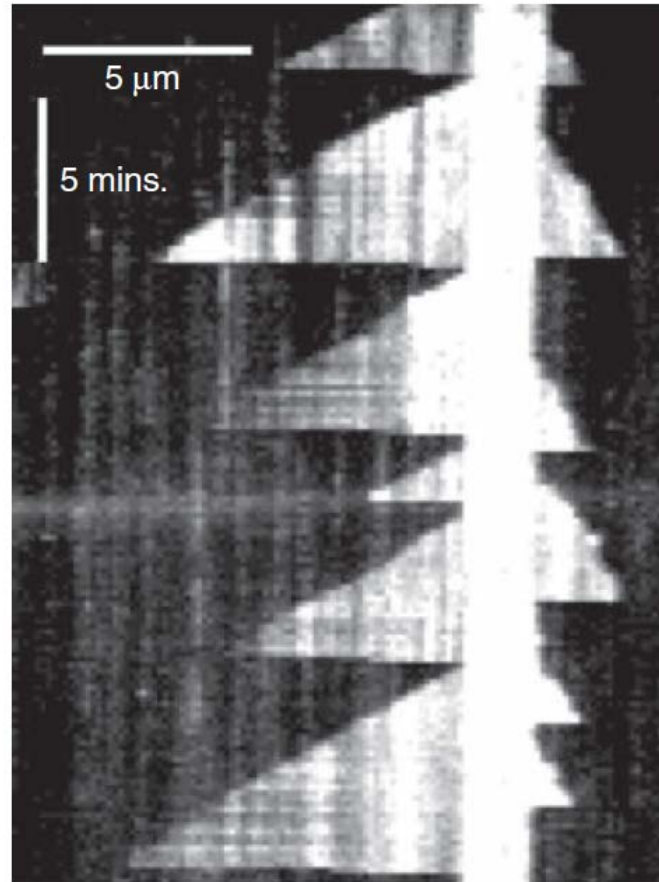


Putting theory into practice

Examples from real experiments

Microtubule dynamics



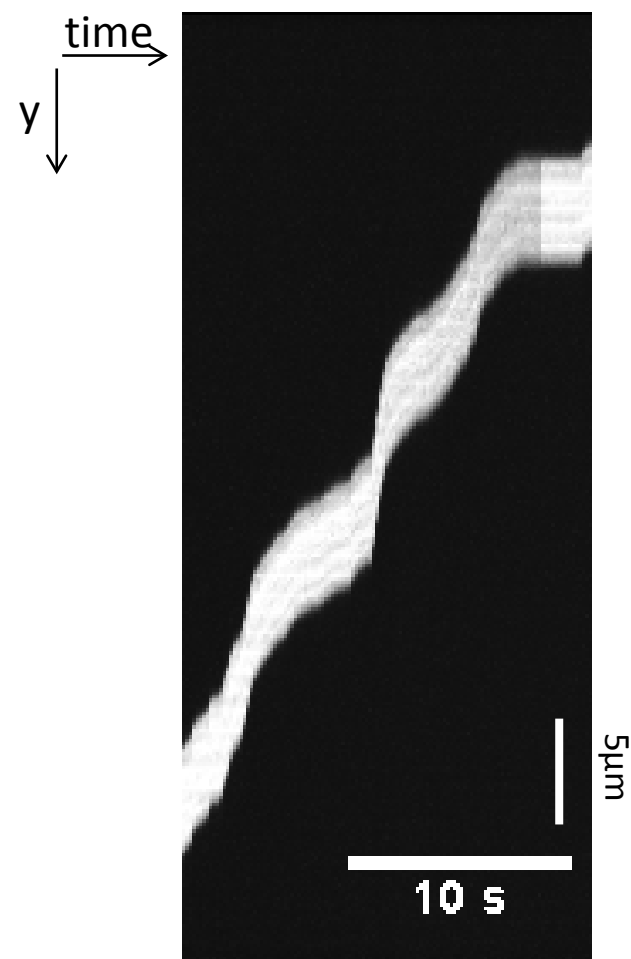
Gliding assay



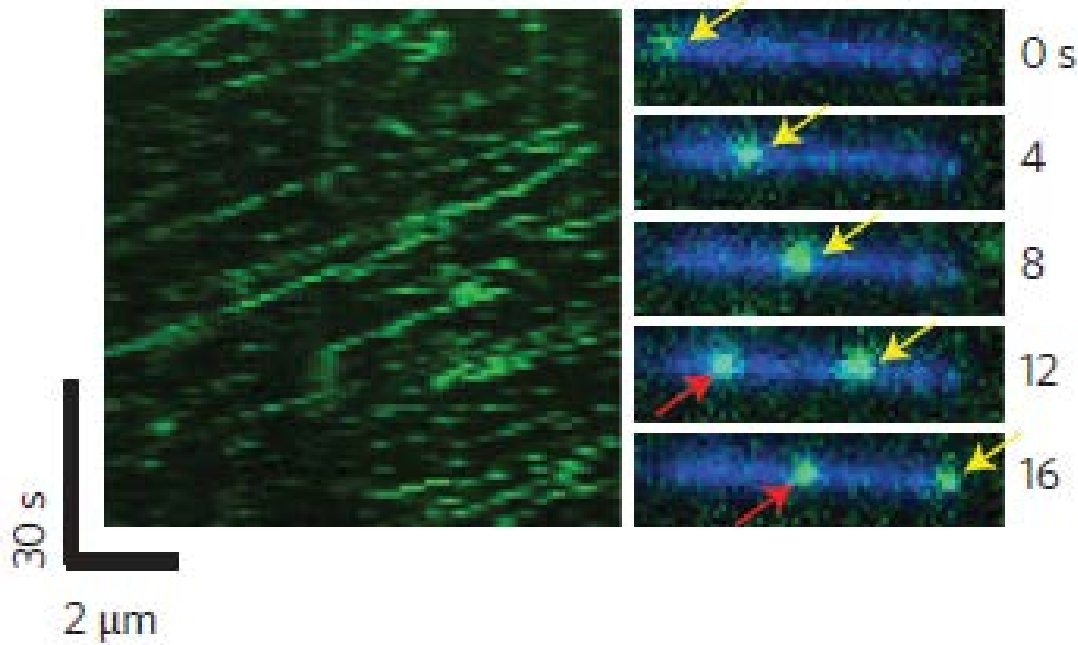
Maximum intensity projection



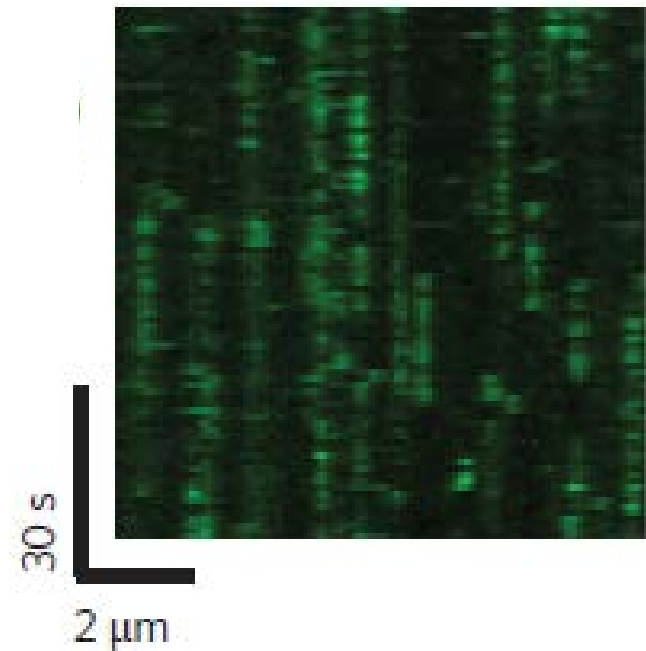
Kymograph

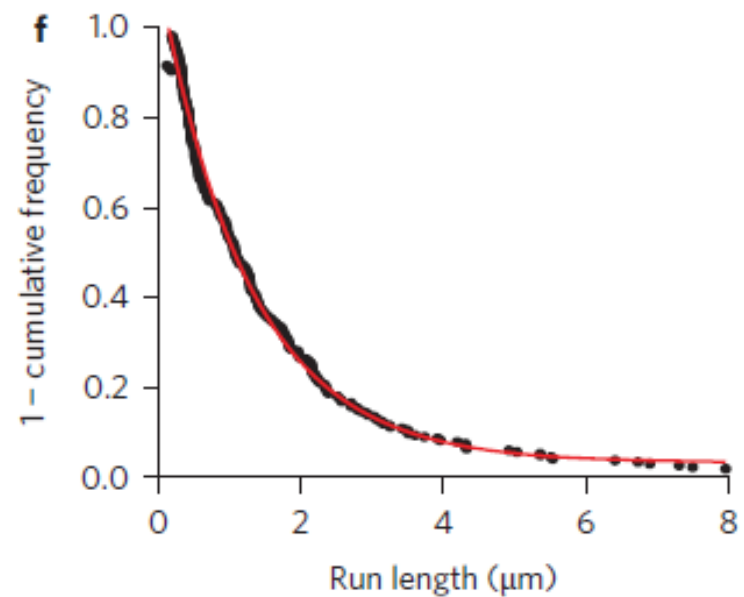
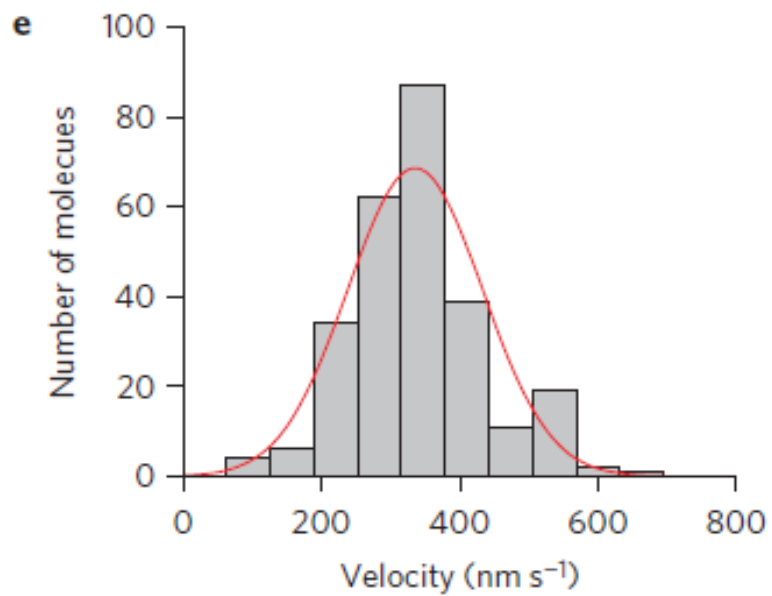


Stepping Assay



Kinesin 14 in plants, retrograde transport





Optical Tweezers

<http://www.umass.edu/musclebiophy/techniques%20-%20laser%20trap.html>

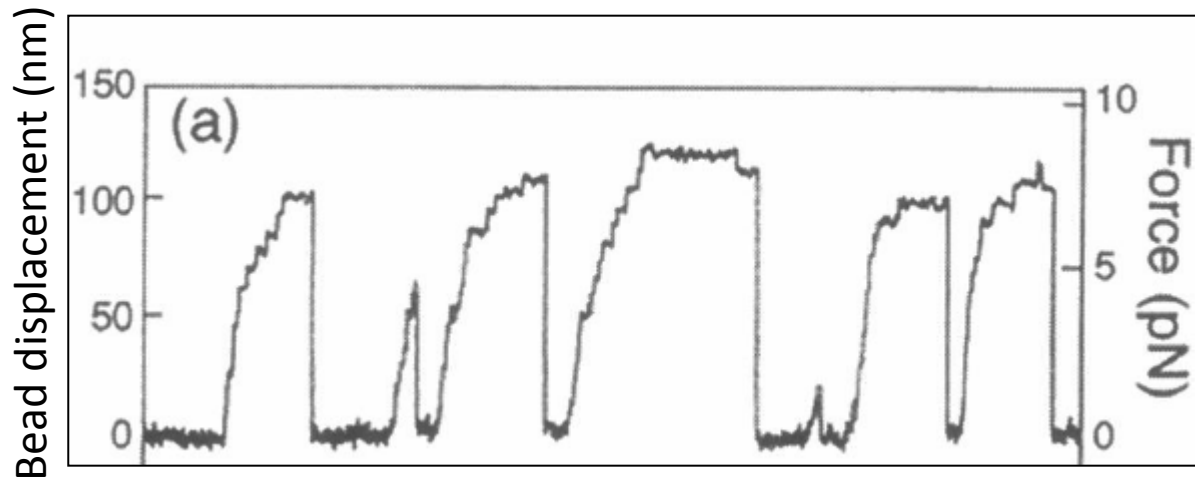
Mechanics of Single Kinesin Molecules Measured by Optical Trapping Nanometry

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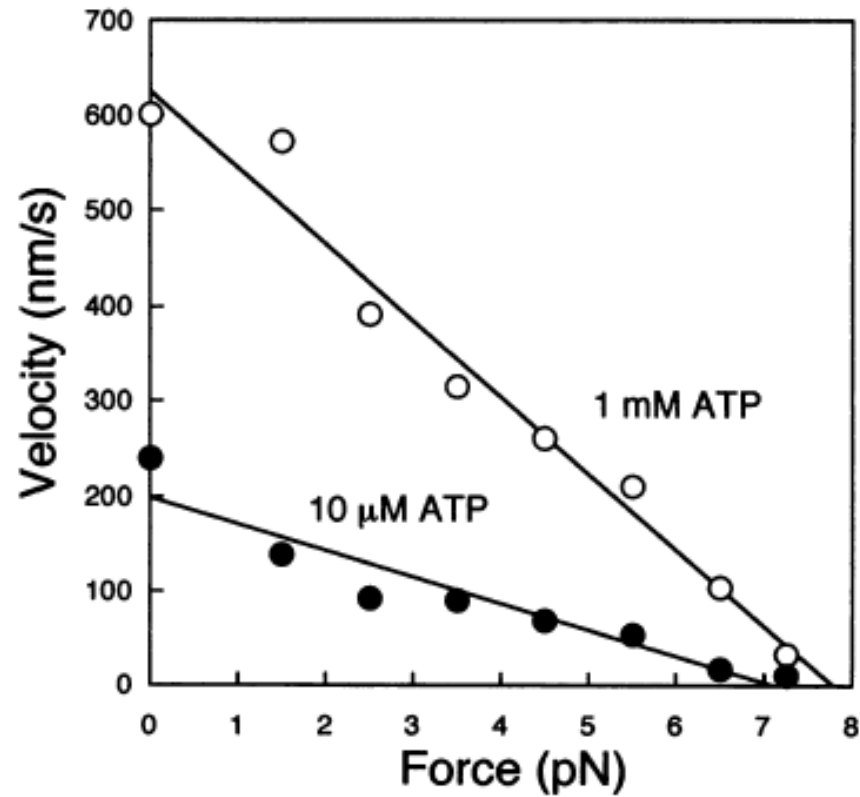
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ABSTRACT We have analyzed the mechanics of individual kinesin molecules by optical trapping nanometry. A kinesin molecule was adsorbed onto a latex bead, which was captured by an optical trap and brought into contact with an axoneme that was bound to a glass surface. The displacement of kinesin during force generation was determined by measuring the position of the beads with nanometer accuracy.

The force-velocity curves were linear from 0 to a maximum force at 10 μM and 1 mM ATP, and the maximum force was ~ 7 pN, which is larger by $\sim 30\%$ than values previously reported. Kinesin exhibited forward and occasionally backward stepwise displacements with a size of ~ 8 nm. The histograms of step dwell time show a monotonic decrease with time. Model calculations indicate that each kinesin head steps by 16-nm, whereas kinesin molecule steps by 8-nm.



Force-velocity relation



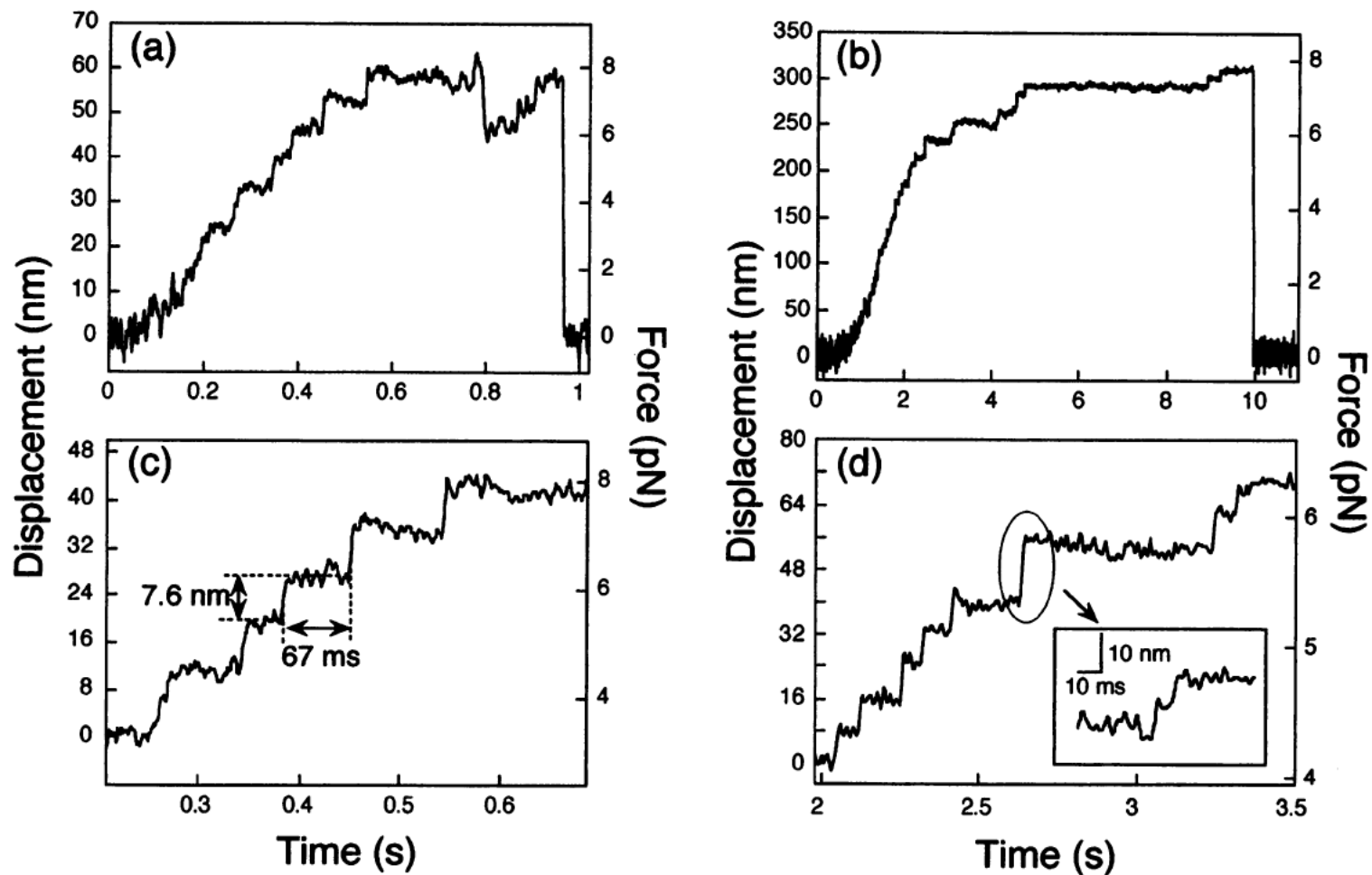
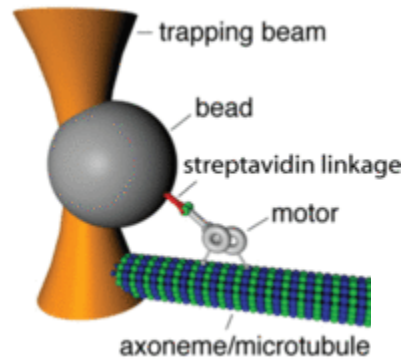


FIGURE 7 Stepwise movements of beads at 1 mM ATP (a) and 10 μ M ATP (b). In c and d, displacements that express kinesin movement are corrected from a and b, respectively, by using the attenuation factor as shown in Fig. 5 e. The force was calculated from the trap stiffness and bead displacement. Trap stiffness: (a) 0.13 pN/nm; (b) 0.025 pN/nm. Filters used in the displacement: (a and c) 100-Hz low-pass filter; (b and d) 50-Hz low-pass filter. (d, inset) 250-Hz low-pass filter. The step size, shown in figures, was measured from the differences in the averages of the displacements between the steps. The time in c indicates the dwell time. Dwell time was measured between steps when displacements were half of the unitary steps.

Dynein stall forces



Dynein Stall Data, Stiffness = 24.841 pN/um, Sensitivity = 1.786 V/um

