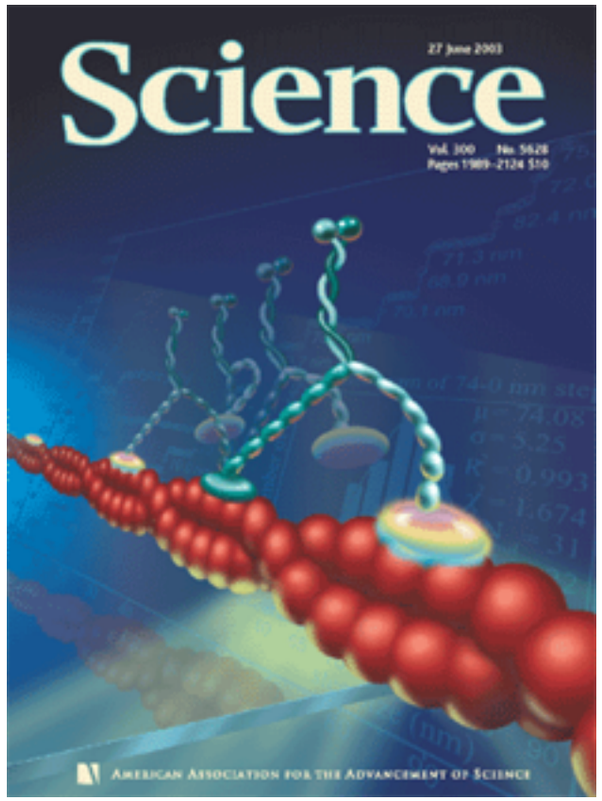
Molecular motors



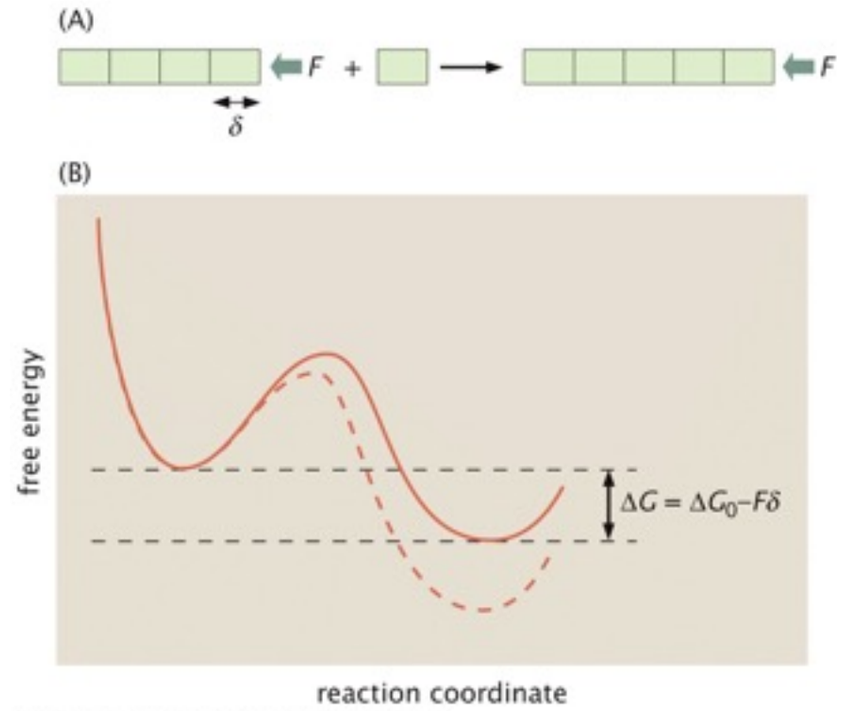


Figure 16.44 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

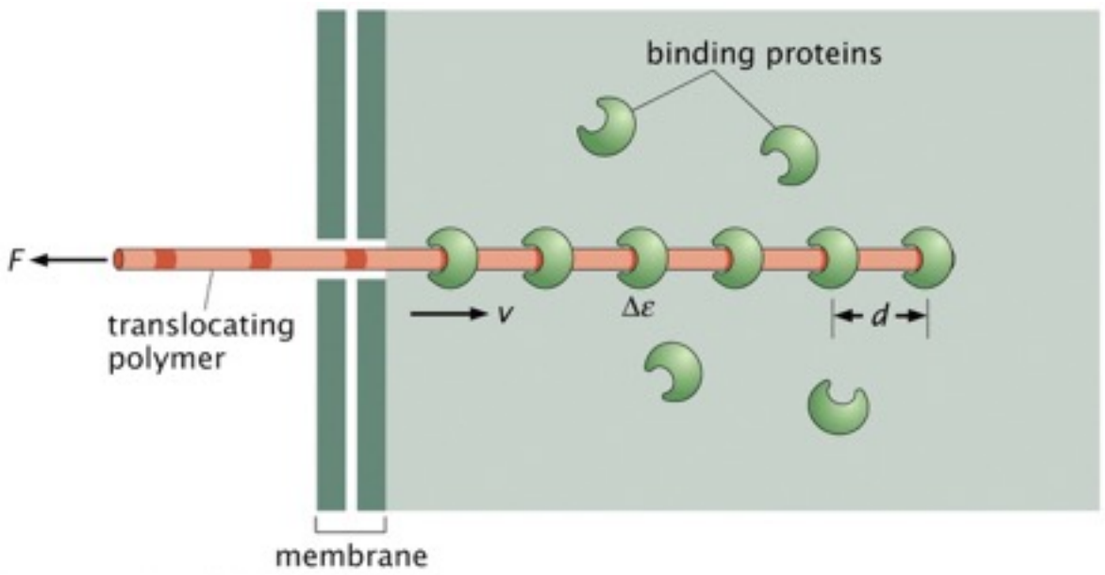
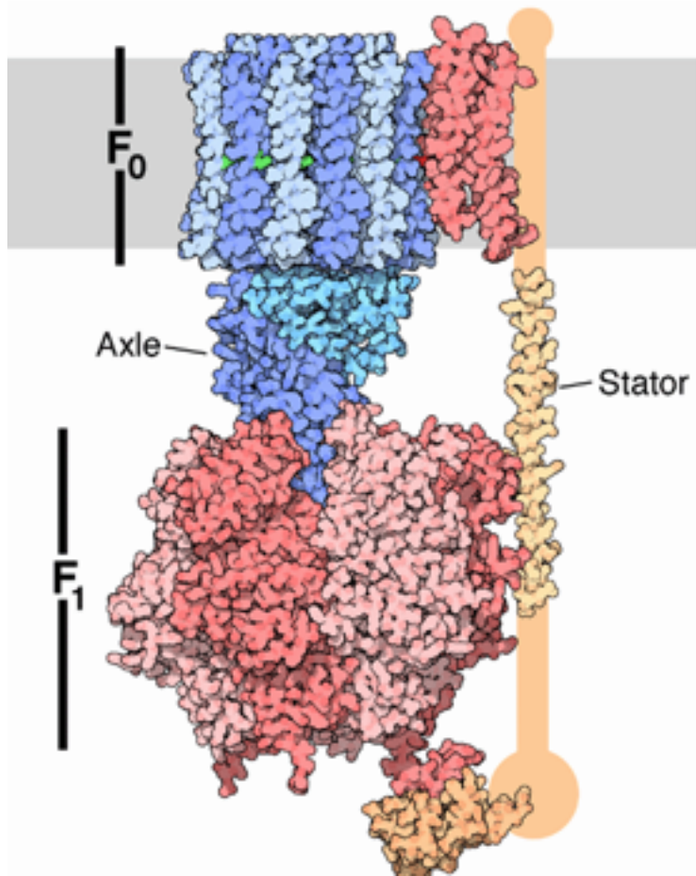


Figure 16.52 Physical Biology of the Cell, 2ed. (© Garland Science 2013)



Molecular **motors**

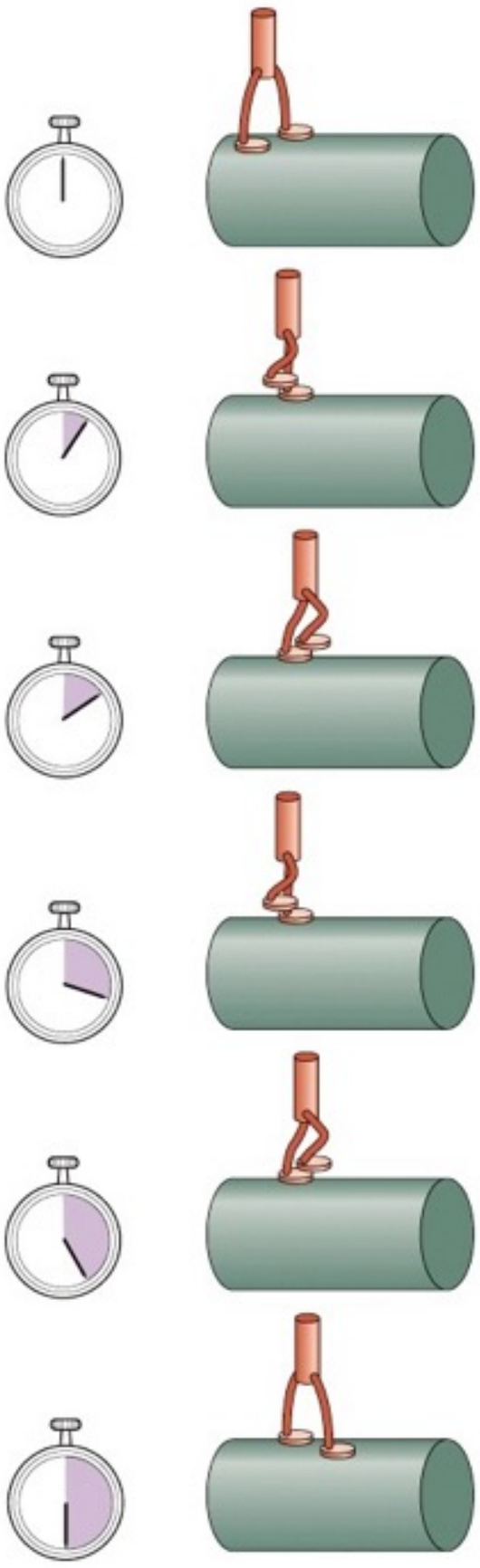


Figure 16.19b Physical Biology of the Cell, 2ed. (© Garland Science 2013)

molecular motors move in fits and starts, in contrast to **macroscopic** ones that appear to move continuously forward

ultimately, they are **stochastic**, due (as usual) to the large effect of thermal forces at molecular scales

biased random walkers, they use energy to **rectify** brownian motion (*driven diffusion*)

"two steps forward, one step back"

PBoC 16.2

Four broad classes

Translational *(ex. myosin)*

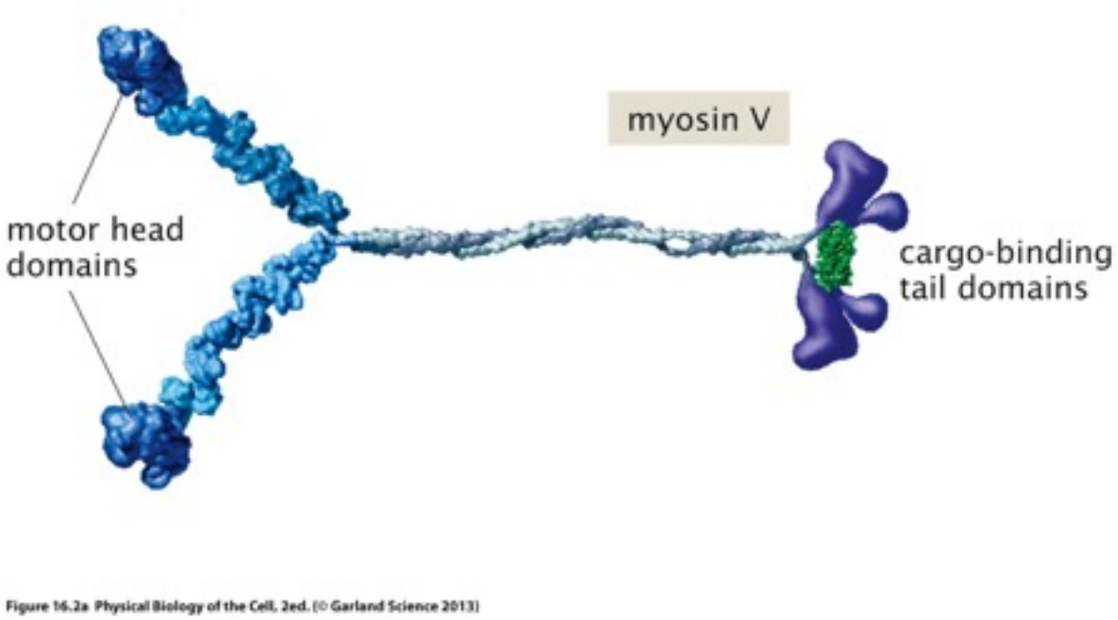
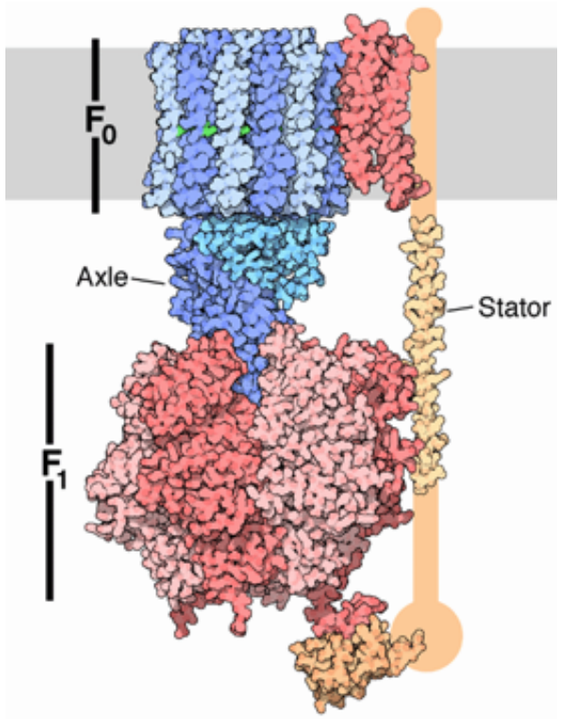


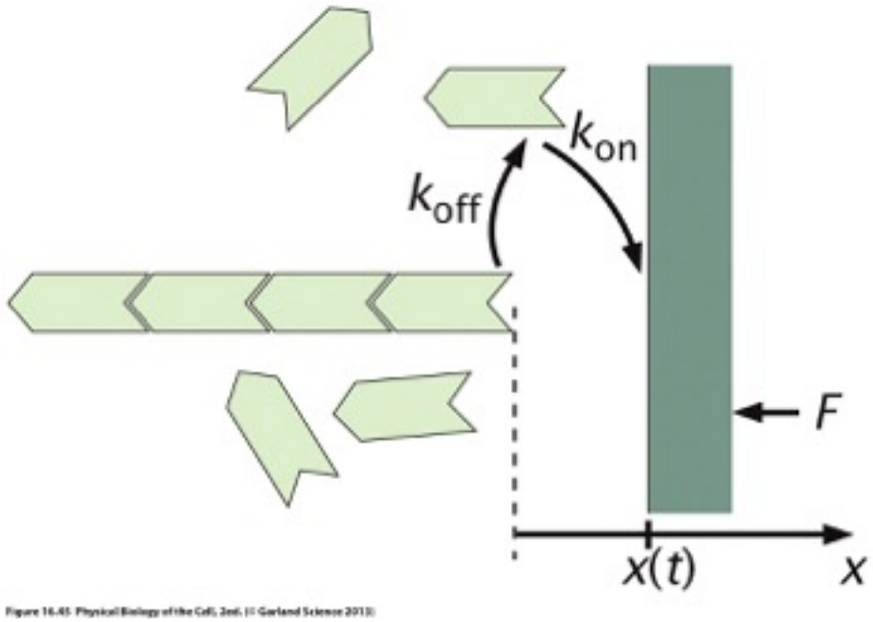
Figure 16.2a Physical Biology of the Cell, 2ed. (© Garland Science 2013)



Rotary *(ex. ATP synthase)*

Polymerization *(ex. actin)*

"pushing by growing"

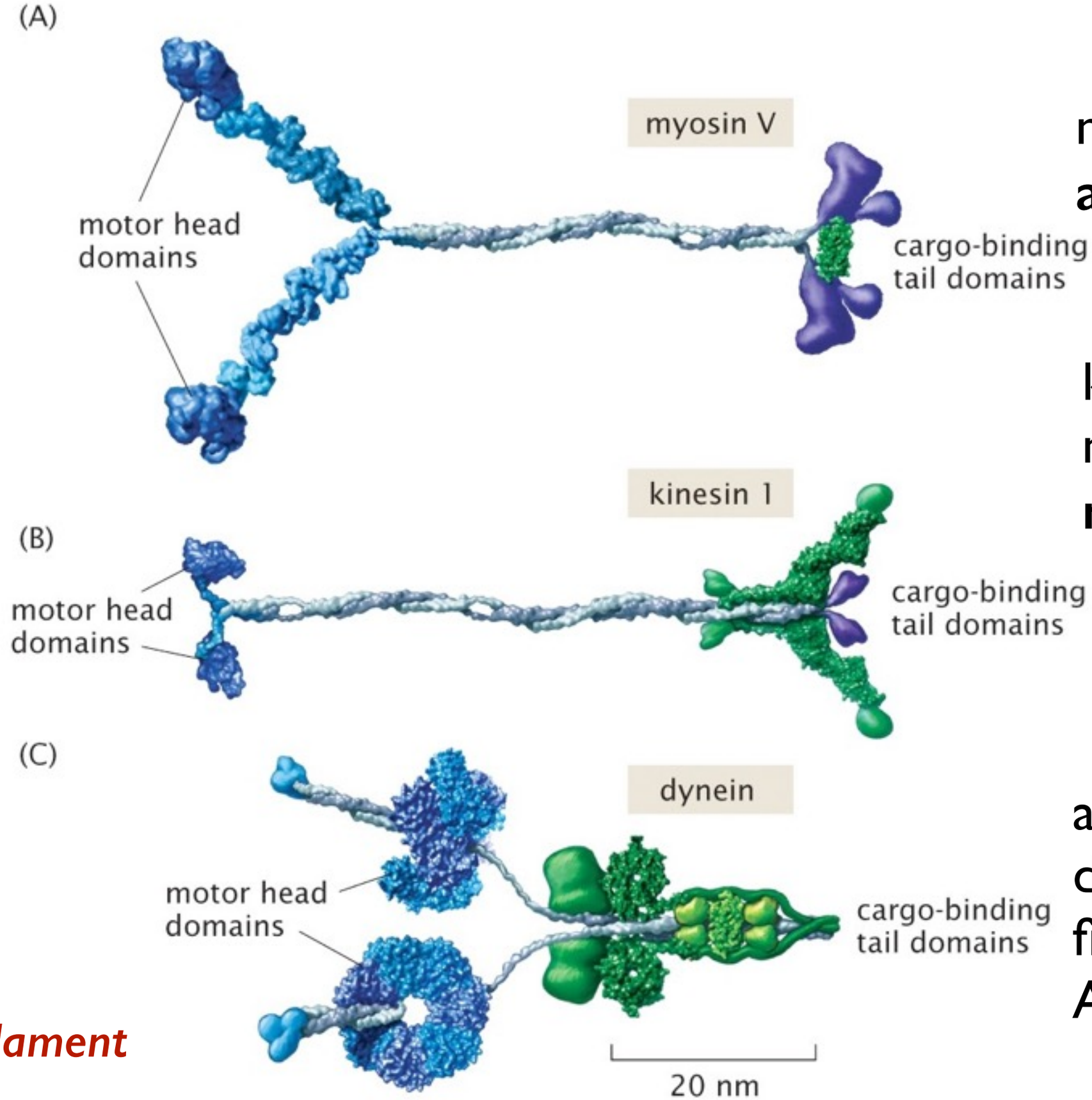


Translocation *(ex. BiP)*

"pushing by pulling"

PBoC 16.1

Translational motors



myosins move along **actin** polymers

kinesins and dyneins move along **microtubules**

all function by taking discrete steps of fixed size, coupled to ATP hydrolysis

filament

PBoC 16.1.1

Figure 16.2 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Translational motors: dynein, kinesin

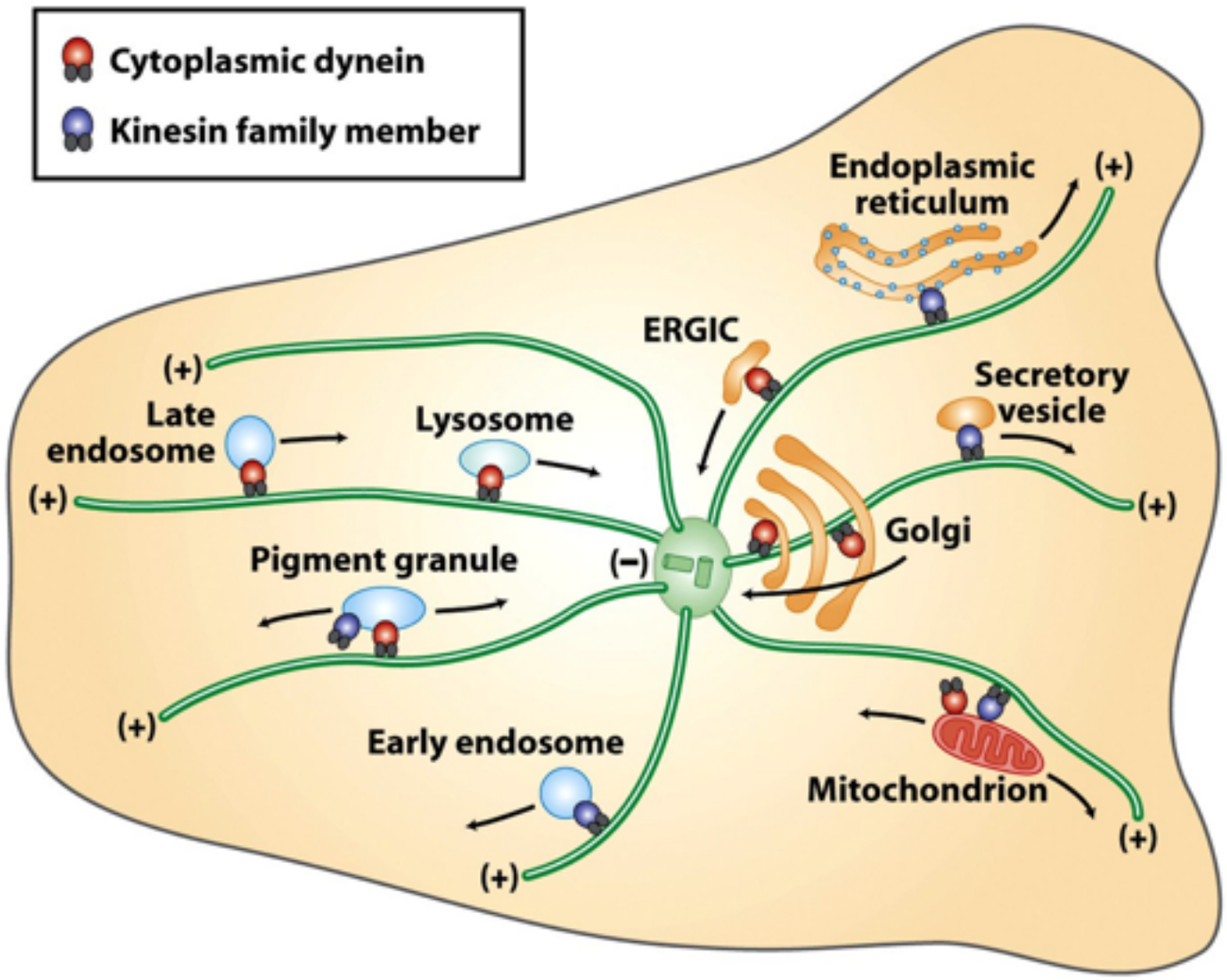


Figure 18-27
Molecular Cell Biology, Sixth Edition

transport large cargos, e.g., vesicles, organelles along **microtubules**, also involved in cell division

kinesins are structurally similar to myosins, dyneins not

most kinesins walk toward **plus end**, i.e., from cell center to periphery

dyneins walk toward the **minus end**, i.e., toward the cell's center

PBoC 16.1.1

translational motor proteins moving along tracks in the cell

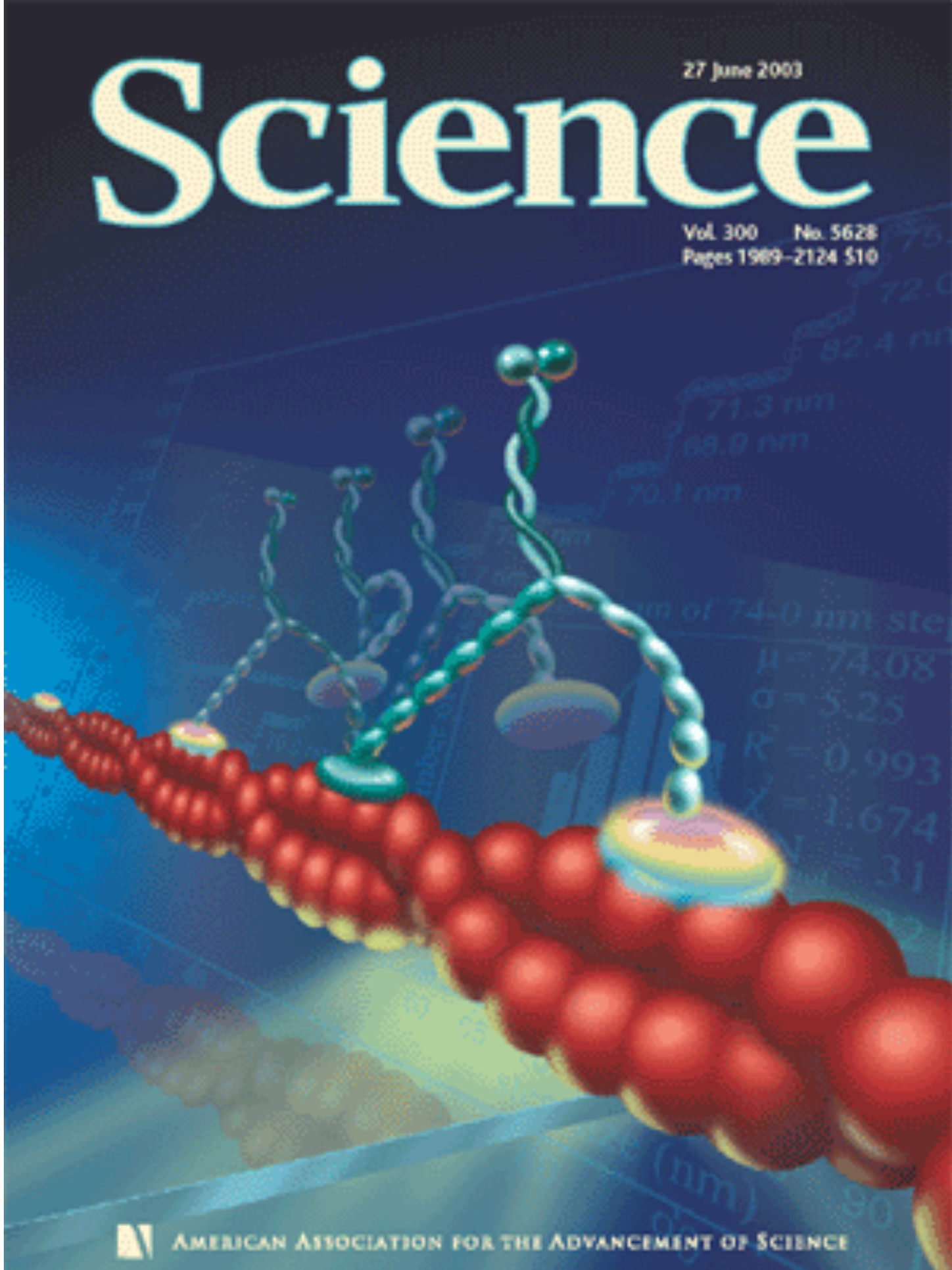
- **Extension of a three-helix bundle domain of myosin VI and key role of calmodulins.** Yanxin Liu, Jen Hsin, HyeongJun Kim, Paul R Selvin, and Klaus Schulten. *Biophysical Journal*, 100:2964-2973, 2011.

further evidence that motor proteins = attractive covers

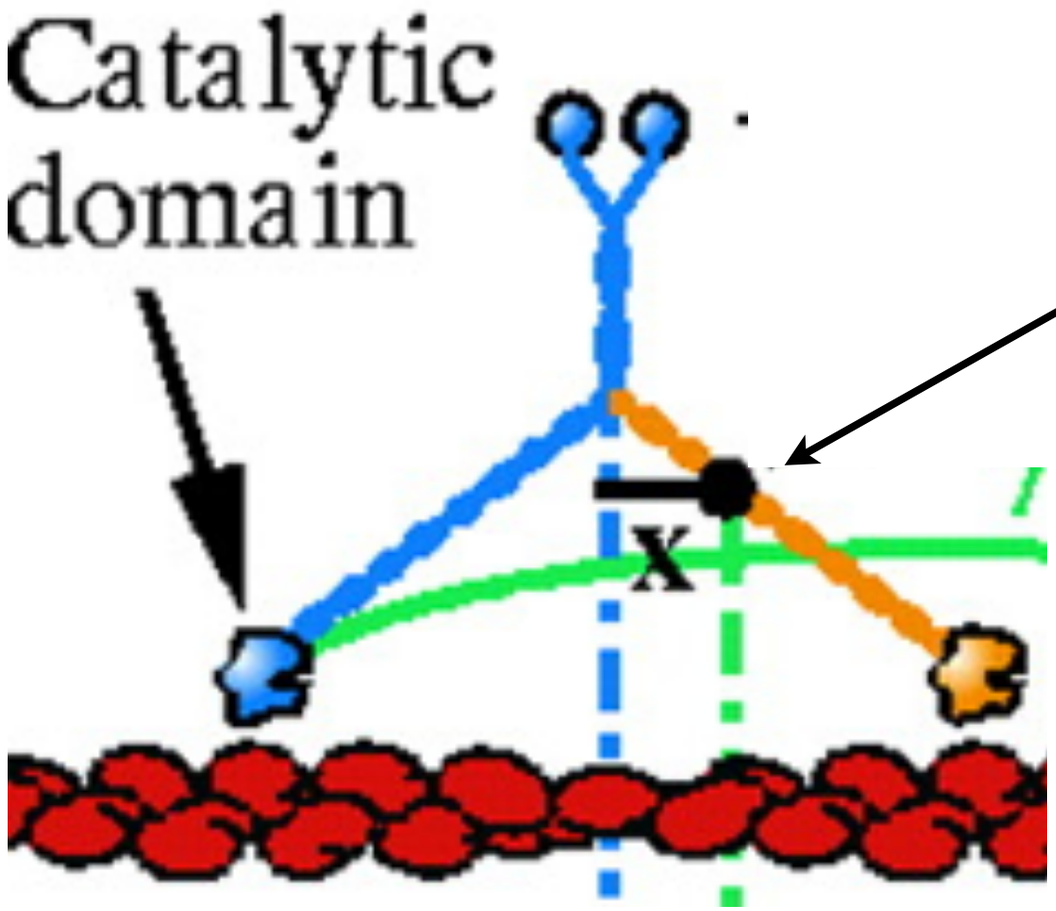
Myosin V Walks Hand-Over-Hand: Single Fluorophore Imaging with 1.5-nm Localization

Ahmet Yildiz, Joseph N. Forkey, Sean A. McKinney, Taekjip Ha, Yale E. Goldman, and Paul R. Selvin

Science 27 June 2003: 2061-2065.



how myosin moves

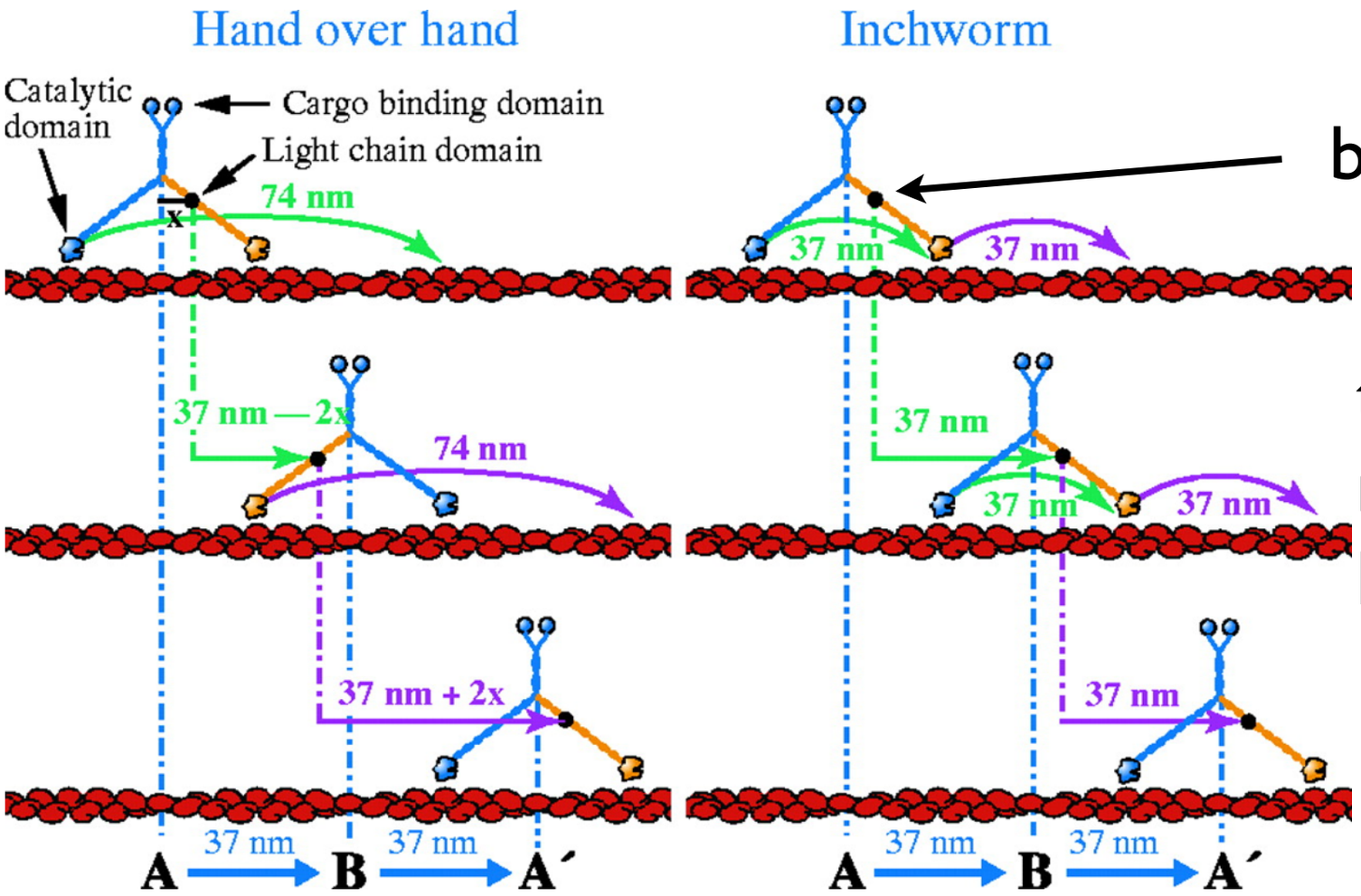


black dot is fluorophore label

two models for how myosin moves along actin, **hand-over-hand** or **inchworm**

let's predict what they see…

how myosin moves



black dot is fluorophore label

two models for how myosin moves along actin, **hand-over-hand** or **inchworm**

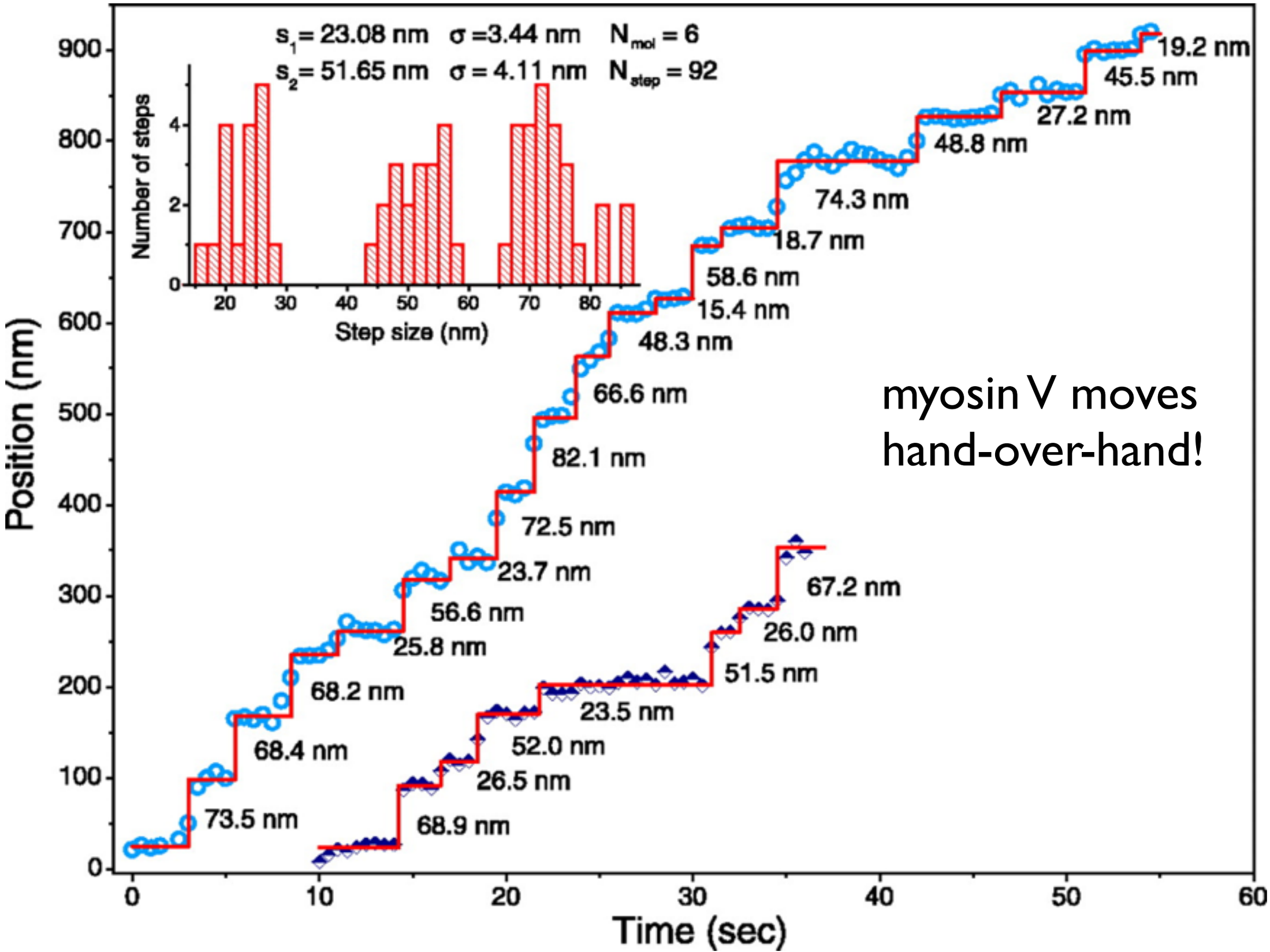
let's predict what they see…

hand-over-hand predicts **pairs** of jumps that **sum** to 74 nm

inchworm model predicts **separate** jumps of 37 nm only

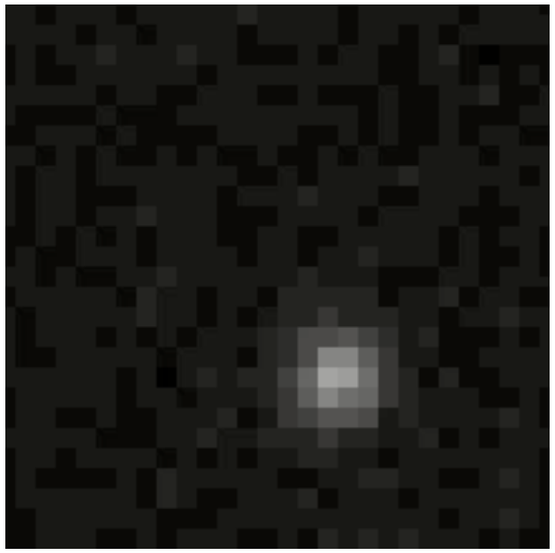
What do the experiments show?

how myosin moves



myosin V moves hand-over-hand!

analyzing the experiments

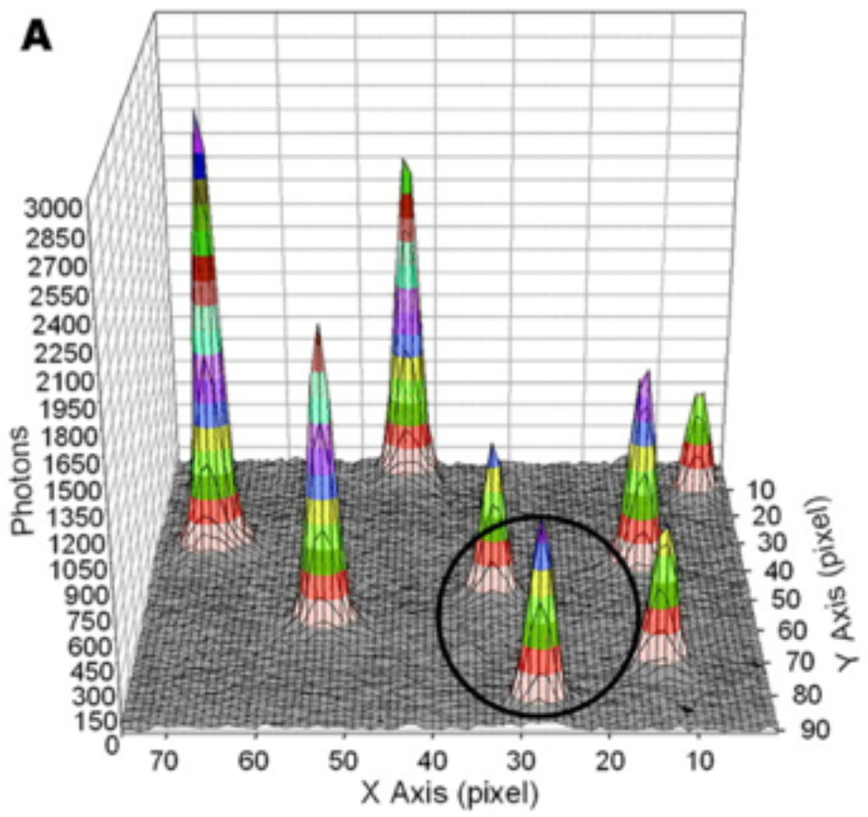


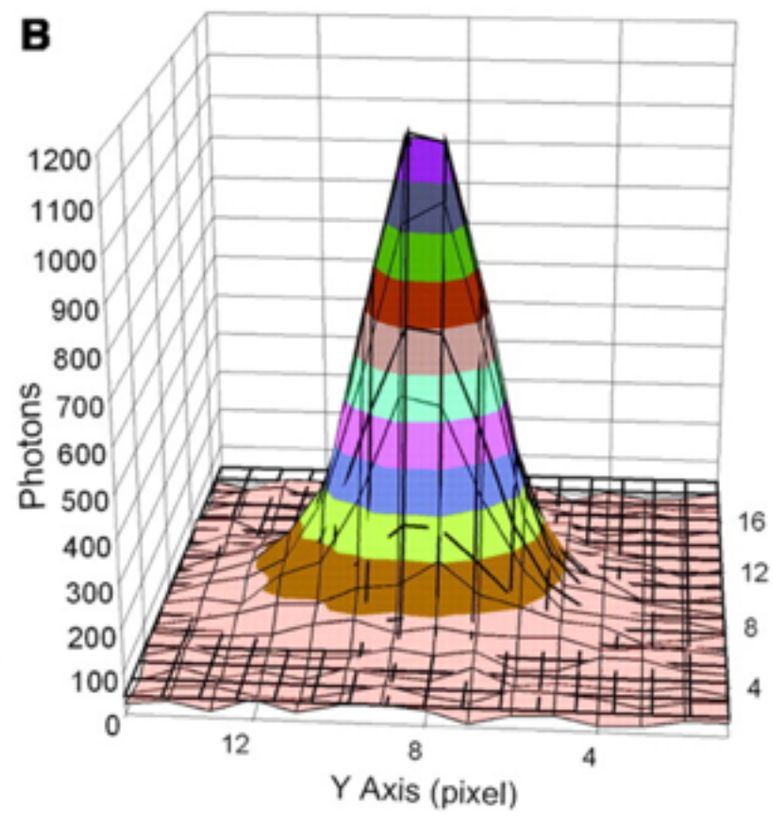
the actual data from the myosin V experiments

FIONA - Fluorescent Imaging with One-Nanometer Accuracy

fit image with a gaussian (point-spread function)

with enough photons, accuracy is **unlimited** (assuming the distribution is correct)

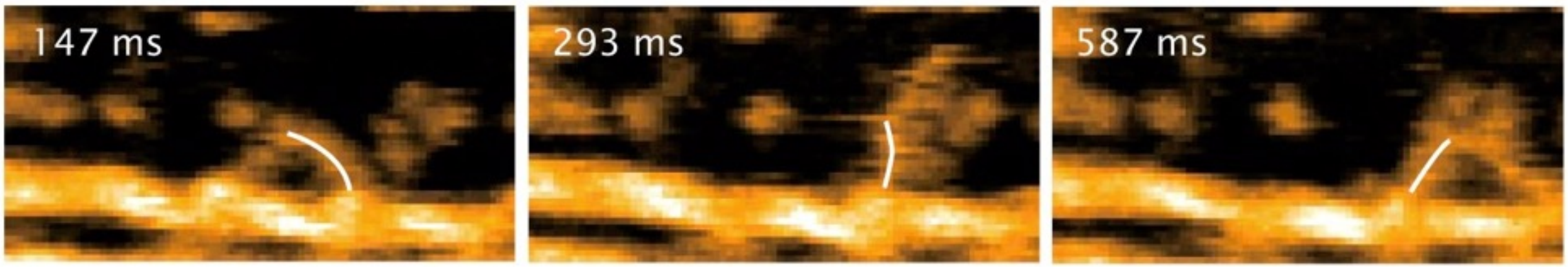




*even for "**real**" experiments, everything is rooted in **modeling**!!!*

analyzing the experiments

High-speed AFM experiments - do you see the myosin?

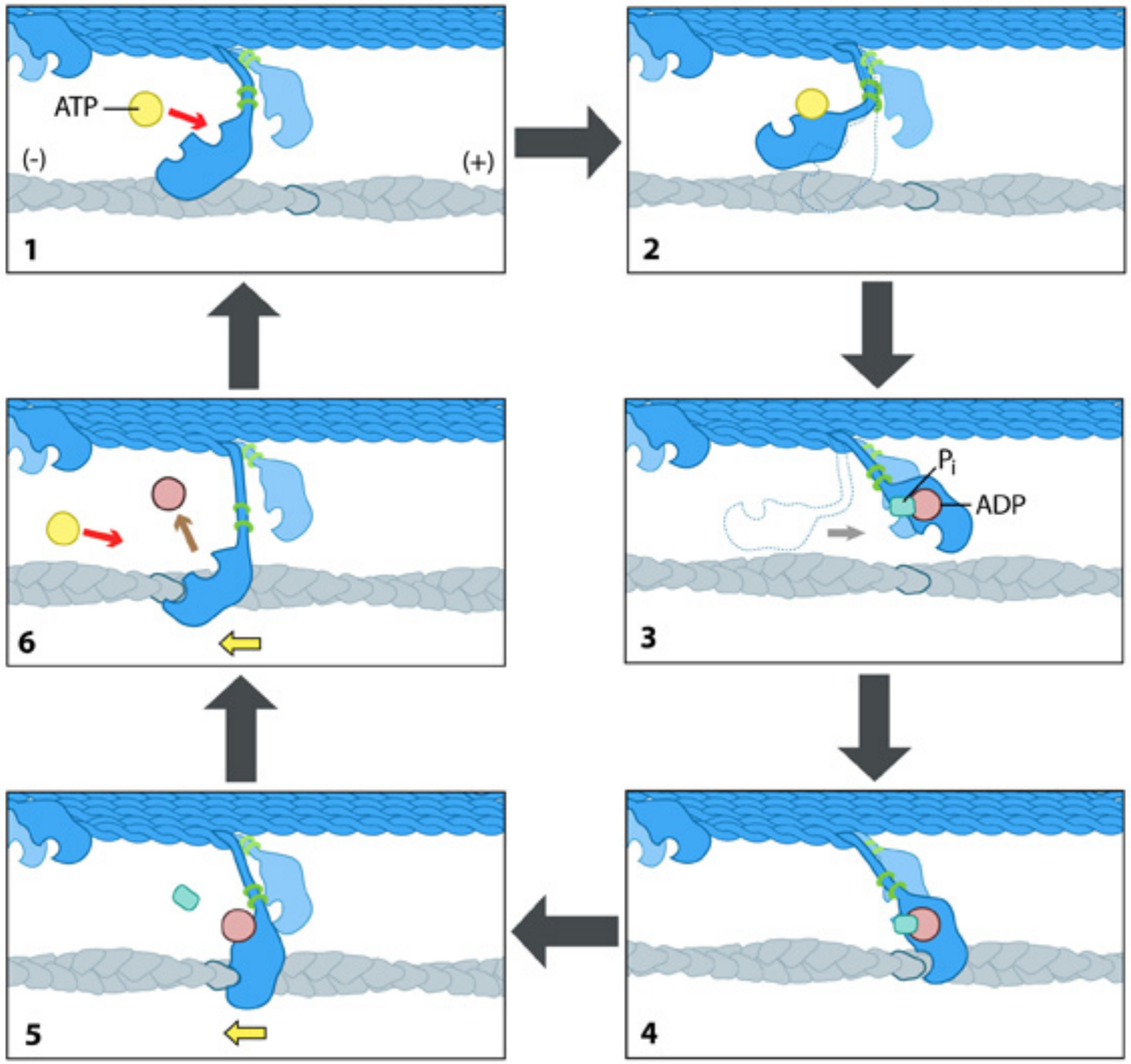


N. Kodera et al. *Nature* 468:72, 2010.

PBoC 16.2.5

myosin motion involves distinct states

head is in rigor state (no ATP or products), bound to actin



ATP binds, head releases and bends forward

ATP is hydrolyzed

head contacts actin again

release of phosphate triggers "power stroke", head moves to original position

ADP is released

http://www.mechanobio.info/modules/go-0008570/2_go-0008570

How many distinct states?

Figure 16.35 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

each step is itself an independent stochastic process that takes an average time τ to occur, e.g., ATP binding, ADP unbinding, etc.

For a single rate-limiting step:

$$p(t) = \frac{1}{\langle t \rangle} e^{-t/\langle t \rangle}$$

For two steps, $p(t)$ is the **convolution** of two independent probabilities

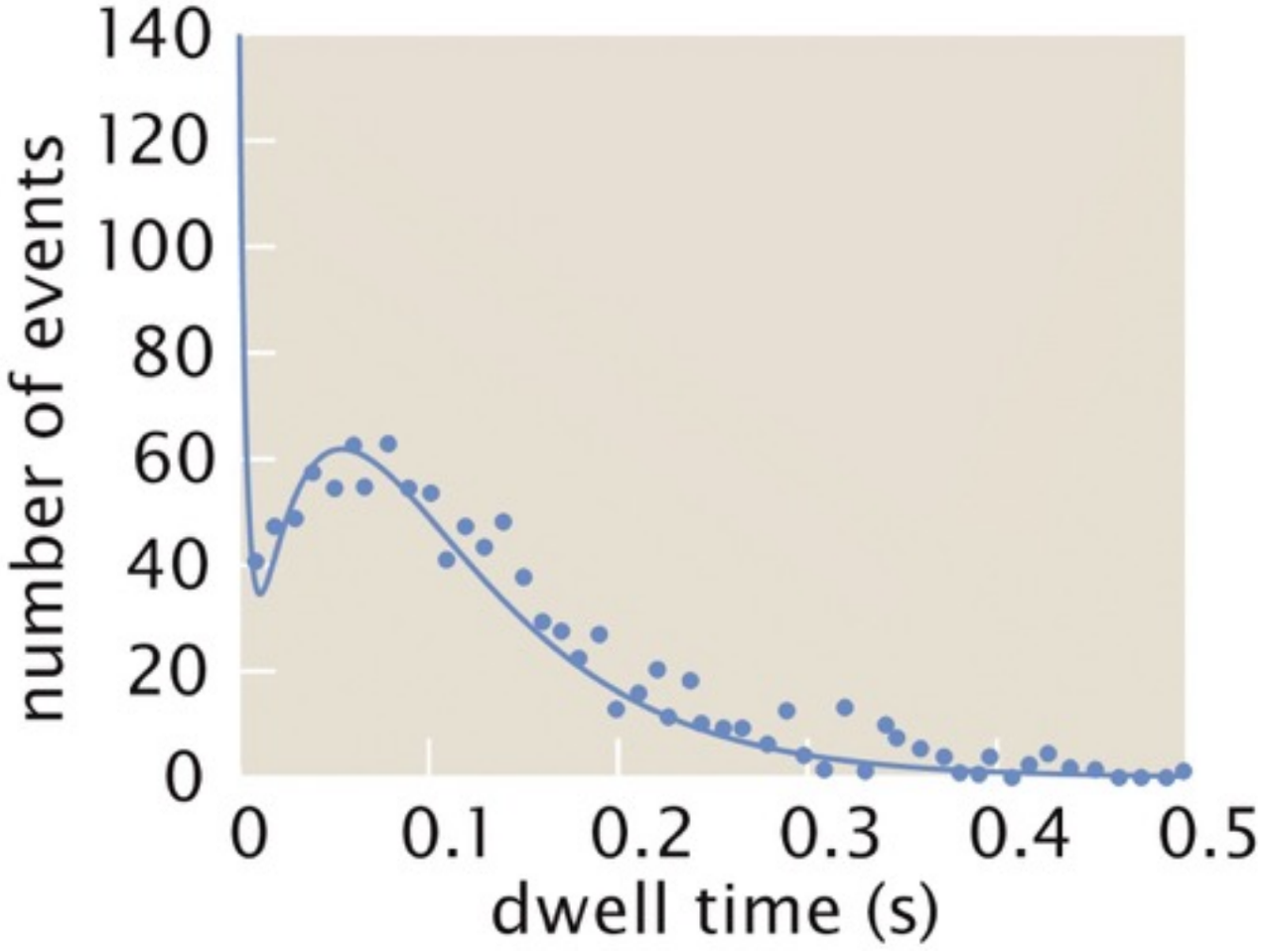
$$p(t) = \int_0^t p_A(\tau) p_B(t - \tau) d\tau$$

PBoC 16.2.4

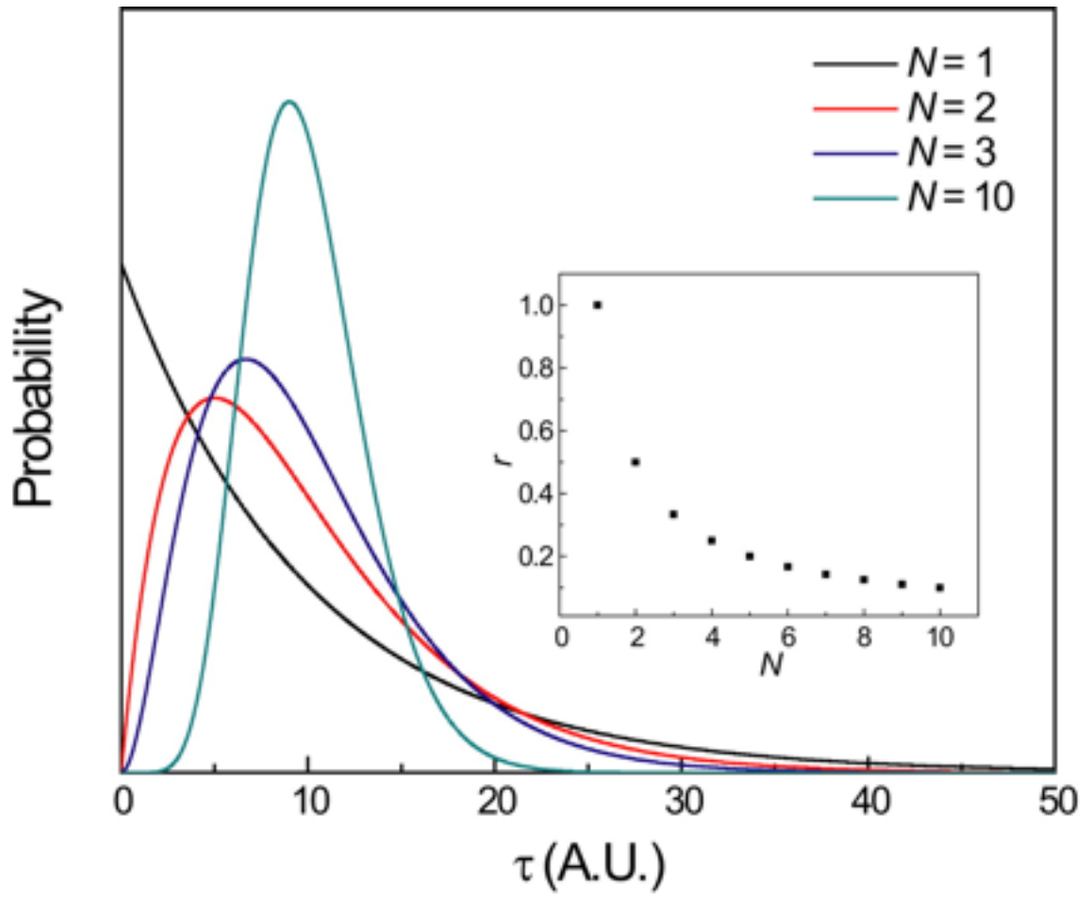
How many distinct states?

$$p(t) = \frac{1}{\tau_B - \tau_A}\left(e^{-t/\tau_B} - e^{-t/\tau_A}\right)$$

Shape of curve and resulting fit can reveal the number of intermediate states, even if hidden from direct observation



*dwell time distribution for **single**-headed myosin V with two-state model fit*



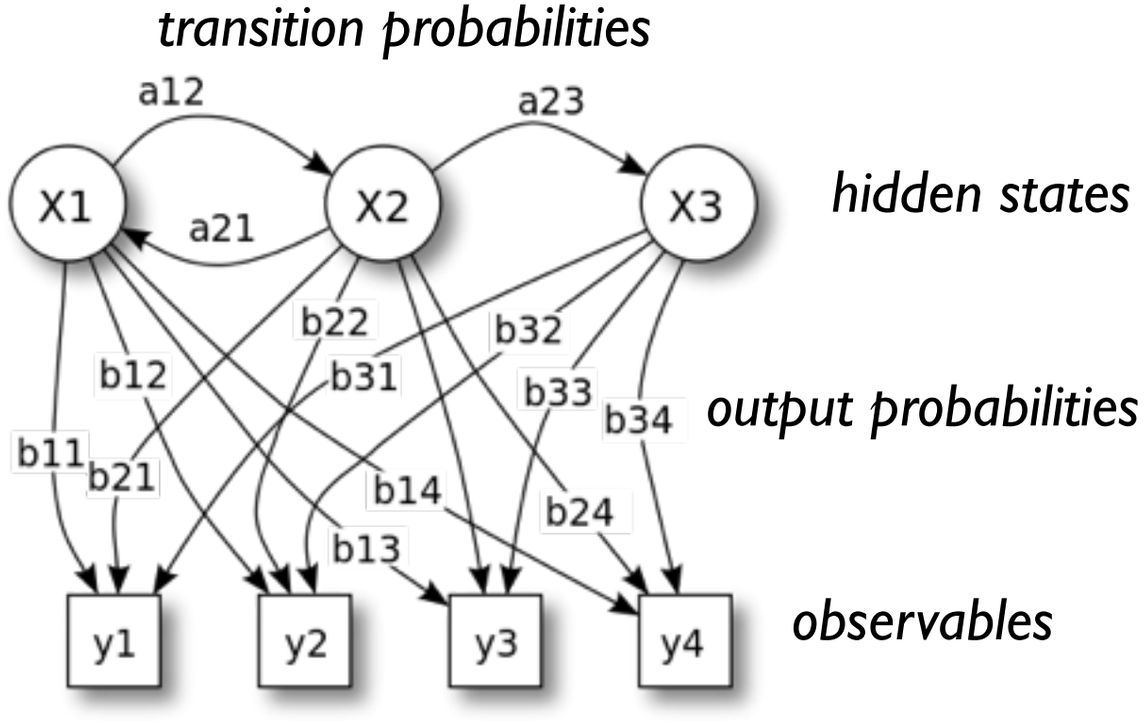
DL Floyd, SC. Harrison, AM van Oijen. Analysis of Kinetic Intermediates in Single-Particle Dwell-Time Distributions. (2010) *Biophys. J.* **99**:360-366.

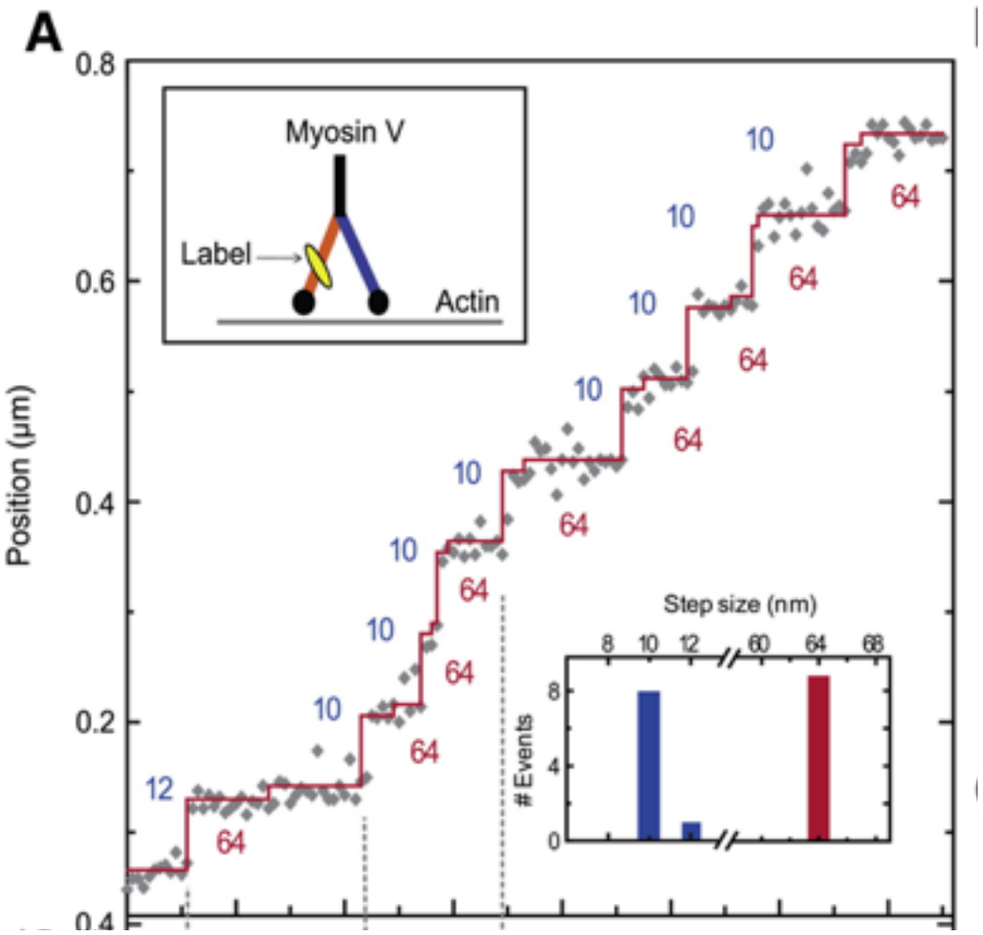
PBoC 16.2.4

hidden Markov models (HMM)

An HMM is a sequence of **hidden** states and corresponding probabilities underlying a connected sequence of observations

Goal is to solve for the most likely hidden states that explain the observed sequence





Ex: Myosin V stepping, don't know dwell times, have only trace of position vs. time

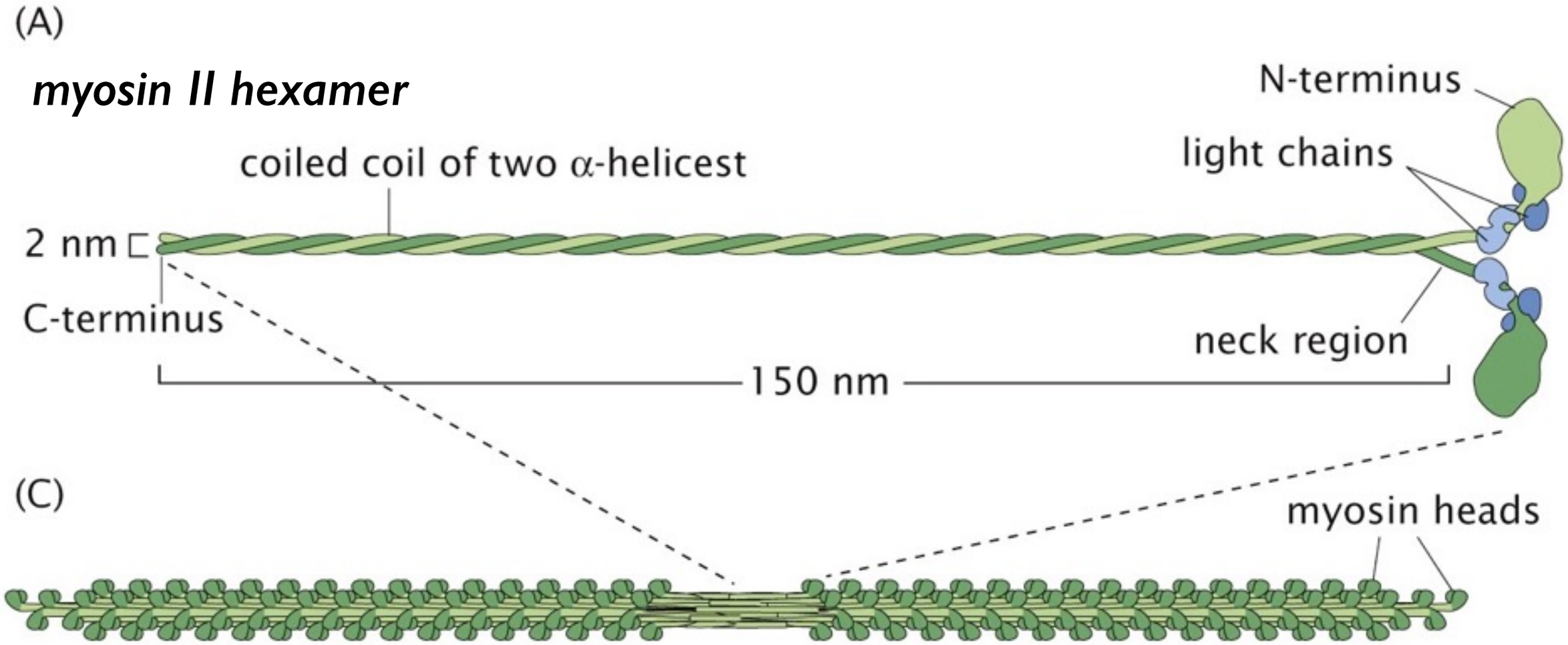
build a model parameterized by (1) *n* kinetic states (hidden) and (2) *m* step sizes

HMM solution reveals two discrete step sizes, allows reconstruction of noise-less trace w/dwell times (**red**)

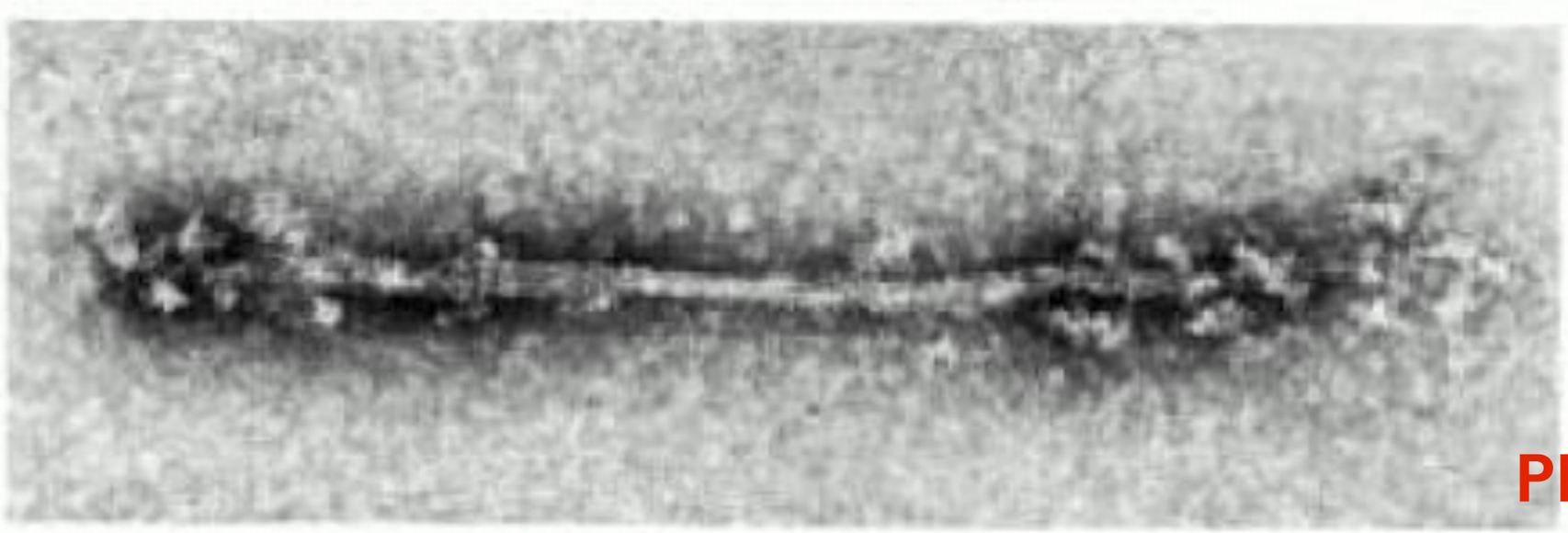
Mullner, Syed, Selvin, Sigworth. Improved Hidden Markov Models for Molecular Motors, Part 1: Basic Theory. (2010) *Biophys. J.* **99**:3684-3695.

Part 2: Extensions and Application to Experimental Data. (2010) *Biophys. J.* **99**:3696-3703.

muscle contraction

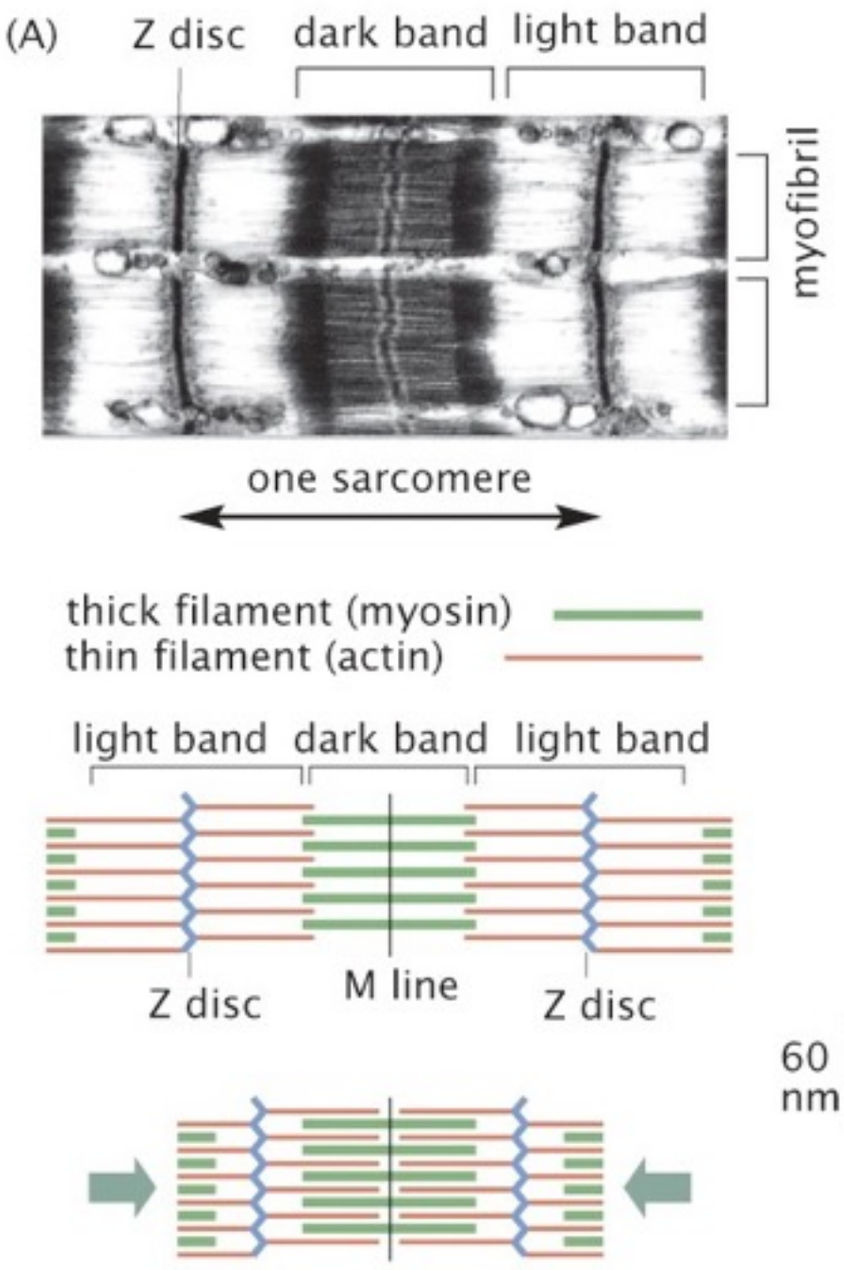


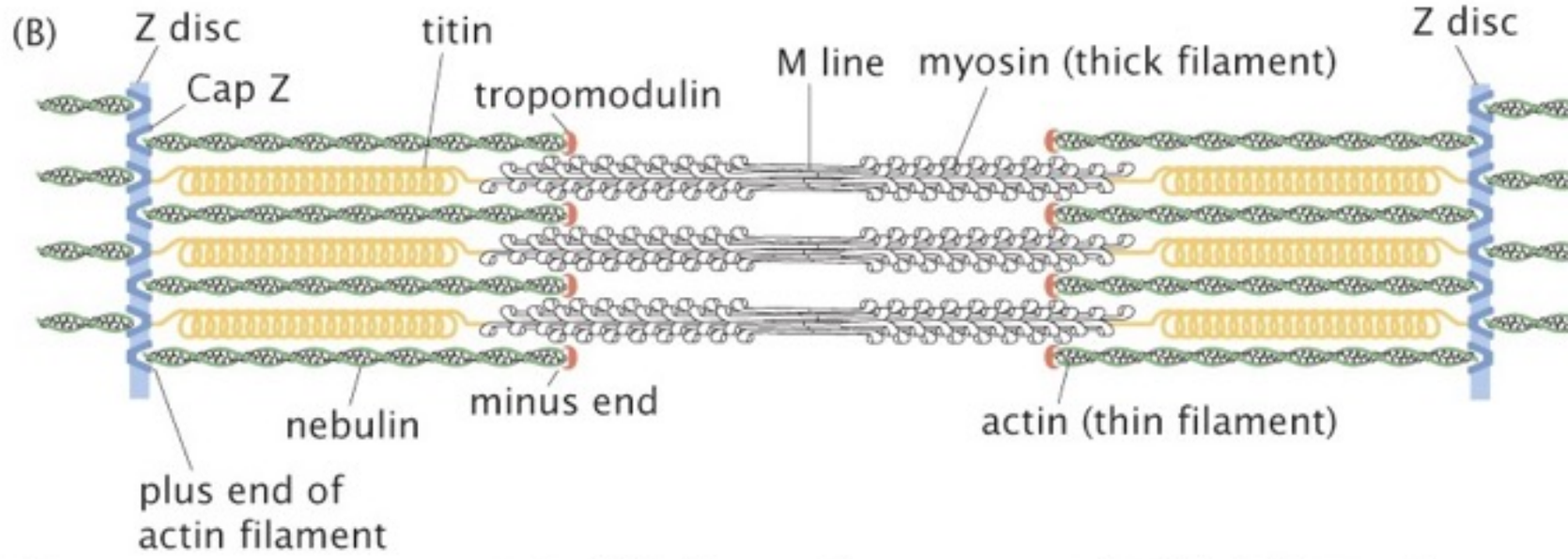
filament of left- and right-pointing myosins



PBoC 16.2.6

muscle contraction





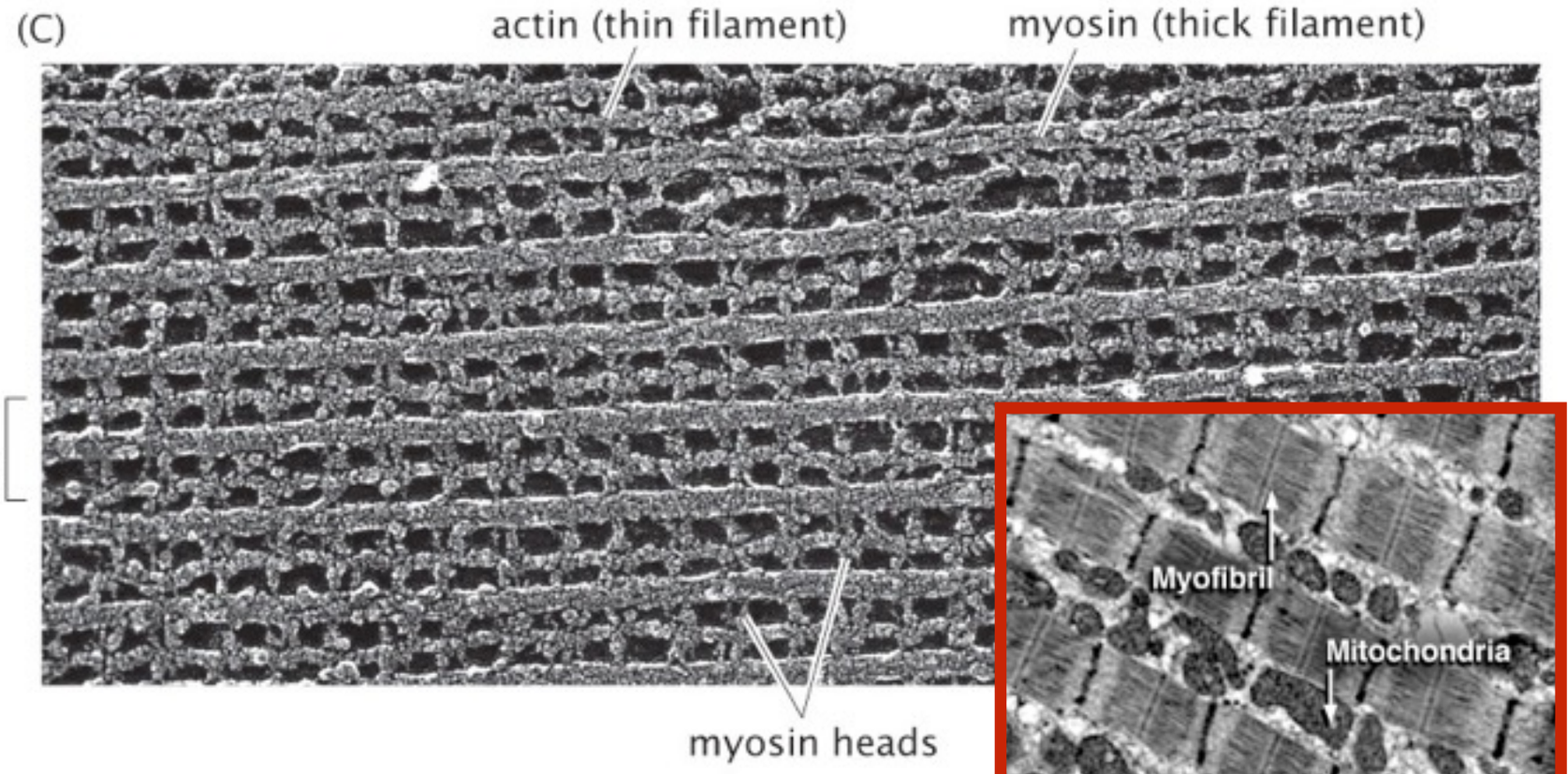
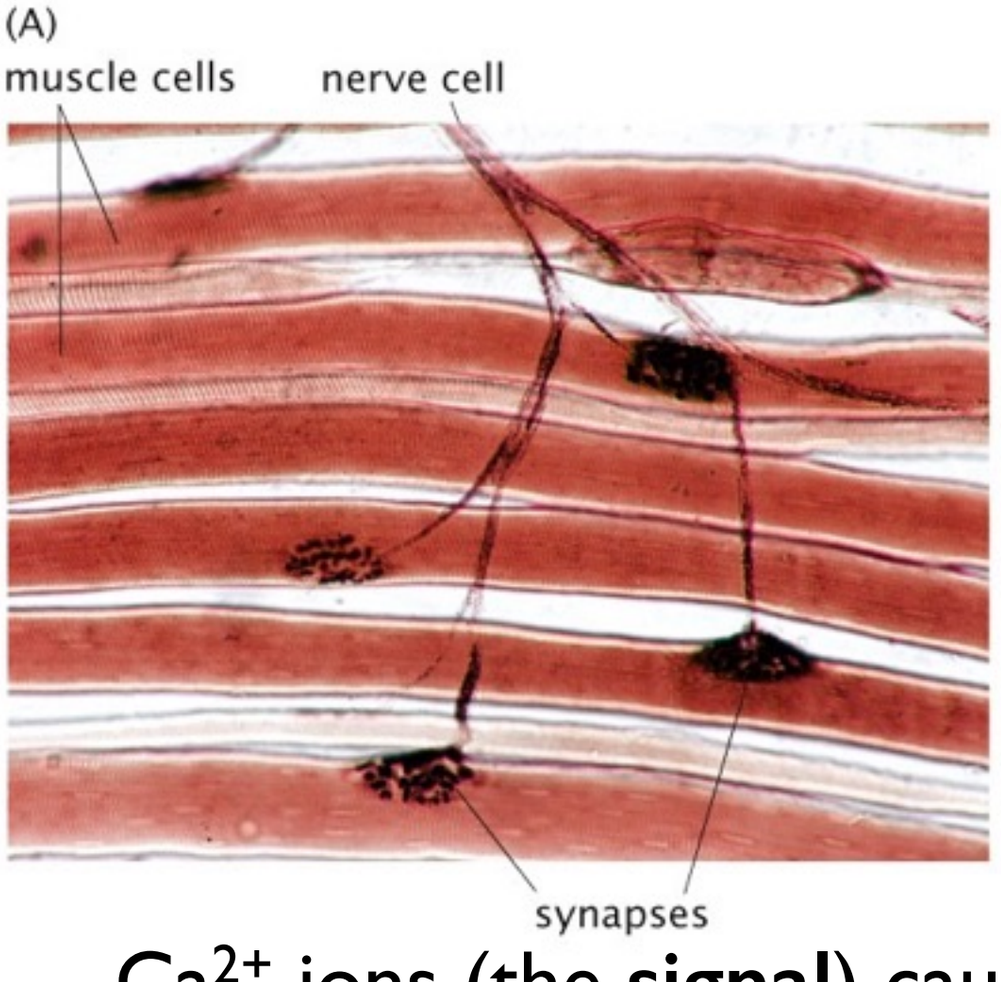


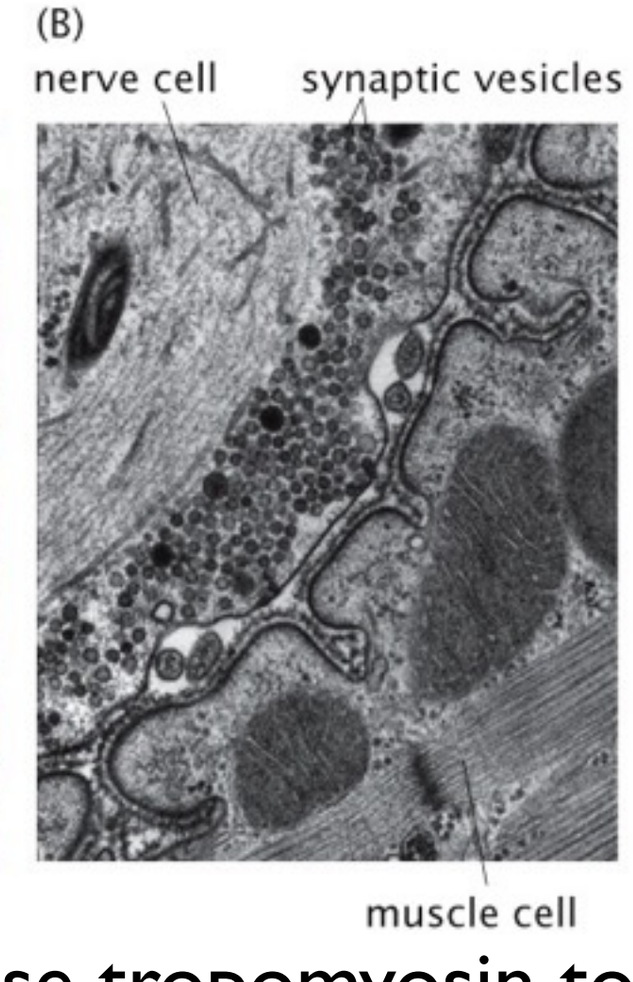
Figure 16.7 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

mediated by three proteins: **myosin** + **actin** (**both** acting as filaments) and **titin** (spring)

PBoC 16.1.1

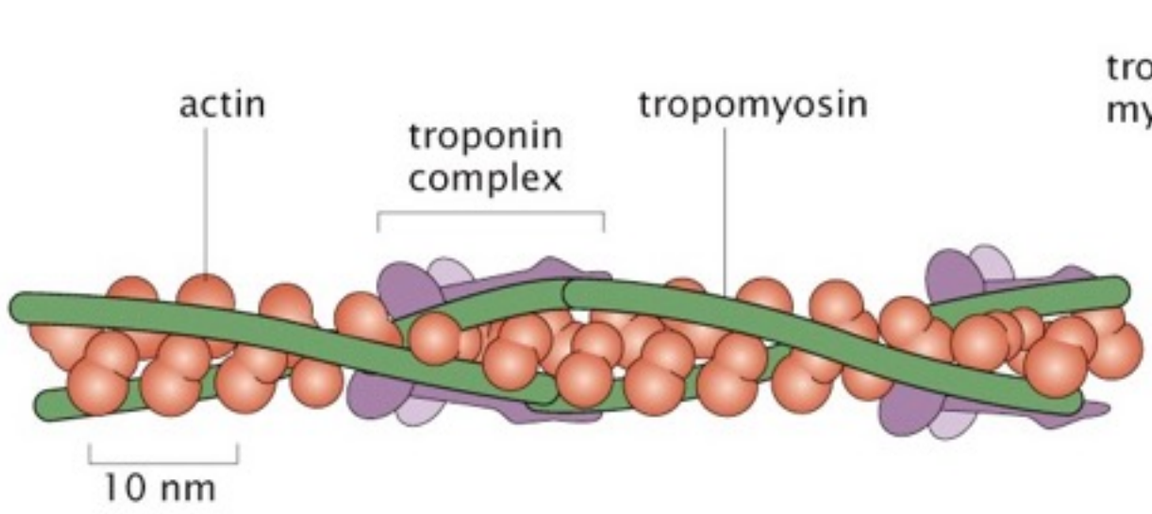
coordinating the signal

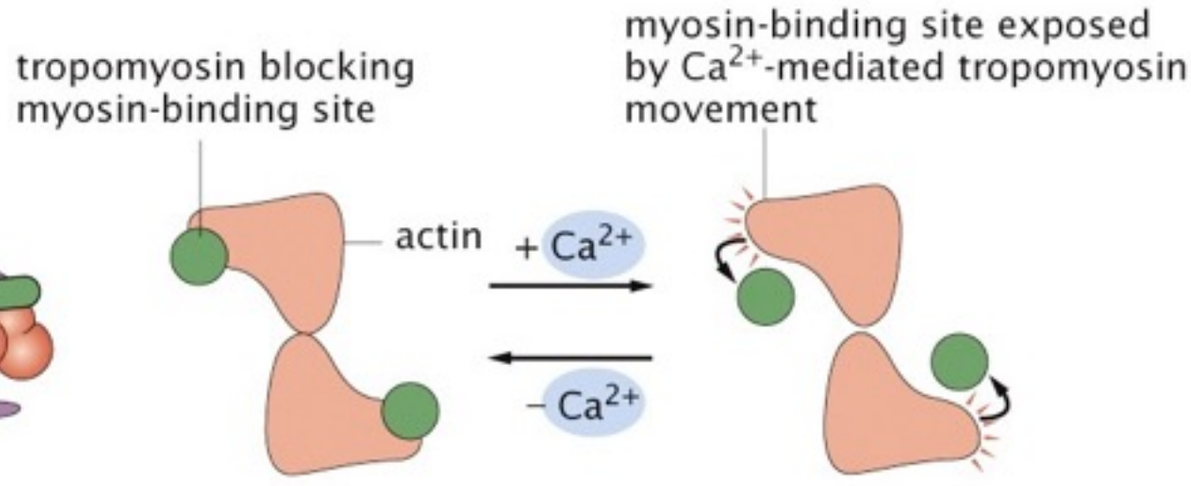




nerve cell releases achetylcholine-containing synaptic vesicles, causes Ca^{2+} channels to open

Ca^{2+} ions (the **signal**) cause tropomyosin to release from actin, exposing myosin-binding sites simultaneously!

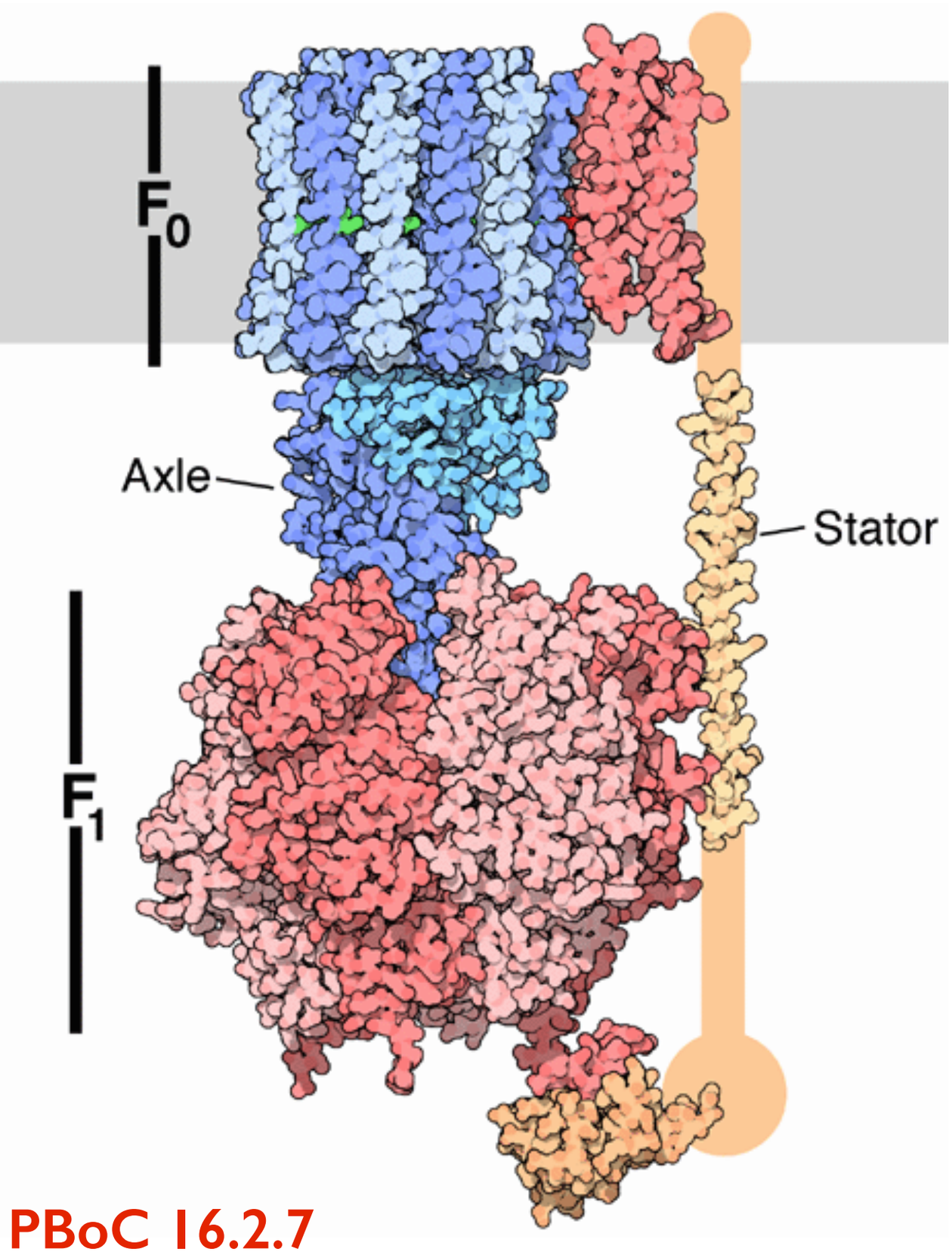




Each myosin moves 11 nm at a rate of ~5/s (55 nm/s), action of hundreds simultaneously increases rate to 8,000 nm/s

PBoC 16.2.6

Rotary motors: ATP synthase



PBoC 16.2.7

ATP is made by two **coupled** rotary motors known as ATP synthase, likely arose through modular evolution

one motor, F_O, sits in the membrane and is driven by a proton gradient (similar to flagellar motor)

the other motor, F_1, is in the cytoplasm and makes (or uses!) ATP (similar to DNA helicase)

the two motors are coupled by an **axle** (rotates) and a **stator** (stationary)

Each motor is *reversible*, one can drive the other!

F_O rotary motor

stator holds **rotor** in place until proton/ion enters from one side through salt bridge (+/- residues)

proton/ion neutralizes charge, rotor then can move freely after which proton/ion leaves to other side

rotation is thermally driven but *rectified* by proton transport across membrane, clockwise/ccw determined by which side proton comes from

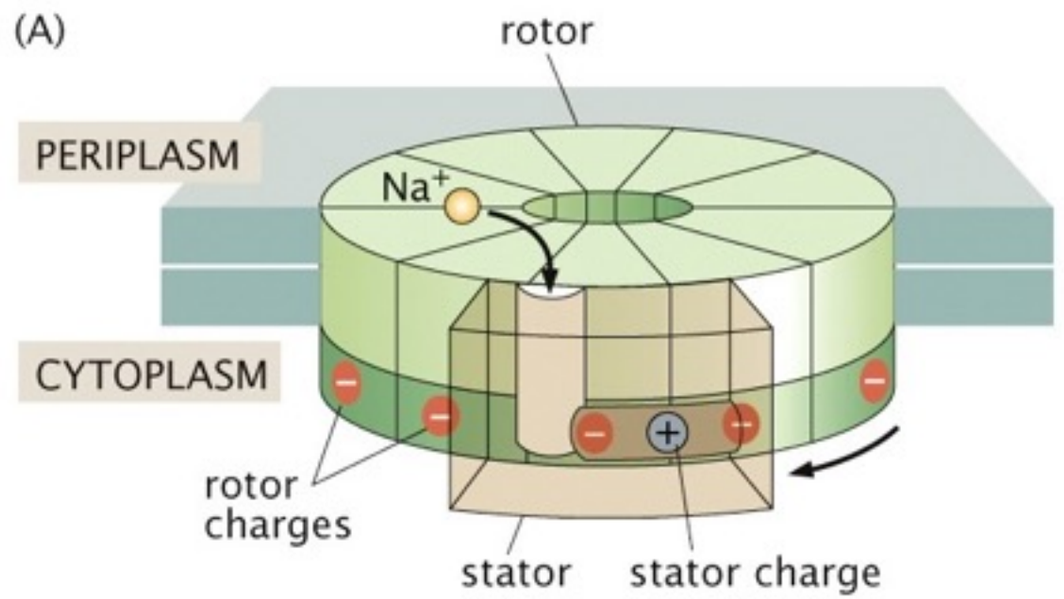
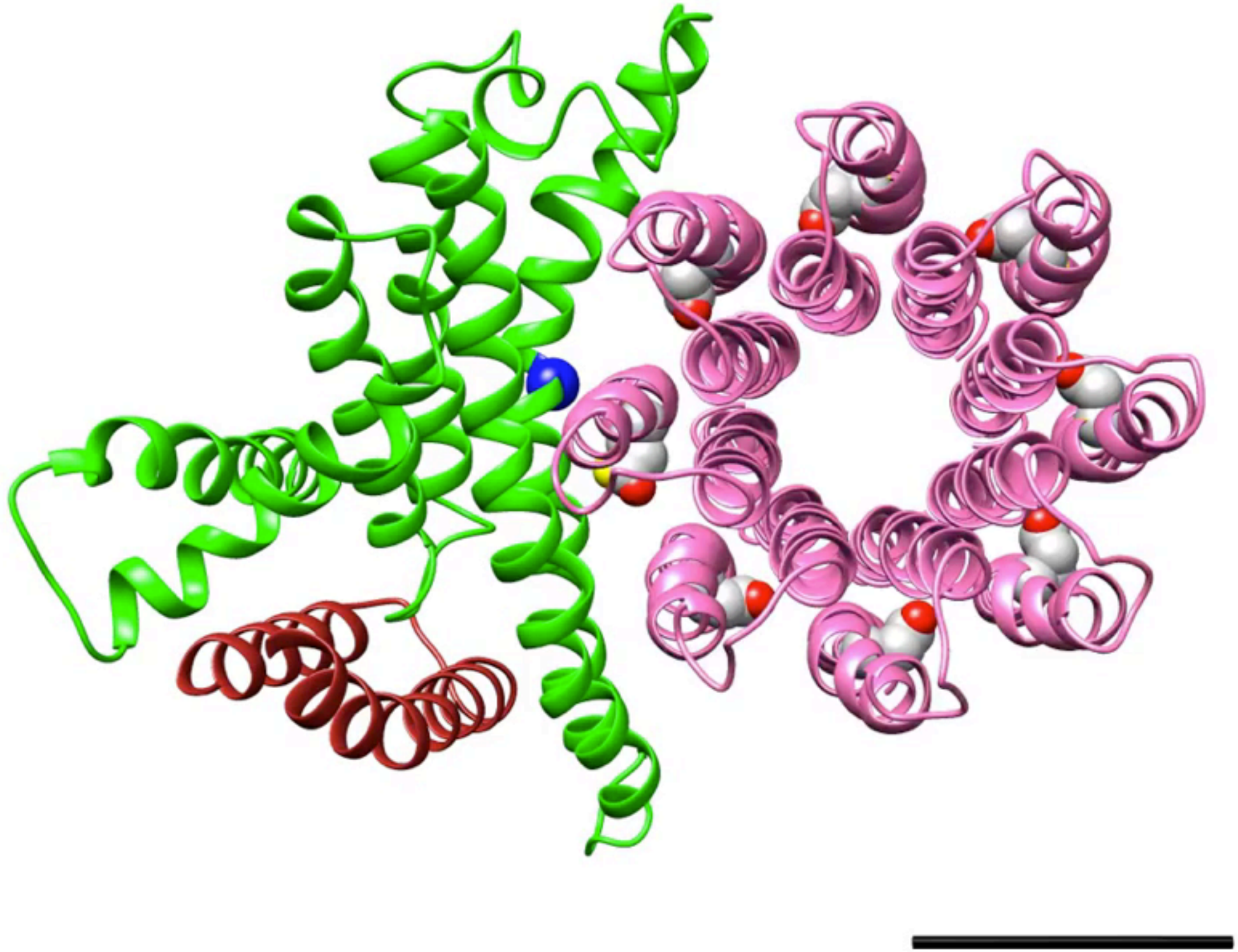


Figure 16.42 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

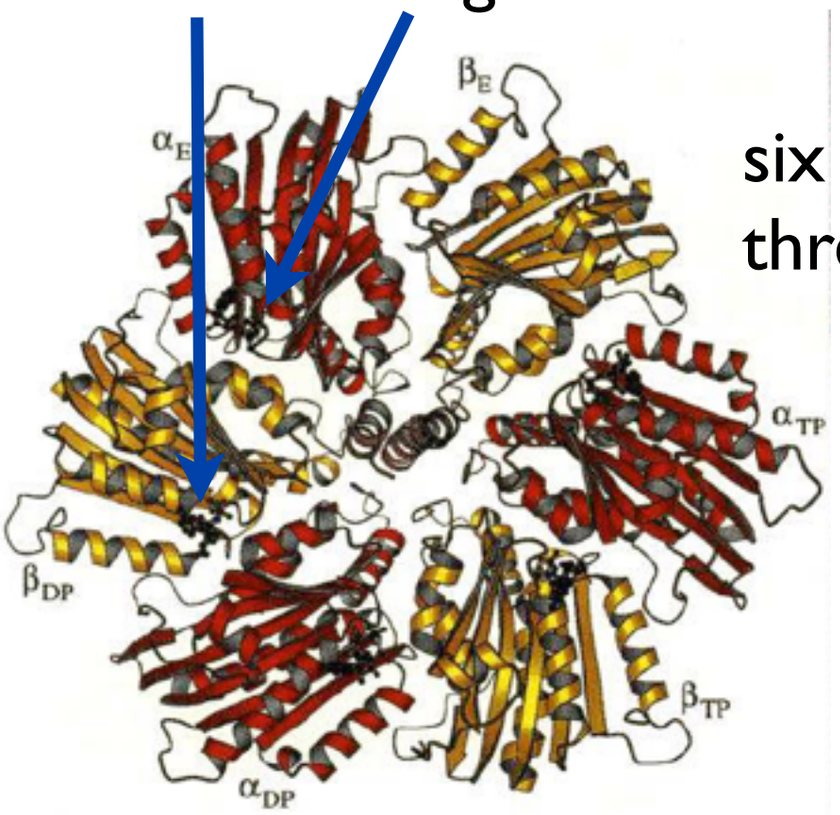
PBoC 16.2.7

F_O rotary motor

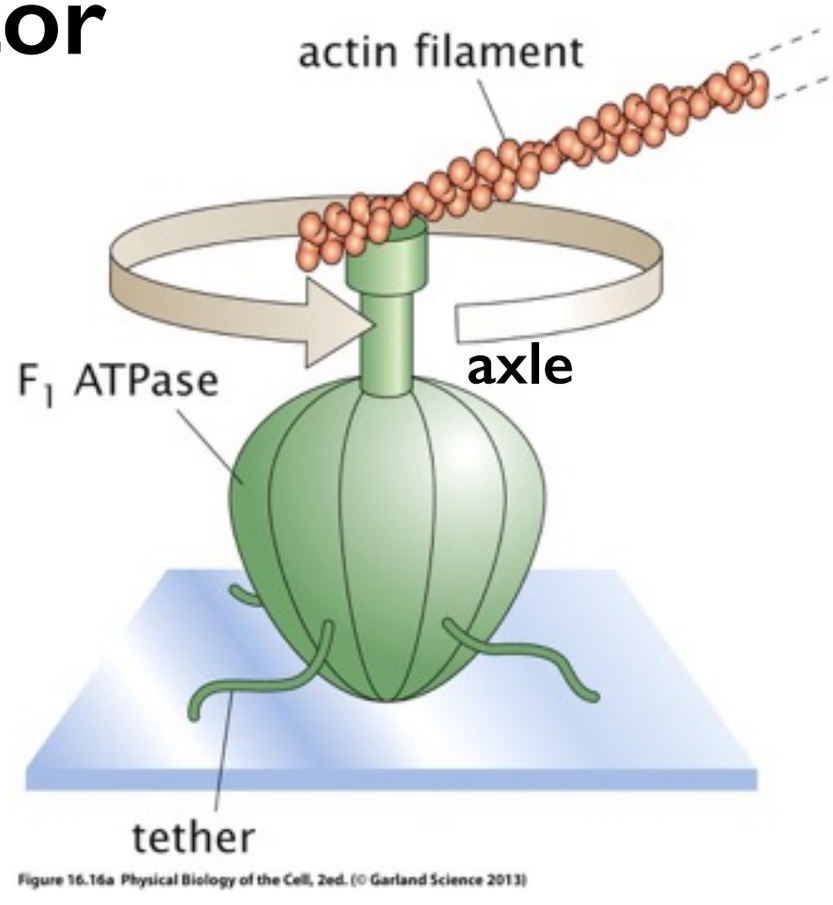


Zhou A, Rohou A, Schep DG, Bason JV, Montgomery MG, Walker JE, Grigorieff N, Rubinstein JL (2015). Structure and conformational states of the bovine mitochondrial ATP synthase by cryo-EM. eLife e10180. doi: 10.7554/eLife.10180.

F_1 rotary motor

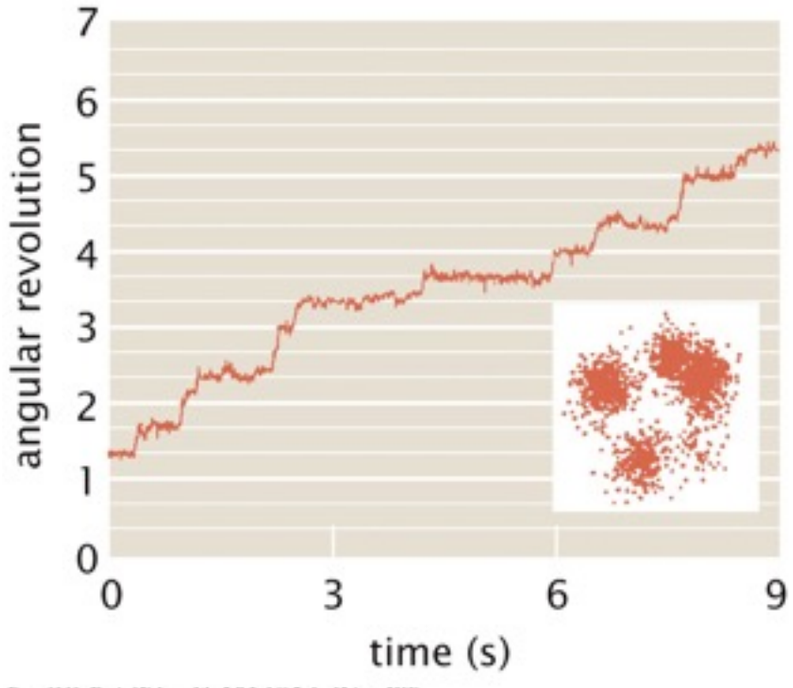


six binding sites, only three (β) are catalytic



b

Hiroyuki Noji, Ryohei Yasuda, Masasuke Yoshida & Kazuhiko Kinosita Jr. (1997) Direct observation of the rotation of F1-ATPase. *Nature*, **386**, 299 - 302.



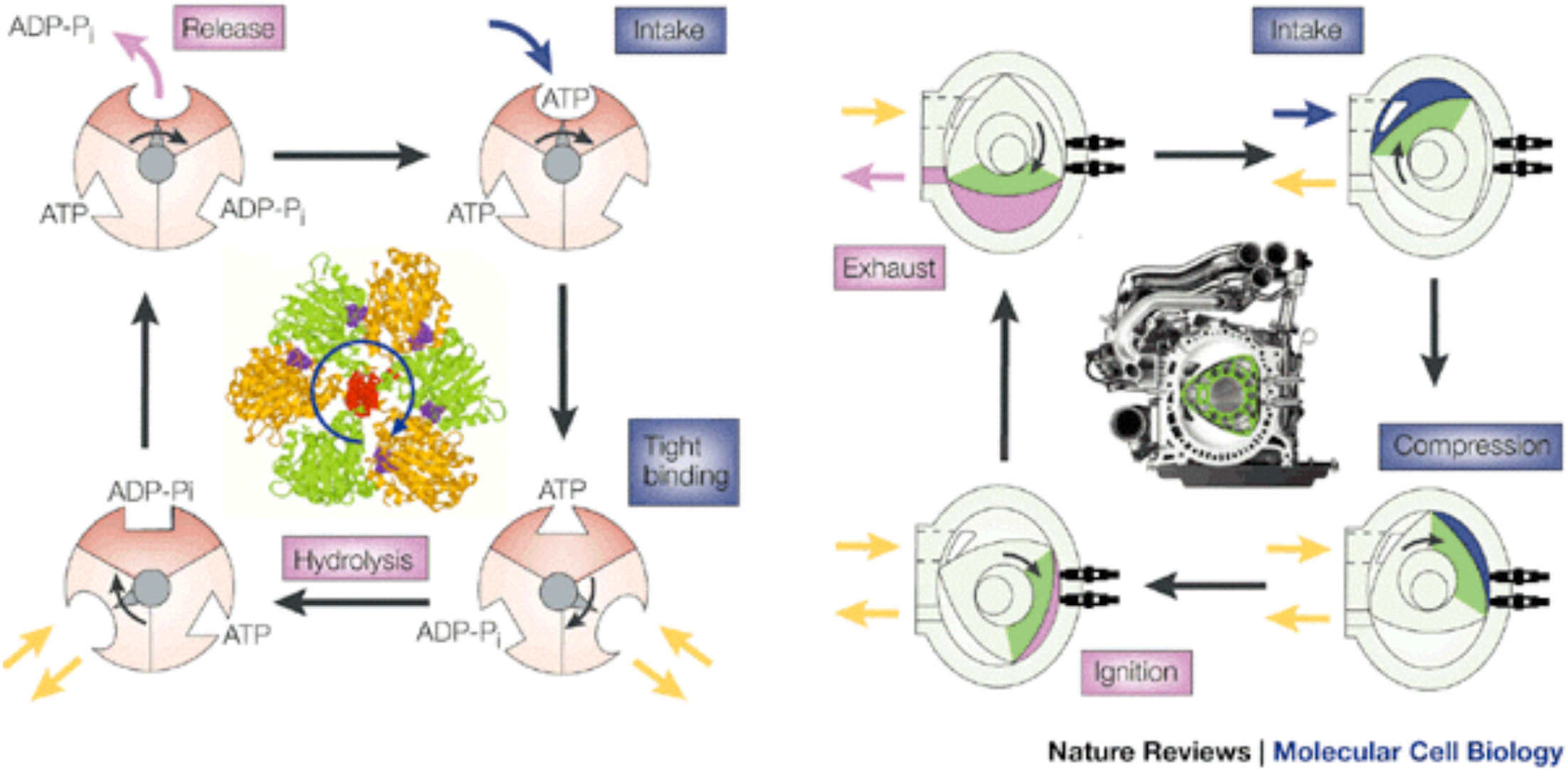
First direct evidence F_1 rotates unidirectionally in discrete (3x) steps

PBoC 16.1.2

F_1 rotary motor

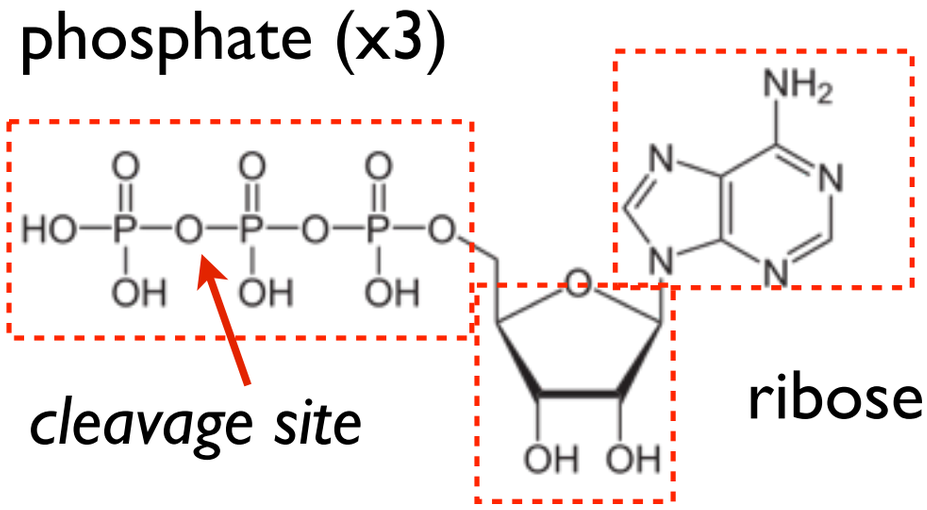
catalytic cycle just like a Mazda rotary combustion engine (convergent evolution???)

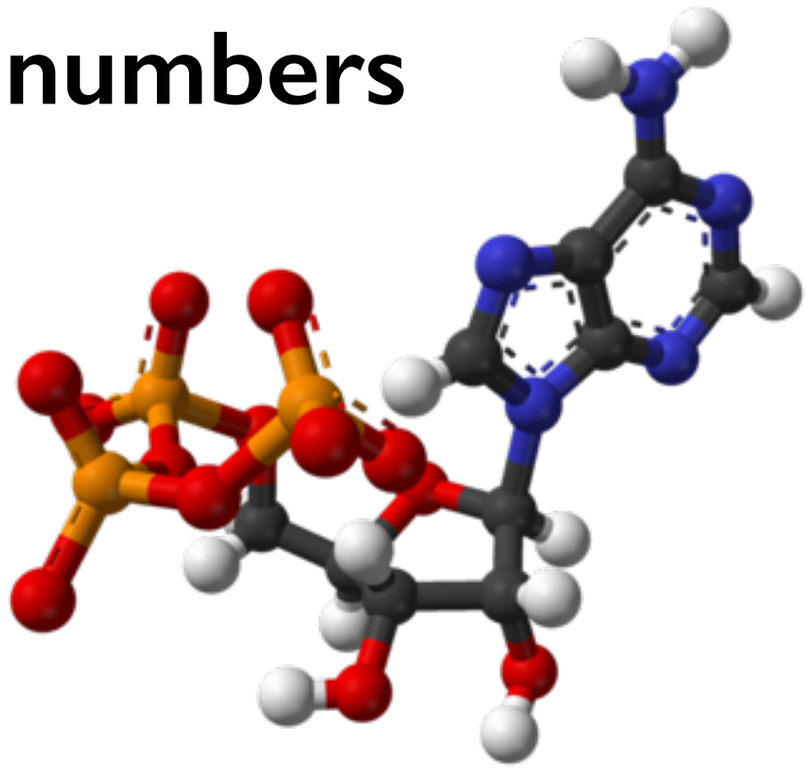
difference is ATP synthase is almost 100% efficient!



ATP synthase — a marvellous rotary engine of the cell Masasuke Yoshida, Eiro Muneyuki and Toru Hisabori *Nature Reviews Molecular Cell Biology* **2**, 669-677 (2001).

ATP synthase by the numbers

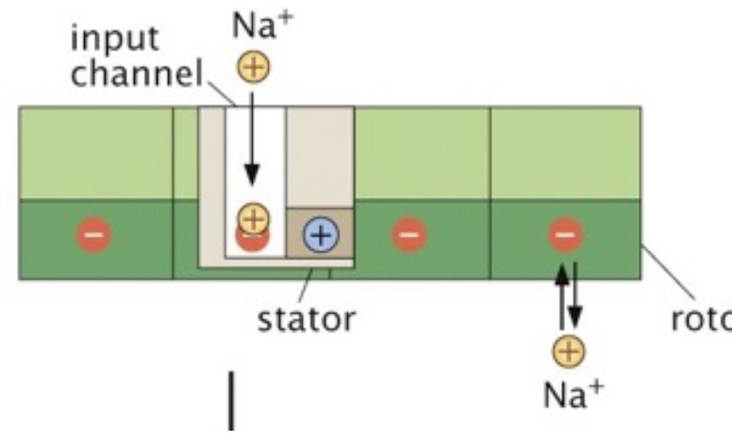


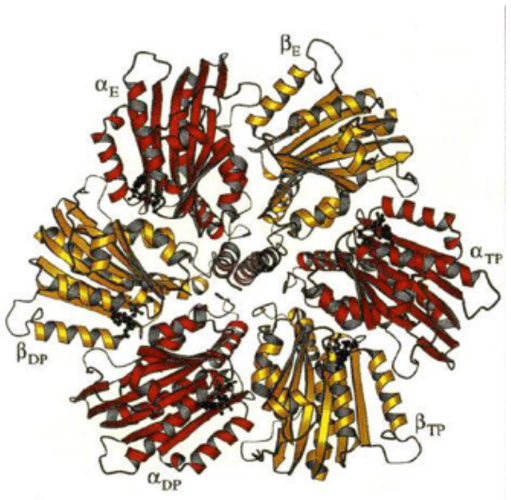


$$\mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{ADP} + \mathrm{P_i}$$

~ -14 kcal/mol in the cell (**23-24 kT**)

typical membrane potential ~ 100 mV
energy gained by moving 1 ion/proton: 0.1 eV = **4 kT**
actual amount is **7 kT,** due to *chemical* (in addition to *electrical*) potential





Each step of the turn (120 degrees) generates 1 ATP
from the numbers above, need at least 3-4 protons to get 1 ATP
and indeed, this is the case (F_o has ~12 subunits)
ATP synthase is practically **100% efficient!!!**

Polymerization motors

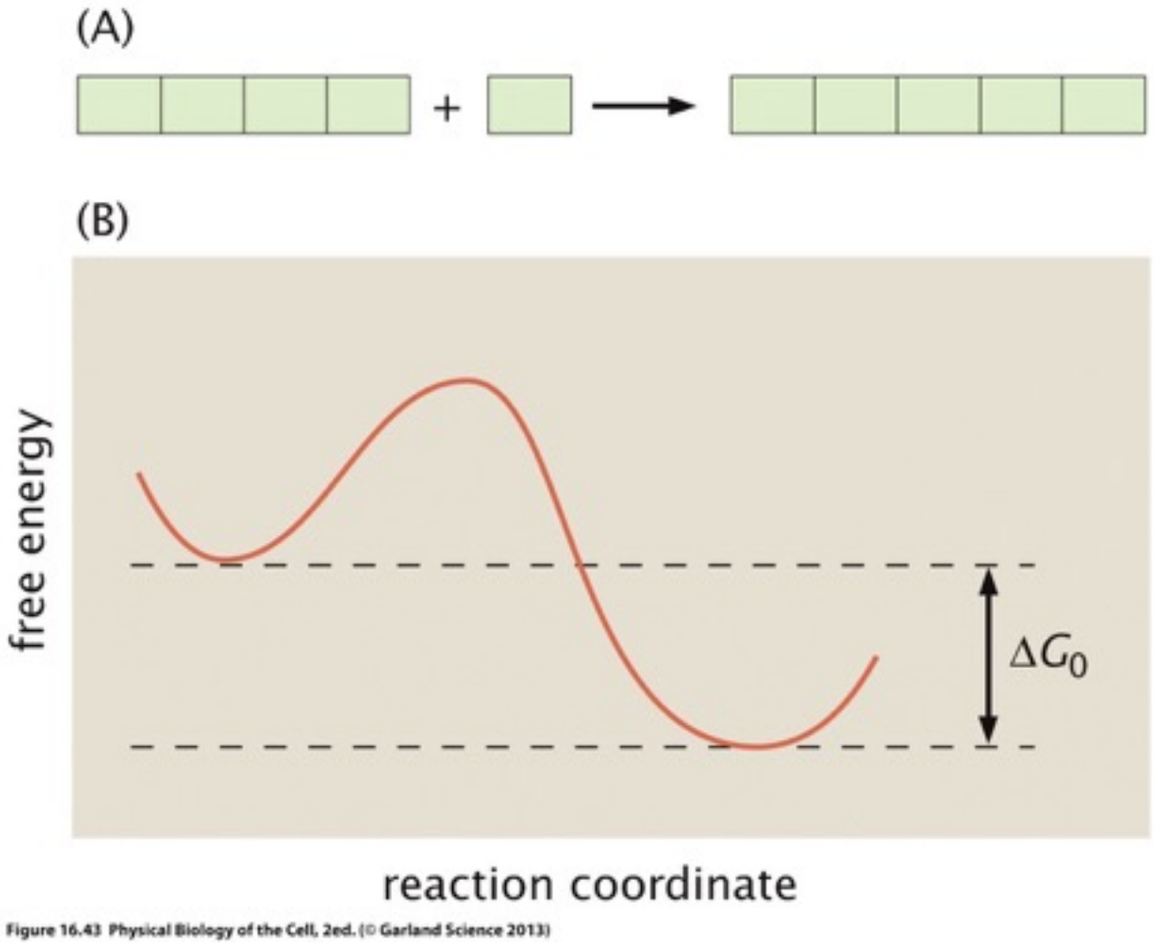


Figure 16.43 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

even the tracks on which translational motors move are motors themselves (e.g., actin, microtubules)

addition of monomer to filament is energetically favorable

$$\Delta G = kT \ln \frac{m}{m^*} \qquad m^* = \frac{\Omega}{V} e^{-\beta \Delta \epsilon}$$

m is monomer concentration, m* = K_d is concentration at equilibrium

this free energy can be harnessed to do useful mechanical work

PBoC 16.3.1

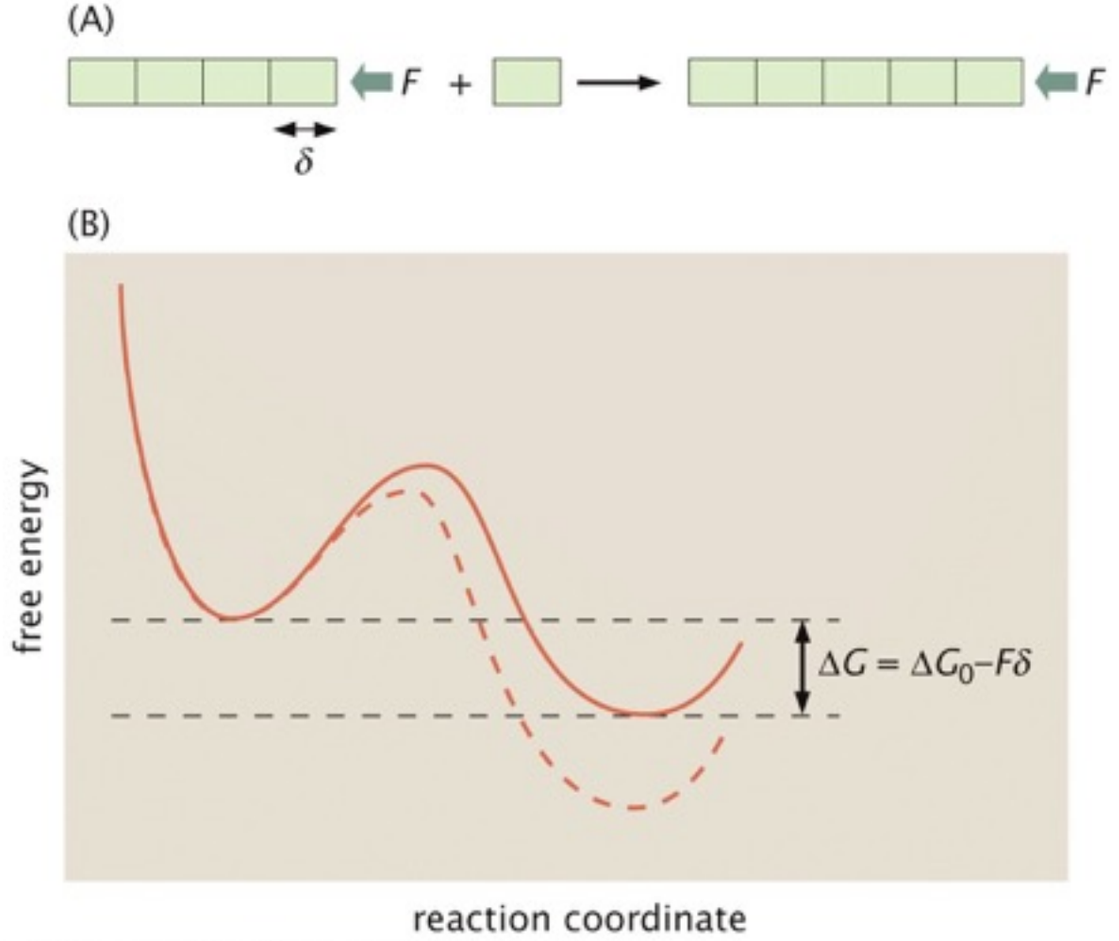
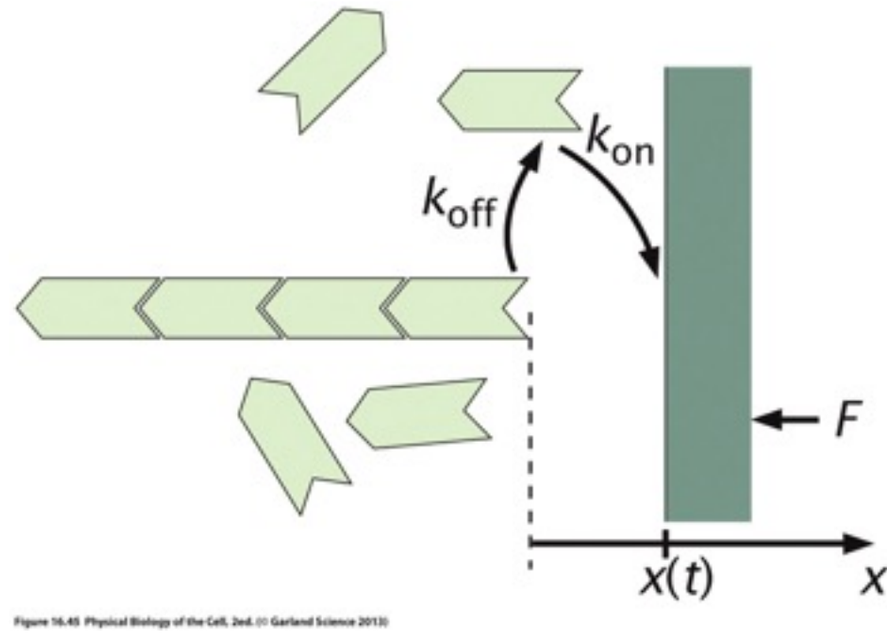


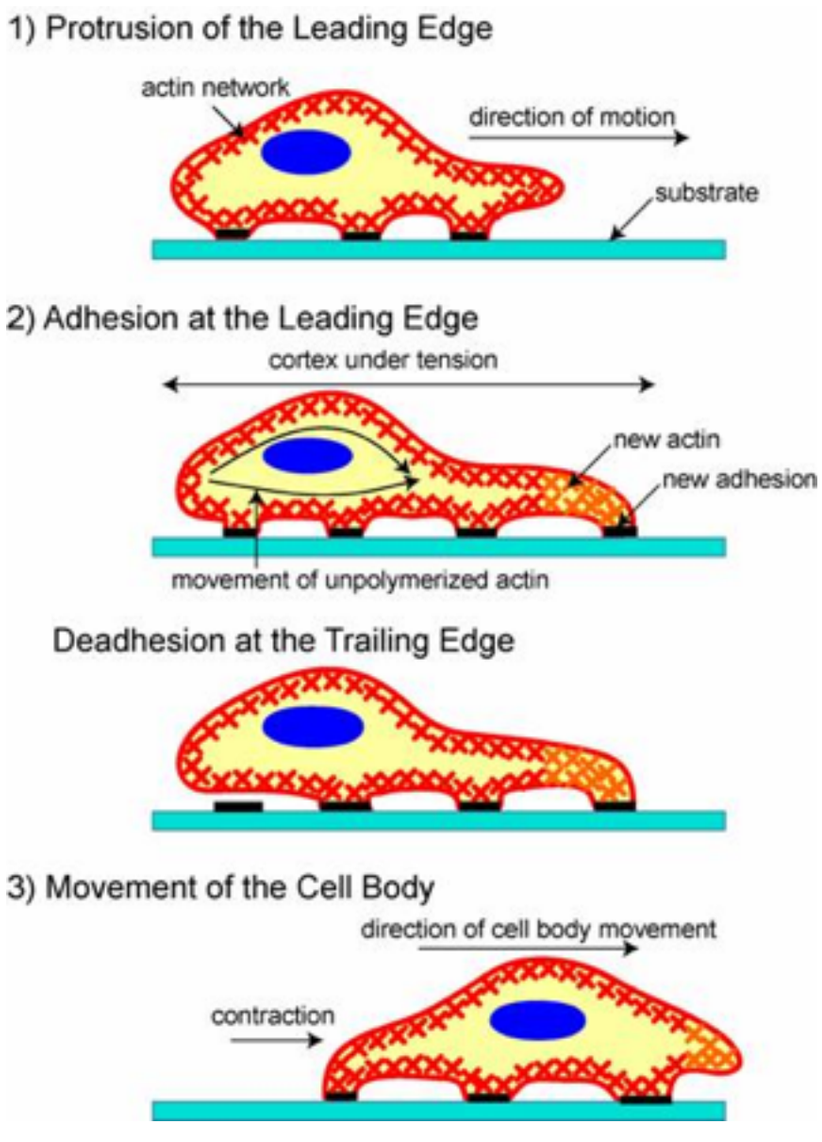
Figure 16.44 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Polymerization motors

random fluctuations of barrier or filament can allow a monomer to come in and bind

the force exerted by the barrier alters the off and on rates

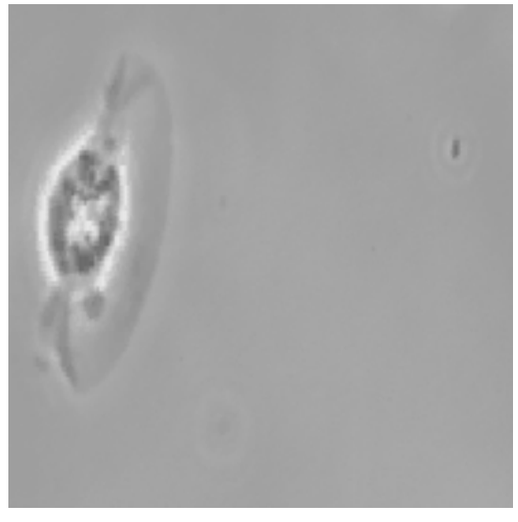




$$F_{\max} = \frac{kT}{\delta} \ln \frac{m}{m^*}$$

estimates for single actin polymer around 5-7 pN

many cells move through actin polymerization forces!



keratocyte (from Julie Theriot lab)

Translocation motors

directed motion generated by ratcheting mechanism, used for, e.g., protein import/export across membranes

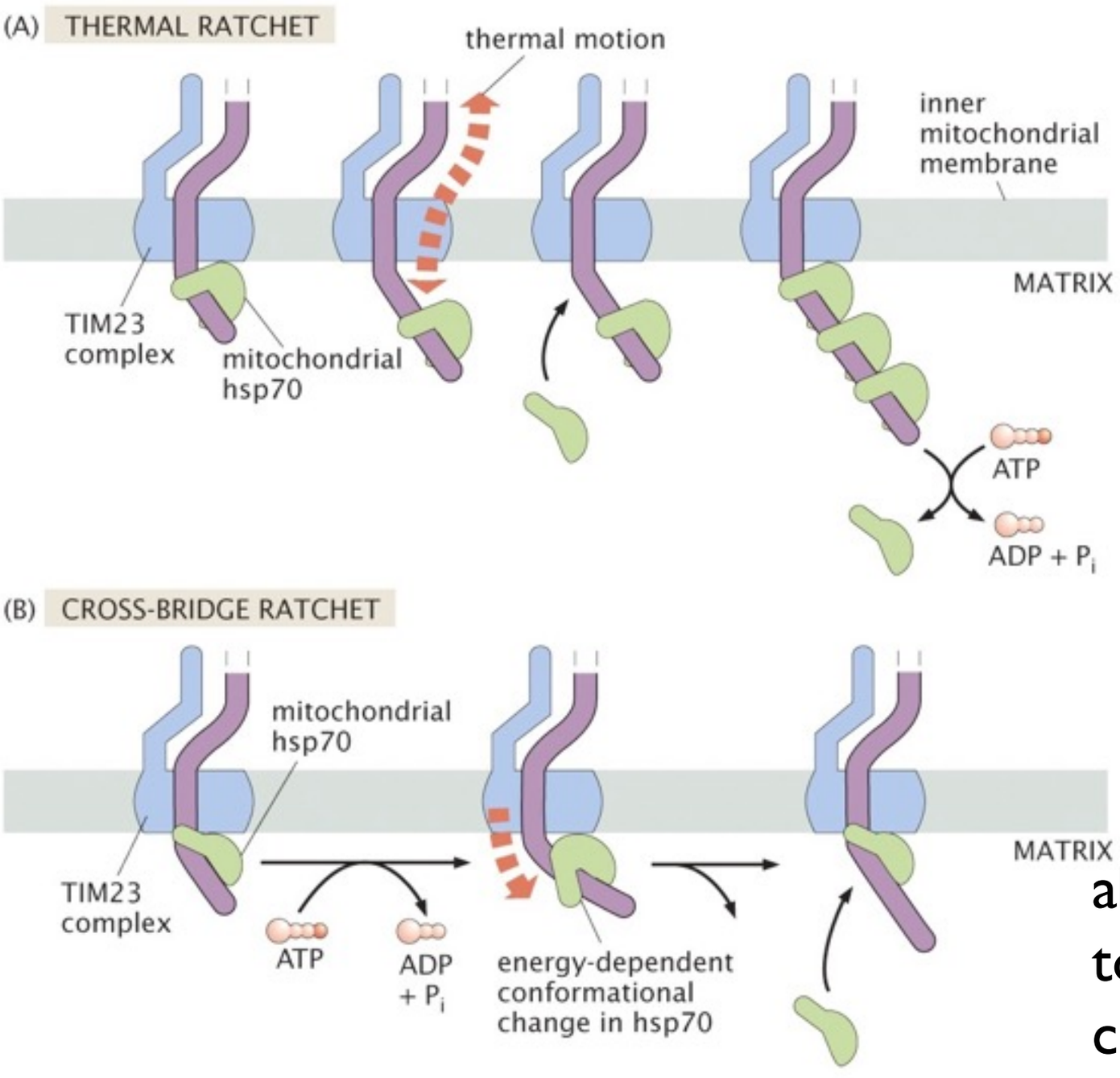


Figure 16.17 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

binding of proteins on one side prevents backsliding, **energy** is utilized to induce **unbinding**

alternatively, **energy** is used directly to **pull** protein through, tight hold in channel prevents backsliding

PBoC 16.1.4

Translocation motors

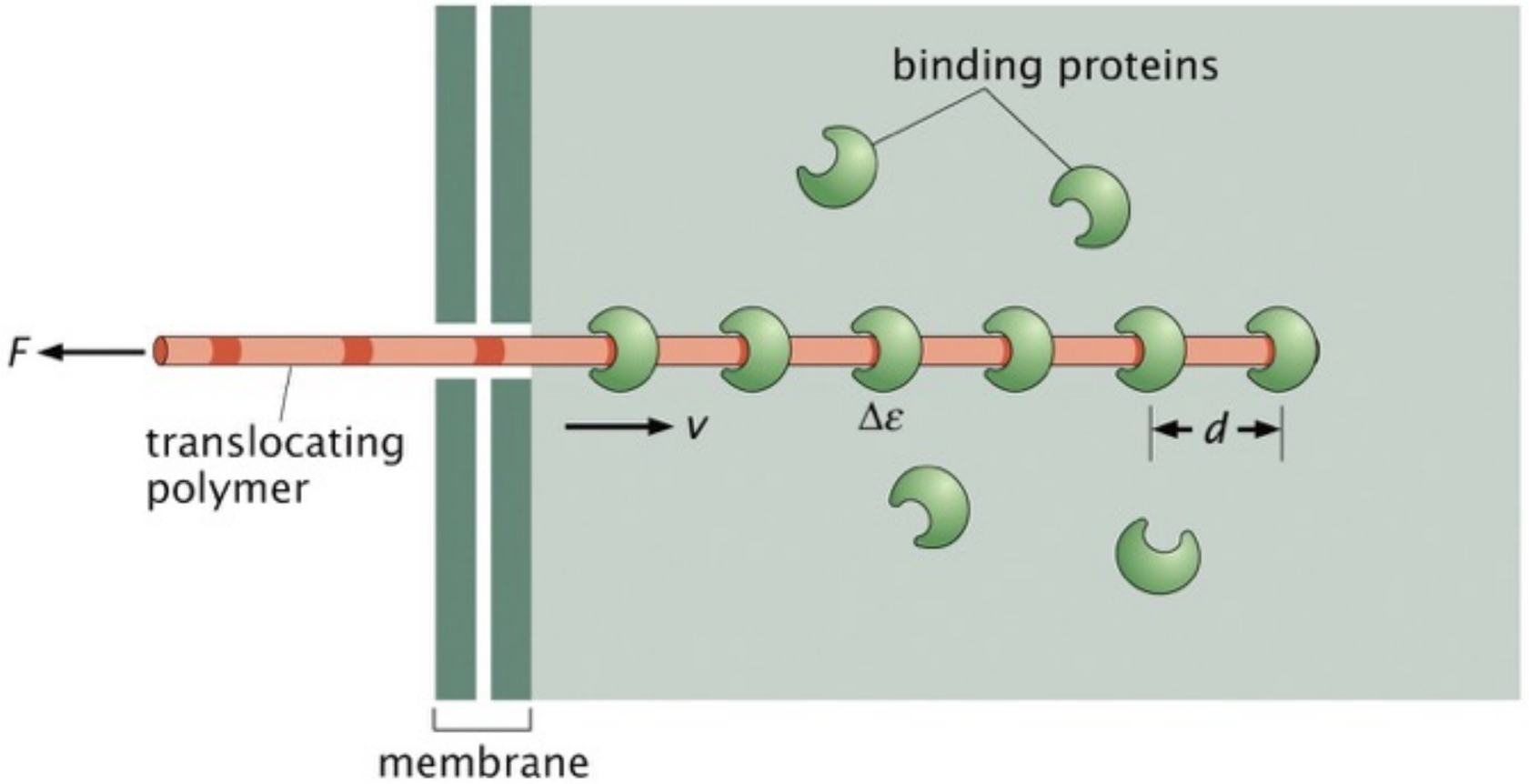


Figure 16.52 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

through diffusion alone, polymer would spontaneously end up on other side in time $t_{\text{diff.}}$

$$t_{\text{diff.}} = \frac{L^2}{D} = \frac{(nd)^2}{D}$$

with binding to prevent backsliding, time is reduced to $t_{\text{trans.}}$

$$t_{\text{trans.}} = n\left(\frac{d^2}{D}\right)$$

$$\frac{t_{\text{trans.}}}{t_{\text{diff.}}} = \frac{1}{n}$$

PBoC 16.3.3