AFM examples2: motor proteins and protein unfolding

Proteins as mechanical devices

Proteins: how rigid? How quickly move and change shape? What happens when struck by a force?

Proteins have similar densities and rigidity to hard plastics and plexiglas.
Their small size makes viscous forces from surrounding fluid larger than inertial forces.
Therefore, they undergoe overdamping motion, they relax monotonically into new conformations.

Proteins are about 40% denser than water. Average density: 1.38×10^3 kg/m^3

Protein mass: express in Dalton (Da) + $1.66\ 10^{-24}$ g. On average: 100 kDa.

Each kDa occupies 1.2 nm^3. 100 kDa is 120 nm^3.

Proteins as mechanical devices

Table 3.2 Young's moduli and tensile strength of materials

Material	Young's modulus, E (GPa)	Tensile strength (GPa)[a]
Carbon nanotube	1300	14
Diamond	1200	—
Steel (stainless)	211	1.1 (wire)
Glass (quartz)	73	1 (fiber)
Wood (fir, along grain)	16	0.06
Plexiglas	3	0.05
Plastic (polypropylene)	2.4	0.035
Teflon (PTFE)	0.34	0.022
Rubber (polyisoprene)	0.02	0.017
Silk (*Bombyx mori*)	5–10	0.3–0.6
Keratin (hair)	2.4	0.2
Actin	2.3	0.03
Collagen	2	0.1
Tubulin	1.9	—
Elastin	0.002	0.002

Source: Data for nonproteins from Tennent, 1971; Kaye and Laby, 1986; Wong et al., 1997. Data on proteins from Table 8.5 (from Wainwright et al., 1976; Kaye and Laby, 1986) and from Fraser and Macrae, 1980; Tsuda et al., 1996.

[a]Note that drawing a material out into a wire or fiber increases its tensile strength (Gordon, 1984).

Proteins as mechanical devices

Hooke's Law for many materials, including proteins, apply only for forces that causes strain up to 0.1-1 %. We should also know the Poisson's ratio (i.e. how much the cross-sectional area changes as the material is stretched).

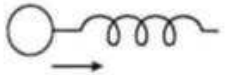
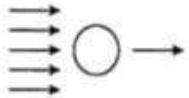
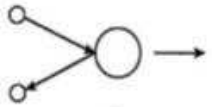
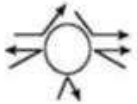
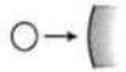
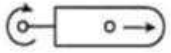
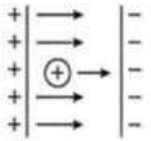
Elastin, titin can be strained up to 100% .

Generally speaking, a protein is neither isotropic nor homogeneous, and a fully atomic description of elasticity should be attempted.

However there are exception: globular proteins as actin and tubulin which form polymers of the cytoskeleton, have Young's moduli independent of the direction of applied force, therefore can be consider isotropic. Moreover, many similar polymers have similar Young's moduli.
We conclude that should exist properties that are connected to the "material" and not to its atomic structure.

Motor proteins and other molecular machines are able to move and do work because **they generate force.**

Table 2.1 Examples of forces acting on molecules

Type of force	Diagram	Approximate magnitude
Elastic		1–100 pN
Covalent		10,000 pN
Viscous		1–1000 pN
Collisional		10^{-12} to 10^{-9} pN for 1 collision/s
Thermal		100–1000 pN
Gravity		10^{-9} pN
Centrifugal		$< 10^{-3}$ pN
Electrostatic and van der Waals		1–1000 pN
Magnetic		$<< 10^{-6}$ pN

Range:
pN -nN

Motor proteins and other molecular machines are able to move and do work because **they generate force.**

Physical forces and their magnitudes at the single-molecule level (Examples)

Elastic

F= k · x , where: k – spring constant (stiffness), x – displacement

Example: motor protein k= 1 pN/1nm, spring strained through distance x= 1 nm → **F= 1 pN**

Viscous

F= ɣ · v, where : ɣ- drag coefficient , v – relative velocity between object and liquid

ɣ= 6πηr, with η – liquid viscosity, r – radius of a spherical particle

Example: for a globular protein (radius r= 3 nm, molecular mass MM= 100 kDa) in water ($\eta \sim 1$ mPa s) → $\gamma \sim 60$ pN s/m

the average thermal speed $v_{rms} = \sqrt{\frac{3KT}{m}}$ 1Da ~ 1.66×10^{-27} kg KT ~ 4.1 pN nm → $v_{rms} \sim 8$ m/s

F ~ 480 pN

Collisional and thermal

Example: Protein – water molecule collision / s: F= Δp/ Δ t

Water molecule : mass m ~ 18 Da, average thermal speed $v_{rms} \sim 600$ m/s, momentum: $p \sim 18 \times 10^{-24}$ kg m/s.

Assuming the interaction is perfectly elastic : $F = \Delta p/\Delta t = \Delta(mv)/\Delta t = 2p/\Delta t \sim 36 \times 10^{-12}$ pN - very small

However, the number of collisions / s is much much bigger ($> 10^{13}$), such that the instantaneous thermal force acting on a the protein is on the order of the viscous force: F **~ 500 pN**, and drives diffusion.

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Motor proteins and other molecular machines are able to move and do work because **they generate force.**

Example 2.1 Physical forces and their magnitudes at the single-molecule level

ELASTIC FORCES. If an object is connected to a spring of stiffness κ that is stretched a distance x beyond its resting length, then the object will experience a force of $F = \kappa x$. For a motor protein, the stiffness might be about 1 mN/m = 1 pN/nm. If the spring is strained through a distance of 1 nm = 10^{-9} m, a distance appropriate to the size of proteins, then the force exerted on the object is 1 pN.

VISCOUS FORCES. If an object is held fixed in a moving liquid or is moving through a stationary fluid, then it will experience a viscous, or drag, force from the liquid. The force is proportional to the relative velocity, v, between the object and the fluid according to $F = \gamma v$. The constant of proportionality, γ, is called the drag coefficient. The drag coefficient is related to the size and the shape of the object as well as the viscosity. For example, for a sphere of radius r moving through a liquid of viscosity η, the drag coefficient is $6\pi\eta r$ (Stokes' law, Chapter 3). The viscous forces on proteins are large. For a globular protein of diameter 6 nm, corresponding to a molecular mass of ~100 kDa (see Table 2.2), the drag coefficient measured by centrifugation studies at 20°C is ~ 60 pN·s/m (Creighton, 1993), in good agreement with Stokes' law. The average instantaneous thermal speed of such a protein in solution at standard temperatures is ~8 m/s (this is a consequence of thermally driven collisions from the surrounding solvent molecules, Chapter 4). The corresponding viscous force is therefore ~480 pN.

COLLISIONAL AND THERMAL FORCES. If an object is struck by another, it experiences a force equal to the rate of change in momentum (mv) of the striking particle, $F = d(mv)/dt$. For example, the mass of a water molecule is $\sim 30 \times 10^{-27}$ kg, the average speed associated with its kinetic energy is ~600 m/s (Chapter 4), and therefore its momentum is $\sim 18 \times 10^{-24}$ kg·m/s. If a protein were struck head-on every second by a water molecule that bounced straight back, then the average force would be equal to 36×10^{-12} pN (twice the momentum for an elastic collision). This is a very small force. However, in solution a huge number of collisions take place per second. The collisions come from all directions, and the resulting randomly directed force, called the thermal force, drives diffusion. The average instantaneous thermal force acting on a 100 kDa protein is on the order of the viscous force, or ~500 pN (Chapter 4).

OPTICAL FORCES. Another example of a collisional force is optical pressure. Because photons have momentum, they exert a force when they are diffracted by an object. The momentum of a photon is $h\nu/c = h/n\lambda$, where h is Planck's constant, ν is the frequency of the light, c is the speed of light, n is the refractive index, and λ is the wavelength (in a vacuum). If an object in water ($n = 1.33$) absorbs one green photon ($\lambda = 500$ nm) per second, the corresponding optical force on it is 1.0×10^{-15} pN (the values for the physical constants can be found in the table on the endpapers). This is a very small force. Even if a molecule adsorbs 10^9 photons per second, which would require very bright laser illumination, the optical force would still be only 10^{-6} pN.

GRAVITY. An object of mass m experiences a gravitational force of magnitude mg, where g is the acceleration due to gravity, equal to ~9.8 m/s^2 at the Earth's surface. With a mass of only 166×10^{-24} kg, a 100 kDa protein experiences a gravitational force of only 1.6×10^{-9} pN. At the single-molecule level, gravitational forces are very small and can be ignored.

CENTRIFUGAL FORCES. An object spinning in a centrifuge experiences a centrifugal force equal to ma_c. Ultracentrifuges are capable of generating centrifugal accelerations, a_c, in excess of 100,000 times that of gravity. The associated centrifugal forces on molecules are still quite modest, $\sim 160 \times 10^{-18}$ N = $\sim 160 \times 10^{-6}$ pN for our 100 kDa protein, but this is large enough to cause the protein to drift at an average speed of ~3 μm/s (using the drag coefficient from Table 2.2). The slow drift is superimposed on the rapid, randomly directed thermal motion. At this speed the protein will sediment through a distance of 100 mm, a typical length of a centrifuge tube, in about 10 hours.

ELECTROSTATIC FORCES. A particle with charge q, in an electric field of strength E, will experience a force $F = qE$. An ion such as sodium experiences an electrostatic force when it moves through an ion channel in the plasma membrane. The charge on the ion is 160×10^{-21} coulombs (see the table of physical constants on the rear endpapers), and the electric field across a typical plasma membrane is 15×10^6 V/m (60 mV potential across the 4-nm-thick membrane). The corresponding force is 2.4 pN. A similar-sized force exists between two monovalent ions in water that are separated by 1 nm (Problem 2.7). The force will be smaller in a salt solution due to charge screening, but will be larger in the interior of proteins where the dielectric constant is low.

Van der Waals forces are also electrostatic: They arise from the charge separation induced by nearby atoms. Van der Waals forces can be as high as 100 pN per nm^2 of protein–protein interface (Appendix 3.1).

MAGNETIC FORCES. Magnetic forces are very small at the molecular level because molecules interact only very weakly with magnetic fields. For example, the maximum force on a proton, the nucleus with the largest magnetic moment, in the strongest nuclear magnetic resonance (NMR) machines is only on the order of 10^{-12} pN. Thus even for a huge protein with 3000 amino acids and 60,000 atoms, subject to a very strong magnetic field, the magnetic force is less than 10^{-6} pN.

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Motor proteins and other molecular machines are able to move and do work because **they generate force.**

Physical forces and their magnitudes at the single-molecule level (Examples) - 2

Electrostatic **F= qE**

Example: force experienced by a potassium ion K^+ , traveling through an ion channel of the plasma membrane.

The charge of the ion q= 160 10^{-21} C; the electric field accross a typical plasma membrane : E= 15 10^{6} V/m

(60 mV potential accross the 4 nm thick membrane) -- **> F= 2.4 pN**

Similar sized force exists between two monovalent ions in water that are separated by 1 nm (*homework)*.

Van der Waals forces are also electrostatic – they arise form the charge separation induced by nearby atoms.

These forces can be as high as 100 pN / nm^2 of protein-protein interface

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Magnetic

Very small at the molecular level because molecules interact very weakly with magnetic fields.

Example: max force on a proton, the nucleus with the largest magnetic moment , in the strongest nuclear magnetic resonance (NMR) machines is only of the order of 10^{-12} pN.

Thus even with a huge protein with 3000 aminoacids and 60000 atoms subject to a very strong magnetic field the magnetic force is < **10^{-6} pN**.

Modeling complex mechanical devices as protein machines by using three elements: Spring, Dashpot, Mass

In a simplified approach, a protein can be thought as a mechancial device composed of

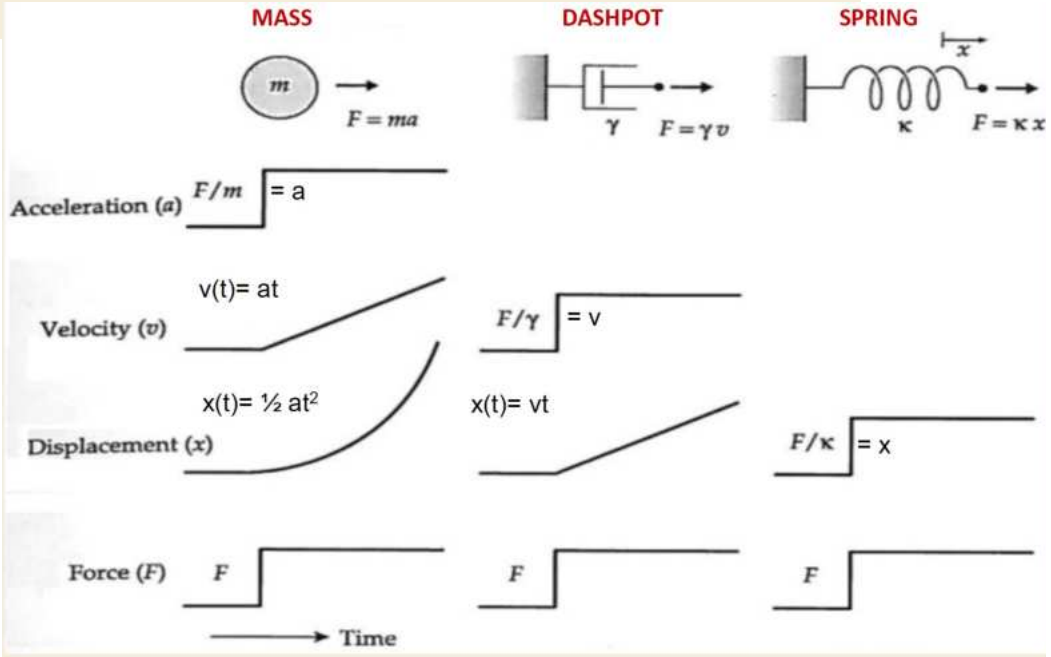
atoms that have mass,

connected by **bonds that have elasticity**, like springs,

and moves **in liquid environment**, facing **viscosity** like dashpots.

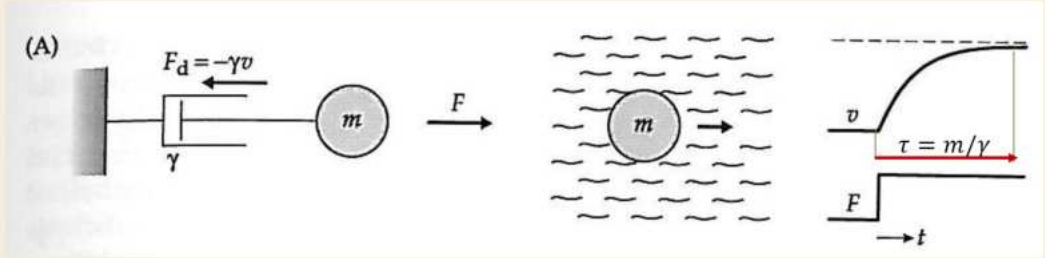
All mechanical devices can be built with three fundamental mechanical elements:

SPRING, DASHPOT, MASS.



Motion of Combinations of Mechanical Elements

A) DASHPOT and MASS. Model for the movement of a cell or a protein through a liquid



Eq of motion

$$m\frac{dv}{dt} + \gamma v = F$$

Solution

$$v(t) = \frac{F}{\gamma}\left[1 - \exp\left(-\frac{t}{\tau}\right)\right]$$

Time constant

$$\tau = \frac{m}{\gamma}$$

Example. The persistance of protein movement through a liquid

For a globular protein of 100 kDa, the time constant **: τ ~ 2.8 ps**

$m_p \approx 166\ 10^{-24}$ kg , $\gamma_p \approx 60 \cdot 10^{-3}$ mPa s µm

$$\tau = \frac{m}{\gamma}$$

After the protein gains speed due to molecular collisions with solvent molecules, the velocity persists for only a very short time as other collisions rapidly randomize the protein's direction of travel.

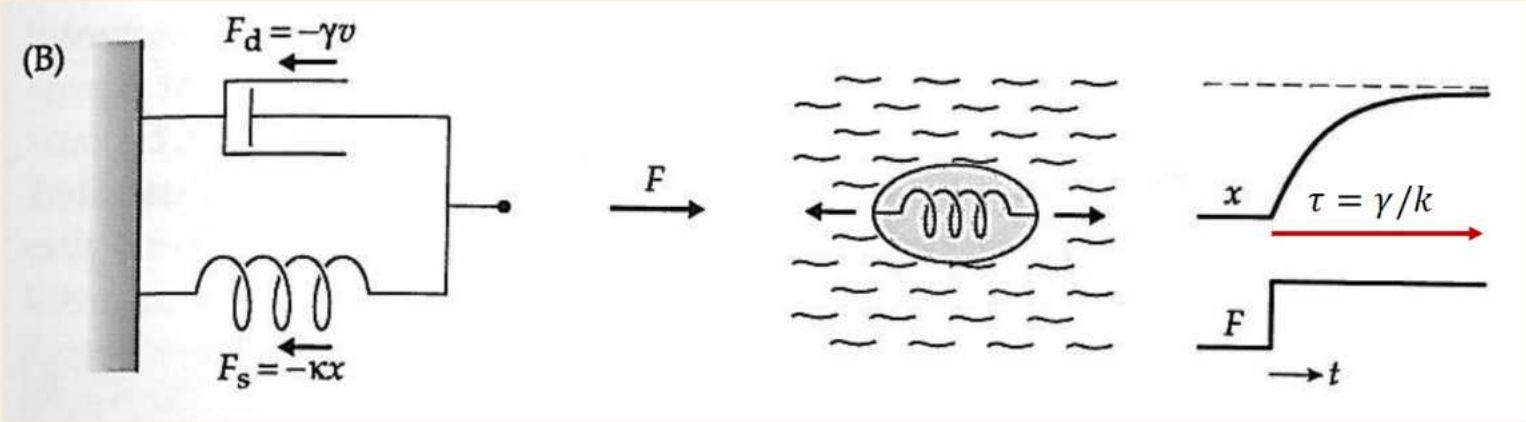
Given that the average instantaneous speed of such a protein is v= 8.6 m/s, the avearge distance that the protein moves before its speed is randomized by molecular collisions is only x= 0.24 A !!!

Motion of Combinations of Mechanical Elements

B) SPRING and DASHPOT in parallel.

Model for a compliant low- mass object that is deformed in a liquid, such as a protein that undergoes a global conformational change.

It can be used also to model a viscoelastic material, such as skin, that takes finite time to adopt a new shape.



$$\gamma \frac{dx}{dt} + kx = F$$

$$x(t) = \frac{F}{k}\left[1 - \exp\left(-\frac{t}{\tau}\right)\right]$$

Time constant

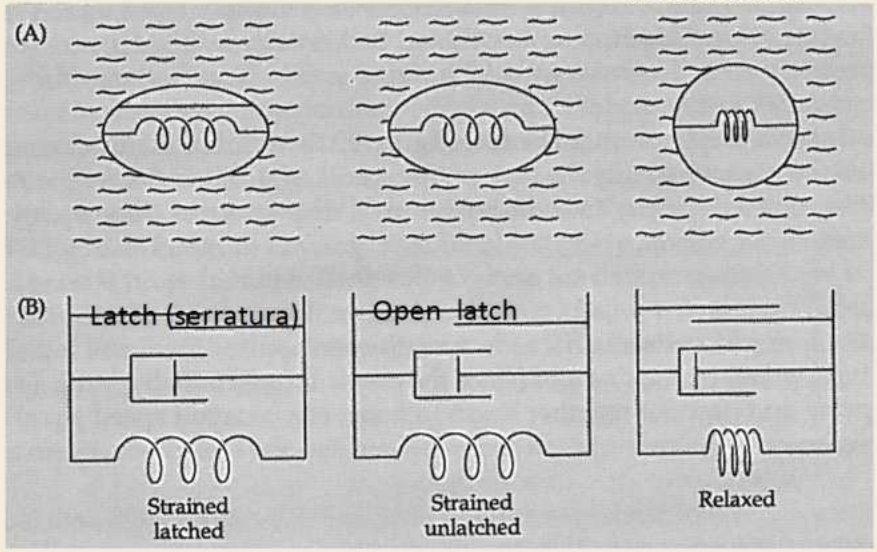
$$\tau = \frac{\gamma}{k}$$

Example. **The timescale of protein conformational changes**.

Globular protein 100 kDa, Local chemical changes, such as breaking of the bonds between two proteins is ps, whereas the global conformational changes of the whole protein occurs much more slowly.

Protein held in a strained conformation due to an internal strut (montante)

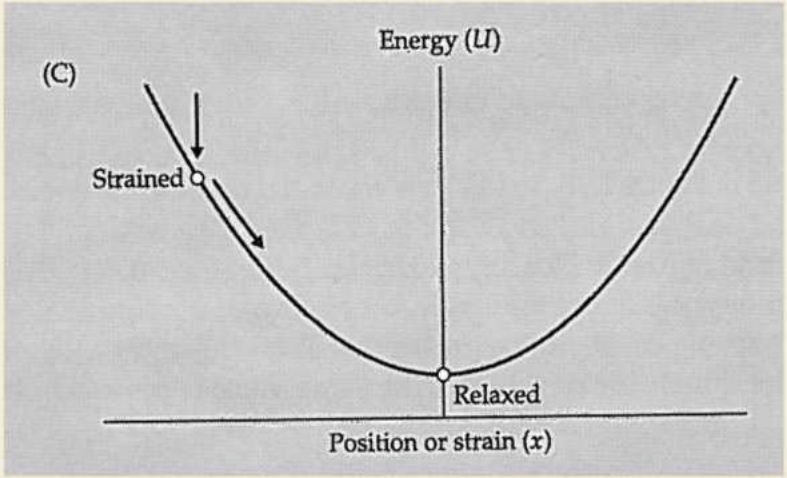
Protein relax, changes shape → unstrained conformation



$\gamma = 60\ pN\ s\ /m \qquad k = 4\ \mathrm{pN/nm}$

$$\tau = \frac{\gamma}{k}$$

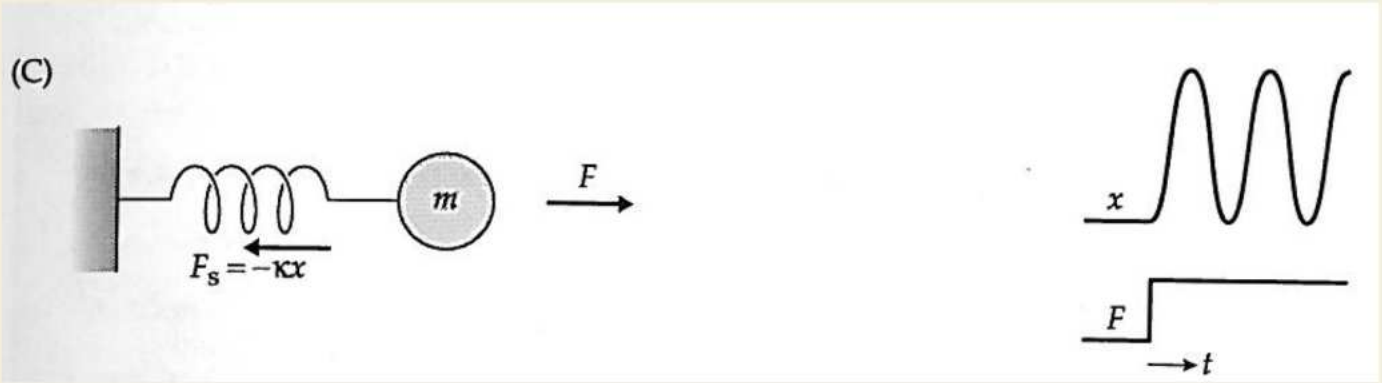
$$\tau = 15\ ns$$



The global conformation changes occur in nanoseconds, while the breaking of the bonds occur in picoseconds

Motion of Combinations of Mechanical Elements

C) MASS and SPRING in serie. Model to describe the vibrations of the atomic bonds.



$$m\frac{d^2x}{dt^2} + kx = F$$

$$x(t) = \frac{F}{k}[1 - \cos(\omega t)]$$

$$\omega = \sqrt{\frac{k}{m}}$$

Harmonic motion

Example. Vibration of chemical bonds.

Chemical bonds can be thought as having stiffness (chemical bonds vibrate at frequency $\omega=2\pi\nu$, which can be detected spectroscopically when the molecule absorbs light of the same frequency as the molecular vibration).

Ex: the fundamental vibration frequency of the **H-Cl** bond in HCl is $\nu = 89.6\ 10^{12}$ Hz ($2990\ cm^{-1}$)

The corresponding wavelength is $\lambda = c / \nu = 3.53\ \mu m$

The appropriate mass **m**~$1.63\ 10^{-27}$ kg (appox mass of the hydrogen nucleus)

Stiffness **k**= $m\ \omega^2 = 517$ N/m – **very stiff !!!**

$$\omega = \sqrt{\frac{k}{m}}$$

Example. Protein vibrations.

Consider the motor protein myosin. Motor domain has a mass **m**~160×10^{-24} kg and stiffness **k** ~ 4 pN/nm.

The vibration frequency is calculated to be: **v** ~ 10^{9} Hz, which means a period of oscillation T= 1 ns.

By contrast, the relaxation time is 15 ns.

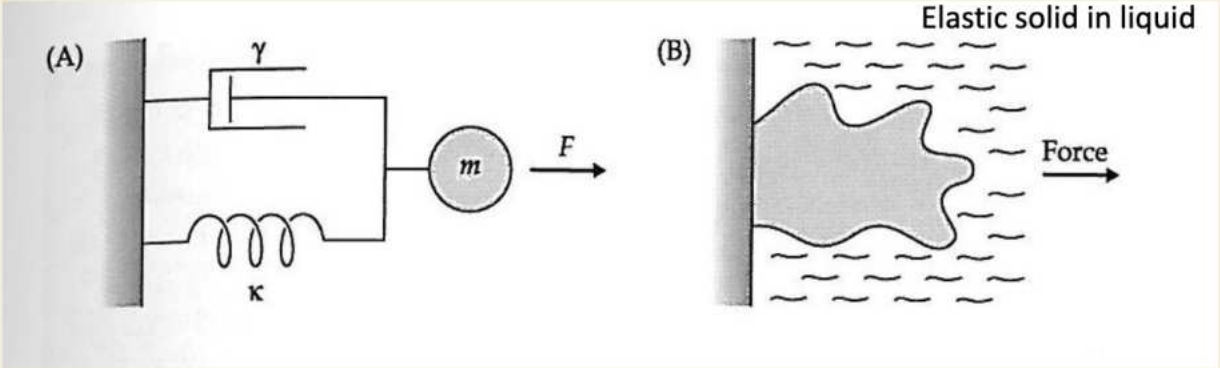
Does the protein oscillate when it detaches from the actin filament or does it creep exponentially into its relaxed state ?

The answer requires solution of the full model, with mass, spring, and dashpot, and the solution shows that the protein creeps rather than rings.

MASS and SPRING with DAMPING.

Simple mechanical model of a protein undegoing a large scale conformational change that is damped by the surrounding fluid, and possibly by internal viscosity.

This model captures the main qualitative features of more complex models in that it can display oscillatory of monotonic motions depending on the strength of the damping.



$$m\frac{d^2x}{dt^2} + \gamma\frac{dx}{dt} + kx = F$$

The solution depends on wether the **damping** is:

small $\frac{\gamma^2}{4mk} < 1$

or

large $\frac{\gamma^2}{4mk} > 1$

Underdamped Motion ($\gamma^2 < 4m\kappa$)

$$x(t) = \frac{F}{\kappa}\left[1 - \exp\left(-\frac{t}{\tau}\right)\frac{\sin(\omega t + \phi)}{\sin\phi}\right] \qquad \text{(A2.1)}$$

where $\tau = \dfrac{2m}{\gamma}$, $\omega^2 = \omega_0^2 - \dfrac{1}{\tau^2}$, $\omega_0^2 = \dfrac{\kappa}{m}$, $\tan\phi = \omega\tau$

Overdamped Motion ($\gamma^2 > 4m\kappa$)

$$x(t) = \frac{F}{\kappa}\left[1 - \frac{\tau_1}{\tau_1 - \tau_2}\exp\left(-\frac{t}{\tau_1}\right) + \frac{\tau_2}{\tau_1 - \tau_2}\exp\left(-\frac{t}{\tau_2}\right)\right] \qquad \text{(A2.2)}$$

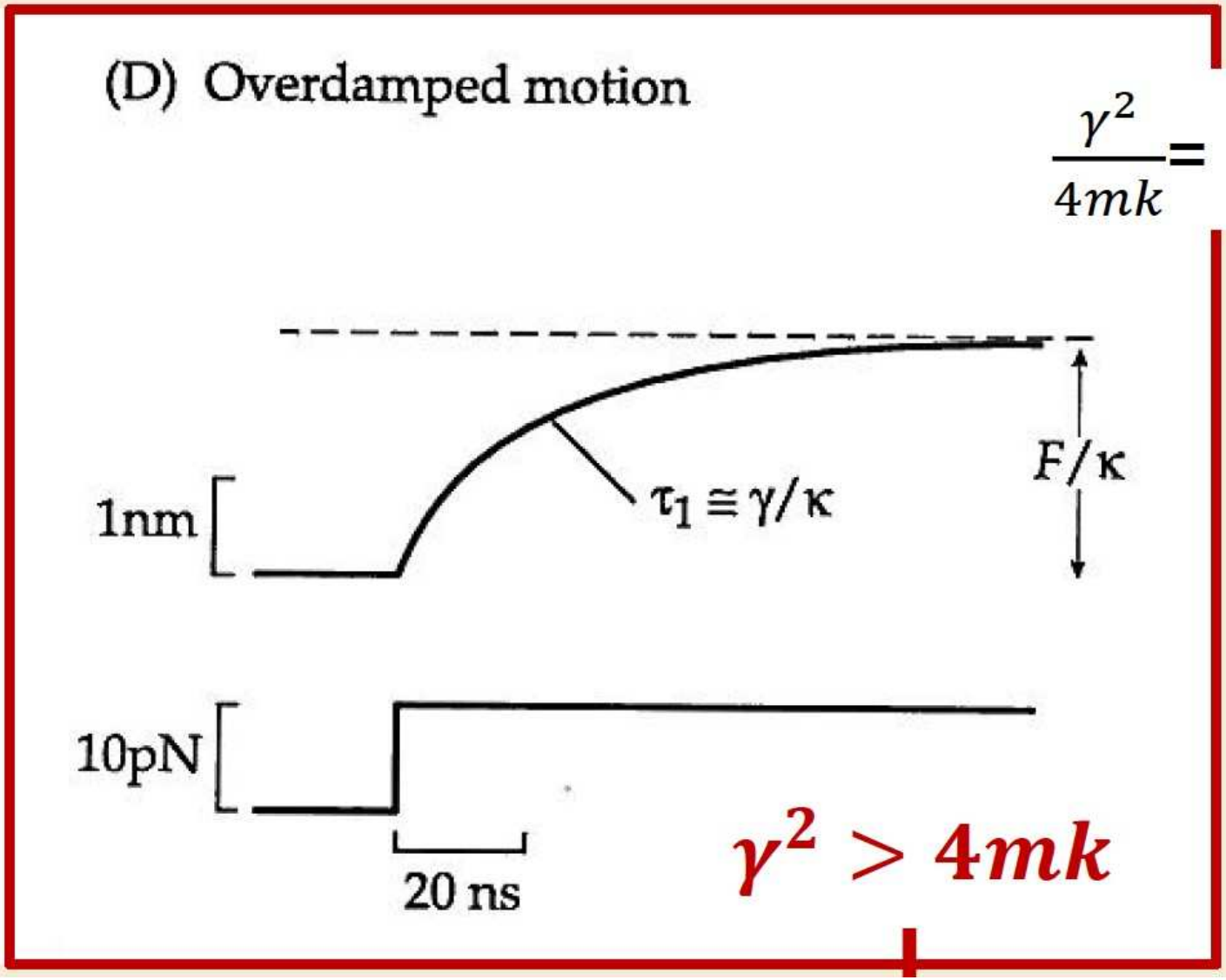
where $\tau_1 = \dfrac{\gamma + \sqrt{\gamma^2 - 4m\kappa}}{2\kappa}$ and $\tau_2 = \dfrac{\gamma - \sqrt{\gamma^2 - 4m\kappa}}{2\kappa}$

Both τ_1 and τ_2 satisfy $(m/\tau) + \kappa\tau = \gamma$. When the motion is highly overdamped ($\gamma^2 >> 4m\kappa$), the time constants become $\tau_1 = \dfrac{\gamma}{\kappa}$ and $\tau_2 = \dfrac{m}{\gamma}$, where $\tau_1 >> \tau_2$.

Protein struck by a force:
- ring like a fork (underdamped motion),
- creep monotonically into a new shape (overdamped motion).

The inertial forces are usually small at microscopic/molecular lever. Usually overdamping applies

protein undergoing a large scale conformational change that is damped by the surrounding fluid, and by internal viscosity.
Globular protein MM=100 kDa ; Stiffness k= 4 pN/nm ;
damping γ= 150 pN s/m



2.2. Thermal forces, diffusion, and chemical forces

In addition to mechanical forces, proteins and cells are subject to **thermal forces**, arising from collisions with water and other molecules in the surrounding fluid

Thermal forces → thermal energy → thermal / Brownian motion

The magnitude of thermal energy is in the range of the energies of chemical reactions driving biological processes, which are just a little bit higher than thermal energy → thermal fluctuations are necessary for proteins to reach their transition states

Molecular machines operate in diffusive environment, differently from macroscopic machines of our everyday world

Boltzmann Distribution Law

describes how the probability of a molecule having a certain energy depends on the surrounding temperature

Principle of Equipartition of Energy

states how much thermal energy a molecule has at a certain temperature

Energy of chemical bonds:

the dissociation energy is seen as being approximately equal with the potential energy in the bond:

$U = \frac{1}{2}kd^2$, where d is the extension required to break the bond, ***d*~ 0.05 nm**.

For H-Cl, the stiffness **k ~ 517 N/m** → U ~ 650 x 10^{-21} J = 650 pN · nm → **U ~ 161 K_BT**

(K_B Boltzmann ct: K_B = 1.38 · 10^{-23} J/K; T- temperatue, e.g. T=300 K ; 1 K_B T ~ 4 · 10^{-21} J= 4 pN · nm)

Energy stored in protein conformational changes:

Myosin molecule. The stifness is about **k ~ 4*10^{-3} N/m** (or 4 pN/nm)

For a conformational change of **d=5 nm** the total energy $U = \frac{1}{2}kd^2$ = 50 pN nm = 50 · 10^{-21} J , **U ~ 12.5 K_BT**

This energy is approximately half of the chemical energy derived from hydrolisis of the gamma phosphate bond of ATP.

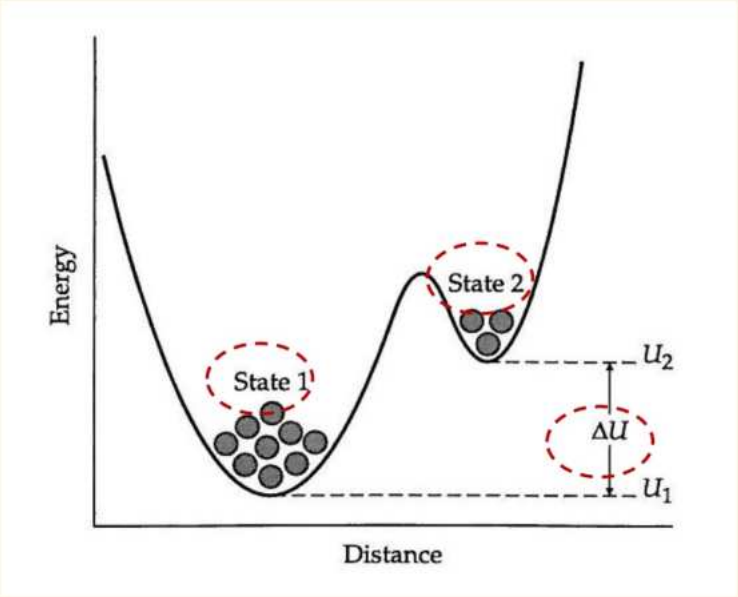
We can generalize this argument to global conformational changes of other protein machines:

The energies are on the order of 10 to 100 x 10^{-21} J (2.5 to 25 K_BT), conformational changes are on the order of 1 to 10 nm.

Therefore the stifnesses are on the order of 0.2 to 200 pN/nm.

Energy landscape

Molecules in a two-state energy landscape:



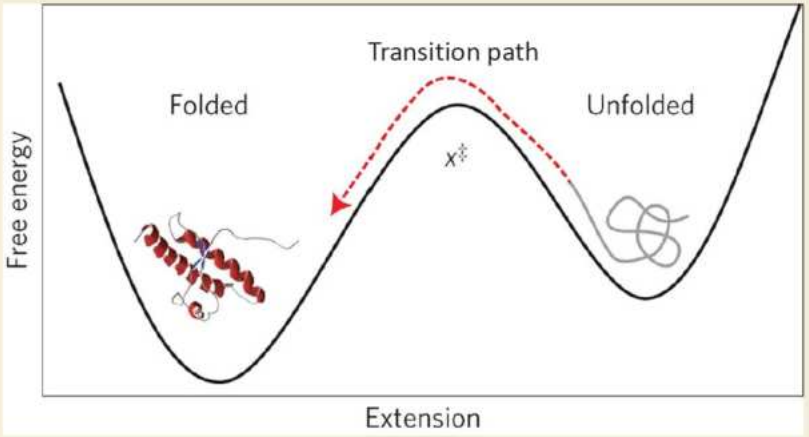
Considering the Boltzmann's equation, the probability of finding a molecule in state 2 relative to state 1 is:

$$\frac{p_2}{p_1} = exp\left[-\frac{\Delta U}{kT}\right]$$

Boltzmann distribution allows to calculate the probability of observing a system at finite temperature in any particular microstate.

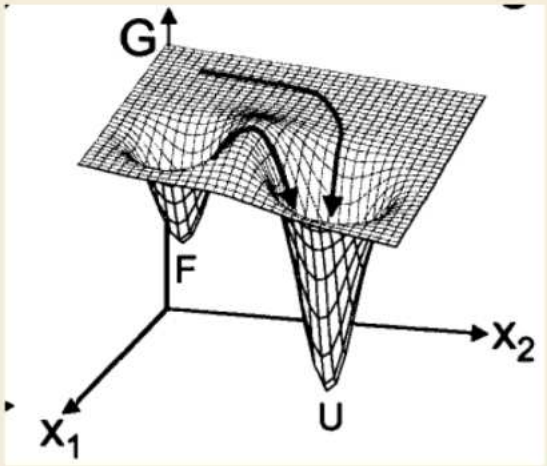
The probability only depends on the energy (free energy) of the state.

Protein unfolding – free energy landscape



A one-dimensional free energy diagram allowing for single unfolding pathway – transition path.

The extension x represents the unfolding reaction coordinates



A two-dimensional free energy diagram allowing for multiple unfolding pathways.

x1 and x2 represent generalized unfolding reaction coordinates.

Boltzmann's Law

Boltzmann's law is very general

The energy could correspond to the particle's potential energy (gravitational, elastic, or electrical) its kinetic energy, or energy associated with its phase, or electronic or chemical state.

The state of a particle (or group of particles) is specified by the position and velocity of the constituent atoms as well their electronic states.

Boltzmann's law is fundamental: we can use it to define **equilibrium** and **temperature:**

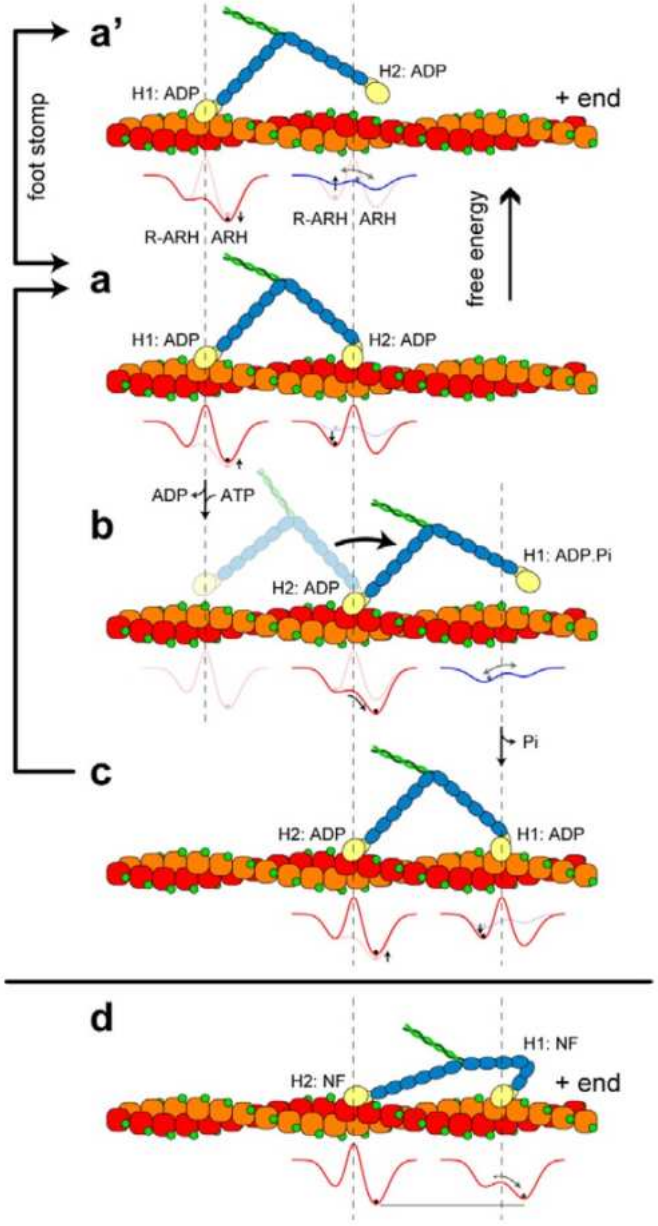
A system is at **equilibrium** if Boltzmann's law holds.

The **temperature** is defined as the corresponding constant in the exponent of the Boltzmann's law formula.

Boltzmann's law is a very important physical law in biology and chemistry.

Single molecule imaging 1

Myosine V walking on actin filaments



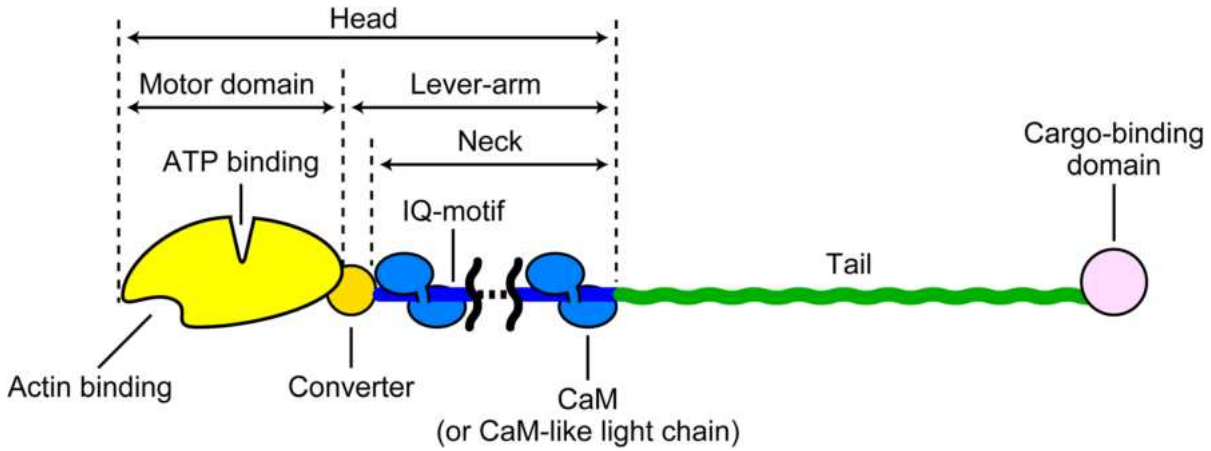
Proteins are dynamic in nature and work at the single-molecule level.

In dynamic HS-AFM the molecule itself is visualized while working and moving on its biological track, providing concomitant structural and dynamic data: not only did the observation confirm the hand-over-hand walking mechanism of myosin-V, it did reveal that the power stroke of this motor is driven by intramolecular mechanical tension

N. Kodera, D. Yamamoto, R. Ishikawa, T. Ando *Nature* **468**, 72 (2010)

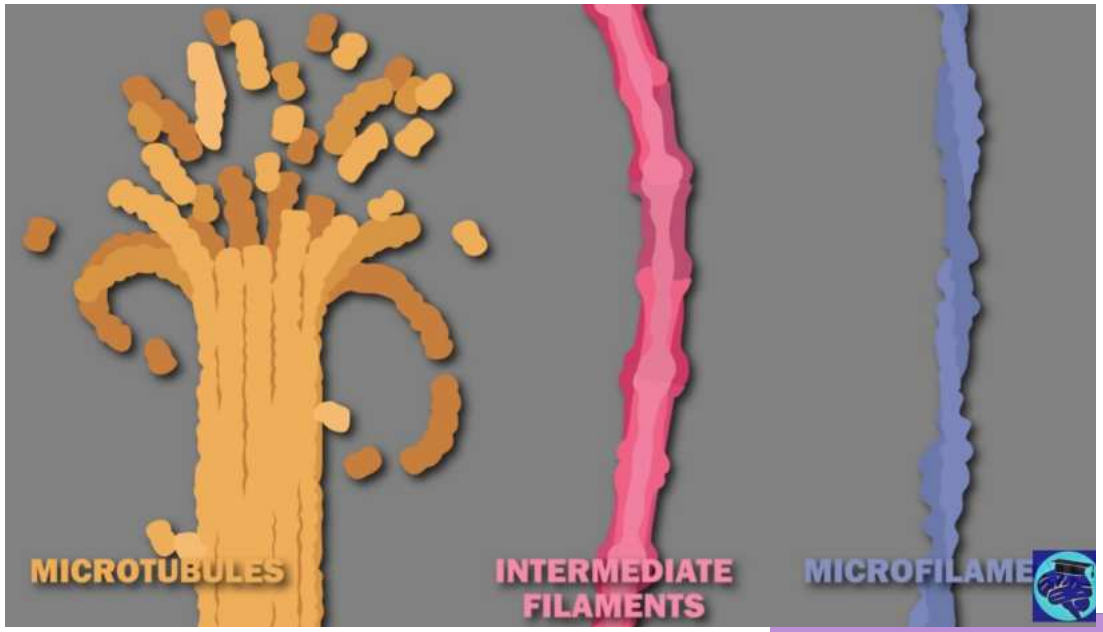
Myosine V walking on actin filaments

Myosine V is a two-headed **processive motor** and functions as a cargo transporter in cells. Each head of the double-headed myosin hydrolyzes ATP into ADP and inorganic phosphate (Pi). The ATPase rate is very low when myosin is alone but is markedly accelerated by its interaction with actin, where the chemical energy liberated by ATP hydrolysis is converted into mechanical work.

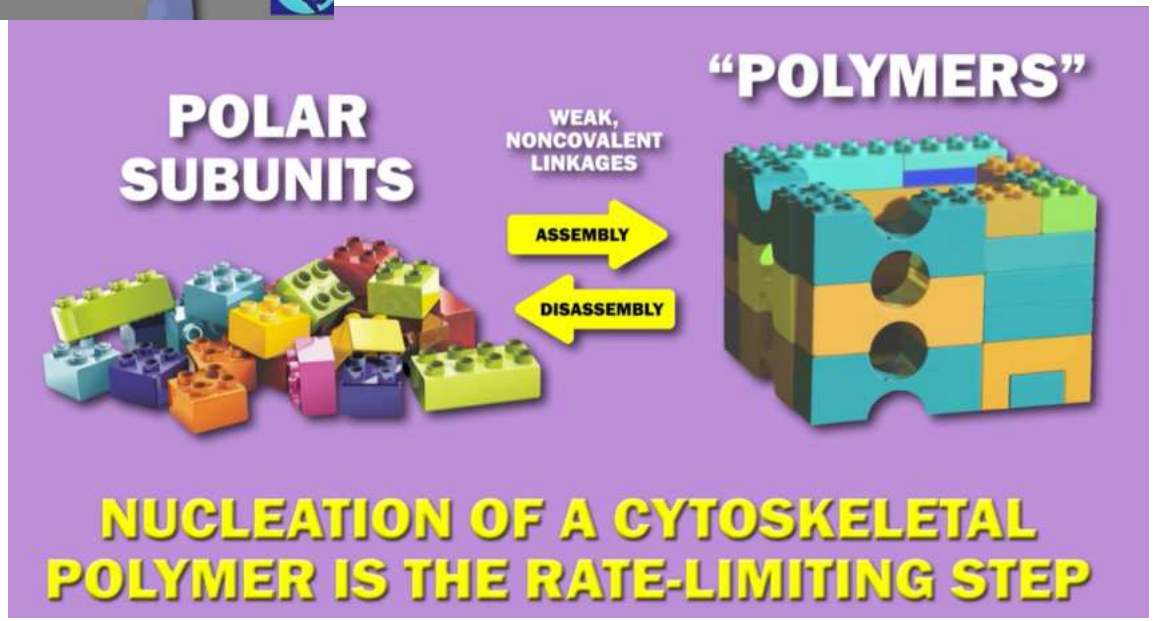


*__processive motor:__ one head is always attached to the actin filament, while the other detaches and moves further along the actin track

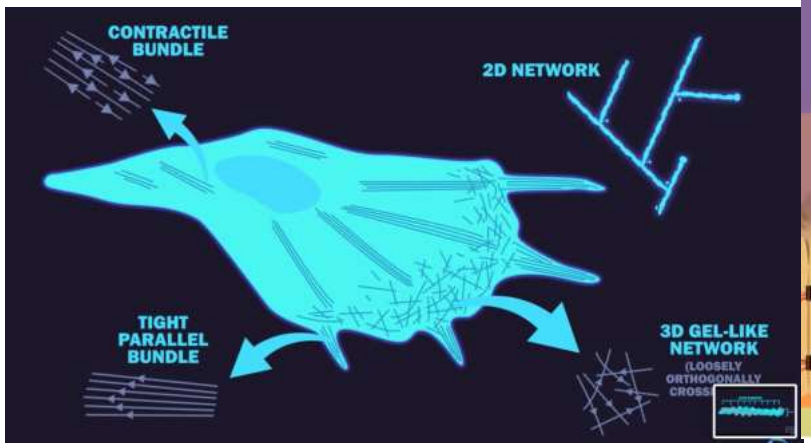
Polymeric fibers: cytoskeleton

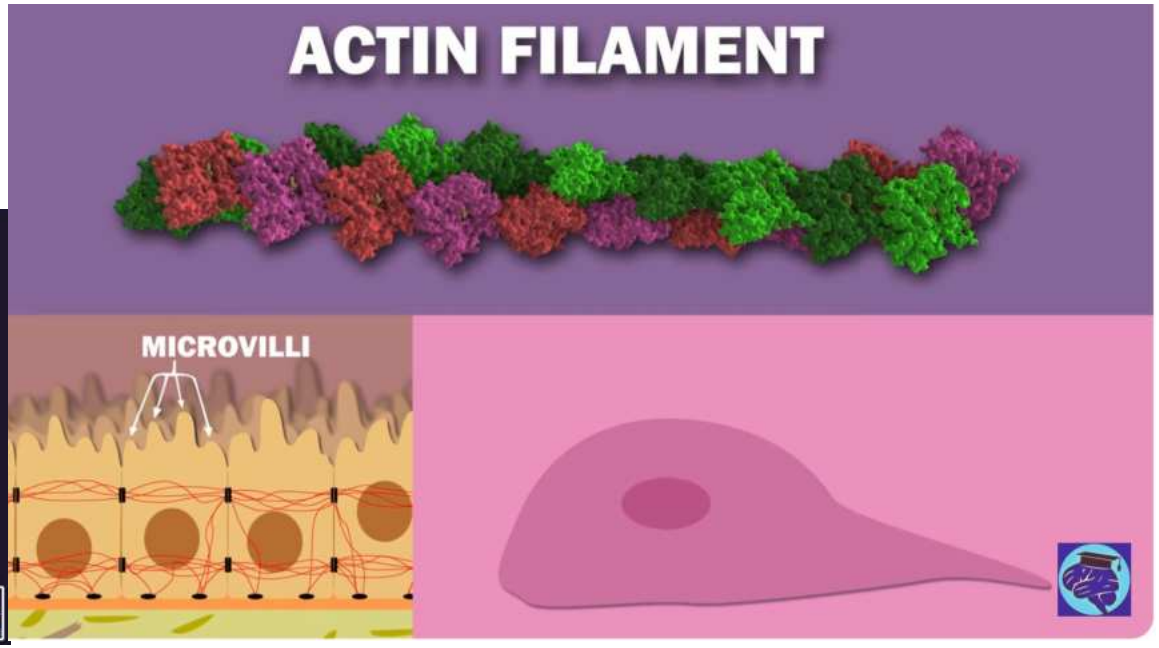


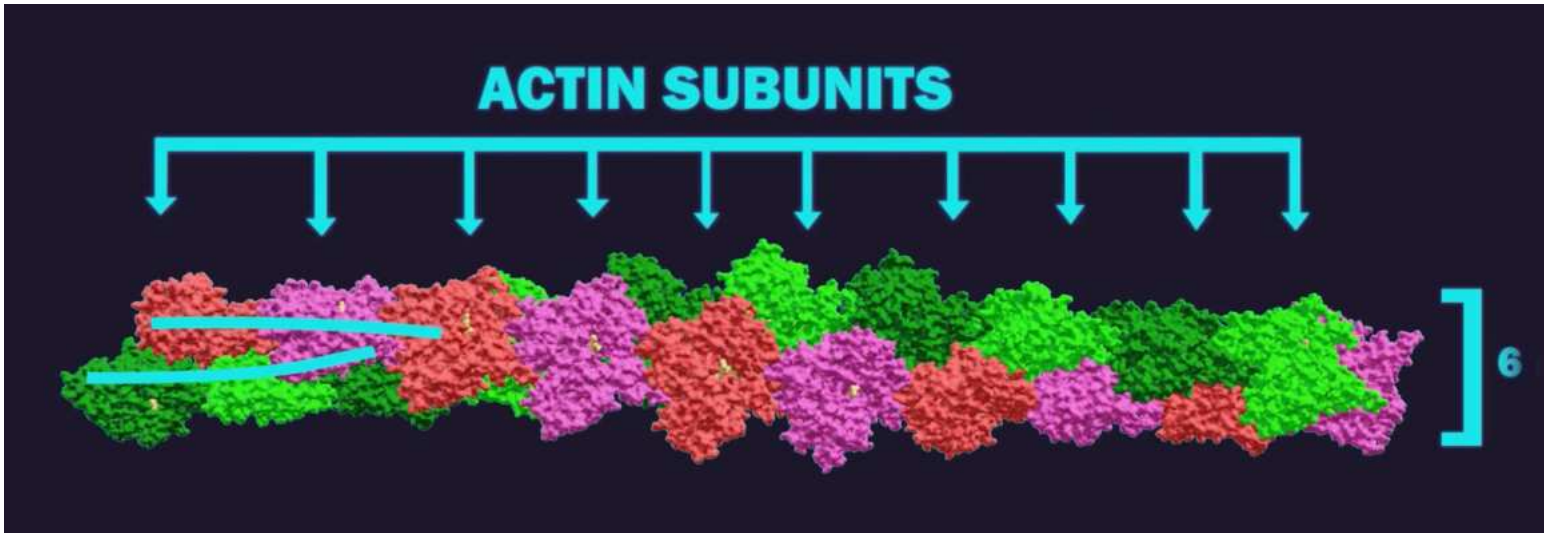
Weak, non covalent interactions
More filaments bind together



Actin filaments







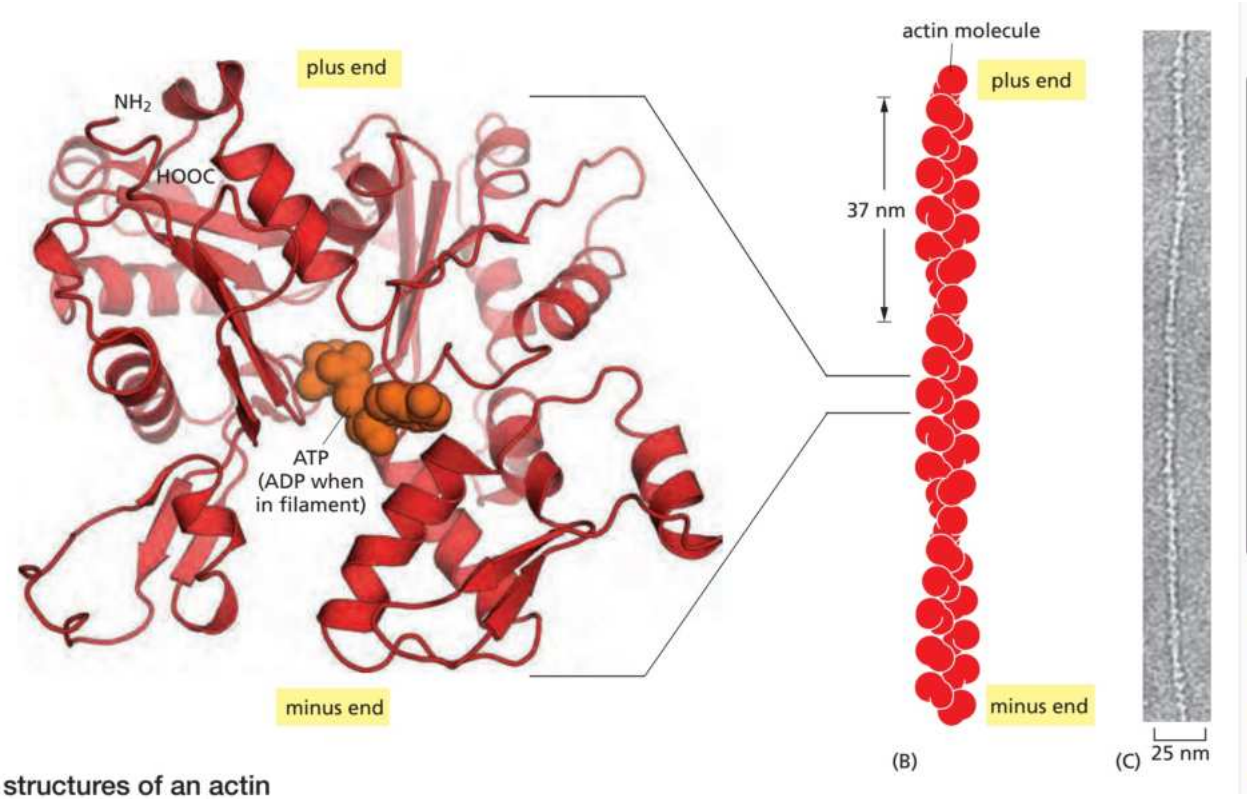
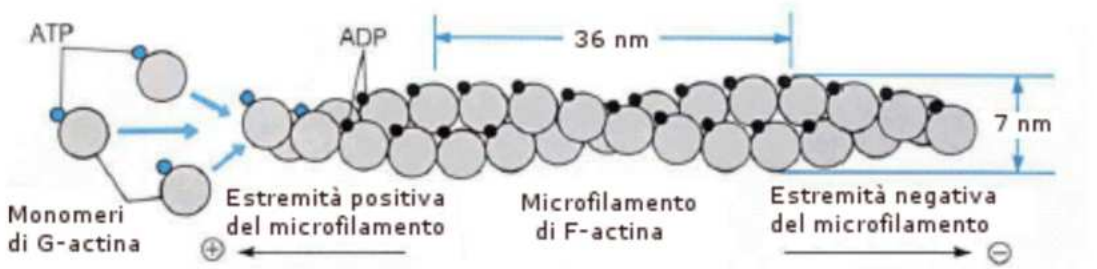


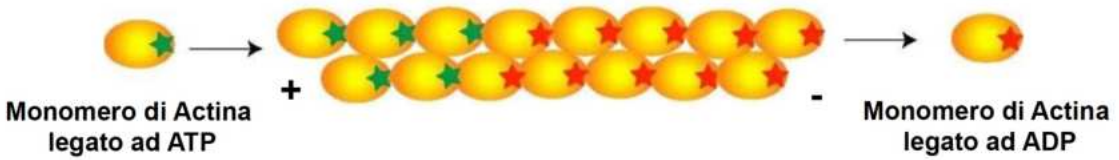
Figure 16–11 The structures of an actin monomer and actin filament. (A) The actin monomer has a nucleotide (either ATP or ADP) bound in a deep cleft in the center of the molecule. (B) Arrangement of monomers in a filament consisting of two protofilaments, held together by lateral contacts, which wind around each other as two parallel strands of a helix, with a twist repeating every 37 nm. All the subunits within the filament have the same orientation. (C) Electron micrograph of negatively stained actin filament. (C, courtesy of Roger Craig.)

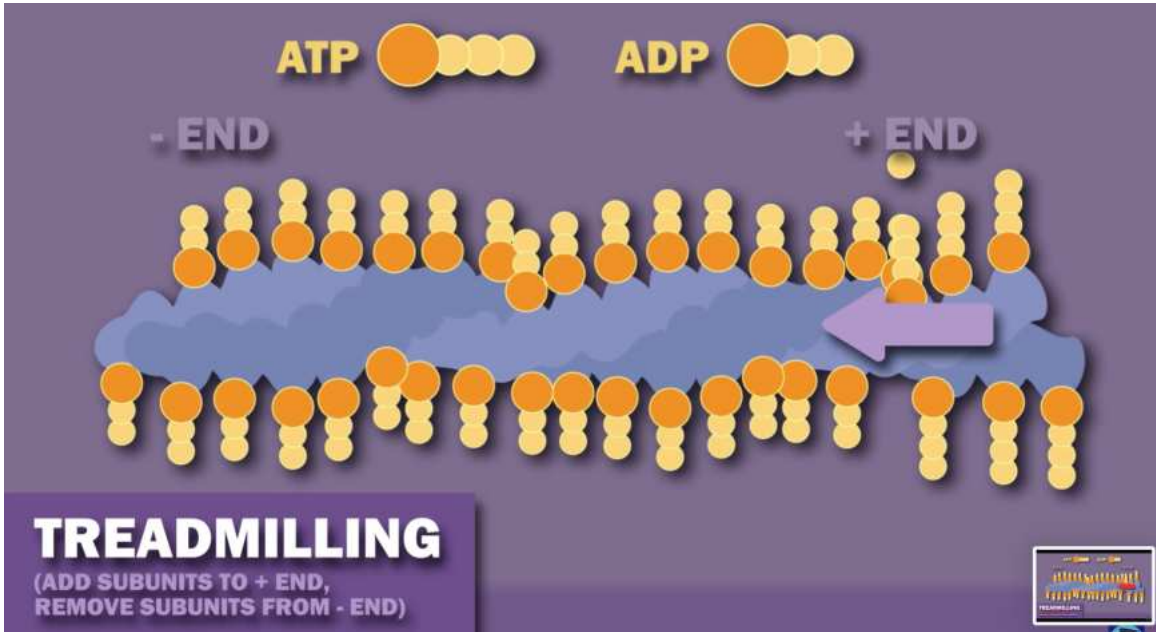
Individual actin filaments are quite flexible. The stiffness of a filament can be characterized by its persistence length, the minimum filament length at which random thermal fluctuations are likely to cause it to bend. The persistence length of an actin filament is only a few tens of micrometers. In a living cell, accessory proteins bundle filament together—more rigid

Alberts, Bruce; Johnson, Alexander; Lewis, Julian; Morgan, David; Raff, Martin; Roberts, Keith; Walter, Peter. Molecular Biology of the Cell (p. 898). W. W. Norton & Company. Kindle Edition.

I filamenti di actina hanno una polarità: per convenzione l'estremità "-" cresce lentamente mentre l'estremità "+" è quella che si accresce più velocemente.







Actin filament dynamics:

actin can catalyze the hydrolysis of the nucleoside triphosphate ATP. For free actin subunits, this hydrolysis proceeds very slowly;

however, it is accelerated when the subunits are incorporated into filaments.

Shortly after ATP hydrolysis occurs, the free phosphate group is released from each subunit, but the ADP remains trapped in the filament structure.

Thus, two different types of filament structures can exist, one with the “T form” of the nucleotide bound (ATP), and one with the “D form” bound (ADP). When the nucleotide is hydrolyzed, much of the free energy released by cleavage of the phosphate–phosphate bond is stored in the polymer. This makes the free-energy change for dissociation of a subunit from the D-form polymer more negative than the free-energy change for dissociation of a subunit from the T-form polymer. Consequently, the ratio of k off/k on for the D-form polymer, which is numerically equal to its critical concentration [Cc(D)], is larger than the corresponding ratio for the T-form polymer. Thus, Cc(D) is greater than Cc(T). At certain concentrations of free subunits, D-form polymers will therefore shrink while T-form polymers grow.

Myosine V walking on actin filaments

Using single-molecule fluorescence microscopy and optical-trap nanometry it has been shown that M5 moves along actin filaments toward the plus end in a "hand-over-hand" manner, advancing 36 nm per ATP hydrolysis cycle.

The 36 nm stride corresponds to a half pitch of the right-handed, double-helical structure of an actin filament, and, therefore, M5 moves approximately on a plane.

The actin monomer is a globular protein called G-actin, with a molecular weight of 41,800 Da. G-actin polymerizes noncovalently into actin filaments, called F-actin. Actin filaments consist of **two strands of globular molecules twisted into a helix with a repeat distance of about 36 nm**.

The mechanism underlying the alternate steps was suggested to arise from asymmetric kinetics of ADP dissociation from the two heads; ADP dissociation at the trailing head is more accelerated than at the leading head and/or ADP dissociation at the leading head is decelerated.

Myosine V walking on actin filaments

Using single-molecule fluorescence microscopy and optical-trap nanometry it has been shown that M5 moves along actin filaments toward the plus end in a "hand-over-hand" manner, advancing 36 nm per ATP hydrolysis cycle.

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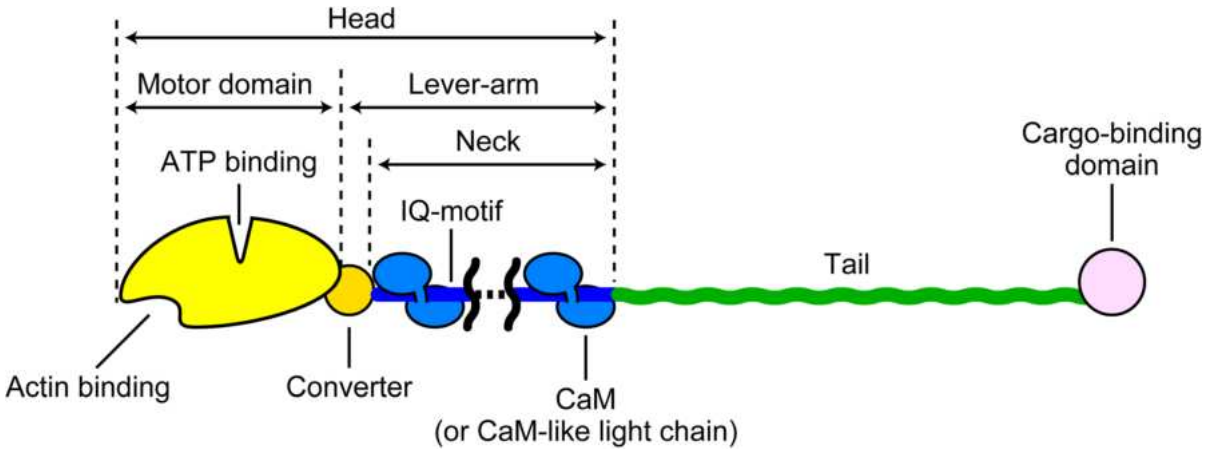
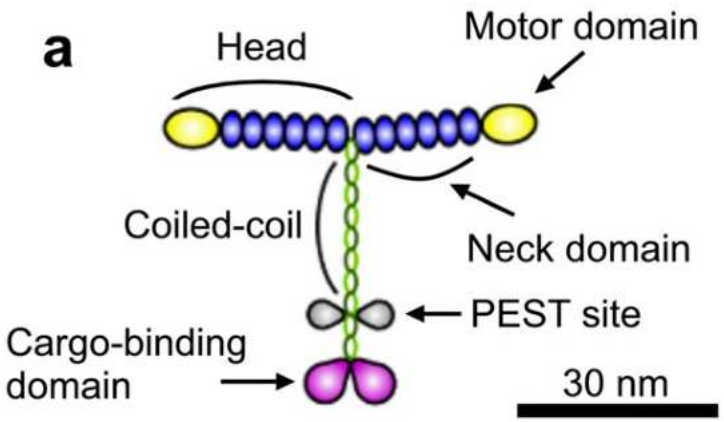


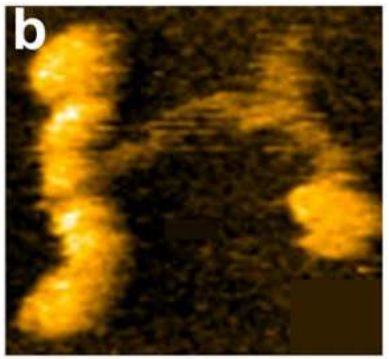
Actin binding and ATP binding sites are interacting

However, despite numerous and extensive studies, the heart of the motor mechanism, that is, how the tension for the forward step is generated in the molecule, coupled with the ATPase reaction, and how the energy liberated by ATP hydrolysis is used, has remained elusive.

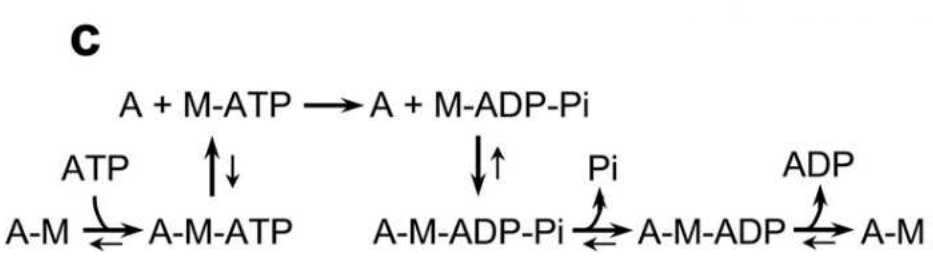
Myosine V walking on actin filaments

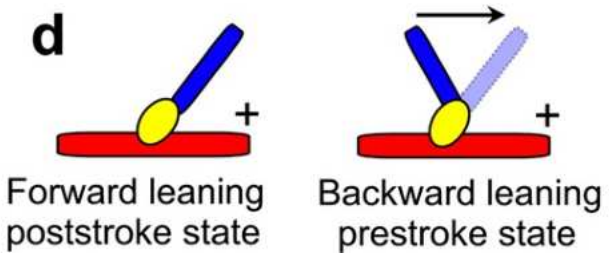
dx.doi.org/10.1021/cr4003837 | Chem. Rev. 2014, 114, 3120−3188





A myosin head has two states with respect to actin binding, i.e. weak (ADP-Pi) and strong (nucleotide-free) bound states.





The nucleotide-free head tightly bound to actin detaches from the actin immediately after binding to ATP, quickly followed by hydrolysis of the bound ATP to ADP−Pi. When the ADP−Pi bound head is attached to actin, the bound Pi dissociates from the head, which is followed by the formation of a strongly bound tertiary complex A−M−ADP (A and M denote actin and myosin, respectively) and then by ADP dissociation, completing one ATPase cycle.

The main role of actin in the ATPase reaction is to accelerate the otherwise very slow Pi and ADP dissociation from a myosin head.

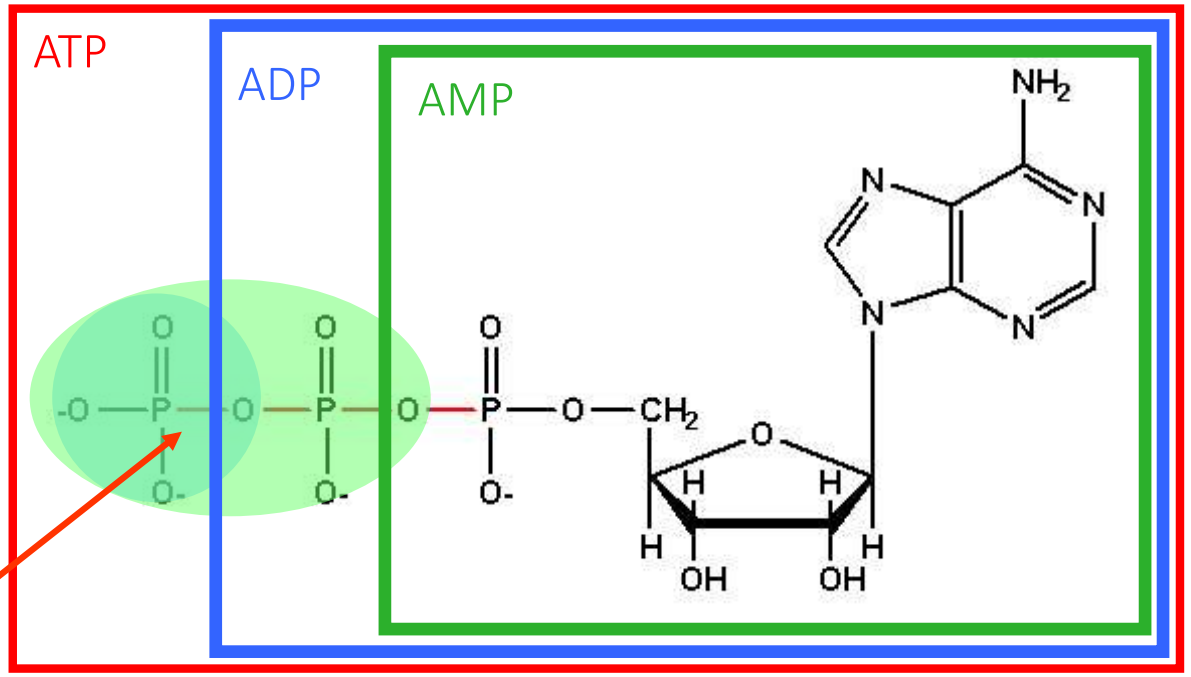
Energy in the cell: ATP

In the cell reactions that require energy are associated with ATP hydrolysis (hydrolysis= breaking down). ATP hydrolysis is an exothermic reaction, and the energy generated can be used to drive a non-spontaneous reaction.

ATP ⟼ ADP + P
(ΔH = -30 kJ/mol)

ATP ⟼ AMP + PP_i
(ΔH =-30 kJ/mol)

Adenosine Tri/Di/Mono phosphate



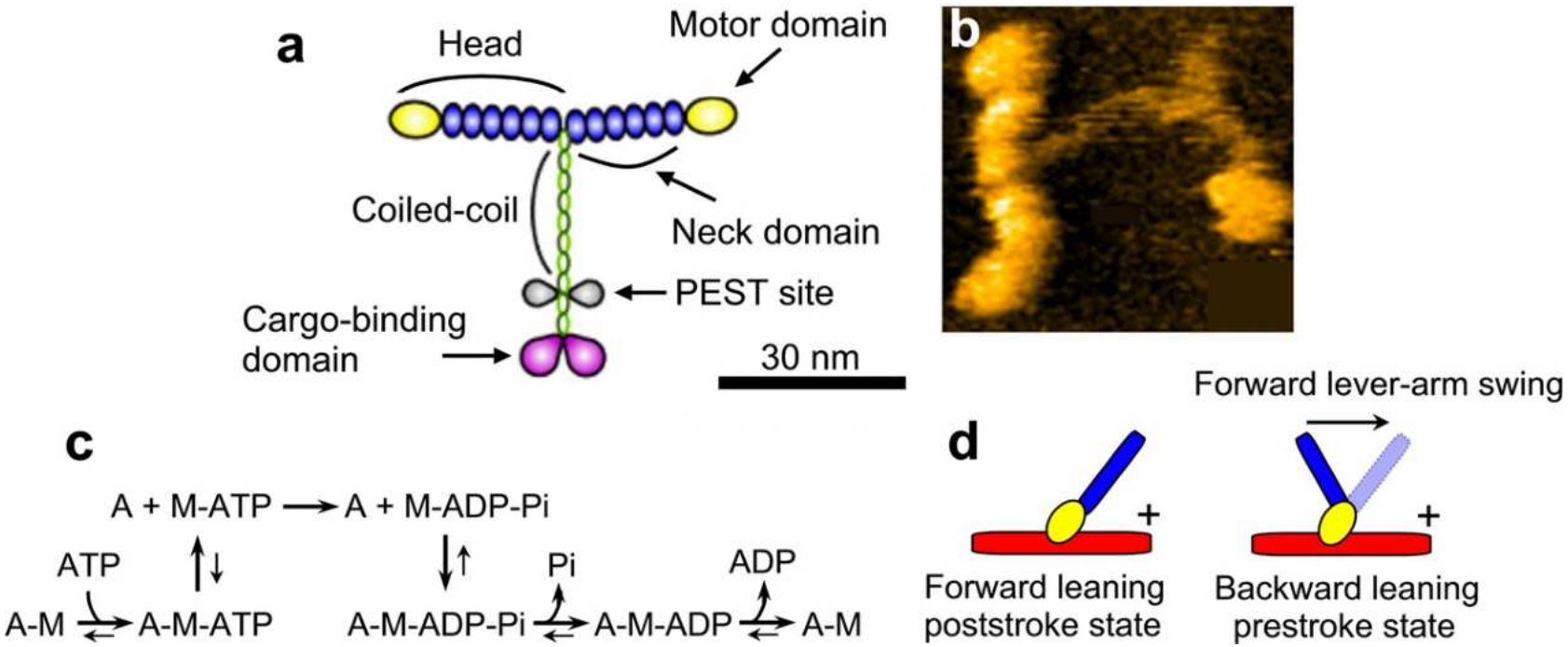
phosphodiester bonds have a large energy of hydrolysis (about 30 kJ/mol)

Energy production: accumulation of ATP

Energy consumption: breaking down (hydrolysis) of ATP ⟼ ADP or AMP

Myosine V walking on actin filaments

dx.doi.org/10.1021/cr4003837 | Chem. Rev. 2014, 114, 3120−3188



The key idea in the prevailing view on the chemo-mechanical coupling in myosin motility, which has been mainly derived from muscle myosin studies, is that the myosin head is supposed to take two different conformations, prestroke and poststroke conformations corresponding to different angles between the motor domain and the neck domain (often called “lever-arm”), depending on the nucleotide states

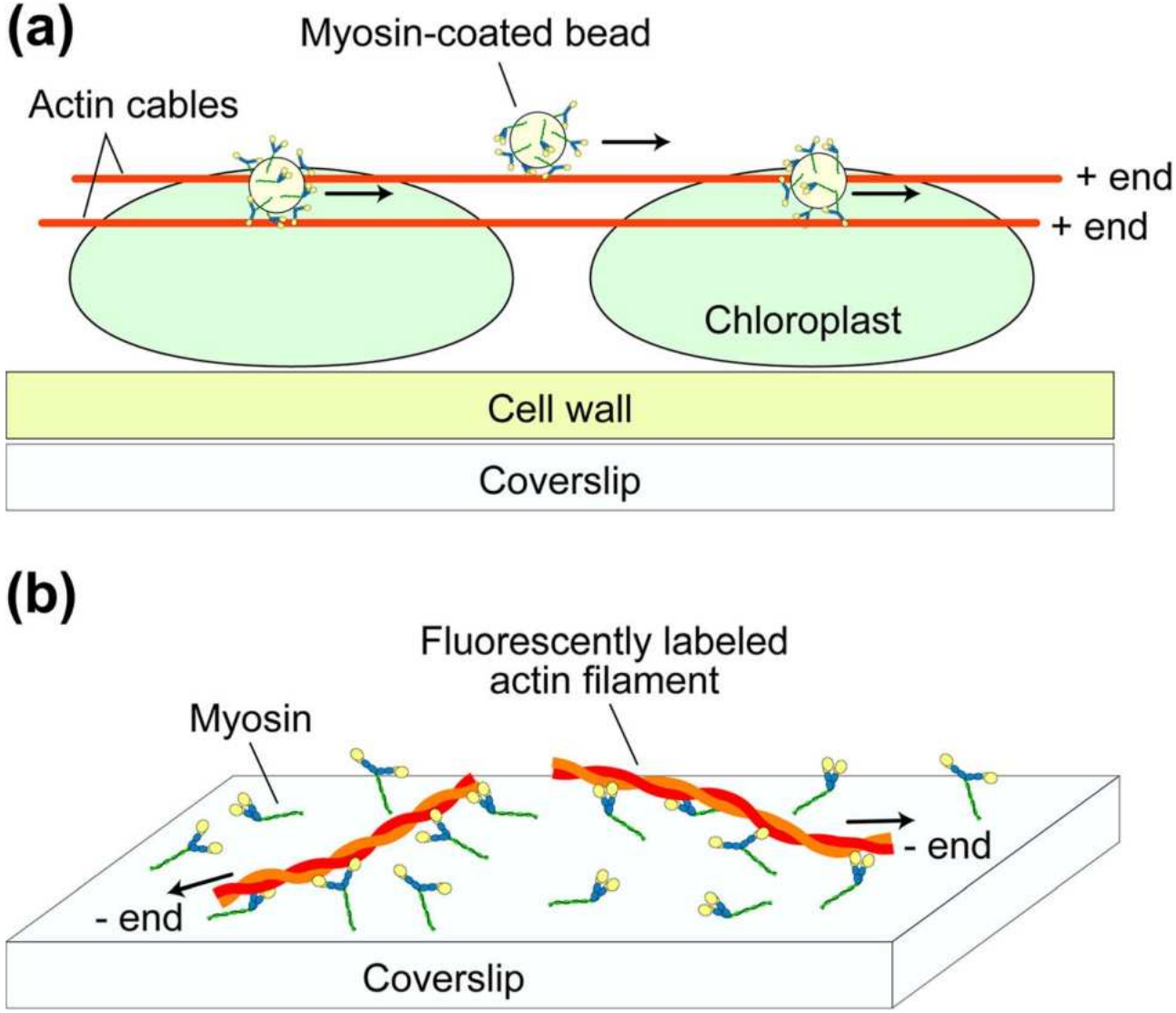
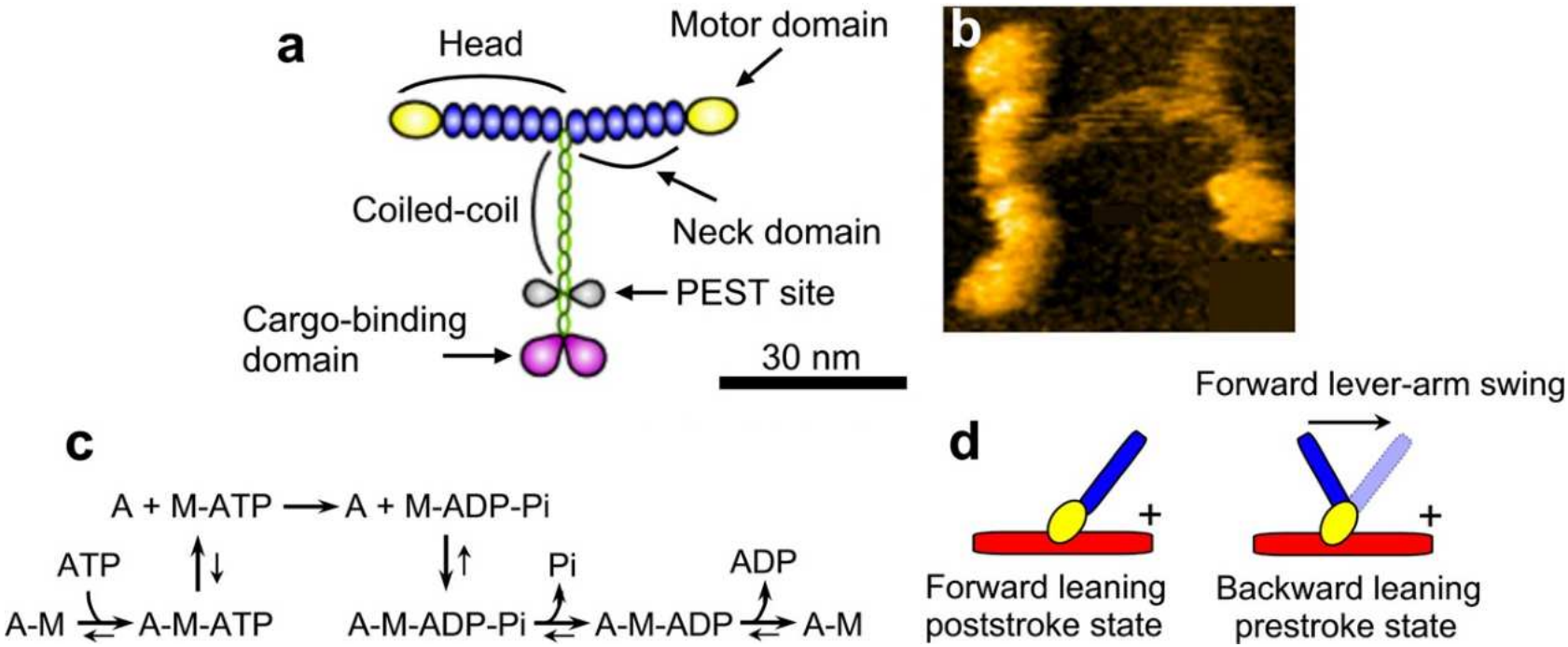


Fig. 4 Schematics showing in vitro motility assay systems for actomyosin. **a** Myosin-coated bead assay. The myosin-coated fluorescent beads are subjected to the polar arrays of actin cables naturally formed on chloroplastd of the alga *Nitella*, and movement of the beads are observed under a florescent microscope. **b** Actin filament gliding assay. Myosin molecules are attached to the surface of a nitrocellulose-coated coverslip and gliding motion of the fluorescently labeled actin filaments are observed under a fluorescence microscope

Myosine V walking on actin filaments

dx.doi.org/10.1021/cr4003837 | Chem. Rev. 2014, 114, 3120−3188

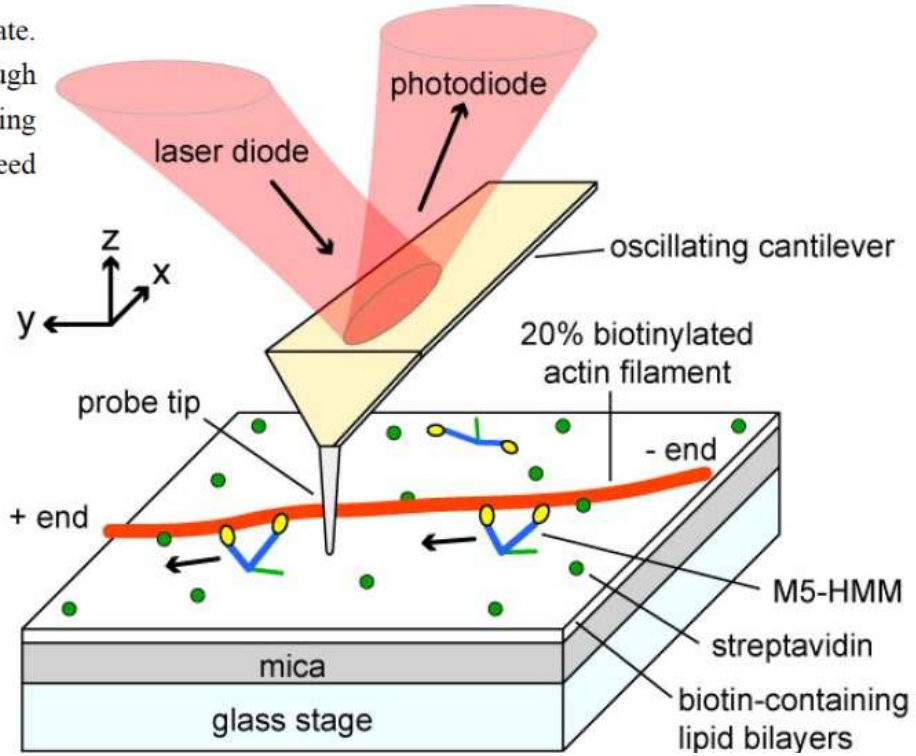


High-speed atomic force microscopy (HS-AFM), allow video-recording the structure and dynamics of functioning biomolecules at single-nanometer resolution, without disturbing their function. It helped to discover that the tension responsible for forward movement can be generated without any chemical transition, meaning that no chemical energy input is required for the tension generation. Moreover, the lever-arm swing (powerstroke) by the leading head spontaneously occurs when the trailing head detaches, thus demonstrating that no chemical energy input is required for the lever-arm swing either.

Myosine V walking on actin filaments

Video imaging by high-speed AFM has been applied to capture the dynamic behaviour of myosin V (two headed motor that functions as cargo transporter in cells) translocating along an acting filament. Moves hand-over-hand, 36 nm per ATP hydrolysis

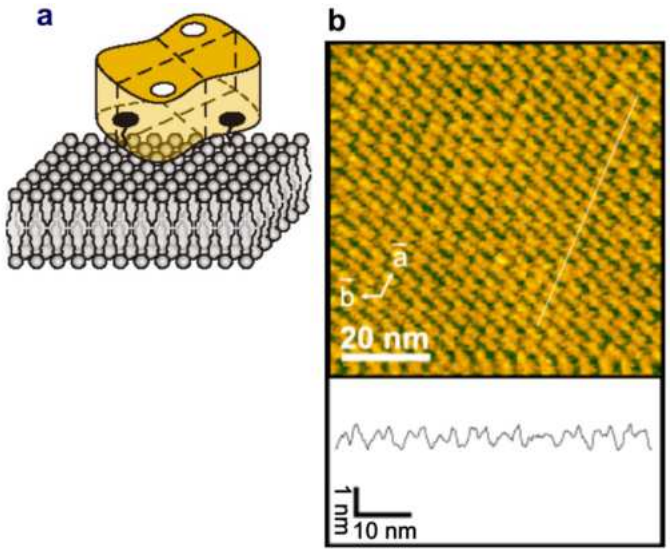
Supplementary Figure 1 | Schematic of assay system for HS-AFM imaging (not scaled). A mica surface was fully covered with biotin-containing lipid bilayers. Streptavidin molecules (green circles) were partially deposited on the substrate. Biotinylated actin filaments were immobilised on the bilayer surface through streptavidin molecules. M5-HMM was deposited on the lipid bilayers. All imaging experiments were performed in the tapping mode using a laboratory-built high-speed AFM apparatus[5,6].



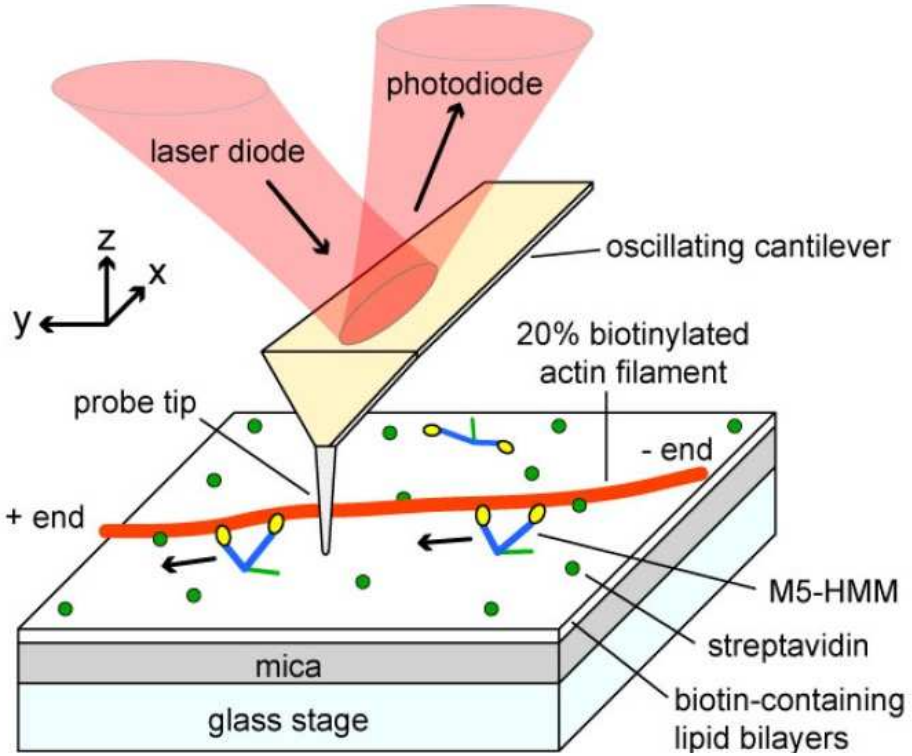
A positively charged lipid in the mixed lipid bilayer was necessary to assure weak interaction with Myosine and translocation along the actin filament

N. Kodera, D. Yamamoto, R. Ishikawa, T. Ando *Nature* **468**, 72 (2010)

Myosine V walking on actin filaments



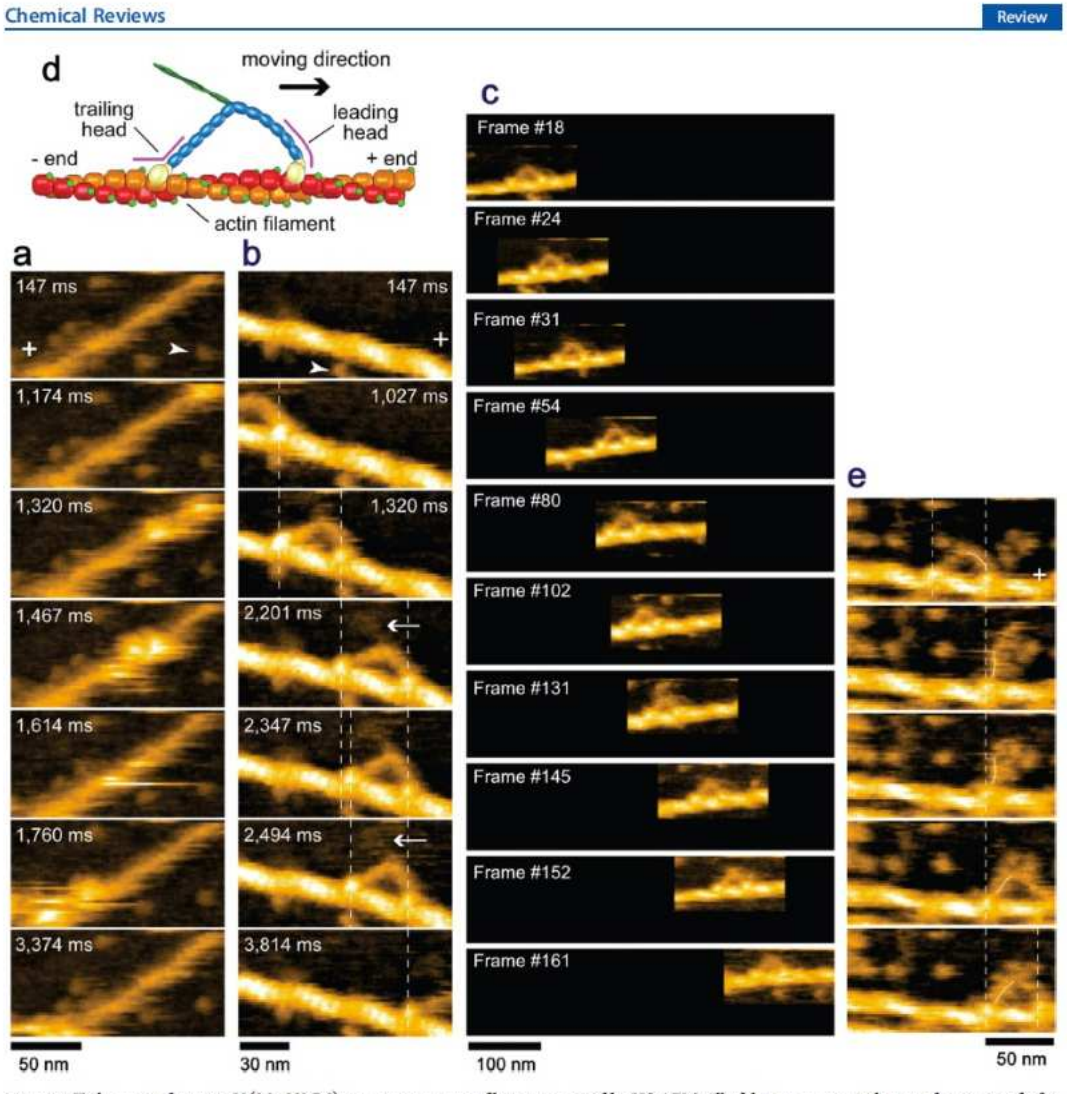
Streptavidin is a homo tetramer with dihedral D2 symmetry. Importantly, it is not favorable to nonspecific binding of many proteins, while each subunit has a high affinity biotin binding site. Streptavidin 2D crystals are easily formed onmthe surface of a fluid SLB containing biotin-lipid.



On the SLBs, two of the four biotin binding sites of streptavidin face the lipid bilayer and are occupied by biotin, whereas the other two are exposed to the aqueous environment and accessible. Therefore, biotinylated samples can be specifically immobilized on the surface of streptavidin 2D crystals

N. Kodera, D. Yamamoto, R. Ishikawa, T. Ando *Nature* **468**, 72 (2010)

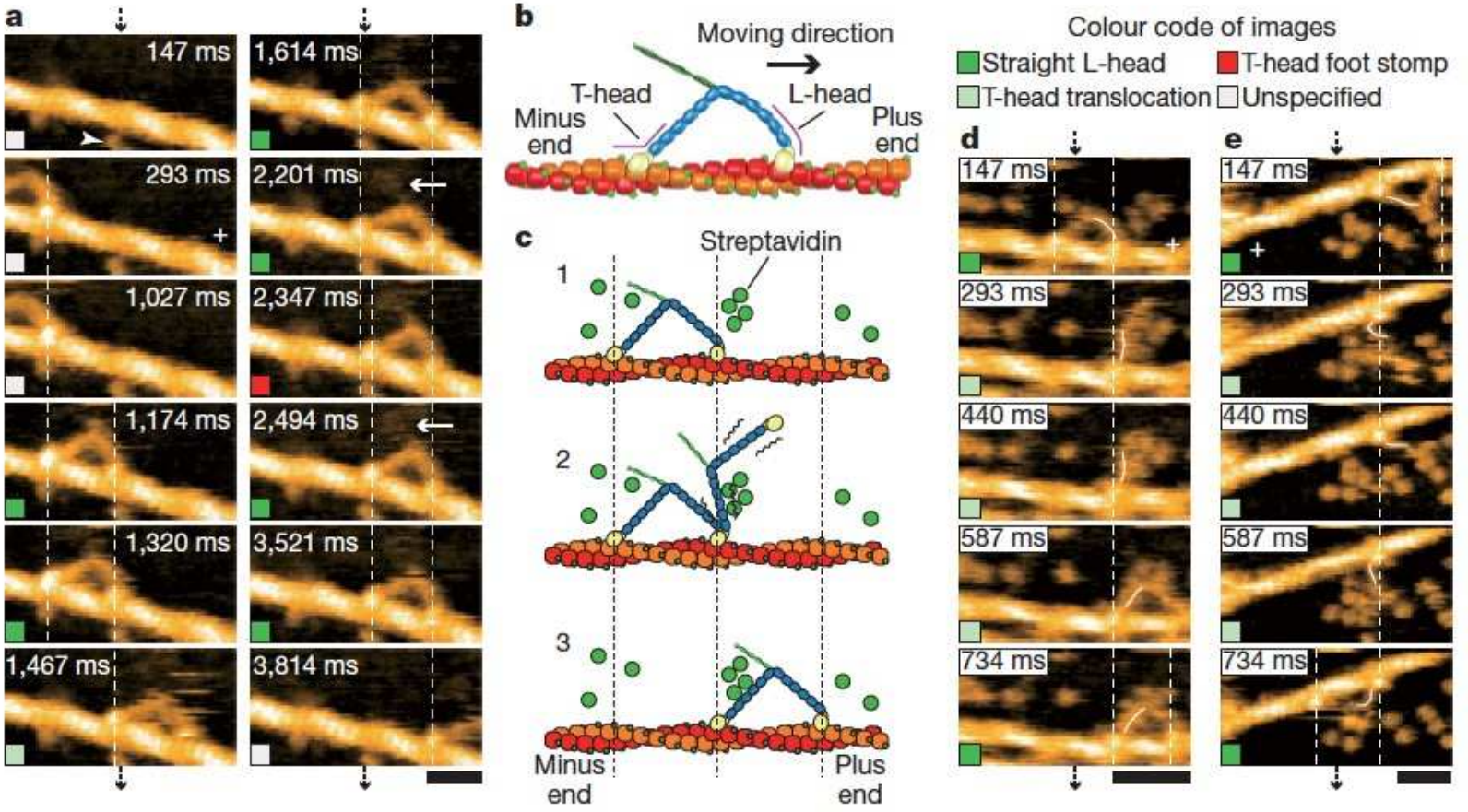
Myosine V walking on actin filaments



N. Kodera, D. Yamamoto, R. Ishikawa, T. Ando *Nature* **468**, 72 (2010)
dx.doi.org/10.1021/cr4003837 | Chem. Rev. 2014, 114, 3120−3188

Myosine V walking on actin filaments

AFM images demonstrate a hand-over-hand movement, with swinging lever-arm motion : the detached T-head rotationally diffused around the advancing neck-neck junction. Extra STV needed as an “obstacle” to slow down the motion to be visualized (100 ms/frame)

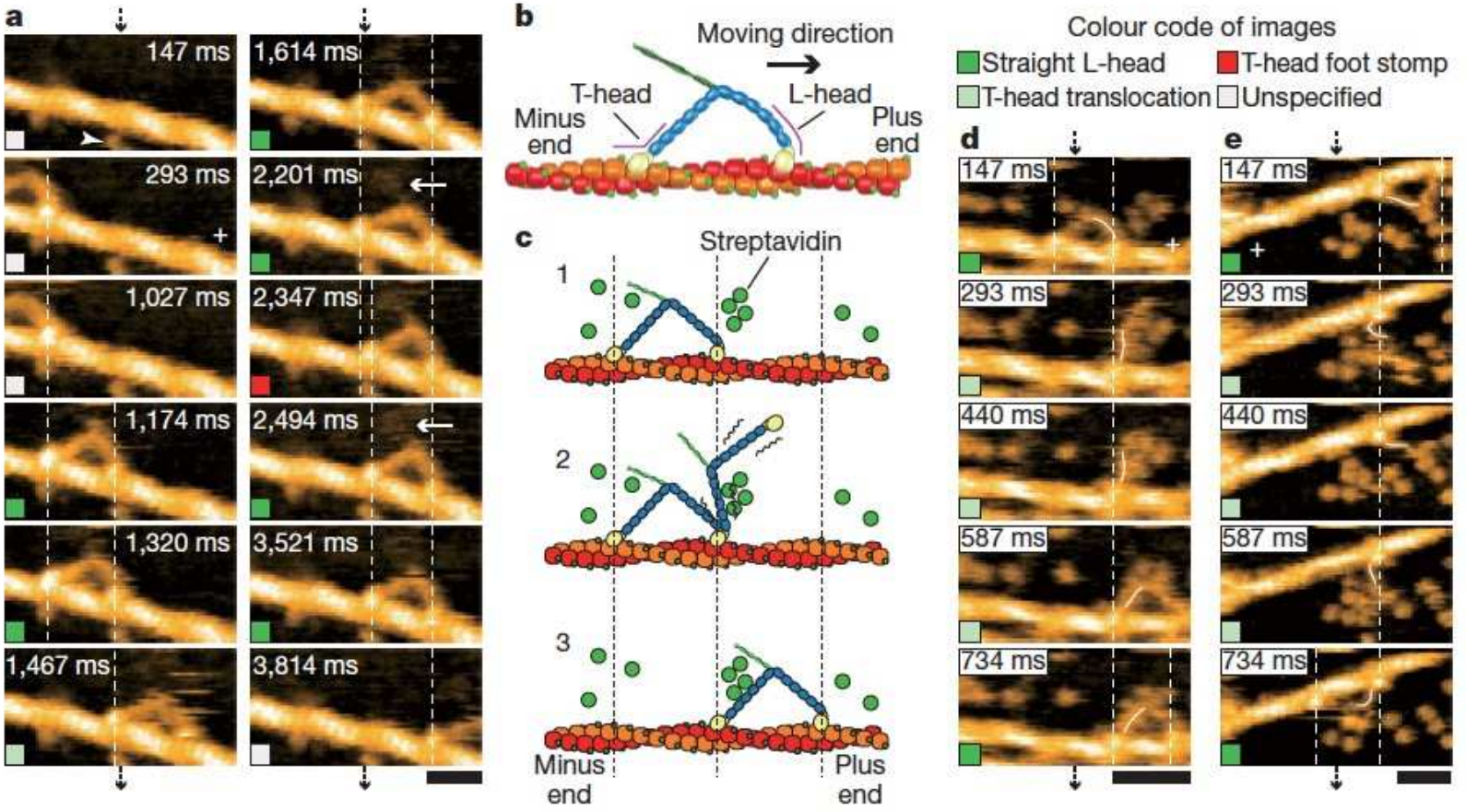


http://biophys.w3.kanazawa-u.ac.jp/M5_movies.htm

N. Kodera, D. Yamamoto, R. Ishikawa, T. Ando *Nature* **468**, 72 (2010)

Myosine V walking on actin filaments

The neck–motor domain junction appears smooth in the leading head (L-head) but is V-shaped in the trailing head (T-head) without exception.
The short coiled coil tail was mostly tilted towards the minus end of actin



N. Kodera, D. Yamamoto, R. Ishikawa, T. Ando *Nature* **468**, 72 (2010)

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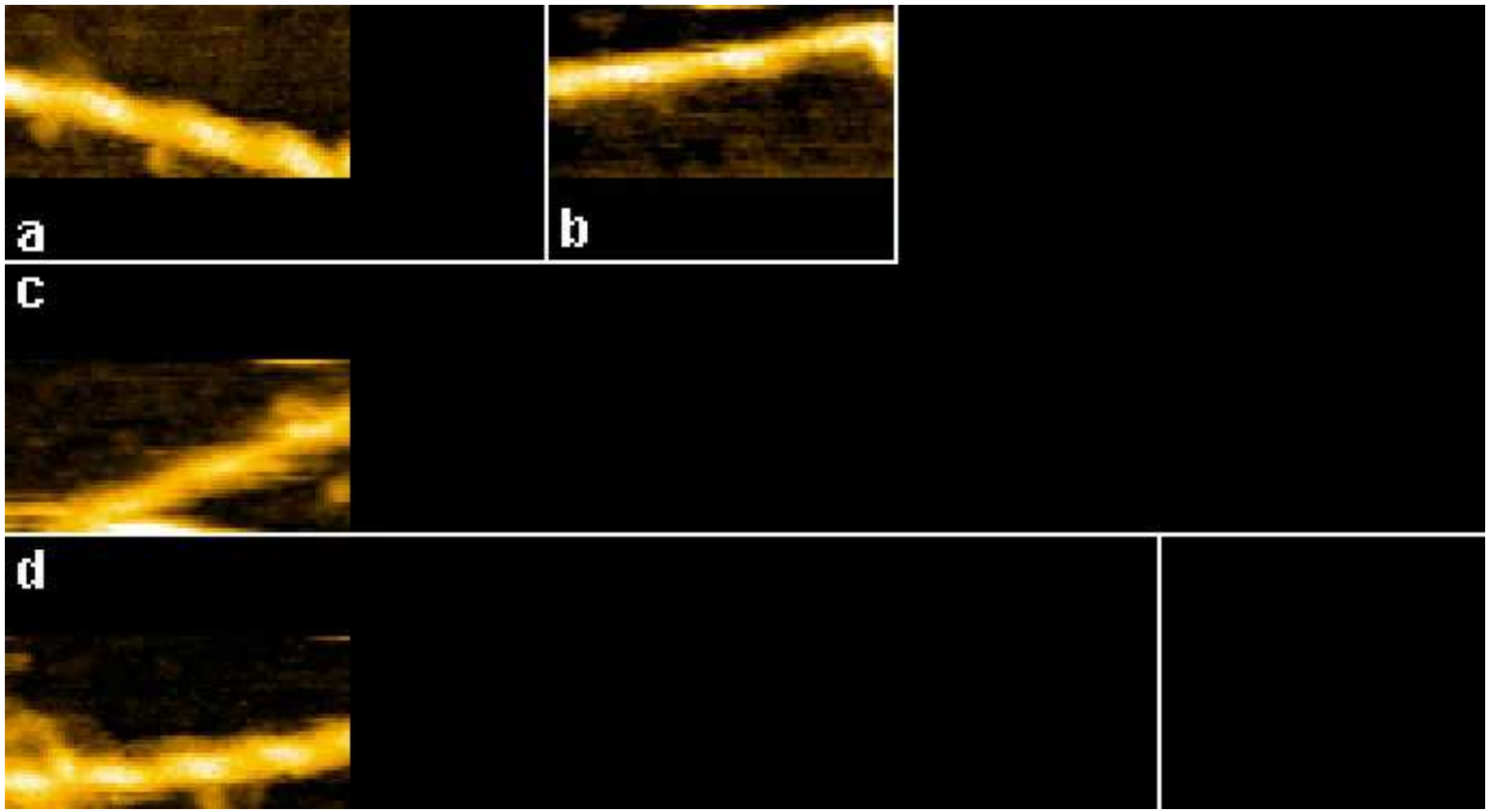
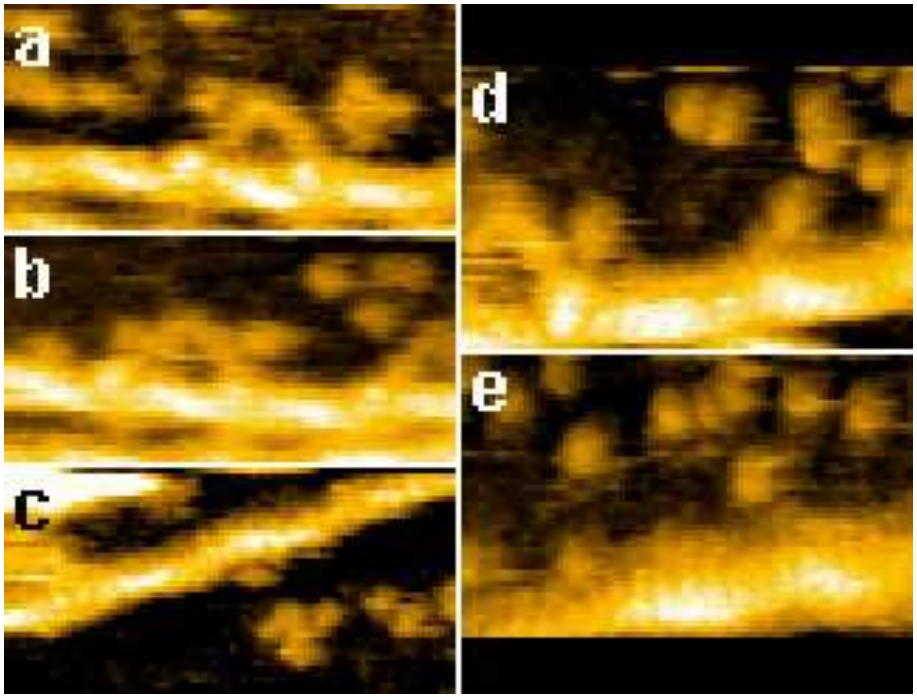


N. Kodera, D. Yamamoto, R. Ishikawa, T. Ando *Nature* **468**, 72 (2010)

http://biophys.w3.kanazawa-u.ac.jp/M5_movies.htm

Myosine V walking on actin filaments

After T-head detachment, the nearly straight leading neck swung from the reverse arrowhead (R-ARH) orientation to the arrowhead (ARH) orientation confirming the swinging lever-arm motion initially proposed for muscle myosin. The detached T-head rotationally diffused around the advancing neck–neck junction (no translational diffusion on the actin occurs) and then bound to a forward site on the actin filament, completing one step.



The captured images show that the forward movement is driven not by bending but by rotation of the L-head. The rotation seems to occur spontaneously after T-head detachment, suggesting that intramolecular tension driving the L-head swing exists in the two-headed bound molecules.

N. Kodera, D. Yamamoto, R. Ishikawa, T. Ando *Nature* **468**, 72 (2010)

Myosine V walking on actin filaments

Moreover, it was observed that the leading head of the two-headed bound M5-HMM was often sharply bent in the nucleotide-free condition while it was mostly straight in ADP and ATP. Therefore, just by looking at the shape of the leading head, we can judge whether or not the leading head contains nucleotides. ADP dissociation rate constant at the leading head is 0.1 s-1. This means that ADP is released from the leading head every 10 s, on average. M5-HMM walks many steps for 10 s. Thus, we can conclude that during walking ADP does not dissociate from the leading head. ADP dissociation, and the subsequent ATP binding, and the resulting detachment from actin solely occurs at the trailing head.

Just before foot stomping at the leading head, the head never showed the sharply bent conformation which is unique to the nucleotide-free leading head. This fact reinforces our conclusion that the leading head performing foot stomp carries ADP and thus the brief detachment from actin (i.e. the initial stage of foot stomp process) is not caused by binding of new ATP to the leading head.

N. Kodera, D. Yamamoto, R. Ishikawa, T. Ando *Nature* **468**, 72 (2010)