CELL MECHANICS

1. Introduction (1h)

2. Physical principles (7h)

2.1. Forces at molecular and cell level
2.2. Thermal forces, diffusion, and chemical forces
2.3. Motor proteins (types, working principles)

3. Mechanics of the Cytoskeleton and Mechnaotransduction (6h)

3.1. Cytoskeleton structure
3.2. Force generation by the cytoskeleton and cell motility
3.3. Cellular mechanotransduction (basic principles and examples)

5. Experimental techniques to study cell mechanics (10 h)

5.1. Optical, magnetic and acoustic tweezers
5.2. Super-resolution optical microscopy techniques (STED, PALM)
5.3. Lab visit and experimental optical tweezers – cell mechanics session at CNR-IOM

- Physical forces and their magnitudes at the single-molecule level
- Modeling complex mechanical devices as protein machines by using three elements:

  Spring, Dashpot, Mass
- Mass, Stiffness and Damping of Proteins

- Motor proteins and other molecular machines are able to move and do work because they generate force. It is the force which drives change and motion.

https://www.mechanobio.info/cytoskeleton-dynamics/what-are-motor-proteins/

- Where this force comes from ? What effect does it have on proteins and cells ?
  Which is the magnitude of forces acting on molecules ?

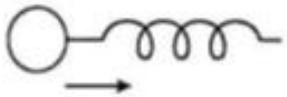
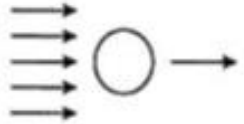
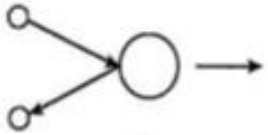
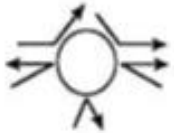
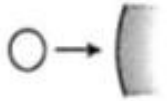
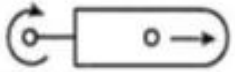
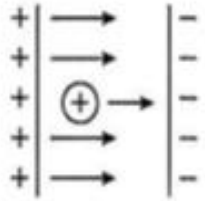
- Modeling complex mechanical devices as protein machines by using three elements:

Spring, Dashpot, Mass

Important NOTE:
Proteins and other biomolecules are so small that the **inertial forces** are comparatively **small** and can usually be ignored, whereas the **viscous forces** from the surrounding fluid are usually **large** and dominate the mechanical responses.

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

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

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

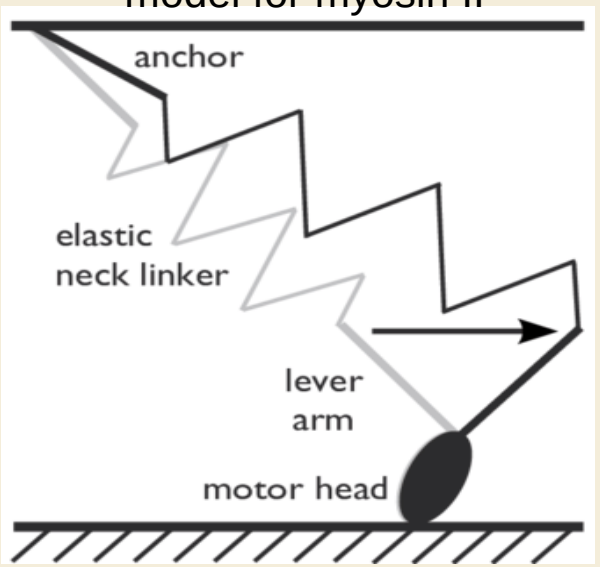
Elastic

$F = k \cdot x$, where: k – spring constant (stiffness), x – displacement

Example: motor protein k= 1 pN/nm,

spring strained trough distance x= 5 nm → **F= 5 pN**

model for myosin II



Viscous

$F = \gamma \cdot v$, where : γ- drag coefficient , v – relative velocity between object and liquid

$\gamma = 6\pi\eta r$, with η – liquid viscosity, r – radius of a spherical particle

Example: globular protein with diameter r= 3 nm, molecular mass MM= 100 kDa, **γ~ 60 pN s/m** (at T= 20 C),

average thermal speed: **v ~ 8 m/s** → **F ~ 480 pN**

consequence of thermally driven collisions from the surrounding solvent molecules

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

Collisional and thermal

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

$$V = \sqrt{\frac{3K_B T}{m}}$$

Water molecule : mass m ~ 0.3 x 10^{-23} g, average speed associated with its kinetic energy : v ~ 600 m/s

→ **momentum: p ~ 18 x 10^{-24} kg m/s**.

Assuming the interaction is perfectly elastic the force for one collision

F= 2 x18 x10^{-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 100 kDa protein is on the order of the viscous force: F **~ 500 pN**, and drives diffusion.

Some Important Constants:

Boltzman constant K_B ~ 1.4 x 10^{-23} m^2 kg / s^2 K

Da = u (unified atomic mass); u ~ 1.66 x 10^{-24} g

Avogadro's number N_A ~ 6 x 10^{23} p/ mol

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Optical forces

Optical pressure due to the momentum of light (photons : p=hν/c)

Example: if an object absorbs one green photon / second, the corresponding force is:

F= Δp/ Δ t ~ **1.3 10 $^{-15}$ pN** - very small

where: E= h ν= 4 10 $^{-19}$ J, the energy of the photon; h= 6.63 10 $^{-34}$ m ^{2} kg / s - Planck constant;

ν = 6 10^{14} Hz - light frequency, c= 3 10^{8} m/s – light speed in vacuum

A laser beam of power P=1 mW has about N ~ 2.5 10 ^{15} photons ! → F (1mW) ~ 3.25 pN

still small but enough to make an effect on small obejcts (e.g. laser pointer beam focused on a black wall)

Gravity

Example: protein 100 kDa = 166 10 $^{-21}$ g, the gravitational force **F= 1.7 10 $^{-9}$ pN** very small

F= mg, m – mass, g – gravitational acceleration; for a Red Blood Cell we have F ~ 1 pN

Centrifugal

Ultracentrifuges → acceleration ac ~ 10 ^{5} g, associated force on protein 100 kDa is still modest: F= 1.7 10^{-6} pN, but this is large enough to cause the protein to drift at an average speed of ~ 3 μm/s → protein sedimentation through a distance of 100 mm (typical length of centrifuge tube) in about 10 h.

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

Electrostatic **F= qE**

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

Charge q= 160 10^{-21} C; plasma membrane – 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 force exists between two monovalent ions in water that are separated by 1 nm .

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

These forces can be as high as 100 pN for 1 nm^2 of protein-protein surface interface

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**.

Homework: calculate / verify the values for the forces

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 $F = \gamma u$ 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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Table 2.2 Physical properties of a globular protein of molecular mass 100 kDa

Property	Value	Comment
Mass	166×10^{-24} kg	Mass of 1 mole/Avogadro constant
Density	1.38×10^{3} kg/m^3	1.38 times the density of water
Volume	120 nm^3	Mass/density
Radius	3 nm	Assuming it is spherical
Drag coefficient[a]	60 pN·s/m	From Stokes' law (Chapter 3)
Diffusion coefficient[a]	67 μm^2/s	From the Einstein relation (Chapter 4)
Average speed[b]	8.6 m/s	From the Equipartition principle (Chapter 4)

Note: 1 nm = 10^{-9} m, but 1 nm^3 = (1 nm)^3 = 10^{-27} m^3.

[a]In water at 20°C

[b]Root-mean-square (the square root of the average value of the square of the velocity)

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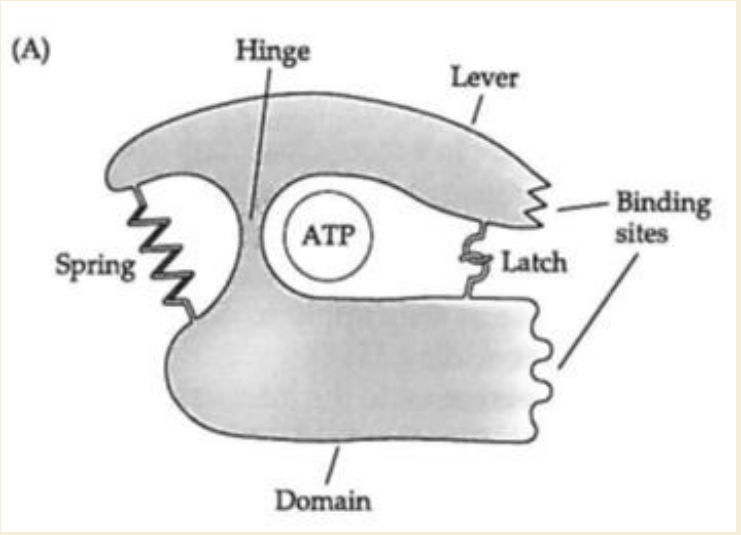
Modeling complex mechanical devices as protein machines by using three elements: Spring, Dashpot, Mass

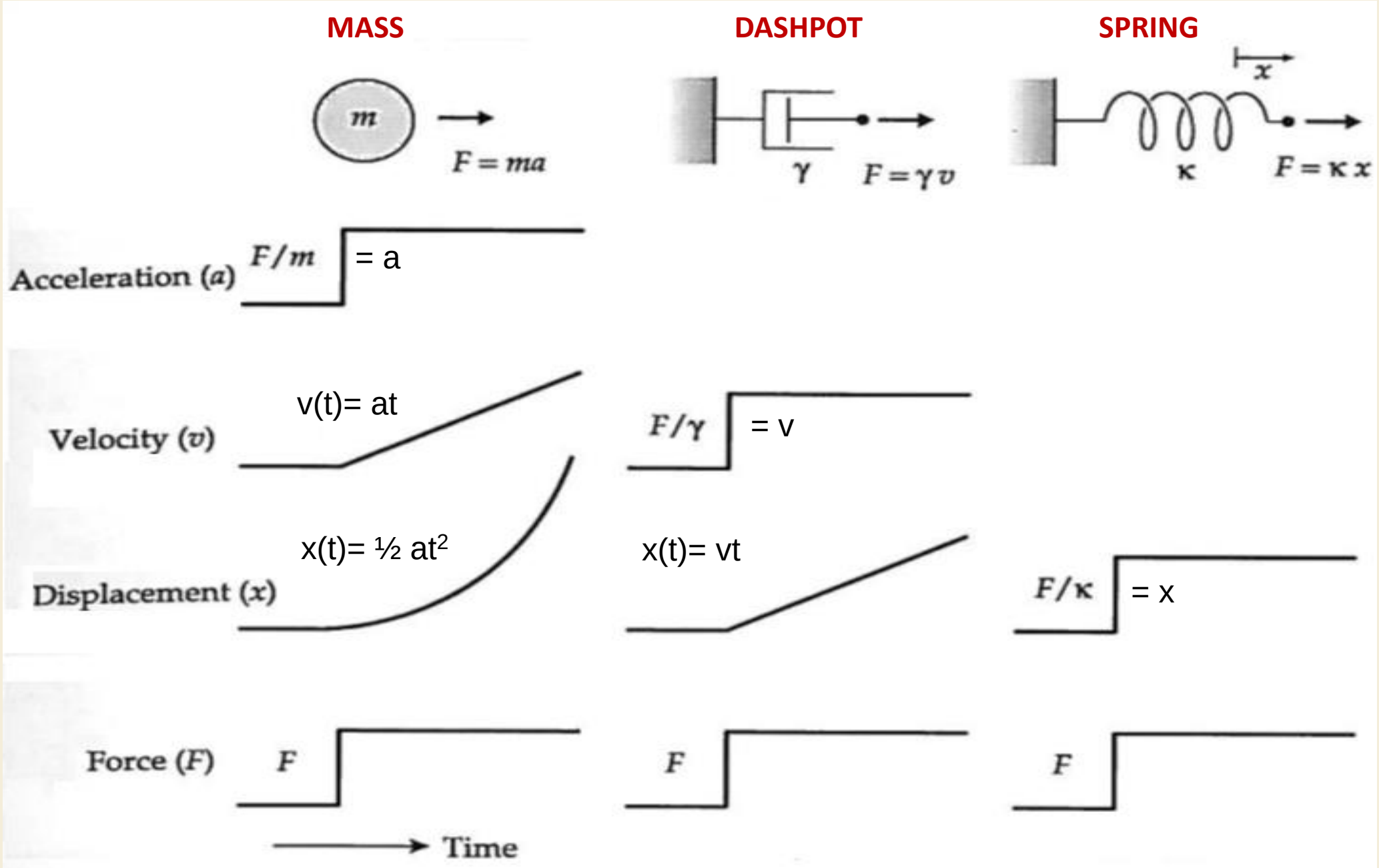
A protein or other molecules can be thought as a mechancial device composed of **atoms that have mass**, connected by **bonds that have elasticity**, like springs, and moves **in liquid environment**, facing **viscosity** like dashpots.

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

SPRING, DASHPOT, MASS.



Motion of a MASS, a DASHPOT and a SPRING under the influence of a constant external force



Example. The force generated by the bacterial motor.

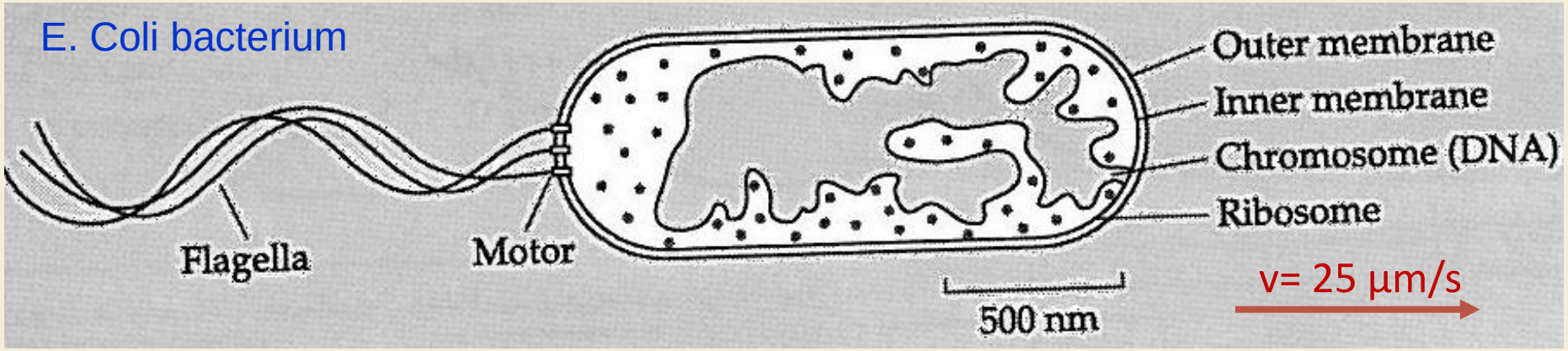
The bacterial flagelar motor should generate a force to move an E. Coli bacterium through water at a constant velocity v= 25 μm/s , which is the force to do this ?

Dashpot:

$F = \gamma \cdot v$ $\qquad\qquad$ $\gamma = 3\pi D\,\eta \sim 10$ mPa s μm

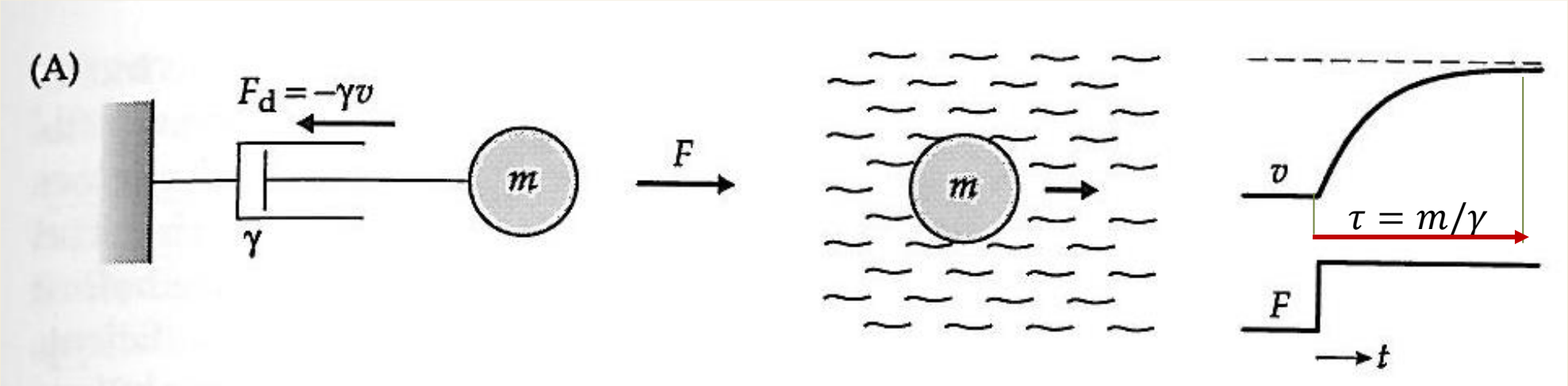
η~ 1 mPa s – water viscosity, D ~ 1 μm (diam of E. Coli)

F ~ 0.25 pN



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

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

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Example. **Inertia of a bacterium**

Consider a bacterium swimming through water at a constant velocity v(0)= 25 μm/s. How long will it continue to coast after its motor has stopped ?

$$v(t) = v(0) \cdot \exp\left(-\frac{t}{\tau}\right)$$

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

$m \approx 0.33 \cdot 10^{-15}$ kg

ɣ = ~10 mPa s μm

$$\boldsymbol{\tau = 0.33 \cdot 10^{-7}\ \mathbf{s}}\ \text{!!!}$$

$$\boldsymbol{x_{stop}} = \int_0^{\tau} \mathrm{v}(0) \cdot \exp\left(-\frac{\mathrm{t}}{\tau}\right) \mathrm{dt} = \mathrm{v}(0) \cdot \tau \approx \mathbf{0.8 \cdot 10^{-3}}\ \mathrm{nm}$$

Less than the diameter of a water molecule

BACTERIA HAS VERY LITTLE INERTIA TO KEEP IT MOVING FORWARD

Example. The persistance of protein movements

For a globular protein of 100 kDa, the time constant τ is:

τ ~ 3 ps

m= 166 10^{-24} kg, ɣ= 60 pN s / m

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

This means that 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.

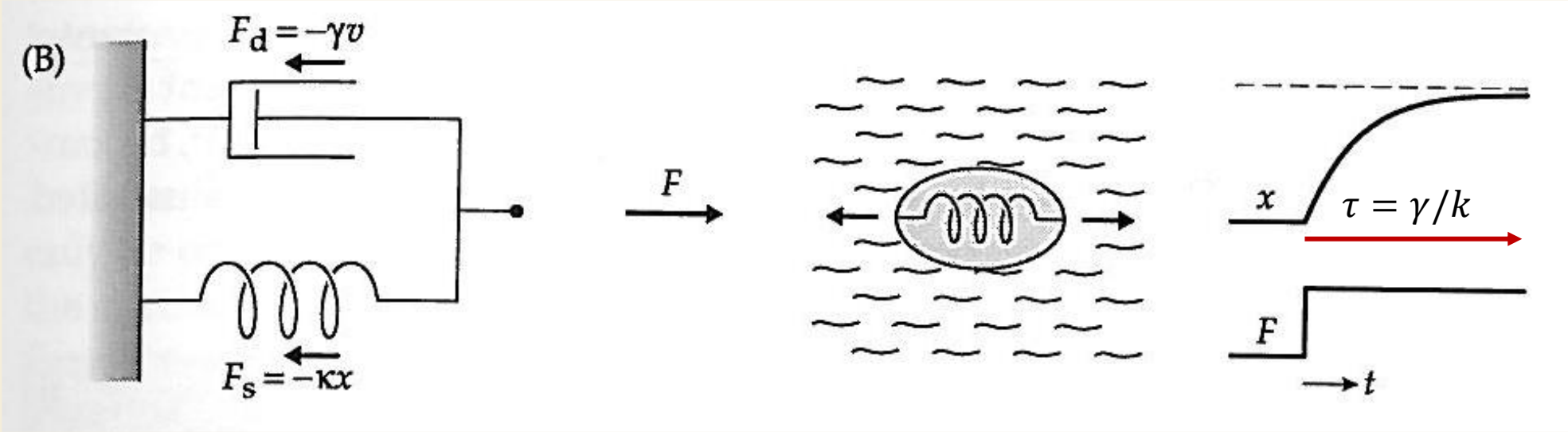
Given that the average instantaneous speed of such a protein is 8.6 m/s, the avearge distance that the protein moves before its speed is randomized by molecular collisions is only 0.24 A.

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 large-scale conformational change.

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



Time constant

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

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

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

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.

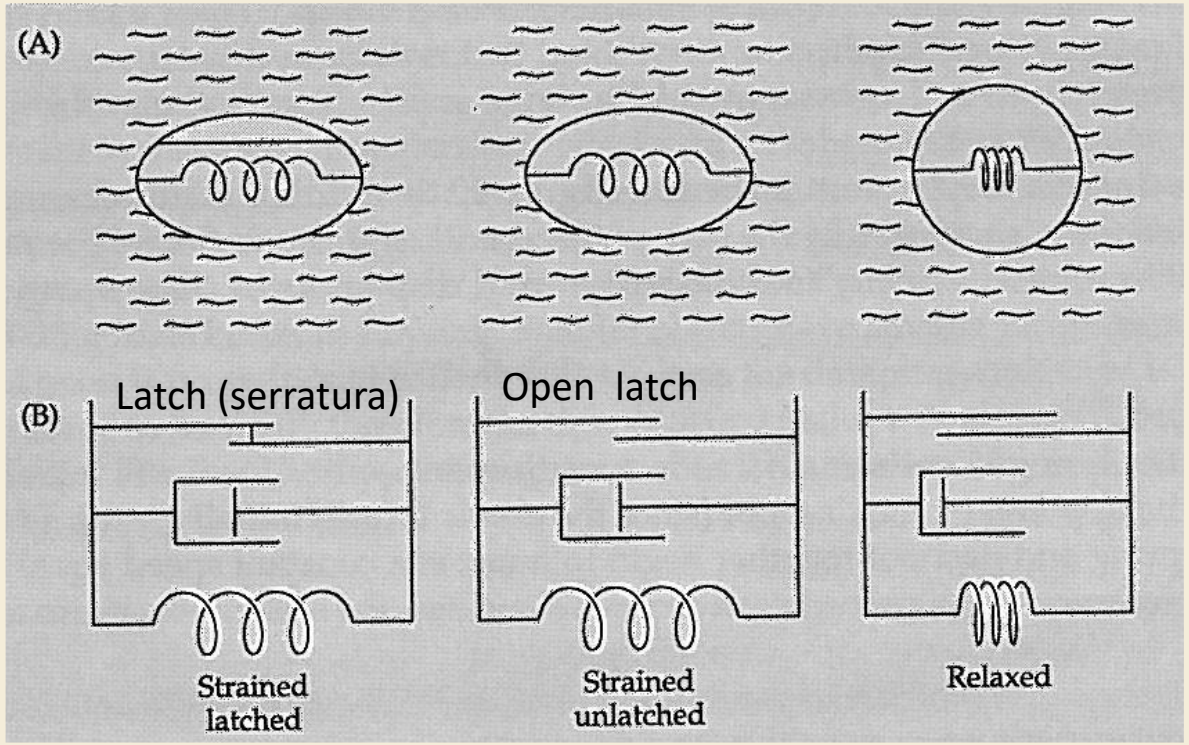
Protein held in a strained conformation due to an internal strut (montante/serratura)

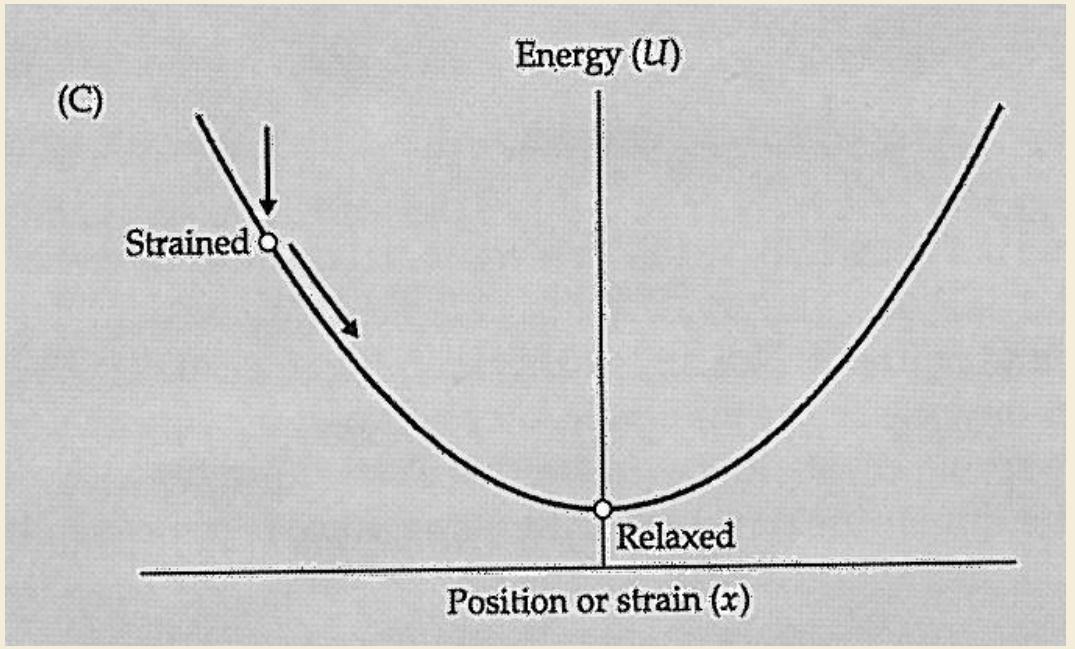
Protein relax, changes shape → unstrained conformation

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

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

$$\boldsymbol{\tau = 15\ ns}$$

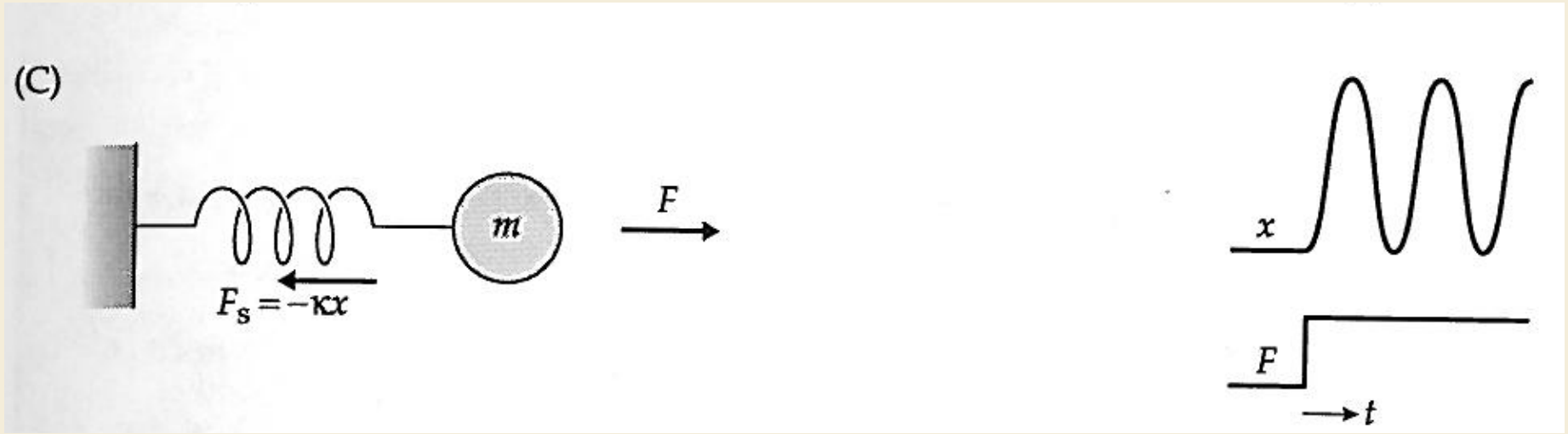




The global conformation changes occur in **nanoseconds,** while the breaking of the bonds occurs in **picoseconds**

Motion of Combinations of Mechanical Elements

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



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

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

Harmonic motion

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

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Example. Vibration of chemical bonds → bonds stiffness

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).

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 → proteins creep rather than ring

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 (calculated before) is 15 ns.

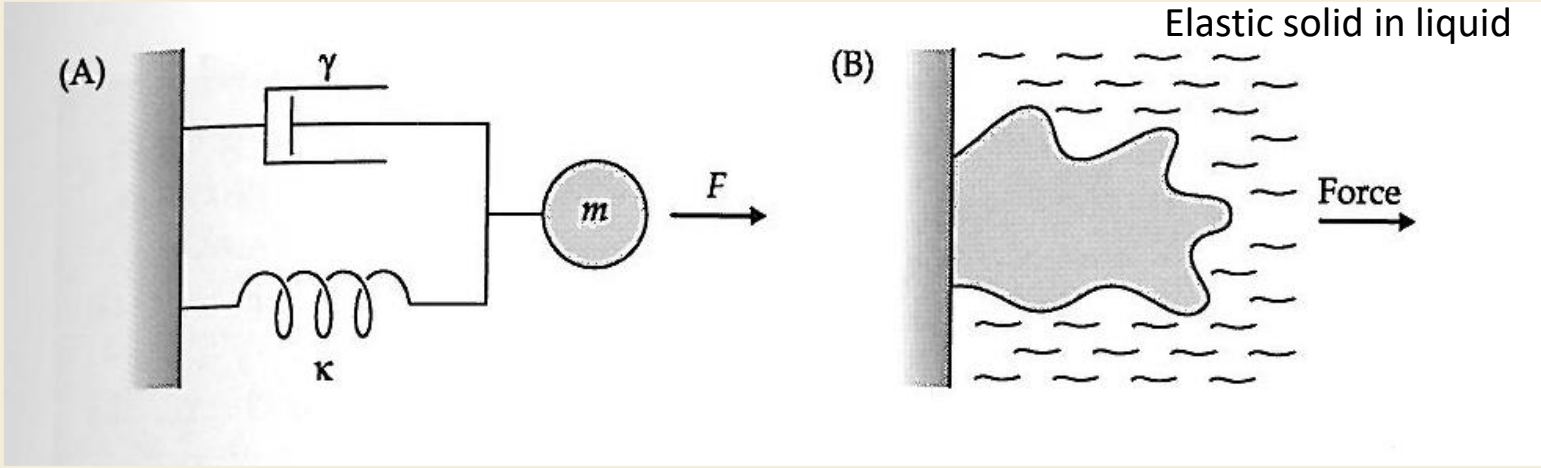
Does the protein oscillate when it detaches from the actin filament or does it creep exponentially into its relaxed state ?

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

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 $$\tau_1 = \frac{\gamma + \sqrt{\gamma^2 - 4m\kappa}}{2\kappa} \quad \text{and} \quad \tau_2 = \frac{\gamma - \sqrt{\gamma^2 - 4m\kappa}}{2\kappa}$$

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 $\tau_1 = \frac{\gamma}{\kappa}$ and $\tau_2 = \frac{m}{\gamma}$, where $\tau_1 >> \tau_2$.

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Critically Damped Motion ($\gamma^2 = 4m\kappa$)

$$x(t) = \frac{F}{\kappa}\left[1 - \left(1 + \frac{t}{\tau}\right)\exp\left(-\frac{t}{\tau}\right)\right] \qquad \text{(A2.3)}$$

where

$$\tau = \frac{2m}{\gamma} = \frac{\gamma}{2\kappa} = \sqrt{\frac{m}{\kappa}}$$

This solution is monotonic, like that in the overdamped case. Note that there is a lag, of duration ~$\tau/2$, before the displacement starts to rise quickly.

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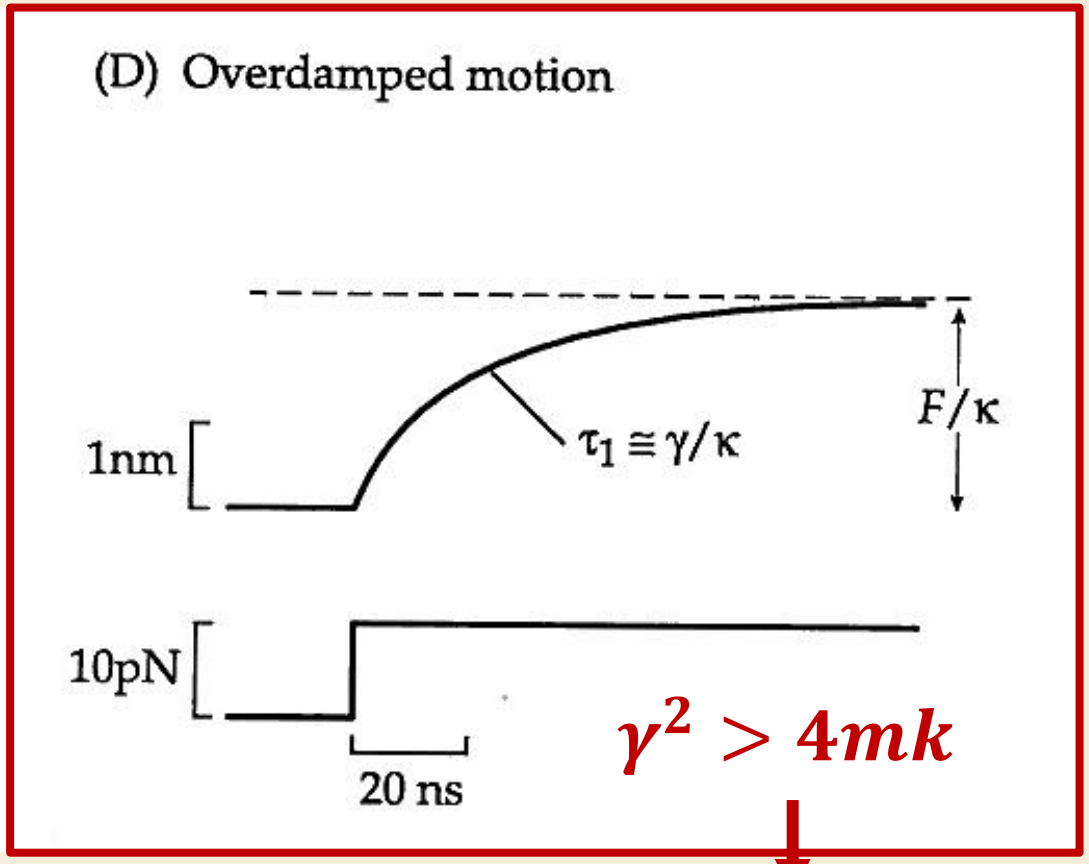
Realistic case:

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= 60 pN s/m



The inertial forces are usually very small at the microscopic and molecular levels, so that the overdamped case usually applies.

Unrealistic case :

Globular protein MM= 10 MDa

Stiffness k= 40 pN/nm (very rigid)

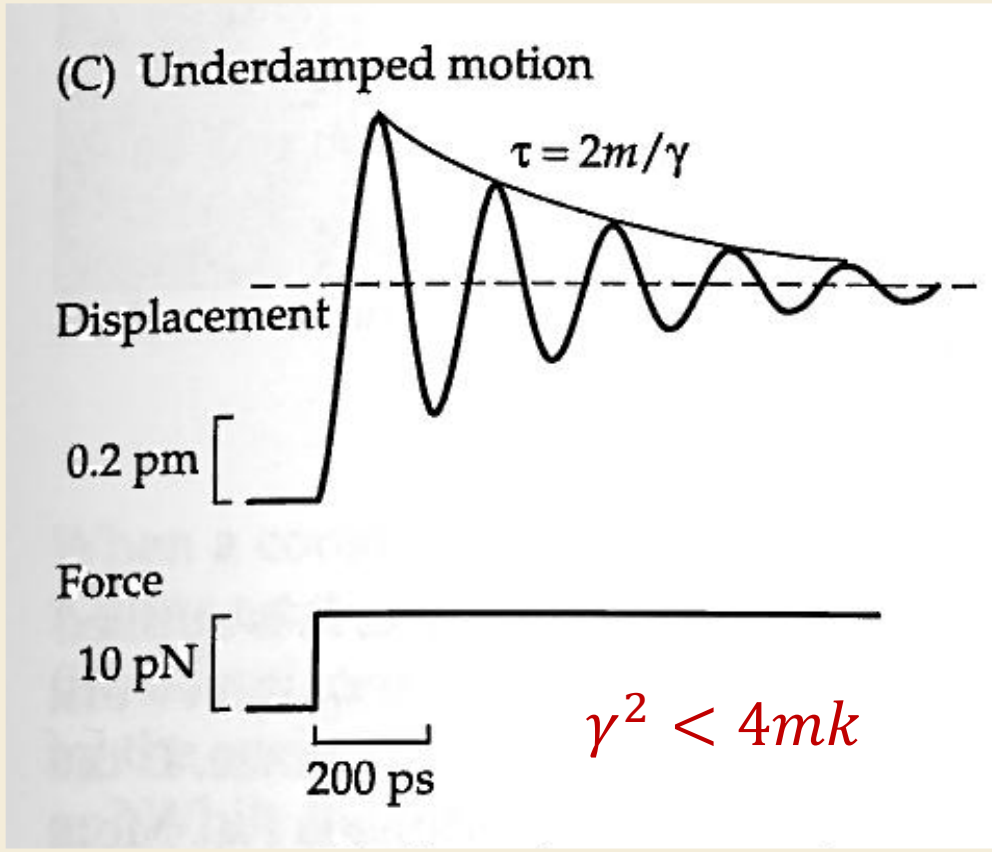
Damping γ= 60 pN s/m

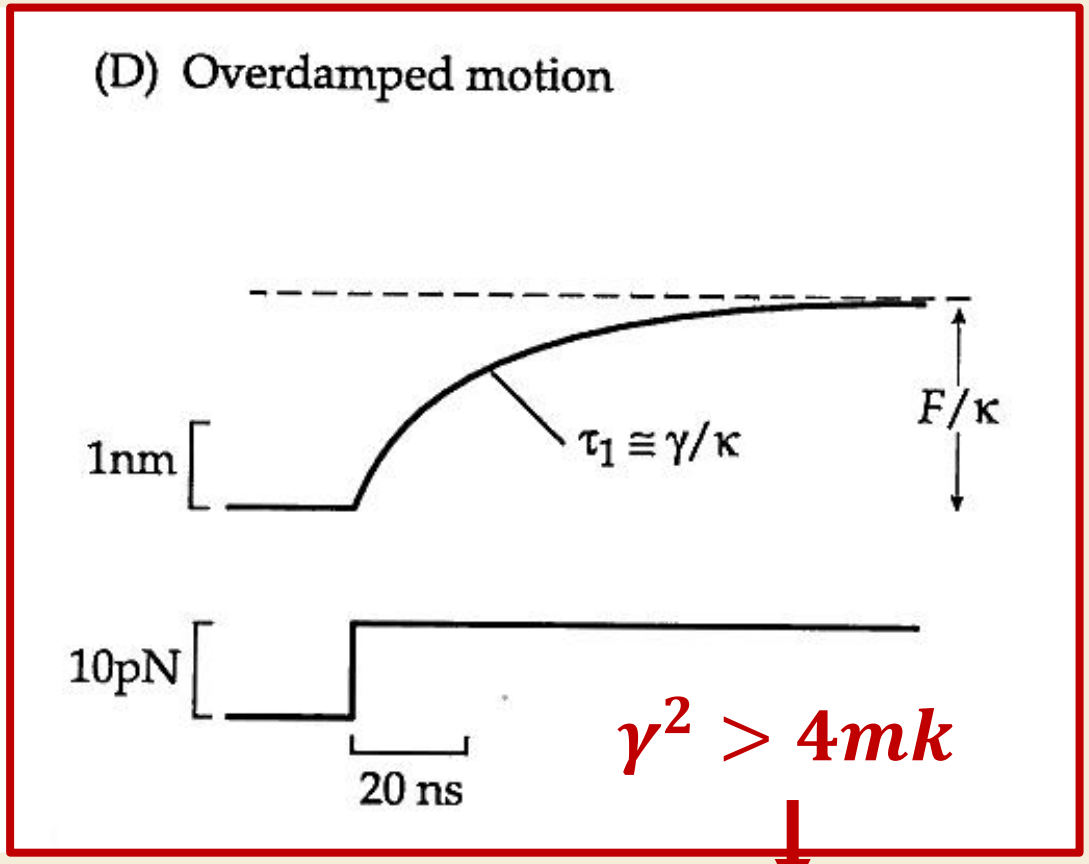
Realistic case:

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= 60 pN s/m





The inertial forces are usually very small at the microscopic and molecular levels, so that the overdamped case usually applies.

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 ~ 646 x 10^{-21} J = 650 pN · nm → ***U* ~ 161 K_BT**

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.

Purpose:

Get a feelling for what proteins are like mechanical devices ?

Questions:

- How rigid the proteins are ?
- How quickly do they move and change shape ?
- What happens when a protein is struck by a force: does it ring like a fork (underdamped motion), or does it creep monotonically into a new shape (overdamped motion ?).

Material properties of the proteins:

density, elasticity / density, viscosity

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Mass of Proteins $m = \rho V$

Proteins are composed of relatively light components:

carbon, oxygen, nitrogen, and hydrogen

Proteins are about **40 % denser than water**, with different proteins having slighlty different densities.

The **average density** of proteins is consider to be:

$\rho = 1.38 \times 10^3$ kg/m^3

The ***SI of mass*** is ***kg***, but in biochemistry the mass of proteins and other biomolecules is usually expressed as **molecular mass**, defined as the mass in grams of a mole of the molecules.

The unit is the **Dalton** : 1 Da = 1.66×10^{-24} g

Ex: A protein of 100 kDa has a mass, $m = 166 \times 10^{-21}$ g

The volume V, occupied by such a protein is: V ~ 120 nm^3 .

The density of proteins is such that each kDa of protein occupies a volume of about 1.2 nm^3 .

Table 3.1 Densities of molecules, proteins, organelles, and cells relative to water

Substance	Density (relative to water[a])
Water	1.00
Glycerol	1.26
Glycine	1.16 (solid)
Alanine	1.40 (solid)
Glutamic acid	1.46 (solid)
Hemoglobin	1.33 (in solution)
Trypsin	1.38 (in solution)
Lysosyme	1.42 (in solution)
Chromosome	1.36
Virus	1.15
Mitochondrion	1.18
Synaptic vesicle	1.05
Erythrocyte	1.10
Fibroblast	1.05

For a homogenous and isotropic solid:

$$\underbrace{\frac{F}{A}}_{\text{pressure}} = E \underbrace{\frac{\Delta L}{L}}_{\text{strain}}$$

E **:** constant [N/m^2] [Pa]

E **- Young's modulus** or **elastic modulus**

- Young's modulus ***E*** is a material property:
  it does not depend on the object size or shape

$$\underbrace{F}_{\text{force}} = k \underbrace{\Delta L}_{\text{extension}} = \frac{AE}{L} \Delta L$$

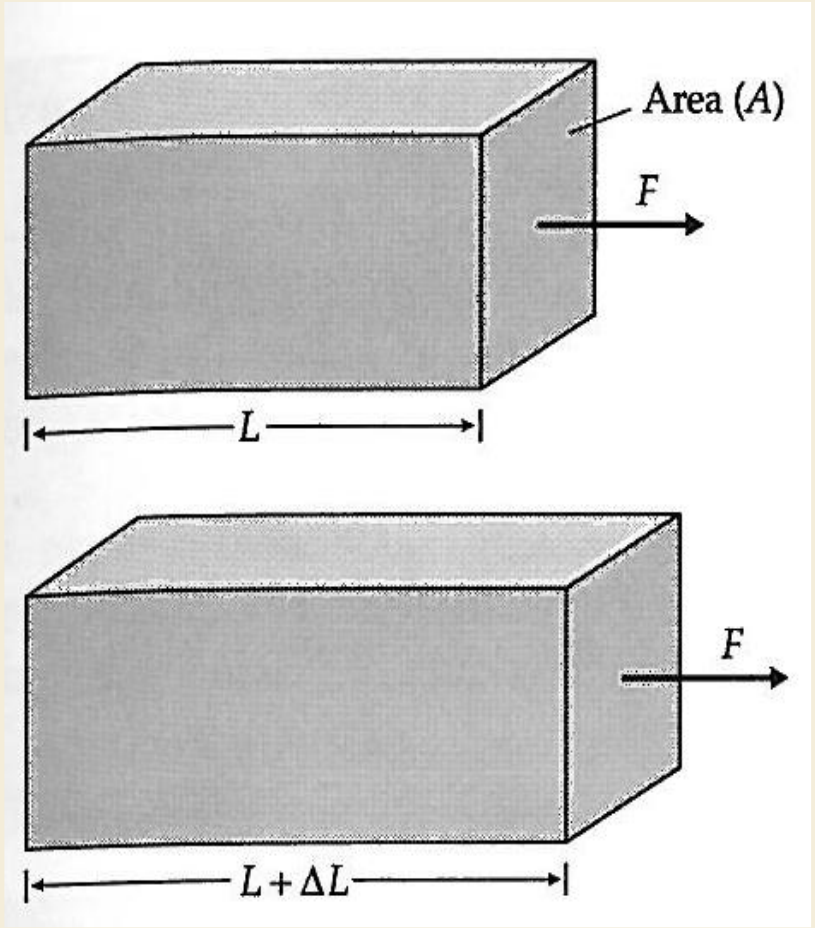
k **:** constant [N/m]

F ~ ΔL ; Hooke's law

K – **stiffness**

- The stiffness, ***k,*** of an object <u>does</u> depend on the size and shape.

A solid strained by a tensile force.



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Material	Young's modulus, E (GPa)
Carbon nanotube	1300
Diamond	1200
Steel (stainless)	211
Glass (quartz)	73
Wood (fir, along grain)	16
Plexiglas	3
Plastic (polypropylene)	2.4
Teflon (PTFE)	0.34
Rubber (polyisoprene)	0.02
Silk (*Bombyx mori*)	5–10
Keratin (hair)	2.4
Actin	2.3
Collagen	2
Tubulin	1.9
Elastin	0.002

For many materials (e.g. metals, plastics and structural proteins) the Hooke's law $F = k\,\Delta L$ applies only for forces that cause strains up to:

$$\frac{\Delta L}{L} = 0.1\text{-}1\ \%$$

At higher forces the material yelds and the yeld pressure is called **tensile strength**.

Other materials such as rubber and proteins like elastin and titin can be strained up to 100 % or more.

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In general **proteins are neither homogenous nor isotropic** due to their complex atomic structure.

Therefore, care must be taken when considering their mechanical properties.

- For a nonhomogeneous, nonisotropic solid, there are as many as 21 elastic parameters for every point in the material. An exact description of the elasticity of a material could therefore be as complex as the full atomic description.
- By contrast, an homogeneous and isotropic material is characterized by just two parameters: the Young's modulus and Poisson's ratio.

Q: **Is it valid to think of proteins as having material properties, or must we always think in terms of their atomic structures?**

This question is related to the domain concept of structural biology in which proteins are thought of as comprising fairly rigid domains joined by more flimsy connecting regions.

In this picture the hinging and twisting of domains is attributed to the less substantial thickness of connections, in the same way that a rubber dumbbell bends about its linking rod not because the rod is composed of a weaker material but because it has a reduced cross-sections.

The domain concept encompasses the idea that protein have material properties.

Are there experiments to support the notion that proteins can be thought of as mechanically isotropic, at least to a first approximation ?

Actin and tubulin: globular proteins which polimerize to form cytoskeletal filaments, Young's moduli are found to be approx independent of the direction of the applied force. Young's moduli of several filamentous proteins are similar despite their quite different atomic structures . This suggests the existence of a material property that is independent of the atomic details.

On the other hand, wet hair shows significant mechanical anisotropy – Young's modulus measured using longitudinal forces is an order of magnitude greater than that measured using transverse forces, due to the orientation of the constituent coiled coils.

The concept that proteins have material properties derives support from both structural and mechanical studies. The simplicity of the material desciption over the atomic one makes it a useful conceptual tool for understanding protein mechanics.

Material description can be readily tested and refined by mechanical experiments on proteins; by contrast the tools necessary for relating mechanical measurements by atomic description of proteins via molecular dynamics simulations are still under development.

The behavior / motion of an object in response to mechanical force can be osciallatory (underdamped) or monotonic (overdamped), depending on the relative magnitudes of the inertial and viscous forces.

These in turn depend on the material properties: mass, stiffness and damping, which were described previously.

To answer which type of motion is, we use a scaling argument: as the dimension of an object gets smaller, the viscous forces increase relative to the inertial forces, and as a result, the global motions of small, comparatively soft objects such as proteins in aqueus solution are expected to be overdamped.

Let us consider a crude mechancial model of a globular protein as a homogeneous and isotropic cube with side ***L***, density ***ρ***, and Young's modulus ***E***, damped by fluid viscosity ***η***.

The mass: $m = \rho V = \rho L^3$. The stiffness: $k = EL$

The drag force associated with a global conformational change that alters the shape of a protein: $F = -\gamma v$, with $\gamma = 3\pi \eta L$.

Overdamped: $\frac{\gamma^2}{4mk} > 1$ → $\frac{\gamma^2}{4mk} \cong 25 \frac{\eta^2}{\rho E L^2} > 1$

Overdamped: $\frac{\gamma^2}{4mk} \cong 25\frac{\eta^2}{\rho E L^2} > 1$

How small must a protein be to ensure that its motion is overdamped and that it does not oscillate when subject to an external force ?

For the middle rigid proteins the Young's moduli, E ~ 1 GPa; the density, ρ ~ 10^3 kg/m^3 , viscosity of water η ~ 1mPa s.

$\frac{\eta^2}{\rho E} \approx 1\ \text{nm}^2$ → $L < 5\ \text{nm}$

This length corresponds to a medium-sized globular protein of ~ 1000 amino acids. Thus the model predicts that global motions of rigid globular proteins or protein domains of molecular weight less than **100 kDa** should be overdamped.

$$\frac{\gamma^2}{4mk} \cong 25\frac{\eta^2}{\rho E L^2} > 1$$

Also the motions of larger proteins is overdamped because:

- The rigidity of allosteric, energy-transducing proteins such as motor proteins and the ribosome is likely to be much less than that of rigid proteins like those of the cytoskeleton. Let us consider a protein undergoing a x=2 nm (modest) conformational change. Assuming this is associated to a large amount of mechancial work, say W=25 K_bT (equal to the free energy of hydrolysis of the gamma phosphate bond of one molecule of ATP) we have W= ½ kx^2 and the stiffness **k= 2W/x= 0.05 N/m**, much smaller than the stiffness of a rigid protein of length 10 nm and Young's modulus 2 Gpa. This value of stiffness leads to a much greater **characteristic length L= 50 nm**, implying that even the motion of a ribosome, one of the largest protein machines, would be overdamped. (!!! k= E L !!!)

  Morover, since we consider a small value for the conformational change and a large value for the work, even this low stiffness is likely to be an overestimate; indeed the stiffness of motor proteins is on the order ot only 1 pN/nm (0.001 N/m) → arguing once more that that protein motions are overdamped.

Example 3.6 Ribosome If a large protein were to oscillate, how fast and how large might these oscillations be? Consider the ribosome, a globular protein–RNA enzyme complex of diameter ~30 nm (Ban et al., 1999, Clemons et al., 1999). The ribosome is the molecular machine that synthesizes proteins. If the ribosome were very rigid ($E = 1$ GPa), and the only damping came from the surrounding fluid, then it would oscillate at a frequency of $\sim(\kappa/m)^{0.5}/2\pi$ Hz $= (E/\rho)^{0.5}/2\pi L \sim 5$ GHz, corresponding to a period of 200 ps. The oscillation would decay quickly, with a time constant of $2m/\gamma \sim (2/3\pi)\rho L^2/\eta \sim 200$ ps (Equation A2.1 in the Appendix). In other words, the oscillations would die out after only a few cycles. The magnitude of the oscillations would depend on the size of the force. Suppose that the force did work on the protein equal to 100×10^{-21} J ($= 25\,kT$), the free energy associated with the hydrolysis of one molecule of ATP (Chapter 14). If we think of this chemical energy as being converted into mechanical potential energy within the protein during the protein synthesis reaction, then the amplitude, x, of the deformation would be only ~0.8 Å (energy $= \frac{1}{2}\kappa x^2$, and we assume that ribosome is as rigid as a cytoskeletal protein with $\kappa = EL = 30$ N/m). The oscillations, if they occurred, would be very small indeed. Considering that the lifetimes of different chemical states are in the order of microseconds to milliseconds, it is unlikely that such small oscillations, even it they were to occur, would play important roles in the chemistry of protein synthesis.

$$\frac{\gamma^2}{4mk} \cong 25\frac{\eta^2}{\rho E L^2} > 1$$

Based on the scaling argument , since cells have linear dimensions about 1000 larger than those of proteins, **one might expect that cells undergo underdamped motions**.

Experimentally it is shown that **this is not the case**: **the motions of the cells are very highly damped**.

For example, the cytoplasm of macrophages that have ingested 1 um diameter magnetic particles can be perturbed using a weak external magnetic field. The particles reorient extremely slowly, with time constant of minutes. The apparent **intracellular viscosity is very high, approx 1000** Pa s and the motion is highly overdamped. Because actin gels crosslinked with the actin binding protein ABP have similar viscoelastic properties to cells, it is likely that the viscoelasticity of cells arises from the stiffness and damping on cytoskeletal filaments. Since the long cytoskeletal filaments are highly damped, so too are cells.

The cytoskeletal filaments **form a gel with a mesh size of about 50 nm**. Small solutes and proteins can readily diffuse through the pores, but the motion of larger particles, such as ribosomes and organelles is severlely restricted.

2.1.1. Physical forces and their magnitudes at the single-molecule level

2.1.2. Modeling complex mechanical devices as protein machines by using three elements:

Spring, Dashpot, Mass

2.1.3. Mass, Stiffness and Damping of Proteins