Introduction to Bioengineering Nov 4, 2009

Single molecule imaging of motor proteins

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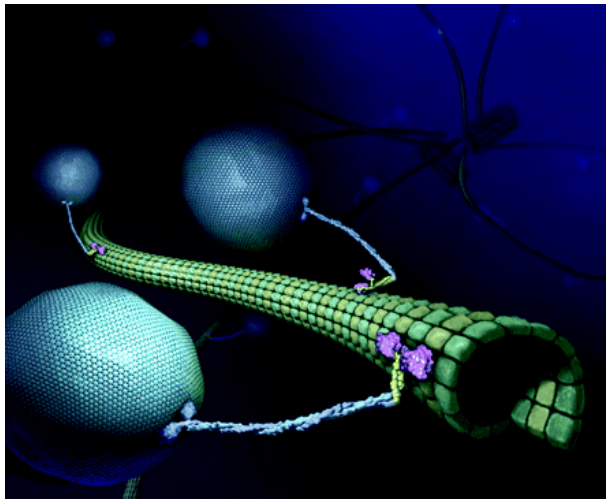
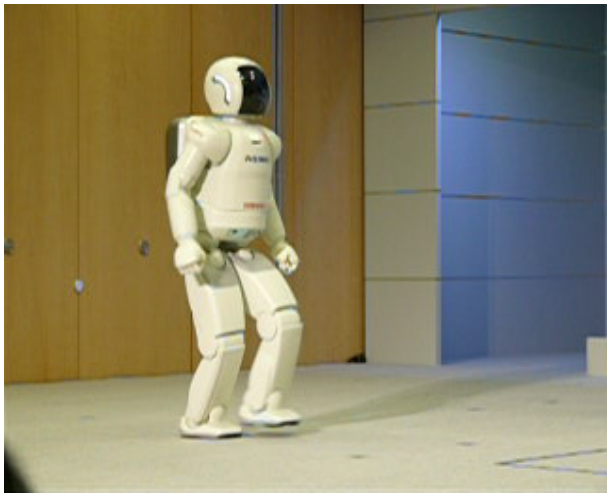


“Walking” macro- and nano-machineries

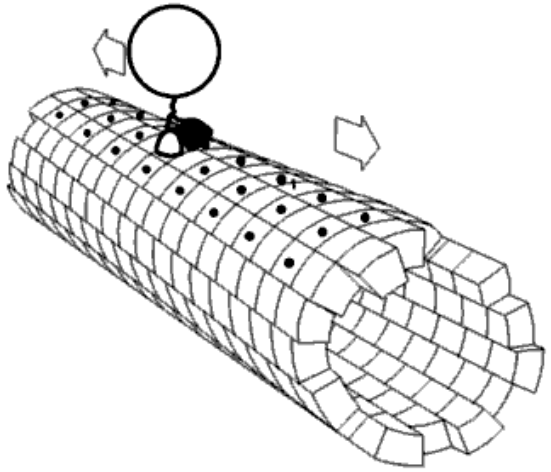
Macro
Robot (Honda ASIMO)



1 m

velocity: 3 km/h

Nano
Kinesin



10 nm

velocity: 500 nm/s
(in human size, 200 km/h)

Simple

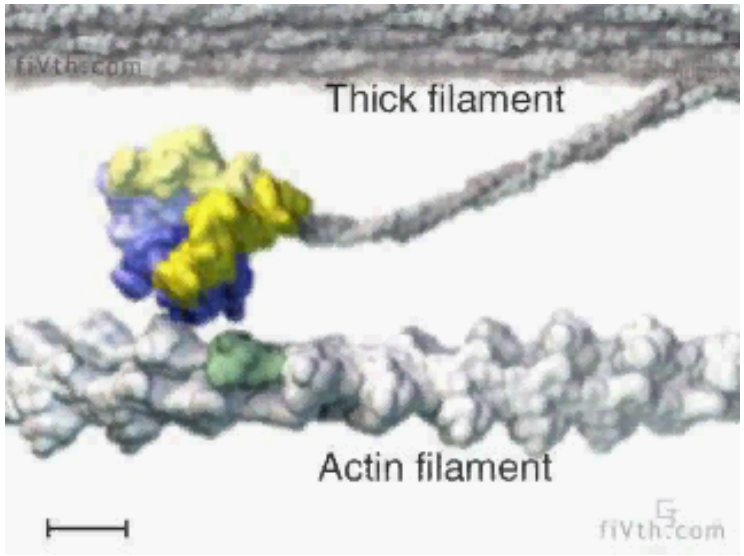
Small

Fast

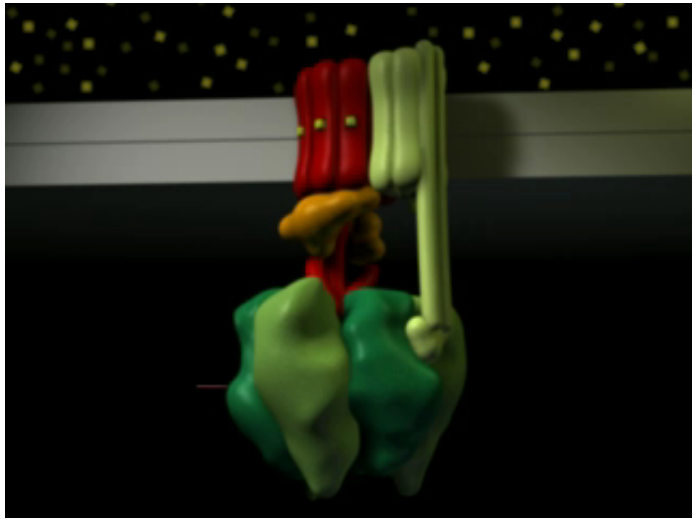
Efficient
(<70%)

Examples of Motor Proteins

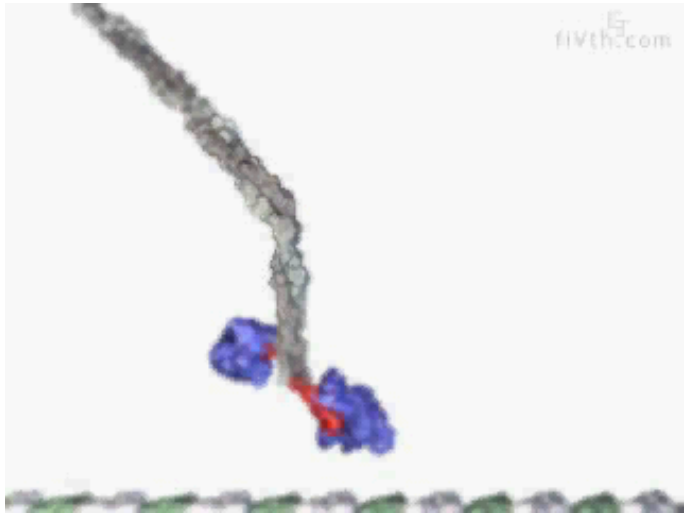
Myosin



ATP Synthase

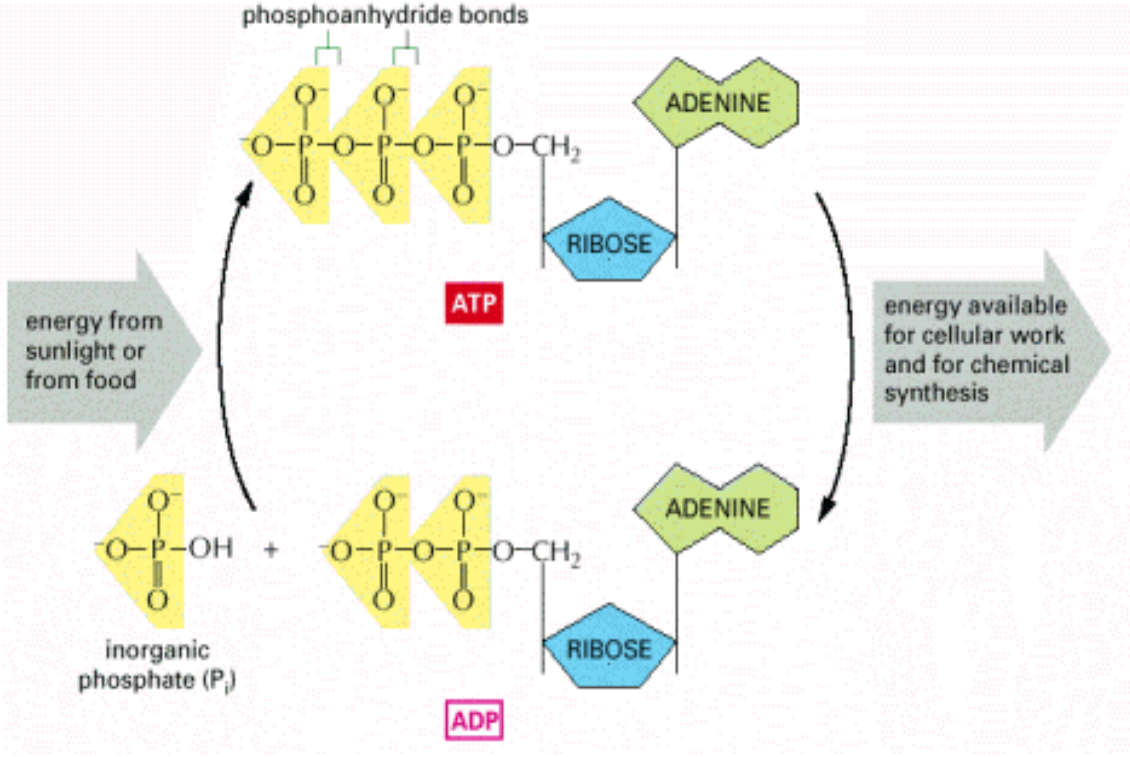


Kinesin



Input energy :

ATP hydrolysis



$$ATP + H_2O \rightleftharpoons ADP + P_i$$

表 5.1 ATP が ADP と Pi に加水分解されるときの自由エネルギーの変化

観察される質量作用比 (Γ)	非平衡度 (Γ/K)	ΔG (kJ mol^{-1})	[ATP]/[ADP] ([Pi]=1 mM のとき)
10^{10}	10^{5}	28.5	10^{-13}
10^{7}	10^{2}	11.4	10^{-10}
10^{5}	1	0	10^{-8}
10^{3}	10^{-2}	−11.4	10^{-6}
10	10^{-4}	−22.8	10^{-4}
1	10^{-5}	−28.5	10^{-3}
0.1	10^{-6}	−34.2	10^{-2}
10^{-3}	10^{-8}	−45.6	10^{0}
10^{-5}	10^{-10}	−57	10^{2}

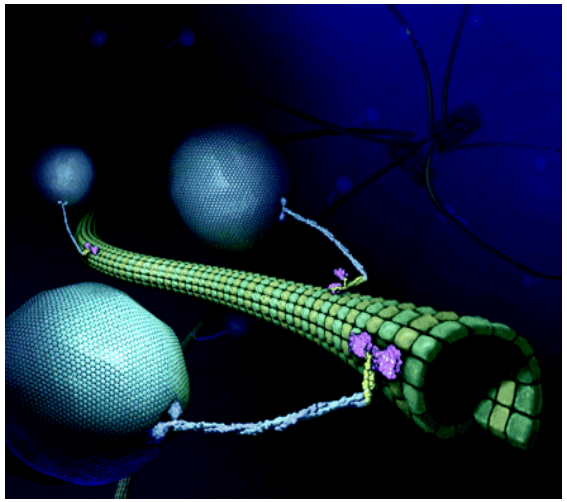
ΔG^0

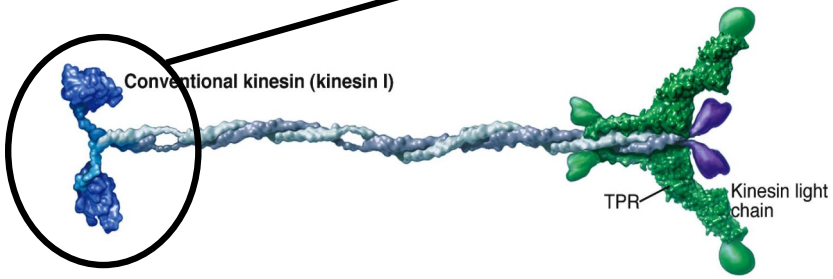
$$K = \frac{[ADP][Pi]}{[ATP]} = 10^{5}\ \mathrm{M}$$

[ATP]/[ADP] ~ 10^2 inside the cells

$$\Delta G = \Delta G^0 + RT \ln \frac{[ADP][Pi]}{[ATP]}$$

Kinesin's structure





10 nm

ATP

Catalytic core ("Head")

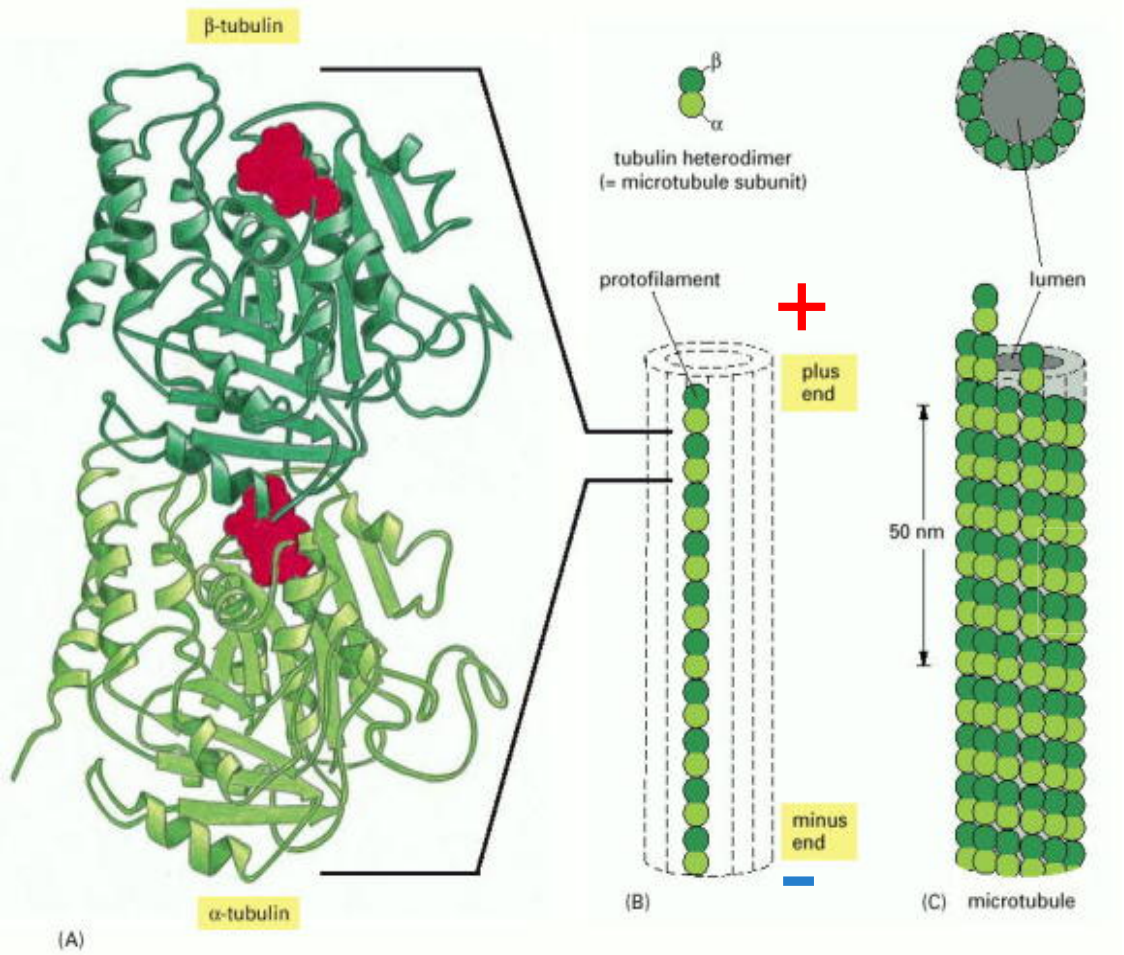
Neck linker

Neck coiled-coil

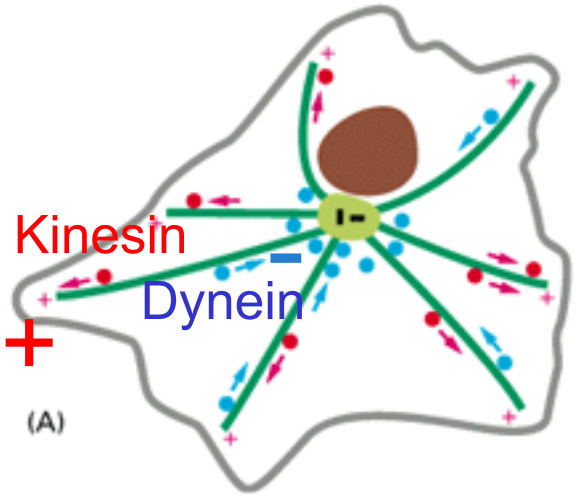
Microtubule -binding

Cargo-binding

Microtubule: track for kinesin



Microtubule organization inside the cells



Molecular Biology of the Cell, 4th ed.

composed of α-tubulin and β-tubulin

Kinesin binds to β-tubulin subunit

has polarity (plus- and minus-ends)

Single molecule imaging using total internal fluorescence microscopy

High-sensitivity camera (ICCD, EMCCD)

Reduce background fluorescence

→reduce the autofluorescence

→<u>reduce the illumination volume</u>

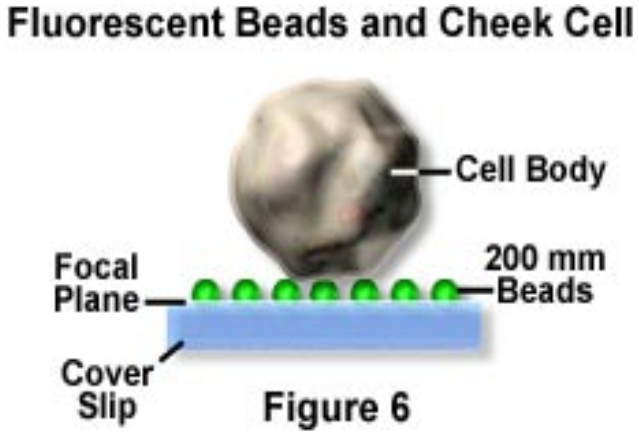
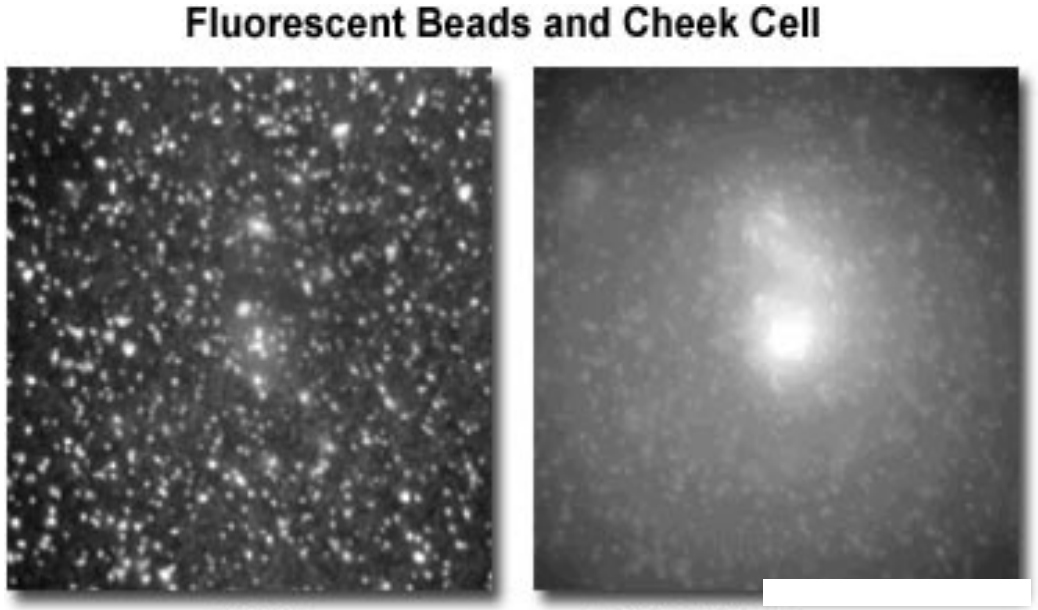
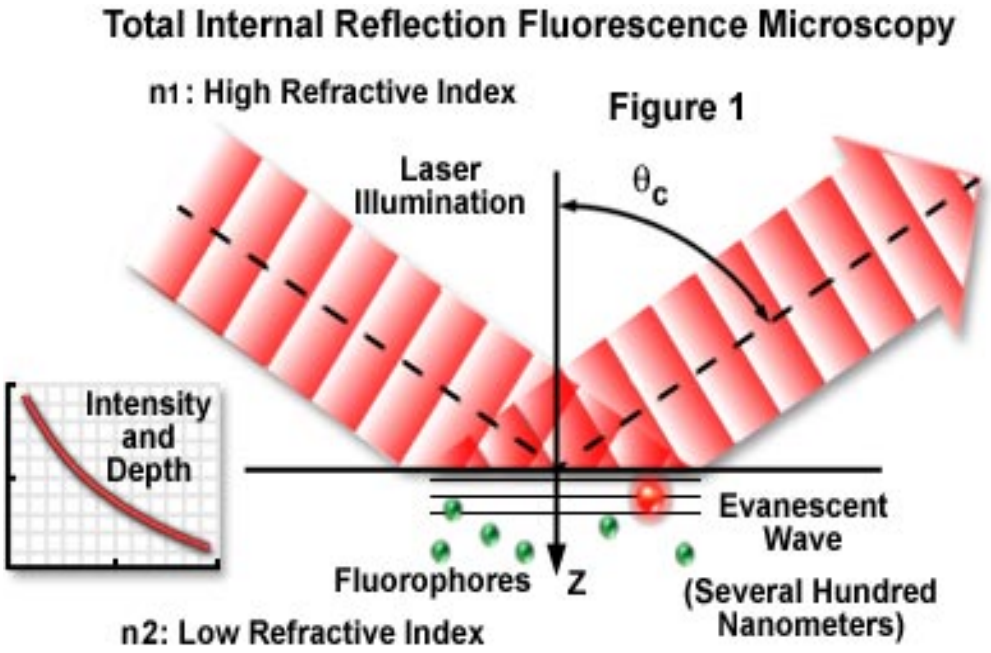
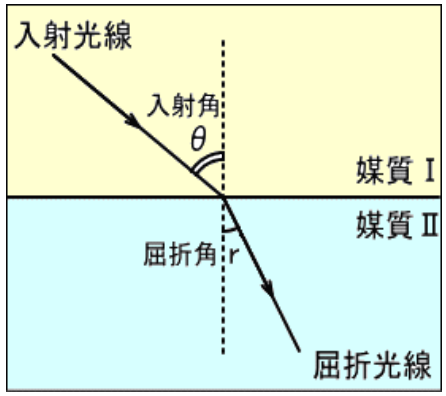


Figure 6



Total internal reflection and evanescence field





Snell's Law

$$n_1 \sin\theta_1 = n_2 \sin\theta_2 \qquad (n_1 > n_2)$$

Critical angle

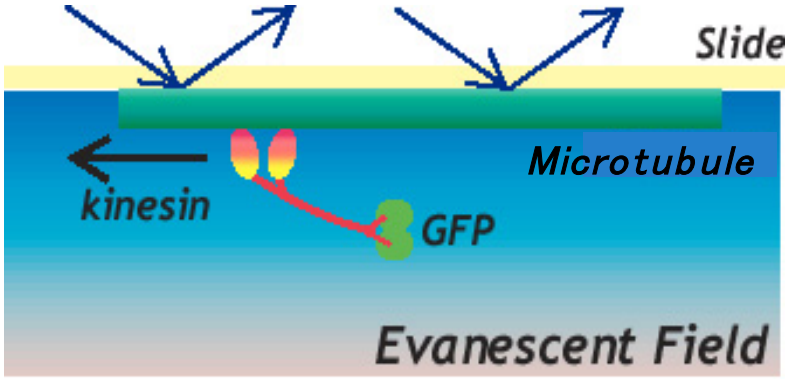
$$\theta_c = \sin^{-1}(n_2 / n_1)$$

$$E(x,z) = T_0 \exp\left[i\left(\omega t - k_2 x \frac{\sin\phi_1}{n}\right)\right] \exp\left[-k_2 z \sqrt{\frac{\sin^2\phi_1}{n_2} - 1}\right]$$

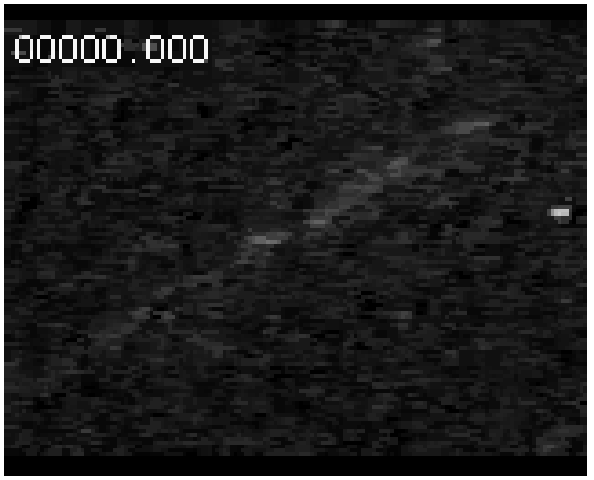
$$I(z) = I(0)e^{-z/d} \qquad d = \frac{\lambda_0}{4\pi}(n_1{}^2 \sin^2\theta - n_2{}^2)^{-1/2}$$

d = 0.25×λ (~150 nm) at θ= 64°

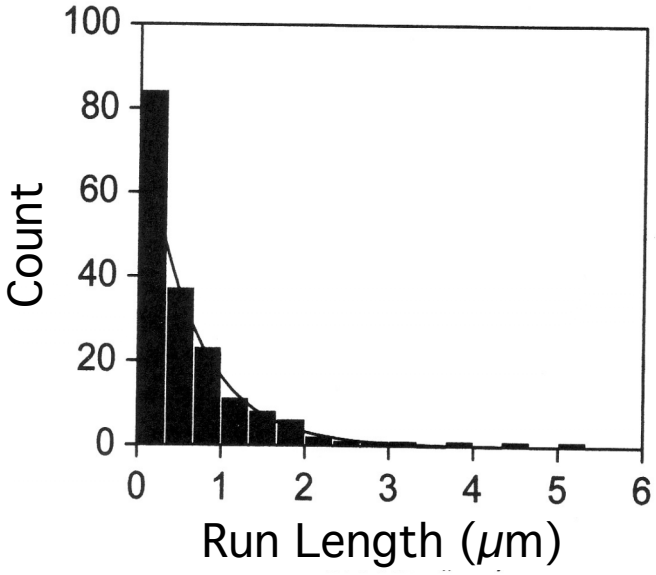
Single molecule fluorescence imaging of kinesin



TIRF (Total Internal Reflection Fluorescence) Microscopy



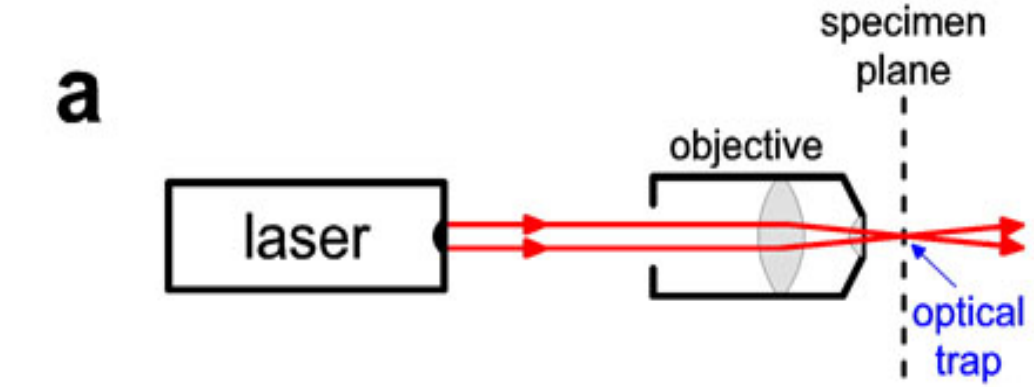
velocity:
~500 nm/s



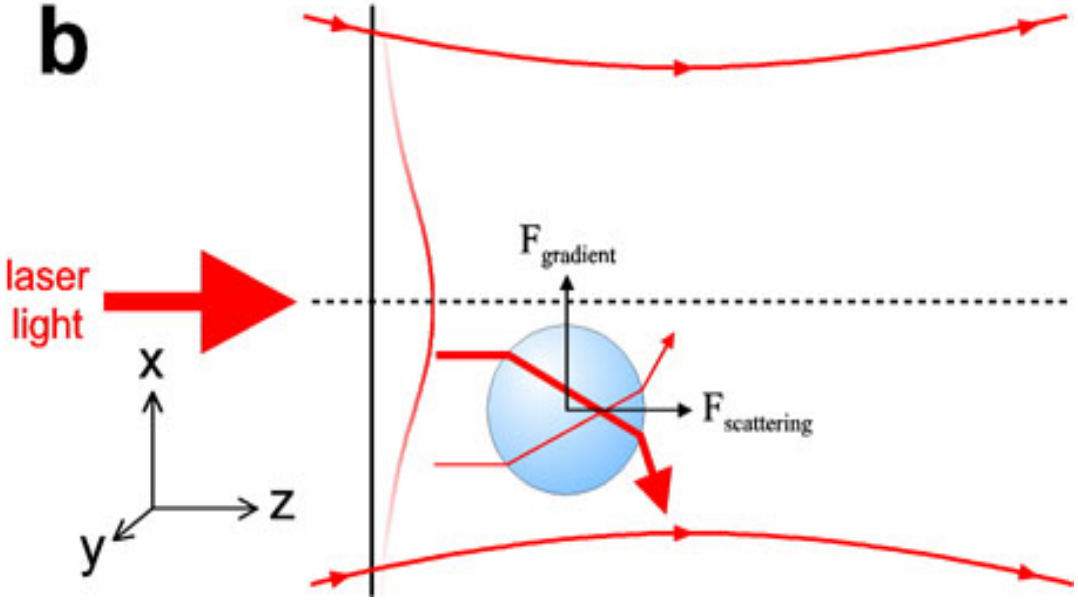
Kinesin moves toward plus-end of the microtubule, processively for ~ 1 μm (100 steps)

Kinesin is a directional and processive motor

Optical trap (Optical tweezers)

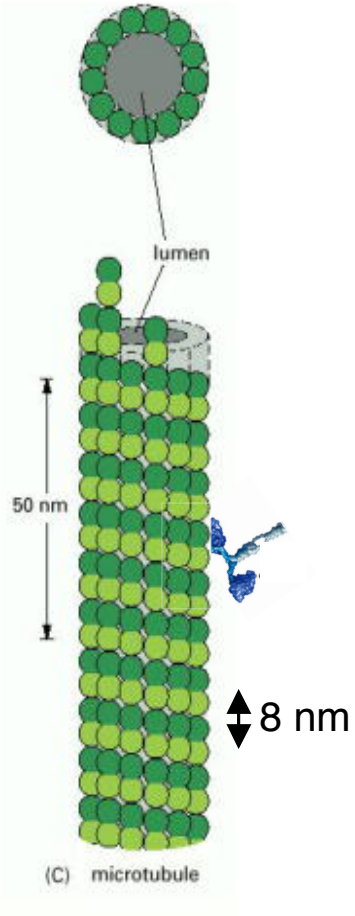


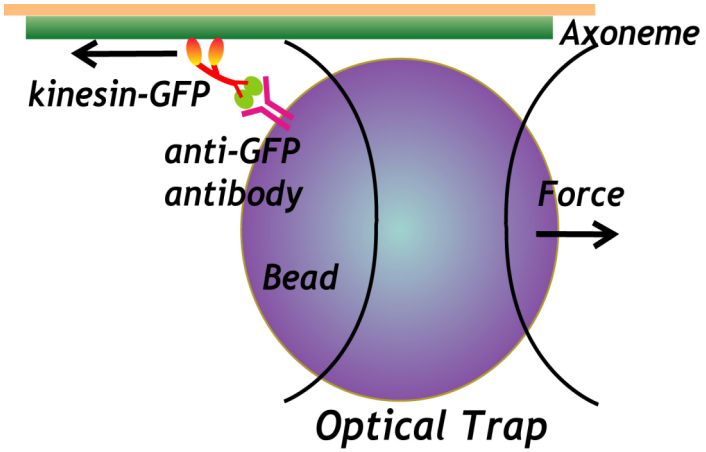
Focused laser beam (passed through the high NA objective lens) can be used to trap small object bear the focus



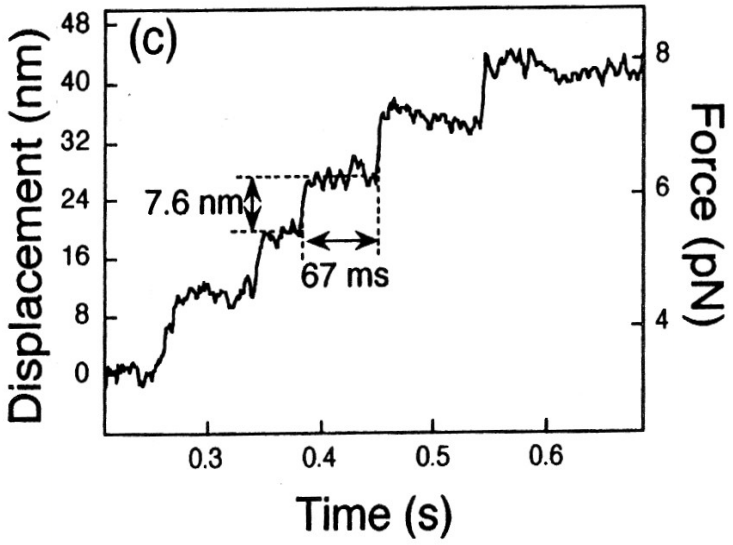
Dielectric particles (10 μm - 25 nm diameter) and cells (eg. bacteria, yeast) can be trapped

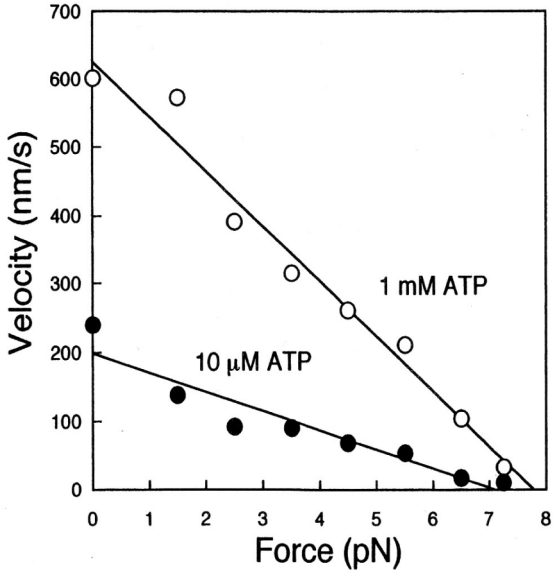
Optical trapping bead assays of kinesin





Kojima et al.
Biophs. J. 1997





take
8 nm steps

×

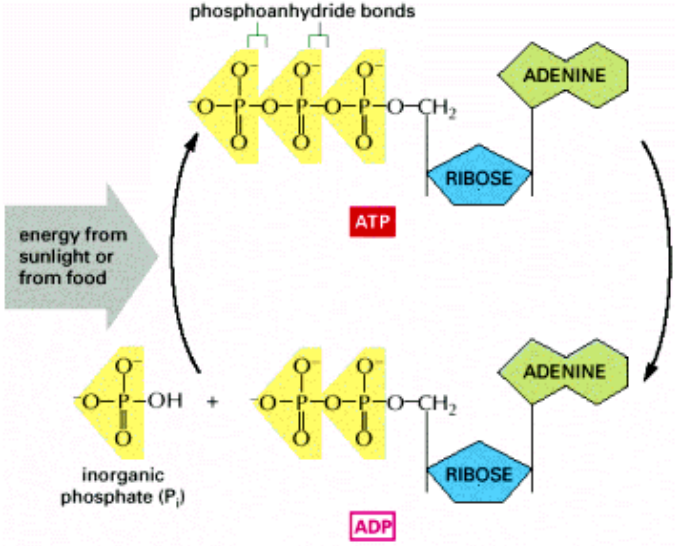
move against load up to
7 pN

56 pN · nm (max. work) ÷ 80 pN · nm (ATP hydrolysis) = 70% efficiency

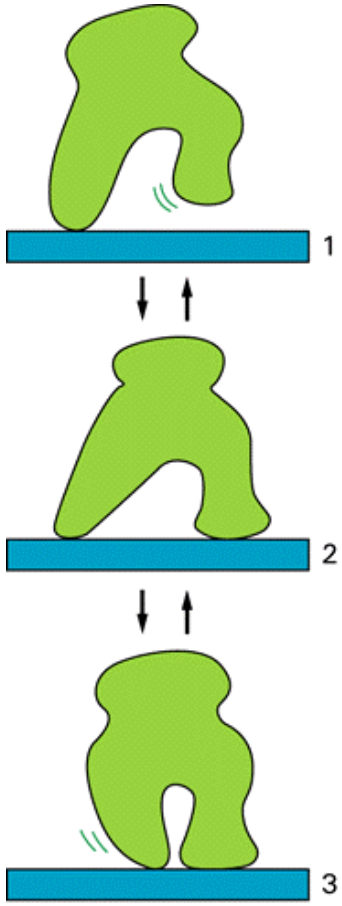
Energy conversion by molecular motors

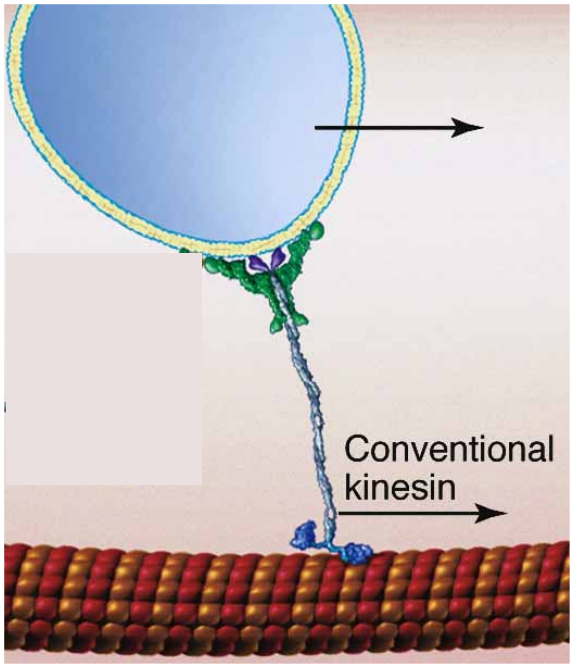
input → output

Chemical energy → Conformational change → Mechanical work



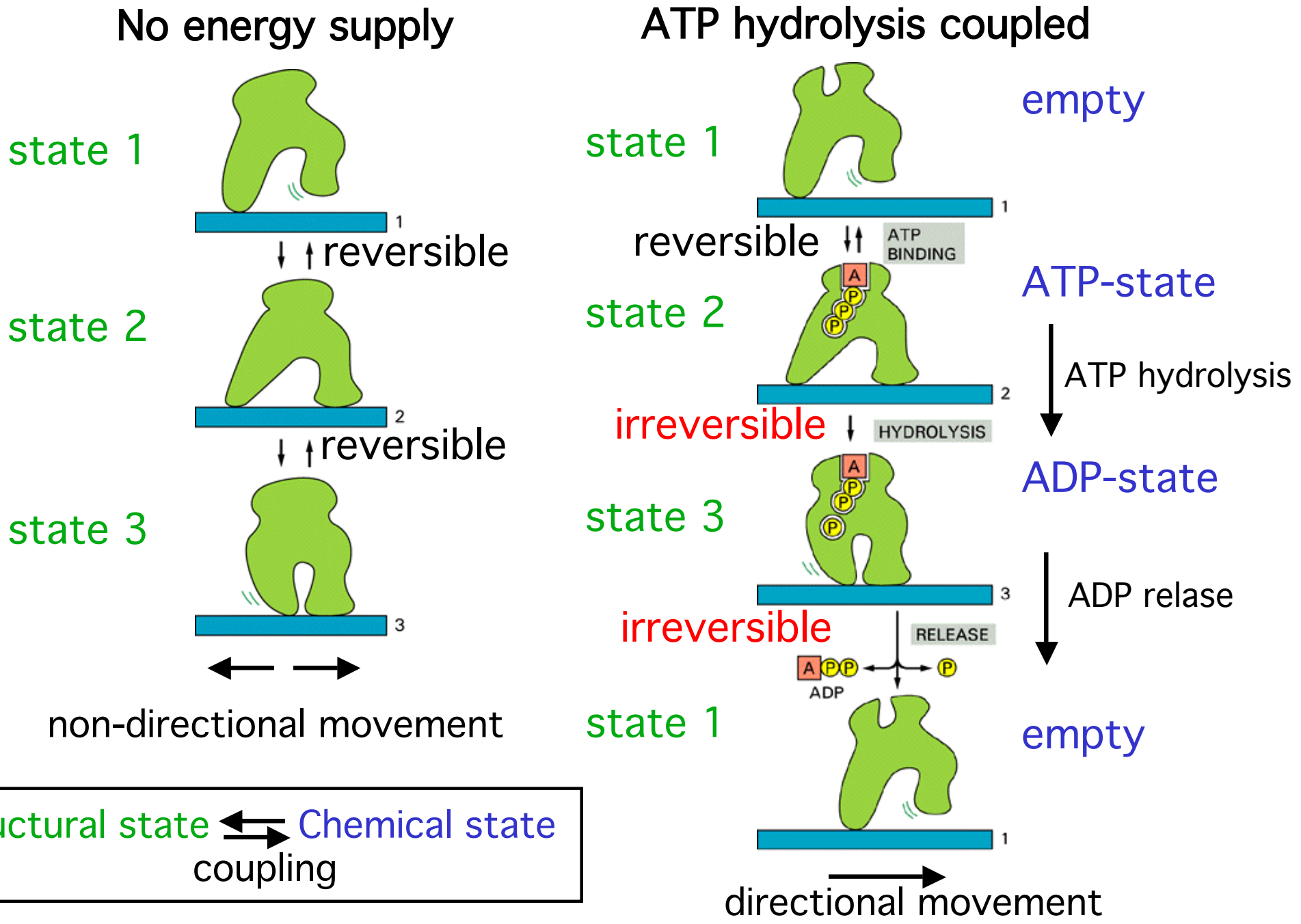
ATP hydrolysis



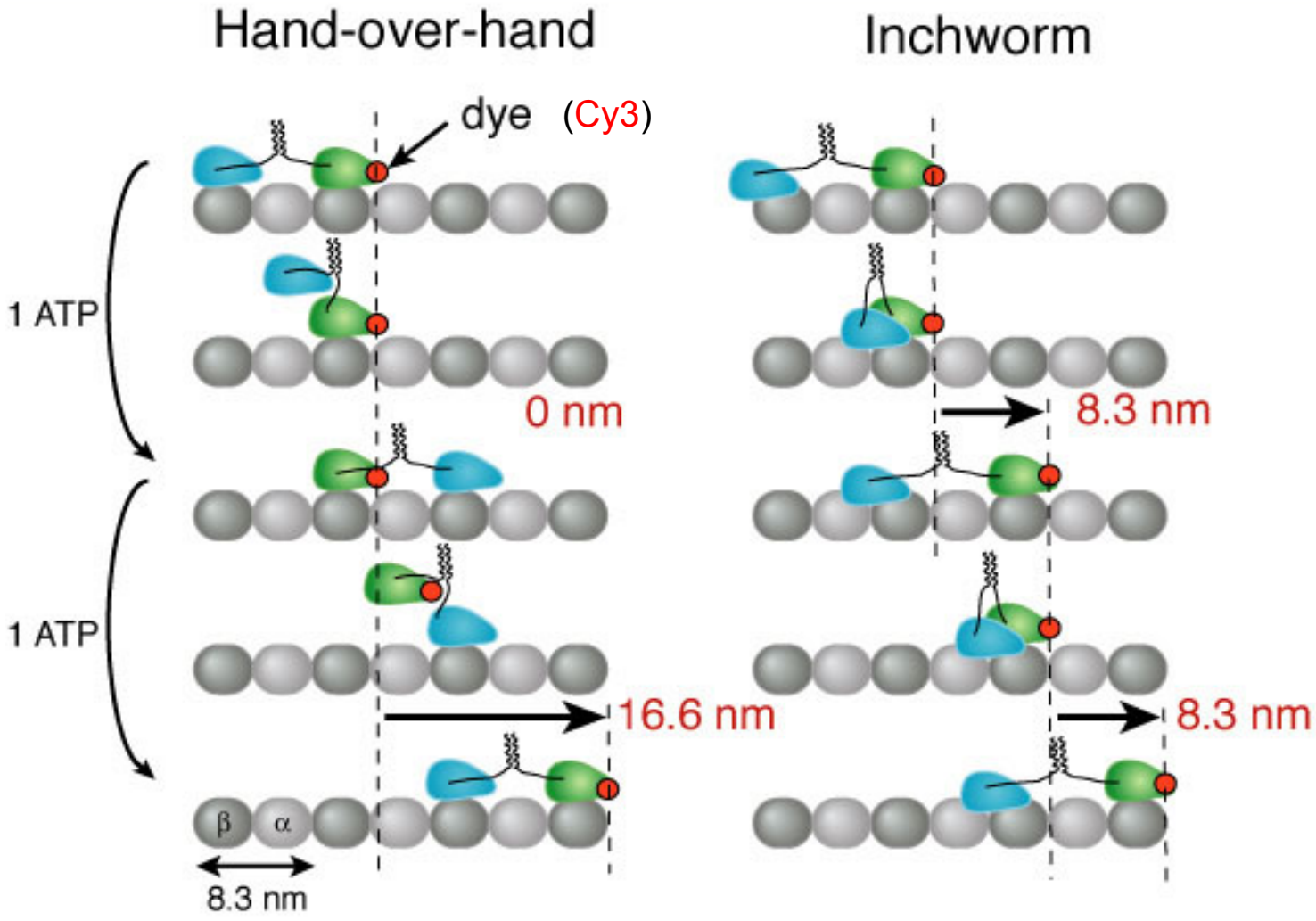


Unidirectinal movement

Structural changes coupled to ATP hydrolysis produce directionality



Observe a moving part of kinesin (FIONA)



Protein engineering: essential tool for single molecule observation

DNA (genetic) → Amino-acids (sequence) → Protein (3D-structure)

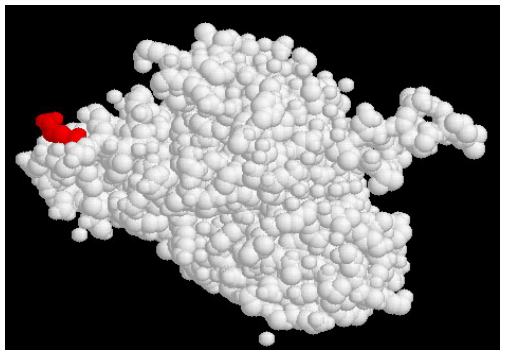
--ATCTCTGCGA--

↑

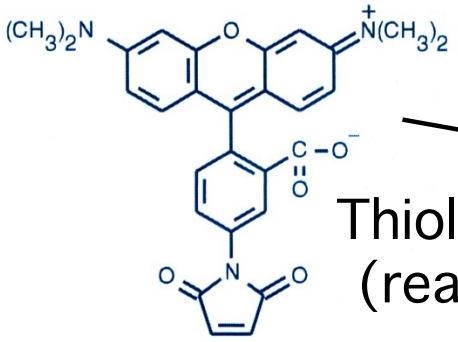
Genetic engineering

--LysGlyCysPro--

(-SH)



Fluorescence labeling ↓



Thiol-reactive dye (react with Cys)

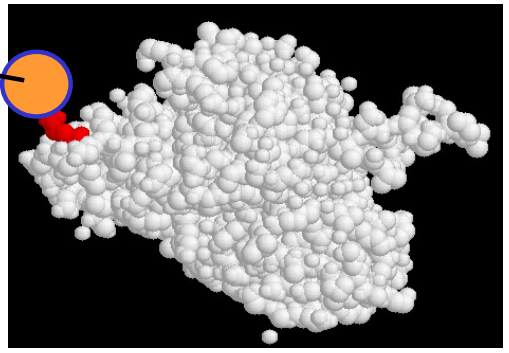
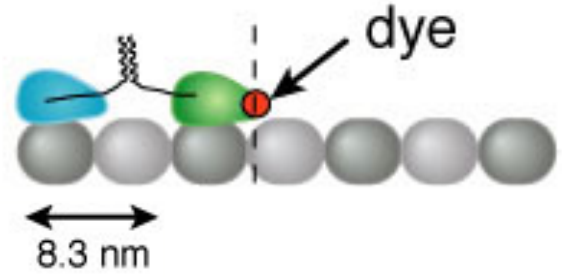
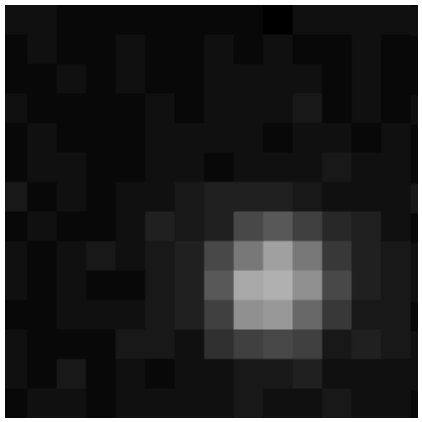


Figure 2.11 T-6027 tetramethylrhodamine-5-maleimide.

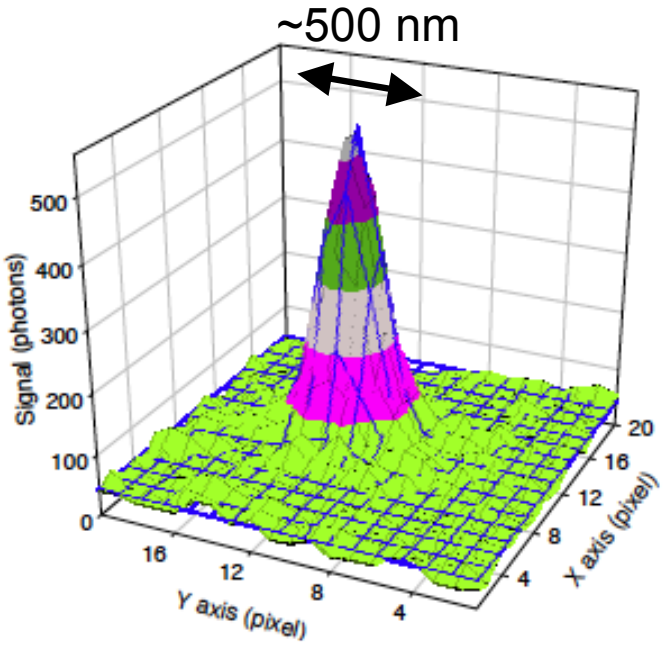
Detect single fluorophore at nanometer precision



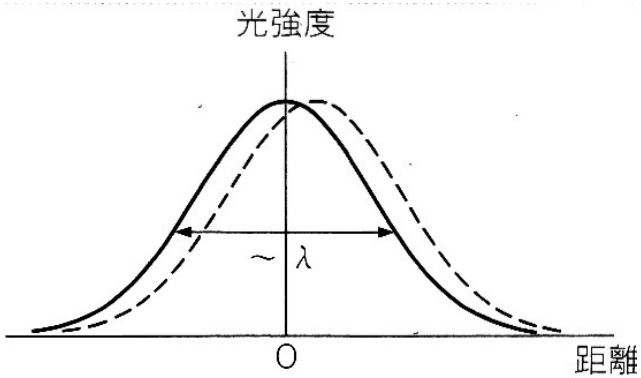
Fluorescence Imaging with One-Nanometer Accuracy (FIONA)



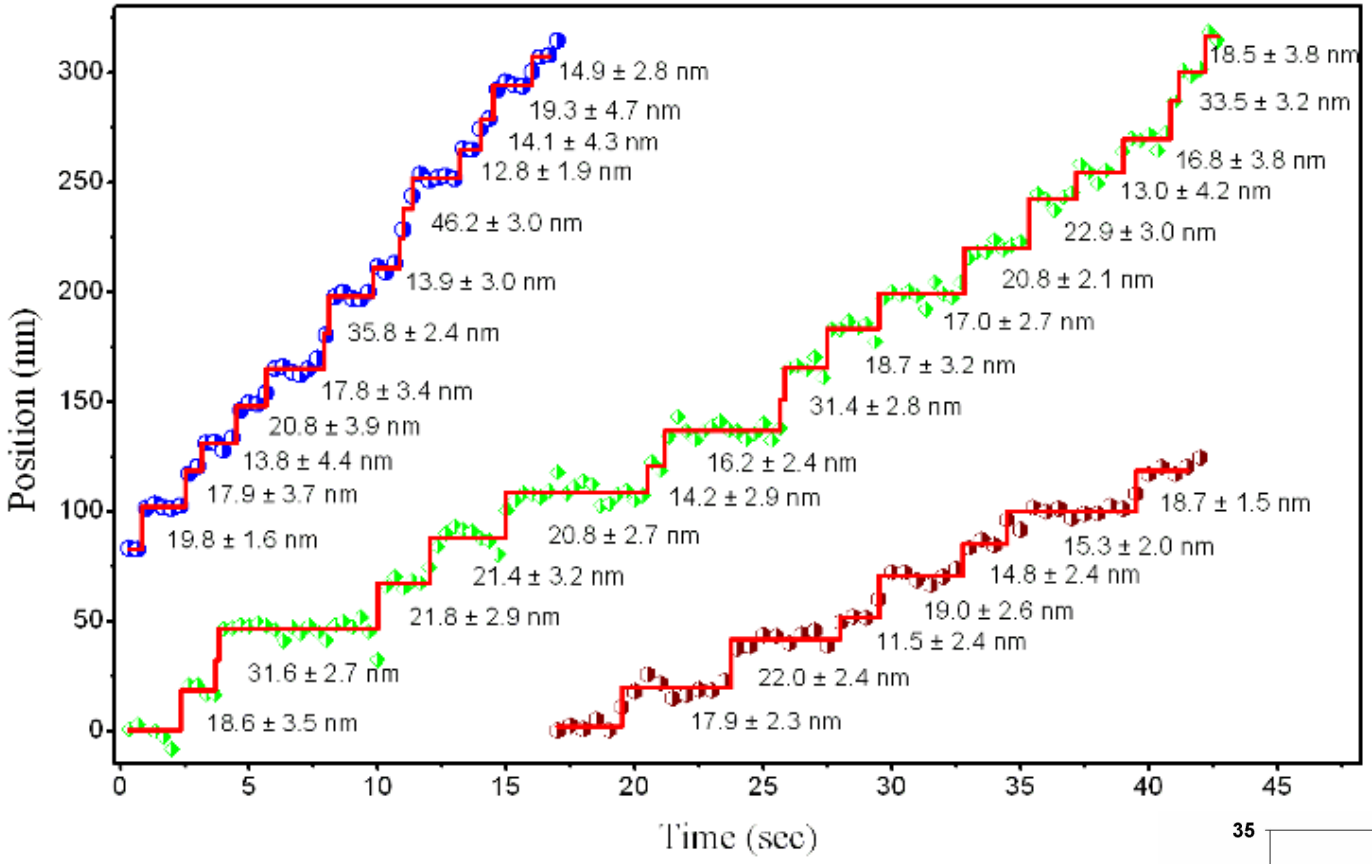
Images were taken using cooled CCD (2 frames/s)

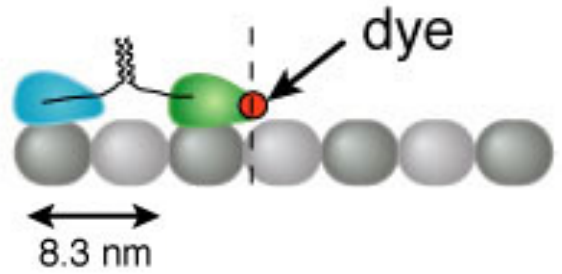


Fitting the image with 2D Gaussian

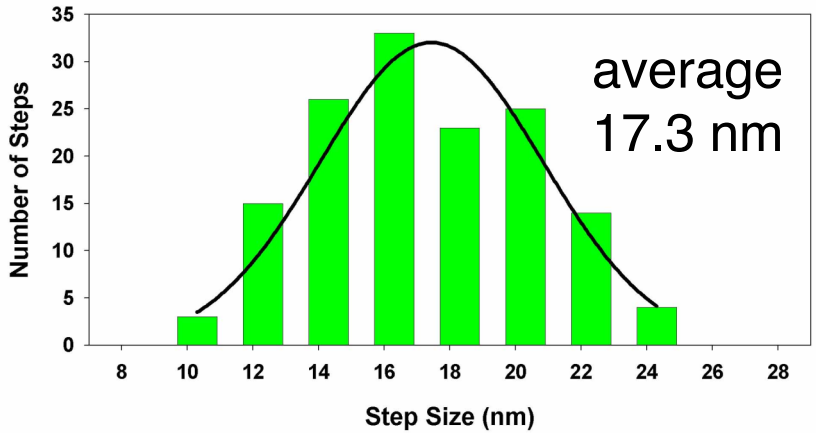


Fluorophores attached on one of the heads showed 16 nm steps





Step size

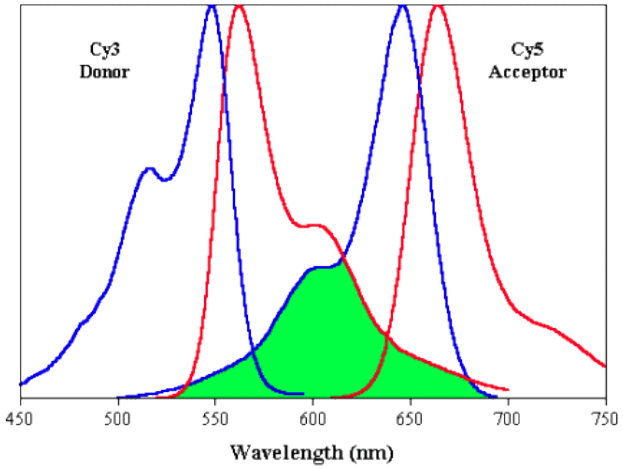


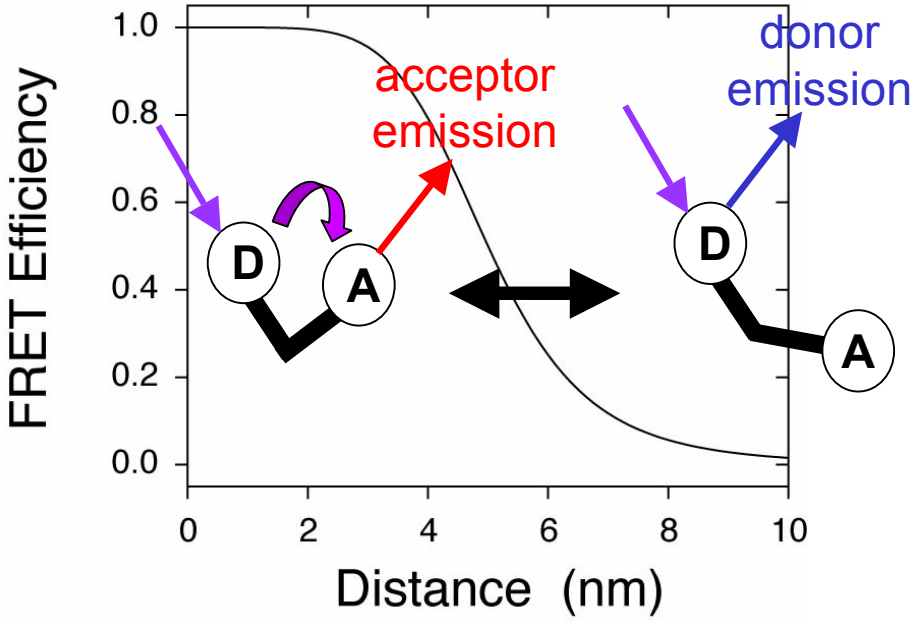
Yildiz et al, Science 303, 676 (2004)

Single Molecule FRET

FRET: Fluorescence Resonance Energy Transfer

Probing the conformational changes in kinesin

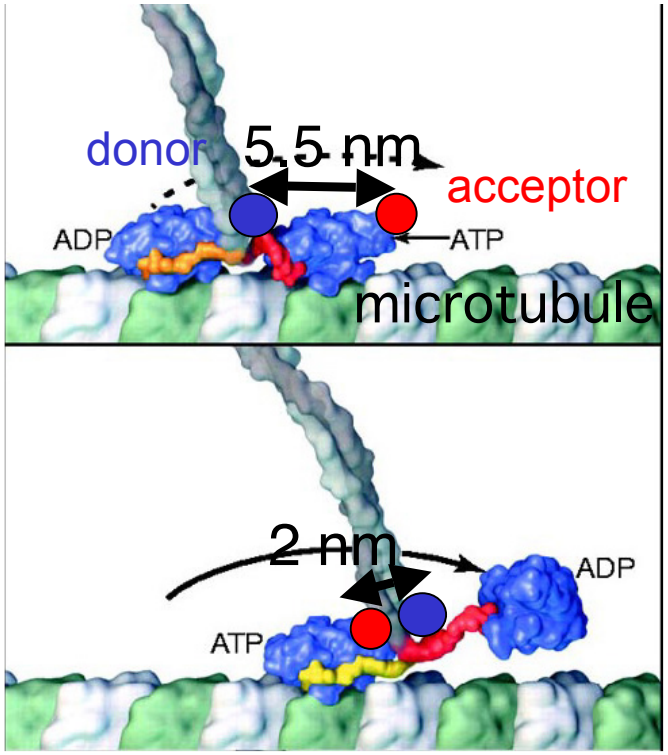




$$E_{FRET} = \frac{I_{Accepter}}{I_{Donor} + I_{Accepter}} = \frac{1}{1 + (R / R_0)^6}$$

R_0 : Foster distance (5.4nm for Cy3&Cy5)

Labeling for neck linker FRET

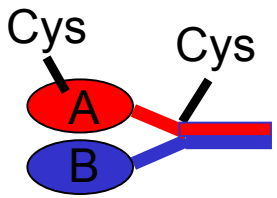


Neck linker structural state		FRET reporter
undock	=	Low FRET (40%)
dock	=	High FRET (100%)

Cysteine-light heterodimer

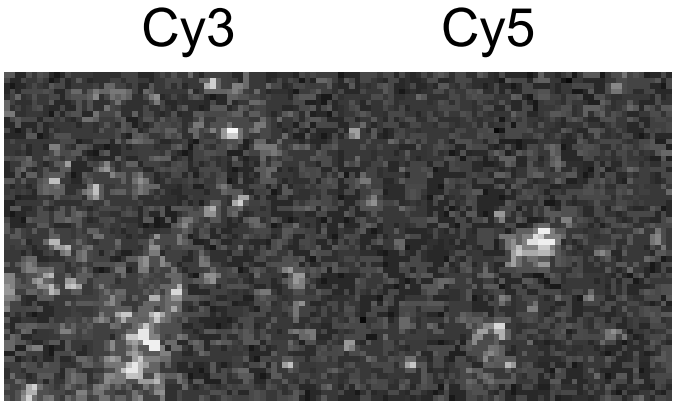
↓

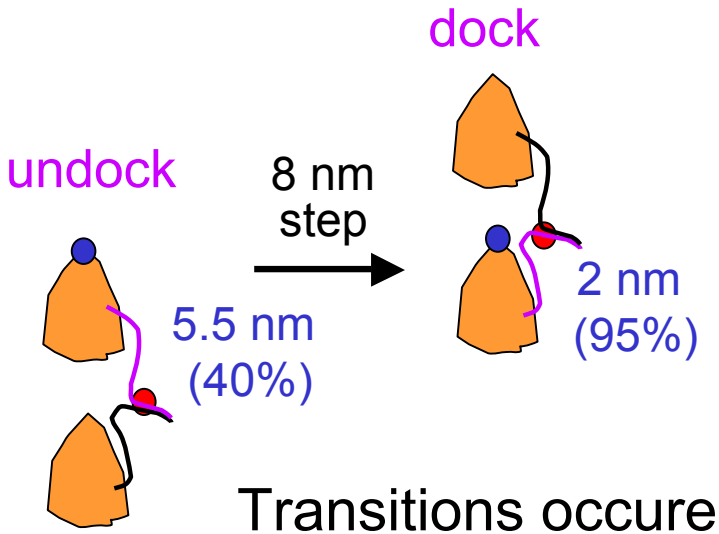
Labeling with Cy3, Cy5-maleimide

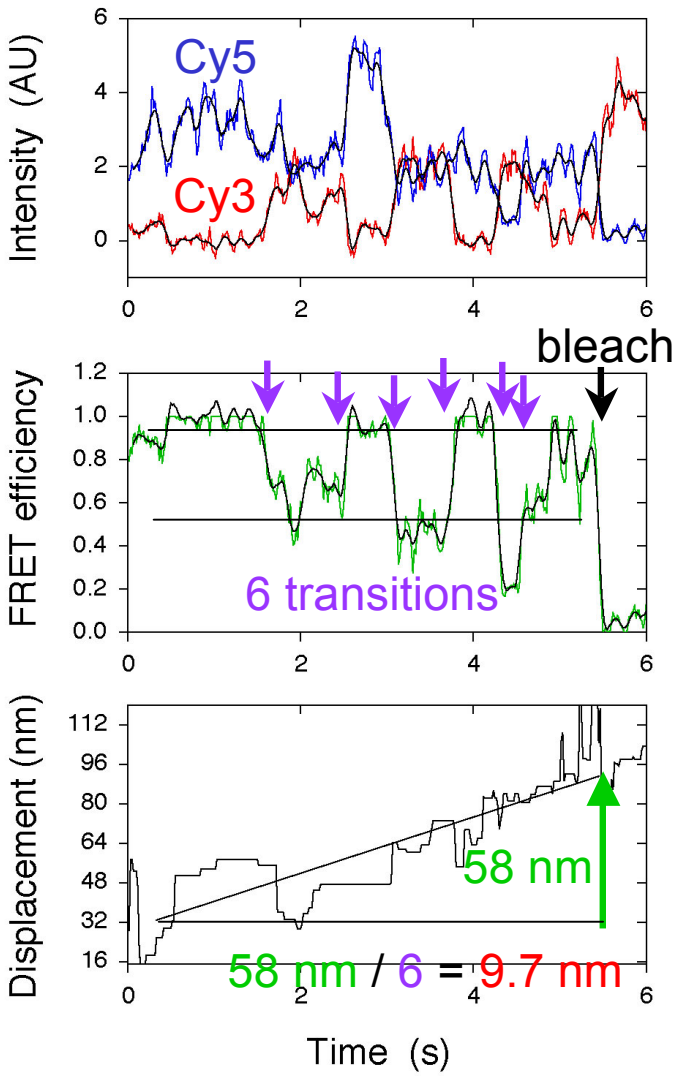


Neck linker FRET: dock-undock transitions were observed during the movement

1 μM ATP



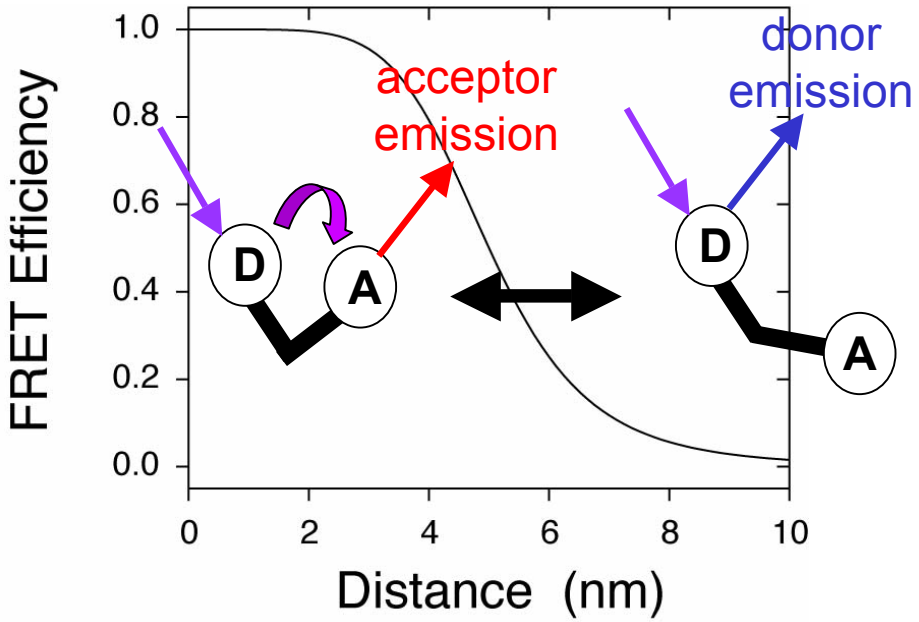


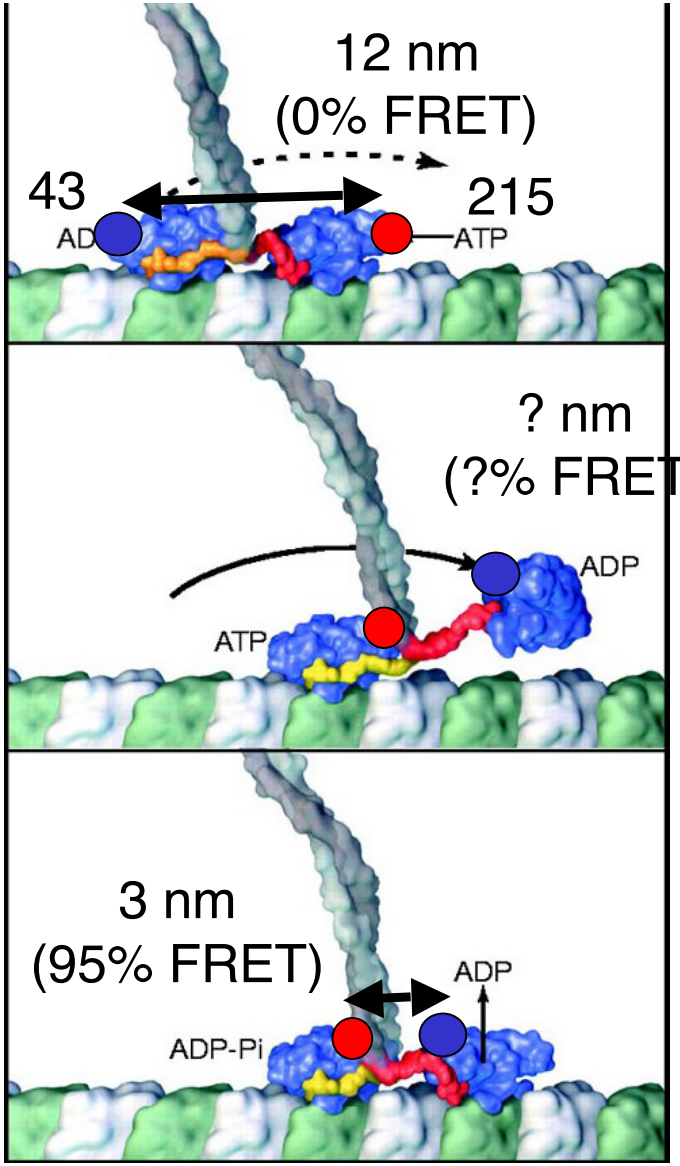


Transitions occured once per ~8 nm step on average

Tomishige et al, Nature Struct. Mol. Biol. 13, 887 (2006)

Donor/acceptor labeling for head-head FRET





Two-head-bound

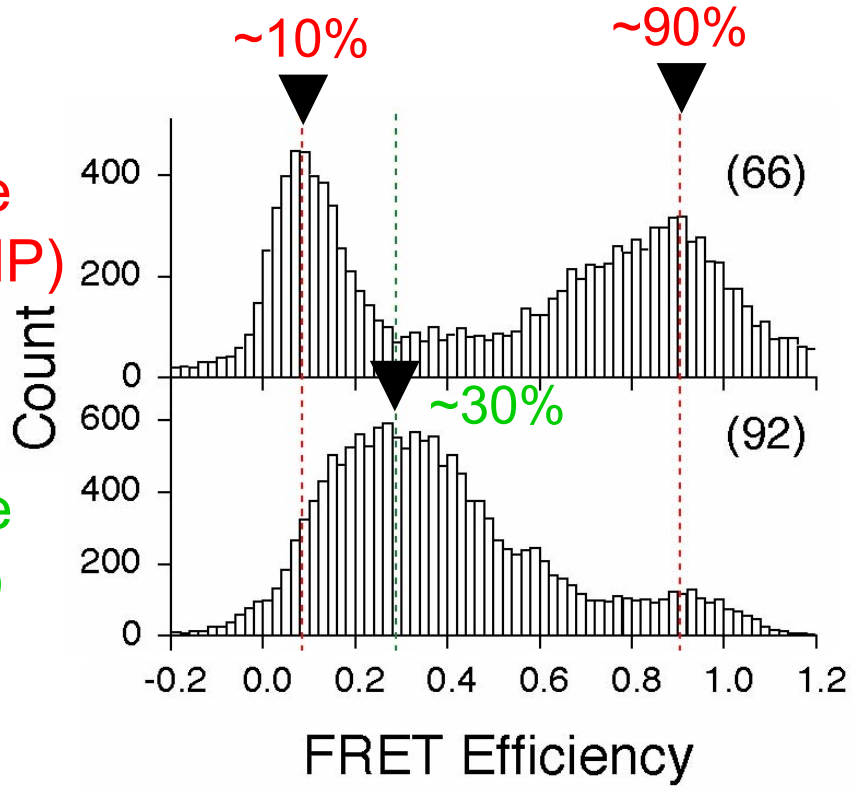
One-head-bound

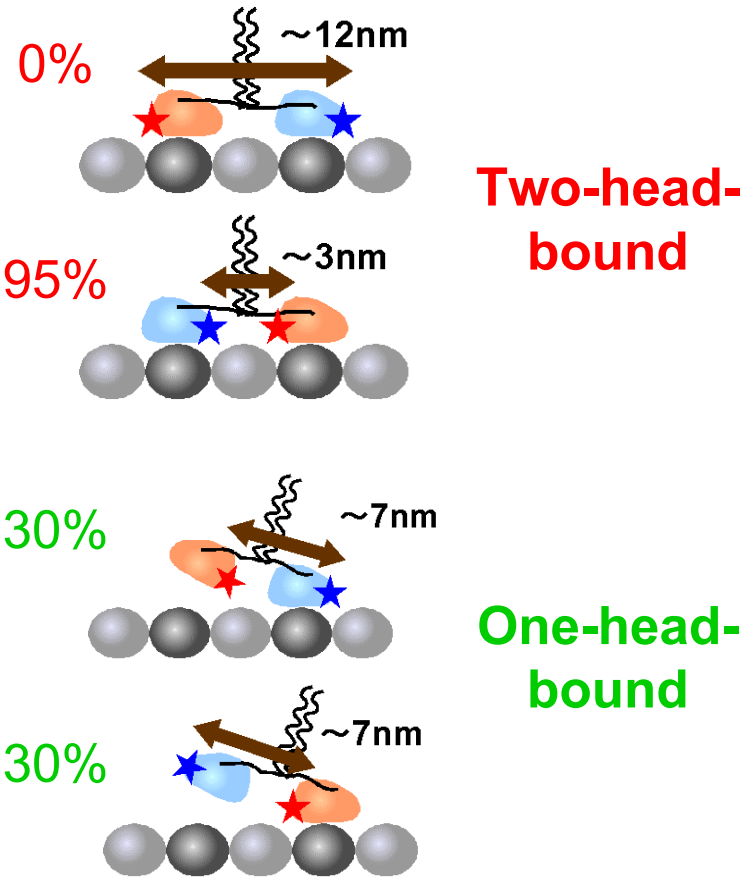
Two-head-bound

Front-to-rear FRET to distinguish one/two-headed states

ATP-like state
(1 mM AMP-PNP)

Low nucleotide
(200 nM ADP)

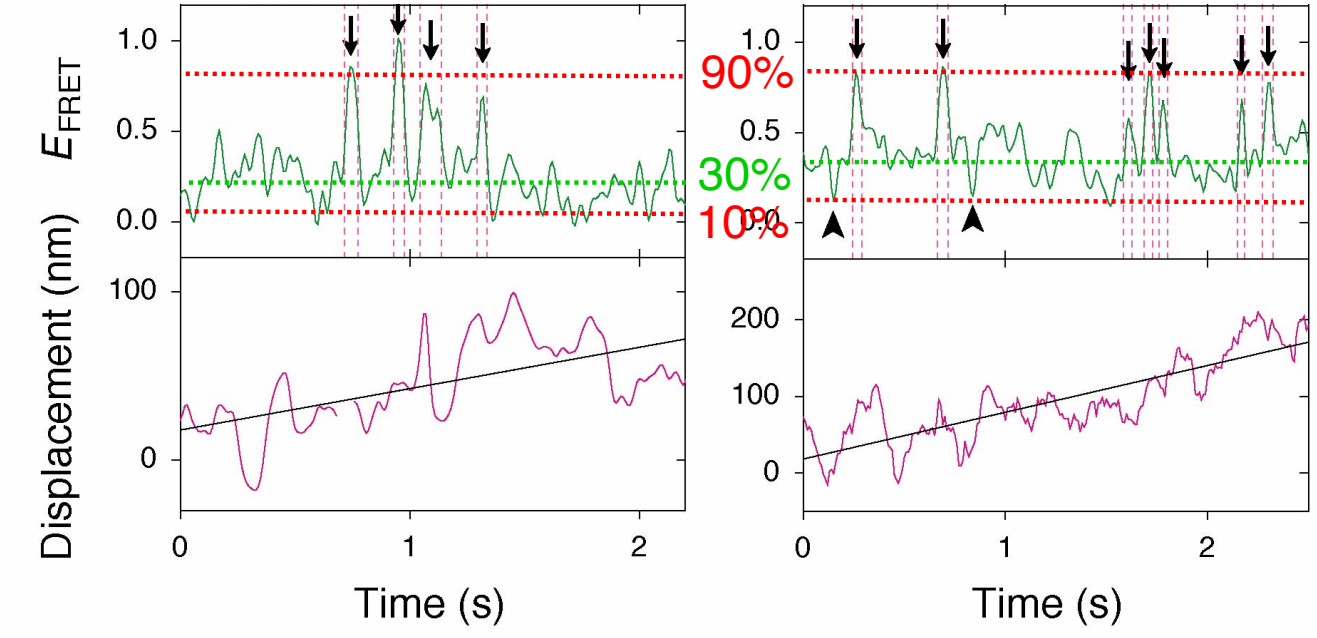


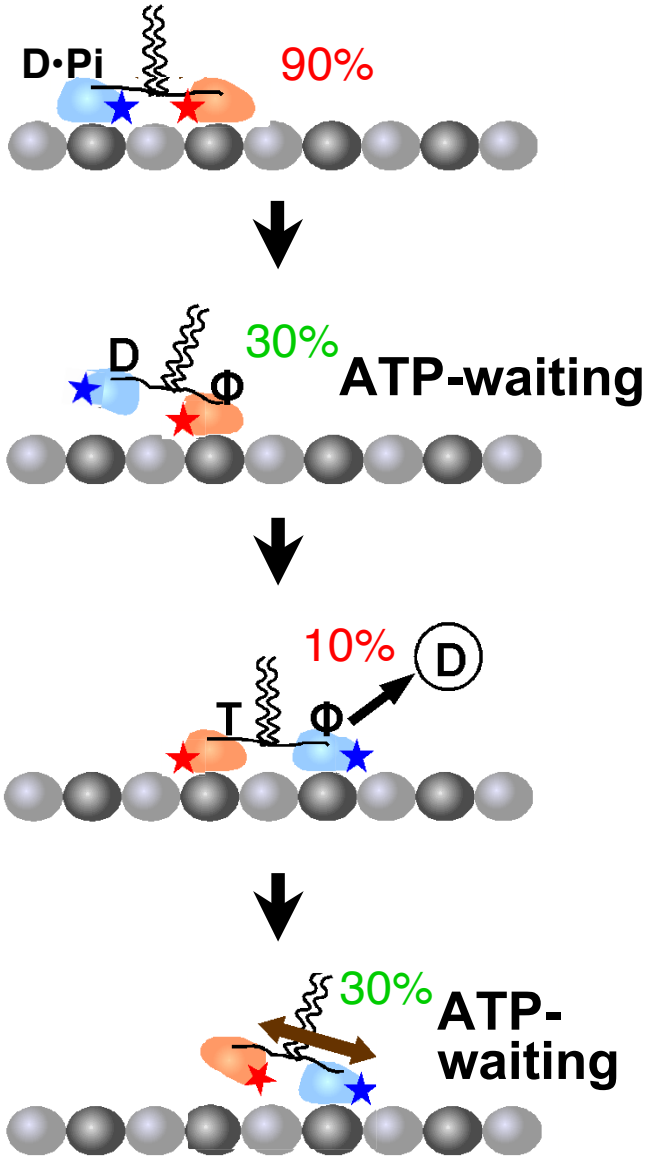


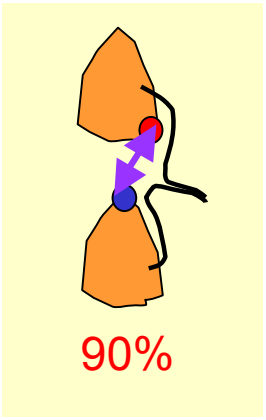
Two-head-bound

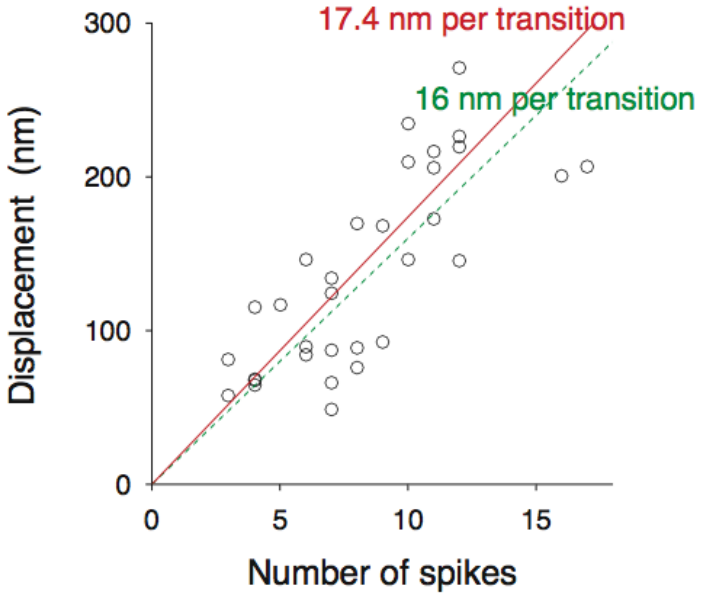
One-head-bound

At low ATP (where ATP-binding is rate-limiting), kinesin spent most of the time in one-head-bound state









Mori et al, Nature **450**, 750 (2007)

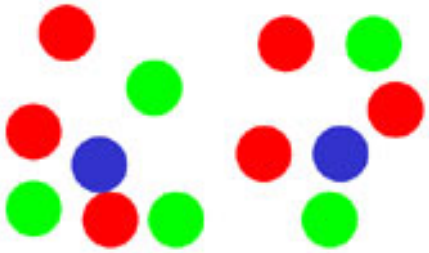
Why “single molecule”?

Advantages against bulk measurements

1) Detection of multiple states

Distribution

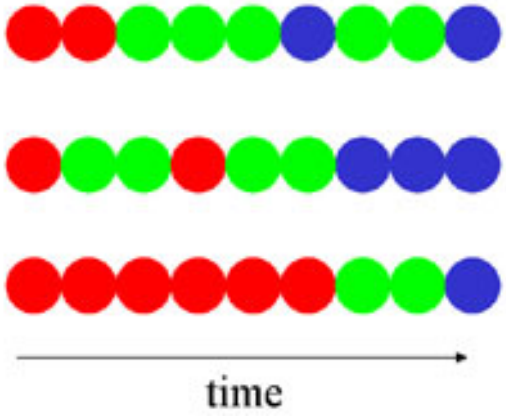
-useful if population is heterogeneous.



2) Transition between states

Time trajectory

-useful if the dynamics is not synchronizable



3) Select specific molecules

Active,
MT bound
doubly labeled
motor

4) Directly linking the function and structure