

CELL MECHANICS

LECTURE 4

2. Physical principles

2.1. Forces at molecular and cell level

- Physical forces and their magnitudes at the single-molecule level
- Modeling complex mechanical devices as protein machines by using three elements: Spring, Dashpot, Mass; example: Mass, Stiffness and Damping of Proteins

2.2. Thermal forces, diffusion, and chemical forces

- Boltzmann Distribution Law and the Principle of Equipartition of Energy
- Diffusion equation - Einstein relation – Stokes law
- Autocorrelation function and Power Spectrum
- The effect of force on the equilibria and rate of chemical reactions
- Example of single molecule force spectroscopy experiments – unbinding, unfolding

In addition to mechanical forces and thermal forces proteins and cells are subject to **chemical forces**, arising from formation of intermolecular bonds

OUTLINE

- Chemical forces; amino acids – protein; types of forces;
- Ligands how they work and examples
- Chemical equilibria and the effect of force on chemical equilibria
- Rate theory of chemical reactions and the effect of force on the chemical rate constant
- Examples of single molecule force spectroscopy experiments – unbinding, unfolding

Strength of Chemical bonds

Type of Bond	Strength (kcal/mole)	~ Strength(KT)
<u>Covalent</u>	50 to 100	150
<u>Ionic</u>	1 to 80	100
<u>Hydrogen</u>	3 to 6	10
<u>Van der Waals</u>	0.5 to 1	2
<u>Hydrophobic</u>	0.5 to 3	5

1 kcal / mole ~ 4.184 kJ / mole ~ 1.6 KT / molecule

1 KT ~ 4.1×10^{-21} J

NA ~ 6×10^{23}

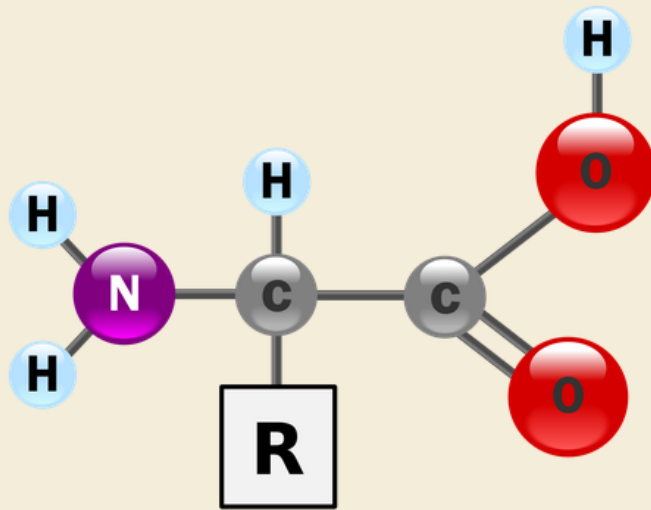
RT= KT x NA

https://earth.callutheran.edu/Academic_Programs/Departments/BioDev/omm/jsmolnew/bonding/chymo.html#top

An Introduction to Chemical Bonds and Protein Structure

Chemical bonds and chemical forces in proteins

Amino Acids – the structural units that make up proteins



chemical structure

Size: 0.4 – 1 nm

Molecular Mass: 75 – 205 Da

Smallest AA: glycine; Biggest AA: thryptophan

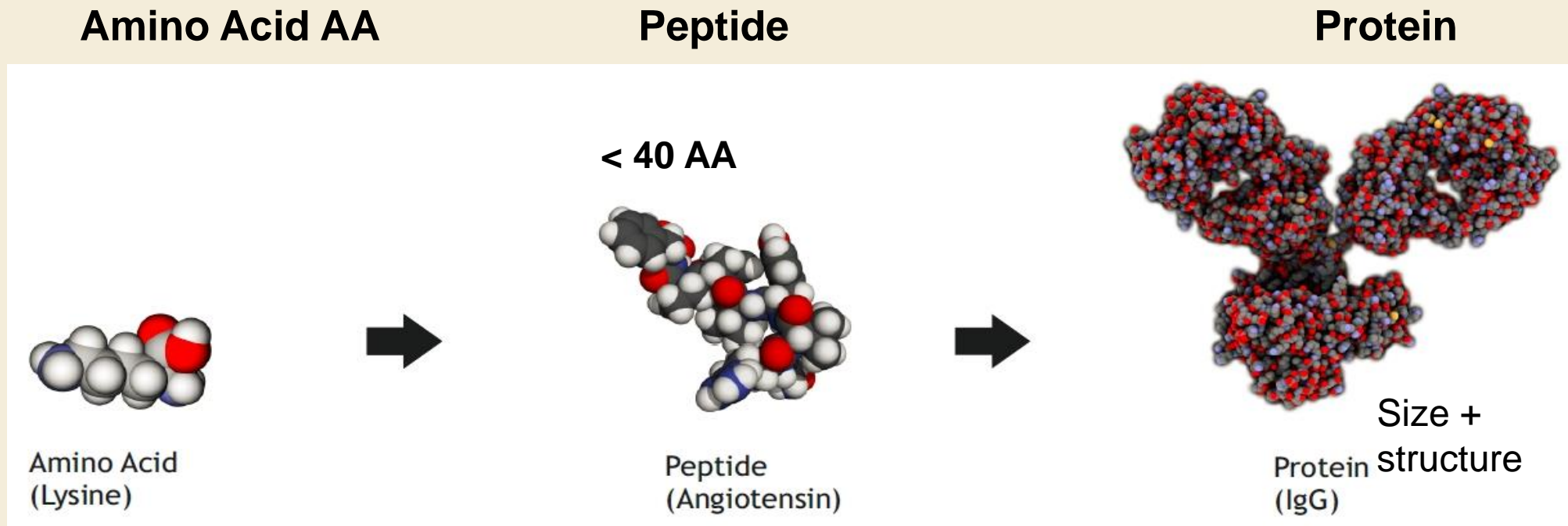
In nature there are more than 100 aa.

20 aa are making up the proteins in the human body

11aa of these 20 aa are synthetized by the human body

A central carbon atom is bonded on four sides with: a carboxyl group COOH; an amino group NH₂; a hydrogen atom H; a side chain R.

R is the only component that differs between amino acids.



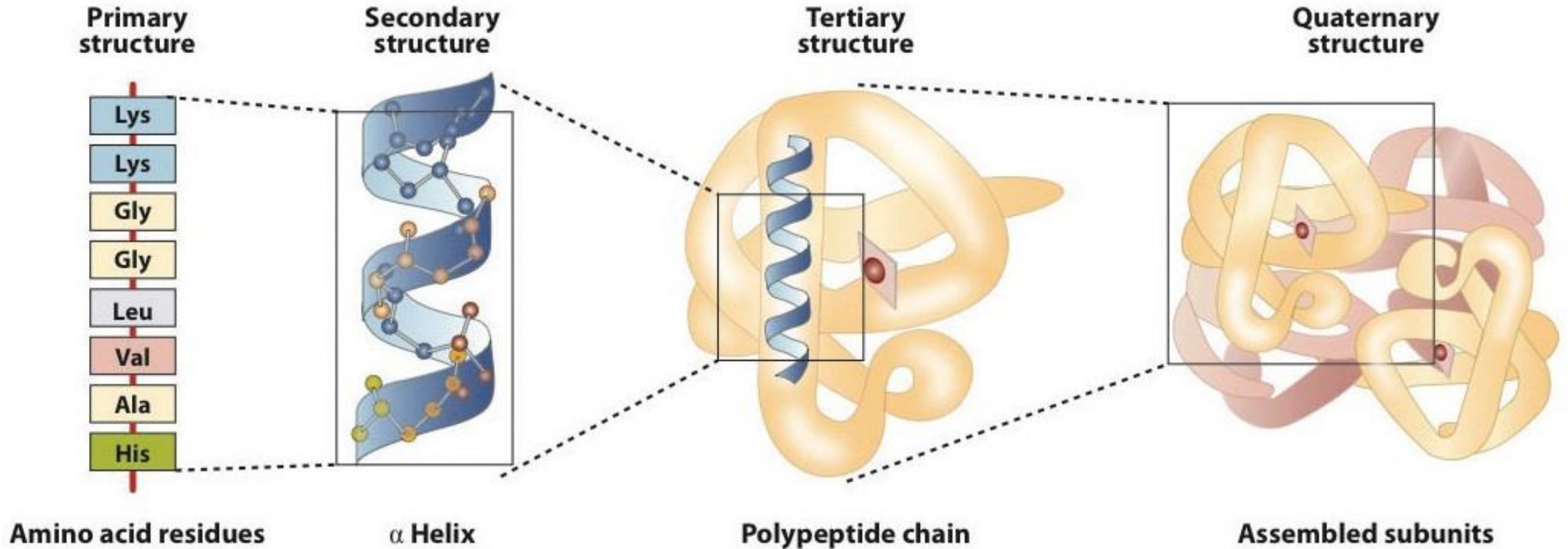
More than 20000 proteins in the human body

How is it possible that from only 20 structural units to get so many proteins and so many functions ?

<https://www.drawittoknowit.com/course/biochemistry/glossary/biochemical-pathway/protein-structure-classes>

[https://chem.libretexts.org/Bookshelves/General_Chemistry/Map%3A_A_Molecular_Approach_\(Tro\)/22%3A_Biochemistry](https://chem.libretexts.org/Bookshelves/General_Chemistry/Map%3A_A_Molecular_Approach_(Tro)/22%3A_Biochemistry)

PROTEIN STRUCTURE



The *primary structure* consists of the specific amino acid sequence.

The resulting peptide chain can twist into an α -helix, which is one type of *secondary structure*.

This helical segment is incorporated into the *tertiary structure* of the folded polypeptide chain.

The single polypeptide chain is a subunit that constitutes the *quaternary structure* of a protein, such as hemoglobin that has four polypeptide chains.

Primary Structure = sequence of amino acids

3-letter code

Lys-Thr-Tyr-Phe-Pro-His-
Phe-Asp-Leu-Ser-His-**Gly** ...

1-letter code

KTYFP~~H~~FDLSHG**G**

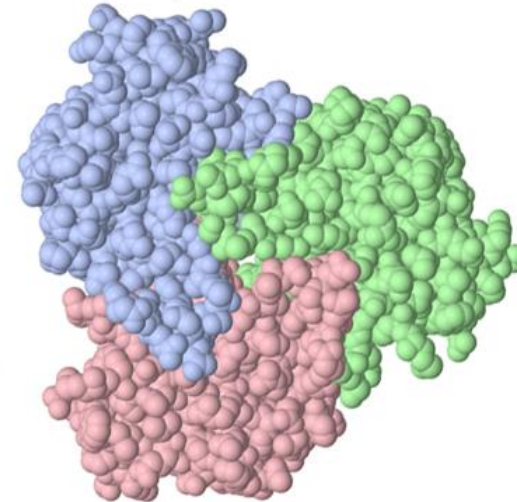
Secondary Structure =
alpha helices, beta strands



Tertiary Structure = fold helices and strands into domains



Quaternary Structure (Biological Units)
= functional assemblies of chains (subunits)

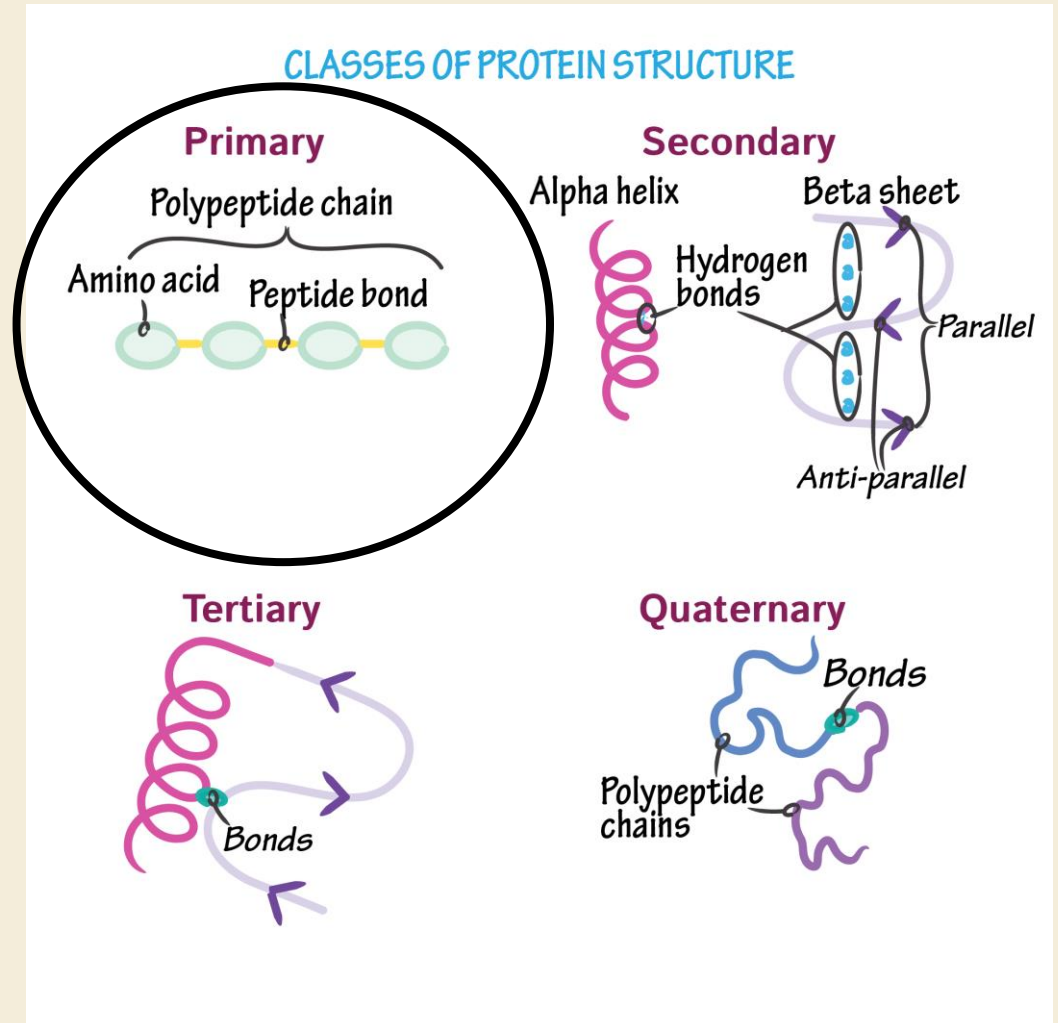


PRIMARY PROTEIN STRUCTURE

Together, peptide bonds and amino acids form a polypeptide chain (i.e. a protein).

The primary structure of a protein determines its secondary and tertiary structures.

As a clinical correlate, in sickle cell anemia, a single substitution of an amino acid in the primary structure of the protein results in a structural defect in hemoglobin.



SECONDARY PROTEIN STRUCTURE

Two basic forms: alpha-helices and beta-sheets

Hydrogen bond interactions within the alpha-helix and beta-sheet provide the stability of secondary structure of proteins.

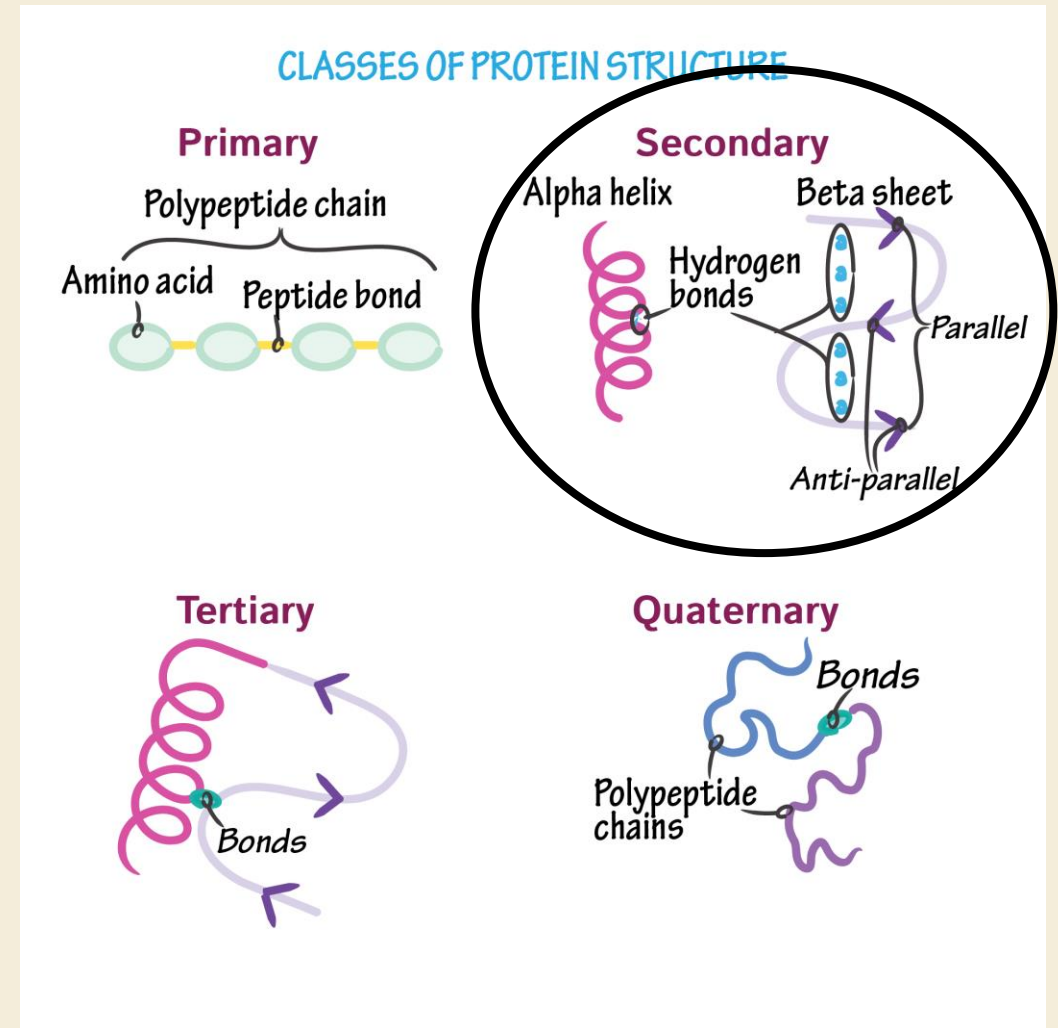
Alpha-helices

Low energy conformations that enable higher-order packing of proteins. Large or charged amino acid groups (such as proline) can disable the alpha helix conformation by manually disrupting the hydrogen bond interactions.

Beta-pleated sheets

More structurally diverse than alpha helices and thus facilitate more diverse protein functions. Create stable, diverse structures within a protein to allow higher order functions.

As a clinical correlate, prions are pathogenic, transmissible agents, which cause conversion from an alpha-helical form to a beta-sheet-rich conformer. Prions accumulate in the brain and cause a variety of spongiform encephalopathies, such as “mad cow disease”.



TERTIARY PROTEIN STRUCTURE

Is the protein's three-dimensional shape (its "native conformation") and the function of a protein is dependent on this three-dimensional globular structure. Primarily comprises alpha helices and beta sheets.

QUATERNARY PROTEIN STRUCTURE

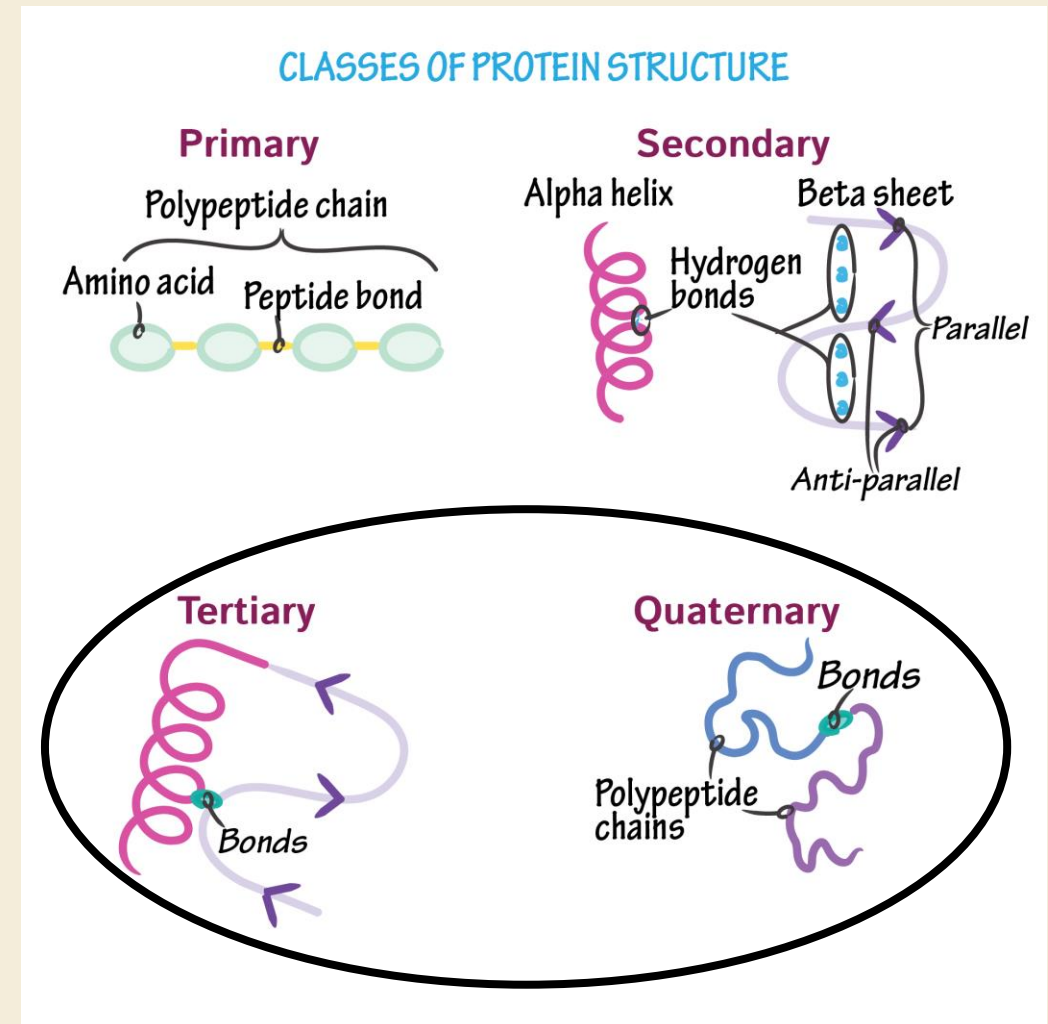
Forms through the interaction of 2 or more separate proteins. Comprises multiple polypeptide chains and occurs in certain protein types, called functional multimeric proteins.

TERTIARY & QUATERNARY PROTEIN BONDING

The most significant stabilizer of tertiary and quaternary protein structures are hydrophobic interactions.

The following additional forces stabilize these structures:

- Hydrophilic interactions.
- Electrostatic interactions.
- Hydrogen bonds between side chains.
- Strong disulfide bonds.



Covalent bonds are the strongest chemical bonds contributing to protein structure. A covalent bond arises when two atoms share a pair of electrons.

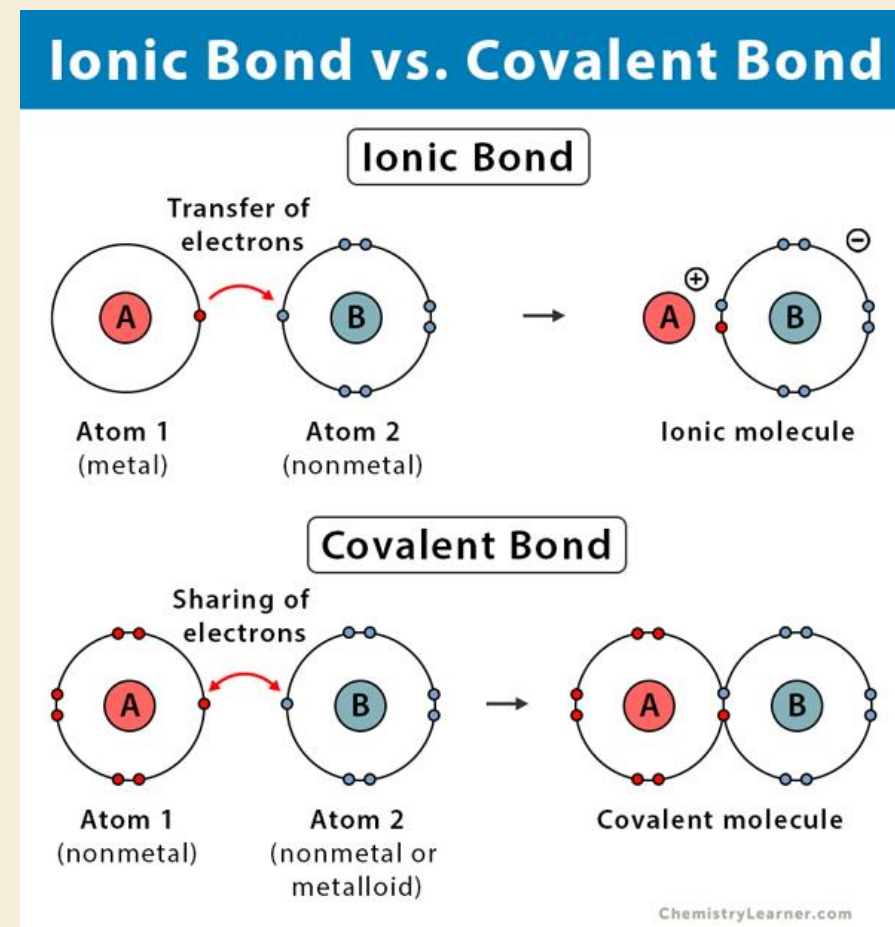
Strength ~ 150 KT / molecule

Electrostatic Interactions

A. Ionic Bonds (salt bridges)

Strength ~ 100 KT / molecule

Ionic bonds are formed as atoms of amino acids bearing opposite electrical charges are juxtaposed. Ionic bonds can be important to protein structure because they are potent electrostatic attractions. In the hydrophobic interior of proteins, ionic bonds can even approach the strength of covalent bonds.

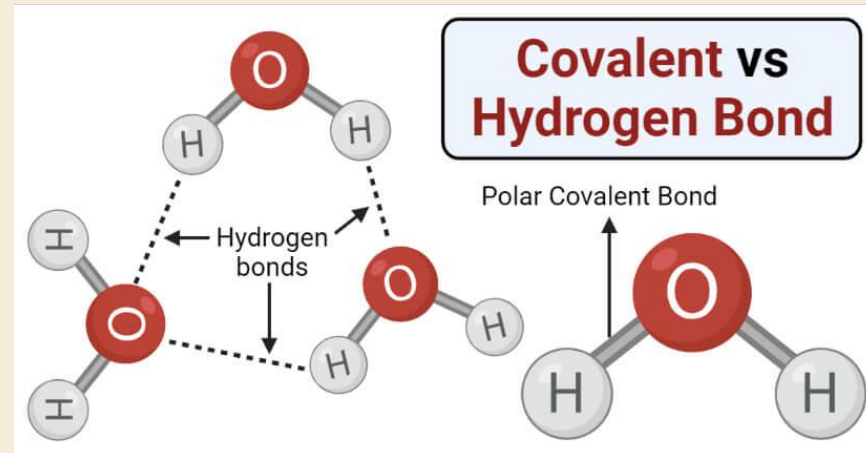


Electrostatic Interactions

B. Hydrogen Bonds

Strength ~ 10 KT / molecule

When two atoms bearing partial negative charges share a partially positively charged hydrogen, the atoms are engaged in a hydrogen bond (H-bond).

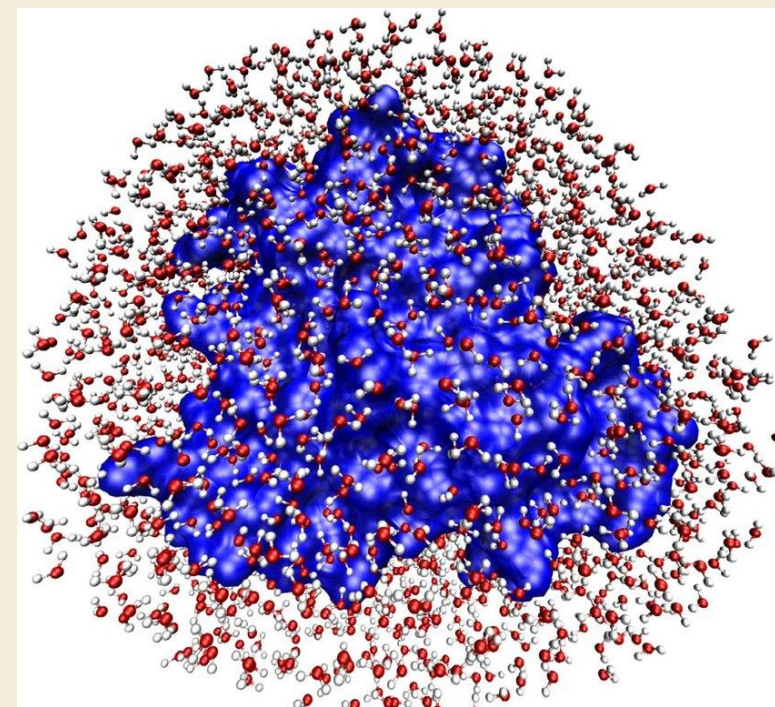


C. Water Shells and Polar Surface Residues

Strength ~ 10 KT / molecule

Polar amino acids, mostly found on protein surfaces, promote appropriate folding by interacting with the water solvent.

Polar water molecules can form shells around charged or partially charged surface residue atoms, helping to stabilize and solubilize the protein.



The hydration shell of myoglobin

Van der Waals Forces

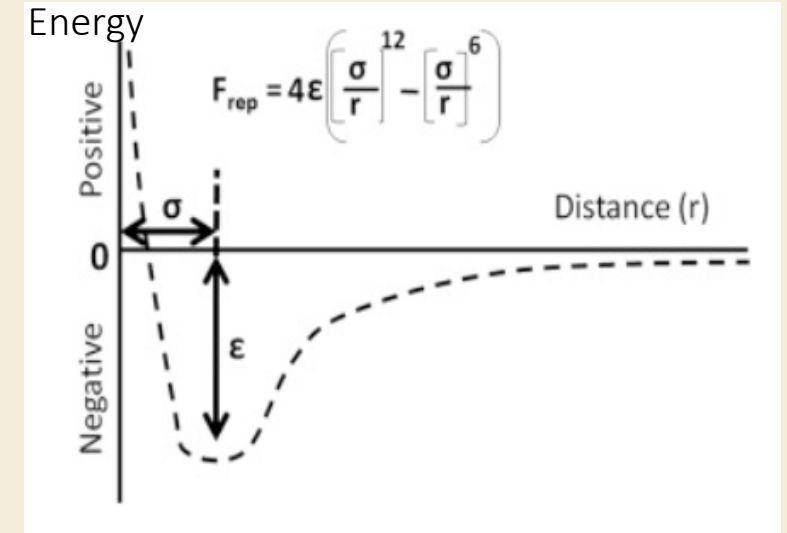
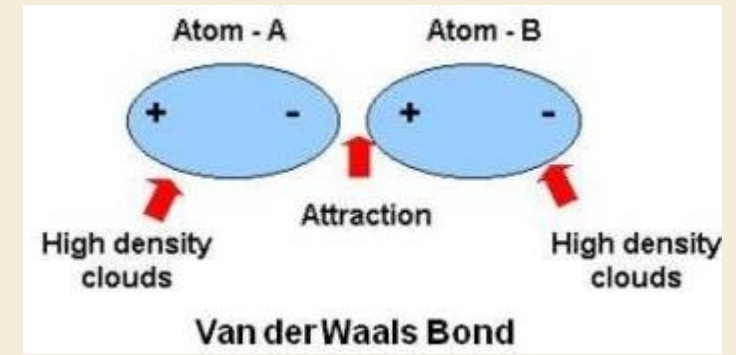
Strength ~ 5 kT / molecule

Van der Waals force is a transient, weak electrical attraction of one atom for another.

This attraction exists because every atom has an electron cloud that can fluctuate, yielding a temporary electric dipole. The transient dipole in one atom can induce a complementary dipole in another atom, provided the two atoms are quite close.

These short-lived, complementary dipoles provide a weak electrostatic attraction, the Van der Waals force.

If the two electron clouds of adjacent atoms are too close, repulsive forces come into play because of the negatively-charged electrons. The appropriate distance required for Van der Waals attractions differs from atom to atom, based on the size of each electron cloud, and is referred to as the Van der Waals radius.



Van der Waals attractions, although transient and weak, can provide an important component of protein structure because of their big number. Most atoms of a protein are packed sufficiently close to others to be involved in transient Van der Waals attractions.

Van der Waals forces can play important roles in protein-protein recognition when complementary shapes are involved.

An example is the case of antibody-antigen recognition, where a complementary fit of the two interacting molecules across a broad surface yields extensive Van der Waals attractions.

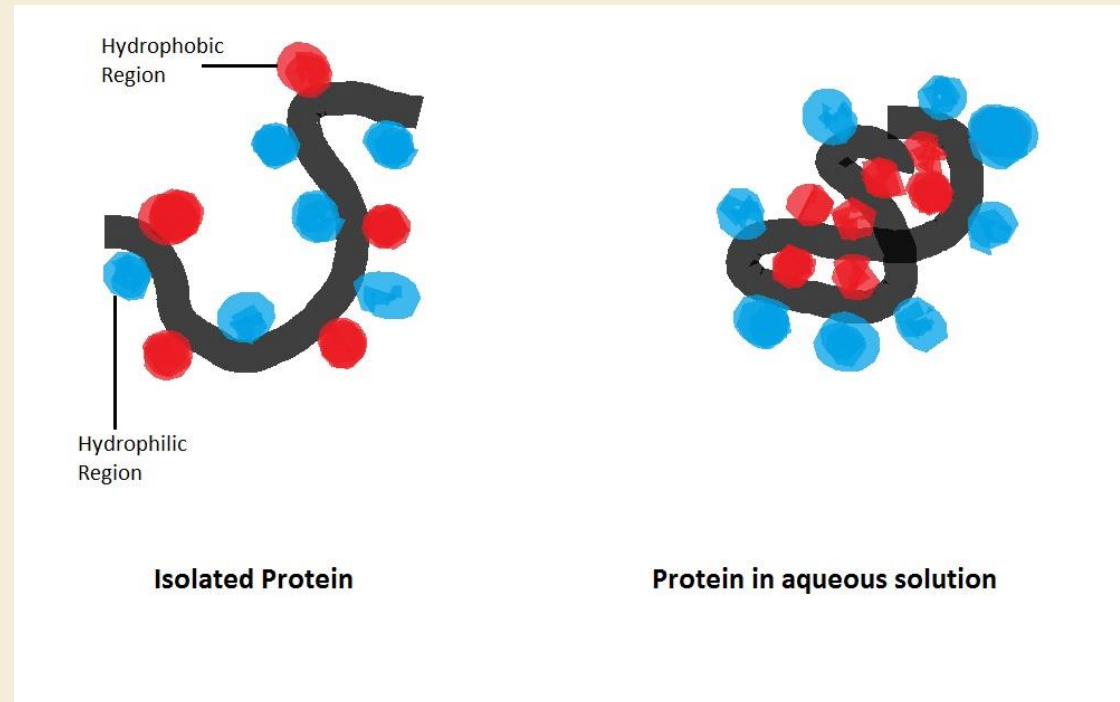
Hydrophobic Interactions

Strength $\sim 5 \text{ KT} / \text{molecule}$

Hydrophobic interactions ("bonds") are a major force driving proper protein folding.

They juxtapose hydrophobic sidechains by reducing the energy generated by the intrusion of amino acids into the H_2O solvent, which disrupts lattices of water molecules.

Hydrophobic bonding forms a hydrophobic protein core, where most hydrophobic sidechains can closely associate and are shielded from interactions with solvent .



Chemical forces arising from changes in bound ligands

What is a LIGAND ?

Any molecule or atom which binds **reversibly** to a protein producing a conformational change of the target / receptor protein.

The ligand travels through the fluids of an organism, within the blood, tissues, or within a cell itself.

It can be natural, as an organic or inorganic molecule. Some ligands can be made synthetically → drug synthesis.

Ligands are typically used in cellular signaling and cellular regulation.

How LIGANDS work and which is their function

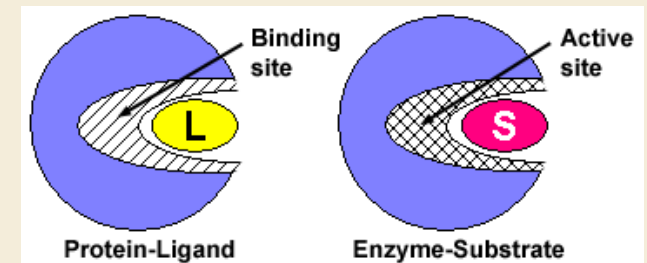
The ligand binds to the protein by weak interactions, inducing a conformational change of the protein. The physical action of the ligand changes the overall shape of the entire structure without breaking or forming new chemical bonds.

The movement /change of the protein itself activates another chemical pathway, or triggers the release of another messenger ligand, to carry the message to other receptors.

Biologically active proteins are active because of their shape. This shape interacts with the chemistry of the ligand to create a stable connection between the two molecules, which will eventually reverse, leaving both molecules the same.

Difference between protein-ligand and enzyme-substrate reaction:

- The ligand binds reversibly and leaves the protein unchanged when it leaves.
- In a substrate and enzyme reaction, the substrate is permanently changed.



How LIGANDS work and which is their function

The ligand activates a protein for a short amount of time and then it is recycled, which allows for the biological control of many interactions. The amount of time a ligand spends attached to its receptor or specific protein is a function of the affinity between the ligand and the protein. The affinity of a particular ligand for a particular protein is determined entirely by its chemical makeup and that of the binding site of the protein.

At the binding site, amino acids will be exposed which tend to complement the desired ligand.

E.g If the ligand is positively charged, the binding site should be negatively charged. This creates the strongest interaction and the protein can obtain a certain degree of specificity for a ligand.

While this is the basis for how cells can begin to distinguish different molecules, it is also at the heart of one of an organism's biggest problems. Many poisons and toxic substances are so toxic because of their ability to interfere with the protein-ligand binding process. Either the toxin directly binds to the protein itself, because it has a higher affinity, or the toxin otherwise prevents the normal bonding of a ligand to its target protein.

Examples of a Ligand: the Oxygen

In the body tissues, oxygen must reach all the mitochondria in the body if the organism is to survive. All organisms of a certain size must contain some sort of circulatory system and use specialized proteins for this.

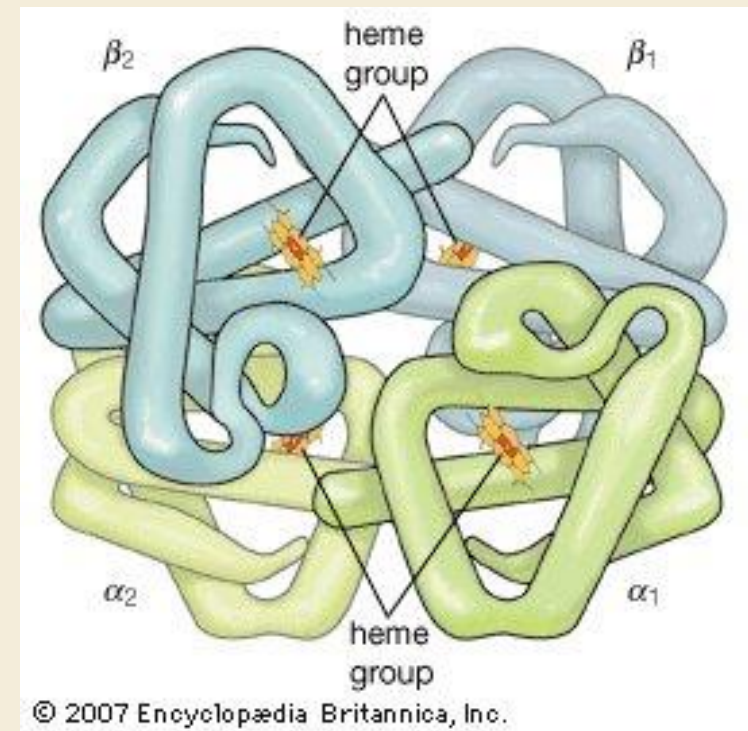
Hemoglobin is the major blood protein responsible for transporting oxygen in humans. Hemoglobin picks up oxygen in the lungs and release it in capillaries. Release is induced by a conformation change of hemoglobin, induced by a lower pH level because of CO₂ increase and red blood cells (RBC) squeezing in capillaries. Oxygen diffuses then in tissue to reach the cells. RBC can then pickup CO₂ and bring it to lungs.

4 oxygen molecules / hemoglobin; 270 million of hemoglobin / RBC;

5×10^9 RBC / mL blood; 0.21 mL oxygen / 1 mL blood

Hemoglobin is a protein made up of four polypeptide chains (α_1 , α_2 , β_1 , and β_2). Each chain is attached to a **heme group** composed of porphyrin (an organic ringlike compound) attached to an **iron atom**. These iron-porphyrin complexes coordinate oxygen molecules reversibly, an ability directly related to the role of hemoglobin in oxygen transport in the blood.

A main competitor of oxygen is carbon monoxide. It has a higher affinity for hemoglobin than oxygen has. Once carbon monoxide is bound to the hemoglobin, it won't come off. This means that someone exposed to large amounts of carbon monoxide will soon have all their hemoglobin saturated by the wrong ligand. Their body will have no ability to transfer oxygen to the brains and tissues.



Examples of a Ligand

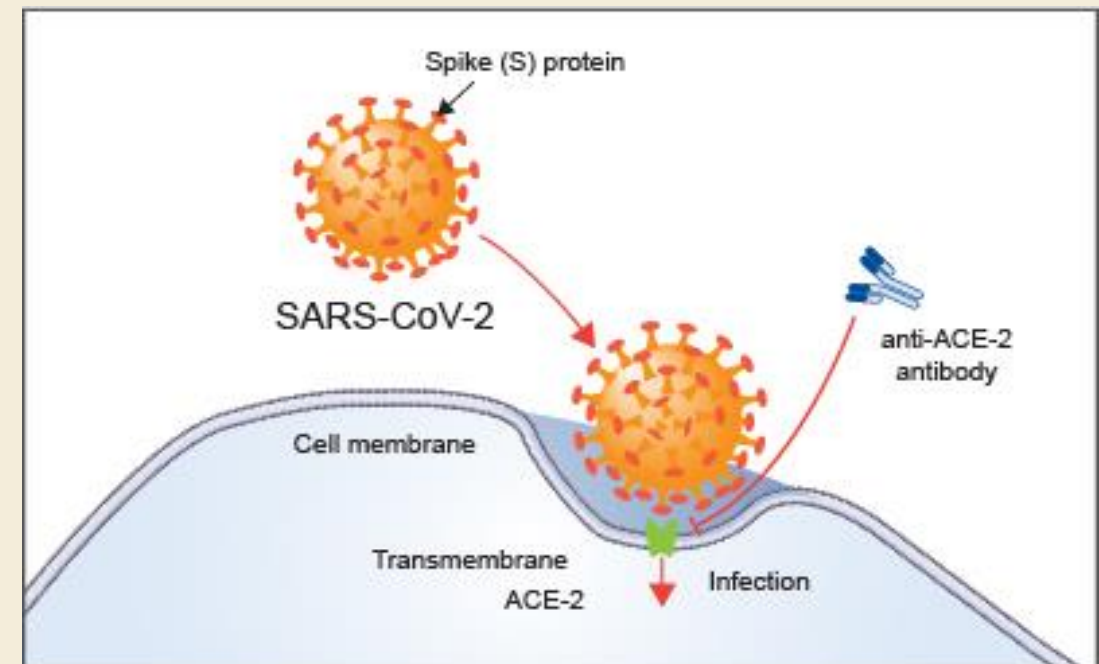
Dopamine

Dopamine is a ligand used heavily in the brain. When the brain releases dopamine, it is as a signal of a pleasure coming from success. In other words, dopamine is tied to the sensation of motivation. The dopamine receptors in the brain are activated when the ligand dopamine is released by the brain. When the receptors are full of dopamine, the brain feels as if you have done something good.

Drugs such as cocaine and methamphetamine increase the effectiveness of dopamine, limiting the amount of dopamine which can be recycled. Thus, the brain stays in a constant state of feeling "rewarded". This is the dangerous feeling which can easily lead to drug addiction.

Bacteria and virus proteins

ACE-2 is the host cell receptor responsible for mediating infection by SARS-CoV-2, the novel coronavirus responsible for coronavirus disease (COVID-19). Treatment with anti-ACE-2 antibodies disrupts the interaction between virus and receptor.



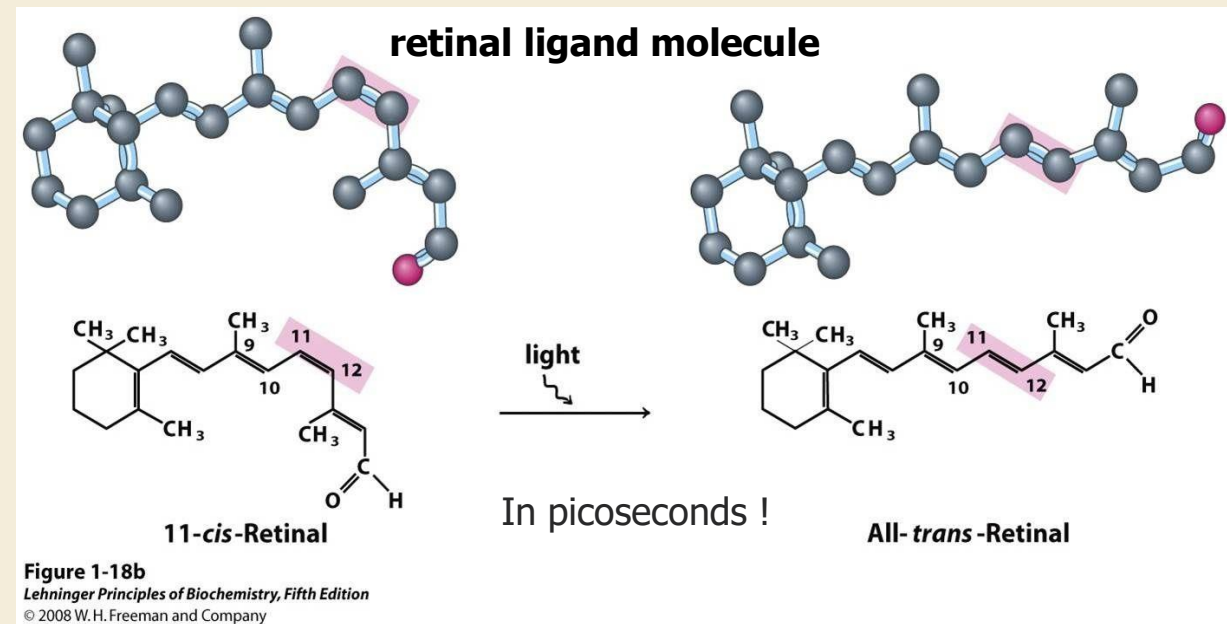
Examples of a Ligand

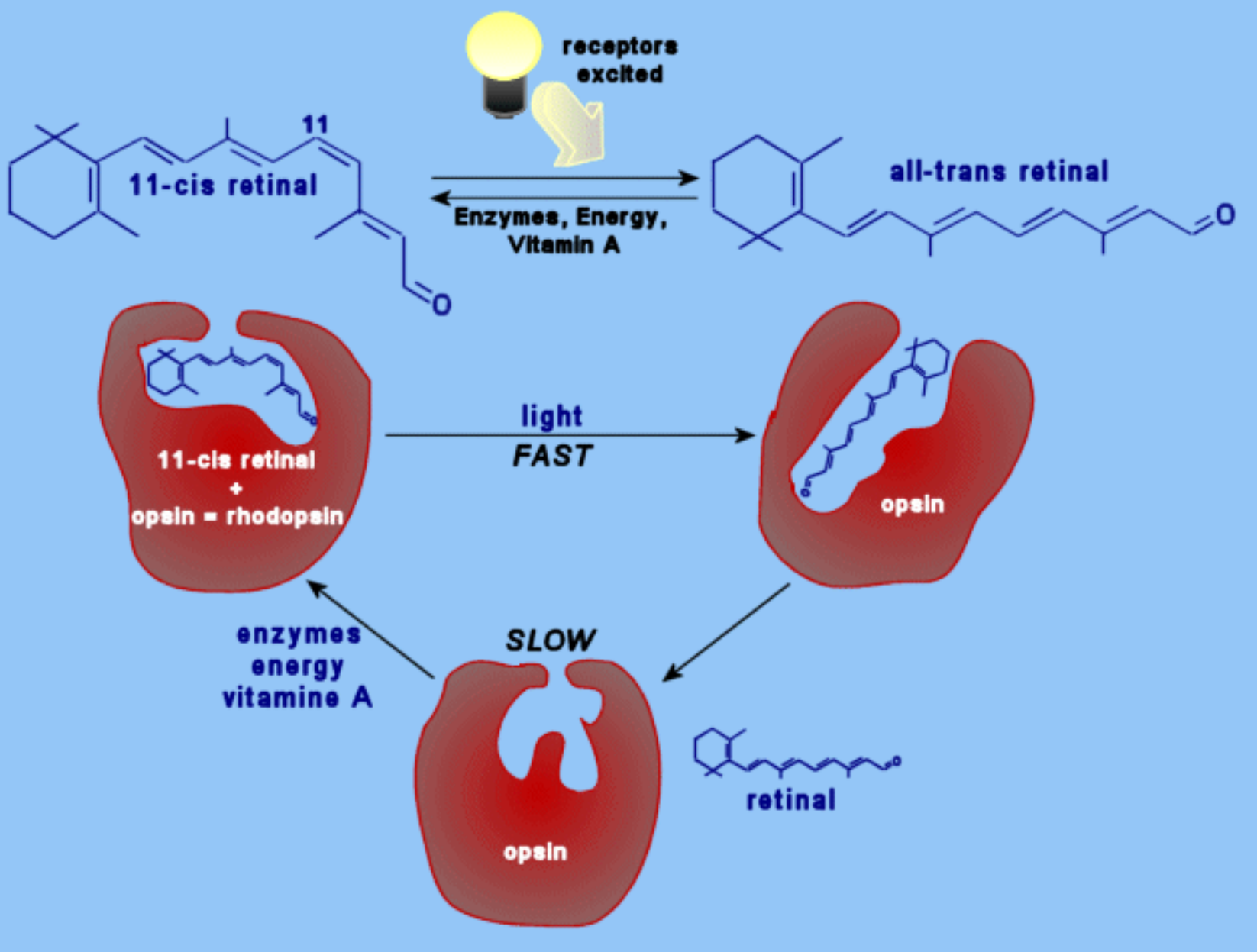
Retinal ligand in rhodopsin - cis to trans isomerization of retinal bound to the opsin protein

Rhodopsin is a light-sensitive receptor protein involved in visual phototransduction. It is a biological pigment found in the rods of the retina and is a G-protein-coupled receptor. **Rod** (and **cone**) are photoreceptor cells in the retina.

Retinal is a small molecule derived from retinoic acid, vitamin A. It has two interesting properties:

- The structure of retinal is such that the electrons be excited easily by light: they can absorb photons in the visible range of wavelengths (400 - 800nm)
- The double bonds can isomerize upon absorption of photons. Isomerization means that for a short moment the atoms can freely rotate along a double bond, thereby changing the geometry of the whole molecule. When retinal is bound to opsin (rhodopsin without retinal is called "opsin"), only one isomerization is possible: 11-cis retinal is transformed into all-trans retinal. This leads to a conformation change of the whole protein, thereby activating a signal pathway





Cis to trans isomerization

Isomerization process in vision:

irradiation of Rh 11-cis-retinal isomerizes to all-trans-retinal in 200 fs

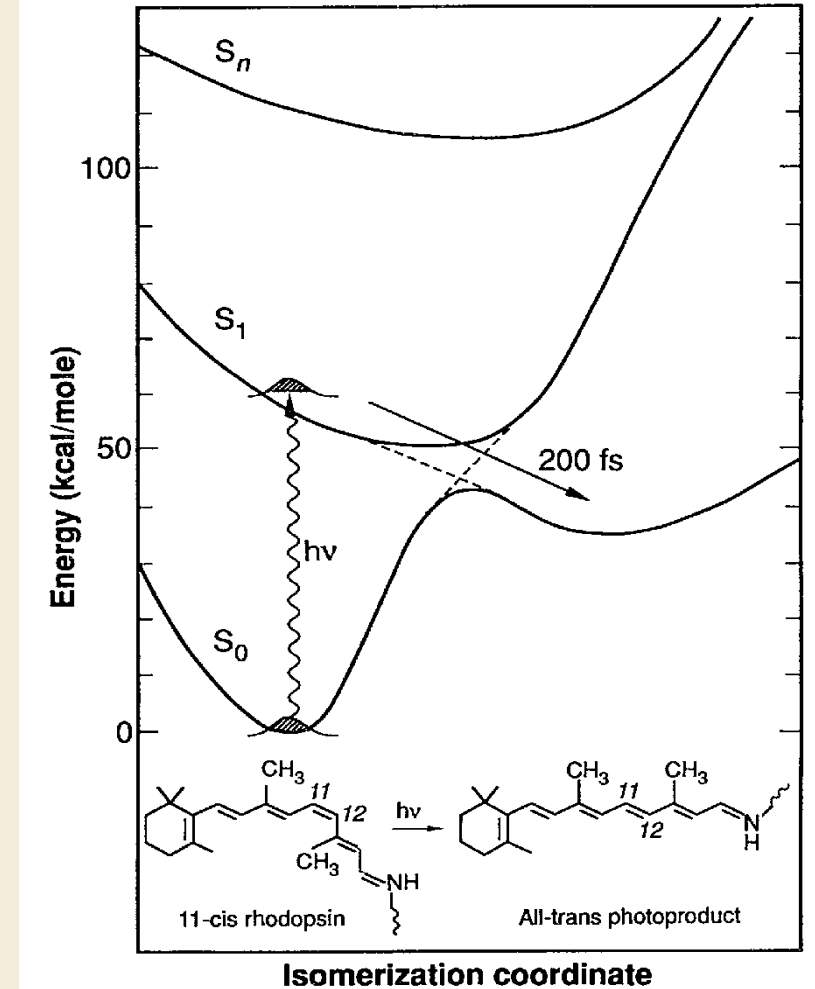
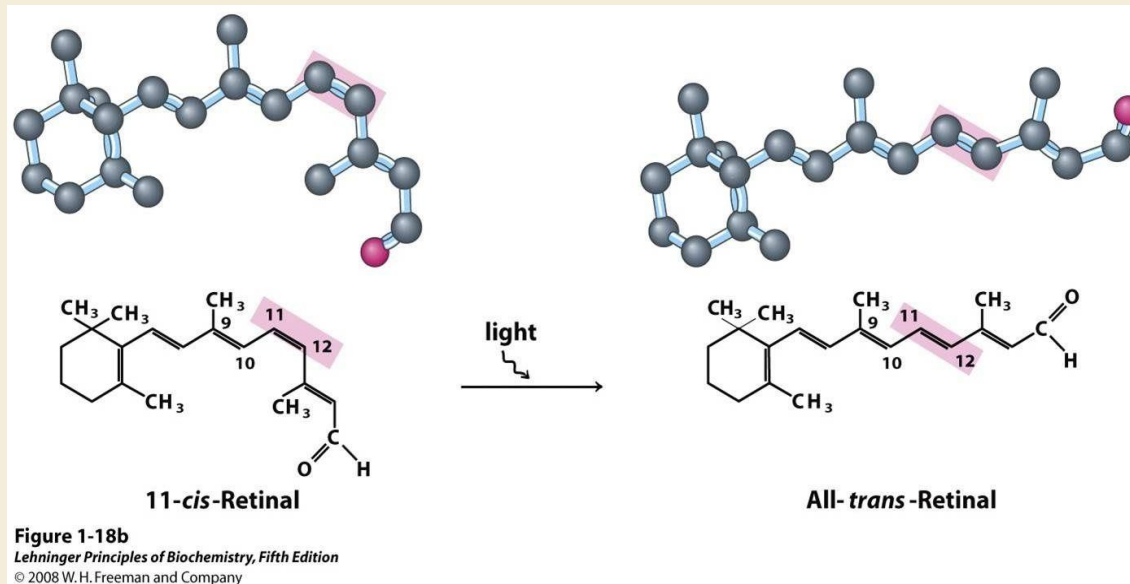
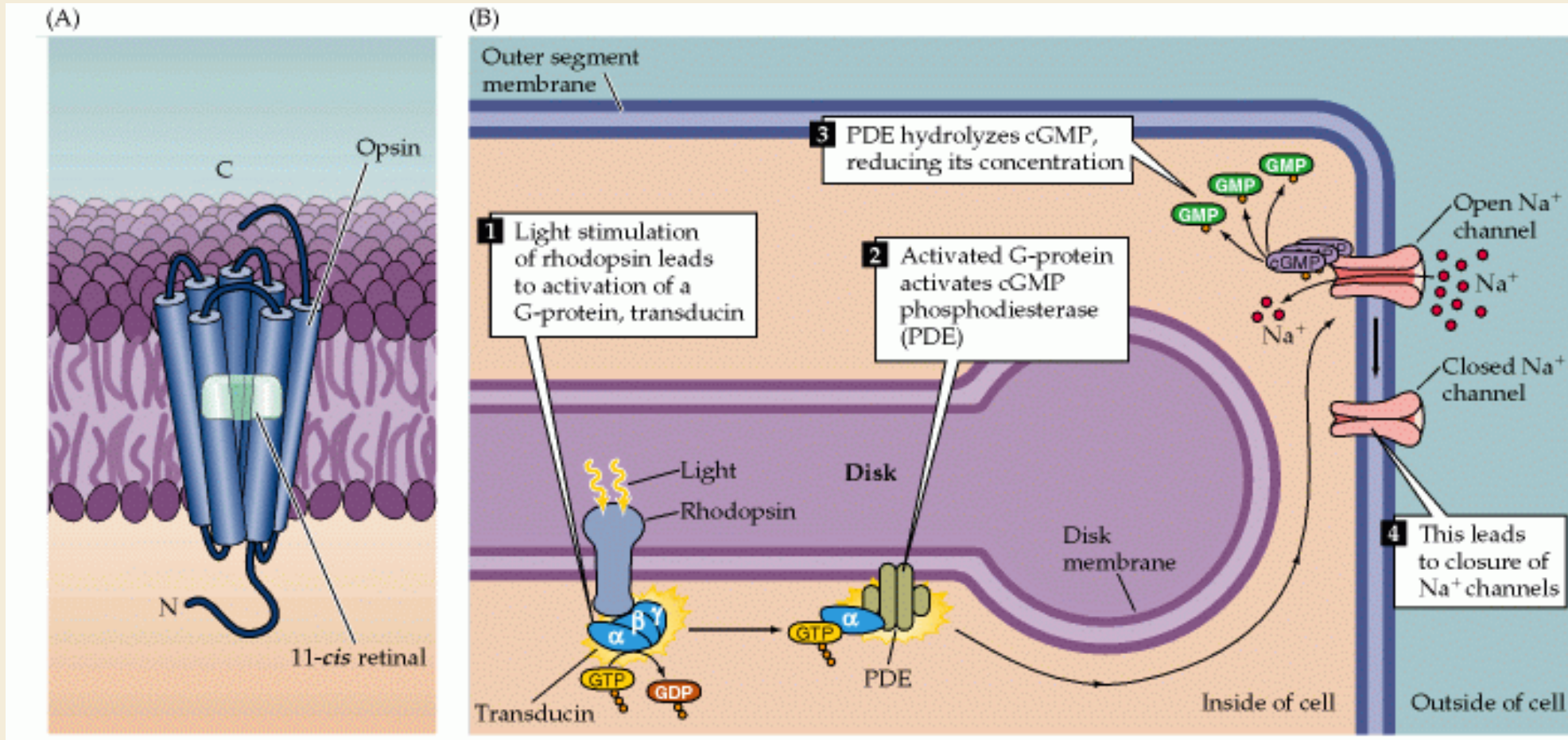


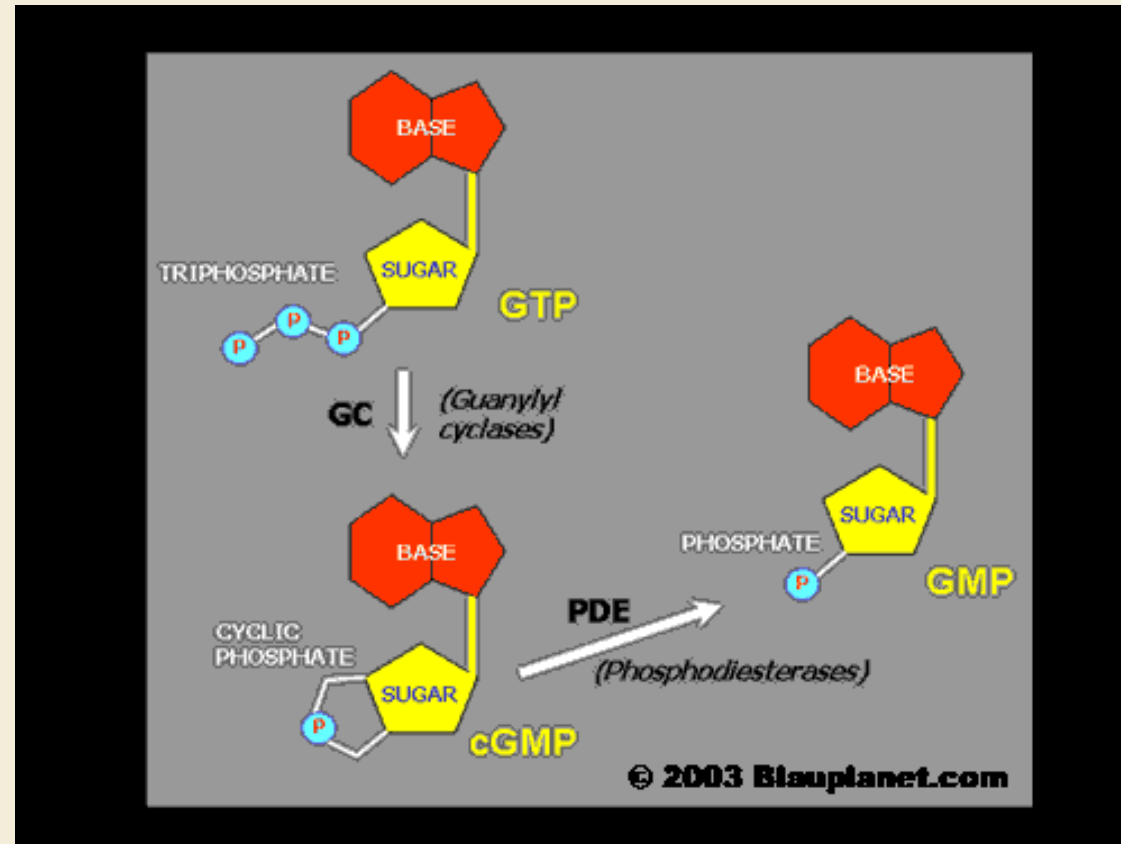
Fig. 1. Schematic ground-state and excited-state potential energy surfaces for the 11-cis \rightarrow 11-trans isomerization in rhodopsin, adapted from (14). The reaction path of the photoisomerization is indicated by the nonadiabatic potential surfaces (broken lines).

Phototransduction pathway occurs via a 4 step process that uses a **2nd messenger cascade** to **amplify the signal**. **Activation of rhodopsin** ultimately results in the **closure of cyclic nucleotide gated Na⁺ channels**, and **hyperpolarization** of the photoreceptor.



<https://openwetware.org/wiki/BIO254:Phototransduction>

phosphodiesterase (PDE)
Cyclic guanosine monophosphate (cGMP)



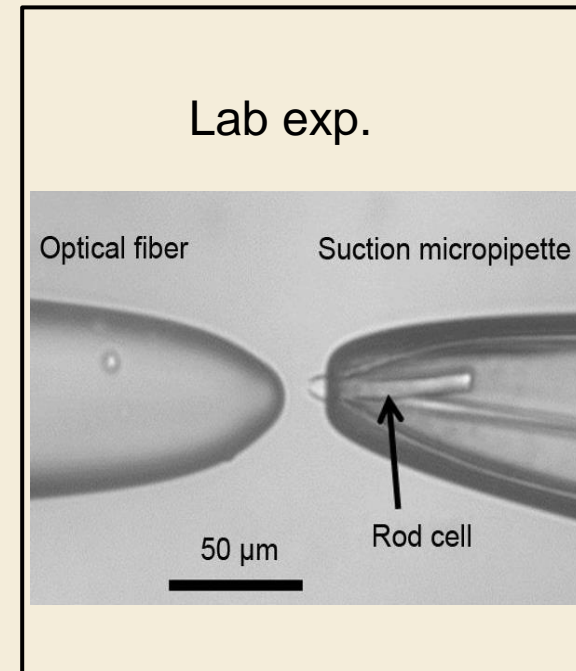
A phosphodiesterase (PDE) is an enzyme that breaks a phosphodiester bond in the second messenger molecule cGMP

Cyclic guanosine monophosphate (cGMP) is a cyclic nucleotide derived from guanosine triphosphate (GTP). Its most likely mechanism of action is activation of intracellular protein kinases in response to the binding of membrane-impermeable peptide hormones to the external cell surface

Lab Experiment Stimulating a single Rod cell

Stimulating Rod cell
with light and measure current

A single rod cell is illuminated and the current
generated is measured in the micropipette



Mechanical force affects chemical reactions

To understand how molecular machines work we need to understand how molecules (proteins) move/deform in response to these chemical forces.

Just as a chemical force might cause a protein to move in one direction, an external mechanical force might cause the protein to move in opposite direction. Thus, mechanical forces can oppose chemical reactions and conversely chemical reactions can oppose mechanical ones. If the chemical force is strong enough, the chemical reaction will proceed even in presence of a mechanical force → reaction generates force.

How force affects ligand-receptor bond equilibrium,

or the equilibrium between two structural states of a protein $E1 \rightleftharpoons E2$?

What is a structural state of a protein ?

A **structural state** refers to an ensemble of a large number of individual **conformational states** that do not vary too much from mean state (stable minimum energy state).

Due to the thermal fluctuations, a complex molecule like a protein can occupy an enormous number of different conformational states.

Conformational state: is defined by a set of coordinates of all the atoms.

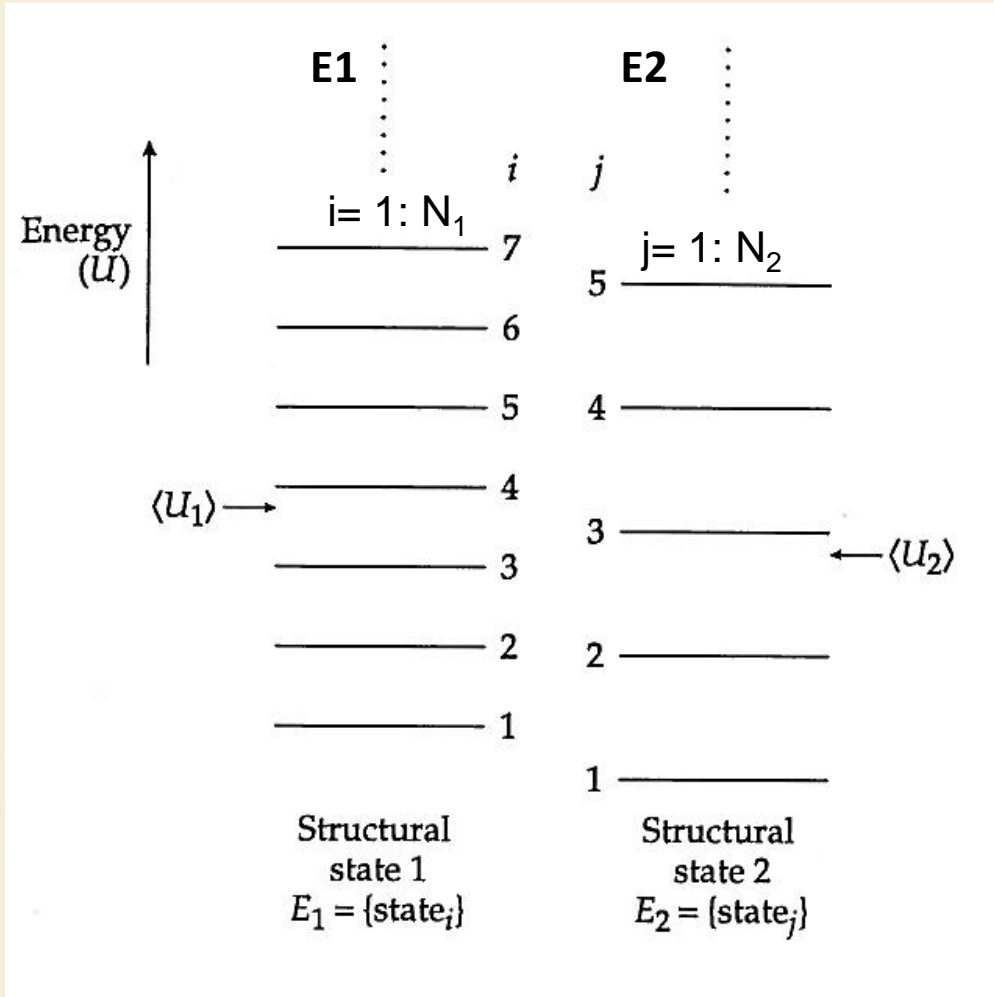
Examples of different structural states:

folded vs unfolded protein, closed vs open ion channel, ligand – receptor unbound vs bound

The probability of finding the protein in a certain **conformational state** is given by Boltzmann's law

The probability of finding the protein in a certain **structural) state** can be also determined using the Boltzmann's law.

Two structural states:



Ensembles of states

Suppose a molecule can be in one of two **structural states E1 and E2**.

The probability to find the molecule in **conformational state i** or **j** is:

$$p_i = \frac{1}{Z} \exp \left[-\frac{U_i}{kT} \right] \quad Z = \sum_i^N \exp \left[-\frac{U_i}{kT} \right] \quad N = N_1 + N_2$$

The probabilities of finding the molecule in the structural state E1 or in the structural state E2 are:

$$p_1 = p(E1) = \sum_{i=1}^{N_1} p_i = Z_1 / Z \quad p_2 = p(E2) = \sum_{j=1}^{N_2} p_j = Z_2 / Z$$

$$P_2 / P_1 = Z_2 / Z_1$$

$$Z_1 = \sum_{i=1}^{N_1} \exp \left[-\frac{U_i}{kT} \right] \quad Z_2 = \sum_{j=1}^{N_2} \exp \left[-\frac{U_j}{kT} \right]$$

For a structural state E we define the free energy as: $G = \langle U \rangle - TS$, where:

U is the potential energy comprising the internal energy associated with all the bonds (covalent, electrostatic) + the external energy corresponding to external variables such as pressure, force, electrical field or gravity.

The entropy S is a measure of disorder: $S = K \ln \Omega$ with Ω number of microscopic configurations; the larger the number of conformations in an ensemble, the greater the entropy S

Free Energy associated with ensembles of conformational states

$$(1) \quad \langle U \rangle = \sum_{i=1}^N u_i p_i$$

The average energy, N -number of different conformational states of a molecule
 u_i - potential energy for conf. state i

$$(2) \quad p_i = \frac{1}{Z} \exp\left[-\frac{u_i}{kT}\right], \quad Z = \sum_{i=1}^N \exp\left[-\frac{u_i}{kT}\right]$$

$$(3) \quad \text{The entropy } S = -K \sum_{i=1}^N p_i \ln p_i$$

(4) The free energy $G = \underline{\langle U \rangle - TS} =$
 $= \sum_{i=1}^N P_i (U_i + kT \ln P_i) =$
 $= \underline{-kT \ln Z}$

in classical thermodynamics, $\langle U \rangle = \Delta H$ - enthalpy
 free energy is expressed in terms of S and T
 entropy + T

in statistical thermodynamics, P_i - probabilities
 free energy is expressed in terms of energy levels U_i
 and energy levels U_i

$$\frac{[E_1]}{Z} = P_1 = \frac{z_1}{Z} \quad [E_2] = \frac{z_2}{Z} \quad \frac{[E_2]}{[E_1]} = \frac{P_2}{P_1} = \frac{z_2}{z_1} = \exp\left[-\frac{\Delta G}{kT}\right]$$

The Boltzmann's law holds for ensembles of conformational states E1 and E2,
if the energy term is replaced by the Gibbs free energies, G1 and G2.

It relates probabilities to free energy.

$$\frac{[E_2]}{[E_1]} = \frac{p_2}{p_1} = \exp\left[-\frac{\Delta G}{kT}\right] = \text{constant} \equiv K_{\text{eq}}$$

[E1], [E2] concentrations of E1 and E2

$\Delta G = G_2 - G_1$ **K_{eq} – equilibrium constant**

Law of Mass Action

If E1 and E2 are in equilibrium and more protein in the E1 form is added, then the amount of E2 will increase as the system returns to the equilibrium ratio. Likewise, adding E2 pushes the reaction back toward E1.

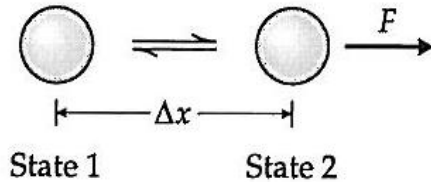
Force can influence the equilibrium between two (or more states).

Boltzmann's law allows to calculate how force influences the equilibrium.

Displacements associated with structural changes

(A) Ex: motor moves along a filament

Translation

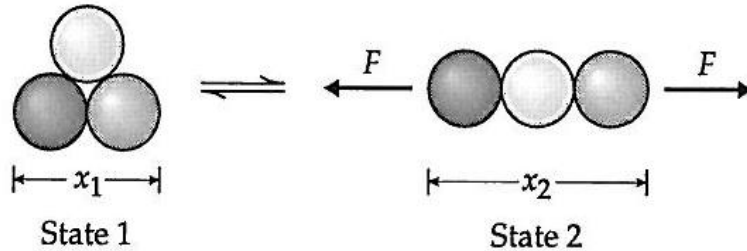


The difference in free energy G

$$\Delta G = -F \cdot \Delta x$$

(B)

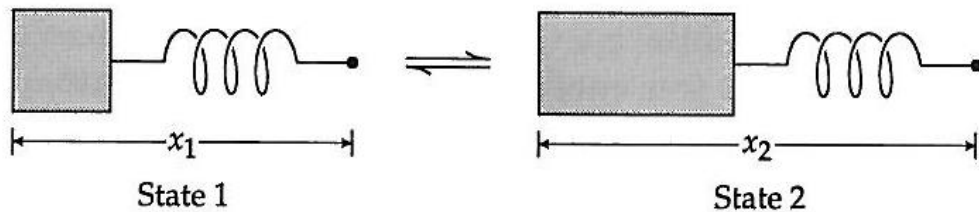
Lengthening



$$\Delta G \cong \Delta G^0 - F\Delta x$$

ΔG^0 The free energy difference in the absence of tension

(C)



Lengthening with a constant stiffness

At equilibrium

$$\frac{[E_2]}{[E_1]} = \exp\left[-\frac{\Delta G}{kT}\right] \cong \exp\left[-\frac{\Delta G^0 - F\Delta x}{kT}\right] = K_{eq}^0 \exp\left[\frac{F\Delta x}{kT}\right]$$

$$\frac{[E_2]}{[E_1]} = \exp\left[-\frac{\Delta G}{kT}\right] \cong \exp\left[-\frac{\Delta G^0 - F\Delta x}{kT}\right] = K_{eq}^0 \exp\left[\frac{F\Delta x}{kT}\right]$$

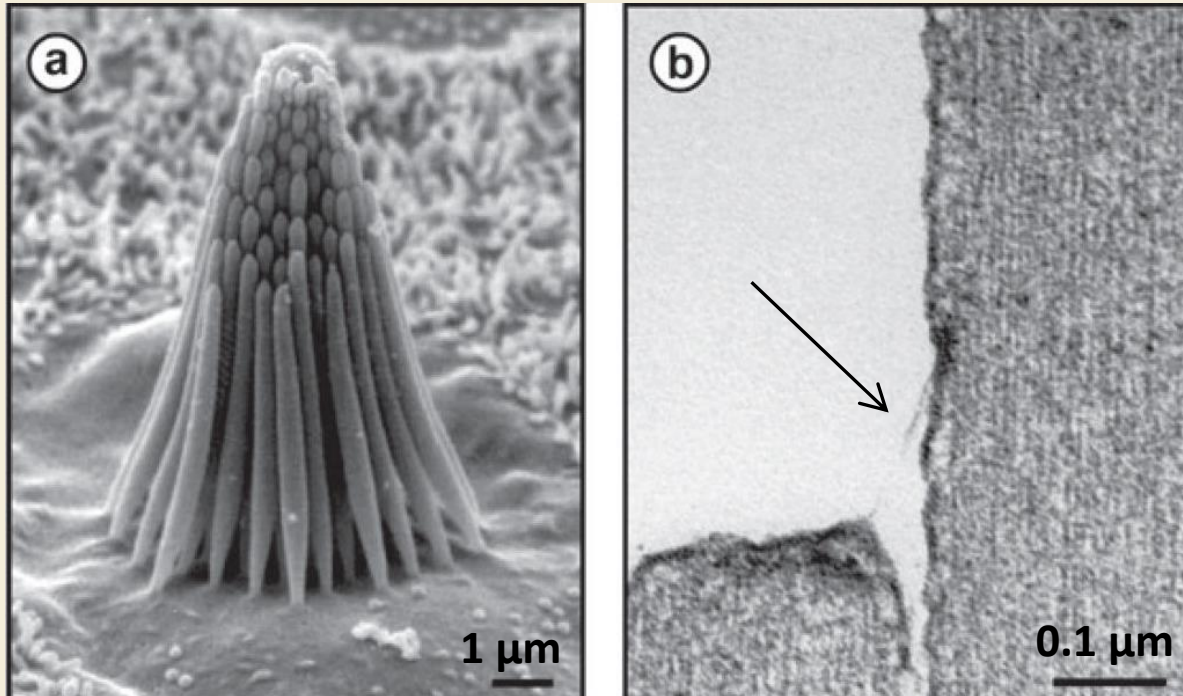
K_{eq}^0 is the equilibrium constant in the absence of force.

An external force couples to a structural change if it is associated with a length change in the direction of the force.

Example:

If the change Δx in the length of molecule is $\Delta x = 4 \text{ nm}$, then a force $F=1 \text{ pN}$ will change the free energy by $\Delta G = 4 \text{ pN nm} \approx 1 \text{ kT}$. **This will lead to an e-fold change in the ratio of concentrations !**

The sensory hair cells of the inner ear underlie the **perception of sound, linear and angular accelerations, and gravity !**



Hair cell anatomy:

(a) Hair bundle in a bullfrog sacculle, comprising ~60 stereocilia

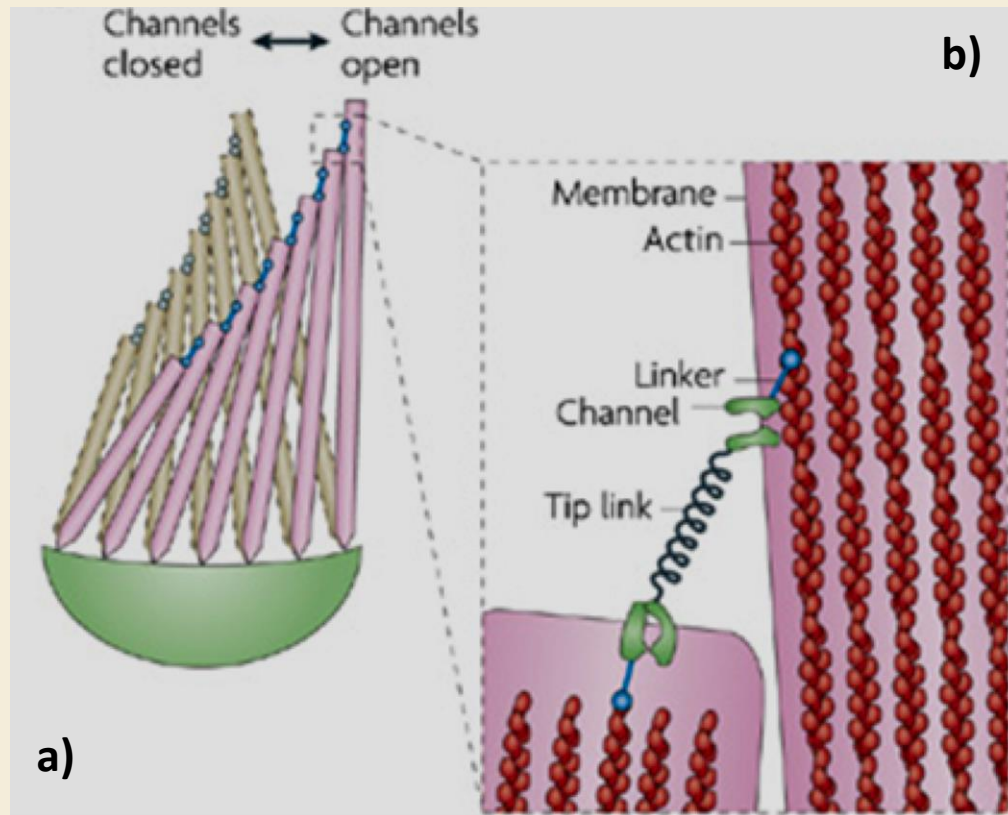
(b) Two stereocilia and the tip link extending between them



The Micromachinery of Mechanotransduction in Hair Cells

Annu Rev Neurosci. 2007 doi:10.1146/annurev.neuro.29.051605.112917.

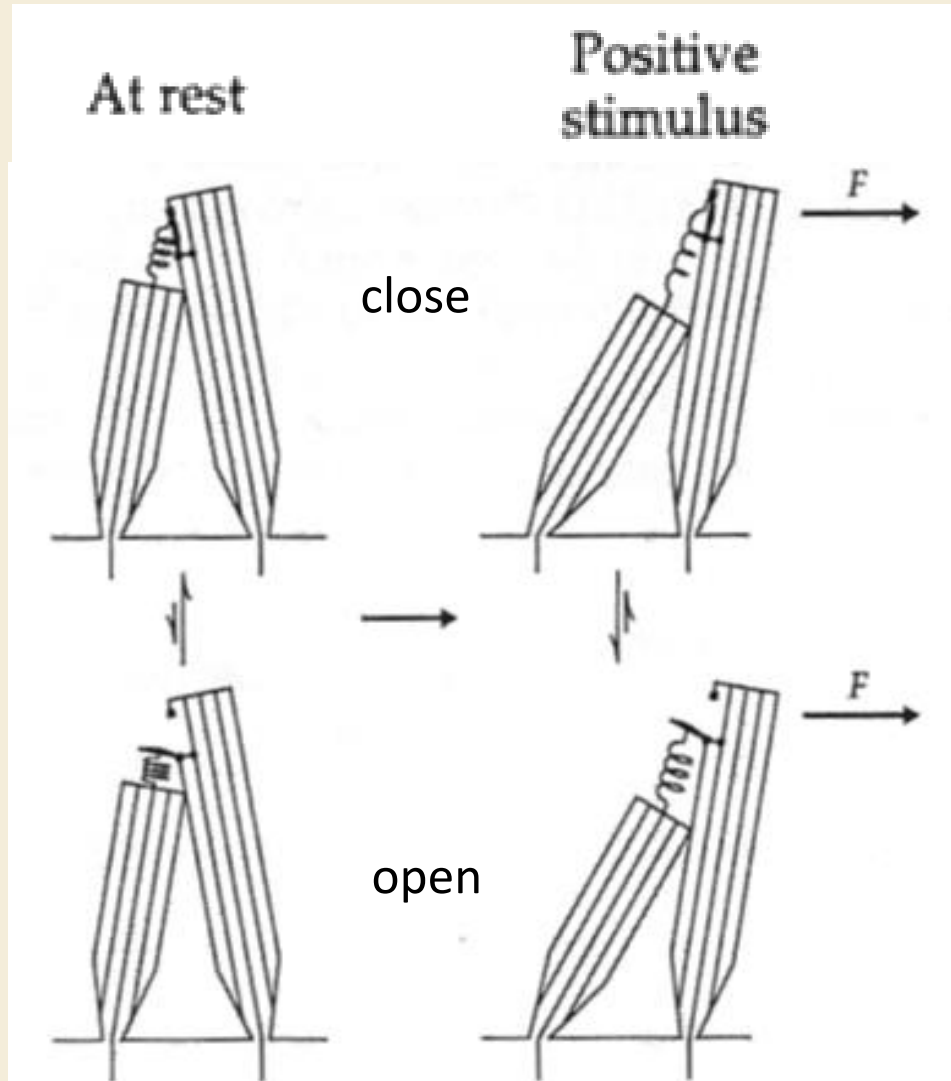
Simplified scheme of stereocilia bundle deflection and ion channel opening



a) Hair bundle in resting (yellow) and deflected (pink) configurations.

b) Deflection, i.e. shearing of the stereocilia relative to each other, causes the tip links to pull directly on K^+ (and Ca^{2+}) channels in the stereocilia, causing the channels to open.

Myosin motors (blue circle) that link the channels to the actin core of the stereocilia can adjust the position to restore resting tension in the tip link, allowing adaptation to persistent stimulation.



External Force \rightarrow deflection of hair bundle \rightarrow shear between adjacent stereocilia \rightarrow tension in the elastic tip link \rightarrow pull and open ion channels.

The opening of a channel shortens the tip link

\rightarrow the open state is stabilized by deflection that increases the tension in the tip link

\rightarrow the open probability increases as the hair bundle is displaced to the right.

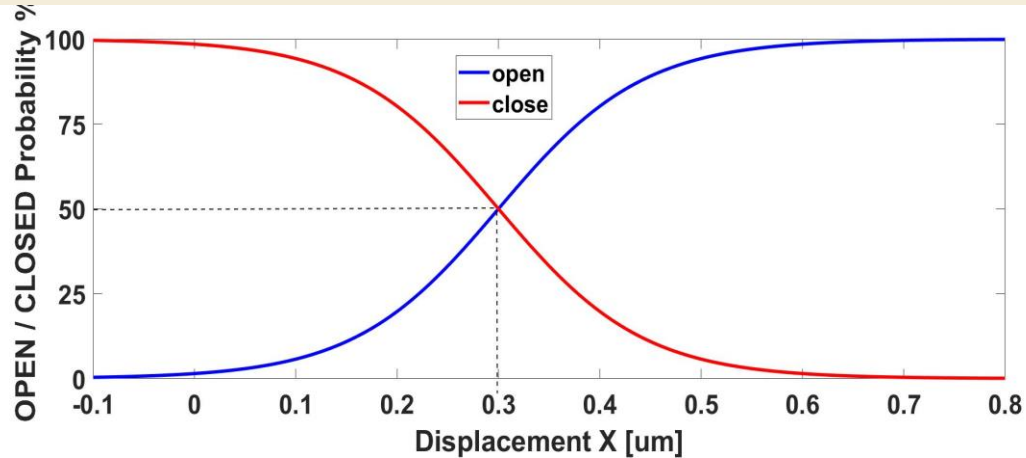
$$\frac{P_{open}}{P_{close}} = k_{eq}^0 \cdot \exp\left(\frac{F\Delta x}{kT}\right) \quad P_{open} + P_{close} = 1$$

$$P_{open} + P_{open} \cdot \frac{1}{k_{eq}^0} \cdot \exp\left(-\frac{F\Delta x}{kT}\right) = 1$$

$$k_{eq}^0 = \frac{P_{open}^{rest}}{P_{close}^{rest}}$$

Open probability increases as the hair bundle is displaced to the right.

Model



$$p_{\text{open}} = \frac{1}{1 + \exp\left[-\frac{F\Delta x}{kT}\right]}$$

$$F = a\kappa(X - X_0)$$

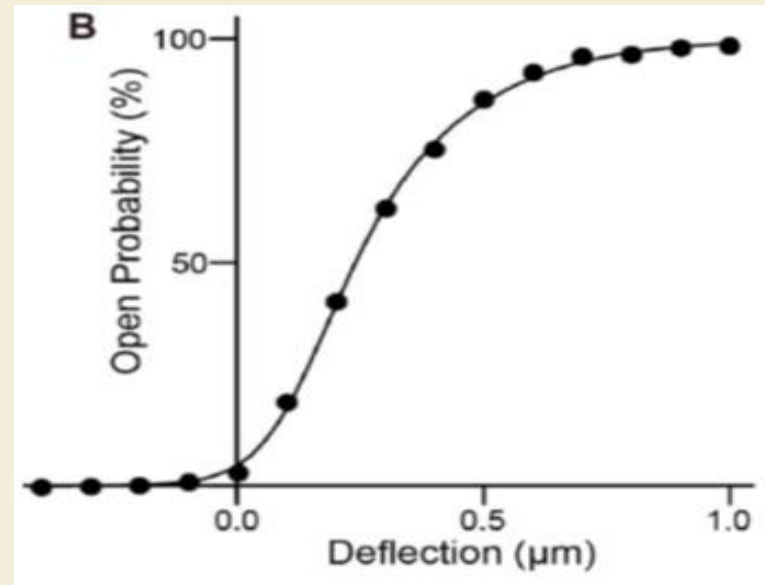
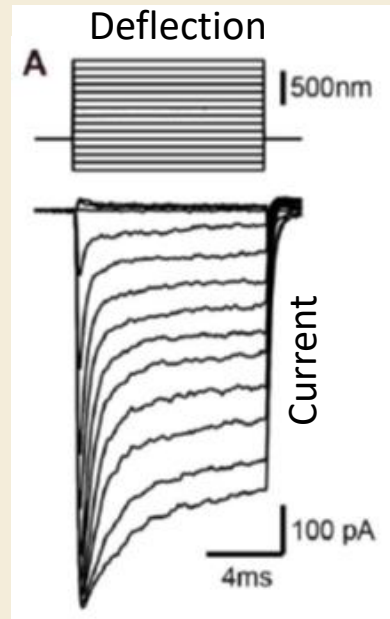
k - the stiffness of the tip link $k = 2 \text{ pN/nm}$

X - the displacement of the hair bundle ($X = -0.1 - 1 \text{ um}$),

X_0 - the displacement at which the channels are open 50% of the time, $X_0 = 0.3 \text{ um}$; a - geometric factor ($a > 1$)

Δx is the swing of the gate, $\Delta x = 2 - 4 \text{ nm}$, $F\Delta x = 200 \text{ pN nm} \sim 50 \text{ kT}$
 ($a = 0.1$, $X - X_0 = 500 \text{ nm}$, $\Delta x = 2 \text{ nm}$)

Experiment



Problems

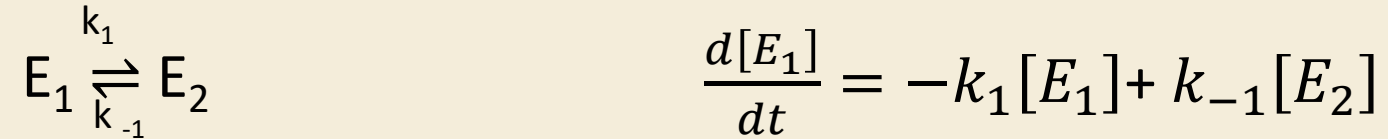
(force influencing the chemical equilibrium defined by the probability of a protein to be in one of two possible states)

home work

- 1 Suppose that one could pull directly on the gate of an ion channel, and that the gate swings through 2 nm as it goes from the closed to the open position. If, in the absence of force, the channel spends half its time open and half its time closed, how much force is needed to increase the open probability to 0.9?
- 2 Suppose that a protein has a stiffness of 2 pN/nm in state 1 and a stiffness of 1 pN/nm in state 2, but that the two states have the same resting length (the length in the absence of a force). If there is initially a very low probability of being in state 2, how much force is needed to increase the open probability e -fold?

Forces also affect the rates of chemical reactions.

The simplest chemical reaction is the conversion between two species that satisfies:



This reaction obeys first-order kinetics because the rate of change depends linearly on the concentrations of species. k_1 and k_{-1} = forward and reverse rate constants [s^{-1}]. (Association and dissociation constants)

When the reaction reaches equilibrium, ($d[E_1]/dt=0$), and:

$$\frac{k_1}{k_{-1}} = \frac{[E_2]}{[E_1]} = K_{eq} = \exp\left[-\frac{\Delta G}{KT}\right]$$

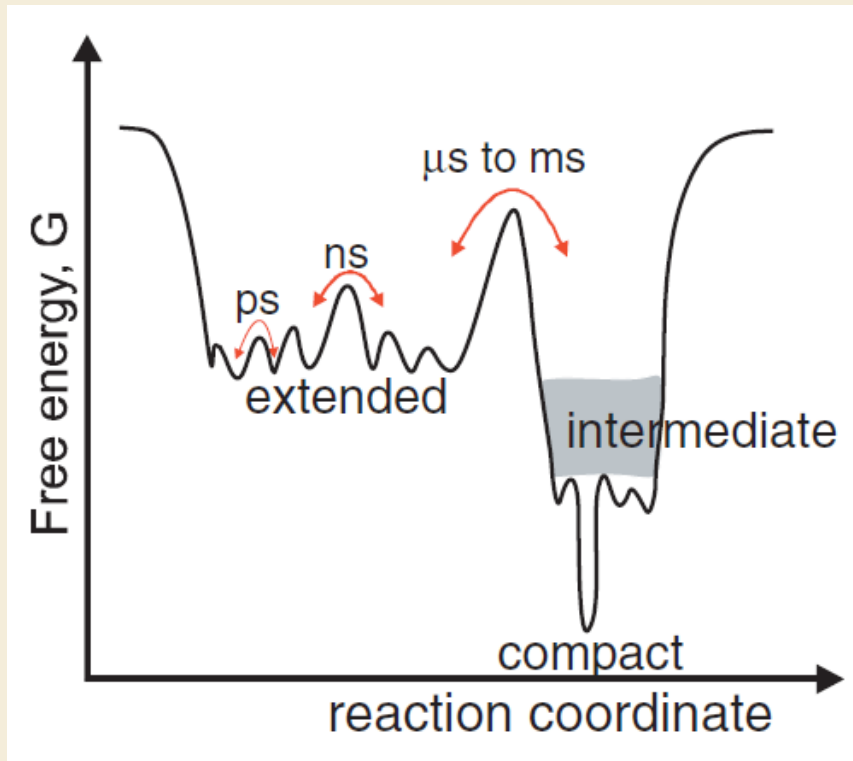
The equilibrium constant K_{eq} , defined with the concentrations, is equal to the ratio of forward and reverse rate constants.

If the free energy difference between product and reactant ΔG depends on the force, then either the forward or the reverse rate (or both) must depend on force.

The transition between two structural states in a first order reaction is very fast,
i.e. the duration of the transition is very much shorter than the average lifetimes of the states
($1/k_1$ for E1, and $1/k_{-1}$ for E2).

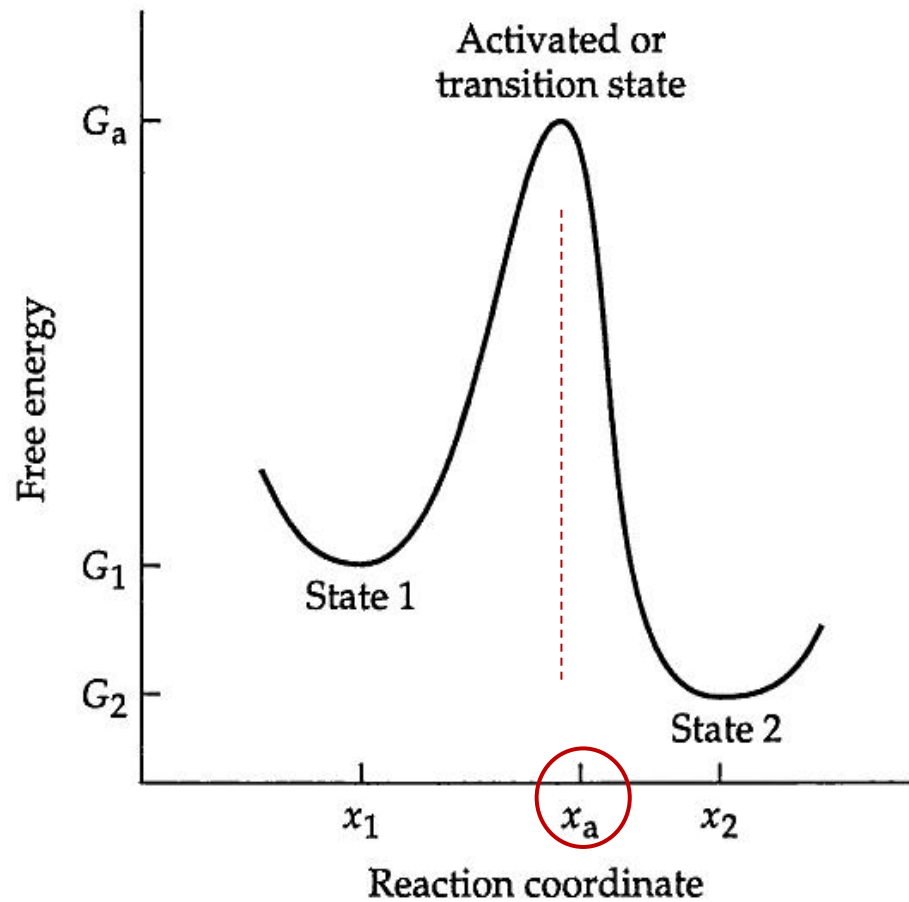
How fast might the transition be ?

- Covalent chemical changes : about 0.1 picosecond;
- Global conformational changes: about 10 nanoseconds;
- Lifetime of the structural states : > 1 milliseconds .



Energy landscape of protein structures and protein motion time scales

a protein's atoms' intramolecular positional fluctuations result in rapid loop and side chain motions at fast time scales
nanosecond (ns) and picosecond (ps)



Some properties of the first-order reactions can be understood using the idea that the reaction proceeds via a high-energy **activated state**, or **transition state**.

The activated state corresponds to a position (x_a) in the reaction coordinate, intermediate between the initial (x_1) and final (x_2) positions.

Assumptions:

- the reactant is in equilibrium with the activated state
- The activated state is equally likely to break to reactant or product with some rate A.

$$k_1 = A \exp \left[-\frac{\Delta G_{a1}}{KT} \right] \quad \Delta G_{a1} = G_a - G_1$$

Arrhenius equation; A- frequency factor

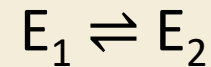
Arrhenius equation provides no information about the frequency factor **A**:

$$k_1 = A \exp \left[-\frac{\Delta G_{a1}}{KT} \right]$$

$$\Delta G_{a1} = G_{a1} - G_1$$

more detailed theories are **Eyring** rate theory and **Kramers** rate theory

Both require that the **reaction coordinate**, i.e. the parameter that measures the progression of the reaction,



be specified.

E.g. the length of a protein, if this changes as a result of the transition.

If the protein is subject to a force, then a natural reaction coordinate is the length of the protein in the direction of the force. For a chemical bond, it is the distance corresponding to unbinding.

In the **Eyring rate theory**,

the reaction is assumed to correspond to the breakdown of a single quantum-mechanical vibration of the protein.

The frequency factor is $A \sim kT/h \approx 6 \times 10^{12} \text{ [s}^{-1}\text{]}$, where h is the Planck constant.

E.g. A reaction with a rate constant $k_1 = 2 \times 10^3 \text{ s}^{-1}$, would have an activation energy : $\Delta G_{a1} = 22 kT$

$$k_1 = A \exp \left[-\frac{\Delta G_{a1}}{kT} \right]$$

Handwritten derivation on lined paper:

$$\frac{A}{k_1} = \exp \left[\frac{\Delta G_{a1}}{kT} \right] \quad A = 6 \cdot 10^{12} \left[\frac{1}{s} \right]$$

$$k_1 = 2 \cdot 10^3 \left[\frac{1}{s} \right]$$

$$\underline{\underline{\Delta G_{a1}}} = kT \ln (3 \cdot 10^9) \approx \underline{\underline{22 kT}}$$

The **Eyring theory** is expected to apply to **covalent changes of proteins and their ligands** but it is not expected to apply to global conformational changes of proteins in which a large number of bonds are made and broken, because in this case the reaction does not correspond to a single mode of vibration of the protein.

$$k_1 = A \exp \left[-\frac{\Delta G_{a1}}{KT} \right] \quad \Delta G_{a1} = G_{a1} - G_1$$

Kramers rate theory

is more adequate as a model for protein conformational changes.

The protein diffuses into the transition state with a rate that is the reciprocal of the

diffusion/relaxation time: $A \approx \frac{1}{\tau} \sqrt{\frac{\Delta G_{a1}}{KT}}$ with $\tau = \gamma/k$ (*relaxation time*)

The protein is sampling a different energy level every τ seconds, because τ is the time over which the protein's shape becomes statistically uncorrelated.

The protein can react only when it attains the energy of the transition state, and the probability of

this occurring is proportional to $\exp \left[-\frac{\Delta G_{a1}}{KT} \right]$.

$$k_1 = A \exp \left[-\frac{\Delta G_{a1}}{KT} \right]$$

The Eyring and Kramers rate theories represent two extreme views of the mechanism of global conformational changes of proteins.

$$A \approx KT/h$$

$$A_{\text{Eyring}} \approx 10^{12} \text{ -- } 10^{13} \text{ [s}^{-1} \text{]}$$

In the **Eyring model**, a sudden, local chemical change (such as the binding of a ligand or the chemical change in a bound ligand) creates a highly strained protein that then relaxes into a new stable conformation. The relaxation is along the quadratic energy curve and has time constant $\tau = \gamma/k$.

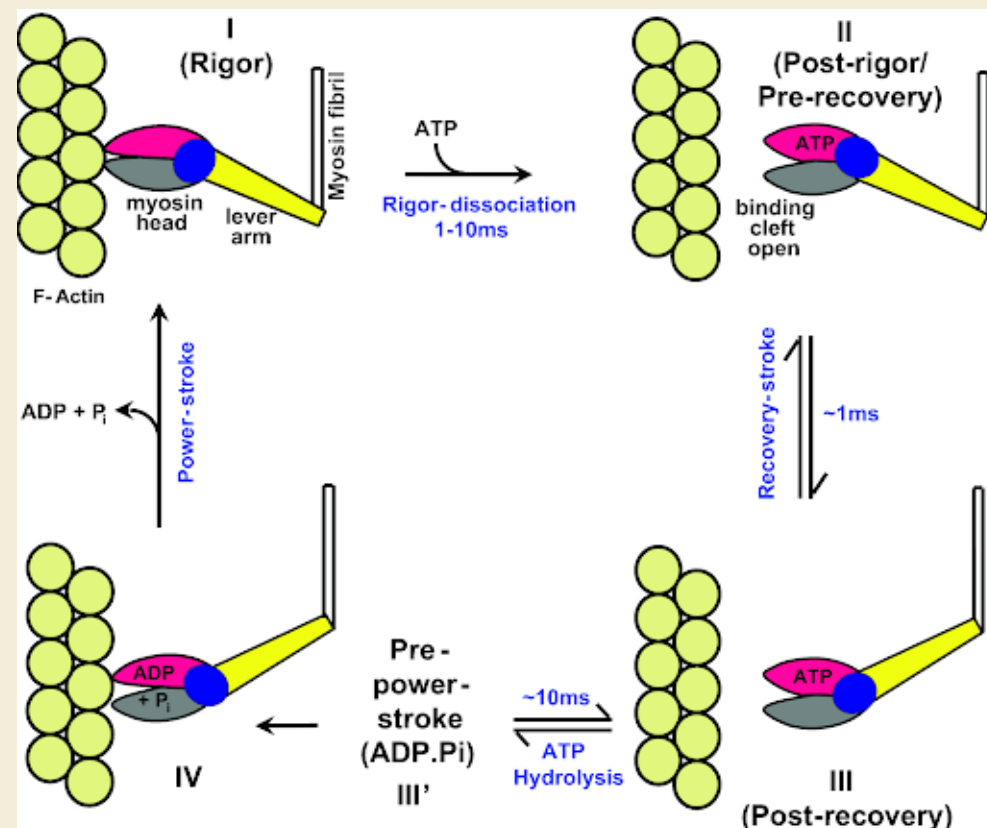
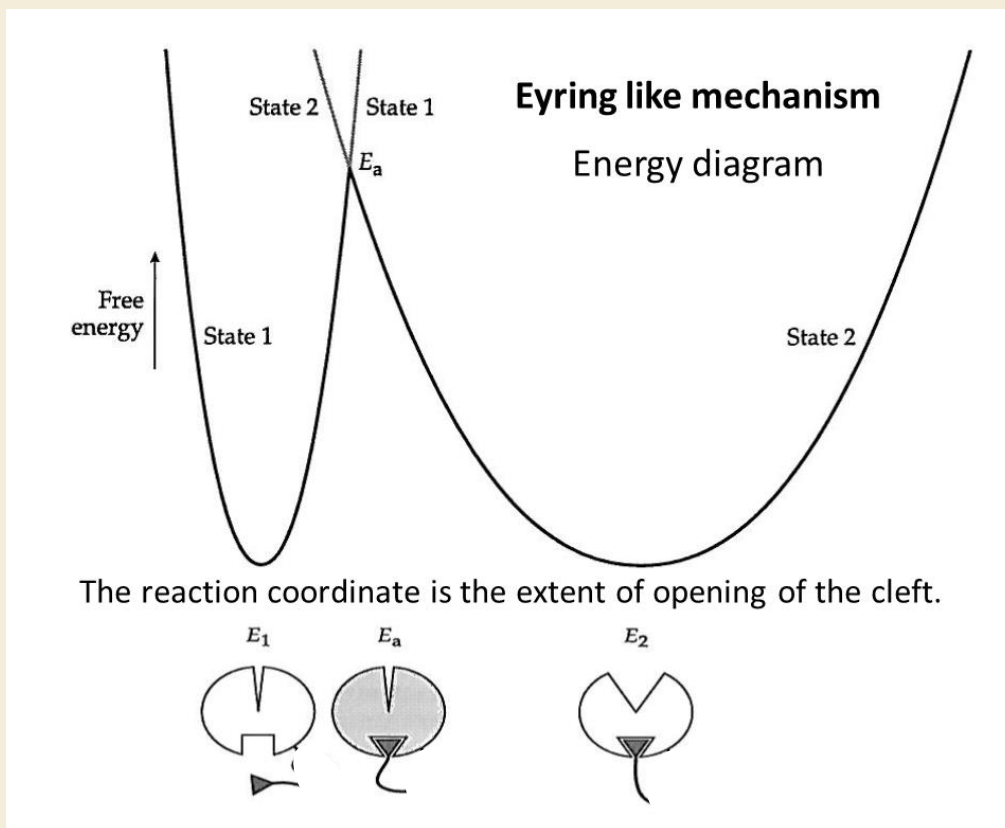
In the **Kramers** theory, the protein undergoes a global diffusion into the activated state.

When a sufficiently large conformational change has been achieved, the protein converts to the final state.

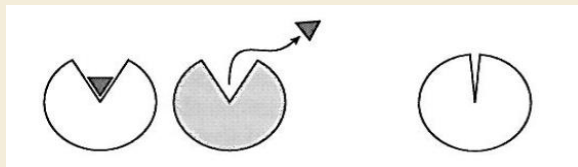
In the extreme, the protein diffuses all the way to the final state, which is then locked in by a subsequent chemical change.

$$A \approx 1/\tau \nu(\Delta G_{a1}/KT) \quad \text{with} \quad \tau = \gamma/k, \quad A_{\text{Kramers}} \approx 10^8 \text{ -- } 10^9 \text{ [s}^{-1} \text{]}$$

The model of the working stroke of myosin



Steps I-II-III : ATP binding to myosin (State 1) → Myosin unbinding and cleft opening (state 2)

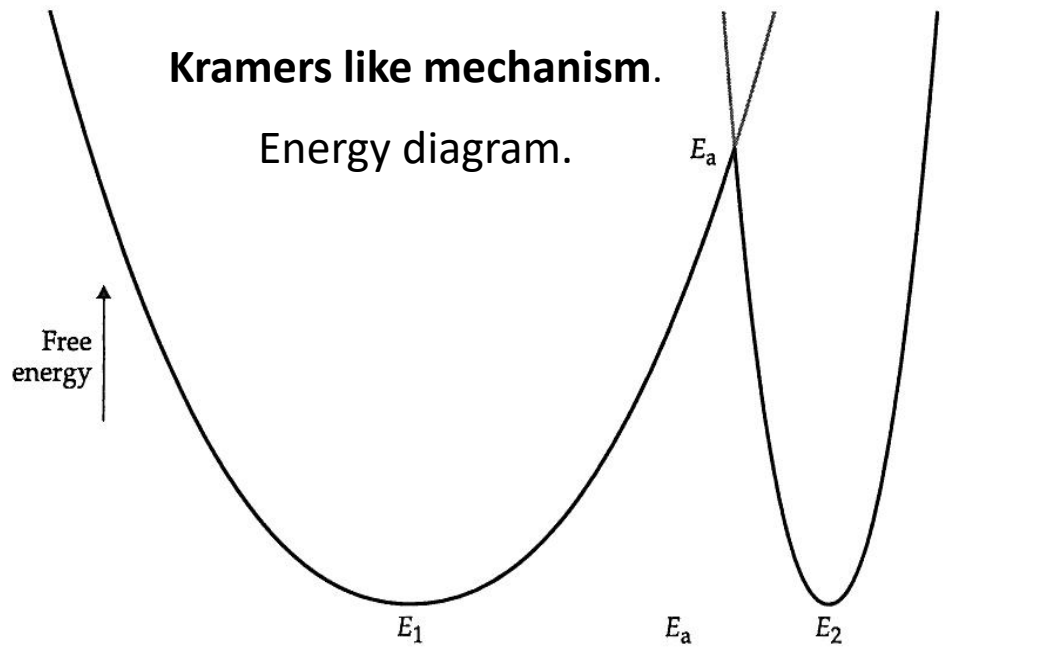


Step III-IV : Phosphate rapidly dissociates (State 1), leaving the protein in a highly strained state.

The relaxation (state 2) of this highly strained state drives the sliding of the actin filaments and the shortening of the muscle

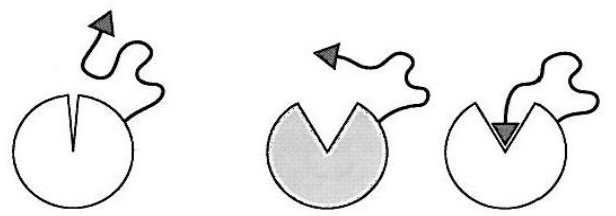
The principle for proteins is that global structural or 'physical' changes of proteins are much slower than local chemical changes, because structural changes are slowed by protein and solvent viscosity.

The fast local changes leave the protein in an unstable state which then relaxes more slowly into a new stable state.



The reaction coordinate is the extent of opening of the cleft.

Monomolecular model



The protein undergoes a global diffusion into the activated state and then converts to the final state.

The free energy available from ATP hydrolysis is 25 KT. The drag coefficient $\gamma = 60 \text{ pN s/m}$, and the relaxation time: $\tau = \gamma/k = 15 \text{ ns}$

It takes about 10 s to pick up 20 KT of energy by a purely diffusive process:

$$k_1 = A \exp \left[-\frac{\Delta G_{a1}}{KT} \right] \quad A \approx \frac{1}{\tau} \sqrt{\frac{\Delta G_{a1}}{KT}}$$

But for myosin, the complete ATP hydrolysis reaction only takes about 50 ms. Therefore, if the ATP hydrolysis reaction has an efficiency of 80% (20KT/25KT), such a diffusive step could not be on the myosin's reaction pathway. However, if the efficiency is 50% the time necessary to pick up 12.5 KT is reduced to about 7 ms.

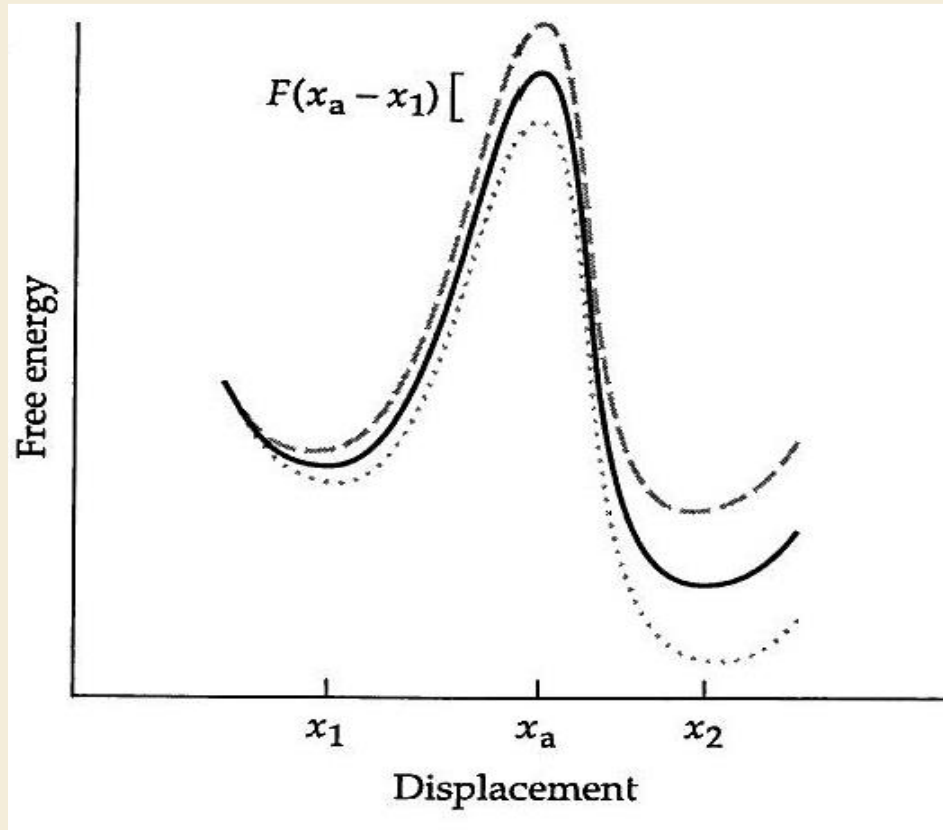
If the state transitions $E_1 \rightarrow E_a \rightarrow E_2$ are associated with displacements x_1 , x_a , and x_2 in the direction of the force, F , then the energies of the states will be decreased by Fx_1 , Fx_a , and Fx_2 , respectively.

This implies:

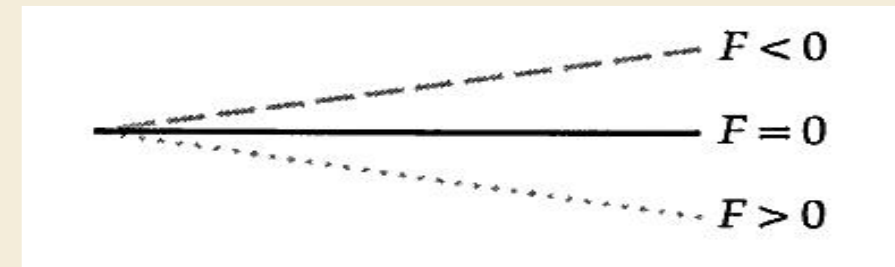
$$k_1 = A \exp\left[-\frac{\Delta G_{a1} - F\Delta x_{a1}}{kT}\right] = k_1^0 \exp\left[\frac{F\Delta x_{a1}}{kT}\right]$$

where $\Delta G_{a1} = G_{a1} - G_1$ and $\Delta x_{a1} = x_{a1} - x_1$.

An analogous expression holds for k_{-1} .

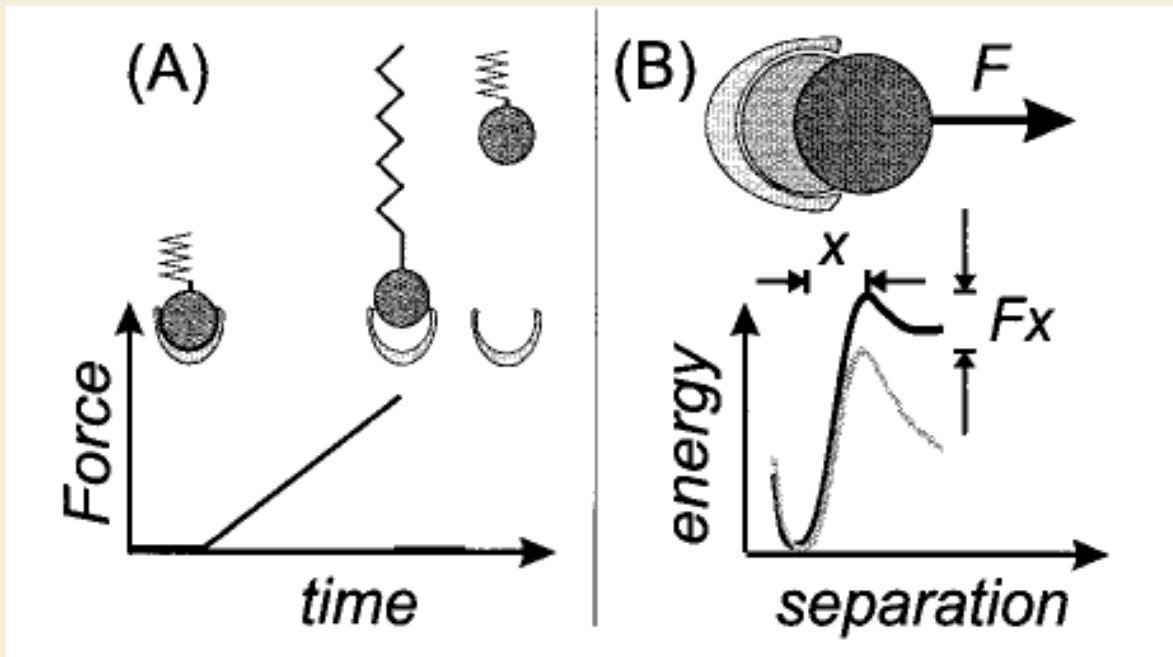


The effect of force on the reaction rates:
tilting the free energy diagram.



How to determine the dissociation rate of a ligand – receptor bond using force

Model and design experiment



(A) Direct observation of the dissociation under a mechanical force. The force on a single complex increases until it dissociates. The dissociation is monitored by an abrupt relaxation of the macroscopic spring of a force probe.

(B) The dissociation over a sharp energy barrier is characterized by a decrease of the barrier, giving rise to a characteristic length scale x .

Eyring theory → Reaction rate without force

$$k_1^0 = \frac{KT}{h} \exp \left[-\frac{\Delta G a_1}{KT} \right]$$

$k_1^0 = k_{off}$ - dissociation rate without force

(1) $k_1 = k_{off} \exp \left[\frac{F \cdot x}{KT} \right]$ with $x = x_a - x_1$ - dissociation with force

Evans-Ritchie model, assumption:

(2) $F = r \cdot t$ with r - loading rate in $\left[\frac{N}{s} \right]$, t - time [s]

The stochastic nature of the dissociation events is captured by solving the master equation for the probability $N(t)$ to be in the bound state, under increasing load $F = r \cdot t$

(3)
$$\frac{dN(t)}{dt} = -k_1 \cdot N(t)$$

This results in a distribution of unbinding forces:

(4)
$$P(F) = \frac{k_1(F)}{r} N\left(\frac{F}{r}\right)$$

The goal is to determine the dissociation rate k_{off}

In the limit of large statistics, the distributions of rupture times and forces follow a first-order (Markov) process where time and force are tied together through the loading dynamics.

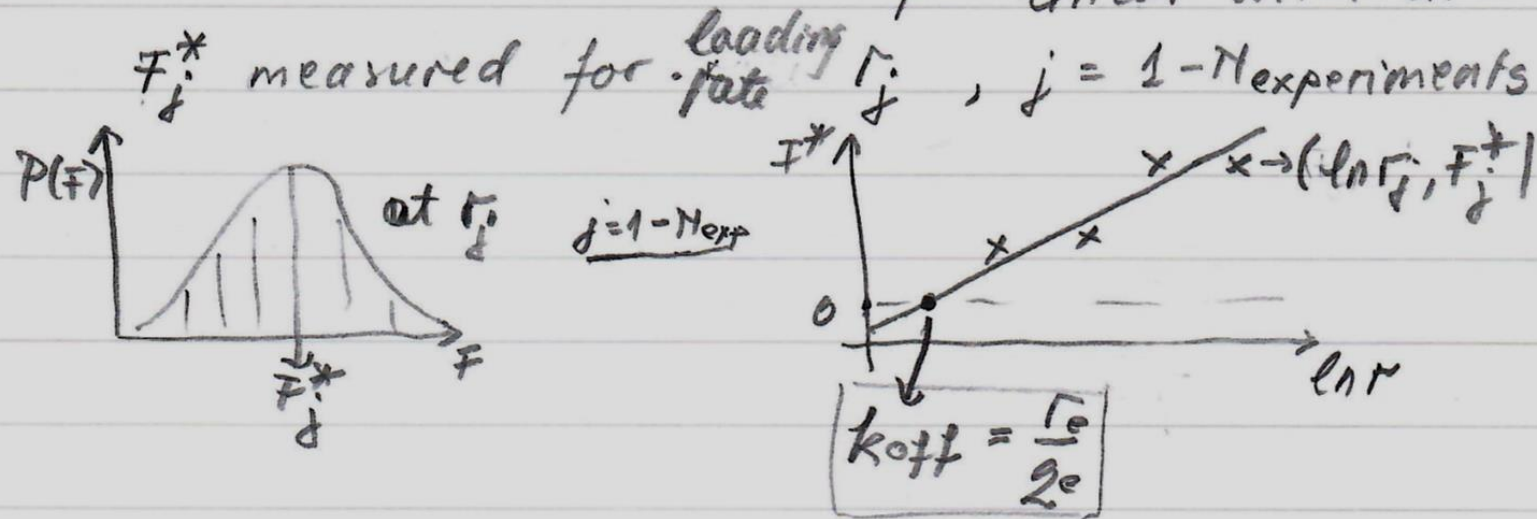
$$(5) \quad P(F) = \frac{k_{\text{off}}}{\Gamma} \cdot \exp \left\{ \frac{F x}{kT} + k_{\text{off}} \frac{kT}{r} \left(\frac{1}{\Gamma} - \exp \frac{F x}{kT} \right) \right\}$$

(6) The most probable unbinding/dissociation force

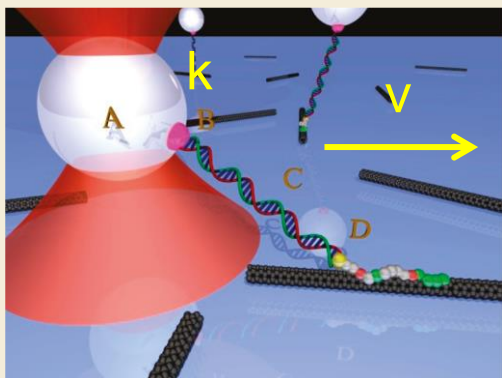
$$F^* = \frac{kT}{x} \ln \left(\frac{\Gamma}{k_{\text{off}}} \frac{r}{kT} \right) \quad \frac{kT}{x} = 2$$

$$(7) \quad F^* = 2 \ln \left(\frac{\Gamma}{2 \cdot k_{\text{off}}} \right) = 2 \ln \Gamma - 2 \ln(2 \cdot k_{\text{off}})$$

F^* linear with $\ln \Gamma$



How it works in practice ?



1. We need a tool to exert force ($F = k_{probe} x$): AFM, OT, MT, AT; the choice depends on the strength of the bond. The probe exerts force on the ligand-receptor bond.
2. We need linkers to connect the ligand with the probe (e.g. OT bead, AFM tip) because the probe is much bigger than the ligand molecule.

* Measurement procedure:

1. Pull the "construct" (ligand + linker) with a force F :

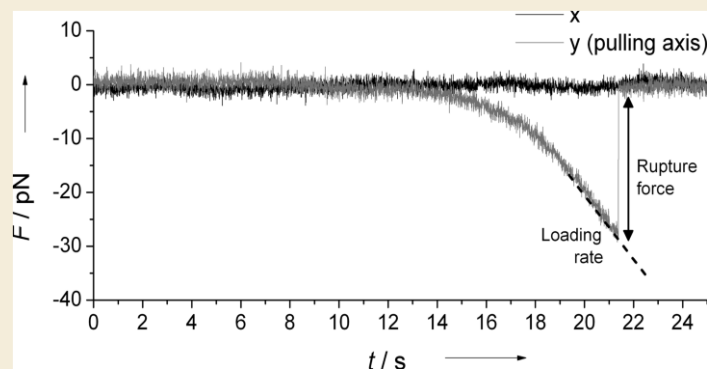
$$F = r \cdot t, \quad r - \text{load rate } \left[\frac{\text{pN}}{\text{s}} \right], \quad t - \text{time } [\text{s}]$$

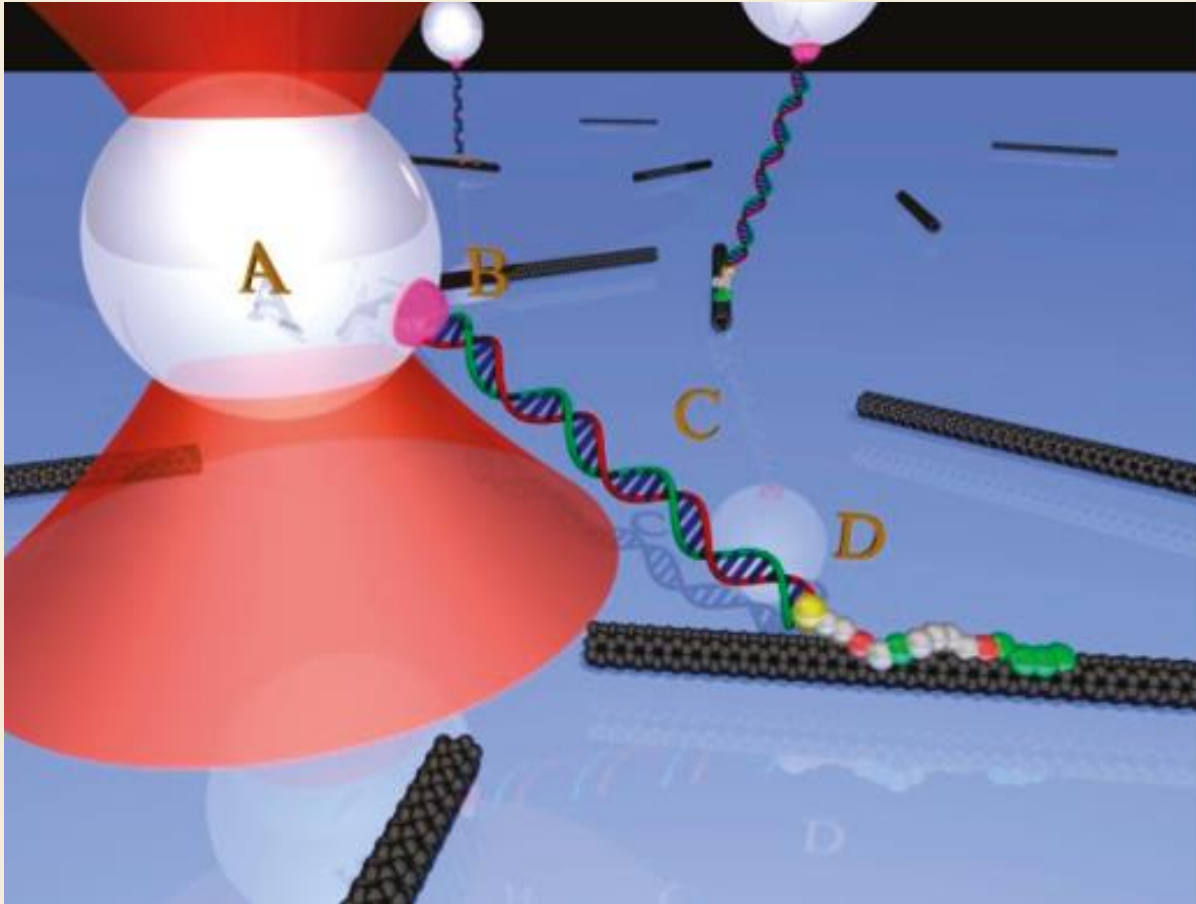
eg. for OT: stiffness of the trap $k_{OT} = 0.001 - 0.5 \left[\frac{\text{pN}}{\text{nm}} \right]$ $F = k \cdot x_B$

Force is applied by moving the ligand (fixed ^{on} substrate)

with a constant velocity v ; $v = 20 - 2000 \left[\frac{\text{nm}}{\text{s}} \right]$

The load rate, r , will be then $r = v \cdot k$ $r = 0.02 - 1000 \text{ [pN/s]}$





Schematics of optical tweezers pulling on a single peptide aptamer molecule linked to a carbon nanotube. The optical trap captures a bead (A) that is linked to an aptamer (D) via a DNA molecule (C) and a biotin/streptavidin linkage (B).

Adhesion through Single Peptide Aptamers

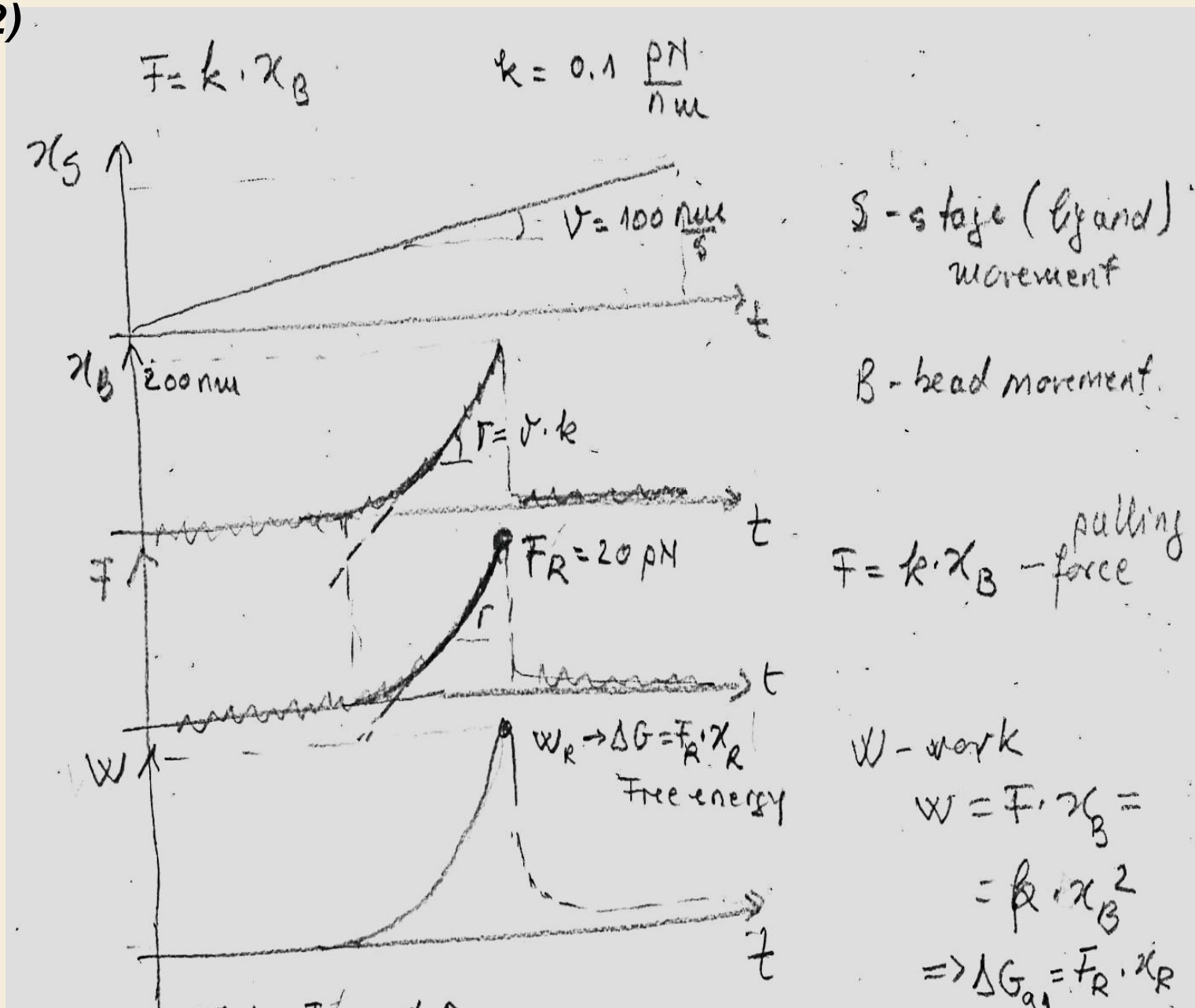
Aptamers are biomolecules with specific binding affinity, enabling applications in sensing, diagnostic, drug delivery, imaging, and therapy.

Peptide aptamers typically contain 8-20 amino-acids and bind materials or biomolecules.

They can be engineered via selection from large libraries of random sequences ($\sim 10^{10}$) by directed evolution techniques such as phage display.

Aubin-Tam et al, Adhesion through single peptide aptamers
[dx.doi.org/10.1021/jp1031493](https://doi.org/10.1021/jp1031493) | J. Phys. Chem. A 2011

2)



S - stage (ligand) movement

B - bead movement

Force ramp approach

The force F increases with time t :
 $F(t) = r t$

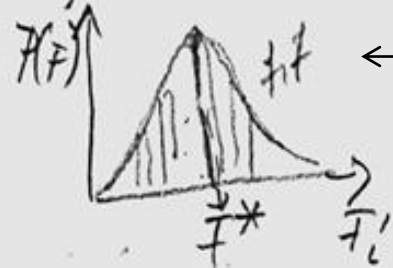
Force is calculated measuring the displacement x_B : $F(x_B) = k x_B$

The load rate $r = v k$ and is the slope of the tangent to the measured displacement of the bead.

3) Take F_R and r .
 → One measurement is not enough (stochastic behavior)
 → $N \gg 50$ measurements necessary → repeat:

$$\Rightarrow \{F_R^i\}_{i=1-N} \sim \Gamma(2, 1)$$

plot the probability distribution $P(F)$ and determine F^*



k_{off} and x as free parameters

$$P(F) = \frac{k_{off}}{\Gamma} \cdot \exp\left\{ \frac{Fx}{kT} + k_{off} \frac{kT}{R} \left(1 - \exp\left(-\frac{Fx}{kT} \right) \right) \right\}$$

The most probable unbinding/dissociation force
 $F^* = \frac{kT}{x} \ln\left(\frac{x}{kT} \frac{\Gamma}{k_{off}} \right) \quad \frac{kT}{x} = 2$

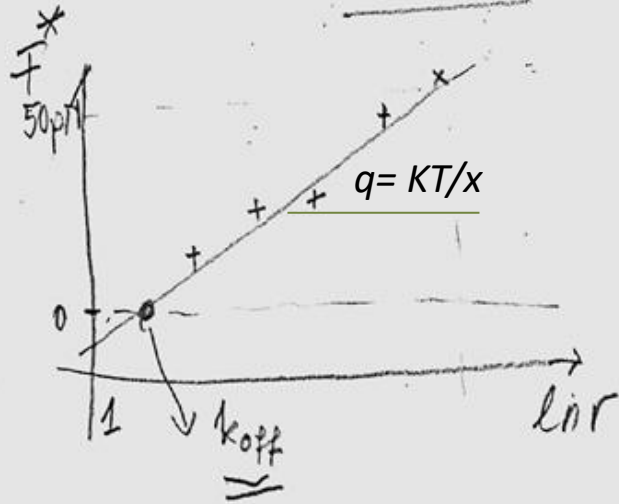
$$k_{off}(F^*) = k_{off} \cdot \exp(F^*x/kT)$$

Having more than one dataset at different load rates r is crucial to extrapolate the value of k_{off} in absence of load $F=0$, (i.e. natural thermal off-rate), that is the most relevant parameter the assay can return.

4)

→ Repeat for different load rates r : r_j $j=1-M$
 $M=5-7$

you get: $(F_j^*, r_j) \rightarrow$ plot $F^* - \ln(r_j)$



$$F^* = q \ln\left(\frac{r}{q \cdot k_{\text{off}}}\right) = q \ln r - q \ln(q \cdot k_{\text{off}})$$

F^* linear with $\ln r$

The slope is $q = KT/x$

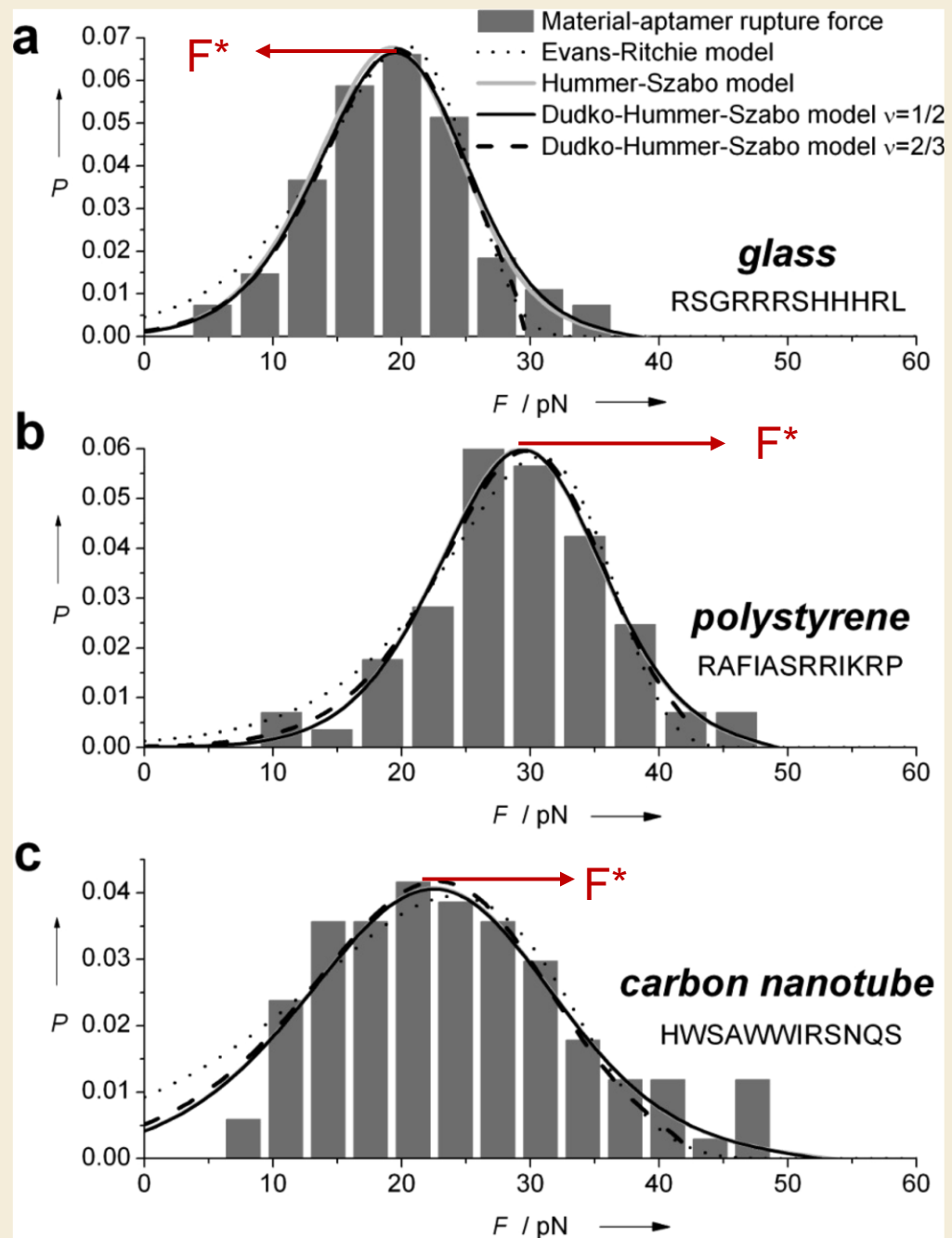
$$F^* = 0 \rightarrow k_{\text{off}} = r_0 / q$$

r_0 – extrapolated load rate r for $F^* = 0$

$$k_{\text{off}}(F^*) = k_{\text{off}} \cdot \exp(F^*x/KT)$$

model prediction

batch	$\Gamma \left[\frac{M}{3} \right]$	$F^* (\text{pM})$	$\left[\frac{\text{nm}}{\text{cal}} \right]$	$k_{\text{off}} (F) \dots^{1/s}$	fit F^* parameters $k_{\text{off}}, x_{\text{cal}}$
1	10	20	0.3	0.13	
⋮	⋮	⋮	⋮	⋮	
j	40	50	0.2	0.21	
⋮	⋮	⋮	⋮	⋮	
M	80	70	0.15	0.34	



Rupture-force probability $P(F)$ distributions
 for peptide aptamer binding to :

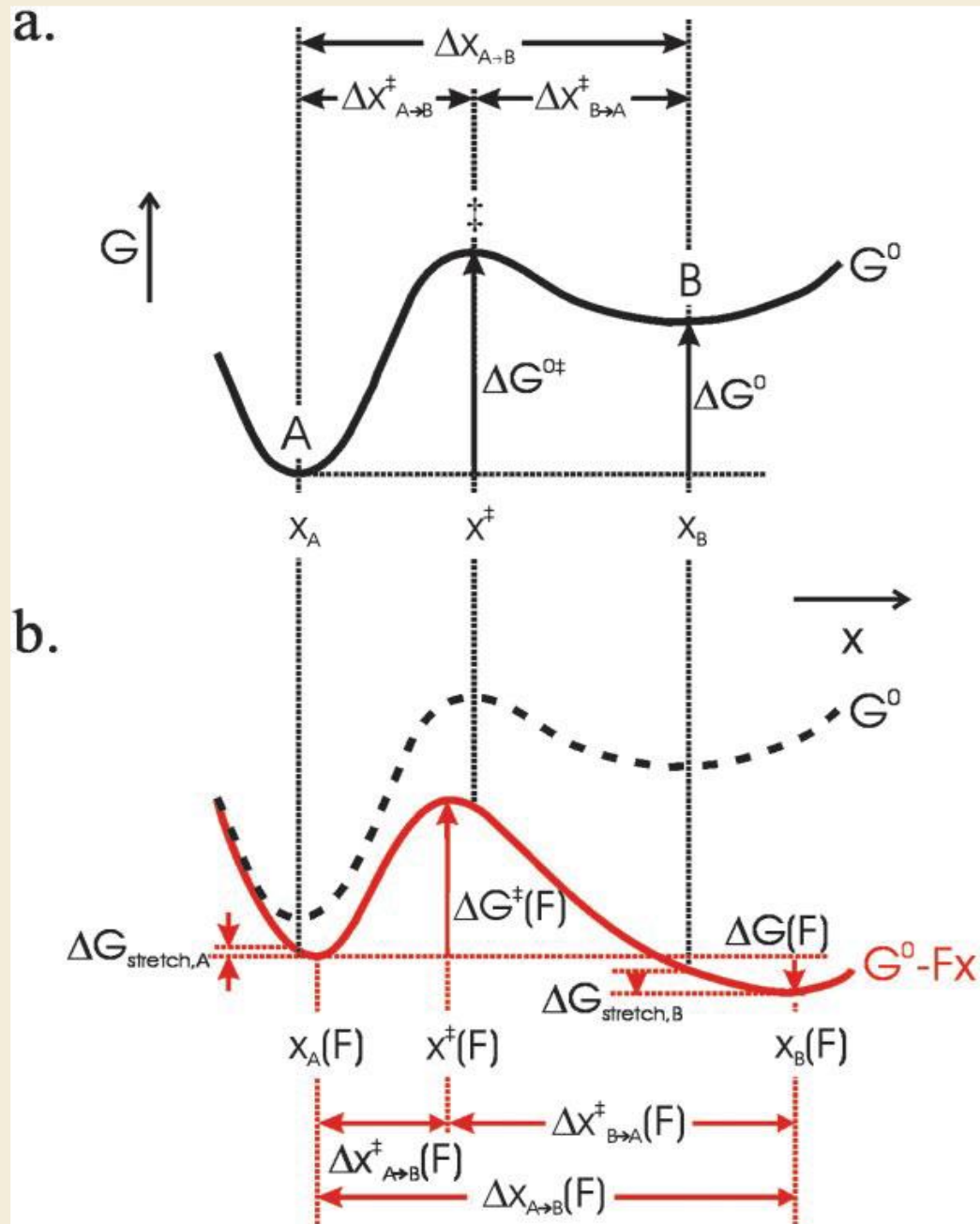
- (a) glass,
 (b) polystyrene, and
 (c) carbon nanotubes

$$\tau_0^a = 1/k_{off}$$

Evans-Ritchie model

interaction	τ_0^a	$x^{\ddagger b}$
glass/aptamer	96.9	0.747
polystyrene/aptamer	109.5	0.652
CNTs/aptamer	20.7	0.404

^a Units are s. ^b Units are nm. ^c ΔG^\ddagger is in $k_B T$ units.



The effect of force on the free energy of a two-state system, where x represents the mechanical reaction coordinate.

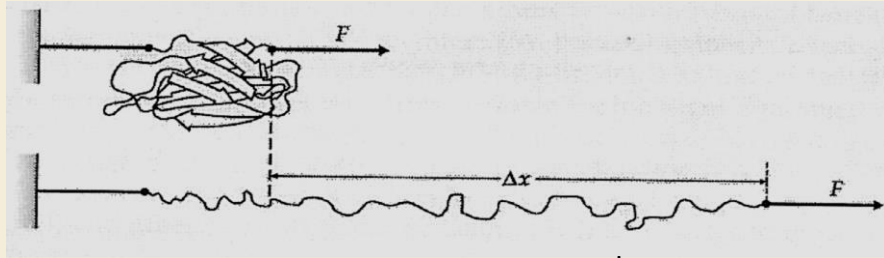
(a) No applied force.

(b) Red curve: positive applied force.

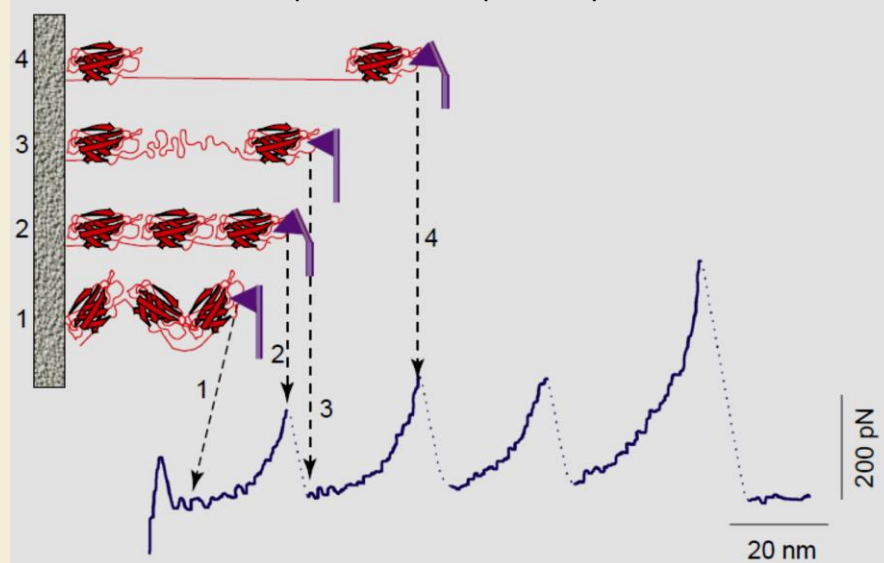
The application of force lowers the energy of both the transition state \ddagger and state B relative to state A, which increases the rate of the forward reaction and the population of state B, respectively.

The positions of the free energy minima (x_A and x_B) and maximum (x_{\ddagger}) shift to longer and shorter x , respectively, with a positive applied force. Their relative shifts in position depend on the local curvature of the free energy surface.

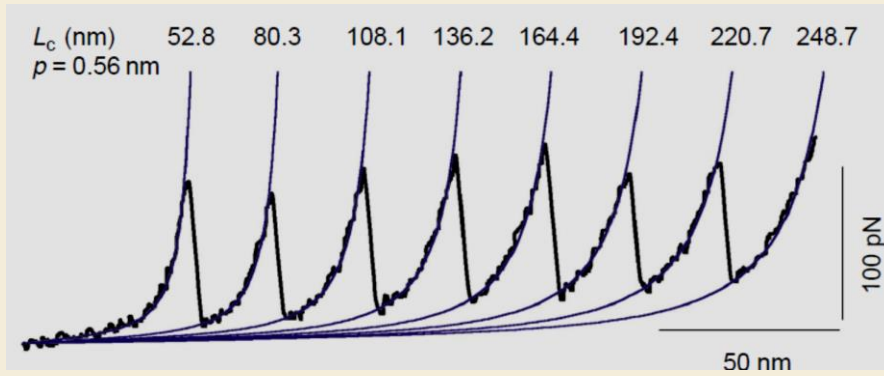
The free energy change of states A and B upon stretching is $\Delta G_{\text{stretch}, A, B}$



Experiment principle



Force-extension relationship for recombinant poly(I27)

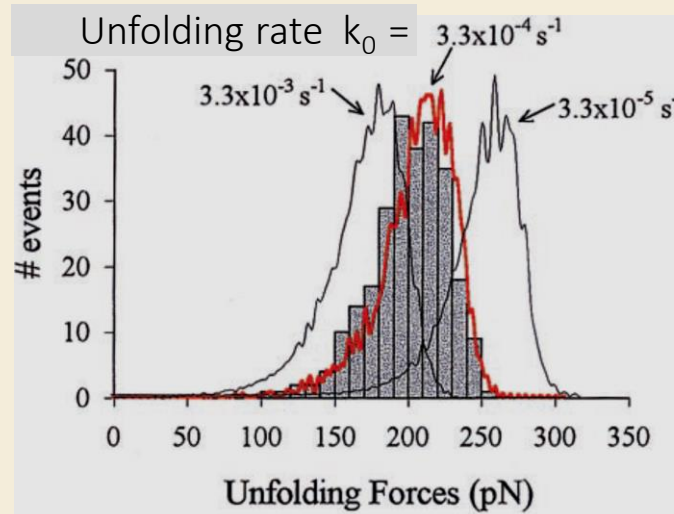


Carrion-Vasquez et al, PNAS 96:3494, 1999

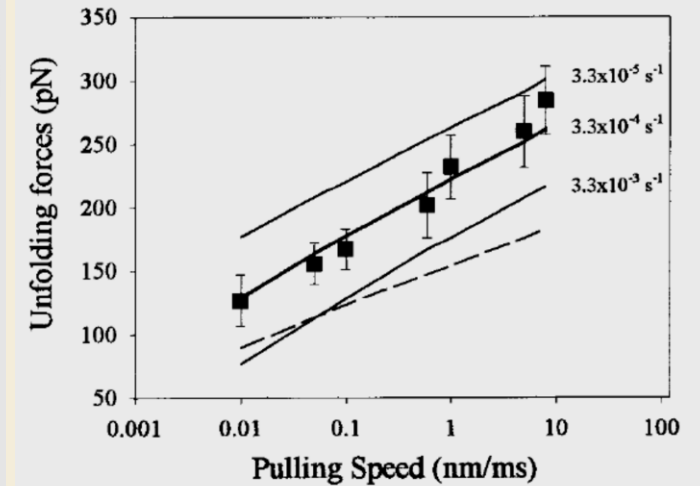
Mechanical and chemical unfolding of a single protein a comparison

AFM was used to reversibly unfold immunoglobulin modules (IG27) domain. And measure the unfolding rate, which was compared with that obtained from chemical unfolding using a denaturant reaction.

Unfolding force frequency histogram



Unfolding force vs pulling speed



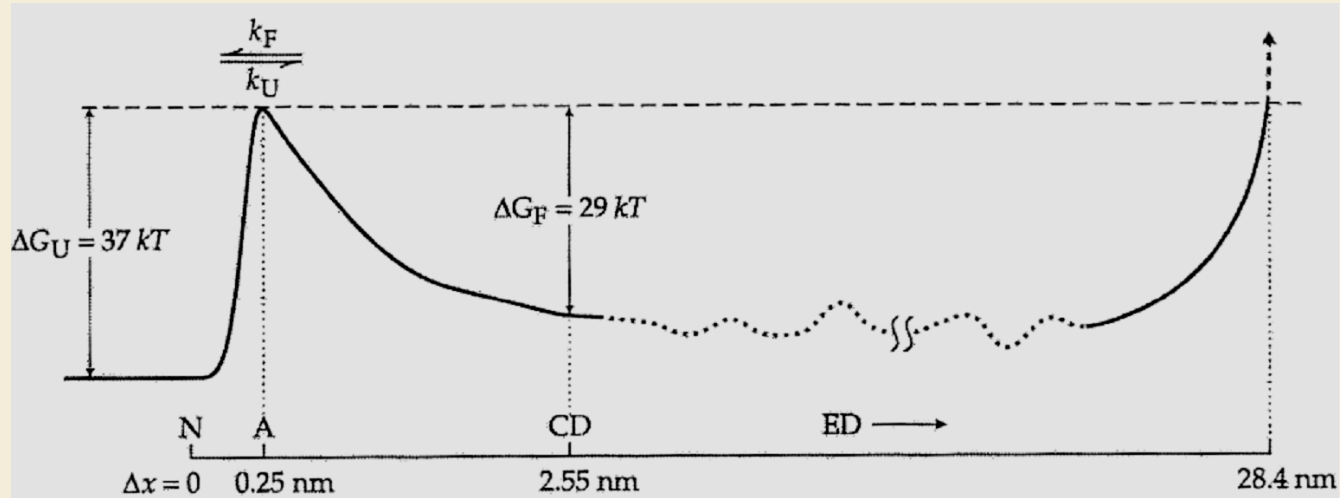
WLC model (Worm Like Chain)

$$F(x) = \frac{kT}{p} \left[\frac{1}{4} \left(1 - \frac{x}{L_c} \right)^2 - \frac{1}{4} + \frac{x}{L_c} \right]$$

unfolding distance $\Delta x = 0.25 \text{ nm}$,
pulling rate $v = 0.6 \text{ nm/ms}$.

Chemical unfolding rate $k_0 = 4.9 \times 10^{-4} \text{ s}^{-1}$

Diagram of the unfolding pathway for an Ig domain as determined by using AFM



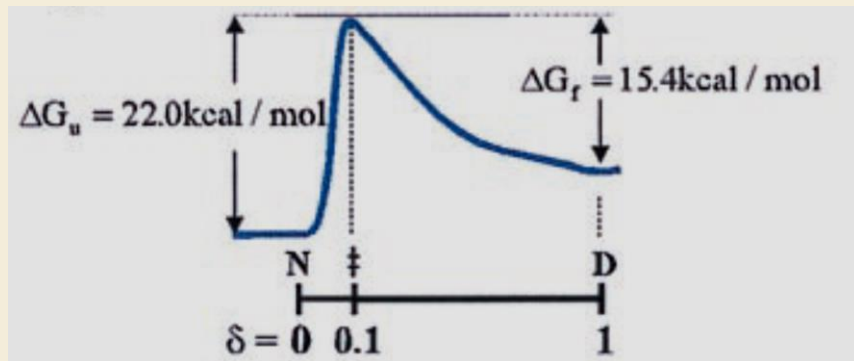
Free energies are calculated from the rates constant using Eyring rate theory.

A small strain of 0.25 nm, about 5% of the length of the folded protein (5.1 nm), is enough to completely destabilize the structure and lead to unfolding.

The folding of the protein requires the formation of a nearly fully folded transition state.

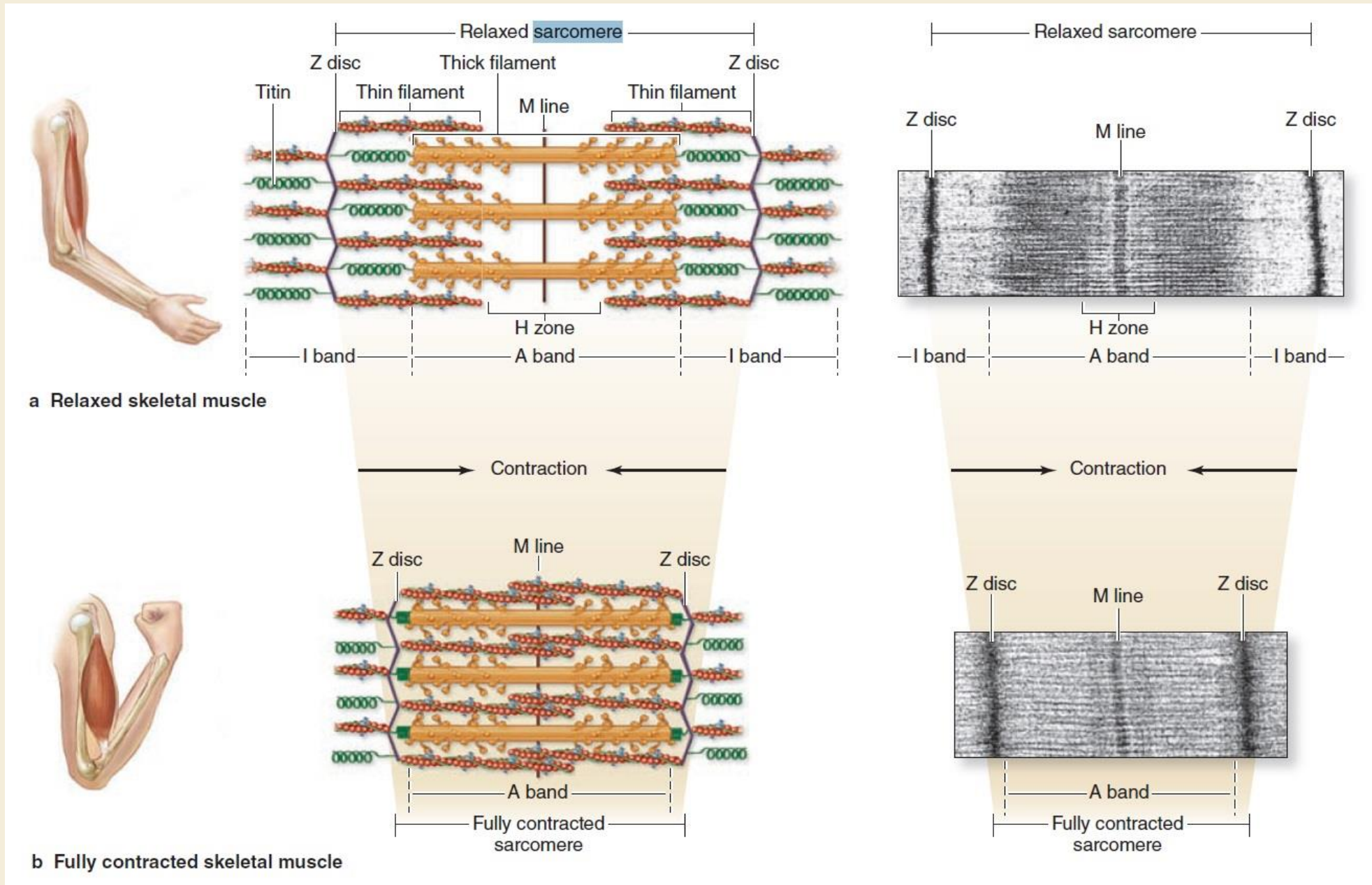
N = native state, A = activated state, CD = compact disordered state, ED = extended state

Diagram of the unfolding pathway for an Ig domain as determined by using chemical denaturants



Carrion-Vasquez et al, PNAS 96:3494, 1999

Mechanical and chemical unfolding of a single protein a comparison



a Relaxed skeletal muscle

b Fully contracted skeletal muscle

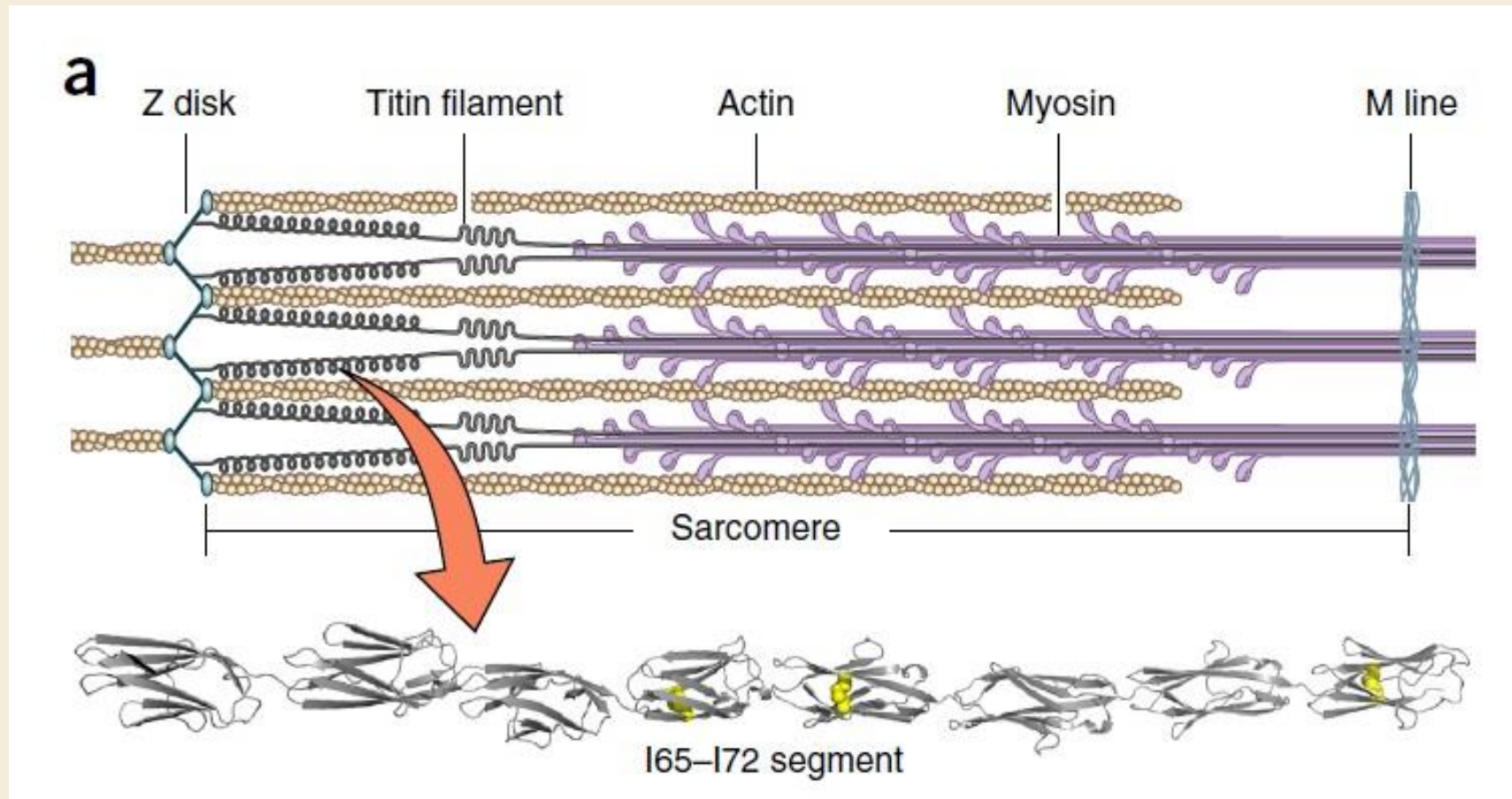
The sarcomere is the smallest functional unit of striated muscle tissue.

Sarcomere shortening during skeletal muscle contraction.

(a) In the relaxed state the sarcomere, I band, and H zone are at their expanded length. The springlike action of titin molecules, which span the I band, helps pull thin and thick filaments past one another in relaxed muscle.

(b) During muscle contraction, the Z discs at the sarcomere boundaries are drawn closer together as they move toward the ends of thick filaments in the A band. Titin molecules are compressed during contraction.

Scheme of one half of the sarcomere from Z disk to M line



The three main sarcomeric proteins: actin, myosin and titin.
Domains I65-I72 from the elastic part of titin is shown.

Why TITIN ?

Titin has nearly 250 Ig immunoglobulin-like and fibronectin domains arranged in a linear fashion, for a total molecular weight of almost 3 MDa. It is big ! (length up to 1 μm).

This morphology greatly facilitates its grabbing and tethering between the surface of two beads in an optical tweezers instrument, or between the tip of an AFM cantilever and a surface, making it possible for the first time to investigate the response of a protein to mechanical denaturation.



Rief M, Gautel M, Oesterhelt F, Fernandez JM, Gaub HE. 1997.
Reversible unfolding of individual titin immunoglobulin domains by **AFM**.
Science 276:1109–12

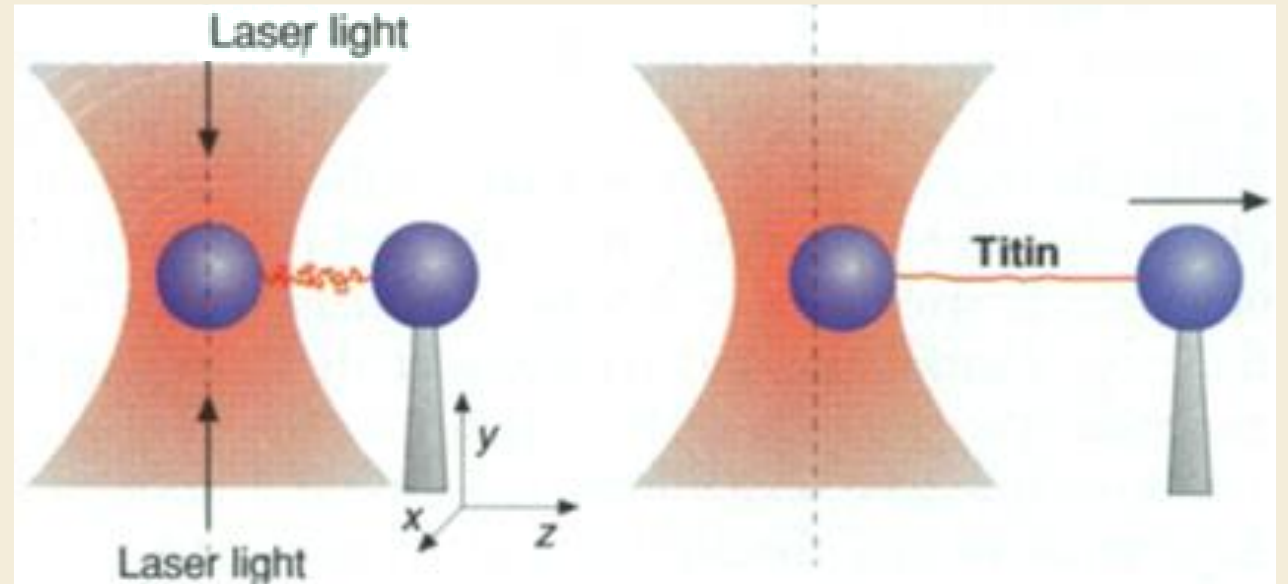
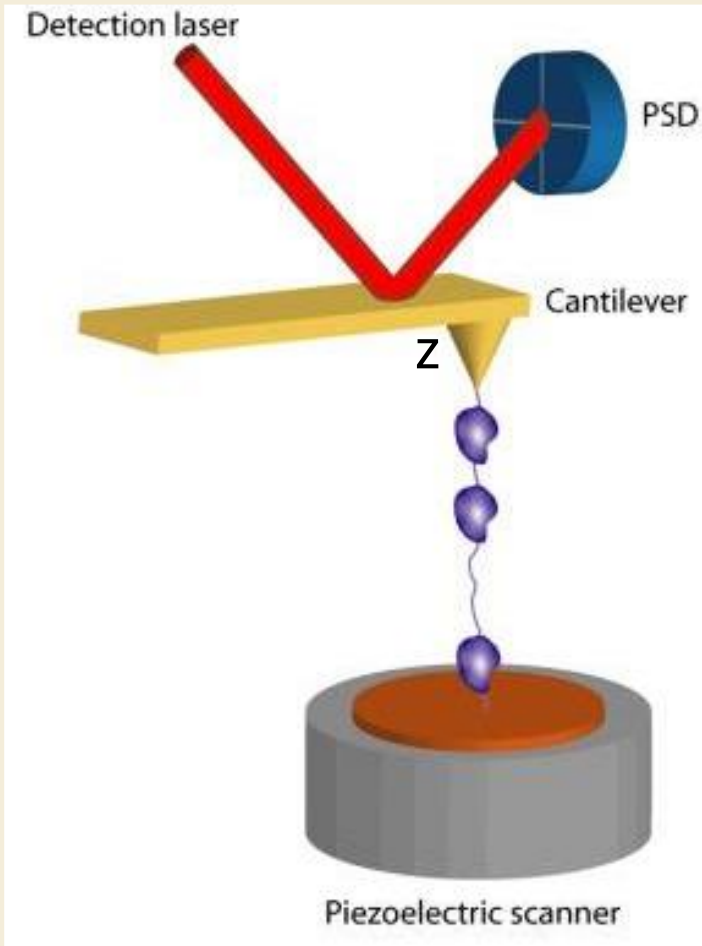
!! Published in the same issue of Science

M. S. Z. Kellermayer, S. B. Smith, H. L. Granzier, C. Bustamante,
Folding-Unfolding Transitions in Single Titin Molecules Characterized with **Laser Tweezers**, Science 276, 1112–1116 (1997).

Tskhovrebova L, Trinick J, Sleep JA, Simmons RM. 1997.
Elasticity and unfolding of single molecules of the giant muscle protein titin. **AFM**
Nature 387:308–12

AFM

Laser /Optical Tweezers



$$F = k z$$

Spatial resolution /
displacement detection

$\delta z \sim 1 \text{ nm}$ for both

Stiffness or spring constant

$$k_{\text{AFM}} \gg k_{\text{OT}}$$

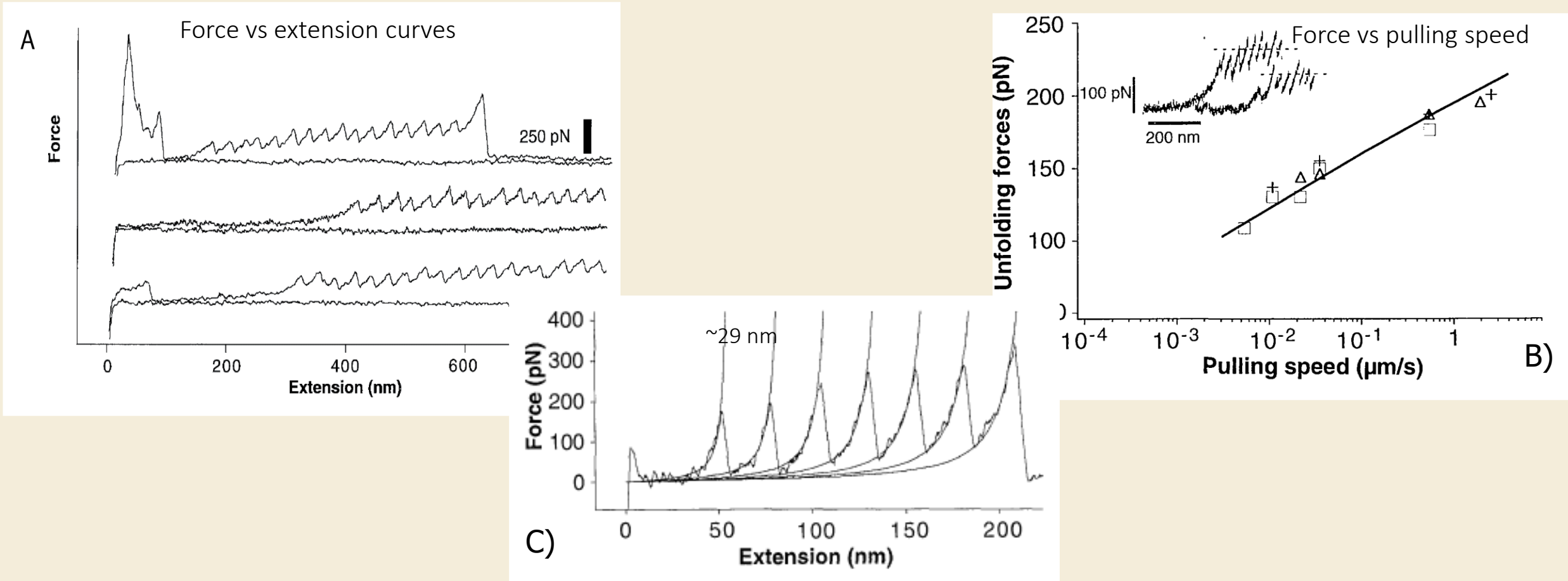


Force resolution: AFM < OT

$$\delta F_{\text{AFM}} \gg \delta F_{\text{OT}}$$

Load rate = probe stiffness x pulling speed

Load rate AFM > Load rate OT for the same pulling speed

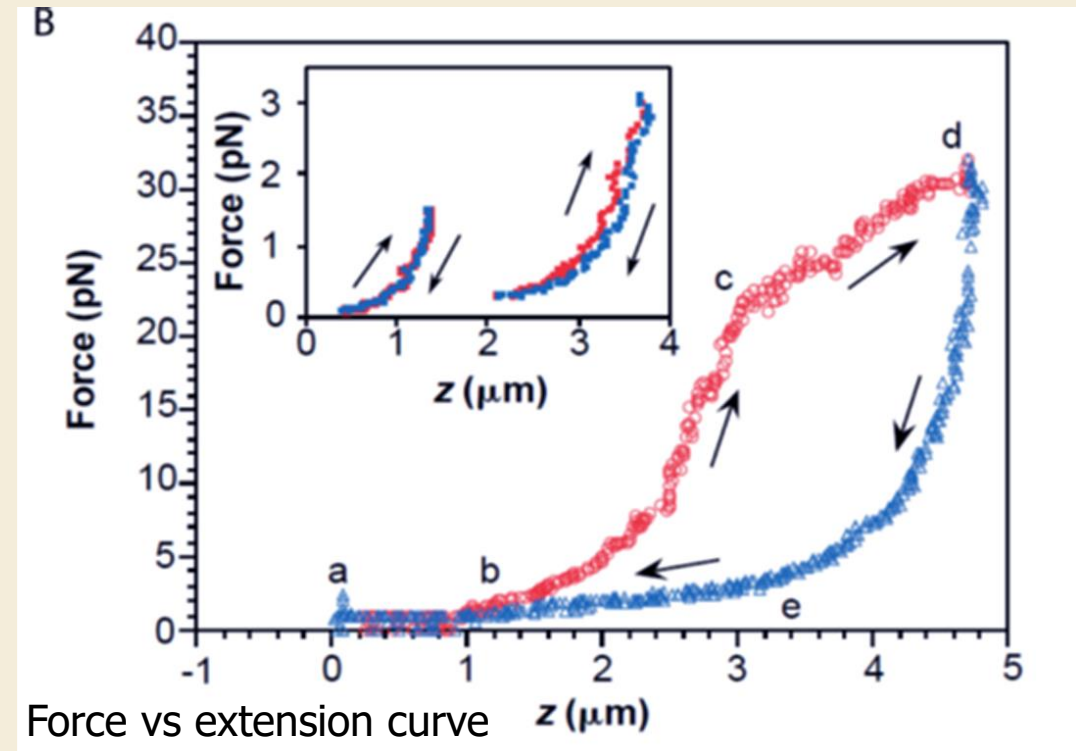
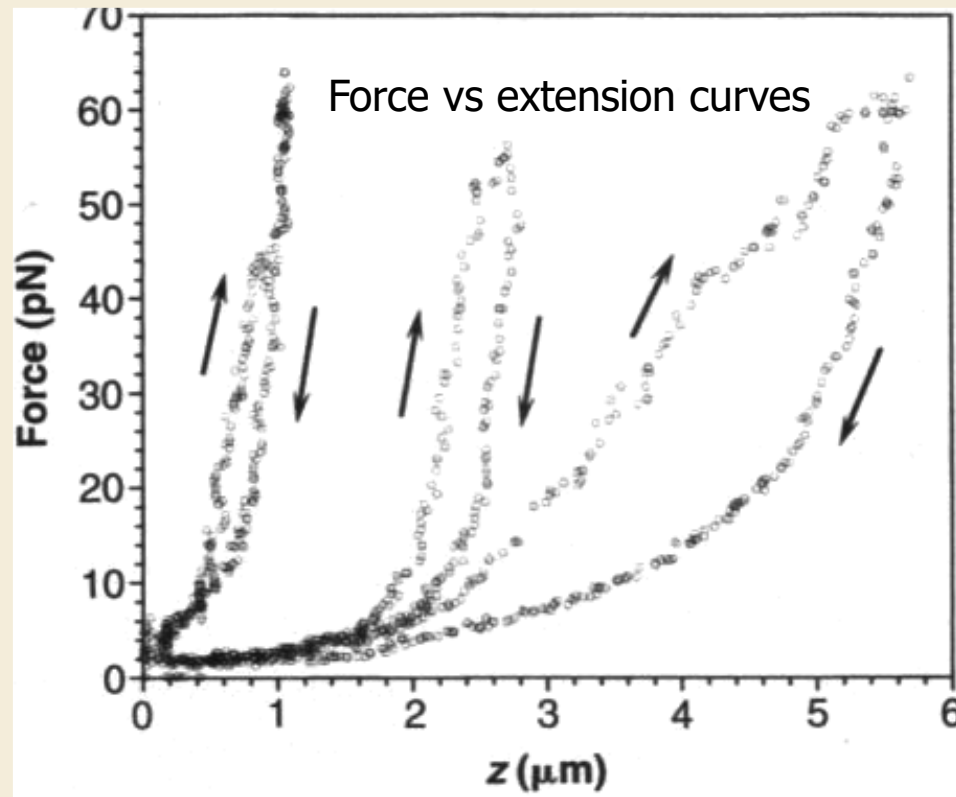


A) Individual titin molecules were repeatedly stretched, and the applied force was recorded vs elongation.

Unfolding of individual Ig domains was observed (shark tooth pattern). AFM tip attachment to the protein is at random points (the observation starts at different extensions in the three curves shown).

B) Unfolding forces vs pulling speed . If we consider a tip stiffness $k = 10 \text{ pN/nm}$, the range of the corresponding load rate is: $0.1 - 10 \text{ nN/s}$.

C) Fitting with the WLC (Worm Like Chain) model for each Ig domain unfolding



A) Stretch and release of single titin molecules at a load rate of max 0.01 nN/s. Hysteresis is observed (the rate of stretch is different from the rate of release). The differences between the three curves are assigned to the different contact point on the titin.

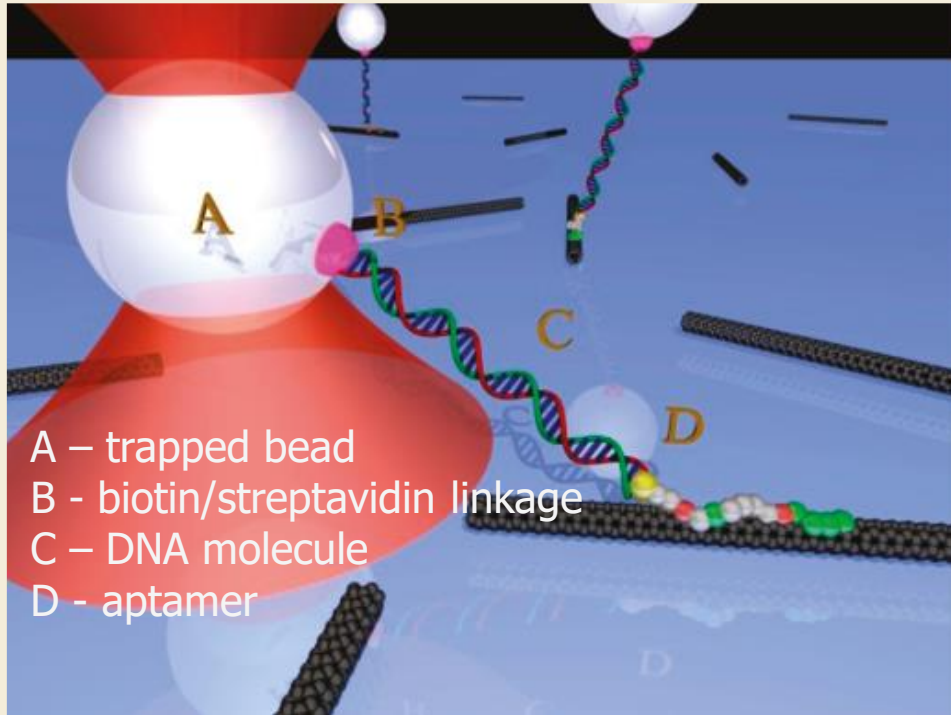
B) The points at the beginning and at the end of the transitions are highlighted. At the beginning of the stretch 5-4% of the molecule is already unfolded. a-c : WLC model ; at higher force (after c) transition fold – unfold ; d-e WLC model, no refolding; e-b refolding;

Force denaturant vs chemical or temperature denaturant

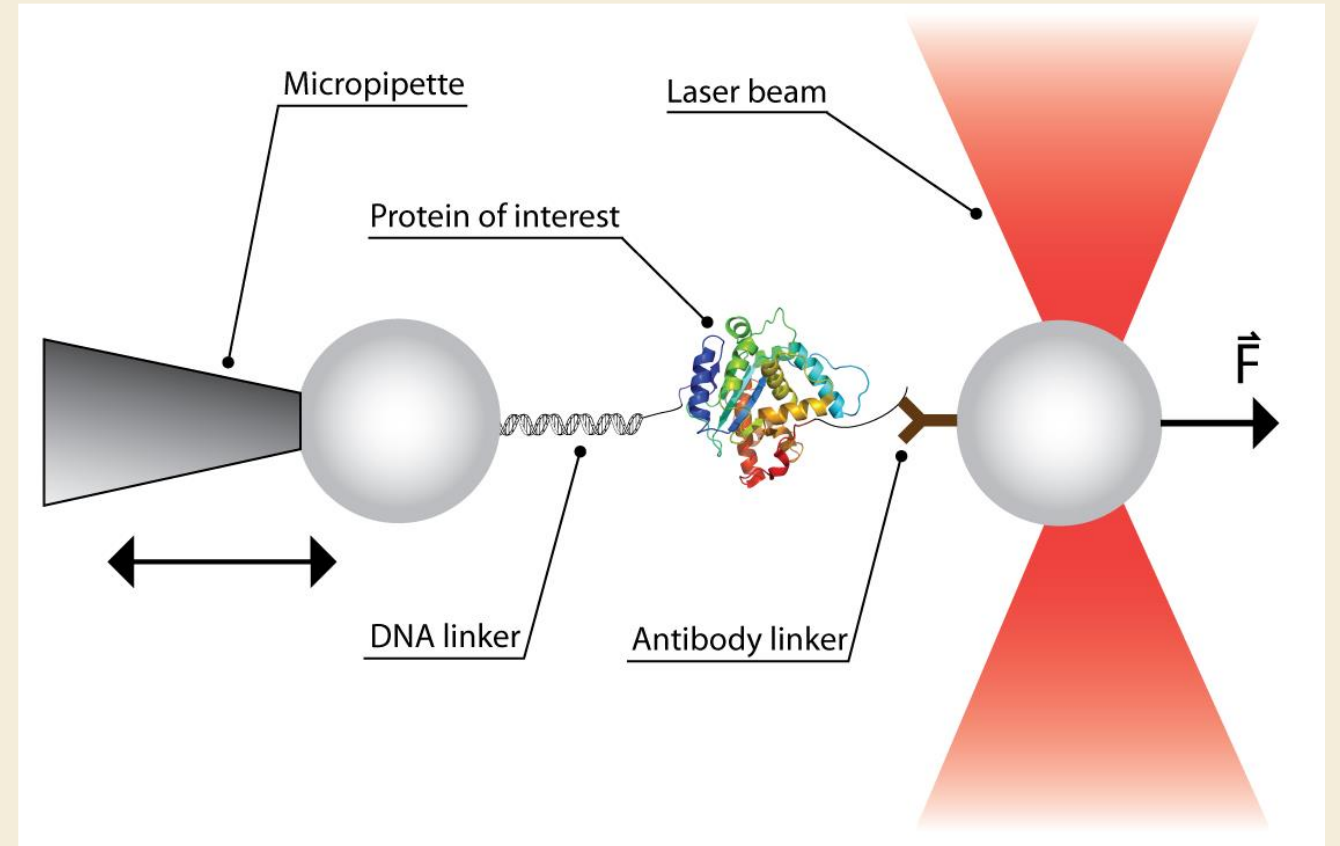
Advantages of studying protein folding by using force, one molecule at a time:

1. the direction along which the force is applied in a mechanical unfolding experiment establishes a well-defined reaction coordinate. Along this privileged spatial direction, parameters of the reaction such as the distance to the transition state, the height of the energy barrier, and the energy difference between the initial (folded) and the final (unfolded) states can be determined.
2. unlike its bulk counterparts (urea, temperature), force is a selective denaturant capable of acting on one part of the molecule without directly affecting another. This locality has made it possible to investigate, for example, the energetic coupling during the folding or unfolding of different regions of a protein.
3. the use of force as a denaturant, which typically requires studying the folding process at the single-molecule level, makes it possible to avoid complications such as aggregation that often plague folding studies in bulk.
4. Studies in bulk – average the parameters.

Examples of OT configuration for force spectroscopy



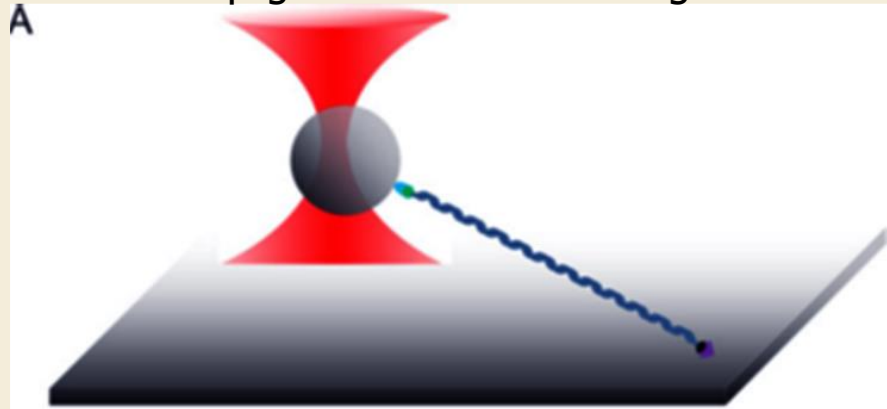
Substrate



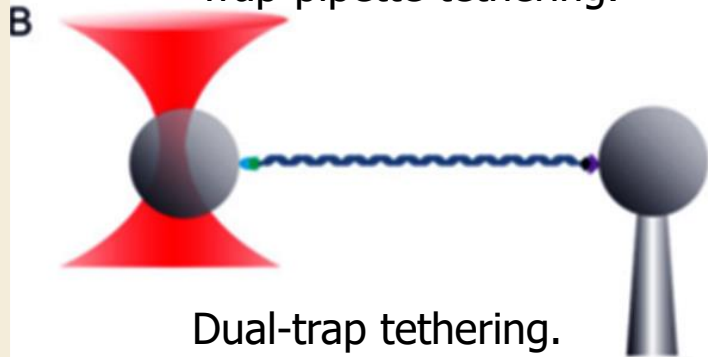
Bead- micropipette.

$\mathbf{F} = \mathbf{k} \mathbf{x}$ – for the moment we consider OT as a Hook spring with elastic constant $k = 0.001 - 0.5 \text{ pN/nm}$ (1-3 order of magnitudes lower than thar of AFM cantilever)

Trap-glass surface tethering



Trap-pipette tethering.



Dual-trap tethering.



Weak trap

Stiff trap

Most frequent: Single or two beads.

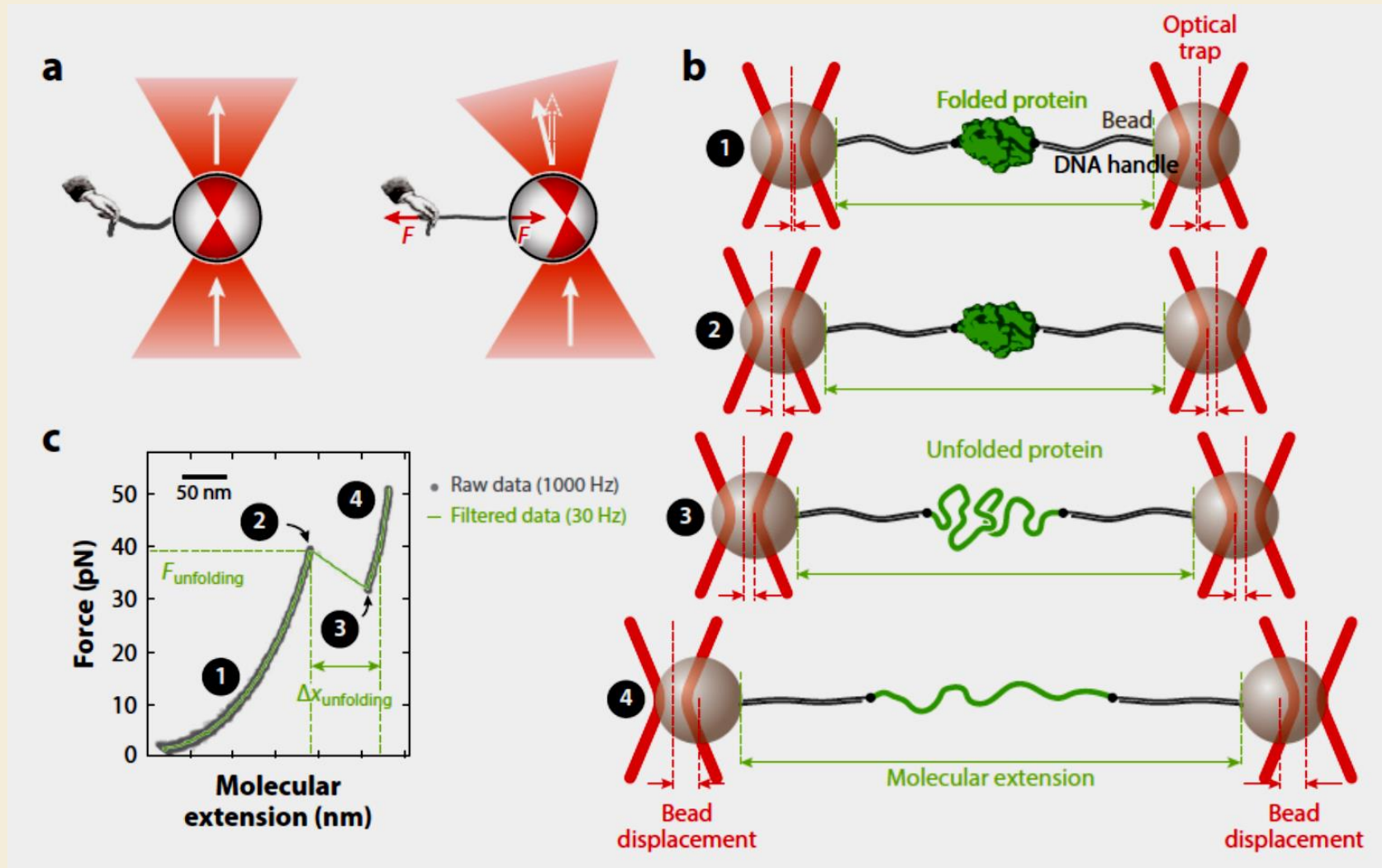
Three beads is also proposed.

Each of these geometries can be implemented as:

- Force ramp $F(t) = r \cdot t$
- Force clamp $F(t) = ct$
- Position clamp $x(t) = ct$

Using feedback system to adapt the position of the bead / trap

Manipulation of single molecules using dual OT.

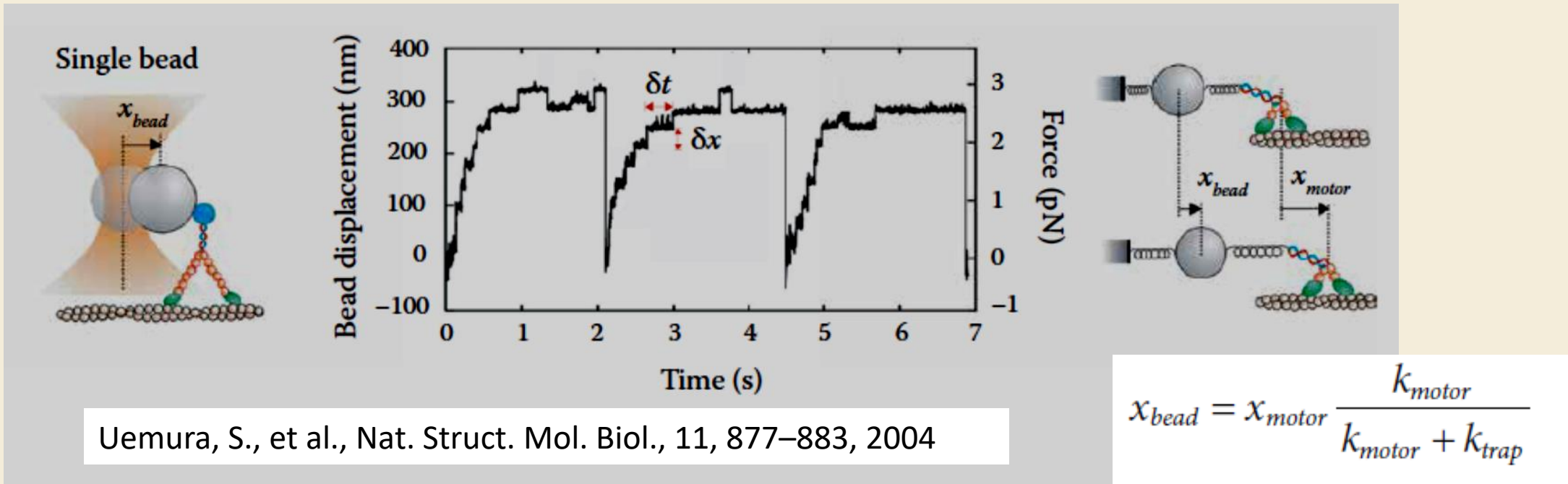


Manipulation of single molecules using optical tweezers.

- a) Light from a tightly focused laser beam (*red*) passes through a bead. When the center of the bead is aligned with the laser focus (*left*), all rays hit the bead surface at a right angle, and the light does not change direction. When the center of the bead is not aligned with the laser focus (*right*), the change in momentum of the light elicits an equal and opposite change in momentum of the bead, resulting in a force F that attracts the bead to the laser focus.

- a) Schematic of a protein tethered between two trapped beads via DNA handles. At low forces, the protein remains in the folded state. However, an increase in force results in stretching of the DNA handles, increasing the molecular extension of the assembly between the beads. Unfolding of the protein results in a further increase in extension. Further increasing the force results in stretching of the DNA and the unfolded protein. Note that the bead displacement is proportional to the applied force, because the traps behave as harmonic springs.

- b) Example of a typical force–extension curve, generated by applying a continuously increasing force to a tethered protein; gray dots represent data at 1,000 Hz, and the green curve represents data filtered to 30 Hz. The numbers are as in panel *b*. The curvature is due to the entropic elasticity of the DNA (region 1) and DNA plus unfolded protein. Unfolding of the tethered protein is apparent as a discontinuity in the curve (rip; from point 2 to point 3).



A myosin V molecule is attached to a trapped bead which proceeds along an actin filament stuck on the coverslip surface.

The optical trap position is fixed and the bead displacement x_{bead} measures protein displacement.

Example showing stepwise movement of a single myosin V motor in a single-bead assay. Consecutive 36 nm stepwise movements (δx) and dwell times between steps (δt) are clearly visible.

Backward steps are also visible at high force.

The force was calculated as: $F = k x_{bead}$

Trap stiffness $k = 0.009$ pN/nm

Stall force is about 2.5 pN.

Measured bead displacements (x_{bead}) do not correspond to motor displacements (x_{motor}) but depend on the motor protein displacement and on the values of the trap and protein stiffness.

More flexible and precise

Double-trap assay.

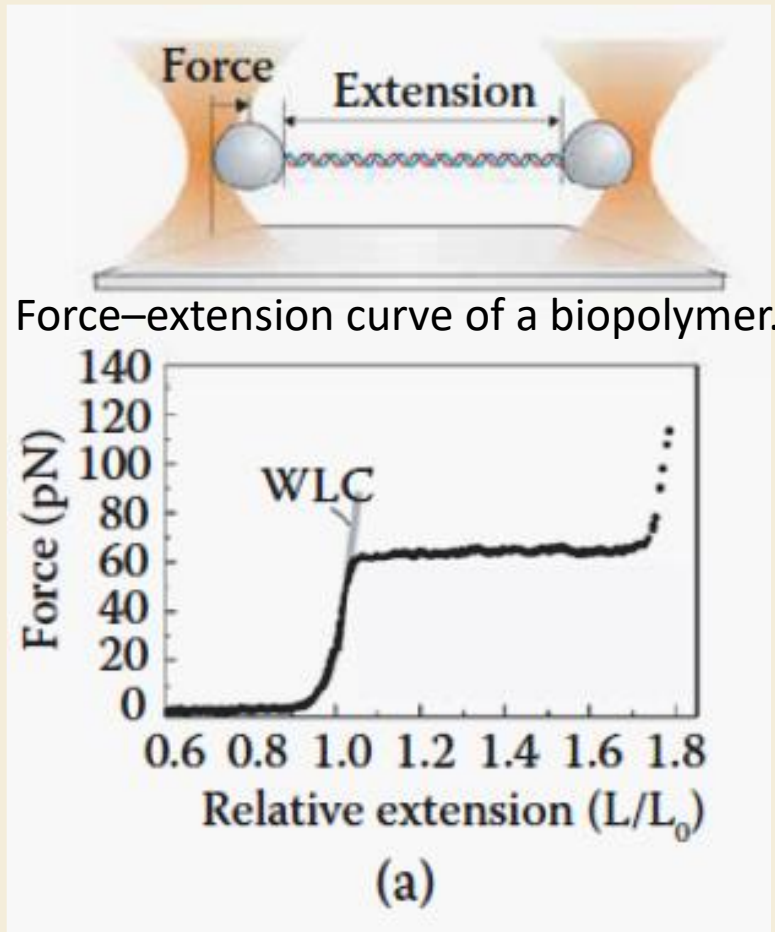
Example

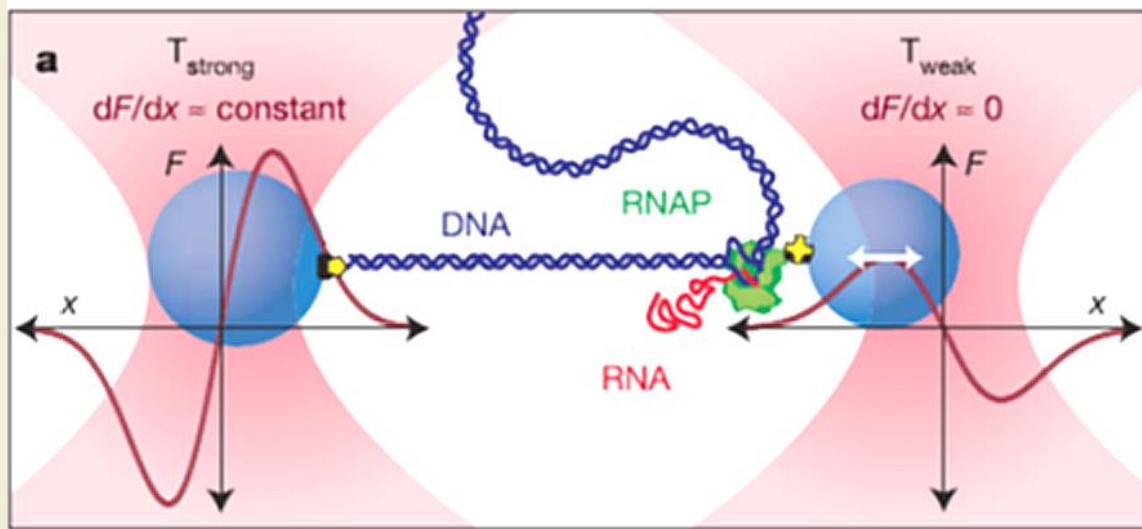
Left trap is stationary and measures the force applied to the polymer.

The right bead (OT) moves in steps or ramps and, for each displacement, the force applied to the polymer and its extension are measured.

The elastic properties of DNA below the overstretching force of 65 pN are well described by the extensible worm-like chain (WLC) model (gray line).

At 65 pN, the DNA molecule undergoes the overstretching transition, during which the intrinsic contour length of the DNA increases from 100% to about 170%.



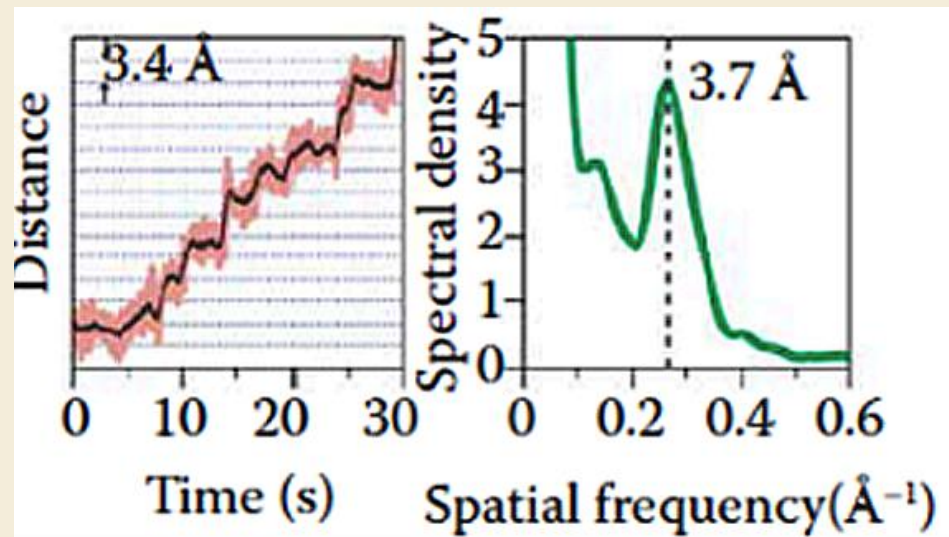


Stiff trap

Weak trap

Example: Dynamics of DNA-processing enzymes.

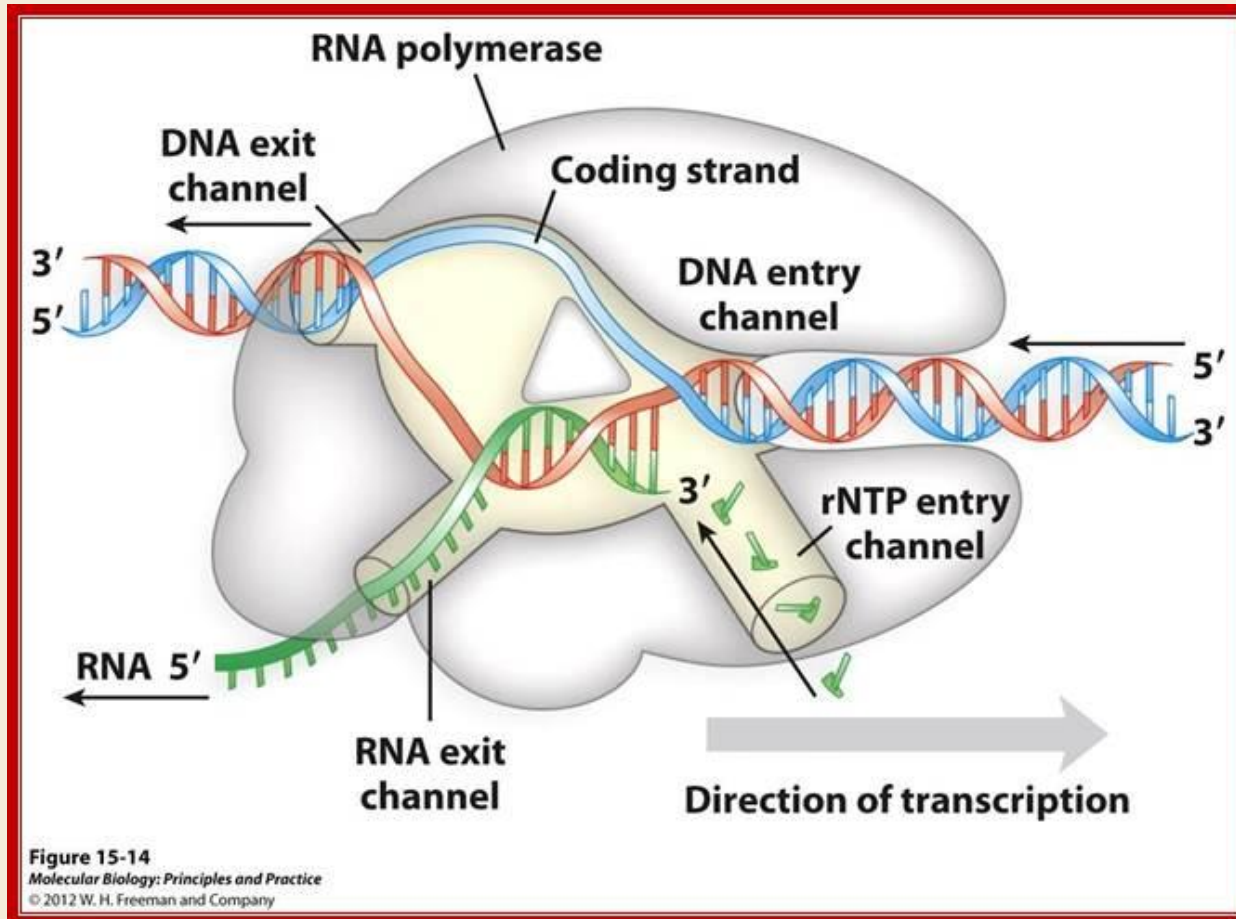
Single, transcriptionally active molecule of RNA polymerase (RNAP, green) attached to a bead held in a trap and tethered via the upstream DNA to another trapped bead. During elongation, the DNA tether lengthens and the beads move apart.



A representative record for a single RNAP molecule transcribing under 18 pN of assisting load. Horizontal lines (dotted) are spaced at 3.4 Å intervals (distance between base sets)

The power spectrum of the average autocorrelation function derived from position histograms shows a peak at the spatial frequency corresponding to the inverse of the fundamental step size, $3.7 \pm 0.6 \text{ \AA}$.

RNA polymerases and DNA transcription



RNA polymerase is the main transcription enzyme.

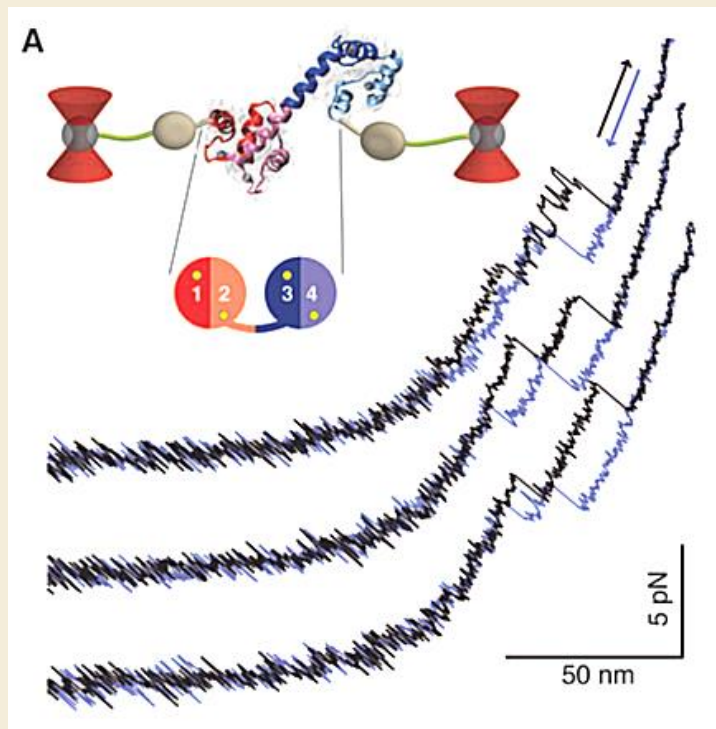
It transcribes DNA into RNA

Transcription begins when RNAP binds to a promoter sequence near the beginning of a gene .

RNAP uses one of the DNA strands as a template to make a new, complementary RNA molecule.

RNAP needs to have the substrate nucleoside triphosphate (NTP) diffuse into its deeply buried active site.

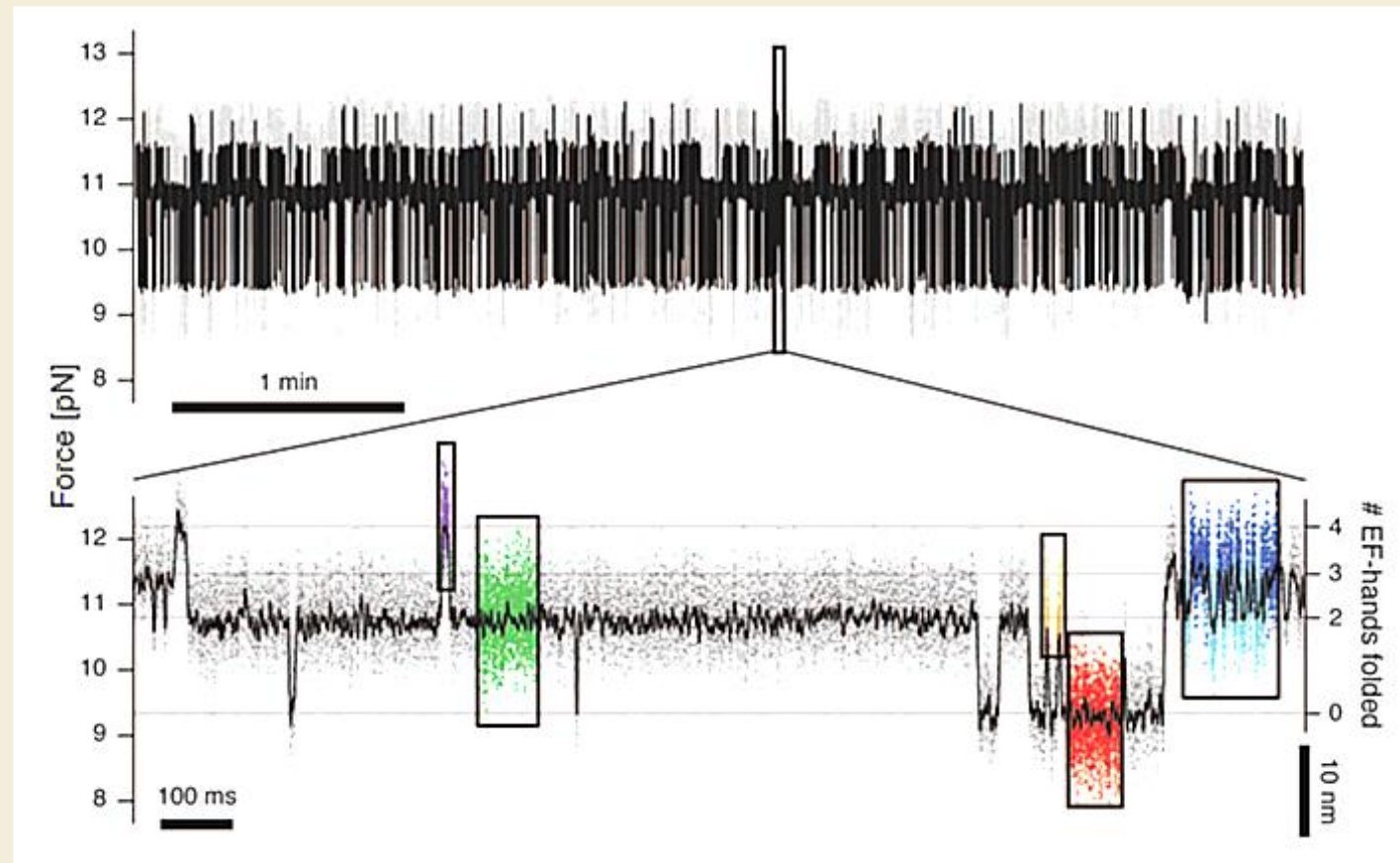
Force ramp



Folding and unfolding of single CaM molecules.

Representative stretch-and-relax cycles for WT-CaM at velocity $v = 500 \text{ nm/s}$.

(Inset) Sketch of the experimental setup with the protein linked with ubiquitin-DNA handles to functionalized silica beads. (Expanded section) Cartoon representation of CaM with the EF hands numbered.

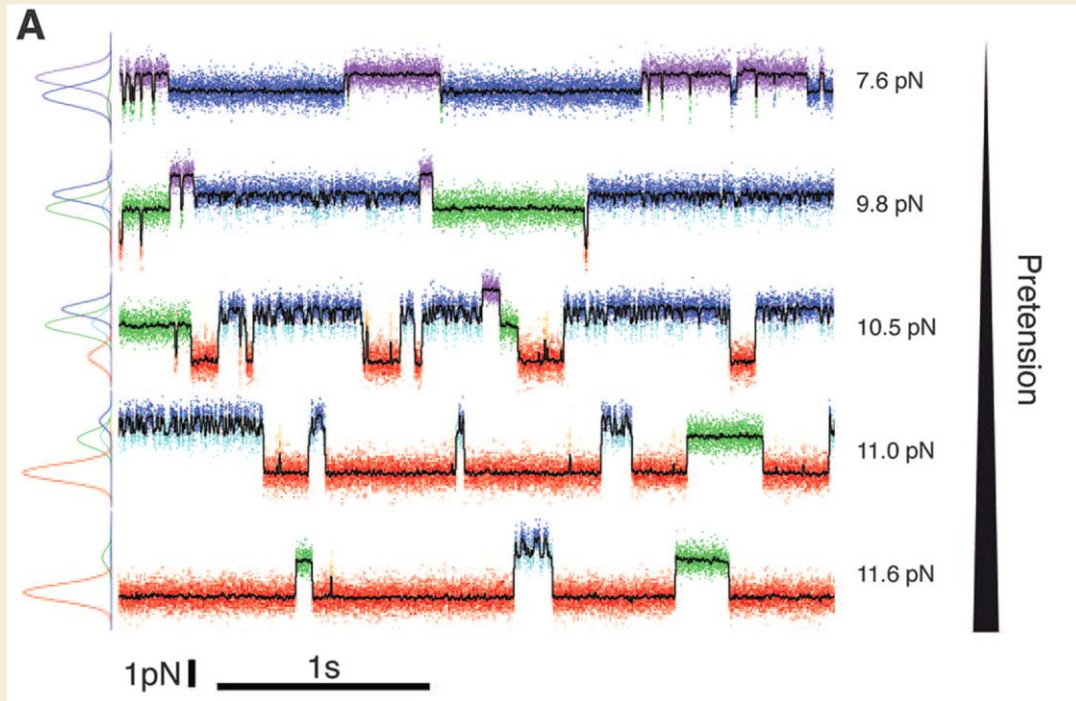


Sample trace during 5min of the fluctuations of a single WT-CaM molecule at a constant trap separation.

The vertical scale denotes the force acting on the molecule as measured by the deflection of the beads from the trap center.

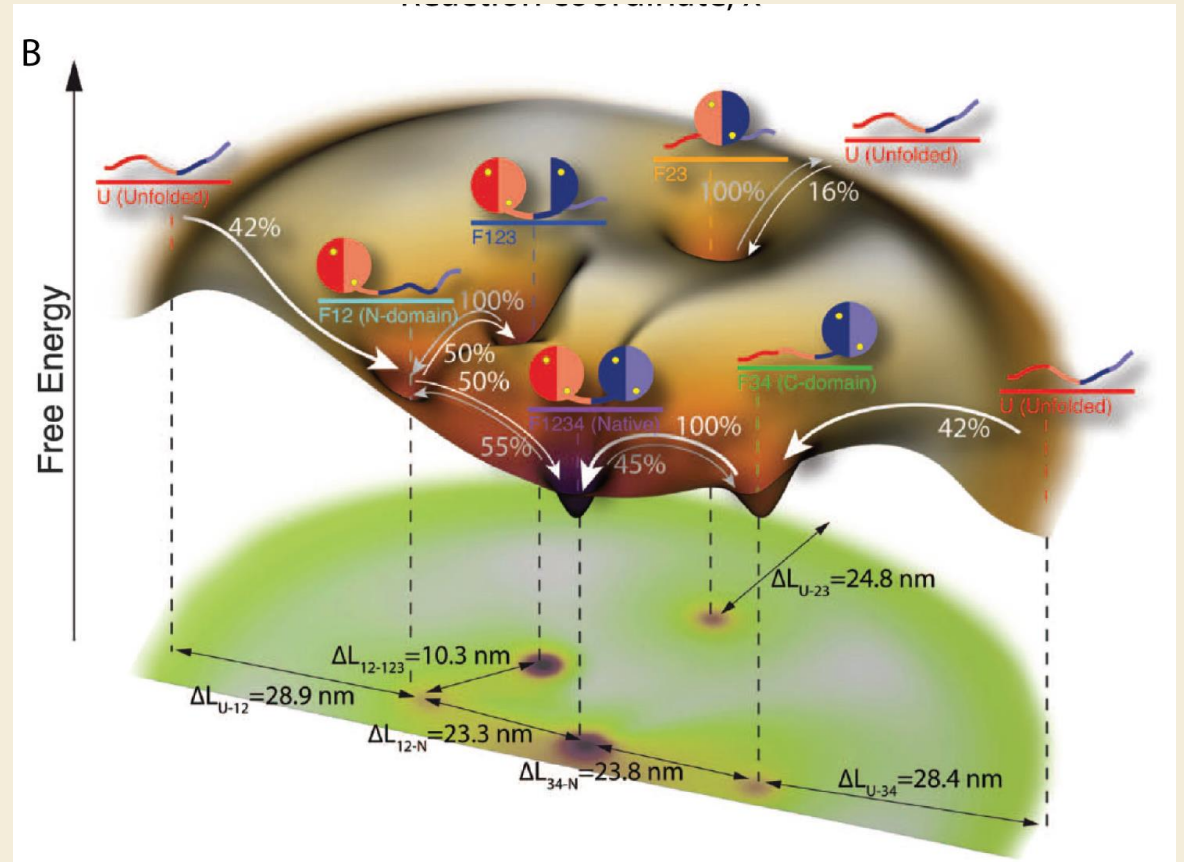
Expanded section: Six different states (see colored regions) can be identified using hidden Markov modeling.

Force clamp



Traces of WT-CaM at different pretensions (Left) Gaussian fits to histograms of each respective state.

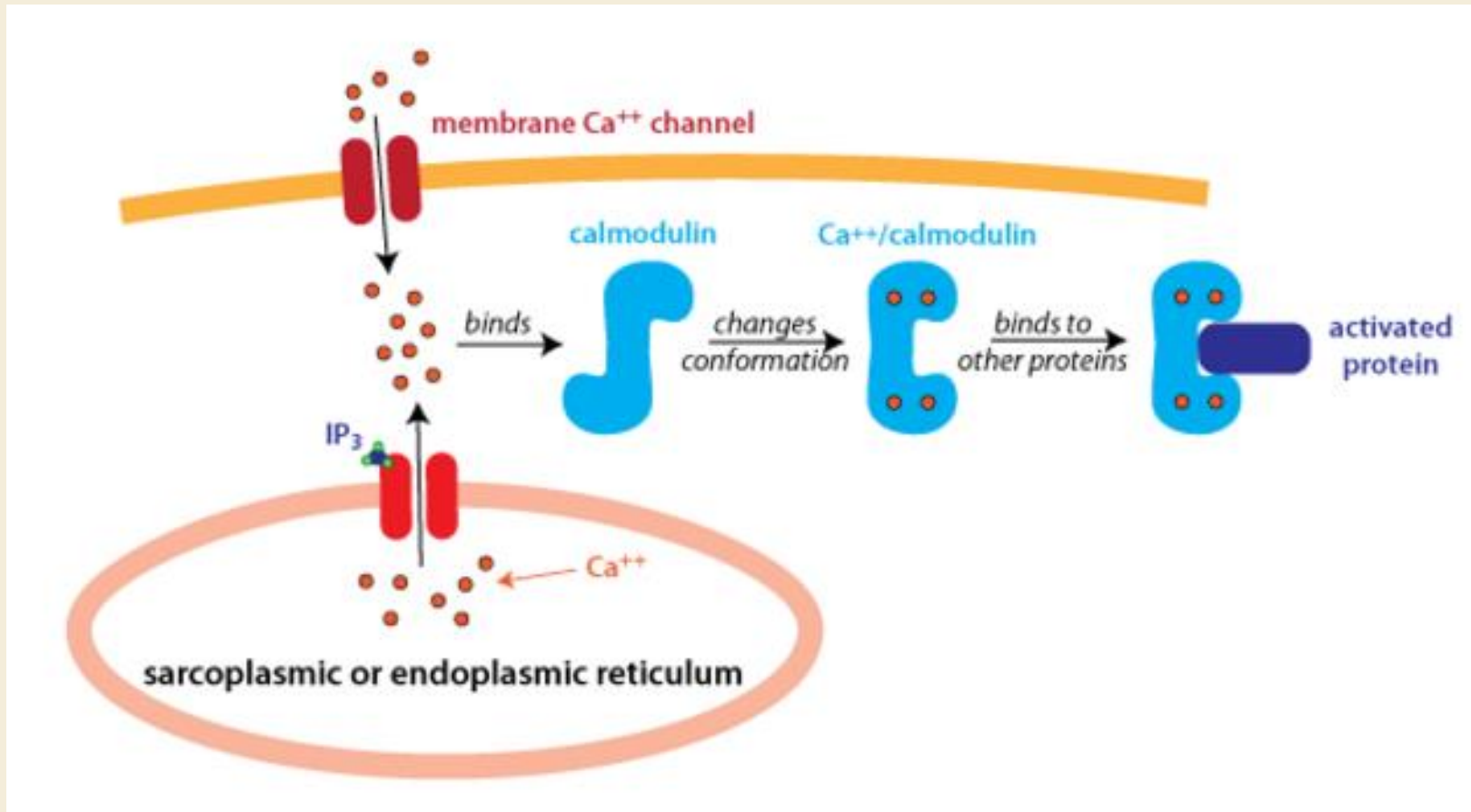
Calmodulin folding-unfolding energy landscape
Stigler, J., et al., Science, 334, 512–516, 2011



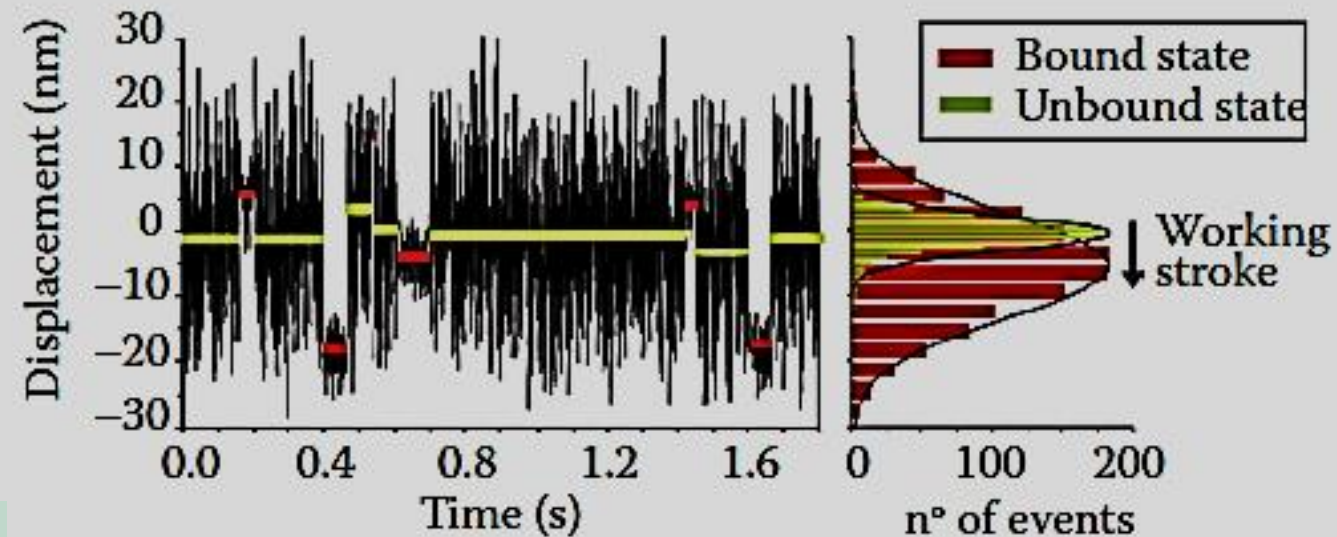
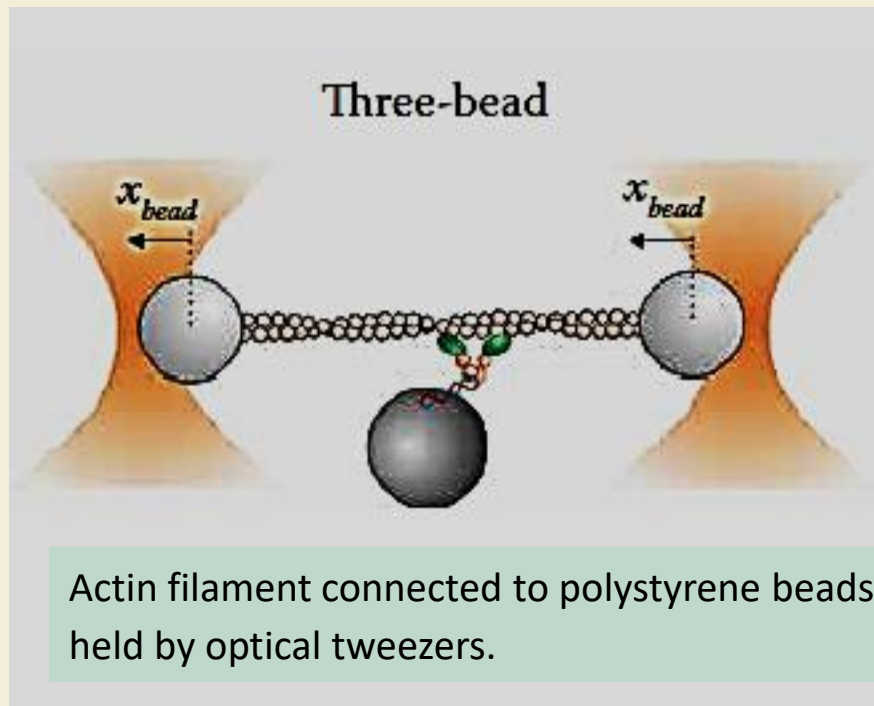
Full kinetic network of WT-CaM folding and unfolding at zero load. Arrows show all observed transitions. The percentage values provided for each transition give the fraction of transitions along the respective pathways out of each state. Distances in the lower part are differences in contour length.

Calmodulin Pathway - briefly

Calmodulin binds to 4 Calcium Ions and Undergoes Conformational Changes



Three-bead assay. Example: working stroke of myosin



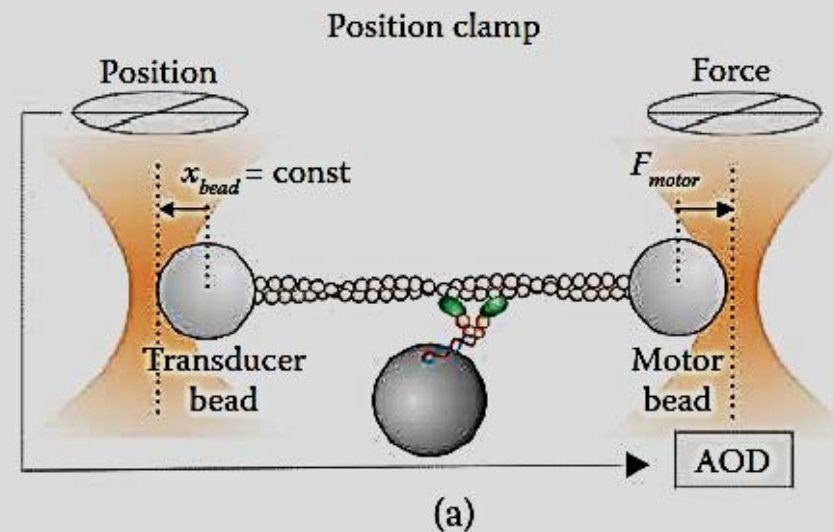
Position recording while myosin is interacting with actin.

A single myosin molecule is attached onto a third bead stuck to the coverslip surface. Movements of the actin filament produced by the attached myosin are measured through bead displacements (x_{bead})

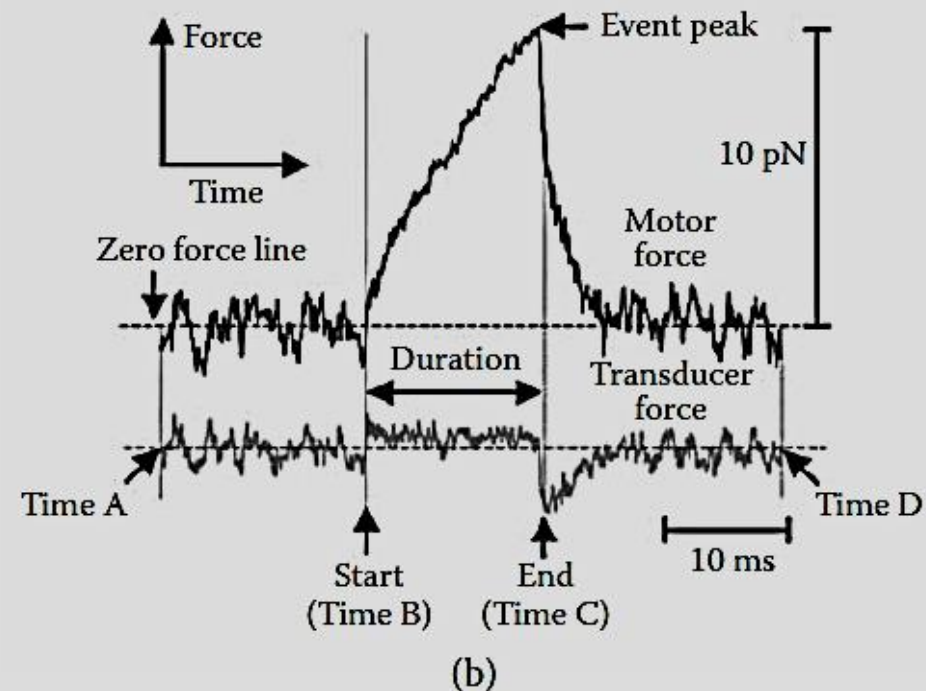
Position recording while myosin is interacting with actin. Red and green lines indicate the average position of bound and unbound events, respectively. Distributions of the average position of bound and unbound events of a 100 s position recording containing several hundreds of interactions. The working stroke is obtained from the displacement between the centers of the two distributions

Position (or isometric) clamp. Example

(a) The left bead detects movements of the dumbbell (x_{bead}), whereas the right bead moves using an AOD (Acoustic Optical Deflector) to oppose the detected movements. The right bead measures the force applied by the motor protein (F_{motor}).



(b) A single acto-myosin interaction in the position clamp. After the force peak the force rapidly declines. The time between the start (Time B) and the point at which the rate of force declines is fastest (Time C) is defined as the duration of an episode.



Takagi, Y., et al., Biophys. J., 90, 1295–1307, 2006.

OT for single molecule spectroscopy; spatial and temporal resolution.

- measure conformational changes and displacements produced by single biological molecules.

Such movements range from several nanometers (molecular motors) down to one base pair (0.35 nm, for DNA and RNA processing enzymes) -- > **high spatial resolution** detection based on Interferometry (see next section).

Actually, position detectors do not set a limit on spatial and temporal resolution of OT.

Thermal noise sets instead fundamental limits on displacement and force measurements with single molecules.

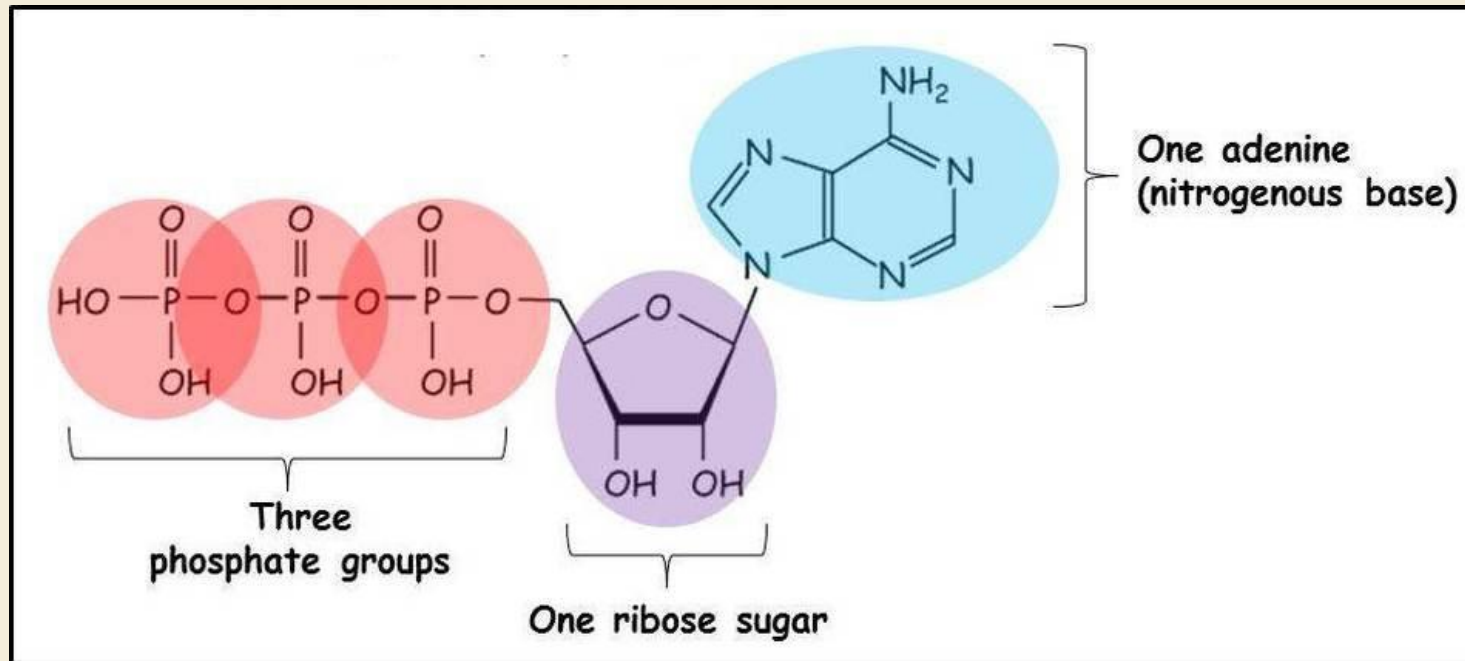
Temporal resolution limit due to relaxation time for bead position.

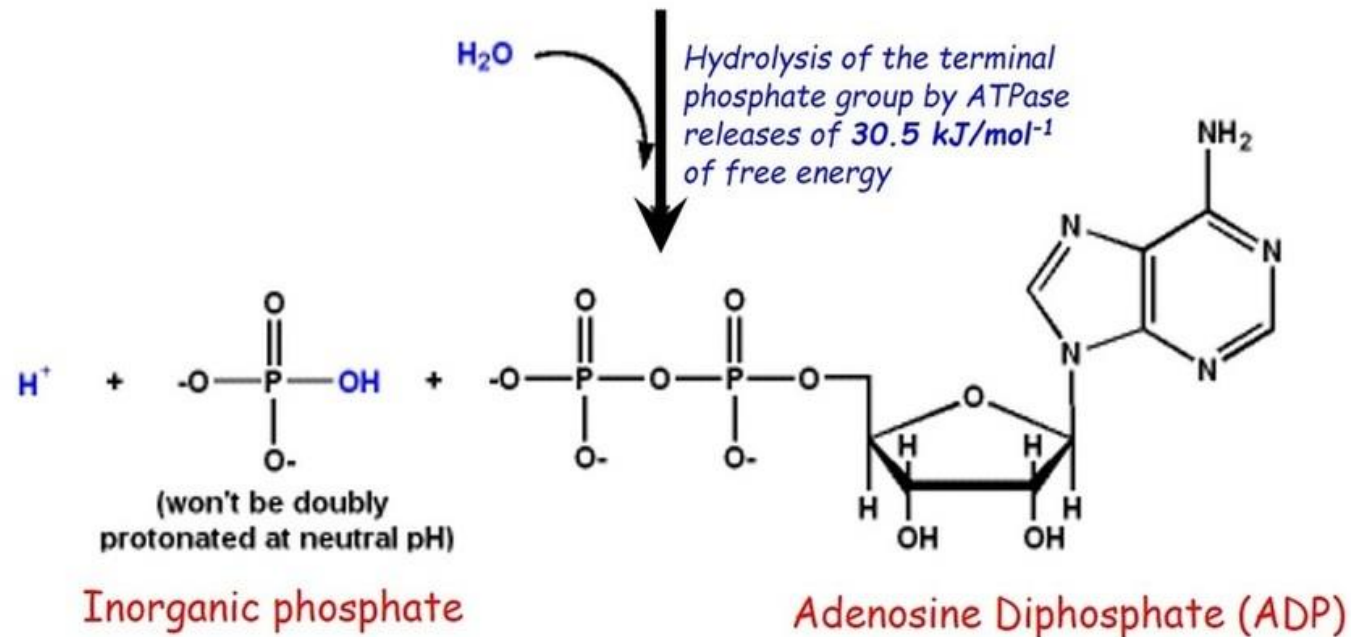
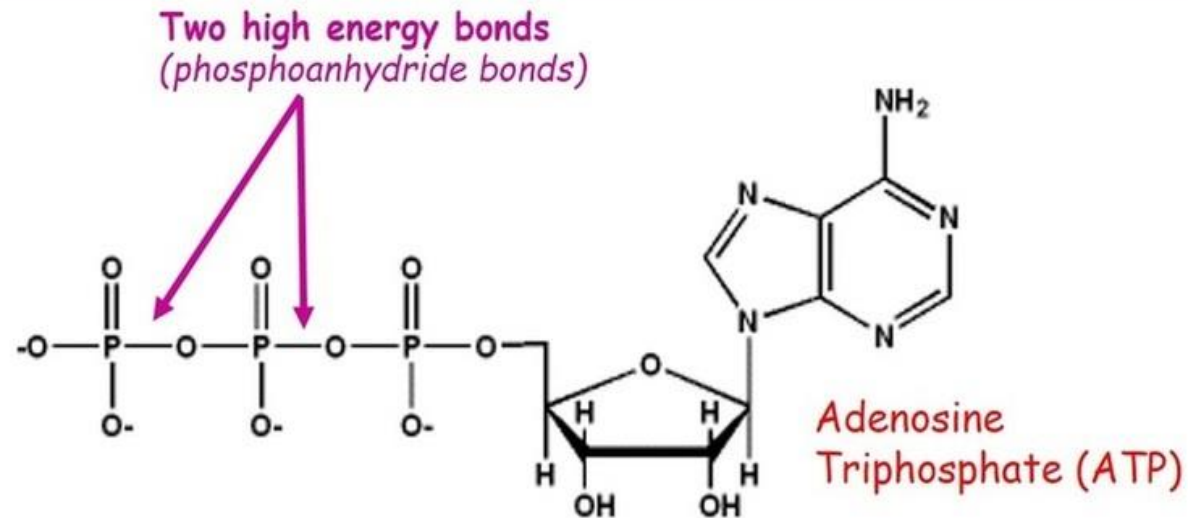
When a single bead trapped in optical tweezers is perturbed from equilibrium, for example, by protein conformational changes or by trap displacements, it moves exponentially to a new equilibrium position with a time constant (relaxation time) $\tau = \gamma/k$, where γ is the viscous drag coefficient and k the stiffness of the system. Therefore, systems with higher stiffness attached to small probes exhibit faster responses to perturbations. If the perturbation develops faster than τ , the bead moves with the same relaxation time τ , filtering out all the movements that occur on shorter time scales.

AFM : $k = 1 \text{ pN/nm} \rightarrow \tau - \text{microseconds}$, OT : $k = 0.001 \text{ pN/nm} \rightarrow \tau - \text{milliseconds}$

Another example of ligand – Adenosine triphosphate – ATP enzyme

ATP is an organic compound that provides energy to drive many processes in living cells, e.g. muscle contraction, nerve impulse propagation, condensate dissolution, and chemical synthesis.





ATP and energy release

When energy is needed inside living cells, the enzyme **ATPase** hydrolyses the bond between the second and third phosphate group in ATP, removing the third group and leaving only two.

The ATP molecule is hydrolysed into **adenosine diphosphate (ADP)** and an **inorganic phosphate ion** with the **release of chemical energy**.

Every **mole of ATP** that is hydrolysed releases **30.6 kJ** when the bond is broken.

