2} \sqrt{\gamma} \right] \right\}.$$

Similarly, J_{-d} and J_0 are also obtained, as detailed in main text.

Following the probability density function of a diffusing particle, the standard deviation of diffusion, σ , is given by $\sqrt{2/\gamma}$. When γ is 160 s^{-1} , $\sigma \approx 0.11$, which is equal to the value of σ in the Monte Carlo simulations (see below).

Monte Carlo simulations. We performed Monte Carlo simulations to model the observed rotational and helical motion. In the simulation, each particle is linked to a rigid body as a cargo with a spring (spring constant, k) and the joint points linking the particles and the cargo are fixed. The particles stochastically attach and dissociate from the rail (on-time constant, τ_{on} ; off-time constant, τ_{off}). A detached particle diffuses around the joint point (standard deviation of diffusion, σ) in the X - Y -plane. When the diffusing particle overrides the asymmetric barrier (asymmetric factors, α_X and α_Y) at the last time of diffusion, the particle takes a step in the X - Y -direction. The force- and moment-balance equations are given by $\sum_i^{N_{\text{on}}} k \vec{d}_i = 0$ and $\sum_i^{N_{\text{on}}} k \vec{d}_i \times \vec{l}_i = 0$, where k is the

spring constant; $\vec{d}_i$ is an extension vector of the spring of the i -th particle that is bound to the rail; $\vec{l}_i$ is the position vector of the i -th joint relative to the center of the joint points connecting the particles that are bound to the rail; and N_{on} is the number of the particles bound to the rail. In the simulation, $\vec{d}_i$ is the displacement between the i -th particle and the i -th joint position. Furthermore, torque rotating the cargo is exerted by the particle when it takes a step. A twist angle ($\Delta\theta$) of the cargo by the torque generated by the particle taking a step is given by $\Delta\theta = (N_{\text{step}}\theta_{\text{step}} + b_{\theta})/N_{\text{on}}$ and $\theta_{\text{step}} = T_{\text{step}}L/KG$, where N_{step} is the number of particles taking steps; N_{on} is the number of particles bound to the rail; θ_{step} is the twist angle of the spring; b_{θ} is the rotational diffusion of the cargo as a normal distribution with zero mean and a standard deviation of σ_{θ} ; T_{step} is the torque force generated by the single particle; L is the length of the spring; G is the modulus of rigidity of the spring; and K is the polar moment of inertia of area of the spring. We thus assume that the twist angle θ_{step} is proportional to the torque force T_{step} . The value of θ_{step} as the torque force was given in the simulation. After calculating the force and moment balance equations, the cargo additionally rotates $\Delta\theta$ degrees about the center of the joint points connecting the particles that are bound to the rail.

A roadblock in the simulation allowed the off-state particles to diffuse above it, whereas the on-state particles could not attach to the roadblock except at its edges. We used the roadblock with a width of 40 lattices and a length of 30 lattices.

The simulation was developed in LabVIEW (National Instruments). The values for the parameters used in the simulation were: $\tau_{\text{on}} = 40$ units of time; $\tau_{\text{off}} = 10$ units of time;

$\alpha_x = 0.12$ units of lattice size; $\alpha_y = 0.58$ units of lattice size; $\sigma = 0.11$ units of lattice size; $\theta_{\text{step}} = 4^\circ$; $b_\theta = 0$. Note that the value of σ was determined by the evaluation of particle flux in the noise-driven ratchet model mentioned in the previous subsection, and that the rotational diffusion of the cargo (b_θ) is negligible for the particle formation in Figure 7a, compared the results of Fig. 7c and Fig. S8b.

Reproducibility

Data for all the GNR assay experiments were acquired during at least three independent experimental days.

Reporting summary

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

Data availability

The data that support this study are available from the corresponding author upon reasonable request.

Code availability

LabVIEW source code and executable software for the Monte Carlo simulations used in this study are available <https://github.com/MitsuSGW/MonteCarlo-MotorTeam-Sim.git>

645

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REFERENCES

1. Vale, R. D. The Molecular Motor Toolbox for Intracellular Transport. *Cell* **112**, 467–480 (2003).
2. Hirokawa, N., Noda, Y., Tanaka, Y. & Niwa, S. Kinesin superfamily motor proteins and intracellular transport. *Nat. Rev. Mol. Cell Biol.* **10**, 682–696 (2009).
3. Verhey, K. J. & Hammond, J. W. Traffic control: Regulation of kinesin motors. *Nat. Rev. Mol. Cell Biol.* **10**, 765–777 (2009).
4. Yajima, J., Mizutani, K. & Nishizaka, T. A torque component present in mitotic kinesin Eg5 revealed by three-dimensional tracking. *Nat. Struct. Mol. Biol.* **15**, 1119–21 (2008).
5. Nitzsche, B., Ruhnnow, F. & Diez, S. Quantum-dot-assisted characterization of microtubule rotations during cargo transport. *Nat. Nanotechnol.* **3**, 552–556 (2008).
6. Brunnbauer, M. *et al.* Torque Generation of Kinesin Motors Is Governed by the Stability of the Neck Domain. *Mol. Cell* **46**, 147–158 (2012).
7. Bugiel, M. & Schäffer, E. Three-Dimensional Optical Tweezers Tracking Resolves Random Sideward Steps of the Kinesin-8 Kip3. *Biophys. J.* **115**, 1993–2002 (2018).
8. Mitra, A., Ruhnnow, F., Girardo, S. & Diez, S. Directionally biased sidestepping of Kip3/kinesin-8 is regulated by ATP waiting time and motor–microtubule interaction strength. *Proc. Natl. Acad. Sci.* **115**, E7950–E7959 (2018).
9. Ramaiya, A., Roy, B., Bugiel, M. & Schäffer, E. Kinesin rotates unidirectionally

- 677 and generates torque while walking on microtubules. *Proc. Natl. Acad. Sci.* **114**,
678 10894–10899 (2017).
- 679 10. Yildiz, A., Tomishige, M., Gennerich, A. & Vale, R. D. Intramolecular strain
680 coordinates kinesin stepping behavior along microtubules. *Cell* **134**, 1030–41
681 (2008).
- 682 11. Maruyama, Y. *et al.* CYK4 relaxes the bias in the off-axis motion by MKLP1
683 kinesin-6. *Commun. Biol.* **4**, (2021).
- 684 12. Pan, X., Acar, S. & Scholey, J. M. Torque generation by one of the motor
685 subunits of heterotrimeric kinesin-2. *Biochem. Biophys. Res. Commun.* **401**, 53–
686 57 (2010).
- 687 13. Bormuth, V. *et al.* The highly processive kinesin-8, Kip3, switches microtubule
688 protofilaments with a bias toward the left. *Biophys. J.* **103**, L4–L6 (2012).
- 689 14. Walker, R. A., Salmon, E. D. & Endow, S. A. The Drosophila claret segregation
690 protein is a minus-end directed motor molecule. *Nature* **347**, 780–782 (1990).
- 691 15. Nitzsche, B. *et al.* Working stroke of the kinesin-14, ncd, comprises two substeps
692 of different direction. *Proc. Natl. Acad. Sci.* **113**, E6582–E6589 (2016).
- 693 16. Mitra, A. *et al.* Kinesin-14 motors drive a right-handed helical motion of
694 antiparallel microtubules around each other. *Nat. Commun.* **11**, 1–11 (2020).
- 695 17. Yajima, J. & Cross, R. A. A Torque Component in the Kinesin-1 Power Stroke.
696 *Nat. Chem. Biol.* **1**, 338–341 (2005).
- 697 18. Yamagishi, M., Fujimura, S., Sugawa, M., Nishizaka, T. & Yajima, J. N-terminal
698 β -strand of single-headed kinesin-1 can modulate the off-axis force-generation

- 699 and resultant rotation pitch. *Cytoskeleton* **77**, 351–361 (2020).
- 700 19. Mitra, A. *et al.* A Brownian ratchet model explains the biased sidestepping of
701 single-headed kinesin-3 KIF1A. *Biophys. J.* **8**, 1–9 (2019).
- 702 20. Yamagishi, M., Maruyama, Y., Sugawa, M. & Yajima, J. Characterization of the
703 motility of monomeric kinesin-5/Cin8. *Biochem. Biophys. Res. Commun.* **555**,
704 115–120 (2021).
- 705 21. Konopka, C. A. & Bednarek, S. Y. Variable-angle epifluorescence microscopy:
706 A new way to look at protein dynamics in the plant cell cortex. *Plant J.* **53**, 186–
707 196 (2008).
- 708 22. Tokunaga, M., Imamoto, N. & Sakata-Sogawa, K. Highly inclined thin
709 illumination enables clear single-molecule imaging in cells. *Nat. Methods* **5**,
710 159–61 (2008).
- 711 23. Ueno, H. *et al.* Simple dark-field microscopy with nanometer spatial precision
712 and microsecond temporal resolution. *Biophys. J.* **98**, 2014–2023 (2010).
- 713 24. Watanabe, T. M. *et al.* Four-dimensional spatial nanometry of single particles in
714 living cells using polarized quantum rods. *Biophys. J.* **105**, 555–564 (2013).
- 715 25. Okada, Y. & Hirokawa, N. A processive single-headed motor: Kinesin
716 superfamily protein KIF1A. *Science (80-.)*. **283**, 1152–1157 (1999).
- 717 26. Hutterer, A., Glotzer, M. & Mishima, M. Clustering of Centralspindlin Is
718 Essential for Its Accumulation to the Central Spindle and the Midbody. *Curr.*
719 *Biol.* **19**, 2043–2049 (2009).
- 720 27. Yajima, J., Alonso, M. C., Cross, R. a & Toyoshima, Y. Y. Direct long-term

- observation of kinesin processivity at low load. *Curr. Biol.* **12**, 301–6 (2002).
28. Berliner, E., Young, E. C., Anderson, K., Mahtani, H. K. & Gelles, J. Failure of a single-headed kinesin to track parallel to microtubule protofilaments. *Nature* **373**, 718–721 (1995).
29. Chrétien, D. & Fuller, S. D. Microtubules switch occasionally into unfavorable configurations during elongation. *J. Mol. Biol.* **298**, 663–676 (2000).
30. Astumian, R. D. & Bier, M. Fluctuation driven ratchets: Molecular motors. *Phys. Rev. Lett.* **72**, 1766–1769 (1994).
31. Kikkawa, M. & Hirokawa, N. High-resolution cryo-EM maps show the nucleotide binding pocket of KIF1A in open and closed conformations. *EMBO J.* **25**, 4187–4194 (2006).
32. Cao, L. *et al.* The structure of apo-kinesin bound to tubulin links the nucleotide cycle to movement. *Nat. Commun.* **5**, (2014).
33. Hwang, W., Lang, M. J. & Karplus, M. Kinesin motility is driven by subdomain dynamics. *Elife* **6**, 1–25 (2017).
34. Davies, T. *et al.* CYK4 Promotes Antiparallel Microtubule Bundling by Optimizing MKLP1 Neck Conformation. *PLoS Biol.* **13**, 1–26 (2015).
35. Can, S., Dewitt, M. a & Yildiz, A. Bidirectional helical motility of cytoplasmic dynein around microtubules. *Elife* e03205 (2014) doi:10.7554/eLife.03205.
36. Hwang, W. & Karplus, M. Structural basis for power stroke vs . Brownian ratchet mechanisms of motor proteins. (2019) doi:10.1073/pnas.1818589116.
37. Kaplan, L., Ierokomos, A., Chowdary, P., Bryant, Z. & Cui, B. Rotation of

743 endosomes demonstrates coordination of molecular motors during axonal
744 transport. *Sci. Adv.* **4**, (2018).

745 38. Enoki, S. *et al.* High-Speed Angle-Resolved Imaging of a Single Gold Nanorod
746 with Microsecond Temporal Resolution and One-Degree Angle Precision. *Anal.*
747 *Chem.* **87**, 2079–2086 (2015).

748 39. Yamagishi, M. *et al.* Structural Basis of Backwards Motion in Kinesin-1-
749 Kinesin-14 Chimera: Implication for Kinesin-14 Motility. *Structure* **24**, 1322–
750 1334 (2016).

751 40. Castoldi, M. & Popov, A. V. Purification of brain tubulin through two cycles of
752 polymerization-depolymerization in a high-molarity buffer. *Protein Expr. Purif.*
753 **32**, 83–8 (2003).

754 41. Ray, S., Meyhöfer, E., Milligan, R. A. & Howard, J. Kinesin follows the
755 microtubule's protofilament axis. *J. Cell Biol.* **121**, 1083–1093 (1993).
756

Figure 1. GNR motility assay for KIF1A. **(a)** Schematic drawing of the optics for 3D tracking and polarization measurements of a gold nanorod (GNR) using laser dark-field imaging with variable-angle epi-illumination. OBJ, objective lens; PDM, perforated dichroic mirror; QWP, quadruple wave plate; pBD, polarizing beam displacer; CL, cylindrical lens; CAM, CMOS camera; L, lens. **(b)** Calibration for polarization measurements. (upper) Series of pairs of scattered-light spots of microbeads (0.1 μm) modulated by a polarizer, which was located in front of L3, for emulating GNR spots with various orientations on the surface. (lower left) Intensity profiles of a pair of scattered-light spots (I_{bottom} , I_{upper}) shown in the upper images. The plots of I_{bottom} and I_{upper} were fitted by intensity functions of the GNR orientation. (lower right) Degree of polarization (DOP) $(I_{\text{bottom}} - I_{\text{upper}})/(I_{\text{bottom}} + I_{\text{upper}})$ as a function of the normalized summed-intensity of two scattered-light spots ($I_{\text{bottom}} + I_{\text{upper}}$). See

770 Methods for details. **(c)** (top) Schematic drawing of the GNR motility assay in which a
771 kinesin-coated GNR moves along a microtubule suspended with fluorescent microbeads
772 (0.5 μm) attached to a glass substrate. (bottom) Fluorescence image of Cy5-labelled
773 microtubules suspended on fluorescent microbeads. The bright spots are the fluorescent
774 microbeads. **(d)** Montage of scattered-light images of one KIF1A-coated GNR (KIF1-
775 GNR) moving around a microtubule every 0.2 s in the presence of 2 mM ATP. The frame
776 rate was 100 frames/s. Scale bar, 2 μm . **(e)** DOP as a function of the summed-intensity of
777 the two KIF1-GNR spots shown in (d). The red line represents an ellipse fit. **(f)** 3D
778 trajectory of the KIF1-GNR shown in (d) through a moving-average filter of 15 frames.
779 **(g)** The time trajectories of the X - and Y -displacements (upper), the DOP and summed-
780 intensity of the two spots (middle), and the estimated angle (bottom) of KIF1-GNR shown
781 in (d). The value of r is Pearson's correlation coefficient between the Y -displacement and
782 the angle. **(h)** Cross-correlation functions (CCFs) between the Y -displacement and the
783 polarization signals.

784

785 **Figure 2.** Typical data of the GNR motility assay for KIF1A. **(a, b)** The time trajectories
786 of the X - and Y -displacements (upper), the degree of polarization (DOP) and summed-
787 intensities of the two spots (middle), and the estimated angles (bottom) of KIF1-GNRs.
788 The time trajectories of the polarization in the image plane show clockwise rotation (a)
789 and oscillatory rotation (b). The value of r is Pearson's correlation coefficient between
790 the Y -displacement and the angle. **(c)** Distribution of the helix and polarization pitches of
791 KIF1-GNRs. The helix and polarization pitches are the displacements during one period
792 of the Y -displacement and the polarization fluctuations, respectively. The red line
793 represents a linear fit ($y = ax$) of which the slope (a) is 0.97 ($R^2 = 0.95$).

Figure 3. GNR motility assay for ZEN-4 and kinesin-1. **(a, c)** Typical time trajectories of the X - and Y -displacements (upper), the degree of polarization (DOP) and summed-intensity of the two spots (middle), and the estimated angles (bottom) of ZEN-4-coated gold nanorods (ZEN4-GNRs) (a) and single-headed kinesin-1 coated GNRs (sK1-GNRs) (c). The three trajectories represent counterclockwise (left), clockwise (middle), and oscillatory (right) rotations of the GNR polarization in the sample plane. The value of r is Pearson's correlation coefficient between the Y -displacement and the angle. **(b, d)** Distribution of the helix and polarization pitches of ZEN4-GNRs (b) and sK1-GNRs (d). The red lines represent linear fits ($y = ax$) of which the slopes (a) are 0.99 ($R^2 = 0.97$) (b) and 0.97 ($R^2 = 0.98$) (d). **(e)** Typical time trajectories of the X - and Y -displacements (upper), the DOP and summed-intensity of the two spots (middle), and the estimated angle (bottom) of double-headed kinesin-1 coated GNRs (dK1-GNRs).

Figure 4. Interpretation of the observed polarization and translational movement of the kinesin-coated gold nanorods (GNRs). (Left) Montage of the projection images of a GNR in the X-Y plane, obtained by a mathematical model of a motor-coated GNR rotating 180° about its yaw axis in the clockwise yawing, in one period of the helical motion. (Right) Time trajectories of the Y-displacement and angle of a motor-coated GNR with different initial phase angles ($\varphi = 0^\circ$ (180°), 45°, 90°, and 135°). The value of r is Pearson's correlation coefficients between the Y-displacement and the angle. Trajectories of a motor-coated GNR in cases of the counterclockwise yawing and non-yawing are shown in Fig. S5. See also Supplementary movies 2–4.

Figure 5. Surface-gliding assay for ZEN-4 coated gold nanorods (ZEN4-GNRs). (a) Schematic drawing of the surface-gliding assay, whereby the generation of force and torque rotate a Cy5-labelled microtubule (Cy5-microtubule) on the ZEN4-GNR. (b) Montage of fluorescence images of Cy5-microtubules every six frames (600 ms), showing that the short Cy5-microtubule continuously rotated (yellow arrowhead). Scale bar, 2 μm . GNR image of the same region-of-interest of the Cy5 images is also shown (right upper). (c) Time trajectory of the microtube orientation.

Figure 6. Mechanistic model for the clockwise rotation and helical motion of kinesin-coated gold nanorods (GNRs). (a) By rotation of the GNR, motors in the posterior and anterior positions relative to the rotation axis move laterally with displacement of $\pm d$. (b) This situation can be modeled using the noise-driven ratchet model with asymmetry potentials (α) and three different starting positions of the particles ($-d, 0, +d$). (c, d) Fluxes of three particles at the starting positions of diffusion in the noise-driven ratchet with asymmetry potentials after $1/\gamma$ units of time (Methods). (c) Fluxes of three particles as a function of d with $\alpha = 0.53$ and $\gamma = 160 \text{ s}^{-1}$. (d) Net flux, $J_{-d} + J_0 + J_{+d}$, as a function of d .

Figure 7. Monte Carlo simulation of the model for kinesin-coated gold nanorods.

(a) Schematic description of the model. Particles (blue balls) are linked to a rigid body as a cargo (orange square) with a spring (green line), and the formation of their joint points (yellow circle or square) are fixed relative to the cargo axis. Particles repeatedly detach and attach to the 2D lattice rail with rate constants of $1/\tau_{on}$ and $1/\tau_{off}$, respectively. A detached particle diffuses around the joint points with a standard deviation of σ . When the particle passes over the barrier with an asymmetric factor (α), at the last time of diffusion, it will attach to the neighboring binding site and generate torque, twisting the spring by θ_{step} . Translational and rotational motions of the cargo are driven by the balances of force and moment (torque). (b, c) Simulation results without and with torque generation ($\theta_{step} = 0^\circ$ in (b) and $\theta_{step} = -4^\circ$ in (c)). The parameters are specified in

847 Methods. (left) Typical X - Y -trajectories of three representative particles (black, magenta,
848 and green filled circles) represent biased forward, sideward and rotational motions of the
849 cargo. (right) Time trajectories of the angle and Y -displacement, and their cross-
850 correlation function (CCF). The axes of the angle and the Y -direction are converted to
851 180° - and 13-lattice periods, respectively. **(d)** Typical examples of simulations of
852 bypassing an obstacle. α_Y and θ_{step} are the sideward asymmetric and torque
853 parameters, respectively (see the main text). The percentages represent the probabilities
854 of success in bypassing the obstacle (blue rectangle) within a calculation time of 55,000
855 and out of 300 trials.