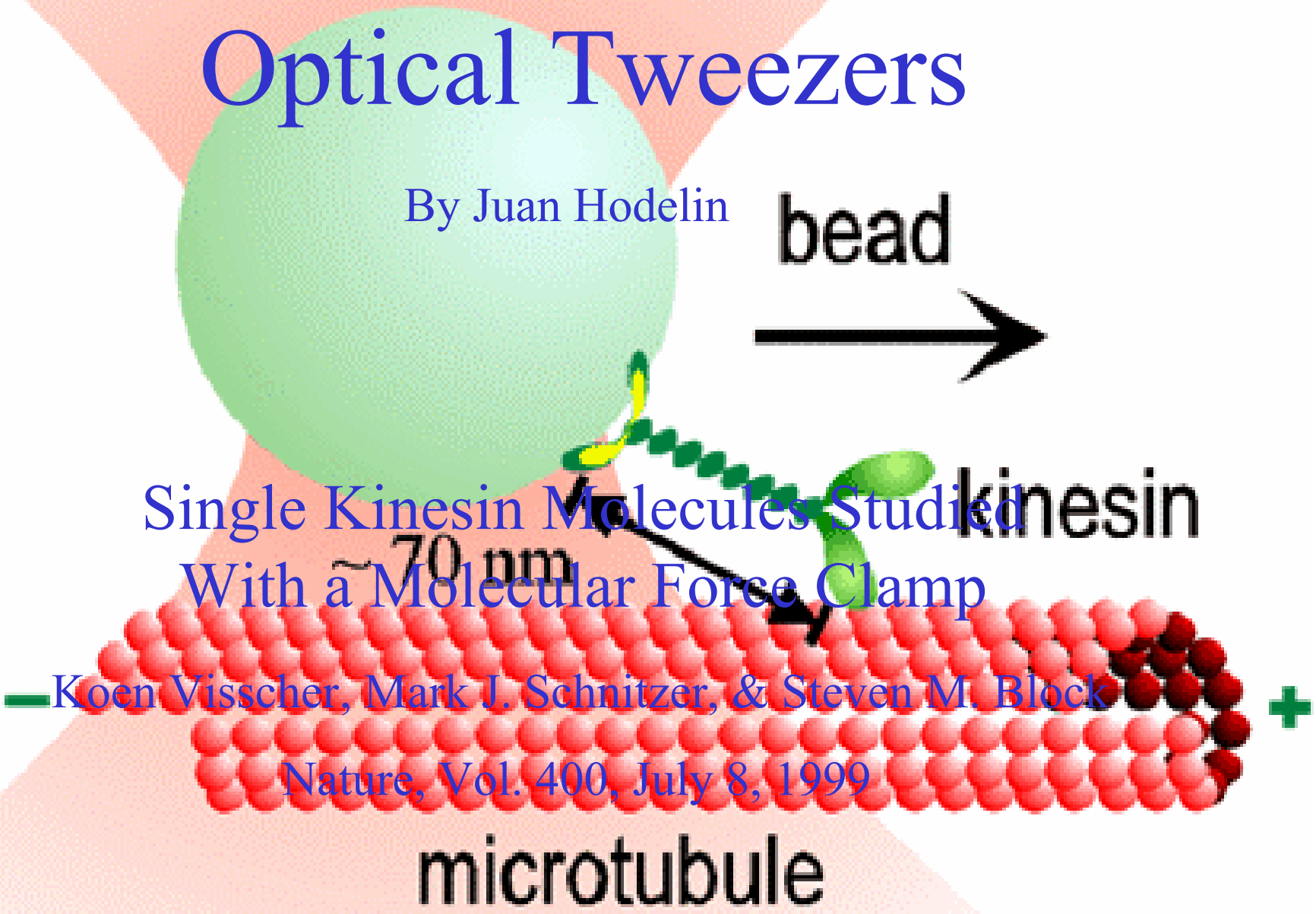
Optical Tweezers

By Juan Hodelin



Single Kinesin Molecules Studied With a Molecular Force Clamp

—Koen Visscher, Mark J. Schnitzer, & Steven M. Block

Nature, Vol. 400, July 8, 1999

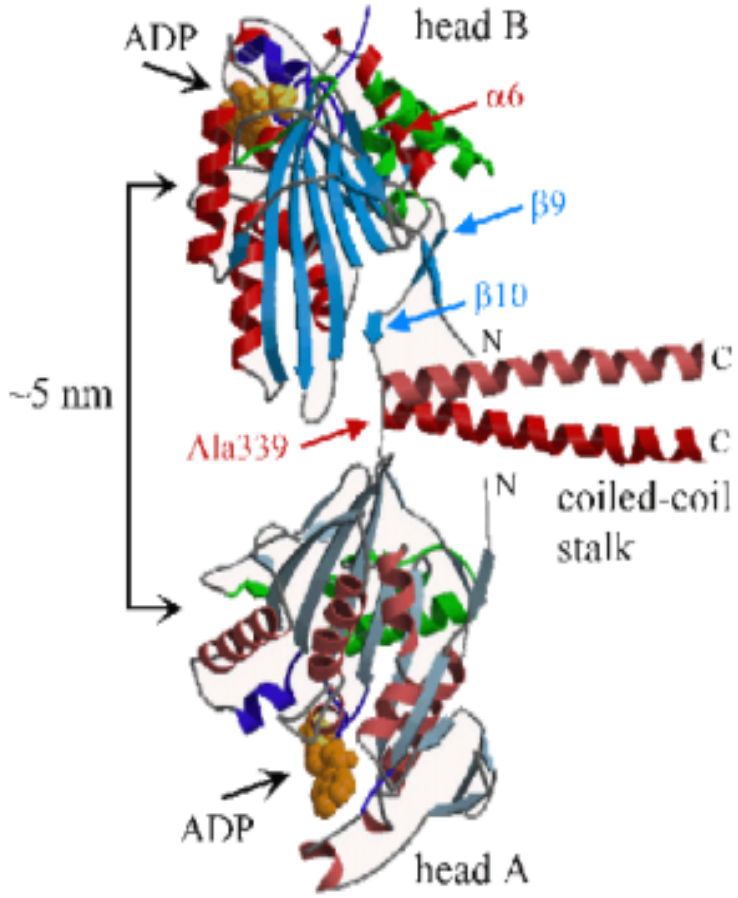
What do they study?

Kinesin is a two-headed, ATP-driven motor protein that moves processively along microtubules in discrete steps of 8 nm, probably by advancing each of its heads alternately in sequence. Molecular details of how the chemical energy stored in ATP is coupled to mechanical displacement remain obscure. To shed light on this question, a force clamp was constructed, based on a feedback-driven optical trap capable of maintaining constant loads on single kinesin motors. The instrument provides unprecedented resolution of molecular motion and permits mechanochemical studies under controlled external loads. Analysis of records of kinesin motion under variable ATP

First, kinesin stepping appears to be tightly coupled to ATP hydrolysis over a wide range of forces, with a single hydrolysis per 8-nm mechanical advance. Second, the kinesin stall force depends on the ATP concentration. Third, increased loads reduce the maximum velocity as expected, but also raise the apparent Michaelis-Menten constant. The kinesin cycle therefore contains at least one load dependent transition affecting the rate at which ATP molecules bind and subsequently commit to hydrolysis. It is likely that at least one other load-dependent rate exists, affecting turnover number. Together, these findings will necessitate

Step by Step

- Kinesin: “one of world’s tiniest motors” consists of ~800 amino acids
- Moves processively in 8-nm steps along microtubules
- Essential for vesicle transport in neurons, and chromosome movement in dividing cells

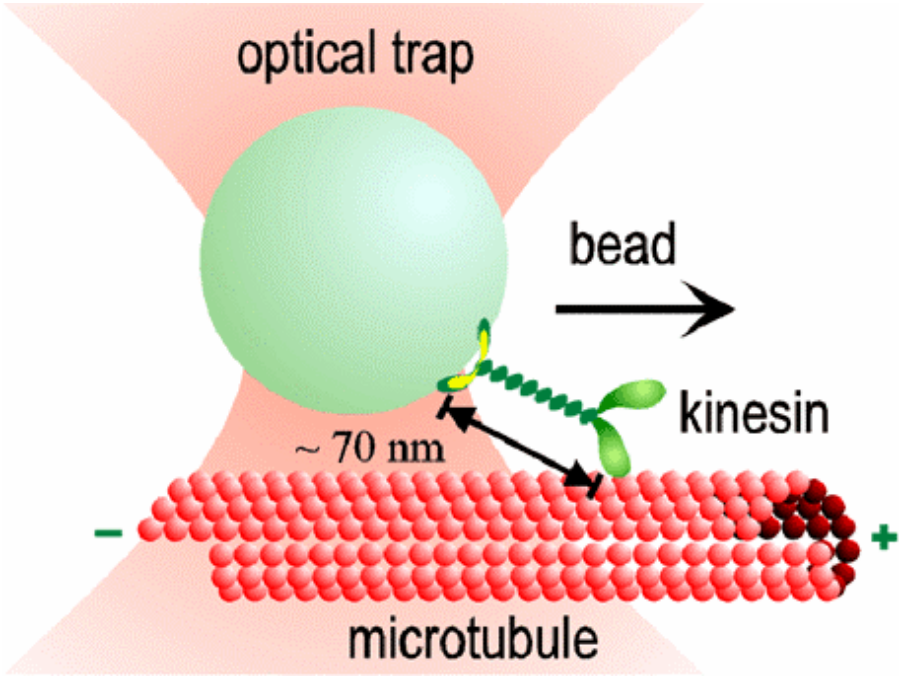


Crystal Structure of Dimeric Kinesin, (Adapted from Kozielski et al., Cell 1997)

http://www.stanford.edu/group/blocklab/ResearchMain.htm#kinesin

How do they study it?

- Adsorb silica bead to kinesin
- Trap the bead with feedback sensitive optical trap to provide constant force or position to kinesin
- Measure the position or force with this apparatus while varying [ATP]



Bead-Trap Schematic: Not to scale

http://www.stanford.edu/group/blocklab/ResearchMain.htm#kinesin

Optical Traps: Quick Facts

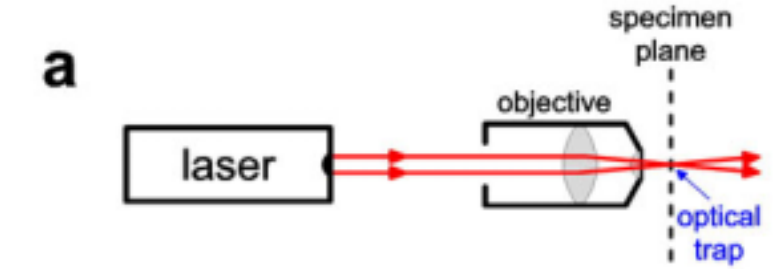
- Optical Force Clamp aka "optical tweezers"
- Traps can manipulate objects as small as a single atom using momentum of light (radiation pressure)
- Forces: 1 pN
- Distances: 1 nm
- Object Size: 10nm -100μm

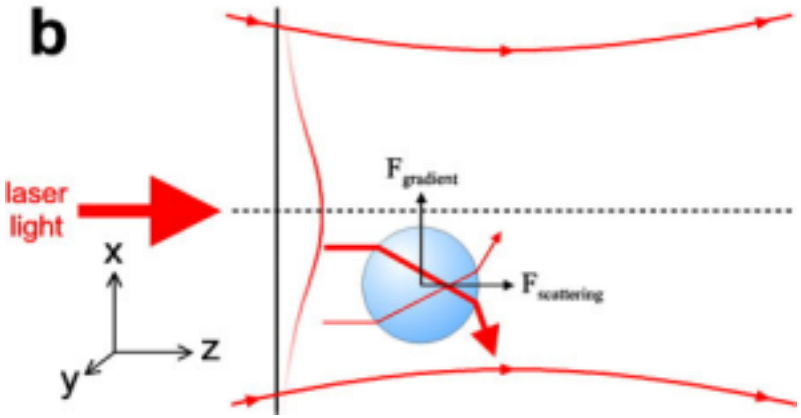
History:

- 1972- Askin: gradient force trapping
- ~1986 – Ashkin & Dziedzic: first biological application (bacteria, protozoa)
- 1992- Steven Chu: elastic props of DNA

The Physics

- Focus laser light to diffraction limited spot with objective (to max gradient force)
- $p = hk/2\pi \Rightarrow \Delta k$
- $F_{gradient}$ toward largest intensity (~ grad(I))
- $F_{scattering}$ in direction of incident ray (~I)
- Traps when $F_{grad} > F_{scat}$

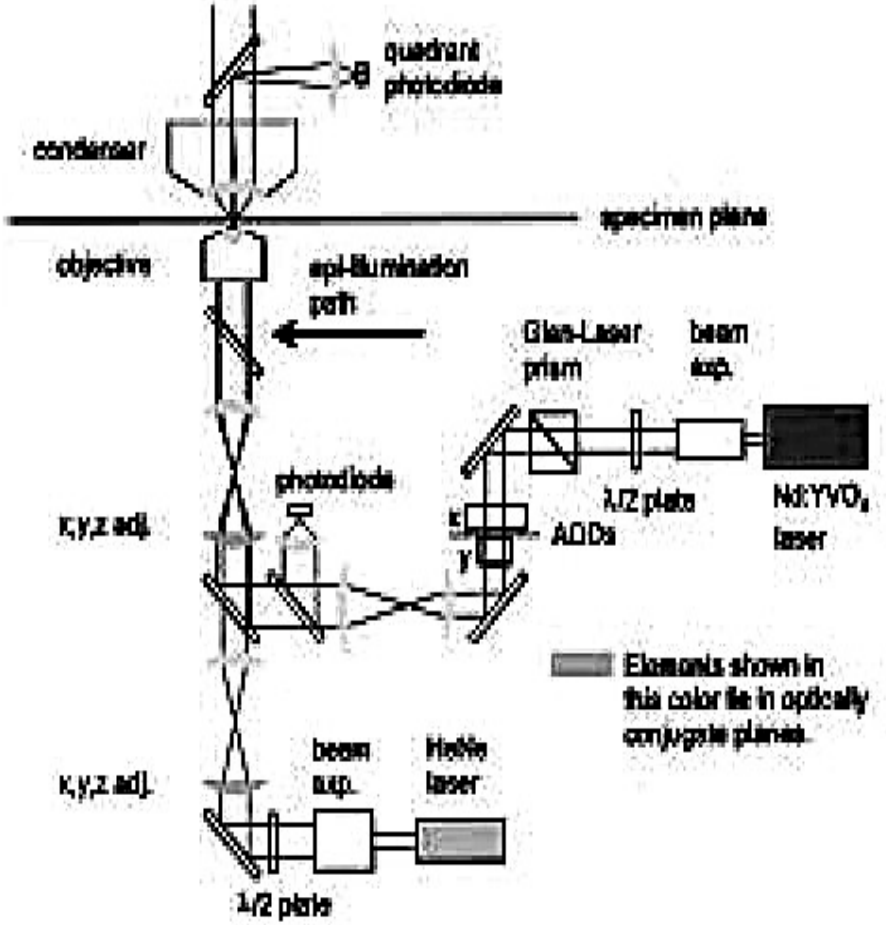




Particle pushed toward center of Gaussian profile and beam waist

Optical Set-up

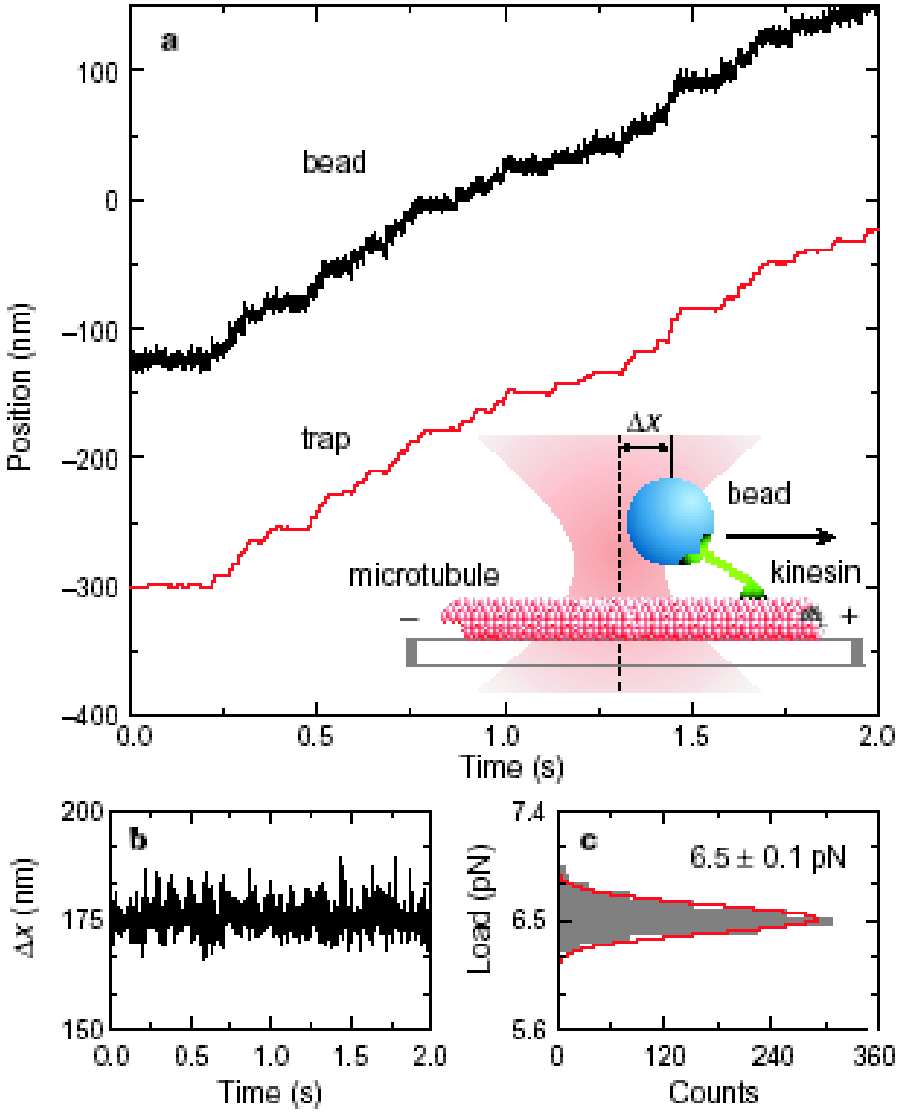
- "Tweezer" beam (Nd:$YV0_4$) and "detector" beam (HeNe) coincident
- Quadrant photodiode detects scattered HeNe light
- Digitally connected to acousto-optical deflectors (AODs) that vary tweezer position
- Creates "force clamp" by moving trap along with bead



From: *Construction of Multiple-Beam Optical Traps with Nanometer-Resolution Position Sensing* ,Koen Visscher, Steven P. Gross, and Steven M. Block

Displacement = Force

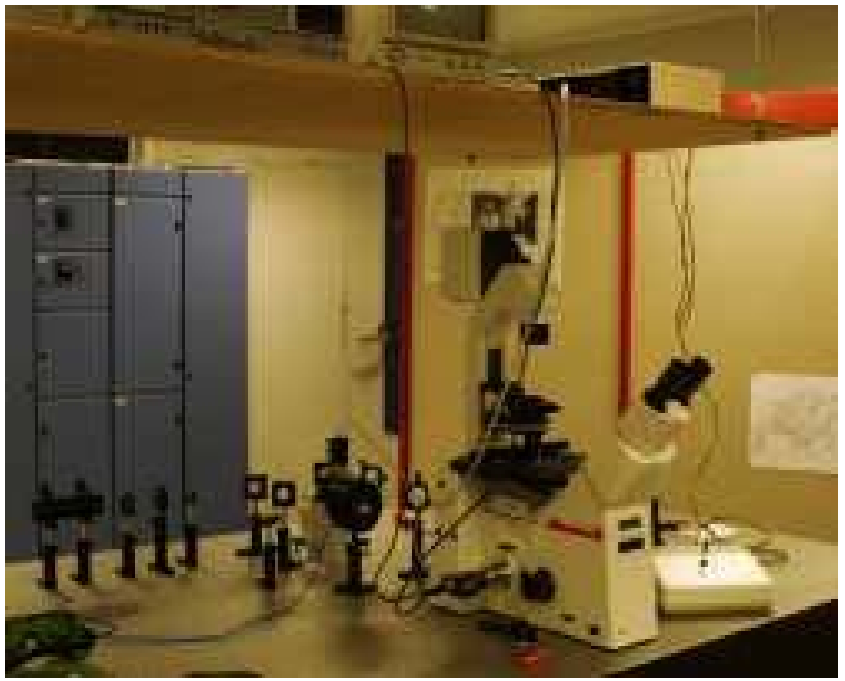
- The kinesin moves along an immobilized microtubule while the trap follows
- Displacement between the center of the trap and the bead was maintained at 175 nm
- This can be converted to a force by multiplying by the trap stiffness (0.037 pN/nm) as in **c.**



Why do they do it this way?

- Previously studied in solution ATPase where no external loads are possible
- Single-molecule flourescence difficult: low ATP affinity
- Low [ATP] does not produce motion
- Fluctuations in position of single molecules requires application of constant force: previously problematic

Now they are able to apply load to fractions of a pN while moving over 200 nm



http://www.nbi.dk/~tweezer/

Experimental Considerations

- Bead attachment
- Assays
- Calculation/Calibration of Trap stiffness
- Calibration of Feedback control mechanism
- Isolation of system from external fluctuations
- Possibility of backward steps

Velocity and Reaction Rates

- **For negligible loads**:

$$v = \frac{V_{max}[ATP]}{[ATP] + K_m}$$

v = velocity

V_{max} = max velocity

K_m = Michaelis-Menten constant

- Expect V_{max} to fall for increasing loads, but *how K_m will change?*

- $V_{max} = \varepsilon(F)\, d\, k_{cat}$

$\varepsilon(F)$ = the coupling efficiency between ATP hydrolysis and mechanical stepping

k_{cat} = max rate of hydrolysis

In practice coupling has been shown to be independent of [ATP] and 1:1 for negligible loads, indicating one hydrolysis per 8 nm step

Loose vs.Tight Coupling

Loose Coupling Model:

- ε depends on F but not [ATP]
- k_{cat} independent of F
- $K_m = k_{cat}/k_b$

where k_b, the rate of ATP binding, is also indep. of load

=> *K_m should be independent of force*

Tight Coupling:

- Predicts a single mechanically sensitive rate that affects k_{cat}, but leaves ε and k_b unchanged

=> *decreasing k_{cat} causes K_M to decrease also with load*

Kinesin Velocity Curves

Observing the average velocities at separate [ATP] and loads revealed:

- V_{max} decreased with increased load as expected
- K_m also increased with increased load
- Both of these together imply a decrease in the binding rate, k_b

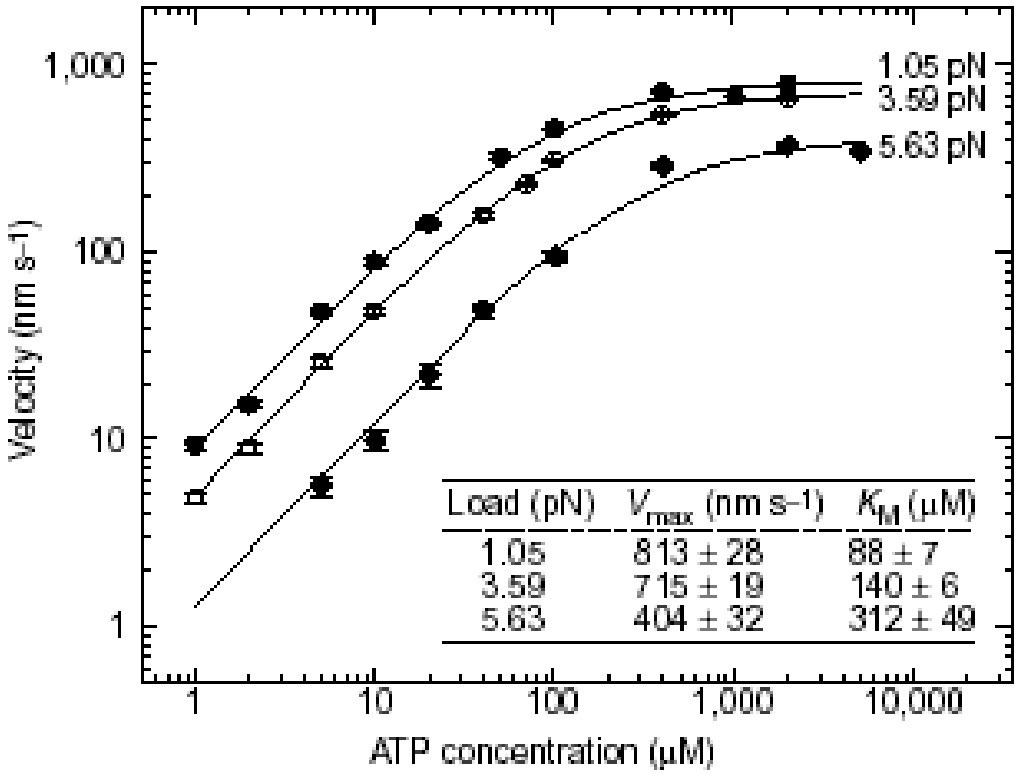
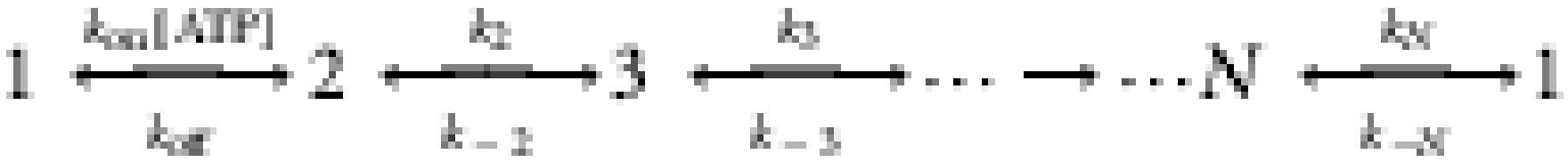


Figure 2 Michaelis–Menten kinetics under load. Double logarithmic plot of the average bead velocity, v (mean ± s.e.m.), versus ATP concentration for various loads (filled circles, 1.05 ± 0.01 pN, N = 11–102 runs; open circles, 3.59 ± 0.03 pN, N = 8–79 runs; diamonds, 5.63 ± 0.06 pN, N = 19–58 runs). Data were fitted to Michaelis–Menten curves (lines), $v = V_{max}[ATP]/([ATP] + K_m)$. Inset, fit parameters, V_{max} and K_m.

3 Possible Mechanisms for decreasing k_b



For a general N intermediate ATPase pathway:

- Second order binding rate, k_{on}, decreases
- Increase in ATP unbinding rate, k_{off}
- Reduction in a general forward rate, k_2, where "2" is generally any state before hydrolysis and any irreversible processes

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They favor: *two, independent mechanically sensitive rates, one of which affects ATP binding through* k_{on} *and/or* k_{off}.

Stall Force Measurements (To further explore the hypothesized 2 mechanochemical limiting rates)

"earlier studies lacked the improved resolution afforded by the force clamp, and [subtle differences] were not distinguished within experimental error"

- Stall force: Hold the trap in position and see where the bead gets relative to the trap center before it stalls for >2s
- Force-velocity curves show a lower stall force for lower [ATP]
- *They conclude that load lowers k_b* => at limiting [ATP] $k_b << k_{cat}$ (stall force becomes ind. of k_{cat}) but at higher [ATP] it depends on both

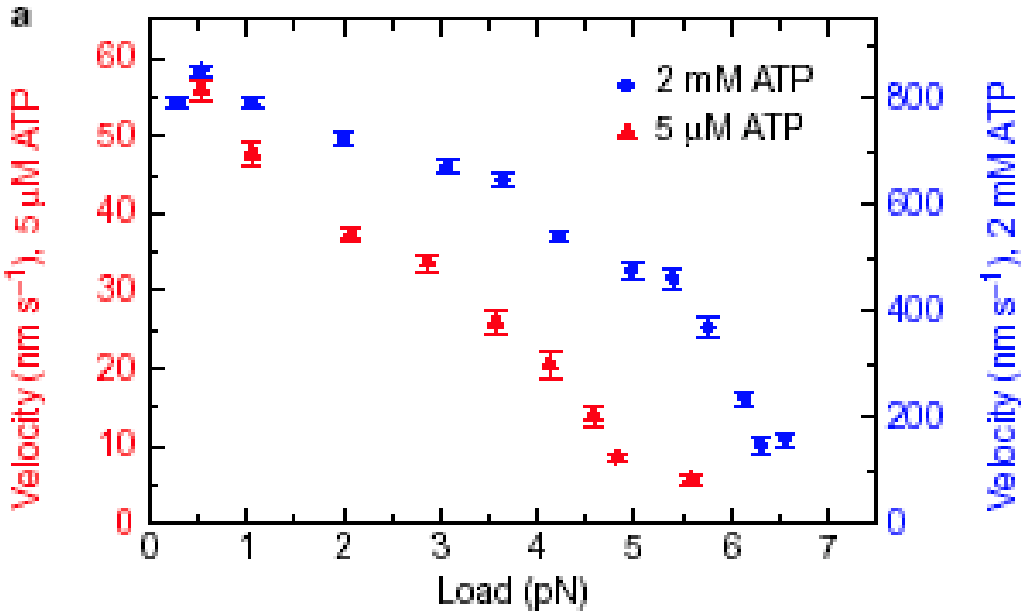


Figure 3a: Force – Velocity Curves

To test further…

- Measured stall force as a function of [ATP]
- Used both force and position clamps
- Bead detachment caused "experimental scatter"
- Also shows increased stall force with concentration, consistent with above results

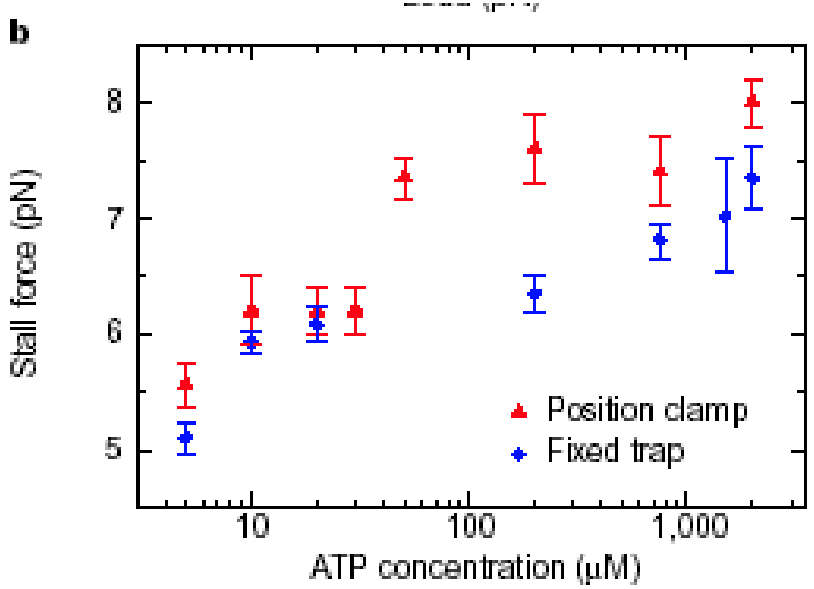


Figure 3 Load dependence of motility. **a**, Average bead velocity, *v* (mean ± s.e.m.), versus applied load for fixed ATP concentrations (red triangles, left axis, 5 μM ATP, $N = 19$–57; blue circles, right axis, 2 mM ATP, $N = 37$–87). The velocity point at (5.6 pN, 5 μM ATP) is likely to represent an overestimate because beads which stalled completely ($v = 0$) were indistinguishable from beads lacking active motors, and so were not included in the analysis. **b**, Stall force (mean ± s.e.m.) versus ATP concentration, measured either with the position clamp[TM] (red triangles) or with a fixed optical trap (blue circles). Stalls had to last a minimum of 2 s to be included in the analysis. Data points represent an average of either 12–29 (position clamp) or 6–70 (fixed trap) stalls.

Randomness parameter

1) Tight coupling: “no futile hydrolysis events that fail to generate movement, even under load”
2) Mix of tight and loose elements with both load dependent transitions and unproductive hydrolyses
3) Loose coupling with increased frequency of backward steps with load—discounted b/c few backwards steps small (~5-10%)

$$r = \lim_{t \to \infty} \frac{\langle x^2(t) \rangle - \langle x(t) \rangle^2}{d\langle x(t) \rangle}$$

- r = randomness parameter, measures temporal irregularities of mechanical advances
- Clocklike motor: r =0
- Poisson motor with 1 biochemical transition: r=1
- r-1 provides a measure of rate-limiting transitions in the overall cycle

Determination of Randomness, r

- Low [ATP]: r~1 so, 1:1 coupling in this regime
- Saturating [ATP] for small loads: r~1/2, corresponding to 2 ATP-indep. rate limiting reactions per step
- Change governed by drop of [ATP] through K_m
- Few pts. at 5.69 pN due to loss of processivity at low [ATP]

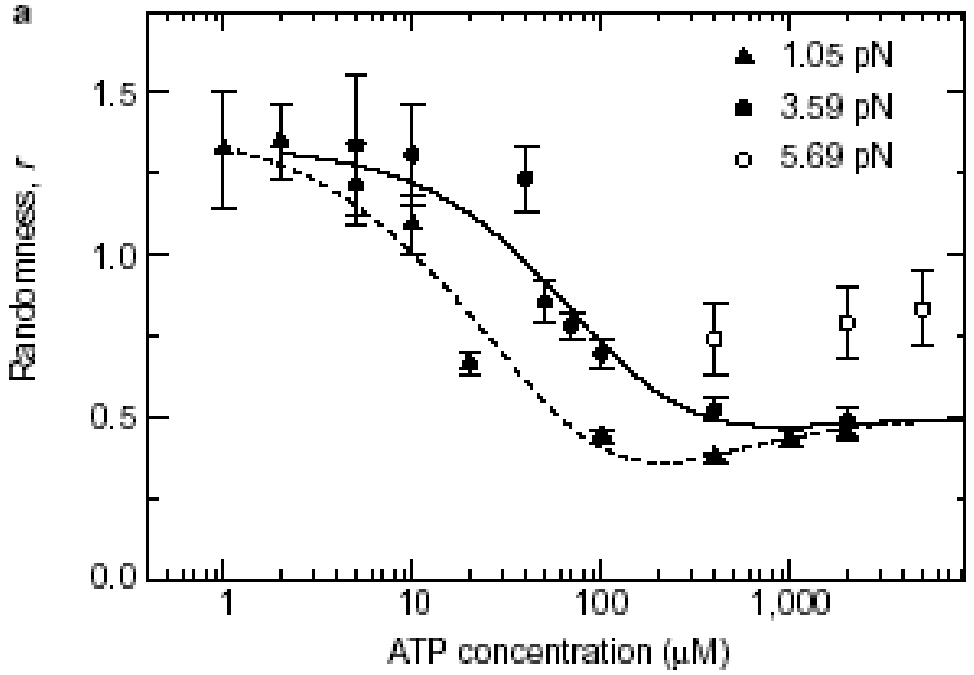


Figure 4a: Randomness vs. [ATP]

What happens to randomness with increased load?

- Tight coupling: by definition r should be invariant
- Loose Coupling: r should increase with load, and this will be even sharper for increased back steps
- At extreme loads the two scenarios become identical, since even in the tightly coupled scheme one of the reactions will become rate limiting (compared to the other)

Randomness vs. Load at Saturating [ATP]

- Results showed constant r ~1/2 until extreme loads caused an increase

⇒ *Indicative of tight coupling*

- Theoretical considerations of the number of backward steps indicate "the fraction of futile hydrolyses must be < 10-15% of all events"

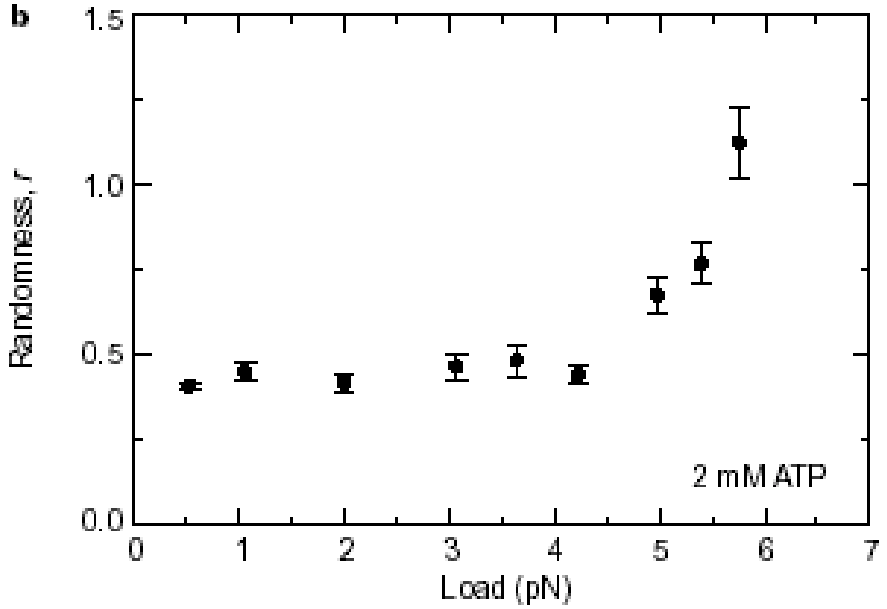


Figure 4 Displacement fluctuations. **a**, Semi-logarithmic plot of the randomness parameter, r (mean ± s.e.m.), versus ATP concentration for fixed loads (filled triangles, 1.06 pN ± 0.01, $N = 11$–102; filled circles, 3.59 ± 0.03 pN, $N = 30$–79; open circles, 5.69 ± 0.03 pN). The rise in r from $\sim\frac{1}{2}$ to ~ 1 begins at a higher ATP level for 3.59 pN than for 1.06 pN, reflecting the higher K_m at the former load. To guide the eye, data are compared to two-parameter analytical fits (dashed line, 1.06 pN; solid line, 3.59 pN). These fits were performed using the Michaelis–Menten parameters from Fig. 2, assuming two rate-limiting transitions at saturating ATP and one ATP hydrolysis per 8-nm step. Backward steps were assumed to occur in an ATP-dependent manner (see Methods). **b**, The randomness parameter, r (mean ± s.e.m.), versus load at 2 mM ATP (circles). Data points represent an average of 43–87 runs.

Implications

"The finding of tight coupling between ATP hydrolysis and mechanical stepping would seem to rule out many current theoretical models for force generation by kinesin"

- Many other models had proposed loose coupling
- Thermal ratchets ruled out since coupling would depend on load
- Models with only one force dependent transition

Conclusions

- Kinesin stepping appears to be tightly coupled to ATP hydrolysis under many loads
- Stall force depends on ATP concentration
- Under increased loads not only does the maximum velocity fall, but also the Michaelis-Menten constant
- 2 load-dependent rates affect the total rate, one of which is the ATP binding rate, k_b

Future Directions

Had previously done msmts. With force in forward direction, which in conjuction with this seem to show:

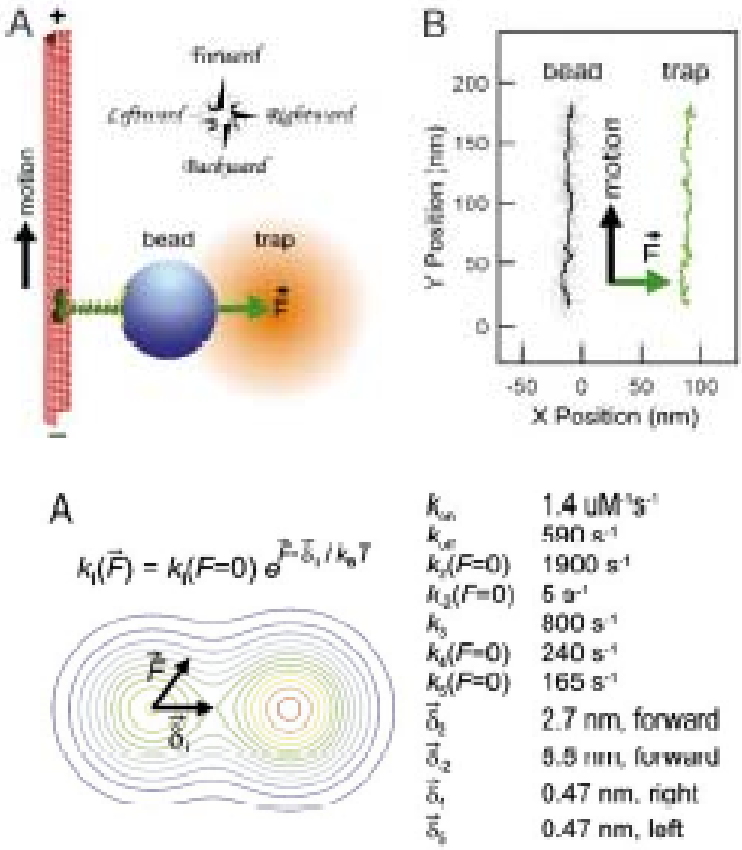
- Velocity increased with forward force
- There are 2 load dependent transitions

Questions:

- Which are load dependent?
- Are substeps a possibility?
- Where do the load dependent transitions take place? Each head?

Recent Research

- Recently created a “2D optical trap” to apply forces in entire specimen plane
- Pinpoint r~1/3, but conjecture at least 4 rate limiting transitions
- Directional bias shows that working stroke is likely along the microtubule axis



Probing the kinesin reaction cycle with a 2D optical force clamp, Block, Asbury, Shaevitz, and Lang, PNAS, 100.