Supplementary Material

for

High-Throughput Force Measurement of Individual Kinesin-1 Motors during Multi-Motor Transport

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Supplementary information

Supplementary Text S1-S4

Methods

Supplementary Figures S1-S17

Supplementary movie 1-4

Supplementary Text

S1: Details of control experiments

We performed a control experiment where we flowed the assembly of d-ssDNA-QD-kinesin in the imaging chamber without anti-digoxigenin, whose presence is essential for specific binding of kinesin. Without anti-digoxigenin, we did not see any quantum dots on the surface. This signifies that there is no non-specific binding of kinesin-quantum dots on the surface and every kinesin is linked to the surface with a d-ssDNA molecule during data acquisition. We also did not observe any microtubule movement when there was no kinesin visible under the microtubule which demonstrated that there were no unlabeled kinesins present on the surface (data not shown). To establish the baseline activity of our purified kinesin-1 construct, we also performed a standard kinesin walking assay on surface immobilized microtubules. Kinesins labeled with quantum dots

walked with 1 micron/s velocity in the presence of saturating ATP, which is typical in kinesin walking assays[1] (Fig. S3 and Supplementary Movie 1).

S2: Effect of vertical distance of microtubule on the d-ssDNA extension

In the simple microtubule gliding assay (without a d-ssDNA linker and with, kinesin directly immobilized on the surface), the distance between the microtubule and the surface was measured to be 17 nm[2]. The radius of gyration (R_g) represents the radius of any polymer chain in its equilibrium position. The vertical length of the d-ssDNA molecule will be $2*R_g$.

R_g for ssDNA is given by[3]:

$$R_g = \left(\frac{L \cdot p}{3}\right)^{\frac{1}{2}}$$

where L is the contour length, and p is the persistence length of the molecule.

R_g of 1180 bp long d-ssDNA comes out to be 21 nm. Therefore, the approximate distance of the microtubule from the surface in the FSIM assay would be 38 nm (17+21 nm). 38 nm vertical distance will have minimal effect on large kinesin displacements (and hence forces) in our assay. For small kinesin displacements, the force exerted by the kinesins will be relatively small (<1 pN), and therefore, the vertical distance will not alter forces significantly. Therefore, we have assumed that the displacement of kinesin is approximately equal to the d-ssDNA extension.

S3: Drag force on a microtubule

Typical drag force values on the microtubules (MTs) are very low (0.01-0.1 pN). Drag force on microtubule can be calculated from the following equation:

$$F = C_{\parallel}\eta L v$$

where F is the drag force, $C_{\parallel}$ is the dimensionless drag coefficient (the drag coefficient per unit length and unit viscosity), η is viscosity, L is the length of the microtubule, and v is MT velocity[4].

As a test case: assuming viscosity of water, 10-micron length of MT, and 1 micron/s velocity of MT: The drag forces comes out to be 0.06 pN. Therefore, what we are observing agrees with the typical drag forces on the MT.

S4: Sensitivity and dynamic range of FSIM assay

A force sensor with a dynamic range of 0-10 pN is appropriate for the experiments of molecular motors as the stall forces of kinesin and dynein are below 7 pN. FSIM assay works well in this force range. Optical trap experiments with d-ssDNA molecule inform us that FSIM assay can measure forces up to 20 pN (Fig. 2c). We note that the standard deviation of the force-extension curve increases as we go above 10 pN, and it may therefore introduce uncertainty in the force calculation. We predict that the FSIM assay could be useful for other systems with higher force ranges but will need improvements in d-ssDNA preparation, purification, and theoretical modeling of the force-extension characteristics.

In the 0-7 pN force range (relevant for molecular motors), some uncertainty in the force calculation is introduced by the localization precision of the quantum dot-kinesin (i.e., the calculation of the d-ssDNA extension). In our acquired datasets, the localization uncertainty of kinesin-QD is 14 nm (Fig. S17). It translates to the force error of <0.1 pN for forces below 1 pN. The error of force calculation due to localization uncertainty increases gradually in the higher force range with the error of 0.5 pN in 4-5 pN force range. Further improvements in the localization precision of the kinesin-quantum dots can reduce the error of force calculation in the FSIM assay.

Supplementary figures

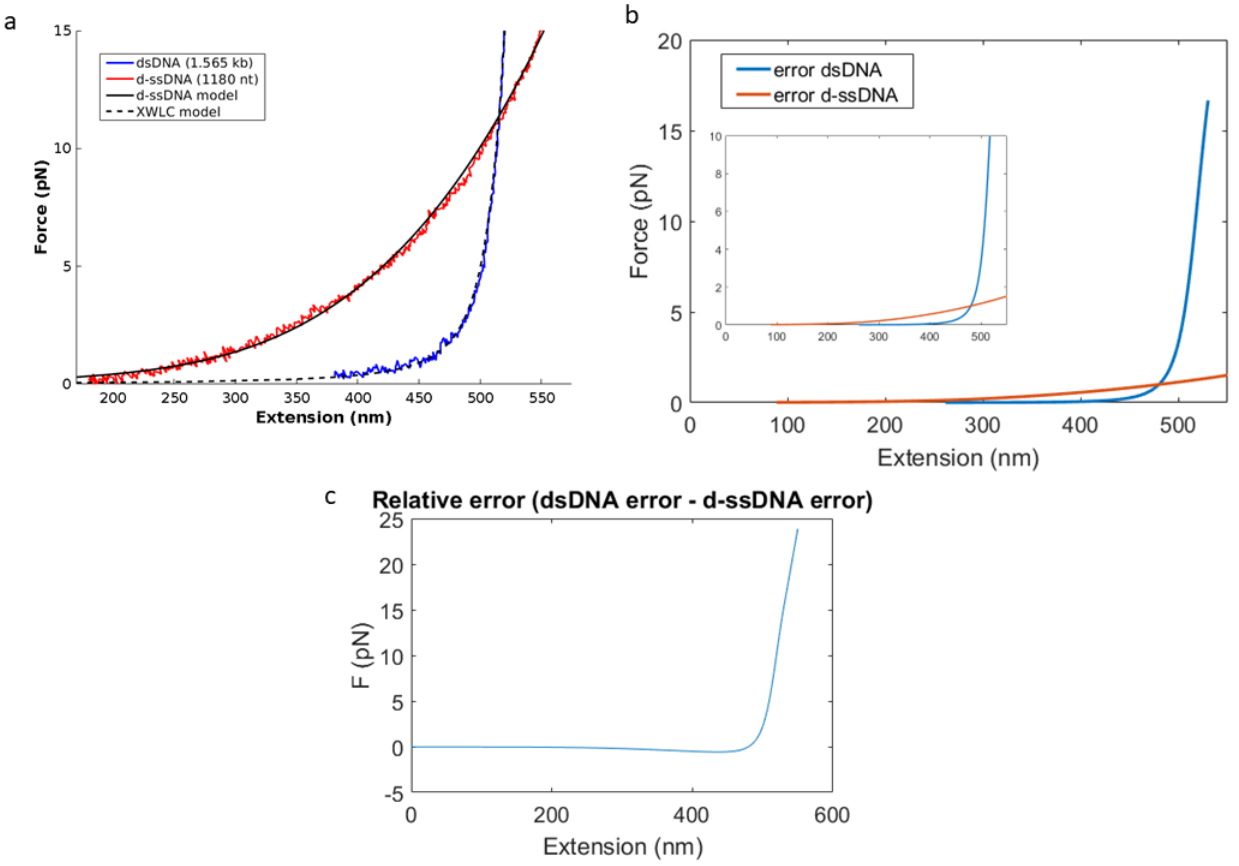


Figure S1. dsDNA force-extension model and the error in force measurement. (a) Representative force-extension curves of dsDNA (blue) and d-ssDNA (red) with theoretical models for each (black solid line and dashed line). The force required to stretch a 1.5-kb dsDNA molecule remains very small up to an extension of 450 nm and shoots up near its contour length. **(b)** Error in the calculation of the force given the average localization precision of 14 nm (see Fig. S17), plotted for both dsDNA and d-ssDNA. After the extension of 450 nm, there is a large variation in force with a small change in extension (eWLC model)[5]. dsDNA is not a reliable force sensing molecule because of the large errors in force calculation. Inset shows the error in force calculation in the range of 0-10 pN. The error was calculated as a product of localization uncertainty of the microscope and the derivative of force-extension curves. (c) Relative error of force calculation. Relative error was defined as the difference between dsDNA error and d-ssDNA error.

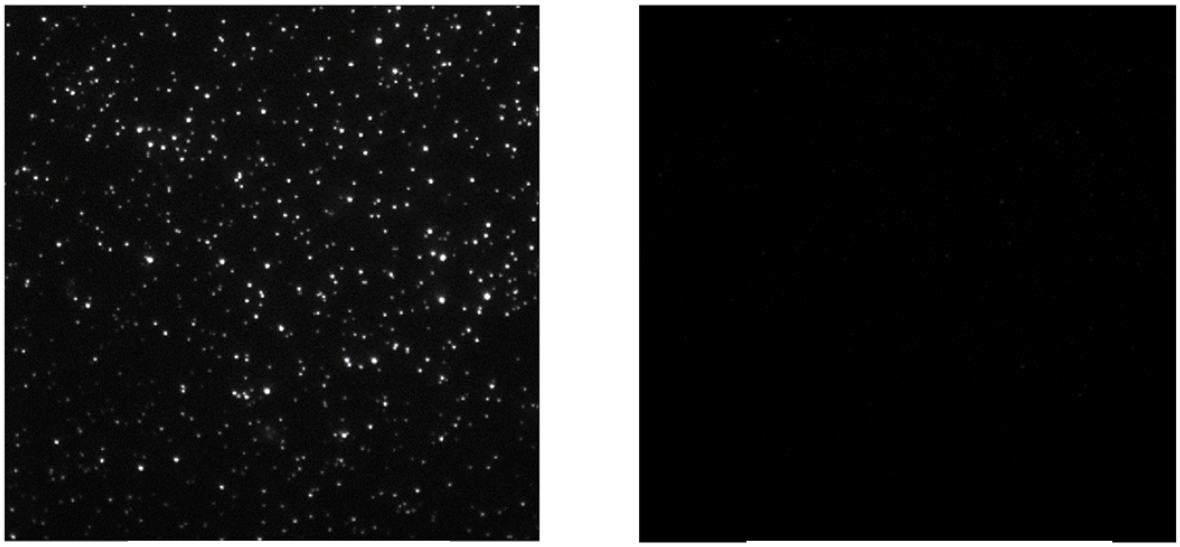


Figure S2. Control for negligible non-specific binding of QDs. The left image shows the concentration of kinesins on the surface when 1 nM of kinesin-QD is flowed in the imaging chamber with anti-digoxigenin-biotin. Anti-digoxigenin-biotin is immobilized on the surface using its biotin end, and the anti-digoxigenin end attaches to the digoxigenin end of the ssDNA-QD-kinesin. When kinesin-QD-ssDNA is flown in the chamber without anti-digoxigenin on the surface, ideally, there should be no kinesin attachment on the surface. This is what we observe in the right-side microscope image, proving there is the negligible non-specific binding of kinesin-QDs on the surface. In our force-sensing experiment, every kinesin has a d-ssDNA molecule attached to it.

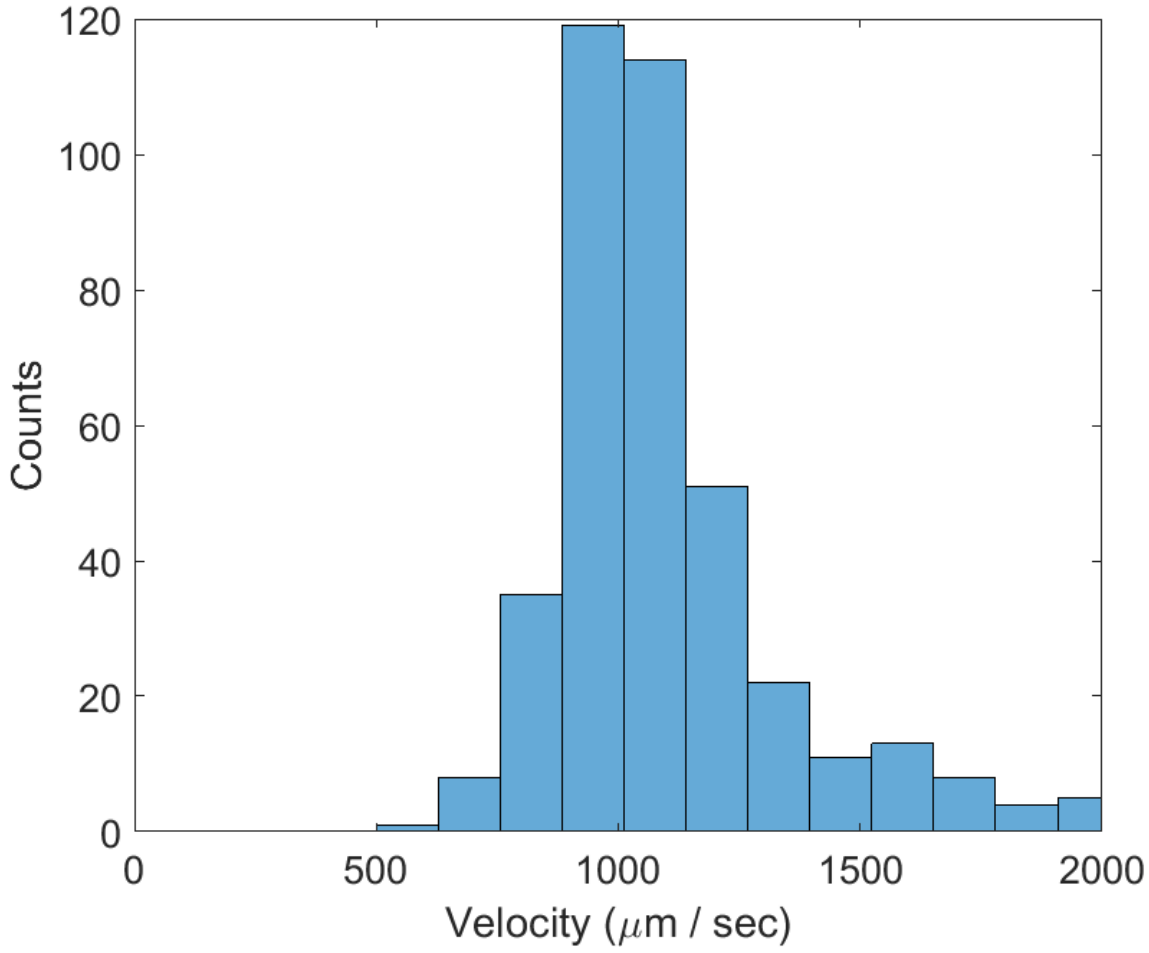


Figure S3. Velocity distribution of kinesin-QD in walking assay. MiCA purified kinesins labeled with quantum dots walked with 1000 nm/s velocity on surface immobilized microtubules. ATP concentration is 1 mM in the imaging buffer.

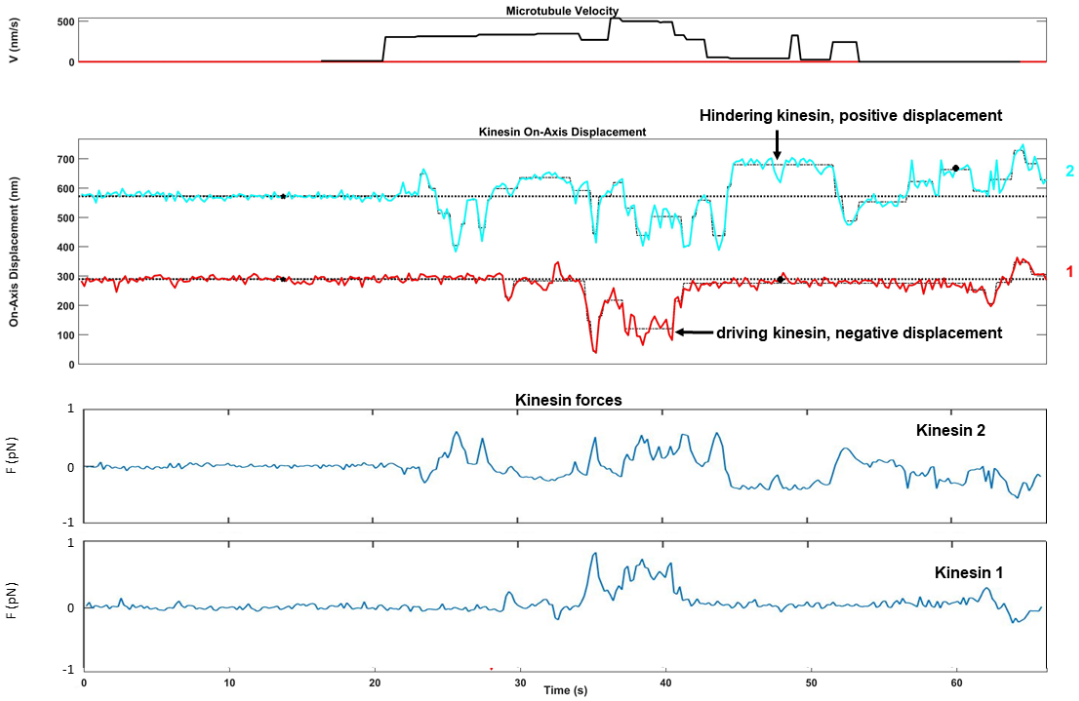


Figure S4. Two kinesins transporting a microtubule. Microtubule velocity, kinesin displacements, and kinesin forces are plotted with time. Kinesin can exhibit both negative and positive displacement and can adopt driving or hindering states. From kinesin displacement, we calculate kinesin forces. Kinesin can apply forces in both positive and negative directions. We have designed our experiments carefully to minimize the presence of unlabeled kinesins using the magnetic purification protocol of quantum-dot labeled kinesins. However, there is a possibility of unlabeled kinesins on the surface which affect the forces of visible kinesins during the cargo transport. Because of this effect, kinesin forces do not seem to be balanced during cargo transport at certain timepoints.

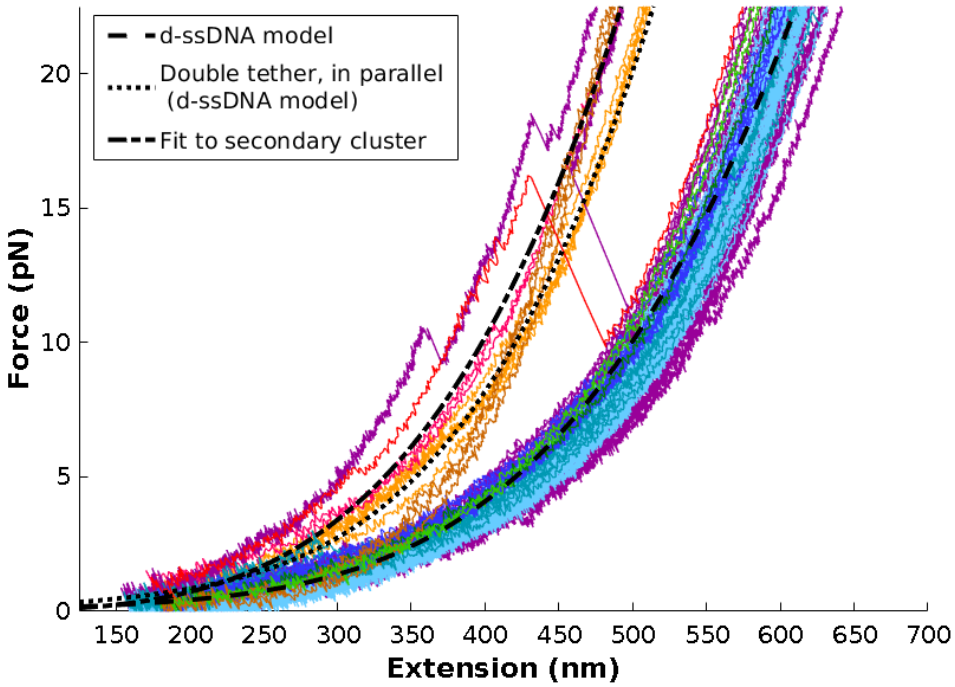


Figure S5. Force extension curves of d-ssDNA. Plot of all force-extension curves of d-ssDNA ($N = 72$) used for modeling its elastic behavior. The curves are color-coded according to bead pair (10 total, same color code as in Fig. 2c), denoting sets of independent measurements. The majority of curves ($N = 64$) comprise a 'primary' cluster, interpreted as the typical stretching behavior of d-ssDNA. Fitting the force-extension curves in the primary cluster determines an analytical model for d-ssDNA elasticity (black dashed line; see Methods). The remaining force-extension curves ($N = 8$) fall into a 'secondary' cluster. Fitting the curves in the secondary cluster, excluding ruptures, using the same analytical model yields a force-extension behavior (black dash-dot line; see Methods) similar to that predicted for two d-ssDNA molecules tethered in parallel (black dotted line).

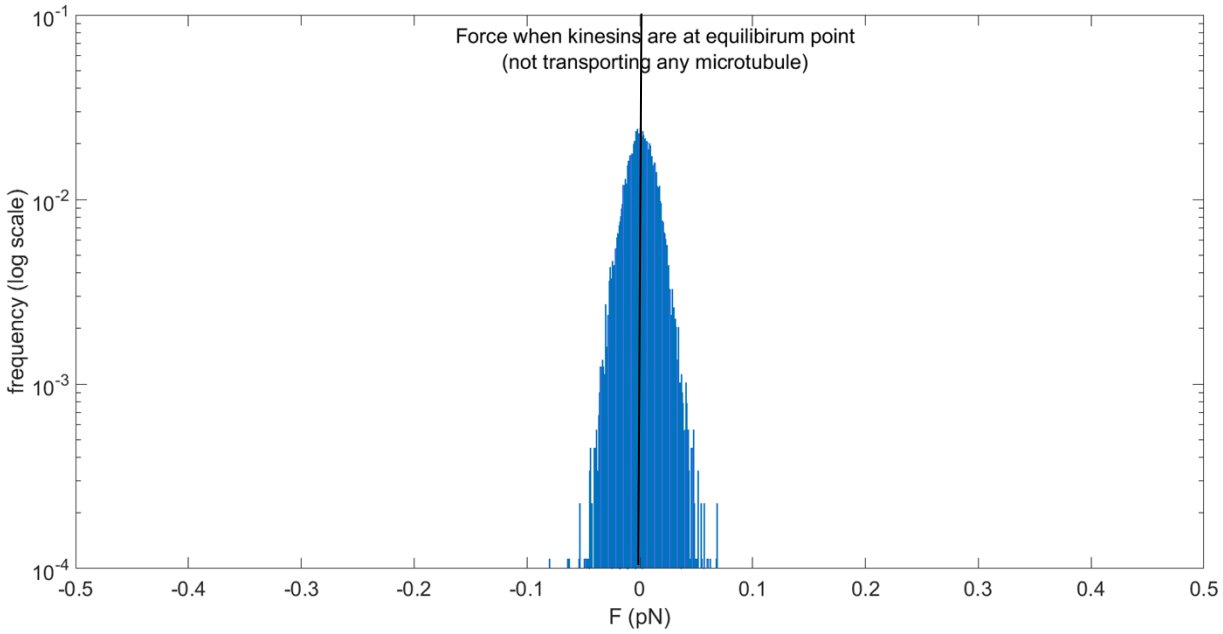


Figure S6. Force by kinesins at the equilibrium position. A large peak at zero forces is observed when there is no microtubule attached to the kinesin, and kinesins remain at their equilibrium positions.

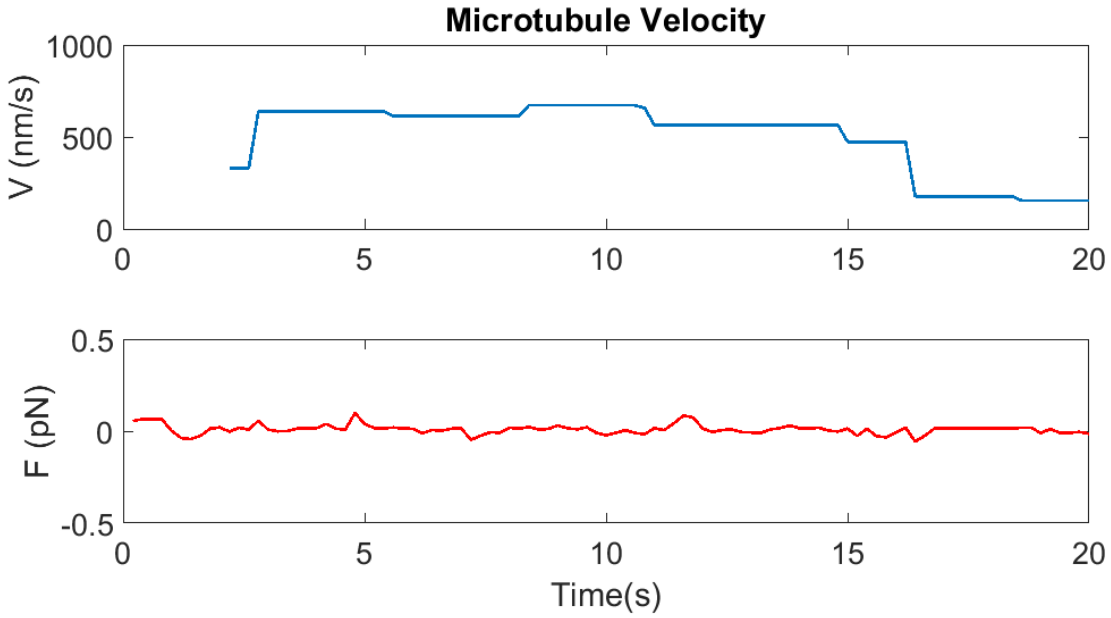


Figure S7. Single kinesin transporting a microtubule. Forces remain low when one kinesin transports a microtubule. Kinesin needs to act against the drag force on the microtubule, which is very low in the solution (see supplementary text 2).

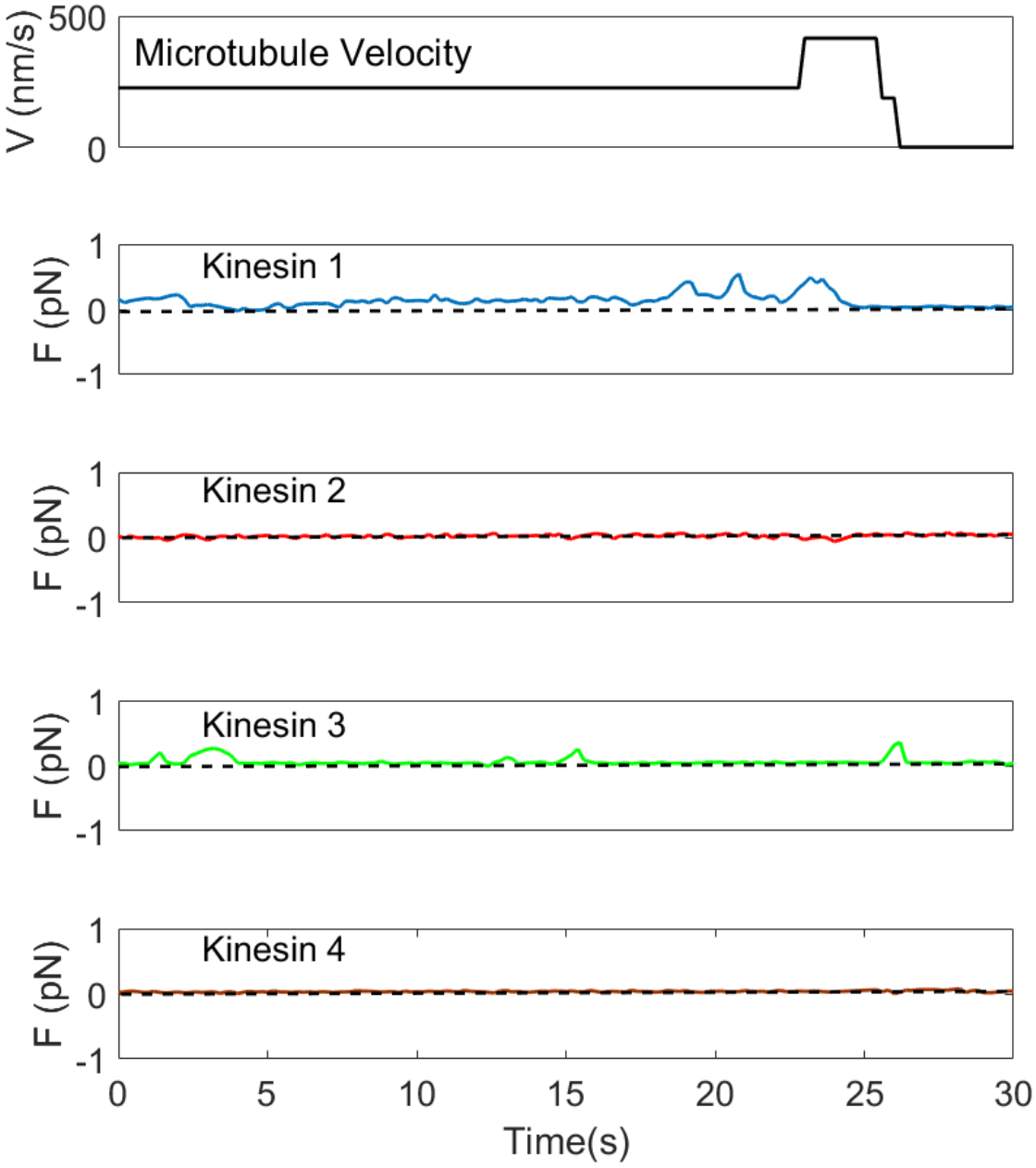


Figure S8. Microtubule transported by four kinesins. Individual forces by four kinesins along with the microtubule velocity are shown. Kinesin #1 and #3 occasionally exert positive forces, whereas kinesin #2 and #4 remain at the equilibrium position.

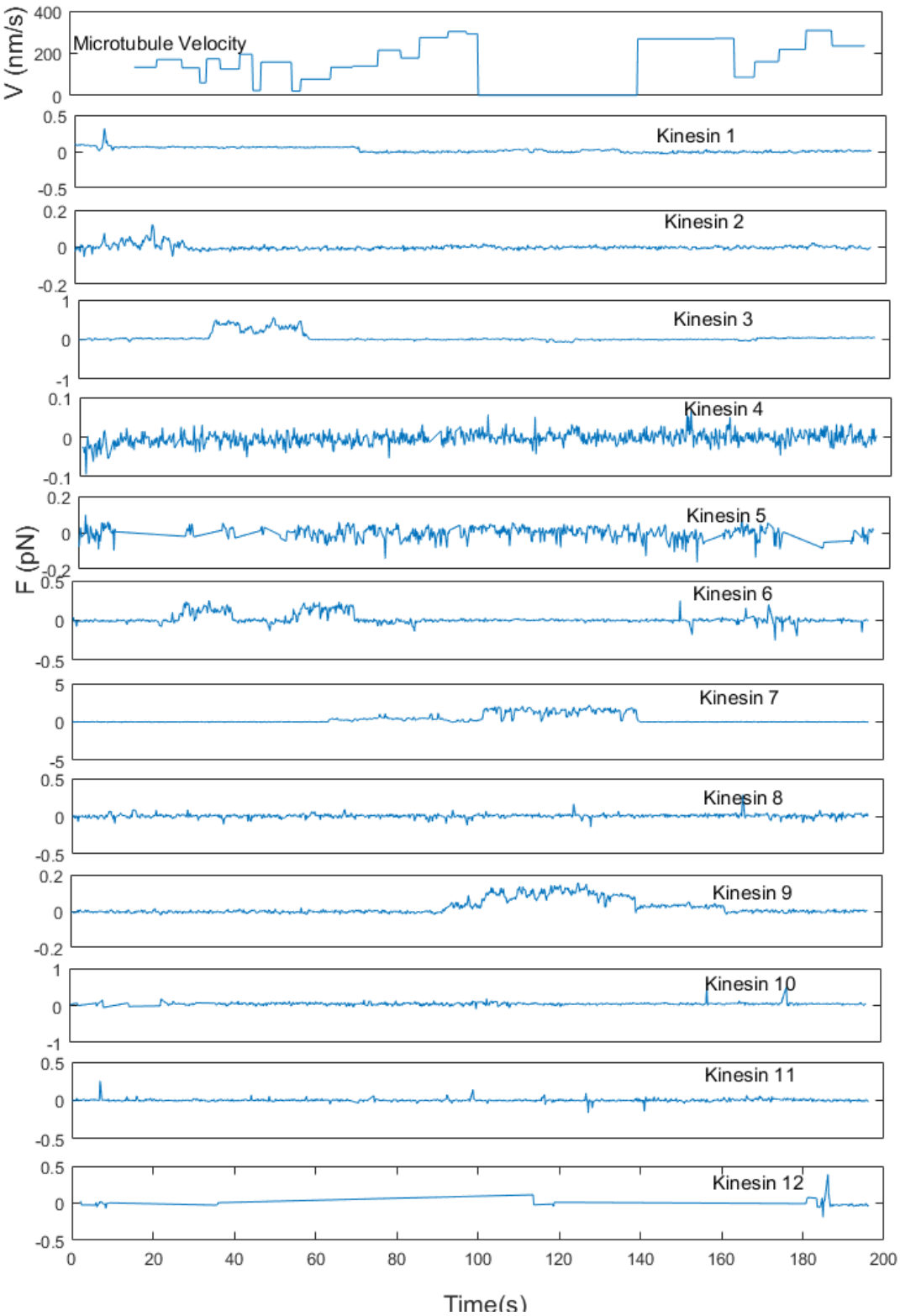


Figure S9. Kinesin transported by 12 kinesins. Forces of individual kinesins are plotted along with the microtubule velocity. Kinesin forces remain mostly below 1 pN during the cargo transport.

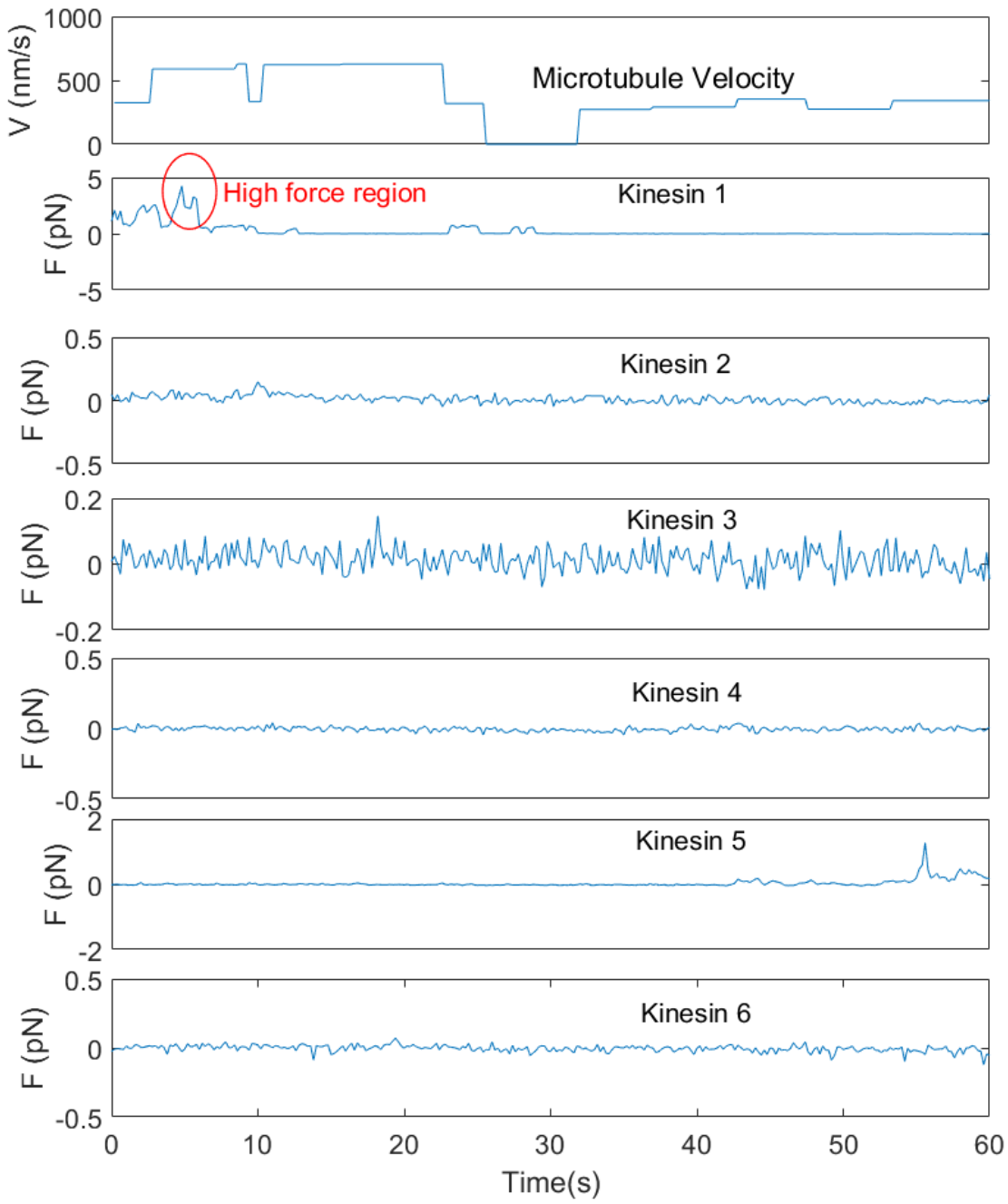


Figure S10. Kinesin exerts more than a pN force. A case where a microtubule is being transported by six kinesins (no roadblocks). Kinesin #1 produces 4 pN of force at t=5 s. This is one of the rare cases where a kinesin exerts more than one pN during cargo transport.

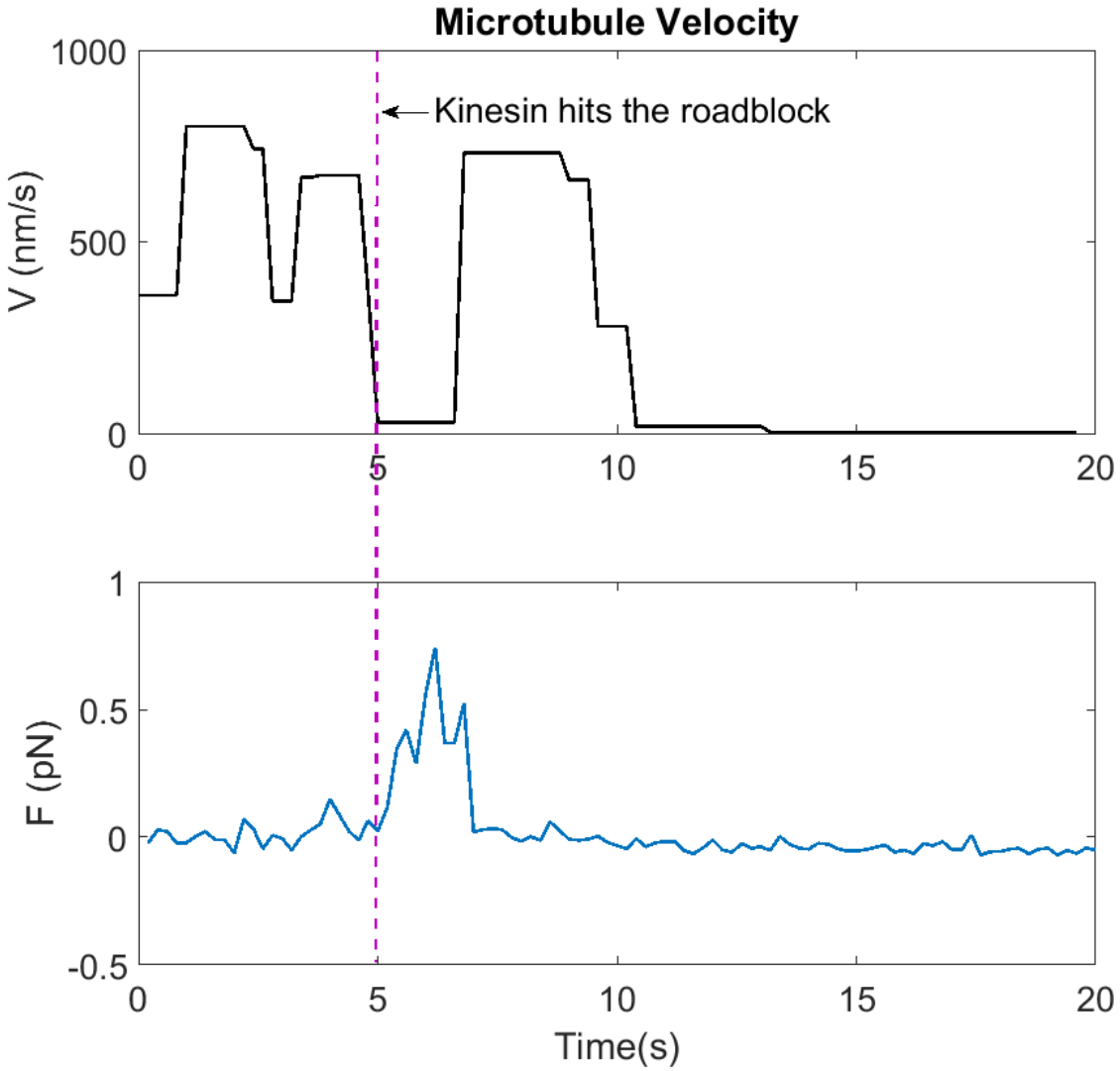


Figure S11. One kinesin overcomes the roadblock. A case where one kinesin is transporting the microtubule. Kinesin hits the roadblock (20 nm quantum dot roadblock) at t=5 s. It increases its force to overcome the roadblock.

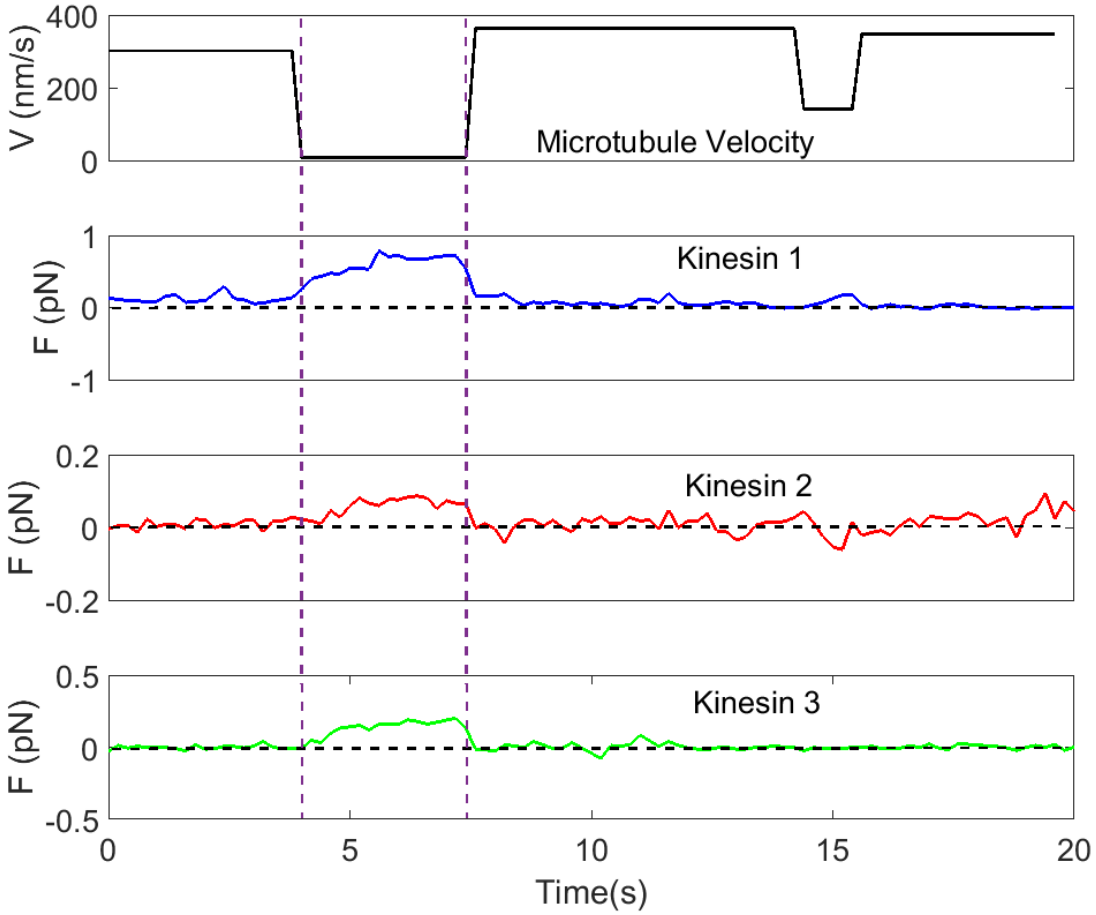
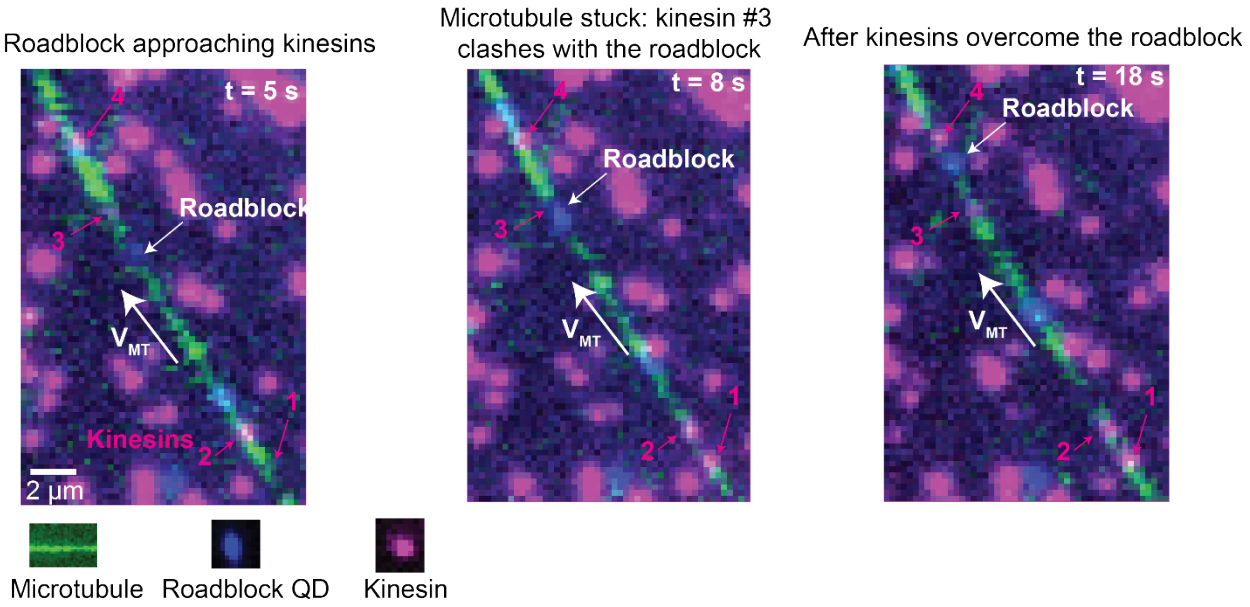


Figure S12. Three kinesins resume microtubule motion. In this example, the microtubule is stuck at the roadblock (20 nm quantum dot roadblock) for a long time (from 4-7 seconds). After t=4 s, all three kinesins exert positive force on the microtubule, resuming its velocity at t=7 s. Purple vertical lines represent when microtubule stops and resumes its motion.



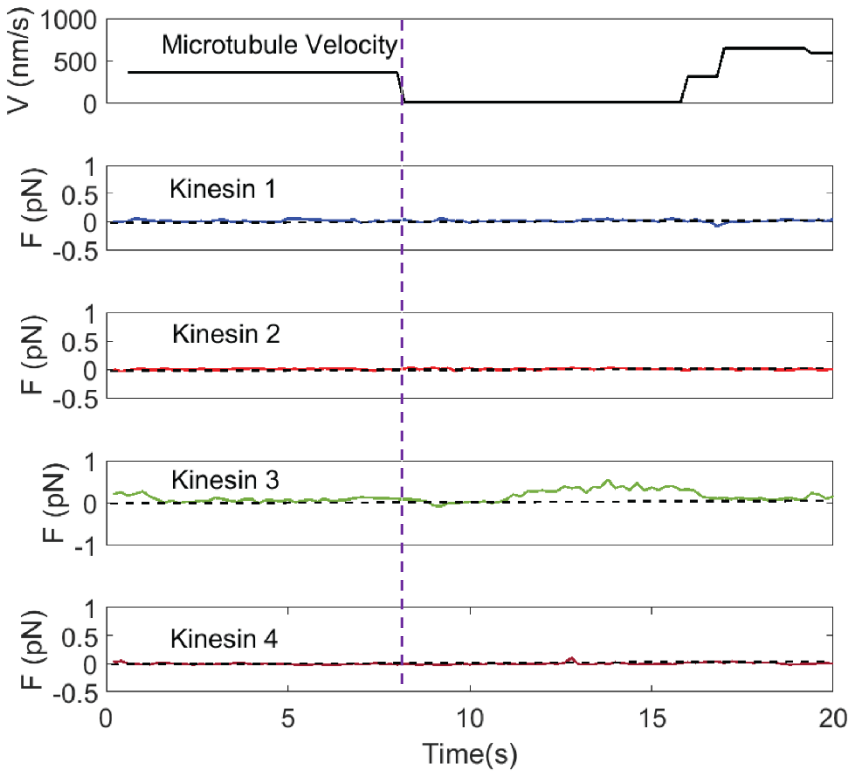


Figure S13. Four kinesins overcome a roadblock. Three microscope images (top) of an example are shown where a microtubule (shown in green) is transported by four kinesins (shown in magenta). Microtubule velocity and forces of individual kinesins are plotted (bottom). Kinesin#3 gets stuck at the roadblock (20 nm quantum dot roadblock, shown in blue) for a long time (starting at 8 s, vertical purple line). After t=16 s, kinesin 3 exerts a positive force on the microtubule, resuming its velocity. The other three kinesins exert low forces.

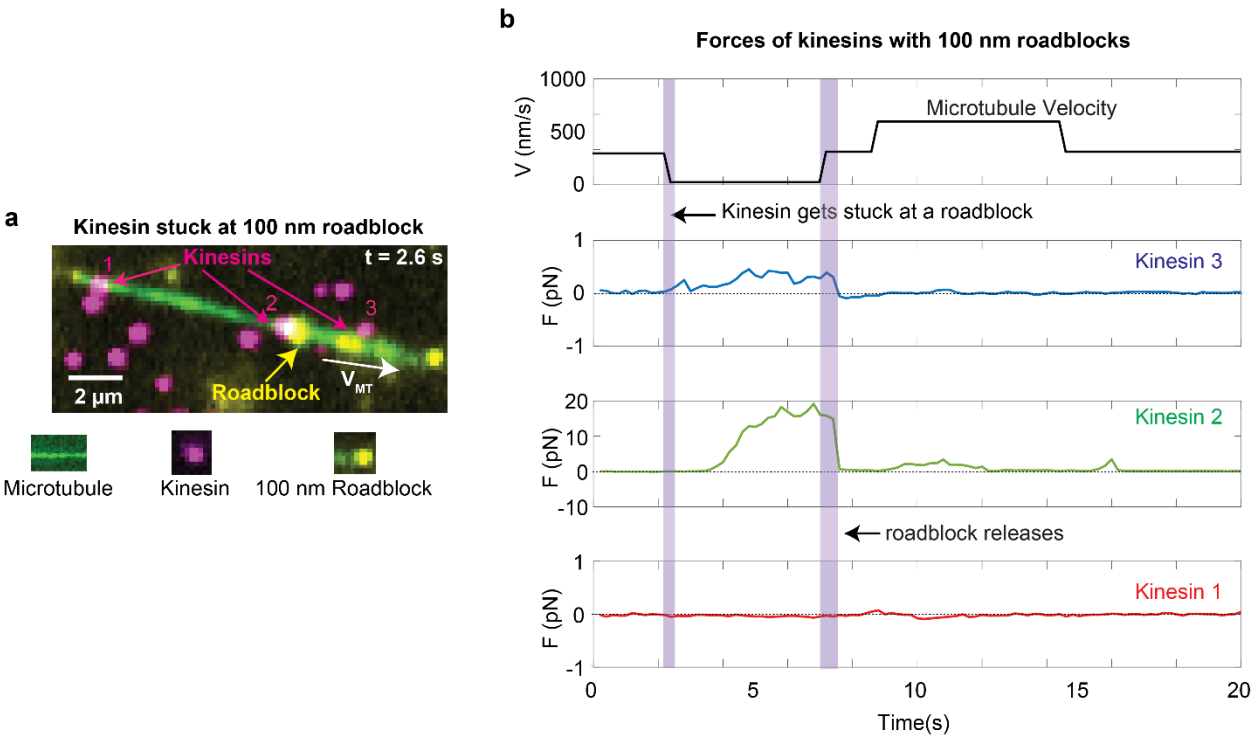


Figure S14. Kinesin forces in the presence of 100-nm roadblocks. (a) Microscope image where kinesins overcome a 100-nm roadblock. The microtubule (green) is driven by three kinesins (magenta). The roadblocks attached to the microtubules are yellow. Kinesin #2 becomes stuck at the roadblock. **(b)** Microtubule velocity (top) is plotted with kinesins' forces for the case shown in Fig. S14a. At t = 2.6 s, kinesin #2 becomes stuck at the roadblock, and the microtubule stops. Kinesin #2 and #3 increase their forces and overcome the roadblock at t = 7.5 s. Purple rectangles indicate the regions when the kinesin becomes stuck at the roadblock and are released. Kinesin #1 does not increase its force and does not play a role in overcoming the roadblock. It is one of the rare cases where forces in the system go beyond the stall force.

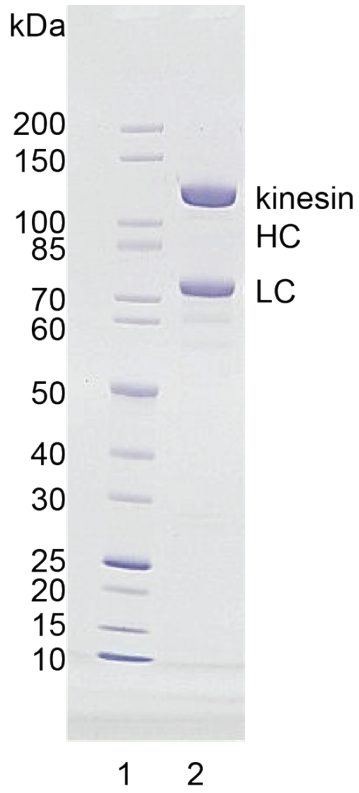


Figure S15: Representative gel of kinesin preparation. SDS-PAGE gel of (lane 1) molecular mass markers and (lane 2) a representative protein preparation of mouse kinesin-1 heavy chain 1-888 (112.2 kDa) with bound light chain (68.4 kDa). NuPAGE 4-12% Bis-Tris Gel (NP0321 Invitrogen) with NuPAGE MOPS SDS running buffer (NP0001 Invitrogen).

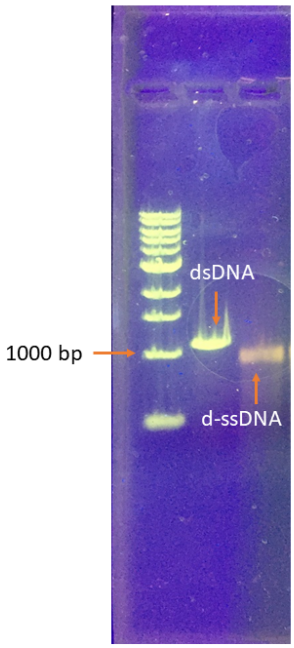


Figure S16. Glyoxal denatured ssDNA (d-ssDNA). The first lane is 1 kb dsDNA ladder. The second lane is control dsDNA (1080 bp). The third lane is d-ssDNA (1080 bp). The gel was stained with SYBR Gold dye.

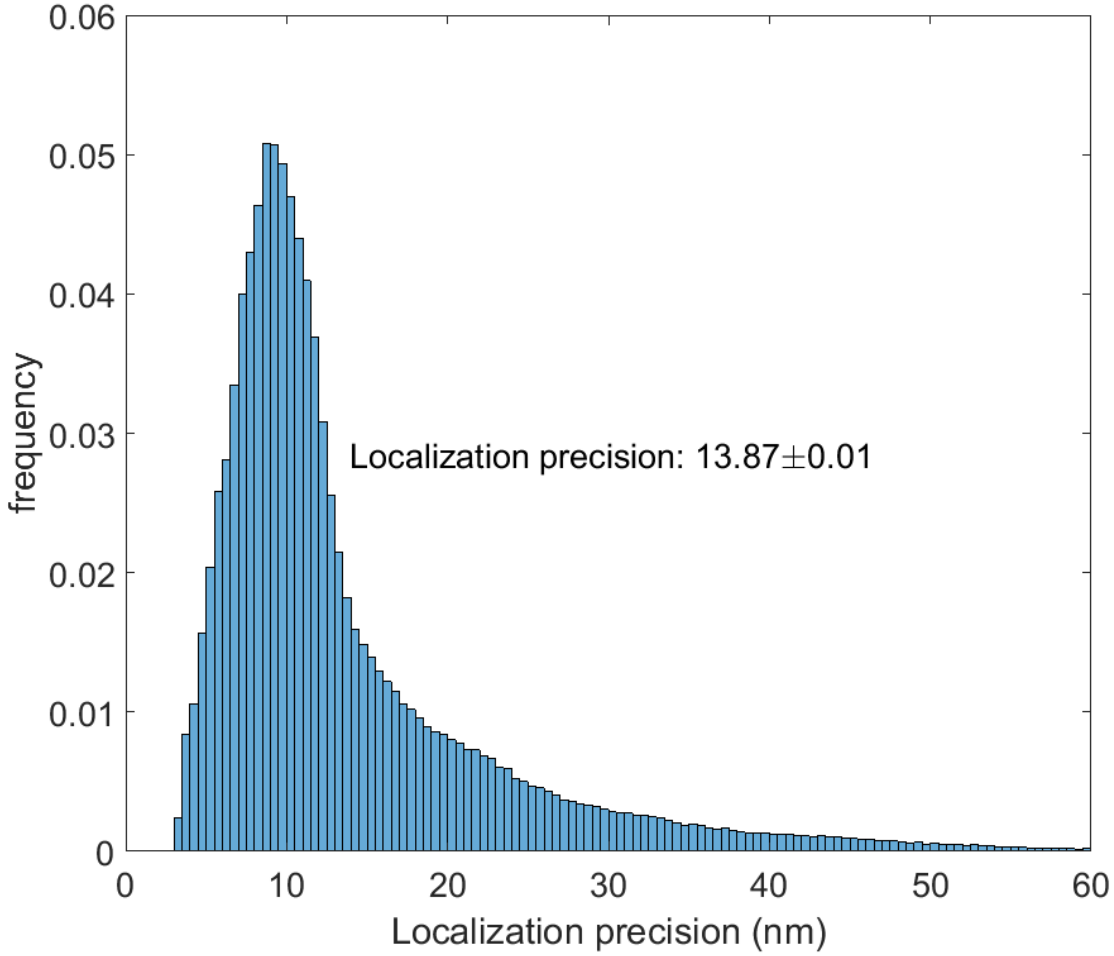


Figure S17. Localization precision of kinesin-quantum dot. The average localization precision of kinesin-QD is 14 nm in our acquired data for FSIM assay **(**number of molecules analyzed=466,464) (Calculated by Thunderstorm Plugin[6] of Fiji[7]).

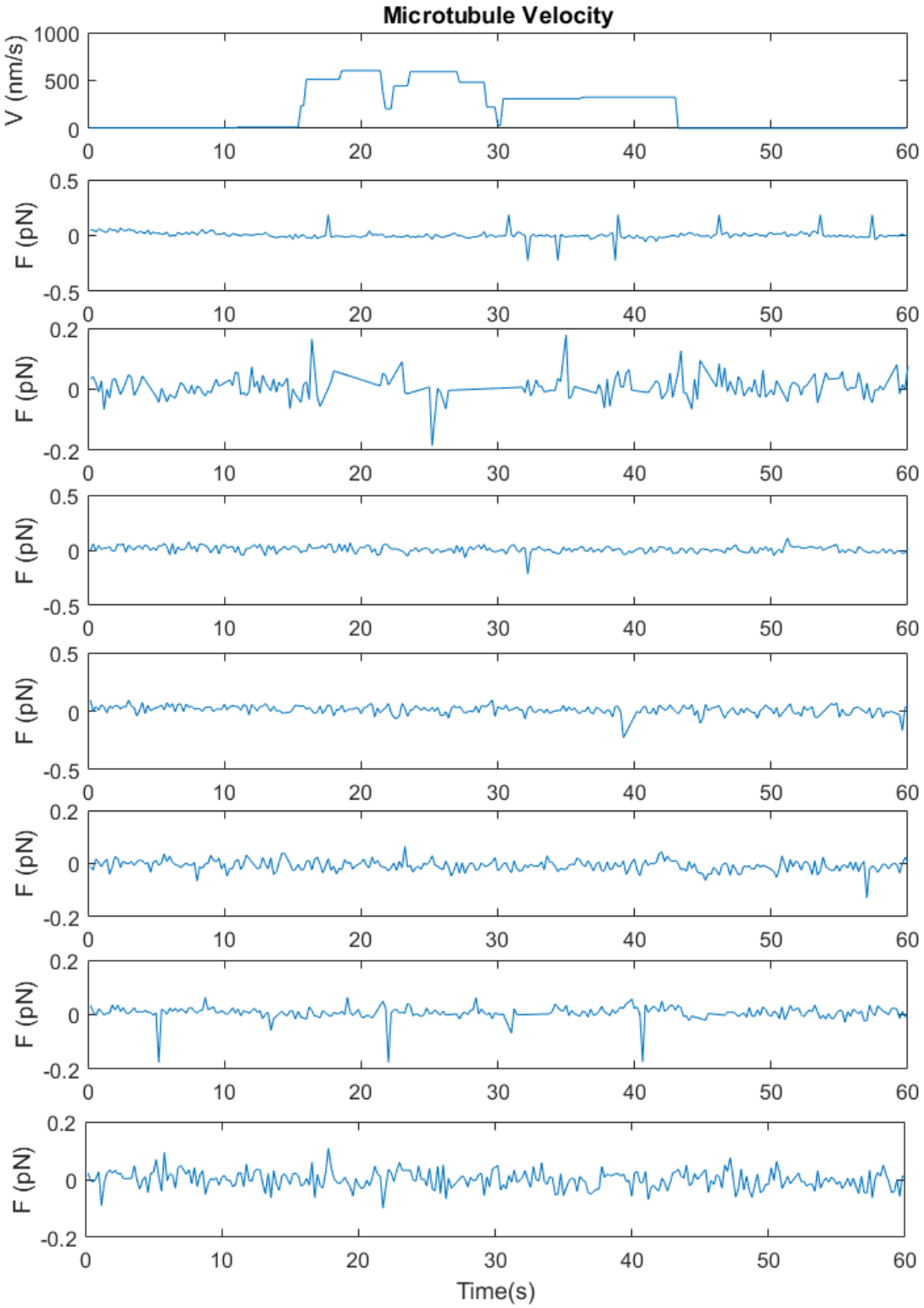


Figure S18. Seven kinesins drive a microtubule in the presence of roadblocks. In the presence of roadblocks, we could measure forces of up to seven kinesins simultaneously transporting a microtubule. Forces of individual kinesins mostly remains less than a pN.

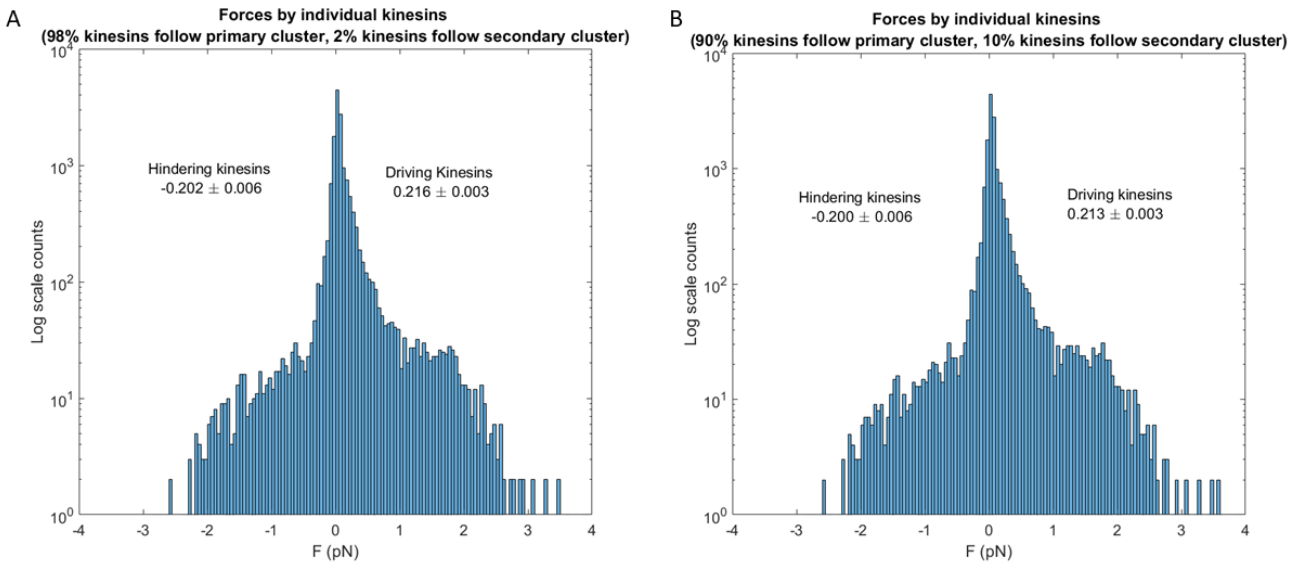


Figure S19: Average force characteristics of kinesins with the assumption that (A) 98% of the kinesin molecules follow the primary cluster and 2% of the kinesins follow secondary cluster force-extension characteristics. (B) 90% of the kinesin molecules follow the primary cluster and 10% of the kinesins follow secondary cluster force-extension characteristics. In both the cases, average forces of kinesins remain comparable to the force values in figure 3.

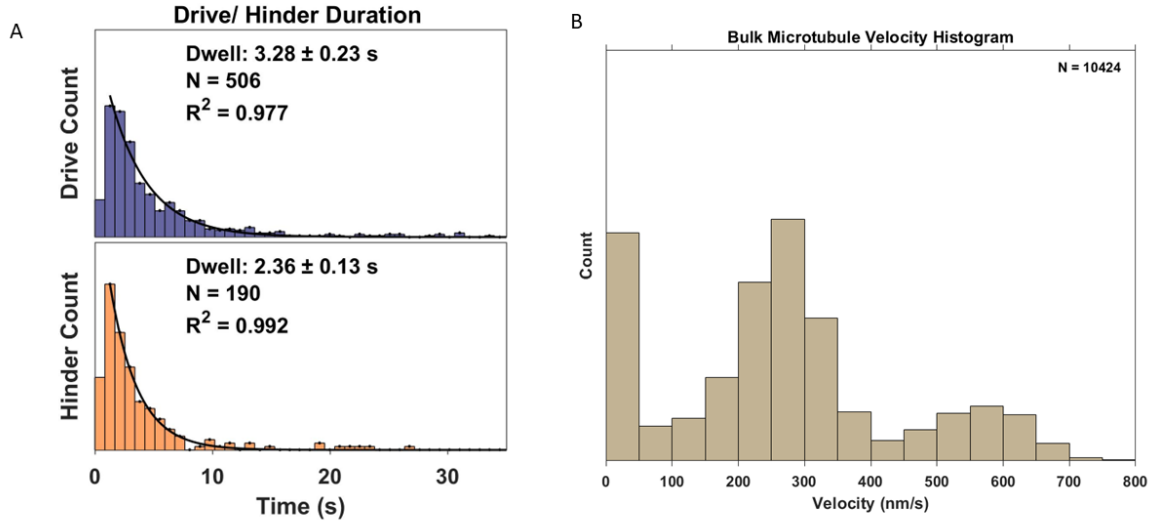
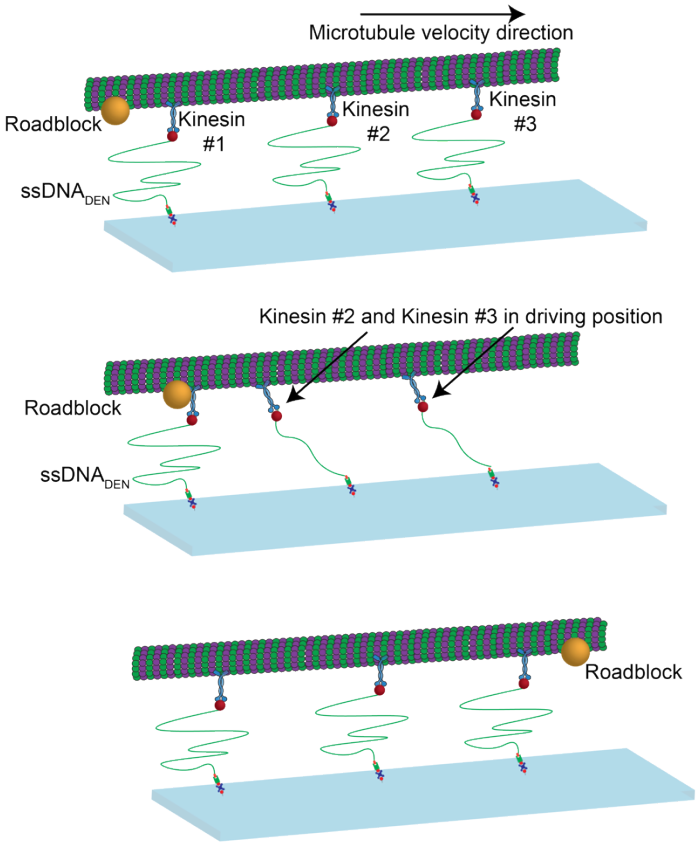


Figure S20: Effect if ssDNA on the kinesin duration and velocity. (A) Duration of driving and hindering kinesins in the FSIM assay with d-ssDNA. Driving kinesin's duration is 3.28±0.23 s and hindering kinesin's duration is 2.36±0.13 s. These durations are comparable to the kinesins duration when a dsDNA is used (as

shown in Tjioe *et al*., eLife, 2019). (B) Microtubule velocity in the case of FSIM assay (with d-ssDNA) is lower than when a dsDNA is used in the assay.

A. How kinesins overcome roadblocks

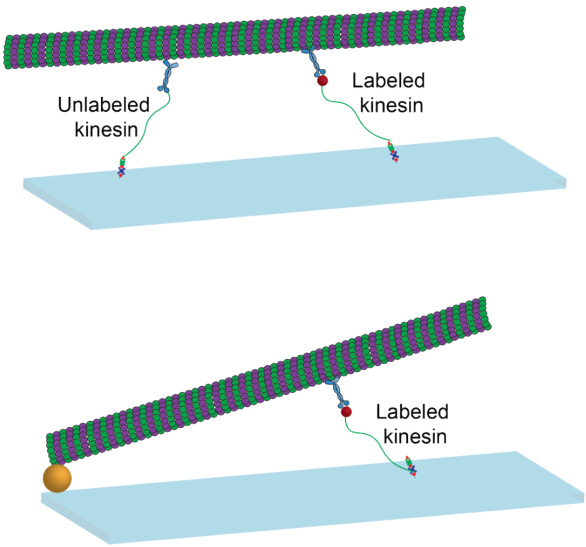


Before roadblock got stuck at kinesin #1

Roadblock got stuck at kinesin #1

After kinesins overcame the roadblocks

B. Potential sources of unbalanced forces



Occasionally, unlabeled kinesins may interfare with the measured forces.

Roadblocks or microtubules may also interact with the glass surface,

Figure S21: A. Cartoon of kinesin positions with respect to the roadblocks for the example in Fig. 4 of the main text. Initially all three kinesins are at their equilibrium position. Then the roadblock hits the kinesin #1. Kinesin #2 and kinesin #3 increase their forces by coming to the driving position and rescue the microtubule motion by overcoming the roadblock. B. Due to the presence of unlabeled kinesins or interaction of microtubule and roadblocks, opposing forces can be present during the microtubule transport that are not detected by the sensor. The schematic depicts the presence of such forces.

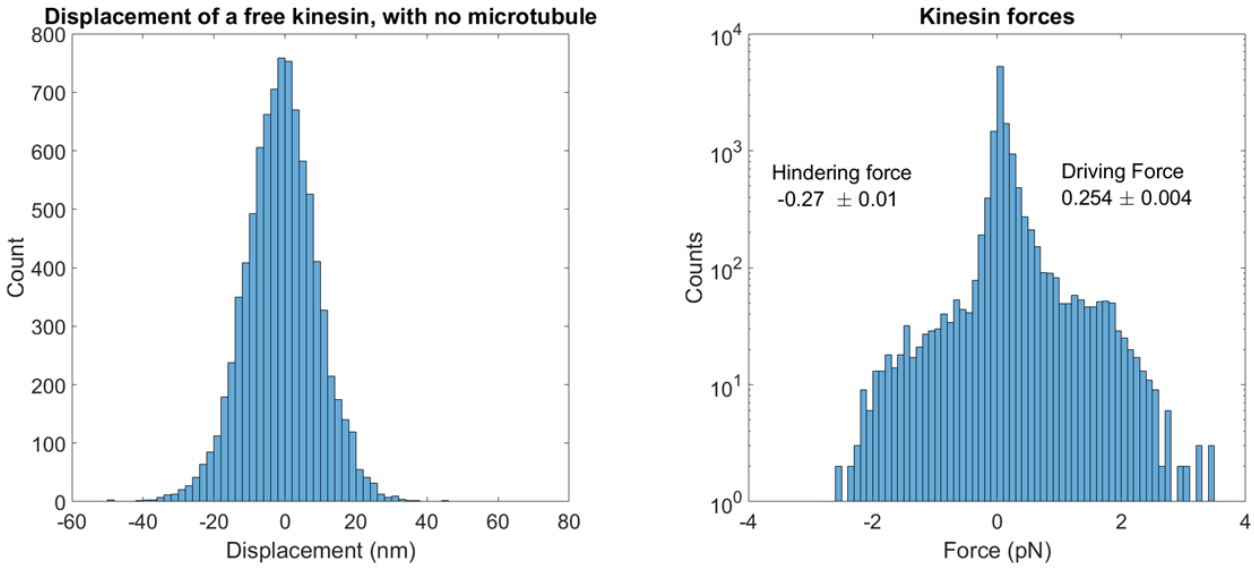


Figure S22: Force distribution of kinesins with displacement more than 20 nm. (A) displacement histogram of free kinesins when there is no microtubule is around. Displacement is primarily below 20 nm. (B) force histogram of kinesins that have displacement more then 20 nm. Average forces are slightly more than what we report in Fig. 3 because of elimination of low force datapoints.

Table S1

S. No.	Primer Name	
1	P1	5'-/5DigN/CAG TGC TGC AAT GAT ACC GC-3'
2	P2	5'-GTA CCG GCA TAA CCA AGC CT -3'
3	P3	5'-/5Phos/CCT GGT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTG AAC AGG CAG ACA TGA AAG A/3Bio/-3'

SI References:

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from the Microtubule Surface Measured by Fluorescence Interference Contrast Microscopy. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (43), 15812–15817. https://doi.org/10.1073/pnas.0510400103.

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